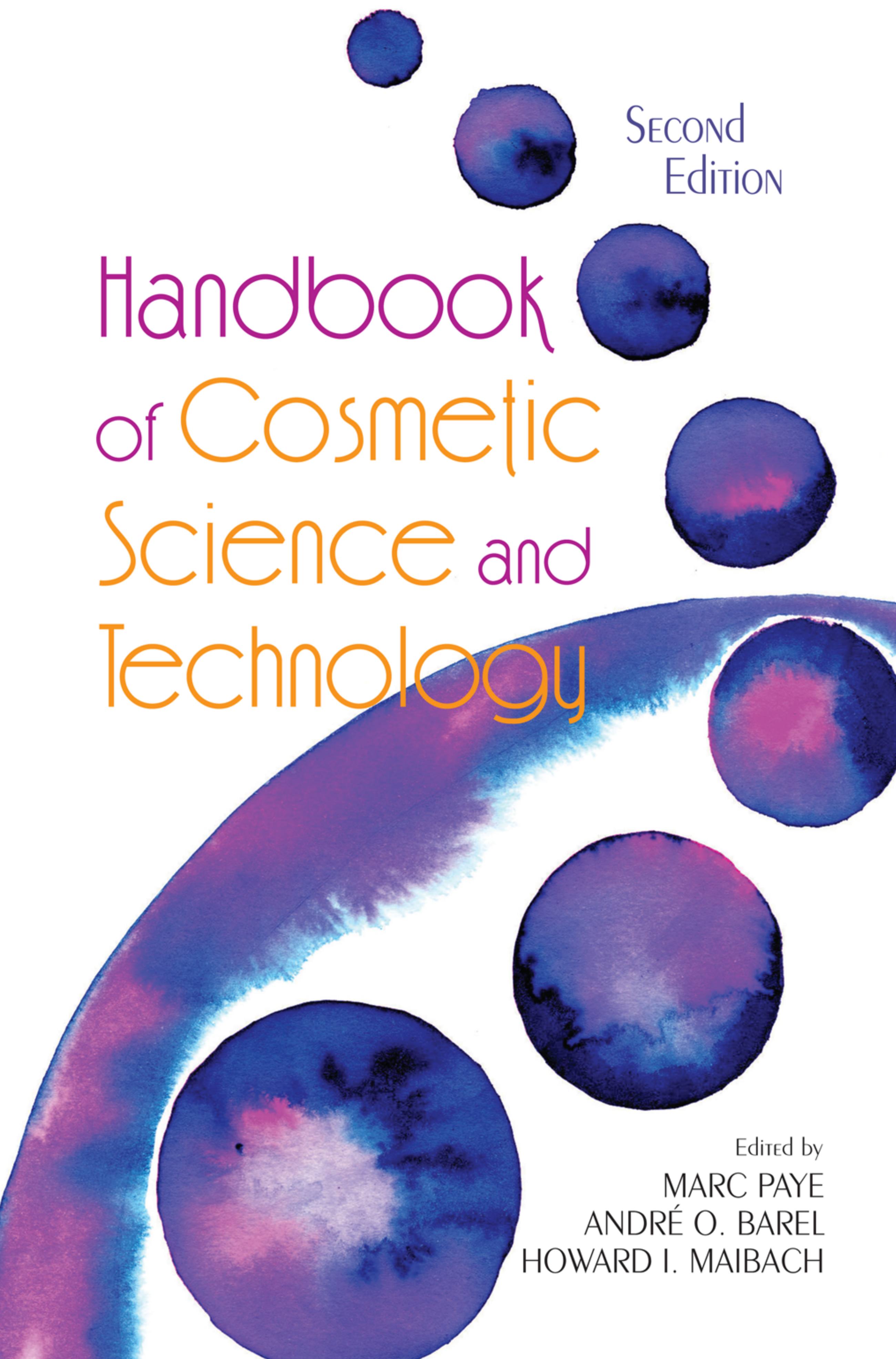

Second Edition
Handbook of Cosmetic Science and Technology
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Handbook of Cosmetic Science and Technology

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Preface

In 2001, we published the first edition of the *Handbook of Cosmetic Science and Technology* with 71 chapters written by leading experts in their field of cosmetology, who have largely contributed to the international success of the Handbook.

Since publication, comments were collected from readers and reviewers to detect improvements that could be added to this edition. Most feedback was highly positive as illustrated by some of the following: "...*excellent overall coverage of most aspect of cosmetology*...;" "... *contains a lot of scientific information about the physical properties of cosmetic ingredients* ...;" "... *an excellent balance of authors from major cosmetic houses and with many academic leaders coming from a huge range of countries provides an international view of cosmetics*...;" "... *an extensive and comprehensive index can be considered as a measure of the book's value*...." This feedback was highly appreciated and motivated us in continuing the adventure and in initiating a second edition that, we hope, will receive the same success as the first one.

Like in all first editions, a few improvements were suggested and were taken into account; it was mainly to develop a more systematic chapter organization as well as making some chapters more accessible and readable for nonexpert readers. Furthermore, cosmetology is, today, a fast moving science with new ingredients, new technologies, and changing regulations. Thus, it was necessary to publish a second edition to remain an up-to-date and practical *Handbook of Cosmetic Science and Technology*.

The objectives pursued with the second edition are multiple. Most chapters, recognized as essential for the cosmetologist, were kept but simplified, reviewed for overlapping with others, made more readable, and mainly updated with new developments or new anticipated trends. Some chapters had to be largely revisited such as in Part VI: Regulatory and Safety Considerations, that is probably the fastest changing field. Many chapters were added to cover new ingredients and technologies identified by the editors. That is mainly evident in Part III: Cosmetic Ingredients where many new, active, and promising ingredients have emerged. Testing the products has also improved, in terms of physicochemistry as well as in cell culture models or in skin measuring techniques. Chapters were added or re-designed to reflect such an evolution. Finally, some gaps in the first edition were filled with chapters on additional product types, adding more emphasis on ethnic skin and its differences in cosmetics requirements.

The editors are grateful to the authors, who contributed to the previous edition and updated their chapters, and to the new authors who agreed to share their experiences on emerging subjects, sometimes with unpublished information.

Finally, it is anticipated that future editions will benefit in the same way as this edition, from readers' suggested additions, deletions, and improvements.

Marc Paye
André O. Barel
Howard I. Maibach

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1
Introduction

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Although cosmetics for the purpose of beautifying, perfuming, cleansing, or for rituals have existed since the origin of civilization, only in the 20th century great progress has been made in the diversification of products and functions and in the safety and protection of the consumer.

Before 1938, cosmetics were not regulated as drugs, and cosmetology could often be considered as a way to sell dreams rather than objective efficacy; safety for consumers was also sometimes precarious. Subsequently, the Food and Drug Association, through the Federal Food Drug and Cosmetic Act, regulated cosmetics which were required to be safe for the consumer.

With industrialization, many new ingredients from several industries (oleo- and petrochemical, food, etc.) were utilized in preparation of cosmetics offering a list of new functions and forms. For a better control of these ingredients, U.S. laws required ingredient classification and product labeling since 1966.

Finally, the latest innovation in the field of cosmetics is the development of active cosmetics (cosmeceuticals in the United States). Currently, cosmetics not only intend to improve the appearance or odor of the consumer but also intend to benefit their target, whether it is the skin, the hair, the mucous membrane, or the tooth. With this functional approach, products became diversified and started to claim a multitude of biologic actions. The cosmetic market then greatly extended with millions of consumers worldwide. The competitive environment pushed manufacturers to promise more to the consumers and to develop cosmetic products of better quality and higher efficacy. Today, many cosmetic products aim at hydrating skin, reducing or slowing the signs of aged skin, or protecting the skin against the multitude of daily environmental aggressions. In order for cosmetic products to support these activities, raw materials became more efficacious, safe, bioavailable, and innovative, while remaining affordable. With the continuous improvement of the basic sciences and

the development of new sciences (e.g., molecular biology), new sources for pure raw materials have been found. Raw materials are not only produced from natural sources and highly purified, but they can also be specifically synthesized or even produced from genetically manipulated microorganisms. However, the availability and use of these sophisticated and active ingredients are not always sufficient for them to be optimally delivered to their targets and to sustain their activity. The cosmetic vehicle is also crucial to obtain this effect, and the role of the formulator is to combine the right ingredient and the appropriate vehicle.

Additional sciences also developed in parallel to active cosmetology and contributed significantly to its rise; this is the case for biometric techniques, which have been developing for more than three decades and allow a progressive and noninvasive investigation of many skin properties. Instruments and methods are now available to objectively evaluate and measure cutaneous elasticity, topography, hydration, turnover rate, or even to see directly in vivo inside the skin through microscope evolution. Major innovations in the field are reported by the International Society for Bioengineering and the Skin. Guidelines for the appropriate usage of instrumental techniques and for the accurate measurement of skin function and properties are regularly published by expert groups such as the Standardization Group of the European Society of Contact Dermatitis or the European Group for Efficacy Measurement of Cosmetics and Other Topical Products. Today, any claimed effect of a cosmetic on the skin should find appropriate techniques for a clear demonstration.

For better protection of the consumer against misleading claims, National or Federal laws prohibit false advertisements on cosmetic products. In Europe, the Sixth Amendment of the European Directive on Cosmetic Products requires manufacturers to have readily available a dossier with the proof of the claims made on their products. The Seventh Amendment of the European Directive, published in March 2004, among several other requirements explained later in this book, also made information about the product more easily accessible to the public by any appropriate means, including electronic means.

Finally, the recent evolution of cosmetic products and the constraints imposed on the cosmetic manufacturer lead cosmetology to largely increase its credibility in front of scientists, physicians, and consumers. Cosmetology has become a science based on the combination of various expertise domains: chemistry, physics, biology, bioengineering, dermatology, microbiology, toxicology, statistics, and many others.

Because of such a complexity in cosmetic science, it was not possible to cover in a useful manner all the aspects in one book. Details of most of the above fields are covered in the different volumes of the "Cosmetic Science and Technology" series. In the first edition of the "Handbook of Cosmetic Sciences and Technologies," we especially aimed at producing a useful guide and a source of ideas for the formulation of modern cosmetics. Four years later, new ingredients, more sophisticated products, more functional vehicles, and more sensitive testing methods have emerged. About 20 chapters were added to those of the previous edition, while about 80% of the others were updated. The outstanding contributors reviewed the major ingredients, the major technologies, and the up-to-date regulations throughout the world that the formulator needs to know. For more experienced scientists, recent innovations in terms of ingredients and cosmetic vehicle forms are described, which should orient the type of products of tomorrow. Finally, the large overview of cosmetic formulations should serve the dermatologist who is daily faced with patients who request recommendation for the most appropriate product for their skin type or who have

specific intolerance to an ingredient. This should help them to better understand cosmetics.

For easier access to the information contained within, the second edition of the handbook has been reorganized and subdivided into six parts, including several chapters written by different authors. It could seem to some a large number of contributors, but it is intentionally that the editors chose that form, to guarantee that each subject be described by a recognized expert in its field and well aware of the latest development in that topic. Also, authors were selected worldwide. Indeed, cosmetology is universal, but there exists some regional specificity, which needs to be addressed.

The first part introduces the reader with a description of the anatomy and physiology of the body targets for cosmetics: skin, hair, and nails.

The second part covers cosmetic vehicles with a special emphasis on a few types of recently introduced delivery systems, such as cosmetic patches, encapsulation of actives, special liposomes, and iontophoresis. The third part describes cosmetic ingredients. For some categories of ingredients, the most useful information is a list of ingredients that exist with a critical analysis of the advantages and disadvantages. For others, however, a good understanding of the role of an ingredient in a product is needed, of its limitations, mechanism of action, and regulatory constraints.

Part four, the largest one, is the core of the handbook and provides guidance to the formulation of skin cleansing products, skin care products, hair products, oral care products, and decorative products. Finally specific chapters cover the special cosmetics for baby and elderly consumers or for men.

In the fifth part, the stability control of cosmetic products is described, as well as an overview on the in vitro and clinical tests used for proving the efficacy and tolerance of the products. Finally, the last part compares the cosmetic legislation in the United States, Europe, and Japan, and provides useful information about safety terminology and description of the principles and mechanism of unwanted interactions of cosmetics with their targets.

Given the number of contributions, it has been a challenge to edit this second edition, only four years after the first; if it has been possible, this is due to the dedication of the authors and to the continuous follow-up made with the authors by Mrs S. Beberman and A. Tucker from Marcel Dekker Inc. We thank all of them for making this enormous task easy, enjoyable, and mainly feasible.

2
The Microscopic Structure of the Epidermis and Its Derivatives

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A general review of the microscopic structure of the epidermis and those epidermal derivatives that are distributed widely over the skin and, therefore, of interest in considerations of mechanisms of percutaneous absorption, will be presented here. Both light and electron microscopic information will be discussed to give an integrated brief summary of the basic morphological picture.

The epithelial component of the skin, the epidermis, is classified histologically as a stratified squamous keratinizing epithelium. It is thickest on the palms and soles (Fig. 1) and thinner elsewhere on the body (Fig. 2). It lies on the connective tissue component of the skin, the dermis, in which are located the blood vessels and lymphatic vessels. Capillary loops in the dermis come to lie in close apposition to the underside of the epidermis. The epidermis, in common with other epithelia, is avascular. The living cells of the epidermis receive their nutrients by diffusion of substances from the underlying dermal capillaries through the basement membrane and then into the epithelium. Metabolic products of the cells enter the circulation by diffusion in the opposite direction.

For other epithelia, the epidermis lies on a basement membrane (basal lamina). This extracellular membrane, interposed between the basal cells of the epidermis and the connective tissue of the dermis, serves the important function of attaching the two tissues to each other. The point of contact of the epidermis with this structure is the basal cell membrane of the basal cells. Along this surface the basal cells show many hemidesmosomes, which increase the adherence of the basal cells (and therefore of the entire epidermis) to the basement membrane (and therefore to the dermis). In some locations, such as the renal glomerulus, the basal lamina also has been shown to play a role as a diffusion barrier to certain molecules.

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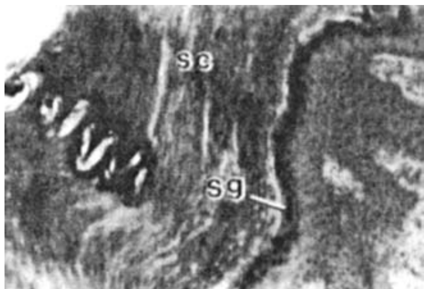

Figure 1 Thick epidermis from sole. The spiral channel through the extremely thick stratum corneum (sc) carries the secretion of a sweat gland to the surface. The stratum granulosum (sg) stands out clearly because its cells are filled with keratohyalin granules that stain intensely with hematoxylin. Hematoxylin and eosin, $\times 100$.

The plane of contact between the epidermis and dermis is not straight, but is an undulating surface, more so in some locations than others. Upward projections of connective tissue, the dermal papillae, alternate with complementary down growths of the epidermis. This serves to increase the surface area of contact between the two and presumably, therefore, the attachment.

Within the epidermis are found four different cell types with different functions and embryologic origins, namely, keratinocytes, melanocytes, Langerhans cells, and Merkel cells. These will be considered in turn.

The keratinocytes are derived from the embryonic surface ectoderm and differentiate into the stratified epithelium. Dead cells are constantly sloughed from the upper surface of the epidermis and are replaced by new cells being generated from the deep layers. It is generally considered that the basal layer is the major source of cell renewal in the epidermis. Lavker and Sun (1) distinguish two types of basal cells, a stem cell type and a type that helps anchor the epidermis to the dermis, and an actively dividing suprabasal cell population. The basal cells have desmosomes connecting them to the surrounding cells and, as mentioned earlier, hemidesmosomes along the basal lamina surface. They have tonofilaments coursing through the cytoplasm and coming into close apposition to the desmosomes. These protein filaments are of the intermediate filament class and are made up principally of

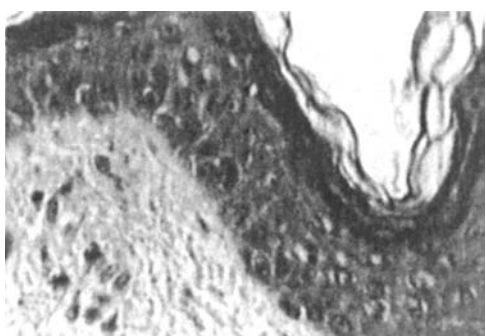

Figure 2 Thin epidermis. The strata spinosum, granulosum, and corneum are considerably thinner than in Figure 1. Hematoxylin and eosin, $\times 200$.

keratin. Basal cells have the usual cell organelles and free ribosomes, the site of synthesis of intracytoplasmic proteins.

As a result of the proliferation of cells from the deeper layers, the cells move upward through the epidermis toward the surface. As they do, they undergo differentiative changes which allowed microscopists to define various layers. The cells from the basal layer enter the stratum spinosum, a layer whose thickness varies according to the total thickness of the epidermis. The layer derives its name because, with light microscopic methods, the surface of the cell is studded with many spiny projections. These contact similar projections from adjacent cells and the structure was called an intercellular bridge by early light microscopists (Fig. 3). Electron microscopy showed that the so-called "intercellular bridges" were really desmosomes, and the light microscopic appearance is an indication of how tightly the cells are held by each other at these points. The number of tonofilaments increases in the spinous cells (prickle cells) and they aggregate into coarse bundles—the tonofibrils—which were recognizable to light microscopists using special stains.

Electron microscopy reveals the formation within the spinous cells of a specific secretory granule. These small, membrane-bound granules form from the Golgi apparatus and are the membrane-coating granules (MCG; lamellar bodies; Odland bodies). They contain lipids of varying types, which have become increasingly characterized chemically (2,3).

As the cells of the stratum spinosum migrate into the next layer there appear in their cytoplasm large numbers of granules that stain intensely with hematoxylin. These are the keratohyalin granules and their presence characterizes the stratum granulosum. Electron microscopy shows that the granules are not membrane-bound but are free in the cytoplasm. Histidine-rich proteins (4,5) have been identified in the granules. The tonofilaments come to lie in close relationship to the keratohyalin granules. The membrane-coating granules are mainly in the upper part of the granular cell.

When observed by either light or electron microscopy there is an abrupt transformation of the granular cell into the cornified cell with a loss of cell organelles. In thick epidermis, the first cornified cells stain more intensely with eosin and this layer has been called the stratum lucidum. The interior of the cornified cell consists of the keratin filaments, which appear pale in the usual electron microscopic preparations, and interposed between them is a dark osmiophilic material. The interfilamentous

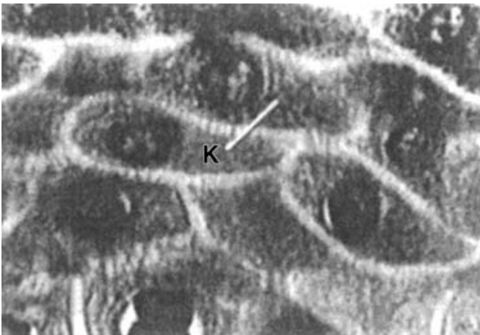

Figure 3 High power view of upper part of stratum spinosum and lower part of stratum granulosum. Note that the many "intercellular bridges" (desmosomes) running between the cells, gives them a spiny appearance. When the cells move up into the stratum granulosum, keratohyalin granules (k) appear in their cytoplasm. Hematoxylin and eosin, ×1000.

matrix material has been shown to have derivations from the keratohyalin granule and is thought to serve the function of aggregation of the keratin filaments in the cornified cell (4,5).

In the uppermost cells of the granular layer the membrane-coating granules move toward the cell surface, their membrane fuses with the cell membrane, and their lipid contents are discharged into the intercellular space. Thus, the intercellular space in the cornified layer is filled with lipid material, which is generally thought to be the principal water permeability barrier of the epidermis (2,3). The stratum corneum has been compared to a brick wall, with the bricks representing the cornified cells, surrounded completely by mortar, representing the MCG material (6).

The cornified cell is further strengthened by the addition of protein to the inner surface of the cell membrane. Two proteins that have been identified in this process are involucrin (7,8) and keratolinin (9). A transglutaminase crosslinking of the soluble proteins results in their fusion to the inner cell membrane to form the tough outer cell envelope of the cornified cell. Desmosomes between the cells persist in the cornified layer.

It can be seen that formation of an outer structure (stratum corneum) which can resist abrasion from the outside world and serve as a water barrier for a land-dwelling animal has proven incompatible with the properties of living cells. The living epidermal cells, therefore, die by an extremely specialized differentiative process that results in their nonliving remains having the properties that made life on land a successful venture for vertebrates.

Distributed among the keratinocytes of the basal layer are cells of a different embryologic origin and function, the melanocytes. In the embryo, cells of the neural crest migrate from their site of origin to the various parts of the skin and take up a position in the basal layer of the epidermis. They differentiate into melanocytes and extend long cytoplasmic processes between the keratinocytes in the deep layers of the epidermis. Because they contain the enzyme tyrosinase, they are able to convert tyrosine to dihydroxyphenylalanine (dopa) and the latter to dopaquinone with the subsequent formation of the pigmented polymer melanin. The tyrosinase is synthesized in the rough endoplasmic reticulum and transferred to the Golgi body. From the latter organelle, vesicles with an internal periodic structure containing tyrosinase are formed. These are the melanosomes, the melanin-synthesizing apparatus of the cell. Melanin is formed within the melanosome, and as it accumulates the internal structure of the melanosome becomes obscured. In light microscope the pigmented melanosome appears as the small brown melanin granule. The melanin granules are then transferred from the melanocyte's cytoplasmic extensions to the keratinocytes, and become especially prominent in the basal keratinocyte's cytoplasm. In this position their ability to absorb ultraviolet radiation has a maximal effect in protecting the proliferating basal cell's DNA from the mutagenic effects of this radiation. Within the keratinocyte, varying numbers of melanosomes are often contained within a single membrane-bound vesicle. The classic method of demonstrating melanocytes is the dopa test. Sections of skin are placed in a solution of dopa and only the melanocytes turn into a dark brown color (Fig. 4).

Within the epidermis is another population of cells which were first demonstrated by Langerhans in 1868. By placing skin in a solution of gold chloride, he showed that a number of cells in the epidermis, particularly in the stratum spinosum, turned black. The cytoplasmic extensions of the cell give them a dendritic appearance. For many decades the nature of this cell type was unknown, including whether it was a living, dead, or dying cell. Electron microscopy showed that it was a viable

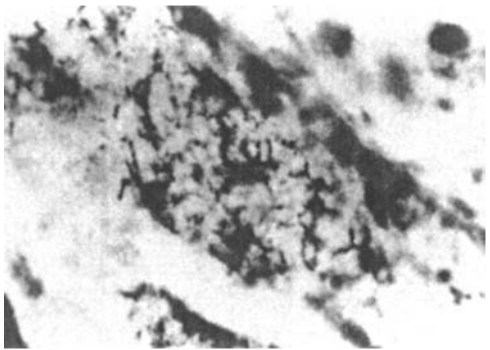

Figure 4 A thick section of the epidermis was made with the plane of section running parallel to the surface of the skin and including the deep layers of the epidermis. Dopa reaction shows whole melanocytes on surface view, illustrating their branching, dendritic nature (×340).

cell in appearance, lacked desmosomes, and possessed a very unusual cytoplasmic structure—the Birbeck granule.

With the development of methods for identifying cell membrane receptors and markers in immune system cells, it was shown that Langerhans cells originate in the bone marrow. They are now thought to be derived from circulating blood monocytes, with which they share common marker characteristics. The monocytes migrate into the epidermis and differentiate into Langerhans cells. Considerable evidence shows that these dendritic cells capture cutaneous antigens and present them to lymphocytes in the initiation of an immune response. Their population in the epidermis is apparently constantly replenished by the blood-borne monocytes.

Finally, a fourth cell type, the Merkel cell, can be found in the epidermis. These appear to be epithelial cells and are found in the basal layer. A characteristic feature is the presence of many small, dense granules in their cytoplasm. Sensory nerve endings form expanded terminations in close apposition to the surface of Merkel cells.

Hair follicles begin their formation as a down growth of cells from the surface epidermis into the underlying connective tissue. The growth extends into the deep dermis and subcutaneous tissue and forms in the deepest part of the structure a mass of proliferative cells—the hair matrix. The cells of the outermost part of the hair follicle, the external root sheath, are continuous with the surface epidermis. The deepest part of the hair follicle is indented by a connective tissue structure, the hair papilla, which brings blood vessels close to the actively dividing hair matrix cells (Fig. 5). As the cells in the matrix divide, the new cells are pushed upward toward the surface. Those moving up the center of the hair follicle will differentiate into the hair itself. The structure of the hair, from the center to the outer surface, consists of the medulla (when present), the cortex, and the cuticle. The cortex forms the major part of the hair. These cells accumulate keratin to a very high degree. They do not die abruptly as in the case of the surface epidermis. Instead, the nucleus of the cell gradually becomes denser and more pyknotic and eventually disappears. Keratohyalin granules are not seen with the light microscope. Cells moving up from the matrix into the region between the hair and the external root sheath form the internal root sheath. Here, the cells adjacent to the hair form the cuticle of the internal root sheath. Next is Huxley's layer and, adjacent to the external root sheath, Henle's

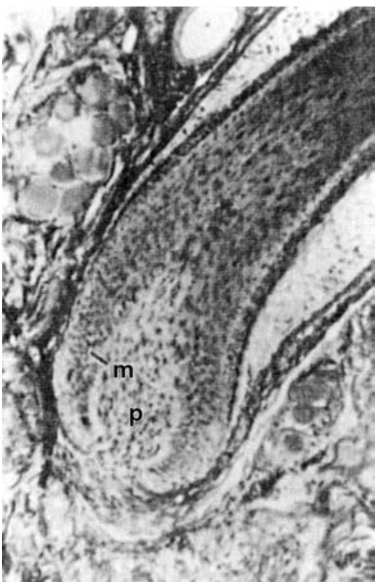

Figure 5 The connective tissue hair papilla (p) indents into the base of the hair follicle. The follicle cells in the hair matrix region (m) show many mitotic figures. Iron hematoxylin and aniline blue, ×150.

layer. These cells accumulate conspicuous trichohyalin granules in their cytoplasm in the deeper part of the internal root sheath. The cells of the internal root sheath disintegrate higher up in the hair follicle and disappear at about the level of the sebaceous gland. Thereafter, the hair is found in the central space of the hair follicle without a surrounding internal root sheath.

When viewed with the light microscope, the hair follicle is surrounded by an exceedingly thick basement membrane called the glassy membrane. Scattered among the keratinocytes in the hair matrix are melanocytes, which transfer pigment to the forming hair cells and give the hair color. Hair growth is cyclic, with each follicle having alternating periods of growth and rest.

About a third of the way down the hair follicle from the surface epidermis, the sebaceous glands connect to the hair follicle. The sebaceous alveoli consist of a rounded, solid mass of epithelial cells surrounded by a basement membrane. The outer cells proliferate and the newly formed cells are pushed into the interior of the sebaceous alveolus. As they move in this direction they accumulate a complex of lipids and lipid-like substances. As the lipids fill the cell it begins to die and the nucleus becomes often pyknotic. The cells eventually disintegrate, releasing their oily contents via a short duct into the space of the hair follicle (Fig. 6). This is the classic example of holocrine secretion where the entire gland cell becomes the secretion. In some scattered locations (e.g., nipple) sebaceous glands can be found independent of the hair follicle. In other areas their size relative to the hair follicle is very large (Fig. 7). As the lipids are extracted in the usual histologic preparations, the cells typically appear very pale.

The major type of sweat gland in the human, the eccrine sweat gland, is distributed over, practically, all parts of the body. It produces a watery secretion which is conveyed to the surface of the skin where its evaporation plays an important

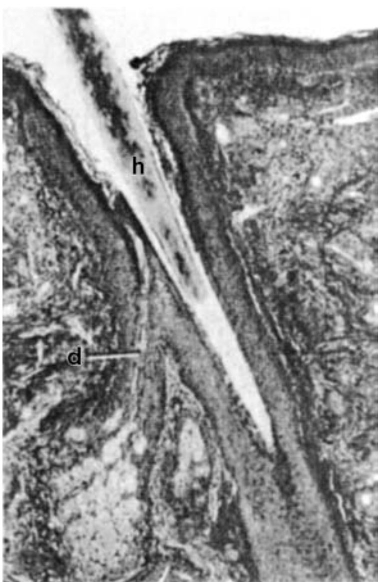

Figure 6 Upper part of hair follicle. The hair (h) is shown emerging from the follicle (the lower part of the hair passed out of the plane of section). The sebaceous gland is shown emptying its secretion via the duct (d) into the space of the follicle. Iron hematoxylin and aniline blue, ×50.

thermoregulatory role. The eccrine glands arise as tubular down growths from the surface epidermis independent of hair follicles. The tubule extends deep into the dermis or the subcutaneous tissue level where it becomes coiled. The eccrine gland, therefore, is a simple coiled tubular gland.

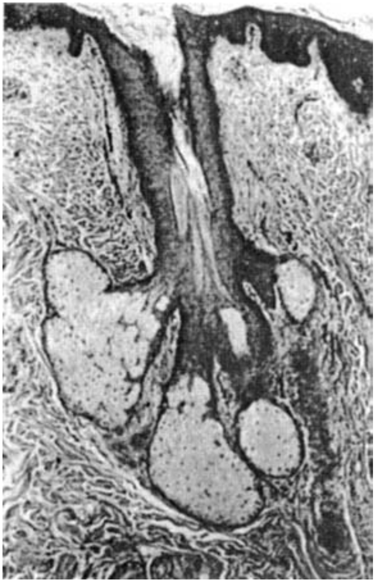

Figure 7 Sebaceous glands in skin of forehead. Hematoxylin and eosin, ×50.

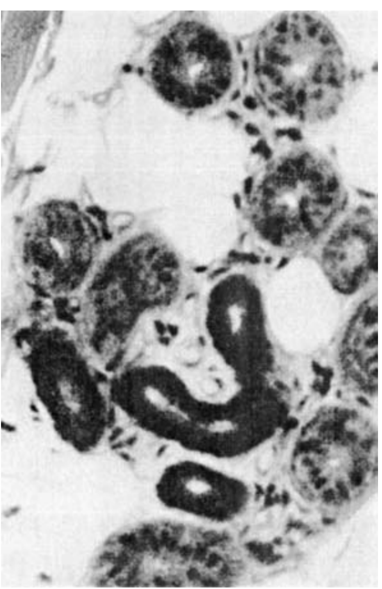

Figure 8 Section through a sweat gland. The pale structures are part of the secretory-coiled tubule, and the dark ones are part of the duct. Hematoxylin and eosin, ×250.

The coiled segment at the blind-ending terminus represents the secretory portion of the gland. This leads to the duct portion of the gland, which is also coiled. The duct then ascends toward the surface. When it reaches the underside of the epidermis a spiralling channel through it conveys the secretion to the skin surface (Fig. 1). It is not understood how this channel remains patent in an epidermis whose keratinocytes are constantly proliferating and migrating.

When viewed with the light microscope, the two parts of the gland can be easily distinguished from each other (Fig. 8). Compared to the duct, the secretory portion is wider, has a larger lumen, its epithelial lining cells appear pale, and many myoepithelial cells are present. The latter are contractile cells that are part of the epithelium, lying within the basement membrane. Their contraction is thought to forcefully expel the secretion towards the skin surface. With the electron microscope, two types of epithelial lining cells are seen in the secretory portion. The so-called dark cells have an extensive contact with the lumen of the tubule and have secretory granules containing glycoprotein substances. The clear cells are distinguished by abundant glycogen in their cytoplasm. Continuous with the tubule lumen are many intercellular canaliculi between the clear cells. It is thought that the clear cells secrete a more or less isotonic solution via these channels into the lumen. The duct portion is lined by two layers of epithelial cells and lacks myoepithelial cells. It is thought that electrolytes are absorbed from the lumen here, making the sweat hypotonic by the time it reaches the surface of the skin.

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REFERENCES

1. Lavker RM, Sun T. Heterogeneity in epidermal basal keratinocytes: morphological and functional correlations. Science 1982; 215:1239–1241.
2. Grayson S, Elias PM. Isolation and lipid biochemical characterization of stratum corneum membrane complexes: implications for the cutaneous permeability barrier. J Invest Dermatol 1982; 78:128–135.
3. Wertz PW, Downing DT. Glycolipids in mammalian epidermis: structure and function in the water barrier. Science 1982; 217:1261–1262.
4. Lynley AM, Dale BA. The characterization of human epidermal filaggrin: a histidine-rich, keratin filament-aggregating protein. Biochim Biophys Acta 1983; 744:28–35.
5. Murozuka T, Fukuyama K, Epstein WL. Immunochemical comparison of histidine-rich protein in keratohyalin granules and cornified cells. Biochim Biophys Acta 1979; 579:334–345.
6. Elias PM. Stratum corneum lipids in health and disease. Fleischmajer R, ed. Progress in Diseases of the Skin. Vol. 2 San Diego: Grune and Stratton, 1984:1–19.
7. Banks-Schlegel S, Green H. Involucrin synthesis and tissue assembly by keratinocytes in natural and cultured human epithelia. J Cell Biol 1981; 90:732–737.
8. Simon M, Green H. Participation of membrane-associated proteins in the formation of the cross-linked envelope of the keratinocyte. Cell 1984; 36:827–834.
9. Zettergren JG, Peterson LL, Wuepper KD. Keratolinin: the soluble substrate of epidermal trans-glutaminase from human and bovine tissue. Proc Natl Acad Sci USA 1984; 81: 238–242.

3
Racial (Ethnic) Differences in Skin Properties

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INTRODUCTION

Racial (ethnic) differences in skin properties may explain racial disparities seen in dermatologic disorders and provide insight into appropriate differences in the management of these disorders. However, racial differences in skin have been minimally investigated by objective methods and the data are often contradictory. Objective methods studied include transepidermal water loss (TEWL), water content (WC), corneocyte variability, blood vessel reactivity, elastic recovery/extensibility, pH gradient, lipid content, surface microflora, microscopic evaluation of mast cell granules, and confocal microscopy.

The majority of the evidence (six out of eight studies) indicates that TEWL is greater in black skin compared with white skin. TEWL measurements of Asian skin are inconclusive, as they have been found to be equal to those of black skin and greater than those of Caucasian skin, equal to those of Caucasian skin, and less than all those of other ethnic groups in different studies. Racial differences in WC, as measured by resistance, capacitance, conductance, and impedance, are also inconclusive as the data are contradictory. Whereas the evidence regarding corneocyte desquamation is minimal, one clinically provocative observation is that blacks have a 2.5 times greater spontaneous desquamation rate compared with Caucasians and Asians, possibly accounting for an increased frequency of xerosis seen clinically in blacks.

With regard to blood vessel reactivity, studies cannot be compared to each other because each uses different vasoactive substances. However, each study, except for one study comparing Hispanics and whites and another comparing Japanese and German women, reveals some degree of racial variation in blood vessel reactivity. It has been demonstrated that the pH of the black skin is less than that of the white skin; however, the studies that have demonstrated this have done so under different skin conditions and on different anatomic sites. Studies on racial differences in lipid content are inconclusive. Additionally, there is insufficient and conflicting evidence to make conclusions regarding racial differences in both skin biomechanics and skin microflora. Microscopic evaluation reveals that black skin contains larger mast cell

granules, and there are differences in its structural properties and enzymes of mast cells compared with white skin, possibly accounting for differences in pruritus experienced by the individuals of these racial groups.

There exists substantial evidence to support that black skin has a higher TEWL, variable blood vessel reactivity, decreased skin surface pH, and larger mast cell granules compared with white skin. Although some deductions have been made about Asian and Hispanic skin, further evaluation needs to be done. Differences in WC, corneocyte desquamation, elastic recovery/extensibility, lipid content, and skin microflora, although statistically significant, are inconclusive.

Racial (ethnic) differences in skin physiology have been minimally investigated. The current experimental human model for skin is largely based upon physical and biochemical properties known about Caucasian skin. Thus, anatomical or physiological properties of skin in different races that may alter a disease process or treatment of that disease are not being accounted for.

Early studies show similarities in black and white skin. For example, Thomson (1) and Freeman et al. (2) conclude that the stratum corneum is of equal thickness in blacks and whites. However, in 1974, Weigand et al. (3) demonstrated a difference in black and white skin with regard to a variable other than color. They demonstrated that the stratum corneum of black skin contains more cell layers and black skin requires more cellophane tape strips to remove the stratum corneum than white skin. Greater variability in the number of tape strips used within the black subject pool was also found compared with the white subject pool, but this variability was not correlated with degree of skin pigmentation. Since stratum corneum thickness is believed to be equal (1,2), the data reflected greater intercellular adhesion among the black individuals (3). Recently developed quantitative techniques for determining stratum corneum mass are yet to be utilized for this purpose (4).

While Weigand et al. (3) objectively demonstrated differences in physical properties in black and white skin, some other studies demonstrating differences used more subjective methods. For example, erythema has been used as a measure of demonstrating skin irritation (5–7). Since erythema is difficult to assess in a person with dark skin, such subjective methods are not sufficient in evaluating racial disparities. Thus, in order to accurately report racial differences in skin properties, objective methods should be utilized. Objective measurements that have been studied are TEWL, WC (via conductance, capacitance, resistance, and impedance), corneocyte variability, blood vessel reactivity, elastic recovery/extensibility, pH gradient, lipid content, surface microflora, electron microscopy and immunoelectron microscopy of mast cell granules, and confocal microscopy of epidermal innervation. Even though these objective methods have been used to compare skin of different races, the data that exist remain minimal. Additionally, the data are often confusing and difficult to interpret. We explore and attempt to clarify the objective data, other than color, available in differentiating skin properties of different races.

We searched MEDLINE, MD Consult, Science Citations Index, the Melvyl Catalogue in the CDL-Hosted Database of the University of California (San Francisco, California, U.S.), Yahoo®, Google®, standard dermatology textbooks, and the University of California (San Francisco, California, U.S.) surge building library files from 1967 to October 2002. Keywords in searches included words pertaining to race (i.e., race, ethnicity, black, African, white, Caucasian, Asian, and Hispanic) and dermatology (i.e., skin, skin physiology, and skin function). The references of each study were then reviewed for other studies that examined racial differences with

objective methods. Studies pertaining to racial differences in skin color and hair were excluded to keep the review focused on skin function/physiology. Words used to describe race/ethnicity of study individuals are the same as those used by the authors in the respective texts.

TRANSEPIDERMAL WATER LOSS

One role of the skin is to maintain an effective barrier against loss of body fluids and absorption of externally applied substances (8). The total amount of water vapor passing through the skin can be classified into water vapor passing the stratum corneum by passive diffusion and water vapor loss as a result of sweating (9). Baseline water diffusion (imperceptible or unnoticed perspiration) amounts to 2.25 $\mu L/m^2/sec$ and is distinct and separate from sweat gland secretion (10). Originally, the term "TEWL" was used to indicate the amount of water vapor passing through the stratum corneum by passive diffusion (9). Current literature, however, refers to TEWL as the total amount of water vapor loss through the skin and appendages, under nonsweating conditions (9). Therefore, note that TEWL is a true reflection of stratum corneum barrier function only when there is no sweat gland activity. In addition to characterizing the water barrier function of skin, measurement of TEWL has been utilized widely in studies to perform predictive irritancy tests and to evaluate the efficacy of therapeutic treatments on diseased skin (11). To date, TEWL is the most studied objective measure in defining differences among the skin of different ethnicities.

Wilson et al. (12) demonstrated higher in vitro TEWL values in black compared with white skin. Water evaporation measured from skin, taken from 10 African-American and 12 Caucasian cadavers matched for age and gender, was then converted to TEWL using an equation. In addition to finding differences in black and white skin physiology, the investigators also found that the TEWL of both races increased with skin temperature. These results were explained on the basis of a prior in vivo study from 1941 showing that blacks had a lower skin and rectal temperature during exercise (13). Thus, in maintaining equal temperatures between black and white skins, they concluded that it would be expected that black skin would have a greater rise in temperature to achieve the same endpoint temperature and, therefore, a higher TEWL (12). Although comparisons between in vitro and in vivo studies are frequently made in medicine, note that the in vitro study may not have accounted for some physiological functions, such as sweating. Also, accounting for physiologic temperature differences by race in skin may be difficult in an in vitro study. As TEWL depends on passive water vapor loss, and based on laws of physics with regard to passive diffusion, the rate of water vapor diffusion across the stratum corneum is theoretically directly related to the ambient relative humidity and temperature (14), and it is reasonable to assume that the increased TEWL in black skin is associated with an increase in temperature if, in fact, a difference in black and white skin temperature does exist.

A subsequent in vivo study by Berardesca and Maibach (20) supported the findings of the in vitro study. The investigators determined the difference in irritation between young black and white skins. They applied 0.5% and 2.0% sodium lauril sulfate (SLS), a water-soluble irritant (surfactant), to untreated, preoccluded, and predelipidized skin and quantified the resulting level of irritation using WC, TEWL, and laser Doppler velocimetry (LDV) of the stratum corneum. No statistical difference was found in irritation between the two groups based on WC and LDV; however,

a statistical difference in the TEWL results of 0.5% SLS applied to the preoccluded skin was found. In that test, blacks had 2.7 times higher TEWL levels than whites ($p = 0.04$), suggesting that blacks in the preoccluded state are more susceptible to irritation than whites. This theory opposes the traditional clinical view, based on observations of the erythema (7), that blacks are less reactive to irritants than whites.

Berardesca and Maibach (21) used the same model to compare differences in irritation between Hispanic and white skins. Although there were no significant differences in TEWL, WC, or LDV among the groups at baseline, the data showed higher values of TEWL for Hispanics compared with whites after SLS-induced irritation. However, these values were not statistically significant. The investigators noted that the reaction of Hispanic skin to SLS resembles that of black skin when irritated with the same substance (20). As skin pigmentation varies greatly within the Hispanic and black communities, the degree of skin pigmentation, according to Fitzpatrick's model (24), could represent an important variable.

However, in a later study, Berardesca et al. (15) found no significant difference in vivo in the TEWL between race or between anatomic site for baseline observation. The investigators examined 15 blacks, 12 whites, and 12 Hispanics to account for degree of skin pigmentation, matched for age and gender, and measured TEWL, WC (via skin conductance), skin thickness, and biomechanical properties, such as skin extensibility, at two sites, the dorsal and the volar forearm. Skin sites that vary in sun exposure were used to highlight the protective effects of melanin from ultraviolet-induced damage. Racial differences in skin conductance (blacks > whites) and skin elasticity were found and are discussed in sections "Water content" and "Elastic Recovery/Extensibility." However, even though the investigators expected a higher TEWL in blacks based on previous studies (12,20) and based on a higher WC (skin conductance) in blacks found in their current study, no significant difference in TEWL was found between races or between anatomic sites. They accounted for the higher WC in black skin with no racial differences in TEWL on the basis that black skin might have increased intercellular cohesion (3) and increased lipid content (25), keeping the water in.

In contrast, Kompaore et al. (17) found significantly higher TEWL values in blacks and Asians compared with whites. After an application of methyl nicotinate (a vasodilator), the investigators evaluated TEWL and lag time to vasodilatation by LDV, before and after removal of the stratum corneum by tape stripping. The participants were seven black men, eight white subjects (six males and two females), and six Asian men all living in France, aged 23 to 32 years, without skin disease. Before tape stripping, TEWL was 1.3 times greater in blacks and Asians compared with whites ($p < 0.01$); no difference was found between blacks and Asians. After eight and 12 tape strips, TEWL values were highest in Asians overall (1.7 times greater in Asians than in whites) ($p < 0.05$). The investigators concluded that, similar to in previous studies (12,20), skin permeability measured by TEWL was higher in blacks than in Caucasians. However, they also concluded that Asian skin had the highest permeability among the groups studied. Although the methods of this study were impressive and well documented, this finding has not yet been duplicated.

Sugino et al. (19) (abstract only) also included Asians in their study but found that baseline TEWL was, in decreasing order, blacks > Caucasians = Hispanics = Asians. Aramaki et al. (18) compared TEWL, stratum corneum hydration, sebum secretion, laser Doppler flowmetry, content of melanin, and erythema on forearm at baseline and after SLS-induced irritation in 22 Japanese women (mean age 25.84 years) and 22 German women (mean age 26.94 years). There were no significant

differences in TEWL between Japanese and German women before or after SLS stress. Only one other study (unpublished data), referenced in a review article (26) about Asian skin, has compared TEWL in Asians with that in Caucasians and also found no statistically significant differences at baseline or after tape stripping; however, no vasoactive substance was applied.

In an attempt to compare degree of skin pigmentation as opposed to race, Reed et al. (22) compared seven subjects with skin types V and VI (four African-Americans, two Filipinos, and one Hispanic) with 14 subjects with skin types II and III (six Asians and eight Caucasians). The investigators used TEWL to assess the ability of the stratum corneum to withstand or recover from insults to the epidermal permeability barrier (i.e., tape stripping). Subjects with skin type V/VI required more tape strippings (66.7 ± 6.9) compared to those with skin type II/III (29.6 ± 2.4) to achieve the same TEWL; i.e., skin type V/VI had increased barrier strength (integrity). These findings correlate with those of Weigand et al. (3) that black skin has more cell layers and increased intercellular adhesion. Furthermore, it was also found that water barrier function (measured by TEWL) in skin type V/VI recovered more quickly. This study demonstrated the differences in stratum corneum barrier function as measured by TEWL among different skin types possibly independent of race. As the sample size with skin types V and VI was small, further studies with larger sample sizes should be conducted to support these findings.

Warrier et al. (16) recognized the discrepancies in data comparing the skin of blacks and whites. Thus, in an attempt to clarify the data, the investigators studied TEWL, electrical capacitance, skin pH, elasticity, dryness/scaling, and skin microflora in 30 black and 30 white women aged 18 to 45 years. In contrast to all previous studies, which found an increase in TEWL in blacks compared with whites (12,17,20), Warrier et al. (16) found TEWL to be significantly lower on the cheeks (20% less) and legs (17% less) in blacks compared with whites ($p < 0.05$). TEWL was also lower on the forearms in blacks, but this was not statistically significant. Prior studies examined the forearm, the inner thigh, and the back. Does the anatomic site act as a confounding variable in obtaining TEWL values? In a study on Caucasian subjects, TEWL values of the posterior auricular and forehead stratum corneum were higher than that of the stratum corneum of the arm, the forearm, or the abdomen (27). Thus, perhaps there are also differences in TEWL when comparing the sites examined (cheeks and lower legs) by Warrier et al. (16) to those of prior studies (forearm, inner thigh, and back) (12,15,17,20,21). Although this study used a larger sample size, the discrepancy in data warrants further studies with large sample sizes and comparisons of various anatomic sites.

Berardesca et al. (23) examined differences in TEWL as well as pH in 10 Caucasian (skin types I and II) and eight African American (skin type VI) women at baseline and after tape strippings. TEWL increased for both races with each tape stripping. Interestingly, even though black women had a higher TEWL at baseline and after each tape stripping compared with Caucasian women, the differences were only statistically significant (1.2 times greater) after three ($p < 0.05$) and six ($p < 0.03$) tape strips. Similar to the study by Reed et al. (22), it was also found that recovery of water barrier function, as measured by TEWL 48 hours after stripping, was greater in blacks as compared with Caucasians, but the difference was not statistically significant.

While the data regarding TEWL (summarized in Table 1) are conflicting, the overall evidence, except for the 1991 study by Berardesca et al. (15), supports some difference between black and Caucasian skin. Most studies using the forearm, back, and inner thigh (12,17,19,20,22,23) showed a greater TEWL in blacks compared with

Table 1 Racial Differences in Skin Properties: Transepidermal Water Loss (TEWL)[a]

Study	Technique	No. of subjects	Site	Results
Wilson et al. (12) (1988)	In vitro	black 10 (mean age 38.6 yr) Caucasians 12 (mean age 41.1 yr)	Inner thigh	TEWL blacks 1.1× > Caucasians (mean corrected log TEWL 2.79 and 2.61 μg/cm^2/h, respectively) ($p < 0.01$ for both value)
Berardesca and Maibach (20) (1988)	In vivo—topical application of SLS (irritant)	black men 10 (age 29.9 ± 7.2 yr) white men 9 (age 30.6 ± 8.8 yr)	Back	No significant difference in TEWL between blacks and whites at baseline After SLS stress: TEWL blacks (untreated, pre-occluded, and pre-deplipidized) > whites but only statistically significant (2.7 × greater) for 0.5% SLS applied in the pre-occluded area ($p < 0.04$)
Berardesca and Maibach (21) (1988)	In vivo—topical application of SLS (irritant)	Hispanic men 7 (age 27.8 ± 4.5 yr) white men 9 (age 30.6 ± 8.8 yr)	Upper back	No significant differences in TEWL between Hispanics and whites at baseline After SLS stress: TEWL Hispanics (untreated, pre-occluded, and pre-delipidized) > whites, but not statistically significant
Berardesca et al. (15) (1991)	In vivo	blacks 15 (mean age 46.7 ± 2.4 yr) whites 12 (mean age 49.8 ± 2 yr) Hispanics 12 (mean age 48.8 ± 2 yr)	Volar and dorsal forearm	No significant difference in TEWL between site or race at baseline
Kompaore et al. (17) (1993)	In vivo—topical application of MN–vasodilator	blacks 7 Caucasians 8	Volar forearm	MN given Before tape stripping: TEWL blacks & Asians 1.3× > Caucasians ($p < 0.01$); no difference between blacks and Asians
		Asians 6 (ages 23–32 yr all)		After eight and 12 tape strips: TEWL Asians > blacks > Caucasians ($p < 0.05$) [Asians 1.7× > Caucasians]

Sugino et al. (19) (1993)	In vivo	blacks, Caucasians, Hispanics, Asians (no. of subjects ages not specified)	Not documented	Baseline TEWL blacks > Caucassians ≥ Hispanics ≥ Asians
Reed et al. (22) (1995)	In vivo	Skin type V/VI: African-America 4 Filipino 2 Hispanic 1 Skin type II/III: Asian 6 Caucasian 8 (ages 22–38 yr, all)	Volar forearm	Skin type V/VI required more tape strippings (66.7 ± 6.9) compared with skin type II/III (29.6 ± 2.4) to achieve the same TEWL, i.e., skin type V/VI had increased water bar, strength (integrity) Barrier function in skin type V/VI recovered more quickly
Warrier et al. (16) (1996)	In vivo	black women 30 white women 30 (ages 18–45 yr, all)	Left and right medical cheeks, mid-volar forearms, lateral mid-lower legs	TEWL blacks < whites on cheeks (20% less) and legs (17% less) at baseline ($p < 0.05$); also lower on forearm but not statistically significant
Berardesca et al. (23) (1998)	In vivo	black women Caucasian women 10 (mean age 42.3 ± 5 yr, both)	Mid-volar forearm	After tape stripping: TEWL blacks 1.2 × > Caucasians after 3 ($p < 0.05$) and 6 tape strips ($p < 0.03$)
Aramaki et al. (18) (2002)	In vivo–topical application of SLS (irritant)	Japanese women 22 (mean age 25.84 yr) German women 22 (mean age 26.94 yr)	Forearm	No significant difference at baseline or after SLS stress

[a]All of the evidence supports TEWL blacks > whites, except for Berardesca et al. (15) which found no significant difference and Warrier et al. (16) which found TEWL blacks < whites. TEWL measurements of Asian skin are inconclusive as they have been found to be equal to black skin and greater than Caucasian skin [Kompaore et al. (17)], equal to Caucasian skin [Aramaki et al. (18)], and less than all other ethnic groups [Sugino et al. (19)].

Abbreviations: MN, methyl nicotinate; SLS, sodium laurilsulfate.

whites; however, the only study that used a larger sample size by Warrier et al. (16) found TEWL to be less in blacks than whites when measured on the cheeks and legs. Perhaps, the anatomic site examined causes the discrepancies in TEWL values. Also, TEWL measurements with regard to Asian skin may be deemed inconclusive as baseline measurements have found Asian skin to have TEWL values that are equal to those of black skin and greater than those of Caucasian skin (17), less than for all other ethnic groups (19), and no different from those of other ethnic groups (18,26). Additionally, investigators should include more races in their studies and also compare degree of skin pigmentation, as opposed to race, as Reed et al. (22) had done. If water barrier function truly depends on degree of pigmentation, this has implications as to whether the stratum corneum gains or loses barrier integrity in cases of acquired hyper- or hypo-pigmentation. Further, differences in barrier integrity/function, as measured by TEWL, also have implications in the ability of people with different skin types and colors to withstand and recover from environmental insults as well as the ability to absorb topical therapeutic agents. Furthermore, TEWL may vary under different pathologic and physiologic conditions. Thus, the health and physiologic state of the subjects should be noted in future studies.

WATER CONTENT

WC or hydration of the skin can be measured by several methods including skin capacitance, conductance, impedance, and resistance. Using capacitance to measure WC is based on the high dielectric constant of water compared with other substances (28). Conductance is also based on the changes in the electrical properties of the stratum corneum when the skin is hydrated (29). Dry stratum corneum is a medium of weak electrical conduction, whereas the hydrated stratum corneum is more sensitive to the electrical field (28). Resistance is the reciprocal of conductance. In general, skin capacitance and conductance show similar behaviors with regard to measuring WC of the skin, while resistance and impedance show opposing behaviors (28). Possible sources of error or variation in measurement include sweat production, filling of the sweat gland ducts, the number of hair follicles, the electrolyte content of the stratum corneum, and artifacts from applied topical agents (29).

In 1962, Johnson and Corah (30) found that blacks had higher levels of skin resistance at baseline than whites ($p < 0.01$) at two different laboratories in St Louis (Missouri, U.S.) and San Diego (California, U.S.). The St Louis study examined 174 children (22 black boys, 32 black girls, 65 white boys, and 55 white girls) aged 83 to 92 months, while the San Diego study examined 42 subjects (16 black men, five black women, 16 white men, and five white women); mean age of 23 years. While the investigators in this study did not correlate their measurements of skin resistance to WC in the skin, by knowing the relationship of skin resistance to WC, we can deduce that a higher resistance in blacks may be correlated with a lower WC.

In addition to comparing TEWL, Berardesca and Maibach (20,21) also compared WC by capacitance before and after topical administration of SLS in blacks and whites and in another study, in Hispanics and whites. There were no significant differences in WC between blacks and whites at baseline or after SLS stress (20). When comparing Hispanics and whites, there was an increase in WC in Hispanics at baseline, but the difference was not significant; however, after SLS application, they found a significant increase in WC in Hispanics compared with that in whites when a negative visual score (i.e., no erythema) was given for irritation

($p < 0.01$) (21). In reviewing the data, however, we found that although the mean values for WC in Hispanics was greater than in whites, the standard deviations were also large. When an irritant reaction was visually detectable, the WC was proportionally increased in both races, eradicating any difference between them.

Berardesca et al. (15) examined WC by conductance on the volar and dorsal forearm of 15 blacks, 12 whites, and 12 Hispanics in addition to examining TEWL, skin thickness, and extensibility. Within each race studied, significant differences existed in WC between the volar and dorsal forearms (Table 2). Whites and Hispanics demonstrated decreased WC on the dorsal aspect of each arm compared with the volar side (22% less and 11% less, respectively), whereas blacks demonstrated a 13% decrease in WC on the volar aspect compared with the dorsal side. The differences, however, were statistically more relevant for white skin ($p < 0.001$) and less for blacks ($p < 0.02$) and Hispanics ($p < 0.05$). In comparing the races with each other, blacks and Hispanics had increased WC compared with whites on the dorsal forearm. On the volar forearm, however, Hispanics demonstrated greater WC than blacks and whites. Their findings do not correlate with those of the prior studies; however, this study measured WC at baseline using conductance, whereas the prior studies measured WC at baseline using resistance (30), and at baseline and after SLS stress using capacitance (20,21). The variability in WC observed between site and race is difficult to interpret. The investigators noted, however, that the white subjects had an increased amount of hair on the forearms compared with the other two groups, possibly accounting for some differences in the results.

Sugino et al. (19) measured WC with an impedance meter in blacks, whites, Hispanics, and Asians. They found that WC was highest in Asians compared with Caucasians, blacks, and Hispanics. The exact values and study size were not documented. The investigators correlated high WC with high ceramide and low TEWL values also measured in their study.

Warrier et al. (16) examined WC by capacitance at baseline in 30 black and 30 white women aged 18 to 45 years. black women had a significantly higher WC on the cheeks ($p < 0.05$), but there were no significant differences at baseline between blacks and whites on the forearms and the legs. They proposed that the difference found on the cheeks might be related to the evidence of a more elaborate superficial vasculature and more apocrine and mixed eccrine–apocrine glands in facial skin of blacks (32), as well as to the differences in melanin content, the packaging of melanocytes, and their ability to prevent epidermal photodamage (33–35).

Manuskiatti et al. (31) studied seven black and five white women (mean age 25.8 ± 4.2 years) and five black women and five white women (mean age 64.7 ± 3.8 years) and measured WC (by capacitance) as well as desquamation index, as a measure of skin scaling, on the preauricle, posterior neck, dorsal upper arm, dorsal forearm, volar forearm, lower back, abdomen, thigh, and lower leg. The results of desquamation index are discussed in section "Corneocyte Variability." They found no racial differences in WC, but did find significant differences between the younger and the older women (younger had higher WC than older women).

The WC results of each study are summarized in Table 2. The SLS-induced irritation studies by Berardesca and Maibach (20,21) revealed no significant differences in WC between the races at baseline or after SLS stress, except for a questionable difference (high standard deviations) in Hispanics greater than whites after SLS stress. As it is believed that artifacts from topically applied substances may alter values measured by capacitance or conductance (28), this may play a role in some of the values obtained by the SLS irritant-induced study. Additionally, Manuskiatti

Table 2 Racial Differences in Skin Properties: Water Content[a]

Study	Technique	No. of subjects	Site	Results
Johnson and Corah (30) (1962)	In vivo—resistance	St. Louis study: black boys 22: black girls 32 white boys 65; white girls 55 (age 83–92 mo, all) San Diego study: black men 16: black women 5 white men 16: white women 5 (age 23 yr, all)	First and third fingers of right hand	Skin resistance blacks > whites at baseline ($p < 0.01$); i.e., blacks have lower water content
Berardesca and Maibach (20) (1988)	In vivo—topical application of SLS (irritant)—capacitance	black men 10 (age 29.9 ± 7.2 yr) white men 9 (age 30.6 ± 8.8 yr)	Back	No significant difference between blacks and whites at baseline or after SLS stress
Berardesca and Maibach (21) (1988)	In vivo—topical application of SLS (irritant)—capacitance	Hispanic men 7 (age 27.8 ± 4.5 yr) white men 9 (age 30.6 ± 8.8 yr)	Upper back	No significant differences between Hispanics and whites at baseline After SLS stress: Hispanics > whites when negative visual score was given for irritation ($p < 0.01$) (large standard deviations)
Berardesca et al. (15) (1991)	In vivo—conductance	blacks 15 (mean age 46.7 ± 2.4 yr) whites 12 (mean age 49.8 ± 2 yr) Hispanics 12 (mean age 48.8 ± 2 yr)	Volar and dorsal forearm	blacks (13% less) volar < dorsal forearm ($p < 0.02$) whites (22% less) dorsal < volar forearm ($p < 0.02$) Hispanic (11% less) dorsal < volar forearm ($p < 0.001$) black and Hispanics > whites on dorsal forearm at baseline Hispanics > blacks and whites on volar forearm at baseline

Sugino et al. (19) (1993)	In vivo—impedance	blacks, Caucasians, Hispanics, and Asians (no. of subjects, ages not specified)	Not documented	Asians > Caucasians, blacks, and Hispanics
Warrier et al. (16) (1996)	In vivo—capacitance	black women 30 white women 30 (age 18–45 yr, both)	Left and right medical cheeks, mid-volar forearms, lateral mid-lower legs	blacks > whites on cheeks at baseline ($p < 0.05$) No significant difference between races on forearms and legs
Manuskiatti et al. (31) (1998)	In vivo—capacitance	black women 7 white women 5 (mean age 25.8 ± 4.2 yr, both) black women 5 white women 5 (mean age 64.7 ± 3.8 yr, both)	Preauricle, posterior neck, dorsal upper arm, dorsal forearm, volar forearm, lower back, abdomen, thigh, lower leg	No significant differences between blacks and whites at baseline

[a]Racial differences in water content, as measured by resistance, capacitance, conductance, and impedance are inconclusive.
Abbreviation: SLS, sodium laurilsulfate.

et al. (31) also found no difference in WC between blacks and whites, but did find differences based on age. Berardesca et al. (15) and Warrier et al. (16), however, did demonstrate racial variability in WC but the values varied by anatomic site. Sugino et al. (19) also demonstrated racial variability with Asians having a higher WC than other ethnic groups, but they use impedance, a technique that is currently less widely used than capacitance and conductance because it is more sensitive to many environmental and technical factors that affect the stratum corneum (28). While Johnson and Corah (30) did not correlate resistance to WC in their study, it can be inferred from their data that racial variance was found in WC. These findings, obtained by measuring skin capacitance, conductance, impedance, and resistance, are difficult to interpret in terms of stratum corneum WC because other physical factors, such as skin micro-relief, sweat production, and the presence of hair on the measuring site, may modify the quality of skin electrode contact (28). Thus, it seems as though there may be factors other than race in the determination of WC and no conclusions with regard to race and WC can be made. Studies with more subjects and the use of more than one method of measuring WC for accuracy should be considered in the future.

CORNEOCYTE VARIABILITY

Corneocytes differ in shape from the keratinocytes that produce them. The disk-like shape of corneocytes allows them to present with a large surface area in the horizontal position (27). In Caucasians the surface area of corneocytes differs by body site (27,36) and age (27,37). It has also been demonstrated in Caucasians that corneocyte surface area is an important factor in the permeability of the skin to water loss and to percutaneous absorption of topically applied substances (27).

Corcuff et al. (38) compared corneocyte surface area and spontaneous desquamation (via corneocyte count) on the upper outer arm in black African Americans, white Americans of European origin, and Asian Americans of Chinese origin. There were 18 to 25 age-matched subjects per group who were free from dermatological disorders. No difference in corneocyte surface area was found between the groups. However, spontaneous desquamation (corneocyte count) was increased in black skin by a factor of 2.5 compared with white and Asian skins ($p < 0.001$). The investigators felt that their findings were not consistent with earlier studies that showed increased intercellular adhesion (3) or increased TEWL (12,20) in black skin. This enhanced desquamation may (partially) account for "ashing," frequently seen clinically in black people.

In contrast, Warrier et al. (16) conducted a study that included corneocyte desquamation and had different results. The investigators studied 30 black and 30 white subjects, matched for age, and found that the desquamation index was greater on the cheeks and forehead of white subjects compared with black subjects. No difference was found on the legs. The investigators attributed the lower corneocyte desquamation on the cheeks and foreheads of blacks compared with whites to possible differences in moisturizing properties of sebum. These findings did not correlate with dry skin frequently seen clinically in black people. As it is believed that corneocyte surface area varies by anatomic site in Caucasians (27), perhaps corneocyte desquamation also varies by site. Corcuff et al. (38) studied the upper outer arm, whereas Warrier et al. (16) examined the cheeks, forearms, and lower legs. More studies of corneocytes desquamation should be conducted on the anatomic areas where dry skin is more frequently experienced. Additionally, the climate

of the area where the study is done should be considered as it may influence desquamation. Warrier et al. (16) conducted their study over a six-week period in winter, from December through February in Cincinnati, Ohio, U.S., when temperatures and relative humidity are low and frequency of dry skin (winter xerosis) is high. In contrast, the city and climate are not documented in the study by Corcuff et al. (38).

In addition to measuring WC, Manuskiatti et al. (31) also examined the desquamation index in seven black and five white women (mean age 25.8 ± 4.2 years) and five black women and five white women (mean age 64.7 ± 3.8 years) on the preauricle, posterior neck, dorsal upper arm, dorsal forearm, volar forearm, lower back, abdomen, thigh, and lower leg. There were no differences in desquamation index between blacks and whites at all areas measured, except at the preauricular area ($p = 0.02$). However, whether blacks or whites had a higher desquamation index at this area was not specified. The investigators discounted the difference found at the preauricular area and attributed the difference to the small sample size used. Like the results found for WC, they also found significant differences in desquamation index based on age (older individuals had higher desquamation index than younger individuals at the preauricle). Overall, they concluded that age and anatomic site but not race, demonstrate a significant influence on skin roughness and scaliness.

Overall, Corcuff et al. (38), Warrier et al. (16), and Manuskiatti et al. (31) reveal statistically significant results, but the findings are contradictory and, therefore, inconclusive (Table 3). Corcuff et al. (38) demonstrate greater corneocyte desquamation in blacks compared with whites on the upper outer arm. In contrast, Warrier et al. (16) found a greater desquamation index on the cheeks and forehead of whites compared with blacks. Additionally, Manuskiatti et al. (31) found a difference on the preauricular area only out of the numerous areas examined, but whether blacks or whites have a higher desquamation index is not specified.

Does the site of measurement of corneocyte desquamation, the WC and TEWL at that site, and the climate of the area where the study was done act as confounding variables for these results? In light of what is now known about WC and TEWL, the issue of corneocyte desquamation should be revisited as these may be contributing variables. Corneocyte desquamation may have clinical implications in issues regarding the diagnosis and treatment of xerosis frequently seen in African Americans.

BLOOD VESSEL REACTIVITY

Cutaneous blood flow has been examined on numerous occasions to assess skin physiology, irritation, evaluation of dermatologic pathology/treatments, effects/delivery of drugs, and wound healing among other areas of interest (39). The visual assessment of cutaneous microcirculation has been measured for centuries by the degree of erythema or pallor/blanching (visual scoring). However, the introduction of objective techniques for the evaluation blood flow has shown that the human eye is rather unreliable. Two techniques utilized by the papers to be discussed are laser doppler velocimetry (LDV) and Photoplethysmography (PPG).

LDV is a noninvasive method that continuously follows the flow of red blood cells. It is based on measurement of the Doppler frequency shift in monochromatic laser light backscattered from moving red blood cells. It detects the frequency-shifted signal and derives an output proportional to the number of erythrocytes multiplied by their velocity in the cutaneous microcirculation (39,40). LDV has been applied to skin

Table 3 Racial Differences in Skin Properties: Corneocyte Variability[a]

Study	No. of subjects	Site	Results
Corcuff et al. (38) (1991)	black (mean age 33.5 ± 7.5 yr) Caucasian (mean age 31 ± 7 yr) Asian (mean age 26.5 ± 7.5 yr) (18–25 subjects per group)	Upper outer arm	No difference in corneocyte surface area Spontaneous desquamation (corneocyte count) blacks 2.5 × > Caucasians & Asians ($p < 0.001$)
Warrier et al. (16) (1996)	black women 30 white women 30 (age 18–45 yr, both)	Left and right medial cheeks, mid-volar forearms, and lateral mid-lower legs	Desquamation index blacks > whites on cheeks (18% less) and forearms (20% less) ($p < 0.05$); but no significant differences on the legs
Manuskiatti et al. (31) (1988)	black women 7 white women 5 (mean age 25.8 ± 4.2, both black women white women 5 (mean age 64.7 ± 3.8, both)	Preauricle, posterior neck, dorsal upper arm, dorsal forearm, volar forearm, lower back, abdomen, thigh, and lower leg	No difference in desquamation index between blacks and whites except at preauricular area ($p = 0.02$) (which race greater not specified)

[a]Racial differences in corneocyte desquamation are inconclusive. The most clinically provocative observation is that of Corcuff et al. (38)–a 2.5 times greater spontaneous desquamation rate in blacks compared with Caucasians and Asians.

physiology, diagnostics (especially scleroderma, Raynaud disease, and patch-test reactions), predictive testing of irritancy (topical drugs, cosmetics, detergents, cleansing agents, and products used in industry), and effects of drugs [vasodilators, minoxidil, sunscreens and ultraviolet light, and topical corticosteroids (blanching)] (39).

PPG can be defined as the continuous recording of the light intensity scattered from a given source by the tissues and collected by a suitable photodetector (41). Specific to the skin, it allows the registration of pulsative changes in the dermal vasculature and is synchronized with heartbeat. Infrared light from a transducer is absorbed by hemoglobin, and the backscattered radiation is detected and recorded. The backscattered light depends on the amount of hemoglobin in the skin, and the result obtained will, therefore, reflect the cutaneous blood flow. PPG has been used for studies of skin physiology, dermatological disorders, as well as systemic diseases (39).

Guy et al. (42) enrolled six black subjects aged 20 to 30 years, six white subjects aged 20 to 30 years, and six white subjects aged 63 to 80 years, with good general health, no recent skin disease, and taking no prescription medications, and studied their response to topically applied vasodilator methyl nicotinate. The substance was applied to the volar forearm and blood vessel reactivity was measured by LDV and PPG. There was no significant difference between the ethnic groups in time to peak response, area under the response–time curve, or time for response to decay to 75% of its maximum value. However, the PPG maximum response was 40% less in the young black group than in the young white group ($p < 0.05$). The authors made note of the fact that the sensitivities of the two methods of study (LDV and PPG) were not equivalent. They concluded that, overall, the data suggested a similarity in response among races and ages. The investigators failed to discuss the significance or implications of the lower maximum PPG seen in the black subjects.

Berardesca and Maibach (20) performed a study to determine the difference in irritation between young black and Caucasian skin. They applied 0.5% and 2.0% SLS to untreated, preoccluded, and predelipidized skin and then quantified the resulting level of irritation using LDV, TEWL, and WC of the stratum corneum. There were no significant differences between black and white skin for LDV at baseline or after application of SLS. The authors did note, however, that in blacks, application of the 0.5% SLS to untreated skin revealed minimal changes in cutaneous blood flow (as measured by LDV) compared with baseline. They used this finding to explain the decreased irritant-induced perceptible erythema in blacks (6). However, after re-examining the data, we might consider that there was about the same degree of minimal change from baseline to application of 0.5% SLS in untreated skin in the Caucasian group.

Berardesca and Maibach (21) used the same model to compare differences in irritation between Hispanic and Caucasian skin. Like the SLS-induced irritation study comparing blacks and whites, the same study comparing Hispanics and whites revealed equivalent blood vessel responses between the two groups.

Berardesca and Maibach (43) performed a subsequent study using LDV, but this time examined racial differences induced by corticosteroid application (a vasoconstrictive stimulus) (43). They examined six black and eight Caucasian men, matched for age, and measured cutaneous hyperemia using LDV before and after the application of 0.05% clobetasol ointment to the forearm. The following parameters were analyzed: (i) The area under the curve response from the starting point of the hyperemic response to the return of blood flow to basal values; (ii) The magnitude of the maximum peak response; (iii) the slope of the rise from immediate post occlusion to peak reactive hyperemic flow; and (iv) the slope of the decay from peak reactive hyperemic flow to resting levels.

After the vasoconstrictive stimulus was given, the black subjects showed a 40% decreased area under the curve response ($p < 0.04$), a 50% decreased peak response ($p < 0.01$), and a decreased decay slope after peak blood flow ($p < 0.04$) compared with the whites. Overall, their data were consistent with a decrease in blood vessel reactivity of blacks compared with whites.

Gean et al. (44) also found differences in blood vessel reactivity among different racial groups; however, their data conflict with the findings of Berardesca and Maibach (43). Gean et al. (44) examined five black subjects (skin types V or VI), five Asian subjects (skin type IV), and five Caucasian subjects (skin type II), aged 20 to 35 years, with no history of skin disease, who were nonsmokers and were not taking prescription medications, and applied three different concentrations of methyl nicotinate to the upper third of the ventral forearm. Methyl nicotinate–induced vasodilatation was assessed visually and also by LDV. At three different dose levels, the following parameters were compared: (i) the diameter of the maximum visually perceptible erythematous area; (ii) the area under the erythematous diameter versus time curve; (iii) the maximum LDV response; and (iv) the area under the LDV response versus time curve. As we are reviewing only the objective data, the first two parameters measuring erythema revealed no significant differences and will not be discussed. The investigators observed that the area under the curve for LDV response versus time was greater in blacks than Caucasians for all methyl nicotinate concentrations ($p < 0.05$). This contrasts with prior studies, which found either no difference (20,42) or a decrease (43) in the area under the curve response in blacks. It has to be noted that in this study a vasodilator (methyl nicotinate) is given, whereas in the prior study by Berardesca and Maibach (43) a vasoconstrictor was given. They also found that the area under the curve response versus time was greater in Asians compared with Caucasians for higher dose levels of methyl nicotinate ($p < 0.05$).

Kompaore et al. (17) evaluated TEWL and lag time to vasodilatation by LDV, before and after removal of the stratum corneum by tape stripping in seven black men, eight Caucasian subjects (six male and two female) and six Asian men. After application of methyl nicotinate, but before tape stripping, there was no difference between the groups in basal perfusion flow (by LDV), but lag time before vasodilatation was greater in blacks and less in Asians compared with Caucasians ($p < 0.05$). After eight and 12 tape strips, lag time before vasodilatation decreased in all three groups, but decreased significantly more in Asians compared with Caucasians and blacks ($p < 0.05$). The order of sensitivity to methyl nicotinate was Asian > Caucasian > black. After topical application of methyl nicotinate, TEWL measurements indicated that black and Asian skin was more permeable to water than Caucasian skin (Table 1); however, LDV-recorded lag time of vasodilatation results revealed that Asian skin had a higher permeability to methyl nicotinate than Caucasian and black skins. This study confirmed the importance of the stratum corneum in barrier function, but could not explain the reason behind the racial differences in TEWL and lag time to vasodilatation.

Aramaki et al. (18) evaluated LDV at baseline and after SLS-induced irritation in 22 Japanese and 22 German women. There was no difference in LDV at baseline and after SLS-induced irritation. Few studies measuring LDV have examined persons of Asian descent. Although it is difficult to compare a study that used tape stripping (17) with one that used a vasoactive substance (18), note that Aramaki et al. (18) had a larger sample size than Kompaore et al. (17) and found no baseline difference in LDV.

The results of the studies on blood vessel reactivity are summarized in Table 4. As each study administered different vasoactive substances, these could not be compared with each other because each substance may act on different receptors on the blood vessels (45). However, note that each study, except for the SLS study comparing Hispanics and whites (21) and the SLS study comparing Japanese and German women (18), revealed some degree of variation in blood vessel reactivity in different races whether it be increased reactivity in blacks (44), decreased reactivity in blacks (20,42,43), or increased lag time to reactivity in blacks at baseline and in Asians after stratum corneum removal (17). This is important when considering disparities in irritation, dermatotoxicology, and dermatopharmacology among people of different ethnic groups.

ELASTIC RECOVERY/EXTENSIBILITY

In addition to examining TEWL and skin conductance, Berardesca et al. (15) also examined biomechanical properties, such as elastic recovery and skin extensibility, on the dorsal and volar forearm in 15 blacks, 12 whites, and 12 Hispanics. These biomechanical properties were determined by applying a specific torque parallel to the skin's surface and then measuring how stretchable the skin was (skin extensibility) and recording the time required for the skin to return to its original state after release of the torque (elastic recovery). For skin elastic recovery they found no significant difference between the races on the dorsal forearm (blacks > whites, but not significant). However, elastic recovery was 26% less in blacks compared with whites on the volar forearm ($p < 0.001$). There was no significant difference in elastic recovery between whites and Hispanics. The authors explained the significantly decreased elastic recovery in blacks compared with whites on the volar forearm, with a higher recovery in blacks on the dorsal side (although not significant), on the basis of greater actinic damage on the dorsal side of whites, with melanin as a photoprotective factor in blacks.

For skin extensibility, within each race, Berardesca et al. (15) found significant differences between dorsal and volar forearms in Hispanics and whites (dorsal < volar; $p < 0.0002$ and $p < 0.0001$, respectively), but extensibility was the same on both sides of the forearm in blacks. Blacks had greater extensibility than whites on the dorsal forearm, but decreased extensibility than whites on the volar forearm ($p < 0.01$ for both). Skin elasticity overall is defined as elastic recovery divided by extensibility. The investigators found no significant differences between the races for this ratio. They explained the variability in these biomechanical properties of skin based on the protective role of melanin against ultraviolet rays. They believed that blacks did not show differences in skin extensibility between the dorsal and volar forearm because they were more photoprotected. Furthermore, they believed that blacks had greater extensibility on the dorsal forearm compared with whites for the same reason. However, if blacks are presumed to also be more photoprotected on the volar forearm compared with whites, this reasoning does not explain why whites were found to have a greater extensibility than blacks on the volar side.

Warrier et al. (16) examined elastic recovery in 30 black and 30 white women, but did not record skin extensibility. There was no significant difference between blacks and whites on the legs, but elastic recovery on the cheeks was 1.5 times greater in blacks than in whites ($p < 0.05$). These findings contradicted those of Berardesca et al. (15) who found a 26% decrease in elastic recovery on the volar forearm of blacks. Warrier et al. (16) explained their findings of higher elastic recovery on the

Table 4 Racial Differences in Skin Properties: Blood Vessel Reactivity[a]

Study	Technique	No. of subjects	Site	Results
Guy et al. (42) (1985)	Topically administered MN (vasodilator); LDV and PPG	blacks 6 (age 20–30 yr) whites 6 (age 20–30 yr) whites 6 (age 63–80 yr)	Volar forearm	MN given: No significant difference in time to peak response, area under response–time curve, or time for response to decay to 75% of its max value PPG max response young black (40% less) $<$ young white ($p < 0.05$)
Berardesca and Maibach (20) (1988)	Topically administered SLS (irritant): LDV	black men 10 (age 29.9 ± 7.2yr) white men 9 (age 30.6 ± 8.8yr)	Back	SLS stress: No significant difference between blacks and whites Blood vessel reacitvity minimal in blacks from baseline to application of 0.5% SLS on untreated skin
Berardesca and Maibach (21) (1988)	Topically administered SLS (irritant); LDV	Hispanic men 7 (age 27.8 ± 4.5yr) white men 9 (age 30.6 ± 8.8yr)	Upper back	SLS stress: Similar LDV response in Hispanics and whites
Berardesca and Maibach (43) (1989)	Topically administered corticoid (vasoconstrictor); LDV	black men 6 Caucasian men 8 (mean age 27 ± 3yr, both)	Forearm	After vasoconstrictor given: 40% decreased area under the curve response blacks compared with whites ($p < 0.04$) 50% decreased peak response in blacks compared with whites ($p < 0.01$) Decreased decay slope after peak blood flow in blacks compared with Caucasians; in blacks, y = 3.3672 – 0.0737× after treatment compared with y = 2.5347 – 0.0367× after treatment ($p < 0.04$) i.e., less blood vessel reactivity in blacks

Gean et al. (44) (1989)	Topically administered MN (vasodilator); LDV	blacks 5 Caucasians 5 Asians 5 (age 20–35yr, all)	Upper 1/3 volar forearm	MN given: Area under the curve for LDV response versus time blacks > Caucasians for all MN concentrations ($p < 0.05$) Area under the curve for LDV response versus time Asians > Caucasians for higher dose levels of MN ($p < 0.05$)
Kompaore et al. (17) (1993)	Topically administered MN (vasodilator); LDV	blacks 7 Caucasians 8 Asians 6 (age 23–32yr, all)	Volar forearm	MN given: Before tape stripping: no difference between the groups in basal perfusion flow, but lag time before vasodilatation was blacks > Caucasians > Asians ($p < 0.05$) After 8 and 12 tape strips: lag time before vasodilation decreased in all three groups, but significantly decreased in Asians > Caucasians > blacks ($p < 0.05$)
Aramaki et al. (18) (2002)	Topically administered SLS (irritant); LDV	Japanese women 22 (mean age 25.84yr) German women 22 (mean age 26.94yr)	Forearm	No significant difference at baseline or after SLS stress

[a]Studies cannot be compared with each other because each uses different vasoactive substances. However, each study, except for Berardesca and Maibach (21) comparing Hispanics and whites and Aramaki et al. (18) comparing Japanese and German women, reveals some degree of racial variation in blood vessel reactivity.

Abbreviations: Corticoid, clobetasol propionate 0.05% ointment; LDV, laser Doppler velocimetry; MN, methyl nicotinate; PPG, photoplethysmography; SLS, sodium laurilsulfate.

cheeks of blacks based on the higher WC that they found on the same anatomic area, thus presumably resulting in a higher elastic deformation.

The data on skin biomechanics, specifically elastic recovery and extensibility, vary by anatomic site and by race. However, the conclusions drawn by Berardesca et al. (15) contradict those of Warrier et al. (16). The data not only vary by race and by site, but may also vary by age. In the study by Berardesca et al. (15), the subjects were all within the same age range (mean age 46.7–49.8 years). However, even though Warrier et al. (16) had a larger number of study subjects, the age range was 18 to 45 years. Overall, the racial differences in skin biomechanics are inconclusive and warrant further study (Table 5).

pH GRADIENT

Racial differences in pH of the skin have also been explored. In addition to examining TEWL, Berardesca et al. (23) also examined differences in pH in 10 Caucasian (skin types I and II) and eight African American (skin type VI) women at baseline and after tape strippings. They found no significant differences between the two races in pH at baseline. However, they found a significantly lower pH in blacks compared with whites after three tape strippings, but no significant differences after 9, 12, and 15 strippings. Thus, there was a lower pH in black skin compared with white skin in the superficial layers of the stratum corneum, but not in the deeper layers. The investigators stated that the data were difficult to explain. It was hypothesized that since the TEWL was also found to be increased after three and six tape strippings, the increased TEWL might allow for an increase in the hydrogen ion concentration in a normally hydrophobic stratum corneum. It is to be noted that although the difference between the races in pH was not significant at deeper layers of the stratum corneum, the pH in both races did decrease with more tape strippings, but the TEWL did not follow the same trend. Thus, an increase in TEWL does not fully explain the findings based on pH.

Warrier et al. (16) also included pH in their study of 30 black and 30 white women; however, they only examined pH at baseline, not after tape stripping. There was a decreased pH on the cheeks of blacks compared with whites, pH = 5.15 versus $p = 5.52$, respectively ($p < 0.05$). There was also a decreased pH in blacks on the legs, but the difference was not significant. The authors attributed the decreased pH in blacks to lactic acid and dicarboxylic amino acids in sweat secretions mixed with sebum, and evaporation of sweat causing acidity to increase (49) suggesting that there might be a higher number of sweat glands in blacks (32).

pH has been found to be lower in blacks compared with whites in two different studies, but under different circumstances. Berardesca et al. (23) demonstrate this difference only in the superficial layers of the stratum corneum on the volar forearm, but not at the baseline, while Warrier et al. (16) demonstrate the significant difference at the baseline on the cheeks but not on the legs. Thus, it can be inferred that some difference between whites and blacks in stratum corneum pH may exist but the etiology of this finding and its confounders remain to be explored (Table 5).

LIPID CONTENT

Sugino et al. (19) correlated high WC values with high ceramide (lipid) levels and low TEWL. They studied WC (by impedance), TEWL, and ceramide levels in black, white, Hispanic, and Asian subjects. The number of subjects, age, and sample site

Table 5 Racial Differences in Skin Properties: Skin Elastic Recovery, pH Gradient, Lipid Content, Surface Micorflora, and Mast Cell Granules.

Study	Technique	No. of subjects	Site	Results
Skin elastic recovery[a]				
Berardesca et al. (15) (1991)	In vivo	blacks 15 (mean age 46.7 ± 2.4 yr) whites 12 (mean age 49.8 ± 2yr) Hispanics 12 (mean age 48.8 ± 2yr)	Volar and dorsal forearm	No significant difference between races on dorsal forearm Elastic recovery blacks (26% less) $<$ whites on volar forearm ($p < 0.001$)
Warrier et al. (16) (1996)	In vivo	black women 30 white women 30 (age 18–45yr, both)	Left and right medial cheeks, mid-volar forearms, lateral mid-lower legs	No significant difference between races on the legs Elastic recovery blacks $1.5 \times >$ whites on cheeks ($p < 0.05$)
Skin extensibility[a]				
Berardesca et al. (15) (1991)	In vivo	blacks 15 (mean age 46.7 ± 2.4 yr) whites 12 (mean age 49.8 ± 2yr) Hispanics 12 (mean age 48.8 ± 2yr)	Volar and dorsal foream	Significant dorsal $<$ volar extensibility within whites and Hispanics ($p < 0.0001$ and $p < 0.0002$, respectively) black $>$ white extensibility dorsal forearm ($p < 0.01$) black $<$ white extensibility volar forearm ($p < 0.01$)
pH gradient[b]				
Berardesca et al. (23) (1998)	In vivo	black women 8 Caucasian women 10 (mean age 42.3 ± 5yr, both)	Mid-volar forearm	No significant difference in pH at baseline After tape stripping: pH significantly decreased in blacks after three tape strips, i.e., superficial SC layers No differences between races after 9, 12, and 15, tape strips, i.e., deeper SC layers

(*Continued*)

Table 5 Racial Differences in Skin Properties: Skin Elastic Recovery, pH Gradient, Lipid Content, Surface Micorflora, and Mast Cell Granules. (*Continued*)

Study	Technique	No. of subjects	Site	Results
Warrier et al. (16) (1996)		black women 30 white women 30 (age 18–45yr, both)	Left and right medial cheeks, mid-volar forearms, lateral mid-lower legs	pH blacks (pH = 5.15) < whites (pH = 5.52) on cheeks at baseline ($p < 0.05$) no significant difference in pH on the legs at baseline
Lipid content[c]				
Reinertson and Wheatley (25) (1959)		Cadavers: black man 1 white man 3 Living: black man 1 white man 1 (age 49–68, all)	Cadavers: Abdomen Living Back and thigh	Lipid and sterol content in total epidermis blacks > whites
Sugino et al. (19) (1993) [Abstract only]		black, white, Hispanic, and Asian (no. of subjects, age not specified)	Not documented	Ceramide levels blacks (50% less) < whites and Hispanics ($p < 0.05$)
Harding et al. (46) (2002)		UK 41 Thai (dry season) 31 Thai (humid season) 31 (age 20–40 yr, all)	Scalp	U.K. and Thai subjects demonstrated similar levels of total lipids

Skin surface microflora[d]				
Rebora and Guarrera (47) (1988)		black men 10	Forearm	Candida albicans blacks (150% greater) > whites ($p < 0.025$)
		white men 10 (age 21–59 yr, both)		Aerobes blacks (650% greater) > whites ($p < 0.025$)
Warrier et al. (16) (1996)		black women 30	Left and right medial cheeks, mid-volar forearms, lateral mid-lower legs	Density of Propionibacterium acnes blacks > whites, but not statistically significant
		white women 30 (age 18–45 yr, both)		No Significant difference in aerobes
Mast cell granules[e]				
Sueki et al. (48) 2001	EM of biopsy specimen	black men 4 (mean age 29.2 ± 3 yr)	Medial-lateral buttock	Mast cells contain 1.5× larger granules in black skin compared with white skin ($p < 0.0001$)
		Caucasian men 4 (men age 29.4 ± 1.2 yr)		Mast cells contain 15% more PLS in blacks compared with whites ($p < 0.05$)
				Mast cells contain 30% less curved lamellae in blacks compared with whites ($p < 0.05$)
				Tryptase immunoreactivity localized to PLS regions in black skin, compared with curved lamellae regions in white skin ($p < 0.0001$)
				Cathepsin G localized to electron-dense amorphous subregions in both black and white skin

[a]unable to draw conclusions regarding racial differences in skin biomechanics (skin eleastic recovery and extensibility) due to insufficient and conflicting evidence.
[b]both studies demonstrate pH of black less than white skin. However, Berardesca et al. (23) demonstrate this difference after superficial tape stripping of the volar forearm, but not at baseline; while Warrier et al. (16) demonstrate the difference at baseline on the cheeks but not on the cheeks but not on the legs.
[c]racial differences in lipid content are inconclusive.
[d]insufficient and conflicting evidence to draw conclusions regarding racial differences in skin microflora.
[e]larger mast cell granules, increased PLS, and increased tryptase localized to PLS in black compared to white skin.
Abbreviations: EM, electron microscopy; PLS, parallel-linear striations; SC, stratum corneum.

were not documented in the abstract. Ceramide levels were 50% lower in blacks compared with whites and Hispanics (total ceramides: 10.7 ± 4.7, 20.4 ± 8.1, and 20.0 ± 4.3 μg/mg, respectively; $p < 0.05$). Although they noted that WC levels were highest in Asians, they did not document the ceramide levels of Asians (according to their hypothesis, Asians should have the highest ceramide levels). Thus, the correlation that was made between WC, TEWL, and ceramide levels was not fully exemplified.

The finding of low ceramide levels in blacks by Sugino et al. (19) is important, because several studies base their findings of increased WC in blacks (15,16) on a 1959 study by Reinertson and Wheatley (25) which, in contrast to Sugino et al., showed higher total epidermis lipid and sterol content in blacks compared with whites. They took abdominal skin from four cadavers (one black man and three white men), and back and thigh skin from one black and one white man, all aged 49 to 68 years, and examined lipid and sterol content. Although they found that lipid and sterol levels were higher in blacks, they had a small sample size and compared skin from both deceased and living subjects at different anatomic sites.

Harding et al. (46) analyzed scalp stratum corneum lipid content in 41 United Kingdom, 31 Thai (dry season) and 31 Thai (humid season) subjects, aged 20 to 40 years, in an attempt to evaluate ethnic differences in dandruff. They observed that decreased levels of scalp stratum corneum free fatty acids, cholesterol, and ceramides were found in subjects with dandruff. However, the overall levels of scalp lipids were similar in United Kingdom and Thai subjects.

Overall, it seems as though racial (ethnic) differences in skin lipid content are inconclusive because one study finds decreased lipids in blacks compared with whites (19), another finds increased lipids in blacks (25), and still another finds no difference between people from the United Kingdom and Thailand (Table 5) (46).

SURFACE MICROFLORA

Racial differences in skin microflora have also been examined. Rebora and Guarrera (47) inoculated the forearm skin of 10 black men and 10 white men (age 21–59 years) with *Candida albicans* and examined the severity of ensuing dermatitis as well as the population of Candida and other aerobes at the inoculum site. The severity of dermatitis was scored subjectively by observation of pustules. However, population of microflora was assessed objectively by colony counts after aerobic incubation at 95°F (35°C) for two days. Black skin harbored 150% more yeast after inoculation with *C. albicans* and 650% more aerobes both at baseline and after inoculation than white skin ($p < 0.025$).

In addition to investigating TEWL, capacitance, desquamation index, elastic recovery, and skin pH, Warrier et al. (16) also examined facial skin microflora in 30 black and 30 white women aged 18 to 45 years. They found no significant differences in the density of aerobes (mostly *Staphylococcus* spp.) between blacks and whites. In contrast, although not statistically significant, there was a higher density of *Propionibacterium acnes* in blacks compared with whites. They felt that this might be due in part to a believed increase in sebum output in blacks(50).

Both studies demonstrated increased skin microflora in blacks in that Rebora and Guarrera (47) found that blacks harbor significantly more *C. albicans* after inoculation, and Warrier et al. (16) found higher density of *P. acnes*, but the values were not statistically significant. However, Rebora and Guarrera (47) found blacks

to have significantly higher levels of aerobes both at baseline and after inoculation with *C. albicans*, while Warrier et al. (16) found no significant racial differences in the density of aerobes. As the minimal data that exist are conflicting, no conclusions regarding skin microflora can be made until investigators examine the issue further (Table 5). Perhaps the age of subjects, anatomic site, and humidity of the geographic environment where the study was conducted cause variation in skin microflora and should be accounted for in future studies.

MAST CELL GRANULES

Based on frequent clinical observations of pruritus and scratching in African Americans, Sueki et al. (48) evaluated differences in mast cells between black and white skins (Table 5). They took 4-mm punch biopsies of normal buttock skin from four African American males (mean age 29.2 ± 3.0 years) and four white males (mean age 29.4 ± 1.2 years) with no prior history of skin disease or atopy and processed the biopsies routinely for electron microscopy. Mast cells in black skin contained 1.5 times larger granules ($p < 0.0001$), 15% more parallel–linear striations (PLS) ($p < 0.05$) and 30% less curved lamellae ($p < 0.05$) compared with white skin. In addition, the investigators also examined the subgranular distribution of mast cell proteases, tryptase, and cathepsin G by immunoelectron microscopy. They found that tryptase immunoreactivity localized to PLS regions in black skin, compared with curved lamellae regions in white skin ($p < 0.0001$). In contrast, Cathepsin G localized to electron-dense amorphous subregions in both black and white skin.

The investigators attributed the larger mast cell granules in black skin to possible increased fusion or division of the granules in blacks. On the other hand, they noted that the larger percentage of PLS and smaller percentage of curved lamellae in blacks was more difficult to explain. They hypothesized that it might be influenced by the mediator content, especially the amount of tryptase. Based on other studies suggesting the participation of mast cells in aberrant fibrosis in skin disorders such as keloid scars (51) and hypertrophic scars (52), the investigators suggested the involvement of tryptase in these disorders. Keloid scarring is frequently observed in black individuals and blacks were found to have increased amounts of tryptase in this study compared with whites. Even though the study had a small sample size and only examined skin from one anatomic region, the researchers still found significant structural differences in mast cells between black and white skins. Further investigation of proinflammatory mediators should be done to corroborate these findings. This discovery should also prompt further electron microscopic evaluation of other cells involved in dermatologic disorders.

EPIDERMAL INNERVATION

While TEWL, WC, and blood vessel reactivity have been used as measures of irritancy, Reilly et al. (53) sought to explain racial differences in irritancy in terms of differences in skin innervation and nociceptor activity. They utilized confocal microscopy to examine epidermal innervation of the volar forearm pretreated with capsaicin in 20 European Caucasian, eight Japanese-American, and eight

Table 6 Summary of Evidence for Racial Differences in Skin Properties

Evidence supports	Insufficient evidence	Inconclusive
TEWL black > white skin	Deductions regarding Asian and Hispanic skin	Racial differences
Variable racial blood vessel reactivity	Racial differences in skin elastic recovery/ extensibility[a]	Water content, corneocyte desquamation,
pH lack < white skin		
Larger mast cell granules, increased PLS, and increased tryptase localized to PLS in black compared with white skin	Skin microflora,[a] epidermal innervation	Lipid content

[a]Skin elastic recovery/extensibility, skin microflora, epidermal innervation were labeled as "insufficient evidence for" racial differences rather than "inconclusive" because only two studies or less examined these variables.
Abbreviations: PLS, parallel-linear striations; TEWL, transepidermal water loss.

Chinese-American volunteers. However, no differences in innervation, including the biochemical properties of the nerve fibers, were found.

CONCLUSION

In conclusion (Table 6), there exists reasonable evidence to support that black skin has a higher TEWL, variable blood vessel reactivity, lower skin surface pH, and larger mast cell granules compared with white skin by means of objective measurements. Although some deductions have been made about Asian and Hispanic skin, the results are contradictory and further evaluation of Asian and Hispanic skin needs to be done.

Table 7 Considerations for Future Research of Racial Differences in Skin Properties Differences

Baseline versus "stress" test differences
Anatomic site examined
Open versus occluded stresses
Ethnic groups in the same versus varying geography
Comparable climatic conditions
Presentation of hard data and statistical analysis
Large sample sizes
Relationship of study parameters to degree of pigmentation
Definition of ethnicity/race
Comparable diets (e.g., controlled diets)
Socioeconomic factors
Prior dermatologic disease
Skin care prior to study
Body mass relationship

Source: From Ref. 55.

A review by Robinson (54) also supported the notion that the evidence comparing Asian and Caucasians is insufficient and less than compelling. Perhaps more specificity about the origin of their heritage should also be included since "Asian" and "Hispanic" encompasses a broad spectrum of people. Racial (ethnic) differences in skin WC, corneocyte desquamation, skin elastic recovery/extensibility, lipid content and skin microflora, although statistically significant, are minimal and contradictory. Thus, no conclusions regarding these objective data can be made.

While there exist relatively few studies that address racial (ethnic) differences in skin properties, and most of these studies used small numbers of subjects, future studies with larger sample sizes are warranted. Investigators should also consider presenting the hard data in these studies in addition to the type of statistical analysis utilized. Other considerations for future research include accounting for: baseline versus "stress" test differences, different anatomic sites, open versus occluded sites, ethnic groups in the same versus varying geography, using comparable climatic conditions, degree of skin pigmentation, diet, socioeconomic factors, prior dermatologic disease, skin care prior to the study, and body mass index as these may all be confounding variables in studies based on racial (ethnic) differences (Table 7).

One issue that must be raised when interpreting these studies is the definition of race or ethnicity. Anthropologists divide racial groups into Caucasoid (e.g., Europeans, Arabs, and Indians), Mongoloid (e.g., Asians), Australoid (e.g., Australian aborigines), Congoid or Negroid (e.g., most African tribes and descendants) and Capoid (e.g., the Kung San African tribe) with the idea that racial variations were selected for to facilitate adaptations to a particular environment (56,57). On the other hand, ethnicity has been defined as how one sees oneself and how one is seen by others as part of a group on the basis of presumed ancestry and sharing a common destiny, often with commonalities in skin color, religion, language, customs, ancestry, and/or occupation or region (58). Thus, ethnicity encompasses a set of categories that overlaps with race but also depends on more subjective and cultural factors, while race seems to encompass genetic variations based on natural selection. With these obscure definitions based on both biology and the subjective manner in which one labels oneself, the basis of objective research on racial or ethnic differences is already somewhat subjective and, therefore, questionable. However, studies show that differences, whether based on genetic variations or on subjective labels, do exist. Perhaps, future studies in dermatology should also address how one defines oneself as of that particular race or ethnic group in addition to examining degree of skin pigmentation. This will help to determine whether the differences are truly the result of genetic variations that were selected for by race or of biologic variations in melanin content that vary between and within each race. Further research in both genetics and dermatology are warranted to draw any final conclusions with regard to race/ethnicity as the etiology for differences in skin physiology.

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REFERENCES

1. Thomson ML. Relative efficiency of pigment and horny layer thickness in protecting the skin of Europeans and Africans against solar ultraviolet radiation. J Physiol (Lond) 1955; 127:236–246.
2. Freeman RG, Cockerell EG, Armstrong J, et al. Sunlight as a factor influencing the thickness of the epidermis. J Invest Dermatol 1962; 39:295–298.
3. Weigand DA, Haygood C, Gaylor JR. Cell layers and density of Negro and Caucasian stratum corneum. J Invest Dermatol 1974; 62:563–568.
4. Dreher F, Arens A, Hostynek JJ, et al. Colorimetric method for quantifying human stratum corneum removed by adhesive tape-stripping. Acta Derm Venereol (Stockh) 1988; 78(3):186–189.
5. Marshall EK, Lynch V, Smith HV. Variation in susceptibility of the skin to dichloroethylsulphide. J Pharmacol Exp Ther 1919; 12:291.
6. Weigand DA, Gaylor JR. Irritant reaction in Negro and Caucasian skin. South Med J 1974; 67:548–551.
7. Basketter DA, Griffiths HA, Wang XM, et al. Individual, ethnic and seasonal variability in irritant susceptibility of skin: the implications for a predictive human patch test. Contact Dermatitis 1996; 35:208–213.
8. Pillsbury DM, Shelley WB, Kligman AM, eds. Dermatology. Chapter 1. Philadelphia (PA): WB Saunders Co, 1956.
9. Rothman S. Insensible water loss. Physiology and Biochemistry of The Skin. Chicago: The University Chicago Press, 1954: 233.
10. Larsen TH, Jemec GBE. Skin mechanics and hydration. In: Elsner P, Berardesca E, Wilhelm KP, eds. Bioengineering of The Skin: Skin Biomechanics. Boca Raton (FL): CRC Press LLC, 2002: 199–200.
11. Distante F, Berardesca E. Transepidermal water loss. In: Berardesca E, Elsner P, Wilhelm KP, eds. Bioengineering of the Skin: Methods and Instrumentation. Boca Raton (FL): CRC Press Inc, 1995:1–4.
12. Wilson D, Berardesca E, Maibach HI. In vitro transepidermal water loss: differences between Black and White human skin. Br J Dermatol 1988; 199:647–652.
13. Robinson S, Dill D, Wilson J, et al. Adaptations of white men and Negroes to prolonged work in humid heat. Am J Trop Med 1941; 21:261.
14. Baker H. The skin as a barrier. In: Rook A, ed. Textbook of Dermatology. Oxford: Blackwell Scientific, 1986:355.
15. Berardesca E, Rigal J, Leveque JL, et al. In vivo biophysical characterization of skin physiological differences in races. Dermatologica 1991; 182:89–93.
16. Warrier AG, Kligman AM, Harper RA, et al. A comparison of black and white skin using noninvasive methods. J Soc Cosmet Chem 1996; 47:229–240.
17. Kompaore F, Marly JP, Dupont C. In vivo evaluation of the stratum corneum barrier function in Blacks, Caucasians, and Asians with two noninvasive methods. Skin Pharmacol 1993; 6(3):200–207.
18. Aramaki J, Kawana S, Effendy I, et al. Differences of skin irritation between Japanese and European women. Br J Dermatol 2002; 146:1052–1056.
19. Sugino K, Imokawa G, Maibach HI. Ethnic difference of stratum corneum lipid in relation to stratum corneum function (abstract). J Invest Dermatol 1993; 100(4):587.
20. Berardesca E, Maibach HI. Racial differences in sodium lauryl sulphate induced cutaneous irritation: black and white. Contact Dermatitis 1988; 18:65–70.
21. Berardesca E, Maibach HI. Sodium-lauryl-sulphate-induced cutaneous irritation: comparison of White and Hispanic subjects. Contact Dermatitis 1988; 18:136–140.
22. Reed JT, Ghadially R, Elias PM. Skin type, but neither race nor gender, influence epidermal permeability function. Arch Dermatol 1995; 131(10):1134–1138.

23. Berardesca E, Pirot F, Singh M, et al. Differences in stratum corneum pH gradient when comparing white Caucasian and Black African-American skin. Br J Dermatol 1998; 139:855–857.
24. Fitzpatrick TB. The validity and practicality of sun reactive skin type I through VI. Arch Dermatol 1988; 124:869–871.
25. Reinerston RP, Wheatley VR. Studies on the chemical composition of human epidermal lipids. J Invest Dermatol 1959; 32:49–59.
26. Yosipovitch G, Theng CTS. Asian skin: its architecture, function, and differences from Caucasian skin. Cosmet Toilet 2002; 117(9):57–62.
27. Rougier A, Lotte C, Corcuff P, et al. Relationship between skin permeability and corneocyte size according to anatomic site, age, and sex in man. J Soc Cosmet Chem 1988; 39:15–26.
28. Distante F, Berardesca E. Hydration. In: Berardesca E, Elsner P, Wilhelm KP, eds. Bioengineering of the Skin: Methods and Instrumentation. Boca Raton (FL): CRC Press Inc, 1995:5–12.
29. Triebskorn A, Gloor M. Noninvasive methods for the determination of skin hydration. In: Frosch PJ, Kligman AM, eds. Noninvasive Methods for the Quantification of Skin Functions. Berlin; New York (NY): Springer-Verlag, 1993:42–55.
30. Johnson LC, Corah NL. Racial differences in skin resistance. Science 1962; 139:766–767.
31. Manuskiatti W, Schwindt DA, Maibach HI. Influence of age, anatomic site and race on skin roughness and scaliness. Dermatology 1998; 196:401–407.
32. Montagna W, Carlisle K. The architecture of Black and White skin. J Am Acad Dermatol 1991; 24:929–937.
33. Montagna W, Prota G, Kenney JA. Black Skin: Structure and Function. San Diego: Academic Press, 1993:1–12.
34. Fitzpatrick TB, Szabo G, Wick MM. Biochemistry and physiology of melanin pigmentation. In: Lowell AG, ed. Biochemistry and Physiology of the Skin. New York: Oxford University Press, 1983:687–712.
35. Kaidbey KH, Poh AP, Sayre M, et al. Photoprotection by melanin: a comparison of Black and Caucasian skin. J Am Acad Dermatol 1979; 1:249–260.
36. Plewig G, Marples BM. Regional differences of cell sizes in the human stratum corneum. J Invest Dermatol 1970; 54:13–18.
37. Leveque JL, Corcuff P, De Rigal J, et al. In vivo studies of the evolution of physical properties of the human skin with age. Int J Dermatol 1984; 23:322–329.
38. Corcuff P, Lotte C, Rougier A, et al. Racial differences in Corneocytes: a comparison between black, white, and oriental skin. Acta Derm Venereal (Stockh) 1991; 71:146–148.
39. Wahlberg JE, Lindberg M. Assessment of skin blood flow: an overview. In: Berardesca E, Elsner P, Maibach HI, eds. Bioengineering of the Skin: Cutaneous Blood Flow and Erythema. Boca Raton (FL): CRC Press, 1995:23–27.
40. Oberg PA. Laser-doppler flowmetry. Crit Rev Biomed Eng 1990; 18:125.
41. Bernardi L, Leuzzi S. Laser doppler flowmetry and photoplethysmography: basic principles and hardware. In: Berardesca E, Elsner P, Maibach HI, eds. Bioengineering of the Skin: Cutaneous Blood Flow and Erythema. Boca Raton (FL): CRC Press, 1995:31–55.
42. Guy RH, Tur E, Bjerke S, et al. Are there age and racial differences to methyl nicotinate-induced vasodilatation in human skin? J Am Acad Dermatol 1985; 12:1001–1006.
43. Berardesca E, Maibach HI. Cutaneous reactive hyperemia: racial differences induced by corticoid application. Br J Dermatol 1989; 129:787–794.
44. Gean CJ, Tur E, Maibach HI, et al. Cutaneous responses to topical methyl nicotinate in Black, Oriental, and Caucasian subjects. Arch Dermatol Res 1989; 281:95–98.
45. Katzung BG. Introduction to autonomic pharmacology. In: Katzung BG, ed. Basic and Clinical Pharmacology. Los Altos (CA): McGraw-Hill, 2001:75–91.
46. Harding CR, Moore AE, Rogers JS, et al. Dandruff: a condition characterized by decreased levels of intercellular lipids in scalp stratum corneum and impaired barrier function. Arch Dermatol Res 2002; 294:221–230.

47. Rebora A, Guarrera M. Racial differences in experimental skin infection with *Candida albicans*. Acta Derm Venereal (Stockh) 1988; 68:165–168.
48. Sueki H, Whitaker-Menezes D, Kligman AM. Structural diversity of mast cell granules in Black and White skin. Br J Dermatol 2001; 144:85–93.
49. Dikstein S, Zlotogorski A. Skin surface hydrogen ion concentration (pH). In: Leveque JL, ed. Cutaneous Investigation in Health and Disease. New York (NY): Marcel Dekker, 1989:59–78.
50. Kligman AM, Shelly WB. An investigation of the biology of the human sebaceous gland. J Invest Dermatol 1973; 30:99–125.
51. Craig SS, DeBlois G, Schwartz LB. Mast cells in human keloid, small intestine, and lung by immunoperoxidase technique using a murine monoclonal antibody against tryptase. Am J Pathol 1986; 124:427–435.
52. Kischer CW, Bunce H, Sheltar MR. Mast cell analysis in hypertrophic scars, hypertrophic scars treated with pressure and mature scars. J Invest Dermatol 1978; 70:355–357.
53. Reilly DM, Ferdinando D, Johnston C, et al. The epidermal nerve fibre network: characterization of nerve fibres in human skin by confocal microscopy and assessment of racial variations. Br J Dermatol 1997; 137:163–170.
54. Robinson MK. Population differences in skin structure and physiology and the susceptibility to irritant and allergic contact dermatitis: implications for skin safety testing and risk assessment. Contact Dermatitis 1999; 41:65–79.
55. Modjtahedi SP, Maibach HI. Ethnicity as a possible endogenous factor in irritant contact dermatitis: comparing irritant response among Caucasians, Blacks, and Asians. Contact Dermatitis 2002; 47(5):272–278.
56. Taylor SC. Understanding skin of color, skin of color: biology, structure, function, and implications for dermatologic disease. J Am Acad Dermatol 2002; 46:S41–S62.
57. Coon CS. The Origin of Races. New York, NY: Alfred A Knopf, 1962.
58. Oppenheimer GM. Paradigm lost: race, ethnicity, and the search for a new population taxonomy. Am J Public Health 2001; 91(7):1049–1055.

4

The Human Periorbital Wrinkle

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Wrinkles are the most obvious—perhaps one of the most disliked—aspects of facial aging. Considering this, it is surprising that relatively little is known about what causes a wrinkle, what is its physical structure, and why it is so hard to erase a wrinkle once it is formed.

Many people, scientists and laymen alike, considered wrinkles to be non-medical and so obvious a phenomenon as not to be worthy of research. There was a common belief that as the skin aged, collagen stiffened, elastic networks collapsed, and the mechanical properties of the skin degraded. Wrinkles were just seen as the "points of weakness" where the degraded skin creased.

Nothing has served to make a larger impact on this simplistic model than the remarkable success of Botulinum toxin in reducing facial wrinkles. Botulinum toxin is injected into the muscles underneath the skin and causes a long lasting paralysis and relaxation of those muscles. The fact that this has such a dramatic effect on the appearance of the wrinkle proves that deep wrinkles are not simply a phenomenon of changed properties of the skin but rather are an effect of the entire integumentary system including not only the dermis but also the subcutaneous fat layer and muscle layer.

In this chapter, we briefly review what is known about the wrinkle and then set out some recent work which casts new light on how deep periorbital wrinkles ("crow's feet") form and are maintained and which may point to new therapeutic approaches to treat wrinkles.

WRINKLES AS AN ASPECT OF AGED SKIN

There are considerable literatures describing the differences between youthful and aged skin. In most cases, no attempt is made to differentiate between the area of the wrinkle and the unwrinkled skin around it. In large part this is because it is sometimes difficult to locate a wrinkle in a histological section—a surprising factor to which we will return later.

For a review of this literature, we would guide the reader to the excellent reviews by Kligman (1), Contet-Audonneau et al. (2), Lavker (3), and Tsuji et al. (4). The key findings are, however, as follows:

- The epidermis thins with age and the properties of the stratum corneum change in a multitude of ways including reduced barrier repair properties and decreased elasticity. These changes are particularly important in causing "fine lines" and are the changes that are countered by the vast majority of effective topical moisturizing products.
- The intricate, interconnected, elastic fiber network in the dermis degrades and is replaced (especially in the case of sun-exposed skin) by large deposits of poorly organized elastin deep in the dermis. This leads directly to loss of resiliency—the ability of the skin to quickly return to its original shape after distortion. In extreme cases, the large elastin deposits (so-called elastosis) can cause the skin to look sallow and yellow in color.
- Collagen fibers become less well organized and the collagen itself undergoes chemical changes that reduce its mechanical flexibility. Repeated imperfect collagen repair can lead to "scar like" patches of stiff, aligned collagen.
- Glycosaminoglycans composition changes. In sun-exposed skin with chronic (i.e., years) of photodamage there is a marked increase in certain types of sulfated glycosaminoglycans (versican) at the expense of others (decorin). Since these molecules can hold as much as 1000 times their own weight of water in an elastic gel these changes hugely impact the water content of the dermis.
- The subcutaneous fat layer (especially in women) decreases dramatically in thickness.

That skin which has undergone all these deleterious changes is more prone to wrinkles is self-evident—however, these changes are *not sufficient* to cause some types of wrinkling to occur. It is quite possible to find individuals and areas of skin where all these histological and biochemical changes can be seen but no wrinkles are visible. The extra factor that seems to be necessary is the repeated movement of the skin causing folding of the skin. Over time, and combined with the overall aging changes set out above, wrinkles form and progressively get worse. Thus, wrinkling can be seen on the face where "expression lines" form but not on the upper body even though the histological degradation of the skin may be worse in areas of the body. Around the mouth, smoking also makes a marked and very adverse contribution to wrinkling.

LOCAL DIFFERENCES IN THE WRINKLE COMPARED TO SURROUNDING SKIN

Considering what an obvious feature a wrinkle represents, there is a surprising lack of published data contrasting the actual wrinkle with the surrounding skin. Such studies that have been done suggest in fact that the classic features of solar elastosis are *less* in the base of the wrinkle than in the surrounding skin (2,4).

One reason for the paucity of these studies is that it is very difficult to identify wrinkles—even deep ones—in histological sections. When the skin is excised, the wrinkle partially disappears—evidence, if that were needed, that the wrinkle is an aspect of the whole skin. To overcome this problem a novel technique was used

whereby a line of cyanoacrylate glue was introduced into the wrinkle and allowed to set prior to taking a biopsy of the wrinkle. With this precaution, the shape of the wrinkle was preserved through processing, allowing a better correlation of histological changes to the architecture of the wrinkle (5).

Sections of these biopsies, which were taken from the periorbital "crow's feet" area were stained by a variety of techniques using both conventional and immunostaining techniques. We confirmed the finding (2,4) that the elastic fiber network was far less damaged at the base of the wrinkle than on the sides of the wrinkle or adjacent skin (Fig. 1). This is thought to be a consequence of the base of the wrinkle being less exposed to ultraviolet light than the surrounding skin—it suggests, however, that the damage to the elastic fiber network is unlikely to be a primary cause of the wrinkle.

In contrast to the normal appearing elastin at the wrinkle base, the "elastin" at the sides of the wrinkle is heavily disorganized (elastotic), a phenomenon that is worse on the lower, more sun-exposed side, as viewed when wrinkle would have been positioned in situ on the face. The collagen fibers were quite abnormal. In stark contrast to the sides of the wrinkles, where the collagen fibers tended to run parallel to the wrinkle and were in places relatively normal, at the wrinkle base there was a dense band of highly aligned collagen fibers running perpendicular to and underneath the wrinkle (Fig. 2).

This band of highly aligned collagen was one of the few features of the wrinkle that correlated clearly with the periorbital wrinkle location and gave any clue to what dictated the shape of the wrinkle. However, predicting how a complex material like skin will behave under different force regimes is a difficult challenge and intuitively "obvious" phenomena often turn out to be very different from what intuition would suggest.

To investigate this, a computer model of the skin was assembled (5), incorporating the structural information derived from the histology, and building in physical material constants for the different parts of the skin.

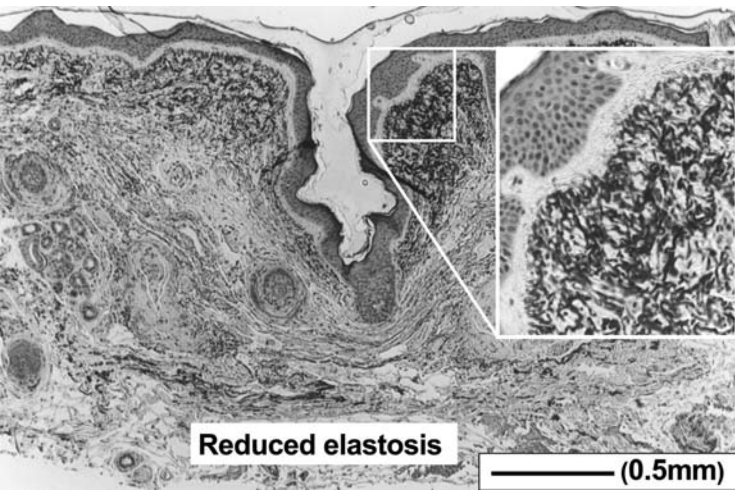

Figure 1 Elastic fibers in the periorbital wrinkle (orcein stain).

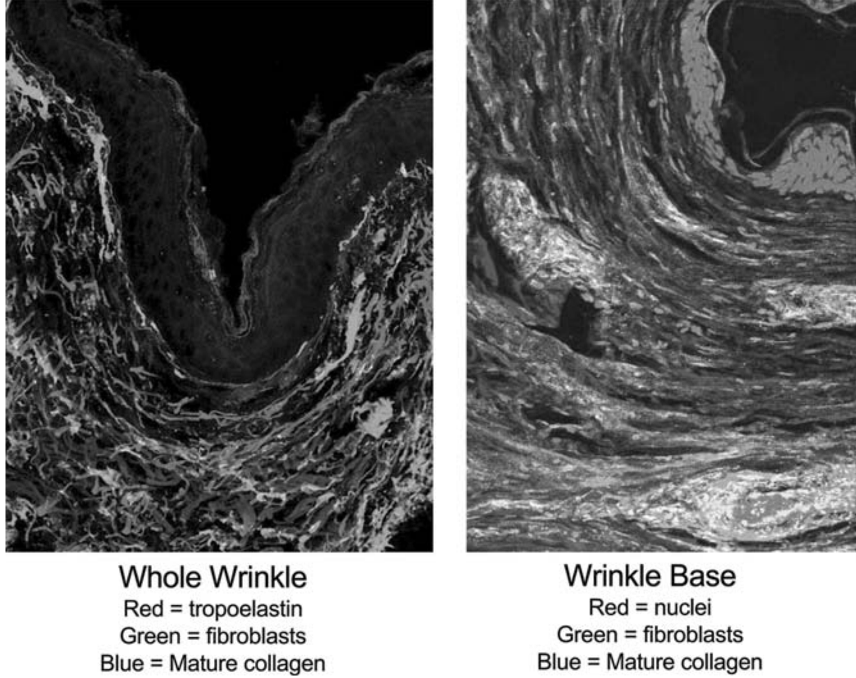

Figure 2 Highly aligned collagen at the base of the wrinkle.

COMPUTER MODEL OF THE PERIORBITAL WRINKLE

Figure 3 shows the basic structure of the computer model. It incorporates four different zones of material—the epidermis, the papillary dermis, the reticular dermis,

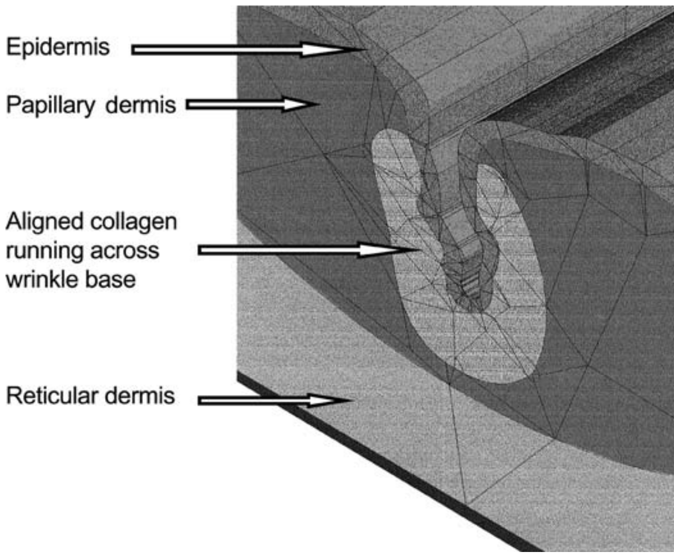

Figure 3 Computer model of the wrinkle.

and the highly aligned zone of collagen. Material properties of these zones were estimated from literature values as shown in Table 1. The model was based on finite element analysis using ProMechanica software. The base of the skin in the model was anchored to an inflexible substrate—no attempt to simulate the muscle or fat layer was made.

The first, and critical, finding was that the wrinkle could only maintain its shape with compressive forces pushing the wrinkle closed. Three possible sources of the compressive forces exist.

- Overall pressure from the surrounding skin:
  - If the skin has a larger natural area than it is constrained to by its attachment to the underlying tissue, then the whole skin will be under compression. This is probably a significant factor in older skin especially where there has been a significant loss of the subcutaneous fat layer leading to shrinkage of the attachment area for the skin. Conventional "face lift" procedures act on this compression effect by reducing the natural area of the skin so that the compression is replaced by a tension. Skin is usually maintained under tension as is evident from its behavior when cut (it will gape) or from instrumental measurements (6–8).
- Action of subcutaneous muscles:
  - The skin is extensively attached to muscle groups that can actively create tensions in the skin. This is particularly so on areas such as the forehead and around the mouth. Where muscle groups act to compress areas of skin, the compression can generate and sustain wrinkles. Botulinum toxin injections act on these muscle groups causing them to relax for as long as six months. The fact that in certain types of wrinkle such injections virtually eliminate the visible wrinkle proves that for these wrinkles the muscle generated compressive forces are the primary cause of the wrinkle.
- Tension within the aligned collagen at the wrinkle base:
  - Figure 4 shows the computer wrinkle model when the wrinkle has been allowed to open up, as if it were excised. The color scale shows the amount of stress in the different elements of the model. A major stress is found in a narrow zone at the base of the wrinkle, in the aligned collagen domain. This stress arises because of the removal of the compressive force on the wrinkle sides, and gives an indication of what might happen during day to day flexing of the wrinkle on the face. Put simply, the aligned collagen band is stiff but under little stress when the wrinkle is closed. As the wrinkle opens, the stiff collagen band deforms and

Table 1 Physical Constants Used in the Wrinkle Model

	Epidermis	Papillary dermis	Reticular dermis	Aligned collagen
Young's modulus	0.06 MPa	0.05 MPa	0.048 MPa	0.065 MPa
Poisson's ratio	0.45	0.49	0.49	0.49

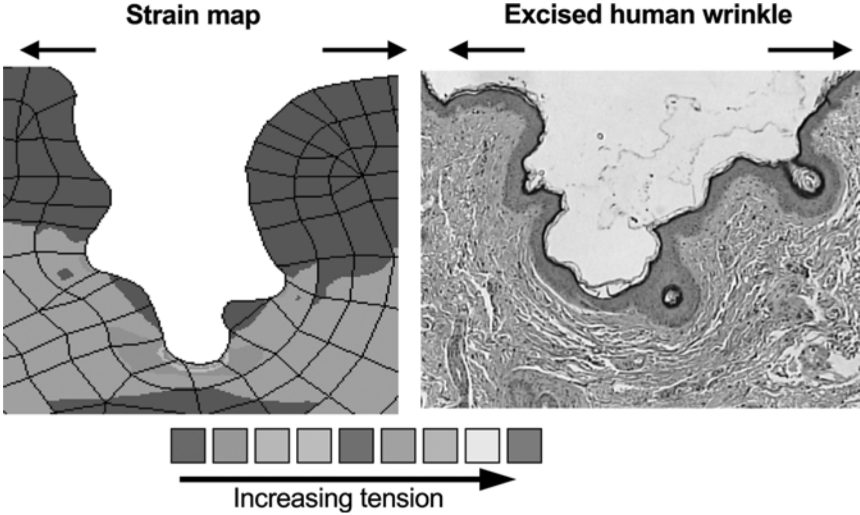

Figure 4 Strains in the periorbital wrinkle allowed to open up.

resists the opening of the wrinkle. This strongly implies that the aligned collagen band is a major factor in maintaining the shape of the periorbital wrinkle.

ORIGIN OF THE ALIGNED COLLAGEN LAYER

Why does the wrinkle possess this zone of highly aligned "scar like" collagen? While it is clearly important to the maintenance of the wrinkle once formed, it is unlikely to have a role in the initial formation of the wrinkle—indeed if such a zone were present in an area of unwrinkled skin it would probably cause the wrinkle to form elsewhere rather than in the strengthened zone.

To understand how the zone could form it is necessary to consider the interplay of forces within the tissue and the behavior of fibroblasts within the tissue.

Fibroblasts are richly endowed with surface molecules that will bind to components of the connective tissue—collagen, fibronectin, etc. When these surface molecules bind to the connective tissue matrix, they transduce mechanical forces acting through the binding sites into biological changes in the cell. One outcome of this is that fibroblasts within a connective tissue matrix that is under tension increase their synthesis of collagen and deposit it preferentially in the direction of the tension (9–11).

What does this mean for the skin? When unwrinkled skin is flexed so as to form a temporary "expression" fold the greatest tension is experienced by the tissue at the base of the fold. In response to that tension fibroblasts deposit collagen in the direction of the tension—perpendicular to the fold. The next time the skin is flexed and the fold forms, the tissue at the base of the fold is just a little stiffer due to the deposited collagen. That makes the tension at the wrinkle base just a little higher and induces the deposition of still more aligned collagen. Over time this vicious circle continues until the aligned collagen comes to dominate the stress field in the skin to the point where it maintains a permanent wrinkle. Sunlight, particularly UVB radiation, exaggerates this effect by activation of proteases and the triggering of further collagen realignment (12).

IMPLICATIONS FOR TREATMENT OF WRINKLES

It is clear from the discussion above that there are multiple distinct factors (e.g., acute and chronic sun exposure, natural skin tension and flexing, intrinsic aging changes including the thinning of the dermis and loss of subcutaneous fat) causing and sustaining wrinkles. In all body and facial sites it is likely that all the factors contribute to the overall wrinkling effect, but the relative contribution of the different factors will differ, resulting in a different optimum treatment regime.

On the forehead, the dominant factor sustaining wrinkles appears to be the action of the subcutaneous muscles. Botulinum toxin injections are therefore highly effective.

On the cheeks and around parts of the mouth, loss of subcutaneous fat and elastic fibers leads to a general loss of tension of the skin. In these cases, Botulinum toxin is less effective, and surgical removal of flaps of skin or generalized contraction of the skin using laser treatment is necessary to have a substantial effect.

The crow's feet area is characterized by wrinkles maintained by the stiffened aligned collagen zone at the wrinkle base. Botulinum toxin has a relatively little effect on these wrinkles, and while surgical "face lift" procedures may have temporary benefits, the analysis above suggests that stretching the wrinkles out may well cause yet more deposition of aligned collagen at the wrinkle base, ensuring that the wrinkles will return and possibly making the problem worse in the long term.

The analysis above suggests that a promising route for treatment of such periorbital wrinkles would involve stimulation of collagen remodeling, to put into reverse the vicious cycle that caused the aligned collagen zone to form. If collagen turnover could be stimulated while the skin was exposed to tension parallel to the long edge of the wrinkle, then the existing perpendicular collagen fibers would be destroyed while new fibers deposited parallel to the wrinkle would no longer sustain the wrinkle shape. Retinoic acid might be effective when used in this way as it has been shown to increase both the collagenase expression in skin and the deposition of newly synthesized collagen. To our knowledge, such an experiment has yet to be attempted.

ACKNOWLEDGMENTS

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REFERENCES

1. Kligman AM. The classification and treatment of wrinkles. In: Kligman AM, Takase Y, eds. Cutaneous Ageing. Japan: University of Tokyo Press, 1986:547–555.
2. Contet-Audonneau JL, Jeanmarie C, Pauly G. A histological study of human wrinkle structures: comparison between sun-exposed areas of the face, with or without wrinkles, and sun-protected areas. Br J Dermatol 1999; 140:1038–1047.
3. Lavker RM. Structural alterations in exposed and unexposed aged skin. J Invest Dermatol 1979; 73:59–66.

4. Tsuji T, Yorifuji T, Hayashi Y, Hamada T. Light and scanning electron microscopic studies on wrinkles in aged persons' skin. Br J Dermatol 1986; 114:329–335.
5. Green MR, Parish WE, Eastwood M, Wares J, Simon M, Siegel DM. The human periorbital wrinkle: immunohistology and computer modelling suggest key roles for directional collagen fibres, and mechanical force in wrinkle maintenance. Int J Cosmet Sci Submitted.
6. Gibson T, Kenedi RM. The significance and measurement of skin tension in man. In: Trans. 3rd Int. Cong. Plast. Surg. Washington, Baltimore: Williams & Wilkins, 1963: 387–395.
7. Alexander A, Cook TH. Accounting for natural tension in the mechanical testing of human skin. J Invest Dermatol 1977; 69:310–314.
8. Diridollou S, Vabre V, Berson M, Vaillant L, Black D, Lagarde JM, Gregoire JM, Gall Y, Patat F. Skin ageing: changes of physical properties of human skin in vivo. Int J Cosmet Sci 2001; 23:353–362.
9. Eastwood M, Mudera VC, McGrouther DA, Brown RA. Effect of precise mechanical loading on fibroblast populated collagen lattices: morphological changes. Cell Motility Cytoskeleton 1998; 40:13–21.
10. Wang JHC, Grood ES. The strain magnitude and contact guidance determine orientation response of fibroblasts to cyclic substrate strains. Connect Tiss Res 2000; 41:29–36.
11. Lindahl GE, Chambers RC, Papakrivopoulou J, Dawson SJ, Jacobsen MC, Bishop JE, Laurent GJ. Activation of fibroblast procollagen 1(1) transcription by mechanical strain is transforming growth factor β dependent and involves increased binding of CCAAT-binding factor (CBF/NF-Y) at the proximal promoter. J Biol Chem 2002; 277:6153–6161.
12. Takema Y, Nishijima A, Ohsu H, Fujimura T, Hattori M. Skin morphology at the time of UV irradiation is important for wrinkle formation. J Soc Cosmet Chem 1997; 48: 297–306.

5
Filaggrin and Dry Skin

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INTRODUCTION

Dry skin is usually considered a very complex condition involving changes in many aspects of the skin (1).

- The lipid barrier is deranged both in structure and composition in those suffering from dry skin, resulting in a vicious circle of reduced barrier quality and increased damage from environmental stresses.
- The desmosomes that hold cells of the stratum corneum fail to complete their programmed self-destruction in people suffering from dry skin, resulting in large flakes of incompletely desquamated cells.
- The stratum corneum has reduced water-binding capacity, resulting in loss of elasticity when the skin is exposed to dry conditions.
- A host of inflammatory changes are seen in dry skin ranging from changed levels of interleukin 1a to elevated prostaglandins.

Overlying this mechanistic complexity is the fact that few people suffer from dry skin in the absence of environmental stress—cold, dry air, wind, detergent damage, and so on. Thus, there is always a complex interplay between intrinsic and extrinsic factors at work.

Against this background of huge complexity it would seem unlikely that any one factor would be the single determinant of whether a person suffers from dry skin or not. However, very recent work suggests that that is indeed the case, and the genetic inheritance of the gene for the unique epidermal protein filaggrin is the dominant determinant of whether an individual will or will not suffer from dry skin.

This chapter starts with a description of this recent work and then reviews what is known about filaggrin in an attempt to provide context and understanding to this extraordinary discovery—though a complete explanation awaits further research, so those expecting a complete and final story may be disappointed!

FILAGGRIN GENOTYPE AS THE MAJOR DETERMINANT OF SUSCEPTIBILITY TO DRY SKIN

Filaggrin is encoded by the profilaggrin gene that is located in the epidermal differentiation complex (2) at the 1q21 locus on human chromosome 1 along with many other genes involved in the epidermal differentiation process. The gene codes for a large precursor protein called profilaggrin that consists of 10 to 12 filaggrin proteins joined by linking peptides (3).

This gene is highly polymorphic, that is, it varies significantly between individuals with many single nucleotide differences found. The most significant polymorphism however is not any change in a single nucleotide but is instead the presence of either 10, 11, or 12 filaggrin repeats in the profilaggrin. At some point in evolution of mankind, replication and fusion of some filaggrin precursor gene must have occurred more than once to give rise to these different forms of profilaggrin. The different genes have spread throughout the human race so that people are now found with all the possible combinations of these different profilaggrin genes. Because each person has two copies of the gene (one from the father and one from the mother) there are six different combinations found. These are referred to as 10,10; 10,11; 10,12; 11,11; 11,12, and 12,12, where the number refers to the number of filaggrin repeats in that copy of the gene (3).

The importance of this genetic makeup became apparent from a very simple experiment, shown in Figure 1. A large panel of volunteers had their filaggrin genotype measured and then answered a simple question. "Do you suffer from frequent dry skin?"

Only two people who had a copy of the 12 repeat profilaggrin gene reported that they suffered from frequent dry skin compared to 15 who had only 10 or 11 repeat genes—a difference that was statistically significant at the 98% level.

Because it is inevitable that many people in both groups would be exposed to the kind of environmental stress that will cause dry skin it must be concluded that possessing a copy of the 12 repeat gene strengthens the skin so much that it can withstand those stresses without suffering damage. Lacking the 12 repeat gene does not make it inevitable that the person will suffer from dry skin—other factors must presumably be present as well—but it does very strongly predispose the person to suffering from dry skin.

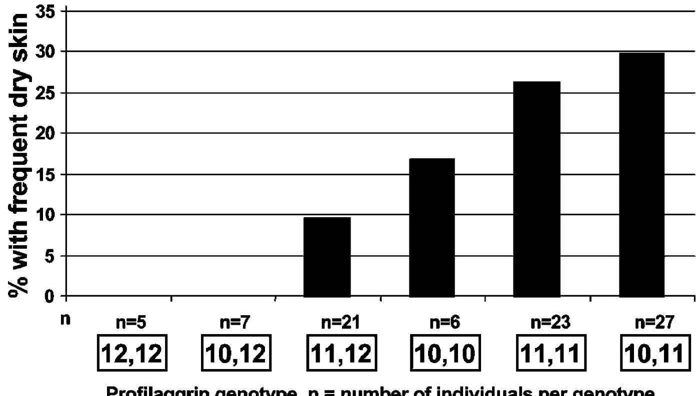

Figure 1 Correlation of profilaggrin phenotype with susceptibility to frequent dry skin.

Filaggrin is clearly a vitally important protein in the skin to have such a dominant effect on the skin's resistance to stress. Exactly why that should be is still not known—in the sections that follow, the current state of knowledge is summarized but no clear mechanism whereby the different profilaggrin genes could have such a dominant effect on the skin is yet apparent.

THE LIFE CYCLE OF FILAGGRIN

Profilaggrin is synthesized in large amounts in the uppermost cells of the epidermis. It accounts, in fact, for as much as 33% of all the protein made in the epidermis and a still larger fraction of that made in the uppermost cells of the granular layer of the epidermis (4) (Fig. 2).

It is one of the largest proteins known, having a molecular weight of about half a million. It consists of an N terminal domain followed by 10, 11 or 12 repeating filaggrin domains joined by short linker peptides and finally a C terminal domain (3).

The filaggrin domains which represent most of the molecule have a most unusual amino acid composition with a preponderance of basic amino acids such as histidine and arginine. This would make these regions very strongly positively charged, or basic, if it were not for the fact that as many as 1 in 15 of the amino acids (usually serine) is phosphorylated. These large number of acidic or negatively charged groups make profilaggrin overall neutral in charge (5).

Profilaggrin aggregates very strongly in the cells where it is made—presumably because of the strong attraction between the many positive and negative charged groups it carries. These aggregates form the keratohyalin granules that give their name to the granular layer of the epidermis.

The transition of a cell from the granular layer into a stratum corneum cell is dramatic. As cells intermediate in appearance between granular layer and stratum corneum are rarely seen in electron micrographs, an upper limit of about six hours has been estimated for the transition process to take place. The granular layer cell contains a nucleus, has a normal outer membrane, has endoplasmic reticulum and

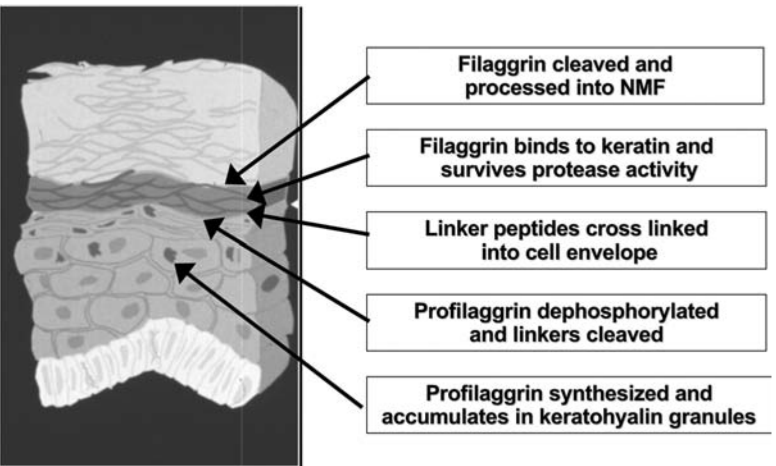

Figure 2 The life cycle of profilaggrin and filaggrin. *Abbreviation*: NMF, natural moisturizing factor.

ribosomes to make proteins, has mitochondria to generate energy—in fact, it has all the components of a living cell. All of these components are broken down and are absent from the stratum corneum cell. There is a massive hydrolytic phase involved, where proteins, nucleic acids, and other macromolecules are broken down and reabsorbed by the body.

Profilaggrin is one of the most protease-sensitive proteins in existence. It is so labile that for many years only partially degraded fragments of it could be purified. Nonetheless, it survives the intense hydrolysis phase of the transition. It does this by virtue of a complex series of molecular changes. The first stage is a complete loss of phosphate coupled to an apparently simultaneous cleavage of the C and N terminal domains and cleavage of the short peptides linking the filaggrin repeats (5).

The different parts of the profilaggrin protein then undergo different fates.

- The linker peptides become incorporated into the thickening cell envelope, joining several other proteins in forming this strong outer layer of the stratum corneum cell (6).
- The N terminal domain, which contains two specific calcium-binding domains of unknown function, translocates into the nucleus shortly before the nucleus is dissolved (7).
- The filaggrin domains, now intensely basic having lost their neutralizing phosphates, bind tightly to the keratin filaments that are the major surviving protein in the cell. The complex of keratin and filaggrin fills the new stratum corneum cell in a close packed, so called, keratin matrix (8).
- Under the influence of the filaggrin, cysteine amino acid side chains from the keratins cross link to permanently lock the keratin filaments through disulfide bonds into a single immensely strong keratin macromolecular assembly (9). In a very real sense, at this stage the keratins and the cross-linked cell envelope together form a single enormous disk-shaped molecule 80 μm wide and 1 to 2 μm thick!

At the end of this process the stratum corneum cell is fully formed, and the rapid changes come to an end. Filaggrin is the second most abundant protein, after keratin in these newly formed stratum corneum cells, but it will not remain so for more than a day or two!

FILAGGRIN AND THE NATURAL MOISTURIZING FACTOR

So long as the stratum corneum cell remains well hydrated—technically so long as the cell has a water activity greater than 0.95—filaggrin changes only slowly. This change is a gradual conversion of the basic arginine amino acids in filaggrin to uncharged citrulline by a specific enzyme called peptidyl arginine deiminase (10). As the stratum corneum cell gradually moves toward the surface of the skin (a process that typically takes about 14 days) this process can continue until the highly basic filaggrin protein becomes neutral again—as it was when it was part of the profilaggrin precursor. This time, however, neutrality is achieved not by matching the positively charged arginine amino acids with negatively charged phosphorylated serines but by conversion of the basic arginines to neutral citrulline.

This reneutralized filaggrin no longer has the ability to aggregate keratin filaments but that is no longer important to the cell as the disulfide cross-linked keratins have no need of filaggrin to maintain their aggregation.

This process of deimination to form neutral filaggrin rarely gets to this level however. As the stratum corneum cell moves toward the skin surface it becomes exposed to increasingly dry conditions. As soon as the water activity (or relative humidity) falls below 0.95 or 95% a new and irreversible process commences (11).

Proteases in the stratum corneum become active and completely degrade the filaggrin down to its individual amino acids (12). The mechanism of this action is not yet known but may be triggered by increased concentrations of ions as water is lost from the cell. Once liberated from the filaggrin, the free amino acids in their turn undergo certain changes. Glutamine loses ammonia and becomes pyrrolidone carboxylic acid through a non-enzymic process (13). Histidine also loses ammonia under the influence of an enzyme histidine ammonia lyase, producing urocanic acid (14).

These processes are critical to the stratum corneum. The amino acids, in general, and the pyrrolidone carboxylic acid, in particular, are very hygroscopic—that is, they attract water even out of relatively dry air. The generation of this complex of amino acids [often called the natural moisturizing factor or (NMF)] thus allows the stratum corneum to remain hydrated when it is exposed to drying conditions. This production of NMF is now generally accepted to be the most vital known function of filaggrin.

It is important to the function of the stratum corneum that conversion of filaggrin to NMF does not occur until the cell has begun to dry out. This is because the very concentrated pool of small molecules, that is the NMF, is capable of generating a huge osmotic pressure if the cell is exposed to high relative humidity. For example, stratum corneum in pure water would generate osmotic pressures within the cell in excess of 200 psi or 15 times the atmospheric pressure. Generation of NMF from filaggrin, only after the cell has moved close enough to the skin surface to partially dehydrate, ensures that such high osmotic pressures are rarely experienced and then only after the immensely strong cross-linked protein structure of the stratum corneum cell has been stabilized.

This control mechanism for conversion of filaggrin to NMF results in the thickness of the filaggrin layer in the varying stratum corneum depending on the humidity of the environment. When the environment is dry, the stratum corneum cells experience the "trigger RH" of 95% humidity as soon as they have moved two to three cell layers up in the stratum corneum. When, however, the environment is humid, or the skin is occluded, they may move almost all the way to the skin surface before the conversion to NMF is triggered. This is illustrated in Figure 3.

Generation of the NMF has yet another effect on the stratum corneum. The large amounts of ammonia liberated from conversion of histidine and glutamine to urocanic acid and pyrollidone carboxylic acid easily diffuse out of the stratum corneum, either to the air or back into the body. This leaves the weak acids behind, which causes the stratum corneum itself to become weakly acidic. This well-known "acid mantle" is important in protecting the stratum corneum from microbiological attack.

PERSPECTIVE ON PROFILAGGRIN AND FILAGGRIN FUNCTIONS

It may seem from all the discussion above that filaggrin is not short of roles because it has been shown to

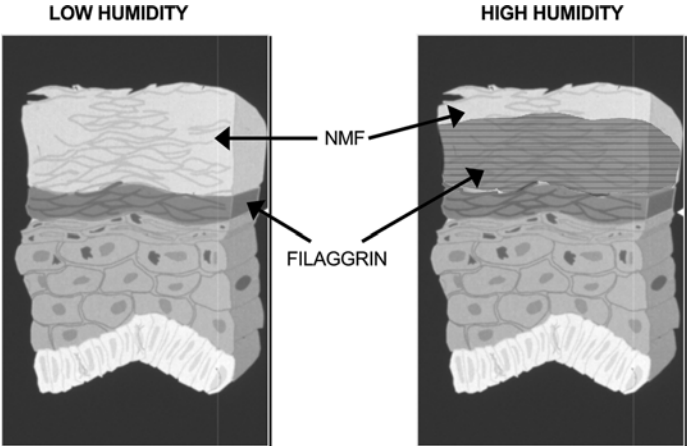

Figure 3 Illustration of the effect of differing environmental humidity of filaggrin conversion to the NMF. *Abbreviation*: NMF, natural moisturizing factor.

- help in aggregating keratin into a close packed matrix structure,
- catalyze disulfide bond formation between keratins,
- create the NMF,
- create the acid mantle of the skin,
- generate urocanic acid which has potential roles as sunscreen and immunomodulator (15,16).

It is probable however that one or more vital roles for the profilaggrin/filaggrin system have yet to be discovered and will provide critical new insights into the complex process of epidermal differentiation.

The large gap in our understanding is illustrated by two key unexplained observations.

Profilaggrin Polymorphisms Correlate to Susceptibility to Dry Skin

This relates to the new findings with which this chapter started. It is tempting to ascribe the major impact that lacking a 12 repeat profilaggrin gene has on susceptibility to dry skin as being due to the impact of the polymorphism on the generation of the NMF. Unfortunately, this superficially attractive hypothesis is unlikely to be valid. The difference in size of the 12, 11, and 10 repeat versions of profilaggrin would account for only, at most, a 20% difference in the NMF level—much smaller than the normal variation between individuals.

None of the other known or hypothesized functions of filaggrin can explain why the different genotypes make such a difference.

Profilaggrin Is Not Always Converted into Filaggrin

All the proposed functions of the profilaggrin/filaggrin system have focused on filaggrin or its breakdown to NMF. Profilaggrin has been seen as just a storage form of the protein keeping it safe and inactive until it is needed.

However, histochemical and biochemical studies of the filaggrin system in oral epithelia [(17), Fig. 4] have shown that in the hard palette of the mouth, profilaggrin

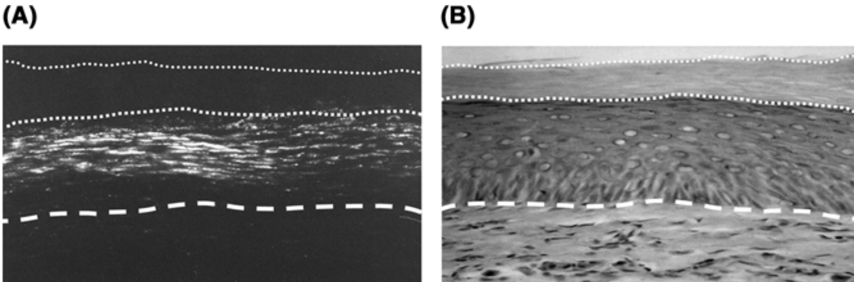

Figure 4 Presence of profilaggrin but absence of filaggrin in hard palette tissue.

is made and aggregated into keratohyalin granules, but not converted to filaggrin at all—presumably it is broken down and reabsorbed along with most other cellular proteins when the hard palette granular layer transits into the stratum corneum. What function does the profilaggrin fulfill in this tissue? Is it related to the translocation of the N terminal domain with its two calcium-binding domains into the nucleus?

Clearly, despite all the progress made, the scientific community is missing a key insight not only into the role of profilaggrin but also into the totality of the process of epidermal differentiation, as profilaggrin and filaggrin were isolated almost 25 years ago. The extraordinary findings with which this chapter started are sufficient to conclude that discovering this insight will mark a great advance in the understanding of how our skin's vital surface layer is formed and how it can sometimes go wrong.

REFERENCES

1. Rawlings AV, Harding CR, Watkinson A, Scott IR. Dry and xerotic skin conditions. In: Leyden, Rawlings, eds. Skin Moisturisation. New York: Marcel Dekker, 2002:119–143.
2. Mischke D, Korge BP, Marenholz I, Volz A, Ziegler A. Genes encoding structural proteins of epidermal cornification and S100 calcium-binding proteins form a gene complex (“epidermal differentiation complex”) on human chromosome 1q21. J Invest Dermatol 1996; 106:989–992.
3. Gan SQ, McBride O, Idler WW, Markova N, Steinert PM. Organisation, structure and polymorphisms of the human profilaggrin gene. Biochemistry 1990; 29:9432–9440.
4. Scott IR, Harding CR. Studies on the synthesis and degradation of a high molecular weight histidine-rich phosphoprotein from mammalian epidermis. Biochim Biophys Acta 1981; 669:65–78.
5. Harding CR, Scott IR. Histidine rich proteins (filaggrins): structural and functional heterogeneity during epidermal differentiation. J Mol Biol 1983; 170:651–673.
6. Takahashi M, Tezuka T, Katunuma N. Filaggrin linker segment peptide and cystatin alpha are parts of the cornified envelope of epidermis. Arch Biochem Biophys 1996; 329:123–126.
7. Zhang D, Karunarratne S, Kessler M, Mahoney D, Rothnagel JA. Characterisation of mouse profilaggrin: evidence for nuclear engulfment and translocation of the profilaggrin B domain during epidermal differentiation. J Invest Dermatol 2002; 119:905–912.
8. Steinert PM, Cantieri JD, Teller JD, Lonsdale-Eccles JD, Dale BA. Characterisation of a class of cationic proteins that specifically interact with intermediate filaments. Proc Natl Acad Sci USA 1981; 78:4097–4101.
9. Steinert PM. Epidermal keratin: filaments and matrix. In: Marks R, Plewig G, eds. Stratum Corneum. New York, Berlin, Heidelberg: Springer-Verlag, 1983:25–38.

10. Harding CR, Scott IR. Histidine rich proteins (filaggrins): structural and functional heterogeneity during epidermal differentiation. J Mol Biol 1983; 170:651–673.
11. Scott IR, Harding CR. Filaggrin breakdown to water-binding compounds during development of the rat stratum corneum is controlled by the water activity of the environment. Dev Biol 1986; 115:84–92.
12. Scott IR, Harding CR, Barrett JG. Histidine-rich-proteins of the keratohyalin granules: source of the free amino acids, urocanic acid and pyrrolidone carboxylic acid in the stratum corneum. Biochim Biophys Acta 1982; 719:110–117.
13. Barrett JG, Scott IR. Pyrrolidone carboxylic acid synthesis in guinea pig epidermis. J Invest Dermatol 1983; 81:122–124.
14. Scott IR. Factors controlling the expressed activity of histidine ammonia lyase in the epidermis and the resulting accumulation of urocanic acid. Biochem J 1981; 194:829–838.
15. Olivarius FD, Wulf HC, Crosby J, Norval M. The sunscreening effect of urocanic acid. Photoderm Photoimmunol Photomed 1996; 12:95–99.
16. DeFabo EC, Noonan FP. Mechanism of immune suppression by ultraviolet irradiation in vivo. J Exp Med 1983; 157:84–98.
17. Harding CR, Scott IR. Stratum corneum moisturising factors. In: Leyden, Rawlings, eds. Skin Moisturisation. New York: Marcel Dekker, 2002:70–72.

6
Hair

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INTRODUCTION: WHAT IS HAIR?

Hair is a keratinized fiber that emerges from the skin surface. Hair fiber have many qualitative characteristics such as it may be thick or thin, straight or curly, long or short. This material has been taken care of since the earliest stages of evolution (grooming). The activity has been organized into professions (hairdresser salons, hair removal institutes, etc.). After the era of industrial revolution, hair, initially the exclusive battlefield of cosmetic companies, has now become a focus of great interest to the medical community. New research and measurement technologies are being developed on a day-to-day basis as the scientific community has invested a lot of effort in the recent years (1). In this chapter we shall provide the reader with some basic numbers and statements. At this stage of the reading, we stress the fact that we consider skin and hair so intimately interacting that they are part of a system. Any compound that is supposedly applied only on the hair may sooner or later come into contact with the skin. At first glance, this may seem trivial but an often-neglected dimension! Who would ever think that a shampoo or a hair dye might induce an allergic reaction that in turn results in hair shedding? As safety is addressed in other sections of this manual, let us focus on the fiber and its life cycle.

In spite of appearance, there is as much hair potential on the skin of a newborn baby as in the adult: The hair potential is structurally fixed even before birth!

There are as many hair follicles in the female as in the male; at least one domain, hardly conceivable in our sex-driven cultures, where man and woman are biologically equipotent!

When no hair is visible at the skin surface, the skin is also named glabrous skin. The only areas of skin that are definitely unable to make natural hair, i.e., palms and soles represent less than 10% of the body surface. The other way round we are hairy animals and you, as a reader, are deemed to remain happy with that knowledge!

Hair thickness ranges from almost nonperceptible—less than 8 μm in diameter—to coarse and wiry fibers over 100 μm in diameter. Hair comes out from the skin with an angle usually between 20° and 60°. In more extreme situations, it may remain in the skin for a while before emerging (ingrown) or stay as a pillar at right angle on the skin surface. The length varies a lot from almost nothing visible above skin level to over 1 m.

A transverse section in black African hair is almost flat elliptical, while Oriental hair is closer to circular and Caucasian hair in between. In the latter, the section of the thinnest hair is circular. The fiber itself contains exclusively dead cells. The cells are still visible (Fig. 1) after completion of a specific differentiation program. The cells stick together and form a fiber that, at some stage, will be shedding as a whole. Breakage may occur after severe weathering. Breakage, a haircut, or shaving will remove only the distal—nonliving—parts of the hair fiber. Hair is biochemically distinct from the horny layer of the epidermis. As a matter of fact hair derives from a special organ where it takes its "roots," i.e., the hair follicle. The number of follicles appears to be determined before birth, and there is no evidence that new roots or follicular neogenesis does occur after the embryonic follicular initiation phase. This organ formation results from subsets of epithelial cells that have migrated at an early stage of the embryonic development and are located deeper inside the skin. The distribution of follicles and their functional activity are responsible for the global appearance and patterning of hair (see illustrations). These, all together, reflect the complexity of the mechanisms involved in the maintenance of these apparently simple fibers; a minimal change in the geometry of the hair root will result in clinically significant changes of the aspect of the hair (Fig. 2). The purpose of the following sections is to describe in greater detail some of these aspects.

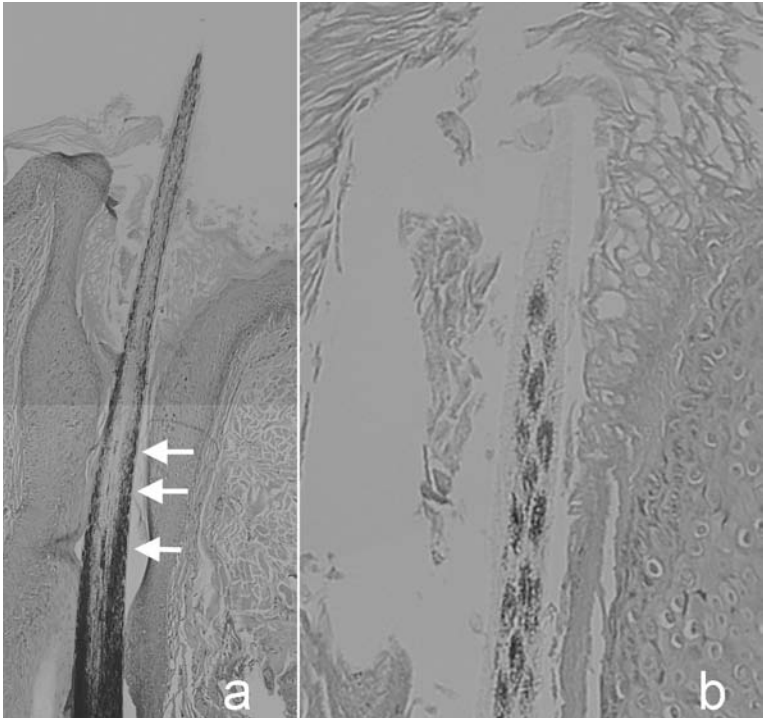
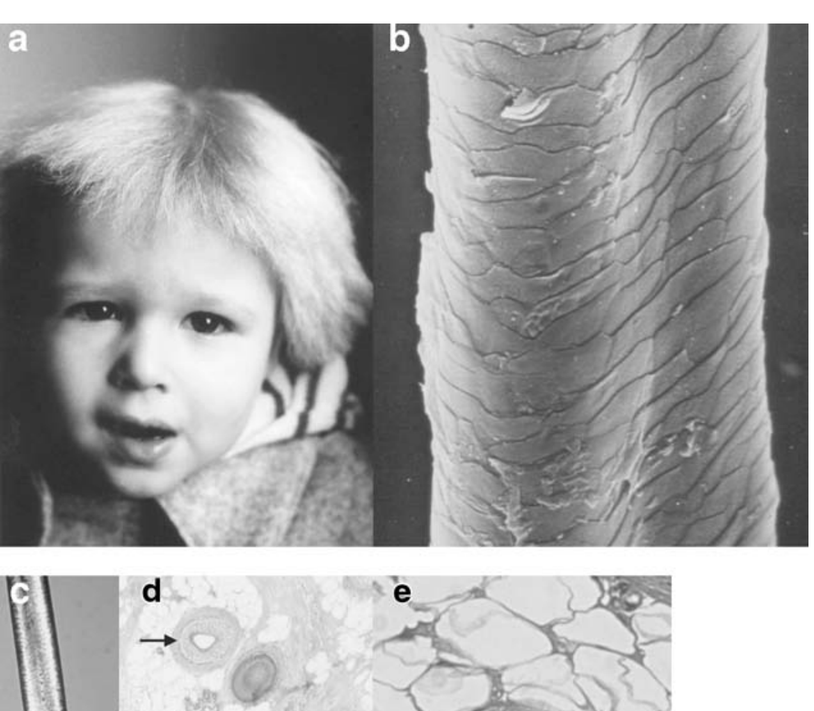


Figure 1 Pigmented black African hair. This section of the upper part (acroinfundibulum, **a**) of a hair follicle is typical of African scalp. The hair fiber shows the unstained hair cuticula that surrounds the hair cortex. Cortical cells are outlined by the hair cuticula and, almost in the center and lower part of the hair fiber, there is evidence of formation of a hair medulla (*arrows*). At higher magnification (**b**) cells in the hair cortex appear as elongated fusiform elements fully packed with dark stained melanin granules. The follicular opening clearly shows epidermal type keratinization of the acroinfundibulum.



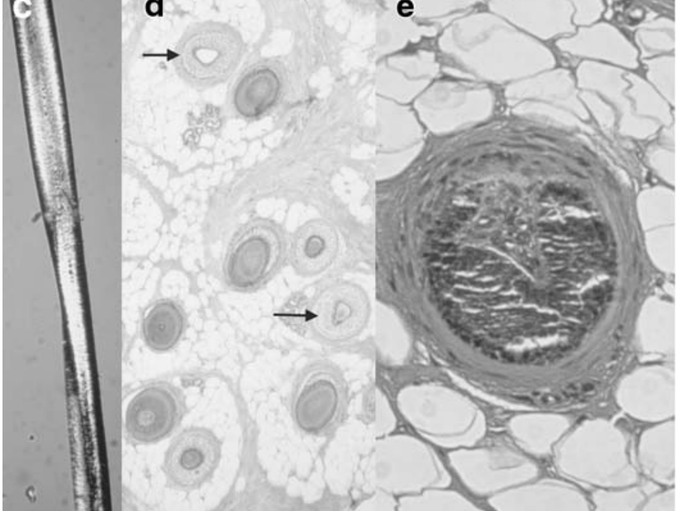


Figure 2 Spun glass hair or uncombable hair syndrome: from the clinic to the hair root. Clinically, this young patient's hair is unmanageable due to the peculiar shape of the fiber (**a**). Scanning electron microscopy (**b**) shows a nice cuticular surface but the shape of the hair is abnormal. The presence of lateral groove provides special physical properties to such fibers that do not lie flat after combing. This grooved hair shows some torsion under polarized light microscopy (**c**). The triangular shape of the hair shaft is due to an abnormality of the fiber constitution deeper inside the hair follicle (**d**). In the deepest part, at higher magnification, the hair root clearly shows the abnormal shape of the dermal papilla (**e**). Instead of an ovoid shape it has a triangular aspect in this section. This in turn is responsible for the abnormal geometry of the inner root sheath (IRS) which is the first structure to become rigid after terminal differentiation. The IRS forms a molding funnel with a triangular shape (**d**, *arrows*). As a consequence the yet undifferentiated hair cells in the center of the hair follicle (future cuticle, cortex, and eventually medulla forming cells) move into and mold to the deformed IRS and generate the triangular shape of the hair fiber.

The Structure of the Hair Fiber

Hair can be compared to a cylinder with three typical layers. The bulk of any hair is the cortex. The mature cortex is made of adjacent, closely packed spindle-shaped cells. Intercellular membranes that play a key role in cementing the cells together unite these cells. Within the cells one finds special keratin molecules. Keratins are a group of insoluble helicoidal protein complexes. Because of the resistance of these protein complexes, hairs have been said to contain hard keratins as opposed to the soft keratins of desquamating tissues (2). These coiled proteins are embedded into an amorphous matrix. This matrix is rich in high-sulfur proteins. Disulfide bonds between molecules make the whole protein build-up cohesive, but the ultimate resisting layers are on the outside and form the hair cuticle (3).

The hair cuticle (Fig. 2) covers the cortex and confers strength and protection to it. Cuticle layer consists of five to ten overlapping cells that look like the tiles on a roof. The cuticle cell-free endings are oriented toward the distal end of the hair. Immediately adjacent to the exposed surface is an exocuticle alpha or A-layer. This layer is rich in high-sulfur proteins and confers much of the resistance to this layer. Nevertheless, the nice looking scale margins of the original fiber are gradually damaged as the hair emerges from the skin. The free edges of these microscopic tiles erode and break off. This peculiar arrangement of overlapping cells appears very efficient to provide long-lasting protection. It prevents premature breakdown of the cortex. As there is no repair, any new alteration imposed upon the exposed cuticle will result in cumulative damage. At last after repeated chemical and physical insults or when genetically abnormal, the cuticle becomes functionally ineffective or is structurally absent. The hair cortex becomes exposed. Cortex does barely resist to minimal insults occurring at the skin or scalp surface, and the hair breaks off. The only cure is a haircut and prevention of renewed physical–chemical insults (2,3).

The hair medulla is usually located in the middle of the thicker hair. The protein content is essentially trichohyalin. The degradation and metabolization of these proteins contribute to the water holding properties of the hair and at the same time results in the appearance of an empty channel in the middle of the hair. Typically, segments of small or larger empty space alternate with protein filled medulla, and air spaces may become almost continuous in the thicker hair fibers. Under the light microscope, hair medulla is usually visible as dark air-filled spaces; these may be mistaken as pigment. For more detailed information about chemical composition of hair we refer the interested reader to more specific textbooks (4,5).

The thickness of hair has been used to classify hair fibers into two broad categories. Thin hair (less than 40 μm) also named vellus hair appears as a soft nonpigmented one and seldom exceeds a length of 2 cm. During embryonic life, the thin hair that is being formed is called lanugo. Under the microscope, it does not show a prominent medulla. Thick hair (more than 40 μm) is considered as a terminal, which is also longer, coarser, and often pigmented and medullated as opposed to the thinner hair (3).

Before puberty, terminal hair is normally present on the scalp, eyebrows, and eyelashes. After puberty, secondary sexual terminal hair is developed from pre-existing vellus hair follicles that respond to circulating and locally transformed hormones, especially androgens. This maturation process is due to specific structural and functional modifications affecting the hair roots in some predefined and limited territories that show hormone sensitivity. Accordingly, a local response reflects a systemic modification.

Hair looks and feels different from the skin from which it derives due to its specific chemical composition and the ensuing physical properties.

The physical properties may be described briefly in terms of optical, mechanical, and electric properties. All together this will condition the general appearance of hairiness, which is explained in physiological terms and in great detail in the Camacho and Montagna textbook (6).

Optical Properties of Hair

Optical properties are largely explained by the peculiar structural organization of the hair shaft. When light hits the hair surface, it is partly reflected, and there is some absorption when the light penetrates the hair fiber. The reflection of visible light on the hair surface or its internal structures is largely dependent on the presence and type of genetically determined pigmentation. In African hair, most of the light is absorbed, and it looks black (Fig. 1).

The cells responsible for skin pigmentation, in general, are also at work in the hair root. The pigment granules in the hair fiber are contained mainly in the hair cortex. This explains why cosmetics that are used to irreversibly modify the natural hair color need to penetrate inside the hair cortex. This job can only be achieved after significant irreversible alterations of the protective layers at the hair surface. The repeated application of these harsh procedures will result in cumulative damage of the hair surface. After some time, the hair feels rough and looks dull. The chemistry of other physical–chemical hair procedures including the surface effects of conditioners and hair dyes is discussed at large in other sections in this textbook.

In some cases, when the pigment load is lighter the hair looks white. On top, such hair may appear very bright as air-filled spaces in the medulla work like microscopic mirrors and reflect the incident light (Fig. 3). This may be present all along the fiber or show up only in some segments. Hence, thick and less pigmented hair of aged people appears even whiter. So-called gray hair, usually referred to as "salt and pepper," is in fact a mixture of white and normally pigmented hair. More detailed structural–functional considerations on white and pigmented hair fibers have been reported in a recent review by Van Neste and Tobin (7) along with original data in a study on the interrelation growth-pigmentation in females during aging (8).

Mechanical Properties of Hair

The hair shaft is a rather strong fiber behaving like reinforced wire. Resistance to longitudinal deformation, bending, and torsion stiffness and hold of set hair are related to fiber diameter (7,8). The relationship between the constraint and the elongation obtained follows a curve of three regions (preyield, yield, and postyield) according to the stretching force (9). Fiber breakage occurs mainly in the postyield region. All factors diminishing this cohesion between cells and ultimately between the protein compounds of the hair cells bring the load value down, e.g., wet hair or in the case of a deficiency of high-sulfur proteins. Examination of load elongation curves helps in studying how hair behaves in the course of various hair dressing procedures including the wide range of temperature, humidity, and chemical agents involved. Tightly curled African hair is fairly fragile, due to the highly twisted configuration and flattening as opposed to Asiatic hair.

One should not confuse the intrinsic physical strength of the fiber itself with the cohesive forces that bind the fiber to the surrounding structures in the hair roots; these hair follicle aspects shall be discussed in the section dealing with the hair cycle and its abnormalities. At this stage however, we want to mention that growing hair is

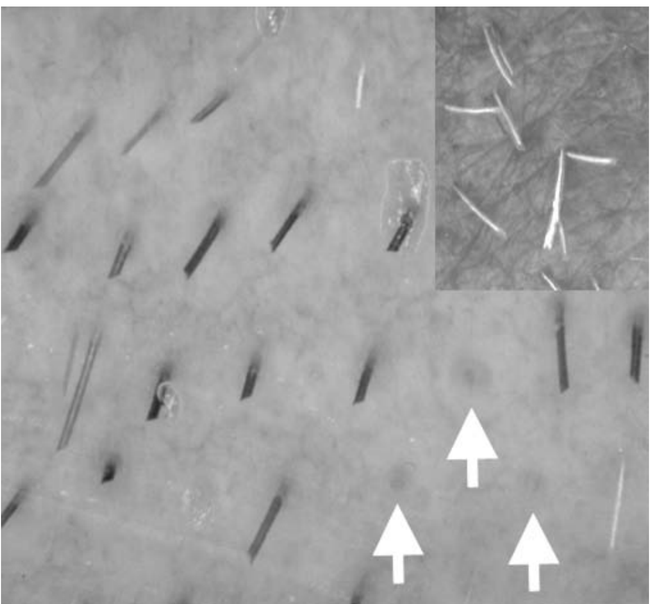


Figure 3 In vivo close-up view of beard. This surface view of facial skin of a male adult shows various thick hair fibers and empty follicular openings (*three arrows*). The presence of a medulla in the unpigmented thick hair is highlighted (*insert*) by light reflection the intensity of which depends much on the type incident light used for the epiluminescence microscopy.

firmly anchored to the skin. At the end of the growth period, hair roots form a club that detaches from the living structures and leads to physiological shedding. This must be distinguished from hair breakage.

The absolute length of the hair as it emerges from the skin surface significantly modifies its physical perception and has led to a lot of misconceptions about hair growth. A typical example is when the hair is cut short or shaved. After some time, people perceive the raspy feeling as more and more short stubs rise on the skin surface. An often-reported interpretation is that hair is now thicker and as a consequence should grow faster. In fact, thickness and growth remain unchanged. The feeling and/or visual perception has changed but neither the composition nor the biology of the hair growth has changed. Modified perception induced misconceptions, and consequently attitudes such as not shaving or epilating women's legs because it will aggravate growth—it would get "worse" spontaneously—or cut the bad hair so as to improve the regrowth.

Electrical Properties of Hair

The electric charges at the surface of hair may be modified along with environmental changes such as rubbing and shampooing (10,11). Heavily charged hair may cause repulsion between individual hair fibers and make hair unmanageable. This fly-away phenomenon should not be confused with other reasons for less manageable hair such as changes in the bending of the fiber as may occur in some hair dysplasias, e.g., straight hair nevus, steel hair or uncombable hair syndrome (Fig. 2).

On the one hand, hair conditioning—during shampooing in the two-in-one technology (12) or in a two-step sequential application modality—helps styling the hair by moderating static charges between the hair itself and also between hair

and the materials used for combing. On the other hand, such hair care procedures do not appear to influence the physiological hair shedding rates or hair dye or curling effects (12,13). Besides positive cosmetic effects on hair (e.g., untangling), compound shampoos or additional "leave-on" compositions may negatively affect perception of hair globally (unpleasant feel, faster greasiness, . . .) or induce unpleasant scalp sensations reported as itch or sensitive scalp (personal unpublished data) or in the most sensitive scalps induce allergic contact dermatitis (see elsewhere in this book).

Inappropriate hair care may result in opposed (positive and negative) electric charges on hair fibers. Attraction of hair segments face to face will fix them together in a randomized way with hair matting. A similar arrangement of felt-like fibers may be obtained on purpose and was trendy in the Rastafarian culture. Whatever the cause, there is no way of untangling these masses of fibers, and a hair cut is the only solution. Newly synthesized and yet unaffected hair fibers will grow and remain manageable as long as the styling habits and hair care are appropriate.

WHERE DOES HAIR COME FROM?

In the previous section we have alluded to "roots and follicles." In this section the structure and morphogenesis of the hair follicle are being reviewed. The formation of the hair follicle as an organ is the result of subtle interactions between dermal cells and epithelial cells. This paired interaction is vital not only for the morphogenetic stages in the embryo but throughout the whole life when the hair follicle structure partially vanishes and is reconstructed periodically along a cycling process. Before we describe when and how the hair follicle starts to work, let us consider some numbers.

The total number of hair roots is probably over one million on the body, of which around 100,000 are on the scalp. Cumulative scalp hair production amounts to 30 to 40 m every day. After a two-year period of continuous growth (e.g., female hair style with long uncut hair) a full head of hair may represent a surface of $6\,m^2$. This is quite something to take care of several times a week or per day! The only really bare areas on the human body (male and female) are the soles of our feet and the palms of our hands because they do not contain hair follicles.

Embryology of the Hair Follicle

The embryo is covered by a continuous epithelial bilayer also named periderm. It is only after the hair follicle will have completed its maturation that follicular openings will become visible. Hair follicles derive from the primitive epithelium covering the human fetal skin. This happens during the eighth and the twelfth week after conception. The morphogenetic steps are very well described by Holbrook who reviewed the work illustrated with refined microscopical techniques (4). It is a synchronized process of mutual induction and differentiation of selective epidermal and dermal cells (14). This takes place initially in some areas like the eyebrows, the upper lip, and the chin. Then the process extends as a wave to other body regions during the fourth month. This finally ends up with the constitution of small follicular units, each of which contains three hair follicles. Under the microscope, the initial steps of this process appear as a crowding of cells in the basal layer of the epidermis face-to-face with aggregated mesenchymal cells. Cells in the basal layer elongate to form the hair peg, which grows obliquely downwards. The final angle formed within the skin is subject to a wide variation according to body region. The broad tip of the hair peg will invaginate so as to almost completely engulf the nugget of

mesenchymal cells, which now forms the dermal papilla. During the downward course of the hair peg, two buds appear at the posterior side of the follicle. The upper one will transform into the sebaceous gland, while the lower bud will contain a privileged population of stem cells. This so-called bulge area is also the place where the arrector pili muscle connects with the hair follicle. In some body sites as the axilla, groins, and skin of genitalia and face, a third swelling is going to develop above the sebaceous gland bud, and this will form the apocrine gland (3,6).

Hair follicle development proceeds in a cephalo-caudal direction and is completed by the twenty-second week of intrauterine life. During the down-growth process, cells in the middle of the epithelial cord differentiate and form the first hair cone. This is made of terminally differentiated inner root sheath (IRS). The IRS surrounds the primitive hair fiber. Due to combined cell proliferation and differentiation, the fiber now starts to grow. The primitive lanugo hair appears to be pushed outside, creates an elevation of the primitive epithelium and in some way is freed from bursting out of the surrounding cells lifted up by desquamation. This is the first time that the lanugo hair becomes visible at the cutaneous surface around the twenty-eighth week. The first hair coat of fine lanugo hair is shed in utero, and the first hair cycle is initiated. The hair formed during these initial cycles is being shed in the amniotic fluid before birth at full term. The second coat of lanugo hair is going to shed from all areas during the first three to four months of life. Now as the follicle has been initiated we shall successively describe its structure and cycling. The first cycle terminates as waves of synchronized hair shedding along a cephalo-caudal direction. This means at birth the frontal hair follicles are usually engaged in an active hair cycle while occipital hair follicles are still in or will soon enter into a resting phase of that cycle. This will be completed after birth, and it explains the usual baldness of the occipital site in newborn babies. The appearance may be more dramatic as friction will more easily remove these hair fibers which are in the shedding phase: Friction unravels the actual synchronization stage of cycling, many resting hairs in the occipital area shed but friction is not the cause of shedding!

Histology of the Hair Follicle

The deepest part of the hair follicle is the hair bulb; it is composed of a central dermal papilla and a surrounding hair matrix. That is the place where cells divide, begin to elongate, and migrate to the skin surface; the undifferentiated cells at the basal layer of the matrix now have adopted several shapes and differentiation pathways that make the hair and the surrounding protective layers.

As this cellular activity (cell division and migration) is very intense and requires a lot of energetic supply, the hair follicle is functionally a very sensitive organ and hair growth closely reflects health and disease.

The hair shaft is enclosed in two sheaths, i.e., the IRS and the outer root sheath.

The IRS consists of three layers.

The cuticle layer of the IRS lies inside, and the free endings are tightly interlocked with those of the cuticle of the adjacent hair, which constitutes the middle of the hair follicle. Next to it is Huxley's layer with usually a two-cell-thick layer in the middle and the one-cell-thick Henle's layer outside.

The IRS is readily engaged in differentiation—for Henle's layer already at the level of the matrix—i.e., well before the presumptive hair cells contained in it hardens. Consequently it appears that this hard cylinder made by the IRS (3,6) determines the definitive shape of the hair shaft.

As opposed to the eosinophilic compact or granular corpuscles (trichohyalin granules) in the IRS, the outer root sheath cells have a characteristic vacuolated aspect. The vitreous membrane and the two connective tissue sheaths surround this outermost epithelial structure. These connective sheaths seem to lay down connective tissue fibers oriented almost vertically or parallel with the follicular longitudinal axis. These sheaths appear to contain various cell populations but essentially myofibroblasts that may be important in migration and contractions of the follicle when dynamic changes occur in a rather short period of time. Examples of such situations are the cycling and restoration of a new dermal papilla after some microtrauma (follicular trans-section experiments). Such repair potential may explain why hair regrows after procedures thought to permanently remove the hair. In the thinnest hair, the smooth muscle creates a kind of hamac supporting the upper part of the isthmus. This has not been clearly demonstrated for thicker follicles. When this muscle is activated the follicle is raised up, and the hair fiber stands erect on the skin surface centering what is known as goose bump skin (or cutis anserina). Elastic fibers seem to play a role in the anchoring of these muscles. The level where the arrector pili muscle appears to merge with the hair follicle is the bulge area, which is considered to be the site where most of the stem cells concentrate. These stem cells enter rarely into cell division, and they enter only after receiving the appropriate signals from the dermal papilla when a new hair cycle is initiated. The bulge is however not the exclusive harbor where these cells can reside, and some found them all along the isthmus area suggesting that there may be some dilution of the stem cell population all along the outer root sheath.

Cyclical Activity of the Hair Follicle

Production of a hair segment by a hair follicle undergoes a cyclical activity. Growth (anagen) is followed by a relatively short transitional phase (catagen) and a resting phase (telogen or club hair) (Fig. 4). To stimulate your imagination think of yourself as a cell sitting at the base of the hair matrix. If you are a member of the "club" you will be squeezed upwards during catagen until you get in touch with the opening of the sebaceous duct. Then you will stay there for a while until the completion of a terminal differentiation path that will lead you to "shedding" together with the hair shaft. The duration of anagen varies greatly with species, body region, season, age, and with the type of hair, whether terminal or vellus. The maximal duration of anagen is the most important factor determining hair length and replacement kinetics.

In adult humans the activity of each follicle is independent of its neighbors (asynchronous). However synchronization characterizes situations during the initial steps of hair growth stimulation (pregnancy, hair growth promoters, ...) or during regrowth when a subject recovers from an acute period of hair loss (after cytotoxic chemotherapy for cancer).

Each follicle goes through the hair cycle a variable number of times during a lifetime. At any one time, around 10% to 15% of the scalp hair follicles are in telogen, only 1% or less are in catagen. Increase in certain functional disorders of the scalp hair such as post partum effluvium, seasonal hair loss, or chronic telogen effluvium (3,15,16). To monitor dynamics of hair replacement various approaches have been proposed, and this will be discussed in the next section. Some are the result of sound and thorough reflection, others are to be considered as historical markers of technological evolution. The latter would better be discarded from our armamentarium because they include flaws with sampling errors and do not appropriately document the phenomenon they

are supposed to. For those who are more curious and interested in the genetic control of hair follicle formation and cycling we refer to recent hair research meeting proceedings (1,17) containing excellent review papers on this and related subjects (18,19) and other sources (20,21) such as regularly updated websites, where more links to hair in general, and hair science in particular can be found.

CLINICAL HAIR GROWTH EVALUATION METHODS

Subjective evaluation and personal satisfaction of people using hair growth modulators and/or cosmetics on a wide scale is the most important factor for the survival of these products in the market as a final outcome. This evaluation will be based on whether they are perceived as efficacious, especially when the benefit is cosmetic in

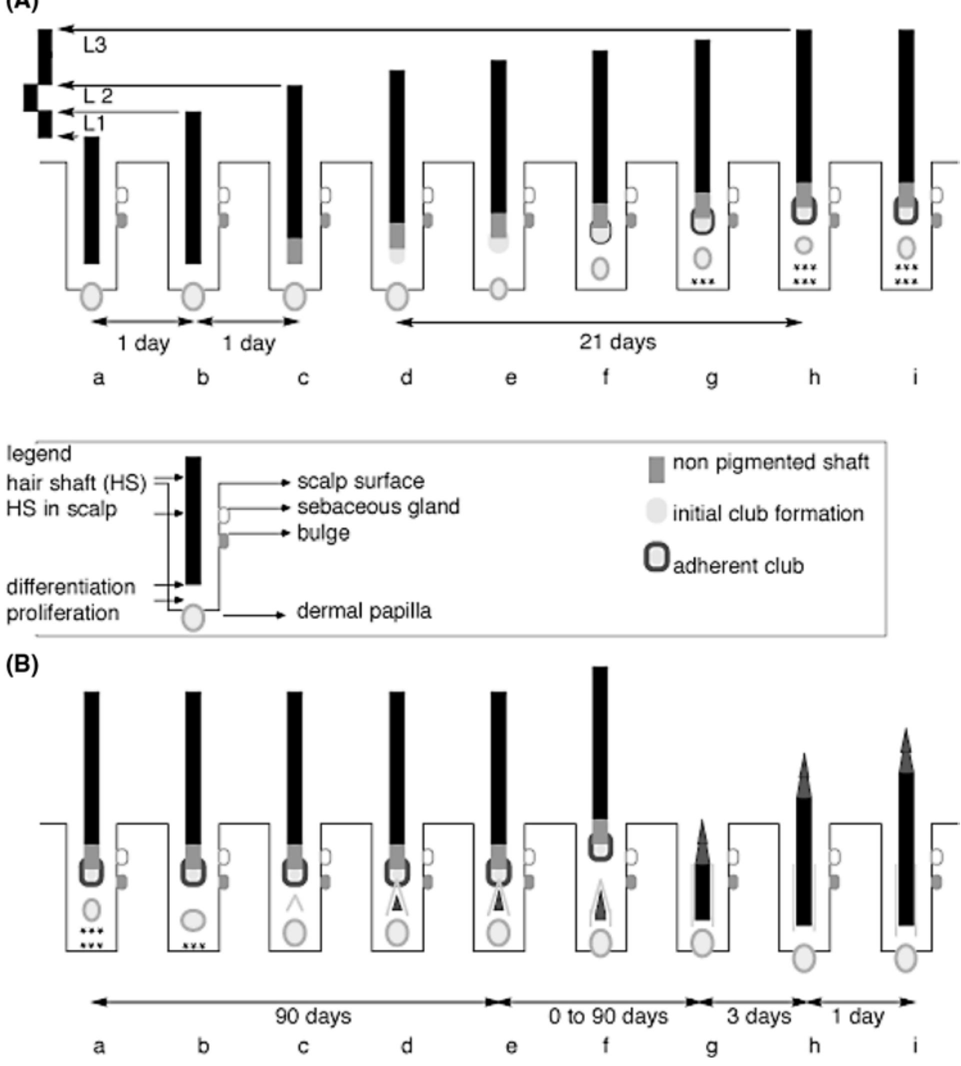


Figure 4 (*Caption on facing page*)

Figure 4 Schematic view of hair cycling of a human hair follicle. The latest steps of the hair growth phase (anagen 6) during which hair is visible at the skin surface and growing are shown in (**A**) while the apparent rest phase of the hair cycle (telogen phase) is shown in (**B**) during which a new hair cycle can be initiated. The legend helps the reader to orient himself within the various components of the human hair follicle which are essential to understand growth and rest. (**A**) From growth to rest: The same hair follicle is represented at various times (days) at the very end of the growth phase. At the skin surface, there is normal pigmented hair production (days a to b and b to c) representing the constant daily hair production (L1 and L2). Then, the pigmentation of the newly synthesized hair shaft (appearing at the bottom of the hair follicle) is decreased (c). This early event announces the regression of the impermanent portion of the hair follicle and is followed by terminal differentiation of cells in the proliferation compartment (d) and shrinkage of the dermal papilla (e). The latter starts an ascending movement together with the hair shaft as this characterizes the catagen phase (d to h; 21 days). The apparent elongation of the hair fiber (L3) reflects the outward migration of the hair shaft. What is left after disappearance of the epithelial cells from the impermanent portion of the hair follicle is first basement membranes followed by dermal connective tissue usually referred to as streamers or stelae (∗∗∗). The true resting stage begins when catagen is completed, i.e., when the dermal papilla abuts to the bottom of the permanent portion of the hair follicle. In the absence of physical interaction between dermal papilla and bulge the next cycle (see **B**) is definitely compromised. As from now no hair growth is observed at the surface (h to i). (**B**) From rest to growth: During this stage, one notices absence of hair growth at the skin surface (a to g) but significant changes occur in the deeper parts of the hair follicle. The dermal papilla expands and attracts epithelial cells from the bulge (stem cell zone) in a downward movement (a to b). To create space, previously deposited materials have to be digested (a to b, ∗∗∗) The epithelial cells then start differentiation in an orderly fashion starting with the IRS (c) and the tip of the cuticle and hair cortex of the newly formed unpigmented hair fiber (d). The resting hair remains in the hair follicle for approximately one to three months (a to e), then the detached hair is shed (f), and the follicle proceeds into the exogen phase. The shiny root end of the shed hair is the club. Before, during, or after hair shedding there may be replacement by a new hair shaft (e to f to g). Indeed, under physiological conditions, the follicle proceeds immediately or only slowly with new hair production (from f to g; max. 90 days). In conditions like AGA a much longer interval before regrowth may be recorded. At the earliest stage of anagen (stage VI), a nonpigmented hair tip is seen first (h) followed by a thicker, more pigmented, and faster growing hair fiber (i) depending on the many regulatory factors controlling the hair follicle.

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nature. One has to acknowledge that the regulatory agencies require such criteria but do not orient researchers or companies as to how to validate or calibrate the criteria.

For an evaluation method to be considered as valuable, it should provide information about the following variables that are directly related to the performance of the hair follicle including the duration of the hair cycle. Hair density, the number of hairs per unit area, is usually expressed as number/cm^2. Linear hair growth rate (LHGR) is the amount of hair produced per unit time and is usually expressed as mm per day. The percentage of anagen hair reflects the time during which hair follicles are engaged in the growth phase and actually produce a hair fiber that is visible at the skin surface (%A6). Thickness is measured on hair diameter and is expressed in micrometers. Last but not least time to hair regrowth after completion of telogen phase (22). For many evaluation techniques, the methodology details are lacking information about sensitivity and reproducibility usually required for clinical investigative techniques (23).

For classification purposes these methods can be divided into three types: invasive, semi-invasive, and noninvasive. Each of these methods will be qualified as analytical if it gives access to most variables determining the hairiness.

Invasive Methods

Scalp biopsy examination is the best example of an invasive method. Taking a scalp biopsy requires local anesthesia. Then the scalp sample requires special processing in a laboratory that is used to scalp histology. In addition to the ordinary vertical sectioning of skin biopsies which permits the study of longitudinal follicular sections, horizontal sectioning (parallel to the skin surface) of scalp biopsies offers further diagnostic opportunities (24–31).

Horizontal sectioning allows the study of a larger number of follicular structures. Inflammatory infiltrates are more easily seen, and their relationship to the follicular structures is more obvious than in vertical sectioning. Fibrous tracts, which are often difficult to visualize on vertical sectioning, become much more apparent on horizontal sectioning. A trained observer will easily stage the hair follicles into anagen, telogen, or catagen. Because of its high magnification, quantitative microscopic analysis of scalp biopsy specimens may serve the purpose of validating other measurement methods (32). In such a study, we demonstrated that total serial sectioning—from top to bottom, i.e., several hundreds of sections—is required for the full picture. By doing so, one realizes also that a hair fiber may remain for some time in the follicular ostium—at the end of telogen and exogen stage—which further complicates analysis between hair counts and hair follicle counts. This has been clearly identified in clinical situations like trichostasis spinulosa (33). The phenomenon also occurred but remained largely unnoticed on the scalp until recently. Indeed, when serial sectioning of scalp samples from the bottom up to the top was performed along with hair counting and staging some discrepancies were unraveled (32). As those exogen hairs blur the picture when hair counts are concerned, we devised a new skin and scalp sampling method that samples and removes specifically those less or eventually nonadherent elements (PCT/EP02/06434, June 2002).

Hair density or number of hair follicles per unit area is usually expressed as number/cm^2. It reflects the number of functionally active follicular units whether growing or not. Under physiological conditions, we know that most follicles will produce visible hair at the scalp surface but this may change dramatically in scalp and hair disorders. Hair follicles remain in the scalp, and a follicular opening tells the presence of a potential follicle. The percentage of anagen follicles properly reflects the time during which hair follicles are engaged into the growth phase. This is true only when an exhaustive count of follicles is made, i.e., including those that are not visible at the scalp surface (anagen III–V) and those that are subject to miniaturization. As mentioned already, this requires complete serial sectioning and accounting of hair and hair follicles, which is generally a painstaking exercise. Thickness is measured sometimes on hair follicle roots as such but most of the time reflects the diameter of the hair fiber. In other cases, the ratio between the diameter of the hair and its follicle (or its inner and outer root sheets) is used to differentiate thinning from thicker hair follicles.

The biopsy is very valuable to search for interfollicular inflammatory changes and to identify subtle changes in the structure of the skin. It has more limited interests, however, in the repeated functional exploration of dynamic processes of individual hair follicles. Indeed, as the scalp samples are small (a 4-mm punch biopsy has an area of 0.12 cm^2) they contain a small number of hair follicles, and lastly, as the tissue is removed it cannot, for obvious reasons, be re-examined later on for functional modifications.

Semi-Invasive Methods

Hair root analysis, also named trichogram, is a typical example of the semi-invasive methods still popular in some European Countries (9). To examine hair root status, it is necessary to diagnose hair disorders, 50 hairs, at least, should be plucked to reduce sampling errors. Even then one does not secure an exhaustive sampling. After sampling, the roots are examined under a low power microscope and classified into anagen and telogen; anything that goes beyond that interpretation is subject to caution due to many artifacts of extracted root morphology. The plucked hairs can be kept for many weeks in dry packaging before analysis.

According to the promoters of the method, the test should be performed almost five days after shampooing. This is a rather unpleasant situation for the patients with the current standards of scalp hygiene. On top of this, it means that the method requires passive accumulation of shedding hair to detect hair loss. We speculate that the method is too highly sensitive to the transit from telogen to exogen and much more reflects the latter transitions rather than supposedly measuring transitions between anagen and telogen. Due to the relative values generated (T/A ratio) and the inherent sampling errors we believe that this technique is a poor indicator of disease activity and/or disease severity in androgen dependent alopecia in women. This has been documented (34). We discourage its use in the clinic and have always rejected the use of the trichogram for efficacy trials. In our center this method has long been abandoned because it is painful and does not generate absolute values of hair density (thick or thinning) as opposed to previously published techniques using surface microscopy (35,36) or the method described in the following paragraph.

The unit area trichogram is performed after one month of regular shampooing (e.g., twice a week), the last shampoo is usually performed the day of the visit. It is a technique in which all the hairs within a defined area (usually 60 mm^2) are plucked and mounted onto double-sided tape attached to a glass slide. Optical microscopy establishes various hair measures such as hair density, anagen %, hair length, and hair diameter. An interesting dimension is "cosmetically significant hair." This is a hair with a defined thickness and length (e.g., $>40\,\mu$m and at least 3 cm). Fashion may however limit the application of the method depending on what hair style is desirable for the patient (e.g., a close hair cut was the favorite hair style for many people in 2000 as compared with longer hair in 1970).

Technically, the scalp area to be sampled should first be degreased (with an acetone/isopropanol mixture) and then delineated with a roller pen. All hairs contained in the area are depilated individually (one by one). Each hair is grasped at a uniform point above the scalp, and the forceps are rotated to ensure firm grasp. Epilation should be performed rapidly in a single action in the direction of hair growth orientation, to minimize trauma to the root (37).

The unit area trichogram has been evaluated in terms of reproducibility and clinical relevance. As an example of its use in open drug trials in men (38), the UAT data tend to confirm hair maintenance with minoxidil topical solution as opposed to the natural progression of androgenetic alopecia (AGA) in men. The induction of hair regrowth with finasteride in men confirms other hair growth measurement methods (39,40). After compensation of initially depleted iron stores in women with chronic telogen effluvium the UAT also effectively demonstrated reduced telogen rates (38).

The technique has also been used for comparative purposes. Most hair growth variables that are estimated through unit area trichogram and the phototrichogram (PTG) appear to be comparable. However, the unit area trichogram has the

advantage; in that, it can be used reliably in subjects in whom there is almost no contrast between hair and skin color (41).

In the next section, we will describe the stepwise improvements that have been implemented into the various close-up photographic techniques including the PTG method. Also after various comparative assays including the more invasive techniques, the latest improvements of the PTG method have now provided, in our hands, a standard for calibration of other technological approaches. But before proceeding, an important point has to be raised.

The question of where to make hair measurements—whether with invasive or less invasive sampling methods—has never been unequivocally resolved. Indeed, hair growth is largely modulated by local factors. Any body site including the scalp is a patchwork of preconditioned lots where subgroups of hair follicles appear to be sensitive to certain factors and evolve independently from their neighbors. This may vary depending on the physiological and pathological conditions. Hence, trials on scalp hair growth, as an example, would require the inclusion of a more global appreciation of the scalp response to the test compound and a detailed description of the site where detailed analysis of hair growth was performed.

Noninvasive Methods

In the description of noninvasive methods we propose to distinguish between global and analytical methods. Global methods apprehend at once various factors responsible for the hair area under examination but cannot resolve the details as opposed with the analytical methods that shall be described later on. The latter have a unique advantage on the former, because they provide a series of individual measures that reflect the structure/function of the hair follicle as an organ. Such data are subject to critical analysis. By combination of all the analytical data one can generate a global value but the reverse is untrue. Also another disadvantage of published global methods is that they are usually not calibrated. From the mathematical point of view differences in categories are unequal. As an example, differences such as between minimal and moderate or between moderate and great improvement may not be equal in terms of amount of hair. When noncalibrated instruments are used it remains difficult to derive clinical relevance of statistically significant findings. In the next section we will see that some answers have been proposed to that question.

Global Methods

Categorical Classification Systems. Distinctive patterns of defective scalp skin coverage or alopecia have been identified by clinicians as patterns more or less specific for male and female patients (42–45).

Though static by definition such diagrams can be enriched by more gradual variations, and there are as many classifications as there are interested observers; one such version for male patterns appears in Figure 5. Such diagrams have been used as references for epidemiological studies (42,46–50) including quality of life and self-perception evaluation as they correlate or not with the severity (51–53). Such a categorical appreciation appears to reflect globally defective hair replacement (54–59) but these schemes generate one number (category I or II and so on up to VII or VIII depending on the classification) that do not help measuring the dynamics of hair changes—whether growth or loss—over time. In summary, if there is too much information in that picture why should we not consider fragmentation, and how to achieve this?

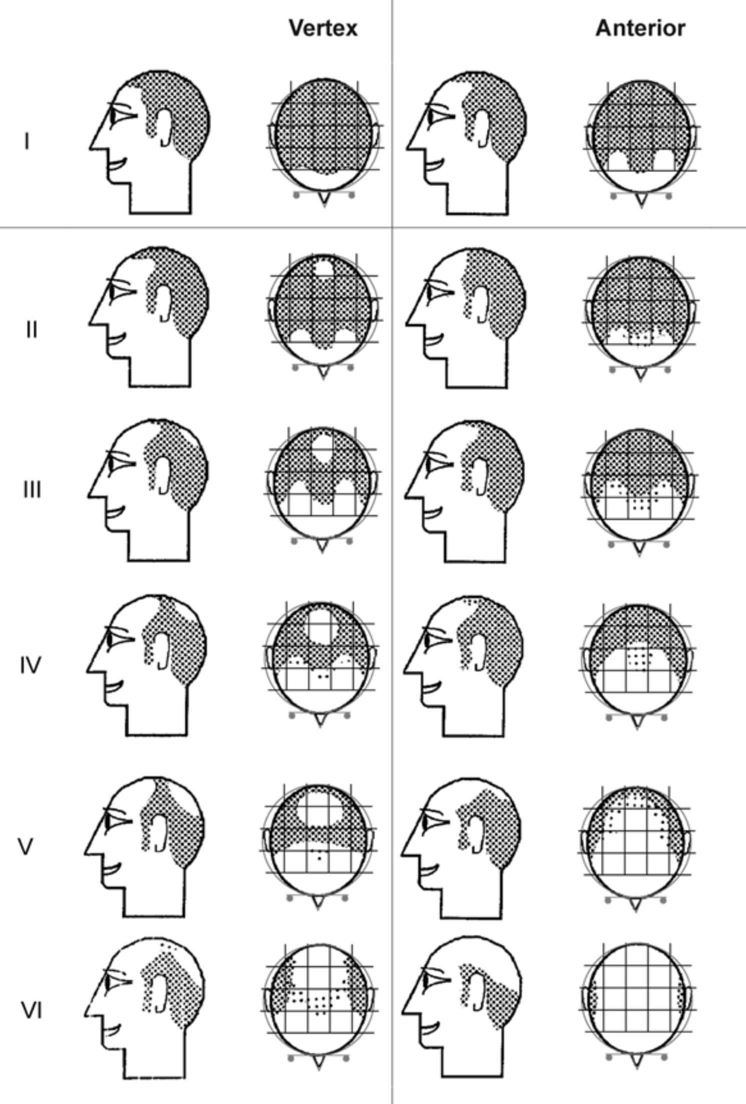

Figure 5 Schematic representation of androgenetic alopecia (male pattern balding) in men. The present classification shows AGA patterns that affect the scalp of genetically susceptible male subjects after puberty when deficient hair replacement ultimately results in bald appearance. They are subdivided into six categories of increasing severity (I–VI) from mild to severe balding. The anterior pattern indicates a backward progression of hair follicle miniaturization that starts from the frontal hairline. The vertex type indicates isolated regression occurring on the vertex but this is usually combined with some involvement of the frontal temporal areas. The application of a standardized grid on those patterned alopecia allows fragmentation of the projected scalp areas and attribution of coverage scores. While looking at the top of the head a continuum of coverage can be evaluated in each square and kept in the patients record. *Abbreviation*: AGA, androgenetic alopecia.

In line with this thought, we proposed, as shown in Figure 5, to fragment the top of the head into a number of fixed and predefined subunits. This helps focusing the attention of the observer on clearly outlined spaces where patterning results in various degrees of defective scalp coverage. We will describe this novel approach in the next section.

Calibrated Scoring Systems. A system has been developed where examination of the scalp surface proceeds through fixed external standards. The top of the head is separated into small units (equal size in the projection plane) with no anatomical correspondence of the bones of the skull (e.g., frontal, parietal, etc. ...). The relative difficulty (score 0 when there is no difficulty and 5 when it is barely possible) to detect scalp skin between the hairs is translated into scalp coverage scores (SCS). This is rated against objective rulers of density (Method and equipment for measuring coverage density of a surface—Skinterface, application patent n° PCT/EP 01/06970, June 2001). The reproducibility (intra- and inter-observer) is very high (correlation factors > 0.89), and more studies using scores generated in the clinic (real time measurement) and on global photographs are now in progress. When all identified sources of variation are kept under control, the variation of scores is less than 5%, and the clinician is able to detect clinically relevant changes due to treatment (personal data; submitted for publication).

Amazingly, this SCS method allowed us to detect subtle changes in distance or angle of vision between pairs of before and after photographs. This aspect is very interesting because these differences remained unnoticed to a panel of independent experts looking at the same paired photographs (personal unpublished data). During calibration studies, the SCS was correlated with clinically relevant hair parameters such as proportion of anagen hair or density of thinning hair (60) as measured with refined analytical methods (58). The main advantage of the SCS method is its easiness to use in the hair clinic where it helps quantifying clinically relevant changes in subjects who respond to specific treatment regimens (1).

Global Vision of the Skin or Scalp. Global photography (and other global vision technologies) has been a significant step forward in scalp hair documentation by creating a permanent record especially for scalp hair. Global photography apprehends all factors involved in hairiness at once and can be used for drug efficacy evaluation providing that adequate scalp preparation and hair style are maintained throughout the study. This is the most patient friendly photographic method. This method is used in the clinic under standardized conditions of exposure (61). Processing and rating have to be performed under controlled (e.g., blinded as to treatment and/or time) conditions. Trained investigators could generate reproducible data. It appears that paired comparison of global photographs is more realistic in its appreciation of hair growth after drug treatment as compared with subjective evaluations of investigators and patients (62).

As sets of photographs can also be scored individually with the SCS system—one photograph at a time and in circumstances one usually encounters in the day-to-day practice (see earlier)—the SCS could provide real time scalp coverage values. Such a quality control before inclusion of subjects into clinical trials may be of great value. Indeed quantifying disease severity and precise evaluation of distribution patterns are immediately available as opposed to global photographs that need expert evaluation. Indeed, in practice, the quality control happens only after processing in a special laboratory. The quality of photographs is evaluated in terms of optics (focus, contrast, ...) but not in terms of hair patterning or alopecia. The techniques of global picturing and density documentation appear as an important issue for future

development, and work is in progress in our laboratory to get real time imaging and quality control as well as density reading of scalp coverage.

Daily Collection of Shed Hair. The cyclic hair growth activity results in a daily shedding process in which telogen hairs are shed and leave an exogen follicle which is, ideally, to be replaced by the anagen stage resulting in visible hair at the scalp surface. The reported normal average daily loss of hair ranges somewhere between 40 and 180 hairs per day but our average in control subjects are closer to the lower values (unpublished data).

In a study of 404 females without hair or scalp disease, daily-lost hair was collected over six weeks in the aim of comparing two shampoos. Results showed mean hair loss rates ranging from 28 to 35 per day. No significant differences were noted in the mean daily hair loss rates during the two weeks baseline and the four weeks treatment period (13).

Quantitating daily hair loss in women was assessed in another study of 234 women complaining of hair loss among which 89 had apparently normal hair density. They have found that subjects with normally dense hair (though complaining of hair loss) shed less than 50 hairs a day (34). So the magic number of 100 so often referred to in textbooks and found in the lay press should be seriously revisited. Less than 50 hairs can be significantly abnormal in a patient having lost 50% of his hair (personal unpublished data).

Hair Weight and Hair Count Methods. The efficacy of hair growth promoting agents can be evaluated by comparing the total hair mass (weight) of hair that is produced during a certain period of time. Mass reflecting production and hair counts of growing hair can also be evaluated in a small, carefully maintained area of the scalp. To be complete, we mention that there is no published comparison with the results obtained with other techniques and the present method which has never been used outside the context of drug trials.

A plastic sheet with a 1.2 cm^2 hole was placed over the selected site. All hairs within the square hole were pulled through it and hand clipped to 1-mm length. Although it does not generate staging of growth phases of individual follicles, the advantage of this method is that it provides a global measurement of growth which under control conditions showed a regression in men with AGA. On a small group of subjects, the technique reveals the drug effects and between treatment regimen differences (example, 2 vs. 5% minoxidil) (63,64). One must be aware of the technical skills necessary to handle the samples in the proper way avoiding the loss of some hairs between the clinic and the laboratory. Again, as for many of these techniques, the methodological comparisons are lacking, and there are no evaluations of the reproducibility and sensitivity, usually required for laboratory evaluation methods. Since the introduction of this method, the authors never alluded to the possible edge effects. In a small rectangle, e.g., 1 cm^2 that should be the strict target area according to protocol, a small drift of 1 mm will result in the exploration of an area exceeding the target by 44%. It is conceivable at baseline that the rather ill-defined edges (marked by two tattoos in the corners of the square) contains a significant proportion of nonproductive hair follicles. The latter might also start production after active treatment. As a consequence these follicles and hair fibers may be mistakenly included in the study sample while the inactive treatment will further regress in terms of hair production in most instances. This will inevitably result in a sampling error with more extra hair under treatment as compared with placebo. This edge effect by the way will dim the relative importance of the therapeutic effect and has never been directly related to the clinical impression.

As a conclusion, the major limitation of this method is that it generates a global index of growth in a supposedly well-controlled site, the individual components of which escape control and cannot be analyzed separately.

As mentioned earlier, hair counts can be made on the clipped hair sample but as well in the hands of the promoter of the method (40) as in our hands (32), the lack of control of the sampling and displaying procedures may complicate the interpretation of the final measurements when precise targets are being monitored over time. Numbers of hair do not reflect the clinical picture as thickness, and LHGR significantly contribute to the mass of hair that is being produced over time (mass = global global index = time × number × thickness × linear hair growth × physical density of the hair fiber).

An improvement of hair counts, historically and controversially taken directly on the head of the patient, was obtained by close-up macrophotography of a clipped scalp site (65). Hair counts reflected "visible" hair even though the exact meaning of "visible" was neither provided nor measured against other methods (61). The method can be repeated over time, and a six-month interval appears as standard from published reports. Using such a time lapse, one does not differentiate between growing and resting hair. Increased hair counts as observed with finasteride in AGA in men is correlated with global perception of improved hair growth with other subjective and objective measurement methods (62) and most probably reflects anagen induction and maintenance as shown when close-up photographs are taken repeatedly at a shorter time interval, e.g., 72 hours (39). This latter method is yet another variant of the PTG that will be discussed in greater detail below.

Hair Pull Test. It is based on the idea that "gentle" pulling of the hair brings about the shedding of telogen hairs (34). Besides the fact that this concept remains speculative, it is a very rough method and difficult to standardize as it is subject to so much interindividual variations among the investigators. Physically speaking the pulling force is not distributed uniformly all over the whole hair bundle creating so much variation in the pulling force from one hair to another. It seems to be useful only in acute phases of hair loss in the more severe conditions (drug induced hair loss, alopecia areata, etc.). At least, we do not advise to make important diagnostic, prognostic or therapeutic decisions on a hair pull test in the most common conditions like chronic telogen effluvium or patterned (male or female) alopecia.

Analytical Methods

Phototrichogram. The basic principle of the PTG consists of taking a close-up photograph of a certain area of the scalp. The hair is cut very close in preparation for the first photograph, followed by repeat photographic documentation after a certain time period. This period of time should be long enough to permit the evaluation of the growth of a hair segment (which is usually between 24 and 72 hours) but not too long to prevent outgrowth or too much overlapping of growing hair. The growth is then evaluated by comparing the two pictures. Hairs that appear longer have grown and are in anagen phase while those, which have not, are in telogen phase (Fig. 6).

Analytical methods that document major aspects of the hair cycling process have been developed over the years (34,54,66–70) and are subject to continuous re-evaluation and improvement (16,41,71). Some PTG data have been computed for mathematical modeling so as to predict patterns of defective hair replacement similar to those observed in the hair clinic (72). Nevertheless all productive hair follicles are not taken into account during the usual PTG procedures. Accordingly, we (32)

recently used contrast enhanced (CE) pictures and showed that the phototrichogram technique (CE-PTG) had an equal resolution as transverse microscopy of scalp biopsies, which are usually considered as the golden standard (24–29). The CE-PTG is the only method that has documented on a follicular basis all transitions of thick and thinning follicles from anagen through catagen into telogen phases (32). In early stages of AGA in men, this sensitive method (58) was able to detect a subclinical phase of AGA with obvious shortening of the anagen phase in the absence of hair miniaturization. This preclinical stage evolves into patterning, i.e., the full-blown phenotype associated with a further shortening of the growth phase along with reduction in hair diameter (16,54–56,73–75). The follicular regression process finally results in production of clinically nonvisible hair (58). The CE-PTG method, as a refined, noninvasive, and validated technique, could be used for calibration purposes for any new method that would be developed for use in the skin and hair clinic. Hence, we identified a new global measurement method that integrates cumulative hair growth and reflects clinically relevant scalp skin coverage as described earlier (patent application n° PCT/EP 01/06970, June 2001). Let us first consider what we can learn to measure with the PTG.

The assessment is made on one or a number of predefined scalp sites considered representative of the condition. The data that can be generated from a PTG are total number of hair present in a certain area, i.e., hair density (n/cm^2), the percentage of hair in the growth phase (anagen %), the LHGR, and with certain restrictions, the hair thickness. Indeed, hair diameter evaluation is more precise with the microscope, but the sample collection and processing of clipped hair may be difficult to standardize and is at risk of error (personal unpublished data). The new systems of epiluminescence microscopy combined with appropriate image processing appear to be a valuable tool for hair growth evaluation.

One of the main advantages of the PTG is that first of all it is a patient friendly method. Secondly it is a totally noninvasive method so it does not affect by itself the natural process of hair growth/loss.

Although many patients report they were afraid at the idea of having their hair cut at one or more given scalp surface sites (area $\pm 1\,cm^2$ in our protocol), most of them confess that this process did not prevent them from enjoying a normal private and social life. Finally, PTG whether or not with CE also permits the follow-up of the same area over long periods of time. This has brought about a lot of valuable information (54). After comparison with UAT (41) weak points of the method have been considered with great care and using photography in combination with hair micrometry results in a valid method for global hair perception but allowing an analytical description of all variables intervenes in hair quality evaluation. The most recent techniques are able to pinpoint the earliest changes of hair growth in AGA in men which appear to evolve in sequence from shortening of anagen of thick hair into a phase of further shortening and thinning turning finally into a stage of reversible miniaturization without production of any visible hair (32). At this stage, one speculates that drug response is still possible before the follicle drops into total miniaturization (irreversible hypotrophy or atrophy) though without scarring.

Variants of PTG Methods. Subtle modifications in the preparation of target sites can help identify the hair in the growing from resting phase especially when less than optimal magnification lenses are used [e.g., less than $\times 3$ (70)]. Indeed after clipping the hair short (first step of the PTG as control for density or hair counts), a close shave will further reduce the visible length of the hair fiber. Then, usually three rather than two days later, the second photograph is taken. A new hair count of the

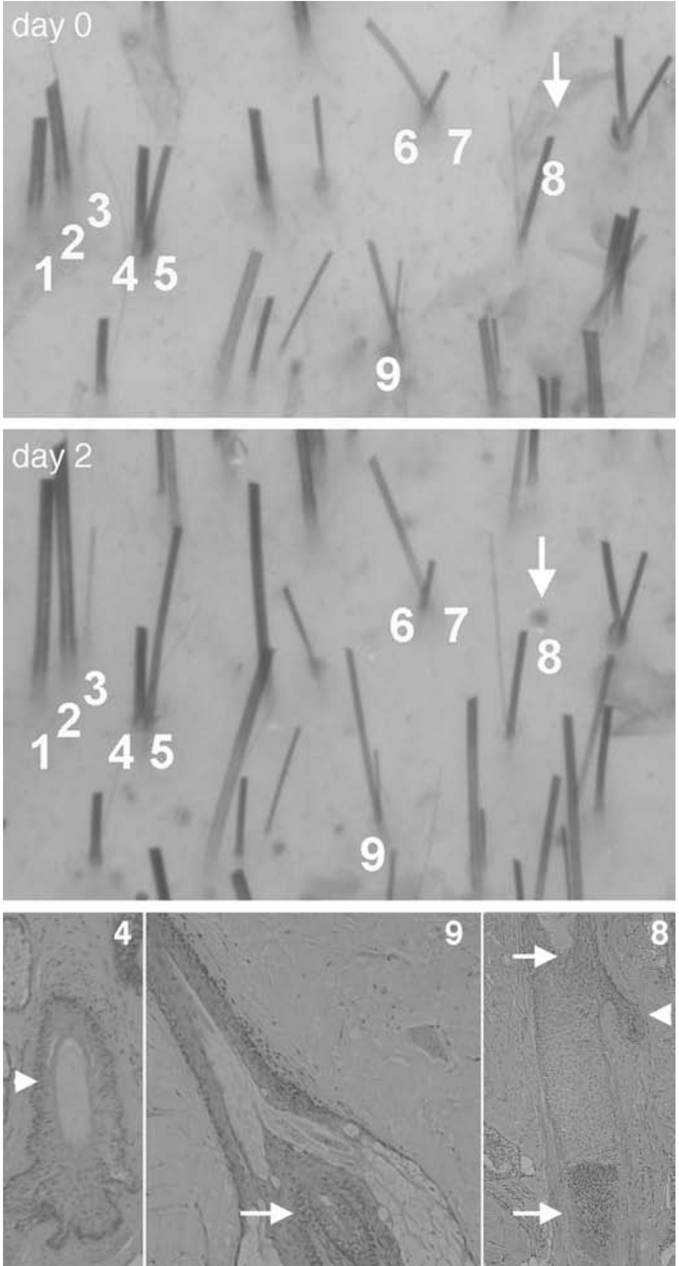

Figure 6 (*Caption on facing page*)

long hair fibers reflects anagen hair follicles. This procedure has been used to monitor changes occurring after finasteride in men with AGA demonstrating a significant induction of growth as compared with placebo (39).

When the photographic camera is replaced by a video or CCD camera equipped with specific lenses other variants of PTG recording are obtained. In fact, reports in Asians and Caucasians were published. In the latter subjects the contrast between hair and scalp seems favorable for the application of this method, and the

Figure 6 Correlation between contrast-enhanced phototrichogram and scalp histology. CE-PTG photographs were taken from the same scalp site immediately after clipping (day 0) and 48 hours later (day 2). Pairwise observation of the same hairs (numbered at day 0 and day 2) deserve some comment: Long fibers on (day 2) are growing hairs from follicles in anagen phase (thick hair 1 to thin hair 2). Neighboring hair follicles are usually not synchronized (telogen 4 and anagen 5). Anagen is clearly different from follicular regression or catagen (6) and telogen or resting stage (7). In catagen, migration rather than growth is reflected by minimal elongation of the visible part of the hair (6), and there is no change in length during telogen (7). After a while the hair will exit (shedding) and leaves an exogen follicle (8) as the follicular ostium appears empty. Finally miniaturized hair follicles (9) characterize male AGA; hair production may not even become visible at the scalp surface. The microscopic correlation is shown in the lower part for a terminal telogen hair (4, *arrowhead* on the adherent club), a resting hair from a miniaturized (or vellus like) telogen follicle (9, *arrow* on resting miniaturized club) and exogen hair follicle (8, *top arrow*). Histology also shows very early stages of regrowing thick (8, *arrow*) and thin (*arrowhead*) of anagen follicles that do not yet produce hair that could be visible at the scalp surface. *Abbreviations*: CE-PTG, contrast-enhanced phototrichogram; AGA, androgenetic alopecia.

low figures of hair density could possibly be racial in origin. However, the authors advise to take these factors into account to keep the biological variation as low as possible (67). The use of CE is advisable for Caucasian hair, and the use of computer applications for textile fiber analysis (76) or hair recognition software—already developed for other applications (77)—has been proposed (78). Promoters of the method recognize that the explored area is small (less than 0.25 cm^2), and by looking somewhat closer to pictures that were posted on the website we found (Fig. 7) that a 30% error might occur even though the error is promoted as "highly reproducible." On top, a small area is yet another source of variability because the number of hair so analyzed is definitely less than 100 (22). Every single hair fiber "weighs" more than 1% of the sample. As active drugs may show an effect in the order of 10% increase in hair counts let us—for the sake of exercise—assume that such a small area—say 0.25 cm^2 as shown in Figure 7—containing 40 hairs in a balding subject is evaluated. In such a situation it is clear that a "one single hair change" represents 25% of the therapeutic effect. This casts serious doubts on the clinical relevance of the reported changes. Therefore, fully automated analysis systems (76,78) remain generally unsatisfactory when detailed description of hair variables such as hair counts, diameter—especially for thinning hair—and growth rate is required. They are not useful when transitions through the hair cycle are to be monitored. Biologically significant source data in terms of follicle distribution and productivity are not reported. Many problems identified in 1989 (76) appear to remain unsolved in 2003—commercially available automation does not mean quality—especially when diagnostic criteria and therapeutic monitoring are concerned! As stated in our initial publications (76) more work needs to be done before such automated systems reach the status of medically acceptable diagnostic–prognostic and therapeutic monitoring tools.

Traction PTG is based on the hypothesis that hairs, which can be easily pulled from the scalp, are telogen while those resisting pulling are anagen (79). This is unproven and not documented as already stated in the hair pull test.

Traction PTGs have been performed on a surface area of 0.25 cm^2. Hairs present at this surface area are held gently between the thumb and index fingers and pulled repeatedly. Hairs that can be easily pulled are counted, and their number is considered as the number of telogen hairs. Those resisting pulling are clipped and

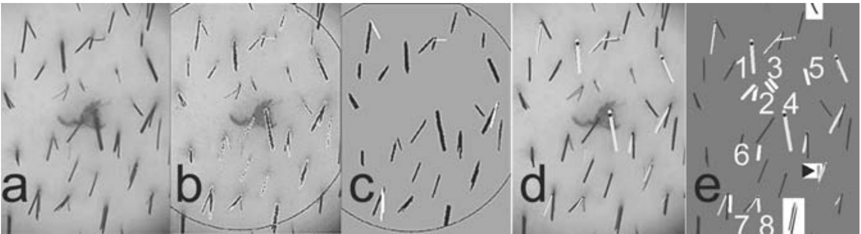

Figure 7 Quality process evaluation comparing manual versus automated hair analysis software. Here we show a typical example of quality process evaluation—as it is usually practiced in our laboratory—comparing results of our routine hair processing method versus those generated by the automated hair analysis software. Image processing results: An image (**a**: source image) was downloaded in July 2003 from a website displaying a full automatic system for hair analysis. Accordingly, after the automated hair selection (**b**: yellow outline of selected elements by software) and analysis (**c**: binary image of selected elements). The same "source" document (**a**) was also submitted to a technician for routine processing in our laboratory. The technician had no information about the origin of the document or results of automated analysis. This modality is part of our routine processing quality control (**d**). Our technician identified 43 hair segments in the circular outline of that image. Fourteen hair fibers had a thickness estimated to be less than 40 μm, and 29 were considered as thicker hair ($\geq$40 μm). Quality analysis of process: At the final revision by the author, the discrepancies were noted between the two processing modalities. They are outlined visually in (**e**) against a pink background. One very thin hair partly hidden within a trio (*white rectangle* with *arrowhead*) was missed by both measurement systems. A display of hair results from automated analysis of image (**a**) is shown in (**f**): 29 hairs were counted in the 0.227 cm^2 area (parts of circular outline) generating a density of 127.8 hair per cm^2. The automated system also missed seven other hairs that were identified by our technician [empty numbered spaces in (**e**)]. Automated counts also included three hairs crossing the edges [*white rectangles top* (1 hair) and *bottom* (2 hair) of (**e**)]. Hairs that stick together were not individualized by the automated system. Results: While the automated analysis counted 29 hairs we found 42 in the same area. Technician's error (1/43) is much lower than automation error (12 or 13/43), i.e., 2.3% instead of 30.2% of the total sample. Conclusion: Hair evaluation as performed with automated systems does not match the quality standards prevailing in our laboratory. Many problems identified in 1989 remain unsolved in 2003! *Source*: From Refs. 76, 78.

counted, and their number represents the anagen hairs. Through this method one can supposedly calculate the hair density per unit area as well as the anagen %.

It is necessary to evaluate this method more critically to check the assumptions made earlier and test many weak points (small surface area, lack of control on traction forces, ability to trace transitions during early stages of catagen–telogen, etc.).

Conclusion for Clinical Hair Growth Evaluation Methods. A bold statement would be to say that the assessment of hair loss requires some experience and a lot of technological effort to grasp all the parameters involved in hair measurement. There was a time, some colleagues argued, that measurement methods were not necessary as the patient could tell whether hair is growing or not. It is obvious that this is untrue as soon as one enters the continuum of the hair replacement process especially when some hairs are still present. Such a statement also looks outdated when one acknowledges the importance of the placebo effect. Indeed, subjective evaluation may reach 60% or more satisfaction while significantly decreased hair counts clearly document the natural worsening of the condition (62). Our experience points to the fact that a combination of a high resolution analytical approach with a global calibrated method seems advisable in the context of kinetic monitoring of hair growth and hair loss in the hair clinic.

BASICS ABOUT PSYCHOSOCIAL ASPECTS OF HAIR

Hair is happiness. This is not today's fashion "motto" or meant to be restricted to the behavior of today's human beings. All mammals keep themselves busy—at least to some extent—and care about their hair coat. The development of appendageal structures has been "the way" evolution has chosen to allow progress. There is evidence that gene mutations affecting "grooming" may lead to a catastrophe. Indeed, the coat of the affected animal is not properly taken care of and turns into a real mess. Hair is yet another way of well being and a very efficient modality of nonverbal communication with the environment.

Where appropriately located, hair is a symbol of nice looking and natural beauty while in other areas like the beard it may be socially inconvenient to have hair (females' chin or lips or sideburns, for example) or to let it grow naturally. In western culture, daily care by shaving is necessary for the majority of males while some religious rituals impose leaving the beard grow and even untouched as a sign of respect for the divine creator. In females, abundant scalp hair is very much welcomed, unlike leg hair, facial hair, and armpits (axillary) hair. Hair distribution in certain body regions is a secondary sexual characteristic. Under the influence of hormonal changes that start puberty, hairs become visible as a result of root thickening such as the beard, moustache, and body hair in males and pubic and axillary hair in both sexes.

Enforced shaving of scalp hair has long been used as a sign of punishment and in certain religious practices as a sign of obedience. Shaving the scalps of prisoners, adulterers, and traitors is a longstanding behavior in the human culture especially during or after wartime. Scalping and hair cutting were meant to express victory and symbolize integration of the animals' or enemies' physical strength or moral force.

Self-supported hair styling is also a mode of expression. Rebellion of youth against the existing social order is often manifested as a change in appearance especially change of hair style, e.g., long haired, skinheads, colorful hair of punks, etc. (80).

Hair also plays a role as a distinguishing sign of the ethnic background, varying from straight to curly in form and from dark to blond in color. There is also a great difference in the amount of response of scalp and body hair to the puberty hormonal changes between ethnic groups. Generally hair is subject to so much interindividual variation that it can be said that apart from the hair follicle there is no organ in the human body that is morphologically so much variable and environmentally (external and internal) sensitive as hair.

Hair fibers definitely play a role as it senses the environment. Patients with a total lack of hair (alopecia areata, for example) complain sensationwise that they live in a different world. Nasal hair protects against dust and acts as an air filter. Axillary and perineal hair reduce the friction during body movement and serve also for the wider or prolonged dissemination of the odor of the apocrine glands. Pubic hair is said to have some excitatory functions during sexual intercourse.

Although hair is not vital to human bodily survival it means a lot in terms of personality and psychological equilibrium (51,81,82). Psychological problems of hair loss would be expected in both sexes, and more among women due to the relevance of physical attractiveness (83). Hair is closely related to physical attractiveness and the difference between male/female hair patterning provides a recognition phenomenon. In general, baldness leads to overestimation of age of affected males (80).

In addition to the aesthetic function of hair, it has more natural functions although becoming less important due to the anthropologic evolution and technical progress of mankind. Scalp hair protects against certain environmental conditions

like sunrays and cold. Lack of scalp protection by natural hair is undoubtedly predisposed to the development of scalp skin cancer including melanoma. Humans also have body language and nonverbal communication with hair. All along the evolution of mankind hair patterns were depicted in great detail. Some psychiatric disorders express specifically on hair sites (trichotillomania and other manias), and the physiological and pathological aspects related to hair grooming may be genetically conditioned (84). In short, hair is a real universe. Those who become interested in the universe of hair may spend all their lives in learning more about its many dimensions.

REFERENCES

1. Van Neste D, Blume-Peytavi U, Grimalt R, Messenger A. Hair Science and Technology. Tournai: Skinterface, 2003.
2. Zviak C. The Science of Hair Care. Paris: Marcel Dekker, Inc., 1986.
3. Dawber R, Van Neste D. Hair and scalp disorders. In: Common Presenting Signs, Differential Diagnosis and Treatment. London: Martin Dunitz, 1995.
4. Goldsmith LA. Biochemistry and Physiology of the Skin. Vol. 1. . USA: Oxford University Press, , 1983.
5. Goldsmith LA. Biochemistry and Physiology of the Skin. Vol. 2. . USA: Oxford University Press, , 1983.
6. Camacho F, Montagna W. Trichology. In: Diseases of the Pilosebaceus Follicle. Spain: Egraf S.A., 1980.
7. Van Neste D, Tobin DJ. Hair cycle and hair pigmentation: dynamic interactions and changes associated with aging. Micron. In press.
8. Van Neste D. Thickness, medullation and growth rate of female scalp hair are subject to significant variation according to pigmentation and scalp location during ageing. Eur J Dermatol. In press.
9. Orfanos CE, Happle R. Hair and Hair Disorders. Germany: Springer-Verlag, 1990: 1–1057.
10. Rook A, Dawber R. Diseases of the Hair and Scalp. 2nd ed. Oxford: Blackwell Scientific Publications, 1991.
11. Al Ghani MA, Geilen CC, Blume-Peytavi U, Orfanos CE. Matting of hair: a multifactorial enigma. Dermatol 2000; 201:101–104.
12. Rushton H, Gummer CL, Flasch H. 2-in-1 shampoo technology: state-of-the-art shampoo and conditioner in one. Skin Pharmacol 1994; 7:78–83.
13. Kullavanijaya P, Gritiyarangsan P, Bisalbutra P, Kulthanan R, Cardin CW. Absence of effects of dimethicone- and non-dimethicone shampoos on daily hair loss rates. J Soc Cosmet Chem 1992; 43:195–206.
14. Stenn KS, Paus R. Controls of hair follicle cycling. Physiol Rev 2001; 81:449–494.
15. Whiting DA. Chronic telogen effluvium. Dermatol Clin 1996; 14:723–731.
16. Van Neste DJJ, Rushton DH. Hair problems in women. Clin Dermatol 1997; 15: 113–125.
17. Hair and hair diseases. Proceedings of the Annual Meeting of the European Hair Research Society, 15–17 September 2000, Marburg, Germany. Eur J of Dermatol, Vol. 11, 2001. Montrouge, France: John Libbey Eurotext, 2001:285–361.
18. Chuong CM, Hou L, Chen PJ, Wu P, Patel N, Chen Y. Dinosaur's feather and chicken's tooth? Tissue engineering of the integument. Eur J Dermatol 2001; 11:286–292.
19. Sundberg JP, King LE, Bascom C. Animal models for male pattern (androgenetic) alopecia. Eur J Dermatol 2001; 11:321–325.

20. Asplund A, Guo Z, Hu X, Wassberg C, Ponten F. Mosaic pattern of maternal and paternal keratinocyte clones in normal human epidermis revealed by analysis of X-chromosome inactivation. J Invest Dermatol 2001; 117:128–131.
21. Robinson M, Reynolds AJ, Gharzi A, Jahoda CA. In vivo induction of hair growth by dermal cells isolated from hair follicles after extended organ culture. J Invest Dermatol 2001; 117:596–604.
22. Van Neste DJJ. Hair growth evaluation in clinical dermatology. Dermatology 1993; 187:233–234.
23. Van Neste D, Randall VA. Hair Research for the Next Millenium. Amsterdam: Elsevier Science, 1996:1–485.
24. Headington JT. Histological findings in androgenic alopecia treated with topical minoxidil. Br J Dermatol 1982; 107(suppl 22):20–21.
25. Headington JT, Novak E. Clinical and histologic studies of male pattern baldness treated with topical minoxidil. Curr Therap Res 1984; 36:1098–1106.
26. Headington JT. Telogen effluvium. New concepts and review. Arch Dermatol 1993; 129:356–363.
27. Whiting DA. Scalp biopsy as a diagnostic and prognostic tool in androgenetic alopecia. Dermatol Therapy 1998; 8:24–33.
28. Whiting DA. The value of horizontal sections of scalp biopsies. J Cutan Aging Cosm Dermatol 1990; 1:165–173.
29. Whiting DA. Diagnostic and predictive value of horizontal sections of scalp biopsy specimens in male pattern androgenetic alopecia. J Am Acad Dermatol 1993; 28:755–763.
30. Whiting DA. Cicatricial alopecia: clinico-pathological findings and treatment. Clin Dermatol 2001; 19:211–225.
31. Sperling LC. Scarring alopecia and the dermatopathologist. J Cutan Pathol 2001; 28:333–342.
32. Van Neste DJJ. Contrast enhanced phototrichogram (CE-PTG): an improved non-invasive technique for measurement of scalp hair dynamics in androgenetic alopecia—validation study with histology after transverse sectioning of scalp biopsies. Eur J Dermatol 2001; 4:326–331.
33. Pagnoni A, Kligman AM, El Gammal S, Stoudemayer T. Determination of density of follicles on various regions of the face by cyanoacrylate biopsy: correlation with sebum output. Br J Dermatol 1994; 131:862–865.
34. Guarrera M, Semino MT, Rebora A. Quantitating hair loss in women: a critical approach. Dermatology 1997; 194:12–16.
35. Barman JM, Astore I, Pecoraro V. The normal trichogram of the adult. J Invest Dermatol 1965; 44:233–236.
36. Barman JM, Pecoraro V, Astore I. Method, technic and computations in the study of the trophic state of the human scalp hair. J Invest Dermatol 1964; 421–425.
37. Rushton DH. Chemical and morphological properties of scalp hair in normal and abnormal states. UK: University of Wales, 1988:1–247.
38. Rushton DH, Norris MJ, Dover R, Busuttil N. Causes of hair loss and the developments in hair rejuvenation. Int J Cosmet Sci 2002; 24:17–23.
39. Van Neste D, Fuh V, Sanchez-Pedreno P, Lopez-Bran E, Wolff H, Whiting D, Roberts J, Kopera D, Stene JJ, Calvieri S, Tosti A, Prens E, Guarrera M, Kanojia P, He W, Kaufman K. Finasteride increases anagen hair in men with androgenetic alopecia. Br J Dermatol 2000; 143:804–810.
40. Price VH, Menefee E, Sanchez M, Ruane P, Kaufman KD. Changes in hair weight and hair count in men with androgenetic alopecia after treatment with finasteride, 1 mg, daily. J Am Acad Dermatol 2002; 46:517–523.
41. Rushton DH, De Brouwer B, De Coster W, Van Neste D. Comparative evaluation of scalp hair by phototrichogram and unit area trichogram analysis within the same subjects. Acta Derm Venereol (Stockh) 1993; 73:150–153.

42. Hamilton JB. Male hormone stimulation is prerequisite and an incitant in common baldness. Am J Anat 1942; 71:451–480.
43. Hamilton JB. Patterned loss of hair in man: types and incidence. Ann N Y Acad Sci 1951; 53:708–728.
44. Norwood OT. Male pattern baldness: classification and incidence. Br J Dermatol 1975; 68:1359–1370.
45. Ludwig E. Classification of the types of androgenetic alopecia (common baldness) occurring in the female sex. Br J Dermatol 1977; 97:247–254.
46. Venning VA, Dawber RP. Patterned androgenic alopecia in women. J Am Acad Dermatol 1988; 18:1073–1107.
47. Birch MP, Messenger AG. Genetic factors predispose to balding and non-balding in men. Eur J Dermatol 2001; 11:309–314.
48. Ellis JA, Stebbing M, Harrap SB. Male pattern baldness is not associated with established cardiovascular risk factors in the general population. Clin Sci 2001; 100:401–404.
49. Ellis JA, Stebbing M, Harrap SB. Polymorphism of the androgen receptor gene is associated with male pattern baldness. J Invest Dermatol 2001; 116:452–455.
50. Olsen EA. Female pattern hair loss: clinical features and potential hormonal factors. J Am Acad Dermatol 2001; 45:S69–S80.
51. Girman CJ, Rhodes T, Lilly FRW, Guo SS, Siervogel RM, Patrick DL, Chumlea WC. Effects of self-perceived hair loss in a community sample of men. Dermatology 1998; 197:223–229.
52. DeMuro-Mercon C, Rhodes T, Girman CJ, Vatten L. Male-pattern hair loss in Norwegian men: a community-based study. Dermatology 2000; 200:219–222.
53. Paik JH, Yoon JB, Sim WY, Kim BS, Kim NI. The prevalence and types of androgenetic alopecia in Korean men and women. Br J Dermatol 2002; 145:95–99.
54. Courtois M, Loussouarn G, Hourseau C, Grollier JF. Hair cycle and alopecia. Skin Pharmacol 1994; 7:84–89.
55. Courtois M, Loussouarn G, Hourseau C, Grollier JF. Ageing and hair cycles. Br J Dermatol 1995; 132:86–93.
56. Ishino A, Uzuka M, Tsuji Y, Nakanishi J, Hanzawa N, Imamura S. Progressive decrease in hair diameter in Japanese with male pattern baldness. J Dermatol 1997; 24:758–764.
57. Birch MP, Messenger JF, Messenger AG. Hair density, hair diameter and the prevalence of female pattern hair loss. Br J Dermatol 2001; 144:297–304.
58. Leroy T, Van Neste D. Contrast enhanced phototrichogram pinpoints scalp hair changes in androgen sensitive areas of male androgenetic alopecia. Skin Res Technol 2002; 8: 106–111.
59. Vecchio F, Guarrera M, Rebora A. Perception of baldness and hair density. Dermatology 2002; 204:33–36.
60. Van Neste D, Leroy T, Sandraps E. Validation and clinical relevance of a novel scalp coverage scoring method. Skin Res Technol 2003; 9:64–72.
61. Canfield D. Photographic documentation of hair growth in androgenetic alopecia. Dermatol Clin 1996; 14:713–721.
62. Kaufman KD. Long-term (5-year) multinational experience with finasteride 1 mg in the treatment of men with androgenetic alopecia. Eur J Dermatol 2002; 12:38–49.
63. Price VH, Menefee E. Quantitative estimation of hair growth: comparative changes in weight and hair count with 5% and 2% minoxidil, placebo and no treatment. In: Van Neste D, Randall VA, eds. Hair Research for the Next Millenium. Amsterdam: Elsevier Science B.V., 1996:67–71.
64. Price VH, Menefee E, Strauss PC. Changes in hair weight and hair count in men with androgenetic alopecia, after application of 5% and 2% topical minoxidil, placebo, or no treatment. J Am Acad Dermatol 1999; 41:717–721.
65. Forslind B, Baden H, Ogawa H. Physical methods for human hair evaluation. In: Hair Research for the Next Millenium. Amsterdam: Elsevier Science B.V., 1996:83–85.

66. Guarrera M, Ciula MP. A quantitative evaluation of hair loss: the phototrichogram. J Appl Cosmetol 1986; 4:61–66.
67. Hayashi S, Miyamoto I, Takeda K. Measurement of human hair growth by optical microscopy and image analysis. Br J Dermatol 1991; 125:123–129.
68. Van Neste DJJ, Dumortier M, De Brouwer B, De Coster W. Scalp immersion proxigraphy (SIP): an improved imaging technique for phototrichogram analysis. J Eur Acad Dermatol Venerol 1992; 1:187–191.
69. Van Neste D, De Brouwer B, De Coster W. The phototrichogram: analysis of some technical factors of variation. Skin Pharmacol 1994; 7:67–72.
70. Guarrera M, Rebora A. Anagen hairs may fail to replace telogen hairs in early androgenic female alopecia. Dermatology 1996; 192:28–31.
71. Blume U, Ferracin I, Verschoore M, Czernielewski JM, Schaefer H. Physiology of the vellus hair follicle: hair growth and sebum excretion. Br J Dermatol 1991; 124:21–28.
72. Halloy J, Bernard BA, Loussouarn G, Goldbeter A. Modeling the dynamics of human hair cycles by a follicular automaton. Proc Natl Acad Sci USA 2001; 97:8328–8333.
73. Tsuji Y, Ishino A, Hanzawa N, Uzaka M, Okazaki K, Adachi K, Imamura S. Quantitative evaluations of male pattern baldness. J Dermatol Sci 1994; 7:136–141.
74. Courtois M, Loussouarn G, Hourseau S, Grollier JF. Periodicity in the growth and shedding of hair. Br J Dermatol 1996; 134:47–54.
75. Rushton DH. Androgenetic alopecia in men: the scale of the problem and prospects for treatment. Int J Clin Pract 1999; 53:50–53.
76. Van Neste D, Dumortier M, De Coster W. Phototrichogram analysis: technical aspects and problems in relation to automated quantitative evaluation of hair growth by computer-assisted image analysis. In: Van Neste D, Lachapelle JM, Antoine JL, eds. Trends in Human Hair Growth an Alopecia Research. Lancaster: Kluwer Academic Publishers, 1989:155–165.
77. Lee T, Vincent NG, Gallagher R, Coldman A, McLean D. Dullrazor®: a software approach to hair removal from images. Comput Biol Med 1997; 27:533–543.
78. Hoffmann R. TrichoScan: combining epiluminescence microscopy with digital image analysis for the measurement of hair growth in vivo. Eur J Dermatol 2001; 11:362–368.
79. Bouhanna P. Le tractiophototrichogramme, méthode d'appréciation objective d'une chute de cheveux. Ann Dermatol Venereol 1988; 115:759–764.
80. Van der Donk A, Rijnvos CJ. Psychosocial aspects of androgenetic alopecia. Rotterdam: CIP, 1992.
81. Passchier J. Quality of life issues in male pattern hair loss. Dermatology 1998; 197: 217–218.
82. Cash TF. The psychological effects of androgenetic alopecia in men. J Am Acad Dermatol 1992; 26:926–931.
83. Cash TF, Price VH, Savin RC. Psychological effects of androgenetic alopecia on women: comparisons with balding men and with female control subjects. J Am Acad Dermatol 1993; 29:568–575.
84. Greer JM, Capecchi MR. Hoxb8 is required for normal grooming behavior in mice. Neuron 2002; 33:23–34.

7

The Normal Nail

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ANATOMY

The nail plate also abbreviated as "nail," is a hard keratin plate, slightly convex in the longitudinal and the transverse axes. It is set in the soft tissues of the dorsal digital extremity, from which it is separated by the periungual grooves (proximal, lateral, and distal) (Fig. 1) (1–5). It stems from the nail matrix located in the proximal part of the nail apparatus. The nail plate and matrix are partly covered by a skin fold called the proximal nail fold (PNF). The lunula, also known as "half-moon," is a whitish crescent, visible at the proximal part of some nails and more specifically those of the thumbs and big toes. It corresponds to the distal part of the matrix. From the latter, the nail plate grows toward the distal region sliding along the nail bed to which it adheres closely and from which it only separates at the distal part, called hyponychium. The latter and overhanging free nail provide a crevice, which is a reservoir for microbes.

Two other structures deserve our attention:

1. The cuticle, which is the transparent horny layer of the proximal nail groove. It adheres to the nail surface and acts as a seal between the nail plate and the PNF. Its disruption allows water, foreign bodies, bacteria and fungi to penetrate under the PNF, which favors paronychia (periungual inflammation).
2. The onychodermal band or better known as the onychocorneal band, which is "orangey," is located in the distal region of the nail. It can be partly blanched by pressure, thus exsanguinating the region. It provides a zone of rugged attachment of the nail-to-nail bed. As for the cuticle, disruption of the onychocorneal attachment will severely affect the nail function, leading to onycholysis (detachment of the nail from its bed).

The upper surface of the nail plate is smooth and has discrete longitudinal ridges becoming more obvious with age (Fig. 2) and in some pathological states. This is a frequent cause of nail brittleness.

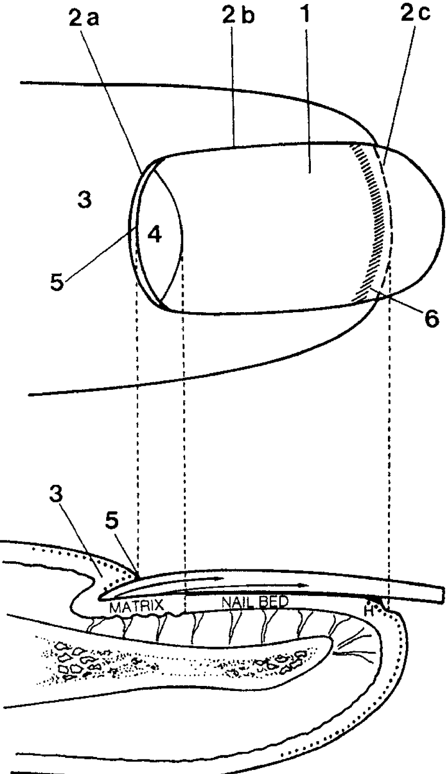

Figure 1 The normal nail: (1) Nail plate, (2) nail grooves [(2a) proximal nail groove, (2b) lateral nail groove, (2c) distal nail groove], (3) proximal nail fold, (4) lunula, (5) cuticle, (6) onychodermal band, (H) hyponychium. Small dots represent the stratum granulosum.

The under surface is corrugated with parallel longitudinal grooves that interdigitate with the opposite ones of the nail bed surface, enhancing the adhesion of the nail plate to the nail bed. The most important adhesion is located in the distal, central part of the nail.

HISTOLOGY

The Nail Plate

The nail plate is made up of parallel layers of keratinized, flat, and completely differentiated cells, called onychocytes. The latter are, in contrast with the corneocytes, firmly adherent and are not desquamated. Nuclear remnants can be observed but they disappear completely, near the distal free edge.

Three zones (characterized by different staining affinities) can be identified at the distal part of the nail: the upper (or dorsal) nail plate, which makes up one-third of the nail; the lower nail plate, which makes up two-thirds of the nail; and the subungual keratin. The latter corresponds to the thick, dense, horny layer of the hyponychium (Fig. 3) (6,7).

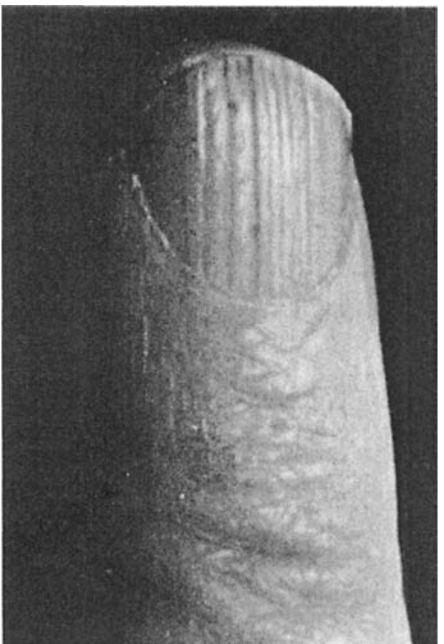

Figure 2 Obvious longitudinal ridges on the nail surface noticed in older people.

In electron microscopy (Fig. 4) (8), the nail plate cells appear to be made of a regular weft of keratin filaments within an interfilamentous matrix. In the upper (or dorsal) nail plate, cells are flat, their cellular membranes are discreetly indented and they are separated from each other by ampullar dilatations. At the surface, those cells are piled up like roof tiles, which give the nail surface its smooth aspect. In the lower nail plate, cells are thicker, their cellular membranes are anfractuous and they interpenetrate through extensions, making real anchoring knots that seem to be partly responsible for nail elasticity.

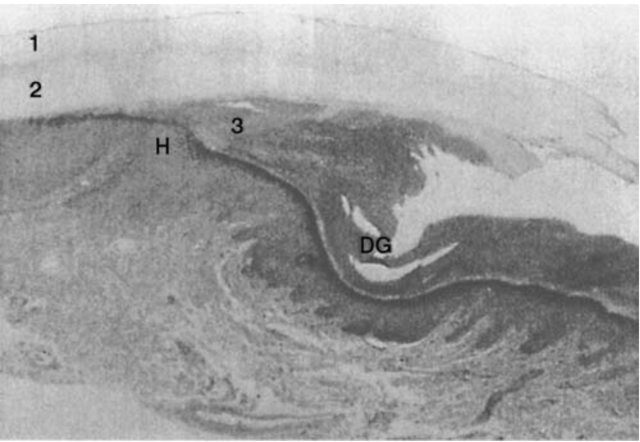

Figure 3 Longitudinal section of the distal part of the nail apparatus: (1) Upper or dorsal nail plate, (2) lower nail plate, (3) subungual keratin. (H) hyponychium, (DG) distal groove.

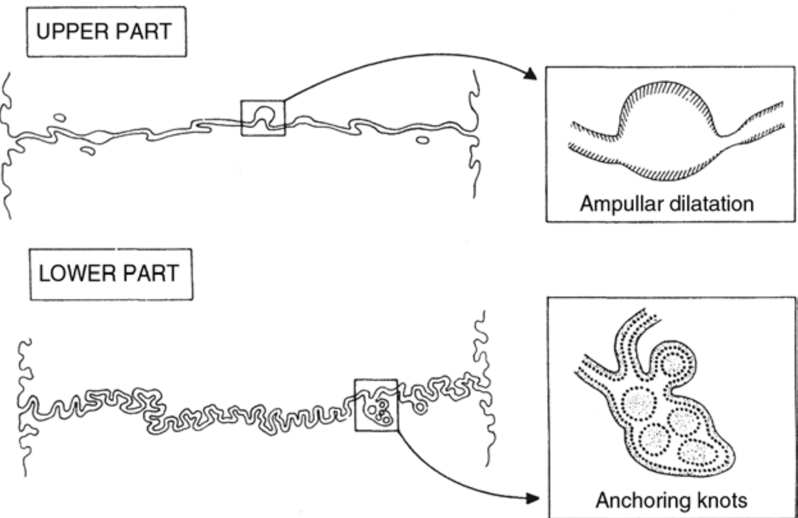

Figure 4 Schematic drawing of the cell membranes in the dorsal and ventral part of the nail plate, as observed in electron microscopic examination. *Source*: From Ref. 8.

Recently, the histological structure of the nail plate has also been studied by synchrotron x-ray micro diffraction (9). Three transversal layers (characterized by different orientations of the keratin molecules) are also identified. The outer or dorsal nail plate, which makes up the one-fourth of the nail, contains epidermal-type keratin filaments, perpendicular or parallel to the nail growth axis. The intermediate nail plate accounting for approximately two-thirds of the nail, is the only one containing hair-like type α-keratin filaments, perfectly orientated perpendicularly to the growth axis. The very thin (one-twelfth of the nail plate only) ventral nail plate is made up of epidermal type keratin filaments, perpendicular or parallel to the nail growth axis.

In the latter study (9), and in those previously mentioned, it should be pointed out that the denominations given to the three parts of the nail are different, which could lead to confusion. In Table 1, the correspondence between the different terms used are shown.

Other Nail Structures

A longitudinal section of the nail apparatus enables us to visualize most characteristics of the other ungual structures (Fig. 1). From the proximal to the distal region, the following are identified:

- The PNF (Fig. 5). Its dorsal part is in continuity with the epidermis of the digit back. Its vascularization is noticeable. The capillary loops are parallel to the skin surface, which allows their in vivo examination, under a special microscope with epi-illumination. This technique, called capillaroscopy, is useful in the diagnosis of Raynaud's phenomenon and connective tissue diseases. The ventral part of the PNF is a flat and rather thin epithelium that keratinizes with a stratum granulosum. The latter can disappear in the most proximal part of the PNF that is the proximal matrix. The cuticle corre-

Table 1 Nail Plate in Transversal Section

LIGHT AND ELECTRON MICROSCOPY (6,8)

	DENOMINATION (Thickness)	ULTRASTRUCTURAL CHARACTERISTICS
NAIL PLATE	Upper Nail Plate (1 / 3)	Flat cells, Discreetly indented cellular membranes, Ampullar dilatations
NAIL PLATE	Lower Nail Plate (2 / 3)	Thick cells, Anfractuous cellular membranes, Anchoring Knots
	Subungual Keratin	Polyhedral cells Desmosomes

X RAY DIFFRACTION (9)

	DENOMINATION (Thickness)	KERATIN (K) Filaments TYPE-ORIENTATION
NAIL PLATE	Outer or Dorsal Nail Plate (1 / 4)	Epidermal type k ⊥ or // to the nail growth axis
NAIL PLATE	Intermediate Nail Plate (2 / 3)	Hard K ⊥ to the nail growth axis
NAIL PLATE	Ventral Nail Plate (1 / 12)	Epidermal K ⊥ or //

Symbols: ⊥, perpendicular; //, parallel.

sponds to the modified stratum corneum of the distal part of the PNF, at the angle of the dorsal and the ventral part.

- The nail matrix is a multilayered epithelium. Its keratinization process is characterized by an onychogenous zone devoid of keratohyaline granules (Fig. 5). It gives birth to the nail plate: the proximal part of the matrix gives

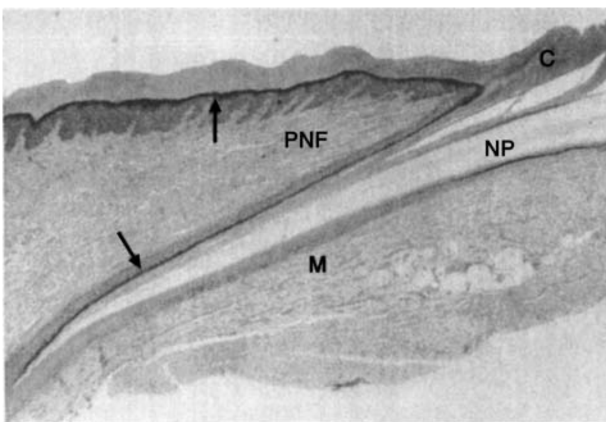

Figure 5 Longitudinal section of the proximal part of the nail apparatus. A stratum granulosum (*arrows*) is present in the dorsal and ventral part of the proximal nail fold epithelium but is absent in the matrix epithelium. *Abbreviations*: PNF, proximal nail fold; C, cuticle; NP, nail plate; M, matrix.

birth to its dorsal part and the distal part of the matrix gives birth to its ventral part. Immunohistochemical studies have shown that the nail matrix epithelium is the sole site of hard keratin synthesis (10).

The epithelium of the matrix also contains melanocytes and Langerhans cells. Melanocytes are about 200/mm^2 in number (about 1150/mm^2 in the epidermis). Most of them are dormant (11) and do not produce pigment. However, in dark-skinned individuals, longitudinal pigmented bands can be observed in nails. This racial physiological pigmentation is attributable to the activation of the matrix melanocytes and to the melanin incorporation in the nail plate (longitudinal melanonychia). It usually affects several nails and tends to become more frequent with aging; this can only be observed in 2.5% of newborn to 3-year-old black children, but in 96% of blacks older than 50 years of age (Fig. 6) (12).

- The nail bed epithelium is thin, reduced to few cellular layers. It keratinizes without any granular layer. The stratum granulosum reappears only at the hyponychium, which represents the distal thickened part of the nail bed and is bordered by the distal groove and the digital pulp (Fig. 3).

Melanocytes are rare (47/mm^2) or may be absent in the nail bed (11).

In an immunohistological point of view, the nail bed is distinguished by the expression of basal keratin markers throughout the epithelium thickness and absence of markers of epidermis or mucosal differentiation. It has been suggested that the nail plate could act as a suprabasal layer for the nail bed. Additionally, expression of keratin 17, which is usually found with myoepithelial differentiation and epithelial mobility, could play a role in the sliding of the nail plate over the nail bed (10).

Finally, strong expression of a carcinoembryonic family antigen has also been described in the upper epithelial cell layers of the major central portions of the nail bed. It may play a part in the adhesion of the nail plate to the nail bed (13).

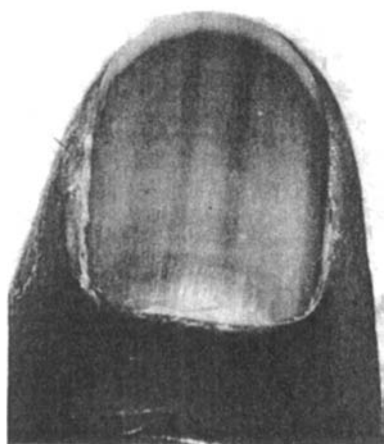

Figure 6 Multiple longitudinal melanonychia in an adult black patient.

- The basal membrane of the nail apparatus is almost identical to that of the skin (14).
- The nail matrix and nail bed mesenchyme ("dermis") does not contain pilosebaceous units. In the distal matrix the connective tissue is loose and edematous. In the proximal matrix and the nail bed, it is characterized by dense collagen bundles, vertically orientated linking the nail apparatus to the periosteum. Elastic fibers are rare. Eccrine sweat glands are usually absent. Glomus bodies, which are specialized arteriovenous anastomosis involved in the regulation of temperature, can also be observed in the dermis. In pathology, they give rise to glomus tumors, characteristically associated with paroxysmal pain. They represent one of the most frequent benign tumors of the nail apparatus.
- No genuine hypodermis is present in the nail but some adipose islets can be observed (7).

PHYSICOCHEMISTRY

The nail is highly rich in keratins, especially in hard keratins, which are close to those of hair and have a high content of disulfide linkage (cystine) (1,3). The high-sulfur–containing keratins play an important role in the nail toughness and presumably in its good barrier property as well.

Sulfur represents 10% of the nail's dry weight; calcium represents 0.1% to 0.2%. The latter, contrary to conventional wisdom, does not intervene in the nail toughness.

Lipid content (particularly cholesterol) is low in nails: from 0.1% to 1% compared with 10% in the stratum corneum of the skin.

Water concentration varies from 7% to 12% (15–25% in the stratum corneum) but the nail is highly permeable to water: when its hydration level increases, it becomes soft and opaque and when its hydration level drops, it becomes dry and brittle.

Studies carried on nail permeability are important for the development of cosmetic and pharmaceutical products specifically devoted to nails (15). As a permeation barrier, it has been shown that the nail plate reacts like a hydrogel membrane, unlike the epidermis that reacts like a lipophilic membrane (16).

The normal nail is hard, flexible and elastic, which gives its good resistance to the microtraumatisms it undergoes daily. Those properties are attributable to the following factors: the regular arrangement and important adhesion of onychocytes, the anchoring knots, the high-sulfur–containing keratins and their regular orientation, and the hydration level of the nail.

PHYSIOLOGY

The nail growth is continuous. In a month, fingernails grow about 3 mm and toenails grow about 1 mm. A complete renewal, therefore, takes four to six months for normal fingernails whereas 12 to 18 months are needed for toenails (1,3).

The origin of the nail plate production is still a debatable point. However, most studies agree and show that at least 80% of the nail plate is produced by the matrix. Indeed, studies based on cell kinetics realized on squirrel monkey (17) or on human nails (18) showed a cell proliferation largely limited to the matrix. This was further supported by later immunohistochemical markers of epidermal proliferation (19).

Finally, the use of keratin antibodies showing the production of hard keratin restricted to the matrix matches the notion that the bulk of the nail plate derives from the matrix (10). It should be added that the main source of nail plate production is the proximal part of the matrix, 80% of nail plate cells being generated within the proximal 50% of the matrix. This probably explains why distal matrix surgery or nail bed surgery have a low potential for scarring compared with proximal matrix surgery (19).

Some studies suggest that the nail bed produces 20% of the nail plate, whereas others suggest that the nail bed hardly participates in the making of the nail plate (19,20).

It is not totally excluded that the ventral part of the PNF on the one hand and the nail bed on the other, could contribute to the dorsal and ventral part of the nail plate, respectively, where soft keratin is observed.

AESTHETICS

For centuries, the nail has played an important aesthetic role. Having clean nails is essential to looking well-groomed and refined, and among women nails also need to be long and painted.

A "good-looking nail" has a smooth and shiny surface. It is transparent and adheres to its bed. Regarding the proximal groove, the cuticle has to be intact and thin. The distal and the lateral grooves have to be clean and the periungual tissues must be without hangnails and sores. The free border has to be smooth; its shape can be round, pointed, oval, or square. Women often wear long fingernails cut oval, which makes fingers look longer and thinner. Yet, square nails are in fashion. Too-long nails can look unpleasant and can even be a nuisance.

Men wear short fingernails cut square. Both women and men have short toenails cut square. A normal nail structure and appropriate cosmetic care are necessary to obtain such "good-looking" nails.

REFERENCES

1. Dawber RPR, de Berker DAR, Baran R. Science of the nail apparatus. In: Baran R, Dawber RPR, de Berker DAR, Haneke E, Tosti A, eds. Diseases of the Nails and their Management. 3rd ed. Oxford: Blackwell Science, 2001:1–47.
2. González-Serva A. Structure and function. In: Scher RK, Daniel CR, eds. Nails: therapy, diagnosis and surgery. Philadelphia: WB Saunders Company, 1990:11–30.
3. Fleckman PH. Basic science of the nail unit. In: Scher RK, Daniel CR, eds. Nails: Therapy, Diagnosis and Surgery. Philadelphia: WB Saunders Company, 1990:36–51.
4. Morgan AM, Baran R, Haneke E. Anatomy of the nail unit in relation to the distal digit. In: Krull EA, Zook EG, Baran R, Haneke E, eds. Nail Surgery: A Text and Atlas. Philadelphia: Lippincott Williams & Wilkins, 2001:1–28.
5. Zais N. Anatomy and Physiology. In: Zaias N, ed. The Nail in Health and Disease. 2nd ed. Norwalk: Appleton & Lange, 1990:3–14.
6. Achten G, André J, Laporte M. Nails in Light and Electron Microscopy. Seminars in Dermatology 1991; 10:54–64.
7. Perrin CH. Anatomie microscopique de l'appareil unguéal. Histologie et histopathologie. In: Dumontier CH, ed. L'Ongle. Paris: Elsevier, 2000:19–28.
8. Parent D, Achten G, Stouffs-Vanhoof F. Ultrastructure of the normal human nail. Am J Dermatopathol 1985; 7:529–535.

9. Garson JC, Baltenneck F, Leroy F, Riekel C, Müller M. Histological structure of human nail as studied by synchrotron X-ray microdiffraction. Cell Mol Biol 2000; 46:1025–1034.
10. de Berker D, Wojnarowska F, Sviland L, Westgate GE, Dawber RPR, Leigh IM. Keratin expression in the normal nail unit: markers of regional differentiation. Br J Dermatol 2000; 142:89–96.
11. Perrin CH, Michiels JF, Pisani A, Ortonne JP. Anatomic distribution of melanocytes in normal nail unit. An immunohistochemical investigation. Am J Dermatopathol 1997; 19:462–467.
12. Leyden JJ, Spott DA, Goldschmidt H. Diffuse and banded melanin pigmentation in nails. Arch Dermatol 1972; 105:548–550.
13. Egawa K, Kuroki M, Inoue Y, Ono T. Nail bed keratinocytes express an antigen of the carcinoembryonic antigen family. Br J Dermatol 2000; 143:79–83.
14. Sinclair RD, Wojnarowska F, Leigh IM, Dawber RPR. The basement membrane zone of the nail. Br J Dermatol 1994; 131:499–505.
15. Sun Y, Liu J-C, Wang JCT, De Doncker P. Nail penetration. Focus on topical delivery of antifungal drugs for onychomycosis treatment. In: Bronaugh RL, Maibach HI, eds. Percutaneous Absorption. Drugs-Cosmetics-Mechanisms-Methodology. 3rd ed. New York: Marcel Dekker, 1999:759–778.
16. Mertin D, Lippold BC. In-vitro permeability of the human nail and of a keratin membrane from bovine hooves: influence of the partition coefficient octanol/water and the water solubility of drugs on their permeability and maximum flux. J Pharm Pharmacol 1997; 49:30–34.
17. Zaias N, Alvarez J. The formation of the primate nail plate. An autoradiographic study in the squirrel monkey. J Invest Dermatol 1968; 51:120–136.
18. Norton LA. Incorporation of thymidin-methyl-H3 and glycin-2-H3 in the nail matrix and bed of humans. J Invest Dermatol 1971; 56:61–68.
19. de Berker D, Mawhinney B, Sviland L. Quantification of regional matrix nail production. Br J Dermatol 1996; 134:1083–1086.
20. Johnson M, Shuster S. Continuous formation of nail along the bed. Br J Dermatol 1993; 128:277–280.

8
Main Cosmetic Vehicles

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INTRODUCTION

The aim of this chapter is to treat the topic of cosmetic vehicles in a conceptual way. It is not the purpose to present a lot of formulations or types of vehicles that are used for all the different cosmetic products and sites of application. Neither will the topic be presented in a comprehensive way, because of its complexity. There are many good examples of formulation compositions described in cosmetic literature and brochures of companies offering cosmetic excipients. In this chapter an overview of various selected aspects is given that should be taken into account when cosmetic preparations are to be formulated. The critical issues for formulation development will be pointed out.

FUNCTION OF VEHICLES

Direct Intrinsic Effect

The term vehicle is used in pharmaceutics as well as in cosmetics in the area of formulation. In general, this term implies differentiation between active and inactive principles. The active principle is embedded into a matrix, the vehicle. With the aid of the vehicle the active principle is delivered to the application site or to the target organ, where the desired effect is achieved. As a matter of fact, however, when dermatological and cosmetic preparations are applied, sharp differentiation between active and inactive principles is generally not possible because of the so-called vehicle effect.

The aim of applying both the pharmaceutical preparation and the cosmetic topical care product is to achieve a desired effect. Pharmaceutical preparations are effective because of a pharmacologically active compound delivered with the aid of a vehicle, whereas cosmetic formulations are not allowed to contain such compounds. Nevertheless, an effect is also achieved by a cosmetic preparation—not any systemic or central or curative effect, but a caring or preventing effect mainly on skin, hair, or nails. This effect may be achieved either by cosmetically active ingredients or by the vehicle itself on the site of application, i.e., on the skin in most cases. In contrast to pharmaceutics, in cosmetics the vehicle is of greater importance.

Depending on the composition, a vehicle is used to exert mainly five types of effects on the skin, briefly described in the following sections.

Cleansing

The most common and probably the oldest use of cosmetic preparations is to clean the human body. In our modern time and society, not just soaps but a variety of sophisticated cosmetic cleansing products are available.

Decoration

Decoration serves to produce a pleasing appearance by minimizing facial defects of color or shape and unobtrusively enhancing and directing attention toward better points (1). Decorative cosmetic preparations are not the main objective of this chapter on vehicles, although similar principles have to be considered for decorative cosmetic preparations.

Care

Probably more cosmetic preparations are applied to care for the outermost organs of the body, i.e., skin, hair, and nails, than to decorate these organs. Care of skin, hair, and nails and improvement of their state is an important function of an applied cosmetic product. Application of an appropriate vehicle may be fully sufficient for the care of the body.

Hydration

The state of dry skin may be treated by applying a cosmetic product. In this case, the skin is hydrated by application of an appropriate vehicle containing specific components that are able to reduce the transepidermal water loss. This results in an increase in water in the stratum corneum and a smoother surface of the skin.

Protection

A further important function of cosmetic vehicles is to build up a protective layer against external potentially damaging factors that could come into contact with the body. Especially in recent years, the protective and preventive function of vehicles has become increasingly important, because of an increase of various external harmful factors or at least higher awareness about them (e.g., air pollution, UV radiation).

Delivery of Actives

From stringent medicinal and legal points of view, a cosmetic preparation must not contain any (pharmacologically) active substance or ingredient that treats or prevents disease or alters the structure or function of the human body (2). That just means that the vehicle is effective directly at the site of application. This is in contrast to pharmaceutical vehicles, which in principle should serve as pure vehicles delivering active substances to the target organ and showing no effect on the body. However, in reality there are no such distinct but floating boundaries. Therefore, cosmetic vehicles can also be considered as means containing cosmetic actives that are applied to the outermost layer of the body. Furthermore, many cosmetically used substances

are bifunctional: First they constitute the vehicle structure, and second they show a positive effect on the skin status when applied.

Carrying Actives to Targets (Targeting)

Going even one step further, cosmetic vehicles can also be considered and used as carriers for cosmetic actives, which, after application, are carried and delivered to the specified target sites, i.e., to the legally allowed targets in deeper regions of the skin. However, this is allowed only if no systemic, physiological, or pharmacological effect is achieved, and the product is shown to be safe.

Delivering active substances to these targets requires the right concentration of actives in the formulation to achieve the optimal release rate and desired distribution of active substances between the vehicle and the target site. That means the vehicle should penetrate (superficially) into the stratum corneum and release the active substance at the optimal rate (immediate or sustained for depot effect) at the target site where the desired effect is achieved.

CLASSIFICATION SYSTEMS OF VEHICLES

There are many types of classification systems based on various principles described in the literature. But one has to be aware that cosmetic preparations are rather complex systems. Most of the various classification systems are unsatisfactory, and it is difficult to set up a comprehensive system. In most cases, it is problematic to make clear distinctions for classifying the vehicles in a proper and unambiguous way. This is because of various possible points of view and characterization criteria used. The state of matter, e.g., depends on temperature, and therefore a lipid-based vehicle might exist either in liquid or semisolid form.

A few systems are discussed in this chapter. For modern formulation development the physicochemically based systems have been found to be the most useful and practical for understanding and explaining formulation issues.

Appearance

The most obvious and simple classification may be performed according to the appearance of the preparations or vehicles. Based on the macroscopic physical state of matter, three types of preparations are distinguished: liquid, semisolid, and solid forms. This classification is not of great interest for rational formulation design and development. However, for many practical issues it is quite useful, e.g., for manufacturing, packaging, and application on the body.

A further classification system is based on state of matter and optical discrimination, be it macroscopic or microscopic. That means vehicles can be classified into monophasic, isotropic systems on the one hand and into anisotropic, heterophasic systems on the other. For example, the term "solution" is commonly used to describe a liquid form with isotropic appearance. However, solutions also occur in solid form, so-called solid solutions. With regard to macroscopic appearance, colloidal systems (e.g., mixed micellar solutions, microemulsions) are also isotropic, whereas, e.g., coarse dispersions belong to the anisotropic systems. Unlike solutions, most cosmetic vehicles are anisotropic, heterophasic systems (mixtures). Thus, a more

Table 1 Junginger's Physical–Chemical Classification System

System	Brief description (examples)
Liquid systems	
Monophasic systems	
Aqueous solutions	Molecular disperse systems of solute in solvent (water, alcohol); liquid, transparent
Alcoholic, alcoholic–aqueous solutions	
Oily systems	Solutions based on (mixtures of) liquid lipids as solvent, e.g., oils for massage
Micellar systems	Solubilisates of low soluble substances due to aggregation formation of surfactants in solution
Microemulsions	Optically isotropic liquid: gel composed of water, lipid, and surfactant in distinct ratio
Multiphasic liquid systems	
o/w emulsions	Internal lipid phase dispersed in the external (continuous) aqueous phase stabilized by surfactants
w/o emulsions	Internal aqueous phase dispersed in the external (continuous) lipid phase stabilized by surfactants
Suspensions	Solid particles dispersed in a liquid phase
Aerosols	
Semisolid systems	
Water-free systems, ointments	
Apolar systems, hydrocarbon gels	Petrolatum
Polar systems	
Polar systems without surfactants	
Lipogels	For example, hydrogenated vegetable oils
Oleogels	Colloidal silica in oils
Polyethylene glycol gels	
Polar systems with surfactants	
w/o absorption bases	Simple ointment (British Pharmacopoeia 1993): emulsifying system (cetostearyl alcohol, wool fat) in paraffin–petrolatum base
o/w absorption bases	Cetomacrogol emulsifying ointment (British Pharmacopoeia 1993) cetomacrogol 600, cetostearyl alcohol in paraffin–petrolatum base
Water-containing systems	
Monophasic systems: hydrogels	
Hydrogels with anorganic gelating agents	Colloidal silica in water (high concentration, labile gel structure)
Hydrogels with organic gelating agents	Hydroxyethylcellulose gel Polyacrylate gel
Multiphasic water-containing systems: creams	
o/w creams	
w/o creams	
Amphiphilic systems	

(*Continued*)

Table 1 Junginger's Physical–Chemical Classification System (*Continued*)

System	Brief description (examples)
Amphiphillic systems with crystalline gel matrix[a]	
Amphiphillic systems with liquid crystalline gel matrix[a]	
Liposomes	Phospholipid vesicles in aqueous medium
Niosomes	Nonionic surfactant vesicles (analogous to liposomes) in aqueous medium
High-concentrated suspensions, pastes	
Powders	

[a]See discussion on mesophases, p. 161.
Abbreviations: o/w, oil-in-water; w/o, water-in-oil.
Source: From Ref. 3.

sophisticated system is needed to describe and classify the heterogeneity of possible vehicle forms in a satisfactory way (Table 1).

Application, Use

Classification of vehicles may also be performed as a function of their use and application site, i.e., preparations used for the following:

- hairs, e.g., shampoo, depilatory agents, hair colorant
- nails, e.g., polish, lacquer
- mouth, e.g., toothpaste, lipstick, lip-protection stick
- skin, e.g., moisturizing product, body lotion, aftershave, deodorant, antiperspirant, sunscreen

On the one hand, it is obvious that for the different application sites and modes different vehicles and forms with appropriate characteristics are needed. On the other hand, different types of vehicles may also be used for the same purpose, e.g., an aqueous–alcoholic solution or a balm for application after shaving.

Physical Chemical

In the development of cosmetic care products, a practical physical–chemical classification system that describes the principal properties and structural matrix of vehicles is preferred. Of course, there is no perfect and comprehensive classification system. A good example of a physical–chemical system is described by Junginger (3) and slightly modified in Table 1. Although not comprehensive, such a system is a useful tool for rational formulation design and development, in particular when controlled and targeted delivery of active principles has to be achieved. Such a vehicle classification system is also a practical basis for production, use, and understanding of cosmetic vehicles. However, the boundaries between the different classes are flexible, and changing with the state of the art and science. More important than pure classification of a cosmetic vehicle is its exact characterization, based on physical, chemical, and biological principles that may eventually lead to a variety of classification possibilities.

In a physical–chemical classification system, various characterization criteria are used for classification of the vehicles:

- Polarity: hydrophilicity, lipophilicity
- State of matter: solid, semisolid, liquid, gaseous
- Size/dimensions of particulates dispersed in the mixtures (dispersions)
  - true solution, molecular dispersion: particle size <1 nm
  - colloidal dispersion: particle size 1–500 nm
  - coarse dispersion: particle size >500 nm
- Solubility characteristics
- Rheology, viscosity
- Composition: physical–chemical characteristics of the main vehicle components (water-free, oily, aqueous, hydrophilic, nonaqueous solvents)

For clarification of the terminology, a selection of definitions or descriptions of the major systems is given in Table 2 (4–9).

DESCRIPTION AND DEFINITION OF MAIN VEHICLES

Solutions

The term "solution" may be used in a narrow sense, describing true solutions (molecular dispersions; Table 2), or in a broader sense, also comprising colloidal solutions, i.e., more or less transparent liquids, e.g., micellar solutions and vesicular systems (media containing liposomes, niosomes).

In general, true solutions used in cosmetics are either based on aqueous, or aqueous–alcoholic media or on inert oily vehicles. Most organic solvents cannot be used because of their local or systemic toxicity, which causes skin irritation or permeation across the skin barrier into the body, respectively. Although good solvents for lipophilic substances, oils may not be used in every case because of their grassy characteristic, low acceptance, and exclusion for hairy application sites. However, for special applications oils are preferred, e.g., for massage. "Massage oils" contain essential oils and fragrances, compounds that are easily dissolved in the oily vehicle because of their lipophilic properties.

Prerequisite for solution formulation is a sufficiently high solubility of the solute in the solvent. Classical examples for solutions used in cosmetics are "eau de parfums" and "eau de toilettes." To enable solubilization of the lipophilic fragrances, alcohol or aqueous–alcoholic solutions are prepared. The addition of alcohol to water, or other suitable hydrophilic but less polar solvents (e.g., glycerol, polyethylene glycol), decreases the polarity of the solvent and thus increases the solubility of the lipophilic solutes. Frequently, a solute is more soluble in a mixture of solvents than in one solvent alone. This phenomenon is known as cosolvency, and the solvents that in combination increase the solubility of the solute are called cosolvents (10).

Another classical example is preparations for mouth washes. They usually contain essential oils or liquid plant extracts like peppermint or myrrh extract, which are kept in solution by the added ethanol (ca. 70%). When used for application, these concentrates are diluted with water. Then, turbidity occurs because of overstepping saturation solubility. To prevent turbidity, solubilizing agents (surfactants, e.g., PEG-40 hydrogenated castor oil) may be added. The solubilization effect is

Table 2 Definitions of Selected Vehicle Systems

Systems	Definitions
Aerosol	Dispersion of liquid or solid in gas
Colloidal	Colloidal systems are dispersions with particle size range of 1–500 nm. They may be classified into the following three groups: 1. Lyophilic colloids: particles interact with the dispersion medium (e.g., gelatin) 2. Lyophobic colloids: composed of materials that have little attraction (e.g., gold in water); 3. association colloids: amphiphiles or surfactive agents aggregated to micelles (4)
Dispersion	Dispersed systems consist of paniculate matter (dispersed phase) distributed throughout a continuous, or dispersion, medium (5).
Emulsion	According to IUPAC, emulsion is defined as liquid droplets and/or fluid crystals dispersed in a liquid. The dispersed phase is also called the internal phase, in contrast to the external or continuous phase. If the internal phase is lipophilic, e.g., vegetable oil or paraffin oil, and dispersed in the external hydrophilic aqueous phase, an emulsion of type o/w is obtained. On the other hand, there are w/o emulsions with the hydrophilic aqueous phase dispersed in the continuous lipophilic phase. For formation and stabilization of emulsions, emulsifiers are required. Emulsions may show liquid or semisolid consistency. Further related aspects are treated in p. 151.
Foam	Dispersion of gas in liquid phase, i.e., structure of air pockets enclosed within thin films of liquid, stabilized by a foaming agent (6)
Gel	A gel is a solid or semisolid system of at least two constituents, consisting of a condensed mass enclosing and interpenetrated by a liquid (7)
Solution	A true solution is defined as a mixture of two or more components that form a homogeneous molecular dispersion, a one-phase system (8)
Suspension	A suspension is a coarse dispersion in which insoluble solid particles are dispersed in a liquid medium (9)

Abbreviations: IUPAC, International Union of Pure and Applied Chemistry.

attributed to aggregation formation of surfactants when in solution. In aqueous solutions surfactants form micelles, small aggregates, when the concentration of the surfactant exceeds the critical micelle concentration (CMC) (11). With the aid of those micelles, the solubility of low soluble, apolar compounds may be increased because of an association or incorporation of the apolar compounds with the apolar region of the micelle. Thus, solubilization or formation of micelles is a favorable means for formulation of solutions.

Finally, salt formation or adjustment of pH also results in improved solubility of originally low soluble, ionizable solutes. Thus, e.g., addition of sodium hydroxide may be used to improve the solubility of hyaluronic acid or preservatives such as

sorbic or benzoic acid. Accordingly, appropriate acids, e.g., lactic acid and citric acid, may be added when solubility of a basic substance must be increased. Although not the main type of formulation used in cosmetics, solutions have the following advantages:

1. They remain physically stable (if true solution and not oversaturated),
2. are easily prepared: simple mixing, under heating if necessary,
3. are transparent, clear, and have a "clean" appearance, and
4. are especially suitable for rinsing and cleaning body surfaces.

However, it must be kept in mind that many compounds are chemically less stable when in a dissolved state.

In summary, whenever a solution has to be formulated, the optimal solvent must be selected, which (1) guarantees sufficient solubility and stability for the solute(s) and (2) is acceptable and safe for application to the body. Solubility may be improved by (1) adaptation of the solvent's polarity with regard to the solute, (2) salt formation/pH adjustment (ionizable compounds), (3) using mixtures of suitable solvents and cosolvents, and (4) solubilization with the aid of surfactants.

Emulsions: Lotions and Creams

Out of the range of cosmetic care products, the emulsion is the form that is probably the most used. For reasons of skin feeling, consumer appeal, and ease of application, emulsions are preferred to waterless oils and lipids along with gels. The main components of emulsions are lipids (lipophilic compounds) and water (and/or hydrophilic compounds). These two immiscible phases are allowed to remain in a metastable mixed state by an amphiphilic component, an emulsifier. This biphasic system may be regarded in analogy to the skin or even to the skin cells, which, simply put, consist of lipophilic and hydrophilic components. Emulsions can either be of the water-in-oil (w/o) or oil-in-water (o/w) types. Showing very similar structural principles, both lotions and creams are discussed in this chapter. If emulsions are liquid, they are generally called lotions. Creams are emulsions occurring in semisolid form. Under gravitation, creams do not flow out through the orifice of reversed containers because of the heavier consistency in comparison with lotions.

Emulsions are prepared by dispersion of the internal in the external phase. For this energy-consuming process, emulsifiers that decrease the interfacial tension between the two immiscible phases are required. Emulsifiers are not only used for formation but also for stabilizing emulsions. Emulsions are metastable systems, and the two phases tend to separate because of coalescence, i.e., when the dispersed droplets fuse. This process may be slowed by the addition of appropriate emulsifiers, which are ionic or anionic surfactants. The emulsifiers are thought to be located at the interfaces between the two phases, the hydrophilic part of the molecule in contact with the water phase and the lipophilic domain of the emulsifier contacting/touching the lipid phase. Large molecules may even dig into the lyophilic phase and serve as stabilizing anchors. Being adsorbed at the interfaces, the emulsifying substances form a film—monomolecular or multimolecular, depending on the substances' structures—that stabilizes the emulsion (12). The addition of viscosity-increasing substances further results in an improved consistency and consequently more stable emulsions.

Except for the emulsifiers, the following types of ingredients are usually added to cosmetic emulsions:

- *Emollients*: They improve the sensory properties of the emulsions. Addition of an emollient results in better spreading when the emulsion is applied to the skin. Examples: isopropyl myristate, silicon oils.
- *Moisturizers and humectants*: They increase and control the hydration state of the skin. Examples: glycerol, urea.
- *Viscosity-increasing agents* are added to increase the viscosity of the external phase, if desired. Examples: xanthan gum, cellulose esters.
- *Active substances* such as UV sunscreens and vitamins;
- *Preservatives* to prevent microbial growth, particularly in o/w emulsions;
- *Perfumes and coloring agents* for aesthetic purposes.

Oil-in-Water Emulsions

The high acceptance of o/w emulsions is based on the following reasons:

- They feel light and not greasy when applied.
- They show good skin spreadability and penetration and an active hydration effect by the external water phase.
- They cause a cooling effect because of the evaporation of the external aqueous phase.

However, o/w emulsions show a lower effect in preventing dry skin in comparison with w/o emulsions. A typical o/w emulsion is composed as follows:

1. Lipid(s) + lipophilic thickening agent (optional, e.g., microcrystalline wax): 10% to 40%;
2. Emulsifier system with optimal hydrophilic–lipophilic balance (HLB) value [approximately 9–10 (13)]: 5%;
3. Coemulsifier (e.g., cetostearyl alcohol, behenyl alcohol): 2%;
4. Preservatives (antimicrobial, antioxidants): q.s.
5. Water + hydrophilic thickening agent (optional; e.g., carbomer) ad: 100%.

Depending on the desired product effect, different types of lipids may be used for formulation. Addition of nonpolar, occluding lipids (e.g., paraffin oil) improves retention of moisture in the skin but lowers spreading on the skin. A good spreading effect is achieved by formation of a low-viscosity emulsion containing polar oils that show a high spreading coefficient (e.g., macadamia nut oil, wheat germ oil, isostearyl neopentanoate) (14).

Selection of the lipophilic ingredients and the excipients of the water phase determine the emulsifier system to be used and additional adjuvants, e.g., viscosity-increasing thickening agents. There is no universal emulsifier system, and a huge variety of combinations might be used. Today, complex emulgator systems that consist of one or more surfactants and a cosurfactant are commonly used. That means at least two surfactants with different HLB values are combined. For example, steareth-21 (HLB = 15.5) may be combined with PEG-5-glyceryl stearate (HLB = 8.7). The latter emulsifier is especially suitable when nonpolar oils are to be incorporated. In recent years, selected polymeric excipients have been used for emulsion stabilization, e.g., crosslinked and linear polyacrylates, polyacrylamides, and derivates of cellulose.

In selecting a coemulsifier, the following general guidelines apply:

- For the same fatty residue, the viscosity decreases if the degree of ethoxylation increases.
- For the same degree of ethoxylation, the viscosity increases if the fatty carbon chain length increases (14).

The degree of viscosity (consistency) of o/w emulsions depends on various factors (15):

- Volume ratio of internal to external phase: increasing lipid percentage results in higher viscosity, but not necessarily in a semisolid cream.
- Type of lipid: incorporation of high melting lipophilic compounds, e.g., solid paraffin and petrolatum, may result in soft semisolid o/w creams.
- Presence of thickening agents in the lipid phase: addition of cetostearyl alcohol generally results in ("hard") semisolid creams.
- Presence of thickening agents in the external aqueous phase: the ultimate means to increase the consistency of a thin o/w emulsion. Addition of hydrocolloids, e.g., carbomers or hydropropyl guar (Jaguar 8600, Rhodia Inc., Cranbury, New Jersey, United States), is the most efficient method to increase the viscosity of o/w emulsions. However, depending on the properties of the added polymer, the skin feeling of the emulsion may become negatively influenced because of the stickiness.

An interesting phenomenon is the occurrence of liquid crystal structures (mesophases) in emulsions under certain conditions. This has been investigated and has become of interest more and more during the last 10 to 20 years. This subject is treated on p. 161.

Water-in-Oil Emulsions

Water-in-oil (w/o) emulsions may still be regarded as heavy, greasy, and sticky although during recent years great progress has been achieved in the preparation of pleasant w/o emulsions. Therefore, the w/o emulsion type is not only the basis for water-resistant sun protection, baby creams, or night creams, but also for protective day creams. This is because during recent years better excipients have become available. The advantages of w/o emulsions are:

- Close resemblance to the natural protective lipid layer in the stratum corneum,
- Efficient skin protection attributable to formation of a continuous layer of lipids on skin after application,
- Sustained moisturization because on skin a continuous semiocclusive barrier is formed that reduces evaporation of skin water and that in addition actively releases the incorporated water from the internal phase, generally several times more efficient than o/w emulsions,
- Improved penetration into the lipophilic stratum corneum coupled with improved carrier function of lipophilic active substances, and even of hydrophilic substances incorporated in the internal aqueous phase,
- Lowered risk of microbial growth,
- Liquid at very low temperatures (beneficial for winter sport products).

A typical w/o emulsion is composed as follows:

- Lipid component: 20%
- Lipophilic thickening agent (e.g., wax; optional): 1%
- Emulsifier system with optimal HLB value (3–8): 7% to 10%
- Preservatives (antimicrobial, antioxidants): q.s.
- $MgSO_4 \bullet 7H_2O$ 0.5%
- Water (+ hydrophilic thickening agent, optional): ad 100%

To avoid the heavy feel of w/o emulsions, appropriate excipients must be selected to get products with well-accepted sensory properties. This heavy feel of w/o emulsions is directly related to the spreading characteristics of the external oil phase. Therefore, polar oils with a high spreading coefficient (16) are preferably used, e.g., macadamia nut oil, isopropyl isostearate, isostearyl neopentanoate. Addition of low-viscosity silicone fluids or volatile cyclomethicone also improves the spreading effect. The physicochemical nature of the lipid components not only determines the spreading on the skin, the degree of occlusivity, and skin protection, but also influences the selection of the emulsifier system. Therefore, choosing an optimal emulsifier system is crucial. For example, glyceryl sorbitan unsaturated fatty acid ester (Arlacel 481) and glyceryl sorbitan saturated fatty acid ester (Arlacel 986) are better suited to emulsify apolar lipids, whereas more hydrophilic emulsifiers like the analogous ethoxylated sorbitan fatty acid esters (Arlacel 581, saturated, and Arlacel 582, unsaturated) or fatty acid esters of polyols (Arlacel 1689, saturated, and 1690, unsaturated) are designed for more polar lipids. A combination of PEG-7-hydrated castor oil and polyglyceryl-3-diisostearate may also be used. Skin feel may be improved by causing thixotropic behavior of the product, which is achieved by addition of a thixotropic agent or by reduction of the emulsifier content.

Multiple Emulsions

Multiple emulsions are triphasic systems or emulsions of emulsions. That means there is a primary emulsion dispersed in an external phase, e.g., water-in-oil-in-water (w/o/w). The dispersed phase in the resulting system contains smaller droplets having the same composition as the external phase (17). The inner aqueous phase is separated from the outer aqueous phase by the oil phase, and therefore the composition of the two aqueous phases may be different, at least after preparation and for a certain storage time. Preparation and stabilization of multiple emulsions is a challenging task. They may either be prepared by a two-step method or by the relatively new one-step process "Partial Phase Solu-Inversion Technology (PPSIT)" (18). The two-step method includes preparation of the primary emulsion, which thereafter is dispersed in the external phase. In the PPSIT, the lipid and electrolyte-containing water phase are heated and mixed above the phase inversion temperature (PIT), where the hydrophilic emulsifier forms w/o emulsions. By cooling down, a w/o/w system occurs at the PIT for a short time period. Then the system is immediately fixed by salting out and forming a lamellar matrix structure based on the emulsifier (19). The advantage of w/o/w emulsions is that they comprise both the light feeling and positive sensory characteristics of o/w emulsions and the skin hydration effect of w/o emulsions.

Gels

Gels are dispersed systems, originally liquids (solutions), that have a certain consistency useful and practical for topical application. In contrast to emulsions, gels generally do not comprise two immiscible phases of opposite lyophilicity. Therefore, the polarity and solubility characteristics of the incorporated substances are either hydrophilic—in hydrogels—or lipophilic—in lipogels (or oleogels). The consistency of gels is caused by gelling (thickening) agents, usually polymers, building a three-dimensional network. Intermolecular forces bind the solvent molecules to the polymeric network, and thus the reduced mobility of these molecules results in a structured system with increased viscosity. Pure gels are transparent and clear or at least opalescent. Transparency is only achieved if all ingredients are dissolved or occur at least in colloidal form, i.e., the size of particles is in the submicron range. Transparency in particular is an attractive property of gels. Gel products have positive esthetic characteristics and are thus becoming more and more popular in cosmetic care products today. Gels can also serve as the basis for more complex formulations:

- Solid particles can be incorporated, resulting in stabilized suspensions.
- Incorporation of oily lipids results in so-called hydrolipid dispersions or quasiemulsions.

Hydrogels

Hydrogels are hydrophilic, consisting mainly (85–95%) of water or an aqueous–alcoholic mixture and the gelling agent. The latter is usually an organic polymeric compound such as polyacrylic acid (Carbopol), sodium carboxy methylcellulose, or nonionic cellulose ethers. Hydrogels have to be preserved against microbial growth.

After application, hydrogels show a cooling effect caused by evaporation of the solvent. They are easily applicable and humidify instantaneously, but if applied over a long time they desiccate the skin. For that reason, humectants such as glycerol may be added. After evaporation, the polymer residue may cause a sticky or "tearing" feel on the skin if inappropriate thickening agents have been used. Careful selection and testing of the needed adjuvants is therefore recommended.

Hydrophobic Gels

Lipogels or oleogels are obtained by adding a suitable thickening agent to an oil or a liquid lipid. For example, colloidal silica may be used for that reason. A special type of hydrophobic gels is silicone-based systems.

Hydrolipid Dispersions

Hydrolipid dispersions are a special type of emulsion and are therefore treated separately in this chapter. They are disperse systems with a hydrophilic continuous phase and a lipophilic internal phase. The concentration of lipids lies between 2% and 20%. In principle, such a system is thermodynamically unstable. For stabilization, suitable large polymers are added, which are hydrated lyophilic colloids in the aqueous medium. Because of their molecular structure these polymeric emulsifiers are able to form mono- to multilamellar films at the interfaces and hence stabilize the emulsion. Typical examples are acrylates/C10–30 alkyl acrylate crosspolymers. These polymers must have a sufficient surface activity that enables them to interact between

the two different phases, resulting in a "quasiemulsion," alternatively called balm, costabilized by hydroxypropyl methylcellulose or polyacrylate. The dispersed oil droplets may show a relatively large size of 20 to 50 μm, but such a quasiemulsion remains stable (20). The great advantage of hydrolipid dispersions is their lack of conventional emulsifiers, surfactants with skin irritation potential.

Microemulsions

According to the definition of Danielsson and Lindman (21), a microemulsion is defined as a system of water, oil, and amphiphile, which is a single optically isotropic and thermodynamically stable liquid solution. "This definition should be widened, however, to include metastable states, spontaneous emulsions of long-lived kinetic stability" (22). The term microemulsion may be a misnomer, because microemulsions consist of large or "swollen" micelles containing the internal phase, much like that found in a solubilized solution (23). Microemulsions contain oil droplets in a water phase or water droplets in oil with diameters of about 10 to 200 nm. Therefore they appear as isotropic, optically clear liquid or gel-like systems. Unlike micellar solubilized systems, microemulsions may not be thermodynamically stable; nevertheless, they are more stable than ordinary emulsions. They are a type of ternary system composed of water, lipid, and surfactant mixture in a distinct ratio. The latter is usually a surfactant, such as Brij 96 [polyoxyethylene (10) oleyl ether] combined with a cosurfactant such as propylene glycol or ethylene glycol. Microemulsions may be used to incorporate or dissolve active substances and have been found to improve skin penetration and permeation (24).

The disadvantage of microemulsions is their rather high concentration of surfactants, which is a risk for increased skin irritation and sensitization. Nevertheless, modern microemulsion formulation is based on alkyl polyglycosides which are regarded to be milder than conventional nonionic surfactants with polyoxyethylene chains.

Nanoemulsions and Nanoparticles

During the last years, special dispersion formulations have been developed and described that contain ultrasmall particles used as carriers for active substances. The particles have a size in the range of 10 to a few hundred nanometers. This group of formulations shows a large heterogeneity and very often various terms or trade names have been created naming the same or similar systems. Generally the particles are dispersed in an aqueous medium.

For example, solid lipid nanoparticles possess a solid matrix composed of physiological lipids or lipoids with a mean diameter in the range of approximately 50 to 1000 nm (25). Active substances may be incorporated into these lipid nanoparticles serving as carriers, provided that the active substances are released after application on the skin.

Alternatively, the core of nanoparticles may either be a liquid lipid functioning as a carrier or a lipophilic agent being directly effective, e.g., an emollient or occlusive agent. For stabilization, a monolayer of surfactants surrounding/covering the lipid droplet is used, e.g., phospholipids combined with a selected cosurfactant in a defined ratio (26,27). Instead of a lipid, lipophilic active substances may be incorporated, e.g., vitamin A or E, UV filters, fragrances, etc. This type of nanoparticle is thought to be relatively insensitive toward the presence of additional surfactants in

contrast to liposomes; therefore, they can be mixed with conventional emulsions, and the size of the nanoparticles remains in the submicron range.

Suspensions

Strictly considered, suspensions are not just vehicles but products consisting of particles, generally actives or functional excipients, that are dispersed in a liquid or semisolid medium that functions as a vehicle. Nevertheless, a suspension is also a type of formulation that may be used for application on the skin and to deliver substances to a target. In this way, a suspension can be regarded as a vehicle entity affecting the application site. Examples are sun-protection products or pearlescent nail lacquers containing pigments.

In suspension, sedimentation of insoluble particles may happen because of difference in density. To guarantee a homogeneous product when applied, the particles must be redispersible by shaking before use. Alternatively, sedimentation must be hindered or at least reduced during storage. This is achieved by reduction of particle size and/or by increasing the viscosity of the vehicle, ideally creating a thixotropic system. The vehicle effect of the suspension on the skin is primarily caused by the liquid or semisolid phase of the vehicle comparable to solutions and emulsions.

Sticks

A stick is a solid delivery vehicle cast in an elongated form. By rubbing a stick onto skin, a variety of cosmetic ingredients can be delivered, such as fragrances, coloring agents, and emollients. In particular, sticks are ideally suited to deliver insoluble substances, e.g., pigments. The most popular cosmetic sticks are lipsticks and antiperspirant/deodorant sticks.

There are mainly three basic vehicle types of sticks:

1. mixture of waxes (e.g., beeswax, carnauba) and oils (e.g., mineral, castor oil) that are cast into solid form, containing dissolved or undissolved active ingredients,
2. hydrophilic or aqueous sticks: solutions based on aqueous, propylene glycol, alcohol mixtures, solidified usually by sodium stearate, containing, e.g., aluminum chlorohydrate as antiperspirant,
3. matrix consisting of a high-boiling volatile silicone (e.g., cyclomethicone) gelled by fatty alcohol (e.g., stearyl alcohol).

In recent years, clear sticks have become popular. As a gelling agent, dibenzylidene sorbitol is used in propylene glycol or other related polyols (28).

FUNCTIONAL DESIGN, COMPOSITION, AND RESULTING EFFECT

There is no universal cosmetic vehicle available that can simply be mixed with an active cosmetic substance to get the cosmetic care product of choice, nor is there a general principle that could be observed to perform development of such a product. But a cosmetic care product has to be developed, and whenever this is the case, various issues and aspects have to be considered, and many problems must be solved step-by-step. Although formulation (galenical development) of cosmetic products is still rather empirical today, a rational approach is suggested. This section discusses

the main issues that are to be considered when a functionally designed cosmetic product is being developed.

Target Profile

First, a clear target profile of the product must be defined. This includes the following:

1. Site of application: Depending on the site, certain forms may not be adequate, e.g., a w/o cream is not at all suitable for application on hair.
2. Area of application: A sticky, greasy cream cannot be applied on the whole body surface.
3. Target site: for example, the uppermost layer of stratum corneum or viable epidermis,
4. sensory properties: for example, foaming shampoo or a light, smooth, low-viscosity cream.
5. optical aspect: clear, transparent, or milky, mono- or multiphasic,
6. state of matter: liquid, semisolid, or solid,
7. basic type of form: solution or emulsion,
8. active substances: selected vegetable oils, vitamins, UV screens,
9. storage stability and conditions,
10. packaging,
11. comparable, competitor products.

Selection of Vehicle Type

The type of vehicle may already be determined by the product target profile. If various types are possible, the most suitable should be selected. The following selection criteria are important: function or desired effect of the vehicle on the skin, ease of formulation feasibility, and physical and chemical stability. Furthermore, solubility, polarity, saturation solubility, vehicle interactions, and formation of mesophases are subjects to be considered when dealing with development and selection of vehicles. These topics are discussed later.

True Solution Versus Disperse System

Whenever the target of an active substance lies in deeper regions of the skin or even in skin cells, the substance must be present in molecular form for successful and efficient delivery, i.e., it must be dissolved in the vehicle or it must be able to dissolve, at least, after application. In other words, dissolution of a substance is a prerequisite for its delivery to a biological viable target (e.g., cell, enzyme). It is only in the dissolved state that fast and efficient penetration and transport into the deeper skin layers and cells is possible.

Thus, the first goal in formulation development is to dissolve the active substance in the vehicle. Therefore, the vehicle should be an ideal solvent for the active substance. If a substance cannot be dissolved in the vehicle—this may happen because of low solubility properties or stability reasons—then the substance has to be incorporated in paniculate form; the smaller the size, the better. Fine particles in the order of 1 μm can be delivered onto or even into the uppermost layers of the skin, as close as possible to the target site. There they may dissolve, faster or slower, depending on their solubility in the skin. In vehicle systems containing particulate matter, homogeneous distribution of the undissolved substances must be guaranteed.

In summary, if the first goal—dissolution of active substance in the vehicle—is not achieved, the first alternative in formulation development must be targeted: The substance to be delivered must occur in particulate form as fine as possible. This is the prerequisite for fast and efficient delivery of insoluble matter into the skin close to the target site.

Polarity

To achieve dissolution of a substance (solute), the adequate vehicle (solvent) has to be selected. The solubility of a substance is attributable in a large measure to the polarity of the solvent, and it generally depends on chemical, electrical, and structural effects that lead to mutual interactions between the solute and solvent (29). Polar solvents dissolve ionic solutes and other polar substances, whereas nonpolar substances are dissolved in nonpolar, lipophilic solvents. Solubility properties determine the selection of the appropriate vehicle for both, for solid as well as for liquid substances. Only nonpolar liquids are mutually completely miscible and thus can be used to make a nonpolar liquid vehicle. Accordingly, the same is true for polar liquids (e.g., water and alcohol).

Solubility characteristics of a compound used in formulation is one of the most important factors to be considered. Solubility data can be found in the literature; very often they are delivered by suppliers of the substances or they must be determined experimentally. In formulation, the solubility parameter 5, according to Hildebrand and Scott (30), is a useful tool for selection of appropriate solvents. The more alike the 8-values of the compounds, the greater is their mutual solubility. A list of solubility parameters of cosmetic ingredients is given (31). Very apolar substances have a low 8-value, and water has the highest value (23). A rule of thumb states that mutual solubility is given if the difference between the two specific 5-values is at maximum 2 units $(\text{cal/cm}^3)''^2$.

Particularly in cosmetic formulation, where oils and lipids play a dominating role, polarity of oils is a factor to be considered. According to ICI Surfactants (16), the polarity may also be expressed by the polarity index based on the surface tension between the oil and water. Another interesting and simple characterization method is based on the bathochromic effect of a suitable dye dissolved in oils. The absorption maximum in the visible light—and therefore the color—of a nil-red-oil solution depends on the polarity of the oil; the higher the absorption maximum, the more polar is the oil or oil mixture (32).

In conclusion, if a monophasic system has to be formulated, only substances with mutual solubility can be combined. In contrast, if multiphasic systems such as emulsions and suspensions are made, the phase-forming components must be mutually insoluble. Nevertheless, preparation and solubilization of multiphasic systems require the addition of amphiphilic substances (emulsifiers in emulsions, surfactants for wetting and repulsing the particles in suspensions). In emulsions, polar as well as nonpolar substances can be dissolved in the hydrophilic or lipophilic phase, respectively. This is one reason for the popularity of emulsions.

Saturation, Supersaturation

Theoretically, a solute can be dissolved in a solvent up to the saturation solubility. Beyond this concentration, precipitation of the solute or phase separation usually occurs. Some substances are able to remain transiently in solution above saturation solubility. This phenomenon is known as supersaturation, a metastable condition.

Supersaturated solutions can be caused to return to saturation equilibrium by triggers such as agitation, scratching the wall of containers, or addition of seeding crystals.

The driving force for delivery of substances, i.e., release from vehicle and penetration into skin, is thermodynamic activity, which is maximal at saturation concentration (33). Consequently, to achieve maximal penetration rate into the skin, a substance must be dissolved in a vehicle at saturation concentration. Moreover, saturated or supersaturated systems are necessary, but not the only prerequisites for optimal topical delivery. For example, the skin–vehicle partition coefficient of the solute also plays a role. The partition coefficient may be raised because of the vehicle–skin interaction yielding in increased skin penetration. In conclusion, achieving the highest possible concentration in the dissolved state is the second goal to be aimed for in formulation development if delivery into the skin is targeted.

Vehicle Interactions

Sun-protection products are a good example of showing interactions between vehicles, active substances, and the skin. The absorption of UV radiation not only depends on the molecular structure and the concentration of the protecting agent, but on the solvent as well. Also, water resistance may be influenced by selection and composition of the vehicle.

Vehicle components may penetrate into the stratum corneum and interact with the stratum corneum lipids. This may result in disturbance of their lamellar structures and increased and faster penetration of compounds in the stratum corneum. Alternatively, presence of vehicle components in the stratum corneum may cause a depot effect for certain compounds.

Substantivity

The term substantivity describes adherence properties of materials to keratinous substrates in the upper skin layers, in particular regarding deposition and retention capacity when in contact with water, which could deplete the material (34). High substantivity is especially important for sun-protection products. It is primarily a function of the physicochemical properties of the active molecules but may also be influenced by the vehicle. For example, addition of film-forming, skin-adherent polymeric substances to the vehicle may increase retention of sunscreens in the skin and thus result in an improved water-resistant product. Another means is creating formulations that contain phospholipids, enabling the formation of vesicular, liposomal structures in the vehicle or in the upper layers of stratum corneum and thus yielding in a depot effect.

An interesting model to assess substantivity has been presented (34). The investigators used human callus to simulate and quantify solute sorption to human skin, which was found to be more suitable than octanol or animal keratin. However, water resistance still has to be determined in vivo to know the true quality of the product.

Mesophases

Not only the type of vehicle, e.g., solution or o/w emulsion, but also occurrence and type of mesophases (liquid crystal structures) determine the properties and behavior of a vehicle. At certain concentrations and combinations of specific emulsifying agents in liquids, associations may be formed, resulting in liquid crystal structures,

also called mesomorphic state or mesophase. The mesophase shows anisotropy and is thermodynamically stable. Different types of mesophases have been described: middle phase (hexagonal), cubic phase, and neat phase (lamellar).

Fatty amphiphiles (e.g., long chain alcohols, acids, monoglycerides) that are dispersed in water in the presence of a high HLB surfactant form lamellar phases. They are able to swell at an elevated temperature close to the melting point of the hydrocarbon chain. These swollen lamellar liquid crystalline phases can incorporate significant quantities of water. The hydrocarbon chains are liquid like, i.e., disordered. If the temperature decreases, the lamellar liquid crystalline phases of fatty amphiphiles are transformed to so-called lamellar crystalline gel network phases, which build complex gel networks. Such networks not only stabilize creams and lotions/but also control their consistency because of their viscoelastic properties. Such mesophases provide the following advantages to emulsions (35):

1. increased stability
2. prolonged hydration properties
3. controlled release of active ingredient
4. easy to formulate
5. well-liked skin feel

Metamorphosis of Vehicles

Most vehicles undergo considerable changes during and after application to the skin because of mechanical stress when spread over the surface and/or evaporation of volatile ingredients. Mechanical stress and skin temperature may influence the viscosity of the vehicle and consequently the release rate of active ingredients. Uptake of water from the skin may alter the composition of the vehicle. All these factors may also cause phase inversion or phase separation. And last but not least, as a consequence of these alterations the thermodynamic activity of an active ingredient within its vehicle will change as well. Thus, by controlling or changing the thermodynamic activity, release of a substance from the vehicle and penetration into the skin can be modulated. For example, if after application the volatile component of the vehicle, being an excellent solvent of the active substance, evaporates, saturation concentration of the active in the remaining vehicle or even supersaturation may be achieved. This results either in improved release and delivery as previously mentioned ("Saturation, Supersaturation") or in precipitation and deposition of the active substance. Another interesting example is given by an optimally composed sun-protecting o/w emulsion; after application the emulsion has transformed to the w/o type because of water evaporation and the mechanical stress caused by spreading. The remaining lipophilic protective film yields in improved water resistance.

In conclusion, the optimally designed and developed vehicle not only demonstrates excellent properties after manufacturing and storage, but also after application and metamorphosis at the application site.

Rheology

The term rheology describes the flow characteristics of liquids and the deformation of solids. Viscosity is an expression of the resistance of a fluid to flow. Rheological properties are crucial for liquid and semiliquid cosmetic formulations because they determine the product's properties meaningful in mixing and flow when produced, filled into containers and removed before use, as well as sensory properties when applied, such as

consistency, spreadability, and smoothness. Furthermore, the rheology of a product may also affect the physical stability and the biological availability of the product (36).

Regarding rheological characteristics, there are two main types of systems: Newtonian and non-Newtonian. The former show constant viscosity when stressed, i.e., the rate of shear (flow velocity) is directly proportional to the shearing stress, e.g., water, mineral oil, etc. In non-Newtonian systems (most cosmetic products), however, viscosity changes with varying stress, i.e., viscosity depends on the degree of shearing stress, resulting either in plastic, pseudoplastic, or dilatant flow or in thixothropy, characteristics that are not discussed in depth here although they are of practical significance. An ideal topical product, e.g., shows optimal thixotropic properties; it does not flow out of a tube's orifice unless slightly pressed, and when on the skin it does not immediately flow and drop off unless easily spread over the application area, where under a certain stress it becomes more fluid because of the thixotropy. The rheological properties of semisolid products are determined first for general characterization in the development phase and second for quality-control reasons after manufacturing. There are various instrumental methods used to measure rheology or viscosity. Today, apparatus based on rotation or oscillation are commonly used for non-Newtonian systems.

To adjust the rheology of products, various means and excipients are available. If the viscosity has to be increased, addition of viscosity-increasing agents is needed. Addition or increase in concentration of electrolytes may influence viscosity. Many systems, e.g., polyacrylates, are sensitive to the presence of ions, and the viscosity is reduced.

In particular, emulsions are susceptible to rheological issues. Various factors determine the rheological properties of emulsions, such as viscosity of internal and external phases, phase volume ratio, particle size distribution, type and concentration of emulsifying system, and viscosity-modifying agents. However, this topic is too complex to be treated comprehensively in this context. It is further discussed in a review by Sherman (37). It is important to realize that small changes in concentrations or ratio of certain ingredients may result in drastic changes of the rheological characteristics. Emulsified products may undergo a wide variety of shear stresses during either preparation or use. Thus, an emulsion formulation should be robust enough to resist external factors that could modify its rheological properties or the product should be designed so that change in rheology results in a desired effect.

Preservation

Antimicrobials

Most cosmetic care products must be protected against microbial growth. Not only for the protection of consumers against infection but also for stability reasons. Growth of microorganisms might result in degradation of ingredients and consequently in deterioration of physical and chemical stability. In general, presence of water in the vehicle as well as other ingredients susceptible to microbial metabolism require adequate preservation.

There are various ways to protect a product against microbial growth:

1. addition of an antimicrobial agent, which is a common practice,
2. sterile or aseptic production and filling into packaging material, preventing microbial contamination during storage and usage,
3. reduced water activity, i.e., controlling growth of spoilage microorganisms by reducing the available amount of water in cosmetic preparations (38).

It is not only mandatory to add antimicrobials but also to test their efficacy after manufacturing and after storage until the expiry date. Nowadays, performance of the preservative efficacy test (PET), also known as the challenge test, is state of the art (39). Today more and more in-use tests are performed to simulate the usage by the consumer and to show efficacious protection against microbial growth after contamination.

Addition of preservatives to complex, multiphasic systems, in particular, is a critical formulation issue for the following reasons:

1. Many preservatives interact with other components of the vehicle, e.g., with emulsifiers, resulting in change of viscosity or in phase separation in the worst case.
2. Depending on the physicochemical characteristics, preservatives are distributed between the different phases which might result in too-low-effective concentration in the aqueous phase.
3. Adsorption of the preservatives to polymers in the formulation and/or packaging material; complexation or micellization might also result in too-low-antimicrobial activity.

In conclusion, it is not sufficient to add a preservative at recommended concentration. To protect the vehicle sufficiently, a properly designed preservative system is required that must be tested in the formulation regarding efficacy and safety. It is a great formulation challenge to achieve sufficient protection against microbial growth in the product, especially, as many antimicrobials are discredited because of their irritation and sensitization potential.

Antioxidants

Protection against oxidation may also be a formulation issue although not so relevant as antimicrobial efficacy. It is achieved by addition of antioxidants or by manufacturing and storing in an inert atmosphere. In particular, modern formulations containing oxidation-sensitive compounds, such as certain vitamins and vegetable oils with unsaturated fatty acid derivatives, must be sufficiently protected against oxygen.

Development Strategy and Rationale

Having considered the aforementioned issues, formulation development is preferably conducted according to a suitable, rational procedure. The complex formulation development process may be represented symbolically by the "magic formulation triangle" (Fig. 1), showing the mutual interaction and dependency of the following:

1. feasibility of preparation or formulation of the active substance(s) in the vehicle,
2. stability (chemical and physical) of the product, and
3. effectivity or activity of the product when applied.

First, the feasibility of preparation and formulation has to be checked. For example, if a low-water-soluble compound should be dissolved in an aqueous vehicle, solubility-enhancing studies are performed. Or, if an emulsion is desired, it has to be checked whether the phases can be emulsified with the selected emulsifying system.

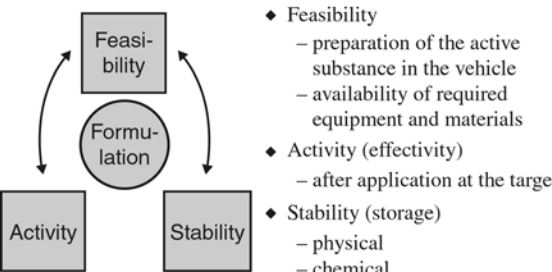

Figure 1 Magic triangle of formulation: mutual interaction and dependency.

After having prepared the desired formulation, both stability and effect must be assessed, preferably more or less in parallel. It does not make any sense to have a stable but ineffective product, or to develop a very effective system that remains stable for a few days or that contains an ingredient that is irritating or sensitizing. Such a product cannot be marketed. For example, if a relatively unstable active substance (e.g., ascorbic acid) must be delivered in dissolved form to be effective or bioavailable at the target site, then a suitable vehicle with good solvent properties must be used. However, the chemical stability of compounds is generally low in solutions. Therefore, not every suitable solvent can be used as a vehicle, but an optimum has to be found, a vehicle enabling both, keeping the active to remain dissolved and in a chemically stable state.

Having in mind those three cornerstones of the formulation triangle, formulation development to find the right vehicle is performed stepwise, addressing the following issues:

1. objective, definition of target profile (p. 113);
2. preformulation investigation: determination of physicochemical properties of (active) substances to be formulated, such as solubility data, partition coefficient, dissociation constant, pH, crystal morphology, particle size distribution, and assessment of their stability and incompatibility;
3. selection of appropriate excipients to be used for formulation;
4. Based on the outcome of these three working steps the feasibility of preparation is checked, and modifications are made if necessary. All of these together prepare the next step.
5. Formulation screening on a small-scale basis with many possible and feasible variations in composition, excipients, preparation methods, and so on.
6. Selection of the best formulations and preparation methods from the screening program for technical scaling-up as well as for confirmation and validation of the results obtained with the formulations: The selection of the formulations is based on criteria such as physical stability or absence of precipitation in solutions, no sedimentation or phase separation or recrystallization in multiphasic systems; chemical stability or degradation, respectively; PET; biological assessment, e.g., skin-hydration effect, sun-protecting effect, and antioxidant or radical scavenger effect in cells and
7. safety evaluation in human beings with formulation chosen for introduction into markets.

PREPARATION METHODS

It is not the intention to present a review on preparation methods and equipment for the manufacturing of cosmetic vehicles and products in this chapter. But it is the common sense which suggests that the preparation method may influence a product's quality. Thus, not only the composition but also the way of preparation should be in the scope of development and preparation work. There are many types and variations of mixing, dispersion, emulsification, and size-reduction equipment that can be used to prepare vehicles that are used in cosmetics. For example, size reduction of the internal phase droplets in an emulsion depends on the mechanical principle of the used equipment, and best results are achieved with a valve homogenizer. In every case the goal is to get a homogeneous product of specified and reproducible quality. Only with a product of specified and constant quality a reproducible effect can be achieved when applied. Standard, basic operations are dissolution, blending and mixing, dispersion and homogenization, and size reduction, which may all be associated by energy transfer involving cooling or heating.

It is of paramount importance that in early development phases preparation is performed under well-defined and known conditions, otherwise scaling-up and reproducibility of product quality become a risky task. Closely related with the preparation method is testing and characterization of the product. This is treated in the following section.

CHARACTERIZATION

Physical Characterization

Appearance

Assessment and description of appearance are one of the easiest, most practical, and nevertheless powerful tests. It may be performed macroscopically, describing color, clearness, transparency, turbidity, and state of matter. In addition, microscopic investigation is recommended; taking microphotographs is useful for documentation.

Rheology

Rheological properties (viscosity, consistency) are important characteristics of most types of cosmetic care products because they have an impact on preparation, packaging, storage, application, and delivery of actives. Thus these properties should be assessed for characterization and quality control of the product.

Most disperse systems and thus cosmetic care products show non-Newtonian flow behavior, namely pseudoplastic, plastic, or dilatant behavior. A wide variety of techniques and methods have been developed to measure viscosity properties. These procedures can be classified as either absolute or relative. The absolute either directly or indirectly measures specific components of shear stress and shear rate to define an appropriate rheological function. Methods used for absolute viscosity measurements are flow through a tube, rotational methods, or surface viscosity methods. Methods used for relative viscosity measurements are those using orifice viscometers, falling balls, or plungers. Such instruments, although they do not measure stress or shear rate, offer valuable quality-control tests for relative comparison between different materials (40). Apparatus based on rotational or even oscillating principles to assess viscoelastic properties are state of the art.

pH

Measurement of pH value (concentration of hydrogen ions) in aqueous vehicles (solutions, suspensions, o/w emulsions, gels) is a valuable control mean. First of all, if possible, a pH value in the physiological range is generally targeted, ideally similar to that of the skin or the specific application site, to prevent irritation. Many reactions and processes depend on pH, e.g., efficacy of antimicrobial preservatives, stability and degradation of substances, and solubility. Thus, pH measurement is a "must," and it is easily performed with the available measurement systems.

Homogeneity

In many cases, at a first step, homogeneity may be assessed visibly; precipitation in a solution or distinct phase separation in an emulsion is easily detected. Nontransparent, multiphasic systems are more difficult to check. In these cases, microscopic investigation of representative samples is suggested along with quantitative assays regarding active ingredients (uniformity of content).

Droplet or Particle Size and Distribution

The physical stability of colloidal systems as well as emulsions or suspensions partially depends on the particle size. In particular, preparations containing small particles with identical electrical charge are more resistant to flocculation and sedimentation than systems containing larger or uncharged entities. Similarly, reduced particle size is an indicator of improved kinetic stability of emulsions or suspensions. For that reason, determination of particle size and size distribution is an important characterization method. Various optical methods are available: A minireview is given (41), and a selection is listed as follows:

1. Perhaps the most commonly used method today is based on laser diffraction, suitable to measure solid particles and also dispersed droplets under special conditions, size range 1 to 600 (μm).
2. Dynamic light scattering, also known as photon correlation spectroscopy, is used for measuring micelles, liposomes, and submicron suspensions (size range 0.003–3 μm).
3. Optical or electron microscopy is a further method of choice.

Chemical Characterization

Besides physical characterization, chemically based investigations are indispensable to assess the quality of a product. It is well known that the quality and composition of a vehicle can influence the chemical stability of ingredients. Many reactions, such as ester hydrolysis or other degradations, may be enhanced or sustained by a change in pH, presence of catalytic or stabilizing agents, respectively. Thus, development and optimal selection of the best vehicle are supported by chemical stability investigations.

Biological Characterization

Further important assessment methods are based on biological tests. This is to evaluate and validate the desired targeted effects in vivo after application of the product. Examples include hydration of the skin, protection against sun radiation, and

protection against skin irritating substances during work. This subject is treated in other chapters of this textbook.

Sensory Assessment

The sensory assessment is a useful tool for product and concept development and for quality control in the cosmetic industry. Although a very subjective and liable method, valuable data are obtained if sensory assessment is conducted in a systematic way. Terms like pick-up, consistency, peaking, cushion, absorption, smoothness, stickiness, tackiness, oiliness, and greasy are used. An interesting paper on that subject has been published by Busch and Gassenmeier (42).

Barry et al. (43) carried out sensory testing on topical preparations and established rheological methods for use as control procedures to maintain uniform skin feel and spreadability. The consistency of a material can be assessed by using three attributes: smoothness, thinness, and warmth (44).

REFERENCES

1. Wilkinson JB, Moore RJ, eds. Harry's Cosmeticology. New York: Chemical Publishing, 1982.
2. Rieger MM. Cosmetics and their relation to drugs. In: Swarbrick J, Boylan JC, eds. Encyclopedia of Pharmaceutical Technology. 3. New York: Marcel Dekker, 1990; 361–373.
3. Junginger HE. Systematik der dermatika—kolloidchemischer aufbau. In: Niedner R, Ziegenmeyer J, eds. Dermatika. Stuttgart: Wissenschaftliche Verlagsgesellschaft mbH, 1992:476.
4. Martin A, Bustamante P, Chun AHC. Physical Pharmacy. Philadelphia: Lea & Febiger, 1993:393–396.
5. Martin A, Bustamante P, Chun AHC. Physical Pharmacy. Philadelphia: Lea & Febiger, 1993:393.
6. Martin A, Bustamante P, Chun AHC. Physical Pharmacy. Philadelphia: Lea & Febiger, 1993:386.
7. Martin A, Bustamante P, Chun AHC. Physical Pharmacy. Philadelphia: Lea & Febiger, 1993:496.
8. Martin A, Bustamante P, Chun AHC. Physical Pharmacy. Philadelphia: Lea & Febiger, 1993:101.
9. Martin A, Bustamante P, Chun AHC. Physical Pharmacy. Philadelphia: Lea & Febiger, 1993:477.
10. Martin A, Bustamante P, Chun AHC. Physical Pharmacy. Philadelphia: Lea & Febiger, 1993:234.
11. Martin A, Bustamante P, Chun AHC. Physical Pharmacy. Philadelphia: Lea & Febiger, 1993:396.
12. Martin A, Bustamante P, Chun AHC. Physical Pharmacy. Philadelphia: Lea & Febiger, 1993:488.
13. Martin A, Bustamante P, Chun AHC. Physical Pharmacy. Philadelphia: Lea & Febiger, 1993:490.
14. ICI Surfactants, brochure 41-IE. Personal Care. Middlesbrough, Cleveland, United Kingdom, 1996.
15. Herzog B, Marquart D, Miiller S, Pedrussio R, Sucker H. Einfluss von zusammensetzung und phasenverhaltnis auf die konsistenz von cremes. Pharm Ind 1998; 60:713–721.

16. ICI Surfactants, brochure 42-4E. Personal Care, emulsifiers for water in oil emulsions. Middlesborough, Cleveland, United Kingdom, 1996:5.
17. Rosoff M. Specialized pharmaceutical emulsions. In: Liebermann HA, Rieger MM, Banker GS, eds. Pharmaceutical Dosage Forms: Disperse Systems. 3. New York: Marcel Dekker, 1998; 11.
18. Gohla SH, Nielsen J. Partial phase solu-inversion technology (PPSIT). Seifen Oele Fette Wachse J 1995; 121:707–713.
19. Kutz G, Friess S. Moderne Verfahren zur Herstellung von halbfesten und fliissigen Emulsionen—eine aktuelle Uebersicht. Seifen Oele Fette Wachse J 1998; 124:308–313.
20. Daniels R. Neue anwendungsformen bei sonnenschutzmitteln. Apotheken J 1997; 19(5):22–28.
21. Danielsson L, Lindman B. Colloids Surf 1981; 3:391.
22. Rosoff M. Specialized pharmaceutical emulsions. In: Liebermann HA, Rieger MM, Banker GS, eds. Pharmaceutical Dosage Forms: Disperse Systems. 3. New York: Marcel Dekker, 1998; 20.
23. Martin A, Bustamante P, Chun AHC. Physical Pharmacy. Philadelphia: Lea & Febiger, 1993:495.
24. Martin A, Bustamante P, Chun AHC. Physical Pharmacy. Philadelphia: Lea & Febiger, 1993:496.
25. Miiller RH, Weyhers H, zur Mu'hlen A, Dingier A, Mehnert W. Solid lipid nanoparticles—ein neuartiger Wirkstoff-carrier fur Kosmetika und Pharmazeutika. Pharm Ind 1997; 59:423–427.
26. Ziilli F, Suter F. Preparation and properties of small nanoparticles for skin and hair care. Seifen Oele Fette Wachse J 1997; 123:880–885.
27. Herzog B, Sommer K, Baschong W, Roding J. Nanotopes™: a surfactant resistant carrier system. Seifen Oele Fette Wachse J 1998; 124:614–623.
28. Schueller R, Romanowsky P. Gels and sticks. Cosmet Toilet Mag 1998; 113:43–46.
29. Martin A, Bustamante P, Chun AHC. Physical Pharmacy. Philadelphia: Lea & Febiger, 1993:215.
30. Hildebrand JR, Scott RL. Solubility of Nonelectrolytes. New York: Dover, 1964 (chap. 23).
31. Vaughan CD. Using solubility parameters in cosmetics formulation. J Soc Cosmet Chem 1985; 36:319–333.
32. Dietz Th. Solvatochromie von Nilrot. Parfiimerie Kosmetik 1999; 80:44–49.
33. Flynn GL, Weiner ND. Topical and transdermal delivery—provinces of realism. In: Gurny R, Teubner A, eds. Dermal and Transdermal Drug Delivery. Stuttgart: Wissenschaftliche Verlagsgesellschaft mbH, 1993:44.
34. Hagedorn-Leweke U, Lippold BC. Accumulation of sunscreens and other compounds in keratinous substrates. Eur J Pharm Biopharm 1998; 46:215–221.
35. Loll P. Liquid crystals in cosmetic emulsions. Reprint RP 94–93E. ICI Europe Limited, Everberg, B, 1993.
36. Martin A, Bustamante P, Chun AHC. Physical Pharmacy. Philadelphia: Lea & Febiger, 1993:457.
37. Sherman P. Rheology of Emulsions. Oxford: Pergamon Press, 1963.
38. Enigl DC, Sorrells KM. Water activity and self-preserving formulas. In: Kabara JJ, Orth DS, eds. Preservative-Free and Self-Preserving Cosmetics and Drugs. New York: Marcel Dekker, 1997:45.
39. Sabourin JR. A Perspective on Preservation for the New Millennium, Cosmetics and Toiletries Manufacture Worldwide. Hemel Hempstead, United Kingdom: Aston Publishing Group, 1999:50–59.
40. Hanna SA. Quality assurance. In: Liebermann HA, Rieger MM, Banker GS, eds. Pharmaceutical Dosage Forms: Disperse Systems. 3. New York: Marcel Dekker, 1998; 460.

41. Haskell RJ. Characterization of submicron systems via optical methods. J Pharm Sci 1998; 87:125–129.
42. Busch P, Gassenmeier Th. Sensory assessment in the cosmetic field. Parfiimerie Kosmetik 1997; 7/8:16–21.
43. Barry BW, Grace AJ. J Pharm Sci 1971; 60:1198, J Pharm Sci 1972; 61:335. 43b. Barry BW, Meyer MC. J Pharm Sci 1973; 62:1349.
44. Martin A, Bustamante P, Chun AHC. Physical Pharmacy. Philadelphia: Lea & Febiger, 1993:471.

9

Encapsulation to Deliver Topical Actives

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INTRODUCTION

Cosmetic technology is constantly developing raw materials and formulation with active ingredients. The new surfactant molecules, the search for original active substances and efficient combinations, and the design of novel vehicles or carriers have led to the implementation of new cosmetic systems in contrast to the classic forms such as creams or gels.

The achievements of recent extensive research have resulted in the development of controlled delivery systems. Some of these systems have been extensively investigated for their therapeutic potential while simultaneously being examined for their possible cosmetic uses. One objective in the design of novel drug delivery systems is controlled delivery of the active to its site of action at an appropriate rate. Novel polymers and surfactants in different forms, sizes, and shapes can aid in this goal. Encapsulation techniques are used in pharmaceuticals, cosmetics, veterinary application, food, copying systems, laundry products, agricultural uses, pigments, and other lesser well-known uses to control the delivery of encapsulated agents as well as to protect those agents from environmental degradation.

DESIGN ASPECTS OF A VECTOR

Microparticles

Microencapsulation is a process by which very thin coatings of inert natural or synthetic polymeric materials are deposited around microsized particles of solids or droplets of liquids. Products thus formed are known as microparticles, covering two types of forms: microcapsules, micrometric reservoir systems, microspheres, and micrometric matrix systems (Fig. 1).

These systems consist of two major parts. The inner part is the core material containing one or more active ingredients. These active ingredients may be solids,

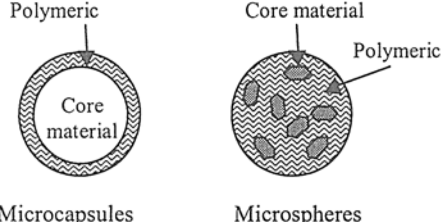

Figure 1 Schematic representation of microparticles.

liquids, or gases. The outer part is the coating material that is usually of a high-molecular weight polymer or a combination of such polymers. The coating material can be chosen from a variety of natural and synthetic polymers, and must be nonreactive to the core material, preferably biodegradable, and nontoxic. Other components, such as plasticizers and surfactants, may also be added.

Initially, microparticles were produced mainly in sizes ranging from 5 μm to as much as 2 mm, but around 1980 a second generation of products of much smaller dimensions was developed. This includes nanoparticles from 10 to 1000 nm in diameter (1), as well as 1 to 10 μm microspheres, overlapping in size with nonsolid microstructures such as liposomes. Commercial microparticles typically have a diameter between 1 and 1000 μm and contain 10 to 90 wt% core. Most capsule shell materials are organic polymers, but fat and waxes are also used. Various types of physical structures of the product of microencapsulation such as mononuclear spheres, multinuclear spheres, multinuclear irregular particles, and so on can be obtained depending on the manufacturing process.

Recently, a polymeric system consisting of porous microspheres named Microsponge has been developed [Microsponge System (2); Advanced Polymer System Inc., Redwood City, California]. These systems are made by suspension polymerization and typically consist of cross-linked polystyrene or polymethacrylates.

No encapsulation process developed to date is able to produce the full range of capsules desired by potential capsule users. The methods, which are significantly relevant to the production of microparticles used in pharmaceutical products and cosmetics, are shown in Table 1. Many techniques have been proposed for the production of microparticles, and it was suggested (9) that more than 200 methods could be identified in the literature. A thorough description of the formation of microparticles are given by several reviews (4,6,10,11).

Table 1 Microencapsulation Methods

Type	Reference
Coacervation-phase separation procedures using aqueous vehicles	3
Coacervation-phase separation procedures using nonaqueous vehicles	4
Interfacial polymerization	5
In situ polymerization	6
Polymer–polymer incompatibility	3
Spray drying, spray congealing, spray embedding, and spray polymerization	4
Droplet extrusion	7

Table 2 Nanoparticles Obtained by Polymerization of a Monomer

Type	Reference
Nanospheres	
Poly(methylmethacrylate) and polyalkylcyanoacrylate nanoparticles	12
Polyalkylcyanoacrylate nanospheres	13
Nanocapsules	
Polyalkylcyanoacrylate nanocapsules	14, 15

Nanoparticles

Nanoparticles can generally be defined as submicron (<1 μm) colloidal systems, but are not necessarily made of polymers (biodegradable or not). According to the process used for the preparation of nanoparticles, nanocapsules or nanospheres can be obtained. Nanocapsules are vesicular systems in which the drug is confined to a cavity surrounded by a unique polymeric membrane; nanospheres are matrix systems in which the drug is dispersed throughout the particles.

Several methods have been developed for preparing nanoparticles. They can be classified in two main categories according to whether the formation of nanoparticles requires a polymerization reaction (Table 2) or whether it is achieved from a macromolecule or a preformed polymer (Table 3). De Vringer and Ronde (25) proposed a water-in-oil (w/o) cream containing nanoparticles of solid paraffin to obtain a topical dermatological product with a high degree of occlusivity combined with attractive cosmetic properties. Kim et al. (26) reported the encapsulation of fat vitamin series in nanospheres prepared with soybean lecithin coated with a nonionic surfactant. Muller (27,28) believes that the solid lipid nanoparticles (SLN) appear as an attractive carrier system for cosmetic ingredients—unloaded and loaded. In the case of unloaded particles, the SLN themselves represent the active ingredient, e.g., when made from skin-carrying lipids. Alternatively, the SLN can be blended with special lipids, e.g., ceramides. Finally, good reviews with methods of preparation for nanoparticles can be found in the literature, by Kreuter (12) and Couvreur et al. (29).

Table 3 Nanoparticles Obtained by Dispersion of Preformed Macromolecules

Type	Reference
Nanospheres prepared by emulsification	
Solution emulsification	16
Phase inversion	17
Self-emulsification	18
Nanospheres of synthetic polymers	19, 20, 21
Nanospheres of natural polymers	21
Nanospheres prepared by desalvation	
Nanospheres of synthetic polymers	22
Nanospheres of natural polymers	23, 24
Nanocapsules	14, 22

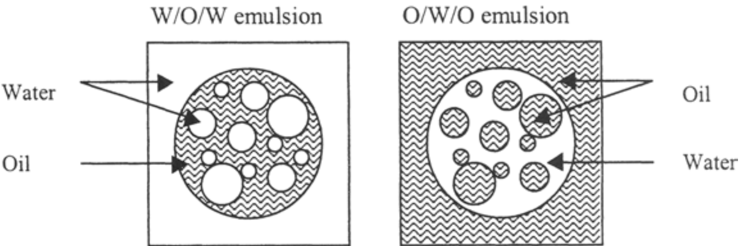

Figure 2 Schematic representation of multiple emulsions.

Multiple Emulsions

Multiple emulsions are emulsions in which the dispersion phase contains another dispersion phase. Thus, a water-in-oil-in-water (w/o/w) emulsion is a system in which the globules of water are dispersed in globules of oil, and the oil globules are themselves dispersed in an aqueous environment. A parallel arrangement exists in oil-in-water-in-oil (o/w/o) type of multiple emulsions in which an internal oily phase is dispersed in aqueous globules, which are themselves dispersed within an external oily phase (Fig. 2).

Multiple emulsions, first described by Seifriz in 1925, have recently been studied in detail. The operational technique plays an even more important role in the production of multiple emulsions than in the production of simple emulsions (30–35). Multiple emulsions have been prepared in two main modes: one-step and two-step emulsification.

One-step emulsification is prepared by forming w/o emulsion with a large excess of relatively hydrophobic emulsifier and a small amount of hydrophilic emulsifier followed by heat treating the emulsion until, at least in part, it gets inverted. At a proper temperature, and with the right hydrophilic lipophilic balance (HLB) of the emulsifiers, w/o/w emulsion can be found in the system. In most recent studies, multiple emulsions are prepared in a two-step emulsification process by two sets of emulsifiers: a hydrophobic emulsifier I (for the w/o emulsion) and a hydrophilic emulsifier II (for the oil-in-water (o/w) emulsion). The primary emulsion is prepared under high shear conditions (ultrasonification, homogenization), whereas the secondary emulsification step is carried out without any severe mixing (an excess of mixing can rupture the drops, resulting in a simple emulsion).

The composition of the multiple emulsions is of significant importance, because the different surfactants along with the nature and concentration of the oil phase will affect the stability of the double emulsion. Parameters such as HLB, oil phase volume, and the nature of the entrapped materials have been discussed and optimized. Several reviews and studies include Florence and Whitehill (36–38), Matsumoto et al. (39,40), Frenkel et al. (41), Csoka and Eros (42), and Opawale and Burgess (43).

Microemulsions

Miocroemulsions are stable dispersions in the form of spherical droplets whose diameter is in the range of 10 to 100 nm. They are composed of oil, water, and usually surfactant and cosurfactant. These systems show structural similarity to micelles and inverse micelles, resulting in o/w or w/o microemulsions, respectively. They are highly dynamic systems showing fluctuating surfaces caused by forming

and deforming processes. The main characteristics of microemulsions are the low viscosity associated with a Newtonian-type flow, a transparent or translucid appearance, and isotropic and thermodynamic stability within a specific temperature setting. Certain microemulsions may thus be obtained without heating, simply by mixing the components as long as they are in a liquid state. One of the conditions for microemulsion formation is a very small, gather than a transient negative, interfacial tension (44). This is rarely achieved by the use of a single surfactant, usually necessitating the addition of a cosurfactant. The presence of a short chain alcohol, e.g., can reduce the interfacial tension from about 10 mN/m to a value less than 10^2 mN/m. Exceptions to this rule are provided by nonionic surfactants which, at their phase inversion temperature, also exhibit very low interfacial tensions.

A microemulsion is usually created by the establishment of a pseudoternary diagram for which a ratio of surfactant/cosurfactant is fixed, representing a sole constituent. The establishment of a ternary diagram is generally accomplished for locating the microemulsion or the microemulsion zones by titration. Using a specific ratio of surfactant/cosurfactant, various combinations of oil and surfactant/cosurfactant are produced. The water is added drop by drop. After the addition of each drop, the mixture is stirred and examined through a crossed polarized filter. The appearance (transparence, opalescence, isotropy) is recorded, along with a number of phases. In this way, an approximate delineation of the boundaries can be obtained in which it is possible to refine through the production of compositions point by point beginning with the four basic components.

Nanoemulsions (Submicron Emulsions)

Emulsions are heterogeneous systems in which one immiscible liquid is dispersed as droplets in another liquid. Such a system is thermodynamically unstable and is kinetically stabilized by the addition of one further component or mixture of components that exhibit emulsifying properties. Depending on the nature of the diverse components of the emulsifying agents, various types of emulsions can result from the mixture of immiscible liquids. The main characteristic of nanoemulsions or submicron emulsions (SMEs) is the droplet size, which must be inferior to micrometers.

Emulsions prepared by use of conventional apparatus, e.g., electric mixers and mechanical stirrers, show large droplet sizes and wide particle distribution. The techniques usually used to prepare SMEs involve the use of ultrasound, evaporation of solvent (45), two-stage homogenizer (46,47), and the microfluidizer (48,49). The nanoemulsion preparation process involves the following steps:

1. Three approaches can be used to incorporate the drug and/or the emulsifiers in the aqueous or oil phase. The most common is to dissolve the water-soluble ingredients in the aqueous phase and the oil-soluble ingredients in the oil phase. The second approach, which is used in fat emulsion preparations (46), involves the dissolution of an aqueous-insoluble emulsifier in alcohol, the dispersion of the alcohol solution in water, and the evaporation and total removal of the alcohol until a fine dispersion of the alcohol solution of the emulsifer in the aqueous phase is reached. The third, which is mainly used for amphotericin B incorporation into an emulsion, involves the preparation of a liposome-like dispersion. The drugs and phospholipids are first dissolved in methanol, dichloromethane,

chloroform, or a combination of these organic solvents, and then filtered into a round-bottom flask. The drug–phospholipid complex is deposited into a thin film by evaporation of the organic solvent under reduced pressure. After sonication with the aqueous phase, a liposome-like dispersion is formed in the aqueous phase. The filtered oil phase and the aqueous phase are heated separately to 70°C and then combined by magnetic stirring.

2. The oil and aqueous phases are emulsified with a high-shear mixer at 70 to 80°C.
3. The resulting coarse emulsion (1–5 μm) is then rapidly cooled and homogenized into a fine monodispersed emulsion.

Bangham (50) clearly shows that the dispersion of natural phospholipids in aqueous solutions leads to the formation of "closed vesicles structures," which morphologically resemble cells. Since 1975 (51), vesicles have been prepared from surfactants. In 1986, the first commercial product incorporating liposomes identical to those described by Bangham appeared on the market (Capture). At the same time, a synthetic one made by nonionic surfactants (52) was also launched (niosomes). Several different compositions, for scientific, economic, and business reasons, prevailed in cosmetic vesicles. None of them really resembles the liposomes we have seen in medical applications. These main groups include (1) liposomes made from soya phospholipids, (2) sphingosomes, i.e., liposomes made from sphingolipids, and (3) nonionic surfactant vesicles (niosomes) which are a proprietary product of L'Oreal and other synthetic amphiphiles. In the 1990s, transfersomes, i.e., lipid vesicles containing large fractions of fatty acids, were introduced. Transfersomes (53–55) consist of a mixture of a lipidic agent with a surfactant. Consequently, their bilayers are much more elastic than those of most liposomes.

This chapter focuses on nonionic surfactant vesicles and transfersomes. Nonionic surfactant vesicles (NSVs or niosomes) consist of one or more nonionic surfactant bilayers enclosing an aqueous space. NSVs consisting of one bilayer are designed as small unilamellar vesicles or large unilamellar vesicles. Vesicles with more bilayers are called multilamellar vesicles.

Niosomes can be prepared from various classes of nonionic surfactants, e.g., polyglycerol alkyl ethers (52,56), glucosyl dialkyl ethers (57), crown ethers, and polyoxyethylene alkyl ethers and esters (58). The preparation methods used should be chosen according to the use of niosomes, because the preparation methods influence the number of bilayers, size, size distribution, entrapment efficiency of the aqueous phase, and membrane permeability of the vesicles (56,59). NSVs can be formed using the same methods that are used for the preparation of liposomes (Table 4).

Table 4 Vesicles Preparation Methods

Method	Reference
Sonication	56, 58, 60
Ether injection	56
Handshaking	56
Reversed phase evaporation	61
Method as described by Handjani-Vila	52

PROPERTIES OF A VECTOR

Microparticles

Microencapsulation has been applied to solve problems in the development of pharmaceutical dosage forms as well as in cosmetics for several purposes. These include the conversion of liquids to solids, separation of incompatible components in dosage form, taste masking, reduction of gastrointestinal irritation, protection of the core materials against atmospheric deterioration, and enhancement of stability and controlled release of active ingredients.

For drug follicular targeting, microspheres were envisaged mainly as site-specific drug delivery systems because they present several advantages: (1) good stability of the microspheres when applied on the skin, (2) easy preparation of microspheres with a defined size in a narrow size distribution, (3) protection of the active incorporated, (4) controlled release of the active in the hair follicles from the microspheres, and (5) the possibility of incorporating either lipophilic or hydrophilic actives into the microspheres (62). Concerning the Microsponge system, each microsphere is composed of thousands of small beads wrapped together to form a microscopic sphere capable of binding, suspending, or entrapping a range of substances. The outer surface is porous, allowing the controlled flow. Microsponges can be incorporated into gels, creams, liquids, powders, or other formulations, and can release ingredients depending on their temperature, moisture, friction, volatility of the entrapped ingredient, or time.

Nanoparticles

Nanoparticles are attractive delivery systems. In most cases the advantages are (1) the solid matrix gives flexibility to modify the drug release profile, (2) the relatively slow degradation allows long release times, and (3) the protection of incorporated compounds against chemical degradation. Drug release from colloidal carriers is dependent on both the type of carrier and the loading mechanisms involved.

Nanospheres

Release from nanospheres may be different according to the drug-entrapment mechanism involved. When the drug is superficially adsorbed, the release mechanism can be described as a partitioning process (rapid and total release if sink conditions are met). When the drug is entrapped within the matrix, diffusion plus bioerosion will be involved with a biodegradable carrier, whereas diffusion will be the only mechanism if the carrier is not biodegradable. From this, it can be inferred that entrapment within the matrix of nanospheres may lead to sustained release, the rate of which may be related to the rate of biodegradation of the polymer.

Nanocapsules

Release from nanocapsules is related to partitioning processes within immiscible phases. The equilibrium between the carrier (loaded drug) and the dispersing aqueous medium (free drug) is dependent both on the partition coefficient of the molecule between the oily and the aqueous phases and on the volume ratio of these two phases. This means that the amount released is directly related to the dilution of the carrier and that the release is practically instantaneous when sink conditions exist. Diffusion of the drug through the polymeric wall of nanocapsules does not seem

to be a rate-limiting step (63). Coating the polymeric wall with an outer layer of phospholipids can advantageously reduce drug leakage from nanocapsules.

Multiple Emulsions

Double emulsions are an excellent and exciting potential system for slow or controlled release of active entrapped compounds. The fact that the inner w/o emulsion serves as a large confined reservoir of water is a very attractive property for dissolving it in significant amounts of water-soluble drugs. The oil membrane seems to serve as a good transport barrier for the confined ionized and/or nonionized water-soluble drugs. The two amphiphilic interfaces are yet an additional barrier. The possibility to manipulate transport and release characteristics of the formulations seems to be feasible. However, despite 20 years of research, no pharmaceutical preparation using the multiple emulsion technology exists in the marketplace. It seems that the main reasons are the droplet instability and the uncontrolled release.

Although the release of the encapsulated active substance is complicated, because of the existence of different mechanisms, the multiple emulsion's behavior after application to the skin appears to be relatively simple because it is similar to the behavior observed with simple emulsions.

Microemulsions

Miocroemulsions are effective vehicle systems for dermal as well as for transdermal drug delivery because of their high drug-loading capacity of their colloidal structure. Furthermore, thermodynamic stability and simple preparation process favor them to be considered as vehicles for skin applications.

Several workers have reported studies in which the lipophilicity of the drug has been increased to enhance its solubility in the dispersed oil droplets. In this way, a reservoir of the drug is produced, and a sustained-release effect is achieved as the drug continuously transfers from the oil droplets to the continuous phase to replace drug release from the microemulsion.

Nanoemulsions

Nanoemulsions have been gaining more and more attention in the last few years, mainly as vehicles for the intravenous administration of lipophilic drugs. In the skin, the patents claimed that these systems could penetrate through the skin to a greater extent compared with usual topical compositions. Nanoemulsions are so strongly compressed that they become ultralight and, like vesicular systems, constitute a new form that could prove extremely fruitful for the release of substances.

Vesicles

Vesicles appear to be promising transdermal drug-delivery systems. The major advantages of topical vesicle drug formulations are

- hydrophilic, lipophilic as well as amphiphilic substances can be encapsulated in the vesicles,
- for the lipophilic and amphiphilic drugs the liposomes serve as "organic" solvent, and as a result, higher local drug concentrations can be applied,

- the vesicles can act as depot, releasing their drug content slowly and in a controlled manner,
- systemic effect of a dermal active compound can be reduced, and the systemic effect of a transdermal drug can be increased depending on the vesicle composition,
- the vesicles may serve as a penetration enhancer,
- the vesicles can interact with the skin because of the amphiphilic character of the bilayer,
- liposomes are biocompatible and biodegradable and have a low toxicity and lack antigenicity status as well,
- vesicle formulations are cosmetically accepted.

There are also some disadvantages of vesicles as drug carriers:

- low encapsulation efficiencies for lipophilic or amphiphilic drugs,
- no drug release from the vesicle,
- low-molecular weight drugs can leak out of the vesicle,
- instability of vesicles during shelf life,
- sterilization of liposome formulations.

DERMATOLOGICAL AND COSMETIC USES OF ENCAPSULATION

Microparticles

In recent years, numerous vectors have been proposed and used in topical formulations as drug-carrier vehicles. It has been claimed that these drug vehicles can improve and control the drug release from conventional topical formulations. Although the application of these colloidal particles in dermatology is of great interest, there are few articles about the characteristics of these vehicles for topical formulations, and most of the background is based on different patents.

Miocroparticles can serve as a drug reservoir in skin products. Rolland et al. (62) investigated in vitro and in vivo the role of 50:50 poly(DL-lactic-co-glycolic acid) microspheres as paniculate carriers to improve the therapeutic index of adaptalene. The percutaneous penetration pathway of the microspheres was shown to be dependent on their mean diameter. Thus, after topical application onto hairless rat or human skin, adaptalene-loaded microspheres (5 μm diameter) were specifically targeted to the follicular ducts and did not penetrate via the stratum corneum. A reduction of either the applied dose (0.01%) or the frequency of administration (every day) was shown to give pharmacological results in the animal model comparable to a daily administration of 0.1% free adaptalene-containing aqueous gel.

Egg albumin microspheres of size 222 ± 25 μm, containing vitamin A ($15.7 \pm 0.8\%$), were used to prepare o/w creams. The in vitro and in vivo drug release of a microencapsulated vitamin A cream was studied and compared with a nonmicroencapsulated vitamin A cream. The in vitro study showed that, during the first three hours, the microspheres could remain on the surface of the skin, and as a consequence, were able to prolong the release of vitamin A. The relative bioavailability of the microencapsulated formulation was $78.2 \pm 7.3\%$ (64).

Mizushima (65) reported that lipid microspheres containing prostaglandin Ei (PGEi), delivered preferentially to specific lesion sites, increased local action and prevented systemic side effects. Sakakibara et al. (66) evaluated the potential of topical application of lipid microspheres containing PGEi to treat ischemic ulcers. Nine

of the 10 patients responded to the treatment, and at the sixth month of follow-up six patients had healed ulcers, and recurrence was noted in three patients.

Skin absorption of benzoyl peroxide from a topical lotion containing freely dispersed drug was compared with that from the same lotion in which the drug was entrapped in a controlled-release styrene–divinylbenzene polymer system (Microsponge). The studies done by Wester et al. (67) showed the following: (1) in vivo, less benzoyl peroxide was absorbed through rhesus monkey skin from the polymeric system, (2) reduced skin irritation in cumulative irritancy studies on rabbits and human, and (3) when the experimental formulations were evaluated for antimicrobial activity in vivo, their efficiency was in line with that of conventional products.

A formulation containing 0.1% tretinoin was tested on 360 patients during 12 weeks for antiacne efficacy in a multicenter, double-blind, placebo-controlled study. Compared with placebo, statistically significant greater reductions in inflammatory, noninflammatory, and the total number of lesions was obtained with the entrapped retinoic acid formulation (68). Encapsulation of deet in liposphere microdispersion resulted in improved efficacy and reduced dermal absorption. Deet-containing lipospheres (10%) were effective against mosquitoes for at least 3.5 hours. The deet absorption through skin from these formulations was a third of that from alcoholic solution for the same concentration (69).

Nanoparticles

Although cosmetic applications of nanoparticles proliferate (numerous patents have been granted), publications, studies, or reports on the skin after topical application have been rare. The incorporation of active substances in the nanospheres attempts to modulate the release of the substances in the skin. When nanocapsules are concerned, the active substances are usually of a lipophilic nature, and they can be composed of an oily compound or dispersion. Here again the objective is to control the release of the actives because the molecule is protected. The release profile of the actives depends on the nature of the constituents.

Recently, Lancome launched a cosmetic product containing nanocapsules of vitamin E (Primordiale). They claim that the vitamin is widely distributed throughout the outer layers of the skin in the form of a gradient. The effectiveness of vitamin E protection when it is incorporated into nanoparticles has been shown in vivo. Dingier et al. (70) reported that the incorporation of vitamin E into SLN enhances the stability. The ultrafine particles possess an adhesive effect. This leads to a formation of fine adhesive film on the skin leading to occlusion and subsequent hydration. Hydration of the skin promotes penetration of actives and enhances their cosmetic efficiency. In another publication of the same research group (71), drug release of encapsulated material as well as nonencapsulated material was measured by tape stripping assay. The drug (RMAD 95) was released into the skin at approximately 53%, whereas the control (RMAD 95/isopropanol) was at 31%.

Immobilization of nanoparticles (polyamide) on the skin for prolonged periods of time has been proved feasible (72). It has been shown to be dependent on formulation because particle retention was increased from 40% up to 98% when embedding the particles into an emulsion. Particle size, surface charge, and payload determine the properties of the nanoparticles and their application. Ziilli et al. (73) encapsulated Uvinil T 150 (UV-B filter) into lipid nanoparticles. They observed an almost 100-fold higher affinity of Uvinil T to hair from positively charged particles compared with negatively charged particles. The same group also showed the application of a gel

containing nanoparticles loaded with vitamin A and vitamin E derivatives enhances the skin humidity compared with controls.

In a 1997 patent, De Vringer (74) showed that the size of particles can change the occlusion factor. Lipoid microparticles are greatly inferior to solid lipoid nanoparticles in their occlusive effect, and the addition of solid lipoid microparticles in a cream lowers the cream's occlusivity, whereas the addition of solid lipoid nanoparticles in a cream raises the cream's occlusivity. Nanospheres containing beta carotene and a blend of UV-A and UV-B sun filters were prepared by Olivier-Terras (75). The results clearly show the synergistic effect resulting from the combination of nanospheres and filters. They obtained with this formulation better bioavailability, better efficacy, and lastly a synergy that possesses an inhibitory effect on tyrosinase as a result of the cinnamic nature of the UV-B screening agents.

The effect of poly(methylmethacrylate) and poly(butylcyanoacrylate) nanoparticles on the permeation of methanol and octanol through hairless mouse skin was reported by Cappel and Kreuter (76). Nanoparticles increase the permeability of methanol through hairless mouse skin, and the permeability of lipophilic octanol is either unaffected by nanoparticles or decreases as a function of nanoparticle concentration depending on the lipophilicity of the polymer material. The potential use of nanoparticles as an ophthalmic drug-delivery system has been shown in numerous studies for either hydrophobic or hydrophilic drugs (77–79). Despite the promising in vivo results, many issues must be resolved before an ophthalmic product can be developed using this technology.

Tobio et al. (80) encapsulated a model protein antigen, tetanus toxoid, into PLA-PEG nanoparticles and evaluated the potential of these colloidal carriers for the transport of proteins through the nasal mucous. The results showed that PLA-PEG nanoparticles have a great potential for delivery of proteins, either to the lymphatic system or to the blood circulation, after nasal administration. Regarding the mode of action of nanoparticles, one might hypothesize that they are associated with the skin surface, facilitating drug transport by changing the vehicle/stratum corneum partition coefficient.

Multiple Emulsions

The first commercial use of a w/o/w type multiple emulsion is Unique Moisturizing® by Lancaster, which was marketed in 1991. Cosmetic application of multiple emulsions have been reported in the patents issued for their composition. One example of an application is perfume encapsulated in the internal phase; very small amounts of it are released over a long period of time. The patents show that multiple emulsions are recommended for all kinds of cosmetic applications: sunscreens, makeup removers, cleansers, and nutritive, hydrating, and cooling products. Kamperman and Sallis (81) show that a highly charged small water-soluble molecule such as phosphocitrate can be presented in the form of a liposome or multiple emulsion and be capable of exerting a positive action against dystrophic calcification. In a rat calcergy model, both vehicles effectively reduced the formation of induced subcutaneous calcified plaques at doses for which the phosphocitrate salt alone was inactive. Three types of emulsion (w/o/w, o/w, and w/o) containing a water-soluble molecule (glucose) were obtained with the same formula (82,83). The release of glucose from the o/w emulsion was the fastest, and the w/o emulsion was the slowest, whereas the release obtained from the w/o/w emulsion was intermediate. The w/o/w emulsion showed some tendency

toward steady state during the first 3 to 12 hours, and the flux was found to be 1.7 times greater than that from the w/o emulsion.

In vivo release of 2.5% lidocaine hydrochloride from simple and multiple emulsion systems was compared with that from aqueous and micellar solution, and anesthetic effects such as duration of action and tolerability were also compared. The double emulsions showed a longer duration of action, less eye irritation, and improved efficacy compared with aqueous solutions (44).

Microemulsions

Over the last 15 years, many studies have been performed with the percutaneous absorption of various actives carried by microemulsions. There are numerous cosmetic products in the form of microemulsions. These products range from body care to facial and hair treatments. They include bath oils, body-thinning products, fixatives for hair, hardeners for nails, hydrating products, antiwrinkle products, seborrhea preventive products, and antiaging serums marketed principally in Europe, the United States, and Japan. In biopharmaceutics, microemulsions were used to solubilize drugs and to improve systemic and topical drug availability.

Gasco et al. (84) ascertained concentrations of timolol in aqueous humor after multiple instillation in rabbit eyes. The microemulsion, a solution of the ion pair, and a solution of timolol alone were used. The bioavailability of timolol from the microemulsion and the ion-pair solution was higher than that obtained from timolol alone. Transport of glucose across human cadaver skin was shown (85) using microemulsions containing up to 68% water. A 30-fold enhancement of the glucose transport was achieved. The enhancing effect for drugs contained in microemulsions in comparison to a cream gel formulation consisting of the same components was shown by Ziegnmeyer and Fiihrer (86). The in vitro permeation across skin membranes as well as the in vivo penetration of tetracycline hydrochloride was higher from a microemulsion than from conventional systems. Thus it can be shown that in addition to the composition, the structure of each of the typically applied vehicles may play a dominant role in the process of penetration.

Fevrier (87) has reported in vitro experiments designed to simulate the percutaneous penetration of tyrosine when administered using an o/w microemulsion composed of a betaine derivative as surfactant, benzyl alcohol, hexadecane, and water. The release of radiolabeled tyrosine from this vehicle was compared with that from a liquid-crystal system and an emulsion using a diffusion cell equipped with rat skin. Both the microemulsion and the liquid-crystal formulation enhanced the penetration of tyrosine through the epidermis when compared with the emulsion. However, cutaneous irritation studies showed a strongly irritant effect from the liquid-crystal formulation but none from the microemulsion.

The penetration of the hydrophilic diphenhydramine hydrochloride from a w/o microemulsion into human skin under ex vivo conditions was studied by Schmalfub et al. (88). Modifications of the vehicle components clarified the extent to which it is possible to control the penetration of a hydrophilic drug incorporated in a microemulsion system. A standard microemulsion showed an accumulation of penetrated drug in the dermis, indicating a potential after high absorption rate. Incorporation of cholesterol into the system leads to an even higher penetration rate and a shifting of the concentration profile further toward the epidermis. The addition of oleic acid had no effect.

Wallin et al. (89) showed that high concentrations of lidocaine base included in a microemulsion produced peripheral nerve block of long duration, compared with solutions as a consequence of slow release of lidocaine. The effect of polysorbate 80 concentration on the permeation of propanolol incorporated into micelles of polysorbate 80 in water, o/w microemulsions of isopropyl myristate–polysorbate 80–sorbitol water, and o/w emulsions of isopropyl myristate–polysorbate 80–sorbitan monooleate-water has been investigated by use of an artificial double-layer membrane, composed of a barrier foil and a lipid barrier, in Franz-type diffusion cells (90). For each system, the apparent permeability coefficient of propanolol decreased with increasing polysorbate 80 concentration. Moreover, for a given polysorbate 80 concentration, the apparent permeability coefficient of propanolol increased when the system was changed from emulsion to a microemulsion and then to a solubilized system because of the increasing interfacial area of total disperse phase.

Microemulsions may exert irritative effects, often by their high content of surfactants. It is possible to overcome this problem by the use of physiologically compatible nonionic and polymeric surfactants. The irritation potential of the formulation depends strongly on its structure. Because of an equilibrium between microemulsions and liquid crystals, when brought into contact microemulsions may dissolve skin structures that are organized in liquid crystalline form. Thus, an irritation is produced. Deduced from this, the nature of the system formed during the penetration process and the residue remaining on the skin surface are of importance in this regard. Acute and cumulative tests were performed on human subjects in vivo with lecithin microemulsion gels using a unilamellar soybean lecithin liposome preparation and the solvent isopropyl palmitate as comparison (91). The study showed a very low acute and a low cumulative irritancy potential for the soybean lecithin microemulsion gel. In general, microemulsions undergo structural changes after an application to the skin because of the penetration and/or evaporation of constituents and under occlusion by the uptake of water from the skin surface. The formed substances and their penetration behavior finally influence the effectiveness of the systems for dermal drug transport.

Nanoemulsions

Many formulations of nanoemulsion are available in patents. Recently, Lancome launched a nanoemulsion rich in ceramides, Re-source. The scientific studies, however, are orientated mainly in the parenteral use of these formulations. Amselem and Friedman (92) indicated that the actives incorporated in SMEs (diameter between 100 and 300 nm) can penetrate through the skin to a greater extent compared with the usual topical compositions. Improved efficacy of different steroidal and nonsteroidal anti-inflammatory drugs and local anesthetics has been observed.

Anselem and Zwoznik (93) determined drug penetration through the skin, local tissue (muscle and joint), and plasma levels of ketoprofen and diclofenac after topical administration in SME creams compared with peroral administration. Compared with peroral drugs, SME-diclofenac and SME-ketoprofen showed 60- to 80-fold more drug in muscle tissue, about ninefold more drug in joints, and four- to sixfold less drug in plasma. The improved skin penetrative properties of the solvent-free SME delivery make this topical carrier very promising to achieve increased transcutaneous penetration of lipophilic drugs and site specificity.

Diazepam was formulated in various regular topical creams and SMEs of different composition (94). The different formulations were applied topically on

mice. The efficacy of diazepam applied topically in emulsions strongly depends on the oil droplet size and, to a lesser degree, on the formulation and oil type. The SMEs as vehicles for transdermal delivery of diazepam generate significant systemic activity of the drug as compared with regular creams or ointments. Transdermal delivery of diazepam via SME is effective, and the activity may reach the range of parenteral delivery. A single application of diazepam in SME cream to mice skin provides pronounced transdermal drug delivery and prolonged protective activity up to six hours.

Using a nanoemulsion composed of lanolin, polyethylene glycol ether of lanolin's alcohol, and water (95), the investigators showed the transdermal delivery of a number of pharmaceutically active ingredients (testosterone, ibuprofen, 5-fluorouracil, verapamil hydrochloride, metronidazole, vincristine sulfate, fentanyl citrate) across isolated stratum corneum. The studies indicated that nanoemulsions derived from lanolin and its derivatives are capable of being developed into useful drug-delivery systems.

Vesicles

The effectiveness of vesicles has been investigated by several research groups (Table 5). Liposomes in particular have received considerable attention (103). In several studies the diffusion of a drug was facilitated or achieved certain selectivity into human and nonhuman skin by vesicle encapsulation. Other studies show that the influence of vesicles on drug transport is negligible. The conflicting results can be understood in terms of vesicle characteristics or in terms of protocol of investigation. Special surface characteristics of vesicle hydration and electrostatic forces, in addition to Van der Waals, can govern the short and long range of repulsive or attractive forces between vesicles and biological media.

Table 5 Effect of Vesicles on the Permeation of Drugs Through the Skin

Reference	Year	Drug	Type of vesicle NSV	Results
96	1995	Retinyl palmitate		Augmentation of the retention of hydrophobic substances in stratum corneum
97	1998	Gap junction	Transferosomes	Protein transported across the intact murine skin and processed immunologically
98	1998	Estradiol	Transferosomes	Augmentation of the flux in eight-fold
99	1998	Cu, Zn-superoxide dismutase	Transferosomes	Reduced local inflammation
55	1998	Insulin	Transferosomes	Transported into the body between the intact skin with a bioefficiency of at least 50% of subcutaneous penetration-enhancing effect
100	1994	Estradiol	NSV	
101	1996	Lidocaine	NSV	The flux was not influenced by the encapsulation
102	1998	Levonorgestrel	Niosomes	Penetration-enhancing effect

Abbreviation: NSV Nonionic surfactant vesicles.

The particle sizes, the physical state (liquid or gel) of the bilayers, the number of bilayers, the electrostatic nature of drugs and vesicles, and the stability of the vesicles face to face with biofluids in different ranges of pHs, temperatures, and degrees of dehydration can also play an important role in the phenomenon. An important contribution to the understanding of the interactions between vesicles and human skin was made by Junginger and his group (100,104). They used freeze fracture electron microscopy and small-angle radiograph scattering to study the effects that vesicle formulations have on the stratum corneum. They identified two types of liposome–skin interactions: (1) adsorption and fusion of loaded vesicles on the surface of the skin leading to increased thermodynamic activity and enhanced penetration of lipophilic drugs and (2) interaction of the vesicles within the deeper layers of the stratum corneum promoting impaired barrier function of these strata for the drug. Recent approaches in modulating delivery through the skin are the design of two novel vesicular carriers: the ethosomes and the transferosomes. The ethosomes are soft phospholipid vesicles; their size can be modulated from tens of nanometers to microns. These vesicular systems have been found to be very efficient for enhanced delivery of molecules with different physical–chemical characteristics to/through the skin. They can be modulated to permit enhancement into the skin strata as far as the deep dermis or to facilitate transdermal delivery of lipophilic and hydrophilic molecules (105).

Transferosomes have been shown to be versatile carriers for the local and systemic delivery of various steroids, proteins, and hydrophilic macromolecules (106). The mechanism proposed by the investigator for transferosomes is that they are highly deformable, thus facilitating their rapid penetration through the intercellular lipids of the stratum corneum. The osmotic gradient, caused by the difference in water concentrations between the skin surface and skin interior, has been proposed as the major driving force for transferosome penetration (54).

THE FUTURE OF ENCAPSULATION

What can we expect from encapsulation in the future? Trying to predict what the future will be is not easy. When one addresses future developments in the field of encapsulation, one has to realize that, at present time, application-oriented research is mainly focused to solve problems. If the number of published articles on encapsulation (liposomes, nanoparticles, microparticles, microemulsions, multiple emulsions, and nanoemulsions) under the heading of drug therapy is a reliable indicator of the state of knowledge, then the field has made progress over the last two decades. Between 1975 and 1980, the Medline Data Base registered about 20 articles per year with the term "liposomes" in their title in the domain of drug therapy. This number has grown to over 100 per year. Because many of these publications dealt directly with new experimental data, we must conclude that our experience has expanded dramatically.

The skin has been "in the picture" since Mezei and his collaborators reported around 1980 on their early work on the liposomal delivery of drugs. Through the efforts of the cosmetic industry, liposomal formulations and nanoparticle formulations on the skin have definitively been an economic success. However, many unanswered questions remain. Molecular biology has provided us with tools to identify and build genetic materials that can be used for the treatment of hereditary diseases. Developing a carrier for gene therapy is one of the main challenges that the

encapsulation field faces today. With respect to gene therapy for the skin, both molecular biology and encapsulation technology are in their debut, and much progress may and should be made in the coming years.

Again, what will the future bring us? We have already indicated where, on the basis of our present knowledge, encapsulation in many vectors offer a rational advantage as active carrier systems to the skin. Therefore, efforts should be made to obtain a better understanding concerning the mechanisms of formulations of these systems at the molecular and supramolecular level. This could lead to new formulation processes and could open new prospects in the area of active delivery by means of encapsulated systems. The field will develop in a more useful fashion when appropriate well-controlled biological and percutaneous penetration studies accompany the advances in chemistry.

REFERENCES

1. Kreuter J. Evaluation of nanoparticles as drug-delivery systems. I. Preparation methods. Pharm Acta Helv 1983; 58:196–201.
2. Won R. U.S. Patent 4,690,825, 1987.
3. Bakan J. Microencapsulation using coacervation/phase separation techniques. In: Controlled Release Technologies: Methods, Theory and Applications. Vol. 2. Boca Raton: CRC Press, 1980:83–105.
4. Deasy P. Microencapsulation and Related Drug Processes. New York: Marcel Dekker, 1984.
5. Chang TMS. Artificial Kidney, Artificial Liver and Artificial Cells. New York: Plenum Press, 1978.
6. Thies C. A survey of microencapsulation processes. In: Benita S, ed. Microencapsulation, Methods and Industrial Applications. New York: Marcel Dekker, 1996:1–9.
7. Lim F, Moss RD. Microencapsulation of living cells and tissues. J Pharm Sci 1981; 70:351–356.
8. Matsumoto S, Kabayashi H, Takashima Y. Production of monodispersed capsules. J Microencaps 1986; 3:25–31.
9. Finch CA. Ullman's Encyclopedia of Industrial Chemistry. . Vol. A 16. 5th ed. New York: VCH Publishers, , 1990:575–588.
10. Kondo A. Microcapsule Processing and Technology. New York: Marcel Dekker, 1979.
11. Jacobs IC, Mason NS. Polymeric delivery systems. In: Elnolkaly MA, Piatt DM, Charpentier BA, eds. ACS Symposium Series 520. Washington, DC: American Chemical Society, 1993:1–17.
12. Kreuter J. Nanoparticles—preparation and applications. In: Donbrow M, ed. Microcapsules and Nanoparticles in Medicine and Pharmacy. Boca Raton: CRC Press, 1992: 125–148.
13. Couvreur P, Kante B, Rolland M. Polycyanoacrylate nanocapsules as potential lysosomotric carriers: preparation morphological and sorptive properties. J Pharm Pharmacol 1979; 31:331–338.
14. Al Khoury FN, Roblot-Treupel L, Fessi H. Development of new process for the manufacture of polyisobutylcyanoacrylate nanocapsules. Int J Pharm 1986; 28:125–132.
15. Rollot JM, Couvreur P, Roblot-Treupel L, Puisieux F. Physicochemical and morphological characterization of polyisobutylcyanoacrylate nanocapsules. J Pharm Sci 1986; 75(4):361.
16. Aleony D, Wittcoff H. U.S. Patent 2,899,397, 1959.
17. Cooper W. U.S. Patent 3,009,891, 1961.
18. Judd P. Brit. Patent 1,142,375, 1969.

19. Gurny R, Peppas NA, Harrington DD, Banker GS. Development of biodegradable lattices for controlled release of potent drugs. Drug Dev Ind Pharm 1981; 7:1–12.
20. Rhone-Poulenc Rorer. Fr Patent 2,660,556, 1990.
21. Kramer PA. Albumin microspheres as vehicles for achieving specificity in drug delivery. J Pharm Sci 1974; 63:1646–1652.
22. Fessi H, Devissaguet JP, Puisieux F, Thies C. Fr Patent 8,618,446, 1986.
23. Marty JJ, Oppenheim RC, Speiser PP. Nanoparticles—a new colloidal drug delivery system. Pharm Acta Helv 1978; 53:17–24.
24. Stainmesse S, Fessi H, Devissaguet JP, Puisieux F. 1st add to Fr Patent 8,618,446, 1988.
25. De Vringer T, de Ronde HAG. Preparation and structure of a water-in-oil cream containing lipid nanoparticles. J Pharm Sci 1995; 84(4):466–472.
26. Kim SY, Lee YM, Lee SI. Preparation and evaluation of in vitro stability of lipid nanospheres containing vitamin A and vitamin E for cosmetic application. Proc Int Symp Cont Rel Bioact Mater 1997; 24:483–484.
27. Miiller RH. Paniculate systems for the controlled delivery of active compounds in pharmaceutics and cosmetics. In: Diederichs JE, Miiller RH, eds. Future Strategies for Drug Delivery with Paniculate Systems. Stuttgart: CRC Press, 1998:73–90.
28. Miiller RH, Mehnert W, Dingier A, Runge SA, zur Miihlen A, Freitas C. Solid lipid nanoparticles (SLN™, Lipopearls™). Proc Int Symp Cont Rel Bioact Mater 1997; 24:923–924.
29. Couvreur P, Coarraze G, Devissaguet JP, Puisieux F. Nanoparticles: preparation and characterization. In: Benita S, ed. Microencapsulation, Methods and Industrial Applications. New York: Marcel Dekker, 1996:183–211.
30. Matsumoto S, Kita Y, Yonezava D. An attempt at preparing water-in-oil-in-water multiple phase emulsion. J Colloid Interf Sci 1976; 57:353–361.
31. Matsumoto S, Sherman P. A preliminary study of w/o/w emulsions with a view to possible food applications. J Texture Studies 1981; 12:243–257.
32. Matsumoto S. Development of w/o/w type dispersion during phase inversion of concentrated w/o emulsions. J Colloid Interf Sci 1983; 94:362–368.
33. Kavaliunas DR, Franck SG. Liquid crystal stabilization of multiple emulsion. J Colloid Interf Sci 1978; 66:586–588.
34. Magdassi S, Frenkel M, Garti N. On the factors affecting the yield of preparation and stability of multiple emulsions. J Dispersion Sci Technol 1984; 5:49–59.
35. De Luca M. Les emulsions multiples H/L/H. Obtention, validation, et liberation. These de l'Universite de Paris XI, Paris, 1991.
36. Florence AT, Whitehill D. Some features of breakdown in w/o/w multiple emulsions. J Colloid Interf Sci 1981; 79:243–256.
37. Florence AT, Whitehill D. The formulation and stability of multiple emulsions. Int J Pharm 1982; 11:277–308.
38. Florence AT, Whitehill D. Stability and stabilization of w/o/w multiple emulsions. In: Shah DO, ed. Macro and Micro Emulsions, Theory and Applications. Washington, DC: American Chemical Society, 1985:359–380.
39. Matsumoto S, Inoue T, Khoda M, Ikurak K. Water permeability of oil layers in w/o/w emulsion under osmotic pressure gradients. J Colloid Interf Sci 1980; 77:555–563.
40. Matsumoto S, Koh J, Michura A. Preparation of w/o/w emulsions in edible form on the basis of phase inversion technique. J Dispos Sci Technol 1985; 6:507–521.
41. Frenkel M, Schwartz R, Garti N. Multiple emulsions. I. Stability inversion, apparent and weighed HLB. J Colloid Interf Sci 1983; 94:174–178.
42. Csoka I, Eros I. Stability of multiple emulsions. I. Determination of factors influencing multiple drop breakdown. Int J Pharm 1997; 156:119–123.
43. Opawale FO, Burgess DJ. Influence of interfacial rheological properties of mixed emulsifier films on the stability of w/o/w emulsions. J Pharm Pharmacol 1998; 50:965–973.

44. Garti N, Aserin A. Pharmaceutical emulsions, double emulsions and microemulsions. In: Benita S, ed. Microencapsulation, Methods and Industrial Applications. New York: Marcel Dekker, 1996:412–534.
45. Yu W, Tabosa do Egito ES, Barrat G, Fessi H, Devissaguet JP, Puisieux F. A novel approach to the preparation of injectable emulsions by a spontaneous emulsification process. Int J Pharm 1993; 89:139–146.
46. Hansrani PK, Davis SS, Groves MJ. The preparation and properties of sterile intravenous emulsions. J Parenter Sci Technol 1983; 37:145–150.
47. Yalabik-Kas HS, Erylmaz S, Hincal AA. Formation, stability and toxicity studies of intravenous fat emulsions. STP Pharm 1985; 1:12–19.
48. Washington C, Davis SS. The production of parenteral feeding emulsions by microfluidizer. Int J Pharm 1988; 169–176.
49. Lidgate DM, Fu RC, Fleitman JS. Using a microfluidizer to manufacture parenteral emulsions. Pharm Technol 1990; 14:30–33.
50. Bangham AD, Standish MM, Watkins JC. Diffusion of univalent ions across the lamellae of swollen phospholipids. J Mol Biol 1965; 13:238–252.
51. Gebicki JM, Hicks M. Preparation and properties of vesicle enclosed by fatty acid membranes. Chem Phys Lipids 1975; 16:142–160.
52. Handjani-Vila RM, Ribier A, Rondot B, Valenberghe G. Dispersions of lamellar phases of non-ionic lipids in cosmetic products. Int J Cosmet Sci 1979; 1:303–314.
53. Planas ME, Gonzalez P, Rodriguez L. Non invasive percutaneous induction of topical analgesia by a new type of drug carriers and prolongation of the local pain-insensitivity by analgesic liposomes. Anesth Analg 1992; 95:614–621.
54. Cevc G, Glume G. Lipid vesicles penetrate into the skin owing to the transdermal osmotic gradients and hydration force. Biochem Biophys Acta 1992; 1104:226–232.
55. Cere G, Gebauer D, Stieber J, Schatzlein A, Blume G. Ultraflexible vesicles, transfersomes, have an extremely low pore penetration resistance and transport therapeutic amounts of insulin across the intact mammalian skin. Biochem Biophys Acta 1998; 1368:201–215.
56. Baillie AJ, Florence AT, Hume LR, Muirhead GT, Rogerson A. The preparation and properties of niosome non-ionic surfactant vesicles. J Pharm Pharmacol 1985; 37:863–868.
57. Van Hal DA, Bowstra JA, Junginger HE. Preparation and characterization of new dermal dosage form for antipsoriatic drug, dithranol, based on non ionic surfactant vesicles. Eur J Pharm Biopharm 1992; 38:47.
58. Hofland HEJ, Bowstra JA, Ponec M, Bodde HE, Spies F, Verhoef JC, Junginger HE. Interactions of non-ionic surfactant vesicles with cultured keratinocytes and human skin in vitro. J Control Release 1991; 16:155–168.
59. Hofland HEJ, Bowstra JA, Verhoef JC, Buckton G, Chowdry BZ, Ponec M, Junginger HE. Safety aspects of non-ionic surfactant vesicles. A toxicity study related to the physiochemical characteristics of non ionic surfactants. J Pharmacol 1992; 44:287–294.
60. Carafa M, Al Haique F, Coviello T, Murtas E, Riccieri FM, Lucania G, Torrisi MR. Preparation and properties of new unilamellar non-ionic/ionic surfactant vesicles. Int J Pharm 1998; 160:51–59.
61. Kiwada H, Nimura H, Fujisali Y, Yamada S, Kato Y. Application of synthetic alkyl glycoside vesicles as drug carriers. (1) Preparation and physical properties. Chem Pharm Bull 1985; 33:753–759.
62. Rolland A, Wagner N, Chatelus A, Shroot B, Schaefer H. Site-specific drug delivery to pilosebaceous structures using polymeric microspheres. Pharm Res 1993; 10(12): 1738–1744.
63. Ammoury N, Dubrasquet M, Fessi H. Indomethacin-loaded poly(d,l-lactide) nanocapsules: protection from gastrointestinal ulcerations and anti-inflammatory activity evaluation in rats. Clin Mater 1993; 13:121–127.
64. Torrado S, Torrado JJ, Cadorniga R. Topical application of albumin microspheres containing vitamin A. Drug release and availability. Int J Pharm 1992; 86:147–152.

65. Mizushima Y. Lipid microspheres as novel drug carriers. Drug Exp Clin Res 1985; 11: 595–600.
66. Sakakibara Y, Jikuya T, Mitsui T. Application of lipid microspheres containing prostaglandin Ei ointment to peripheral ischemic ulcers. Dermatology 1997; 195:252–257.
67. Wester RC, Rajesh P, Nacht S, Leyden J, Melendres J, Maibach HI. Controlled release of benzoyl peroxide from a porous microsphere polymeric system can reduce topical irritancy. J Am Acad Dermatol 1991; 24(5):720–726.
68. Embil K, Natch S. The Microsponge® delivery system (MDS): a topical delivery system with reduced irritancy incorporating multiple triggering mechanisms for the release of actives. J Microencaps 1996; 13(5):575–588.
69. Domb AJ, Marlinsky A, Maniar M, Teomim L. Insect repellent formulations 6f *n*,*n*- diethyl-*m*-toluamide (deet) in a liposphere system: efficacy of skin uptake. J Am Mosquito Control Ass 1995; 11(1):29–34.
70. Dingier A, Hildebrand G, Niehus H, Miiller RH. Cosmetic anti-aging formulation based on vitamin E-loaded solid lipid nanoparticles. Proc Int Symp Cont Rel Bioact Mater 1998; 25:433–434.
71. Miiller RH, Dingier A, Hildebrand G, Gohla S. Development of cosmetic products based on solid lipid nanoparticles (SLN). Proc Int Symp Cont Rel Bioact Mater 1998; 25:238–239.
72. Deniau N, Ponchel G, Bonze F, Meybeck A, Duchene D. Immobilization of paniculate systems on the skin by the mean of emulsions. Drug Dev Ind Pharm 1993; 19(13): 1521–1540.
73. Ziilli F, Suter F, Birman M. Cationic nanoparticles: a new system for the delivery of lipophilic UV-filters to hair. Drug Cosmet Ind 1996; 4:46–48.
74. De Vringer T. U.S. Patent 5,667,800, 1997.
75. Olivier-Terras J. U.S. Patent 5,554,374, 1996.
76. Cappel MJ, Kreuter J. Effect of nanoparticles on transdermal drug delivery. J Microencaps 1991; 8(3):369–374.
77. Calvo P, Vila-Jato JL, Alonso MJ. Comparative in vitro evaluation of several colloidal systems, nanoparticles, nanocapsules, and nanoemulsions, as ocular drug carriers. J Pharm Sci 1996; 85(5):530–536.
78. Calvo P, Alonso MJ, Vila-Jato JL, Robinson JR. Improved ocular bioavailability of indo-methacin by novel ocular drug carriers. J Pharm Pharmacol 1996; 48:1147–1152.
79. Heussler LM, Sirbart D, Hoffman M, Maincent P. Poly(e-caprolactone) nanocapsules in carteolol ophthalmic delivery. Pharm Res 1993; 10(3):386–390.
80. Tobio M, Greef R, Sanchez A, Langer R, Alonso MJ. Stealth PLA-PEG nanoparticles as protein carriers for nasal administration. Pharm Res 1998; 15(2):270–275.
81. Kamperman H, Sallis JD. Liposome and multiple emulsion formulations augment the anticalcifying efficacy of phosphocitrate in a cutaneous calcergy model. J Pharm Pharmacol 1995; 47:802–807.
82. Ferreira LAM, Seiller M, Grossiord JL, Marty JP, Wepierre J. Vehicle influence on in vitro release of glucose: w/o, w/o/w and o/w systems compared. J Control Release 1995; 33:349–356.
83. Ferreira LAM, Doucet J, Seiller M, Grossiord JL, Marty JP, Wepierre J. In vitro percutaneous absorption of metronidazole and glucose: comparison of o/w, w/o/w and o/w systems. Int J Pharm 1995; 121:169–179.
84. Gasco MR, Gallarate M, Trotta M, Bauchiero L, Gremmo E, Chiappero O. Microemulsions as topical delivery vehicles: ocular administration of timolol. J Pharm Biomech Anal 1989; 7(4):433–434.
85. Osborne DW, Ward AJI, O'Neill KJ. Microemulsions as topical drug delivery vehicles: in-vitro transdermal studies of a hydrophilic model drug. J Pharm Pharmacol 1991; 43: 451–455.
86. Ziegnmeyer J, Fiihrer C. Mikroemulsionen als topishe arzneiform. Acta Pharm Technol 1980; 26(4):273–275.

87. Fevrier F. Formulation de microemulsion cosmetiques. Nouv Dermatol 1991; 10:84–87.
88. Schmalfub U, Neubert R, Wohlrab W. Modification of drug penetration into human skin using microemulsions. J Control Release 1997; 46:279–285.
89. Wallin R, Dyhre H, Bjorkman S, Fyge A, Engstrom S, Renck H. Prolongation of lidocaine induced regional anaesthesia by a slow release microemulsion formulation. Proc Int Symp Cont Rel Bioact Mater 1997; 24:555–556.
90. Kristis G, Niopas I. A study on the in vitro percutaneous absorption of propanolol from dispersed systems. J Pharm Pharmacol 1998; 50:413–418.
91. Dreher F, Walde P, Luisi PL, Eisner P. Human skin irritation studies of a lecithin microemulsion gel and of lecithin liposomes. Skin Pharmacol 1996; 9:124–129.
92. Amselem S, Friedman D. U.S. Patent 5,662,932, 1997.
93. Amselem S, Zwoznik E. Enhanced skin penetration and site specificity of ketoprofen and diclorofenac formulated in submicron emulsion topical creams. Pharm Sci 1998; (suppl):65.
94. Schwarz JS, Weisspapir MR, Friedman DL. Enhanced transdermal delivery of diazepam by submicron emulsion (SME) creams. Pharm Res 1995; 12(5):687–692.
95. Flockart IR, Steel I, Kitchen G. Nanoemulsions derived from lanolin show promising drug delivery properties. J Pharm Pharmacol 1998; 50(suppl):141.
96. Guenin EP, Zatz J. Skin permeation of retinyl palmitate from vesicles. J Soc Cosmet Chem 1995; 46:261–270.
97. Paul A, Cevc G, Bachawat BK. Transdermal immunisation with an integral membrane component, gap junction protein, by means of ultradeformable drug carriers, transfersomes. Vaccine 1998; 16(2/3):188–195.
98. El Maghraby GMM, Williams AC, Barry BW. Optimization of deformable vesicles for epidermal delivery of oestradiol. J Pharmacol 1998; 50(suppl):146.
99. Simes SI, Marins MBF, Cruz MEM, Cevc G. Anti-inflammatory effects of Cu, Zn-superoxide dismutase in liposomes, transfersomes or micelles in the acute murine ear edema model. Perspec Percutan Penetration 1997; 5b:50.
100. Hofiand HEJ, Van der Geest R, Bodde HE, Junginger HE, Bowstra JA. Estradiol permeation from non-ionic surfactant vesicles through human stratum corneum in vitro. Pharm Res 1994; 11(5):659–664.
101. Van Hal DA, Jeremiasse E, de Vringer T, Junginger HE, Bowstra JA. Encapsulation of lidocaine base and hydrochloride into non-ionic surfactant vesicles (NSVs) and diffusion through stratum corneum in vitro. Eur J Pharm Sci 1996; 4:147–157.
102. Vora B, Khopade AJ, Jain NK. Proniosome based transdermal delivery of levanorgestrel for effective contraception. J Control Release 1998; 54:149–165.
103. Bowstra JA, Junginger HE. Non-ionic surfactant vesicles (niosomes) for oral and transdermal administration of drugs. In: Puisieux F, Couvreur P, Dellatre J, Devissaguet JP, eds. Liposomes, New Systems and New Trends in Their Applications. 1995:101–121.
104. Hofland HEJ, Bowstra JA, Bodde HE, Spies F, Junginger HE. Interactions between liposomes and human stratum corneum in vitro: freeze fracture electron microscopic visualization and small angle X-ray scattering studies. Br J Dermatol 1995; 132:853–866.
105. Touitou E, Alkabes M, Dayan N, Eliaz N. Ethosomes: novel vesicular carriers for enhanced skin delivery. Pharm Res 1997; 14(11)(suppl):305.
106. Cevc G. Material transport across permeability barriers by means of lipid vesicles. In: Powsky RL, ed. Handbook of Physics of Biological Systems. Vol. I. Elsevier Science, 1995:441–466 (Chap. 9).

10
Encapsulation Using Porous Microspheres

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INTRODUCTION

Encapsulation can be broadly defined as the formation of small, spherical particles that incorporate an active agent. The first commercial application of encapsulation was by the National Cash Register Company, who developed an improved copying paper using two dyes that were coated with clay. When these capsules were ruptured by the application of pressure, a colored imprint was produced. This successful application triggered other uses in agriculture, pharmaceuticals, oil industries, food industries, and consumer products (1).

Because such spherical particles are very small, usually about 20 μm, the process of forming such particles is referred to as microencapsulation. However, we need to distinguish between microcapsules and microspheres. Microcapsules have a core containing the active agent surrounded by a membrane, whereas microspheres are solid particles that contain an active agent homogeneously dispersed within the solid matrix. Microspheres can be either solid or porous. These three types are shown schematically in Figure 1.

Release of agents incorporated into microcapsules can occur abruptly either as in the National Cash Register Company product or as the "scratch and sniff" product manufactured by the 3M Company, where the outer membrane is ruptured by the application of pressure or the rupture can occur in a controlled manner by the diffusion of the active agent from the core through the outer rate-limiting membrane.

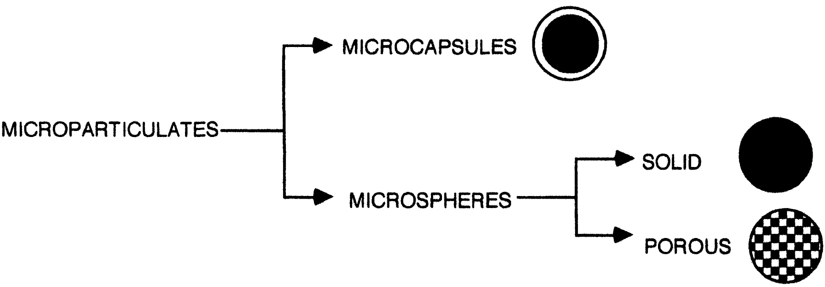

Figure 1 Schematic representation of various microparticulates.

In the latter case, if the thermodynamic activity of the drug in the core remains constant and the drug is removed rapidly from the aqueous environment surrounding the microcapsule, constant release kinetics, referred to as zero order, are obtained. No such products have been applied to the cosmetics and cosmeceutical field, but have been extensively investigated in controlled-release applications, particularly in contraception (2) and narcotic addiction (3).

Agents incorporated into microspheres are released by kinetics that are typical of matrix systems and follow t^2 kinetics as predicted by the Higuchi equation (4). Thus, the initial release rate is rapid and declines as the thickness of the drug-depleted layer increases. Studies of release kinetics from biodegradable porous microspheres indicate that release kinetics similar to those noted for matrix-type microspheres are obtained (5).

Other than liposomes, which are discussed in chapter 17, only one type of microparticle has found important applications in cosmetics and skincare technology and these are porous microspheres. This chapter will discuss the application of porous microspheres in cosmetics and skincare applications.

POROUS MICROSPHERES

Preparation

A special kind of porous microsphere is a patented (6,7), highly cross-linked polymer sphere having a size that can vary from about 3 to 3000 μm. The porous spheres are produced by an aqueous suspension of polymerization of monomer pairs consisting of a vinyl and a divinyl monomer, e.g., methyl methacrylate (the vinyl monomer) and ethylene glycol dimethacrylate (the divinyl monomer), or styrene and divinylbenzene. The divinyl monomer functions as a cross-linker, and because it is used in concentrations as high as 50% to 60%, the copolymer is a very highly cross-linked material. As a consequence of their chemical structure and high cross-link density, the microspheres are totally inert and do not degrade in the body, nor do they dissolve or swell, when exposed to any organic solvent. They have been found to be stable between pH 1 and 11, and at temperatures as high as 135°C.

To prepare the copolymer, the vinyl and divinyl monomers, initiator, suspending agent (emulsifier), and a porogen that produces the porous structure, are dispersed in water and the copolymerization started by thermally activating the initiator. The porogen must be miscible with the monomers and function as a precipitant for the polymer. Polymer particle size is controlled by the size of the suspended monomer droplets, which in turn is a function of the nature and amount of the suspending agent and the shear induced by the stirring process. When all variables are carefully controlled, a uniform batch of particles having the desired size and the desired porosity can be obtained. Typically, the surface area of such porous microspheres can be varied between 20 and 500 m^2/g, and the pore volume can be varied from 0.1 to 3.4 cm/g.

A scanning electron micrograph of a porous microsphere magnified 5000 times is shown in Figure 2. A view of the interior, in this case magnified 6000 times and obtained by freeze–fracture, is shown in Figure 3. As can be seen, the internal structure comprises small polymer particles enclosed in a porous membrane. The porosity of the microspheres is attributable to the interstitial volumes between the polymer particles, and because the membrane that surrounds the solid polymer particles is porous, the interstitial volume is open to the outside.

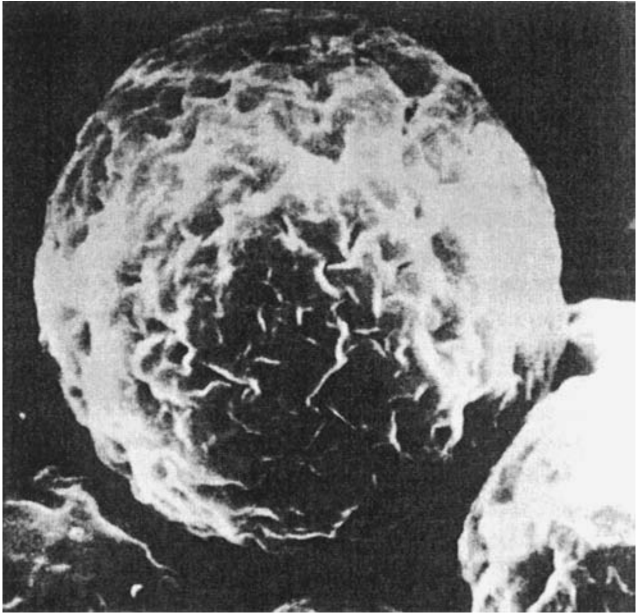

Figure 2 Electron scanning micrograph of a porous microsphere. Magnification 5000×.

Loading of Active Agents

These can be incorporated by two different procedures. In one procedure, referred to as the one-step procedure, the active agent functions as the porogen and is incorporated during the polymerization process. However, this method has some limitations

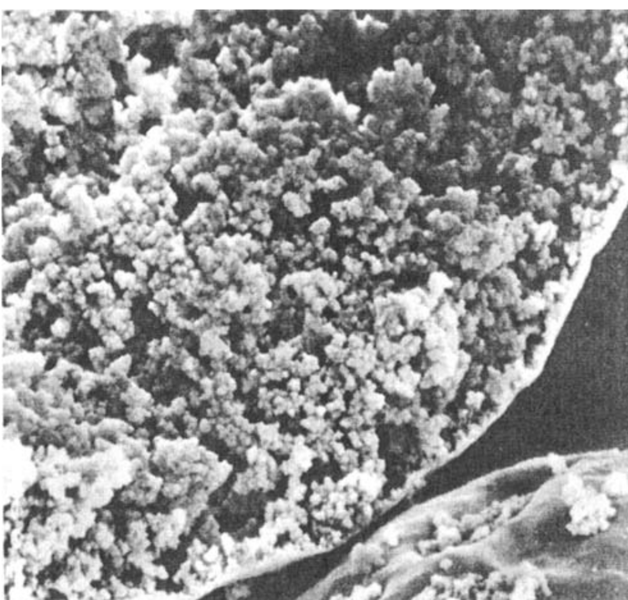

Figure 3 Freeze-fracture micrograph of a single porous microsphere. Magnification 6000×.

because the active agent has to satisfy the requirements of a porogen, it must be stable toward free radicals generated during the copolymerization process, and it must not inhibit the copolymerization process. For this reason, a procedure where porous microspheres are produced first, and subsequently loaded with the active agent, is more generally applicable. Such a process is known as the two-step procedure.

Loading is achieved by stirring empty porous microspheres in a solution of the active agent, which diffuses into the microsphere particles. The solvent is then evaporated to obtain microspheres with the active agent loaded within the pores. If the agent is soluble in the polymer, some may partition into the matrix. Should a high loading be desired, or if the active agent is only sparingly soluble in the solvent, the process can be repeated any number of times. Clearly, when using such a procedure some of the active agent will also be found on the outside of the microspheres particles.

The incorporation of an active agent into these microspheres can be investigated by environmental scanning electron microscopy (ESEM). This method has the advantage over conventional scanning electron microscopy (SEM) in that no metallic coating is required and samples can be analyzed at ambient pressures in water vapor. Samples are sprinkled lightly onto a metallic stub, 1 cm in diameter, bearing conductive double-sided adhesive tape, and then analyzed using a Phillips XL30 ESEM FEG instrument operated with greater than 99% relative humidity (Davies M, and Patel N, private communication). Using this procedure, a good visualization of the microspheres and any free drug, if present, can be achieved.

Such a visualization method is important because loading efficiency depends on the nature of the active agent, primarily, its solubility and the partition coefficient between the microspheres and the solvent used in the entrapment procedure. Both lipophilic and hydrophilic materials can be loaded into such microspheres, and range from water to petrolatum to silicone oil. Extensive studies have shown that the active agent is not bound to the microspheres and can be completely extracted.

Release of Active Agents

Although porous microspheres can function in a limited way as a sustained-release delivery vehicle, they are best viewed as a reservoir. However, the combination of microspheres with incorporated active agents dispersed in a vehicle can function as a controlled-release device if a vehicle in which the drug is only poorly soluble is chosen. When such a formulation is applied to the skin, only the amount of the drug that is dissolved in the vehicle is presented to the skin. Then, as the drug diffuses from the vehicle into the skin, the saturation concentration of the drug in the vehicle is maintained by diffusion of the drug from the microspheres into the vehicle. This process is shown schematically in Figure 4.

APPLICATIONS

Porous microspheres have been used in two major applications. One application takes advantage of the high porosity of the microspheres to entrap liquid materials, such as silicone oil, to convert a liquid into a free-flowing powder. This allows significant formulation flexibility, and a babywipe product has been developed where silicone in porous microspheres has been formulated in an aqueous medium.

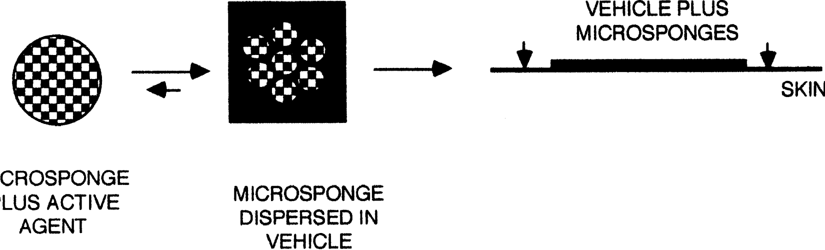

Figure 4 Schematic representation of controlled release of active agent from porous microspheres dispersed in a vehicle.

In the other application, microspheres with incorporated active agents are dispersed in a suitable vehicle for topical applications. As already discussed, when active agents that are normally skin irritants are used, and a vehicle in which the active agent is only poorly soluble is chosen, a significant reduction of irritation, when compared with ordinary formulation, is noted. Such a reduction in irritancy will be illustrated with two products, one incorporating benzoyl peroxide and the other incorporating *trans*-retinoic acid (RA).

Benzoyl Peroxide

Benzoyl peroxide () is clinically effective in acne, primarily because of its bactericidal activity against *Proprionibacterium acnes* and possibly because of its mild keratolytic effects (8–10). The main site of pharmacological action is the pilosebaceous canal (11). BPO penetrates through the follicular opening, probably by dissol-

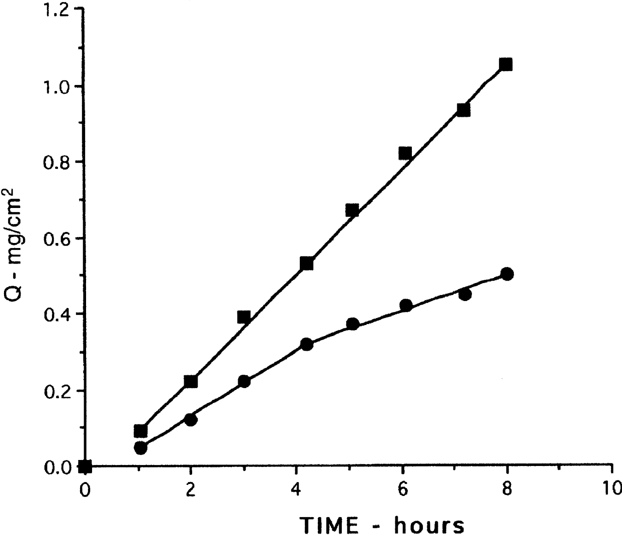

Figure 5 Release of BPO dispersed in vehicle (■) and BPO entrapped in porous microspheres and dispersed in vehicle (●). Results are the average of two determinations. Formulations applied to silastic membrane. Receiving fluid 1:1 mixture of water and acetone. *Source*: From Ref. 14.

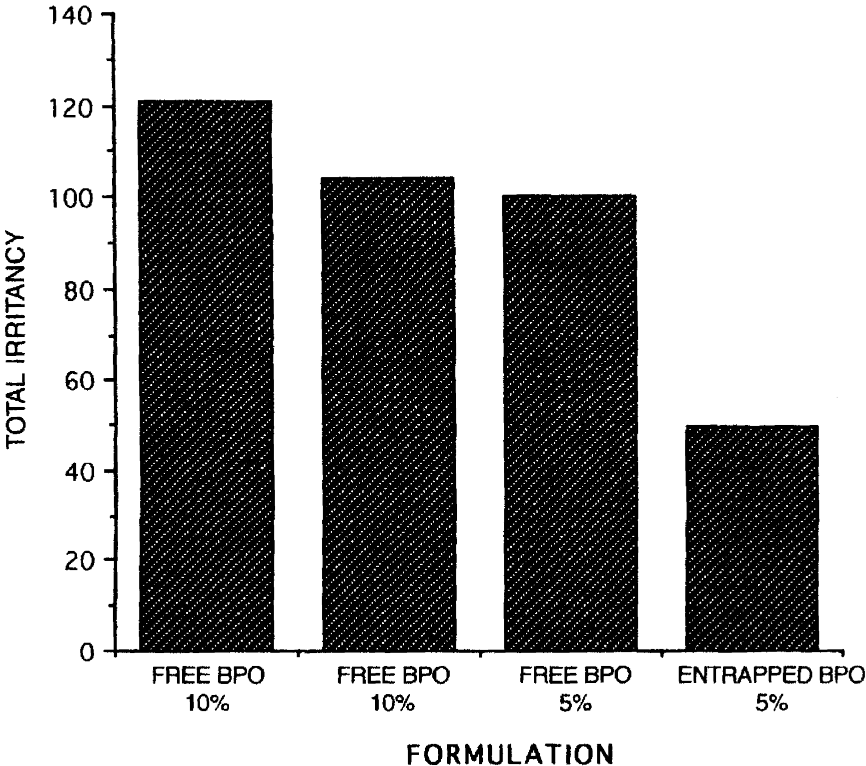

Figure 6 Fourteen-day cumulative irritancy test on BPO formulations in human volunteers comparing three commercial products containing BPO dispersed in a vehicle and one commercial formulation containing BPO entrapped in porous microspheres at BPO concentrations shown.

ving into sebaceous lipids, and then exerts its antimicrobial activity (12). Skin irritation is a common side effect and a dose relation seems to exist between efficacy and irritation (13). Thus, a controlled-release formulation would clearly be advantageous.

In vitro release kinetics was determined by applying formulations to silastic membranes mounted in static diffusion cells, and by using excised human skin. Release of BPO from two formulations applied to a silastic membrane, one incorporating free BPO and one incorporating BPO entrapped in porous microspheres as shown in Figure 5. Initial release of BPO dispersed in the vehicle shows good-linearity, but with further release would decline as expected for t^2 kinetics. The calculated flux for the initial release is 0.09 mg/cm^2/h. The release of BPO entrapped in the porous microspheres shows a discontinuity. Initial flux is about 0.1 mg/cm^2/h, very close to the release from BPO dispersed in the vehicle, followed by a slower release with a flux of 0.04 mg/cm^2/h. These data indicate that not all BPO have been entrapped in the porous microspheres, and that the formulation contains some free BPO. Initial release is attributable to release of the free BPO, followed by the release of entrapped BPO.

The topical irritancy of a BPO controlled-release formulation has been determined in rabbits, in rhesus monkeys, and in human volunteers (14) using

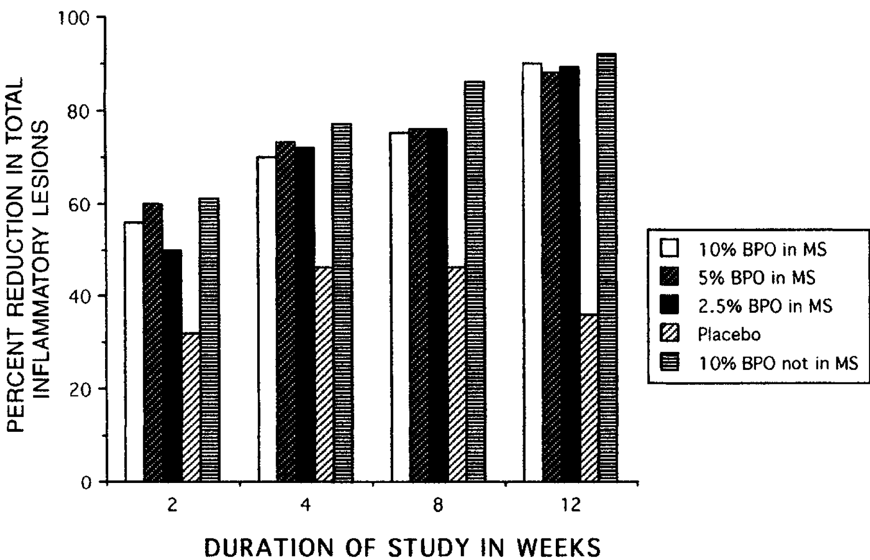

Figure 7 Percent reduction in total inflammatory lesions (papules/pustules) in human volunteers at 2, 4, 8, and 12 weeks, using the formulations shown.

formulations with BPO dispersed in a vehicle and BPO entrapped in porous microspheres dispersed in a vehicle.

The Cumulative 14-day irritancy scores in human volunteers are shown in Figure 6 and Table 1. In this study involving 29 patients, total irritancy of four commercial products, three containing free BPO and one containing entrapped BPO at the BPO concentrations shown, were compared. Clearly, the entrapped BPO product is significantly less irritating. A 12-week human trial, comparing the efficacy of entrapped BPO formulations at various concentrations, a placebo formulation and a free BPO formulation have also been carried out. The total reduction of inflammatory lesions shown in Figure 7 and the total reduction of noninflammatory lesions shown in Figure 8 clearly indicate that the entrapped BPO are as efficacious as

Table 1 A 14-day Cumulative Irritancy in Human Volunteers

Formulation	% Total subjects with postive response	Cumulative response index[a]
2.5% BPO		
Commercial product	36	1.04 (1)
Entrapped BPO	12	0.24 (2)
Vehicle	0	0.0 (3)
10% BPO		
Commercial product	52	2.59 (4)
Entrapped BPO	24	1.64 (5)
Vehicle	0	0.0 (6)

[a]Duncan's Multiple Range tests showed significant difference ($p < 0.05$) between (1) and (2), (1) and (3), (4) and (6), (5) and (6), but no significant difference ($p > 0.05$) between (2) and (3).
Source: Ref. 14.

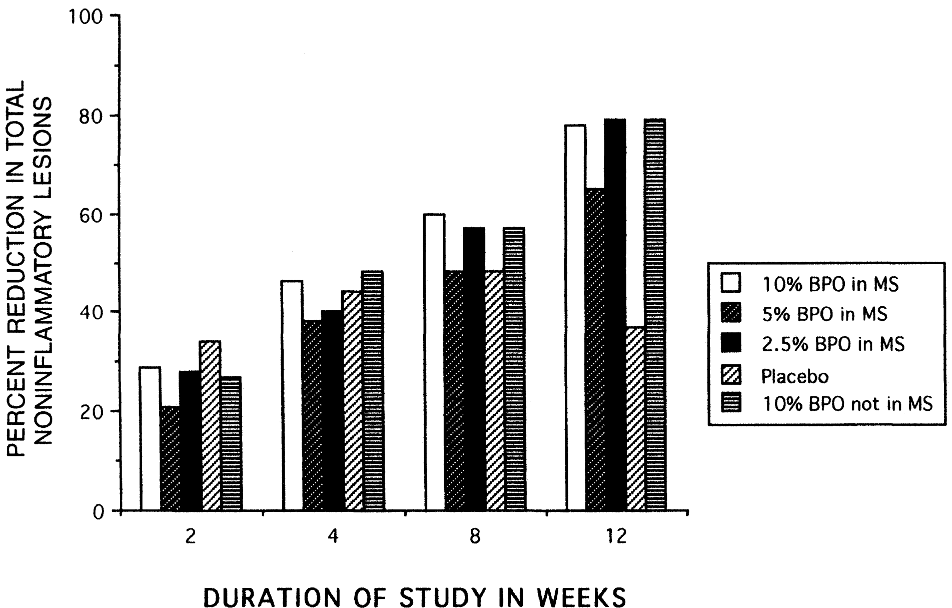

Figure 8 Percent reduction in total noninflammatory lesions (open and closed comedones) in human volunteers at 2, 4, 8, and 12 weeks, using the formulations shown.

the free BPO. These results support evidence also obtained independently, that most, if not all, BPO entrapped in the porous microspheres are released.

Retinoic Acid

All *trans*-RA are highly effective topical treatment for acne vulgaris. However, cutaneous irritation reduces patient compliance, and thus clinical effectiveness. A gel formulation with 0.1% RA entrapped in a porous microsphere has been developed and a single-center, double-blind, positive-controlled, randomized Phase I study was carried out. The formulation with entrapped RA was designated as 0.1% tretinoin microsphere gel (TMG), and the one with free RA was designated 0.1% RA cream. The study formulation was assigned to be applied to the right side of a subject's face on a randomized basis and the alternate formulation to the left side of the face. The dose for each formulation was 0.1 g, which was applied to the cheek areas once daily for up to 14 days. The subjects were evaluated daily by an expert grader for dryness and erythema. Results of subjects' self-assessment are shown in Table 2 and in Figure 9. Clearly, a formulation with RA entrapped in porous

Table 2 Subject Self-Assessment

	0.1% TMG[a]	0.1% RA cream	*p* value
Number who prefer	23	2	
Preference score	1.88	0.10	0.0002

[a]TMG is Retin-A® Micro cream 0.1%. *t* Preference score perceived as less burning and/or stinging graded on a scale from 0 (no difference) to 4 (maximal difference).

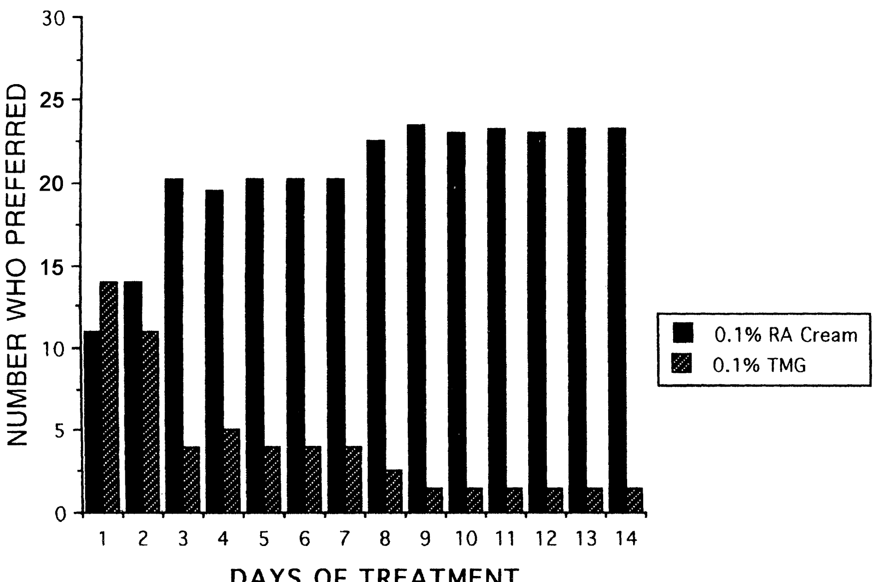

Figure 9 Daily self-assessment of preference for mildness. Single-center, double-blind, randomized, half-face study comprising 25 adult Caucasian women selected for having sensitive skin. 0.1% TMG is retinoic acid entrapped in porous microspheres and 0.1% RA cream in a commercial formulation. 0.1% TMG and 0.1% RA cream applied to corresponding side of subject's face, once a day for up to 14 days by a blinded technician.

microspheres resulted in a statistically significant preference for the TMG formulation, which was perceived as causing less burning and stinging. In an independent, controlled-multicenter trial, this TMG formulation has also proven effective for the treatment of acne and is now commercially available.

CONCLUSIONS

Porous microspheres are highly cross-linked and highly porous copolymers, which have found extensive use in the skincare arena. The nature of the polymer allows the loading of a wide range of chemical entities with subsequent release dependent on the vehicle into which the porous microspheres have been dispersed. This polymer has found widespread acceptance as a means of reducing irritation without decreasing efficacy when used appropriately.

REFERENCES

1. Luzzi LA. Microencapsulation. J Pharm Sci 1970; 59:1367–1376.
2. Beck LR, Tice TR. Poly(lactic) and poly(lactic acid-co-glycolic acid) contraceptive delivery systems. In: Mishell DR, ed. Long-Acting Steroid Contraception. New York: Raven Press, 1983:175–199.
3. Nuwayser ES, Gay MH, DeRoo DJ, Blackovich PD. Sustained release injectable naltrexone microcapsules. Proc Intern Symp Control Rel Bioact Mater 1988; 15:201–202.

4. Higuchi T. Rates of release of medicaments from ointment bases containing drugs in suspension. J Pharm Sci 1961; 50:874–875.
5. Sato T, Kanke M, Schroeder HG, DeLuca P. Porous biodegradable microspheres for controlled drug delivery. I. Assessment of processing conditions and solvent removal techniques. Pharm Res 1988; 5:21–30.
6. Won R. Method for delivering an active ingredient by controlled time release utilizing a novel delivery vehicle which can be prepared by process utilizing the active ingredient as a porogen. U.S. Patent 4,690,825. September 1, 1987.
7. Won R. Two step method for preparation of controlled release formulations. U.S. Patent 5,145,675, September 8, 1992.
8. Nacht S. Comparative activity of benzoyl peroxide and hexachlorophene. In vivo studies against Proprionibacterium acnes in humans. Arch Dermatol 1983; 119:577–579.
9. Fulton JE, Bradley S. The choice of vitamin A, erythromycin and benzoyl peroxide for the topical treatment of acne. Cutis 1976; 17:560–564.
10. Kligman AM, Leyden JJ, Stewart R. New uses of benzoyl peroxide: a broad spectrum antimicrobial agent. Int J Dermatol 1977; 16:413–417.
11. Nacht S. Methods to assess the transepidermal and intrafollicular penetration of anti-acne agents. In: Proceedings of the 1980 Research and Scientific Development Conference, New York, 1981:88–91.
12. Leyden JJ. Topical antibiotics and topical antimcrobial agents in acne therapy. In: Julin LA, Rossman H, Strauss H, eds. Symposium in Lund, Uppsala, Sweden: Uppland GrafiskerAB. 1980;151–164.
13. Fulton JE, Bradley S. Studies on the mechanism of action of topical benzoyl peroxide in acne vulgaris. J Cuta Pathol 1974; 1:191–194.
14. Wester RC, Patel R, Nacht S, Leyden J, Melendres J, Maibach H. Controlled release of benzoyl peroxide from a porous microsphere polymeric system can reduce topical irritancy. J Am Acad Dermatol 1991; 24:720–726.

11 Liposomes

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INTRODUCTION

Publications about and patents on liposomes, along with their different chemical components, preparation, and use in skincare products, have often been reviewed (1–4). The reviews do not need any additional comments. Of interest are general questions, such as why liposomes should be used in cosmetics, what functionalities are expected from them, and what advantages they do provide compared with alternative formulations.

The properties of the widely used main component of liposomes, phosphatidylcholine, play a key role in answering these questions. Other compounds such as niotensides and ceramides, which are naturally predestined for the preparation of liposomes, are less important today. Niotensides do not offer superior claims, and ceramides are not available in sufficient quantities and qualities at affordable prices.

PHOSPHATIDYLCHOLINE

In the horny layer, which is the barrier against external materials, phospholipids and phosphatidylcholine, in particular, play a minor role. The lipid bilayers contain only traces of phospholipids, and the main components are free fatty acids, cholesterol, triglycerides, hydrocarbons, and ceramides. But deeper in the living part of the epidermis, phosphatidylcholine is usually found as the most important constituent of all biological membranes, especially of plasma cell membranes. Over and above that, phosphatidylcholine is the source of phosphocholine to transform ceramides into sphingomyelins. In this context, phosphatidylcholine stands for living tissues, whereas the increase of ceramides in the cells means that their death by apoptosis is imminent (Fig. 1).

Phosphatidylcholine of both human and vegetable origin shows a fatty acid composition, which is dominated by unsaturated fatty acids. The fatty acid content of soy phosphatidylcholine, which is readily available and mostly used in cosmetic formulas, is characterized by a proportion of linoleic acid up to 70% of the total fatty acids. Consequently, soy phosphatidylcholine has a very low phase-transition temperature of below 0°C in water-containing systems. This may be the reason behind its ability to fluidize the lipid bilayers of the horny layer, which can be determined by measuring the increase in the transepidermal water loss (TEWL) after application

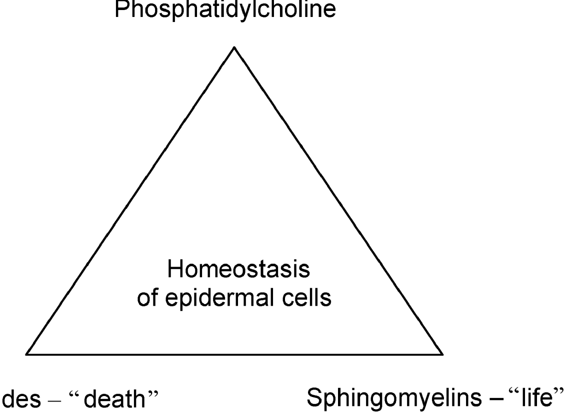

Figure 1 Homeostasis of epidermal cells.

for a short while. The slight increase of TEWL coincides with the penetration of phosphatidylcholine and active agents, which are co-formulated with phosphatidylcholine. Because of its high content of linoleic acid and penetration capability, soy phosphatidylcholine delivers linoleic acid very effectively into the skin, resulting in antiacne properties (5).

By adhering very strongly to surfaces containing proteins like keratin, phosphatidylcholine shows conditioning and softening effects, which have been known from the beginning of skincare products' development. So, for example, shampoos were formulated in the past very often with egg yolk to soften hair and prevent it from becoming charged with static electricity. Egg yolk is very rich in lecithin. The main compound of egg lecithin is phosphatidylcholine.

In a given mixture it is not relevant in which form the phosphatidylcholine is incorporated. However, when phosphatidylcholine is formulated, it is practically inevitable that bilayer-containing systems like liposomes will occur, because this is the most natural form of the material. For example, phosphatidylcholine swollen by water transforms spontaneously into liposomes when "disturbed" by little amounts of salts or water-soluble organic compounds like urea. In contrast, it has been known for a long time that horny layer pretreated by phosphatidylcholine can be penetrated much more easily by nonencapsulated materials. So liposomes are not really needed to turn out the functionalities of phosphatidylcholine, but they are very convenient, because the handling of pure phosphatidylcholine requires a lot of experience and sometimes patience as well.

Phosphatidylcholine is known as a penetration enhancer; this enhancing property is usually associated with liposomes. Liposomes are the vesicles said to transport cosmetic agents better into the horny layer. That is true and, moreover, the conditioning effect causes the horny layer to become a depot for these agents. Measurements of systemically active pharmaceuticals revealed that an increase of penetration is not synonymous with an increase of permeation. Actually, permeation of active agents is often slowed by phosphatidylcholine in such a way that a high permeation peak in the beginning of the application is prevented. Instead, a more continuous permeation takes place outside the horny layer depot into the living part of the body over a longer period of time. This property makes phosphatidylcholine and liposomes very attractive for the application of vitamins, provitamins, and other substances influencing the regenerating ability of the living epidermis.

$$
\begin{array}{l}
CH_2\text{-}O\text{-}CO\text{-}(CH_2)_n\text{-}CH_3 \\
| \\
CH\text{-}O\text{-}CO\text{-}(CH_2)_n\text{-}CH_3 \\
| \\
CH_2\text{-}O\text{-}\overset{-}{P}O_2\text{-}O\text{-}CH_2\text{-}CH_2\text{-}\overset{+}{N}(CH_3)_3
\end{array}
$$

Figure 2 Hydrogenated phosphatidylcholine ($n = 14,16$).

In contrast, liposomes consisting of unsaturated phosphatidylcholine have to be used with caution in barrier creams, because they do not strengthen the natural barrier function of the skin with an exception of its indirect effect of supporting the formation of ceramide I. Ceramide I is known for containing linoleic acid and for being one of the most important barrier-activating substances. Instead of unsaturated phosphatidylcholine, a fully hydrogenated phosphatidylcholine (Fig. 2) should be selected for products designed for skin protection.

Hydrogenated phosphatidylcholine stabilizes the normal TEWL similarly to ceramides when the horny layer is attacked by hydrophilic or lipophilic chemicals (6). Table 1 shows a summary of the properties of unsaturated and hydrogenated phosphatidylcholine. Hydrogenated phosphatidylcholine is synonymous with hydrogenated soy phosphatidylcholine, which contains mainly stearic and palmitic acids and semisynthetic compounds like dipalmitoylphosphatidylcholine (DPPC) and distearoylphosphatidylcholine (DSPC). Because of their special properties, it can make sense to combine unsaturated with saturated phosphatidylcholine in one and the same cosmetic or dermatological product.

LIPOSOMES

Liposomes are spherical vesicles, whose membranes consist of one (unilamellar) or more (oligolamellar, multilamellar) bilayers of phosphatidylcholine. Sometimes,

Table 1 Properties of Phosphatidylcholines

Parameter	Soy phosphatidylcholine	Hydrogenated soy phosphatidylcholine
Skin barrier function	Penetration enhancement; conditioning the horny layer	Stabilizing the barrier function; conditioning the horny layer
Barrier compatibility	Yes, slightly enhancing TEWL	Yes, stabilizing normal TEWL
Phase-transition temperature (aqueous system) (°C)	Below 0	50–60
Fatty acid composition	Unsaturated fatty acids: predominantly linoleic acid and oleic acid	Saturated fatty acids: predominantly stearic and palmitic acids
Solubility	Soluble in triglycerides, alcohols, and water (lamellar)	Insoluble in triglycerides, alcohols, and water
Toxicity	CIR-report (7); anticomedogenic	CIR-report (7)
Dispersing ability	Hydrophilic and lipophilic compounds	Hydrophilic and lipophilic compounds

Abbreviations: TEWL, transepidermal water loss; CIR, cosmetic ingredient review.

especially in patents, reference is made not about liposomes but about "vesicles with an internal aqueous phase." The vesicles can differ in size (diameter about 15–3500 nm) and shape (single and fused particles). At a given chemical composition, these parameters strongly depend on the process of preparation. Very often the preparations are metastable. That means the state of free enthalpy is not in an equilibrium with the environment. As a result the vesicles change their lamellarity, size, size distribution, and shape with time. For example, small vesicles tend to form larger ones and large vesicles smaller ones. Fortunately, this is mostly not critical for quality, because the properties of the phosphatidylcholine, which the vesicles are based on, remain unchanged as a rule. Nevertheless the stability seems to be the best in a range of about 100 to 300 nm. That is the case of pure aqueous dispersions of highly enriched (80–100%) soy phosphatidylcholine.

In a complete formulation together with further ingredients, other influences like compatibility, concentration of salts, amphiphilics, and lipophilics play an important role. Therefore, it is often very difficult to prove the existence of liposomes, for example, in a gel phase or a creamy matrix. However, this is more a marketing problem than a problem of effectiveness of the formulation. Today, we can assume that the effectiveness of phosphatidylcholine is based more on the total chemical composition of the cosmetic product and less on the existence or nonexistence of the added liposomes. This may seem curious, but it is in fact the reality.

Of course, formulations are very effective, in particular, when consisting of pure liposomal dispersions bearing lipophilic additives in the membrane spheres and/or hydrophilics in the internal and external aqueous phases within the range of their bearing capacity. In this respect, there has been an intensive search to increase the encapsulation capacity of liposomes for lipids because consumers are used to applying lipid-rich creams. Efforts were made to add an emulsifier to the liposomal dispersions to stabilize higher amounts of lipids. Formulators now know that the compatibility of liposomes for emulsifiers is generally limited. In contrast, additional emulsifiers have a weakening effect on the barrier affinity of phosphatidylcholine. They cause the phosphatidylcholine and the lipids to be more easily removed from the skin while washing. In this respect, there is only one rational consideration: to make use of nanoparticles ("nanoemulsions," "nanodispersions") consisting of phosphatidylcholine and lipids instead of liposomes. Nanoparticles are a consequence of the observation that oil droplets can fuse with liposomes, when the capacity of bilayers for lipids is exhausted (8). Further increasing the lipid/phosphatidylcholine ratio and using high-pressure homogenizers lead to nanoparticles. Nanoparticles consist of emulsion-like oil droplets surrounded by a monolayer of phosphatidylcholine. The advantage of nanoparticles is that they allow formulations to tolerate more lipids and remain stable. Also, additional emulsifiers are not needed.

Liposomal dispersions based on unsaturated phosphatidylcholine lack in stability against oxidation. Like linoleic esters and linoleic glycerides, these dispersions have to be stabilized by antioxidants. By thinking naturally, a complex of vitamins C and E (respectively, their derivatives like acetates and palmitates) can be used with success. In some cases, phosphatidylcholine and urea seem to stabilize each other (9,10). Moreover, agents that are able to mask traces of radical-forming ions of heavy metals, like iron, can be added. Such additives are chelators like citrates, phosphonates, or ethylene diamine tetraacetic acid (EDTA). Alternatively, the unsaturated phosphatidylcholine can be substituted by a saturated one like DPPC or hydrogenated soy phosphatidylcholine, which should be favored for its price. Because of the higher phase-transition temperature, liposomal dispersions based

on hydrogenated material are more sophisticated in their preparation and are reserved for pharmacological applications as a rule. An interesting new development in the field of cosmetic compositions with hydrogenated soy phosphatidylcholine is the derma membrane structure (DMS) technology (11). DMS stands for cream bases (technically the creams are gels) containing hydrogenated soy phosphatidylcholine, sebum-compatible medium chain triglycerides (MCT), shea butter, and squalane. In addition to liposomal dispersions and nanoparticles, DMS is a third way to formulate phosphatidylcholine with hydrophilic and lipophilic compounds free of further emulsifiers (Fig. 3). DMS is water- and sweat-proof and therefore suitable for skin protection and sun creams without using silicones or mineral oil additives. It can easily be transformed into other final products by stirring at room temperature together with liquid lipids and/or aqueous phases.

As previously mentioned, DMS is predestined for skin protection, but by addition of nanoparticles and/or liposomal dispersions, it can easily be enriched by unsaturated phosphatidylcholine containing esterified linoleic acid. The resulting products are creamy, stable, and anticomedogenic. The effects of pure DMS basic creams on skin moisturizing, smoothing, and tightening are still significant several days after finishing the application.

Liposomes, nanoparticles, and DMS have to be preserved. This may be a problem, because phosphatidylcholine (lecithin) inactivates most of the conventional preservatives (12). In contrast, preservatives should not be penetrated into the skin to prevent irritation and sensitization. Therefore, glycols like propyleneglycol, glycerol, butyleneglycol, pentyleneglycol, hexyleneglycol, sorbitol, and their mixtures are the compounds of choice. In contrast to ethanol, which can also be applied up to a certain extent, these polyols show a moisturizing effect at the same time.

One of the reasons for substituting phosphatidylcholine by polyglycerols and other synthetic derivatives at the beginning of the liposomal developments was its hydrolytic instability in aqueous preparations for longer periods of time and at higher temperatures. In fact phosphatidylcholine, like other glycerides, is attacked by water to form lysophosphatidylcholine and free fatty acids. But the cleavage of the glyceride bond occurs mainly at a pH >7; so formulations in the range of pH 5.5 to 7 are sufficiently stable for most purposes. It is possible that hydrolysis depends on the amount of additional surface-active compounds. This is another reason to use liposomal dispersions without additional emulsifiers.

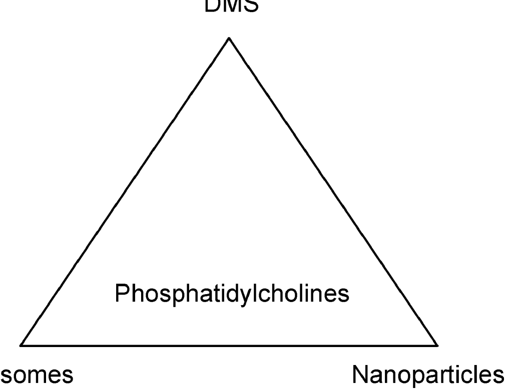

Figure 3 Formulations with phosphatidylcholine free of further emulsifiers. *Abbreviation*: DMS, derma membrane structure.

AVAILABILITY

As previously mentioned, liposomal dispersions are a very comfortable method to use to work phosphatidylcholine into cosmetic formulations to obtain its superior spectrum of multifunctionality. Preliposomal fluid phases with up to 20% phosphatidylcholine and more are commercially available (13). Also, there are references to the use of instant liposomes in combination with carbohydrates as dry powders (1). An interesting consideration is bath oils, which form in situ liposomal dispersions free of additional emulsifiers (14). These compositions are based on mixtures of phosphatidylcholine, triglycerides, and alcohol. By pouring the mixtures into water, liposomal particles are spontaneously formed. These particles strongly tend to adhere to the skin surface and benefit dehydrated and sensible skin. Numerous other methods for preparing liposomes have been described (1).

APPLICATIONS

Today, most of the experts working in the field of liposomal dispersions agree that liposomes do not penetrate as intact vesicles into the skin or permeate through the skin. Liposomes are believed to be deformed and transformed into fragments as a rule. Therefore size, shape, and lamallarity are not so relevant for the application, but for the chemical composition of the total formulation.

The multifunctional properties of phosphatidylcholines lead to a number of different applications. So, formulations with unsaturated phosphatidylcholine are preferred to support skin regeneration, antiaging, acne prevention, and penetrating other active agents, like vitamins and their derivatives, into the skin. Formulations with hydrogenated phosphatidylcholine may be used for skin and sun protection, but it should be emphasized that in this respect nanoparticles and DMS are still more convenient. The main components of choice to prepare "natural" formulations, which are compatible with horny layer, sebum constituents, and their functions, are illustrated in Figure 4. Details about the role of mineral salts can be found in Ref. 15.

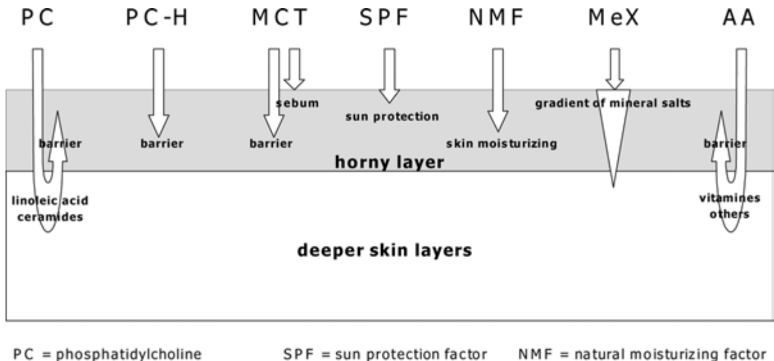

Figure 4 Main components of "natural" formulations.

Table 2 PC-Containing Formulations

Parameter	Liposomes	Nanoparticles	DMS	Conventional emulsions
PC	++	+	Used as additive	(+)
PC hydrogenated	Rarely used	+	++	Rarely used
Lipophilic ingredients	Limited	+	+	+
Hydrophilic ingredients	+	+	+	+
Amphiphilic ingredients	Limited	Limited	+	+
Auxiliary compounds	As few as possible	As few as possible	Rarely used	++
Preparation (usual)	Usual and high pressure homogenizers	High pressure homogenizer	High pressure homogenizer	Phase conversion method
Physical stability	(+)	+	++	++
Chemical stability	Depending on pH	Depending on pH	Depending on pH	Depending on pH
Preservation	Glycols, ethanol	Glycols, ethanol	Glycols, ethanol	Conventional preservatives
Penetration	++	+	+	(+)
Skin protection	– (unsaturated PC)	(+)	++	(+)
Convenient particle size (nm)	100–300	50–200	Not detectable	Usual droplets
Typical cosmetic applications	Antiaging, regeneration	Lotions for sensible skin	Skin protection, sun protection	Versatile
Prevention of skin diseases	++ (e.g., acne)	++ (e.g., encapsulated primrose oil: neurodermatitis)	++ (e.g., neurodermatitis, dehydrated skin)	(+)

Abbreviations: DMS, derma membrane structure; PC, phosphatidylcholine.

THE FUTURE OF LIPOSOMAL PREPARATIONS

Liposomal dispersions have proved not only to be innovative and effective cosmetic ingredients, but also to be a very convenient form to work with phosphatidylcholine. In dermatology, they will be used with success for preventing and treating several skin diseases. Complementary formulations are established where liposomal dispersions come up against limiting factors. Table 2 shows liposomal and complementary formulations in a direct comparison.

Generally, members of the membrane family, like liposomes, nanoparticles, and DMS, are more compatible with the skin structure than usually applied conventional emulsions. "Compatible" means that formulations do not disturb the integrity of the skin lipid bilayers and are not washed out when the skin is cleaned. In the sense of modern strategies of cosmetics, these formulations get by with a minimum of auxiliary compounds, which put only a strain on the skin. Moreover, compatibility means embedding lipids and hydrophilic agents in the horny layer and being in line with the natural situation.

Remarkably, phosphatidylcholine need not be applied in high concentrations, because experience shows that formulations are stable at lower amounts. Also, there is a cumulative effect in the horny layer with repeated application of phosphatidylcholine. In many cases liposomes, nanoparticles, and DMS are compatible with each other in a sense that they can be used as a modular system. So these formulations are believed to still have a great future in cosmetic science.

How far new findings about the importance of the choline moiety of phosphatidylcholine (16) will impact skincare research and development cannot be estimated today.

REFERENCES

1. Lasic DD. Liposomes and niosomes. In: Rieger MM, Rhein LD, eds. Surfactants in Cosmetics. 2nd ed. New York: Marcel Dekker, 1997:263–283.
2. Wendel A. Lecithins, phospholipids, liposomes in cosmetics, dermatology and in washing and cleansing preparations. Augsburg: Verlag fuer chemische Industrie, 1994.
3. Wendel A. Lecithins, phospholipids, liposomes in cosmetics, dermatology and in washing and cleansing preparations. Part II. Augsburg: Verlag fuer chemische Industrie, 1997.
4. Braun-Falco O, Korting HC, Maibach HI, eds. Liposome Dermatics. Berlin: Springer-Verlag, 1992.
5. Ghyczy M, Nissen H-P, Biltz H. The treatment of acne vulgaris by phosphatidylcholine from soybeans, with a high content of linoleic acid. J Appl Cosmetol 1996; 14:137–145.
6. Lautenschlaeger H. Kuehlschmierstoffe und Hautschutz — neue Perspektiven. Mineraloeltechnik 1998; (5):1–16.
7. Cosmetic Ingredient Review. Lecithin and Hydrogenated Lecithin. Washington: The Cosmetic, Toiletry, and Fragrance Association, 1996.
8. Lautenschlaeger H. Liposomes in dermatological preparations. Part II. Cosmet Toilet 1990; 105(7):63–72.
9. Nippon Surfactant Kogyo KK, Japanese Patent 199104364104 (1992).
10. Lautenschlaeger, German Patent 4021082 (1990).
11. Kutz G. Galenische Charakterisierung ausgewaehlter Hautpflegeprodukte. Pharmazeutische Zeitung 1997; 142(45):4015–4019.
12. Wallhaeusser KH. Praxis der Sterilisation, Desinfektion — Konservierung. 5th ed. Stuttgart: Georg Thieme Verlag, 1995:43, 394.

13. Roeding J. Properties and characterisation of pre-liposome systems. In: Braun-Falco O, Korting HC, Maibach HI, eds. Liposome Dermatics. Berlin: Springer-Verlag, 1992: 110–117.
14. Lautenschlaeger, German Patent 4021083 (1990).
15. Feingold KR. Permeability barrier homoeostasis: its biochemical basis and regulation. Cosmet Toilet 1997; 112(7):49–59.
16. Blusztajn JK. Choline, a vital amine. Science 1998; 281:794–795.

12
Novel Liposomes

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INTRODUCTION

Liposomes are microscopic lipid vesicles. They are found naturally in human milk (1) and are dynamic entities on many levels. Liposomes provide a valuable tool for dermatological formulation strategy because of their various proven benefits (2–5). Topically applied drug products using liposome technology were first commercialized in Switzerland when Janssen-Cilag launched Peveryl Lipogel in 1988. A decade later the first topical liposome drug product introduced in the U.S. was ELA-Max®, developed and commercialized by Ferndale Laboratories, Inc. Other pharmaceutical companies have used liposome encapsulation in topical preparations for functional purposes including, improving percutaneous penetration, increasing therapeutic efficacy, and targeting specific sites in the skin. Table 1 shows examples of several dermatological products that have become commercial successes.

Since their introduction into cosmetics by Christian Dior in Capture® (1987), liposomes have become a staple for some and an enigma for others. Although barriers to use have been both spurious and real, ranging from cost, difficulty of formulation, scale-up problems, stability, and a general misunderstanding of the basis for which liposomes have the most value, they have made their way into numerous skin care products over the years (6) and, by all counts, have passed the tests of time and utility.

Conventional liposomes, as we have seen in previous Chapters, are made from purified phospholipids, mostly DPPC alone or DPPC plus cholesterol or another sterol. The ability of these amphiphilic lipids to orient themselves in the polar solvent in which they are suspended, usually water, into bilayer sheets sets the stage for liposome formation. The resultant colloidal aggregate, with the hydrophilic heads positioned outward and the hydrophobic tails inward, is the bilayer which can be assembled into vesicles, but only with the addition of outside energy (Fig. 1). Just as it takes energy from your hands and fingers to physically bend a page of this book into a tube, energy is required to bend these bilayer sheets of phospholipids into vesicles. Contrary to popular belief these lipids do not form vesicles spontaneously, and there is confusion between self-forming and spontaneous vesiculation (7). Phospholipid bilayers will self-close to form liposomes only when outside energy is added to

Table 1

Product name	Drug	Use
Peveryl Lipogel®	Econazole	Mycotic fungal infections
Hametum Crème	Hamamelis	Inflammation
Heparplus EmGel	Heparin	Anticoagulation
L.M.X.	Lidocaine	Dermal anesthesia
Miltrex	Miltefosine	Breast cancer tumors with cutaneous metastases

the system. The source of external energy is process energy in the form of sonication, high-speed vortexing, high-pressure homogenization, and high shear fluid processing.

Important derivatives of conventional liposomes are nonionic surfactant liposomes and nonphospholipid vesicular systems, both are sometimes referred to as synthetic surfactant vesicles. These vesicles are composed of single- and double-chain synthetic surfactants with nonionic polar heads. They form bilayer vesicles with the structure of the lipophilic tail controlling membrane properties. French and Japanese inventions employing these types of technologies surfaced in the patent literature as early as 1980 (8) and were incorporated into cosmetic products by the end of the decade. Niosomes were introduced into Lancôme and other L'Oreal brands in the late 1980s, and others followed.

Because topically applied products containing cosmetically active substances have evolved into "cosmeceuticals" or "cosmetics" a shift in formulation goals from an emphasis on organoleptics to skillfully coupling organoleptics and therapeutics has become the priority. It is well known that working with phospholipids requires a great deal of processing and formulation skill to produce a finished product that is pleasing and elegant, yet maintains liposome integrity and ingredient encapsulation.

COLLOIDAL ORGANIZATION

A fundamental property of lipids is their geometric shape. This spatial configuration is determined by the packing parameter, *P*, of the lipid. Packing parameters are a relative measure of a given lipid, determined by the size of the polar head and the length of the lipid hydrocarbon chain (9). Although empirical, determining the

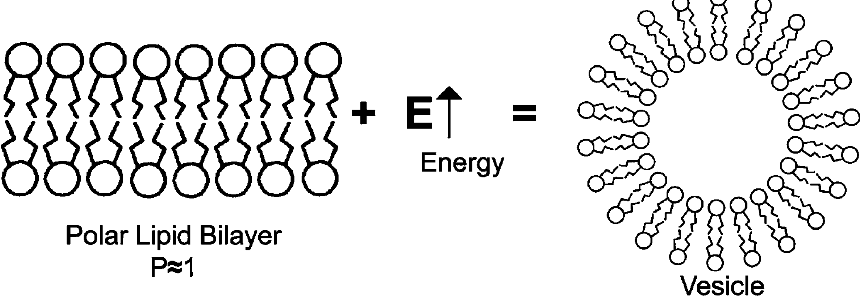

Figure 1 Polar lipid bilayer and resultant vesicle.

packing parameter, P, gives us a very useful guide to the aggregate shape of amphiphilic lipids. P is defined as

$$vP = al,$$

where v is the volume of the molecule, a is its polar head group area, and l is the length of the hydrocarbon chain. For single-chain lipids, detergents, and surfactants the polar head is proportionately larger than the nonpolar tail and $P < 0.75$; so they organize into micelles. When $P \sim 1$ the lipid is best suited for forming liposomes and when $P > 1$, polar heads are smaller relative to the nonpolar chain and inverse micelles form. Some interesting lipids used in cosmetic formulating, their packing parameter range, and their shapes and ultimate organization are shown in Figure 2.

To form a bilayer, lipid head groups and hydrocarbon chains must organize themselves so that the radius of curvature results in a vesicle. If the hydrocarbon chains are too small relative to the head group, the radius of curvature will be too large and micelles will form. If the hydrocarbon chains are too large relative to the head groups, the radius of curvature will be of the opposite sign and an inverse micelle will form.

NEW SPONTANEOUS, THERMODYNAMICALLY STABLE (STS) LIPOSOMES

Most, if not all, known liposome suspensions are not thermodynamically stable (10). Instead, the liposomes are kinetically trapped into higher energy states by the energy used in their formation. Again, energy may be provided as heat, sonication extrusion, or homogenization. Because every high-energy state tries to lower its free energy, known liposome formulations experience problems with aggregation, fusion, sedimentation, and leakage of liposome associate material. However, when well prepared the liposomes and final product can remain stable for years provided they are stored properly and not able to be chemically degraded. A thermodynamically stable liposome suspension, which could avoid some of these difficult to overcome problems, is desirable. Notwithstanding their success, and the progress that has been made in expanding the use of conventional liposomes, there are various disadvantages that prompted the search for novel liposome-forming lipids.

A group of novel lipids, which have fundamental properties that allow thermodynamically stable liposomes to form easily and cost effectively is presented in this chapter. These lipids have the general structure in Figure 3.

This structure has similarities to phospholipids but the fundamental differences are essential to the formation of thermodynamically stable liposomes. The essential hydrophilic head group is a PEG chain, which can range in size anywhere from 8 to 45 subunits, or 300 Daltons to 5000 Daltons, $n = 8$ to 45. PEGylated lipids have been incorporated into the membranes of some liposome compositions (i.e., Stealth®) made from phospholipid liposomes which have a phosphate head group (11). Glycerol, a three-carbon chain, provides the backbone, and the two hydrocarbon chains R1 and R2 can vary in length, $C = 8$ to 25. The size of the PEG head relative to the length of the hydrophilic chain is the fundamental property that allows liposome formation when added to water with the addition of little or no energy. When mixed with water the liposomes form spontaneously and remain in the lowest free energy state.

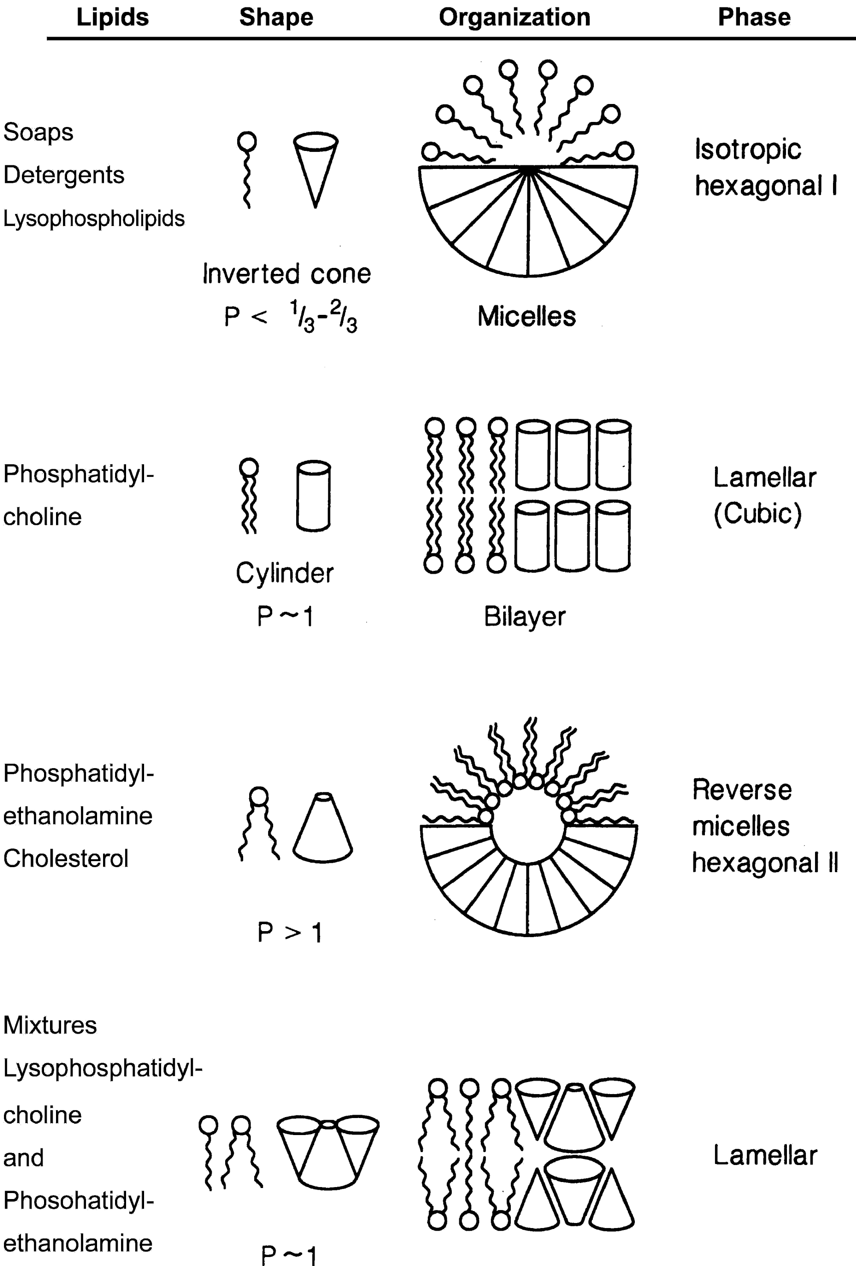

Figure 2 A model of geometric packing of various amphiphilic lipids into colloidal aggregates. *Source*: From Ref. 9.

By using these lipid molecules little or no energy is needed when mixing the lipid and an aqueous solution to form liposomes. When mixed with water the lipid molecules disperse and self assemble into vesicles as the system settles into its natural low free energy state. The resultant suspension is a thermodynamically stable system containing multilamellar lipid vesicles. Figure 4 depicts the formation of vesicles when a lipid is mixed with water at the melting point of the lipid.

$O(CH_2CH_2)nOH$

O O

=O =O

R_1 R_2

Figure 3 General structure of lipids.

Working with these lipids in cosmetic formulations is uncomplicated and has made liposome preparations on both small- and large-scale considerably less labor intensive. Because the melting temperatures of these lipids are low and heating them to temperatures about 75 to 80°C is not denaturing, they display excellent versatility in a variety of dermatological formulations. No organic solvents are necessary to dissolve the lipid prior to incorporating them into a formulation, and simple mixing with conventional processing equipment is all that is required for liposome formation. Table 2 gives examples of the lipids that have been used in cosmetic formulations.

The liposomes formed using these lipids have a size range of 750 to 1500 Å with a mean bilayer thickness of around 40 Å (12). They appear uniform in size upon microscopic analysis and tend to be perfectly round as opposed to oval or anomalous shaped that some liposomes assume. The most likely explanation for this is the purity and uniformity of these synthetic lipids.

The ideal way to view liposomes in a cosmetic laboratory and to validate the presence or absence of liposomes in a formulation is through an optical light microscope with polarized light. Figure 5 shows a photograph of several multilamellar liposomes formed from PEG-12 GDO and cholesterol. These are the perfectly round, white objects with a demarcating cross over the center. They are robust and have been stable under stressors: 40°C and 50°C for 90 days and during freeze thaw cycling. In cosmetic formulations these liposomes are able to withstand higher ionic surfactant levels and a broader pH range, from 2.5 to 9.18 compared to conventional liposomes. Additionally PEG-12 GDO and PEG-12 GDM have been used as an ingredient solubilizer with great success, dissolving difficult ingredients including cholesterol. Because of their thermodynamic stability they will not fuse, aggregate, or destabilize trying to get to the lowest energy state.

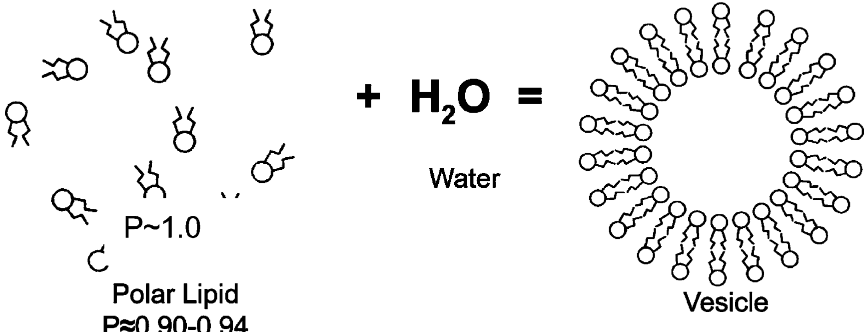

Figure 4 Vesicle formation when lipid is mixed with water at the melting point of the lipid.

Table 2

Lipid	Melting point	Spontaneous liposomes at melting temperature
PEG-12 glyceryl dioleate	Fluid @ 25°C	Yes
PEG-12 glyceryl dimyristate	Fluid @ 25°C	Yes
PEG-23 glyceryl palmitate	31.2°C	Yes
PEG-12 glyceryl disterate	40.0°C	Yes
PEG-23 glyceryl disterate	39.8°C	Yes

Using these nonphospholipid lipids in a cosmetic preparation makes the product less susceptible to microbial over growth owing to the absence of phosphate, an essential microbial nutrient. Contributing to their overall stability, the absence of this type of head group prevents oxidation of the amino group in the polar head of some phospholipids that causes a fishy smell (13).

Toxicity of phospholipids has been reviewed thoroughly (14), and they are essentially nontoxic. An extensive test on the toxicity of the lipids in Table 2 was recently conducted and concluded that they are nonirritating and nontoxic (15). It has also been observed that these lipids are well tolerated with all skin phenotypes in many skin care products.

UTILITY

Cosmetic products incorporating conventional liposomes have become ubiquitous. At times, the practical significance of liposomes has been a secondary consideration. Taking precedence is the label claim and marketing propaganda that publicizes the presence of liposomes. The microscopic system of these vesicles with the entrapped freight in a cosmetically elegant vehicle has a consumer benefit far beyond the limits of cosmetic labeling, however, meaningful therapeutic amounts of liposomes relative to the ingredient concentrations need to be present to achieve payback.

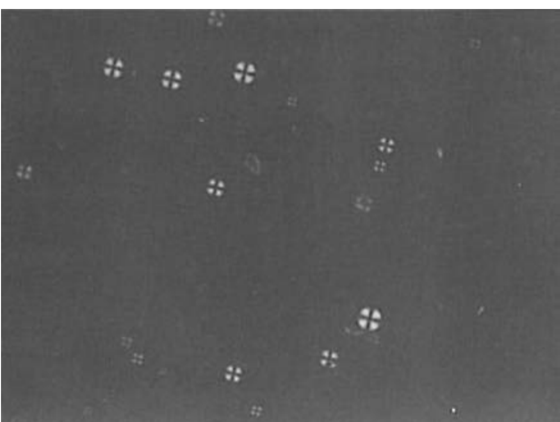

Figure 5 Multilamellar liposomes formed from PEG-12 GDO and cholesterol.

ENCAPSULATION EFFICIENCY

The tremendous advantages of liposomes in dermatological products are improved ingredient solubilization, microencapsulation of the ingredient for improved ingredient stability in the cosmetic system, enhanced skin penetration resulting in longer residence time of the ingredient in the skin, sustained release of the ingredient for prolonged effect, and the beneficial effects of the lipid molecule itself by providing lipid material to the skin to improve barrier function and moisture (16).

In considering the constructs of a cosmetic formulation the art of commingling liquids, solids, oils, water, crystals, cellulosics, and pastes, among others, is required. As mentioned earlier in this Chapter, although the therapeutic value of a particular product has become a focus, overlooking the effects of touch, feel, and smell of a product would be a mistake. Therefore, great time and effort are expended on consideration of the concentrations of each ingredient.

The potency of cosmetically active ingredients is low, and therefore the usage levels are in the 1% to 5% range, generally. In addition, most formulas contain more than one ingredient that has benefits to the skin. To achieve liposomal encapsulation of the proposed active ingredients the physicochemical properties of the materials must be known. Lipophilic ingredients have a higher encapsulation rate and are entrapped in the acyl chains of the lipid whereas hydrophilic compounds reside in the water layer and have a lower degree of encapsulation. The type of lipid, the compounds being encapsulated, the charge of the lipid and the active, all contribute to the encapsulation efficiency of the active ingredients. A simple way to empirically begin formulation and potentially achieve maximum encapsulation is to start with a lipid-to-active molar ratio of 2:1. For hydrophilic ingredients a higher relative amount may be necessary (Fig. 6) (17).

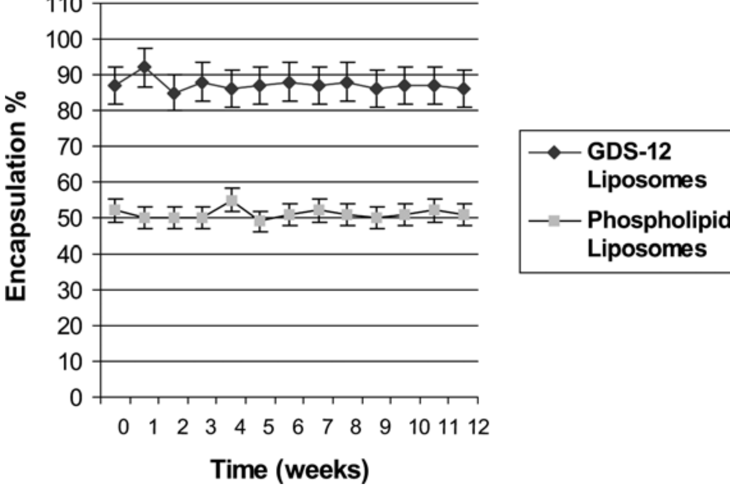

Figure 6 Two formulations with equal molar concentrations of lipids found that the novel PEG lipid that forms STS liposomes had greater encapsulation efficiency than the conventional phospholipid liposomes.

SKIN PENETRATION

One of the chief reasons to use delivery vehicles, even in cosmetic products, is their ability to promote skin penetration. Delivering active ingredients into layers of the skin below the stratum corneum is desirable for many cosmetic purposes including hydration, improving barrier function, scavenging for oxy-radicals to prevent aging, fortifying the epidermis and dermis with nutrients and vitamins such as vitamin A and its congegers, the tocopherols, and vitamin C, and the delivery of other dermally active ingredients for other purported benefits.

Dermal delivery of liposome encapsulated biologically active ingredients for cosmetic and drug applications was well underway by the mid 1990s (18). The ability of vesicles to promote cutaneous permeation and sequester active ingredients in the skin is discussed in numerous studies (19–22). This has become an important benefit of liposomal encapsulation; the use of liposomes not only accelerates the skin permeation of the active but also accelerates and possibly enhances the ''therapeutic'' effect.

A skin penetration study conducted using STS liposome encapsulated caffeine on human cadaver skin and a continuous flow through diffusion cell systems gave interesting results (Fig. 7) (23).

COSMETIC APPLICATIONS

Applications for conventional liposomes continue to show promising outcomes in therapeutic as well as pharmaceutical areas. Treatments for skin discoloration problems, which include lightening products as well as vitiligo therapy (24), have been encouraging. Stabilizing retinol with liposomes and quenching ingredients has potential to solve a use-limiting problem with a valuable cosmetic ingredient (25). Another more intrepid cosmeceutical use of liposomes has been in targeting hair follicles to

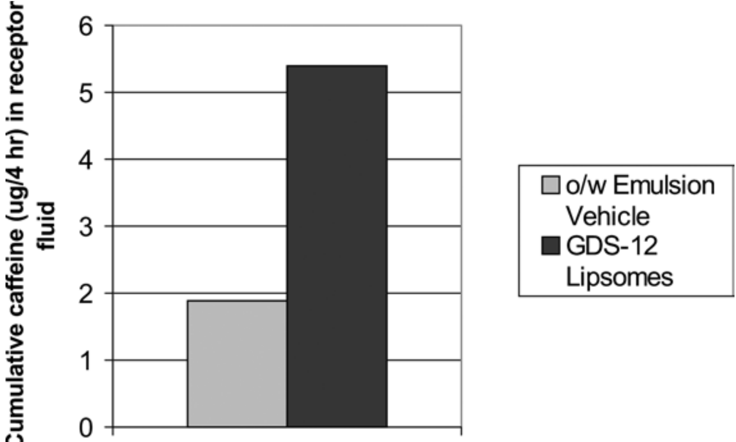

Figure 7 Radiolabeled caffeine encapsulated into a liposome made from PEG-12 GDS compared to a nonencapsulated oil–water emulsion containing the same concentration of [C14]-caffeine. After 8 hours more caffeine penetrated the skin and diffused into the receptor fluid.

deliver hair-growth stimulating molecules and potentially DNA (26–28). The ease of use, cost, stability, and vast utility of STS liposomes has created another viable delivery candidate of these applications as well.

Basic research has established STS liposomes in cosmetic science as a viable delivery system. These lipids, principally PEG-12 GDS and PEG-12 GDO, have been used employing this technology in products for therapeutic uses such as acne, dry skin, inflammation, skin lightening, and photowrinkles. Laboratory testing has demonstrated the pharmaceutical capabilities of ingredient solubilization, ingredient stability, and sequestering to be superior to currently available delivery vehicles. STS liposomes also offer options for the delivery of peptides and growth factors, hydrolyzed wheat proteins, glycolic acids, retinal, cofactors, and coenzymes.

CONCLUSION

Vesicular delivery systems in topical formulations have been available to cosmetic scientists since the late 1980s when conventional phospholipid and, to a limited extent owing to patent protection, nonphospholipid vesicles entered this field. During the past 20 years many success stories have unfolded with topically applied liposome products. There is continued focus on the internal "operating systems" of cosmetic formulations where liposomes are becoming more used and appreciated and consequently seem to be the system of choice.

The ideal cosmeceutical formulation should contain proper concentrations of solubilize "active" ingredients encapsulated in a vesicle and incorporated in a visually appealing cream, gel, lotion, or serum that contains an engaging fragrance which effortlessly rubs into the skin. Laboratory formulation and large-scale manufacturing should not require unconventional processing or expensive equipment to make the resultant product commercial. A novel delivery system that can help achieve this idyllic definition has been discussed. Presently, it has overcome the barriers of entry to liposome use, and adds another tool to the dermatological formulators toolbox.

REFERENCES

1. Keller BC, Lasic DD, Faulkner G. Liposomes in breast milk. Agro-Food Industry Hi-Tech 2000; 11(3):6–8.
2. Lampen P, Pitterman W, Heise HM, Schmitt M, Jungmann H, Kietzmann M. Penetration studies of vitamin E acetate applied from cosmetic formulations to the stratum corneum of an in vitro model using quantifications by tape stripping, UV spectroscopy, and HPLC. J Cosmet Sci 2003; 54(2):119–131.
3. Redziniak G. Liposomes and skin: past present, future. Pathol Biol (Paris) 2003; 51(5): 279–281.
4. Kleiber C, Sorenson M, Whitside K, Gronstal BA, Tannous R. Topical anesthetics for intravenous insertion in children: a randomized equivalency study. Pediatrics 2002; 110(4): 758–761.
5. Kundu S, Achar S. Principles of office anesthesia: part II. Topical anesthesia. Am Fam Physician 2002; 66(1):99–102.
6. Lasic DD. Liposomes and Niosomes. In: Surfactants in Cosmetics. 2nd ed. New York: Marcel Dekker, 1997:280.
7. Lasic DD, Joannic R, Keller BC, Frederik PM. Spontaneous vesiculation. Ad Colloid Interface Sci 2001; 89–90:337–349.

8. Vanlerberghe, Guy, Hadjani, Rose Marie. US Patent 4212344, 1980.
9. Lasic DD. Liposomes: From Physics to Applications. Amsterdam, London, New York, Tokyo: Elsevier, 1993:49–51.
10. Keller. US Patent 6610322, 2003.
11. Hristova K, Needham. Physical properties of polymer-grafted bilayers. In: Stealth Liposomes. Boca Raton: CRC Press, 1995:35–39.
12. Aswal V, Ghosh S. Small angle neutron scattering studies if novel non-ionic surfactant vesicles. Unpublished results.
13. Lasic DD. Liposomes: From Physics to Applications. Amsterdam, London, New York, Tokyo: Elsevier, 1993:35.
14. Fiume Z. Final report on the safety assessment of lecithin and hydrogenated lecithin. Int J Toxicol 2001; 20(suppl 1):21–45.
15. Bidhe RM, Ghosh S. Acute and sub-chronic oral toxicity study in rats fed with novel lipids. AAPS Pharmsci. In press.
16. Handjani-Vila Rm, Ribier A, Vanlerberghe G. Liposomes in the cosmetic industry. In: Gregoridadis G, ed. Liposome Technology. Vol. II. Boca Raton: CRC Press, , 1993: 201–213.
17. Tay K, Raiford AD, Ghosh S, Hassan EE. Oral delivery of liposomes via soft gelatin capsules: I. Characterization and stability studies. AAPS Poster Session, Oct 2003, poster.
18. Weiner N, Lieb N, Niemiec S, Ramachandran C, Hu Z, Egbaria K. Liposomes: a novel topical delivery system for pharmaceutical and cosmetic applications. J Surg Target 1994; 2(5):405–410.
19. Touitou E, Shaco-Ezra N, Dayan N, Jushynski M, Rafaeloff R, Azoury R. Dyphylline liposomes for delivery to the skin. J Pharm Sci 1992; 81(2):131–134.
20. Mezei M. Biodistribution of liposome-encapsulated active ingredients applied to the skin. In: Braun-Falco O, Korting HC, Maibach HI, eds. Liposome Dermatics. Berlin, Heidelberg: Springer-Verlag, 1992:206–214.
21. Verma DD, Verms S, Blume G, Fahr A. Liposomes increase skin penetration of entrapped and non-entrapped hydrophilic substances into human skin: a skin penetration and confocal laser scanning microscopy study. Eur J Pharm BioPharm 2003; 55(3): 271–277.
22. Perugini P, Genta I, Pavanetto F, Conti B, Scalia S, Baruffini A. Study on glycolic acid delivery by liposomes and microspheres. Int J Pharm 2000; 196(1):51–61.
23. Ghosh S, Wester R, Barbadillo S, Choi MJ, Hui X, Maibach H. Comparative study of in vitro cutaneous dispositions of topical caffeine formulations in human skin: enhanced penetration using novel QuSomal® caffeine formulation. Unpublished results.
24. Deleeuw J, Van Der Beek N, Maierhofer G, Neugebauer WD. A case study to evaluate the treatment of vitiligo with khellin encapsulated L-phenylalanin stabilized phosphotidylcholine liposomes in combination with ultraviolet light therapy. Eur J Dermatol 2003; 13(5):474–477.
25. Young AM, Gregoriadis G. Photolysis of retinal in liposomes and its protection with tocopherol and oxybenzone. Photochem Photobiol 1996; 63(3):344–352.
26. Ciotti SN, Weiner N. Follicular liposomal delivery systems. J Liposome Res 2002; 12(1–2): 143–148.
27. Hoffmen RM. Topical liposomes targeting of dyes, melanins, genes and proteins selectively to hair follicles. J Drug Target 1998; 5(2):67–74.
28. Cotsarelis G. The hair follicle as a target for gene therapy. Ann Dermatol Venereol 2002; 129(5 Pt 2):841–844.

13

Elastic Vesicles as Topical/Transdermal Drug Delivery Systems

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INTRODUCTION

The topical/transdermal (TT) delivery route for drug administration has many advantages over other pathways including avoiding the hepatic first pass effect, continuous drug delivery, fewer side effects, and improved patient compliance (1). A major obstacle to TT drug delivery is the low penetration of drugs through skin. The stratum corneum (SC) provides a principle barrier to TT delivery of applied drugs and consists of corneocytes that are embedded in an intercellular lipid matrix composed of ceramides, free fatty acids, and cholesterol (CHOL) (2). Several approaches have been used in order to weaken this skin barrier and to improve TT drug delivery (3–8).

One possibility for increasing the penetration of drugs is the use of vesicular systems such as liposomes. Due to their biocompatibility and capability of incorporating both hydrophilic and lipophilic drugs, liposomes have been investigated as parenteral drug and antigen carrier systems and more recently as TT drug delivery systems (9–18). Despite improvements in TT delivery, conventional liposomes were not efficient at delivering transdermally across skin because they do not deeply penetrate the skin, but rather remain confined to the upper layer of the SC. Thus, several investigators developed novel elastic vesicles to deeply and easily penetrate across skin (19–21).

In the early 1990s, novel series of liquid-state vesicles have been developed and these vesicles could better facilitate drug transport across the skin as compared to conventional vesicles. Novel types of vesicular systems have been recorded to penetrate intact skin if applied non-occlusively in vivo, by virtue of their very high and self-optimizing deformability. Elastic vesicles are classified with phospholipid and detergent-based types (Table 1). Because of high flexibility, elastic vesicles squeeze through small pores in SC less than the vesicle sizes. Elastic vesicles were more efficient at delivering a low and high molecular weight drug to the skin in terms of quantity and depth (22–30). The precise quantity and depth of elastic vesicles' penetration through skin depends on the carrier type, the total mass applied, the entrapment efficiency, and the detailed application conditions such as occlusion,

Table 1 Drug Skin Penetration from Phospholipid- and Detergent-Based Elastic Vesicles

Drugs (comments)	Animal	Composition	Enhancing factor	References
Dipotassium Glycyrrhizinate (KG)	Pig	PC:KG (4:1)	5.9	46
		HPC:KG (4:1)	5.5	
Methotrexate	Pig	PC:KG (2:1)	5.2	50
		HPC:KG (2:1)	5.9	
Dexamethasone	Rat	PC:CHOL (7:3)	1	29
		PC:deoxycholate (85:15)	2.2	
		PC:Tween-80 (85:15)	1.9	
		PC:Span-80 (85:15)	2.3	
Diclofenac	Rat	Commersial form	1	26
		Lotion-like transfersomes	30–100	
Gap junction protein (Antibody production)	Mouse	Soybean PC	1	28
		PC/sodium cholate/SDS	4.7	
Insulin (decrease of blood glucose)	Mouse	PC liposomes or micelle	No change	43
		PC/cholate (8.7:1.3)	20–30%	
Cyclosporin A	Mouse	PC/cholate (10:2.8)	16.2	47
Estradiol	Human	PC/cholate (84:16)	18	44,45
		PC/Span 80 (84:16)	16	
		PC/Tween 80 (84:16)	15	
		PC/oleic acid (84:16)	13	
5-Fluorouracil	Human	PC/cholate (84:16)	6.9–13.2	48
Rotigotine	Human	L-595/PEG-8-L (50:50)	30.6	53
Pergolide	Human	L-595/PEG-8-L (50:50)	2.7	22

pretreatment of vesicles, duration, and application volume. Elastic vesicles prolonged the release and showed better biological activity in comparison with conventional liposomes and ointments.

This review focuses on the effect of elastic vesicles for enhancing the penetration chemicals, and it defines action mechanism and optimal condition of elastic vesicles.

ELASTIC VESICLES–SKIN INTERACTION

Vesicle–skin interactions can occur either at the skin surface or in the deeper layers of the SC. Hofland et al. (31) and Abraham and Downing (32) have shown fusion and adsorption of vesicles onto the SC surface, forming stacks of lamellae and irregular structures on top of the skin. Vesicle-skin interactions are strongly influenced by the composition of the vesicles, resulting in differences in their phase state and elasticity. Liquid-state vesicles have shown to have superior to gel-state vesicles (33). When comparing the interactions of elastic and rigid vesicles with hairless mouse skin in vivo, only the elastic liquid-state vesicles affected the ultrastructure of the viable tissue. No changes in the ultrastructure were observed with any of the conventional liposomes (34).

Cevc and Blume (19) suggested that elastic vesicles (Transfersomes®) were able to penetrate through intact SC under the influence of a transepidermal osmotic gradient. Although the transport of most compounds is increased during occlusive

application, Cevc and Blume (19) have suggested that elastic vesicles (Transfersomes) are most efficient under nonocclusive conditions. Nonocclusive conditions are necessary in order to create a transepidermal osmotic gradient, which is believed to be the driving force for elastic transport into the skin. The osmotic gradient is caused by the difference in water concentrations between the skin surface and skin interior. Transfersomes are highly deformable and this property facilitates their rapid penetration through the intercellular lipid pathway of the SC. Schatzlein and Cevc (35) reported the existence of irregularities within the intercellular lipid packing of murine SC, that can act as virtual channels through which Transfersomes could penetrate.

In the case of detergent-based elastic vesicles, Van den Bergh et al. (34,36) investigated the interaction of elastic and rigid vesicles with murine skin in vivo, and with human skin in vitro. Unlike Transfersomes, these studies did not show any evidence that elastic vesicles could penetrate through the SC. However, Honeywell-Nguyen et al. (37) demonstrated a fast penetration of intact elastic vesicles into human SC and these vesicles were localized within channel-like regions. They also investigated the in vivo interaction of elastic vesicles with human skin, using the tape stripping technique in combination with freeze–fracture electron microscopy and demonstrated a fast penetration of intact elastic vesicles into human SC (38). They proposed that the channel-like regions represent imperfections within the intercellular lipid lamellae in the areas with highly undulating cornified envelopes. Taken together, intact elastic vesicles may penetrate into human SC via channel-like regions.

Ethosomes are also phospholipid elastic vesicles having a high content of ethanol. Ethanol interacts with lipid molecules in the polar head group region, resulting in a reduction in the melting point of SC lipid, increasing their fluidity. The interaction of ethanol into the polar head group environment can result in an increase in membrane permeability. This is followed by a fusion of ethosomes with cell membranes. In addition to the effects of ethanol on SC structure, ethosome itself interacts with the SC barrier and then can penetrate the disturbed SC bilayer (39,40).

TRANSFERSOMES

Skin has small virtual pores (20–40 nm) and this limits passing through intercellular passages in the outer skin layers (20,41). To overcome this problem, Cevc developed a new liposomal system with more deformable aggregates called Transfersomes (20). Transfersomes differ from more conventional liposomes in several respects. Transfersomes resemble liposomes in morphology but not in function (42). The most important is the extremely high and stress-dependent adaptability of such mixed lipid aggregates. Because of high deformability, Transfersomes squeeze through pores in SC that are less than one-tenth in the liposomes's diameter. Thus, sizes up to 200–300 nm can penetrate intact skin (43). Transfersomes contain phosphatidylcholine (PC) and a surfactant (edge activator), and also consist of at least one inner aqueous compartment surrounded by a lipid bilayer. Sodium cholate, Span 80, Tween 80, oleic acid, and dipotassium glycyrrhizinate (KG) (20,44–46) were employed as edge activators. For transdermal DNA delivery, DOTAP (positive charged molecule), as a component, was used in producing Transfersomes instead of PC (30). Subsequent studies have documented that Transfersomes were more effective than conventional

liposomes or ointment in the enhancement of small and large drug molecules across mouse and human skin (24–30,47,48).

Several investigators reported that Transfersomes prolonged the release and improved the biological activity in vivo (24,26,29,49). The in vivo performance of Transfersomes was studied by a carrageenan- and arachidonic acid–induced edema model with dexamethasone and triamcinolone acetonide (TRMA). Jain et al. (29) showed that dexamethasone Transfersomes could provide a maximum of 82.32% inhibition of paw edema, whereas conventional liposomes and ointments prevented approximately 38.32% and 25.35% of paw edema, respectively. With TRMA, the drug dose of 0.2 mg/cm^2 suppressed 75% inhibition of ear edema for 48 hours. In contrast, a conventional formulation of TRMA required a 10-fold higher drug dosage to achieve a similar effect (24). A similar result was obtained with hydrocortisone and diclofenac Transfersomes (24,26,49). Diclofenac association with Transfersomes prolonged the effect and reached 10-fold higher concentrations in the tissue under the skin in comparison with the drug from a commercial hydrogel (26).

In addition to chemical drugs, Transfersomes could deliver large molecules into the body through intact skin. Paul et al. (28) investigated the effect of Transfersomes on the transdermal immunization with protein antigen. They applied Transfersomes to the intact skin surface with gap junction proteins (GJP) and showed that the specific antibody titers were higher than those elicited by subcutaneous injection of GJP in Transfersomes, mixed micelles, or conventional liposomes. Hofer et al. (27) reported the formulation of IL-2 and IFN-α containing Transfersomes for transdermal application. They showed that IL-2 as well as IFN-α were trapped by Transfersomes (75–80%) in biologically active form and in sufficient concentration for immunotherapy. Cevc (25) also reported the transdermal delivery of insulin with Transfersomes. Transfersomes could deliver insulin through skin barrier with a reproducible drug effect that resembled closely to that of insulin injected under the skin. In addition, Kim et al. (30) investigated the effect of Transfersomes (DOTAP and cholate) on the transdermal application of DNA in mice intact skin. They reported that the GFP expression was detected in some organs such as liver and lungs when GFP was complexed with Transfersomes whereas the GFP mixed only with PBS did not observe with GFP expression. Transfersomes were found to be capable of penetrating DNA into intact skin of mice when transdermally applied. Thus, Transfersomes may be developed further as a noninvasive protein and gene delivery system.

Transfersomes have several advantages on the TT drug delivery. They can be entraped and delivered with small and large molecules through TT delivery. When applied on the intact skin, Transfersomes are not detrimental to the skin. Phospholipid, as a component of Transfersomes, even seem to improve the hydration of the aged skin (26). The advantages include a faster onset of drug effect, longer times of action, a biological action that is unaffected by mechanical abrasion, and the ability to reduce the necessary dosage needed to achieve therapeutic effects. Thus, the use of Transfersomes on the skin offers unprecedented opportunities for well-controlled and modern topical medication, not just for low molecular weight, but also for a variety of macromolecular therapeutics.

EFFECT OF TYPE SURFACTANTS AND CONCENTRATION

To prepare Transfersomes vesicles, edge activators (surfactants) were incorporated into the vesicular membranes; sodium cholate or sodium deoxycholate, Span 80,

and Tween 80 have been used for this purpose. El Mghraby et al. (44,45) investigated the effect of surfactants on the formation of Transfersomes and permeation into skin using cholate, Span 80, Tween 80, and oleic acid. Also they investigated the effect of surfactant concentration on the skin permeation.

Transfersomes significantly improved the epidermal delivery of estradiol compared to the aqueous solution. The maximum flux increased by 18-, 16-, 15-, and 13-fold for Transfersomes containing sodium cholate, Span 80, Tween 80, and oleic acid compared with control. The skin deposition also increased by eight-, seven-, and eight-fold for cholate, Span 80, and Tween 80, respectively, compared with control. The efficiencies of Span 80 and Tween 80 were comparable with that of sodium cholate, but efficiency of oleic acid was less than that of sodium cholate. With dexamethasone Transfersomes, Span 80 was more effective as compared with sodium deoxycholate and Tween 80 as edge activators on penetration and edema inhibition assay.

With respect to drug delivery from vesicles, J_{max} first increased with increasing surfactant concentration, then decreased. These results suggested that too low or too high concentrations of surfactants are not beneficial in vesicular delivery of estradiol through skin and also indicated that the possible penetration enhancing effect of surfactants is not mainly responsible for improved estradiol skin delivery from deformable vesicles. The surfactant concentrations in the refined formulation were assessed to be 14.0%, 13.3%, and 15.5% w/w for sodium cholate, Span 80, and Tween 80, respectively (44). A possible explanation for lower drug delivery at high surfactant concentrations may be that surfactant at high concentrations decreased the entrapment efficiency and disrupted the lipid membrane so that it became more permeable to the entrapped drug. This will in turn reduce the delivery. The overall results suggested that there may be an optimum concentration of surfactant in lipid vesicles for maximum skin delivery of drug using transfersome vesicles.

In addition, KG is used in preparing elastic vesicles. KG has emulsifying properties and good solubility. Thus, it is widely used in cosmetics. Trotta et al. (46) evaluated the ability of KG to produce elastic vesicles with soya lecithin (PC) or hydrogenated lecithin (HPC). They compared KG permeation from elastic vesicles and aqueous solution in the pig skin. All systems showed a negligible flux but differed the residual amount of KG in the skin. PC and HPC elastic vesicles promote the transfer of KG into the pig skin (60 ± 8 to $71 \pm 10\,\mathrm{mg\,cm^{-2}}$), while the KG solution failed to achieve transport ($12 \pm 3\,\mathrm{mg\,cm^{-2}}$). There were no significant differences between elastic vesicles containing PC or HPC. The skin deposition increased by five-fold compared with 0.25% KG control solution.

Trotta et al. (50) also reported the effect of KG elastic vesicles on the dermal penetration of methotrexate (MTX) in pig skin. In an estimation of the cumulative amount of MTX permeated after 24 hours through pig skin, aqueous solution and conventional liposomes are quite similar in terms of MTX delivery through skin, whereas elastic vesicles show an increase in the amount of MTX permeated. In a skin deposition after 24-hour application, PC and HPC elastic vesicles promoted the transfer of MTX into pig skin. Skin deposition increased by a factor of 3 compared with either aqueous solution or conventional liposomes. Thus, KG acts as a good edge activator to produce elastic vesicles for TT drug delivery.

Which detergents are the best choice in Transfersomes formulation? The effect of detergents was different from other formulation conditions. The use of cholate seems to be better than other detergents in the case of skin delivery of large molecules. In addition to cholate, KG is a good candidate. KG has chemical stability,

good solubility, emulsifying property, and anti-inflammatory activity. Thus, the use of elastic vesicles containing KG creates new opportunities for the well-controlled and modern topical medication. However, a more extensive study about compositions should be undertaken to define the optimal formulation regardless of drug types.

NON-PHOSPHOLIPID-BASED ELASTIC VESICLES

In addition to phospholipid-based elastic vesicles (Transfersomes and ethosomes), Van den Bergh et al. (21) developed a series of elastic vesicles, consisting of the bilayer-forming surfactant L595 (sucrose laurate ester) and the micelle-forming surfactant PEG-8-L (octaoxyethylene laurate ester). L595 consisted of 100% sucrose laurate ester (30% monoester, 40% diester, and 30% triester). Surfactant-based elastic vesicles consisted of L595, PEG-8-L, and sulfosuccinate as stabilizers in the molar ratio 50/50/5. Several investigators reported that L595–PEG-8-L elastic vesicles were effective in enhancing the skin permeation of various drugs (22,51–53). However, drug transport was influenced by fluidity of elastic vesicles. The most rigid vesicles consisting of L595/PEG-8-L (100:0) significantly reduced the flux by 50% compared to the buffer control ($P < 0.05$). However, increasing the PEG-8-L content—and thereby increasing the vesicle elasticity—resulted in a significant higher flux for the L595/PEG-8-L (70/30 or 50/50) elastic vesicles ($P < 0.01$). This was a three-fold increase to the buffer treatment and a six-fold increase to the most rigid vesicle treatment (22). Similar results were obtained from Rotigotine elastic vesicles (53).

Elastic vesicle transport and the appearance of vesicle material in human SC can be affected by several factors including pH, entrapping efficiency, pretreatment of vesicles, occlusive volume, and duration of application (37,38,53). The optimal pH differs depending on the drugs. The optimal pH was found to be 5.0 in case of pergolide, giving the highest drug incorporation as well as the highest drug transport. There was more than a fourfold difference between the highest flux at pH 5.0 ($371.0 \pm 51.7\,\mathrm{ng\,cm^{-2}}$) and the lowest flux at pH 7.0 ($89.3 \pm 9.1\,\mathrm{ng\,cm^{-2}}$) (22). Unlike pergolide, optimal pH was found to be 9.0, giving the highest drug incorporation ($99.8 \pm 0.02\%$) in case of rotigotine elastic vesicles. At pH 5.0, the entrapment efficiency is very low ($22.1 \pm 9.6\%$). The flux and cumulative amount of vesicle solution at pH 9.0 with high drug entrapment efficiency was 2.7-fold higher than those resulting from the vesicle solution at pH 5.0. Vesicles solution at pH 9.0 ($3251 \pm 902\,\mathrm{ng\,cm^{-2}}$) gave rise to enhancement effect of factor 80 as compared to the corresponding buffer solution ($42 \pm 29\,\mathrm{ng\,cm^{-2}}$). In contrast, vesicles solution at pH 5.0 ($1072 \pm 160\,\mathrm{ng\,cm^{-2}}$) did not significantly enhance the drug transport as compared to its corresponding buffer control ($1133 \pm 241\,\mathrm{ng\,cm^{-2}}$) (53). Table 2 summarizes the effect of elastic vesicles on pergolide and rotigotine delivery into skin. As shown in Table 2, enhancement of rotigotine was much higher than that of pergolide. Entrapment efficiency and drug properties may result in this difference.

Nonocclusive cotreatment with elastic vesicles improved the skin delivery of pergolide compared to nonocclusive buffer control by more than twofold. Occlusion improved drug transport from both elastic vesicles as well as buffer solutions due to the fact that water is an excellent penetration enhancer for pergolide (37). In contrast to nonocclusive application, occlusive treatment with elastic vesicles showed a lower flux compared to occlusive treatment with the buffer control. A higher volume of application could increase the partitioning of vesicles into the skin, thereby

Table 2 Comparison of Pergolide and Rotigotine Skin Delivery with Elastic Vesicles

	Rotigotine	Pergolide
Use	Dopamine agonist	Dopamine agonist
Compositions	L595/PEG-8-L-sulfosuccinate (50/50/5)	L595/PEG-8-L-sulfosuccinate (50/50/5)
Enhancement ratio	30.6-fold	2.7-fold
Occlusion	–	Increase penetration
Optimal pH	9.0	5.0
Entrapment	$99.8 \pm 0.02\%$ at pH 9.0	2.5 mg/mL at pH 5.0 (Saturated)
Average size	117 ± 6 nm	100 ± 5 nm
Pka	7.9	5–6
Lipophilicity	Lipophilic at pH 9.0	Lipophilic and positive charge

increasing the enhancement effect. Pergolide transport from the 40 and 100 μL application were much higher within the first 20 hours as compared to that from the 20 μL application. However, there were no significant differences in the total cumulative amounts of drugs transported (306 ± 47.4, 462.0 ± 112.0, and 509.7 ± 141.9 in 20, 40, and 100 μL application, respectively) (37). They also investigated the effect of co-application and pretreatment on the rotigotine transport. Co-application ($3483 \pm 1067\ \text{ng cm}^{-2}$) significantly enhanced the drug transport by many fold, whereas pretreatment ($126 \pm 18\ \text{ng cm}^{-2}$) clearly had no effect on the drug transport as compared to buffer control ($133 \pm 27\ \text{ng cm}^{-2}$) (53). Similar result was obtained from pergolide elastic vesicles.

From these results, detergent-based elastic vesicles were found to be powerful drug delivery systems across skin. Co-application (co-treatment) and entrapment efficiency were essential factors for an optimal drug delivery by elastic vesicle formulations. The drug entrapment efficiency is strongly dependent on the pH of the drug–vesicular system. Thus, pH was also important factor to deliver drugs with TT pathway.

ETHOSOMES

Ethosomes are phospholipid liposome carriers containing high content of ethanol (20–45%) (23). However, due to the interdigitation effect of ethanol on lipid bilayers, it was believed that high concentrations of ethanol are detrimental to liposomal formulation. Touitou developed an ethosomes for transdermal drug delivery (54). Currently ethanol can only be found in relatively low concentrations in liposomes formulation; 7–10% for Transfersomes, 14% for Mibelle, and 16% for Natipide II. But, high content of ethanol was used in case of proniosomes (30–50%) (55,56). Ethosomes are soft, malleable vesicles tailored for enhanced delivery of various drugs to/through the skin and cellular membranes. Unlike conventional liposomes, that are known mainly to deliver drugs to the outer layers of skin, ethosomes were shown to enhance permeation through the SC barrier. They penetrate skin and enhance drug delivery to deep skin SC (23,39,54,57). They are noninvasive delivery carriers that enable drugs to reach the deep skin layers and the systemic circulation.

Touitou et al. (23) investigated the effect of phospholipid and ethanol concentration on the size distribution of ethosome vesicles. In the ethanol concentration

range of 20–45%, the size of the vesicles increased with decreasing ethanol concentration, with the largest sizes in preparation containing 20% ethanol (193 ± 8 nm) and the smallest in preparations containing 45% ethanol (103 ± 9 nm). The dependence of vesicle size on phospholipid content was determined for ethosomes containing 30% ethanol and PC concentration ranging from 0.5% to 4%. An eightfold increase in PC concentration (from 0.5% to 4%) resulted in a twofold increase in ethosome size (from 118 ± 2 to 249 ± 24 nm). Also, ethanol imparted a negative charge to the vesicles and improved the vesicle stability. Hydrophilic and hydrophobic chemicals can be entrapped into ethosomes and entrapment efficiency of hydrophobic chemicals was higher than that of hydrophilic chemicals. Entrapment efficiencies of minoxidil, testosterone, trihexylphenidyl (THP), and bacitracin were 83 ± 6%, 90 ± 3.5%, 75 ± 0.8%, and 77.4 ± 2.9%, respectively (23,39,58).

Enhanced delivery of chemicals from the ethosomal carrier was observed in permeation experiments with fluorescent probes (for detection of penetration depth) and drugs to nude mouse skin. Hydrophilic calcein penetrated the skin to a depth of 160, 80, and 60 μm from ethosomes, hydroethanolic solution (30% ethanol in water), and liposomes, respectively. Lipophilic rhodamine red (RR) penetrated the nude mouse skin to a depth of 140 μm from both the ethosomal system and from hydroethanolic solution. The probe fluorescence intensity was significantly greater from the ethosomal system (150 AU for ethosomes and 40 AU for hydroethanolic solution). Fluorescence was still visible at the deepest skin layers (20 AU at 260 μm depth) in case of ethosomal system. Deep penetration from liposomes was almost neglible (20 AU at 40 μm depth) (59).

Touitou et al. (23) also investigated the ability of ethosomes to deliver minoxidil to the deep layers of the skin. When it permeated the skin it was 45 and 35 times higher from the ethosomal system than 30% ethanolic solution and absolute ethanol, respectively; the amount of minoxidil in the skin was also seven and five times greater than control systems, respectively. Similar results were obtained using acyclovir®, testosterone, and inoic molecules such as propranolol and THP (23,39,60). Horwitz et al. (60) reported that acyclovir delivered from an ethosomal system performed significantly better than Zovirax® (Glaxo-Wellcome). The amount of testosterone permeated in the rabbit skin in 24 hours was 30 times greater from the ethosomal system than Testoderm® (848.16 ± 158.38 μg versus 27.79 ± 16.23 μg). The amount of testosterone in the skin was also almost seven times greater when the drug was delivered from ethosomal system (130.76 vs. 18.32 μg) (23). THP flux from ethosomes (0.21 mg cm^{-2} h^{-1}) was 87, 51, and 4.5 times higher than that from liposomes, buffer, and hydroethanolic solution, respectively (39). These data indicate that the ethosomal system is a more effective permeation enhancer than ethanol and hydroethanolic solution. Table 3 summarizes the ethosomal drug delivery to skin and cell membranes. Lodzki et al. (61) also reported the cannabidiol® transdermal delivery in a murine model. The flux of cannabidiol differs depending on skin sites. After a 24-hour application, permeated the skin 37.43 ± 13.58 and 110.07 ± 24.15 mg cm^{-2} in hip skin and abdominal skin, respectively. Cannabidiol was also detected in the muscle, liver, pancreas, and blood. Godin and Touitou (58) evaluated the skin depth penetration from bacitracin® ethosomes in vivo in rats. After an 8 hour topical application to rat abdomen, bacitracin penetrated more deeply into the skin from ethosomes than 30% ethanolic solution and liposomes. Taken together, the ethosomal system is effective in delivering drugs deeply into and through the skin.

Ethosomal systems are easy to prepare, nonirritant and composed mainly of phospholipids and ethanol; compounds commonly found in pharmaceutical

Table 3 Drug Skin Permeation from Ethosomasl Vs. Control System

Drugs	Animal model	System	Qr[a] (μg cm^{-2})	Qs[a] (μg cm^{-2})	References
Minoxidil®	Nude mouse	Ethosomes	673.0 ± 92.0	69.6 ± 11.0	23
		30% EtOH	13.1 ± 3.5	10.0 ± 2.3	
Trihexyphenidyl®	Nude mouse	Ethosomes	1750 ± 250	586 ± 77	39
87 times		30% EtOH	500 ± 50	415 ± 21	
Cannabidiol®	Nude mouse	Ethosomes	110.07 ± 24.15	–	61
Bacitracin®	Human	Ethosomes	12.0 ± 1.0	–	58
Testosterone®	Rabbit	Ethosome patch	848.16 ± 158.38	130.76 ± 20.23	23
		Testoderm®	27.79 ± 16.23	18.32 ± 8.34	

[a]The quantity of drugs that permeated the skin (Qr) and the quantity of drugs in the skin (Qs) in 24 or 18 hour (trihexyphenidyl) were measured in diffusion cells from systems, each containing 0.5% minoxidil, 0.1% bacitracin, 1% trihexyphenidyl, 0.25 mg testosterone per cm^2.

preparations. Because of their unique structure, ethosomes are able to entrap and deliver through the skin highly lipophilic molecules such as cannabinoids, testosterone, and minoxidil, as well as cationic drugs such as propanolol® and trihexyphenidil. Enhanced delivery of bioactive molecules through the skin and cellular membranes by means of an ethosomal carrier opens numerous challenges and opportunities for the research and future development of novel improved therapies.

ACTION MECHANISM OF ELASTIC VESICLES ON PENETRATION

How do phospholipid- and detergent-based elastic vesicles enhance drug penetration into skin? Two mechanisms can be proposed. First, the elastic vesicles can act as penetration enhancers, whereby vesicle bilayers enter the SC and subsequently modify the intercellular lipid matrix. This will facilitate the penetration of free drug molecules into and across the SC (Mechanism 1). Second, the elastic vesicles can act as drug carrier systems, whereby intact vesicles can enter the SC carrying vesicle-bound molecules into the skin (mechanism 2) (37,53). In order to assess whether a drug carrier mechanism of action is involved or whether elastic vesicles simply act as penetration enhancers, two important questions should be answered: (1) Is pretreatment of the skin with empty vesicles sufficient (for mechanism 1), or is it essential to incorporate drugs into the vesicle solution (for mechanism 2)? (2) What is the effect of the entrapment efficiency on the drug transport? Does higher entrapment efficiency result in a higher drug transport?

Cevc et al. (19) proposed Transfersomes are drug carrier systems that can deliver across the intact skin. It is believed that the successful passage of such carriers is based on two important factors: the high elasticity (deformability) of the vesicle bilayers and the existence of an osmotic gradient across the skin. Because of high deformability, Transfersomes could—under influence of the transepidermal osmotic gradient—squeeze themselves between the cells in the SC and carry large amounts of drugs across the intact skin. Fang et al. (18) investigated the mechanism of vesicular system across the skin with soybean PC liposomes containing enoxacin®. After a 12 hour pretreatment, drug permeation across PC-treated skin was higher than that across nontreated skin. Also, Verma et al. (17) reported that PC liposomes carry

not only the entrapped hydrophilic drug but also the nonentrapped drug into the SC and possibly into deeper skin layers. These results indicated that PC liposomes could serve as permeation enhancers for drug delivery via the skin. With 5-fluorouracil Transfersomes, the percentage of drug penetrated (13.5%) was higher than the drug entrapment efficiency (8.8%) of Transfersomes (48). This strongly suggested that Transfersomes components may have altered the skin structure, as a penetration enhancer. Taken together, Transfersomes may have two functions to enhance drug transport across skin; as a carrier system as well as a penetration enhancer.

Several investigators reported that pre-treatment of detergent-based elastic vesicles did not improve the transport of pergolide and rotigotine, whereas higher entrapment efficiency resulted in higher drug transport (38,53). These data suggest that a penetration enhancing process is not the main or the only mechanism of action, and detergent-based elastic vesicles may act as a carrier system. Honeywell-Nguyen and Bouwstra (37) proposed that detergent-based elastic vesicles facilitate drug transport by a fast partitioning in the SC, thereby carrying vesicle-bound drug molecules into SC. The vesicles remain in the SC and do not penetrate into the deeper skin layers. Hence, there are four major steps determining the effectiveness of the elastic vesicles system: (i) the drug association to the vesicle bilayers, (ii) the partitioning of vesicles into the SC, (iii) the drug release from the vesicles once in the SC, and (iv) the diffusion of free drugs in the SC and partitioning into the viable skin tissue and subsequently into the systemic circulation (37). Taken together, they proposed that a penetration enhancing effect of the individual surfactant component is not the main or the only mechanism of action for the elastic vesicles and it is essential to apply drug molecules together with the vesicles.

Touitou et al. (23) proposed the action mechanism of ethosomal systems. First, ethanol disturbs the organization of the SC lipid bilayer and enhances its lipid fluidity. The flexible ethosome vesicles can then penetrate the disturbed SC bilayers. The release of the drug in the deep layers of the skin and its transdermal absorption could then be the result of a fusion of ethosomes with skin lipids and drug release at various points along the penetration pathway. Unlike other elastic vesicles, occlusion slightly increased the skin penetration of ethosomes. This result indicated that the existence of an osmotic gradient across the skin was not an important factor (58). These data differ from that observed with elastic vesicles where permeation enhancement occurred only in nonocclusive conditions and points toward different mechanisms of action of the two carriers.

To further investigate mechanism of ethosomal skin permeation, Godin and Touitou (58) used double staining methods; ethosomes coloaded with two fluorescent probes, rhodamine red (RR) and FITC-bacitracin® (FITC-Bac). Both probes were delivered from ethosomes to a maximal possible depth of 200 μm. When the two probes were observed separately at the skin depth of 90 μm, it was clearly seen that the delivery of FITC-Bac from ethosomes was followed by the delivery of ethosomal components in the same area. Skin penetration profile date indicated that penetration of ethosomal vesicles into the skin peaked at approximately 40 μm, while depth of maximum bacitracin penetration was approximately 90 μm, suggesting that the release of the drug in deep skin layers occurred. In a double staining study, the bacitracin delivered from ethosomes entered the skin between the coreocytes through the intercellular lipid domain. High content of ethanol fluidizes the ethosomal memebranes to produce highly deformable vesicles and subsequently ethosomes squeeze drugs between the cells in the SC and carry large amounts of drugs across the intact

skin (40,48). Additionally, we cannot exclude the possibility that ethosomes can be trapped in follicles and delivered to deep layers of the skin.

CONCLUSIONS

Highly deformable elastic vesicles (Transfersomes, ethosomes, detergent-based elastic vesilces) improve the transdermal delivery of low and high molecules in vitro and in vivo systems. The use of elastic vesicles as a vesicular drug carrier could overcome the limitation of low penetration ability of conventional liposomes or commercial ointment across the skin. Penetrating-enhancing effects of phosphlipid- and detergent-based elastic vesicles act as a drug carrier system as well as penetration enhancers. For optimal drug delivery, it is essential that drug molecules are associated with vesicles and applies with co-application (cotreatment) conditions. In vivo study, Transfersomes and ethosomes showed better biological activity in comparison with conventional liposomes or commercial ointment. Thus, many topical drugs may be developed using elastic vesicles. However, a more extensive study about vesicular type and compositions should be undertaken to fully establish the optimal condition, for which elastic vesicles are the most suitable vehicles.

REFERENCES

1. Barry BW. Novel mechanisms and devices to enable successful transdermal drug delivery. Eur J Pharm Sci 2001; 14:101–114.
2. Schurer NY, Elias PM. The biochemistry and function of stratum corneum lipids. Adv Lipid Res 1991; 24:27–56.
3. Banga AK, Bose S, Ghost TK. Iontophoresis and electroporation: comparison and contrasts. Int J Pharm 1999; 179:1–19.
4. Regnier V, Preat V. Localization of a FITC-labeled phosphorothioate oligonucleotide in the skin after topical delivery by iontophoresis and electroporation. Pharm Res 1998; 15:1596–1602.
5. Mezei M, Gulasekharam V. Liposomes—a selective drug delivery system for the topical route administration. Part I. Lotion dosage forms. Life Sci 1980; 26:1473–1477.
6. Yoshioka T, Sternberg B, Florence AT. Preparation and properties of vesicles (niosomes) of sorbitan monoesters 9 (Span 20, 40, 60 and 80) and a sorbitan triester (Span 85). Int J Pharm 1994; 105:1–6.
7. Williams AC, Barry BW. Penetration enhancers. Adv Drug Del Rev 2004; 56:603–618.
8. Elias PM, Tsai J, Menon GK, Hollern WM, Feingold KR. The potential of metabolic interventions to enhance transdermal drug delivery. J Invest Dermatol Sym Proc 2002; 7:79–85.
9. Lasic DD. Doxorubicin in sterically stabilized liposomes. Nature 1996; 380:561–562.
10. Abra RM, Bankert RB, Chen F, Egilnez NK, Huang K, Saville R, Slater JL, Sugano M, Yokota SJ. The next generation of liposome delivery system: recent experience with tumor-targeted, sterically stabilized immunoliposomes and active-loading gradients. J Liposome Res 2002; 12:1–3.
11. Clemons KV, Stevens DA. Comparative efficacies of four amphotericin B formulations—Fungizone, amphotech (Amphocil), AmBisome, and Abelcet—against systemic murine aspergillosis. Antimicrob Agents Chemother 2004; 48:1047–1050.
12. Choi MJ, Maibach HI. Topical vaccination of DNA antigens: topical delivery of DNA antigens. Skin Pharmacol Appl Skin Physiol 2003; 16:271–282.

13. Chang JS, Choi MJ, Cheong HS, Kim K. Development of Th1-mediated $CD8^+$ effector T cells by vaccination with epitope peptides encapsulated in pH-sensitive liposomes. Vaccine 2001; 19:3608–3614.
14. Chang JS, Choi MJ, Kim TY, Cho SY, Cheong HS. Immunogenecity of synthetic HIV-1 V3 loop peptides by MPL adjuvanted pH-sensitive liposomes. Vaccine 1999; 17:1540–1548.
15. Chang JS, Choi MJ. pH-Sensitive liposomes as adjuvants for peptide antigens. Methods Enzymol 2003; 373:127–136.
16. Touitou E, Junginger HE, Weiner ND, Nagai T, Mezei M. Liposomes as carriers for topical and transdermal delivery. J Pharm Sci 1994; 83:1189–1203.
17. Verma DD, Verma S, Blume G, Fahr A. Liposomes increase skin penetration of entrapped and non-entrapped hydrophilic substances into human skin: a skin penetration and confocal laser scanning microscopy study. Eur J Pharm Biopharm 2003; 55:271–277.
18. Fang JY, Hong CT, Chiu WT, Wang YY. Effect of liposomes and niosome on skin permeation of enoxacin. Int J Pharm 2001; 219:61–72.
19. Cevc G, Blume G. Lipid vesicles penetrate into intact skin owing to the transdermal osmotic gradients and hydration force. Biochim Biophys Acta 1992; 1104:226–232.
20. Cevc G. Transfersomes, liposomes and other lipid suspensions on the skin: permeation enhancement, vesicle penetration, and transdermal drug delivery. Crit Rev Ther Drug Carrier Syst 1996; 13:257–388.
21. Van den Bergh BAI. Elastic liquid state vesicles as a tool for topical drug delivery. Thesis. Leiden University, The Netherlands, pp. 49–80 (1999).
22. Honeywell-Nguyen PL, Frederik PM, Bomans PHH, Junginger HE, Bouwstra JA. Transdermal delivery of pergolide from surfactant-based elastic and rigid vesicles: characterization and in vitro transport studies. Pharm Res 2002; 19:992–998.
23. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes-novel vesicular carriers: characterization and delivery properties. J Control Release 2000; 65:403–418.
24. Cevc G, Blume G. Biological activity and characteristics of triamcinolone-actonide formulated with the self-regulating drug carriers, Transfersomes. Biochim Biophys Acta 2003; 1614:156–164.
25. Cevc G. Transdermal drug delivery of insulin with ultradeformable carriers. Clin Pharmacokinet 2003; 42:461–474.
26. Cevc G, Blume G. New, highly efficient formulation of diclofenac for the topical, transdermal administration in ultradeformable drug carriers, Transfersomes. Biochim Biophys Acta 2001; 1514:191–205.
27. Hofer C, Gobel R, Deering P, Lehmer A, Breul J. Formulation of interleukin-2 and interferon-alpha containing ultradeformable carriers for potential transdermal application. Anticancer Res 1999; 19:1505–1507.
28. Paul A, Cevc G, Bachhawat BK. Transdermal immunization with an intergral membrane component, gap junction protein, by means of ultradeformable drug carriers, Transfersomes. Vaccine 1998; 16:188–195.
29. Jain S, Jain P, Umamaheshwari RB, Jain NK. Transferosmes—a novel vesicular carrier for enhanced transdermal delivery: development, characterization, and performance evalution. Drug Dev Ind Pharm 2003; 29:1013–1026.
30. Kim A, Lee EH, Choi SH, Kim CK. In vitro and in vivo transfection efficiency of a novel ultradeformable cationic liposome. Biomaterials 2004; 25:305–313.
31. Hofland HEJ, van der Geest R, Bodde HE, Junginger HE, Bouwstra JA. Estradiol permeation from nonionic surfactant vesicles through human stratum corneum in vitro. Pharm Res 1994; 11:659–664.
32. Abraham W, Downing DT. Interaction between corneocytes and stratum corneum lipid liposoms in vitro. Biochim Biophys Acta 1990; 1021:119–125.
33. Van Kuijk-Meuwissen MEMJ, Junginger HE, Bouwstra JA. Interactions between liposomes and human skin in vitro, a confocal laser scanning microscopy study. Biochim Biophys Acta 1998; 1371:31–39.

34. Van den Bergh BA, Bouwstra JA, Junginger HE, Wertz PW. Elasticity of vesicles affects hairless mouse skin structure and permeability. J Control Release 1999; 62:367–379.
35. Schätzlein A, Cevc G. Non-uniform cellular packing of the stratum corneum and permeability barrier function of intact skin: a high-resolution confocal laser scanning microscopy study using highly deformable vesicles (Transfersomes). Br J Dermatol 1998; 138:583–592.
36. Van den Bergh BAI, Vroom J, Gerritsen H, Junginger HE, Bouwstra JA. Interactions of elastic and rigid vesicles with human skin in vitro: electron microscopy and two photon excitation microscopy. Biochim Biophys Acta 1999; 1461:155–173.
37. Honeywell-Nguyen PL, Bouwstra JA. The in vitro transport of pergolide from surfactant-based elastic vesicles through human skin: a suggested mechanism of action. J Control Release 2003; 86:145–156.
38. Honeywell-Nguyen PL, Wouter Groenink HW, de Graaff AM, Bouwstra JA. The in vivo transport of elastic vesicles into human skin: effects of occlusion, volume and duration of application. J Control Release 2003; 90:243–255.
39. Dayan N, Touitou E. Carriers for skin delivery of trihexyphenidyl HCl: ethosomes vs. liposomes. Biomaterials 2000; 21:1879–1885.
40. Valjakka-Koskela R, Kirjavainen M, Mönkkönen J, Urtti A, Kiesvaara J. Enhancement of percutaneous absorption of naproxen by phospholipids. Int J Pharm 1998; 175: 225–230.
41. Cevc G, Blume G, Schätzlein A, Gebauer D, Paul A. The skin: a pathway for systemic treatment with patches and lipid-based agent carriers. Adv Drug Del Rev 1996; 18: 349–378.
42. Planas ME, Gonzalez P, Rodriguez L, Sanchez S, Cevc G. Noninvasive percutaneous induction of topical analgesia by a new type carrier and prolongation of local pain insensitivity by anesthetic liposomes. Anesth Analg 1992; 75:615–621.
43. Cevc G, Gebauer D, Stieber J, Schätzlein A, Blume G. Ultraflexible vesicles, Transfersomes, have an extrimely low pore penetration resistance and transport therapeutic amounts of insulin across the intact mammalian skin. Biochim Biophys Acta 1998; 1368: 201–215.
44. El Maghraby GMM, Williams AC, Barry BW. Oestradiol skin delivery from deformable liposomes: refinement of surfactant concentration. Int J Pharm 2000; 196:63–74.
45. El Maghraby GMM, Williams AC, Barry BW. Skin delivery of oestradiol from lipid vesicles: importance of liposome structure. Int J Pharm 2000; 204:159–169.
46. Trotta M, Peira E, Debernardi F, Gallarate M. Elastic liposomes for skin delivery of dipotassium glycyrrhizinate. Int J Pharm 2002; 241:319–327.
47. Guo J, Ping Q, Sun G, Jiao C. Lecithin vesicular carriers for transdermal delivery of cyclosporin A. Int J Pharm 2000; 194:201–207.
48. El Maghraby GMM, Williams AC, Barry BW. Skin delivery of 5-fluorouracil from ultradeformable and standard liposomes in-vitro. J Pharm Pharmacol 2001; 53:1069–1077.
49. Cevc G, Blume G, Schätzlein A. Transfersomes-mediated transepidermal delivery improves the regiospecificity and biological activity of corticosteroids in vivo. J Control Release 1997; 45:211–226.
50. Trotta M, Peira E, Carlotti ME, Gallarate M. Deformable liposomes for dermal administration of methotrexate. Int J Pharm 2004; 270:119–125.
51. Honeywell-Nguyen PL, van den Bussche MH, Junginger HE, Bouwstra JA. The effect of surfactant-based elastic and rigid vesicles on the penetration of lidocaine across human skin. STP Pharma 2002; 12:257–262.
52. Bouwstra JA, De Graaff A, Groenik W, Honeywell L. Elastic vesicles: interaction with human skin and drug transport. Cell Mol Biol Lett 2002; 7:222–223.
53. Honeywell-Nguyen PL, Arenja S, Bouwstra JA. Skin penetration and mechanisms of action in the delivery of the D2-agonist rotigotine from surfactant-based elastic vesicle formulation. Pharm Res 2003; 20:1619–1625.

54. Touitou E. Compositions for applying active substances to or through the skin. US patent 5,540,934 (1996).
55. Fang JY, Yu SY, Wu PC, Huang YB, Tasi YH. In vitro skin permeation of oestradiol from various proniosome formulations. Int J Pharm 2001; 215:91–99.
56. Vora B, Khopade AJ, Jain NK. Proniosome based transdermal delivery of levonorgestrel for effective contraception. J Control Release 1998; 54:149–165.
57. Touitou E, Godin B, Weiss C. Enhanced delivery of drugs into and across the skin by ethosomal carriers. Drug Dev Res 2000; 50:406–412.
58. Godin B, Touitou E. Mechanism of bacitracin permeation enhancement through the skin and cellular membranes from an ethosomal carrier. J Control Release 2004; 94:365–379.
59. Touitou E, Godin B, Dayan N, Weiss C, Piliponsky A, Levi-Schaffer F. Intracellular delivery mediated by an ethosomal carrier. Biomaterials 2001; 22:3053–3059.
60. Horwitz E, Pisanty S, Czerninski R, Helser M, Eliav E, Touitou E. A clinical evaluation of a novel liposomal carrier for acyclovir in the topical treatment of recurrent herpes labialis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1999; 87:700–705.
61. Lodzki M, Godin B, Rakou L, Mechoulam R, Gallily R, Touitou E. Cannabidiol-transdermal delivery and anti-inflammatory effect in murine model. J Control Release 2003; 93:377–387.

14
Topical Delivery by Iontophoresis

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INTRODUCTION

Passive permeation of drugs across the skin is limited by the low permeability of the stratum corneum. Transdermal and topical delivery of drugs are presently applicable to only a few drugs with appropriate balance hydro/lipophilicity, small size, no charge, and relatively high potency (1,2).

Strategies have been developed to increase the transport across (transdermal delivery) or into (topical delivery) the skin. They consist in increasing the permeability of the skin or providing a driving force acting on the drug. Chemical methods (e.g., penetration enhancers) or physical methods (e.g., iontophoresis, sonophoresis, or electroporation) have been shown to significantly enhance transdermal transport (2–4).

Iontophoresis is a noninvasive technique which uses a mild electric current to facilitate transdermal delivery of drugs for both systemic and local effects. Iontophoretic transport of drugs has been extensively studied (5–10). It has the potential to overcome many of the barriers to topical drug absorption (8–15).

This chapter will focus on local delivery by iontophoresis as an aid in penetrating topically applied drugs. The mechanisms and the parameters affecting iontophoretic transport will be reviewed and the role of iontophoresis in clinical practice and cosmetics will be discussed.

IONTOPHORESIS

Iontophoresis may be defined as the administration of molecules through the skin by the application of an electric current (5–10).

An iontophoretic system has three basic components: (1) the source of electric current, (2) a reservoir containing the active and an electrode as well as a counter electrode in a return reservoir, and (3) a control unit of parameters. The current used for iontophoretic delivery is applied for minutes or hours with current density ranging from 0.1 to 0.5 mA/cm^2. Miniaturized systems, approximately 10 cm^2, including a battery have been developed for transdermal drug delivery. For topical delivery of active, the current source can be an external power supply and a larger area can be treated by the current.

Iontophoresis has been widely studied for transdermal drug delivery. It has been used to achieve systemic concentration sufficient for a desired therapeutic effect. In the same way, iontophoresis has also been successfully used in clinical medicine to achieve topical delivery of drugs for several decades. It has found widespread use in physical therapy and dermatology.

The rationales for topical drug delivery by iontophoresis are: (i) to deliver locally high concentration of an active: the delivery of a drug is enhanced by iontophoresis by 1 to 3 orders of magnitude as compared to passive diffusion, minimizing the systemic level of medication; (ii) to control delivery of the active by current application: inter- and intraindividual variations can be reduced, (iii) to extend transdermal transport to low and medium (<5000) molecular weight hydrophilic compounds (5–10,16,17). The literature supports the concept that iontophoresis is a method of choice for drug application in the therapy of surface tissue (11–15).

MECHANISMS OF IONTOPHORETIC TRANSPORT

Theoretical Mechanisms of Iontophoretic Transport

The electrically induced transport of an ion across a membrane results from three mechanisms: (1) diffusion related to a chemical potential gradient, the passive diffusion, (2) electrical mobility owing to an electric potential gradient, electromigration, and (3) solute transfer attributable to a convective solvent flow, i.e., electroosmosis. The sum of these contributions represents the total flux (Eq. (1)) (5–10,17,18). The estimation of the steady-state flux is given by the Nernst–Planck equation (Eq. (2)):

$$J_T = J_P + J_E + J_O \qquad (1)$$

J_T = total flux
J_P = passive diffusion flux
J_E = electrical flux
J_O = electroosmotic flux

$$J_{SS} = -D\mathrm{d}c/\mathrm{d}x - DzcF/RT\mathrm{d}\varepsilon/\mathrm{d}x \qquad (2)$$

J_{SS} = steady-state flux
D = diffusion coefficient
c = concentration
z = valence
F = Faraday's constant
R = gas constant
T = absolute temperature
ε = electrical potential
X = distance

For ionic species, the contribution of passive diffusion is negligible and the major iontophoretic mechanism involved in the transport is electromigration. However, the contribution of electroosmotic flow has been reported to be significant for neutral molecules and macromolecules. Due to its negative charges, when submitted to current, the skin undergoes a net convective solvent flow from the anode to the cathode. Hence, neutral molecules can also be delivered into or extracted from the skin by iontophoresis (10,18–20).

The flux of electrons created by the electrodes is exactly equilibrated by the flux of electrons coming in or getting out of the skin. The restoration of cathode

electroneutrality can be accomplished by two mechanisms: one negative charge can migrate into the skin ($Drug^{-}$ or Cl) or one positive charge can move from the skin towards the cathodal compartment. The opposite is true for the anodal compartment (10).

Pathways for Transport

As for conventional transdermal drug delivery, the molecular transport can take place in the stratum corneum by transcellular or paracellular pathways and/or in the appendages (sweat glands and hair follicles) . The major route of iontophoretic transport is believed to be the appendageal pathway because of its low electrical resistance (21,22). However, recent evidences support the existence of a paracellular route (23–25) and to a lesser extent an intercellular route.

PARAMETERS AFFECTING IONTOPHORETIC DELIVERY

Iontophoretic delivery of compounds into or through the skin is affected by the electrical parameters of iontophoresis, as well as the physicochemical parameters and the formulation of the active. The factors affecting iontophoretic transport have been extensively studied and are summarized in Table 1 (5–10,26,27).

The electrical parameters allow control on drug transport. Increasing the current density and/or the duration of current application enhances the delivery of the active into or through the skin. The use of pulsed current rather than constant current can be used to avoid skin polarization but usually decreases active transport.

The design of electrodes is also important. Both inert and active electrodes can be used for the transport of molecules by iontophoresis. Inert electrodes such as platinum or stainless steel induce electrolysis of water and consequently pH shift of the solution requiring the presence of a buffer which increases the number of competitive ions. In an iontophoretic circuit with active electrodes, an oxidation at the anode and a

Table 1 Parameters Affecting Iontophoretic Transport

	Parameters increased	Effect on iontophoretic transport
Electrical parameters of iontophoresis	Current density	↗
	Duration of current application	↗
	Current waveform	↘/↗
	Electrode design	↘/↗
	Area of current application	↗
Physicochemical properties of the active	Molecular weight	↘
	Charge	↗/↘
	Partition coefficient (lipophilicity)	↘
Formulation of the active	pH: ionization	↗/↘
	Competitive ions	↘
	Viscosity	↘

Source: From Refs. 10, 26.

reduction at the cathode occur. In the particular case of the Ag/AgCl electrodes, reduction of AgCl in Ag at the cathode results in the release of chloride ions while oxidation of Ag in Ag^+ at the anode will require chlorides ions to form AgCl (10). The polarity of the electrodes has to be adapted to the charge of the drug: anodal delivery for positively charged or neutral molecules and cathodal delivery for negative compounds.

The physicochemical properties of the molecule have to be evaluated. The charge, the molecular weight, and the lipophilicity are critical in the choice of a candidate. The charge is a criterion difficult to isolate from the other important parameters and has to be evaluated as the case may be. Meanwhile, best results are generally observed with charged molecules. Cationic lipophilic drugs have been reported to decrease or reverse electroosmosis flux (10,19). Most of the time, an inverse relation is found between size and lipophilicity and flux. Hence lipophilic and large molecules (>10,000 g/mol) are not good candidates for iontophoresis (10). Worth noticing is that the physicochemical properties of an ideal candidate for iontophoresis (hydrophilic, ionized) are different from those needed for classical topical formulations as passive delivery is limited to small (<500 g/mol) lipophilic drugs.

The formulation of the active reservoir as well as the counter electrode reservoir also affects iontophoretic transport. Decreasing the amount of competitive ions enhances the transport. Increasing drug ionization by modifying pH usually enhances permeation (9,10).

To enhance the delivery of a drug in the skin, the formulation of the reservoir and the electrode design have to be optimized. Once the formulation has been optimized and fixed, the control of active delivery can be achieved by modifying the current density and the duration of current application (5).

Hence, the prerequisites for efficient delivery by iontophoresis are (i) a good aqueous solubility (>1 mg/ml), (ii) a formulation with a pH allowing ionization of the active and a low concentration of competitive ions, and (iii) a polarity of electrodes allowing electromigration (anodal or cathodal iontophoresis for positive drugs and for negative drugs, respectively) and/or electroosmosis (anodal iontophoresis) (2,26).

EFFECTS OF IONTOPHORESIS ON THE SKIN: SAFETY ISSUES

Evidence for the safety of iontophoresis comes from (i) the long clinical experience with topical iontophoretic delivery, (ii) the noninvasive investigations in animals and humans, (iii) the biophysical studies of the stratum corneum, and (iv) the histological studies.

Effect of Iontophoresis on the Stratum Corneum

The effect of iontophoresis on the stratum corneum structure has been extensively studied by biophysical and histological methods. The effect of iontophoresis on the stratum corneum has been reviewed (28). As shown in Table 2, the major modifications of the stratum corneum induced by iontophoresis include an increased stratum corneum hydration and a disorganization of the lipid lamellae.

Bioengineering Investigations of the Effect of Iontophoresis on the Skin

Noninvasive bioengineering methods have been used in animals as well as in humans to investigate the effect of current applications in vivo (Table 3). The barrier function

Table 2 Influence of Iontophoresis on the Stratum Corneum

Methods	Effect	References
Impedance	Decreased resistance	29,30
ATR-FTIR	Increased hydration	31,32
	No change in lipid fluidity	
X-ray scattering		
small angle	Disorganization of the lipid lamellae spacing	32
wide angle	No change in the lipid packing in the lamellae	32
Freeze fracture electron microscopy	Disorganization of the intercellular lipid lamellae	28

Source: From Ref. 28.

of the skin is hardly modified by iontophoresis as measured by transepidermal water loss. Laser Doppler velocimetry and Chromametry confirm that a mild and reversible erythema is induced by current application. The higher the density or the duration of current application, the higher the erythema (28).

Tolerance and Safety Issues Associated with Iontophoresis

The clinical literature on the application of low intensity current for topical drug delivery supports the fact that iontophoresis is a safe procedure. In general, a minor erythema is observed by visual scoring or bioengineering methods. Draize score indicates in most studies that iontophoresis leads to mild erythema which almost disappears within a maximum of 24 hours after patch removal (36,38). The parameters affecting the sensation of current application have been reviewed (39).

A regional variation in function of the skin and irritation because of iontophoresis has been observed: For example, erythema score and skin reaction were greater at the chest than the abdomen or upper arm (37). There are overall no gender effects or over all ethnic group effect on the bioengineering and clinical changes after iontophoresis (36).

Interestingly, with saline iontophoresis, the erythema at the cathode is usually higher than that at the anode (30,36). The composition of the electrode reservoir could influence the irritation induced by the current and should be optimized to decrease this irritation.

In patients using the Glucowatch biographer® for noninvasive glucose monitoring by reverse iontophoresis for 12 hours (three minutes at $0.3\,mA/cm^2$ every

Table 3 Bioengineering Investigations of the Effect of Iontophoresis on the Skin

Methods	Effect	References
Transepidermal water loss	Transient increase (due to an increased hydration)	(29,30,33–35)
Laser Doppler velocimetry	Transient increase in cutaneous blood flow	(30,33–35)
Chromametry	Transient increase in redness	(33,35)
Capacitance		(36,37)
Skin temperature	No change or minor decrease	(36,37)

Source: From Ref. 28.

10 minutes), none or mild erythema or edema was reported in the vast majority of Glucowatch biographer® applications (40).

In conclusion, the clinical use as well as experimental studies attests to the overall safety of iontophoresis and the absence of long term side effects. Nevertheless, it should be pointed out that iontophoresis is not without potential injury if not used correctly. The major danger in all iontophoretic treatments is the occurrence of skin irritation and burns. Pain sensation can be used as a criterion for the prevention of skin burns as a consequence of excessive densities ($>0.5\,mA/cm^2$). If the electrode metal touches the skin, burns can be caused by excessive current at the site of contact. The solute and the excipients in the solution being delivered can also influence the reaction of the skin.

TOPICAL DELIVERY OF DRUGS AND COSMETICS BY IONTOPHORESIS

Topical Iontophoretic Delivery

The main rationale to use iontophoresis for topical delivery is to achieve a higher concentration of the active in the skin as well as to avoid a systemic distribution of the drugs.

Besides in vitro studies with diffusion cells, several techniques used to study the local concentration in the skin (microdialysis, tape-stripping, confocal scanning laser microscopy, analysis after cryomicrotomization, autoradiography, etc.) confirm the advantage of iontophoresis for topical delivery (25,41,42). It has been shown for many drugs that iontophoresis enhances the amount of permeant in the skin by one to three orders of magnitude.

Clinical Applications of Topical Iontophoretic Transport

Iontophoresis has been successfully used in medicine to achieve topical delivery of drugs and actives. Most of the clinical applications of iontophoresis were developed in physical therapy, cosmetics, and dermatology. The key areas, where efficacy has been demonstrated in clinical studies, include treatment of hyperhidrosis, local anesthesia, local treatment of inflammation, and skin cancer. In some cases, notably for the delivery of cosmetics, the ability of the medication to penetrate the target tissue in sufficient quantities to produce a clinical effect was not studied in controlled clinical trials.

Iontophoretic delivery of pilocarpine is extensively used for the diagnosis of cystic fibrosis. It enhances sweat secretion, allowing the measure of chloride concentration in the sweat (43). Cystic fibrosis indicators are commercially available.

Tap water iontophoresis has been widely used for the treatment of hyperhidrosis. It is effective in the management of hyperhidrosis for the axillae, palms, and soles by reducing sweat production with only mild and temporary side effects. The exact mechanism of action remains unknown (44,45). Current is typically applied in 10 to 20 minutes per session which needs to be repeated two or three times per week followed by a maintenance program (11). Commercial devices have been marketed. Iontophoresis of actives such as anticholinergic agent and aluminum chloride can increase the average remission.

The successful use of iontophoretic delivery of lidocaine for local anesthesia of the skin has been reported in a variety of situations including painless venipuncture, painless dermatological procedures such as pulsed dye ablation of port-wine stains, laceration repairs, IV catheter insertion, laser surgery and shave biopsy (46–63). The advantages of iontophoresis induced anesthesia include the painless procedure, the

adequate local concentration with a very low systemic distribution (57,58), and the quick onset of action as compared to anesthesia using a eutectic mixture of local anesthetics (10 vs. 60 minutes). The depth of anesthesia was also improved (6–10 mm vs. 3–5 mm), allowing more potential applications (59–63). The disadvantages are a higher cost compared to eutectic mixture of local anesthetic and the inability to treat more than one site at the same time. The first drug iontophoresis device combination approved by the FDA is Iontocaine®. This belongs to a first-generation device with a reservoir that has to be refilled. Recently, a new system, developed by Vyteris, prefilled and less cumbersome is on the way to be approved.

Iontophoresis was also investigated for the delivery of nonsteroidal anti-inflammatory agents to avoid side effects and obtain local administration. The transdermal or topical transport of various molecules is enhanced by iontophoresis. Piroxicam, ketoprofen, and diclofenac have already been tested in in vivo human studies (64–69).

Iontophoresis can also facilitate the penetration of active molecules in the deep tissue underlying the skin. In rabbits subcutaneous tissue and muscle beneath the drug application site were significantly greater than plasma concentrations or similar unexposed tissue (66). Iontophoresis of dexamethasone sodium phosphate has been reported to deliver clinically effective doses as high as those obtained when using standard therapies and to be effective for the treatment of patients with musculoskeletal inflammation such as tendinitis, arthritis, or carpal tunnel syndrome (70–73).

For the treatment of skin cancer, miscellaneous drugs were tested. Of particular interest is the iontophoretic delivery of aminolevulinic acid which is associated with photodynamic therapy (74–76).

Methotrexate® (77–79), khellin® (80), and cyclosporin A® (81) were investigated for a topical treatment of psoriasis. All the studies show that their local concentration is increased by iontophoresis but clinical studies are still lacking to confirm the concept.

Antiviral drugs such as idoxuridine®, acyclovir®, or vidarabin® can be delivered topically by iontophoresis (12,13,42,82–85). Promising results could be obtained with this technique in the treatments of pathologies such as herpes simplex virus, postherpetic neuralgia, and active zooster lesions. Skin acyclovir concentrations monitored by microdialysis were shown to be increased following iontophoresis as compared to passive diffusion and were lower than that obtained by IV bolus with high dose. However, systemic exposure to acyclovir was negligible. Increasing current density enhanced acyclovir concentration in the skin (42).

Other studies on topical iontophoresis include the treatment of warts with sodium salicylate® (86), calcium deposit with acetic acid (87), improvement of peripheral microcirculation by PGE_1 (88,89), prevention of skin ageing with L-ascorbic acid (90), evaluation of topical antihistaminic drugs after histamine iontophoresis (91,92), and finally the treatment of acne scars (93) or hypertrophic scars (94,95).

CONCLUSIONS

Iontophoresis has gained a great deal of attention during the last two decades for both systemic and topical delivery. It offers a convenient and safe means to enhance the topical concentration of drugs in the skin and even in deeper underlying tissue as compared to passive diffusion or systemic delivery, this allowing to widen the number of molecules susceptible to be administrated locally. It is particularly attractive for the delivery of low molecular weight (<1000 g/mol) hydrophilic solutes at

the site of action. Moreover, iontophoresis enables precise control of topical delivery by varying electrical current.

The rationale for using iontophoresis to deliver active in cosmetics and the technology for safe, optimized, and controlled iontophoretic transport are well established. However, further double blind clinical studies are needed to confirm the interest of iontophoresis in specific cosmetic uses.

REFERENCES

1. Hadgraft J, Guy RH. Transdermal drug delivery. New York: Marcel Dekker, 2003.
2. Naik A, Kalia YN, Guy RH. Transdermal drug delivery; overcoming the skin's barrier function. PSST 2000; 3:318–326.
3. Walters K, Hadgraft J eds. Pharmaceutical skin permeation enhancement. New York: Marcel Dekker, 1993.
4. Barry B, Williams A. Parmeation enhancement through skin. In: Swarbick J, Boylan J, eds. Encyclopedia of Pharmaceutical Technology. 11. 1995; 449–493.
5. Sage B. Iontophoresis. In: Swarbick J, Boylan J, eds. Encyclopedia of Pharmaceutical Technology. 8. 1993;217–247.
6. Singh P, Maibach H. Iontophoresis in drug delivery: basic principles and applications. Crit Rev Therap Drug Carrier Syst 1994; 11:161–213.
7. Singh P, Maibach H. Iontophoresis: an alternative to the use of carriers in cutaneous drug delivery. Adv Drug Del Rev 1996; 18:379–394.
8. Roberts M, Lai M, Cross S, Yoshida N. Solute transport as a determinant of iontophoretic transport. In: Potts R, Guy RH, eds. Mechanisms of Transdermal Drug Delivery. New York: Marcel Dekker, 1997:291–349.
9. Delgado-Charro MB, Guy RH. Transdermal iontophoresis for controlled drug delivery and non-invasive monitoring. STP Pharm 2001; 11:403–414.
10. Kalia YN, Naik A, Garrison J, Guy RH. Iontophoretic drug delivery. Adv Drug Del Rev 2004; 56:619–658.
11. Banga A. Clinical applications of iontophoresis devices for topical dermatological delivery. In: Banga A, ed. Electrically Enhanced Transdermal Drug Delivery. Taylor & Francis, 1998:57–74.
12. Gargarosa L, Ozawa A, Ohkido M, Shimomura Y, Hill J. Iontophoresis for enhancing penetration of dermatologic and antiviral drugs. J Dermatol 1995; 22:865–875.
13. Gargarosa L, Hill M. Modern iontophoresis for local drug delivery. Int J Pharm 1995; 123:159–171.
14. Singh J, Bhatia K. Topical iontophoretic drug delivery: pathways, principles, factors and skin irritation. Med Res Rev 1996; 16:285–296.
15. Costello C, Jeshe A. Iontophoresis: applications in transdermal medication delivery. Phys Ther 1995; 75:554–563.
16. Green P. Iontophoretic delivery of peptides drug. J Control Release 1996; 41:33–48.
17. Phipps JB, Gyory J. Transdermal ion migration. Adv Drug Del Rev 1992; 9:137–176.
18. Pikal M. The role of electroosmotic flow in transdermal iontophoresis. Adv Drug Del Rev 1992; 9:201–237.
19. Hirvonen Y, Guy RH. Transdermal iontophoresis: modulation of electroosmosis by polypeptides. J Control Release 1998; 50:283–289.
20. Rao G, Guy RH, Glikfeld P, LaCourse W, Leung L, Tamada J, Potts R, Azimi N. Reverse iontophoresis: non invasive glucose monitoring in vivo in humans. Pharm Res 1995; 12:1869–1873.
21. Cullander C. What are the pathways of iontophoretic current flow through mammalian skin? Adv Drug Del Rev 1992; 9:119–135.

22. Scott E, Laplazza A, White H, Phipps B. Transport of ionic species in skin: contribution of pores to the overall skin conductance. Pharm Res 1993; 10:1699–1709.
23. Monteiro-Rivière N. Identification of the pathways of transdermal iontophoretic drug delivery: light and ultrastructural studies using mercuric chloride in pigs. Pharm Res 1994; 11:251–256.
24. Turner N, Ferry L, Price M, Cullander C, Guy RH. Iontophoresis of poly-L-lysines: the role of molecular weight. Pharm Res 1997; 14:1322–1331.
25. Regnier V, Préat V. Localization of a FITC-labeled phosphorothioate oligodeoxynucleotide in the skin after topical delivery by iontophoresis and electroporation. Pharm Res 1998; 15:1596–1602.
26. Préat V, Vanbever R, Jadoul A, Regnier V. Electrically enhanced transdermal drug delivery: iontophoresis vs electroporation. In: Couvreur P, Duchêne D, Green P, Junginger H, eds. Transdermal Administration, A Case Study, Iontophoresis. Paris: Editions de la santé, 1997:58–67.
27. Jadoul A, Mesens J, de Beukelaer F, Crabbé R, Préat V. Transdermal permeation of alnitidan by iontophoresis: in vitro optimization and human pharmacokinetic data. Pharm Res 1996; 13:1347–1352.
28. Jadoul A, Bouwstra J, Préat, V. Effects of iontophoresis and electroporation on the stratum corneum. Review of the biophysical studies. Adv Drug Del Rev 1999; 35:89–106.
29. Kalia Y, Nomato LD, Guy RH. The effect of iontophoresis on skin barrier integrity: non invasive investigation by impedance spectroscopy and transepidermal water loss. Pharm Res 1996; 13:957–961.
30. Craane-vanHinsberg W, Verhoef J, Spies F, Bouwstra J, Gooris G, Junginger H, Boddé H. Electroperturbation on the human skin barrier in vitro (I) the influence of current density on the thermal behaviour of skin impedance. Eur J Pharm Biopharm 1997; 43:43–50.
31. Thysman S, Van Neste D, Préat V. Non invasive investigation of human skin after in vivo iontophoresis. Skin Pharmacol 1995; 8:229–236.
32. Jadoul A, Doucet J, Durand D, Préat V. Modifications induced on stratum corneum by iontophoresis: ATR-FTIR and X-ray scattering studies. J Control Release 1996; 42:165–173.
33. Fouchard D, Hueber F, Teillaud E, Marty JP. Effect of iontophoretic current flow on hairless rat skin in vivo. J Control Release 1997; 49:89–99.
34. Vandergeest R, Elshove D, Danhof M, Lavrijsen A, Boddé H. Non-invasive assessment of skin barrier integrity and skin irritation following iontophoretic current application in humans. J Control Release 1996; 41:205–213.
35. Vanbever R, Fouchard D, Jadoul A, De Morre N, Préat V, Marty JP. In vivo non-invasive evaluation of hairless rat skin after high-voltage pulse exposure. Skin Pharmacol Appl Skin Physiol 1998; 11:23–34.
36. Singh J, Gross M, Sage B, Davis HT, Maibach HI. Effect of saline iontophoresis on skin barrier function and cutaneous irritation in four ethnic groups. Food Chem Toxicol 2000; 38:717–726.
37. Singh J, Gross M, Sage B, Davis HT, Maibach HI. Regional variations in skin barrier function and cutaneous irritation due to iontophoresis in human subjects. Food Chem Toxicol 2001; 39:1079–1086.
38. Ledger P. Skin biological in electrically enhanced transdermal delivery. Adv Drug Del Rev 1992; 9:289–307.
39. Prausnitz M. The effects of electric current applied to skin: a review for transdermal drug delivery. Adv Drug Del Rev 1996; 18:395–425.
40. Tierney MJ, Tamada JA, Potts RO, Jovanovi L, Garg S. Cygnus Research Team. Clinical evaluation of the Glucowatch biographer: a continual, non-invasive glucose monitor for patients with diabetes. Biosens Bioelectron 2001; 16:621–629.
41. Jadoul A, Hanchard C, Thysman S, Préat V. Quantification and localization of fentanyl and TRH delivered by iontophoresis in the skin. Int J Pharm 1995; 120:221–228.
42. Stagni G, Ehsan Ali M, Weng D. Pharmacokinetics of acyclovir in rabbit skin after IV-bolus, ointment and iontophoretic administrations. Int J Pharm 2004; 274:201–211.

43. Gibson L, Cooke R. A test for concentration of electrolytes in sweat in cystic fibrosis of the pancreas utilizing pilocarpine by iontophoresis. Pediatrics 1959; 23:545.
44. Hill A, Baker G, Jansen G. Mechanism of action of iontophoresis in the treatment of palmar hyperhidrosis. Cutis 1981; 28:69–72.
45. Holzle E, Alberti N. Long term efficacy and side effects of tap water iontophoresis of palmoplantar hyperhidrosis-the usefulness of home therapy. Dermatologica 1987; 175:126.
46. Russo J, Lipman AG, Comstock TJ, Page BC, Stephen RL. Lidocaine anesthesia: comparison of iontophoresis, injection and swabbing. Am J Hosp Pharm 1980; 37:843–847.
47. Maloney JM, Bezzant JL, Stephen RL, Petelenz TJ. Iontophoretic administration of lidocaine anaesthesia in office practice an appraisal. J Dermatol Surg Oncol 1992; 18:937–940.
48. Ashburn MA, Gauthier M, Love G, Basta S, Gaylord B, Kessler K. Iontophoretic administration of 2% lidocaine HCl and 1:100.000 epinephrine in humans. Clin J Pain 1997; 13:22–26.
49. Rose JB, Galinkin JL, Jantzen EC, Chiavacci RM. A study of lidocaine iontophoresis for pediatric venipuncture. Anesth Analg 2002; 94:867–871.
50. Zempsky WT, Parkinson TM. Lidocaine iontophoresis for local anesthesia before shave biopsy. Dermatol Surg 2003; 29:627–630.
51. Kennard CD, Whitaker DC. Iontophoresis of lidocaine for anesthesia during pulsed dye laser treatment of port-wine stains. J Dermatol Surg Oncol 1992; 18:287–294.
52. Nunez M, Miralles ES, Boixeda P, Gomez F, Perez B, Abraira V, Ledo A. Iontophoresis for anesthesia during pulsed dye laser treatment of port-wine stains. Pediatr Dermatol 1997; 14:397–400.
53. Bezzant JL, Stephen RL, Petelenz TJ, Jacobsen SC. Painless cauterization of spider veins with the use of iontophoretic local anesthesia. J Am Acad Dermatol 1988; 19:869–875.
54. Greenbaum SS. Iontophoresis as a tool for anesthesia in dermatologic surgery: an overview. Dermatol Surg 2001; 27:1027–1030.
55. DeCou JM, Abrams RS, Hammond JH, Lowder LR, Gauderer MW. Iontophoresis: a needle-free, electrical system of local anesthesia delivery for pediatric surgical office procedures. J Pediatr Surg 1999; 34:946–949.
56. Lener EV, Bucalo B, Kist D, Moy R. Topical anesthetic agents in dermatologic surgery: a review. Dermatol Surg 1997; 23:673–683.
57. Riviere JE, Montiero-Riviere NA, Inman AO. Determination of lidocaine concentrations in skin after transdermal iontophoresis: effects of vasoactive drugs. Pharm Res 1992; 9:211–214.
58. Wallace MS, Ridgeway B, Jun E, Schulteis G, Rabussay D, Zhang L. Topical delivery of lidocaine in healthy volunteers by electroporation, electroincorporation, or iontophoresis: an evaluation of skin anesthesia. Regional Anesth Pain Med 2001; 26:229–238.
59. Irsfeld S, Klement W, Lipfert P. Dermal anaesthesia: comparison of EMLA cream with iontophoretic local anaesthesia. Br J Anaesth 1993; 71:375–378.
60. Bjerring P, Arendt-Nielsen L. Depth and duration of skin analgesia to needle insertion after topical application of EMLA cream. Br J Anaesth 1990; 64:173–177.
61. Galinkin JL, Rose JB, Harris K, Watcha MF. Lidocaine iontophoresis versus eutectic mixture of local anesthetics (EMLA) for IV placement in children. Anesth Analg 2002; 94:1484–1488.
62. Greenbaum SS, Bernstein EF. Comparison of iontophoresis of lidocaine with a eutectic mixture of lidocaine and prilocaine (EMLA) for topically administered local-anesthesia. J Dermatol Surg Oncol 1994; 20:579–583.
63. Wahlgren CF, Quiding H. Depth of cutaneous analgesia after application of a eutectic mixture of the local anesthetics lidocaine and prilocaine (EMLA cream). J Am Acad Dermatol 2000; 42:584–588.
64. Curdy C, Kalia YN, Naik A, Guy RH. Piroxicam delivery into human stratum corneum in vivo : iontophoresis versus passive diffusion. J Control Release 2001; 76:73–79.

65. Fang JY, Sung KC, Hung-Hong L, Fang CL. Transdermal iontophoretic delivery of diclofenac sodium from various polymer formulations: in vitro and in vivo studies. Int J Pharm 1999; 178:83–92.
66. Hui X, Anigbogu A, Singh P, Xiong G, Poblete N, Liu P, Maibach HI. Pharmacokinetic and local tissue disposition of [14C] sodium diclofenac following iontophoresis and systemic administration in rabbits. J Pharm Sci 2001; 90:1269–1276.
67. Garagiola U, Dacatra U, Braconaro F, Porretti E, Pisetti A, Azzolini V. Iontophoretic administration of pirprofen or lysine soluble aspirin in treatment of rheumatic diseases. Clin Ther 1988; 10:553–558.
68. Saggini R, Zoppi M, Vecchiet F, Gatteschi L, Obletter G, Giamberardino MA. Comparison of electromotive drug administration with ketorolac or with placebo in patients with pain from rheumatic disease: a double masked study. Clin Ther 1996; 18:1169–1174.
69. Panus PC, Campbell J, Kulkarni SB, Herrick RT, Ravis WR, Banga AK. Transdermal iontophoretic delivery of ketoprofen through human cadaver skin and in humans. J Control Release 1997; 44:113–121.
70. Hasson S, Daniels J, Schieb, D. Exercise training and dexamethasone iontophoresis in rheumatoid arthritis. Physiotherapy Canada 1991; 43:11–14.
71. Bertolucci LE. Introduction of antiinflammatory drugs by iontophoresis: double blind study. J Orthop Sports Phys Ther 1982; 4:103–108.
72. Glass JM, Stephen RL, Jacobson SC. The quantity and distribution of radiolabeled dexamethasone delivery to tissue by iontophoresis. Int J Dermatol 1980; 19:519–525.
73. Petelenz TJ, Buttke JA, Bonds C, Lloyd LB, Beck JE, Stephen RL, Jacobsen SC, Rodriguez P. Iontophoresis of dexamethasone: laboratory studies. J Control Release 1992; 20:55–66.
74. Lopez RFV, Bentley MVL, Delgado-Charro MB, Guy RH. Optimization of aminolevulinic acid delivery by iontophoresis. J Control Release 2003; 88:65–70.
75. Lopez RFV, Bentley MVL, Delgado-Charro MB, Guy RH. Iontophoretic delivery of 5-aminolevulinic acid (ALA): effect of pH. Pharm Res 2001; 18:311–315.
76. Rhodes L, Tsoukas M, Anderson R, Kollias N. Iontophoretic delivery of ALA provides a quantitative model for ALA pharmacokinetics and PpIX phototoxicity in human skin. J Invest Dermatol 1997; 108:87–91.
77. Alvarez-Figueora MJ, Delgado-Charro MB, Blanco-Mendez J. Passive and iontophoretic transdermal penetration of methotrexate. Int J Pharm 2001; 212:101–107.
78. Alvarez-Figueora MJ, Blanco-Mendez J. Transdermal delivery of methotrexate: iontophoretic delivery from hydrogels and passive delivery from microemulsions. Int J Pharm 2001; 215:57–65.
79. Stagni G, Shukla C. Pharmacokinetics of methotrexate in rabbit skin and plasma after iv-bolus and iontophoretic administrations. J Control Release 2003; 93:283–292.
80. Marconi B, Mancini F, Colombo P, Allegra F, Gioardano F, Gazzaniga A, Orecchia G, Santi P. Distribution of khellin in excised human skin following iontophoresis and passive dermal transport. J Control Release 1999; 60:261–268.
81. Boinpally RR, Zhou SL, Devraj G, Anne PK, Poondru S, Jasti BJ. Iontophoresis of lecithin vesicles of cyclosporin A. Int J Pharm 2004; 274:185–190.
82. Volpato NM, Nicoli S, Laureri C, Colombo P, Santi P. In vitro acyclovir distribution in human skin layers after transdermal iontophoresis. J Control Release 1998; 50:291–296.
83. Volpato NM, Sauli P, Colombo P. Iontophoresis enhances the transport of acyclovir through nude mouse skin by electrorepulsion and electroosmosis. Pharm Res 1995; 12:1623–1627.
84. Park NH, Gangarosa LP, Kwon BS, Hill JM. Iontophoretic application of adenine arabinoside monophosphate to herpes simplex virus type 1-infected hairless mouse skin. Antimicrob Agents Chemother 1978; 14:605–608.
85. Park NH, Gangarosa LP, Hill JM. Iontophoretic application of ara-AMP (9-β-D-arabinofuranosyladenine-5′-monophosphate) into adult mouse skin. Proc Soc Exp Biol Med 1977; 156:326–329.

86. Gordon A, Weinstein M. Sodium salicylate iontophoresis in the treatment of plantar warts. Phys Ther 1968; 49:869.
87. Kahn J. Acetic acid iontophoresis for calcium deposit. Phys Ther 1977; 57:658.
88. Asai J, Fukuta K, Torii S. Topical administration of prostaglandin E_1 with iontophoresis for skin flap viability. Ann Plast Surg 1997; 38:514–517.
89. Saeki S, Yamamura K, Matsushita M, Niishikimi N, Sakurai T, Nimura Y. Iontophoretic application of prostaglandin E1 for improvement in peripheral microcirculation. Int J Clin Pharmacol Ther 1998; 36:525–529.
90. Ebihara M, Akyama M, Ohnishi Y, Tajima S, Komata K, Mitsui Y. Iontophoresis promotes percutaneous absorption of L-ascorbic acid in rat skin. J Dermatol Sci 2003; 32:217–222.
91. Thysman S, Jadoul A, Leroy T, Van Neste D, Préat V. Laser Doppler evaluation of skin reaction in volunteers after histamine iontophoresis. J Control Release 1995; 36:215–219.
92. Leroy T, Tasset C, Valentin B, Van Neste D. Comparison of the effects of cetirizine and ebasine on the skin response to histamine iontophoresis monitored with laser Doppler flowmetry. Dermatology 1998; 197:146–151.
93. Schmidt JB, Binder M, Macheines UV, Bieglmager C. New treatment of atrophic acne scars by iontophoresis with estriol and tretinoin. Int J Dermatol 1995; 34:53–57.
94. Shigeni S, Murakami T, Yata N, Ikuta Y. Treatment of keloid and hypertrophic scars by iontophoretic transdermal delivery of tranilast. Scan J Plast Reconstr Surg Hand Surg 1997; 31:151–158.
95. Zhao L, Hung L, Choy T. Delivery of medication by iontophoresis to treat post-burn hypertrophic scars: investigation of a new electronic technique. Burns 1997; 23(suppl 1): S27–S29.

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Using Iontophoresis to Enhance Cosmetics Delivery

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INTRODUCTION

Bringing active molecules to the target site of action is mandatory to attain a biological effect, thus increasing dermal penetration of active cosmetic agents can be considered as the panacea of modern cosmetology. Iontophoresis is a virtually painless procedure that uses a mild electrical current to deliver water-soluble, ionized compounds into intact skin and the underlying tissue.

This article provides an introduction to iontophoresis and discusses possible applications of iontophoresis in cosmetics. It also describes recent advances in making this technique accessible for consumers at home.

DELIVERING ACTIVES TO THE SKIN

The skin is a multilayer organ, comprised of three major layers, namely, the epidermis, dermis, and hypodermis. The dermis contains connective tissue, blood vessels, occasionally white blood cells, nerve endings, hair follicles, sweat glands, and sebaceous glands. The connective tissue is made up of fibroblasts (cells that are responsible for the production of collagen and elastic fibers).

The epidermis does not contain blood vessels, but has nerve endings and muscle fibers. The epidermis can be further subdivided into the following strata (beginning with the outermost layer): corneum, lucidum, granulosum, spinosum, and basale. Cells are formed through mitosis at the innermost layers. They move up the strata, changing shape and composition, as they differentiate and become filled with keratin. Thus, the dermis and the viable (inner) parts of the epidermis are the target sites for cosmetic active agents aimed to influence biological processes, such as skin aging and pigmentation.

The outermost layer of the epidermis, the stratum corneum (SC) consists of a dense segment of cornified cells (corneocytes), cemented to each other by the epidermal lipids. Typically there are about 15 to 20 cell layers, and the lipid content is 10% to 30%

of the total volume of the SC. Therefore, this horny layer acts as an effective permeability barrier, which prevents the infiltration of certain substances such as micro-organisms, chemicals, and allergens.

This barrier is very effective toward water-soluble compounds, particularly charged molecules that may become suitable for routine dermal delivery only if an external source of energy is provided to drive these active agents into the skin. By contrast, small lipid-soluble molecules can partition into the SC and then diffuse across the lipid bilayer membranes.

IONTOPHORESIS

Iontophoresis is a technique of dermal and transdermal delivery by which charged bioactive molecules (active agents) are transferred into the skin using a weak electrical current (1–5).

In practice, the active agent, in an appropriate carrier (solution, gel, or cream) is placed between the active electrode (also called the "delivery electrode") and the skin. The other electrode (also called the "return electrode") is placed elsewhere on the body. A mild electrical current is applied for a set time period. This process is schematically illustrated in Fig. 1.

It has been observed that for ionic molecules, the major contribution to the overall flux is mainly because of the iontophoretic delivery, whereas the contribution of diffusive delivery and electro-osmosis to ion flux is relatively smaller (6,7).

Electro-osmosis can transport water and also active agents. When a current is applied, there is a flow of water from the electrode's water-containing reservoir into the skin. Any active agent in aqueous solution, ionic or nonionic, will follow the water flow into the skin.

Electro-osmosis is always in the direction of the flow of the counter-ions. Human skin is negatively charged at pH above 4, and the counter-ions are positive ions; therefore, electro-osmotic flow always occurs from anode to cathode. If only nonionic active agents are to be delivered, it may be necessary to add a small quantity of electrolyte, such as sodium chloride, to the solution for conductivity and to establish efficient electro-osmotic flow.

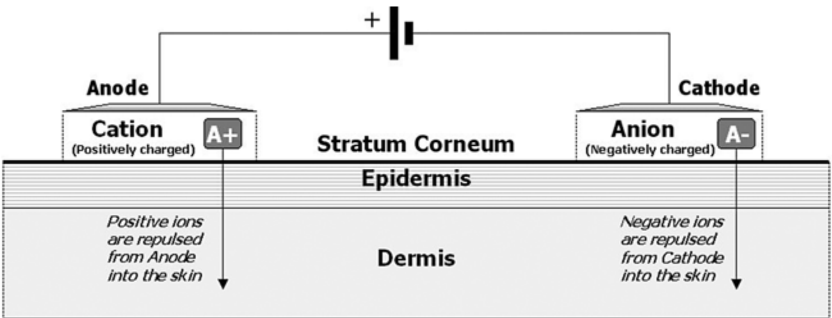

Figure 1 A schematic representation of iontophoretic delivery.

INFLUENCE OF THE ENERGY SOURCE

Numerous variables influence the process of iontophoresis. These factors include the energy source for the process and the characteristics of the active agent and the formulation. Each of these will be discussed in detail.

Current Type

Most iontophoretic devices use a "constant" current. In some devices, the current is initiated at a low level and slowly increased, then lowered again to zero. Some devices have also used a "pulsed" current for delivering the active agent (8). In a few cases and at higher current densities, the use of waved or pulsed currents may reduce irritation and improve tolerability of the users to high current densities.

Current Strength and Duration

The rate at which the ions are introduced into the body with various current strengths can play an important role. When the current is stronger, more ions penetrate at one time, and their accumulation produces the desired local effect and may even build up a reserve of ions that will later be diffused more deeply into the tissues, perhaps resulting in a prolonged effect. In cosmetic iontophoresis, current strength has its "cosmetic acceptability," as the consumer will not accept any significant itching or inflammation following an iontophoretic application.

Therefore, one should not use high current intensities. Notably, Kno et al. (9) have described that the sensation felt by consumers varies on an individual basis.

Size, Charge, and Nature of the Electrodes

The electrode material used should be harmless to the body and sufficiently flexible to be applied close to the body surface. The distribution of the active agent within the skin depends on the size and position of the electrodes. The literature indicates that larger electrodes usually introduce greater amounts of the active agent.

Active Agent's Molecular Size, Charge, and Salt Form

The molecular size, charge, and salt form of an active agent determine its iontophoretic delivery pattern. Monovalent positively charged drugs are delivered with greater efficiency by iontophoresis than monovalent negatively charged anions. This has been ascribed to the net negative charge on the skin.

As the molecular size increases, the diffusivity decreases. Nevertheless, proteins and peptides with high-molecular weight of 3000 to 5000 d have been delivered effectively by iontophoresis (10).

It has been reported that different salt forms have different specific conductivities, which may influence the iontophoretic delivery rate (11). Therefore, it is advisable to carry out conductivity experiments in vitro, which will provide information concerning the general suitability of an active agent for iontophoresis. The salt form of active agents must be considered along with the pH of the solution for determining the amount of active agent in the ionized state.

Active Agent's Concentration

Increased uptake by the skin during and after iontophoresis correlates with an increase in drug concentration (12,13). This is generally true until a plateau level is reached at which no further increase in flux is observed.

Active Agents for Cosmetic Iontophoresis

A variety of active agents can be used in cosmetic iontophoresis. Ionic cosmetic active agents include many anionic molecules (in their salt form), such as alpha- and beta-hydroxy acids (e.g., lactic, glycolic, and salicylic acids), which are important in the treatment of skin aging. Retinoic acid is useful in the treatment of acne and scars. Vocative compounds (such as caffeic, lipoic, and nicotinic acids) are useful in treating cellulite. Vitamins, such as ascorbic acid and its stable salt derivatives, can be used to lighten hyperpigmented skin. These anionic species are delivered under the cathode.

Likewise, cationic active agents used in cosmetics are delivered iontophoretically under the anode. Amino acids and peptides are also favorable candidates for iontophoretic delivery, as long as the pH is in a range that maintains them in ionic state. Many nonionic, yet water-soluble, cosmetic active agents can also be delivered, using electro-osmosis, under the anode.

INFLUENCE OF THE FORMULATION

Formulation variables also influence iontophoretic treatment. These variables include the type of formulation, the pH, and the electrolyte concentration.

Type of Formulation

The migration of the active agent under the influence of the electrical current will differ according to the type of formulation, such as gel or cream or solution. This can be related to the differences in viscosities, material electrical charge, and properties of the formulation excipients.

When creams are used, one should ensure that the conductance of the formulation is sufficient to enable effective current, because transfer of active agent under the influence of the electrical current depends on current strength.

pH

The pH is the determining factor governing the amount of active agent present in the ionized state.

For optimum iontophoresis, it is desirable to have a relatively large proportion of the active agent in the ionized state, and the pH of the formulation should be adjusted accordingly; however at low pH levels, the concentration of hydrogen ions increases, thereby inducing a vascular reaction (vasodilation) through C-fiber activation, which is undesirable (14). Thus, it is preferable to keep the pH close to 7. At pH 5.5 and below, there is an increased risk of vascular reaction and inflammation. Perhaps, pH levels may be lowered owing to the reaction of water electrolysis during iontophoresis. This possibility can be eliminated by the use of a buffer or by selecting appropriate electrode materials.

ELECTROLYTES IN THE FORMULATION

Electrical current is carried by positive and negative ions in solution. There is no major distinction between ions of the same charge, even though they are composed of different chemical elements. In other words, any current that is carried by ions that are not the active agent lessens the effective delivery of the ions one might wish to transport.

Therefore, formulations for iontophoresis should be as pure as practically possible and, in general, contain as few extraneous substances as possible. For water-soluble nonionic active agents that are expected to penetrate through electro-osmosis, electrolytes are required to the level that facilitates the desirable current density.

Any ionic substance in the solution can compete for the current in the iontophoretic process and decrease the effectiveness of delivery of the desirable active agents. So, for most efficient iontophoretic transfer, it is best to have the active agent alone in the solution, unless there is justification to have other ingredients, such as acid or base for pH adjustment, to increase the ratio of ionized/nonionized active agent present.

EXAMPLES OF COSMETIC IONTOPHORESIS

Here are several published examples of uses of iontophoresis and electro-osmosis in cosmetics.

Treatment of Pigmentation Disorders

Vitamin C is known to both inhibit melanin formation and reduce oxidized melanin. However, vitamin C does not easily penetrate the skin. In 2003, Huh et al. reported that iontophoresis treatment using an active form of vitamin C (namely magnesium ascorbyl phosphate or MAP) at 3.6% for 12 weeks resulted in significant reduction of pigmentation. Twelve weeks after iontophoresis, the colorimeter values of the treated site showed a significant decrease in the L value (from 4.60 to 2.78, $p = 0.002$), compared to the control site (from 4.45 to 3.87, $p = 0.142$) (15).

In addition, Power Paper, a developer of thin and flexible iontophoretic patches, has reported that it conducted a controlled human study with its Enhancer® patch and MAP 3% gel, which revealed a 50% and 60% mean reduction of spot size and of pigment intensity within 42 days, respectively. In addition, significant effects were noticeable only after seven days of treatment. This effect was 300% better than the results attained by applying the MAP 3% passively onto the face, without using microelectronic currents.

Treatment of Scars

Atrophic acne scars are a frequent problem after acne, with very limited success of treatment using traditional modalities. In 2002, Schmidt et al. reported on the treatment of postacne scars using iontophoresis with 0.025% tretinoin (vitamin A acid) gel (16). The treatment was performed twice weekly in 32 volunteer patients for a period of three months by applying the substance under a constant direct current of 3 mA for 20 minutes. At the end of treatment, in 94% of patients a significant decrease in the scar depth was observed clinically. In conclusion, tretinoin-iontophoresis was found

to be an effective, noninvasive treatment of atrophic acne scars without causing disturbing side effects. Fernandes (17) has also reported similar results.

Formerly, Schmidt et al. (18) also reported that iontophoresis plus estriol® (mainly topically active estrogen) was also effective in the treatment of postacne scars. In 1980, Tannenbaum (19) reported that iodine iontophoresis reduces scar tissue.

Anti-Wrinkle Effects

Power Paper has recently reported dramatic results of its microiontophoretic patches. The patch is equipped with an integrated electrical cell and a hydrogel interface, intended for use on skin wrinkles.

Human clinical studies on several subjects have shown that a single 20-minute treatment using the patch results in a visible reduction of the number and depth of wrinkles under the eye and at the crow's feet area. The effect was noticed immediately after removal of the patch and lasted for several hours, in some cases for an entire 24-hour period. Repeated treatments resulted in long-lasting effects on skin topography (20).

The short-term effects can be explained by the occurrence of a slight, subclinical inflammatory response, which resulted in skin smoothening. The longer-term

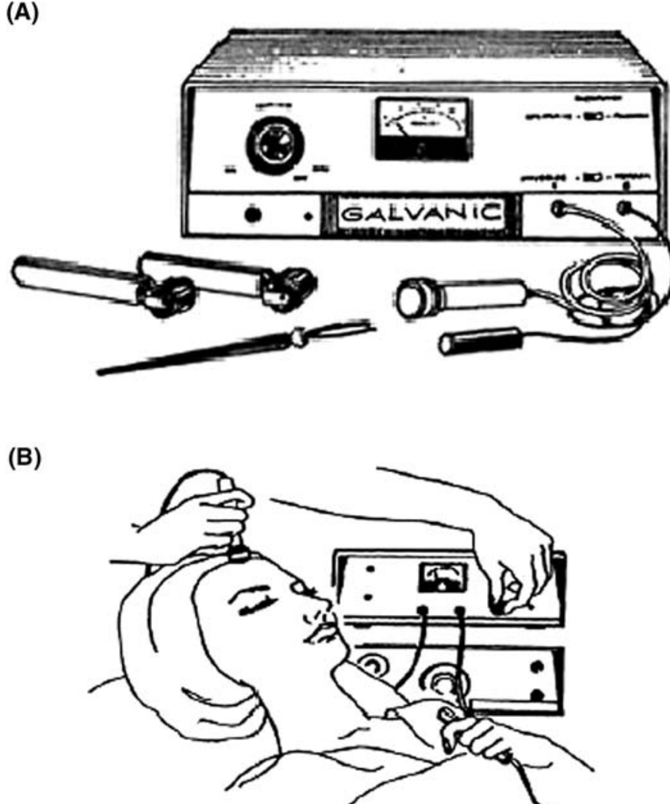

Figure 2 Galvanic treatment equipment used for iontophoretic delivery. (**A**) Stationary cosmetic galvanic equipment, linked to a variety of delivery electrodes. (**B**) The aesthetician holds the delivery electrode and moves it back and forth on the client's face.

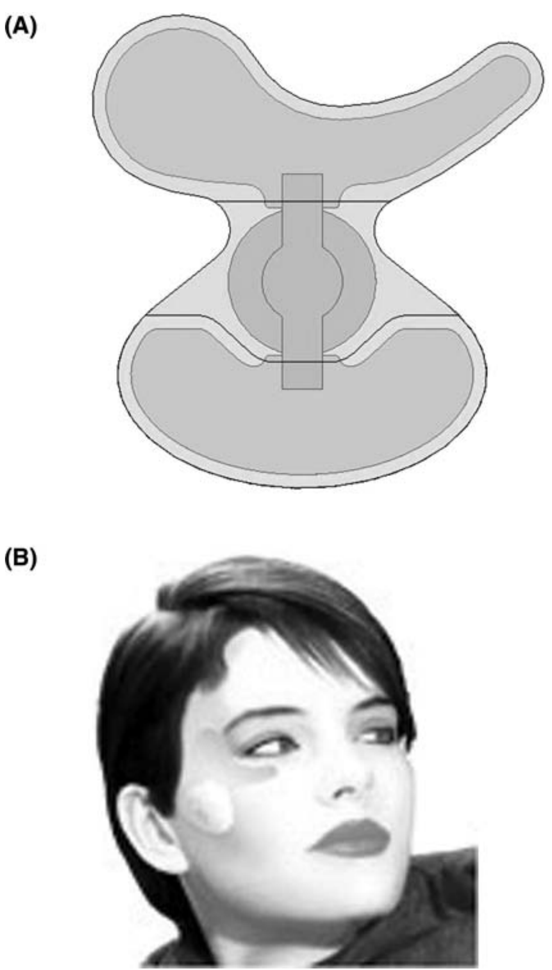

Figure 3 Thin and flexible PowerCosmetics patches. (**A**) An illustration of the "Vitalizer" patch, comprising a thin and flexible battery (in the center) and two electrodes, coated by hydrogel. (**B**) Placement of the "Vitalizer" patch onto the eye contour area.

rejuvenation effects may have resulted from tissue stimulation, enhanced blood flow, improved respiration, and increased cell turnover.

IONTOPHORESIS DEVICES

A typical iontophoresis device consists of an electrical power source (e.g., battery), electrodes, and the active agent, in an appropriate carrier (solution, gel, or cream).

In the iontophoretic process, the current, beginning at the device, is transferred from the electrode through the active agent solution as ionic flow. The active agent ions are delivered to the skin where the repulsion continues moving the active agent through whatever pathways are available, namely, pores and, possibly, through a disrupted SC.

To date, devices used for iontophoresis have been large and cumbersome, and thus have only generally been used in beauty parlors and clinics Fig. 2A (21). Such

instruments include a power supply unit linked to a hand held electrode, usually an anodic one. The esthetician applies an active agent solution, gel, or cream on the skin, and then passes the electrode back and forth on the target area, as illustrated in Fig. 2B. This routine is called "Galvanic treatment."

Smaller devices, ranging from the size of a penlight flashlight to a cellular phone have recently been introduced. Some of the newer units contain the electrodes into the unit itself, thus eliminating the need for additional wiring.

Most recently, a new generation of iontophoretic patches, containing a fully integrated power source, has become available for home use. The patches are enabled through the invention of proprietary thin and flexible, safe and nontoxic, fully disposable electrical power cells and microelectronics incorporated into a simple cosmetic patch Fig. 3. The developer, Power Paper Ltd. has revealed two types of iontophoretic patches. One type[a] boosts the topical delivery of lotions, gels, serum preparations, and other cosmetic formulations. The other type[b] provides immediate effects of wrinkle reduction and skin smoothening. The patches can be designed to suit and target any area of the body.

CONCLUSION

Iontophoresis is an active means to deliver active agents into the skin and to achieve enhanced cosmetic benefits in a variety of skin disorders. Use of the appropriate combination of electrical current and the active agent can provide superior results in the treatment of hyperpigmentation, melasma, aged skin, scars, cellulite, and many other aesthetic disorders of the skin.

Recent developments of more affordable and convenient iontophoretic devices and patches will make this technique available for consumers' use in the home setting and, thus, will probably lead to a significant increase in the usefulness of iontophoresis.

REFERENCES

1. Singh P, Maibach HI. Iontophoresis in drug delivery: basic principles and applications. Crit Rev Ther Drug Carr Syst 1994; 11:161–213.
2. Guy RH, Kalia YN, Delgado-Charro MB, et al. Iontophoresis: electroreplusion and electroosmosis. Adv Drug Deliv Rev 1992; 9:289–307.
3. Banga AK. Electrically Assisted Transdermal and Topical Active Agent Delivery. Bristol, Pennsylvania: Taylor & Francis Group 1998.
4. Singh P, Liu P, Dinh SM. Facilitated transdermal delivery by iontophoresis. In: Bronaugh RL, Maibach HI, eds. Percutaneous Absorption Drugs –Cosmetics – Mechanisms – Methodology. Drugs and Pharmaceutical Sciences. 97. New York: Marcel Dekker, 1999:633–657.
5. Schultz SG. Basic Principles of Membrane Transport. New York: Cambridge University Press, 1980:21–30.
6. Marro D, Delgado-Charro M, Guy RH. Contributions of electromigration and electroosmosis to iontophoretic drug delivery. Pharm Res 2001; 18:1701–1708.

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[a] PowerCosmetics Enhancers, Power Paper Ltd., Petah Tikvah, Israel. PowerCosmetics is a trademark of Power Paper Ltd.

[b] PowerCosmetics Vitalizers, Power Paper Ltd.

7. Srinivasan V, Higuchi WI, Sims SM, Ghanem AH, Behl CR, Pons S. Transdermal iontophoretic drug delivery: mechanistic analysis and application to polypeptide delivery. J Pharm Sci 1989; 78:370–375.
8. Bagniefsky T, Burnette RR. A comparison of pulsed and continuous current iontophoresis. J Cont Rel 1990; 11:113.
9. Kno PC, Cliu J, Chang SF, Chien YW. Proceedings of the Japan-United States Congress on Pharmaceutical Sciences (PD-508), Honolulu, Hawaii, December 2–8, 1987.
10. Bodde HE, Verhoef JC, Ponec M. Transdermal peptide delivery. Biochem Soc Trans 1989; 17:943–945.
11. Gangarosa LP, Park NH, Fong BC, Scott DF, Hill JM. Conductivity of active agents used for iontophoresis. J Pharm Sci 1978; 67(10):1443–1439.
12. Siddiqui O, Roberts MS, Polock AZ. J Pharm Pharmacol 1985; 37:732–735.
13. Abramson HA, Alley A. Skin reactions I: mechanism of histamine iontophoresis from aqueous media. Arch Phys Ther X-Ray Radium 1937; 18:327.
14. Morris SJ, Shore AC. Skin blood flow responses to the iontophoresis of acetylcholine and sodium nitroprusside in man: possible mechanisms. J Physiol 1996; 496(2):531–542.
15. Huh CH, Seo KI, Park JY, Lim JG, Eun HC, Park KC. A randomized, double-blind, placebo-controlled trial of vitamin C iontophoresis in melasma. Dermatology 2003; 206: 316–320.
16. Schmidt JB, Donath P, Hannes J, Perl S, Neumayer R, Reiner A. Tretinoin-iontophoresis in atrophic acne scars. Int J Dermatol 1999; 38:149–153.
17. Fernandes D. 6th Internet World Congress for Biomedical Sciences, 2000.
18. Schmidt JB, Binder M, Macheiner W, Bieglmayer C. New treatment of atrophic acne scars by iontophoresis with estriol and tretinoin. Int J Dermatol 1995; 34:53–57.
19. Tannenbaum M. Iodine iontophoresis in reducing scar tissue. Phys Ther 1980; 60:792.
20. Tamarkin D. Enhancing cosmetic efficacy by orders of magnitude using thin and flexible microelectronic patches. Health and Beauty America, (September 30–October 2, 2003.)
21. Gerson J. Milady's Standard Textbook for Professional Estheticians. 8th ed. New York: Millday Thompson Learning, 1999:292–295.

16
Cosmetic Patches

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INTRODUCTION

The cosmetic patch is a new "cosmetic form" that is the result of the natural evolution of this technology in the pharmaceutical field. It appeared in the market just a few years ago, and although its applications are currently not too many, they have already been established as the new weapon to fight against the natural imperfections of our skin or to prevent the adverse reaction caused by environmental or other external influences. A broad spectrum of companies, including the major players, distributes at least one cosmetic-patch system. L'Oreal®, Estee Lauder®, Beiersdorf®, Cheseborough-Ponds®, Neutrogena®, Lavipharm®, as well as smaller manufacturers, participate in this special market.

HISTORY AND EVOLUTION

There is a close relation between topical pharmaceutical and cosmetic preparations. This relationship has its origin in the ancient years. Not only the forms (creams, ointments, solutions, liposomes, microemulsions), but also technologies and their production conditions are related to each other. By this rationale, the research and development of cosmetic patches started a few years ago. The influence of the pharmaceutical technology is apparent for the cosmetic patches not as simple cosmetic forms but as cosmetic delivery systems. It is not the first time that such a thing has happened. Liposomes and microparticles, for example, had been transferred from other application fields to the pharmaceutical and later to the cosmetic technology fields with successful results. In Figures 1 and 2 we can see the similarities of these two categories about the conventional forms as well as their delivery systems.

Cosmetic patches today, although at the beginning of their evolution and having weaknesses in some cases, represent a convenient, simple, easy, safe, and effective way for cosmetic applications, using one of the most acceptable, modern, and successful delivery technologies.

Pharmaceuticals

'Creams
'Ointments
*Powders
'Lotions
'Solutions
etc.
Casmsfic Forms

Figure 1 Dosage forms "equivalent" for cosmetics and pharmaceutical.

BORDERS BETWEEN PHARMACEUTICAL AND COSMETIC PATCHES

By definition, cosmetic products cannot be used or claimed for the treatment of diseases. Sometimes the companies use claims exceeding the borders between pharmaceutical and cosmetic applications, because the line is very thin between these major classes and/or in the past it was easier to use such terms. The patches could not be the exception to the rule.

Some patches that stand between pharmaceutical and cosmetic fields, for example, acne or acneic conditions, are included in this category; as we will see later, in some countries the actives combining with the claims characterize the classification, although in others products like these are considered to be real cosmetics. We could synopsize some simple rules to differentiate these two classes:

1. Cosmetic patches are not pharmaceutical patches (in the same way cosmetic creams are not pharmaceutical creams).
2. Cosmetic patches are designed for cosmetic applications.
3. Cosmetic patches contain cosmetic ingredients only (at concentrations allowed for cosmetic applications).
4. Cosmetic claims have to be confirmed by cosmetic efficacy tests.
5. Additional tests, patch specific, have to be established for cosmetic patches (e.g., peel force, wearing tests, residual solvents).
6. Safety first and efficacy second have to characterize these new forms.

APPLICATIONS OF COSMETIC PATCHES

In theory, cosmetic patches can be applied in most cases for the same use as classical cosmetic products, for example, wrinkles, aging, dark rings under the eyes, acneic conditions, hydration of specific areas, spider veins, looseness, and slimming. In practice, several of the aforementioned applications have been investigated, with

Drug Delivery Systems

- Liposomes
- Microparticles
- Patches (Topical/Epidermal)
- Microsponges
etc.

Figure 2 Delivery systems "equivalent" for cosmetics and pharmaceuticals.

very positive results and a high degree of acceptability from the consumers. The role of the specific form is not to cannibalize or to fully substitute the existing cosmetic forms. The main mission is to provide a breakthrough proposition for the cosmetic category as problem solvers. Someone could compare the cosmetic patches' role with the one of pharmaceutical patches. Where applicable and feasible, the pharmaceutical patches have almost substituted the classical forms because of their superiority over the conventional forms. But they did it because of, for example, the convenience, better efficacy, less side effects, and the lessened need for use. In contrast, they never substituted all the existing pharmaceutical forms. Each one plays its own important role.

We could synopsize by saying that cosmetic patches are destined mainly as problem-solver cosmetic forms, that is, they are more effective and efficient products with an absolutely and strictly localized action. Applied on the specific site, they limit their action on the specific area (acting topically), protecting at the same time the site and the active(s) itself.

DIFFERENCES BETWEEN CLASSICAL COSMETIC FORMS AND PATCHES

It is known that from the moment classical cosmetics (creams, lotions, etc.) are applied to the skin, they start changing continuously. The air, atmosphere's pollution, humid or dry environment, dust, and anything that can be transferred with it as well as any other factors alter the composition and the form of the product, which results in significant changes to the product's action. Patches, in contrast, are systems of occlusion even if there is sometimes the need, and we have the possibility, to manufacture breathable or porous patches. Because of this, permeation is getting easier, interactions with the environment are being considerably reduced, and we can expect a more "accurate" and "controlled" overall result.

Using the term "permeation," we mean the possibility that is given to several substances to reach the site of action, without of course confusing this term with the capability of a pharmaceutical patch to introduce the therapeutic substances into the systemic circulation at therapeutic levels. In many cases, this permeation makes the difference between an effective and noneffective form of administration of a cosmetic "active."

DEVELOPMENT OF COSMETIC PATCHES

All of the aforementioned pluses concern "good" cosmetic patches. As always happens with the new trends and the products following them, the low level of knowledge and experience guides several organizations to launch products without proofs of the required quality. As you will find later in the text, cosmetic patches are not pieces of Scotch tape containing one or a combination of cosmetic actives. On the contrary, it has to be an "extremely safe and effective scientific product." As such a product, it has to be supported with all the safety and efficacy proofs required.

As a new form or better delivery system, a cosmetic patch requires additional tests not applicable on conventional cosmetic products. Because of the occlusive or

semiocclusive character, these patches require a different level of investigation concerning the percentages of the ingredients, the compatibility with the skin, the possible amplified dermal reactions, and so on. Only special people and companies can formulate cosmetic patches. First, what is required is the full and perfect knowledge of the patch technology combined with the same level of knowledge and experience of the cosmetically acceptable ingredients and synergistically acting combinations. Until now, the experience on the patch technology used to be a monopoly of the scientists in the pharmaceutical field. The scientists in the specific pharmaceutical field know very well the correlation between active ingredient and therapy. They used a specific active to treat a specific illness or symptom. Cosmetic technology is "philosophically" different. Although in recent years there have been cosmetically active ingredients with a specific action, conventional cosmetic products use several components, and it is often difficult to make the distinction between "active" and "excipients." At the same time, because there are not real actives as we mean them in the pharmaceutical terminology or the regulations and we cannot use high concentrations of these actives, the cosmetic formulator is obliged to use, in most cases, "its own cocktail of cosmetic actives" to achieve the expected result. This is a big conceptual difference between the two types of formulators; the pharmaceutical and the cosmetic. This situation is also going to follow the cosmetic patches formulation. It is expected that several "cocktails of synergistically correct combinations" will play the role of the actives included in the pharmaceutical patches. It is obvious that the case of the cosmetic patch development and the required background cannot be found easily.

TYPES AND CONFIGURATION

There are several ways to describe and categorize a cosmetic patch. It can be characterized from the patch form (e.g., matrix, reservoir), the application purpose and the expected result (e.g., moisturizing, antiwrinkle), the type of its structural materials (synthetic, natural, hybrid), and the duration of application (e.g., overnight patch, half-hour patch). Cosmetic marketing is always more inventive in finding attractive terms to characterize a cosmetic product, but even scientifically there is better flexibility regarding the terminology. In practice, this category of patches covers the entire field, starting from the small or larger patch-like "facial masques" and finish to the cosmetic patches similar to their pharmaceutical cousins. In between, we can position some patch-like products or strips for the removal of blackheads from the nose or other problematic areas of the face or for the stretching of the skin. Another way to classify cosmetic patches is the duration of application, the action, and so on. Table 1 presents a different classification.

Table 1 Examples of Cosmetic Patch Categories

Pore cleansers
Blackhead removers
Stretching stripes
Short-term patch-like masks
Short-term treatment patches
Overnight treatment patches

Cosmetic Patches

PROBLEM—"TREATMENT"—INGREDIENTS
APPLICATION SITE—SHAPE

Pimples
Acneic skins
Brown spots
Wrinkles
Oily skin
Dry skin
Aged skin
Spider veins
After sun eryth.
Black circles, etc.

Hydration
Circul. improvement
Discoloration
Free rad. scavenging
Elastine protection
Sebum regulation
Stretching, etc.

G lycols
V itamins
B iotechn prod.
K eratolytics
A ntimicrobials
F R. scavengers
etc.

FRONT-HANDS-NECK-CHEEK, etc.

ROUND-RECTANGULAR-SQUARE-CHEEK-SHAPED, etc

Figure 3 Versatility of use and applications for cosmetic patches.

Regarding the flexibility of cosmetic patches, Figure 3 shows several and numerous combinations concerning applications as problem solvers, shape, ingredients, and site, among others.

Table 2 presents a "map" of cosmetic patches, covering a big part of their world.

It is obvious from all these examples of cosmetic patches that most are designed according to the principle of the matrix patch. This type of patch is thin, has a lightweight, has a reasonable production cost, and represents the trend in our days.

Table 2 Categories of Functional Cosmetic Patches

Category	Description
Antiblemish patch	An extremely popular, very small and thin patch for the treatment of pimples and blemishes. Contains a balanced percentage of salicylic acid, anti-irritant, and antimicrobial agents.
Pore cleansers	Very popular patches applied to the nose; their role is to clean pores and remove sebum plugs.
Pimple patch	A relatively large and thick patch for the care of pimples and blemishes.
Eye-contour patch	Mixture of several beneficial active ingredients for the fast relief of the area under the eyes after a short-term treatment (e.g., half hour).
Antiaging patch	One of the first cosmetic patches developed and sold. It bases its claims on ascorbic acid contained in the adhesive. Several similar patches have been developed.
Antiwrinkle patch	Based mainly on the antioxidant action of Vitamin C, as with the antiaging patch, this patch set is suggested for the prevention and treatment of wrinkles.
Lifting patch	Based on a mixture of glycolic acid, proteins, vitamins, and plant extracts, this large patch is used for the treatment of wrinkles of the neck.
Slimming patch	Thin and transparent, this patch contains a mixture of natural extracts (Fucus vesiculosus, Ginkgo biloba, etc.) and claims a slimming effect.

STRUCTURAL COMPONENTS OF THE COSMETIC PATCHES

Generally speaking, a matrix patch is composed of three discreet layers:

1. The backing film
2. The adhesive layer
3. The release liner

A matrix patch has the form shown in Figure 4.

Backing Film

The backing film is one of the three layers of a matrix patch. It is the layer that is apparent after the adhesion of the patch on the specific site of the skin. Its main role is to protect the adhesive layer from the influence of external factors; it also provides such characteristics as flexibility, occlusivity, breathability, and printability. Several materials have been used as backing films. The selection of a specific film for use in a cosmetic patch may depend on the following factors:

1. Cost
2. Stability
3. Printability
4. Machinability
5. Glossy or matte appearance
6. Compatibility
7. Anchorage to the adhesive
8. Transparency
9. Opacity
10. Occlusivity
11. Breathability

Several materials can be used for these purposes depending on the needs already presented.

One of the first and cheapest cosmetic patches used a simple paper layer.

Most of the pore cleansers use nonwoven materials. The reason is obvious: all these systems require wetting the nose before application of the patch. It means that the system has to dry out to be able to remove the sebum plugs that stick to the dried layer.

Polyethylene or polyester films are used also in most systems. They do not need to dry out after the application. Sometimes the film used is nontransparent. A white, foamy material is the backing layer of the pimple patch.

Figure 4 Typical structure of a matrix-type patch.

In some cases, other more expensive materials have also been tested, such as polyurethane, chlorinated polyethylenes, nylon, and saran. It is very important that the materials used as backing films for cosmetic patches have the same quality specifications with the similar films used for pharmaceutical patches to avoid any adverse reactions of the skin.

Release Liners

The main role of this layer is to protect the product, especially the adhesive layer, before the use of the product. The pharmaceutical patch development has provided a long list of release liners that can be useful for cosmetic patches as well. There are three main classes of release liners according to their composition:

1. paper based: glassine paper, densified kraft super-calendared paper, clay-coated paper, polyolefine coated paper, etc.;
2. plastic based: polystyrene, polyester (plain, metallicized), polyethylene (low and high density), cast polypropylene, polyvinyl chloride, etc.;
3. composite material based on the combination of several films.

All these materials have a common characteristic: one release layer coated on one or both sides depending on the needs of the product and the system itself. This coating is, generally speaking, silicon or polyfluorocarbon. The grade, thickness, coating, and curing methods vary according to the materials and the satisfaction of specific needs.

As mentioned for backing films, this layer has to be compatible with the components of the adhesive layer and should satisfy the specific needs of the product. Sometimes this layer has to be, for example, printed, scored, perforated, or tinted. The selection of the material and the grade are dictated from similar factors to the ones influencing the selection of the backing layer.

Adhesive Layer

This is the most important layer of a matrix cosmetic patch. The adhesive layer contains not only the adhesive that makes the patch stick to the skin, but also in most cases the cosmetic active ingredients and the additives required for correct formulation of a cosmetic product. Starting with the adhesive itself, the majority of adhesives used in cosmetic patches are taken from the general category of pressure-sensitive adhesives (PSAs). This is a class of adhesives used in several applications and in all pharmaceutical patches. As its name reveals, PSAs are adhesives which, in their solvent free form, remain permanently tacky and stick to the skin with the application of very slight pressure. There are three groups of PSAs: (1) acrylics, (2) silicones, and (3) rubbers. There are numerous members in the three main families of PSAs, but only few can be used for the formulation of cosmetic patches. The reason is that, as also happens with pharmaceutical patches, there are so many restrictions on the selection of an adhesive that the useful members are relatively few. The limitations are governed by the mechanical and biomedical properties of the adhesive as well as by the characteristics of compatibility, reactivity, and stability.

The components of the adhesive are also governed by such properties as solvents, monomers, cross-linkers, and emulsifiers.

There is also another category of cosmetic patches with similar structure, but formulated with a dry-adhesive system other than PSA. In this class we can bring the example of pore cleansers. Here the adhesive layer is created in situ, by wetting

the dry adhesive layer with water, the same way we stick a stamp on a letter. The components included in the composition of dry adhesives can be found in the classes of synthetic or natural derivatives, for example, polyvinyl derivatives, starches, celluloses, and sugars.

Pouching Materials

Although this material is not a component of cosmetic patches, its importance for the integrity of the product during its shelf life makes us examine it just after the basic patch components. Almost all cosmetic patches as happens with the pharmaceutical ones, are pouched in pouches. For pharmaceutical patches, the rule is to package one patch in one pouch. With the cosmetic analogues, and in an effort to reduce cost, sometimes patches can be found in the same pouch for more than one application. In this case, it is recommended that the product has stability information for the time interval between the opening of the pouch and the use of the last patch as well as to foresee some kind of resealable pouch. The materials used for the two categories are similar or the same. One of the differences is the number of packaged patches in one pouch. The protection of the product is the main mission of this packaging material, the role of which is critical for long-term stability of the product.

The pouching material, as has been mentioned, influences a lot of the stability of some sensitive molecules. Sometimes the phenomena of adsorption are noticed because of the affinity of some ingredients with the internal, sealable layer of the pouching laminate. In this case, for example, AHAs can escape from the adhesive layer and, passing the edge, can be absorbed from the ionomers plastic film of the pouching material. Another protection the pouching material provides is protection from UV radiation by using at least one opaque layer in case of light-sensitive materials, along with protection from oxygen.

Production

The production of cosmetic patches depends on the type of patch, the component characteristics, and the overall configuration of the final product. Because most cosmetic patches are matrix patches, it is useful to follow the general steps of typical production concerning this type of patch. Practically, production starts from the weighing of raw materials and other components, and ends with packaging of the product in the final carton. It is not within the scope of this chapter to go into details in this field, but we can mention the basic steps of the production sequence. Some information is required regarding the critical steps of production, or better the steps that could influence the quality of the product itself. The mixing of cosmetic ingredients and adhesives has to take place under a very slight nitrogen atmosphere (pressure) to avoid oxidation of the ingredients during this phase, but not too high (to avoid inclusion of nitrogen in the mass of the mixture and bubble formation during the drying cycle). Drying is also a critical step because, during this process, the temperature of the coating goes up and the ingredients have to be stable at these conditions. During drying, some of the ingredients are evaporated and/or sublimated. An accurate validated process has to be defined to finally take the patch as it had been designed. The exposure to light has to be limited as well, and the web has to be protected and kept in the predefined conditions before packaging. Of course, all the technology for production of pharmaceutical patches is applicable, but found outside the scope of this chapter.

PRODUCTION STEPS

Production of Casting Solution

This involves the mixing of active ingredient(s), additive(s), and other adjuvants, in the mass of the adhesive in the appropriate size and design production vessel and in the appropriate space.

The bulk could be a solvent or waterborne system, and the basic steps are as follows:

1. Weighing
2. Mixing
3. Deaeration
4. Release
5. Filtration and transfer to pressure vessel
6. Final bulk release

Coating—Drying—Lamination

The casting solution is prepared, released, coated, dried, laminated, and formed to the final rolls according to the specific standard operating procedures (SOPs), and the production records as follows:

1. feeding of the dosing pump, and through this the coating station;
2. casting on the release film;
3. drying of the coated solution passing through the drying tunnel;
4. continuous thickness control and recording;
5. lamination with the backing material;
6. winding in rolls;
7. splitting of the rolls;
8. quarantine;
9. final control;
10. release.

Packaging

The process involved in packaging is described as follows:

1. Roll feeding
2. Punching
3. Pouching
4. Cartoning
5. Boxing

REGULATORY ISSUES

As always happens with new forms, there is some confusion regarding the regulatory status of cosmetic patches. The main reason is that cosmetic patches are not included, for the time being, in the approved forms of cosmetic preparations. Considering the Directive 76/768/European Economic Community, August 1993, which is the official regulation of cosmetic products in the European Union, a cosmetic product "shall mean any substance or preparation intended to be placed in

contact with the various external parts of the human body (epidermis, hair system, nails, lips, and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance, and/or correcting body odors and/or protecting them or keeping them in good condition.'' According to this definition, cosmetic patches, acting similarly to conventional cosmetics, are included with cosmetic products. The confusion starts from the second paragraph of the same article, stating that: ''The products to be considered as cosmetic products within the meaning of this definition are listed in Annex I.'' In Annex I are included all the conventional forms, but not patches because, at the time of issuing, patches did not exist. So, because cosmetic patches conceptually, according to the cosmetic definition, comply with it, and because cosmetic patches are reality in our days, Annex I has to be revised with the addition of this new category.

Another reason for this confusion is the common origin of patches and transdermal systems. As previously mentioned before, all transdermals are not patches and all patches are not transdermals.

It is true that the first patches were dedicated to transdermal delivery of actives. At the same time, it is true and correct that not all transdermal systems are patches and that not all patches are by definition transdermals. We have the case of Nitro-Bid ointment for the transdermal delivery of nitroglycerin, but at the same time we have ''patches'' stuck to the skin for diagnostic purposes or for delivering the active to the opposite direction, for example, to the air to repel mosquitoes or for the topical treatment of pain.

To achieve transdermal delivery and effectiveness, several other factors are required:

1. the intrinsic properties of the molecule;
2. its concentration in the system;
3. the appropriate permeation enhancers;
4. the application site;
5. the surface area.

and other factors play a very significant role in

1. the rate and extent of absorption;
2. the ability of the specific active to reach the blood stream;
3. its efficacy and toxicity.

Without forgetting the peculiarity of cosmetic patches as cosmetic delivery systems or forms, we could propose that this new system not be encountered with skepticism and to follow the rules governing other cosmetic preparation. It means that the composition of the formula qualitatively and quantitatively has to follow existing cosmetic regulations, followed by specific tests and controls required especially for patches (e.g., residual solvents, adhesion on the steel, wearability), as well as tests regarding the safety parameters of an occlusive or semiocclusive system.

FUTURE TRENDS

The evolution of cosmetic patches is something expected after the warm acceptance of new cosmetic delivery systems from consumers. There are three axes for their expansion:

1. *The technological field.* It is expected that any new progress on patches, generally speaking, will strongly influence cosmetic patches as well. Even nonpassive cosmetic patches, like the iontophoretic ones, will find in the future several applications for the administration of more sophisticated cosmetic ingredients and actives.
2. *The applications.* For the time being, the applications of cosmetic patches cover a small part of the overall cosmetic applications. It is expected in the future to have a coverage of almost the whole spectrum of cosmetic applications.
3. *The ingredients.* The cosmetic patches, as previously explained, need to present a more potent solution for the cosmetic treatment of skin problems. Thus, there is the need for the use of very potent ingredients or extracts, that are probably especially designed for the patches to achieve a very fast and effective action.

17
Antibacterial Agents and Preservatives

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INTRODUCTION

The term "antibacterial agent" is largely used to qualify chemical agents, which are included in cosmetics or household products to provide them with either a specific bactericidal or a bacteriostatic activity during usage. The second function of antibacterial chemicals is to protect the product during its life by providing a preservative efficacy against microbial insults. A given chemical agent can be used as an active ingredient in antibacterial product or as a preservative to protect the formula from microbial contamination.

Taking into account that not only bacteria but also fungi or yeast can be concerned, and to simultaneously cover all germs, the word "antimicrobial" will be used.

Historically, the first antibacterial products developed were skin wash products such as soap bars, derived from deodorant soap bar. The purpose was not only to clean the skin but also to reduce its microbial flora (1).

During the last 25 years, many different antibacterial or antimicrobial products were marketed. They include: toothpastes and mouthwashes, liquid antibacterial soaps, deodorants, and even antibacterial products for dishwashing.

In Europe, cosmetic antibacterial products are regulated by the European Cosmetic Directive (2) providing their intended primary function, presentation, and positioning is cosmetic. Dishwashing products are under the scope of the Dangerous Preparation Directive (3), the European Biocidal Directive (4), and the Regulation on Detergents (5).

The new *Biocidal Products Directive* 98/8/EC (BPD) (4) regulates the use of antibacterial molecules. The new Directive was enforced as a national legislation in all European Union member countries by May 14, 2000.

The main features are:

- Establishment of a positive list of biocide active substances.
- National authorization of biocidal products containing active substances on the positive list.

- Mutual recognition by Member States of biocidal product authorizations.
- Ten-year review program to evaluate existing active substances.

Product Authorization

Under the scope of the Directive, each product must have a "dossier" that contains information on its biocidal efficacy, physical, chemical, and analytical properties, toxicological, and ecotoxicological properties, environmental effects, and where appropriate, any other effects it has if brought into contact with food. The aim is to establish that the biocide is sufficiently efficacious without having unacceptable effects on human health. Data must be submitted in accordance with the data requirements in Annexes II and III of the Directive. All new active substances are required to follow this approval process from May 14, 2000.

Existing biocidal products can continue to be marketed if identified or notified by January 31, 2003. These are listed in Annex I of the Directive. Annex III lists the identified substances, which are allowed until September 1, 2006.

When products are notified, their active substances have to be reviewed under the European Union review program, before maximum fixed dates depending on the classification of the substance (in Annex V of the Directive).

Article 1(2) excludes cosmetic products from the scope of the BPD as they are under the rule of the Cosmetic Products Directive 76/768/EEC (CPD) (2). In summary, the exclusion provision contained in Article 1(2) of the BPD in relation to the CPD covers the following cases:

- Cosmetic ingredients—as they are within the scope of the CPD—(including preservatives listed in the CPD Annex VI) under the conditions specified in the preamble and in the Annex itself.
- Cosmetic products with a secondary biocidal activity and claim, as long as the primary function of the products is cosmetic and they comply with the requirements of the CPD.

However, certain antibacterial cosmetic products are difficult to classify under the rule of either Directive. These "borderline" products include, for example, mouthwashes with antibacterial activity and others. Lachout has provided a detailed list of the borderline products (6).

The decision to classify a product into one of the two directives has to be taken by the competent authorities within the European Member States, on a case-by-case approach, taking into account the claims, the presentation, and the ingredient of the product. The two directives have different rules regarding the safety, proof of efficacy, and use of the antibacterial product; so the classification into one of the two product groups defines the requirements that the product has to follow.

The first part of this chapter will review the different kinds of antibacterial products and the methods to demonstrate their efficacy, in line with the existing legislations.

The second part of this chapter will review the preservative systems and how to build a well-preserved formula. The purpose of preservation is to protect all aspects of a product against microbial attack before and during consumer use. Integrity of products in terms of efficacy, fragrance, appearance, and stability must be maintained. The tests methods for preservative efficacy can be found in chapter 54 of this book.

ANTIBACTERIAL PRODUCTS

Topical Antimicrobial Products

Most antibacterial soap bars contain triclocarban® (TCC) as the active ingredient. In the past, antibacterial soap bars were also formulated with formaldehyde. These were very effective for hospital use, but the use of formaldehyde declined due to toxicity reasons. Currently, a maximum of 1% of the liquid soaps are formulated with triclosan. Safety of the regular use of TCC and triclosan in hand-washing products has been extensively discussed by the Food and Drug Administration (1).

The FDA prepared a tentative final monograph in 1994 (1) in which topical antimicrobial products were classified in three categories:

1. Antiseptic hand wash or health care personnel hand wash.
2. Patient preoperative skin preparation.
3. Surgical hand scrub.

Whereas this means that products intended to be used in home care would have to meet the requirements of products for healthcare. In response, the industrial associations: Cosmetic, Toiletry, and Fragrance Association (CTFA), and the Soap and Detergent Association (SDA) proposed another classification, based on a Healthcare Continuum Model (HCCM), in which the antimicrobial products were related to six categories; two to be used by the general population (antimicrobial hand washes and body washes), three for use by healthcare professionals (preoperative preparation, surgical scrubs, and health care personnel hand washes) and one category for the food handlers.

Since then, the industry has submitted data to the FDA showing the efficacy of various active ingredients used in the six categories; among these ingredients are: TCC, chloroxylenol (PCMX), povidone–iodine, surfactant iodophor, alcohol, and quaternary ammonium compounds (7).

Extensive studies have also been carried out with essential oils as antibacterial agent in soaps. Data showed that the minimal inhibitory concentration (MIC) for antimicrobial soaps formulated with different essential oils were more than 100 times higher than the MIC obtained on TCC-based soaps when tested against *Staphylococcus aureus* (8).

Deodorants and Antiperspirants

The first antiperspirants appeared on the market at the beginning of the 20th century. They were based on aluminum chloride, which induced skin irritation and fabric damage, because of the low pH of the solutions (9). Several years later, Shelly et al. (10) showed that underarm odor was provoked by the growth of the axillae bacterial flora, which degraded the apocrine secretions. These bacteria are mainly staphylococci (*Staphylococcus epidermidis*) and diphteroids from the Corynebacteriaceae family.

Antiperspirants can prevent the growth of such bacteria by reducing the available moisture of the axillaries among other mechanisms. Some products used the hexachlorophene as an active ingredient, but its use was discontinued because of neurotoxic properties (11). Currently, many of them contain aluminum salts, or zirconium–aluminum combinations such as Al-Zr-tri-/tetra-chlorohydrex glycinate as active ingredients. Their low pH of approximately 4.0 also helps in the antibacterial activity.

Antiperspirants are deodorants, because they suppress the odor source by reducing the perspiration and bacterial growth. Deodorants mask axillary malodor

through the presence of essentials or a fragrance in the product. They may contain an antibacterial ingredient that is effective in controlling axillary bacteria, such as alcohol or Triclosan, and prevent the production of malodor (11).

Oral Care Products

These are mainly toothpastes and mouthrinses. In general, dental creams serve to clean the teeth, to remove dental stains, and most recently to reduce and/or to prevent gingivitis and to kill the germs responsible for bad mouth odor. Mouthrinses, whether used before or after brushing, are also claimed to sanitize the mouth (12). All of these products contain an antibacterial ingredient to control the oral flora.

Active ingredients used in dental cream are mainly triclosan and chlorhexidine. Other ingredients such as the natural Sanguinarine extract also claim a sanitizing effect on the oral flora.

The same ingredients can be used in mouthrinses, but most of them also contain alcohol to ensure a good antiseptic effect of the product.

It is interesting to observe that fluorinated dental creams without any specific active ingredient also exhibit antimicrobial activity (12). This could be related to their fluoride content, which, in association with the surfactant system in the formula, release active cationic systems that are antibacterial.

Hand Dishwashing Products

Among the antibacterial household products that have recently appeared on the market, antibacterial hand dishwashing liquids have become increasingly popular. Even if these products are not cosmetics, they are in direct contact with the skin for a certain time. From a safety point of view, they can be regarded as rinse-off cosmetics. Furthermore, some products on the market have a double claim: "dishwashing liquid and antibacterial liquid soap." They are classical dishwashing liquids based on anionic and nonionic surfactants, to which one or more antibacterial agents have been introduced. Some of these formulae have been optimized to maintain their cleaning/degreasing performance on dishes while fighting bacteria on the hands, in the washing solution, and washing implements during use. Ingredients used can be essential oils, or triclosan or others. The concentration is chosen to ensure a good balance between a maximum of antibacterial efficacy and a good skin tolerance while keeping good cleaning performances.

METHODS TO DEMONSTRATE ANTIMICROBIAL PRODUCT EFFICACY

In vitro and in vivo tests can be used to demonstrate the efficacy of antimicrobial products. Only the in vitro tests will be considered here, as they are applicable to all antibacterial products. A detailed review of the in vivo tests has been previously published (1).

The Minimal Inhibitory Concentration Test

The principle is to determine the minimal inhibitory concentration (MIC) of the test product by performing serial dilutions of the latter in growth medium and inoculating each dilution with the test strain. Products are generally tested at two-fold serial dilutions. After suitable incubation, the first tube not exhibiting bacterial growth

gives the MIC level, generally expressed in ppm (parts per million) of product. The test can be carried out using either 2 mL of broth in tubes or 0.05–0.1 mL, in microtiter plates (13) or on agar plates. Control samples without any antimicrobials must be included in the test. This test is very useful to compare activities of different products, from the same category (e.g., soaps) with different actives, or to compare the active ingredients themselves. However, MIC data obtained on formulated products are very subjective and should be interpreted carefully.

Usually, the test organisms are: *S. aureus*, *S. epidermidis*, and *Escherichia coli* for topical antimicrobial. *Pseudomonas aeruginosa* and *Salmonella typhimurium* are added for the dishwashing products; for specific claims in the kitchen, *Aspergillus niger* and *Candida albicans* can be used as test strains. To test oral care products, the chosen organisms are *Actynomyces viscosus*, *Streptococcus mutans*, and *Streptococcus sanguis* as representatives of the oral flora (13).

The Zone Inhibition Test

This method can be used to test the susceptibility of bacteria to antibiotics as well as antibacterial ingredients (14). Antibacterial ingredients or products at different concentrations are applied to a substrate, usually a paper disk, or directly to the surface of an agar plate previously seeded with the test bacteria. During the incubation, the test material will diffuse into the agar layer and produce a zone of growth inhibition of the microorganism. The larger the inhibition zone the more susceptible the organism. However, the data are influenced by the diffusion capacity of the product or the active ingredient into the agar; oily products will not diffuse at the same rate as aqueous-based products. Thus, it is very important to use negative and positive controls. The data will be expressed in mm (millimeters) of inhibition zone around the disk. The strains used for this test are usually the same as those used for the MIC test. These two methods give a good idea of the bacteriostatic concentrations of the tested product or ingredient.

The requirements from the FDA monograph of 1994 (15) are the MIC test on the active ingredient, the vehicle, and the final formula, associated with a time-kill test methodology to be carried out at several time points over a period of 30 minutes.

The Time-Kill Test

This test determines both the killing kinetics and the activity spectrum of antibacterial formulations. It is generally performed in a suspension. The principle is to place in contact a dilution of the product or the antibacterial agent and a specified bacterial inoculum during a defined period of time. At the end of the contact time, the antibacterial agent in the mixture is inactivated by dilution into neutralizing broth. Serial dilutions in appropriate broth are performed and the number of survival bacteria enumerated on solid culture media. This method can use different concentrations of test agents and bacterial inocula, and different contact times. In general, the concentrations are chosen so that the final organism/test solution concentration is representative of the used concentration of the product.

In the United States, there is no detailed standardized time-kill test. The FDA has requested that a standard procedure be submitted for their consideration (15). In answer, the American Society for Testing and Materials (ASTM), Subcommittee

of antimicrobial agents, has prepared a draft to standardize the organism inocula, the media, the neutralizers and the contact times (16).

In Europe, the situation is different: to test the antimicrobial efficacy of products and/or agents, standards have been in existence for more than 20 years in France (17), Holland, Germany, and the United Kingdom. The Council of Europe has installed a Commission for the Normalization of European Norms (18) which is writing the European Norms (EN) for testing disinfectants and antiseptics. These normalized tests are annexed to the BPD as soon as they are published. The requirements for disinfection are 99.99% to 99.999% of killing (4–5 log reductions) of the initial inoculum, depending on the test.

These norms are also used by the industry to prove the efficacy of their antibacterial products, but the requirements are less strict: 99% to 99.9% killing (2–3 log reductions). Siquet has published a detailed review of the European Norms (19).

PRESERVATION AND PRESERVATIVE SYSTEMS

Concept of Active Preservation and Self-Preserving Formula

To ensure effective preservation, the method of choice is to add one or more active antimicrobial ingredients to the product. These ingredients must be compatible with the other ingredients of the formula and must retain efficacy for an extended period of time. They have also to be non-toxic for the consumer.

To choose an active antimicrobial molecule as a preservative is not so easy; this molecule must have a good oil–water partition coefficient because the contaminating microbes are living in the aqueous phase of the formula. It must not be inactivated by external factors such as the pH and the manufacturing process (20). Other factors have also to be considered: such as the packaging, which could affect the preservative activity, the adsorption rate on some components of the formula, and the solubility of the preservative molecule and its volatility (20).

Furthermore, the inactivation of the microorganisms by the preservative should be sufficiently fast to prevent any adaptation or resistance to the preservative system (21). So, the ideal preservative system must be selected for each formula, taking into account the possible inactivating ingredients or the potentiation capacity of other ingredients. Among these, EDTA (ethylenediaminetetra-acetic acid) is known to act in synergy with many other chemical preservatives. This potentiation is delivered through the permeation of the cell membrane of gram-negative bacteria: EDTA is a chelating agent and disrupts the outer lipid layer where stability is calcium and magnesium ion dependent. As such, it increases the penetration of the other antimicrobial chemicals into the bacterial cell (22,23).

In general, liquid- and emulsion-based cosmetic products are the most susceptible to the development of microorganisms. Powdered products, such as talc are also susceptible to contamination and need to be preserved (24).

Another way to preserve a product is to build a "self-preserved" formula by using raw materials, which are not supporting germ growth and optimizing their relative content. The use of humectants such as glycerin or sorbitol at a sufficient level increases the formula resistance. In a dental cream, a mixture of sorbitol and glycerine, at respective levels of 10% and 12%, is often enough to protect the formula. This is linked to the decrease of the water activity in the formula due to the presence of these humectants (25).

Other ingredients, such as alcohols, cationic detergents, fragrance components, lipophilic acids (lauric and myrisitic acids) used as emulsifiers, which have intrinsically antibacterial properties, can contribute to the self-preservation of a cosmetic (26).

This is also true for essential oils like tea tree oil or geraniol or eucalyptol, often used as cosmetic ingredients.

Some physical factors, such as the pH and the formula water activity can also contribute to build a self-preserved product. Microorganisms essentially live at pH around five to eight, and any pH outside this range induces more difficult life conditions for bacteria. The water activity or availability is an important factor as the water is a necessary ingredient for bacterial growth. The water availability concept is detailed in chapter? of this book.

Most Commonly Used Preservatives

Table 1 lists the most commonly used chemicals to preserve cosmetic products.

The chemical preservatives are too numerous to be listed here. Details on preservatives have been presented by Wallhäuser (26). These molecules can be used in synergistic mixtures, to improve the activity spectrum. For example, the parabens can be used with the imidazolydinil urea, the formaldehyde can be used with the EDTA, and so on.

Most of the preservative manufacturers have developed their own synergistic mixtures of chemicals; this allows the use of lower levels of each chemical and thus decreases the toxicity potential with increased preservative efficacy.

Attention must be paid to the regulations: in Europe, the Annex VI of the Cosmetic Directive 79/768 lists the chemicals, permanently and provisionally allowed to be used as preservatives in the cosmetic products. For each of them, there

Table 1 Most Commonly Used Preservatives

Preservative name	Activity spectrum	Compatible with	Inactivated by	Optimum pH
Parabens: esters of benzoic acid	Fungi, gram positive	Cationic	Anionic, nonionic, proteins	<7
Imidazolydinil urea	Broad, weak against fungi	Anionic, nonionic, cationic, proteins		4–9
Diazolydinil urea				
isothiazolones	Broad	Anionic, nonionic, cationic	Bleach, high pH	4–8
Formaldehyde	Broad	Anionic, nonionic, cationic	T>60°C	4–9
DMDM hydantoin				
benzalkonium Cl	Gram positive, gram negative, weak against olds	Nonionic, cationic	Anionic, proteins, soaps	4–9
2-Bromo-2-nitropropane 1,3-diol	Broad	Anionic, nonionic, cationic	Heat, high pH, cysteine, aluminum	<6

Abbreviations: DMDM hydantoin, Dimethyloldimethylhydantoin.

is an upper concentration use limit and for several of them, restrictions are mentioned (2). In the United States, the use of preservative molecules is regulated by the FDA.

REFERENCES

1. Morrison BM, Scala DD, Fischler G. Topical antibacterial wash products. In: Rieger MM, Rhein LD, eds. Surfactants in Cosmetics. 2nd ed. Marcel Dekker, 1997:331–356.
2. European cosmetic directive 76/768EEC.
3. Dangerous Substances and Preparations European Directive 1999/45/EC.
4. European biocide directive 98/8/EC.
5. Regulation Nr 648/2004 of the European Parliament and of the Council of 31 March 2004 on Detergents.
6. Lachout P. Cosmetic products-borderline situations. Council of Europe Publishing, November 2000, Strasbourg, France. ISBN 92–871–4450–8.
7. Poppe CJ. Ensuring a future for antimicrobials, Soap/Cosmetics/Chemical Specialities, 1996, 56–58.
8. Morris JA, Khettry A, Seitz EW. Antimicrobial activity of Arome chemicals and essential oils. J Am Oil Chem Soc 1979; 56:595–603, 1979.
9. Jass HE. The history of antiperspirant product development. Cosmet Toilet 1980; 95:25–31.
10. Shelley WB, Hurley HJ, Nichols AC. Arch Derm Shyphilol 1953; 68:430 (cited in ref 4).
11. Orth DS, ed. Cosmetic products that prevent, correct or conceal conditions caused by microorganism. In: Handbook of Cosmetic Microbiology. Marcel Dekker, 1993: 221–323.
12. Settembrini L, Gultz J, Boylan R, Scherer W. Antimicrobial activity produced by six dentifrices. General Dentistry 1998:286–288.
13. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard M7-A2. 2nd ed. Villanova, PA, 1990.
14. Balows A, Hausler WJ, Herrmann KL, Isenberg HD, Shadomy HJ. Manual of Clinical Microbiology, 5th ed. American Society of Microbiology,Washington, DC, 1991.
15. Food and Drug Administration (FDA). Tentative final monograph for health-care antiseptic drug products; proposed rules. Federal Register 59, June 17, 1994:31402–31451.
16. American Society for Testing and Materials (ASTM), E35.15 Subcommittee on Antimicrobial and Antiviral agents Meeting: WK412 Guide for the Assessment of Antimicrobial Activity Using a Time-Kill Procedure.
17. Association Française de Normalisation. Normes antiseptiques et Désinfectants. 2nd ed. Tour Europe, Cedex 7, Paris, France, 1989.
18. European Committee for Standardization, CEN 216, rue de Stassart 36, Brussels, Belgium, 1998.
19. Siquet F. Disinfection and preservation in detergents. In: Stubenrauch J, Broze G, eds. Handbook of Detergents. Vol. 1. New York: Marcel Dekker, 1999.
20. McCarthy TJ. Formulated factors affecting the activity of preservatives. In: Kabara, ed. Cosmetic and Drug Preservation, Principles and Practices. New York: Marcel Dekker, 1984:359–387.
21. Orth DS, Lutes CM. Adaptation of bacteria to cosmetic preservatives. Cosmet Toilet 1985; 100:57–59.
22. Kabara JJ, ed. Food grade chemicals in a system approach to cosmetic evaluation. In: Cosmetic and Drug preservation, Principles and Practices, New York: Marcel Dekker, 1984:339–356.
23. Denyer SP, Hugo WB, Harding VD. Synergy in preservative combinations. Internat J Pharm 1985; 25:245–253.

24. Selleri R, Caldini O, Orzalesi G, Facchini S. La conservation du produit cosmétique. Biol Chim Farm 1974; 113:617–627.
25. Orth DS. Handbook of Cosmetic Microbiology, chapter 4. New York: Marcel Dekker, 1993.
26. Wallhäuser KH. Antimicrobial preservatives used by the cosmetic industry. In: Kabara, ed. Cosmetic and Drug Preservation, Principles and Practices. New York: Marcel Dekker, 1984:605–745.

18
Colorants

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The use of coloring agents for decorative purposes is one of the earliest cultural accomplishments of humankind. Even in prehistoric times, colorants[a] could be found not only for art—the famous cave paintings in southern Europe, for example—but also, especially, for body painting, tattooing, or, to use the modern phrase, for decorative cosmetics. Although there were several historical periods in which those who wore cosmetics were scorned or condemned, its use has nevertheless remained constant among cultures throughout history. In more recent times, decorative cosmetics has been joined by other cosmetic products whose colors are not intended to conceal or change the appearance of something; instead, these colorants must conform to the statement that a given product makes about itself. While it is true that many first-time purchases are heavily influenced by the way the consumer feels about the color of the product and the attractiveness of its packaging, we nevertheless have some very definite associations between certain products and the colors they should have. Blue would certainly be inappropriate for a soap perfumed with sandalwood; the only color that would do for a pine-scented bubble bath is green; and it is logical to give citrus scents psychological reinforcement by coloring them yellow or yellow-green.

Although the use of colorants has a long history, a great deal of time passed before their role in cosmetics was legally established. This happened in Germany in 1887 with the enactment of the so-called Color Law, which banned the use of hazardous colorants. The issue of concern that led to this law was primarily pigments containing heavy metals; products of the then-developing color industry were not a genuine consideration. In 1906, a color law was passed in Austria that included various purity specifications and made the use of some coal-tar dyes illegal. In 1907, the use of the first certified food colorants were legalized in the United States, and at the

[a] *Colorants*: General term for all materials that can be used to color. There are three kinds: (1) colorants that are soluble in the medium being colored (in the case of cosmetics, usually water- or oil-soluble), (2) pigments and color lakes that are not soluble in the medium being colored (the latter are usually aluminum hydroxide lakes of water-soluble colorants), and (3) water-dispersible pigments (pigments that yield stable dispersions in water when excipients are added; they can then be processed like soluble colorants).

same time purity specifications were also determined. The Federal Food, Drug and Cosmetic Act of 1938 first outlined the use of colorants in food, drugs, and cosmetics.

The dramatic boom in the development of the color industry led to numerous new colorants and pigments. Because it had become clear that it was not only heavy metals that were dangerous but also colorants themselves or their initial products could pose a threat as well. After World War II, scientific organizations (2) increased their systematic efforts to compile and publish (3) the results of toxicological and dermatological research and encourage further studies. Unfortunately, international cooperation was less intense then, than it is today. This means that there are significant differences between the approved colorants for cosmetics in the European Union (EU), the United States, and Japan, for example. An illustration of this is the colorant patent blue V (C.I. 42051) (4), which is approved in the EU for all cosmetic products (5), but not in the United States or Japan. The same is true of fast yellow (C.I. 13015) and many other European cosmetic colorants. Furthermore, to some extent even approved colorants have different restrictions on their use,[b] especially for use in the area around the eyes. Table 1 shows the cosmetic colorants in the EU that are also approved for use in the United States and/or Japan. Because they lack fastness, natural colorants (e.g., carotenoids, anthocyans, and chlorophylls) play only a minor role in the process of coloring cosmetics. Carmine is an exception (C.I. 75470); the classic red pigment for lipstick is also the only red pigment in the United States that can be used for the eyes.

By comparison, inorganic pigments are used in large quantities. In coloring decorative cosmetics, several products are of vital importance: titanium dioxide (C.I. 77891) in particular—the most important white pigment—the iron oxides and iron hydroxides for the colors yellow (C.I. 77492), and red (C.I. 77491) and black (C.I. 77499), ultramarine (C.I. 77007)—especially in blue and violet—Prussian blue (C.I. 77510), manganese violet (C.I. 77742), coal black (C.I. 77268:1), pearlescent pigments (mica C.I. 77019), and bismuth oxychloride (C.I. 77163). By combining iron oxides, including the addition of titanium dioxide, various brown tones can be created in makeup and toning creams. The most significant colorant, however, is composed of the organic colorants and pigments, which belong to different chemical classes. Mainly these are azo, triarylmethane, anthraquinone, xanthene, or phthalocyanine colorants or pigments; occasionally they include indigo derivatives (Figs. 1–6 and Table 1).

Regardless of their chemical class, cosmetic colorants are sorted into three groups; this classification is based on their solubility, which determines how they are used: (1) colorants that are soluble in the medium being colored (usually water- or oil-soluble), (2) pigments and color lakes that are not soluble in the medium being colored, and (3) water-dispersible pigments.

Because of the extensive differences in national laws, two major factors must be considered in the development of colored cosmetics: one is technical, and the other is a legal matter. There are three phases to the procedure.

After the formulation of the uncolored product has been developed, the decision must be made about the countries in which the product will be marketed.

[b] In the EU there are four areas of applications: (1) approved for all cosmetic products; (2) not for use around the eyes; (3) not for use near the mucus membranes; and (4) only for brief contact with the skin.

Table 1 Cosmetic Colorants in the EU That Are Also Approved in the United States and/or Japan[a] (as of July 1998)

Color index number or name, color, colorant category, solubility	Japan[a]	U.S.[b]	Application area in the EU (example of use)
10020 green, water-soluble nitrosonaphthol colorant	Green No. 401 approved (category III)	Not approved	EU: 3 tenside products
10316 yellow, water-soluble nitro colorant	Yellow No. 403 approved (category III)	Ext.-D&C yellow No. 7, not for eyes and lips	EU: 2 soap, tenside products
11680 yellow, azo pigment (also water dispersible)	Yellow No. 401 approved (category III)	Not approved	EU: 3 soap
11725 orange, azo pigment	Orange No. 401 approved (category III)	Not approved	EU: 4 soap
12085 red, azo pigment	Red No. 228 approved (category III)	D&C red No. 36, not for use near eyes	EU: 1 lipstick (max. 3%)
12120 red, azo pigment	Red No. 221 approved (category III)	Not approved	EU: 4
14700 red water-soluble azo colorant	Red No. 504 approved (category III)	FD&C red No. 4, not for eyes and lips	EU: 1 soap, alcohol-based perfume products
15510 orange, water-soluble azo colorant	Orange No. 205 approved (category II)	D&C orange No. 4, not for eyes and lips	EU: 2 tenside products, soap
15620 red, water-soluble azo colorant	Red No. 506 approved (category III)	Not approved	EU: 4
15630 red (sodium salt), not easily water-soluble azo colorant	Red No. 205 approved (category II)	Not approved	EU: 1 (max. 3%)
15630: 1 red (barium salt) azo pigment	Red No. 207 approved (category II)	Not approved	EU: 1 (max. 3%) soap, lipstick, makeup
15630: 2 red (calcium salt) azo pigment	Red No. 206 approved (category II)	Not approved	EU: 1 (max. 3%) soap, lipstick, makeup
15630: 3 (strontium salt) azo pigment	Red No. 208 approved (category II)	Not approved	EU: 1 (max. 3%) soap, lipstick, makeup
15800: 1 red (calcium salt) azo pigment	Red No. 219 approved (category II)	D&C red No. 31, not for eyes	EU: 3

(*Continued*)

able 1 Cosmetic Colorants in the EU That Are Also Approved in the United States and/or Japan[a] (as of July 1998) (*Continued*)

olor index number or ame, color, colorant ategory, solubility	Japan[a]	U.S.[b]	Application area in the EU (example of use)
5850 red (sodium salt) not easily water-soluble azo colorant	Red No. 201 approved (category II)	D&C red No. 6, not for eyes	EU: 1
5850: 1 red (calcium salt) azo pigment	Red No. 202 approved (category II)	D&C red No. 7, not for eyes	EU: 1 soap, lipstick, makeup
5865: 2 red (calcium salt) azo pigment	Red No. 405 approved (category III)	Not approved	EU: 1 soap, lipstick, makeup
5880: 1 red (calcium salt) azo pigment	Red No. 220 approved (category II)	D&C red No. 34, not for eyes	EU: 1 soap, lipstick, makeup
5985 orange, water-soluble azo colorant, also as aluminum lake	Yellow No. 5 approved (category I)	FD&C yellow No. 6, not for eyes	EU: 1 (food colorant E 110) alcohol-based perfume products
6035 red, water-soluble azo colorant, also as aluminum lake	Not approved	FD&C red No. 40, also approved for eyes	EU: 1 (food colorant E 129) tenside products, alcohol-based perfume products, mouthwash
6185 red, water-soluble azo colorant, also as aluminum lake	Red No. 2 approved (category I)	Not approved	EU: 1 (food colorant E 123) tenside products
6255 red, water-soluble azo colorant, also as aluminum lake	Red No. 102 approved (category I)	Not approved	EU: 1 (food colorant E 124) tenside products, alcohol-based perfume products
7200 blue-red, water-soluble azo colorant, also as aluminum lake	Red No. 227 approved (category II)	D&C red No. 33, not for eyes	EU: 1 mouthwash, alcohol-based perfume products, tenside products
8820 yellow, water-soluble azo colorant	Yellow No. 407 approved (category III)	Not approved	EU: 4
9140 yellow, water-soluble azo colorant, also as aluminum lake	Yellow No. 4 approved (category I)	FD&C yellow No. 5, also approved for eyes	EU: 1 (food colorant E 102) tenside products

20170 yellow-brown, water-soluble azo colorant	Brown No. 201 approved, also as aluminum lake (category II)	D&C brown No. 1, not for eyes and lips	EU: 3 tenside products
20470 blue-black, water-soluble azo colorant	Black No. 401 approved (category III)	Not approved	EU: 4 tenside products, soap
45410 red, Xanthene colorant, fluorescent, water-soluble salts, also as barium lake and aluminum lake, free acid (45410:1) soluble in ethanol and oils	Red No. 218 free acid, red No. 231 potassium salt, both approved (category II), red. No. 104(1) sodium salt approved (category I)	D&C red No. 27, free acid, D&C Red No. 28, sodium salt, both not for eyes	EU: 1 lipstick
45425 red, xanthene colorant, fluorescent, sodium salt water-soluble, free acid (45425:1) soluble in ethanol and oils, also as aluminum lake	Orange No. 206 free acid, orange No. 207 sodium salt, both approved (category II), No. 206 not approved as aluminum lake	D&C orange No. 10, free acid, D&C orange No. 11, sodium salt, both also approved as color lakes, but not for eyes and lips	EU: 1 lipstick
45430 red, water-soluble xanthene colorant, also as aluminum lake	Red No. 3 approved, also as aluminum lake (category I)	FD&C red No. 3, not approved for cosmetics	EU: 1 (food colorant E 127) aluminum lake in lipstick
47000 yellow, oil-soluble quinophthalone colorant	Yellow No. 204 approved (category I)	D&C yellow No. 11, not for eyes and lips	EU: 3
47005 yellow, water-soluble quinophthalone colorant, also as aluminum lake	Yellow No. 203 approved also as aluminum lake, barium lake and zirconium lake (category II)	D&C yellow No. 10[c], not for eyes	EU: 1 (food colorant E 104) tenside products, soap, permanent and semi-permanent hair products
59040 green fluorescent, water-soluble pyrene colorant, also as aluminum lake	Green No. 204 approved also as aluminum lake (category II)	D&C green No. 8, max. 0.01% not for eyes and lips	EU: 3 tenside products, soap

(*Continued*)

Table 1 Cosmetic Colorants in the EU That Are Also Approved in the United States and/or Japan[a] (as of July 1998) (*Continued*)

Color index number or name, color, colorant category, solubility	Japan[a]	U.S.[b]	Application area in the EU (example of use)
60725 blue-violet, oil-soluble anthraquinone colorant	Purple (Violet) No. 201 approved (category II)	D&C violet No. 2, not for eyes and lips	EU: 1 oil products
60730 violet, water-soluble anthraquinone colorant	Purple (Violet) No. 401 approved (category III)	Ext. D&C violet No. 2, not for eyes and lips	EU: 3 hair, alcohol-based perfume products
61565 green, oil-soluble anthraquinone colorant	Green No. 202 approved (category II)	D&C green No. 6, not for eyes and lips	EU: 1 oil products
61570 green, water-soluble anthraquinone colorant, also as aluminum lake	Green No. 201 approved (category II)	D&C green No. 5, approved for eyes as well	EU: 1 tenside products, soap
73000 blue, pigment (indigo, vatblue colorant)	Blue No. 201 approved (category II)	Not approved	EU: 1
73015 blue, water-soluble indigo colorant	Blue No. 2 approved, also as aluminum lake (category I)	FD&C Blue No. 2, not approved for cosmetics	EU: 1 (food colorant E 132) aluminum lake for eye makeup
73360 red, indigo pigment	Red No. 226 approved (category II)	D&C red No. 30, not for eyes	EU: 1 toothpaste, lipstick
74160 blue, phthalocyanine pigment (also water dispersible)	Blue No. 404 approved (category III)	Not approved	EU: 1 eye makeup, toothpaste, soap, tenside products
75120 yellow to orange, oil-soluble carotenoid (also water-dispersible)	Annatto, approved (category I)	Annatto (no FDA certificate) for eyes as well	EU: 1 (food colorant E 160b) oil products, creams
75130 see 40800			
75170 white, natural organic pigment	Guanine, approved (category I)	Guanine (no FDA certificate) for eyes also	EU: 1 decorative cosmetics
75470 red, natural anthraquinone pigment, also water-soluble	Carmine, approved (category I)	Carmine (no FDA certificate) for eyes also	EU: 1 (food colorant E 120) makeup, lipstick

75810 see 75815			
75815 green, water-soluble porphyrine colorant	Sodium copper chlorophylline, approved (category I)	Potassium sodium copper chlorophylline, (no FDA certificate) max. 0.1%, only approved for oral and dental care products	EU (listed as C.I. 75810) (food colorant E 141): 1, oral and dental care
77000 silver-colored, inorganic pigment	Aluminum powder approved (category I)	Aluminum powder (no FDA certificate) external application, also for eyes (limitation of the particle size)	EU: 1 (food colorant E 173)
77004 white, pigment	Kaolin approved (category I)	Kaolin (no FDA certificate), considered cosmetic raw material and not colorant	EU: 1 No known use as a colorant
77007 blue, violet, pink, red and green inorganic pigments	Ultramarine approved (category I)	Ultramarine (no FDA certificate), also for eyes, but not in products for mouth and lips	EU: 1 makeup, eye cosmetics, lipstick, soap
77019 white to opaque, inorganic pearlescent pigment (mica)	Mica, approved (category I)	Mica (no FDA certificate), also for eyes	EU (summarized in the EC guideline with CL 77891): decorative cosmetics
77120 white, inorganic pigment	Barium sulfate considered cosmetic raw material and not colorant	Barium sulfate considered cosmetic raw material and not colorant	EU: 1 no known use as a colorant
77163 white inorganic pearlescent pigment	Bismuth oxychloride approved (category I)	Bismuth oxychloride (no FDA certificate) also for eyes	EU: 1 decorative cosmetics
77220 white, pigment	Calcium carbonate considered cosmetic raw material and not colorant	Calcium carbonate consider cosmetic raw material and not colorant	EU: 1 no known use as a colorant
77231 white, inorganic pigment	Calcium sulfate considered cosmetic raw material and not colorant	Calcium sulfate considered cosmetic raw material and not colorant	EU: 1 no known use as a colorant

(*Continued*)

Table 1 Cosmetic Colorants in the EU That Are Also Approved in the United States and/or Japan[a] (as of July 1998) (*Continued*)

Color index number or name, color, colorant category, solubility	Japan[a]	U.S.[b]	Application area in the EU (example of use)
77266 black, inorganic pigment	Carbon black approved (category I)	Not approved	EU: 1 decorative cosmetics
77288 green, inorganic pigment	Chromium oxide green, approved for eyes as well, but not around mouth and lips	Chromium oxide green (no FDA certificate), also for eyes, but not around mouth and lips	EU: 1 decorative cosmetics, soap
77289 green, inorganic pigment	Hydrated chromium oxide approved for eyes, but not around mouth and lips	Chromium hydroxide green (no FDA certificate), also approved for eyes, but not around mouth and lips	EU: 1 decorative cosmetics, soap
77400 copper-colored, inorganic pigment	Not approved	Copper powder (no FDA certificate), for external application and also for eyes	EU: 1 decorative cosmetics
77491 red-brown, inorganic pigment	Red oxide for iron approved (category I)	Synthetic iron oxide (no FDA certificate) also for eyes	EU: 1 (all food colorant E 172) creams, makeup, lipstick, soap
77492 yellow, inorganic pigment	Yellow oxide of iron approved (category I)		
77499 black, inorganic pigment	Black oxide of iron approved (category I)		
77510 blue, inorganic pigment	Ferric ferrocyanide approved (category I)	Ferric ferrocyanide (no FDA certificate), also for eyes, but not around mouth and lips	EU: 1 decorative cosmetics especially eye makeup
77713 white, inorganic pigment	Magnesium carbondate approved (category I)	Magnesium carbonate considered cosmetic raw material and not colorant	EU: 1 powder

77742 violet, inorganic pigment	Manganese violet approved for eyes but not around mouth and lips	Manganese violet (no FDA certificate) also for eyes	EU: 1 decorative cosmetics
77820 silver-colored inorganic pigment	Not approved	Silver (no FDA certificate), max. 1% only for use on nails	EU: 1 (food colorant E 174) no known use as a cosmetic colorant
77891 white, inorganic pigment	Titanium dioxide approved (category I)	Titanium dioxide (no FDA certificate) also for eyes	EU: 1 (food colorant E 171) creams, makeup, lipstick, powder, soap, toothpaste
77947 white, inorganic pigment	Zinc oxide approved (category I)	Zinc oxide (no FDA certificate) for external application and also for eyes	EU: 1 no known use as a colorant
Aluminum stearate, calcium stearate, and magnesium stearate white, oil-soluble	Considered cosmetic raw material and not colorant	Considered cosmetic raw material and not colorant	EU: no known use as a cosmetic colorant
Lactoflavin (riboflavin, vitamin B2) yellow, water soluble	Riboflavin approved (category I)	Not approved	EU: 1 (food colorant E 101) no known use as a cosmetic colorant
Caramel sugar brown, water-soluble	Caramel approved (category I)	Caramel (no FDA certificate) also used for eyes	EU: 1 (food colorant E 150a-d) rarely also in creams

[a]Japan: category I—approved for all cosmetic product, catagory II—for external use, category III—not for use on mucus membranes.
[b]Unless otherwise indicated and if chemically possible, the corresponding aluminium color lake is also approved.
[c]Because of its perceptual composition of mono-, di-, and trisulfonic acid, D&C yellow No. 10 does not correspond to the specification of EU-approved food colorant E104, which is also listed under CI 47005.

Figure 1 Azo colorant yellow-orange S (FD&C Yellow No. 6), C.I. 15985.

Figure 2 Triarylmethane colorant brilliant blue FCF (FD&C Blue No. 1), C.I. 42090.

Because not all colorant groups are appropriate for all cosmetics, some are selected (Table 2) and then examined to see which colorant of the respective category is approved in all the countries where the cosmetic product will be marketed.

At this point, the product is colored and stability tests are then conducted (original packaging, light, heat, etc.). Changing the formulation after successful completion of these tests is strongly discouraged. The testing must be repeated if the risk of unpleasant surprises is to be ruled out.

Although there are approximately 160 approved cosmetic colorants in the EU—many more than in the United States, for example—only a limited number of them is really used. Table 3 shows selected cosmetic products and the colorants that are often and usually added in industry.

Figure 3 Xanthene colorant sulforhodamine B, C.I. 45100.

Figure 4 Anthraquinone colorant alizarin cyanine green (D&C Green No. 5), C.I. 61570.

Figure 5 Indigo pigment indanthrene brilliant pink R (D&C Red No. 30), C.I. 73360.

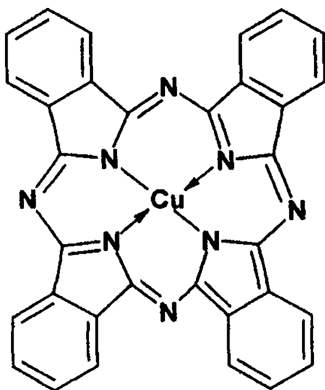

Figure 6 Phthalocyanine pigment heliogen blue B (phthalocyanine blue), C.I. 74160.

Table 2

Colorant group	Cosmetic products
Water-soluble colorants	Bath products (shampoo, shower gel, and bubble bath), creams, soap, toothpaste gel, mouthwash
Oil-soluble colorants	Oil products, soap
Pigments	Makeup, powder, lipstick, toothpaste, soap
Color lakes	Eye makeup, lipstick
Water dispersible pigments	Soap

Table 3

Cosmetic products (selection)	Color	Recommended colorant
Bubble bath	Blue	C.I. 42045, 42051, 42090
	Yellow	C.I. 13015, 19140, 47005, 45350 (fluorescent)
	Green	C.I. 61570, 59040 (fluorescent) as well as by mixing blue and yellow colorings
	Orange	C.I. 16255, 15985 as well as by mixing yellow and red colorants
	Pink/red brown	C.I. 16255, 16035, 16185 can be created by mixing red and yellow or orange and blue colorants
	Violet	By mixing red and blue, especially C.I. 42090 and 16185.
Recommended dose		0.05–0.3%
Shampoo, shower gel, liquid soap	Same colors as Bubble bath and also	
	Blue	C.I. 61585 and
	Pink	C.I. 45100
Recommended dose		0.01–0.05%
Bath salts	Blue	C.I. 42090, 42051
	Yellow	C.I. 47005, 45350 (fluorescent)
	Green	C.I. 61570, also as mixture of blue and yellow colorants
	Pink	C.I. 45430
Recommended dose		0.005–0.01%
Oil products	Blue	C.I. 60725
	Yellow	C.I. 40800
	Green	C.I. 75810
	Orange	C.I. 75120
	Turquoise	C.I. 61565
	Red-orange	C.I. 12150
Recommended dose		0.01–0.05%
Soap	Blue	C.I. 61585, 74160, 77007
	Yellow	C.I. 10316, 11680, 11710, 21108, 47005, 77492
	Green	C.I. 10006, 10020, 59040 (fluorescent), 61570, 74260
	Orange	By mixing red and yellow
	Red	C.I. 12490, 77491
	Black	C.I. 77499, 77268:1
	Violet	C.I. 51319 and by mixing blue and red
	White	C.I. 77891
Recommended dose		Water-soluble colorants or water dispersible pigments 0.01–0.05% Pigments 0.05–0.5%
Toothpaste	Blue	C.I. 74160
	Green	C.I. 74260
	Red	C.I. 73360
	White	C.I. 77891
Recommended dose		0.02–0.05%

(*Continued*)

Table 3 (*Continued*)

Cosmetic products (selection)	Color	Recommended colorant
Toothpaste gels	Blue	C.I. 42051, 42090
Recommended dose		C.I. 0.02–0.05%
Mouthwash	Blue	C.I. 42090
	Green	C.I. 61570 or a mixture of C.I. 42090 and C.I. 47005
	Red	C.I. 16035
Recommended dose		5–20 ppm
Alcoholic perfume products	Blue	C.I. 42051, 42090
	Yellow	C.I. 47005, 13015, 19140
	Orange	C.I. 15985
	Red	C.I. 16035, 17200
Recommended dose		5–20 ppm
Lipstick	All pigments (cosmetic application area 1 in the EU)	
Recommended dose		1–10%
Makeup, powder	Brown	Mixtures, of C.I. 77491, 77492, 77499, 77891
Recommended dose		2–10%
Eye makeup	Blue	C.I. 77510, 77007
	Yellow	C.I. 77492
	Red	C.I. 77491, 75470
	Violet	C.I. 77742
	Black	C.I. 77266, 77268:1, 77499
Recommended dose		5–30%

Hair-toning and hair-coloring products have a special status among the cosmetics in the EU because the EU guidelines for cosmetics do not apply to these products, especially because common cosmetic colorants have little or no affinity to hair.

Two different kinds of colorants are used to color hair: (*i*) Oxidation hair colors that permanently color the hair and (*ii*) Substantive colorants that only affect the outside of the hair and can be washed away again (semipermanent coloring).

In oxidation hair colors, a colorless initial product penetrates the hair, where a reaction takes place with the aid of hydrogen peroxide (hence the term oxidation hair colors) and another colorless initial product. No colorants are used; the color is first created on the inside of the hair.

Substantive colorants are largely cationic and cannot penetrate the hair because their molecules are too large; therefore, they only adhere on the outside and can be removed again comparatively easily.

REFERENCES

1. Colour Index: Vols. 1–4 (1971) 3rd ed., Vols. 5–6 (1975) revised 3rd ed. Yorkshire England: The Society of Dyers and Colourists.

2. DFG-Farbstoff-Kommission (DFG Dyestuffs Commission). Cosmetic Colorants, 3rd ed. Weinheim: VCH, 1991.
3. Hendry GAF, Houghton JD. Natural Food Colorants. Glasgow and London: Blackie, 1992.
4. Lehmann G, et al. Identifizierung von farbstoffen in hautcremes [Identifying colorants in skin creams]. Seifen-Ole-Fette-Wachse [Soaps-Oils-Fats-Waxes] 1986; 16:565.
5. Lehmann G, Binkle B. Identifizierung von farbpigmenten in kosmetischen erzeugnissen [Identifying color pigments in cosmetic products]. Seifen-Ole-Fette-Wachse [Soaps-Oils-Fats-Waxes] 1984; 5:125.
6. Loscher M. Farben—visualisierte gefiihle [Colors—visualized feelings]. DRAGOCO Report 1981:4/5.
7. Marmion DM. Handbook of U.S. Colorants for Foods, Drugs and Cosmetics, 2nd ed. 1984, ISBN 0-471-09312-2.
8. Moschl G, et al. Perlglanzpigmente fur kosmetika [Pearlescent pigments for cosmetics]. Seifen-Ole-Fette-Wachse [Soaps-Oils-Fats-Waxes] 1980; 8:207.
9. Otterstatter G. Die farbung von lebensmitteln, arzneimitteln, kosmetika [Coloring foods, drugs, Cosmetics]. 2nd revised ed. Hamburg: Behr's Verlag, 1995.
10. Schweppe H. Handbuch der naturfarbstoffe—vorkommen, verwendung, nachweis. [Handbook of natural colorants—their presence, use and verification]. Landsberg/Lech: Ecomed, 1992.

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Skin Feel Agents

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INTRODUCTION

Skin feel additives are substances conferring sensorial properties to a skin care product, triggering pleasant perception during application onto the skin and after use. Effectiveness of sensory triggers is governed by their substantivity to the skin which occurs either by hydrophobic interaction or by charge attraction or a combination of these two factors. A large variety of cosmetic ingredients function as skin feel/conditioning additives comprising lipophilic materials, silicones, water soluble polymeric substances (including proteins) and their cationic derivatives, humectants, etc. The Cosmetic, Toiletry and Fragrance Association (CTFA) divides skin conditioning agents into various groups: emollients, occlusive materials, and miscellaneous substances including, among others, cationic macromolecules and several surfactants.

This chapter focuses on skin feel agents for rinse-off products and more particularly for surfactant based skin cleansing products: facial cleansers, soap and syndet bars, shower gels and body washes, foam baths (or bubble baths), and bath oils. Shower gels, bars, and facial cleansers first contact the skin, before being rinsed during the cleaning process; the substantivity of the conditioning agents is crucial to ensure sensory performances otherwise they are washed off, and the end skin benefit is not perceived by the user. For bath products intended to be heavily diluted for use, it is difficult for skin feel agents to be really effective, except perhaps in case of bath oils. Indeed, when bath oils are diluted in water they either float on the surface or lead to a coarse unstable o/w emulsion; when the body emerges from the bath, oils spontaneously stick onto the skin due to their water incompatibility, excluding them out of the "bathing liquor."

The advent of emollients in body cleansing liquids occurred with the emergence in the early 90s of the "body washes" referring to "2-in-1" foaming emulsions; before the development of this new product niche, cationic polymeric materials were the most used skin conditioning agents.

Sensorial performance profile of a body cleansing product comes in a variety of signal attributes:

- feeling on the skin during use: spreading of a liquid (also related to product rheology), feel of a bar (slipperiness or roughness), foam feel related to foam quality (creaminess, density);

- skin feel during rinsing: e.g., slipperiness, roughness of the skin, ‘‘clean feel’’ (squeaky feel) left by soaps;
- feel while drying the skin with a towel and feel on damp skin: softness, roughness, stickiness;
- after feel and long lasting of skin sensations: smoothness, softness, moisturization, etc.

All these product attributes are governed at first by the composition of the cleansing base: the surfactant nature (amphoterics, nonionics, anionics), their total and relative concentrations, the clinical mildness for the skin of the surfactant mixture, etc., and can be further influenced or improved by judiciously chosen skin conditioning agents.

Besides physical, clinical, and organoleptic characteristics of the body cleansing product, several other imponderable parameters can act upon the skin feel performance and perception: environmental conditions, usage habits, water hardness, skin condition of the user, pilosity, etc.

Also, consumer expectations in terms of sensorial profile of a product depend on climatic (relative humidity, temperature) and sociodemographic parameters (sex, occupation, lifestyle, running water availability, etc.), skin type and concerned body part (face, whole body, etc.), product positioning (sport, moisturizing, nourishing, others), etc.

Criteria of selection and technical constraints to be taken into account when choosing skin feel agents are

1. solubility and compatibility with the surfactant system;
2. sensitivity to electrolytes and pH;
3. product physical form: bar, liquid;
4. processibility (bars) and easiness of formulation;
5. sensitivity to temperature;
6. impact on finished product performance profile:
   a. on the foam: foam feel, volume, and stability, creaminess, bubble structure, etc.;
   b. on the product rinseability;
   c. induction of undesirable and unexpected secondary effects on skin feel when skin is damp (e.g., stickiness);
7. impact on finished product esthetic:
   a. on fragrance perception and stability;
   b. on product clarity when relevant;
   c. on viscosity, rheological profile;
   d. on color;
8. origin: animal or vegetal, natural or synthetic;
9. risk of skin sensitization;
10. cost.

EMOLLIENTS AND REFATTENERS

Introduction

CTFA dictionary defines *emollients* as ‘‘cosmetic ingredients which help to maintain the soft, smooth, and pliable appearance of the skin; emollients function by their

ability to remain on the skin surface or in stratum corneum to act as lubricant, to reduce flaking, and to improve the appearance of the skin."

Emollients are also described as *refatting additives* or *refatteners* in case of bath products. The word "refattener" refers to substances improving the lipid content of the upper layers of the skin; they prevent defatting and drying out of the skin.

Several emollients showing strong lipophilic character are identified as *occlusive* ingredients; they are fatty/oily materials which remain on the skin surface and reduce transepidermal water loss. CTFA dictionary defines occlusives as "cosmetic ingredients which retard the evaporation of water from the skin surface; by blocking the evaporative loss of water, occlusive materials increase the water content of the skin."

Overall, emollients and refatteners are oils and fats derived from natural origins or obtained by chemical synthesis; they are classified in nonpolar (paraffins and isoparaffins) and polar substances (esters and triglycerides); their chemical structure influences the interaction with the skin surface and affect their sensorial properties. As a class, they comprise lipids, oils, and their derivatives, fatty acid esters, lanolin derivatives, silicones, and their organofunctional derivatives.

Originally, emollients were developed for use in leave-on skin care products. Formulation technology can aid the deposition of refatting additives on the skin from wash-off products and avoid getting rinsed off with the surfactants; nevertheless, the large dilution factor in bath products remains a significant hurdle for skin end benefit perception (except in bath oils).

Emollients and refatteners will provide after feel but will also influence skin feel during usage, foam feel, and most of the time foam quantity and quality. The more hydrophobic the refattening additive, the more negative its impact on flash foam generation, foam quantity, and stability.

In other respects, the more lipidic the material, the better its skin substantivity, and the easier the efficacy documentation; proof and substantiation of claims is of more and more importance in the frame of European legislation for cosmetics and toiletries and of the competitive environment.

Lipophilic Emollients and Occlusives

Occlusive materials comprise vegetable oils, triglycerides, mineral oil, natural or synthetic waxes, fatty acid esters, lanolin oil, and its derivatives, polydimethylsiloxanes, among others (Table 1).

They form an occlusive layer on the skin, keeping water inside the upper stratum corneum layers and consequently acting as moisturizers.

Mineral oil and vegetable oils as well as waxes generally produce a heavy and greasy feeling on the skin. *Hydrophobic fatty acid esters* are an almost unlimited source of synthetic emollients and refatteners; they provide a lighter and more pleasant skin feel than oils and waxes. Any fatty acid can be esterified either by ethylene glycol or propylene glycol, or glycerin polymers, or isopropyl alcohol, or any longer chain alcohol, etc. The feel they impart and their impact on foam is related to the fatty acid chain length; short chains (isopropyl myristate and octyl octanoate, for example) deliver dryer feel and have lesser impact on foam than longer ones (stearates and isostearates, for example) which are greasier and detrimental to foam quantity and stability (1).

Hydrophobic emollients are efficacious skin refatteners but not easy to incorporate in surfactant mixtures commonly used in liquid skin cleansing products. They must be emulsified which most of the time necessitates hot process. They have a detrimental impact on foam speed, quantity, and stability. To circumvent this weakness

Table 1 Emollients and Refatteners

		INCI names
Fats/oils (triglycerides); hydrocarbons; waxes		Petrolatum
		Ceresin
		Mineral oil
		Wheat germ oil/wheat germ glycerides
		Almond/peach oil
		Coconut oil
		Jojoba oil
		Rape seed/olive/sesame oil
		Sunflower/corn/safflower oil
Fatty acid esters; hydrophobic emollient esters	Ethylene glycol esters	Glycol stearate or palmitate or oleate
	Polyethylene glycol esters	PEG-5 octanoate
	Propylene glycol esters	Propylene glycol myristate or laurate
	Polypropylene glycol esters	PPG-36 oleate
	Isopropyl esters	Isopropyl myristate or laurate or palmitate
	Polyglyceryl esters	Polyglyceryl-10-laurate or myristate
	Alkyl esters	Octyl octanoate
		Cetearyl octanoate
		Octyl hydroxystearate
Fatty acid mono and diglycerides		Glyceryl oleate
		Glyceryl laurate
Ethoxylated triglycerides		PEG-6 caprylic capric triglycerides
		PEG-4 caprylic/capric glycerides
		PEG-45 palm kernel glycerides
		PEG-20 almond glycerides
		PEG-60 corn glycerides
		PEG-18 palm glycerides
		Olive oil PEG-7 esters
		Hydroxylated milk glycerides
Ethoxylated mono and diglycerides; hydrophilic emollient esters	Ethoxylated glyceryl esters	PEG-7 glyceryl cocoate
		PEG-8 glyceryl laurate
		PEG-15 glyceryl laurate
		PEG-30 glyceryl cocoate
		PEG-78 glyceryl cocoate
		PEG-20 glyceryl oleate
		PEG-82 glyceryl tallowate
		PEG-200 glyceryl tallowate
Fatty alcohols		Lauryl alcohol/oley alcohol
		Octyldodecanol
		Dicaprylyl ether

(*Continued*)

Table 1 Emollients and Refatteners (*Continued*)

		INCI names
Emollient ethers	Ethoxylated/propoxylated fatty alcohol's	PPG-5 laureth-5
		PPG-5 ceteth 20
		PPG-8 ceteth 20
	Polypropylene glycol ethers	PPG-14 butyl ether
		PEG-4 lauryl ether
		PPG-15 stearyl ether
		PPG-60 cetyl ether
		PPG-3 myristyl ether

of lathering capacity manufacturers sometimes provide a mechanical foaming device with the product: a puff or massage flower (2).

Highly hydrophobic refattening additives are not meant for foaming preparations but rather for bath oils. Soaps and syndet bars can easily accommodate waxes and oils without impairing their basic foaming and cleaning functions. Besides beeswax, petrolatum or ceresin, lanolin and jojoba oil, cocoa butter or mineral oil are other examples of skin conditioners commonly used in bars. Paraffin wax is often used in soaps and syndets not only for the smooth feel they impart to the finished bar, the mildness they bring to the formulation, but also for the role of plasticizer they play, adding firmness to the bar. Vegetal oils are included as skin nourishing/refattening agents (almond, wheat germ, olive oils, for example).

Fatty acid mono and diglycerides (1,3) are prepared either by transesterification of triglycerides with glycerin or treatment of alkanoates with glycerin. Lipophilic character remains predominant in these esters; depending on chain length, they are soluble in surfactant solutions or they must be emulsified. Besides the improved skin feel they induce, they also reduce defatting of the skin possibly caused by surfactant based cleansers. Monoglycerides of stearic, lauric, and palmitic acids (glyceryl mono stearate, laurate, and palmitate) are part of the composition of natural lipids of the skin. They adsorb and can be detected on skin after application through a skin cleansing product (4).

Several mixtures of monoglycerides and mild foaming surfactants are commercially available; they claim improved foam qualities (bubble size, creaminess, and stability) and documentable skin refattening properties (5,6). On top of skin feel improvement, they also reduce degreasing effect of cleansers, thanks to their lipophilic character, and improve the compatibility of the surfactants with the skin (1). An example of improvement in the skin barrier function and in skin tactile sensations has been demonstrated for glyceryl oleate in a model shower gel composition (5).

Hydrophilic Lipids

Hydrophilic lipids are (Table 1) (1) preferred for foaming skin cleansing preparations.

Ethoxylation and propoxylation make lipids more compatible with water and more easily soluble in aqueous surfactant solutions.

One has to find the right balance between ethoxylation and skin substantivity: the more the lipids are ethoxylated, the more they are soluble and the less the impact on foam but also the less their persistence on the skin and the weaker their refattening properties.

Ethoxylated glycerides are obtained either by reaction of natural triglycerides with ethylene oxide (a complex end mixture is then obtained) or by ethoxylation of monoglycerides. They are often referred to as "water soluble vegetable oils"; their solubility in water will depend on the carbon chain length of starting glycerides and on the degree of ethoxylation.

Low ethoxylates triglycerides are still enough lipophilic to provide good refattening properties, leading to a very pleasant skin feel, perceivable at quite high use levels.

Ethoxylated mono- and diglycerides generally associate various properties beneficial to the skin. They are more or less refattening the skin, depending on chain length and ethoxylation ratio. They act as anti-irritant or mildness additives and confer slipperiness to the foam. Depending on chain length and ethoxylation degree they are either water dispersible or soluble. Amongst the low ethoxylates monoglycerides, PEG-7 glyceryl cocoate is one of the most used. This emollient depresses irritation of anionic surfactants and shows minimum impact on lathering profile. Higher ethoxylates of longer C chain length (PEG-200 glyceryl tallowate) are still substantive to the skin due to their high molecular weight, provide a smooth feel but due to their stronger hydrophilic character, their refatting properties are less obvious to evidence (7).

Ethoxylated/propoxylated fatty alcohols are useful light emollients: through an appropriate selection of optimum combination between parent alcohol chain length, propoxylation, and ethoxylation degree, these emollients can be formulated up to 2% to 3% in surfactant solutions with minimum impact on foam volume.

Lanolin

Lanolin is extracted from sheep wool grease; it is a complex mixture of esters of high molecular weight lanolin alcohols (aliphatic alcohols, sterols, and trimethyl sterols) and of lanolin fatty acids. Free lanolin alcohols, acids, and lanolin hydrocarbons are minors. Lanolin alcohols and lanolin oil are recommended as superfatting agents in soaps. *Ethoxylation* of the hydroxyl groups *of lanolin* or of its derivatives leads to hydrophilic, water soluble lanolin compounds, offering a broad range of useful emollients to the formulator (Table 2) (8,9). Some moderately to highly ethoxylated derivatives, recommended for their good emolliency and moisturization properties, are processable in liquid skin cleansers with limited impact on foam profile; as an example, the *75-mole ethoxylated lanolin* does not depress foam and is recommended as skin conditioner in soaps, liquid body cleansing products, and bubble baths. *Medium ethoxylates lanolin alcohols* have limited impact on foam performances of body cleansing liquids; *lower ethoxylates* can be formulated in bars. *Propoxylated lanolin alcohols* are lipophilic emollients used in soap bars and in other cleansers based on synthetic surfactants.

Alkoxylated lanolin derivatives are obtained by reaction with mixtures of propylene and ethylene oxides in various ratios; they are more soluble than ethoxylated lanolin. They serve as refattening and foam stabilizing agents.

Esterification of *lanolin fatty acid* with isopropyl alcohol provides a range of *esters* of various molecular weights. Medium molecular weight esters are used as superfatting agents in soaps.

The use of animal derived ingredients in cosmetics is regulated in Europe. Lanolin is part of those ruminant derived ingredients considered as noninfective with

Table 2 Emollients and Refatteners

	INCI names
Lecithin	Propylene glycol (and) lecithin (and) sodium lauryl sulfate (and) disodium sulfosuccinate (and) cocamidopropyl hydroxysultaine (and) isopropyl alcohol
Lanolin and its derivatives	Lanolin oil
	Lanolin alcohol
Ethoxylated lanolin	PEG-75 lanolin
Ethoxylated lanolin alcohols	Laneth-16
	Laneth-25
Propoxylated lanolin alcohols	PPG-30 lanolin alcohol ether
Alkoxylated lanolin	PPG-12 PEG-50 lanolin
	PPG-40 PEG-60 lanolin oil

regards to bovine spongiform encephalopathy (BSE), according to the World Health Organization (WHO).

Lecithin

Lecithin (Table 2) is a natural mixture of polar and neutral lipids; the word "lecithin" is also used as the trivial name of a particular phospholipid: phosphatidylcholine.

Main vegetable sources of lecithin used in personal care products are soybean and maize; egg yolk is practically the only animal source of lecithin used in cosmetics and toiletries. The percentage of polar lipids and their fatty acid pattern are characteristic of the lecithin source.

Bare lecithin, a secondary product of Soya oil extraction, typically contains 60% to 70% polar lipids (mainly phospholipids, namely, phosphatidylcholine and glycolipids) and a remaining 25% to 35% Soya oil. This raw lecithin is further fractionated, purified, and chemically modified to allow easier processing and formulation in toiletry products. Emollient, refattening, and moisturizing properties of lecithin are guided by its content in phospholipids.

Lecithin softens, nourishes, and refattens the skin; it provides a nongreasy, long lasting skin feel and improves foam feel and quality (creaminess, slipperiness, richness).

Ready to use mixtures of phospholipids in surfactant solutions, free of residual Soya oil, are commercially available for an easy incorporation in liquids or bars; some of these compounds allow formulation of clear products.

Silicone Derivatives

Only major materials used in body cleansing products will be briefly discussed here (10,11).

Predominant silicones overall used in personal care products are polydimethyl siloxane also named *dimethicones*. They are not soluble in water or in surfactant solutions; their incorporation into liquid cleansers requires an emulsification process.

The length of the dimethylsiloxane polymer chain dictates its molecular weight and hence its viscosity. Most commonly used materials have viscosities ranging from about 100 to several thousand centistokes. High to medium molecular weight dimethicones are occlusive, skin protective emollients; lower molecular weights are dryer emollients, generally preferred for use in skin cleansers. Dimethicones have detrimental effect on foam profile but are good film forming agents, lubricants, imparting a nongreasy, nontacky, and silky feel as compared to "heavier" mineral or vegetable oils. They are used in soap bars, where they also aid mold release, and in 2-in-1 shower gels (body washes).

Polymethylcyclosiloxanes or *cyclomethicones* are tetrameric or pentameric oligomers of the same backbone as polydimethylsiloxane and show the same chemical and physical properties; they are low viscosity fluids with relatively high volatility due to their low molecular weight and the weak intermolecular attractivity. Because they are not substantive, cyclomethicones are often identified as dry emollients; they deliver light, transient, and dry skin feel during product use.

Formulation of these nonpolar insoluble silicones requests hot emulsification process (nonionic emulsifiers) and proper emulsion stabilization.

Dimethicones are modified or functionalized with other organic groups to modulate their solubility in water or in surfactant solutions (and consequently make them easier to formulate) and their skin substantivity properties. By adjusting the type and proportion of hydrophilic substituents, the resulting copolymer is soluble or dispersible in aqueous cosmetic products. The combination of the dimethicone structure with polyoxyalkylated substituents (ethylene or propylene oxide) yields *dimethicone copolyols*: copolymers more soluble in water with surface activity. They are foam boosters and stabilizers; even if they are less film forming than parent polydimethylsiloxanes, they significantly participate in skin sensations during application (use) and provide excellent smooth and silky after feel (12). They can be used to formulate clear aqueous products.

Blends of polydimethylsiloxanes with volatile and/or water soluble derivatives are used to design a sensorial profile adapted to the finished product and its end use.

HUMECTANTS

CTFA dictionary defines humectants as "cosmetic ingredients intended to increase the water content of top layers of the skin." (Table 3).

Humectants are hygroscopic substances generally soluble in water; these "moisture attractants" maintain an aqueous film at the skin surface.

The primary used humectant in personal care products is glycerin; it tends to provide a heavy and tacky feel which can be overcome by using it in combination with other humectants such as sorbitol.

Less expensive than glycerin, propylene glycol is the second most widely used humectant in cosmetics and toiletries' products; it reduces viscosity of surfactant solutions and tends to depress the foam.

Low molecular weight polyethylene glycols (PEGs from about 10 to 200 PEG units), amino acids (AA), and other constituents of skin Natural Moisturizing Factors like sodium pyrrolidone carboxylic acid (PCA) and sodium lactate are also applicable for use in surfactant based skin cleansing products.

Table 3

Humectants	INCI names
	Glycerin
	Glycereth-26 and glycereth-7
	Propylene glycol
	1,3 butylene glycol
	From PEG-8 to about PEG-200
	Sorbitol
	Sorbeth-6 to sorbeth-40
	Xylitol
Ethoxylated methyl glucose	Methyl gluceth-10/methyl gluceth-20
	Amino acids
	Lactic acid/sodium lactate
	Sodium PCA
Substantive conditioning humectants	Steardimonium panthenol
	Lauryl methyl gluceth-10 hydroxypropyl dimonium chloride
	Chitosan-PCA

Humectants are not substantive to the skin, they are easily rinsed off after cleaning; consequently, skin feel improvement is not obvious to perceive, and their efficacy in terms of skin moisturization is difficult to document.

Glycerin, propylene glycol, 1,3-butylene glycol, or sorbitol are typically used in body washes, bubbles baths, shower gels, or soaps to prevent the desiccation of the product itself and the formation of a dry layer at the surface. They also ensure stability and clarity of liquid cleansers at cold temperatures.

Few substantive humectants can be mentioned. They are cationic in nature which makes them adsorbing to the negatively charged skin surface. In the quaternized polyalkoxylated methyl glucose derivative (lauryl methyl gluceth-10 hydroxypropyldimonium chloride) the hydrophilic moiety delivers humectant properties; the hydrophobic chain at the cationic end of the molecule ensures both substantivity and skin conditioning.

Chitosan–PCA is another example. Chitosan is a polycationic (at acidic pH) high molecular weight polymer produced by deacetylation of chitin, the major constituent of invertebrate exoskeletons. Combining chitosan with PCA leads to a highly substantive, film forming humectant material.

POLYMERS

Polymeric materials can interact both with protein of skin surface and with skin lipids. Parameters influencing the interaction between skin surface and polymers are

- the positive charge density: the more cationic the character of the polymer, the better the polymer interaction with negatively charged skin surface;
- the hydrophobicity of polymer: grafting of fatty moieties on the polymer backbone favor hydrophobic interactions with hydrophobic areas of the keratin;

- the molecular weight of the polymer: the higher the polymer size, the more its substantivity to the skin (film forming properties). However, very low molecular weight polymers can easily penetrate the skin surface chinks and as such adsorb into the superficial stratum disjonctum;
- the nature of surfactants neighboring the polymer in the finished product: the polymer can interact with surfactants either through their charges or through hydrophobic interactions; also competition between polymer and surfactants for skin anchoring sites can occur. In both cases, deposition and adsorption of polymer onto the skin surface are weakened.

Natural Polymers and Their Chemically Modified Derivatives

Proteins

Proteins differ by (a) the source, (b) the molecular weight, (c) the AA composition, AA side groups and electrical charge (more of cationic or of anionic AA), (d) the chemically attached moieties (quats, fatty chains, silicone, etc.) on the peptide backbone (Table 4) (13–15).

Proteins can be from vegetable or animal origin. The use of animal derived ingredients in cosmetics is regulated in Europe due to the emergence of BSE infection in ruminants. Hydrolyzed proteins, among others collagen, are considered as safe in cosmetics provided that the supplier implements and certifies the application of an adequate production process.

Collagen from pork or beef is still one of the most widely used *animal* proteins; "marine collagen" (fish) is used as an alternative source of collagen to traditional bovine derived materials. Milk proteins, keratin, and elastin are also considered in cosmetics and toiletries. Concerns related to BSE have initiated a shift away from animal derived ingredients and an increased interest in plant derived proteinic materials.

Vegetable/plant proteins are most of the time associated with significant amounts of soluble and insoluble carbohydrates due to extraction process; soluble carbohydrates confer dark color and strong odor to the raw material; in some commercial grades carbohydrates have been removed. The combination of hydrolyzed vegetable proteins and oligosaccharides produces conditioning additives with synergistic moisturizing action and film forming properties. Major vegetal starting materials are wheat gluten, almond meal, rice, oat, soya, maize, etc.

Proteins are functional over a wide range of pH; nevertheless, as they are amphoteric materials, below their isoelectric point they carry a net positive charge which makes them substantive to the negatively charged skin surface.

Film forming properties of proteins and hydrolyzates are related to their molecular weight (the higher, the better). Overall, proteins convey smoothing and moisturizing effect; they produce soft and silky feel to the skin. They have positive effect on foam profile: they increase foam stability, confer creaminess and density as well as slipperiness to the foam.

Proteins and hydrolyzates are also known for their ability to reduce the irritation caused by anionic surfactants and to combat skin dryness induced by detergents (16–19).

Some native proteins such as elastin, keratin, or vegetable proteins are insoluble. There exist soluble native collagen species; their use is restricted to some specialized applications. To make native proteins suitable for a wide range of applications, they are converted into soluble hydrolyzates by chemical or enzymatic

Table 4 Natural Polymers and Their Chemically Modified Derivatives

Polymers	INCI names
Native proteins	
Solubilized in anionic surfactants	Native wheat protein/lauryl ether sulfate complex
Protein hydrolyzates	
Animal source	Hydrolyzed animal protein
	Hydrolyzed collagen
	Hydrolyzed milk protein
Plant derived	Hydrolyzed vegetal protein
	Hydrolyzed wheat protein/oligosaccharide complex
	Hydrolyzed wheat protein and hydrolyzed wheat starch
	Hydrolyzed oats
	Hydrolyzed wheat gluten
Quaternized protein hydrolyzates	
Animal source	Hydroxypropyl trimonium hydrolyzed collagen
Plant derived	Hydroxypropyl trimonium hydrolyzed wheat protein
Fatty side chains grafted on protein backbone	
Native protein	Wheat extract (and) stearic (and) sodium chloride
Quaternized fatty chains grafted	
Protein hydrolyzate	Steardimonium hydrolyzed wheat protein or collagen
	Lauryl or cocodimonium hydroxypropyl hydrolyzed collagen
	Alkyl quaternary hydrolyzed soya protein
Copolymers	
Protein-PVP	Hydrolyzed wheat protein/polyvinyl pyrrolidone copolymer
Protein-Silicones	Hydrolyzed wheat protein hydroxypropyl polysiloxane copolymer
Quaternized copolymer	Hydroxypropyl trimonium hydrolyzed wheat protein polysiloxane copolymer

degradation. The sizes of resulting peptides depend on the hydrolysis process used. Chemical processes give rise to broader molecular weight distributions and enzymatic digestion to narrower ones. Besides that, native proteins solubilized in various anionic surfactants (by formation of a protein–surfactant complex) are commercially available, allowing easy formulation of these film forming, moisturizing, mildness additives.

A wide range of protein hydrolyzates molecular weights is available, ranging from 500,000 down to 1000 d. Protein hydrolyzates of intermediate molecular weight (average 3000–5000 d) are the most widely used ones; they are less substantive than high molecular weight proteins but provide smooth skin feel, slippery feel during use, and sensation of skin hydration. Hydrolyzates are readily soluble and compatible with all classes of surfactants.

Most commercially available proteins and derivatives have characteristic odor and color. Furthermore, products formulated with proteins or hydrolyzates should be adequately preserved.

Chemically Modified Protein Derivatives

To increase interaction of proteinic material with skin surface, proteins or hydrolyzates are functionalized or chemically modified (Table 4). Protein possesses reactive side chain amino and carboxyl groups which are sites for further modification of their intrinsic properties.

Hydrophobic interactions with the skin surface are favored and reinforced by grafting fatty carbon chains; ionic interactions are maximized by grafting cationic moieties onto the protein backbone.

Hydrolyzed protein copolymers combine substantivity and film forming properties of parent proteins with characteristic sensorial properties of companion conditioning agents; these macromolecular proteinic complexes offer greater moisturizing and conditioning potential as compared to the individual components (20).

Native proteins coupled with fatty acids lead to macromolecular entity with dual hydrophilic/hydrophobic characteristics and physicochemical properties. Skin substantivity is guided both by the size of the starting protein and by the chain length (the hydrophobicity) of the fatty acid. The macromolecules are surface active and can be formulated in bars or liquids; they produce smooth long lasting skin feel; long chain fatty acid derivatives tend to decrease foam volume but confer creaminess, richness, and slipperiness to the lather.

Copolymers of silicone and proteins are obtained by covalent bonding of low molecular weight polydimethylsiloxanes on amino groups of (vegetable) protein hydrolyzate. They combine beneficial properties of proteins (anti-irritant effect, substantivity, film forming, soft after feel) with lubricity of silicone (21,22). Quaternized protein-silicone copolymers are now commercially available.

Polyvinyl pyrrolidone-protein copolymers: proteinic component imparts substantivity and polyvinyl pyrrolidone (PVP) maximizes the moisture retention and film forming properties of the resulting copolymer. PVP/protein ratio will modulate the profile of performance on the skin and the influence on lathering characteristics of surfactant based skin cleanser.

Quaternized protein hydrolyzates: Cationic protein hydrolyzates are obtained by reacting the primary amine sites on the protein backbone with a tertiary amine: hydroxypropyl or propyl trimethyl ammonium or alkyl trimethyl ammonium (23).

Covalent attachment of quaternary groups strongly increases the cationic character of the protein hydrolyzate, making it further skin substantive and resistant to rinsing with water.

Covalent attachment of fatty quaternary groups (alkyl dimethyl ammonium) on peptides greatly improves both ionic and hydrophobic interactions with the skin. Alkyl chain can be lauryl, myristyl, stearyl. Alkyl trimonium hydrolyzed proteins are still water soluble and compatible with all classes of surfactants. These hydrophobically modified cationic protein hydrolyzates are highly adsorbable at all pH levels and offer skin substantivity at minimum concentration. They impart pronounced conditioning effect; the lipophilic moieties provide emollient feel.

Overall, quaternized versions of a protein are many times more substantive than the parent protein hydrolyzate. Quaternization of protein hydrolyzates raises their isoelectric point (IP) to pH 10 regardless of their initial IP values.

Cationic Guar Gum

Guar gum is a galactomannane polysaccharide derived from the endosperm of cyamopsis tetragonolobus seeds (Table 5).

Table 5 Natural Polymers and Their Chemically Modified Derivatives

Polymers	INCI names	Comments
Cellulose derivatives	Polyquaternium 10	Polymeric quaternary ammonium salt of HEC reacted with trimethyl ammonium substituted epoxide
	Polyquaternium 24	Polymeric ammonium salt of HEC reacted with lauryl dimethyl ammonium substituted epoxide; average degree of substitution = 1
	PG-hydroxyethyl cellulose lauryl or coco or stearyl dimonium chloride	Average degree of substitution > 1
Guar derivatives	Guar hydroxypropyl trimonium chloride hydroxypropyl guar hydroxypropyl trimonium chloride	

Depolymerization of the gum by enzymatic or chemical processes allows one to modulate its molecular weight and consequently to impact its solubility, thickening properties, and the transparency of the finished product. Free hydroxyl groups on the polysaccharidic backbone can participate in esterification and etherification reactions. Hydroxypropyl (HP) side groups improve guar compatibility with electrolytes. Cationic guar derivatives are obtained by reaction of HP guar with epoxypropyltrimethyl ammonium chloride; positive charge density of resulting guar hydroxypropyl trimonium chloride depends on substitution degree. Cationic guar derivatives are film forming, they impart soft, smooth, and silky feel to the skin; moreover, they act as antiirritant for anionic surfactants and soaps and have positive effect on foam feel and quality (24,25).

Cationic Cellulose Derivatives

Polyquaternium 10 is a range of polymeric quaternary ammonium salts of hydroxyethyl cellulose (HEC) reacted with trimethyl ammonium substituted epoxide. Polyquaternium 10 solutions are non-Newtonian and are commercially available (a) in several viscosity grades depending on their molecular weights (they contribute to viscosity of formulations) and (b) with "high" to "moderate" cationic substitution. In vivo tests showed that these cationic cellulosic polymers protect the skin from aggression by anionic surfactants (Table 5) (26,27).

Polyquaternium 24 is a polymeric quaternary ammonium salt of HEC reacted with lauryldimethyl ammonium substituted epoxide; it is a hydrophobically modified Polyquaternium 10.

The average degree of substitution with quaternary fatty chain is 1 in Polyquaternium 24; a range of alkyl dimonium hydroxypropyl oxyethyl cellulose with higher proportion of grafted cationic fatty groups (average degree of substitution is 1.2) is also commercially available.

Table 6 Synthetic Quaternized Polymers

Polyquaterniums	INCI names
Polyquaternium 6	Dimethyl diallyl ammonium chloride homopolymer
Polyquaternium 7	Acrylamide/dimethyl diallyl ammonium chloride copolymer
Polyquaternium 11	Poly(vinylpyrrolidone/dimethylaminoethyl methacrylate)
Polyquaternium 22	Acrylic acid/dimethyl diallyl ammonium chloride copolymer
Polyquaternium 39	Acrylamide/acrylic acid/dimethyl diallyl ammonium chloride terpolymer

The presence of fatty side chains on these quaternized cellulose ethers makes them surface active and further participates in their very high skin substantivity and film forming properties. They impart silky smooth after feel.

These alkyl quaternary cellulose polymers are soluble in water (longer C chains must be slightly warmed) and compatible with a wide range of surfactants; they have favorable influence on the lathering properties providing creaminess, density, slipperiness, and stability to the foam.

Synthetic Quaternized Polymers

An array of dimethyl diallyl ammonium chloride (DMDAAC) based polymers and copolymers is commercially available. Their substantivity, film forming properties, and resulting skin feel depend on both the molecular weight (ranging from about 400,000 up to 7 million) and the density of positive charges which also dictates the compatibility of the polymer with anionic surfactants. These polymers generally make foam denser and more stable (Table 6) (28).

DMDAAC homopolymer (Polyquaternium 6) carries the highest positive charge density and is not compatible with anionic surfactants.

Inclusion of acrylamide into DMDAAC homopolymer decreases the positive charge density leading to a skin conditioning polymer more compatible with anionics (Polyquaternium 7) (29,30). Polyquaternium 7 is probably one of the mostly used synthetic cationic polymers in body cleansing products; it is highly substantive to the skin, delivering soft, silky, moisturized after feel (28).

Positive charge density is also decreased by copolymerizing DMDAAC with either acrylic acid (Polyquaternium 22) or with both acrylamide and acrylic acid (Polyquaternium 39). Another widely used synthetic cationic polymer in liquid skin cleansers and in bar soaps is a quaternized copolymer of PVP and dimethylaminoethyl methacrylate (Polyquaternium 11). This PVP copolymer is available in molecular weights ranging from 100,000 to 1,000,000.

SURFACTANTS

Benefits brought by additional skin conditioning agents are sometimes hidden by a mild or very mild cleaning surfactant system delivering by itself very good skin feel properties; the sensorial base line is high to start with and the increment in performance brought by skin feel agent is leveled off, and sometimes even not perceivable (Table 7).

Table 7 Surfactants and Their INCI Names

Nonionics	INCI names
Polyhydric alcohol esters	
Sucrose esters	Sucrose laurate or cocoate
Methyl glucose esters	PEG-120 methyl glucose dioleate
	PEG-80 methyl glucose laurate
Glucose ethers	Alkyl polyglucosides
Fatty acid alkanolamides	Cocodiethanolamide
Amphoterics	
Ampholytes	Cocamidopropyl betaine
	Olivamidopropyl betaine
	Sesamidopropyl betaine
	Isostearamidopropyl betaine
	Cocamidopropyl hydroxysultaine
	Cocamidopropyldimethyl aminohydroxypropyl hydrolyzed collagen
	Dimethicone propyl PG-betaine
Propionates	Alkylamino propionates
	Alkyliminodipropionates
Imidazoline derivatives	Acylamphoacetate
Anionics	
Phosphoric acid esters and salts	C9-C15 alkyl phosphate
	PPG-5 ceteth-10 phosphate
	Oleth-3 phosphate
Acyl amino acids and salts	
Acyl peptides	Sodium cocoyl hydrolyzed protein
	Sodium lauroyl oat amino acids
	TEA or sodium lauroyl animal collagen amino acids
Acyl glutamates	Sodium cocoyl glutamate
Sarcosinates	Sodium cocoyl or lauroyl sarcosinate
Taurates	Sodium methyl cocoyl taurate
Sulfonic acids and salts	
Sulfosuccinates	Disodium laneth-5 sulfosuccinate
	Disodium ricinoleamido MEA-sulfosuccinate
	Disodium laureth sulfosuccinate
	Disodium PEG-8 palm glycerides sulfosuccinate
Isethionates	Sodium cocoyl isethionate
Carboxylates	Alkyl glucose carboxylate
	Sodium PEG-7 olive oil carboxylate

It is however important to notice that several mild anionic and most of the nonionic surfactants, if they provide a pleasant after feel, are characterized by a "water feel" (feel in solution) often unpleasant, with rough and drag feel sensations.

Amphoteric surfactants are amino acid derivatives; their net charge varies with the pH in solution; at pH below the isoelectric point they are positively charged in aqueous solution and can consequently adsorb more easily onto the skin. Alkyl chain length also significantly acts on the skin feel; some betaines based on C16/C18 cuts provide greasier refattened feel, but also have detrimental effect on foam.

Table 8 Exfoliants/Scrubbing Agents

Apricot/walnut shells powder or flour
Corn cob
Jojoba beads
Polyethylene/styrene beads
Almond meal
Apricot/peach seed powder
Loofah
Maize scape powder
Kaolin

Polydimethylsiloxane grafted with a betaine moiety leads to an amphoteric surfactant combining substantivity, refattening properties as well as silicone typical skin feel profile.

Some *nonionics* are used for their emolliency properties and excellent after feel: sucrose and methyl glucose esters as well as sucrose ethers are mentioned as examples. Fatty acid alkanolamides are often referred to as refatteners; these are not lipids but they confer a greasy slippery feel to the foam and impart a particular after feel to the skin which subjectively compares to refatting.

Several mild *anionic surfactants* are known to provide improved skin feel (after feel) by themselves: sarcosinate, taurate, acylglutamate, and isethionate are cited as examples. Fatty acids-protein condensates salts act also as a conditioning aid imparting a pleasant smooth feel to the skin. The inclusion of fatty acids in soap and syndet bars contributes to enhance skin feel during and after use and produce creamier lather. Phosphoric acid fatty esters deliver soap like skin feel: slipperiness during use, very good rinseability leaving skin feeling "clean," and powdery like after feel.

EXFOLIATING AGENTS

Skin scrub agents or body polishers are solid materials from natural origin (clay, fine powder of seeds, or shells of different vegetables) or obtained by chemical synthesis (tiny beads of styrene or polyethylene) (Table 8). When the scrub agent containing the body cleansing product is rubbed or massaged onto the skin, fine solid particles remove the superficial skin horny layer by mechanical abrasion, leaving behind a fresh smooth skin surface. They are the easiest additives for the consumer to perceive. Scrubbing particles can be suspended in liquid body cleanser thanks to structuring polymers like xanthan gum or carraghenan which build a viscoelastic network in the surfactant matrix. The scrubbing agent must be very carefully selected when formulating facial cleansers. Skin of the face is more sensitive or delicate than the rest of the body. For facial application, the formulator should orientate his choices toward soft clays or melting jojoba beads, for example.

CONCLUSIONS

The overall skin feel profile provided by a skin cleansing product is conditioned by the huge variety of composition constituents. Many of them have been described

in this chapter but not exhaustively. Other factors can influence the sensations perceived by the consumer like the presence of electrolytes, or of thickening polymers in the product as well as the water hardness in the user dwelling. It will be the responsibility of the formulator to consider all the potential synergisms or antagonisms in his finished product, to deliver the desired skin feel.

REFERENCES

1. Domsch A. Modern bath and shower preparations under dermatological aspects. Seife Öle Fette Wachse 1991; 15:573–576.
2. Gordon G, Schoenberg CO, Winder LC. Personal cleansing system comprising a polymeric diamond-mesh sponge and a liquid cleanser with moisturizer. US patent 5,804,539 (1998). Assigned to The Procter and Gamble Company.
3. Herbe JF. Produits d'hygiène: les tendances. Parfums Cosmétiques Arômes 1993; 18(113):37–41.
4. Domsch A. Rückfettung in bade-und-duschpräparaten. Seifen Öle Fette Wache 1986; 112:163–167.
5. Gassenmeier T, Busch P, Hensen H, Seipel W. Some aspects of refatting the skin; effects oriented to skin lipids for improving skin properties. Cosmetics Toiletries 1998; 113(9): 89–92.
6. Both W, Gassenmeier T, Hensen H, Hörner V, Seipel W, Le Hen Ferrenbach C, Robbe Tomine L. Parfum Cosmetiques Actualités 1998; 23(142):63–65.
7. Fuller JG. Ethoxylated mono and diglycerides in skin and hair care applications. 15th IFSCC International Congress, London, 1988, Vol. A, paper A5:43–55.
8. Barnett G. Lanolin and derivatives. Cosmetics Toiletries 1986; 101(3):23–44.
9. Whalley GR. Take a closer look at lanolin. Household Personal Products Ind 1998; 36(5):115–118.
10. Wendel SR. Utilisation des silicones dans les cosmétiques et produits de toilette. Parfums, Cosmétiques, Arômes 1984; 9(59):67–68.
11. Alexander P. Oils in water. Manufacturing Chemist 1989; 60(3):33–35.
12. Wendel SR, DiSapio AJ. Organofunctional silicones for personal care applications. Cosmetics Toiletries 1983; 98(5):103–106.
13. Gallagher KF. Hydrolyzed vegetable proteins: a formulator's guide (part 1). Drug Cosmetic Ind 1991; 151(8):34–66.
14. Gallagher KF, Jones RT. Hydrolyzed vegetable proteins: a formulator's guide (part 2). Drug Cosmetic Ind 1992; 152(12):26–36.
15. Chvapil M, Eckmayer Z. Role of proteins in cosmetics. Int J Cosmet Sci 1985; 7:41–49.
16. Teglia A, Secchi G. New protein ingredient for skin detergency: native wheat protein-surfactant complexes. Int J Cosmet Sci 1994; 16:235–246.
17. Tavss EA, Eigen E, Temnikow V, Kligman AM. Effect of protein cationicity on inhibition of in vitro epidermal curling by alkylbenzene sulfonate. J Am Oil Chem Soc 1986; 63(4):574–579.
18. Eigen E, Weiss S. Skin protecting composition containing a water-soluble partially degraded protein. US Patent 3,548,056 (1970). Assigned to Colgate Palmolive Company.
19. Marsh RA, Mackie GJ, Hale P. Detergent composition comprising modified proteins. US Patent 4,195,077 (1980). Assigned to The Procter & Gamble Company.
20. Gallagher KF, Jones RT. Emerging technology in protein copolymerization. Cosmetics Toiletries 1993; 108(3):97–104.
21. Jones R. Protein potential. Soap Perfumery Cosmetics 1992; 65(4):33–34.
22. Jones R. Dérivés de protéines greffés aux silicones. Parfums Cosmétiques Arômes 1993; 18(109):69–71.

23. Stern ES, Johnsen VL. Cosmetic proteins: a new generation. Cosmetics Toiletries 1983; 98(5):76–84.
24. Marti ME. Phyto-active cosmetics. Drug Cosmetic Ind 1992; 152(2):36–46.
25. Pugliese P, Hines G, Wielinga W. Skin protective properties of a cationic guar derivative. Cosmetics Toiletries 1990; 105(5):105–111.
26. Faucher JA, Goddard ED, Hannan RB, Kligman AM. Protection of the skin by a cationic cellulose polymer. Cosmetic Toiletries 1977; 92(6):39–44.
27. Goddard ED. Cationic cellulosic derivatives. In: Kennedy JF, Phillips GO, Williams PA, eds. Cellulosic Chemical Biochemical and Material Aspects. London: Horwood, 1993: 331–336.
28. Alexander P. Cationic polymers for skin & hair conditioning. Manufacturing Chemist 1987; 58(7):24–29.
29. Jack S. The use of Merquat in hair and skin care. Soap Perfumery Cosmetics 1985; 58(11):633–636.
30. Sykes R, Hammes PA. The use of Merquat polymers in cosmetics. Drug Cosmetic Ind 1980; 126(2):62–136.

20
Hydrating Substances

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INTRODUCTION

Hydrating substances are used in cosmetic products to retard moisture loss from the product during use and to increase the moisture content in material that is in contact with the product. This function is generally performed by hygroscopic substances, or humectants, which are able to absorb water from the surroundings. In the International Cosmetic Ingredient Dictionary, approximately 125 substances are listed as humectants and almost 200 hygroscopic materials are used to increase the water content of the skin (1).

Target areas in the body for treatment with humectants are dry hair and dry skin. Sometimes mucous membranes also benefit from application of humectants. Dry hair is brittle, rough, has a tendency to tangle and has hardly any luster. Humidity of the atmosphere is the only source of moisture to hair, except shampooing, and the addition of humectants to the hair will, therefore, facilitate its retention of water. The same is true for the skin, although it is constantly supplied with water from inside of the body. The skin forms a critical structural boundary for the organism and is frequently compromised as a result of under hydration. The water held by the hygroscopic substances in the stratum corneum is a controlling factor in maintaining skin flexibility and desquamation (2,3).

The special blend of humectants found in the stratum corneum is called natural moisturizing factor (NMF) (4). NMF can make up about 10% of the dry weight of the stratum corneum cells (4). Substances belonging to this group are amino acids, pyrrolidone carboxylic acid (PCA), lactates, and urea (Tables 1 and 2) (4). NMF is formed from the protein filaggrin and this formation is regulated by the moisture content in the stratum corneum (2). In skin diseases such as ichthyosis vulgaris (5,6) and psoriasis (7), there is a virtual absence of NMF. In ichthyosis vulgaris, the stratum granulosum is thin or missing due to a defect in the processing of profilaggrin, which also is noticed as tiny and crumbly keratohyalin granules (8).

Glycerin is another humectant suggested to be important for the stratum corneum hydration (Tables 1 and 2). Skin dryness in sebaceous gland deficient mice has been found to be linked to reduced levels of glycerin because of absence of triglycerides, which are the primary source for glycerin (17). This type of dryness may also be applicable to clinical situations where sebaceous glands are absent or involuted,

Table 1 Chemistry of Hygroscopic Substances

Name	CAS-No	Mw	Other names	Natural source
Butylene glycol	107-88-0	90.1	1,3-butanediol, 1,3-butylene glycol	
Glycerin	56-81-5	92.1	Glycerol, 1,2,3-propanetriol	Hydrolysis of oils and fats
Lactic acid	50-21-5	90.1	2-hydroxypropanoic acid	Sour milk and tomato juice
Panthenol	81-13-0	205.3	Dexpanthenol, pantothenol, provitamin B_5	Plants, animals, bacteria
PCA	98-79-3	129.11	L-pyroglutamic acid, DL-pyrrolidonecarboxylic acid, 2-pyrrolidone-5-carboxylic acid	Vegetables, molasses
Propylene glycol	57-55-6	76.1	1,2-propanediol	
Hyaluronic acid	9004-61-9	5×10^4–8×10^6	Hyaluronan	Cock's combs, biofermentation
Sorbitol	50-70-4	182.17	D-glucitol	Berries, fruits
Urea	57-13-6	60.08	Carbamide, carbonyl diamide	Urine

Abbreviations: MW, molecular weight; PCA, pyrrolidone carboxylic acid.
Source: From Refs. 1, 9–11.

such as in prepubertal children showing eczematous patches, which disappear with the onset of sebaceous gland activity. Moreover, xerosis in the distal extremities of aged skin and in patients receiving systemic isotretinoin for treatment of acne may be linked to glycerin depletion because of the lower sebaceous gland activity (17).

Physiologically occurring and synthetic substances are used as humectants in cosmetic products (Tables 1 and 2). The water binding capacity of the sodium salts of lactic acid and PCA appears to be higher than that of glycerin and sorbitol (Table 3) (12,13). Treatment of solvent-damaged guinea pig footpad corneum with humectant solutions shows that the water held by the corneum decreases in the following order: sodium PCA > sodium lactate > glycerin > sorbitol (18). Urea also has strong osmotic activity (19,20). However, which of these substances most efficiently reduces xerosis or other dry skin conditions is not known. Besides differences in water binding capacity, their absorption into the skin is important for the effect. Hence, the in vitro humectancy should be distinguished from the in vivo moisturizing effect (21). Some factors to consider during product development are highlighted in Table 4 .

This chapter will provide basic information about some commonly used humectants, which are primarily used for treatment of the skin. Moreover, safety information will also be provided.

BUTYLENE GLYCOL

Description

Butylene glycol usually means 1,3-butanediol but the term can also be used for 2,3-butanediol (Tables 1 and 2). The alcohol is a viscous, colorless liquid with sweet

Table 2 Chemical Formulas of Humectants

Humectant	Formula
Butylene glycol	
Glycerin	
Lactic acid	
Panthenol	
PCA	
Propylene glycol	
Sorbitol	
Urea	

flavor and bitter aftertaste (9). It is soluble in water, acetone, and castor oil, but practically insoluble in aliphatic hydrocarbon (9).

General Use

Butylene glycol is used as humectant for cellophane and tobacco (10). It is also used in topical products and as solvents for injectable products. Butylene glycol is claimed to be most resistant to high humidity and is often used in hair sprays and setting lotions (22). The alcohol also retards loss of aromas and preserves cosmetics against spoilage by microorganisms (22).

Safety

Butylene glycol is considered safe by the Cosmetic Ingredient Review (CIR) Expert Panel (23). Human skin patch test on undiluted butylene glycol produced a very low

Table 3 Moisture-Binding Ability of Humectants at Various Humidities

Humectant	31%	50%	52%	58–60%	76%	81%
Butylene glycol						38[e]
Glycerin	13[c] 11[b]	25[a]	26[b]	35–38[c,f]	67[b]	
Na–PCA	20[c] 17[b]	44[a]	45[b]	61–63[c,f]	210[b]	
Na–lactate	19[b]	56[a]	40[b]	66[f]	104[b]	
Panthenol	3[d]		11[d]		33[d]	
PCA	<1[c]				<1[c]	
Propylene glycol					32[f]	
Sorbitol			1[a]		10[f]	

Abbreviation: PCA, pyrrolidone carboxylic acid.
[a]*Source*: From Ref. 12.
[b]*Source*: From Ref. 13.
[c]*Source*: From Ref. 14.
[d]*Source*: From Ref. 15.
[e]*Source*: From Ref. 9.
[f]*Source*: From Ref. 16.

order of primary skin irritation and a repeated patch test produced no evidence of skin sensitization (23). The substance is reported to be less irritating than propylene glycol (24,25). Few reports of contact allergy exist, but the substance does not seem to cross-react with propylene glycol (24).

GLYCERIN

Description

In 1779, the Swedish scientist, C.W. Scheele discovered that glycerin could be made from a hydrolyzate of olive oil. The alcohol is a clear, colorless, odorless, syrupy, and hygroscopic liquid (Tables 1 and 2) (9,11), approximately 0.6 times as sweet as cane sugar (9,11). It is miscible with water and alcohol; slightly soluble in acetone; practically insoluble in chloroform and ether (9,10).

General Use

Glycerin can be used as a solvent, plasticizer, sweetener, lubricant, and preservative (11). The substance has also been given intravenously or by mouth in a variety of

Table 4 Parameters to Consider During Product Development to Obtain the Desired Effect

Formulation related	Effect on the target area
Price and purity?	Product claim?
Chemical stability during production and shelf life?	Substantivity in rinse-off products?
Sensitive to heat? UV-light? pH?	Penetration characteristics?
Incompatibilities with other ingredients?	Hygroscopicity?
Adsorption to the packaging material?	Adverse effects?
Effects on the preservation system?	

clinical conditions in order to benefit from its osmotic dehydrating properties (10). This effect can also be used topically for the short-term reduction of vitreous volume and intra-ocular pressure of the eye (10,11). Moreover, concentrated solutions of glycerin are used to soften ear-wax (10) and suppositories with glycerin (dose 1–3 g) promote fecal evacuation (10).

Effects on Skin

The importance of glycerin in skin care products is well established. To explain its benefits, studies have focused on its humectant and protecting properties. Levels ranging between a few percent and 20% to 25% are used in moisturizers for treatment of dry skin conditions (26). Glycerin not only attracts water but has also been suggested to modulate the phase behavior of stratum corneum lipids and to prevent crystallization of their lamellar structures in vitro at low relative humidity (27). Incorporation of glycerin into a stratum corneum model lipid mixture enables the lipids to maintain the liquid crystal state at low humidity (27). The biochemical consequences of these properties may be due to the influence of the activity of hydrolytic enzymes crucial to the desquamatory process in vivo (28). Thereby the rate of corneocyte loss from the superficial surface of human skin increases, probably due to an enhanced desmosome degradation (2,28).

Repeated tape stripping taken from skin treated with 15% glycerin cream indicates that glycerin diffuses into the stratum corneum to form a reservoir (29). During some hours after application a decrease in transepidermal water loss (TEWL) has been noted (29–32) followed by increased values after some hours in animal skin (32). No evidence of deterioration of the skin barrier function has been noted after long-term treatment of normal and atopic skin with 20% glycerin (33,34). Instead glycerin has been found to accelerate barrier recovery after acute external perturbations (35). Moreover, in human skin its surface profile, electrical impedance, and increase in the coefficient of friction were found to accompany an improvement in the skin condition, as assessed by an expert (30).

Safety

Very large oral or parenteral doses can exert systemic effects because of the increase in the plasma osmolality resulting in the movement of water by osmosis from the extravascular spaces into the plasma (10). Glycerin dropped on the human eye causes a strong stinging and burning sensation, with tearing and dilatation of the conjunctival vessels (36). There is no obvious injury (36), but studies have indicated that glycerin can damage the endothelial cells of the cornea (36). Glycerin has been shown to have excellent skin tolerability and treatment with up to 20%. Glycerin did not show any signs of adverse effects in atopic dry skin (26).

HYALURONIC ACID

Description

Hyaluronic acid is a member of the class of amino-sugars containing polysaccharides known as the glycosaminoglycans widely distributed in body tissues. The polymer provides the turgor for the vitreous humor of the eye and the name hyaluronic acid derives from the Greek *hyalos* (glossy, vitreous) and uronic acid. Another name

for the substance is hyaluronan. Molecular weight is within the range of 50,000 to 8×10^6 depending on source, methods of preparation, and determination (9). Hyaluronic acid is a regulator of cell behavior and influences cellular metabolism. Moreover, the molecule binds water and functions as a lubricant between the collagen and the elastic fiber networks in dermis during skin movement. A 2% aqueous solution of pure hyaluronic acid holds the remaining 98% water so tightly that it can be picked up as though it was a gel (37).

During manufacturing, the large, unbranched, noncross-linked, water-containing molecule is easily broken by shear forces (37). The carbohydrate chain is also very sensitive to breakdown by free radicals, ultraviolet radiation, and oxidative agents (37).

General Use

A viscous solution of the sodium salt is used during surgical procedures on the eye and intra-articular injections have been tried in the treatment of osteoarthritis (10). Topical application of 0.1% solution in patients with dry eye increased tear film stability and alleviated symptoms of irritation and grittiness (10).

Effects on Skin

High molecular weight hyaluronic acid solutions form hydrated viscoelastic films on the skin (37). The larger the molecular size, the greater the aggregation and entanglement of the molecules and hence, the more substantial and functional the viscoelastic film associated with the skin surface (37). Owing to the high molecular weight, hyaluronic acid will not penetrate deeper than the crevices between the desquamating cells. The polymer may also be injected to obtain a smoother surface and reduce the depth of wrinkles.

Safety

Sodium hyaluronate is essentially nontoxic. When the substance is used as an ophthalmic surgical aid, transient inflammatory ocular response has been described (36).

LACTIC ACID

Description

Lactic acid is colorless to yellowish crystal or syrupy liquid, miscible with water, alcohol, glycerol, but insoluble in chloroform (10). Lactic acid is an α-hydroxy acid (AHA), i.e., an organic carboxylic acid in which there is a hydroxy group at the two, or alpha (α), position of the carbon chain (Table 2). Lactic acid can exist in a DL, D, or L form. The L and the D forms are enantiomorphic isomers (mirror images). Lactate is also a component of the natural hygroscopic material of the stratum corneum and constitutes about 12% of this material (Table 1) (4).

Formulations containing lactic acid have an acidic pH in the absence of any inorganic alkali or organic base. The pH is increased in several formulations by partial neutralization.

General Use

Lactic acid has been used in topical preparations for several decades because of its buffering properties and water-binding capacity (18). Lactic acid and its salts have been used for douching and to help maintain the normal, acidic atmosphere of the vagina. Lactic acid has also been used for correction of disorders associated with hyperplasia and/or retention of the stratum corneum, such as dandruff, callus, keratosis, and verrucae (viral warts) (10). Moreover, lactic acid has been suggested to be effective for adjuvant therapy of mild acne (38). Also, ethyl lactate has been proposed to be effective in the treatment of acne, due to its penetration into the sebaceous follicle ducts with subsequent lowering of pH and decrease in the formation of fatty acids (39).

Investigators have also reported increases in the thickness of viable epidermis (40,41) as well as improvement in photoaging changes (40,42). Lactic acid in combination with other peeling agents is used to produce a controlled partial-thickness injury to the skin, which is believed to improve the clinical appearance of the skin (43).

Effects on Skin

In guinea pig footpad corneum, it has been shown that both lactic acid and sodium lactate increase the water holding capacity and skin extensibility (18). When the pH increases, the adsorption of lactic acid decreases, due to the ionization of the acid (18). In another study on strips of stratum corneum from human abdominal skin, the uptake of water by sodium lactate was greater than that by lactic acid, but the stratum corneum was plasticized markedly by lactic acid and not by sodium lactate (12).

The concentrations used for treatment of ichthyosis and dry skin have ranged up to 12% (44). After treatment with 5% lactic acid combined with 20% propylene, glycol increased TEWL has been noted in patients with lamellar ichthyosis (45). However, lactic acid has been suggested to stimulate the ceramide synthesis and improve skin barrier function (46,47).

Safety

Lactic acid is caustic to the skin, eyes, and mucous membranes in a concentrated form (36). Compared to other acids, lactic acid has no unusual capacity to penetrate the cornea, so its injurious effect is presumably attributable to its acidity (36).

Immediately after the application of an AHA, stinging and smarting may be noticed; this is closely related to the pH of the preparations and the substances themselves (48–50). In normal skin, irritation and scaling may be induced when the acids are applied in high concentrations and at low pH (51). At a fixed lactic acid concentration, the desquamative effect is highly pH dependent, while at fixed pH, the turnover rate of skin is concentration dependent (49).

PANTHENOL

Description

D-Panthenol is a clear, almost colorless, odorless, and viscous hygroscopic liquid, which may crystallize on prolonged storage (Tables 1 and 2) (10). Panthenol is an alcohol, which is converted in tissues to D-pantothenic acid (Vitamin B_5), a

component of coenzyme A in the body. The substance can be isolated from various living creatures, which gave the reason for its name (Table 1) (Panthoten is Greek for everywhere) (52). Panthenol is very soluble in water, freely soluble in alcohol and glycerol, but insoluble in fats and oils (15). The substance is fairly stable to air and light if protected from humidity, but it is sensitive to acids and bases and also to heat (15). The rate of hydrolysis is lowest at pH 4 to 6 (15).

General Use

Panthenol is widely used in the pharmaceutical and cosmetic industry for its moisturizing, soothing, and sedative properties (52,53). It is also found in topical treatments for rhinitis, conjunctivitis, sunburn, and for wound healing (ulcers, burns, bed sores, and excoriations) (10,52); usually 2% is used (10). The mechanisms of action are only partly known.

The hygroscopic alcohol can further be used to prevent crystallization at the spray nozzles of aerosols (15).

Effects on Skin and Hair

Topically applied panthenol is reported to penetrate the skin and hairs and to be transformed into pantothenic acid (52,54). Treatment of sodium lauryl-sulphate (SLS)-induced irritated skin with panthenol accelerates skin barrier repair and stratum corneum hydration (53). Moreover, skin redness decreased more rapidly by panthenol treatment (53).

Pantothenic acid can be found in normal hair (15). Soaking of hair in 2% aqueous solution of panthenol has been reported to increase the hair diameter up to 10% (55).

Safety

Panthenol has very low toxicity and is considered safe to be used in cosmetics (54). Panthenol and products containing panthenol (0.5–2%) administered to rabbits caused reactions ranging from no skin irritation to moderate-to-severe erythema and well-defined edema (54). Low concentrations have also been tested on humans and those formulations did not induce sensitization or significant skin irritation (54). Contact sensitization to panthenol present in cosmetics, sunscreens, and hair lotion has been reported, although allergy to panthenol among patients attending for patch testing is uncommon (52,56).

PCA AND SALTS OF PCA

Description

PCA is the cosmetic ingredient term used for the cyclic organic compound known as 2-pyrrolidone-5-carboxylic acid (Tables 1 and 2). The "L" form of the sodium salt is a naturally occurring humectant in the stratum corneum at levels about 12% of the NMF (4) corresponding to about 2% by weight in the stratum corneum (14). The sodium salts of PCA are among the most powerful humectants (Table 3). PCA is also combined with a variety of other substances, like arginine, lysine, chitosan, and triethatnolamine (1).

Effects on Skin

A significant relationship has been found between the moisture-binding ability and the PCA content of samples of stratum corneum (14). Treatment with a cream containing 5% sodium-PCA also increased the water-holding capacity of isolated corneum compared to the cream base (57). The same cream was also more effective than a control product containing no humectant, and equally effective as a similar established product with urea as humectant, in reducing the skin dryness and flakiness (57).

Safety

In animal studies, no irritation in the eye and the skin was noted at concentrations up to 50% and no evidence of phototoxicity, sensitization, or comedogenicity was found (58). Minimal, transient ocular irritation has been produced by 50% PCA (58). Immediate visible contact reactions in back skin have also been noted after application of 6.25% to 50% aqueous solutions of sodium PCA (59). The response appeared within five minutes and disappeared 30 minutes after application. PCA should not be used in cosmetic products in which *N*-nitroso compounds could be formed (58).

PROPYLENE GLYCOL

Description

Propylene glycol is a clear, colorless, viscous, and practically odorless liquid having a sweet, slightly acrid taste resembling glycerol (Tables 1 and 2) (11). Under ordinary conditions it is stable in well-closed containers and it is also chemically stable when mixed with glycerin, water, or alcohol (11).

General Use

Propylene glycol is widely used in cosmetic and pharmaceutical manufacturing as a solvent and vehicle, especially, for substances unstable or insoluble in water (11,60). It is also often used in foods as antifreeze and emulsifier (9,11). Propylene glycol is also used as an inhibitor of fermentation and mold growth (9).

Effects on Skin

Propylene glycol has been tried in the treatment of a number of skin disorders including ichthyosis (45,61,62), tinea versicolor (63), and seborrheic dermatitis (64), due to its humectant, keratolytic, antibacterial, and antifungal properties (11,60).

Safety

Propylene glycol has been given an acceptable daily intake (ADI) value of 25 mg/kg by the Joint FAO/WHO Expert Committee of Food (11,65). Poisoning has been found after oral doses around 100 to 200 mg/kg to children (66–68) and after topical treatment with high concentrations in burn patients (69), but the alcohol is considered safe for use in cosmetic products (70).

Clinical data have shown skin irritation and sensitization reactions to propylene glycol in normal subjects at concentrations as low as 10% under occlusive conditions and in dermatitis patients as low as 2% (25,70). The nature of the cutaneous response remains obscure and, therefore, the skin reactions have been classified into four mechanisms: (a) irritant contact dermatitis, (b) allergic contact dermatitis, (c) nonimmunologic contact urticaria, and (d) subjective or sensory irritation (50). This concept allows a partial explanation of effects observed by different authors (71).

PROTEINS

Description

Proteins and amino acids for cosmetics are based on a variety of natural sources. Collagen is the traditional protein used in cosmetics. Collagen has a complex triple helical structure, which is responsible for its high moisture retention properties. Vegetable-based proteins have during recent years grown in importance as an alternative to using animal by-products. Suitable sources include wheat, rice, soybean, and oat.

In cosmetics, native proteins can be used, but perhaps the most widely used protein types are hydrolyzed proteins of intermediate molecular weight with higher solubility. An increased substantivity is obtained by binding fatty alkyl quaternary groups to the protein. Improved film-forming properties can be obtained by combining the protein and polyvinylpyrrolidone into a copolymer. Such modifications may increase the moisture absorption compared with the parent compound. Potential problems with proteins are their odor and change in color with time. Furthermore, as they are nutrients their inclusion in cosmetics may require stronger preservatives.

Efficacy and Safety

Amino acids belong to the NMF and account for 40% of its dry weight (4). Because of their relatively low-molecular weight they are capable of penetrating the skin and cuticle of the hair more effectively than the higher molecular weight protein hydrolyzates.

Salts of the condensation product of coconut acid and hydrolyzed animal protein (72), and wheat flour and wheat starch (73) are considered safe as cosmetic ingredients by CIR. The most frequent clinical presentation of protein contact dermatitis is a chronic or recurrent dermatitis (74). Sometimes an urticarial or vesicular exacerbation has been noted a few minutes after contact with the causative substance (74,75). Hair conditioners containing quaternary hydrolyzed protein or hydrolyzed bovine collagen have induced contact urticaria and respiratory symptoms (75). Atopic constitution seems to be a predisposing factor in the development of protein contact dermatitis (75).

SORBITOL

Description

Sorbitol is a hexahydric alcohol appearing as a white crystalline powder, odorless, and having a fresh and sweet taste (Tables 1 and 2) (9,11). Sorbitol is most commonly available as 70% aqueous solution, which is clear, colorless, and viscous. It

occurs naturally in fruits and is easily dissolved in water, but not so well in alcohol. It is practically insoluble in organic solvents.

Sorbitol is relatively chemically inert and compatible with most excipients, but it may react with iron oxide and become discolored (11).

General Use

Sorbitol is used in pharmaceutical tablets and in candies when noncariogenic properties are desired. It is also used as sweetener in diabetic foods and in toothpastes. Sorbitol is also used as laxative intrarectally and believed to produce less troublesome side effects than glycerin (10). Its hygroscopic properties are reported to be inferior to that of glycerin (Table 3) (12,76).

Safety

When ingested in large amounts (>20 g per day) it often produces a laxative effect (10,11).

UREA

Description

Urea is another physiological substance occurring in human tissues, blood, and urine (Tables 1 and 2). The amount is of the order of 2% in urine. The extraction of pure urea from urine was first accomplished by Proust in 1821 and pure urea was first synthesized by Wöhler in 1828 (77).

Urea is a colorless, transparent, slightly hygroscopic, odorless or almost odorless, prismatic crystal, or white crystalline powder or pellet. Urea is freely soluble in water, slightly soluble in alcohol and practically insoluble in ether (10). Urea in solution hydrolyzes slowly to ammonia and carbon dioxide (10).

General Use

Urea is used as a 10% cream for the treatment of ichthyosis and hyperkeratotic skin disorders (77,78) and in lower concentrations for the treatment of dry skin. In the treatment of onychomycosis, urea is added to a medicinal formulation at 40% as a keratoplastic agent to increase the bioavailability of the drug (79).

Effects on Skin

An increased water-holding capacity of scales from psoriatic and ichthyotic patients has been observed after treatment with urea-containing creams (78,80).

Concern has been expressed about the use of urea in moisturizers, with reference to the risk of reducing the chemical barrier function of the skin to toxic substances (19). The increase skin permeability by urea has been shown in several studies, where it has been found to be an efficient accelerant for the penetration of different substances (81–83). Not all studies, however, support the belief that urea is an effective penetration promoter (84,85), and treatment of normal skin with moisturizers containing 5% to 10% urea has been found to reduce TEWL and also to diminish the irritative response to the surfactant SLS (86,87). One moisturizer

with urea also reduced TEWL in atopic patients (33,88) and made skin less susceptible against irritation to SLS (89). TEWL has also been reduced in dry skin (90) and in ichthyotic patients (78).

Safety

Urea is a naturally occurring substance in the body, as the main nitrogen containing degradation product of protein metabolism. Urea is an osmotic diuretic and has been used in the past for treatment of acute increase in intracranial pressure due to cerebral edema (10). No evidence of acute or cumulative irritation has been noted in previous studies on urea-containing moisturizers, but skin stinging and burning are reported after treatment with 4% to 10% urea creams in dry and lesioned skin (90–92).

CONCLUSIONS

A number of interesting humectants are available as cosmetic ingredients. Most of them have a long and safe history of use, and several are also naturally occurring in the body or accepted as food additives. The low-molecular weight substances are easily absorbed into the skin, providing a potential drawback of stinging sensations from some of them. The high-molecular weight substances usually do not penetrate the skin, but instead they are suggested to reduce the irritation potential of surfactants. However, case reports of urticarial reactions have been reported after exposure to modified proteins (75).

The advantage with the larger and chemically modified materials are that they have an increased substantivity to target areas, whereas it is apparent that small amounts of several low-molecular weight hygroscopic substances have a questionable contribution to the water content of hair and stratum corneum in rinse-off products (Table 4).

Another issue worth considering is whether the obtained humectancy is the only mode of action. Some humectants may modify the surface properties and increase the extensibility of stratum corneum without influencing the water content. Furthermore, humectants may also modify skin barrier function and influence specific metabolic processes in the skin. One should also keep in mind that humectants can improve the cosmetic properties of the formulation and some of them also facilitate marketing of the product just because of their names.

REFERENCES

1. Pepe RC, Wenninger JA. International Cosmetic Ingredient Dictionary and Handbook. Washington, DC: The Cosmetic, Toiletry, and Fragrance Association, 2002.
2. Rawlings AV, Scott IR, Harding CR, Bowser PA. Stratum corneum moisturization at the molecular level. J Invest Dermatol 1995; 103:731–740.
3. Blank IH. Factors which influence the water content of the stratum corneum. J Invest Dermatol 1952; 18:433–440.
4. Jacobi OK. Moisture regulation in the skin. Drug Cosmet Ind 1959; 84:732–812.
5. Horii I, Nakayama Y, Obata M, Tagami H. Stratum corneum hydration and amino acid content in xerotic skin. Br J Dermatol 1989; 121:587–592.

6. Sybert VP, Dale BA, Holbrook KA. Ichthyosis vulgaris: identification of a defect in filaggrin synthesis correlated with an absence of keratohyaline granules. J Invest Dermatol 1985; 84:191–194.
7. Marstein S, Jellum E, Eldjarn L. The concentration of pyroglutamic acid (2-pyrrolidone-5-carboxylic acid) in normal and psoriatic epidermis, determined on a microgram scale by gas chromatography. Clin Chim Acta 1973; 43:389–395.
8. Vahlquist A. Ichthyosis—an inborn dryness of the skin. In: Lodén M, Maibach HI, eds. Dry Skin and Moisturizers; Chemistry and Function. Boca Raton: CRC Press, 2000: 121–133.
9. Budavari S. The Merck Index. Rahway: Merck & Co, 1989.
10. Reynolds JEF. Martindale The Extra Pharmacopoeia. 30th ed. London: The Pharmaceutical Press, 1993:597.
11. Rowe RC, Sheskey PJ, Weller PJ. Handbook of Pharmaceutical Excipients. 4th ed. London: Pharmaceutical Press, 2003.
12. Takahashi M, Yamada M, Machida Y. A new method to evaluate the softening effect of cosmetic ingredients on the skin. J Soc Cosm Chem 1984; 35:171–181.
13. Rieger MM, Deem DE. Skin moisturizers. II. The effects of cosmetic ingredients on human stratum corneum. J Soc Cosm Chem 1974; 25:253–262.
14. Laden K, Spitzer R. Identification of a natural moisturizing agent in skin. J Soc Cosm Chem 1967; 18:351–360.
15. Huni JES. Panthenol. Basel Roche 1981.
16. Huttinger R. Restoring hydrophilic properties to the stratum corneum—a new humectant. Cosmet Toilet 1978; 93:61–62.
17. Fluhr JW, Mao-Qiang M, Brown BE, Wertz PW, Crumrine D, Sundberg JP, Feingold KR, Elias PM. Glycerol regulates stratum corneum hydration in sebaceous gland deficient (asebia) mice. J Invest Dermatol 2003; 120:728–737.
18. Middleton J. Development of a skin cream designed to reduce dry and flaky skin. J Soc Cosmet Chem 1974; 25:519–534.
19. Hellgren L, Larsson K. On the effect of urea on human epidermis. Dermatologica 1974; 149:89–93.
20. Miettinen H, Johansson G, Gobom S, Swanbeck G. Studies on constituents of moisturizers: water-binding properties of urea and NaCl in aqueous solutions. Skin Pharmacol Appl Skin Physiol 1999; 12:344–351.
21. Sagiv AE, Marcus Y. The connection between in vitro water uptake and in vivo skin moisturization. Skin Res Technol 2003; 9:306–311.
22. Rietschel RL, Fowler JF. Fisher's contact dermatitis. 4th ed. Baltimore: Williams & Wilkins, 1995.
23. Final report of the safety assessment of butylene glycol, hexylene glycol, ethoxydiglycol, and dipropylene glycol. J Am Coll Toxicol 1985; 2:223–248.
24. Sugiura M, Hayakawa R. Contact dermatitis due to 1,3-butylene glycol. Contact Dermat 1997; 37:90.
25. Fan W, Kinnunen T, Niinimäke A, Hannuksela M. Skin reactions to glycols used in dermatological and cosmetic vehicles. Am J Contact Dermat 1991; 2:181–183.
26. Lodén M, Andersson AC, Anderson C, Bergbrant IM, Frödin T, Öhman H, Sandström MH, Särnhult T, Voog E, Stenberg B, Pawlik E, Preisler-Häggqvist A, Svensson Å, Lindberg M. A double-blind study comparing the effect of glycerin and urea on dry, eczematous skin in atopic patients. Acta Derm Venereol 2002; 82:45–47.
27. Froebe CL, Simion FA, Ohlmeyer H, Rhein LD, Mattai J, Cagan RH, Friberg SE. Prevention of stratum corneum lipid phase transitions in vitro by glycerol—an alternative mechanism for skin moisturization. J Soc Cosmet Chem 1990; 41:51–65.
28. Rawlings AV, Harding C, Watkinson A, Banks J, Ackerman C, Sabin R. The effect of glycerol and humidity on desmosome degradation in stratum corneum. Arch Dermatol Res 1995; 287:457–464.

29. Batt MD, Fairhurst E. Hydration of the stratum corneum. Int J Cosm Sci 1986; 8: 253–264.
30. Batt MD, Davis WB, Fairhurst E, Gerreard WA, Ridge BD. Changes in the physical properties of the stratum corneum following treatment with glycerol. J Soc Cosmet Chem 1988; 39:367–381.
31. Wilson DR, Berardesca E, Maibach H. In vivo transepidermal water loss and skin surface hydration in assessment of moisturization and soap effects. Int J Cosmet Sci 1988; 10:201–211.
32. Lieb LM, Nash RA, Matias JR, Orentreich N. A new in vitro method for transepidermal water loss: a possible method for moisturizer evaluation. J Soc Cosmet Chem 1988; 39:107–119.
33. Lodén M, Andersson AC, Andersson C, Frodin T, Oman H, Lindberg M. Instrumental and dermatologist evaluation of the effect of glycerine and urea on dry skin in atopic dermatitis. Skin Res Technol 2001; 7:209–213.
34. Lodén M, Wessman C. The influence of a cream containing 20% glycerin and its vehicle on skin barrier properties. Int J Cosm Sci 2001; 23:115–120.
35. Fluhr JW, Gloor M, Lehmann L, Lazzerini S, Distante F, Berardesca E. Glycerol accelerates recovery of barrier function in vivo. Acta Derm Venereol 1999; 79:418–421.
36. Grant WM. Toxicology of the eye. 3rd ed. Springfield: Charles C Thomas, 1986.
37. Balazs EA, Band P. Hyaluronic acid: its structure and use. Cosmet Toilet 1984; 99:65–72.
38. Berson DS, Shalita AR. The treatment of acne: the role of combination therapies. J Am Acad Dermatol 1995; 32:S31–S41.
39. Prottey C, George D, Leech RW, Black JG, Howes D, Vickers CF. The mode of action of ethyl lactate as a treatment for acne. Br J Dermatol 1984; 110:475–485.
40. Ditre CM, Griffin TD, Murphy GF, Sueki H, Telegan B, Johnson WC, Yu RJ, Van Scott EJ. Effects of alpha-hydroxy acids on photoaged skin: a pilot clinical, histologic, and ultrastructural study. J Am Acad Dermatol 1996; 34:187–195.
41. Lavker RM, Kaidbey K, Leyden JJ. Effects of topical ammonium lactate on cutaneous atrophy from a potent topical corticosteroid. J Am Acad Dermatol 1992; 26:535–544.
42. Stiller MJ, Bartolone J, Stern R, Smith S, Kollias N, Gillies R, Drake LA. Topical 8% glycolic acid and 8% L-lactic acid creams for the treatment of photodamaged skin. A double-blind vehicle-controlled clinical trial. Arch Dermatol 1996; 132:631–636.
43. Glogau RG, Matarasso SL. Chemical face peeling: patient and peeling agent selection. Facial Plast Surg 1995; 11:1–8.
44. Wehr R, Krochmal L, Bagatell FWR. A controlled two-center study of lactate 12% lotion and a petrolatum-based creme in patients with xerosis. Cutis 1986; 37:205–209.
45. Gånemo A, Virtanen M, Vahlquist A. Improved topical treatment of lamellar ichthyosis: a double blind study of four different cream formulations. Br J Dermatol 1999; 141: 1027–1032.
46. Rawlings AV, Davies A, Carlomusto M, Pillai S, Zhang AR, Kosturko R, Verdejo P, Feinberg C, Nguyen L, Chandar P. Effect of lactic acid isomers on keratinocyte ceramide synthesis, stratum corneum lipid levels and stratum corneum barrier function. Arch Dermatol Res 1996; 288:383–390.
47. Berardesca E, Distante F, Vignoli GP, Oresajo C, Green B. Alpha hydroxyacids modulate stratum corneum barrier function. Br J Dermatol 1997; 137:934–938.
48. Frosch PJ, Kligman AM. A method for appraising the stinging capacity of topically applied substances. J Soc Cosmet Chem 1977; 28:197–209.
49. Thueson DO, Chan EK, Oechsli LM, Hahn GS. The roles of pH and concentration in lactic acid-induced stimulation of epidermal turnover. Dermatol Surg 1998; 24:641–645.
50. Smith WP. Comparative effectiveness of alfa-hydroxy acids on skin properties. Int J Cosm Sci 1996; 18:75–83.
51. Effendy I, Kwangsukstith C, Lee JY, Maibach HI. Functional changes in human stratum corneum induced by topical glycolic acid: comparison with all-trans retinoic acid. Acta Derm Venereol 1995; 75:455–458.

52. Schmid-Grendelmeier P, Wyss M, Elsner P. Contact allergy to dexpanthenol. A report of seven cases and review of the literature. Dermatosen 1995; 43:175–178.
53. Proksch E, Nissen HP. Dexpanthenol enhances skin barrier repair and reduces inflammation after sodium lauryl sulphate-induced irritation. J Dermatol Treat 2002; 13:173–178.
54. Final report on the safety assessment of panthenol and pantothenic acid. J Am Coll Toxicol 1987; 6:139–163.
55. Driscoll WR. Panthenol in hair products. D&CI 1975:45–149.
56. Stables GI, Wilkinson SM. Allergic contact dermatitis due to panthenol. Contact Dermat 1998; 38:236–237.
57. Middleton JD, Roberts ME. Effect of a skin cream containing the sodium salt of pyrrolidone carboxylic acid on dry and flaky skin. J Soc Cosmet Chem 1978; 29:201–205.
58. PCA and sodium PCA. Cosmetic Ingredient Review, Washington. CIR Compendium, 1997:106–107.
59. Larmi E, Lahti A, Hannuksela M. Immediate contact reactions to benzoic acid and the sodium salt of pyrrolidone carboxylic acid. Contact Dermat 1989; 20:38–40.
60. Catanzaro JM, Smith JG Jr. Propylene glycol dermatitis. J Am Acad Dermatol 1991; 24:90–95.
61. Goldsmith LA, Baden HP. Propylene glycol with occlusion for treatment of ichthyosis. JAMA 1972; 220:579–580.
62. Gånemo A, Vahlquist A. Lamellar ichthyosis is markedly improved by a noval combination of emollients. Br J Dermatol 1997; 137:1011–1031.
63. Faergemann J, Fredriksson T. Propylene glycol in the treatment of tinea versicolor. Acta Derm Venereol 1980; 60:92–93.
64. Faergemann J. Propylene glycol in the treatment of seborrheic dermatitis of the scalp: a double-blind study. Cutis 1988; 42:69–71.
65. TNO. Toxicity profile—Propylene glycol. Surrey, UK: BIBRA International Ldt, 1996.
66. Glover ML, Reed MD. Propylene glycol: the safe diluent that continues to cause harm. Pharmacotherapy 1996; 16:690–693.
67. LaKind JS, McKenna EA, Hubner RP, Tardiff RG. A review of the comparative mammalian toxicity of ethylene glycol and propylene glycol. Crit Rev Toxicol 1999; 29: 331–365.
68. Mortensen B. Propylene glycol. Nord 1993; 29:181–208.
69. Drugs AAoP-Co. "Inactive" ingredients in pharmaceutical products: Update (Subject review). Pediatrics 1997; 99:268–278.
70. Final report of the safety assessment of propylene glycol and polypropylene glycols (PPG-9, -12, -15, -17, -20, -26, -30, and 34). J Am Coll Toxicol 1994; 13:437–491.
71. Funk JO, Maibach HI. Propylene glycol dermatitis: re-evaluation of an old problem. Contact Dermat 1994; 31:236–241.
72. Final report on the safety assessment of potassium-coco-hydrolyzed animal protein and triethanolamine-coco-hydrolyzed animal protein. J Am Coll Toxicol 1983; 2:75–86.
73. Final report on the safety assessment of wheat flour and wheat starch. J Environ Pathol Toxicol 1980; 4:19–32.
74. Janssens V, Morren M, Dooms-Goossens A, Degreef H. Protein contact dermatitis: myth or reality? Br J Dermatol 1995; 132:1–6.
75. Freeman S, Lee MS. Contact urticaria to hair conditioner. Contact Dermat 1996; 35:195–196.
76. Rovesti P, Ricciardi DP. New experiments on the use of sorbitol in the field of cosmetics. P&EOR 1959.
77. Rosten M. The treatment of ichthyosis and hyperkeratotic conditions with urea. Aust J Derm 1970; 11:142–144.
78. Grice K, Sattar H, Baker H. Urea and retinoic acid in ichthyosis and their effect on transepidermal water loss and water holding capacity of stratum corneum. Acta Derm Venereol (Stockh) 1973; 54:114–118.

79. Fritsch H, Stettendorf S, Hegemann L. Ultrastructural changes in onychomycosis during the treatment with bifonazole/urea ointment. Dermatol 1992; 185:32–36.
80. Swanbeck G. A new treatment of ichthyosis and other hyperkeratotic conditions. Acta Derm-Venereol (Stockh) 1968; 48:123–127.
81. Wohlrab W. The influence of urea on the penetration kinetics of vitamin-A-acid into human skin. Z Hautkr 1990; 65:803–805.
82. Beastall J, Guy RH, Hadgraft J, Wilding I. The influence of urea on percutaneous absorption. Pharm Res 1986; 3:294–297.
83. Kim CK, Kim JJ, Chi SC, Shim CK. Effect of fatty acids and urea on the penetration of ketoprofen through rat skin. Int J Pharm 1993; 99:109–118.
84. Lippold BC, Hackemuller D. The influence of skin moisturizers on drug penetration in vivo. Int J Pharm 1990; 61:205–211.
85. Wahlberg JE, Swanbeck G. The effect of urea and lactic acid on the percutaneous absorption of hydrocortisone. Acta Derm Venereol 1973; 53:207–210.
86. Lodén M. Urea-containing moisturizers influence barrier properties of normal skin. Arch Dermatol Res 1996; 288:103–107.
87. Lodén M. Barrier recovery and influence of irritant stimuli in skin treated with a moisturizing cream. Contact Dermat 1997; 36:256–260.
88. Andersson A-C, Lindberg M, Lodén M. The effect of two urea-containing creams on dry, eczematous skin in atopic patients. I. Expert, patient and instrumental evaluation. J Dermatol Treat 1999; 10:165–169.
89. Lodén M, Andersson A-C, Lindberg M. Improvement in skin barrier function in patients with atopic dermatitis after treatment with a moisturizing cream (Canoderm®). Br J Dermatol 1999; 140:264–267.
90. Serup J. A double-blind comparison of two creams containing urea as the active ingredient. Assessment of efficacy and side-effects by non-invasive techniques and a clinical scoring scheme. Acta Derm Venereol 1992; 177(suppl):34–43.
91. Lodén M, Andersson A-C, Lindberg M. The effect of two urea-containing creams on dry, eczematous skin in atopic patients. II. Adverse effects. J Dermatol Treat 1999; 10:171–175.
92. Gabard B, Nook T. Tolerance of the lesioned skin to dermatological formulations. J Appl Cosmetol 1991; 9:25–30.

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Ceramides and Lipids

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HISTORICAL PERSPECTIVES

Many published accounts of the composition of lipids from human stratum corneum have been complicated by the almost inevitable presence of sebaceous lipids as well as exogenous contaminants. When stratum corneum samples are obtained from excised skin, there is almost always massive contamination with subcutaneous triglycerides as well as fatty acids derived from the subcutaneous fat. In addition precautions must be taken to avoid contamination with environmental contaminants such as alkanes and cosmetic components. As a result of these complications, much work has been done with pig skin as a model (1–6). Young pigs, if properly housed and tended, can be kept clean, and the sebaceous glands are not active. By direct heat separation of epidermis from an intact carcass, it is possible to avoid subcutaneous fat. In terms of general structure, composition, and permeability barrier function, the pig appears to provide a good model for humans. An alternative approach is to use the contents of epidermal cysts (7,8). This material represents exfoliated stratum corneum lipid that is free of sebaceous and environmental contaminants. If the contents are carefully expressed from the capsule, a contaminant-free sample of stratum corneum lipid can be obtained. Cholesterol sulfate is partially hydrolyzed during the desquamation process; however, this is only a minor stratum corneum component. In either the pig or cyst model, the major lipid components are ceramides, cholesterol, and fatty acids, which represent approximately 45%, 27%, and 12% of the total lipid, respectively (9). Other minor components include cholesterol sulfate and cholesterol esters. The fatty acids in either model are predominantly straight-chain saturated species ranging from either 14 (cyst) or 16 (pig) carbons through 28 carbons in length with the 22 and 24 carbon species being the most abundant. The main focus in the rest of this chapter will be on the stratum corneum ceramides.

The first analysis of stratum corneum lipids per se was performed in 1932 by Kooyman (10) who showed a dramatic reduction in the proportion of phospholipids

in stratum corneum compared to the inner portion of the epidermis. Subsequently Long (11), using the very thick epidermis from cow snout as a model, analyzed lipids from horizontal slices of epithelial tissue. He observed a gradual accumulation of cholesterol and fatty acids in progressing from the basal region toward the surface. Phospholipids initially accumulated, but were degraded as the stratum corneum was approached. In 1965, Nicolaides (12) identified ceramides as a polar lipid component of stratum corneum. This fact was included in a footnote and was largely ignored until the pioneering work of Gray and Yardley in the mid- to late 1970s (1,2,13,14). Among other things, these investigators demonstrated that the ceramides are structurally heterogeneous and contain normal fatty acids, α-hydroxyacids, sphingosines, and phytosphingosines as components. However, individual ceramide types were not well resolved and no definitive structures could be proposed. The first attempt to isolate individual ceramide types and to determine the identities of the individual fatty acid and long-chain base components was conducted in 1979 using neonatal mouse epidermis as a source of lipids (15). Eight putative ceramide fractions were isolated, and six of these were analyzed. The remaining two were too minor for any analysis. Unfortunately, only normal fatty acids, sphingosines, and dihydrosphingosines were reported for each fraction analyzed. This suggests extensive cross-contamination sufficient to preclude recognition of the actual structural diversity. In 1983, the detailed structures of the ceramides from porcine epidermis were published (3). Six structurally different types of ceramides were identified, and these included sphingosines, dihydrosphingosines, and phytosphingosines as the base components, normal, α-hydroxyacids, and ω-hydroxyacids as the amide-linked fatty acids, and one ceramide type included an ester-linked fatty acid. Subsequently, it was demonstrated that the same ceramide structural types are present in human stratum corneum, although the proportions are somewhat different (8,15). In addition to the sphingosine- and phytosphingosine-containing ceramides found in pig stratum corneum, humans also contain a series of ceramides that contain 6-hydroxysphingosine (16). One member of this series contains amide-linked normal fatty acids, a second one contains amide-linked α-hydroxyacids, and the third is an acylceramide. More recently, a third acylceramide has been reported in human stratum corneum (17). All three of the acylceramides contain long-chain ω-hydroxyacids with ester-linked linoleate on the ω-hydroxyl group (17).

In 1987 it was discovered that porcine epidermal stratum corneum contains significant levels of covalently bound lipid, the major component of which is an ω-hydroxyceramide (4). Small amounts of saturated fatty acid and ω-hydroxyacid are also present. A similar situation was demonstrated for human stratum corneum; however, in this case there was a second hydroxyceramide that was shown to contain a variant phytosphingosine (18). This subsequently proved to be 6-hydroxysphingosine (16). The free and covalently bound ceramides are discussed in detail below.

CERAMIDES FROM EPIDERMIS

As noted above, the first comprehensive study of epidermal ceramide structures was directed at the porcine ceramides, which were separated into six chromatographically distinct fractions (3). Each fraction was analyzed by a combination of chemical, chromatographic, and spectroscopic methods, and representative structures are included in Figure 1.

The least polar of the porcine ceramides, ceramide fraction 1, consists of 30- through 34-carbon ω-hydroxyacids amide-linked to a mixture of sphingosines

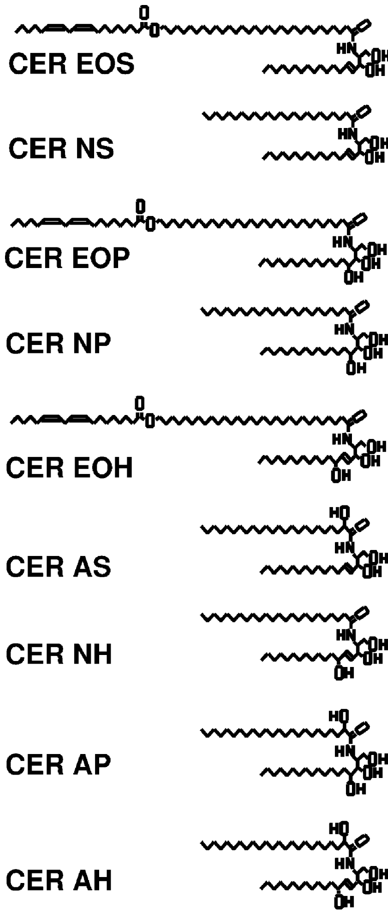

Figure 1 Representative structures of the free ceramides from human stratum corneum.

and dihydrosphingosines. The long-chain base component of this ceramide ranges from 16 through 22 carbons in length with 18:1, 20:1, and 22:1 being the most abundant. There is also a fatty acid ester-linked to the ω-hydroxyl group, 75% of which consists of linoleic acid. This species has often been referred to as ceramide 1 or acylceramide, but in the more systematic nomenclature system proposed by Motta et al. (19) this becomes Cer[EOS]. (In this system, the amide-linked fatty acid is designated as N, A, or O to indicate normal, α-hydroxy, or ω-hydroxy, respectively. The base component is designated S or P for sphingosine or phytosphingosine, respectively. It is understood that sphingosines are generally accompanied by dihydrosphingosines in the ceramides. The presence of an ester-linked fatty acid is designated by the prefix E.) Cer[EOS] is unusual in two aspects: (i) the very long ω-hydroxyacyl portion of the molecule is long enough to completely span a typical bilayer, and (ii) a high proportion of the ester-linked fatty acid is linoleic acid. It is thought that this ceramide along with an analogous glucosylated Cer[EOS] in the living layers of the epidermis accounts for the essential role of linoleic acid in formation and maintenance of the barrier function of the skin (3,19,20). Specific roles for Cer[EOS] have been proposed in the organization of the intercellular lipid lamellae of epidermal stratum corneum (21–24). In the formation of the intercellular lamellae of the

stratum corneum, flattened lipid vesicles are initially extruded from the lamellar granules into the intercellular space (25). These flattened vesicles fuse in an edge-to-edge manner to produce paired bilayers. Much, if not all, of the Cer[EOS] associated with the paired bilayers is oriented with the polar head group in the center and the linoleate in the outer leaflets of the bilayers. The linoleate chains from adjacent pairs of bilayers are thought to evert to form the main portion of an intervening bilayer, with some of the other lipids filling in the free space. It has been suggested that the everted linoleate chains interdigitate so that the once adjacent bilayers containing the ω-hydroxyacylsphingosine portions of the Cer[EOS] molecules are connected by a narrow intervening layer, thus producing the alternating broad–narrow–broad pattern seen in transmission electron micrographs, and accounting for the major 13-nm periodicity seen in micrographs and X-ray diffraction patterns (5,6,22,23,26,27). However, more recently it has been pointed out that the concentration of linoleate in the central lamella of the trilamellar units would result in greater reduction of ruthenium by this central lamella (24). The additional reduced ruthenium would accumulate beneath the plane of the polar head groups of the central lamella, thereby producing a broad–narrow–broad pattern of lucent bands without having interdigitation in the central lamella. In this view, all of the lamellae would be approximately 4.3-nm thick. Possible roles of human Cer[EOH] and Cer[EOP] in lipid organization are presently unknown.

Porcine ceramide fraction 2 has proven to be Cer[NS]. The fatty acid component is saturated and straight chained, and ranges from 16- through 32-carbons in length. C20:0, C22:0, C24:0, C26:0, and C28:0 are the most abundant, constituting 9% to 19% of the total fatty acid mass each. The long-chain bases again consist of a mixture of sphingosines and dihydrosphingosines ranging from 16- through 22-carbons in length. The most abundant bases are 18:0, 18:1, 20:0, and 20:1.

Porcine ceramide fraction 3, Cer[NP], contains the same range of fatty acids found in Cer[NS], but the long-chain base component is now a phytosphingosine with no double bond and a third hydroxyl group on carbon 4. The phytosphingosines found here range from 16- through 24-carbons in length, and the most abundant are 20:0 and 22:0.

Porcine ceramide fractions 4 and 5 both proved to be Cer[AS], but they differed in terms of the chain-length distributions of the α-hydroxyacid component. The chromatographically more mobile fraction 4 contained 24- through 28-carbon α-hydroxyacids amide linked to sphingosines and dihydrosphingosines, while ceramide fraction 5 contains α-hydroxypalmitic acid amide linked to sphingosines and dihydrosphingosines. Ceramide fraction 4 also contains somewhat longer bases with major amounts of 20:0 and 20:1, whereas ceramide fraction 5 contains mainly 16- through 18-carbon bases. This difference in carbon content results in chromatographic separation into two fractions, even though the basic structural type is the same in each.

Finally, the most polar of the pig ceramide fractions consists of α-hydroxyacids amide-linked to phytosphingosine, Cer[AP]. The α-hydroxyacids present in Cer[AP] range from 16- through 28-carbons in length, but the 24- and 26-carbon entities account for approximately 70% of the total fatty acid mass. The phytosphingosines have a chain-length distribution similar to that described for Cer[NP] above.

Subsequently, the human stratum corneum ceramides were investigated and were shown to produce a similar, though not identical, pattern on thin-layer chromatograms (15). Notably, the human fraction most closely matching porcine ceramide fraction 3 is somewhat broader and less symmetrical. The material most closely

matching porcine ceramide fractions 4 and 5 merged into one broad peak, and was designated ceramide 4/5. This was shown to reflect a more continuous chain-length distribution among the α-hydroxyacid components of Cer[AS] as opposed to the bipolar distribution found in the pig. The most polar human fraction similar to porcine ceramide fraction 6 appeared as an incompletely resolved doublet. These two components were designated ceramides 6I and 6II. Subsequently it has been shown the ceramide fraction 6II contains the variant phytosphingosine—6-hydroxysphingosine (16). The Motta system of nomenclature has been extended to include this new long-chain base as H (16). So ceramide 6I is Cer[AP], and ceramide 6II becomes Cer[AH]. Human ceramide fraction 3 has been shown to contain a minor amount of 6-hydroxysphingosine–containing acylceramide, Cer[EOH] (16) as well as Cer[EOP] (17), in addition to Cer [NP]. Likewise, ceramide fraction 4/5 contains Cer[NH] (28) in addition to Cer[AS] (15). Cer[EOP], Cer[EOH], and Cer[NP] are generally resolved by high performance thin layer chromatography, and sometimes by conventional thin layer chromatography; however, Cer[NH] and Cer[AS] have only been separated after acetylation (17).

In addition to the extractable lipids, there are covalently bound lipids coating the outer surface of the cornified envelope in epidermal stratum corneum. This consists mainly of ceramides. In porcine stratum corneum the principal covalently bound lipid is Cer[OS] derived from Cer[EOS] (4). In human stratum corneum, in addition to covalently bound Cer[OS], a second more polar covalently bound ceramide was found (18). This was later shown to be Cer[OH] (16). Representative structures of Cer[OS] and Cer[OH] are presented in Figure 2.

LIPIDS FROM OTHER KERATINIZED TISSUES

The hair and nails contain cholesterol sulfate and ceramides generally similar to those in the stratum corneum as their principal polar lipid components (29). The ceramides from hair have been partially characterized (30).

Hair contains 18-methyleicosanoic acid covalently bound to the outer surface of the cuticle cells in humans as well as in other mammals (22). The attachment is

HO O HN OH OH

CER OS

HO O HN OH OH OH

CER OH

Figure 2 Representative structures of the covalently bound ceramides from human stratum corneum.

apparently through thioester linkages. This covalently bound lipid layer provides a hydrophobic outer surface for the hair shaft.

In the oral cavity, the regions of the hard palate and gingiva are covered by a keratinizing epithelium that closely resembles the epidermis in many ways (5). The stratum corneum in these regions, like epidermal stratum corneum, contains ceramides, cholesterol, and fatty acids as major lipid components; however, unlike epidermal stratum corneum, the oral stratum corneum also contains relatively high proportions of phospholipids and glycosylceramides. The ceramides in the oral stratum corneum include the same structural types found in epidermal stratum corneum in similar relative proportions, except that in the oral tissue the proportion of Cer[EOS], the acylceramide, is much lower. It is thought that this lowered proportion of Cer[EOS] accounts for the fact that the broad–narrow–broad lamellar pattern that is characteristic of the intercellular lipids of epidermal stratum corneum is never seen in oral stratum corneum.

COMMERCIALLY AVAILABLE CERAMIDES

There are presently no commercial sources of the ceramides based on 6-hydroxysphingosine.

A variety of ceramides based on phytosphingosine produced by a fermentation technique are commercially available from Cosmoferm, a group company of Gist-brocades based in Delft, The Netherlands. These include an acylceramide, Cer[EOP], which consists of a 27-carbon ω-hydroxyacid amide linked to phytosphingosine and bearing ester-linked stearic acid on the ω-hydroxyl group. There are also two ceramides of the type Cer[NP]. One of these contains stearic acid and the other oleic acid amide linked to phytosphingosine. Finally, this supplier also produces N-2-hydroxystearoyl-phytosphingosine, Cer[AP]. These specific ceramides are routinely available; however, it is also possible to customize any of these general structural types to include different fatty acids.

There are several commercial sources of ceramides or ceramide analogues similar to the ceramide type Cer[NS]. For example, SEDERMA of Parsippany, New Jersey, produces a synthetic ceramide consisting of *N*-stearoyl-dihydrosphingosine, sold as ceramide 2®. This synthetic ceramide is partially racemic at carbon 3 of the base component; however, the stereochemical configuration at this carbon is at least 70% R, which is the configuration in natural dihydrosphingosine.

FUTURE DIRECTIONS

Presently, ceramides are being used in skin moisturizers and at least one line of hair care products. It has been documented that ceramides are important in the permeability barrier of the skin and the water holding properties of the stratum corneum (31,32). It seems likely that the interest in ceramides for incorporation into cosmetic products will result in the introduction of additional, novel ceramide formulations for use in skin and hair care. In addition, it can be anticipated that ceramides will eventually be incorporated into other personal care products, such as stick deodorants, or cosmetic products, such as lipstick. This will likely lead to commercial availability of additional ceramide structural variants that more

closely resemble all of the ceramide types that have been identified in human stratum corneum.

REFERENCES

1. Gray GM, Yardley HJ. Different populations of pig epidermal cells: isolation and lipid composition. J Lipid Res 1975; 16:441–447.
2. Yardley HJ, Summerly R. Lipid composition and metabolism in normal and diseased epidermis. Pharmacol Ther 1981; 13:357–383.
3. Wertz PW, Downing DT. Ceramides of pig epidermis: structure determination. J Lipid Res 1983; 24:759–765.
4. Wertz PW, Downing DT. Covalently bound ω-hydroxyceramide in the stratum corneum. Biochim Biophys Acta 1987; 917:108–111.
5. Law S, Wertz PW, Swartzendruber DC, Squier CA. Regional variation in content, composition and organization of porcine epithelial barrier lipids revealed by thin-layer chromatography and transmission electron microscopy. Arch Oral Biol 1995; 40: 1085–1091.
6. Bouwstra JA, Cheng K, Gooris GS, Weerheim A, Ponec M. The role of ceramides 1 and 2 in the stratum corneum lipid organization. Biochim Biophys Acta 1996; 1300:177–186.
7. Nicolaides N, Levan NE, Fu WC. The lipid pattern of the wen (keratinous cyst of the skin). J Invest Dermatol 1968; 50:189–194.
8. Wertz PW, Swartzendruber DC, Madison KC, Downing DT. The composition and morphology of epidermal cyst lipids. J Invest Dermatol 1987; 89:419–425.
9. Wertz PW, Downing DT. Stratum corneum: biological and biochemical considerations. In: In: Hadgraft J, Guy RH, eds. Transdermal Delivery Systems. New York: Marcel Dekker, 1988:1–22.
10. Kooyman DJ. Lipids of the skin. Some changes in the lipids of epidermis during the process of keratinization. Arch Dermatol Syphilol 1932; 25:444–450.
11. Long VJW. Variations in lipid composition of different depths of the cow snout epidermis. J Invest Dermatol 1970; 55:269–273.
12. Nicolaides N. Skin lipids. II. Class composition of samples from various species and anatomic sites. J Am Oil Chem Soc 1965; 42:691–702.
13. Gray GM, Yardley HJ. Lipid compositions of cells isolated from pig, human, and rat epidermis. J Lipid Res 1975; 16:434–440.
14. Gray GM, White RJ. Glycosphingolipids and ceramides in human and pig epidermis. J Invest Dermatol 1977; 70:336–341.
15. Wertz PW, Miethke MC, Long SA, Strauss JS, Downing DT. The composition of the ceramides from human stratum corneum and from comedones. J Invest Dermatol 1985; 84:410–412.
16. Robson KJ, Stewart ME, Michelsen S, Lazo ND, Downing DT. 6-Hydroxy-4-sphengenine in human epidermal ceramides. J Lipid Res 1994; 35:2060–2068.
17. Ponec M, Weerheim A, Lankhorst P, Wertz P. New acylceramide in native and reconstructed epidermis. J Invest Dermatol 2003; 120:581–588.
18. Wertz PW, Madison KC, Downing DT. Covalently bound lipids of human stratum corneum. J Invest Dermatol 1989; 91:109–111.
19. Motta SM, Monti M, Sesana S, Caputo R, Carelli S, Ghidoni R. Ceramide composition of the psoriatic scale. Biochim Biophys Acta 1993; 1182:147–151.
20. Wertz PW, Downing DT. Glycolipids in mammalian epidermis: structure and function in the water barrier. Science 1982; 217:1261–1262.
21. Kuempel D, Swartzendruber DC, Squier CA, Wertz PW. In vitro reconstitution of stratum corneum lipid lamellae. Biochim Biophys Acta 1998; 1372:135–140.

22. Wertz PW. Integral lipids of hair and stratum corneum. In: In: Zahn H, Jolles P, eds. Hair: Biology and Structure. Basel: Birkhauser, 1996:227–237.
23. Bouwstra JA, Gooris GS, Dubbelaar FE, Weerheim AM, Ijzerman AP, Ponec M. Role of ceramide 1 in the molecular organization of the stratum corneum lipids. J Lipid Res 1998; 39:186–196.
24. Hill JR, Wertz PW. Molecular models of the intercellular lipid lamellae from epidermal stratum corneum. Biochim Biophys Acta 2003; 1616:121–126.
25. Landmann L. The epidermal permeability barrier. Anat Embryol 1988; 178:1–13.
26. Bouwstra JA, Gooris GS, Dubbelaar FE, Ponec M. Phase behavior of stratum corneum lipid mixtures based on human ceramides: the role of natural and synthetic ceramide 1. J Invest Dermatol 2002; 118:606–616.
27. Bouwstra JA, Gooris GS, Dubbelaar FE, Ponec M. Phase behavior of stratum corneum lipid mixtures based on human ceramides: coexistence of crystalline and liquid phases. J Lipid Res 2001; 42:1759–1770.
28. Stewart ME, Downing DT. A new 6-hydroxysph-4,5-sphingenine-containing ceramide in human skin. J Lipid Res 1999; 40:1434–1439.
29. Wix MA, Wertz PW, Downing DT. Polar lipid composition of mammalian hair. Comp Biochem Biophys 1987; 86B:671–673.
30. Hussler GG, Kaba AM, Francois C, Saint-Leger D. Isolation and identification of human hair ceramides. Int J Cosmet Sci 1995; 17:197–206.
31. Lintner K, Mondon P, Girard F, Gibaud C. The effect of a synthetic ceramide-2 on transepidermal water loss after stripping or sodium lauryl sulfate treatment: an in vivo study. Int J Cosmet Sci 1997; 19:15–25.
32. Imokawa G, Akasaki S, Minematsu Y, Kawai M. Importance of intercellular lipids in water-retention properties of the stratum corneum: induction and recovery study of surfactant dry skin. Arch Dermatol Res 1989; 281:45–51.

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Silicones—A Key Ingredient in Cosmetic and Toiletry Formulations

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UNIQUE MATERIALS

Silicone is a generic name for many classes of organosilicone polymer that consist of an inorganic siloxane (Si–O) backbone with pendant organic groups (usually methyl). It is this structure that gives silicones their unique combination of properties and, in particular, their surface properties (Fig. 1).

SILOXANE BACKBONE

The prime role of the siloxane backbone is to present the available methyl groups to their best advantage, and it does this by virtue of its unique flexibility. In most hydrocarbons, the bond angles are very fixed, and steric packing considerations often prevent the available methyls from adopting lowest surface energy orientations. In silicones, the Si–O bond length is significantly longer and the Si–O–Si bond angle flatter than comparable C–C and C–O bonds resulting in a very low barrier to rotation and making the polymer chains very flexible. This flexibility makes many orientations possible and provides "free space" to accommodate different sized substituents or to allow easy diffusion of gaseous molecules, a property useful in the formation of "breathable" films. Coupled with the low intermolecular forces between methyl groups, this flexibility also has a

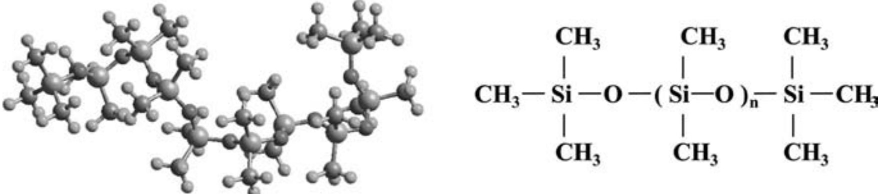


Figure 1 Unique chemical structure of silicones.

profound effect on the bulk as well as the surface properties of silicones, seen in the small variation of physical parameters with temperature and molecular weight, the low freezing and pour points of fluids, the low boiling points, the high compressibility, and the retention of liquid nature to unusually high molecular weights. It also makes a number of structural and compositional variations possible, resulting in many families of silicones, including linear and cyclic structures, a wide range of molecular weights and varying degrees of branching or cross-linking.

Additionally, the siloxane bond is exceptionally strong providing the polymer with a high degree of thermal and oxidative stability and ensuring stability in formulation (1–3).

PENDANT ORGANIC GROUPS

The key function of the organic (methyl) groups is to provide the intrinsic surface activity of the silicones. The order of increasing surface energy for single carbon based groups is –CF3 > –CF2– > –CH3 > –CH2–. Liquid surface tension measurements show that, as expected, the order of increasing surface activity is hydrocarbon, silicone, and fluorocarbon. Interfacial tension measurements against water, however, show the order of increasing interfacial activity to be fluorocarbon, hydrocarbon, and silicone. Silicones do not fit the simple pattern that a reduction in surface energy means an increase in hydrophobicity and interfacial tension because of their backbone flexibility, which allows them to adopt various orientations at different interfaces. The interfacial tension of silicone is also independent of chain length, indicating high molecular chain freedom. In addition, critical surface tension of wetting values for silicones have been found to be higher than their liquid surface tension values, meaning that they are able to spread over their own absorbed film. This has an advantage in achieving complete, uniform surface coverage, facilitates the efficient spreading of other materials, and results in smooth, lubricating films.

In addition, owing to the organic groups, the solubility parameters of silicones are significantly lower than those of water and many organic materials making them useful in forming barriers to wash-off or wear, and increasing the substantivity of formulations.

The introduction of functional groups such as phenyl, alkyl, polyether, amino, etc. onto the backbone expands the properties and benefits of silicones further (1–3).

KEY INGREDIENTS IN THE COSMETICS AND TOILETRIES INDUSTRY

Silicones were first used in the cosmetics and toiletries industry in the 1950s, when low levels of medium-viscosity dimethicone (polydimethylsiloxane) were used to prevent the whitening effect, characteristic of soap-based skin lotions. It was not until the 1970s, when formulators were concerned about the use of chlorofluoro carbons in aerosols, that silicones were considered more seriously as possible ingredients for cosmetic formulations, and their unique properties began to be recognized. Since then, the use of silicones has expanded rapidly to virtually all segments, and today, 57% of all new products being introduced into the United States market contain silicone, with many different types being used (4).

There are five main families of silicones which are used in the cosmetics and toiletries industry today:

1. Cyclomethicones (cyclosiloxanes) are volatile fluids with ring structures. The most commonly used materials are the tetramer, pentamer or hexamer, or blends of these. They are good solvents and serve as good carriers for high molecular weight silicones that would otherwise be very difficult to handle.

In addition, they have very low heats of vaporization compared to water or ethanol giving them a non-cooling feel when drying. Cyclomethicones are classified as non-volatile organic compounds in the United States.

2. Dimethicones (polydimethylsiloxanes, PDMS) are linear structures ranging from volatile to non-volatile with increasing molecular weight. Volatile Dimethicones exist as fluids with viscosities of 0.65 to 2 mm^2/s. Non-volatile Dimethicones exist as fluids with viscosities of 5.0 mm^2/s up to gums. Dimethicone emulsions make handling of the higher molecular weight fluids easier. Specialized emulsion polymer technologies allow the production of ultra–high molecular weight linear PDMS emulsions with an internal dynamic viscosity as high as 200 million mm^2/s.
3. Silicone blends consist of Dimethiconol or Dimethicone gums or Trimethylsiloxysilicates (highly cross-linked resins) dispersed in lower molecular weight Dimethicones or Cyclomethicones. They have been developed to improve ease of formulation and compatibility of high molecular gums or resins, used for their substantivity.
4. Dimethicone and Vinyldimethicone Crosspolymers or blends are silicone elastomers that are cross-linked to different degrees, resulting in different product forms. They exist in powder form (free flowing or suspended in water) or as elastomeric silicone gels that are swollen with solvent (usually cyclomethicone). The introduction of different functionality into such products is also possible. They are used as rheology modifiers in skin care and antiperspirant products, providing a dry, powdery feel to formulations.
5. Functional silicones:

   a. Dimethicone Copolyols (silicone polyethers) are fluids or waxes where some of the methyl groups along the siloxane backbone have been replaced with polyoxyethylene or polyoxypropylene groups. The addition of polyoxyethylene substituents increases the hydrophilicity of silicones. Polyoxypropylene substituents are used to balance out this hydrophilicity by increasing the hydrophobic characteristics of the copolymer.
   b. Phenyl Trimethicones are fluids where some of the methyl groups have been replaced by phenyl groups. The phenyl groups increase the refractive index and improve compatibility with organic materials.
   c. Amodimethicones or Trimethylsilylamodimethicones are fluids where some of the methyl groups have been replaced by secondary and primary amine groups. The polar amine groups have a profound effect on the deposition properties of the silicone, giving it an affinity for negatively charged surfaces, such as the proteinaceous surface of the hair. Emulsions of these fluids are commonly used.
   d. Alkyl Dimethicones are fluids or waxes where some of the methyl groups have been replaced by alkyl groups. This results in a family of silicone–hydrocarbon hybrids with possibilities for variations in viscosities, softening temperatures, and rheological characteristics. They have increased compatibility with organic materials.
   e. Nylon-611/Dimethicone Copolymer is a solid copolymer of polyamide and dimethicone used as an optimal thickener or structurant for silicone and organic-based formulations, retaining a silky, smooth, nongreasy skin feel.
   f. Cyclomethicone and Dimethicone Copolyol or Laurylmethicone Copolyol are silicone emulsifiers. They show ampiphilic behavior

and have been designed to emulsify aqueous phases into silicones, usually Cyclomethicone or low-medium polarity organic oils.

SKIN CARE, SUN CARE, AND DECORATIVE PRODUCTS

Skin Feel/Emolliency

The main reason that silicones are used in all types of skin care product is because of their sensory properties. Studies on the emollient properties of various materials have shown that silicones deliver greater emolliency values than many commonly used cosmetic ingredients both during and after application. They are described as smooth, velvety, and non-greasy or oily and are able to impart this feel to cosmetic and toiletry formulations, improving the negative feel associated with other ingredients (5).

Cyclomethicones are used for transient effects giving slight lubricity, a light texture, fast spreading, and good distribution of the product on application, whilst leaving no residual effects. They are often included in formulations to remove the greasy or oily feel of hydrocarbon-based emollients and are the basis for "oil-free" type claims (6). They are used in light products for daily use such as facial cleansers, day creams, or liquid foundations. Higher molecular weight silicones such as Dimethicone and Dimethiconol are used to give a more lubricious, longer lasting effect in richer, more nourishing skin treatment products such as night creams or after-sun products (7). Silicone elastomers are used to give a dry, powdery feel to skin care formulations (8). Silicones are also non-comedogenic/non-acnegenic unlike many occlusive, lipophilic fatty emollients which can promote comedone/acne formation on the skin (9).

Substantivity (Long Lasting/Durability)

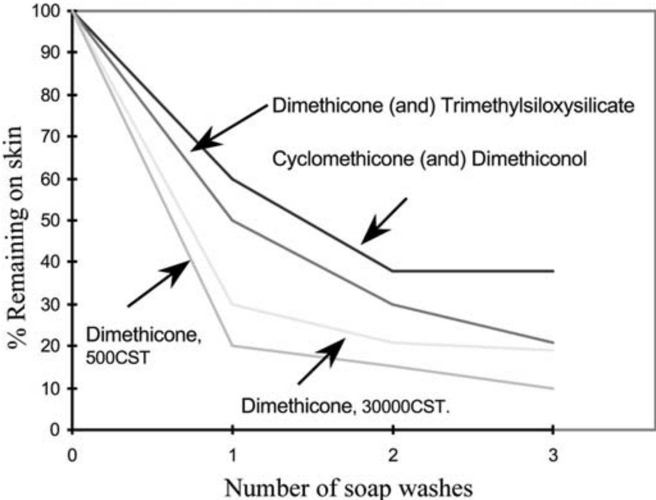
High molecular weight Dimethicones or Cyclomethicone or Dimethiconols form water-resistant films on the skin which can help prolong the effects of skin care, sun care, or decorative products. This substantivity can be improved further by using Alkyl Dimethicones such as Cetyl Dimethicone or C30–45 Alkyl Methicone (7) (Fig. 2).

The use of the substantivity of silicones to improve the substantivity of other ingredients in cosmetic and toiletry formulations has been demonstrated in sun care products. The addition of 2.5 wt% Cetyl Dimethicone to an oil-in-water sunscreen formulation shows excellent in-vivo resistance to wash-off. The formulation has an SPF of 21.1 before immersion which reduces to 19.2 only, after immersion for 80 minutes (7,10).

Cyclomethicones are the basis for long-lasting/non-transfer decorative products, especially lipsticks. They are used to disperse waxes and pigments, improve application, and impart a pleasant skin feel, often replacing non-volatile hydrocarbon oils. When they evaporate, a uniform film of waxes and pigments remains which is resistant to transfer and wear (11).

Permeability/Controlled Moisturization/Protection Against Dehydration

Owing to the flexibility of the Si–O–Si backbone, the majority of silicones are permeable to water vapor, producing "breathable" films. This is an important parameter for cleansing products or color cosmetics to avoid clogging pores. The presence of an alkyl group in the chain, however, reduces this permeability, resulting in semi-permeable/semi-occlusive materials, e.g., Stearyl Dimethicone, or even material with an occlusivity similar to Petrolatum, e.g., C30–45 Alkyl Methicone (7,12).



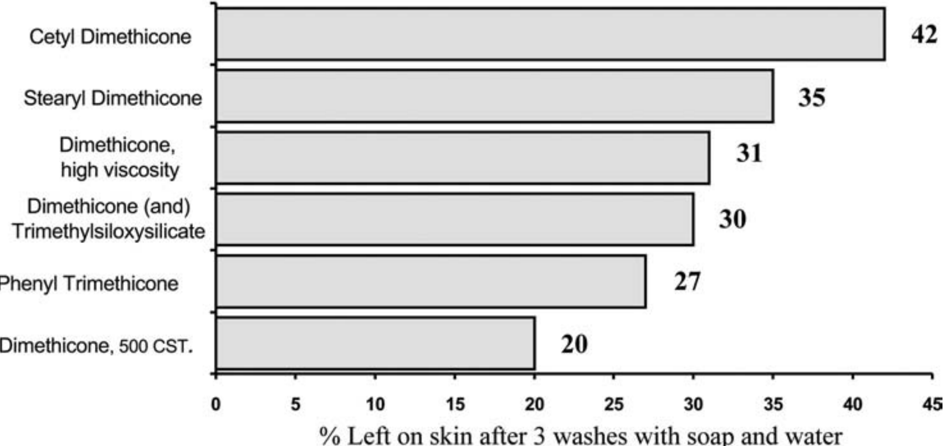


Figure 2 Substantivity of different silicones.

Enhanced Efficacy

Apart from improving the feel and long-lasting benefits of skin care products, silicones can also enhance the efficacy of other ingredients in the formulation. Studies carried out on sun care products have shown that the Alkyl Methicones can enhance the SPF of products containing either organic or inorganic sunscreens. For inorganic sunscreens, a 100% increase in SPF was seen with an oil-in-water system containing 2 wt% Cetyl Dimethicone and a 75% increase in the SPF for a water-in-oil system containing C30–45 Alkyl Methicone (10,12).

Protection

Dimethicone is listed in the FDA Monograph for Skin Protectant Drug Products for OTC Human Use in the United States (12). Owing to their hydrophobicity, silicones are used in protective hand creams to provide a water-resistant barrier against

water-borne contaminants. Recent studies indicate that Cyclomethicone and Dimethicone may also prevent irritation caused by sunscreen agents (13).

Cleansing

The excellent spreading characteristics, dry non-greasy/oily feel, and good solvency of Cyclomethicones make them ideal for use in skin cleansers to help lift and remove dirt without stinging. They can be used alone or in combination with ingredients such as mineral oil. Silicone emulsifiers allow Cyclomethicone to be present in the continuous phase as well as allowing the incorporation of polar ingredients such as water, glycerine, etc., allowing the formulation of rinseable foaming facial washes (14).

Powdered silicone elastomers have the capability of absorbing lipophilic materials such as sebum from the skin making them very useful for greasy skin application (15).

Water-soluble Dimethicone Copolyols have shown benefits in foaming facial washes. They provide a creamy, denser foam as well as improve the foam volume. In liquid body cleansing products, such as foam baths, shower gels, and liquid soaps, can improve foaming and foam stabilization. They have also been recognized as additives that reduce eye and skin irritation from anionic surfactants (14,16).

Emulsions of ultra–high molecular weight linear silicones can be perceived on the skin at very low levels owing to their very low sensory threshold (17).

Rheology Modification/Structural Integrity (Sticks)

In addition to improving the aesthetics of formulations, silicones can also act as rheology modifiers. This is particularly applicable to water-in-oil or water-in-silicone-type systems. One such silicone rheology modifier is the C30–45 Alkyl Methicone where 149% and 93% increase in emulsion viscosity has been observed for water-in-silicone and water-in-oil emulsions, respectively, with 2 wt% of the wax (7). Rheology modification using 2 to 4 wt% Stearyl Dimethicone is believed to be part of the reason for the success of this product in enhancing the SPF of sun care products containing organic sunscreens (9). These waxes are also used to maintain the structural integrity of stick products, improving their feel and application. Silicone elastomers can also be used to modify the rheology of skin care and antiperspirant formulations. Such elastomers have the capacity to absorb large amounts of solvents such as Cyclomethicone or low viscosity Dimethicone without exhibiting any syneresis. It is this property which allows them to successfully thicken formulations. The ability of elastomers to significantly modify the rheology of a formulation combined with their unique powdery feel has led to their use in antiperspirant products.

Silicone polyamides such as Nylon-611/Dimethicone Copolymer have the ability to make crystal clear sticks with blends of organic and silicone materials (18).

Formulating Flexibility

Silicones can be used in all types of skin care products ranging from simple oil-in-water gels or emulsions to water-in-silicone and water-in-oil emulsions, from crystal clear to white in color. Silicone emulsifiers increase this flexibility further. They allow silicones to be present in the continuous phase as well as allowing the incorporation of polar ingredients such as water, glycerine, etc. Matching the refractive index of the water phase with the oil phase in such emulsions makes the formulation of clear gels possible, and adjusting the phase ratio determines the product form from lotions to gels. This technology is the basis for the clear antiperspirant gels seen on the market today. It is

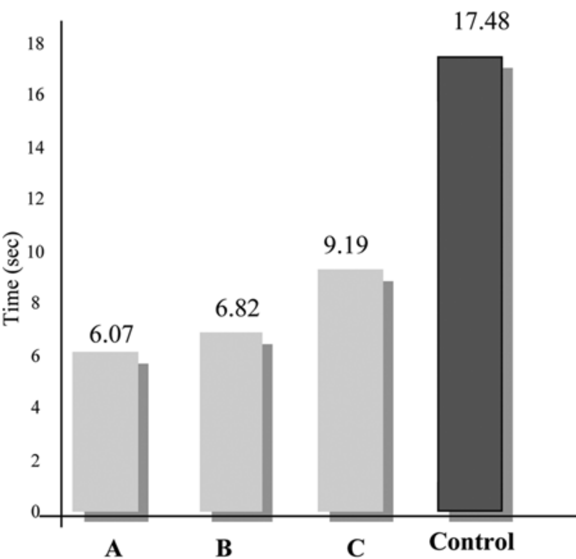
also possible to make non-aqueous emulsions using silicones to deliver hydrophilic ingredients or those that are sensitive to hydrolysis. In addition, the benefits of water-in-oil systems such as good sensory profiles, improved wash-off resistance, and excellent moisturization have been demonstrated. Silicone emulsifiers offer versatility for low or high shear systems as well as cold processing, presenting new opportunities for cost-effective and highly innovative skin care and underarm products (19).

HAIR CARE PRODUCTS

Hair Conditioning/Improved Combing

Various types of silicone are used to give different degrees of hair conditioning. Dimethicone Copolyols provide light conditioning owing to their solubility in water and low level of substantivity (Fig. 3). They can also help reduce eye irritation associated with shampoos and similar products that contain anionic surfactants. Higher molecular weight Dimethicones/Dimethiconols or Trimethylsilylamodimethicones/Amodimethicones provide a higher level of conditioning owing to their insolubility in water and greater substantivity. The latter have an affinity for negatively charged surfaces such as the proteinaceous surface of the hair that contributes to their substantivity.

Evaluation of the average detangling times of Dimethiconol (gum), Amodimethicone, and Dimethicone (high viscosity fluid) emulsions at a 4% level in an illustrative two-in-one shampoo formulation indicates that they all show significant improvement over the untreated control tress with the Dimethiconol emulsion providing the best conditioning effect (20,21).



A: Dimethiconol (and) TEA-Dodecylbenzenesulfonate

B: Amodimethicone (and) Cetrimonium Chloride (and) Trideceth -12

C: Dimethicone (and) Laureth-23 (and) Laureth-4

Figure 3 Hand detangling results on slightly bleached hair for diluted emulsions.

Synergistic effects have been observed between quaternary polymers commonly used in shampoos for conditioning and Dimethicone Copolyols. Better detangling results are observed for shampoos containing Dimethicone Copolyol and quaternary polymers than with the quaternary polymers or Dimethicone Copolyols alone (21).

Similar evaluation of silicones in conditioners indicates that Dimethicone emulsions provide the best conditioning effect in rinse-off products and in permanent waving products. An emulsion of Trimethylsilylamodimethicone significantly reduces the wet and dry combing force. Combinations of silicones such as Cyclomethicone, silicone blends, and Phenyl Trimethicone are the basis for anhydrous leave-in conditioners, sometimes referred to as "cuticle coat" products (20).

Sensory Enhancement

As in skin care, silicones impart a soft smooth feel to the hair. Sensory evaluations of cuticle coat formulations consisting entirely of blends of silicone showed that, in addition to ease of combing, they improve spreadability, silkiness and softness, gloss, and perceived repair of split ends compared to the control (20,22).

Silicones as Drying Aids

Silicones such as Trimethylsilylamodimethicone can help hair dry more quickly in comparison to drying aids such as Stearalkonium Chloride, preventing damage owing to the use of hair dryers etc. (20).

Foam Boosting

Dimethicone Copolyols can be used to boost the foaming properties of shampoos as well as provide a light conditioning effect (20).

Reduced Flyaway

Tests comparing shampoo formulations containing quaternary polymers to those with quaternary polymers and Dimethicone Copolyols show an improvement in static control with the addition of the silicone. Sensory evaluation has also shown a reduction of flyaway with Dimethicone emulsions (20,22).

Improved Shine

Silicones, in particular Phenyl Trimethicone, are recognized for their ability to enhance hair shine and gloss along with adding softness, manageability, and smoothness to the abraded hair cuticle (20,23).

Natural-Look Fixatives

Because of their low surface tension, silicones spread easily to help fixative products distribute evenly on the surface of hair and improving their effectiveness. They are also used in conjunction with or as a replacement for organic plasticizers. Organic materials tend to be hydrophilic, which diminishes the holding power of a resin. In contrast, the hydrophobic nature of silicones helps repel water so there is less opportunity to reduce the resin's holding properties. The use of Dimethicone Copolyol as a resin plasticizer can also help give hair a more natural look (20).

LONGER LASTING PERMANENT WAVE AND COLORING PRODUCTS

Silicones, such as Trimethylsilylamodimethicone, can be used to provide a more durable conditioning effect and a longer lasting permanent wave. Pretreatments containing silicone blends help prevent hair damage during the harsh perming process. In hair color products, blends of volatile and non-volatile silicone (Cyclomethicone and Trimethylsilylamodimethicone) can be used to seal the hair cuticle and hold color in. The volatile silicone evaporates, leaving behind a smooth, uniform film on the surface of the hair (20,24).

Amino-functional silicones can prevent degradation of the hair color owing to the combination of UV exposure and washing and provide conditioning benefits such as ultra-shine, improved drying time, moisturization, and a smooth and light feel without negatively impacting hair body and volume (25).

Evaluation of rinse-off conditioners indicates that silicones show excellent potential to provide color lock properties in permanent hair color products.

ANTIPERSPIRANT AND DEODORANT PRODUCTS

In addition to the benefits which silicones bring to skin care products such as improved feel, delivery of actives, low residue, formulating flexibility, etc., the following advantages are seen in antiperspirant and deodorant formulations (26).

Anti-Whitening

Dimethicones, Phenyl Trimethicone, or Alkyl Dimethicones have been shown to reduce/mask the whitening effect caused by antiperspirant salts by matching the refractive index (27).

Improved Spray Characteristics

Low levels of Cyclomethicone and Dimethiconol have been demonstrated to reduce the spray width, height, and particle size of antiperspirant pump spray and aerosol formulations, leading to a more directional spray with low mistiness and dustiness (26,28). The silicone blend may also contribute to the substantivity of the antiperspirant active and lubricate the spray valve to prevent clogging.

Noncooling

The heat of vaporization of volatile silicones such as Cyclomethicone is much lower than that of water or ethanol meaning that much less energy is required for them to evaporate. This leads to a non-cooling effect in formulation (26).

The multifunctional benefits of silicones make them invaluable in today's cosmetic and toiletry formulations, and with the introduction of more and more new silicones, this is a trend that is expected to continue well into the next millennium.

REFERENCES

1. Owen MJ. The surface activity of silicones: a short review. Ind Eng Chem Prod Res Dev 1980; 19:97–103.
2. Owen MJ. Why silicones behave funny. Chemtech 1981; 11:288–292.

3. DiSapio A. Silicones in personal care: An ingredient revolution. Brussels, Belgium: Dow Corning Publication 22-1547-01, 1994.
4. Cosmetic Research—USA News, 2003.
5. Goldemberg RL, Pela Rosa CP. Int Soc Cosmet Chem 1971; 22:635–654.
6. De Backer G, Ghiradi D. Goodbye to grease. Soap, Perfumery and Cosmetics, June 1993.
7. Blakely J, Van Reeth I, Vagts A. The silicone difference in skincare. Inside Cosmetics October/November 1998:14–17.
8. Van Reeth I, Dahman F, Lau A, Starch M. Novel silicone thickening technologies: Delivering the appropriate rheology profile to optimize formulation performance. Brussels, Belgium: Dow Corning Publication 22-1786-01, 1999.
9. Lanzet M. Comedogenic effects of cosmetic raw materials. Cosmet Toiletries 1986; 101:63–72.
10. Van Reeth I, Dahman F, Hannington J. Alkylmethylsiloxanes as SPF enhancers. Relationship between effects and physico-chemical properties. International Federation of Societies of Cosmetic Chemists (IFSCC) 19th Congress Poster, Sydney, Australia, 1996.
11. Abrutyn E. Translating silicone chemistry to color cosmetics. Midland, Michigan, USA: Dow Corning Publication 25-888-97, 1997.
12. Van Reeth I, Marchioretto S, Dahman F, DeSmedt A, Dupont A. Silicones: enhanced protection across personal care applications. International Federation of Societies of Cosmetic Chemists (IFSCC) 20th Congress Poster, Cannes, France, 1998.
13. Nichols K, Desai N, Lebwohl M. Effective sunscreen ingredients and cutaneous irritation in patients with rosacea. Cutis 1998; 61:344–346.
14. Blakely J. The benefits of silicones in facial and body cleansing products. Brussels, Belgium: Dow Corning Publication 22-1549-01, 1994.
15. Starch M. New Developments in silicone elastomers for skin care. Midland, Michigan: Dow Corning Publication 27-1060A-01, 2002.
16. DiSapio A.J, Fridd P. Dimethicone copolyols for cosmetic and toiletry applications. International Federation of Societies of Cosmetic Chemists (IFSCC) 19th Congress Platform Presentation, London, United Kingdom, 1988.
17. Van Reeth I, Marteaux L, Delvaux M. Silicone in body wash: a new perspective for formulators. In-Cosmetic, Dusseldorf, Germany, April 26, 2001.
18. Urrutia A, Maxon B, Van Reeth I, Gacic G, Courel B, Buckingham A, King D. Dow corning® polyamide: An innovative structurant for personal care applications. Midland, Michigan: Dow Corning Publication 27-1086-01, 2003.
19. Hickerson R, More M, Van Reeth I. New options with silicone emulsifiers. Midland, Michigan: Dow Corning Publication 27-1082-01, 2003.
20. Marchioretto S. Optimising the use of silicones in haircare products. Brussels, Belgium: Dow Corning Publication 22-1720-01, 1998.
21. Marchioretto S, Blakely J. Substantiated synergy between silicone and quats for clear and mild conditioning shampoos. SÖFW October 2, 1997.
22. Thomson B, Vincent J, Halloran D. Anhydrous hair conditioners: silicone-in-silicone delivery systems. Soap, Cosmetics, Chem Specialties 1992; 68:25–28.
23. Reimer BM, Oldinski RL, Glover DA. An objective method for evaluating hair shine. Soap, Cosmetics, Chem Specialties October 1995.
24. Fridd PF, Taylor RM. GB Patents GB2186889 and GB2186890.
25. Van Reeth I, Urrutia A. New silicone-based solutions for suncare. SEPAWA Congress, Bad Durkheim, Germany, October 2003.
26. Abrutyn ES, Bahr BC, Fuson SM. Overview of the antiperspirant market: Technology and trends. Brussels, Belgium: Dow Corning Publication 22-1555-01, 1994.
27. Abrutyn ES, Bahr BC, Legrow GE, Schulz WJ. US Patent 5, 225, 188; 1993.
28. Spitzer J. US Patent 4, 152, 416; 1979.

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UV Filters

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INTRODUCTION

The presence of ultraviolet (UV) filters in skin care and cosmetic products represents a key benefit that cosmetics can provide consumers. The hazards of UV light exposure are well known. It is estimated that the incidence of nonmelanoma skin cancer in the United States exceeds one million cases per year (1). UV induced or photoaging accounts for 80% to 90% of visible skin aging (2). UV radiation damages the skin by both direct effects on DNA and indirectly on the skin's immune system (3).

In animal models sunscreens prevent the formation of squamous cell carcinomas of the skin (4). The regular use of sunscreens has been shown to reduce the number of actinic or precancerous keratoses (5) and solar elastosis (6). Daily sunscreen use on the hands and face reduced the total incidence of squamous cell carcinoma in an Australian study (7). Sunscreens also prevent immunosuppression (8). Double blind photoaging studies show consistent improvement in the "untreated" control groups due in part to the use of sunscreens by all study subjects (9).

The effect of sunscreen use on melanoma is less clear. A meta-analysis of population based studies of population based case control studies found no effect of sunscreen use on risk for melanoma (10). Nevertheless, observational studies suggest that intermittent or intense sun exposure is associated with increased risk for melanoma (11), supporting the hypothesis that preventing sunburn especially in childhood may reduce the lifetime risk of melanoma.

The cosmetic formulator has an expanding menu of active sunscreen ingredients for incorporation into a variety of cosmetic formulations. Selection is restricted by regulatory agencies in the country in which the final product is to be marketed. This chapter will concentrate on reviewing available UV filters.

DEFINITIONS

Ultraviolet radiation (UVR) reaching the Earth's surface can be divided into UVB (290–320 nm) and UVA (320–400 nm). UVA can be further subdivided into UVA I (340–400 nm) or far UVA and UVA II (320–340 nm) or near UVA.

The sun protection factor (SPF) is defined as the dose of UVR required to produce 1 minimal erythema dose (MED) on protected skin after application of 2 mg/cm^2 of product divided by the UVR to produce 1 MED on unprotected skin. A "water-resistant" product maintains the SPF level after 40 minutes of water immersion. A "very water-resistant" or "waterproof" product is tested after 80 minutes of water immersion. If the SPF level is diminished by immersion, a separate SPF level may be listed. A "broad spectrum" or "full spectrum" sunscreen provides both UVB and UVA protection. Ideally this includes both UVA I and UVA II coverage.

HISTORY

Two UV filters, benzyl salicylate and benzyl cinnamate, were first incorporated into a commercially available sunscreen emulsion in the United States in 1928 (12).

In the early 1930s, phenyl salicylate (Salol) was used in an Australian product (13). Aminobenzoic acid (PABA) was patented in 1943 leading to the development of PABA derivative UV filters. During World War II red vetinary petrolatum was used by the U.S. military encouraging the development of further UV filters in the postwar period.

In the 1970s increased interest in commercial sunscreen products led to refinements and consumer acceptance of these products over the next two decades. Facilitated by growing awareness as to the hazards of UVR, higher SPF products became the norm. Daily use consumer products containing UV filters, including moisturizers, color cosmetics, and even hair care products, have become more prevalent in the past decade. Concerns related to the adequacy of sunscreen protection for the prevention of melanoma and photoaging in the last few years have led to greater interest in broad spectrum sunscreen UV protection throughout the entire UVA range.

REGULATORY

United States

Sunscreen products in the United States are regulated by the Food and Drug Administration (FDA) as over the counter drugs. The Final Monograph for Sunscreen Drug Products for Over-the-Counter Human Use (Federal Register 1999: 64: 27666–27693) establishes the conditions for safety, efficacy, and labeling of these products. As active ingredients in drug products, they are listed by their United States Adopted Names Drug Name. The number of allowable sunscreen ingredients has been reduced to 16 (Table 1) reflecting the lack of interest in some of the ingredients in previously issued tentative monographs. Avobenzone and zinc oxide were added expanding the available UVA I blockers. Maximum allowable concentrations are provided. Minimum concentration requirements were dropped providing that the concentration of each active ingredient is sufficient to contribute a minimum SPF of not less than 2 to a finished product. A sunscreen product must have a minimum SPF of not less than the number of active sunscreen ingredients used in combination multiplied by 2. Products with SPF values above 30 are allowed but the SPF declaration for sunscreens with SPF values above 30 are limited to SPF 30 plus. The term "sunblock" is prohibited. It was previously allowed for products that contained titanium dioxide®. Newer labeling requires the listing of UV filters not only as active ingredients but also their concentration in the product. Consideration of labeling

Table 1 FDA Sunscreen Final Monograph Ingredients

Drug name	Conc. %	Absorbance
Aminobenzoic acid	Up to 15	UVB
Avobenzone	2–3	UVAI
Cinoxate	Up to 3	UVB
Dioxybenzone ensulizole	Up to 3 Up to 4	UVB, UVAII UVB
Homosalate	Up to 15	UVB
Meradimate	Up to 5	UVAII
Octocrylene	Up to 10	UVB
Octinoxate	Up to 7.5	UVB
Octisalate	Up to 5	UVB
Oxybenzone	Up to 6	UVB, UVAII
Padimate O	Up to 8	UVB
Sulisobenzone	Up to 10	UVB, UVAII
Titanium dioxide	2–25	Physical
Trolamine salicylate	Up to 12	UVB
Zinc oxide	2–20	Physical

and testing procedures for UVA protection was deferred. An amendment to the Monograph establishing a UVA standard is expected shortly.

Europe

In Europe, sunscreen products are considered to be cosmetics, their function being to protect the skin from sunburn. The Third Amendment of the European Economic Community Directive provides a definition and lists the UV filters that cosmetic products may contain. Table 2 lists UV filters that are fully permitted as amended most recently through commission directive 2003/83/EC. As cosmetic products, cosmetic or International Nomenclature of Cosmetic Ingredients (INCI) nomenclature is utilized as listed in the Cosmetic Toiletry and Fragrance Association International Cosmetic Ingredient Dictionary. The European Union (EU) allows several ingredients not available in the United States (see discussion below). The EU has recently added titanium dioxide to the approved list, but not zinc oxide.

Australia

In 1992 sunscreens were declared to be drugs in Australia. The latest edition of Australian Standard 2604 was published in 1993 as a joint publication of Australia and New Zealand. Sunscreen products are classified as either primary or secondary depending on whether the primary function of the designated product is to protect from UVR as opposed to a product with a primary cosmetic purpose. SPF designations greater than 15 are not permitted (SPF 15+ represents the maximum designation). In general, Australian Approved Names (AAN) for allowed active sunscreen ingredients are the same as FDA Drug nomenclature with a few differences.

Other Countries

Most non-EEC European countries follow the EEC directive. Many other countries follow U.S. trends with their own provisions. In Japan, sunscreens are classified as

Table 2 List of UV Filters Which Cosmetic Products may Contain EEC Directive Annex VII—Part 1

Reference number	Substance	INCI name	Maximum authorized concentration
1	4-Aminobenzoic acid	PABA	5%
2	*N*,*N*,*N*-Trimethyl-4-(2-oxoborn-3-ylidenemethyl) anilinium methyl sulfate	Camphor benzylkonium methosulfate	6%
3	Homosalate (INN)	Homosalate	10%
4	Oxybenzone (INN)	Benzophenone-3	10%
6	2-Phenylbenzimidazole-5-sulfonic acid and its potassium, sodium, and triethanolamine salts	Phenylbenzimidazole sulfonic acid	8% (expressed as acid)
7	3,3′-(1,4-Phenylenedimethylene)bis[7,7-dimethyl-2-oxo-bicyclo-(2,2,1)hept-1-ylmethanesulfonic acid] and its salts	Terephthalidene dicamphor sulfonic acid	10% (expressed as acid)
8	1-(4-*tert*-Butylphenyl)-3-(4-methoxyphenyl) propane-1, 3-dione	Butylmethoxydibenzoylmethane	5%
9	Alpha-(2-oxobron-3-ylidene)toluene-4-sulfonic acid and its salts	Benzylidene camphor sulfonic acid	10% (expressed as acid)
0	2-Cyano-3,3-diphenyl acrylic acid, 2-ethylhexyl ester	Octocrylene	10% (expressed as acid)
1	Polymer of *N*-(2 and 4)-[(2-oxoborn-3-ylidene)methyl] benzyl acrylamide	Polyacrylamidomethyl benzylidene camphor	6%
2	Octyl methoxycinnamate	Ethylhexyl methoxycinnamate	10%
3	Ethoxylated ethyl-4-aminobenzoate	PEG-35 PABA	10%
4	Isopentyl-4-methoxycinnamate	Isoamyl *p*-methoxycinnamate	10%
5	2,4,6-Trianilino-(*p*-carbo-2′-ethylhexyl-1′-oxy)-1,3,5-triazine	Octyl triazone	5%
6	Phenol, 2-(2H-benzotriazol-2-yl)-4-methyl-6-(2-methyl-3-(1,3,3,3-tetramethyl-1-(trimethylsilyl)oxy)-disiloxanyl)propyl)(drometrizone trisiloxane)	Drometrizone trisiloxane	15%

(*Continued*)

Table 2 List of UV Filters Which Cosmetic Products may Contain EEC Directive Annex VII—Part 1 (*Continued*)

Reference number	Substance	INCI name	Maximum authorized concentration
17	Benzoic acid, 4,4-((6-(((1,1-dimethylethyl)amino)carbonyl)phenyl)amino)-1,3,5, triazine-2,4-diyl)diimino)bis-cbis(2-ethylhexyl)ester)	Diethylhexyl butamido triazone	10%
18	3-(4′-Methylbenzylidene)-d-t camphor	4-Methylbenzylidene camphor	2%
19	3-Benzylidene camphor	3-Benzylidene camphor	2%
20	2-Ethylhexyl salicylate	Octyl salicylate	5%
21	4-Dimethyl-amino-benzoate of ethyl-2-hexyl	Octyl dimethyl PABA	8%
22	2-Hydroxy-4-methoxybenzophenone-5-sulfonic acid and its sodium salt	Benzophenone-5	5% (of acid)
23	2,2′-Methylene-bis-6-(2H-benzotriazol-2yl)-4-(tetramethylbutyl-1,1,3,3,-phenol)	Bisoctyltriazol	10%
24	Monosodium salt of 2,2′-bis-(1,4-phenylene) 1*H*-benzimidazole-4,6-disulfonic acid	Bisamidazylate	10% (of acid)
25	(1,3,5)-Triazine-2,4-bis-((4-(2-ethyl-hexyloxyl)-2-hydroxy)-phenyl)-6-(4-methoxyphenyl)	Anisotriazine	10%
26	Dimethicodiethylbenzalmalonate (CAS No. 207574–74-1)		10%
27	Titanium dioxide		25%

cosmetics. Regulations for each individual country need to be consulted for selection of the various UV filters for incorporation into a sunscreen product to be marketed in a given jurisdiction.

MECHANISM OF ACTION

UV filters have been traditionally divided into chemical absorbers and physical blockers based on their mechanism of action. Chemical sunscreens are generally aromatic compounds conjugated with a carbonyl group (14). These chemicals absorb high intensity UV rays with excitation to a higher energy state. The energy lost results in conversion of the remaining energy into longer lower energy wavelengths with return to ground state. The evolution of modern sunscreen chemicals represents a prototype study in the use of structure–activity relationships to design new active ingredients and has been well reviewed elsewhere (15).

Physical blockers reflect or scatter UVR. Newer microsized forms of physical blockers may also function in part by absorption (16). Sometimes referred to as "nonchemical" sunscreens, they may be more appropriately designated as inorganic particulate sunscreen ingredients.

NOMENCLATURE

Sunscreen nomenclature can be quite confusing. They may be referred to by their chemical or trade name. In the United States, individual sunscreen ingredients are also assigned a drug name by the Over-the Counter (OTC) Monograph. Annex VII of the EU may use either a drug or chemical name. Australia has its own approved list of names (AAN). Table 3 lists the most commonly used names including their primary listing in the International Cosmetic Ingredient Dictionary (INCI designation) (17).

INDIVIDUAL UV FILTERS

Sunscreen ingredients may be considered by dividing them into larger overall classes by chemical structure. They may also be classified by their absorption spectrum. Although the lists of UV filters approved by the various regulatory agencies may seem quite extensive, fewer are used with any degree of frequency. The discussion which follows will concentrate on those listed in Table 3.

UVB

PABA and its Derivatives

Aminobenzoic acid or PABA was one of the first chemical sunscreen chemicals to be widely available. Several problems limited its use. It is very water soluble, was frequently used in alcoholic vehicles, stained clothing and was associated with a number of adverse reactions. Ester derivatives of PABA, mainly octyl dimethyl PABA or Padimate O, became more popular with greater compatibility in a variety of more substantive vehicles and a lower potential for staining or adverse reactions. Amyl dimethyl PABA and glyceryl PABA (glyceryl aminobenzoate) are no longer used.

Table 3 Sunscreen Nomenclature

CAS #	Drug name (FDA)	INCI name	Colipa #	EU reference #	Trade names	Solubility	Spectrum
150-13-0	Aminobenzoic acid	PABA	S 1	1	4-Aminobenzoic acid	Hydrophilic	UVB
70356-09-1	Avobenzone	Butyl methoxydibenzyl methane	S 66	8	Parsol 1789	Lipophilic	UVA I
104-28-9	Cinoxate	Cinoxate				Lipophilic	UVB
118-56-9	Homosalate	Homosalate	S 12	3	Eusolex HMS	Lipophilic	UVB
134-09-8	Meradimate	Menthyl anthranilate			Dermoblock MA, Neo Heliopan, Type MA	Lipophilic	UVA II
6197-30-4	Octocrylene	Octocrylene	S 32	10	Escalol 597, Eusolex OCR, Uvinul N-539–50	Lipophilic	UVB
5466-77-3	Octyl methoxycinnamate	Octyl methoxycinnamate	S 28	12	Neo Heliopan AV, Parsol MCX, Eusolex 2292	Lipophilic	UVB
88122-99-0	Octyl triazone	Octyl triazone	S 69	15	Uvinul T-150	Lipophilic	UVB
118-60-5	Octisalate	Octyl salicylate	S 20	8	Escalol 587, Eusolex BS, Uvinul O-18	Lipophilic	UVB
131-57-7	Oxybenzone	Benzophenone-3	S 38	4	Eusolex 4360, Neo Heliopan, Uvinul M40	Lipophilic	UVB, UVA II
21245-02-03	Padimate O	Octyl dimethyl PABA	S 78	17	Escalol 507, Eusolex 6007	Lipophilic	UVB
27503-81-7	Ensulizole	Phenylbenzimidazole Sulfonic acid	S 45	6	Eusolex 232, Neo Heliopan Hydro	Hydrophilic	UVB
4065-45-6	Sulisobenzone	Benzophenone-4	S 78	17	Escalol 577, Uvinul MS 40	Lipophilic	UVB, UVA II

Padimate O or octyl dimethyl PABA is a most potent UV absorber in the mid UVB range. Because of problems with PABA formulations, marketers have emphasized the "PABA-free" claim. Although still used (18), it is confused with PABA limiting its use. The decline in the use of this PABA derivative along with the demand for higher SPF products has led to the incorporation of multiple active ingredients in a single product to achieve the desired SPF.

Cinnamates

The next most potent UVB absorbers, the cinnamates, have largely replaced PABA derivatives. Octinoxate or octyl methoxycinnamate is the most frequently used sunscreen ingredient (18). Octyl or ethylhexyl methoxycinnamate has an order of magnitude less than Padimate O and requires additional UVB absorbers to achieve higher SPF levels in a final product. Cinoxate (ethoxy-ethyl-*p*-methoxycinnamate) is less widely used. Diethanolamine methoxycinnamate is a water soluble cinnamate but is only provisionally listed in the FDA monograph.

Salicylates

Salicylates are weaker UVB absorbers. They have a long history of use but were supplanted by the more efficient PABA and cinnamate derivatives. They are generally used to augment other UVB absorbers. With the trend to higher SPFs, more octisalate or octyl salicylate (ethylhexyl salicylate) is being used followed by homosalte of homomenthyl salicylate. Both materials have the ability to solubilize oxybenzone and avobenzone. Trolamine or triethanolamine salicylate has good water solubility.

Camphor Derivatives

Not approved by the FDA for use in the United States, there are six camphor derivatives approved in Europe. 4-Methylbenzylidene camphor (EU no. 18) is the most widely used (18).

Octocrylene

2-Ethylhexyl-2-cyano-3,3-diphenylacrylate. 2-Ethylhexyl-2-cyano-3,3-diphenylacrylate or octocrylene is chemically related to cinnamates. It can be used to boost SPF and improve water resistance in a given formulation. Octocrylene is photostabile and can improve the photostability of other sunscreens. It is very expensive and can present difficulties in formulation.

Phenylbenzimadazole Sulfonic Acid

Phenylbenzimadazole sulfonic acid or ensulizole is a water soluble UVB absorber that can be utilized in the water phase of emulsion systems, in contrast to most oil soluble sunscreen ingredients, allowing for a less greasy, more esthetically pleasing formulation such as a daily use moisturizer containing sunscreen. Phenylbenzimidazole sulfonic acid boosts the SPF of organic and inorganic sunscreens. It can also be used in clear gels owing to its water solubility.

Triazones

Octyl triazone (EU no. 15) is available in Europe. Methylene bis-benzotriazol tetramethylbutylphenol (EU no. 23) or Tinasorb M which has recently become available

in Europe is predominately a UVB absorber (19). An anisotriazine (EU no. 25) or Tinasorb S is now also available which provides UVA protection.

UVA

Benzophenones

Although oxybenzone or benzophenone-3 absorbs most efficiently in the UVB range, absorption extends well into the UVA II range. It is used primarily as a UVA absorber, but boosts SPF values in combination with other UVB absorbers. Oxybenzone is supplied as a solid material, has poor solubility and a relatively low extinction coefficient. Sulisobenzone or benzophenone-4 is water soluble, somewhat unstable and used with less frequency.

Menthyl Anthranilate

Anthranilates including Meradimate are weak UVB filters and absorb mainly in the near UVA portion of the spectrum. They are less effective than benzophenones in this range and are less widely used.

Butylmethoxydibenzoylmethane

Avobenzone or Parsol 1789 was approved by the FDA for use in OTC sunscreens in the United States after publication of the final monograph having been used quite extensively in Europe for considerably longer. It provides strong absorption in the UVA I range with peak absorption at 360 nm. Because an agreed upon standard for measuring UVA protection in the United States does not exist, a minimum use concentration has been set at 2% with a maximum of 3%.

Avobenzone should not be confused with isopropyl dibenzoylmethane (Eusolex 8020) which had previously been available in Europe. The high incidence of adverse photosensitivity reported with the combination of isopropyl dibenzoylmethane with methylbenzylidene camphor by coupled reactions in the late 1980s led to a decrease in its use in commercial (20). In 1993 its production was discontinued, and it is no longer listed in Annex VII. Reported sensitivity to butylmethoxydibenzoylmethane was on the basis of cross-reactivity to isopropyl dibenzoylmethane. Isolated allergy to butylmethoxydibenzoylmethane is rare (20).

Photostability refers to the ability of a molecule to remain intact with irradiation. Photostability is potentially a problem with all UV filters. This issue has been raised specifically with avobenzone (21) with photolysis demonstrated in a specially designed in-vitro system (22) which simultaneously irradiates and measures transmittance in situ. This effect may degrade other sunscreens in a formulation. The relevance of this testing to the in-vivo situation remains unclear. Overall formulation may be critical in this regard.

Tetraphthalydine Dicamphor Sulfonic Acid

3,3′-(1,4-Phenylenedimethylene)bis[7,7-dimethyl-2-oxo-bicyclo-(2,2,1)hept-1-yl methanesulfonic acid (EU ref. No. 7) or Mexoryl SX is a UVA blocker more recently available in Europe with comparable (23) or superior efficacy to avobenzone (24).

Physical Blockers

Some of the original sunblocks were opaque formulations reflecting or scattering UVR. Color cosmetics containing a variety of inorganic pigments function in this fashion. Titanium dioxide and zinc oxide are chemically inert and protect through the full spectrum of UVR. They offer significant advantages. Poor cosmetic acceptance limited the widespread use of these two ingredients until recently, when microsized forms have become available. By decreasing particle size of these materials to a microsize or ultrafine grade it is less visible on the skin surface.

Micropigmentary sunblocks function differently compared to opaque sunblocks of pigmented color cosmetics by absorbing and not simply reflecting or scattering UVR (16). By varying and mixing particle sizes differing levels of photoprotection are achieved throughout the UV spectrum. In addition to avobenzone, micropigmentary TiO_2 and ZnO offer the best available protection in the UVA II range.

Photoreactivity has been raised as an issue with these materials. Both TiO_2 and ZnO are semiconductors potentially absorbing light and generating reactive species (25). These effects have been demonstrated in vitro (26). Coating these materials reduces their photochemical reactivity. The in vivo relevance of these effects has not been demonstrated, and both materials have a long history of safe use. Physical blockers also have the significant advantage of lowered skin irritancy potential.

Titanium Dioxide

TiO_2 was the first micropigment extensively used. Advantages include a broad spectrum of protection and inability to cause contact dermatitis. The use of rutile as opposed to anatase crystal forms of titanium dioxide lessens photoactivity. Newer materials are amphiphilic designed to be dispersed in both water and oil emulsion phases. Particle size and uniformity of dispersion are key to achieving SPF. Primary particle size may be 10 to 15 nm with secondary particle assembly size to be 100 nm. Particle size needs to be less than 200 nm to achieve transparency.

Despite advances in the technology and understanding of these materials, whitening remains a problem secondary to pigment residue. Adding other pigment simulating flesh tones may partially camouflage this effect. The net effect may be that the user is inclined to make a less heavy application of product effectively lowering SPF (27). "Hybrid" formulations employing a combination of chemical absorbers with inorganic particulates may represent a practical compromise.

Zinc Oxide

Zinc oxide was only recently approved as an active sunscreen agent for the FDA OTC Sunscreen Monograph. Reduced to a particle size of less than 200 nm, light scattering is minimized, and the particles appear transparent in thin films (28). ZnO has a refractive index of 1.9, as opposed to 2.6 for TiO_2, and therefore causes less whitening than TiO_2. ZnO attenuates UVR more effectively in the UVA I range (29) with a peak at 360 nm. Microfine TiO_2 at an equal concentration offers somewhat more protection in the UVB range. Fine particle ZnO is not approved as a sunscreen ingredient in the EU.

ADVERSE REACTIONS—TOXICITY

In a longitudinal prospective study of 603 subjects applying daily either an SPF 15+ broad spectrum sunscreen containing octyl methoxycinnamate and avobenzone or a

vehicle cream, 19% developed an adverse reaction (30). Interestingly, the rates of reaction to both the active and vehicle creams were similar, emphasizing the importance of excipient ingredients in the vehicle. The majority of reactions were irritant in nature. Not surprisingly, a disproportionate 50% of the reacting subjects were atopic. Less than 10% of the reactions were allergic with none of the subjects patch tested actually found to be allergic to an individual sunscreen ingredient.

Subjective irritation associated with burning or stinging without objective erythema from some organic UV filters (31) is the most frequent sensitivity complaint associated with sunscreen use. This is most frequently experienced in the eye area. Longer lasting objective irritant contact dermatitis may be difficult to distinguish from true allergic contact dermatitis. In a postmarket evaluation of sunscreen sensitivity complaints in 57 patients, 20 of the patients had short-lasting symptoms, 26 long-lasting, and 11 mixed or borderline symptoms (32). Half of the patients were patch and photopatch tested, and only three showed positive reactions to sunscreen ingredients.

Contact and photocontact sensitivity to individual sunscreen ingredients have been extensively reviewed (20). Considering their widespread use the number of documented allergic reactions is not high (33). PABA and PABA esters accounted for many of the early reported reactions, but with a decrease in their use reactions to benzophenones may be increasing (34). Reactions to dibenzoylmethanes have been previously discussed. Fragrances, preservatives, and other excipients account for a large number of the allergic reactions seen (20).

Virtually all sunscreen ingredients reported to cause contact allergy may be photoallergens. Although still relatively uncommon, sunscreen actives seem to have become the leading cause of photocontact allergic reactions (35,36). Individuals with pre-existing eczematous conditions have a significant predisposition to sensitization associated with their impaired cutaneous barrier. The majority of individuals who develop photocontact dermatitis to sunscreens are patients with photodermatides (17).

CONCLUSION

A limited menu of UV filters for incorporation into sunscreen products is available to the formulating chemist, depending on regulatory requirements in an individual country or jurisdiction. With the demand for higher SPFs, the trend has been to use more individual and a wider variety of agents in newer products. Recent research in sunscreen efficacy has emphasized the need for products protecting against the full UV spectrum with a limited number of available agents. Regulatory agencies are very slow to approve new ingredients. Rules governing the approval of new ingredients by the European Economic Community appear to be more flexible in this regard.

Sunscreen efficacy remains very dependent on vehicle formulation. Solvents and emollients can have a profound effect on the strength of UV absorbance by the active ingredients and at which wavelengths they absorb (37). Film formers and emulsifiers determine the uniformity and thickness of the film formed on the skin surface which in turn determine SPF level, durability, and water resistance (38). Lastly, product esthetics play a large role in product acceptance, particularly with sunscreens being incorporated into daily use cosmetics. These constraints provide the sunscreen formulator with significant challenges in developing new and improved formulations.

REFERENCES

1. Weinstock MA. Death from skin cancer among the elderly: epidemiological patterns. Arch Dermatol 1997; 133:1207–1209.
2. Yaar M, Gilchrest BA. Aging versus photoaging: postulated mechanisms and effectors. J Invest Dermatol Symp Proc 1998; 3:47–51.
3. Naylor MF, Farmer KC. The case for sunscreens: a review of their use in preventing actinic damage and neoplasia. Arch Dermatol 1997; 133:1146–1154.
4. Gurish MF, Roberts LK, Krueger GG, Daynes RA. The effect of various sunscreen agents on skin damage and the induction of tumor susceptibility in mice subjected to ultraviolet irradiation. J Invest Dermatol 1981; 76:246–251.
5. Thompson SC, Jolley D, Marks R. Reduction of solar keratoses by regular sunscreen use. N Engl J Med 1993; 329:1147–1151.
6. Boyd AS, Naylor M, Cameron GS, Pearse AD, Gaskell SA, Neldner KH. The effects of chronic sunscreen use on the histologic changes of dermatoheliosis. J Am Acad Dermatol 1995; 33:941–946.
7. Green A, Williams G, Neale R, Hart V, Leslie D, Parsons P, Marks GC, Gaffney P, Battistutta D, Frost C, Lang C, Russel A. Daily sunscreen application and beta-carotene supplementation in prevention of basal cell and squamous cell carcinomas of the skin: a randomized controlled trial. Lancet 1999; 354:723–729.
8. Roberts LK, Beasley DG. Commercial sunscreen lotions prevent ultraviolet-radiation-induced immune suppression of contact hypersensitivity. J Invest Dermatol 1995; 105: 339–344.
9. Stiller MJ, Bartolone J, Stern R, Smith S, Kollias N, Gillies R, Drake LA. Topical 8% glycolic acid and 8% L-lactic acid creams for the treatment of photodamaged skin: A double-blind vehicle-controlled clinical trial. Arch Dermatol 1996; 132:631–636.
10. Huncharek M, Kupelnick B. Use of topical sunscreens and the risk of malignant melanoma: a meta-analysis of 9067 patients from 11 case control studies. Am J Public Health 2002; 92:1173–1177.
11. CDC. Counseling to prevent skin cancer: recommendations and rationale of the US Preventative Services Task force. MMWR 2003; 52(No. RR-15):13–17.
12. Shaath NA. Evolution of modern sunscreen chemicals. In: Lowe NJ, Shaath NA, Pathak MA, eds. Sunscreens: Development, Evaluation, and Regulatory Aspects. 2nd ed. New York: Marcel Dekker, 1997:3–31.
13. Rebut, R. The sunscreen industry in Europe: Past, present, and future. In: Lowe NJ, Shaath NA, eds. Sunscreens: Development, Evaluation, and Regulatory Aspects. New York: Marcel Dekker, 1990:161–178.
14. Shaath NA. The chemistry of sunscreens. Cosmet Toilet 1986; 101:55–70.
15. Shaath NA. On the theory of ultraviolet absorption by sunscreen chemicals. J Soc Cosmet Chem 1987; 82:193.
16. Sayre RM, Killias N, Roberts RL, Baker A, Sadiq I. Physical sunscreens. J Soc Cosmet Chem 1990; 41:103–109.
17. Wenninger JA, McEwen GN Jr, eds. International Cosmetic Ingredient Dictionary and Handbook. 10th ed. Washington: The Cosmetic, Toiletry, and Fragrance Association, 2004.
18. Steinberg DC. Frequency of use of organic UV filters as reported to the FDA. Cosmet Toilet 2003; 118(10):81–83.
19. Murphy GM. An update on photoprotection. Photodermatol Photoimmunol Photomed 2002; 18:1–4.
20. Schauder S, Ippen H. Contact and photocontact sensitivity to sunscreens. Review of a 15-year experience and of the literature. Contact Dermatitis 1997; 37(5):221–232.
21. Deflandre A, Lang G. Photostability assessment of sunscreens. Benzylidene camphor and dibenzoylmethane derivatives. Int J Cosmet Sci 1988; 10:53–62.

22. Sayre RM, Dowdy JC. Avobenzone and the photostability of sunscreen products. Presented at the 7th Annual Meeting of the Photomedicine Society, Orlando, Florida, February 26, 1998.
23. Chardoon A, Moyal D, Hourseau C. Persistent pigment-darkening response as a method for evaluation of ultraviolet: A protection assays. In: Lowe NJ, Shaath NA, Pathak MA, eds. Sunscreens: Development, Evaluation, and Regulatory Aspects. 2nd ed. New York: Marcel Dekker, 1997:559–581.
24. Seite S, Colige A, Piquemal-Vivenot P, Montastier C, Fourtanier A, Lapiere C, Nusgens B. A full-spectrum absorbing daily use cream protects human skin against biological changes occurring in photoaging. Photodermatol Photoimmunol Photomed 2000; 16:147–155.
25. Murphy GM. Sunblocks: Mechanisms of action. Photodermatol Photoimmunol Photomed 1999; 15:34–36.
26. Wamer WG, Yin JJ, Wei RR. Oxidative damage to nucleic acids photosensitized by titanium dioxide. Free Radic Biol Med 197; 23:851–858.
27. Diffey BL, Grice J. The influence of sunscreen type on photoprotection. Br J Dermatol 1999; 137:103–105.
28. Fairhurst D, Mitchnik MA. Particulate sun blocks: general principles. In: Lowe NJ, Shaath NA, Pathak MA, eds. Sunscreens: Development, Evaluation, and Regulatory Aspects. 2nd ed. New York: Marcel Dekker, 1997:313–352.
29. Mitchnick MA, Fairhurst D, Pinnell SR. Microfine zinc oxide (Z-Cote) as a photostable UVA/UVB sunblock agent. J Am Acad Dermatol 1999; 40:85–90.
30. Foley P, Nixon R, Marks R, Frowen K, Thompson S. The frequency of reactions to sunscreens: results of a longitudinal population-based study on the regular use of sunscreens in Australia. Br J Dermatol 1993; 128:512–518.
31. Levy SB. Sunscreens for photoprotection. Dermatol Therapy 1997; 4:59–71.
32. Fischer T, Bergstrom K. Evaluation of customers' complaints about sunscreen cosmetics sold by the Swedish pharmaceutical company. Contact Dermatitis 1991; 25:319–322.
33. Dromgoole SH, Maibach HI. Sunscreening agent intolerance: contact and photocontact sensitization and contact urticaria. J Am Acad Dermatol 1990; 22:1068–1078.
34. Lenique P, Machet L, Vaillant L, Bensaid P, Muller C, Khallouf R, Lorette G. Contact and photocontact allergy to oxybenzone. Contact Dermatitis 1992; 26:177–181.
35. Fotiades J, Soter NA, Lim HW. Results of evaluation of 203 patients for photosensitivity in a 7.3-year period. J Am Acad Dermatol 1995; 33(4):597–602.
36. Trevisi P, Vincenzi C, Chieregato C, Guena L, Tosti A. Sunscreen sensitization: a three-year study. Dermatology 1994; 189:55–57.
37. Agrapidis-Paloympis LE, Nash RA, Shaath NA. The effect of solvents on the ultraviolet absorbance of sunscreens. J Soc Cosmet Chem 1987; 38:209–221.
38. Klein K. Formulating sunscreen products. In: Lowe NJ, Shaath NA, eds. Sunscreens: Development, Evaluation, and Regulatory Aspects. New York: Marcel Dekker, 1990: 235–266.

24
Skin Whitening: Ellagic Acid

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Melanin is a key factor determining the color of skin. The enzyme tyrosinase plays the most important role in melanin synthesis (melanogenesis) (1,2). Several tyrosinase inhibitors (chemicals, plant extracts, animal products) have been proposed, based on the view that melanogenesis can be controlled, and skin-whitening products can be developed if tyrosinase activity can be suppressed. However, few have been put to practical use. In practice, it is difficult to develop these candidate materials from in vitro studies to approve for human use, even if inhibitory effects on mushroom-derived tyrosinase or pigment cells can be identified. In addition to showing adequate efficacy and safety, there are many problems to consider, such as stability of the products, production and marketing costs, and perception of the user.

Ellagic acid (EA) (Fig. 1) was approved in 1996, in Japan, as the active ingredient of a quasidrug for prevention of spots and freckles after developing sunburn owing to exposure to excess sunlight. EA, a naturally occurring polyphenol (3,4) containing four hydroxyl groups, is found in many plants such as strawberry, grape, green tea, eucalyptus, walnut, and tara. Generally, EA is produced by hydrolysis and purification from ellagitannin.

GENERAL PROPERTIES

EA is a cream-colored powder slightly soluble in water and ethanol, in alkaline solution and pyridine, and practically insoluble in ether (4). EA has high antioxidant activity (5), and is listed as a food additive in Japan. The hydroxyl groups of EA can chelate with metal ions (6,7).

IN VITRO STUDIES

EA inhibits mushroom-derived tyrosinase competitively and in a dose-dependent manner; the inhibition constant (k_i) is 81.6 μM (8). The decrease in copper concentration and the reduction in tyrosinase activity by EA follow almost parallel patterns. Tyrosinase activity, after inhibition by EA, partially recovers after addition of cuprous or cupric ion (Fig. 2).

Figure 1 Ellagic acid.

Growth of B16 melanoma cells in culture medium was not suppressed by EA at concentrations less than 4 μM. At 4 μM, the inhibition of tyrosinase activity was 38.3%, and the decrease in melanin concentration was 54.4%. Although the color of the cells (reflecting the melanin concentration) became whitened in the presence of EA, cell color reverted to the original shade when EA was removed from the culture medium (Fig. 3). The addition of other metals, in place of the copper compounds, did not lead to recovery of the enzymic activity.

These results show that the inhibitory effect of EA is reversible, effective only in its presence, and specific to copper compounds. It is proposed that EA chelates with copper ion(s) at the active center of tyrosinase, which is a metaloprotein containing copper. Further structural changes then make the tyrosinase inactive. Because the molecular structure of EA is planar, EA may be able to penetrate into the active center of tyrosinase easily. It is clear that EA inhibits tyrosinase because of its molecular structure as well as its ability to chelate with copper.

ANIMAL STUDIES

Brownish guinea pigs have melanocytes in their skin, and the skin pigmentation is enhanced by ultraviolet (UV) light irradiation, similar to the human situation. The

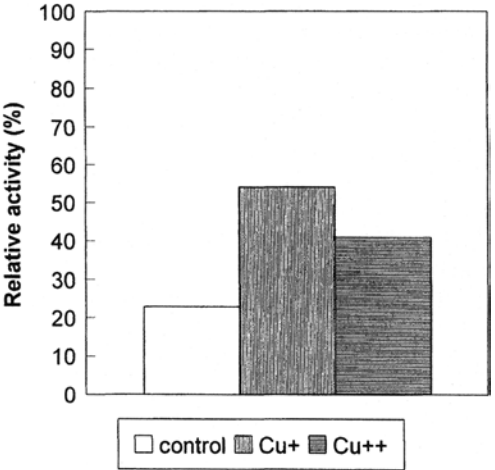

Figure 2 Effects of addition of copper ion on recovery of tyrosinase activity. Cu^{+} or Cu^{2+} (5 μM) were added to tyrosinase during inhibition by EA.

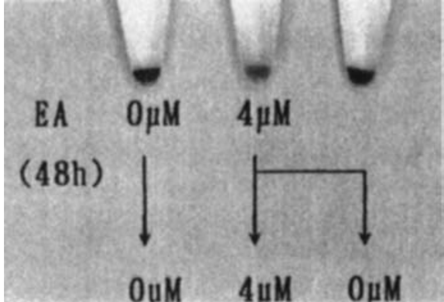

Figure 3 Effect of EA on melanoma cells. Cells were incubated with EA (4 μM) for 48 hours. Culture medium was changed for fresh medium in the presence or absence of EA (4 μM) and incubated for an additional 48 hours.

preventative effect of EA on skin pigmentation was investigated by applying EA topically, on the back, for six weeks and irradiating by UV for the first two weeks (8). The appearance of skin to which EA was applied became similar to normal skin. The melanin content of the skin to which EA had been applied was reduced, not only in the basal layers but also in the stratum spinosum, -granulosum, and -corneum, in comparison with the same structures in control sections to which EA had not been applied. Tyrosinase activity was similar. Furthermore, application of EA to the skin after UV light irradiation had almost the same affect as applying EA concurrently with the initial irradiation.

According to the results of the studies using the brownish guinea pig, EA is a more efficient skin whitener and suppressor of pigmentation than arbutin or kojic acid, other active skin whiteners, at the same dose level (1%) (Fig. 4).

Furthermore, the efficacy of EA was almost the same as that of hydroquinone (HQ), a well-known depigmentation agent (Fig. 5). When the same animals were subjected to UV irradiation again after completion of the application phase, normal skin pigmentation was observed in the EA applied area as well as in the control areas, but only slight pigmentation was seen in the HQ treated skin. The results of

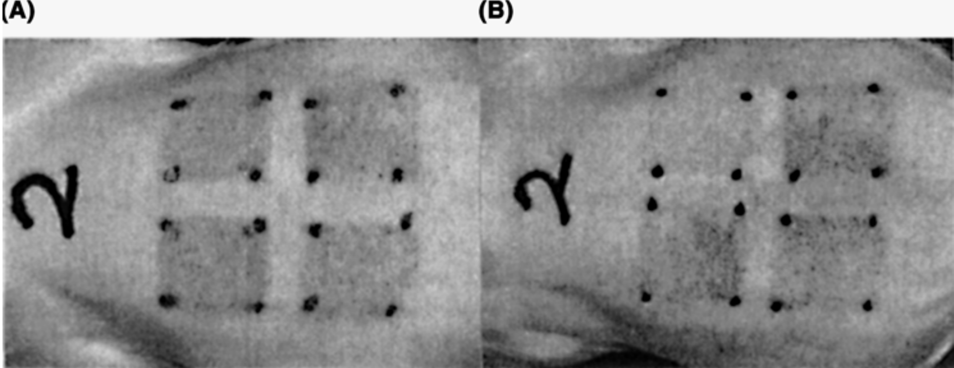

Figure 4 Comparison of effects of some commercially available agents in preventing skin pigmentation induced by UV-light irradiation. Samples were applied for four weeks after UV-light irradiation (eight times): (**A**) before application; (**B**) after application for four weeks; (*upper left*) ellagic acid, (*upper right*) vehicle, (*lower left*) arbutin, and (*lower right*) kojic acid.

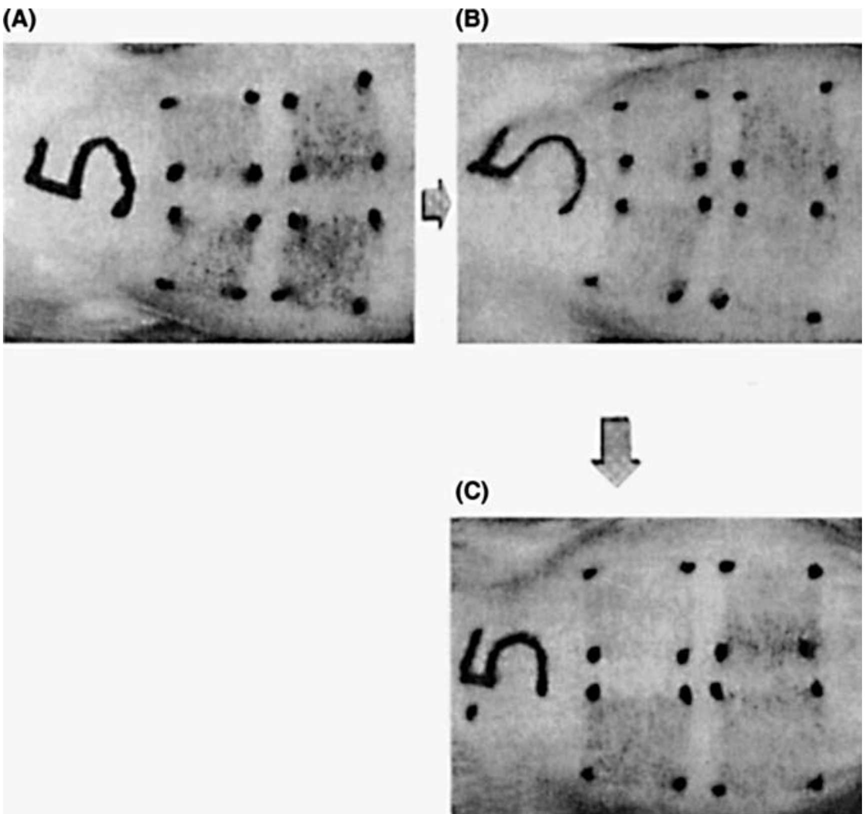

Figure 5 Effects of ellagic acid on UV-light–induced pigmentation. Samples were applied for four weeks (**A**) after UV-light irradiation (eight times): (*upper left*) hydroquinone; (*upper right*) vehicle only; (*lower left*) control—no EA applied; (*lower right*) ellagic acid. After application was terminated (**B**), the same area was irradiated again (**C**).

these investigations indicated that EA was not injurious to melanocytes but was a good inhibitor of tyrosinase activity. In comparison, HQ may be toxic to melanocytes.

EFFECT ON HUMAN SKIN

A skin cream containing EA was applied for six weeks to the brachium before each irradiation by UV light (9). The sites were irradiated three times at 1 MED. Skin pigmentation was partially suppressed after only one week's application, and completely suppressed after three and six weeks' application (Fig. 6). Eighty-six percent of the efficacy of EA evaluated by a double-blind controlled test was rated "moderately preferable" or better (Fig. 7). Similar efficacy rates were calculated by the image analysis method. Side effects such as depigmentation were not observed throughout the application period.

Thus, EA can prevent the buildup of skin pigmentation after sunburn. It can also be expected to improve the appearance of pigmented skin such as melasma or

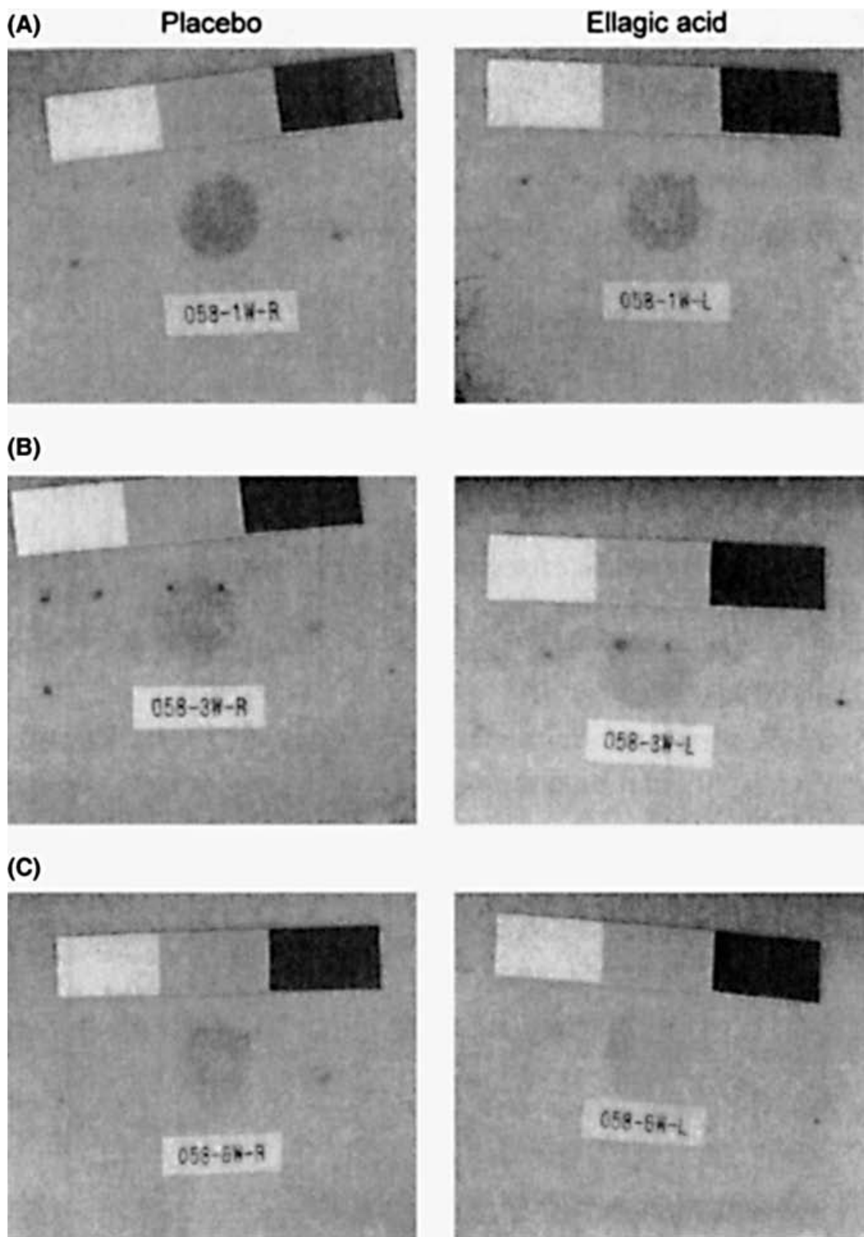

Figure 6 Effect of ellagic acid on UV-light–induced skin pigmentation in human.

freckles, for such skin pigmentation is believed to follow similar mechanisms to that of sunburn, at least from the viewpoint of epidermic disorders, even if the mechanism of melasma and so on are not precisely clear. Many impressions that skin pigmentation appears to be lightened have been gathered from users of products containing EA. In practice, the characteristics of melasma, postinflammatory pigmentation, and other conditions appear to be improved by this application (Table 1) (10). EA is a promising skin-whitening active ingredient.

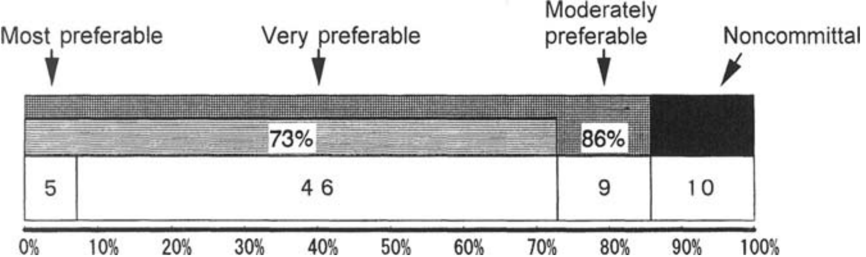

Figure 7 Efficacy for whitening effect on sunburn subjects.

Table 1 Efficacy of Ellagic Acid to Some Skin Pigmentation Conditions

	Chloasma (spot) (15 cases)	Post-inflammatory pigmentation (24 cases)	Ephelides (freckle) (18 cases)	Senile pigment freckle (13 cases)
Most preferable	2	12	0	0
Very preferable	4	7	1	4
Moderately preferable	5	4	5	5
Noncommittal	4	1	12	4
Effective ratio (%)	73.3	95.8	33.3	69.2

REFERENCES

1. Nordlund JJ, Boissy RE, Hearing VJ, King RA, Ortonne J-P, eds. The Pigmentary System (Physiology and Pathophysiology) New York: Oxford University Press, 1998.
2. Tanaka Y, Masuda M. Trends in skin-whitening agents in Japan. INFORM 1998; 9:306.
3. Bate-Smith EC. Chromatography and systematic distribution of ellagic acid. Chem Ind BIF Rev April 1956:R32.
4. Zee-Cheng RK-Y, Cheng CC. Ellagic acid. Drugs Future 1986; 11:1029.
5. Osawa T, Ide A, Su J-D, Namiki M. Inhibition of lipid peroxidation by ellagic acid. J Agric Food Chem 1987; 35:808.
6. Press RE, Hardcastle D. Some physico-chemical properties of ellagic acid. J Appl Chem 1969; 19:247.
7. Zhang N-Z, Chen Y-Y. Synthesis of macroporous ellagitannic acid resin and its chelating properties for metal ions. J Macromol Sci—Chem 1988; A25(10&11):1455.
8. Shimogaki H, Tanaka Y, Tamai H, Masuda M. In vitro and in vivo evaluation of ellagic acid on melanogenesis inhibition. Int J Cosmet Sci 2000; 22:291.
9. Kamide R, Arase S, Takiwaki H, Watanabe S, Watanabe Y, Kageyama S. Clinical evaluation on the effects of XSC-29 preparation on the pigmentation of the skin by exposure to ultraviolet light. Nishinihon J Dermatol 1995; 57:136.
10. Yokoyama M, Itoh Y. Clinical evaluation of the use of whitening cream containing ellagic acid for the treatment of skin pigmentation conditions. Skin Res 2001; 43:286.

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Skin Whitening: New Hydroquinone Combination

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DISORDERS OF PIGMENTATION

Pigmentation disorders arise as a result of an increased production of melanin by melanocytes and/or an elevated transfer of melanosomes from melanocytes to basal and suprabasal keratinocytes (1–3). Melanin (eumelanin and pheomelanin) results from the hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA) through the enzymatic action of tyrosinase, and the subsequent oxidation of DOPA to dopaquinone (4). Once produced, melanin is transferred to keratinocytes or into the dermis via any of the following processes: (1) Damage to melanocytes in the basal layer allows for phagocytization by melanophages, releasing melanin into the dermis; (2) melanosomes are directly deposited, through their dendrites, into the dermis; or (3) macrophages migrate into the epidermis where melanosomes are phagocytized, returning them to the dermis.

Melasma

Difficult to treat, melasma, also known as chloasma or "mask of pregnancy," is a relatively common, chronic pigmentary condition typically seen in women of childbearing age. In fact, it is known to appear at any time during a woman's reproductive years and is often associated with pregnancy or oral contraceptive use. Melasma is more common among women of darker skin types. A small percentage of cases, approximately 10%, occur in men, most frequently in those of Middle Eastern, Caribbean or Asian descent.

Melasma presents as often distinctly demarcated, irregularly shaped light- to dark-brown macules. The blotches usually appear on the upper lip, nose, cheeks, chin, forehead, and, occasionally, the neck. A centrofacial pattern of distribution, involving the cheeks, forehead, upper lip, nose, and chin, is the manifestation most often seen, but there are three identifiable patterns of presentation (2,5). The mandibular pattern and the malar pattern, which affects the nose and cheeks, are rarer. Although it most often occurs in skin routinely exposed to the sun, there are reports

in the literature of melasma appearing on the nipples and around the external genitalia (6,7).

Etiology

The cause of melasma has not yet been clearly identified, but solar exposure, genetic predisposition, and hormonal influences are considered among the most important factors (6,8). Estrogen and progesterone, nutritional deficiency, and certain anti-epilepsy drugs are considered significant causal or exacerbating factors in its development (4). Also, Hydantoin and Dilantin have been reported to contribute to melasma in both women and men (7,9).

A history of frequent, prolonged sun exposure seems to be a necessary precondition for the development of this pigment disorder, and solar exposure is also well known to exacerbate the condition (10,11). Interestingly, in the winter months, when sun exposure is usually less frequent, melasma is typically less noticeable (10). In the absence of other compelling evidence, sun exposure is considered the primary exogenous causal factor in melasma (1,12). Data suggesting a genetic component are sparse, but a few familial cases have been reported (13).

It is not uncommon for women to develop melasma on the upper lip after hot wax application to remove unwanted hair. In fact, this phenomenon is so often reported by patients that the author speculates that heat may play a role in melasma development as it does in erythema *ab igne* (a reticulated erythematous hyperpigmented eruption arising after chronic exposure to heat).

It has been observed that women who use oral contraceptives are those who most often present with melasma (6,7). This painless but often stress-inducing condition also frequently affects pregnant women; together, women in these categories comprise the majority of melasma cases. Occasionally, there are menopausal and premenstrual presentations associated with melasma. The low incidence of melasma among postmenopausal women on estrogen replacement therapy suggests that estrogen alone is an unlikely etiologic root (10). This is a highly idiopathic condition, varying from patient to patient, within individuals, and even from pregnancy to pregnancy (10). It is also characterized by a high degree of recalcitrance. Melasma may subside in the months following a patient's pregnancy or after discontinuing oral contraceptives, but still persists, taking up to five years to resolve (7,9). An endocrine etiology has been posited by some (7), but no such mechanism has yet been proved (9). Some ovarian disorders are also correlated with an increased incidence of melasma, but no causal link has been established. The odds of experiencing initial onset of melasma are lower than the likelihood of recurrence once melasma has developed.

OTHER PIGMENTATION DISORDERS

Solar Lentigos

Long-standing data suggest that as many as 90% of elderly patients have one or more solar lentigos (14). Sun exposure, as the name suggests, is responsible for this condition; both acute and chronic exposure have been associated with inducing these macular brown lesions usually 1 cm in diameter. The face and backs of hands are the areas most often affected. This condition is resistant to the Kligman formula (15).

Postinflammatory Hyperpigmentation

Various skin conditions, such as acne, eczema, and allergic responses, can lead to postinflammatory hyperpigmentation, also known as postinflammatory pigment alteration (PIPA), as can more serious cutaneous events, such as burns, surgeries, and trauma. Certain treatments for skin disease or cosmetic conditions can also engender or exacerbate discoloration (e.g., chemical peels and laser resurfacing). PIPA can occur in any skin color, but it most often affects people with darker skin types (16–18). The condition results from an elevation in melanin synthesis in response to a cutaneous attack and can be diffused or localized—its distribution depends on the location of the original insult to the skin.

PIPA presents in areas of previous inflammation as irregular, darkly pigmented splotches (19). Any area of the skin can be affected, but the disorder is especially stressful to patients when it occurs in the face. Not surprisingly then, PIPA is one of the most common conditions provoking patients to visit a dermatologist. PIPA unfortunately tends to recur in susceptible individuals (20).

Treatment

The goals of therapy are to slow the proliferation of melanocytes, inhibit the formation of melanosomes, and promote the degradation of melanosomes (21). Sun-protective behavior is a necessity. As such, patients must use a good high-SPF sunscreen with UVA protection and make all reasonable efforts to avoid sun exposure. The sunscreen should be worn 24 hours a day. Other practical, behavioral elements of therapy can include UVA screens for car and home windows and protective clothing, such as hats.

The Kligman Formula

The "Kligman formula" is a mixture consisting of 0.1% tretinoin, 5.0% hydroquinone, 0.1% dexamethasone, and hydrophilic ointment (15). The daily application of this combination, through five to seven weeks, resulted in depigmentation of normal adult skin in black males treated for melasma, ephelides, and postinflammatory hyperpigmentation. The removal of one component of the therapeutic regimen led to failure to achieve depigmentation. The formula, which has been very popular as a therapeutic option for melasma since its introduction in 1975, is not commercially available now, but can be formulated by a pharmacy. The success of the Kligman formula led to the status of topical combination therapy as the current mainstay of melasma treatment.

Other Topical Components

The standard products used to produce hypopigmentation include phenolic and nonphenolic derivatives. Hydroquinone and hydroquinone combination formulations are among the phenolic group; tretinoin and azelaic acid are among the nonphenolic agents (22). Typical topical preparations include 2% to 4% of hydroquinone, low potency steroids, kojic acid, arbutin, azelaic acid, hydroxy acids, and retinoids. Tretinoin (0.1%) has been evaluated as a single agent in the treatment of melasma and favorably reviewed (23,24), but such monotherapy took as long as 10 months, in one study, before the condition improved. A 10-month, randomized, vehicle-controlled clinical study did show that topical 0.1% tretinoin lightened melasma in 28 black

patients, with only mild side effects (25). Combination therapy has been considered the mainstay therapy for patients with any skin type, though.

Although the majority of the discussion here will focus on recent research with a novel combination compound, it is worth noting that other tools in the dermatologic armamentarium have achieved favorable results. For example, the addition of glycolic acid to hydroquinone has been shown to promote efficacy by facilitating the penetration of both agents (26). A recently evaluated cream containing 10% buffered glycolic acid, 4% hydroquinone, vitamins C and E, and sunscreen has also been shown to be safe and effective in the treatment of melasma (27). In combination with topical agents, glycolic acid peels and/or Jessner's peels can be used to accelerate the resolution of melasma. Jessner's solution and 70% glycolic acid (combined with tretinoin and hydroquinone between peels) have been shown to work equally well in the treatment of melasma (28). Kojic acid has also been demonstrated to enhance the efficacy of topical agents. A study in Singapore followed 40 Chinese women treated with 2% kojic acid in a gel containing 10% glycolic acid and 2% hydroquinone on one half of the face and the same application without kojic acid on the other half (29). Patients were observed for 12 weeks and showed improvement in melasma on both sides of the face. The side treated with the combination containing kojic acid showed greater improvement; it should be noted. The melasma was cleared in 24 of the 40 patients who received kojic acid as compared to 19 of 40 patients treated with the gel without kojic acid.

It is also worth noting that laser therapy has been used with some degree of effectiveness in treating several pigmentary disorders, but has not yet been established as a first-line therapy for melasma (22).

A NEW PRESCRIPTION COMBINATION THERAPY—TRILUMA

Triluma® is a combination of tretinoin 0.05% (Retin A), hydroquinone 4.0%, and fluocinolone acetonide 0.01% (a mild steroid). Two 8-week, multicenter, randomized, investigator-blind studies were conducted to compare the efficacy and safety of this triple-combination hydrophilic cream formulation with various dual-combination agents. The same drug concentrations and vehicles were used in all formulations. The dual combinations included tretinoin plus hydroquinone, tretinoin plus fluocinolone acetonide, and hydroquinone plus fluocinolone acetonide.

The theoretical basis for this new formulation rests on the prior success of the various first-line components in dual-combination therapies, namely, hydroquinone, tretinoin, and a range of topical corticosteroids. Of particular conceptual importance in the product development are the clinical and experimental data demonstrating the effects of tretinoin and other retinoids in abrogating epidermal atrophy that can be induced by topical corticosteroids (30,31). A total of 641 predominantly white female adults (ranging in age from 21 to 75 years) were randomized to the various treatment groups. In both studies, all formulations were applied once daily, at night. Patients enrolled in the study represented Fitzpatrick skin types I through IV and exhibited moderate to severe hyperpigmentation.

Results

Significantly more of the patients treated with Triluma (26.1%) demonstrated complete clearing compared with the dual-combination therapy groups (4.6%) at the end

of eight weeks (32). Researchers observed complete or nearly complete clearing of hyperpigmentation in 77% of the aggregate Triluma group compared with 42.2% for fluocinolone acetonide, 27.3% for tretinoin and fluocinolone acetonide, and 46.8% for tretinoin and hydroquinone. Side effects associated with Triluma were transient and mild. The most frequently occurring adverse effects included erythema at the application site, desquamation, burning, xerosis, and pruritus.

Some authors have cautioned against the use of hydroquinone in high concentrations because of its association with inducing ochronosis. Nevertheless, hydroquinone is the most effective topically applied hypopigmenting agent approved by the United States Food and Drug Administration for melasma treatment (22). No ochronosis events were observed among patients on any of the treatment regimens containing hydroquinone (4.0%).

The use of topical corticosteroids as therapy for melasma has also been discouraged by some authors because of the association with skin atrophy and telangiectasia (33). In fact, protracted use of potent topical corticosteroids is known to engender cutaneous atrophy. When steroids are used in combination with retinoids, however, the incidence and depth of atrophy appear to be reduced. In fact, the combination of tretinoin application with corticosteroid has been shown to ameliorate the epidermal atrophy induced by the topical corticosteroid while not reducing its activity (34). The combination of a corticosteroid with a retinoid is believed to reduce the risk of steroid-induced atrophy (30). The data from this study seem to support this fact because only one patient, in the dual-therapy group hydroquinone and fluocinolone acetonide, exhibited skin atrophy. This patient did not receive tretinoin. A 12-week open label long term safety study showed a similar safety profile as the previously described eight-week study (32).

The results of the two related studies suggest that the use of this triple-combination agent may be more effective than any of the dual-combination agents in counteracting or inhibiting the pathophysiologic mechanism of melasma. Triluma combines three well-established agents in a formulation that appears to be effective and safe in the treatment of melasma. This triple-combination topical therapy also shows favorable tolerability and represents a significant advance in the dermatologic armamentarium for melasma.

The concept of such a triple combination is also supported by another recent study. Researchers evaluated the efficacy of a formula containing 0.1% tretinoin, 5% hydroquinone, and 1% hydrocortisone in 25 Korean female patients with melasma recalcitrant to therapy. Patients were evaluated before treatment, then instructed to apply hydrocortisone on their faces for four months and were also assessed four weeks and four months after treatment. Overall, investigators reported statistically significant depigmentation in clinical and histological studies and increased subepidermal collagen synthesis, results that were observed as early as four weeks after hydrocortisone treatment (35).

SUMMARY

Many types of skin are susceptible to pigmentation disorders. Such conditions can appear especially prominent in people with dark skin. Traditionally, disorders of pigmentation have been recalcitrant to treatment, frustrating patients and physicians alike. Combination therapy, including prolonged use of topical agents, sun avoidance, and, often, in-office chemical peels, has been the mainstay. Laser treatments have been of limited success. A new topical combination therapy, Triluma, has

shown great promise, though, in simplifying and improving treatment for these intractable disorders, particularly melasma. In addition, this new combination therapy is effective, tolerable, and easy to use.

Financial conflict statement: Dr. Baumann was an investigator for Galderma in several clinical trials.

REFERENCES

1. Barankin B, Silver SG, Carruthers A. The skin in pregnancy. J Cutan Med Surg 2002; 6:236–240.
2. Sanchez NP, Pathak MA, Sato S, Fitzpatrick TB, Sanchez JL, Mihm MC Jr. Melasma: a clinical, light microscopic, ultrastructural, and immunofluorescence study. J Am Acad Dermatol 1981; 4:698–710.
3. Baumann L. Cosmetic Dermatology: Principles and Practice. New York, NY: McGraw-Hill, 2002:63–69.
4. Freedberg IM, Eisen AZ, Wolff K, Austen KF, Goldsmith LA, Katz S. eds. Fitzpatrick's Dermatology in General Medicine. 5th ed. New York: McGraw-Hill, 1999:996.
5. Mandry Pagan R, Sanchez JL. Mandibular melasma. P R Health Sci J 2000; 19:231.
6. Baran R, Maibach HI. Textbook of Cosmetic Dermatology. 2nd ed. London: Dunitz Martin Ltd., 1998:396–397.
7. Arnold HL, Odom RB, James WD, eds. Andrews' Diseases of the Skin: Clinical Dermatology. 8th ed. Philadelphia: W.B. Saunders, 1990:991–994.
8. Ibid.
9. Champion RH, Burton JL, Ebling FJG, eds. Rook, Wilkinson, Ebling: Textbook of Dermatology. 5th ed. London: Blackwell Science, 1992:1596–1597.
10. Personal communication.
11. Personal communication.
12. Mosher DB, Fitzpatrick TB, Ortonne J-P, et al. Hypomelanoses and hypermelanoses. In: Freedberg IM, Eisen AZ, Wolff K, et al., eds. Fitzpatrick's Dermatology in General Medicine. Vol. 1. New York, NY: McGraw-Hill, 1999:945–1017.
13. Ibid.
14. Hodgson C. Senile lentigo. Arch Dermatol 1963; 87:197.
15. Kligman AM, Willis I. A new formula for depigmenting human skin. Arch Dermatol 1975; 111:40.
16. Burns RL, Prevost-Blank PL, Lawry MA, Lawry TB, Faria DT, Fivenson DP. Glycolic acid peels for postinflammatory hyperpigmentation in black patients. Dermatol Surg 1997; 23:171.
17. Grimes PE, Stockton T. Pigmentary disorders in blacks. Dermatol Clin 1988; 6:271.
18. Ruiz-Maldonado R, Orozco-Covarrubias ML. Postinflammatory hypopigmentation and hyperpigmentation. Semin Cutan Med Surg 1997; 16:36.
19. Bulengo-Ransby SM, Griffiths CE, Kimbrough-Green CK, Finkel LJ, Hamilton TA, Ellis CN, Voorhees JJ. Topical tretinoin (retinoic acid) therapy for hyperpigmented lesions caused by inflammation of the skin in black patients. N Engl J Med 1993; 328:1438.
20. Fairley JA. Tretinoin (retinoic acid) revisited. N Engl J Med 1993; 328:1486.
21. Pandya AG, Guevara IL. Disorders of hyperpigmentation. Dermatol Clin 2000; 18:91.
22. Grimes PE. Melasma. Etiologic and therapeutic considerations. Arch Dermatol 1995; 131(12):1453–1457.
23. Griffiths CE, Finkel LJ, Ditre CM, Hamilton TA, Ellis CN, Voorhees JJ. Topical tretinoin (retinoic acid) improves melasma. A vehicle-controlled, clinical trial. Br J Dermatol 1993; 129:415.
24. Kimbrough-Green CK, Griffiths CE, Finkel LJ, Hamilton TA, Bulengo-Ransby SM, Ellis CN, Voorhees JJ. Topical retinoic acid (tretinoin) for melasma in black patients. A vehicle-controlled clinical trial. Arch Dermatol 1994; 130:727.

25. Personal communication.
26. Lim JT, Tham SN. Glycolic acid peels in the treatment of melasma among Asian women. Dermatol Surg 1997; 23:177.
27. Guevara IL, Pandya AG. Safety and efficacy of 4% hydroquinone combined with 10% glycolic acid, antioxidants, and sunscreen in the treatment of melasma. Int J Dermatol 2003; 42(12):966–972.
28. Lawrence N, Cox SE, Brody HJ. Treatment of melasma with Jessner's solution versus glycolic acid: a comparison of clinical efficacy and evaluation of the predictive ability of Wood's light examination. J Am Acad Dermatol 1997; 36:589.
29. Lim JT. Treatment of melasma using kojic acid in a gel containing hydroquinone and glycolic acid. Dermatol Surg 1999; 25:282.
30. Kligman LH, Schwartz E, Lesnik RH, Mezick JA. Topical tretinoin prevents corticosteroid-induced atrophy without lessening the anti-inflammatory effect. Curr Probl Dermatol 1993; 21:79–88.
31. McMichael AJ, Griffiths CE, Talwar HS, Finkel LJ, Rafal ES, Hamilton TA, Voorhees JJ. Concurrent application of tretinoin (retinoic acid) partially protects against corticosteroid-induced epidermal atrophy. Br J Dermatol 1996; 135:60–64.
32. Taylor SC, Torok H, Jones T, Lowe N, Rich P, Tschen E, Menter A, Baumann L, Wieder JJ, Jarratt MM, Pariser D, Martin D, Weiss J, Shavin J, Ramirez N. Efficacy and safety of a new triple-combination agent for the treatment of facial melasma. Cutis 2003; 72:67–72.
33. Giannotti B, Melli MC. Current approaches to the treatment of melasma. Clin Drug Invest 1995; 10(suppl 2):57–64.
34. Personal communication.
35. Kang WH, Chun SC, Lee S. Intermittent therapy for melasma in Asian patients with combined topical agents (retinoic acid, hydroquinone and hydrocortisone): clinical and histological studies. J Dermatol 1998; 25(9):587–596.

26
Alpha Hydroxy Acids

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Hydroxy acids represent a fascinating group of substances, which belong to the category of cosmeceuticals. In the past two decades they have been widely used into a variety of skin care products for moisturizing, keratolytic and antiaging effects. The family of hydroxy acids include α-hydroxy acids (AHAs), β-hydroxy acids, combination hydroxy acids, and polyhydroxy acids. AHAs are organic carboxylic acids characterized by a hydroxy group in the alpha position. They are hydrophilic because of their aliphatic and linear structure. On the basis of the number of hydroxy group, AHAs can be divided into three subcategories: monocarboxylic acids (glycolic acid), dicarboxylic acids (malic acid), and tricarboxylic acids (citric acid) (1).

Glycolic acid (GA), which is the smallest AHA, and lactic acid (LA) are the most frequently used. GA contains two carbons (H2C(OH)COOH); LA contains three carbons and its keto form, pyruvic acid, has recently shown to be particularly effective in the treatment of skin aging and acne (2). Malic acid and tartaric acid consist of four carbon chains, while citric and gluconic acids have six carbon chains (3).

AHAs are naturally found in a variety of species including foods and plants (citric, malic, tartaric, glycolic, etc.), animals (cells and body fluids), and microorganisms such as bacteria, fungi, viruses, and algae. AHAs are involved in many metabolic processes and participate in essential cellular pathways such as Krebs cycle, glycolysis, and serine biosynthesis.

In epidermis, AHAs have been reported to decrease stratum corneum cohesiveness, to increase thickness of the viable epidermis and deposition of hyaluronic acid; they also induce a reversal of basal cell atypia and a restoration of rete ridges to a more undulating pattern (4–6). AHAs also increase the number and secretion of lamellar bodies which are basic for barrier function in the epidermis (7). At higher concentrations AHAs induce epidermolysis.

AHAs reduce corneocyte cohesion by influencing ionic bonds via three mechanisms: the distance between charges, the number of charges, and the medium between charges. When the stratum corneum becomes hydrated the distance between corneocytes is increased, and therefore cohesion is decreased. Another mechanism involved is the AHA-induced enzymatic inhibition of the reactions of sulfate transferase, phosphotransferase, and kinases which leads to fewer electronegative sulfate

and phosphate groups on the outer wall of corneocytes resulting in diminishment of cohesion forces (8).

AHAs probably stimulate epidermal proliferation by improving energy and redox status of keratinocytes.

In the dermis, the structural changes are characterized by increased deposition of mucopolysaccharides, collagen, hyaluronic acid, chondroitin sulfate, and elastic fibers whose quality is also improved (9).

Although the cosmetic and therapeutic benefits of AHAs are well characterized, little is known about their exact biological mechanism of action, which is still partially unclear.

Collagen is known to be responsible for both tensile strength and elasticity of the skin. GA has been reported to have stimulatory effects on collagen production and fibroblast proliferation both in vivo and in vitro. Because keratinocytes are the major type of skin cell, it has been hypothesized that the effects of topical GA could be mediated by keratinocytes, through the induction of cytokines' release. The effect of GA on the dermal matrix metabolism of keratinocytes and fibroblasts has been investigated in a recent study. Okano et al. have shown that GA acts on the fibroblasts both directly and indirectly through keratinocyte-released cytokines (10). They demonstrated that fibroblasts cultured with keratinocyte-conditioned media have an increased mRNA expression of matrix metalloproteinases MMP-1 and MMP-3 which are the most important protein involved in matrix degradation. In addition, they have shown that GA treatment elicits a strong expression of IL-1α in keratinocytes. IL-1α is known to play a role in the induction of MMP-1 in fibroblasts. Rakic et al. have previously found that fibroblasts cultured with the medium conditioned by keratinocytes were treated with the GA release IL-6 (11). Furthermore, it is known that IL-1α released from keratinocytes could induce dermal fibroblasts to produce IL-6. Taken together, these data suggest that IL-1α and IL-6 might stimulate MMP-1 production, resulting in a dermal remodeling. In summary, on one side GA directly promotes collagen synthesis by fibroblasts, on the other side it stimulates matrix degradation mediated by a cytokine network derived from keratinocytes.

Topical AHAs also modulate the production of other important cytokines derived from keratinocytes, such as vascular endothelial growth factor (VEGF) and angiogenin (ANG). Keratinocytes strongly increase the release of VEGF after treatment with AHAs, and this is owing to de novo synthesis. Rendl et al. demonstrated for the first time that keratinocytes produce ANG; in contrast to VEGF, secretion of ANG is decreased after topical application of AHAs (12). VEGF exerts many effects on microvasculature as it promotes the formation of new blood vessels and helps the survival of endothelial cells. The beneficial effect of AHAs treatment on the alterations of cutaneous vessels observed in photoaging is therefore probably mediated by VEGF produced by keratinocytes. Whereas the biological relevance of ANG production by keratinocytes and its downregulation caused by AHAs application remain to be clarified.

Although the mechanism by which AHAs clinically improve photoaging and intrinsic skin aging has not yet been elucidated, these recent findings bring new tools for further investigations.

Clinical studies have indicated that AHAs are not only effective in improving photodamaged skin but also in treating pigmentary lesions such as melasma, solar lentigines, and postinflammatory hyperpigmentations. The mechanism of this effect is not only because of rapid pigment dispersion but also as a consequence of epidermal

remodeling and accelerated desquamation. Recently Usuki et al. found that GA and LA directly inhibit tyrosinase activity (13).

Because cosmetic products containing AHA are widely used, usually for a long time and patients have anecdotally reported an increase in sensitivity to UV light during the use of AHA-containing products, many studies have been performed to assess whether topical GA can enhance photodamage. It has been recently found that GA makes the skin more sensitive to ultraviolet light, with a lowered minimal erythema dose and enhanced formation of sunburn cells; this is reversed within a week after discontinuing the use of AHA-containing products (14). Little is known, however, about the effect of topical AHAs on UV-induced skin tumorigenesis. In sharp contrast, GA has shown to be protective against UV-induced skin cancer development (15). Applied immediately after UV irradiation, GA induces a 20% reduction of skin tumor incidence, a 55% reduction of tumor multiplicity, and a 47% decrease in the number of cancers larger than 2 mm. This inhibitory action is accompanied by a decreased expression of some UV-induced proteins which regulate the cell cycle such as proliferating cell nuclear antigen, cyclin D1, cyclin E, and cyclin dependent kinase 2 and 4 (cdk2 and cdk4). Furthermore, the activation of transcription factors AP-1 and NF-κB is inhibited. GA also inhibits the UVB-induced expression of c-fos and reduces mRNA levels of apoptosis-regulatory genes p53 and p21 (16). These results suggest that GA may play an important inhibitory role on UV-induced tumorigenesis.

Owing to the above-mentioned mechanisms, the clinical effects of AHAs include improvement of skin firmness and elasticity, reduction of wrinkles and hyperpigmentations, decreased alterations of cutaneous vessels, enhanced photosensitivity but protection against UV-induced skin cancer development.

Optimization of the formulation allows improvement of efficacy: hydroxy acids perform better at low pH; therefore pH is of great importance for achieving good therapeutical results. The suggested range is between 3.0 and 5.0, but lower pH values seem to be also very effective. The acidity depends partly on the concentration of the hydroxy acid, and it is determined by electrostatic, inductive and steric effects, and hydrogen bonding. Besides hydroxy acid concentration, free acid content is another important factor for the development of a good product. At pH 3 there is a greater absorption through the skin than at pH 7; as at pH 3 the concentration of free acid is higher. To raise the proper pH and free acid content, the preparation can be either neutralized with an inorganic alkali or organic base or buffered to obtain a formulation resistant to pH changes. When hydroxy acids are applied on the skin the pH of the stratum corneum changes, leading to changes in both epidermis and dermis. The higher the AHA concentration the deeper is the effect of the preparation (1). Chronic treatment with low pH formula is likely to induce changes in the pH of living epidermis. Several enzymes (phosphatases, lipases, transforming growth factor β, etc.) have maximum activity at pH 5 or lower, and it is possible that an acid environment may activate these mechanisms.

Topical retinoids, either occurring naturally such as vitamin A (all-*trans* retinol) and its metabolites (tretinoin) or synthetically such as adapalene and tazarotene, are well known and are accepted agents for treatment of aged and photoaged skin. Retinoids' long-term application shows benefits similar to those assessed with AHAs; they have been reported to induce dermal deposition of reticulin fibers and to promote angiogenesis in the papillary dermis. Retinoids bind specific nuclear receptors, which belong to the steroid/thyroid superfamily of ligand-specific nuclear transcription factors. There are two types of retinoid receptors both present on

human skin (keratinocytes and dermal fibroblasts): retinoic acid receptors α, β, and γ which bind tretinoin and 9-*cis* retinoic acid and retinoid X receptors α, β, and γ which bind 9-*cis* retinoic acid. The binding between retinoid and its nuclear receptor leads to gene transcription and induction of mRNA for cellular retinoic acid binding protein (CRABP)-II (17). Retinoids have several side effects including photosensitivity, teratogenicity, and so-called "retinoid dermatitis" characterized by erythema, scaling and itch at the site of application. Furthermore, from a cosmetic point of view, clinical improvement takes several months to become evident (18). AHAs are generally safer, less irritant, and provide cosmetic results in a shorter time.

During the last decade, AHAs have been used for the management of some pathologic skin conditions characterized by hyperkeratosis such as xeroderma, ichthyosis, and psoriasis. Vignoli et al. (19) showed a reduction in psoriasis severity after treatment with GA as measured by visual scoring and noninvasive instruments (Table 1): in this study, a significant improvement of transepidermal water loss (TEWL), erythema (a^* value), and cutaneous blood flow (CBF) occurred after treatment with either 15% GA or 0.05% betamethasone. No significant differences appear in TEWL and erythema between GA and betamethasone; on the other hand, a significantly decreased CBF is recorded in the sites treated with betamethasone confirming the higher effect of corticosteroid compounds in terms of vasoconstriction and reduction of inflammation.

More recently, Kostarelos et al. (20) have performed a clinical study demonstrating the synergistic action between AHA (10%) and betamethasone (0.1%) against scalp psoriasis. They have observed for the first time that scalp lotion combining AHA and betamethasone was more effective compared to betamethasone alone, furthermore lesions healed in a shorter time. These data suggest that by combining AHAs and steroids, the therapeutic effect can be reached decreasing considerably the dose of the latter, with also fewer side effects.

Prolonged treatment with AHAs can also lead to stratum corneum barrier fortification and increased resistance to chemical irritation; sodium lauryl sulfate irritation has been shown to be reduced in AHAs treated sites. It has been observed that AHAs can modulate stratum corneum barrier function and prevent skin irritation (21); the effect is not equal for all AHAs, being more marked for the molecules characterized by antioxidant properties (Fig. 1). This effect has been shown by other

Table 1 Mean Values (±SE) of CBF (Perfusion Units), TEWL (g m^2/h) and Erythema (a^* value). Significant Differences in CBF are Recorded Between GA and Betamethasone Treated Sites During the Study (17). No Significant Differences Appear Concerning TEWL and Erythema. All Treatments Induced a Significant Decrease of the Parameters Investigated During the Study (TEWL, $P < 0.01$ Glycolic, $P < 0.005$ Betamethasone; CBF, Glycolic $P < 0.001$, Betamethasone $P < 0.0001$; Erythema, Glycolic $P < 0.01$, Betamethasone $P < 0.009$).

	CBF		TEWL		Erythema	
	Glycolic	Betameth.	Glycolic	Betameth.	Glycolic	Betameth.
Baseline	109.9 ± 14.9	101.9 ± 12.7	19.6 ± 3.4	18.5 ± 3.7	17.1 ± 1.0	17.7 ± 0.9
Day 5	78.3 ± 9.9*	52.6 ± 7.5	11.1 ± 1.5	10.8 ± 1.6	15.9 ± 0.7	16.3 ± 0.8
Day 10	82.1 ± 13.9*	38.4 ± 5.4	12.2 ± 1.6	8.8 ± 1.7	16.9 ± 1.1	15.2 ± 0.9
Day 15	57.6 ± 6.5*	35.3 ± 8.6	9.6 ± 1.6	8.6 ± 2.3	14.8 ± 0.8	14.5 ± 0.8

Abbreviations: CBF, cutaneous blood flow; TEWL, transepidermal water loss; GA, glycolic acid.

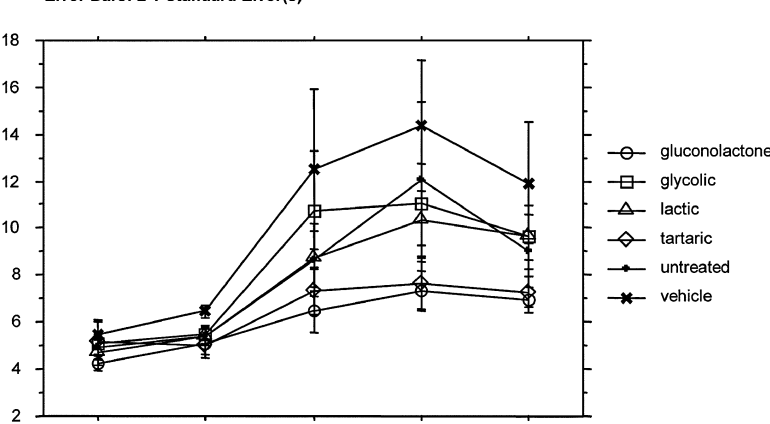

Figure 1 Transepidermal water loss (±SE) after SLS challenge (g/m^2h). Lower barrier damage is detected in AHA treated sites compared to vehicle and untreated areas ($p < 0.006$). Gluconolactone is significantly lower than GA at each time point (hour 0 = $p < 0.01$, hour 24 = $p < 0.03$, hour 48 = $p < 0.04$) and than LA at hour 48 ($p < 0.04$). *Abbreviations*: SLS, sodium lauryl sulfate; AHA, α-hydroxy acids; GA, glycolic acid; LA, lactic acid. *Source*: From Ref. 21.

keratolytic compounds such as urea (22) and can be related to the increased production of stratum corneum lipids such as ceramides induced by treatment (23).

Over the years a number of cosmetic or dermatologic compounds have gained attention for the ability to treat skin disorders and in particularly skin aging. AHAs are certainly the most intriguing class of cosmeceuticals, widely incorporated in products for skin care, and they have assumed an important place in the therapeutic armamentarium of dermatologists. Even though many aspects of their mechanism of action have been clarified, many others are still far from being elucidated, and much work remains to be done. The future seems promising for these simple molecules.

REFERENCES

1. Draelos ZD. α-Hydroxy acids, β- hydroxy acid, and other topical agents. Dermatol Therapy 2000; 13:154–158.
2. Ghersetich I et al. Pyruvic acid peels for treatment of photoaging. Dermatol Surg 2004; 30:32–36.
3. Van Scott E, Yu RJ. Alpha hydroxy acids: therapeutic potentials. Canadian Dermatol 1989; 1:108–112.
4. Sams RL II et al. Basal cell proliferation in female SKH-1 mice treated with alpha- and beta-hydroxy acids. Toxicol Appl Pharmacol 2001; 175(1):76–82.
5. Bernstein EF et al. Glycolic acid treatment increases type I collagen mRNA and hyaluronic acid content of human skin. Dermatol Surg 2001; 27(5):429–433.
6. Hool HL et al. The effects of an alpha hydroxy acid (glycolic acid) on hairless guinea pig skin permeability. Food Chem Toxicol 1999; 37(11):1105–1111.
7. Kim TH et al. The effects of topical alpha-hydroxy acids on the normal skin barrier of hairless mice. Br J Dermatol 2001; 144(2):267–273.

8. Van Scott E, Yu RJ. Hyperkeratinization, corneocyte cohesion and alpha hydroxy acids. J Am Acad Dermatol 1984; 11:867–879.
9. Sang EM et al. The effect of glycolic acid on photoaged albino hairless mouse skin. Dermatol Surg 1999; 25:179–182.
10. Okano Y et al. Biological effects of glycolic acid on dermal matrix metabolism mediated by dermal fibroblasts and epidermal keratinocytes. Exp Dermatol 2003; 12(2):57–63.
11. Rakic L, Nusgens BV. Comparing caustic and biological activity of trichloroacetic and glycolic acids on keratinocytes and fibroblasts in vivo. Skin Pharmacol Appl Skin Physiol 2000:52–59.
12. Rendl M et al. Topically applied lactic acid increases spontaneous secretion of vascular endothelial growth factor by human reconstructed epidermis. Br J Dermatol 2001; 145:3–9.
13. Usuki A et al. The inhibitory effect of glycolic acid and lactic acid on melanin synthesis in melanoma cells. Exp Dermatol 2003; 12(2):43–50.
14. Kays K et al. Topical glycolic acid enhances photodamage by ultraviolet light. Photodermatol Photoimmunol Photomed 2003; 19:21–27.
15. Hong JT et al. Inhibitory effect of glycolic acid on ultraviolet-induced skin tumorigenesis in SKH-1 hairless mice and its mechanism of action. Mol Carcinog 2001; 31(3):152–160.
16. Ahn KS et al. Inhibitory effect of glycolic acid on ultraviolet B-induced c-fos expression, AP-1 activation and p53-p21 response in a human keratinocyte cell line. Cancer Lett 2002; 186(2):125–135.
17. Griffiths CEM. The role of retinoids in the prevention and repair of aged and photoaged skin. Clin Exp Dermatol 2001; 26:613–618.
18. Hermitte R. Aged skin, retinoids and alpha hydroxy acids. Cosmet Toilet 1992; 107:63–67.
19. Vignoli GP et al. Effects of glycolic acid on psoriasis. Clin Exp Dermatol 1998; 23:190–191.
20. Kostarelos K et al. Double-blind clinical study reveals synergistic action between alpha-hydroxy acid and betamethasone towards topical treatment of scalp psoriasis. JEADV 2000; 14:5–9.
21. Berardesca E et al. Alpha hydroxy acids modulate stratum corneum barrier function. Br J Dermatol 1997; 137:934–938.
22. Loden M. Urea-containing moisturizers influence barrier properties of normal skin. Arch Dermatol Res 1996; 288:103–107.
23. Rawlings AV et al. Effect of lactic acid isomers on keratinocyte ceramide synthesis, stratum corneum lipid levels and stratum corneum barrier function. Arch Dermatol Res 1996; 288:382–390.

27
Surfactants

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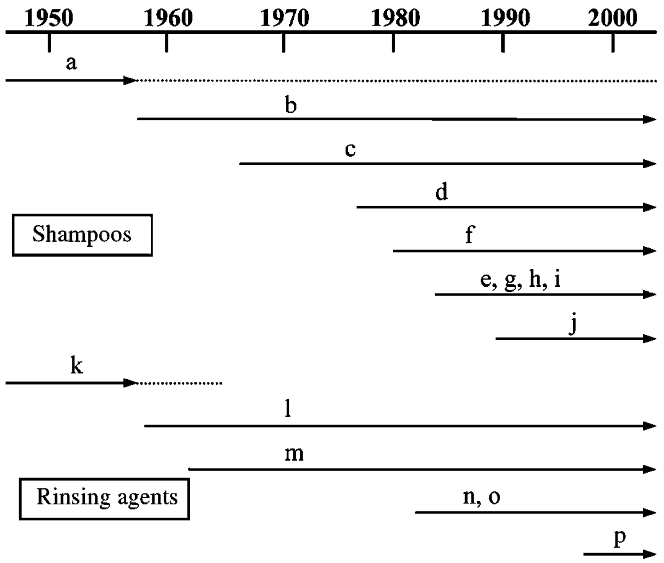
SOLUTION PROPERTIES OF SURFACTANTS

Surfactants for cosmetic use may be grouped into the following six categories: cleaning agents, emulsifying agents, foam boosters, hydrotropes, solubilizing agents, and suspending agents (1). Most cosmetic products are formulated through use of these surfactants as main ingredients. This section briefly surveys major surfactants for shampoos and rinses presently on the Japanese market. Basic solution properties of surfactants are then discussed.

Anionic Surfactants

Soaps for detergent have been in use since 3000 BC. Primary detergents in shampoos before the 1950s were mainly potassium or ammonium salts of fatty acids. These soaps have good foaming performance in pure water, though only slightly so in hard water owing to the formation of insoluble metal soaps (2). Various synthetic surfactants have been developed during the past 50 years. They have come to replace soaps and are soluble even in hard water. The most common synthetic surfactants are alkyl sulfate (AS) and alkyl ether sulfate (AES). These initially appeared on the U.S. market more than 50 years ago, and liquid shampoos subsequently came to use throughout the country in the 1960s. Ammonium or ethanolamine salts of AS and sodium or ammonium salts of AES were used on a particularly large scale for the preparation of many products. Through use of ethylene oxide (EO) groups, AS increases solubility and foam volume and reduces precipitate of Ca salt. Increase in solution viscosity is essential for enhancing the appeal of shampoos to customers. Alkanol amides of fatty acids are effective for viscosity and foam enhancement. Alphaolefin sulfonate (AOS) is commonly used as an anionic surfactant in shampoos (3). A surfactant is a mixture of hydroxyalkane and alkene sulfonetes whose structures are shown in Figure 1. AOS exhibits excellent stability at low pH compared with AS or AES and is more soluble in hard water than AS. Increase in solution viscosity has been shown possible through the use of alkanol amides and anionic surfactants in combination.

Various surfactants as supporting ingredients are used in the absence of complete functional performances. Alkyl sulfosuccinates exhibit excellent foaming capacity,



a: Carboxylic acid	R-COO-X
b: Alkyl sulfate	$R\text{-}OSO_3\text{-}X$
c: Alkyl ether sulfate	$R\text{-}O(C_2H_4O)_nSO_3\text{-}X$
d: ••-Olefin Sulfonate	$R\text{-}CH_2{=}CHCH_2SO_3\text{-}X$
	$R\text{-}CH_2CH(OH)CH_2CH_2SO_3\text{-}X$
e: Sulfosuccinate	$R\text{-}NHCOCH(CH_2COO\text{-}X)SO_3\text{-}X$
f: N-Acyl glutamate	$RCONHCH(C_2H_4COO\text{-}X)COO\text{-}X$
g: N-Acyl-••-alaninate	$RCON(H \text{ or } CH_3)C_2H_4COO\text{-}X$
h: N-Acyl methyl taurate	$RCON(CH_3)C_2H_4SO_3\text{-}X$
i: Alkylpolyglycoside	$RO\ \{Glucose\}_nH$
j: Acyl amidopropyl betain	$RCONHC_3H_6N(CH_3)_2CH_2COO$
k: Citric acid	$HOOCCH_2C(OH)(COOH)CH_2COOH$
l: di-Alkyl dimethylammonium salt	$R(R')N(CH_3)_2\text{-}X$
m: n-Alkyl trimethylammonium salt	$RN(CH_3)_3\text{-}X$
n: g-Alkyl trimethylammonium salt	$R(R')\ CHCH_2N(CH_3)_3\text{-}X$
o: N-Acyl Arginine ethyl ether	$RCONHCH(COOC_2H_5)C_3H_6NHC(NH_2){=}NH$
p: N-Acyl Amidobutyl guanidium salt	$RCONHC_4H_8NHC(NH_2){=}NH$

Figure 1 Surfactants for shampoos and rinsing agents on the Japanese market.

and their use is attended with low skin irritation provided AS is present (3). In the 1980s, surfactants with low skin irritation came into popularity. Several amino acids have been developed for surfactant use, such as acyl glutamate (4). These have excellent foaming, good biodegradability, and low skin irritation. Acyl amino acids such as lauroyl β-alaninate (5) and the N-methyl β-alaninate (6) are presently in use. N-acyl methyltaurate (7) is also available and has been proven ideal for shampoo use with low skin irritation.

Nonionic and Amphoteric Surfactants

Nonionic surfactants are preferable to those that are anionic, but have found limited use owing to poor foaming capacity for shampoos. Alkanol amides and alkyl amine-oxides are used primarily as foam boosters and stabilizers (3). Alkyl glucoside may be obtained through reaction of fatty alcohol with glucose; it is mild to the skin and has good foam stability (8).

Amphoteric surfactants are used in combination with anionic and nonionic surfactants to achieve greater shampoo mildness. A typical amphoteric surfactant is N-acyl amidopropyl betaine (3) featured by low skin irritation and foaming enhancement. Alkyl iminodiacetates may be obtained from fatty amines as mild surfactants (9). The cocoylarginine ethyl ester (CAE) is prepared from arginine and shows high affinity to hair (10,11). A new mild amphoteric surfactant, Amisafe, is derived from arginine (12) and functions as a cationic surfactant at weakly acidic pH and is readily adsorbed onto hair.

Cationic Surfactants

Because of the negative charge on the surface of hair, cationics strongly bind to hair and are difficult to remove by rinsing. When a shampoo containing soap has been used, acidic rinse containing citric acid may be applied to remove the alkali and metal soaps. Dialkyl ammonium salts are used in rinse formulations for shampoos containing AS and AES as main ingredients (13). Quaternary ammonium salts containing mono- or dialkyl groups with 16 to 22 carbon atoms are presently in wide use. At the start of the 1980s, a milky lotion-type rinse came into prominent use. Adding oils to a gel comprising cationic surfactant, fatty alcohol, and water produced it. Novel cationic surfactants are presently being produced. Quaternary ammonium salts are made using long-chain Guarbet alcohol form lamellae liquid crystals even in cold water and are readily adsorbed onto hair (14). Amido guanidine cationic surfactants (AG) with methylene groups as spacers between amide and guanidino groups (15) are available, and there is a hair conditioner containing AG with excellent moisturizing properties even at low humidity.

Micelle Formation and Surfactant Solubility

The high solubility of surfactants in water is very important in the preparation of cosmetic products. Surfactants show characteristic solubility because of the presence of hydrophobic groups, which squeeze out the hydrocarbon chains of surfactants to bring about micelle formation (16). A phase diagram of the two-component system is shown in Figure 2 (17). At dilute surfactant concentrations, micelle formation occurs above a critical temperature and at surfactant concentration above the critical micelle concentration. In region I, surfactant concentration is too low for micelle aggregation to occur, and consequently the surfactants dissolve into monomers. In region II, surfactant micelles are equilibrated with monomers. In region III, surfactant monomers are present along with precipitated hydrated solid surfactants. That is, the micelles comprise melting hydrated solid surfactants beyond the phase boundary curve between regions II and III. The point where the two phase boundary curves intersect is the Krafft point of a surfactant solution.

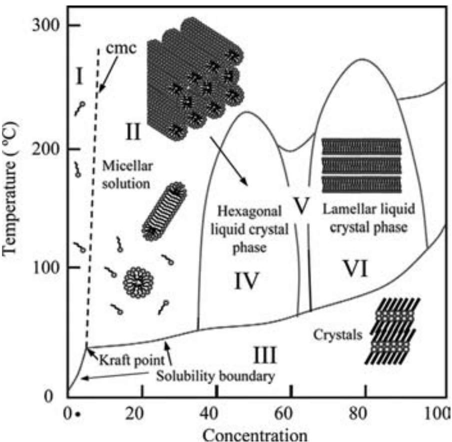


Figure 2 Schematic phase diagram of an ionic surfactant. *Source*: From Ref. 17.

Liquid Crystals and Gels

Various intermediate phases may exist between solid and liquid states. At high surfactant concentration in Figure 2, several liquid crystalline phases can be seen to have formed. The liquid crystalline phases of surfactant–water systems are in the liquid state with a long-range repulsive order of 1, 2, or 3 (18,19). With increase in surfactant concentration, the hexagonal (IV), cubic liquid crystalline (V), and lamellae phase (VI) are produced. The hexagonal phase consists of long rod micelles of surfactants hexagonally arranged. The lamellae phase comprises surfactant bilayers separated by water layers. The water layers vary in thickness from 10 Å to several 100 Å. The hexagonal and lamellae phases are optically anisotropic, whereas the cubic liquid crystalline phase is optically isotropic. The cubic phase may take on various structures such as packed spherical micelles in a cubic array, surfactant rods connected in a complex manner to form a continuous network, and bicontinuous networks with positive and negative curvature interfaces (19,20).

In liquid crystalline phases, hydrocarbon chains are in a liquid-like state. When these phases are cooled, a coagel phase consisting of hydrated crystals and a gel phase are formed as shown in Figure 3 (21,22). The gel phase contains fairly ordered intermediate water, except for hydrated water, between surfactant bilayers. This phase is produced on warming the coagel phase when hydration interaction occurs between counter ions. Phase diagrams for octadecyltrimethyl ammonium salts show the stability of the gel phase.

Phase Behavior of Nonionic Surfactants

Increase in nonionic surfactant aqueous solution temperature causes the development of two isotropic phases in solution, above what is called the cloud point. The hydrophilic/hydrophobic balance of a nonionic surfactant may differ considerably at this temperature, and consequently there is a characteristic phase behavior in nonionics/hydrocarbon/water ternary systems, as is the case when using a plane of fixed 1:1 weight ratio of oil to water, as shown in Figure 4 (23). At lower temperatures, nonionic surfactants are highly soluble in water and form O/W microemulsions in a water-rich phase with excess oil. At higher temperatures, they are highly

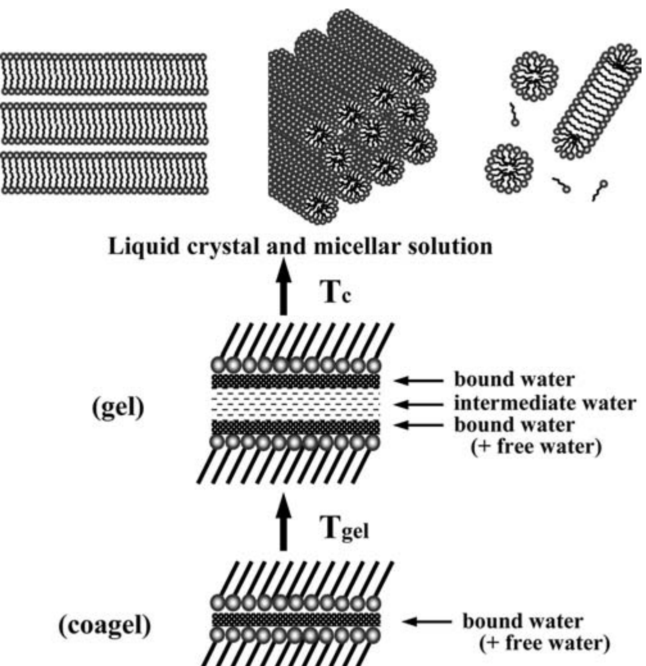


Figure 3 Change in the aggregation of surfactants and water molecules in response to increase in temperatures. *Source*: From Ref. 21.

soluble in oil and form W/O microemulsions in an oil-rich phase with excess water. At the phase inversion temperature, a three-phase system comprises a middle phase microemulsion, a nearly pure water phase, and an oil phase. Phase transition with temperature is an indication of potential for cosmetic use.

FOAMING PROPERTIES OF SURFACTANTS

Foaming is an essential property of shampoos, skin cleansers, aerosols, shaving cream, mouthwash, and toothpaste, and mechanisms of foam formation and stabilization

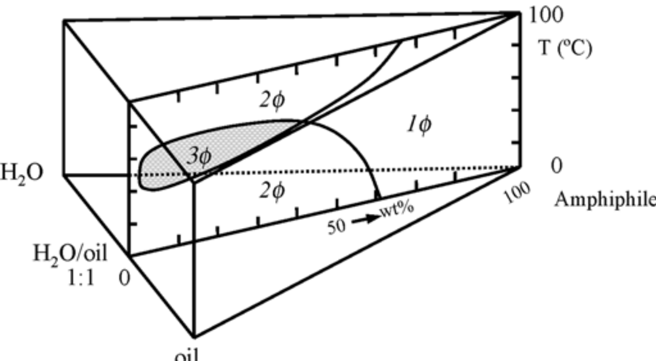


Figure 4 Vertical section of the phase prism of a ternary system for H_2O/oil = 1/1. *Source*: From Ref. 23.

(breakdown) have been studied (24–26). Persistence of bubble collapse is enhanced by the following (27): (1) high viscosity in the liquid phase to retard hydrodynamic drainage; (2) high surface viscosity to retard liquid loss between interfaces; (3) surface effects to prevent thinning of liquid film, such as the Gibbs–Marangoni effect; (4) electrostatic and steric repulsion between adjacent interfaces to prevent drainage caused by disjoining pressure and (5) gas diffusion from smaller to larger bubbles.

Methods for Foaming Assessment

Foam is a dispersion of gas bubbles in a liquid, and the liquid film of each bubble is colloidal in size. Surfactant solutions often have the important feature of foaminess. This property may be defined as foam volume produced from a unit volume of solution and may be evaluated based on pressure or temperature and the particular method of formation (28,29). Standard methods of formation are listed in Table 1. According to the principle of foam generation, foaming methods have been classified into two groups: static and dynamic. Foaminess in this study was evaluated based on foam volume and lifetime. These factors are difficult to assess independently by conventional methods. Because of the complexity of the foam system, better methods are being sought.

Dynamic Surface Tension

Surface elasticity is a major factor determining thin liquid film stability (24). Foam contains many bubbles separated by liquid films that are continuously enforced by dynamic change in the liquid, such as liquid drainage and bubble motion. In the case of surfactant-stabilized aqueous film, stretching causes local decrease in the surface concentration of the adsorbed surfactant. This decrease causes an increase in the local surface tension (the Gibbs elasticity), which acts in opposition to the original stretching force. In time, the original surface concentration of the surfactant is restored. This time-dependent restoring force in thin liquid film is referred to as the Marangoni effect. Dynamic adsorption at the gas/liquid interface must thus be considered in the assessment of foam stability. Although there are various techniques for measuring dynamic surface tension (30), the maximum bubble pressure method is mostly used for this measurement to monitor foam generation on a short time scale.

A typical curve of dynamic surface tension shows induction, rapid fall, mesoequilibrium, and equilibrium (31). The first three regions are important in high-speed dynamics. Data for surface tension values for aqueous solution of polyoxyethylene dodecyl ethers (C12En), $C_{12}H_{25}O(C_2H_4O)_nH$, where $n = 5$ to 53, as a function of time, are presented in Figure 5 (32). Maximum rate of the decrease in surface tension, $(d\gamma_t/dt)_{max}$, was determined based on the data (33). Dynamic surface tension (γ_t) at constant surfactant concentration may be obtained as

$$\gamma_t = \gamma_m + (\gamma_0 - \gamma_m)/\{(1 + (t/t^*)^x\}, \qquad (1)$$

where γ_t is the surface tension of the solution at time t; γ_m is the mesoequilibrium surface tension of the solution (where γ_t shows little change—<1 mNm^{-1} per 30 seconds—with time), γ_0 is the equilibrium surface tension of the solvent, and t^* and x are constants for a given surfactant. The parameter t^* is the time for γ_t to reach a value midway between γ_0 and γ_m, and decrease in surfactant concentration. The

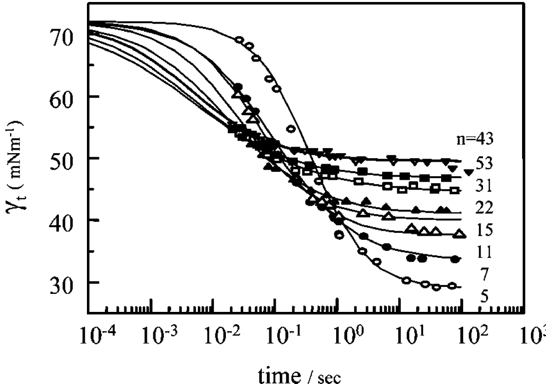


Figure 5 Effects of EO units on dynamic surface tension, γ_t, versus bubble surface lifetime, t, for 1 mM aqueous C12En solution at 25°C.

curves obtained with Eq. (1) are widely fitted for the observed time scale, as shown in Figure 5. The $(d\gamma_t/dt)_{\max}$ may be derived from Eq. (2) as

$$(d\gamma_t/dt)_{\max} = -x(\gamma_0 - \gamma_m)/4t^*. \tag{2}$$

Foamability and Foam Stability

Methods for foam formation and stability were established based on various sources of data, such as dynamic surface tension and liquid film movement, respectively, using laminometer (L_{lamellae}). Ross–Miles foam behavior of aqueous C12En solutions is shown in Figure 6. Initial foam height increased linearly with EO. Residual foam height decreased sharply with increase in EO. Dynamic surface properties of aqueous C12En solutions are shown in Figure 7. The $(d\gamma_t/dt)_{\max}$ increased linearly with EO, whereas L_{lamellae} decreased sharply with EO. Dynamic foam behavior by these methods was found consistent with conventional foam test results. Initial foam height in the Ross–Miles test was in good agreement with $(d\gamma_t/dt)_{\max}$, and residual foam height in good agreement with L_{lamellae}. Foam formation would thus appear to

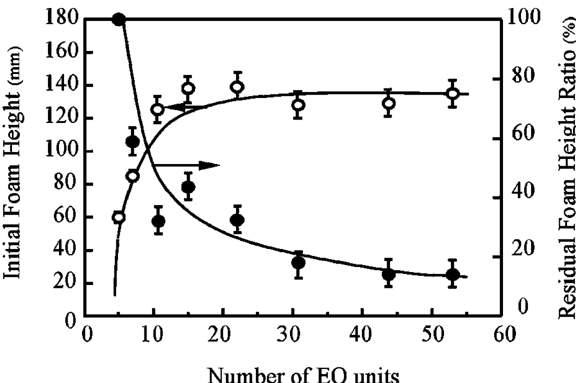


Figure 6 Effects of EO units on the Ross–Miles foam behavior for 1 mM aqueous C12En solution at 26°C.

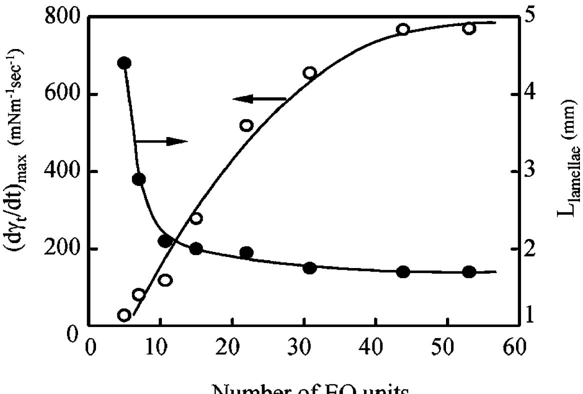


Figure 7 Effects of EO units on dynamic parameters for 1 mM aqueous C12En solution at 26 C.

depend primarily on the rate of adsorption of surfactants onto a gas/liquid interface and foam stability may also be a factor. For nonionic surfactants, initial foam height and stability are less compared with ionic surfactants in aqueous solution because of the large surface area per molecule of surfactant molecule.

ADSORPTION OF SURFACTANTS

Adsorption at the solid/liquid interface is an important feature requiring consideration in mechanics, electronics, biological systems, agriculture, foods, and cosmetics. When the adsorption isotherms of surfactants on a water soluble protein and hair are measured, several quantitative aspects of surfactant adsorption can be clarified.

Adsorption of Surfactants on Water Soluble Protein

Since the correlation between protein denaturation and skin roughness was shown in the early 1970s, protein denaturation has been used as a model for estimating skin roughness by surfactants and detergents (34). Regarding protein denaturation induced by surfactants, the interaction between surfactants and proteins has been studied by analysis of the characterization of surfactant binding and protein unfolding since the 1940s. Especially, concerning the interaction between SDS and bovine serum albumin (BSA), many studies have been done, for example, the binding isotherm, thermodynamics, and the change in structure (35–37). It is also well known that SDS-induced denaturation of BSA can be counteracted by dodecyltrimethylammonium chloride (DTAC) or *N*,*N*′-dimethyldodecylaminoxide (37). They hinder anionic surfactants from binding to BSA by forming a complex of two or more surfactants and neutralizing the electrical charge of the anionic surfactants such as SDS. The nonionic surfactants of the alkylene oxide (EO) adduct type, typically represented by alcohol ethoxylate (AE), can also reduce SDS-induced BSA denaturation (38). The higher the concentration of AE or the larger the adduct number of EO, the greater was the inhibitory effect of AE on SDS-induced denaturation of BSA. The binding isotherm of SDS in the absence or presence of C12E8 onto BSA immobilized-agarose (BSA-agarose) as a function of the equilibrium SDS concentration is shown in

Figure 8. In the absence of C12E8, the binding isotherm of SDS to BSA-agarose showed a pattern similar to that of SDS and BSA. Under saturation binding condition, about 100 molecules of SDS bind to 1 molecule of BSA-agarose, whereas typical saturation binding of SDS to BSA is more than 160 molecules of SDS/molecule of BSA. In the presence of C12E8, the higher the C12E8 concentration, the lower the amount of SDS bound to BSA-agarose. The effect of C12E8 indicates that C12E8 is adsorbed on the alkyl chain of SDS, which is electrostatically bound to BSA. The adsorbed C12E8 reduced the amount of electrostatic SDS binding, so that cooperative binding of SDS as well as the denaturation of BSA was dramatically suppressed by the addition of C12E8. The amount of SDS binding to BSA-agarose tended to be smaller as the average EO adducting number increased. BSA denaturation by SDS closely paralleled the adsorption behavior (Figure 9). As for the adsorption of AS on BSA, when the average EO adduct number was more than 9, the amount of every AE adsorbed on BSA was almost the same.

Binding of Surfactant to Human Hair

The binding of a surfactant to human hair or wool has been well studied. The thermodynamic aspects of surfactant binding are thus considered in this section. The binding of ionic surfactants to globular proteins has been extensively investigated by thermodynamic analysis of binding interactions (35–41). In consideration of the fine structure of human hair, surfactants should bind to the cuticle, cortex, and fibrils, all comprising proteins. Thus, continuous binding of a surfactant with human hair would appear the same as that of surfactants with globular proteins.

Binding isotherms of SDS for normal and damaged hair are shown in Figure 10 (42). SDS bound to cold-waved hair increased remarkably compared with normal and bleached hair. Each isotherm has two regions. Region I shows Langmuir binding attributable to interactions of SDS with ionic sites on hair. For Region II, there was a noted sharp increase in adsorption as a result of surfactant aggregation at the surface brought about by lateral interactions between hydrocarbon chains. Damaged hair may possibly be an indication of disruption of disulfide cross-links. This

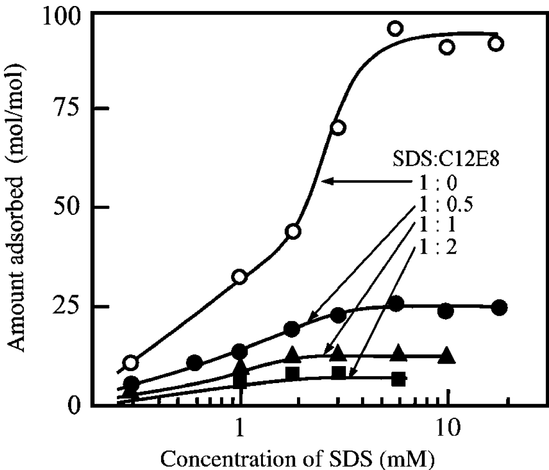


Figure 8 Effect of C12E8 on adsorption of SDS on BSA-agarose at 25°C.

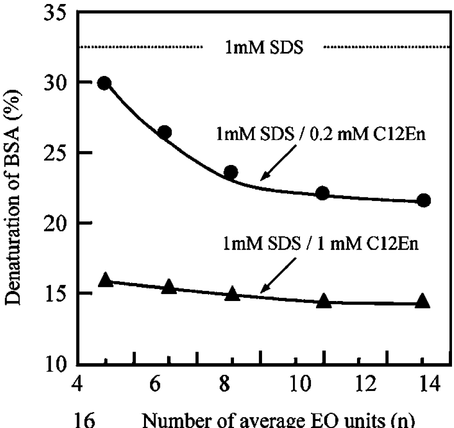


Figure 9 Denaturation of BSA by SDS and C12En mixed system at 25°C.

increase involves the consequent binding of SDS on polypeptides in the hair because of electrostatic repulsion among micelle-like clusters. Rigid disulfide bonds are maintained, and thus such binding was noted to a slight degree for the isotherms of normal hair. The binding isotherms of DTAC for normal and damaged hair indicated no increase in binding.

In the Langmuir binding region, the equation of Klotz [Eq. (3)] has quantitative application, as

$$1/\gamma = (1/K \quad n)\ (1/C) + 1/n, \tag{3}$$

where γ is the total bound surfactants, n, the total number of binding sites, K, the binding constant, and C is the concentration of surfactants at equilibrium. n and K may be obtained from the plot of $1/\gamma$ versus $1/C$. The free energy change, $-\Delta G$, is related to the binding constant as

$$-\Delta G = R \quad T \ \ln K. \tag{4}$$

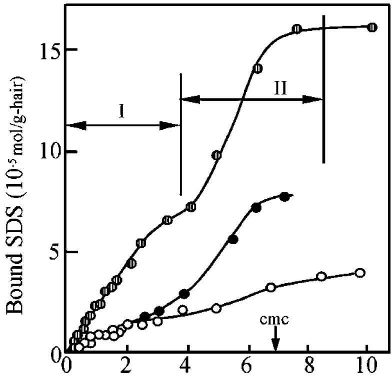
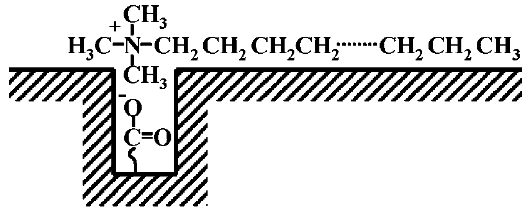


Figure 10 Binding of SDS to normal hair (), bleached hair (•, and cold-waved hair (○) at 25°C.



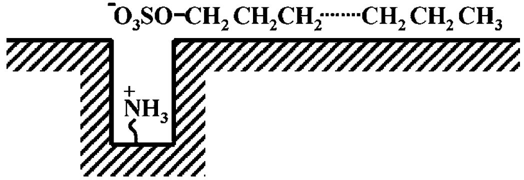


Figure 11 Schematic diagrams of the binding of surfactants human hair.

Thermodynamic parameters for binding between the surfactants and normal hair are listed in Table 2. n and $-\Delta G$ for anionic surfactants were the same in all regardless of the alkyl chain length. $-\Delta G$, when SDS was bound to BSA, was twice that in the case of SDS binding to hair. In the case of BSA, electric and hydrophobic interactions contribute to the free energy change of binding. Electrostatic interactions between anionic surfactants would thus appear quite weak, and no alkyl chains at all would be in a hydrophobic area. n and $-\Delta G$ for cationic surfactants were also the same regardless of the alkyl chain length. $-\Delta G$, in the case of DTAC binding to BSA and cationic surfactant binding to keratin powder, was the same as for binding to hair. The force of cationic surfactant binding to hair would thus appear to arise mainly from hydrophobic interactions, and alkyl chains would not be present in a hydrophobic area on the surface of hair, as also in the case of anionic surfactants. Binding sites for ionic surfactants on hair are shown in Figure 11 (42). Dissociated carboxyl and amino groups of polypeptides may possibly be present just inside the surface of the hydrophobic layer.

REFERENCES

1. Rieger MM. Cosmetics. In: Kroschwitz, JI, Howe-Grant M, eds. Encyclopedia of Chemical Technology. Vol. 7. 4th ed. New York: Wiley, 1992:572–619.
2. Porter MR. Anionics. In: Handbook of Surfactants. London: Blackie, 1994:99–104.
3. Reich C. Hair cleansers. In: Rieger MM, Rhein LD, eds. Surfactants in Cosmetics: Revised and Expanded. 2nd ed. New York: Marcel Dekker, 1997:357–384.
4. Sakamoto K. Application and effect of derivatives of amino acids for hair. Fragrance J 1979; 39:57–62.
5. Yoshimura M, Jokura Y, Hanazawa H, Nozaki T, Okuda M, Imokawa G. Biological characterization of a unique amino acid derivative-surfactant, lauroyl beta-alanine in relation to its cutaneous effect. J Soc Cosmet Chem Jpn 1993; 78:249–254.
6. Ishii M, Takizawa H, Usuba Y, Ishikawa K, Morimoto K, Akiba H. Synthesis and properties of N-acyl-N-alkyl-β-alanine. J Jpn Oil Chem Soc 1968; 17:616–622.
7. Miyazawa K, Tamura U, Katsumura Y, Uchikawa K, Sakamoto T, Tomita K. Anionic surfactants as detergents for scalp and hair. J Jpn Oil Chem Soc 1989; 38:297–305.

8. Kamegai J, Watanabe H, Hanazawa H, Kobayashi H. Properties and applications of nonionic surfactants derived from saccharides to shampoo. J Soc Cosmet Chem Jpn 1993; 27:255–266.
9. Takeguchi K, Shimada M. Solution properties of alkyliminoacetate. J Jpn Oil Chem Soc 1997; 46:1375–1381.
10. Nakanishi N, Matsuzawa Y, Mikami N. Moisturizing effect of amino acids and their derivatives. Fragrance J 1995; 23:71–80.
11. Infante MR, Perez L, Pinazo A. Novel cationic surfactants from arginine. In: Krister H, ed. Novel Surfactants: Preparation, Application, and Biodegradability. New York: Marcel Dekker, 1998:87–114.
12. Tabohashi T, Ninomiya R, Imori Y. A novel amino acid derivative for hair care products. Fragrance J 1998; 26:58–63.
13. Scott GV, Robbins CR, Barnhurst JD. Sorption of quaternary ammonium. J Soc Cosmet Chem 1969; 20:135–152.
14. Yahagi K, Hoshino N, Hirota H. Solution behavior of new cationic surfactants synthesized by using long-chain Guarbet alcohols in water and their application to hair conditioners. J Soc Cosmet Chem Jpn 1990; 23:301–309.
15. Mitamura J, Suzuki N, Onuma K, Miyake M, Nakamura T, Kiyomiya A. Development of new cationic surfactant "AG" and application for hair conditioners. J Soc Cosmet Chem Jpn 1996; 30:84.
16. Degiorgio V. Introduction. In: Degiorgio V, Corn M, eds. Physics of Amphiphiles: Micelles, Vesicles, and Microemulsions. Amsterdam: North-Holland, 1985:7–23.
17. Raney KH. Surfactant requirements for compact powder detergents. In: Showell MS, ed. Powder Detergents. New York: Marcel Dekker, 1998:241–284.
18. Lindman B. Amphoteric systems. Some basic aspects. In: Degiorgio V, Corn M, eds. Physics of Amphiphiles: Micelles, Vesicles, and Microemulsions. Amsterdam: North-Holland, 1985:7–23.
19. Laughlin RG. The structures and properties of surfactant phases. In: The Aqueous Phase Behavior of Surfactants. London: Academic Press, 1994:181–237.
20. Fontell K. Cubic phases in surfactant and surfactant-like liquid systems. Colloid Polym Sci 1990; 268:264–285.
21. Kodama M, Seki S. Coagel-gel-liquid crystal phase transition and heat properties of amphiphiles. Hyomen 1990; 22:61–67.
22. Kodama M, Seki S. Thermodynamical investigations on phase transition of surfactant–water systems: thermodynamic stability of gel and coagel phases and the role of water molecules in their appearance. Adv Colloid Interface Sci 1991; 35:1–30.
23. Kahlweit M, Strey R. The phase behavior of H_2O–oil-nonionic amphiphile ternary systems. In: Rosano HL, Clausse M, eds. Microemulsion Systems. New York: Marcel Dekker, 1987:1–13.
24. Malhotra AK, Wasan DT. Interfacial rheological properties of absorbed surfactant films with applications to emulsion and foam stability. In: Ivanov IB, ed. Thin Liquid Films: Fundamentals and Applications. New York: Marcel Dekker, 1988:829–890.
25. Pugh RJ. Foaming, foam films, antifoaming and defoaming. Adv Colloid Interface Sci 1996; 64:67–142.
26. Aveyard R, Clint JH. Foam and thin film breakdown processes. Curr Opin Colloid Interface Sci 1996; 1:764–770.
27. Myers D. Foams. In: Mysels D, ed. Surfaces, Interfaces, and Colloids: Principles and Applications. New York: VCH, 1991:251–270.
28. Domingo X, Fiquet L, Meijer H. Foam ability/stability of surfactants. Tenside Surf Deterg 1992; 29:16–22.
29. Tamura T. The test methods for measuring foaming and antifoaming properties of liquid. J Jpn Oil Chem Soc 1993; 42:737–745.

30. Dukhin SS, Kretzschmar G, Miller R. Experimental techniques to study adsorption kinetics. In: Möbius D, Miller R, eds. Dynamics of Adsorption at Liquid Interfaces. Amsterdam: Elsevier, 1995:140–201.
31. Hua H, Rosen M. Dynamic surface tension of aqueous surfactant solutions: I. Basic parameters. J Colloid Interface Sci 1988; 124:652–659.
32. Tamura T, Kaneko Y, Ohyama M. Dynamic surface tension and foaming properties of aqueous polyoxyethylene *n*-dodecyl ether solutions. J Colloid Interface Sci 1995; 173: 493–499.
33. Rosen MJ, Hua XY, Zhu ZH. Dynamic surface tension of aqueous surfactant solutions: IV. Relationship to foaming. In: Mittal KL, Shah DO, eds. Surfactants in Solution. Vol. 11. New York: Plenum, 1991:315–327.
34. Imokawa G, Sumura K, Katsumi M. Study on skin roughness caused by surfactants: II. Correlation between protein denaturation and skin roughness. J Am Oil Chem Soc 1975; 52:484–489.
35. Reynolds JA, Gallagher JP, Steinhardt J. Effect of pH on the binding of N-alkyl sulfates to bovine serum albumin. Biochemistry 1970; 9:1232–1238.
36. Takagi T, Tsujii K, Shirahama K. Binding isotherms of sodium dodecyl sulfate to protein polypeptides with special performance to SDS-polyacrylamide gel electrophoresis. J Biochem 1975; 77:939–947.
37. Ohbu K, Jona N, Miyajima N, Mizushima N, Kashiwa I. Evaluation of denaturation property of surfactants onto protein as measured by circular dichroism. J Jpn Oil Chem Soc 1980; 29:866–871.
38. Tadenuma H, Yamada K, Tamura T. Analysis of protein-mixed surfactant system interactions; The BSA-SDS and polyoxyethylenealkylether system. J Jpn Oil Chem Soc 1999; 48:207–213.
39. Schwuger MJ, Bartnik G. Interaction of anionic surfactants with proteins, enzymes, and membranes. In: Gloxhuber C, ed. Anionic Surfactants: Biochemistry, Toxicology, Dermatology. New York: Marcel Dekker, 1980:1–49.
40. Nozaki Y, Reynolds JA, Tanford C. The interaction of a cationic detergent with bovine serum albumin and other proteins. J Biol Chem 1974; 249:4452–4459.
41. Hiramatsu K, Ueda C, Iwata K, Aoki K. The interaction of bovine plasma albumin with cationic detergent. Studies by binding isotherm, optical rotation and difference spectrum. Bull Chem Soc Jpn 1977; 50:368–372.
42. Ohbu K, Tamura T, Mizushima N, Fukuda M. Binding characteristics of ionic surfactant with hair. Colloid Polym Sci 1986; 264:798–802.

28
Classification of Surfactants

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INTRODUCTION

The term "*surfactant*" applies to a group of molecules having both a hydrophilic part and a hydrophobic (or lipophilic) part. Surfactants modify the interfacial properties of the liquids in which they are incorporated; this property stems from their tendency to concentrate at the interfaces separating immiscible phases.

Depending on the nature of the hydrophilic moiety ensuring the water affinity of the molecule, surfactants are distributed in anionic, cationic, amphoteric, and nonionic classes. Regarding the hydrophobic moiety of the molecule, it is a hydrocarbon chain in most common surfactants; however, in some more specialized surfactants, this hydrophobic part can be a nonhydrocarbon chain such as a polydimethylsiloxane or a perfluorocarbon. The selection of surfactants in the frame of cosmetic products development is a delicate task in which numerous factors have to be taken into account. Among others, one should consider those directly related to functions to be fulfilled (detergency, emulsification, foam quality, rinsability, mildness for skin, skin feel, etc.), and also those related to cost, toxicity, and biodegradability.

The aim of this chapter is to provide a classification of various commercially available surfactants.

IONIC SURFACTANTS

Anionic Surfactants

In aqueous solution, anionic surfactants form a negatively charged ion provided the composition pH is neutral to alkaline. The ionized moiety can be a carboxylate, sulfate, sulfonate, or phosphate. Among most frequently used surfactants in skin care products, the alkyl sulfates and alkyl ethoxylated sulfates can be mentioned for their high-foaming capacity. Anionics are generally used in association with other surfactants (nonionics or amphoterics) which bring improvements in the skin tolerance, in the foam quality or in the product viscosity.

Other anionics are also used in personal products, however as secondary surfactants, often for their milder profile and their low-foaming properties (isethionates, sulfosuccinates, taurates, sarcosinates, phosphoric acid esters, acylglutamates, etc.).

Carboxylates

Carboxylate salts. Surfactants belonging to this class generally derive from oleochemistry. Carboxylate salts (or soaps) are directly produced by the alkaline hydrolysis (or saponification) of animal and vegetable glycerides and result from the neutralization of fatty acids.

Saturated sodium soaps are extremely soluble in water up to C_8; those with chain lengths approaching C_{18} become less soluble and they are insoluble above C_{20}.

Starting from C_{16} chain lengths, the fatty acids can be either saturated or unsaturated. Unsaturated fatty acids are prone to undergo oxidation and form oxides and peroxides, which cause rancidity and yellowing.

Potassium soaps and salts of alkanolamines are more fluid and also more soluble than sodium salts.

The extremely low solubility of alkaline earth and heavy metal fatty acid salts makes this class of surfactants less appropriate for use in hard water.

$$R-C(=O)O^{\ominus} \quad M^{\oplus} \qquad M = Na, K, NH_4, \text{etc.} \qquad R = CH_3\text{-}(CH_2)_x\text{-}$$

Alkyl carboxylate

The main application of fatty carboxylates is found in the soap bars widely used in the world for fabric hand wash (generally based on tallow/coconut oil mixtures).

Water-soluble soaps are mainly used in skin cleansers (soap bars or liquids), shaving products (sticks, foams, or creams), and deodorant sticks.

Mixtures of fatty acids and their salts are used in "acid soaps."

Water-insoluble soaps form gels in nonaqueous systems and, because of their hydrophobicity, they can be appropriate surfactants for w/o emulsions.

Ester carboxylates. This class of surfactants is a subcategory of the previously discussed surfactant group based on carboxylic acids; they are monoesters of di- and tricarboxylic acids.

These esters are produced by condensation reactions involving different types of molecules; either an alcohol with a polycarboxylic acid (e.g., tartric or citric acid) or a hydroxyacid (e.g., lactic acid) with a carboxylic acid.

The reacting alcohol may have been previously ethoxylated.

$$\begin{array}{l} \quad\;\; CH_2C(=O)-O(CH_2CH_2O)_7-C_{12}H_{25} \\ HO-C-COONa \\ \quad\;\; CH_2C(=O)-O(CH_2CH_2O)_7-C_{12}H_{25} \end{array}$$

Sodium dilaureth-7 citrate

Because of their good foaming properties and substantivity on the hair, ester carboxylates are especially suitable in shampoos; in combination with alcohol ethoxy sulfates (AEOS), they provide reduced skin irritation.

Short-chain lactylates (i.e., issued from lactyllactic acid) are substantive on the skin and show humectant properties.

Ether carboxylates. Alkyl polyglycol ether carboxylates are the best known surfactants in this category.

These surfactants are formed by the reaction of sodium chloracetate with ethoxylated alcohols.

Because of the addition of ethoxylated groups, ether carboxylates are more soluble in water and less sensitive to water hardness compared to conventional soaps. Also, keeping the best properties of nonionic surfactants, they do not exhibit any cloud point and show good wetting and foam stability.

Ether carboxylates do not undergo hydrolysis in presence of alkali or acids.

$$RO - (CH_2CH_2O)_n - _2 - COONa$$

Alkyl polyglycol ethercarboxylate, sodium salt

Ether carboxylates are used as general emulsifier and emulsion stabilizers. In personal care, they impart mildness, creamy foaming, skin-feel and hair-conditioning benefits. Therefore, they are especially suitable in shampoos in combination with alcohol ether sulfates and possibly with cationics.

More recently, a new generation of alkyl glucose carboxylates is emerging. These surfactants exhibit both the high mildness of alkyl polyglucosides (APG) surfactants and additional attributes such as foaming and sensory benefits. A typical surfactant of this class is the sodium lauryl glucose carboxylate.

Sulfates

Alkyl sulfates. Alkyl sulfates are organic esters of sulfuric acid; they vary by the length of the hydrocarbon chain and by the selected counterion.

Alkyl sulfates are produced by sulfation of the corresponding fatty alcohols.

The properties of alkyl sulfates depend mainly upon the chain length and on the degree of branching of the hydrocarbon chain, as well as, to a smaller extent, on the nature of the counterions.

They are generally good foamers, more especially in hard water; best foam characteristics are obtained in the C_{12}–C_{14} chain length range.

Sodium lauryl sulfate (SLS) has a 12 carbon chain length and is one of the most common surfactants. It is not well tolerated by the skin. When the chain length increases, i.e., in the C_{14}–C_{18} range, surfactant penetrability through the stratum corneum decreases along with its irritation potential; but the foaming capacity is accordingly depressed. Chains with carbon number lower than 12 are better tolerated by the skin than SLS but exhibit more a pronounced smell.

Combination with other surfactants allows considerable improvement of the lauryl sulfate compatibility with skin while keeping a good foam. SLS is however less frequently used than its ethoxylated counterpart. Lauryl sulfate is available under the form of various salts: SLS, ammonium lauryl sulfate (ALS), magnesium lauryl sulfate [$Mg(LS)_2$], and triethanolamine lauryl sulfate (TEALS). Skin tolerance of lauryl sulfates is as follows: $Mg(LS)_2 > TEALS > SLS > ALS$.

$$R{-}CH_2{-}O{-}SO_2{-}O^- \; Na^+$$

Sodium alkyl sulfate

Alkyl sulfates are used in cosmetics and personal-care areas (e.g., diethanolamine lauryl sulfate in shampoos); they are associated with other surfactants and improve foaming characteristics of detergent systems.

Pure SLS is also used in oral care and incorporated in dental creams, essentially as a foaming agent.

Alkyl ether sulfates. Alkyl ether sulfates which are also called AEOS result from the sulfation of an ethoxylated alcohol.

Compared with alkyl sulfates, the ether sulfates show higher water solubility, improved foam stability in hard water, and better skin tolerance. The viscosity of surfactant solutions of ether sulfates is much more sensitive to the presence of electrolytes than alkyl sulfates; formulators often take advantage of this opportunity to bring liquid formulations to the desired viscosity by simply adjusting the salt level (e.g., NaCl).

The higher the number of ethoxy groups (EO) in the molecule, the lower the surfactant ability to penetrate the stratum corneum, and the less irritant for skin it will be. Similar ranking is true for eye irritation. Also, the foaming capacity decreases as ethoxylation degree increases.

$$R - CH_2 - O - (CH_2 - CH_2 - O)_n - SO_3\ Na$$

Sodium alkyl ether sulfate

Alkyl ether sulfates are extensively used in personal products such as liquid soaps, shower gels, foam baths and, more especially shampoos. Sodium lauryl ether sulfate (SLES) is today the most currently used primary tensioactive and more especially, under the forms SLES-2 EO and SLES-3 EO, which combine good foaming and skin compatibility properties.

Amide ether sulfates. The amide ether sulfates are obtained by sulfation of the corresponding ethoxylated amide.

The magnesium salts foam well and their skin compatibility is excellent.

$$\left[R-\underset{\underset{O}{\|}}{C}-NH(CH_2CH_2O)_3-SO_3 \right]_2 Mg^{2+}$$

Magnesium PEG-3 cocamide sulfate

Owing to their weak lipid removal effect, amide ether sulfates are used in very mild personal cleaners.

Alkyl glyceride sulfates. The best known surfactant of this class is the coco-monoglyceride sulfate (CMGS). It is obtained by transesterification of coconut oil with glycerol followed by a sulfation with sulfur trioxide and a neutralization with sodium hydroxide.

$$\begin{array}{l} H_2C-O-\overset{\overset{O}{\|}}{C}-R \\ \quad | \\ HC-OH \\ \quad | \\ H_2C-O-SO_4Na \end{array}$$

Sodium alkyl monoglyceride sulfate

This surfactant is very well designed for cosmetic and personal-care products. Compared with the corresponding alkyl ether sulfate, it shows similar foaming power. Because this surfactant acts as a foam booster, it can be advantageously

combined with APG. Such mixtures also show a thickening ability induced by salt addition. CMGS is said to present a better skin compatibility profile than ether sulfate or other anionic surfactants.

Sulfonates

On a chemical standpoint, there is an important difference between the previously discussed alkyl sulfates and the alkyl sulfonates: in the former, the sulfur atom is linked to the carbon chain via an oxygen atom, and in the latter, the sulfur atom is directly linked to the carbon atom.

Alkyl sulfonates. Three major types of alkyl sulfonates must be considered: the primary and secondary paraffin sulfonates (PS and SAS) and the α-olefin sulfonates (AOS).

The paraffin sulfonates are very water-soluble surfactants, good foamers, and good o/w emulsifiers. Their solutions do not thicken easily upon salt addition. Therefore, they are particularly appropriate to formulate fluid liquids or highly concentrated products.

The AOS have general properties fully comparable to linear alkylbenzene sulfonate (LAS) (see next section); they are good o/w emulsifiers, wetting, and foaming agents.

$$CH_3-(CH_2)_m-CH_2-SO_3Na$$

Primary sodium alkyl sulfonate

$$CH_3-(CH_2)_m-CH(CH_3(CH_2)_n)-SO_3Na$$

Secondary sodium alkyl sulfonate

± 35 %:

$$R-CH_2-CH_2-CH=CH-CH_2-SO_3Na$$

$$R-CH_2-CH=CH-CH_2-CH_2-SO_3Na$$

± 65 %:

$$R-CH_2-CH_2-CH(OH)-CH_2-CH_2-SO_3Na$$

Constituents of AOS: sodium alkene sulfonates and sodium hydroxy alkane sulfonate

Alkane sulfonates (PS and SAS) are mainly used in Europe in detergent products.

AOS have been mainly used in Asia as surfactants for heavy and light duty laundry detergents, synthetic soap bars, and household products. Because they are less irritating than alkyl–aryl sulfonates, they have also been used in the United States in several personal products (liquid soaps, bubble baths, and shampoos) as alternatives to alcohol ether sulfates. They are also marginally used in oral care formulations.

Alkyl–aryl sulfonates. Today, LAS is the most important surfactant on a volume basis, but its use in personal care is very limited.

It is worth mentioning that some methyl or methyl–ethyl substituted aryl sulfonates, i.e., sodium xylene, toluene, or cumene sulfonates, although not showing typical surfactant properties are used as hydrotropes (i.e., solubilizing agents which decrease hydrophobic effects in aqueous systems).

$$CH_3-(CH_2)_x-CH(C_6H_4SO_3Na)-(CH_2)_y-CH_3$$

Sodium Linear Alkylbenzene Sulfonate (LAS)

LAS is a very cost-effective surfactant that is extensively used in a broad variety of detergents for household, fabric care, institutional, and industrial products.

Because of its too high detersive action, LAS has a relatively low compatibility with skin and is only scarcely used in cosmetics except in some antiseborrheic preparations.

Sulfosuccinates. Sulfosuccinates are the sodium salts of alkyl esters of sulfosuccinic acid; they generally result from the condensation of maleic anhydride with a fatty alcohol, followed by a sulfonation with sodium bisulfite, $NaHSO_3$. Some variants of sulfosuccinates are derived from other substituted fatty molecules such as fatty alcohol ethoxylates, fatty amines (yielding sulfosuccinamates), or fatty alkanolamides.

$$NaO_3S-CH(C(=O)-OR)-CH_2-C(=O)-OR$$

Sodium dialkyl sulfosuccinate

Monoester disodium salts are the most common sulfosuccinates used in cosmetic applications. Monoesters of alkanolamines (sulfosuccinamates) are milder than monoesters of fatty alcohols (sulfosuccinates). Monoesters derived from ethoxylated alcohols or alkanolamides are extensively used in personal products and especially in shampoos; they are known for their mildness and skin-irritation reduction when used in association with other anionic surfactants.

Sulfo fatty acid esters. These surfactants are sometimes known under their abbreviated names: FES, MES, and ASME, for fatty ester sulfonate, methyl ester sulfonate, and alpha sulfo (or α-sulfo) methyl ester, respectively. Most of α-sulfo fatty acid esters derive from fatty acid methyl esters.

$$R-CH(SO_3Na)-C(=O)-O-CH_3 \qquad R-CH(SO_3Na)-C(=O)-O-R'$$

Methyl ester of α-Sulfo fatty acid, sodium salt. Alkyl ester of α-Sulfo fatty acid, sodium salt.

α-Sulfo methyl ester surfactants deriving from C_{16}–C_{18} fatty acid (e.g., ASMT, the tallowate) are appropriate for use in laundry detergents. ASME is also used in the formulation of syndet bars (laundry bars based on synthetic surfactants).

To our knowledge, these surfactants are not used in personal care.

Fatty acid isethionates and taurides. Fatty acid isethionates are usually prepared by reaction of a fatty acid chloride with sodium isethionate ($HO–CH_2–CH_2–SO_3–Na$), itself resulting from the addition of sodium bisulfite to ethylene oxide. These surfactants are insensitive to water hardness and show good wetting, foaming, and emulsifying properties. In addition, they are very mild and have excellent compatibility with the skin. Taurides (or taurates) are acyl-amino alkane sulfonates that have chemical structures close to isethionates. They can be used in association with other surfactants to increase the viscosity.

$$R-C(=O)-OCH_2CH_2-SO_3Na$$

Fatty acid isethionate

$$R-C(=O)-N(CH_3)-CH_2CH_2-SO_3Na$$

Sodium methyl acyl tauride

Acyl isethionates have been used in shampoos and personal cleansers. They are also incorporated in syndet bars together with various soaps. The most currently used is the cocoyl isethionate.

Taurides (or taurates), which have properties similar to soaps (except the sensitivity to water hardness), have been extensively used in shampoos but are now replaced by AEOS. Today they are limitedly used in cosmetics mainly in foam baths and toilet bars.

Taurides are also used in soap bars especially designed for laundering with seawater, in agriculture, and textile dying.

Phosphates Esters

This class of surfactants includes alkyl phosphates and alkyl ether phosphates.

$$RO-P(=O)(OH)-OH$$

Alkyl phosphoric ester

$$RO-P(=O)(OR)-OH$$

Dialkyl phosphoric ester

The use of phosphate esters as surfactants is especially useful in applications for which a particular tolerance to pH, heat, or electrolytes is required. They are also used in acidic cleaning products for household as well as industrial applications. Mild for the skin, alkyl phosphates sometimes enter the composition of facial and cleansing products.

Acyl-amino Acids and Salts

Acyl glutamates. These surfactants are formed by acylation of a natural amino acid, the glutamic acid $HOOC–CH_2–CH_2–CH(NH_2)–COOH$ (or α-aminoglutaric acid).

These surfactants are mild for the skin and the eyes, deliver improved skin feel, but are poor foamers.

$$NaOOC-CH_2CH_2CH(NH-C(=O)-R)-COONa$$

Sodium acyl glutamate

Acyl glutamates are mainly used in personal products such as shampoos.

Acyl peptides. These surfactants are formed from hydrolyzed proteins (e.g., animal collagen).

Depending upon the protein hydrolysis process (chemical or enzymatic), the average polypeptide molecular weight can vary from about 350 to 2000 and some free aminoacids may be present in the hydrolysate. An acylation reaction occurs on the amine terminal functions and, possibly, on some side groups (e.g., the hydroxyls) and thus leaves the carboxyl groups free which must be neutralized.

Products containing such surfactants are prone to be contaminated by various germs and have to be properly preserved.

$$R-\overset{\overset{O}{\|}}{C}-[NH\,\overset{\overset{X}{|}}{CH}\,\overset{\overset{O}{\|}}{C}]_x-NH\overset{\overset{X}{|}}{CH}\,\overset{\overset{O}{\|}}{C}-ONa$$

Sodium acyl polypeptide (X= amino acids side groups)

Acyl peptides are mild surfactants designed for the personal-care area; they are especially used in shampoos owing to their substantivity on the keratin of hair and, therefore, they effectively deliver the expected benefits of conditioning agents.

Acyl sarcosides. Sarcosinates (or salts of acyl-amino acids) are the condensation products of fatty acids with *N*-methylglycine $CH_3–NH–CH_2–COOH$ (or sarcosine).

$$R-\overset{\overset{O}{\|}}{C}-\underset{\underset{CH_3}{|}}{N}-CH_2COONa$$

Sodium acyl sarcosinate

Sarcosinates are good surfactants for cosmetic usage because of their mildness to skin, substantivity on skin and hairs when incorporated in formulations around neutral pH, conditioning action, and foaming resistance in the presence of soaps or sebum. Incorporated in shampoos with alkyl sulfates, they boost the lather.

Sarcosinates are also used as corrosion inhibitors.

Cationic Surfactants

From a very general standpoint, cationic surfactants differ from anionic and nonionic ones by the fact that they carry a positive charge. Their major interest in cosmetic industry resides in hair care; in this frame, they are used as hair conditioners and antistatic agents.

Cationics are also found in the personal-care area as emulsifiers in some cosmetic preparations and as bactericidal agents.

Alkylamines

Primary, secondary, and tertiary alkyl amines, and more especially their salts, are included in this surfactant class.

$$R-NH_2$$

Alkyl amine

$$R-N\begin{matrix}\diagup CH_3\\ \diagdown CH_3\end{matrix}$$

Dimethyl alkyl amine

$$R-\overset{\overset{O}{\|}}{C}-NH-(CH_2)_3-N\begin{matrix}\diagup CH_3\\ \diagdown CH_3\end{matrix}$$

Alkylamido dimethyl propylamine

Amines and their salts are mainly used in textile treatment and occasionally in rinse fabric softeners. Salts of amines are used in cosmetics together with other surfactants. Their usage is restricted to specialties; they exhibit conditioning and antistatic properties in hair care applications. Amido-amines are also used in cosmetic products.

Alkylimidazolines

Reaction of a fatty acid with a substituted ethylene diamine forms imidazoline. Heating the resulting amido-ethylamine yields the imidazoline with a five-member substituted ring.

The tertiary nitrogen atom can be quaternized.

```
R — C —— N — R'
    ||       |
    N       CH2
     \     /
      CH2
```

R' = $CH_2CH_2NH_2$ => alkyl aminoethyl imidazoline

R' = CH_2CH_2OH => alkyl hydroxyethyl imidazoline

Imidazolines are cationic o/w emulsifiers.

Considered to be irritating, they are scarcely used in cosmetics as substantive hair-conditioning agents.

Quaternary Ammonium Compounds

Quaternary ammonium compounds form a class of surfactants that contain a positively charged nitrogen atom linked to four alkyl or aryl substituents.

The positive charge is permanent, regardless of pH.

Tetra alkyl(–aryl) ammonium salts. Tetra alkyl ammonium salts have the structure $[R_1R_2R_3R_4N^+]\ X^-$ where R_1, R_2, R_3, and R_4 are alkyl or aryl groups and X^- represents an anion. The water solubility of quaternaries mainly depends upon the nature of R substituents.

Low solubility quaternaries can adsorb on various substrates and impart various useful conditioning effects (softening, antistat, corrosion inhibition . . .).

With the exception of *N*-alkyltrimethyl ammonium salts, quaternary surfactants usually show poor detergency, wetting, and emulsifying capacities. Quaternaries are generally not compatible with anionics because of the formation of a water-insoluble complex.

```
[      CH3       ]
[       |        ]
[ R — N+ — R'    ]   X-
[       |        ]
[      CH3       ]
```

Quaternary compound

The major usage of quaternaries is related to their ability to adsorb on natural or synthetic substrates and fibers. They are widely used as softening agents in rinse fabric softeners.

Their softening and antistatic properties are similarly exploited in hair-conditioning shampoos or after-shampooing rinses.

It is worth noting that, in cosmetic applications, quaternaries may cause ocular and local irritation. Among quaternaries, some are used as germicides and disinfectants (e.g., didecyl dimethyl ammonium chloride and benzalkonium chloride).

Heterocyclic ammonium salts. Heterocyclic quaternaries are derived from heterocyclic aliphatic or aromatic compounds in which a nitrogen atom constitutive of the cycle is quaternized.

$$\left[C_{17}H_{35}-\text{(imidazoline ring)}-N(CH_2CH_2\,NH\,CO\,C_{17}H_{35})-CH_3 \right]^+ \; CH_3OSO_3^-$$

Imidazolinium quaternary compound

The quaternaries derived from imidazoline and morpholine are used as hair conditioners and antistatic agents. Those derived from aromatic heterocycles are used as germicides.

Alkyl betaines. Alkyl betaines, which are *N*-trialkyl derivatives of amino acids ($[R_1R_2R_3]N^+CH_2COOH$), are classified as cationics because they exhibit a permanent positive charge. Because they also have a functional group able to carry a negative charge in neutral and alkaline pH conditions, they are often regarded, although this position is questionable, as amphoterics.

The positive charge is always carried by a quaternized nitrogen while the anionic site can be a carboxylate (betaine), a sulfate (sulfobetaine or sultaine), or a phosphate (phosphobetaine or phostaine).

Betaines are good foaming, wetting, and emulsifying surfactants, especially in the presence of anionics. Alkylamido betaines deliver more stable foam and are better viscosifiers than alkyl dimethyl betaines. Betaines are compatible with other surfactants, and they frequently form mixed micelles; these mixtures often deliver unique properties that are not found in the individual constitutive surfactants.

Betaines have low eye and skin irritation; moreover, the presence of betaines is known to decrease the irritation effect of anionics.

$$R-N^+(CH_3)_2-CH_2COO^-$$

Alkyl dimethyl betaine

$$R-C(=O)-NH-(CH_2)_3-N^+(CH_3)_2-CH_2CH(OH)CH_2SO_3^-$$

Alkylamidopropyl hydroxysultaine

Because of their ability to improve the skin tolerance against irritating anionic surfactants, and also, because of their high price, betaines are usually used in association with other surfactants.

Betaines are especially suitable in personal-care applications (e.g., shampoos, foam baths, liquid soaps, shower gels, etc.), fabric hand-wash products, and dishwashing products.

Quaternized APG. These surfactants derived from natural sources are recently available. They are made of alkyl polyglycoside with a cationic backbone. These very mild quaternary compounds are substantive to the skin and hair and provide a soft after feel. Thanks to the sugar moiety, they show low irritation potential, and therefore, they are particularly suitable for personal-care formulations. Major benefits are a longer lasting effect associated with a reduction of irritation compared with traditional quaternary surfactants.

Some potential applications reside in hair care products (e.g., in wet combing benefit, fly-away hair control), water in oil cationic emulsions, formulations in the presence of anionics, foam building in shampoo formulations, or surfactants for baby wipes.

Ethoxylated Alkylamines

These surfactants can be considered as cationic or nonionic depending on the degree of ethoxylation and the pH at which they are used. Polyethoxylated amines are formed by ethoxylation of primary or secondary fatty amines.

$C_{12}H_{25}N\ [(CH_2CH_2O)_3H]_2$

Laurylamine + 6 EO (or POE 6)

$R-N(CH_2CH_2O)_xH-CH_2CH_2CH_2N[(CH_2CH_2O)_yH][(CH_2CH_2O)_zH]$

Alkyl propanediamine ethoxylate

The ethoxylated alkylamines have various application fields; they are generally exploited for their capacity of adsorbing on surfaces.

In personal care, ethoxylated alkyl amines are used as emulsifiers and hair-conditioning agents.

Ethoxylated amidoamines find applications in rinse fabric softeners.

Esterified Quaternaries

Esterified quaternaries (or esterquats) are produced by the esterification of the hydroxyl group(s) of secondary or tertiary amino-alcohols with selected fatty acids.

$$\left[H_3C-N^{\oplus}(CH_2-CH_2-O-C(=O)-R)_2(CH_2-CH_2-OH) \right]\ MeSO_4^{\ominus}$$

Esterquat : N-Methyl-N,N-bis[$C_{16/18}$-acyloxy)ethyl]-N-(2-hydroxyethyl)ammonium-methosulfate salt

The esterquats are suitable substitutes for straight quaternaries; they present an improved environmental profile and comparable softening properties compared with straight quaternaries.

Amphoteric Surfactants

Amphoteric surfactants are characterized by the fact that these surfactants can carry both a positive charge on a cationic site and a negative charge on an anionic site. The use of amphoteric terminology is still more restrictive: the charge of the molecule must change with pH, showing a zwitterionic form at intermediate pH (i.e., around the isoelectric point).

The surfactant properties are accordingly influenced by pH: around the isoelectric point the zwitterionic form takes place, exhibiting the lowest solubility; in alkaline conditions the anionic form is predominant, delivering foam and detergency and in acidic conditions, the cationic form prevails, providing surfactant substantivity.

Although betaines are commonly classified among amphoterics, this classification is improper because these surfactants never exhibit in single anionic form.

Amphoteric surfactants are generally used as secondary tensioactives for their foam stabilizing effect, their thickening capacity, and their skin-irritation reduction capacity on alkyl sulfates and alkyl ethoxy sulfates.

Acyl Ethylenediamines and Derivatives

These surfactants are made by the reaction of an alkyl imidazoline with chloroacetic acid (yielding amphoglycinates) or with acrylic acid (yielding amphopropionates).

$$R-\overset{\overset{O}{\|}}{C}-NH-CH_2CH_2-\overset{\overset{CH_2COONa}{|}}{N}-CH_2CH_2OH$$

Acylamphoacetate

$$R-\overset{\overset{O}{\|}}{C}-NH-CH_2\,CH_2-\overset{\overset{CH_2\,CH_2\,COO\,Na}{|}}{N}-CH_2\,CH_2\,O\,CH_2\,CH_2\,COO\,Na$$

Acylamphodipropionate

Amphoterics of this class are mainly used in personal products (e.g., coco amphocarboxy glycinate). Incorporated in baby shampoos, they reduce eye irritation.

Other applications are fabric softeners, industrial cleaners, and car cleaners.

N-Alkyl Amino Acids or Imino Diacids

These molecules are chemical derivatives of amino acids that can be produced by the reaction of chloroacetic acid or acrylic acid with an alkyl amine. Their compatibility with other surfactants is excellent.

These surfactants are good emulsifiers and show optimal wetting and detergency under alkaline pH. They are good foamers at neutral and alkaline pH but lose their foaming properties under acidic conditions.

They are substantive to surfaces and provide antistatic effects.

They provide skin and eye irritancy reduction in combination with anionics.

$R{-}NH{-}CH_2CH_2{-}COOH$

Alkyl aminopropionic acid

$C_{12}H_{25}{-}NH{-}CH_2{-}COONa$

Sodium coco glycinate

$R{-}N(CH_2CH_2CH_2{-}NH_2)(CH(COOH)CH_2CH_2{-}C(=O){-}NH_2)$

Aminopropyl alkylglutamide

$R{-}N(CH_2CH_2{-}COONa)_2$

Sodium alkyliminodipropionate

Amphoterics of this class are mainly used in personal products.

Polycarboxylates deliver reduced eye irritation and provide hair-conditioning benefits. Their zwitterionic forms are substantive on the hairs.

NONIONIC SURFACTANTS

Nonionic surfactants do not dissociate into ions in aqueous medium. They generally deliver a weak to moderate foam. They are appreciated for their good skin and eye compatibility as well as for their anti-irritant potential when they are combined with anionics in an appropriate concentration ratio. Therefore numerous products for sensitive skin, babies, or the face incorporate nonionics as major surfactant.

Fatty Alcohols

Fatty alcohols are primarily used as a chemical precursor for the production of several other surfactants.

$$R - CH_2 - OH$$

Fatty alcohol

Because they are not water soluble, the use of fatty alcohols is very limited in liquid products. They are mainly used as opacifiers, thickening agents, and foam depressors (e.g., lauric alcohol).

Ethers

Alkoxylated Alcohols

This class of surfactants mainly covers ethoxylated or propoxylated alcohols.

Ethoxylated alcohols (also called "polyethyleneglycol ethers" or "PEG ethers") are produced from the reaction of fatty alcohols with ethylene oxide (EO).

Similarly, propoxylated alcohols (also called "polypropyleneglycol ethers" or "PPG ethers") are obtained with propylene oxide (PO).

The Hydrophilic-lipophilic balance (HLB) of ethoxylated alcohols can be adjusted by properly balancing the hydrophilic ethoxylated chain and the hydrophobic fatty chain.

Ethoxylate nonionics are compatible with all surfactants. Some beneficial associations with ionic surfactants are often shown.

In the frame of personal-care applications, ethoxylated alcohols often result from the transformation of natural lipids. The nomenclature specific to cosmetic chemicals (i.e., INCI names[a]) is applied to these nonionics: they are denominated by using the root of the fatty acid name terminated by the suffix "eth" (contraction of "ethoxylated"), directly followed by the ethoxylation degree (e.g., laureth-4, oleth-5, myristeth-7).

As some raw materials yield on hydrolysis various fatty chain lengths, the names of the derived nonionics are either drawn from the natural source (e.g., laneth-16 for a lanolin derived nonionic) or from the combined abbreviations of the constitutive fatty chains (e.g., ceteareth-20 for a combination of cetyl and stearyl).

$$CH_3(CH_2)_x - O - (CH_2CH_2O)_nH$$

Alkyl polyethyleneglycol ether or alcohol ethoxylate (e.g., laureth 20 for $x = 11$ and $n = 20$)

$$CH_3-[CH_2]_x-[O-CH(CH_3)CH_2]_y-[OCH_2CH_2]_z-OH$$

EO/PO Alkyl Ether (e.g., propyleneglycol capreth-4 for x = 9, y = 1 and z = 4)

Applications of ethoxylated alcohols are numerous in industrial as well as in household products.

When properly selected, alkoxylated alcohols are also useful for personal products as good emulsifiers and solubilizers. The cosmetic applications remain, however, limited because of their rather weak foaming capacity.

Because they are prone to undergo degradation by oxidation, the following precautions can greatly improve the stability of ethoxylate nonionics: storage in the dark, minimal air contact, low temperature storage, avoiding storage of diluted products, and the addition of an antioxidant.

Ethylene oxide/Poly(propylene oxide) Block Polymers

These polymeric surfactants have some similarity with the previously discussed alkoxylated alcohols. They consist in the combination of the assembly of poly-(propylene glycol) (PPG) (hydrophobic part) and poly(ethylene glycol) (PEG) chains (hydrophilic part). Such surfactants are known under the denomination "poloxamers" (INCI name) and are called EO/PO block copolymer nonionics.

A major property of Ethylene oxide (EO)/Poly(propylene oxide) (PO) nonionics is their low-foaming profile.

As straight EO nonionics, EO/PO copolymers exhibit the cloud point phenomena.

EO/PO nonionics are also mild surfactants.

$$HO(CH_2CH_2O)_x[CH(CH_3)CH_2O]_y(CH_2CH_2O)_zH$$

Ethoxylated PPG Ether

[a] The International Cosmetic Ingredient Dictionary provides a nomenclature of conventional names for cosmetic ingredients that are defined by the Cosmetic, Toiletry, and Fragrance Association (CTFA).

These surfactants are especially useful for applications in which foaming must be significantly depressed, such as automatic dishwashing detergents, laundry detergents, and rinse aids.

Owing to their mildness, EO/PO block polymers also find applications in cosmetic products. They are generally used as emulsifying, solubilizing, or fluidizing agents.

APG

Alkyl polyglycosides are most often known as the simple abbreviation APG.

APGs are produced by the alkylation of short-chain glucosides resulting from acidic alcoholysis of polysaccharides such as starch. Commercial products consist of mixtures of mono-, di-, and triglucosides. Accordingly the glucosidic chain varies between 1.2 and 3 depending on the production conditions.

Surfactants of this class are good emulsifiers and provide good wetting and foam profile.

APG are compatible with all other surfactants. They show good chemical stability at neutral and alkaline pH, and are impaired under acidic conditions ($pH < 5$).

Alkylpolyglucoside

APGs are used in detergents and personal-care cleansers (e.g., shampoos). They are claimed to be very mild for skin as well as to reduce the skin-irritation potential of anionics. Additionally, they impart an excellent skin feel.

Their thickening effect in the presence of anionics and their foam stabilization capacity are also exploited in personal-care applications.

Ethoxylated Oils and Fats

This class of surfactants essentially covers ethoxylated derivatives of lanolin (i.e., aliphatic alcohols and sterols, fractionation products of wool fat) and of castor oil (i.e., fatty acids with a high ricinoleic acid fraction, extracted from ricinus seeds).

$$CH_3(CH_2)_5\underset{\displaystyle |\atop \displaystyle O\text{—}(CH_2CH_2O)_x\text{—}H}{CH}CH_2CH{=}CH(CH_2)_7\text{—}\overset{\displaystyle O\atop \displaystyle \|}{C}\text{—}O\text{—}(CH_2CH_2O)_y\text{—}H$$

PEG castor oil derivative

Ethoxylated products of lanolin and castor oil are good and excellent emulsifiers, respectively. These surfactants are mainly used in the cosmetic industry; their major interest is to offer the possibility of claims based on the natural origin of the constitutive surfactant systems.

Alkanolamides

Straight Alkanolamides

Alkanolamides are *N*-acyl derivatives of monoethanolamine and diethanolamine.

$$R\,CO\,NH\,CH_2CH_2OH \qquad R\,CO\,N\begin{cases} CH_2CH_2OH \\ CH_2CH_2OH \end{cases} \qquad R\,CO\,N\begin{cases} CH_2CH_2\;OCOR \\ CH_2CH_2OH \end{cases}$$

Monoalkanolamide   Dialkanol amide   Ester-amide

Alkanolamides have been largely used in household detergent products; their consumption has now significantly declined because of the extensive use of alkyl ethoxylated detergent products.

Because of their foam boosting and viscosity enhancing capacity in the presence of anionics, alkanolamides are also usefully incorporated in personal care, especially in shampoos.

Ethoxylated Alkanolamides

Reaction of an alkanolamide with ethylene oxide leads to an ethoxylated amide.

$$R\;CO\;NH(CH_2CH_2O)_n\;H$$

Polyethoxylated monoalkanolamide

It is more expensive than its corresponding ethoxylated alcohol and has therefore restricted usage. The benefits of thickening, foam stabilization, and dispersibility are exploited in personal-care cleansers.

Ester

In this surfactant class, there are five major subcategories to be considered:

1. ethoxylated fatty acids
2. glycol esters, glycerol esters, and ethoxylated derivatives
3. sorbitan esters and ethoxylated derivatives
4. alkyl carbohydrates esters
5. triesters of phosphoric acid

Ethoxylated Fatty Acids

This class of surfactants comprises mono- and diester that results from the reaction of fatty acids with either ethylene oxide or polyethylene glycol.

$$R-\overset{\overset{\displaystyle O}{\|}}{C}-O-(CH_2CH_2O)_n\text{-}H$$

PEG fatty acid ester

$$R-\overset{\overset{\displaystyle O}{\|}}{C}-O-(CH_2CH_2O)_n-\overset{\overset{\displaystyle O}{\|}}{C}-R$$

PEG fatty acid diester

Given their outstanding emulsifying properties, ethoxylated fatty acids are useful in domestic and industrial detergents, more especially in degreasing compositions.

If properly balanced, combinations of esters with low and high ethoxylation provide excellent emulsifiers for creams and lotions. They are also used as mild cleaners or viscosifying agents (e.g., PEG-150-distearate).

In cosmetics (shampoos), less water-soluble grade (i.e., ethylene glycol monostearate) is used as a pearlescent agent.

Glycol Esters, Glycerol Esters, and Ethoxylated Derivatives

A common point among the surfactants grouped in this class and the following two classes (sorbitan esters and alkyl carbohydrates esters) is that they all derive from the condensation reaction of a polyhydroxyl compound (e.g., glycol, glycerol, sorbitol, sucrose) with a fatty acid. Some of them can be directly extracted from natural sources.

The resulting esters can be additionally ethoxylated to increase their HLB value and, thereby, their solubility in water.

These surfactants show poorer wetting and foaming properties in comparison with alcohol-derived nonionics. Emulsifying properties are excellent.

In general, esters and lower ethoxylates are appropriate for w/o dispersions whereas higher ethoxylates are more suitable emulsifiers for o/w dispersions.

$R{-}C(=O){-}OCH_2CH_2OH$

Ethylene glycol ester

$R{-}C(=O){-}OCH_2CH(CH_3){-}OH$

Propylene glycol ester

$CH_2O{-}C(=O){-}R$ / $CHOH$ / CH_2OH

Monoglyceride

$CH_2O{-}C(=O){-}R$ / $CHOH$ / $CH_2O{-}C(=O){-}R$

1.3-diglyceride

$CH_2O{-}C(=O){-}R$ / $CHO(CH_2CH_2O)_nH$ / $CH_2O{-}C(=O){-}R$

Polyethoxylated 1.3-diglyceride

$HOCH_2CH(OH)CH_2O(CH_2CH(OH)CH_2O)_nCH_2CH(OH)CH_2O{-}C(=O){-}R$

Polyglyceryl monoester

Because of their high compatibility, these surfactants are widely used in the cosmetic and food industry.

Glycol and glycerol esters are used in the pharmaceutical and cosmetic industries either as emulsifying agents or as oily compounds, refatting agents, emollients, and skin conditioners in various products such as creams, lotions, ointments, and gels.

Stearate derivatives also deliver thickening and opacifying properties (e.g., the glyceryl stearate). Some of them are also used as pearlescent agents (i.e., glycol stearate and distearate).

Ethoxylated derivatives are used as solubilizing agents, emulsifiers, and even as emollients. Some show effective thickening effect when combined with other surfactants (e.g., PEG-200 glyceryl stearate).

Sorbitan and Sorbitol Esters and Ethoxylated Derivatives

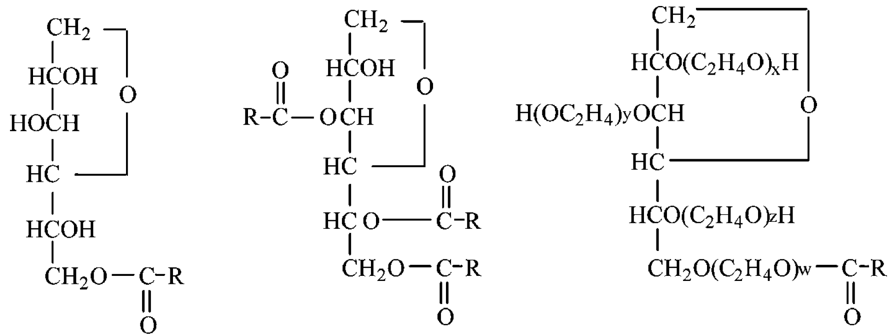
Sorbitan molecule is generated from the dehydration of the sorbitol molecule, which results in an internal ether bond.

CH_2OH
HCOH
HOCH
HCOH
HCOH
CH_2OH
Sorbitol

$-H_2O$ →

CH_2
HCOH
HOCH
HC
HCOH
CH_2OH
O
1, 4 Sorbitan

Sorbitol and sorbitan esters are obtained by acylation of hydroxyl groups, using most frequently natural fatty acids such as lauric, palmitic, stearic, or oleic. These surfactants can be optionally ethoxylated. Acylation (or ethoxylation) can occur on almost all hydroxyl groups present in the original polyol molecule.



1, 4 Sorbitan monoester 1, 4 Sorbitan triester Polyethoxylated Sorbitan

The field of application of sorbitan esters and their ethoxylated derivatives is identical to the one of glycol and glycerol esters (see previous section).

The sorbitol esters with a higher degree of ethoxylation (e.g., sorbitol septaoleate 40 EO) are also used as spreading aids in emollient bath oils.

Alkyl Carbohydrates Esters

Surfactants of this class are better known as "sugar esters" or "sucrose esters."

The sucrose esters are obtained by transesterification of sucrose with fatty acid methyl esters or triglycerides.

Surfactants of this class are good emulsifiers.

Of great interest about such surfactants is their natural origin and good biodegradability.

It is worth noting that some glucoside surfactants, e.g., the so-called *saponins*, are already present in nature and directly available from vegetal sources.

Saccharose fatty acid monoester

Sucrose esters are food-grade ingredients and have similar uses as the previously described glycol, glycerol, and sorbitan esters in the food and cosmetic industries.

They are very mild surfactants and can be used as emulsifiers or as cleansing agents with emollient properties.

Amine Oxides

Amine oxides are produced by the oxidation of tertiary amines using a 35% hydrogen peroxide solution as the oxidizing agent.

Amine oxides remain mainly nonionic in neutral and alkaline conditions ($pH > 7$) but can become weakly cationic under acidic conditions.

In current amine oxides, the initial reactives are alkyl dimethyl amines with chain lengths ranging from C_{12} to C_{18}.

Amine oxides are compatible with all other surfactants.

Amine oxides are also known to increase the skin compatibility of detergent products.

A small amount of amine oxide increases the cloud point of nonionics.

$$R-N(CH_3)_2 + H_2O_2 \xrightarrow{60\text{-}80^{\circ}C} R-N(CH_3)_2 \rightarrow O + H_2O$$

Incorporated in shampoos, amine oxides contribute to impart viscosity, reduce eye and skin irritancy, and enhance foam properties (more creamy). They are especially suitable in slightly acidic or neutral formulas.

NONHYDROCARBON SPECIALTY SURFACTANTS

Alkoxylated Polysiloxanes

Surfactants, which can be classified in the chemical group of organosilicones, are structurally derived from polydimethylsiloxanes in which some methyl are replaced by hydrophilic groups that can be of anionic, cationic, or nonionic nature.

The nonionic derivatives are mostly represented by the polyether–polydimethylsiloxane copolymers.

The general structure of these surfactants is illustrated below. The hydrophilic chain(s) generally contain EO/PO block copolymers.

$$(CH_3)_3\,Si-O-\left[\begin{array}{c} CH_3 \\ | \\ Si-O \\ | \\ CH_3 \end{array}\right]_m\left[\begin{array}{c} CH_3 \\ | \\ Si-O \\ | \\ \end{array}\right]_n Si\,(CH_3)_3$$

$$(CH_2)_p-O-(C_2H_4O)_x-(C_3H_6O)_y-H$$

Polysiloxan-Polyether Copolymer (p generally equals 0 or 3)

These surfactants are specialty ingredients and are used in very different fields (e.g., painting, foam control, phytosanitary products).

They are also used in cosmetics and hair care:

- in cosmetic or personal-care products as emulsifiers in, e.g., protective creams, hydrating body milks, liquid soaps, and shave creams, and
- in hair-care products (e.g., shampoos, conditioners, gels, lotions, foams) to act as combing out auxiliaries, reduce the irritancy of surfactant system, provide improved skin feel, or control the foam. The CTFA adopted name of these surfactants is Dimethicone Copolyol.

Fluorosurfactants

Fluorosurfactants form a distinct group of surfactants besides the conventional surfactants based on hydrocarbon chains.

Fluorosurfactants differ from hydrocarbon surfactants by the hydrophobic moiety of the molecule, which is made of perfluoroalkyls chains $F\text{–}(CF_2\text{–}CF_2)_n\text{–}$, in which n ranges from about 3 to about 8.

As for conventional surfactants, a broad variety of hydrophilic functional groups (e.g., ethoxylated chains, sulfonates, quaternaries, betaines) can be grafted on fluorosurfactants.

Depending on their nature, these surfactants show variable emulsifying and foaming characteristics.

Although fluorosurfactants have some potential prospects in personal care (e.g., improved hair conditioning), we are not aware of any significant application in this field. We can however report their use in barrier creams that require good spreading and stable o/w emulsions.

FURTHER READING

Rieger MM. Surfactant Encyclopedia. 2nd. Carol Stream: Allured Publishing, 1996.

Ash M, Ash I. Handbook of Industrial Surfactants. In: An International Guide to More Than 16,000 Products by Trade Name, Application, Composition and Manufacturer. Aldershot: Gower, 1993.

Falbe J. Surfactants in Consumer products. In: Falbe J, ed. Theory, Technology and Application. Berlin/New York: Springer, 1987.
Lange KR. Detergents and Cleaners: A Handbook for Formulators. München: Hanser Publishers, 1994.
Porter MR. Handbook of Surfactants. London: Blackie Academic & Professional, 1991.

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Anti-Irritants for Surfactant-Based Products

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In the scientific literature, sodium lauryl sulfate (SLS) is regularly used as the 'gold' model to induce skin irritation (1). This is for several reasons:

- SLS is classified as skin irritant, Xi-R38 (2),
- SLS can be obtained in a very pure form which allows different laboratories to work on the same material,
- SLS can be easily formulated in various vehicles,
- allergic reactions to SLS are not common, although a few cases were reported (3),
- the level of induced irritation can be controlled by adjusting the concentration (4,5), and any skin damage is rapidly reversible.

However, SLS is not the only surfactant to be irritant to the skin. Even if some surfactants are not classified as irritants by the Dangerous Substances Directive (2), in certain conditions and concentrations, all surfactants can be regarded as potential irritants to different degrees. This chapter will focus mainly on anionic surfactants, as they are mostly used in toiletries and require the most attention to optimize their skin compatibility in finished products.

Fortunately, nowadays many systems have been developed to minimize the risks of intolerance to hygiene cosmetics or surfactant-based products. This is extremely important because hygiene habits have strongly evolved over the years. Not so long ago, people came in contact with surfactants not more than once a day with the only objective to clean themselves; today it is not unusual to see people having several showers a day not only for hygiene purposes, but also for pleasure and relaxation. Because of their increased use, toilet products must be as mild as possible for the skin. Not only the mildest ingredients are used, but also finished hygiene products have to contain one or more anti-irritant systems.

Owing to their structure and diverse physicochemical properties, surfactants interact with the skin in various ways. When a surfactant comes into contact with the skin, it can:

- bind to the surface of the skin (proteins) and affect the skin feel;
- denature the skin surface proteins;

- interact with the lipidic components of the skin barrier (by extraction or disorganization) and increase its permeability;
- interact with the living cells of the epidermis and initiate the release of chemical messengers responsible for the inflammatory reaction;
- cause an oxidative stress and generate reactive oxygen species;
- interact with neurocomponents of the skin and cause sensorial signs of irritation.

Especially designed anti-irritant systems may be used to counteract the negative effect of surfactants on the skin.

ANTI-IRRITATION BY USING ONLY MILD SURFACTANTS

The first approach to develop a surfactant-based product that is mild to skin is to carefully select the mildest surfactants. Nonionic surfactants are generally considered as the mildest and are usual ingredients in body cleansing products for babies, for sensitive skin subjects, or for face cleansing products. However, several anionic surfactants are also extremely respectful of the skin condition and are often introduced in the same categories of products. These are, for instance, highly ethoxylated (at least 5-EO) alkyl sulfates, sulfosuccinate esters, sarcosinates, fatty acid–protein condensate, alkyl phosphate ester, alkyl glutamate, taurates, and others. Amphoteric surfactants are rarely used alone, but rather as secondary surfactants, thus their intrinsic irritation potential has no real meaning. Cationic surfactants are essentially used for their antibacterial properties rather than their tensioactive properties, and are often described in the literature as the most irritating surfactants. However, like for anionic surfactants, it is also possible to find very mild cationic surfactants. Owing to their low usage, the cationic surfactants will not be described in this chapter.

ANTI-IRRITATION BY AN APPROPRIATE COMBINATION OF SURFACTANTS

The best counter-irritants for surfactants are other surfactants. Several authors have clearly demonstrated such a positive interaction between various surfactants both in vitro (6,7) and in vivo (8–10), with diluted (6–8) or with highly concentrated solutions (9,10). Amphoteric surfactants are well known to decrease the irritation potential of anionic surfactants (11), but nonionic surfactants can display the same effect when used at a sufficiently high concentration. More surprisingly, certain anionic surfactants can reduce the irritation potential of another anionic surfactant, instead of cumulating their effects (9).

In aqueous solutions, surfactants tend to assemble by their hydrophobic tail and form micelles (12). The totality of surfactants is however not entrapped into the micelles and the micelles constantly form and dissociate at a rate that is dependent of the type of surfactants entering into their composition. Importantly, only the monomers of surfactant can directly interact with the skin proteins and cause irritation. When several surfactants are present in the same solution, surfactants form larger and more stable micelles, which can decrease the relative amount of monomers in the solution. This mechanism of how surfactants can be anti-irritants is well

accepted, but there are other potential mechanisms/hypotheses. For instance, the addition of a secondary surfactant milder than the primary one could decrease the binding of the primary surfactant to the skin surface by occupying and competing for the same binding site. A decrease in anionic surfactants' skin surface binding has been demonstrated by Attenuated Total Reflectance–Fourier Transformed Infra-Red Spectroscopy in presence of a secondary surfactant of any type (personal data).

ANTI-IRRITATION BY POLYMERS OR PROTEINS/PEPTIDES

The counter-irritant capability of polymers or proteins on surfactants has been reported in the literature for a long time (13–16). The mechanism by which polymers and proteins function is similar to the one described above for surfactant mixtures. They incorporate into the micelles and thus decrease the relative amount of free monomers in solution. Their skin substantivity can also involve blocking of binding sites at the surface of the skin thus making them nonaccessible to surfactants.

Polymers or proteins differ in their ability to interact with the skin surface and to be incorporated into the micelles. The following parameters should be considered when selecting a polymer/protein:

- a better interaction with the micelles correlates with increased hydrophobicity (13);
- a better substantivity with the skin correlates with higher hydrophobicity, when the polymer is quaternized or is cationic or when the net charge or the size of the polymer/protein increases (14–16).

As stated above, more hydrophobic and/or larger polymers/proteins are much more effective to depress the skin irritation potential of surfactants.

However, in literature the anti-irritant effect of proteins/polymers onto surfactants has been demonstrated mostly in single surfactant solution, and at a high polymer–surfactant ratio not always compatible with other properties of the finished product. When they are formulated into finished products already optimized for skin compatibility through an appropriate combination of surfactants, most polymers or proteins do not bring any further mildness benefit to the product.

ANTI-IRRITATION BY REFATTENING AGENTS

One of the negative effects of surfactants on skin is the alteration of its lipid barrier. This can be easily assessed by measuring the transepidermal water loss (17,18) which increases with an impairment of the barrier. Using refattening ingredients or skin barrier repairing ingredients in surfactant-based products can reduce the disruption of the barrier function if these ingredients are appropriately delivered to the skin surface. Such ingredients are often the basis for the barrier cream effect of creams (lotions) topically applied before or after contact with an irritant. Some of these ingredients can also be formulated into a surfactant system and act directly as anti-irritants in the mixture. The occlusive effect they bring at the surface of the skin delays the water loss and maintains the skin in a less dehydrated state. Furthermore, they can progressively form an additional barrier protecting the skin against the surfactants in repetitive product application conditions. Several types of refattening

ingredients are available and can be formulated into surfactant systems. Among these are ethoxylated mono-, di-, and triglycerides, fatty alcohols, and ethoxylated fatty alcohols, fatty acid esters, lanolin derivatives, or silicone derivatives. A few products containing a high percentage of oil also exist and can possibly play such a role.

ANTI-INFLAMMATORY EFFECT

Anti-inflammatory ingredients are not specific for surfactant-induced irritation, and most of them are used in pharmacology rather than in cosmetology. Due to the complexity of the inflammatory process, several families of anti-inflammatory ingredients have been developed such as glucocorticoids, nonsteroidal anti-inflammatory drugs (tacrolimus, cyclosporin, rapamycin, ascomycin, and leflunomide), flavonoids, essential oils, or alpha-bisabolol (19–21). In order to be effective, such ingredients must be delivered to the skin in a bioavailable form and in a sufficient amount. The case of essential oils, of flavonoids and of alpha-bisabolol, is discussed in more details in other sections of this handbook.

ANTIOXIDANTS

In biological systems, antioxidant processes have a protective role against oxidative stress through three different mechanisms:

- by scavenging the early pro-oxidant species,
- by preventing the initiation or the propagation of the free-radical reactions,
- by returning oxidized groups to their reduced state.

In dermatology and cosmetology applications, antioxidants belong to a relatively new field of investigation and interest. Some of the most important antioxidants with known applications are vitamin E, vitamin C, thiols, and flavonoids. Their mechanism of action in the antioxidant process is reviewed by Weber et al. (chap 31). In surfactant-based products, antioxidants are only occasionally used to reduce the skin irritation potential of the product (22).

ANTISENSORY IRRITATION

Although much less discussed than the clinical irritation which is characterized by observable or functional alterations, subjective irritation also exists. It does not have great interest for the dermatologists, but for cosmetologists it can be the reason for the success or rejection of their product.

Three different categories of sensory signals of irritation have been identified. Briefly:

- stinging, burning, itching signals;
- dryness, tightness perception preceding clinical signs of irritation;
- peculiar "irritated-skin" perceived signals unrelated with a true irritation process.

They will require different "anti-irritant" systems.

Anti-Irritants for Stinging, Burning, and Itching Sensations

Strontium salts have been demonstrated to be effective and selective anti-irritants for chemically induced sensory irritation associated with stinging, burning, or itching manifestations (23). Strontium salts (nitrate or chloride) are claimed to be especially indicated for subjects with sensitive skin in the face, and prone to stinging sensations (23,24). The interest of strontium salts, as described by Hahn (23), is that they are very specific and selective inhibitors of the sensory signals of irritation, without suppressing all the warning signals or the other sensations associated to other receptors (e.g., temperature, tactile, pressure, ...).

Several controlled clinical studies (23,25) were run to show that strontium nitrate or chloride, at a concentration from 5% to 20%, effectively suppresses or reduces sensory irritation caused by chemical or biological irritants over a wide range of pH from 0.6 to 12. In some tests, strontium salts were included in the solution with the irritant, or before or after the application of the irritant as shown in Table 1.

Although not tested in surfactant systems, strontium salts may play a similar beneficial effect on surfactant-induced sensory irritation, mainly in certain classes of sensitive skin subjects.

As described with the mechanism of sensory irritation, it has been observed in some studies that, on top of reducing the sensorial signs of irritation, strontium salts could also decrease the level of erythematous reaction generated by the irritant.

Several hypotheses to explain the mechanism of action of strontium salts were suggested (23,26) but this latter still remains unclear:

- the action of strontium salts being quite immediate after application, it is assumed that they act directly on the type-C nociceptor and suppress the neuronal depolarization that normally transmits the sensory signal to the brain;

Table 1 Clinical Tests Support the Antisensory Irritant Potential of Strontium Salts (23)

Irritant	Test site	Timing of application[a]
Lactic acid, 7.5%, pH 1.9 (solution)	Face	Mixed, pre-, or post-
Lactic acid, 15%, pH 3.0 (solution)	Face	Mixed
Glycolic acid, 70%, pH 0.6 (peeling solution)	Arm	Mixed
Capryloyl salicylic acid, 1% (exfoliant cream)	Cheek	Mixed
Ascorbic acid, 30%, pH 1.7 (solution)	Face	Mixed
Aluminum chloride, 20% (antiperspirant preparation)	Axilla	Pre-
Aluminum/zirconium salt, 25% (antiperspirant solution)	Arm	Mixed
Calcium thioglycolate, pH 9–12 (depilatory lotion)	Leg	Post
Histamine (intradermal injection, 100 μg)	Forearm	Pre-

[a]"Pre-" means that strontium salts were applied to skin prior to the irritant, "Post-" means that the salts were applied after skin had been irritated by the irritant, and "Mixed" means that strontium salts were included in the preparation with the irritant.

- by their analogy to calcium strontium salts could use calcium channels to induce the release of neurotransmitters in synapsis, or could antagonize the usual calcium-induced depolarization;
- it is also not impossible that strontium salts could directly influence keratinocytes or inflammatory cells and regulate the release of some cytokines.

Anti-Irritants for Dryness/Tightness Perception Preceding Clinical Signs of Irritation

Tightness and dryness perceptions are usually the earliest warning signs detected by highly receptive subjects using products which are not irritating by one single use, but which can become slightly irritating or skin drying after multiple exposures. These signs are generally followed, if the product is not discontinued, by the progressive development of clinical signs of intolerance such as scaling, flaking, or even erythema (27).

This kind of subclinical irritation is essentially observed for surfactant-based products, and the anti-irritant systems described for surfactant-induced irritation are thus valid. Additionally, topical skin rehydrating preparations can also be effective in some cases to decrease the dryness/tightness perception.

Antinegative Skin Feel

Negative subjective signals that are translated as "irritated skin" by the consumers whilst totally independent of irritation can be addressed in two ways:

- if these signals are induced by the surfactant-based product, the surfactant system should be reformulated. Indeed, each surfactant is associated with a specific perception to the skin such as slipperiness, smoothness (perception of a mild product) or, at the extreme, roughness and drag (perception of an irritant product). A good combination of surfactants can provide the desired skin feel and signal.
- skin feel additives may be added to the product to deliver smoothness, silkiness, hydrated feel, ... associated with a "nonirritated" skin signal. A review of the skin feel additives has been made by Zocchi in another section of this handbook (chap. 19).

MAGNESIUM IS NOT AN ANTI-IRRITANT FOR SURFACTANTS

Magnesium is frequently described as a depressor of skin irritation. Such a false idea is essentially arising from in vitro data based on protein denaturation tests. In these tests, the more a surfactant solution denatures a protein, the more it is predicted as irritant, for the skin and magnesium clearly depresses surfactant-induced protein denaturation in vitro (29). However, when well-controlled in vivo tests are performed to investigate the effect of magnesium directly on human volunteers, it is confirmed that magnesium does not decrease the skin irritation potential of surfactants or surfactant-based products (28). The in vivo studies included both acute irritation by occlusive patch tests and chronic irritation by repetitive short-term applications of the products. The study compared sodium and magnesium salts of surfactants (e.g., magnesium and SLS) in single solutions or incorporated into

finished products, and investigated the effect of adding magnesium sulfate to a solution of surfactant.

Some preliminary studies with calcium showed a behavior similar to magnesium (personal data) with an inhibition of protein denaturation in vitro while no reduction of irritation in vivo.

CONCLUSION

This chapter briefly reviews several systems by which it is now possible to control the skin irritation potential of surfactant-based products. This can be done

- through a modification of surfactant behavior in solution,
- through a modification of their interaction with skin surface,
- through a protection of the skin surface via ingredients (e.g., lipids, proteins, ...) from the solution,
- through a control of their subjective perception by the consumer using strontium salts or skin feel agents.

These anti-irritant systems combined with a selection of mild surfactants allow the cosmetic formulator to design very mild hygiene products.

Other anti-irritant systems also exist for leave-on cosmetics and in pharmacology such as antioxidants and anti-inflammatory ingredients. They are still not yet commonly used in surfactant-based products but if correctly delivered to the skin during the use of the product they could provide a new field of research for improving the tolerance of cleansing products.

REFERENCES

1. Lee CH, Maibach HI. The sodium lauryl sulfate model: an overview. Contact Dermatitis 1995; 33:1–7.
2. EC Directive 67/548/EEC.
3. Prater E, Goring HD, Schubert H. Sodium lauryl sulfate—A contact allergen. Contact Dermatitis 1978; 4:242–243.
4. Dillarstone A, Paye M. Classification of surfactant-containing products as 'skin irritants'. Contact Dermatitis 1994; 30:314–315.
5. Agner T, Serup J. Sodium lauryl sulphate for irritant patch testing—a dose–response study using bioengineering methods for determination of skin irritation. J Invest Dermatol 1990; 95:543–547.
6. Rhein LD, Simion FA. Surfactant interactions with skin. Surf Sci Ser 1991; 32:33–49.
7. Rhein LD, Robbins CR, Fernee K, et al. Surfactant structure effects on swelling of isolated human stratum corneum. J Soc Cosmet Chem 1986; 37:125–139.
8. Lee CH, Kawasaki Y, Maibach HI. Effect of surfactant mixtures on irritant contact dermatitis potential in man: sodium lauroyl glutamate and sodium lauryl sulphate. Contact Dermatitis 1994; 30:205–209.
9. Dillarstone A, Paye M. Antagonism in concentrated surfactant systems. Contact Dermatitis 1993; 28:198.
10. Hall-Manning TJ, Holland GH, Rennie G, et al. Skin irritation potential of mixed surfactant systems. Food Chem Toxicol 1998; 36:233–238.
11. Dominguez JG, Balaguer F, Parra JL, Pelejero CM. The inhibitory effect of some amphoteric surfactants on the irritation potential of alkylsulphates. Int J Cosmet Sci 1981; 3:57–68.

12. Tamura T, Masuda M. Surfactants: physico-chemistry. In: Barel AO, Paye M, Maibach HI, eds. Handbook of Cosmetic Science and Technology. New York: Marcel Dekker, 2001:417–430.
13. Teglia A, Secchi G. New protein ingredients for skin detergency: native wheat protein–surfactant complexes. Int J Cosmet Sci 1994; 16:235–246.
14. Teglia A, Mazzola G, Secchi G. Relationships between chemical characteristics and cosmetic properties of protein hydrolysates. Cosmet Toilet 1993; 108:56–65.
15. Goddard ED, Leung PS. Protection of skin by cationic cellulosics: in-vitro testing methods. Cosmet Toilet 1982; 97:55–69.
16. Pugliese P, Hines G, Wielenga W. Skin protective properties of a cationic guar derivative. Cosmet Toilet 1990; 105:105–111.
17. Van der Valk PGM, Nater JP, Bleumink E. Skin irritancy of surfactants as assessed by water vapor loss measurements. J Invest Dermatol 1984; 82:291–293.
18. Kawasaki Y, Quan D, Sakamoto K, Cooke R, Maibach HI. Influence of surfactant mixtures on intercellular lipid fluidity and skin barrier function. Skin Res Technol 1999; 5:96–101.
19. Schön MP, Homey B, Ruzicka T. Antiphlogistics (dermocorticoids and topical immunomodulators). In: Gabard B, Elsner P, Surber C, Treffel P, eds. Dermato-pharmacology of topical preparations. Berlin, Heidelberg: Springer-Verlag, 2000:179–190.
20. Li BQ, Fu T, Gong W, et al. The flavonoid baicalin exhibits anti-inflammatory activity by binding to chemokines. Immunopharmacology 2000; 49:295–306.
21. Stanzl K, Vollhardt J. The case of alpha-bisabolol. In: Barel AO, Paye M, Maibach HI, eds. Handbook of Cosmetic Science and Technology. New York: Marcel Dekker, 2001:277–284.
22. Katsarou A, Davoy E, Xenos K, Armenaka M, Theoharides TC. Effect of an antioxidant (quercetin) on sodium-lauryl-sulfate-induced skin irritation. Contact Dermatitis 2000; 42:85–89.
23. Hahn GS. Anti-sensory irritants. In: Barel AO, Paye M, Maibach HI, eds. Handbook of Cosmetic Science and Technology. New York: Marcel Dekker, 2001:285–298.
24. Hahn GS. Strontium is a potent and selective inhibitor of sensory irritation. Dermatol Surg 1999; 25:689–694.
25. Zhai H, Hannon W, Hahn G, Pelosi A, Harper A, Maibach HI. Strontium nitrate suppresses chemically-induced sensory irritation in humans. Contact Dermatitis 2000; 42: 98–100.
26. Brewster B. MDs address sensory irritation from AHAS. Cosmet Toilet 2000; 113(4): 9–10.
27. Simion FA, Rhein LD, Morrison BM Jr, Scala D, Salko DM, Grove GL. Self-perceived sensory responses to soap and synthetic bars correlate with clinical signs of irritation. J Am Acad Dermatol 1995; 32:205–211.
28. Paye M, Zocchi G, Broze G. Magnesium as skin irritation depressor: fact or artifact? Proceedings of the XXVIII Jornadas Anuales del CED, Barcelona, Spain, June 1998: 449–456.
29. Goffin V, Paye M, Piérard GE. Comparison of in vitro predictive tests for irritation induced by anionic surfactants. Contact Dermatitis 1995; 33:38–41.

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Seawater Salts: Effect on Inflammatory Skin Disease: An Overview

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Use of mineral spa water and seawater has been and continues to be a common treatment modality for inflammatory skin conditions such as psoriasis, atopic dermatitis, and irritant contact dermatitis. Spa water and seawater are noted for their relatively high concentrations of minerals such as strontium and selenium and for their high osmolarity relative to physiological saline (Table 1). Despite widespread use, few studies explore what aspect of seawater accounts for its therapeutic effect and what is its mechanism of action. Recent studies are summarized in Table 2.

SEAWATER

Recent in vivo and in vitro studies lend credence to the common practice of applying seawater to inflamed skin. In acute eruptions of atopic dermatitis, seawater exhibited antipruritic effects as evaluated by a significant reduction of visual analogue scores for itching (4). In the setting of irritant contact dermatitis, Pacific ocean water compresses significantly decreased transepidermal water loss (TEWL) and increased skin capacitance compared to the deionized water control when the compresses were applied for 20 minutes at a time for several times over the course of two weeks (12). TEWL measures water barrier disruption, while capacitance measures stratum corneum water content. Thus, the results provide evidence for seawater's ability to inhibit skin barrier disruption and inhibit stratum corneum dryness in irritant contact dermatitis. Seawater has also been shown to be of benefit in psoriasis. In a randomized, double-blinded, controlled study, Dead Sea® salt baths, containing a high mineral composition, were administered daily at 35°C for 20 minutes for three weeks. Relative to the distilled water control, Dead Sea salt baths significantly decreased psoriasis area and severity index (PASI) scores in psoriasis vulgaris patients immediately after treatment with therapeutic effects still significant one month after the treatment ended. However, there was no statistical difference in PASI scores and patient subjective evaluations between the treatment group that received Dead Sea salt baths and the group that received common salt baths (mostly

Table 1 Composition of Seawater at 3.5% Salinity

Element	Atomic weight	Parts per million
Sodium (NaCl)	22.9898	10,800
Potassium	39.102	392
Magnesium	24.312	1290
Strontium	87.62	8.1
Selenium	78.96	0.0009
Bromine	79.909	67.3

Note: Parts per million = mg/liter = 0.001 g/kg.
Source: Adapted from www.cea-life.com.

sodium chloride) of the same osmolality. While this study supports seawater's therapeutic effects, it suggests that osmolality, instead of ion character, may act as the active component in seawater therapy (3).

CATIONS

Sodium

As a substantial component of seawater, sodium has been explored as an explanation for seawater's therapeutic effect. Similar to seawater, compresses with 500 mM NaCl alone have been shown to inhibit the increase in TEWL and increase skin capacitance and, thus, inhibit skin barrier disruption and inhibit stratum corneum dryness in irritant contact dermatitis (12). Sodium compresses for irritant contact dermatitis also decreased blood flow associated with irritation relative to baseline values when applied for 30 minutes twice a day for four consecutive days (6). However, the same study also showed that sodium compresses did not significantly change the skin's clinical appearance as measured by chromametry and clinical scoring. Sodium's role in seawater therapy is further questioned in this study by the finding that there was no significant difference between sodium compresses and cool water compresses, and the idea that neither osmolality nor ions, but temperature explains the therapeutic effect of seawater (6). In vitro psoriasis studies also imply that sodium may not account for seawater's clinical value. Psoriasis is characterized by epidermal hyperplasia and heightened mitotic activity. In vitro, sodium salts failed to significantly affect fibroblast proliferation (5).

Potassium

As with sodium, studies yield conflicting results regarding potassium's role in explaining seawater therapy. The 10 mM KCl compresses inhibited skin barrier disruption by inhibiting an increase in TEWL but had no effect on capacitance and, thus, stratum corneum dryness in irritant contact dermatitis (12). While potassium seems to have some effect on irritant contact dermatitis, its effect on psoriasis is less clear. An in vitro psoriasis experiment showed that potassium salts failed to significantly affect fibroblast proliferation (5), whereas another in vitro study that included two hour incubations with salt solutions of 0, 50, 100, and 300 mM demonstrated that potassium salts were more effective than those of sodium and magnesium in reversibly inhibiting fibroblast

Table 2 Seawater and its Effects on Inflammed Skin

Source	No. of patients	Type of dermatitis	Therapy	Duration	Evaluation	Significant results	Conclusion
Yoshizawa et al. (12)	3	Irritant dermatitis	Sea water (Pacific ocean)	20 mins, 8× over 2 wks	TEWL, capacitance	↓TEWL, ↑capacitance	Inhibits barrier disruption and stratum corneum dryness
			500 mM NaCl			↓TEWL, ↑capacitance	Inhibits barrier disruption and stratum corneum dryness
			10 mM KCl			↓TEWL	Inhibits barrier disruption only
			55 mM $MgCl_2$			Not significant	
			10 mM $CaCl_2$			Not significant	
Levin and Maibach (6)	9	Irritant dermatitis	H_2O compress	30 mins, bid for 4 d	TEWL, LDF, chromametry, clinical score	↓TEWL, LDF	Both compresses *equally* inhibit barrier disruption and microcirculatory blood flow associated with inflammation but do not affect the clinical score or color
			Physiological saline compress			↓TEWL, LDF	
Celerier et al. (1)	In vitro	Atopic dermatitis	Spa water	1 wk continuous immersion	Inflammatory cytokine levels: IL-1α, IL-6, TNF-α	↓IL-1α, ↓↓↓IL-6, ↓TNF-α	Inhibits all three cytokines, but inhibits IL-6 to a greater degree
			260 μg/L $SrNO_3$			↓IL-1α, ↓↓↓IL-6, ↓↓TNF-α	Sr salts inhibit all three cytokines, but inhibit IL-6 to a greater degree. Sr salts

(*Continued*)

Table 2 Seawater and its Effects on Inflammed Skin (*Continued*)

Source	No. of patients	Type of dermatitis	Therapy	Duration	Evaluation	Significant results	Conclusion
							selectively inhibit TNF-α production
			260 μg/L $SrCl_2$			↓IL-1α, ↓↓↓IL-6, ↓↓TNF-α	
			60 μg/L $SeCl_2$			↓IL-1α, ↓↓IL-6, ↓TNF-α	Se salts inhibit all three cytokines, but inhibit IL-6 to a greater degree. Se salts selectively inhibit IL-1α production
			60 μg/L $SeNaO_3$			↓↓IL-1α, ↓↓↓IL-6, ↓TNF-α	
			60 μg/L $SeNaO_4$			↓↓IL-1α, ↓↓↓IL-6, ↓TNF-α	
Hiramatsu et al. (4)	20	Atopic dermatitis	Salt water	4 wks	VAS[a] for itching	5.05 ± 1.53 (pre) to 2.8 ± 2.4 (post)	Decreased itching of acute eruptions
Halevy et al. (3)	30	Psoriasis vulgaris	35°C Dead sea salt bath	20 mins qd for 3 wks	PASI, Patient subjective evaluation	$34.8 \pm 24\%$ reduction after 3 wks, $43.6 \pm 31.1\%$ reduction after 7 wks	Dead sea salt bath significantly decreased PASI over course of treatment; effect lasted for 1 month after treatment. However, the Dead Sea salt bath's effect on PASI did not significantly differ from the common salt bath

Gambichler et al. 2001	10	Psoriasis	NaCl (24%) immersion followed by phototherapy (280–365 nm)	20 mins, 30× over 7.5 wks	Clinical score (desquamation, erythema, infiltration of plaques)	68.4% reduction from baseline	Significant decrease in clinical score from baseline, but effect is not significantly different from tap water control
Levi-Schaffer et al. (5)	In vitro	Psoriasis	75 mM NaCl 75 mM KCl 75 mM $MgCl_2$ 75 mM $MgBr_2$ 75 mM KBr	1, 2, or 3 days	Fibroblast proliferation, cAMP levels	$MgCl_2$ inhibits proliferation by $50.7 \pm 2.2\%$; $MgBr_2$ inhibits proliferation by $55.0 \pm 2.3\%$; No treatments significantly affected cAMP levels	Mg salts had a significantly stronger inhibitory effect on fibroblast proliferation than other salt treatments of the same osmolarity. The inhibitory effect was immediate (within 24 hrs)
Shani et al. (11)	In vitro	Psoriasis	0, 50, 100, 300 mM NaCl	2 hr incubation	Thymidine incorporation	Maximum reduction: 44.4%	Br salts significantly inhibit growth greater than Cl salts. K exerted the greatest growth inhibition with KBr's inhibitory effect similar to that of the diluted Dead Sea brine
			0, 50, 100, 300 mM NaBr			52.4%	
			0, 50, 100, 300 mM KCl			54.3%	
			0, 50, 100, 300 mM KBr			86.5%	
			0, 50, 100, 300 mM $MgCl_2$			32.5%	
			0, 50, 100, 300 mM $MgBr_2$			47.7%	

[a]Visual analog scale.

proliferation and that KBr's inhibitory effect was similar to that of Dead Sea water (positive control) (11).

Magnesium

Recent studies suggest that magnesium has a greater influence on inflammatory processes in psoriasis than in irritant contact dermatitis. The 55 mM magnesium salt compresses showed no significant effects on barrier disruption or stratum corneum dryness in irritant contact dermatitis (12). However, magnesium illustrated significant and immediate (within 24 hours of treatment) inhibition of fibroblast proliferation in the in vitro psoriasis studies (5). Additional in vitro and in vivo studies showed that magnesium ions reduced the antigen-presenting capacity of Langerhans cells and are associated with reduced expression of HLA-DR and costimulatory B7 molecules by Langerhans cells (9). This last study hints at a possible role for magnesium in limiting the initial immune response or ongoing inflammation process in psoriasis.

Strontium and Selenium

Long suspected as the reason for the efficacy of Dead Sea water therapy, strontium and selenium have been shown to possess anti-inflammatory properties.

In a double-blind, vehicle-controlled, random-treatment study on irritant contact dermatitis, strontium salts were applied topically as pretreatment or mixed with irritant and were found to decrease the duration and magnitude of inflammation and sensory irritation (stinging, burning, and itching) without local anesthetic effects. Strontium inhibited total cumulative irritation from 56% to 81% according to a patient report, and these findings held true for the broad range of chemically unrelated irritants such as glycolic acid, lactic acid, aluminum chloride, and calcium thioglycolate that were used in the study (2). In another study, strontium exhibited anti-inflammatory effects on the molecular level.

An in vitro, controlled study looked at the effects of strontium and selenium on cutaneous inflammatory cytokines, IL-1α, IL-6, and TNF-α, at concentrations similar to those found in the Dead Sea. A week long continuous immersion of both healthy and atopic dermatitis skin in 260 μg/L strontium showed that strontium did not significantly affect cytokine levels in healthy skin. However, the study did show that strontium salts significantly inhibited all three cytokines relative to baseline values, but that strontium selectively inhibited TNF-α to a greater degree (1).

A potential role for selenium in reducing inflammatory processes in skin has also been supported by recent studies. In the aforementioned study, healthy and atopic dermatitis skin were also immersed in 60 μg/L selenium solution for one week. In normal, healthy skin, selenium significantly decreased IL-1α cytokine levels but had no effect on IL-6 or TNF-α levels relative to that of the control medium. In atopic dermatitis skin, selenium salts significantly inhibited all three cytokines relative to baseline values but selectively inhibited IL-1α to a greater degree (1). Selenium has also been correlated with the duration and severity of psoriasis and may be related to the protective function of selenoproteins (thioredoxin reductases and glutathione peroxidases) against ultraviolet-induced cell damage and death. Both low plasma selenium and low plasma glutathione peroxidase activity have been seen in psoriasis patients. Patients with a longer history (>3 years) of psoriasis exhibited a significantly lower Se level compared to patients with a shorter history

of psoriasis (<10 months). Selenium also seemed to correlate with the severity of psoriasis in this study. A significant inverse relationship was found between RBC glutathione peroxidase and psoriasis area and severity index scores in individuals with psoriasis of greater than three years (10).

ANIONS

Bromine

Of the few anions in seawater that are studied, bromine has the most evidence for affecting skin disease processes. In an in vitro study of psoriasis with two hour incubation with Dead Sea Brine, NaCl, NaBr, KCl, KBr, MgCl, MgBr at 0, 50, 100, and 300 mM, bromide salts significantly inhibited fibroblast proliferation as compared with chloride salts. When combined with potassium in KBr, bromine's inhibitory effect was similar to that of diluted Dead Sea (positive control) (11). In another in vitro study, magnesium bromide inhibited fibroblast proliferation to a greater extent than magnesium chloride ($55.0 + 2.3\%$ vs. $50.7 + 2.2\%$), lending further evidence that if anions play a part in seawater therapy, bromine, not chlorine, is most likely to be the active anion (5).

With a paucity of studies and small sample sizes in each study, it is difficult to definitively say whether seawater or its individual components offer any clinical benefit in the inflammations of the skin. If seawater is proven to be of therapeutic value, further studies will be needed to explore whether it is the synergism between seawater's various components, the osmolality, or individual ions alone that mediate its effect. As inflammatory skin diseases like eczema and psoriasis are accompanied by a defect in permeability barrier function, improving barrier function results in reduced inflammation. Thus, possible mechanisms of action of seawater salts include putative effects on barrier functions. This effect of ions on barrier function has been studied extensively (7,8).

Currently, Dead Sea salt is sold in many countries and is used in clinical treatments and private bathtubs but in much lower concentrations than the Dead Sea itself. Recent interest in the science of alternative medicines may be a stimulus for a more complete biological analysis of these ancient practices.

REFERENCES

1. Celerier P, Richard A, Litoux P, Dreno B. Modulatory effects of selenium and strontium salts on keratinocyte-derived inflammatory cytokines. Arch Dermatol Res 1995; 287: 680–682.
2. Hahn G. Strontium is a potent and selective inhibitor of sensory irritation. Dermatol Surg 1999; 25:689–694.
3. Halevy S, Giryes H, Friger M, Sukenik S. Dead Sea bath salt for the treatment of psoriasis vulgaris: a double-blind controlled study. J Eur Acad Dermatol Venereol 1997; 9:237–242.
4. Hiramatsu H, Seino M, Nagase A, Arai S, Mukai H. Salt water therapy for patients with atopic dermatitis. Nishinihon J Dermatol 1998; 60:346–349.
5. Levi-Schaffer F, Shani J, Politi Y, Rubinchik E, Brenner S. Inhibition of proliferation of psoriatic and healthy fibroblasts in cell culture by selected Dead Sea salts. Pharmacology 1996; 52:321–328.

6. Levin C, Maibach H. Do cool water or physiologic saline compresses enhance resolution of experimentally induced irritant contact dermatitis? Contact Derm 2001; 45:146–150.
7. Mauro T, Dixon DB, Komuyes L, Hanley K, Pappone PA. Keratinocyte K^+ channels mediate Ca^{2+}-induced differentiation. J Invest Dermatol 1997; 108(6):864–870.
8. Mauro T, Guitard M, Behne M, Crumrine D, Rassner U, Elias PM, Hummler E. The ENac channel is required for normal epidermal differentiation. J Invest Dermatol 2002; 118(4):589–594.
9. Schempp C, Dittmar H, Hummler D, Simon-Haarhaus B, Schulte-Monting J, Schopf E, Simon J. Magnesium ions inhibit the antigen-presenting function of human epidermal Langerhans cells in vivo and in vitro. Involvement of ATPase, HLA-DR, B7 molecules, and cytokines. J Invest Dermatol 2000; 115:680–686.
10. Serwin A, Wasowicz W, Gromadzinska J, Chodynicka B. Selenium status in psoriasis and its relation to the duration and severity of the disease. Nutrition 2003; 19:301–304.
11. Shani J, Sharon R, Koren R, Even-Paz Z. Effect of Dead Sea brine and its main salts on cell growth in culture. Pharmacology 1987; 35:339–347.
12. Yoshizawa Y, Tanojo H, Kim SJ, Maibach H. Seawater or its components alter experimental irritant dermatitis in man. Skin Res Tech 2001; 7:36–39.

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Antioxidants

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INTRODUCTION

In the field of dermatology, antioxidants are widely used and are innovative ingredients in topical applications. This chapter is intended to provide an overview of the current state of research on the use of antioxidants in cosmeceutical applications. The most important antioxidants, vitamin E, vitamin C, thiols, and flavonoids, will be introduced, and their intriguing cooperation as well as their role in signal transduction events will be discussed. The body is continuously exposed to oxidants. Endogenous sources arise as a consequence of normal metabolic pathways. For example, mitochondrial respiration produces superoxide and hydrogen peroxide, whilst enzymes such as lipoxygenases, xanthine oxidase, and NADPH oxidase produce hydroperoxides and superoxide, respectively. Exogenous oxidants arise from environmental pollutants such as smoke, smog, UV radiation, and diet. In response to these oxidants, a number of systemic antioxidants are available whose functions are to scavenge reactive oxygen species preventing damage to macromolecules such as lipids, DNA, and proteins. Antioxidant protection arises from molecules synthesized as part of metabolism, e.g., glutathione (GSH) and uric acid; essential vitamins which must be taken in from the diet, e.g., vitamins E and C; enzymes which decompose reactive oxygen species, e.g., superoxide dismutases, catalase, and the glutathione peroxidases. These systems provide protection in various intra- and intercellular compartments. Usually there is a tight balance between oxidants produced and antioxidant scavenging, however, under certain conditions the balance can be tipped in favor of the oxidants, a condition called oxidative stress. Potentially oxidative stress can be caused either by an increase in the number of oxidants, for example, as a result of cigarette smoking or UV irradiation, or by a deficiency in antioxidants. This is of major concern because oxidative stress has been implicated in a number of conditions including atherosclerosis, skin cancer, and photoaging.

Figure 1 Naturally occurring forms of vitamin E. Tocopherols contain a saturated side chain (A), whereas the isoprenoid side chain of tocotrienols is polyunsaturated (B). The a-forms contain both methyl groups on the chromanol nucleus (1,2), whereas the p-forms contain only methyl group (1), the y-forms only (2), and the 5-forms none.

VITAMIN E

Vitamin E is the major lipophilic antioxidant in skin, and it is the most commonly used natural antioxidant in topical formulations. It is found in all parts of the skin, the dermis, and epidermis, as well as in the stratum corneum, and is believed to play an essential role in the protection of biomolecules from oxidative stress.

Vitamin E is a family of eight naturally occurring isoforms: four tocopherols (a-, p%, y-, 8-form) and four tocotrienols (a-, J-, y-, 8-form) (Fig. 1) (1). All forms consist of a chromanol nucleus that carries the redox-active phenolic hydroxyl group, and a lipophilic tail. While tocopherols contain a phytil side chain, the isoprenoid tail of the tocotrienols is polyunsaturated, making the chain more rigid. The side chain is anchored in lipid membranes while the nucleus is located at the lipid/aqueous interface. Even though the radical-scavenging activity of the different isoforms is essentially identical, their biological activity after oral administration differs dramatically (2). This phenomenon can be explained by the existence of an α-tocopherol transfer protein in the liver that positively selects RRR-α-tocopherol and incorporates it into very low density lipoproteins which leads to recirculation of the oc-tocopherol pool, while this transfer protein does not recognize the other forms, which are therefore excreted more rapidly (3).

In skin, as in the other human organs, α-tocopherol is the predominant form of vitamin E with five to ten higher concentrations than y-tocopherol. Delivery of vitamin E to the SC occurs in two different modes. On the one hand, it stored into differentiating keratinocytes and moves up into the newly formed SC, which leads to a gradient-type distribution of α-tocopherol with decreasing concentrations toward the skin surface (4). On the other hand, vitamin E is secreted by sebaceous glands and reaches the SC from the outside. In sebaceous gland-rich regions like the face, this delivery mechanism is responsible for the enrichment of the outer SC with vitamin E (5).

Various oxidative stressors have been shown to deplete vitamin E, among other antioxidants. In the epidermis, a dose of at least four minimal erythemal doses (MED) of solar simulated UV radiation is needed to deplete vitamin E (6), while doses as low as 0.75 MED are capable of destroying vitamin E in the human SC (4). Mouse experiments have shown that a dose of 1 ppm $\times$ 2 h of ozone (O_3) depletes SC vitamin E (7). Because this concentration of O_3 is higher than the naturally occurring levels of tropospheric O_3 the biological relevance of these findings for

the skin of humans is not yet clear. A one time application of benzoyl peroxide (BPO) (10% w/v), a concentration commonly used in the treatment of acne, depleted most of the SC vitamin E in human volunteers (8).

oc-Tocopherol is widely used as an active ingredient in topical formulations. After topical application, it penetrates readily into skin (9). Because the free form of vitamin E is quite unstable and light sensitive (it absorbs in the UV-B range), the active hydroxyl group is usually protected by esterification with acetate. This increases the stability but renders the compound redox inactive. When administered orally, vitamin E acetate is hydrolyzed quantitatively in the intestines (10). There is some controversy however as to whether α-tocopherol acetate can by hydrolyzed in human skin. Chronic application of α-tocopherol acetate leads to an increase in free vitamin E in both the rat (11) and the mouse (12), where it was recently shown that UV-B increases the hydrolysis of α-tocopherol acetate by induction of nonspecific esterases up to ten- to thirty-fold (13). While one study suggested that bioconversion of α-tocopherol acetate does not occur in human skin (14), significant hydrolysis was demonstrated in recent studies using a human epidermis-tissue culture model (15).

The availability of the free form of vitamin E needs to be considered when analyzing possible health benefits. The majority of studies have been carried out in animal models, while only limited data exist for human studies. Lipid peroxidation is inhibited after topical application of α-tocopherol (16). Several studies indicate that topically applied α-tocopherol inhibits UVB-induced photodamage of DNA in a mouse model (17) and keratinocyte cultures (trolox) (18). Protection against Langerhans cell depletion by UV light was observed after topical application of α-tocopherol in a mouse model (19). a-Tocopherol and its sorbate ester were studied in a mouse model of skin aging. Both antioxidants were found to be effective, sorbate even more so than α-tocopherol (20). Systemic administration of vitamin E in humans (only in combination with vitamin C) increased the MED and reduced changes in skin blood flow after UV irradiation (21,22), Yet several studies indicate that α-tocopherol acetate is not as effective as free vitamin E when applied topically. Inhibition of DNA mutation in mice was five to ten times less effective (18). Also, in a mouse model, unlike free vitamin E, the acetate form seemed to be ineffective (23). In summary, even though some health benefits of vitamin E supplementation have been shown, there is still a need for controlled studies in humans under physiological conditions.

Recently, the tocotrienol forms of vitamin E have become a focus of interest, because they have been found to be more efficient antioxidants in some model systems than tocopherols (24). Even if they are not bioavailable after oral supplementation, topical application circumvents the exclusion by a-TTP in the liver. In fact, free tocotrienols readily penetrate into mouse skin (9), and tocotrienyl acetate is hydrolyzed in skin homogenates and in murine skin in vivo (25). Topical application of a tocotrienol-rich fraction has been demonstrated to protect mouse skin from UV- and O-induced oxidative stress (26,27). In conclusion, tocotrienols bear a potential that yet remains to be explored.

VITAMIN C

Ascorbic acid or vitamin C is one of the most important water soluble antioxidants and present in high amounts in the skin. While most species are able to produce ascorbic acid, humans lack the enzymes necessary for their synthesis. Deficiency in ascorbic acid causes scurvy, a disease already described in the ancient writings

Figure 2 Structural formula of vitamin C as the monoanion ascorbate.

of the Greeks (28). Apart from the pure antioxidant function, ascorbic acid is an essential cofactor for different enzymes. The antioxidant capacity of vitamin C is related to its unique structure (Fig. 2). Owing to its pKal of 4.25 it is present as a monoanion at physiological pH, which can undergo a one electron donation to form the ascorbyl radical with a delocalized electron and can be further oxidized to result in dehydroascorbic acid. Dehydroascorbic acid is relatively unstable and breaks down if it is not regenerated (see antioxidant network). In vitro ascorbic acid can scavenge many types of radicals including the hydroxyl (OH^4), the superoxide (O_2″~), and water soluble peroxyl (ROO") radicals as well as other reactive oxygen species, such as O_3, and quenches singlet oxygen. Owing to their relative reduction potentials, ascorbate can reduce Fe(III) to Fe(II), which in turn can decompose hydrogen peroxide (H_2O_2) to the dangerous hydroxyl radical. Therefore, vitamin C can exert pro-oxidant effects in the presence of unbound iron (Fenton chemistry).

In the skin, vitamin C is found in all layers. In SC it forms a similar gradient as vitamin E with decreasing concentrations toward the outside. Vitamin C is depleted by O_3, UV radiation, and BPO. One of the earliest discoveries of vitamin C benefits in the skin was the observation that it stimulates collagen synthesis in dermal fibroblasts (29). Recently a pretranscriptional role of vitamin C had been described (30). Also, vitamin C is essential in the formation of competent barrier lipids in reconstructed human epidermis (31).

Several studies have investigated protective effects of vitamin C against oxidative stress. UVB-induced immunotolerance, as a marker of damage to the immune system, could be abrogated by topical application of vitamin C to murine skin (32). UVB-induced sunburn cell formation was mitigated by vitamin C in porcine skin (33). While one study reported a postadministrative protective effect of vitamin C-phosphate against UV-induced damage in mice (34), another study found no such effect in humans (35). Systemic application of vitamin C in combination with vitamin E protected against UV-induced erythema in humans (21). In a keratinocyte cell culture system vitamin C reduced UVB-induced DNA damage (18). In mice, an anticarcinogenic effect of vitamin C was described (36). However, no data regarding such benefits exists in humans.

Because vitamin C is not very stable, it is difficult to incorporate it into topical formulations. Esterification with phosphate is used to circumvent this limitation. In vitro experiments demonstrate that Mg-ascorbyl-2-phosphate penetrates the murine skin barrier and is bioconverted into free ascorbate (37).

THIOL ANTIOXIDANTS

Thiols share an oxidizable sulfhydryl (SH) group. Glutathione (GSH) is a tripeptide (Fig. 3) whose SH group at the cysteine can be oxidized, forming a disulfide oxidized

Antioxidants

Figure 3 Chemical structures of thiols: (**A**) GSH consisting of glycine, cysteine, and glutamic acid; (**B**) lipoic acid as in its oxidized form as a disulfide.

glutathione with another GSH molecule. Physiologically, more than 90% of the GSH is in the reduced form. Glutathione peroxidases use GSH oxidation to reduce H_2O_2 and other water soluble peroxides. The synthesis of GSH by the human cell is stimulated by N-acetyl-cysteine (NAC), which is hydrolyzed to cysteine intracellularly. Moreover, NAC acts as an antioxidant itself. Lipoic acid (l,2-dithiolane-3-pentanonic acid or thioctic acid, LA) is a cofactor of multienzyme complexes in the decarboxylation of cc-keto acids. Applied as the oxidized dithiol dehydrolipoic acid it is taken up by cells and is reduced by rhitochondrial and cytosolic enzymes [NAD(P) H dependent]. It thereby forms an efficient cycle, because it can in turn regenerate GSSG to GSH and stimulate the GSH synthesis by improving cysteine utilization (38).

General provisos in the use of thiols in skin applications are the typical smell and the poor solubility of LA in aqueous solutions below pH 7. Yet, several thiol agents have been tested for protective effects in the skin. For oral as well as topical application in mouse models, GSH-ethylesters and GSH-isopropylesters proved to be more efficient than free GSH. Oral supplementation decreased the formation of UV-induced tumors (39) and the formation of sunburn cells (40). Topical treatment partially inhibited UV-induced immunosuppression (41). NAC was able to reduce UVA-induced DNA damage in fibroblasts (42) and protected mice against UVB-induced immunosuppression after topical application (43) in a mode that did not involve de novo GSH synthesis (44). LA was demonstrated to penetrate into mouse skin (45), while oral supplementation of LA has actually been shown to have an anti-inflammatory effect in mice (46), to prevent symptoms of vitamin E deficiency in vitamin E-deficient mice (47), and vitamin C and E deficiency in guinea pigs (48).

POLYPHENOLS

Flavonoids are widely distributed plant pigments and tannins occurring in barks, roots, leaves, flowers, and fruits. Their roles in plants include photoprotecting and contributing to the plant color. Consequently, our diet contains flavonoids which can be found in a variety of foods from green vegetables to red wine (49).

Despite the fact that flavonoids have been used in traditional medicine for several centuries, it was not until 1936 that their first biological activity, the

Figure 4 Chemical structure of catechin, a flavane, as an example of a flavonoid. Flavanes share a common base structure (rings A, B, C) that is hydroxylated in different patterns.

vitamin C-sparing action, was described by Rusznyak and Szent-Gyorgyi (50). As a result, they received the name of "vitamin P." Flavonoids, also referred to as plant polyphenols, have been recognized as potent antioxidants. Their free radical-scavenging and metal-chelating activities have been extensively studied. Nonetheless, given their polyphenolic structure (Fig. 4), the electron- and hydrogen-donating abilities constitute the major feature of their antioxidant properties (51). By opposition to the antioxidants previously described, flavonoids are not part of the endogenous antioxidant system but still interact with it through the antioxidant network (see the following paragraph).

Among the applications found in traditional medicine, flavonoids account for anti-inflammatory, antiphlogistic, and wound-healing functions (52). Their effect on skin inflammation has been thought, for a long time, to be limited to the inhibition of the activity of 5-lipoxygenase and cyclo-oxygenase (49). However, recent studies suggest a more subtle mode of regulation of the inflammatory reaction by flavonoids. In fact, flavonoids such as silymarin, quercetin, genistein, and apigenin are effective inhibitors of NF-kB, a proinflammatory transcription factor, thereby reducing the transcription of proinflammatory genes and preventing inflammation (53–55).

Oral supplementation and topical application of green and black tea polyphenols show beneficial effects against ultraviolet radiation (UVR) -induced skin carcinogenesis in mice (56–58). In addition, these flavonoids and silymarin were found to prevent UVR-induced inflammation as well as ornithine decarboxylase expression and activity (59), all of these events being potential contributors to carcinogenesis (60).

Procyanidins, also named condensed tannins, are flavonoids found in, e.g., pine bark (pycnogenol), grape seeds, and fruits. By direct protein interaction, they were shown to protect collagen and elastin, two dermal matrix proteins, against their degradation (61). Furthermore, some of these procyanidins exhibit a remarkable effect on follicle hair proliferation (62) thus extending the therapeutic applications of flavonoids to alopecia. Although the flavonoids are not part of our endogenous antioxidant defenses, they display a broad spectrum of properties particularly helpful in preventing UVR-caused deleterious effects in human skin.

THE ANTIOXIDANT NETWORK

When an antioxidant reacts with an oxidant, it is converted to a form that no longer functions as an antioxidant, and is said to be consumed. In order for the oxidized product to function again, it needs to be recycled to its native form. The antioxidant

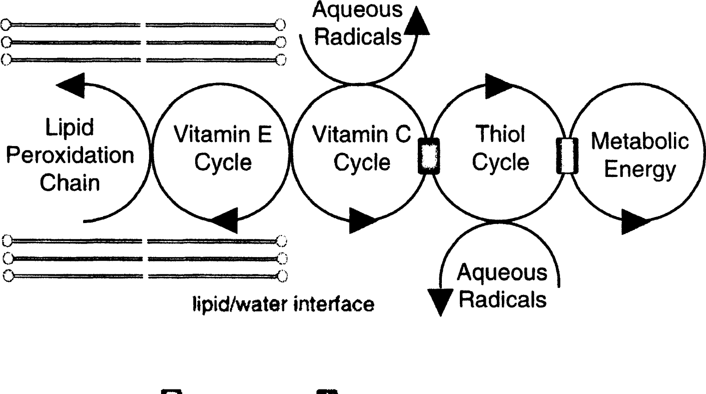

Figure 5 Schematics of the intertwined action of the antioxidant network. An ascorbate molecule can either recycle the vitamin E radical arising from breaking the lipid peroxidation chain, or scavenge an aqueous radical. Glutathione can either regenerate ascorbate or scavenge a radical enzymatically. Glutathione itself can then be regenerated by the cellular metabolism.

network describes the ability of the antioxidants to recycle and regenerate oxidized forms of each other thereby providing extra levels of protection (Fig. 5). Thus the process is synergistic; the net antioxidant protection is always greater than the sum of the individual effects.

The major systemic antioxidants, vitamin E, vitamin C, and GSH, are present in different cellular compartments, and all have the ability to interact with one another. Typically the radicals formed on the antioxidants are more stable and longer lived than the damaging radicals produced in vivo, which is mostly attributable to delocalization of the unpaired electron. Thus they have more chance to interact with each other and be reduced than to react with macromolecules. Vitamin E is the major chain-breaking antioxidant, protecting biological membranes from lipid peroxidation (63), which is a difficult task considering the ratio of phospholipids molecules to vitamin E, about 1500:1. However, vitamin E is never depleted because it is constantly being recycled. When vitamin E becomes oxidized, a radical on vitamin E is formed (chromanoxyl radical). In the absence of networking antioxidants this radical can either become pro-oxidant by abstracting a hydrogen from lipids (64) or react to form nonradical products (consumed). However, a number of antioxidants are known to be able to reduce the chromanoxyl radical and regenerate vitamin E (65). These include vitamin C (66), ubiquinol, and GSH (67); Vitamin C, the most abundant plasma antioxidant and first line of defense, can reduce the tocopheroxyl radical, forming the ascorbyl radical. Interactions between vitamins E and C have been shown in various systems both in vivo (reviewed in Ref. 68) and in vitro (69) (reviewed in Ref. 70). The ascorbyl radical is practically inert and oxidizes further to form dehydroascorbic acid. This can be reduced back to native vitamin C by GSH. This process is known to occur both chemically (71) and enzymatically (72) in both erythrocytes (73) and neutrophils induced by bacteria (74); the latter may relate to a host defense mechanism. GSH is the major intracellular antioxidant. Oxidized GSSG is constantly recycled to GSH enzymatically by glutathione reductase, thus providing a constant pool of GSH. GSH recycling relies

on NAD(P)H as the electron donor. Thus metabolic pathways involved in energy production provide the ultimate electron donors for the antioxidant network. It is also known that GSH can directly recycle vitamin E (65,75), as can ubiquinol (76), another lipophilic antioxidant which itself is recycled in mitochondria as part of the electron transport chain.

Certain supplements are also known to contribute to the network by recycling antioxidants. LA is a prime example because this potent antioxidant can recycle ascorbate, GSH, and ubiquinol in vitro (reviewed in Ref. 77). Recently it has been demonstrated that flavonoids may also play a networking role because they are also able to recycle the ascorbyl radical (78). Thus, there exists a very organized defense system against free radical attack, which ultimately serves to protect and recycle antioxidants in various cellular compartments.

REGULATION OF GENE TRANSCRIPTION BY ANTIOXIDANTS

The skin is the largest human organ and permanently exposed to a variety of stresses. Among these, oxidative insults such as UVR and ozone exposure account for the cause of many skin disorders. However, oxidative damage is not responsible for all biological effects engendered by these stressors in the skin. Indeed, UVR causes changes in the expression of genes encoding, e.g., proinflammatory cytokines, growth factors, stress response proteins, oncoproteins, and matrix metalloproteinases (79). Although the immediate target(s) of UVR is/are still unknown, certain kinases and transcription factors can be activated by UVR thereby increasing gene transcription (80). One transcription factor, NF-kB, appears to be of particular interest for the skin because the lack of its inhibitory protein, IkBoc, is associated with the development of a widespread dermititis in knockout mice (81,82). Furthermore, reactive oxygen species, such as the ones produced after UVR, are suspected to play an important role in the activation of NF-kB (83). Consequently, antioxidants have been found to be among the most potent NF-kB inhibitors. However, clinical studies are required to assess the effectiveness of these antioxidants, including the flavonoid silymarin, a-LA and the GSH precursor N-acetyl-L-cysteine, on skin inflammatory disorders. Using high-throughput procedures such as the cDNA arrays, for instance (84), the evaluation of the antioxidants on the whole genome is henceforth possible. These studies will only confirm the hypothesis that antioxidants are responsible for a much broader action spectrum than their antioxidant functions per se and extend their role on more subtle regulatory mechanisms of the gene expression.

PERSPECTIVES

The general role of antioxidants in the protection against oxidative stress is well established. In skin applications, antioxidants are a promising tool to mitigate oxidative injury. Even though a growing amount of literature deals with skin protection by antioxidants, there is still a need for investigation. In particular, clinical human studies need to be carried out to show the efficacy of antioxidants in topical formulations.

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REFERENCES

1. Brigelius-Flohe R, Traber MG. Vitamin E: function and metabolism. FASEB J 1999; 13:1145–1155.
2. Traber MG, Rader D, Acuff RV, Ramakrishnan R, Brewer HB, Kayden HJ. Vitamin E dose–response studies in humans with use of deuterated RRR-alpha-tocopherol. Am J Clin Nutr 1998; 68:847–853.
3. Traber MG, Ramakrishnan RR, Kayden HJ. Human plasma vitamin E kinetics demonstrate rapid recycling of plasma RRR-alpha-tocopherol. Proc Natl Acad Sci USA 1994; 91:10005–10008.
4. Thiele JJ, Traber MG, Packer L. Depletion of human stratum corneum vitamin E: an early and sensitive in vivo marker of UV induced photo-oxidation. J Invest Dermatol 1998; 110:756–761.
5. Thiele JJ, Weber SU, Packer L. Sebaceous gland secretion is a major physiological route of vitamin E delivery to the skin. J Invest Dermatol 1999; 113:1006–1010.
6. Shindo Y, Witt E, Han D, Packer L. Dose–response effects of acute ultraviolet irradiation on antioxidants and molecular markers of oxidation in murine epidermis and dermis. J Invest Dermatol 1994; 102:470–475.
7. Thiele JJ, Traber MG, Polefka TG, Cross CE, Packer L. Ozone-exposure depletes vitamin E and induces lipid peroxidation in murine stratum corneum. J Invest Dermatol 1997; 108:753–757.
8. Thiele JJ, Rallis M, Izquierdo-Pullido M, et al. Benzoyl peroxide depletes human stratum corneum antioxidants. J Invest Dermatol 1998; 110:674.
9. Traber MG, Rallis M, Podda M, Weber C, Maibach HI, Packer L. Penetration and distribution of alpha-tocopherol, alpha- or gamma-tocotrienols applied individually onto murine skin. Lipids 1998; 33:87–91.
10. Traber MG, Serbinova EA, Packer L. Biological activities of tocotrienols and tocopherols. In: Packer L, Hiramatsu M, Yoshikawa T, eds. Antioxidant Food Supplements in Human Health. New York: Academic Press, 1999.
11. Norkus EP, Bryce GF, Bhagavan HN. Uptake and bioconversion of alpha-tocopheryl acetate to alpha-tocopherol in skin of hairless mice. Photochem Photobiol 1993; 57:613–615.
12. Beijersbergen van Henegouwen GM, Junginger HE, de Vries H. Hydrolysis of RRR-alpha-tocopheryl acetate (vitamin E acetate) in the skin and its UV protecting activity (an in vivo study with the rat). J Photochem Photobiol B 1995; 29:45–51.
13. Kramer-Stickland K, Liebler DC. Effect of UVB on hydrolysis of alpha-tocopherol acetate to alpha-tocopherol in mouse skin. J Invest Dermatol 1998; 111:302–307.
14. Alberts DS, Goldman R, Xu MJ, et al. Disposition and metabolism of topically administered alpha-tocopherol acetate: a common ingredient of commercially available sunscreens and cosmetics. Nutr Cancer 1996; 26:193–201.
15. Nabi Z, Tavakkol A, Soliman N, Polefka TG. Bioconversion of tocopheryl acetate to tocopherol in human skin: use of human skin organ culture models. J Dermatol Sci 1998; 16:S207.
16. Lopez-Torres M, Thiele JJ, Shindo Y, Han D, Packer L. Topical application of alpha-tocopherol modulates the antioxidant network and diminishes ultraviolet-induced oxidative damage in murine skin. Br J Dermatol 1998; 138:207–215.
17. McVean M, Liebler DC. Inhibition of UVB induced DNA photodamage in mouse epidermis by topically applied alpha-tocopherol. Carcinogenesis 1997; 18:1617–1622.
18. Stewart MS, Cameron GS, Pence BC. Antioxidant nutrients protect against UVB-induced oxidative damage to DNA of mouse keratinocytes in culture. J Invest Dermatol 1996; 106:1086–1089.
19. Halliday GM, Bestak R, Yuen KS, Cavanagh LL, Barnetson RS. UVA-induced immunosuppression. Mutat Res 1998; 422:139–145.

20. Jurkiewicz BA, Bisset DL, Buettner GR. Effect of topically applied tocopherol on ultraviolet radiation-mediated free radical damage in skin. J Invest Dermatol 1995; 104:484–488.
21. Eberlein-Konig B, Placzek M, Przybilla B. Protective effect against sunburn of combined systemic ascorbic acid (vitamin C) and d-alpha-tocopherol (vitamin E). J Am Acad Dermatol 1998; 38:45–48.
22. Fuchs J, Kern H. Modulation of UV-light-induced skin inflammation by D-alpha-tocopherol and L-ascorbic acid: a clinical study using solar simulated radiation. Free Radic Biol Med 1998; 25:1006–1012.
23. Yuen KS, Halliday GM. Alphα-tocopherol, an inhibitor of epidermal lipid peroxidation, prevents ultraviolet radiation from suppressing the skin immune system. Photochem Photobiol 1997; 65:587–592.
24. Serbinova EA, Packer L. Antioxidant properties of alpha-tocopherol and alpha-tocotrienol. Methods Enzymol 1994; 234:354–366.
25. Weber SU, Luu C, Traber MG, Packer L. Tocotrienol acetate penetrates into murine skin and is hydrolyzed in vivo. Oxygen Club of California, Book of Abstracts 1999:13.
26. Weber C, Podda M, Rallis M, Thiele JJ, Traber MG, Packer L. Efficacy of topically applied tocopherols and tocotrienols in protection of murine skin from oxidative damage induced by UV-irradiation. Free Radic Biol Med 1997; 22:761–769.
27. Thiele JJ, Traber MG, Podda M, Tsang K, Cross CE, Packer L. Ozone depletes tocopherols and tocotrienols topically applied to murine skin. FEBS Lett 1997; 401:167–170.
28. Sauberlich HE. Pharmacology of vitamin C. Ann Rev Nutr 1994; 14:371–391.
29. Murad S, Grove D, Lindberg KA, Reynolds G, Sivarajah A, Pinnel SR. Regulation of collagen synthesis by ascorbic acid. Proc Natl Acad Sci USA 1981; 78:2879–2882.
30. Davidson JM, LuValle PA, Zoia O, Quaglino D Jr, Giro M. Ascorbate differentially regulates elastin and collagen biosynthesis in vascular smooth muscle cells and skin fibroblasts by pre-translational mechanisms. J Biol Chem 1997; 272:345–352.
31. Ponec M, Weerheim A, Kempenaar J, et al. The formulation of competent barrier lipids in reconstructed human epidermis requires the presence of vitamin C. J Invest Dermatol 1997; 109:348–355.
32. Nakamura T, Pinnel SR, Darr D, et al. Vitamin C abrogates the deleterious effects of UVB radiation on cutaneous immunity by a mechanism that does not depend on TNF-alpha. J Invest Dermatol 1997; 109:20–24.
33. Darr D, Combs S, Dunston S, Manning T, Pinnell S. Topical vitamin C protects porcine skin from ultraviolet radiation-induced damage. Br J Dermatol 1992; 127:247–253.
34. Kobayashi S, Takehana M, Kanke M, Itoh S, Ogata E. Postadministration protective effect of magnesium-L-ascorbyl-phosphate on the development of UVB-induced cutaneous damage in mice. Photochem Photobiol 1998; 67:669–675.
35. Dreher F, Denig N, Gabard B, Schwindt DA, Maibach HI. Effect of topical antioxidants on UV-induced erythema formation when administered after exposure. Dermatology 1999; 198:52–55.
36. Pauling L. Effect of ascorbic acid on incidence of spontaneous mammary tumors and UV-light-induced skin tumors in mice. Am J Clin Nutr 1991; 54:1252S–1255S.
37. Kobayashi S, Takehana M, Itoh S, Ogata E. Protective effect of magnesium-L-ascorbyl-2 phosphate against skin damage induced by UVB irradiation. Photochem Photobiol 1996; 64:224–228.
38. Han D, Handelman G, Marcocci L, et al. Lipoic acid increases de novo synthesis of cellular glutathione by improving cytine utilization. Biofactors 1997; 6:321–338.
39. Kobayashi S, Takehana M, Tohyama C. Glutathione isopropyl ester reduces UVB-induced skin damage in hairless mice. Photochem Photobiol 1996; 63:106–110.
40. Hanada K, Sawamura D, Tamai K, Hashimoto I, Kobayashi S. Photoprotective effect of esterified glutathione against ultraviolet B-induced sunburn cell formation in the hairless mice. J Invest Dermatol 1997; 108:727–730.

41. Steenvoorden DP, Beijersbergen van Henegouwen G. Glutathione ethylester protects against local and systemic suppression of contact hypersensitivity induced by ultraviolet B radiation in mice. Radiat Res 1998; 150:292–297.
42. Emonet-Piccardi N, Richard MJ, Ravanat JL, Signorini N, Cadet J, Beani JC. Protective effects of antioxidants against UVA-induced DNA damage in human skin fibroblasts in culture. Free Radic Res 1998; 29:307–313.
43. Steenvoorden DP, Beijersburgen van Henegouwen GM. Glutathione synthesis is not involved in protection by NAC against UVB-induced systemic immunosuppression in mice. Photochem Photobiol 1998; 68:97–100.
44. Steenvoorden DP, Hasselbaink DM, Beijersbergen van Henegouwen GM. Protection against UV-induced reactive intermediates in human cells and mouse skin by glutathione precursors: a comparison of N-acetylcysteine and glutathione ethylester. Photochem Photobiol 1998; 67:651–656.
45. Podda M, Rallis M, Traber MG, Packer L, Maibach HI. Kinetic study of cutaneous and subcutaneous distribution following topical application of [7,8-14C] rac-alpha-lipoic acid onto hairless mice. Biochem Pharmacol 1996; 52:627–633.
46. Fuchs J, Milbradt R. Antioxidant inhibition of skin inflammation induced by reactive oxidants: evaluation of the redox couple dihydrolipoate/lipoate. Skin Pharmacol 1994; 7:278–284.
47. Podda M, Tritschler HJ, Ulrich H, Packer L. Alpha-lipoic acid supplementation prevents symptoms of vitamin E deficiency. Biochem Biophys Res Commun 1994; 204:98–104.
48. Rosenberg HR, Culik R. Effect of oc-lipoic acid on vitamin C and vitamin E deficiencies. Arch Biochem Biophys 1959; 80:86–93.
49. Pietta P. Flavonoids in medicinal plants. In: Packer L, Rice-Evans C, eds. Flavonoids in Health and Disease. New York: Marcel Dekker, 1998:61–110.
50. Rusznyak SP, Szent-Gyorgyi A. Vitamin P: flavonols as vitamins. Nature 1936; 138:27.
51. Rice-Evans CA, Miller NJ, Paganga G. Structure–antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med 1996; 20:933–956.
52. Middleton E Jr, Kandaswami C. The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer. In: Harborne JB, ed. The Flavonoids: Advances in Research Since 1986. London: Chapman & Hall, 1993.
53. Saliou C, Kitazawa M, McLaughlin L, et al. Antioxidants modulate acute solar ultraviolet radiation-induced NF-kappa-B activation in a human keratinocyte cell line. Free Radic Med 1999; 26:174–183.
54. Gerritsen ME, Carley WW, Ranges GE, et al. Flavonoids inhibit cytokine-induced endothelial cell adhesion protein gene expression. Am J Pathol 1995; 147:278–292.
55. Natarajan K, Manna SK, Chaturvedi MM, Aggarwal BB. Protein tyrosine kinase inhibitors block tumor necrosis factor-induced activation of nuclear factor-KB, degradation of IkBoc, nuclear translocation of p65, and subsequent gene expression. Arch Biochem Biophys 1998; 352:59–70.
56. Gensler HL, Timmermann BN, Valcic S, et al. Prevention of photocarcinogenesis by topical administration of pure epigallocatechin gallate isolated from green tea. 1996; 26:325–335.
57. Wang ZY, Huang MT, Lou YR, et al. Inhibitory effects of black tea, green tea, decaffeinated black tea, and decaffeinated green tea on ultraviolet B light-induced skin carcinogenesis in 7,12-dimethylbenz[a]anthracene-initiated SKH-1 mice. Cancer Res 1994; 54:3428–3435.
58. Javed S, Mehrotra NK, Sukla Y. Chemopreventive effects of black tea polyphenols in mouse skin model of carcinogenesis. Biomed Environ Sci 1998; 11:307–313.
59. Katiyar SK, Korman NJ, Mukhtar H, Agarwal R. Protective effects of silymarin against photocarcinogenesis in a mouse skin model. J Natl Cancer Inst 1997; 89:556–566.
60. Agarwal R, Mukhtar H. Chemoprevention of photocarcinogenesis. Photochem Photobiol 1996; 63:440–444.

61. Tixier JM, Godeau G, Robert AM, Hornebeck W. Evidence by in vivo and in vitro studies that binding of pycnogenols to elastin affects its rate of degradation by elastases. Biochem Pharmacol 1984; 33:3933–3939.
62. Takahashi T, Kamiya T, Hasegawa A, Yokoo Y. Procyanidin oligomers selectively and intensively promote proliferation of mouse hair epithelial cells in vitro and activate hair follicle growth in vivo. J Invest Dermatol 1999; 112:310–316.
63. Burton GW, Ingold KU. Autoxidation of biological molecules. I. The antioxidant activity of vitamin E and related chain-breaking phenolic antioxidants in vitro. J Am Chem Soc 1981; 103:6472–6477.
64. Bowry VW, Stocker R. Tocopherol-mediated peroxidation—the prooxidant effect of vitamin E on the radical initiated oxidation of human low density lipoprotein. J Am Chem Soc 1993; 115:6029–6044.
65. Sies H. Strategies of antioxidant defense. Eur J Biochem 1993; 215:213–219.
66. Packer JE, Slater TF, Willson RL. Direct observation of a free radical interaction between vitamin E and vitamin C. Nature 1979; 278:737–738.
67. Wefers H, Sies H. The protection by ascorbate and glutathione against microsomal lipid peroxidation is dependent on vitamin E. Eur J Biochem 1988; 174:353–357.
68. Gey KF. Vitamins E plus C and interacting conutrients required for optimal health. Biofactors 1998; 7:113–174.
69. Kagan VE, Witt E, Goldman R, Scita G, Packer L. Ultraviolet light-induced generation of vitamin E radicals[1] and their recycling. A possible photosensitizing effect of vitamin E in skin. Free Radic Res Commun 1992; 16:51–64.
70. Kamal-Eldin A, Appelqvist L-A. The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids 1996; 31:671–701.
71. Winkler BS. Unequivocal evidence in support of the nonenzymatic redox coupling between glutathione/glutathione disulfide and ascorbic acid/dehydroascorbic acid. Biochem Biophys Acta 1992; 1117:287–290.
72. Wells WW, Xu DP, Yang YF, Rocque PA. Mammalian thioltransferase (glutaredoxin) and protein disulfide isomerase have dehydroascorbate reductase activity. J Biol Chem 1990; 265:15361–15364.
73. May JM, Qu ZC, Whitesell RR, Cobb CE. Ascorbate recycling in human erythrocytes: role of GSH in reducing dehydroascorbate. Free Radic Biol Med 1996; 20:543–551.
74. Wang Y, Russo TA, Kwon O, Chanock S, Rumsey SC, Levine M. Ascorbate recycling in human neutrophils: induction by bacteria. Proc Natl Acad Sci USA 1997; 94: 13816–13819.
75. Bast A, Haenen GRMM. Regulation of lipid peroxidation of glutathione and lipoic acid: involvement of liver microsomal vitamin E free radical reductase. In: Emerit I, Packer L, Auclair C, eds. Antioxidant in Therapy in Preventive Medicine. New York: Plenum Press, 1990:111–116.
76. Kagan V, Serbinova E, Packer L. Antioxidant effects of ubiquinones in microsomes and mitochondria are mediated by tocopherol recycling. Biochem Biophys Res Commun 1990; 169:851–857.
77. Packer L, Witt E, Tritschler HJ. cc-Lipoic acid as a biological antioxidant. Free Radic Biol Med 1995; 19:227–250.
78. Cossins E, Lee R, Packer L. ESR studies of vitamin C regeneration, order of reactivity of natural source phytochemical preparations. Biochem Mol Biol Int 1998; 45:583–597.
79. Tyrrell RM. UV activation of mammalian stress protein. In: Feige U, Morimoto RI, Yahara I, Polla B, eds. Stress-Inducible Cellular Responses. Basel (Switzerland): Birkhauser Verlag, 1996:255–271.
80. Herrlich P, Blattner C, Knebel A, Bender K, Rahmsdorf HJ. Nuclear and non-nuclear targets of genotoxic agents in the induction of gene expression: shared principles in yeast, rodents, man and plants. Biol Chem 1997; 378:1217–1229.

81. Beg AA, Sha WC, Bronson RT, Baltimore D. Constitutive NF-kappa-B activation, enhanced granulopoiesis, and neonatal lethality in I-kappa-B-alpha deficient mice. Genes Dev 1995; 9:2736–2746.
82. Klement JF, Rice NR, Car BD, et al. I-kappa-B-alpha deficiency results in a sustained NF-kappa-B response and severe widespread dermatitis in mice. Mol Cell Biol 1996; 16:2341–2349.
83. Flohe L, Brigelius-Flohe R, Saliou C, Traber M, Packer L. Redox regulation of NF-kB activation. Free Radic Biol Med 1997; 22:1115–1126.
84. Schena M, Heller RA, Theriault TP, Konrad K, Lachenmeier E, Davis RW. Microarrays: biotechnology's discovery platform for functional genomics. Trends Biotechnol 1998; 16:301–306.

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Dexpanthenol

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INTRODUCTION

Dexpanthenol containing creams and ointments has been widely used for treatment of several skin conditions, in particular for superficial wounds and lesions of the skin and mucous membranes and for conditions characterized by disturbed stratum corneum morphology and altered permeability barrier function (e.g., dry skin). Wounds and skin conditions with impaired barrier function are some of the most common problems in medicine. Millions of dollars are spent every year for treatment of these disorders. Several studies have shown that topical treatment with dexpanthenol containing creams and ointments is effective. These results suggest an important function of the substance in skin physiology and therefore indicate that dexpanthenol is a substance with characteristics of both drugs and cosmeceuticals.

BIOPHYSIOLOGY AND ABSORPTION

Dexpanthenol (D-pantothenyl-alcohol, DAS81-13-0, provitamin B5) is an alcohol analog of the biologically active pantothenic acid (vitamin B5). Pantothenic acid is unstable in topical formulations and, therefore, the stable alcohol dexpanthenol is used in creams and ointments (1). The alcohol is easily absorbed into the skin where it is intracellularly enzymatically oxidized to pantothenic acid in different tissues (2). Dexpanthenol is soluble in water and alcohol, although insoluble in fats and oil-based substances. With an appropriate vehicle, dexpanthenol easily penetrates into the skin. Rate of penetration and total absorption is reduced when dexpanthenol is administered as an oil/water formula (3,4). Pantothenic acid concentrates in hair, hair roots, nails, epidermis, and corium after topical application.

MODES OF ADMINISTRATION

Dexpanthenol is used topically in water-based emulsions or solutions in concentrations of 2% to 5%. Dexpanthenol is administered as a cream, an ointment, a gel, and

as a foam spray for the treatment of skin disorders. The substance is also used for the treatment of several skin conditions and is an active ingredient in a variety of sunscreens, after-sun formulas, and baby products. For the treatment of mucous membrane lesions, nasal and ocular ointments are available. Dexpanthenol is used as a dissolving lozenge for mouth and throat irritations. The substance is also systemically administered in the form of a multivitamin supplement, or locally administered as an injection or enema (5).

Mechanisms of Action

The mechanisms of action are only partly known. Pantothenic acid is an essential vitamin that is ubiquitous in plants and animals. Pantothenic acid is essential for the synthesis of coenzyme A (CoA), which, in turn, is necessary for the metabolism and oxidation of fatty acids, amino acids, proteins, and carbohydrates. The Food and Nutrition Board of the National Research Council (U.S.) has suggested that a safe and adequate intake dose for pantothenic acid is 4 to 7 mg daily. In swine, experimental deficiency of pantothenic acid results in bloody diarrhea and ulceration of the gastrointestinal mucosa (5). Pantothenic acid is also called antigray hair factor of the rat, or chicken antidermatitis factor. Deficiency in animals leads to growth retardation, depigmentation of hair and feathers, inflammation of mucous membranes, and dermatitis. Although deficiencies rarely occur in humans because pantothenic acid is naturally present in many commonly consumed grains, meats, and dairy products, administration of pantothen antagonists, inadequate nutrition, diabetes mellitus, chronic alcohol abuse, and long-term administration of parenteral nutrition intake can cause a deficiency to develop. Immunological and neurological disturbances, impaired growth rates of important organs, depigmentation as well as skin inflammations and impaired wound healing are symptoms of vitamin B5 deficiency. Pantothenic acid deficiency is very unlikely to occur alone and is usually accompanied by other nutritional deficiencies (6).

CoA is an essential component of the fatty acid–synthetase complex where sphingolipids, triglycerides, phospholipids, and cholesterol are synthesized (7). CoA serves as cofactor for the transfer of acetyl groups and plays a role in early steps of the synthesis of fatty acids and sphingolipids (8) which are crucially important for stratum corneum lipid bilayers to provide permeability barrier function (9). Fatty acids and sphingolipids are also abandoned in the cell membranes, and therefore synthesis of pantothenic acid and CoA must be increased for the replenishment of lost or damaged keratinocytes during wound healing and permeability barrier repair. CoA is present in keratinocytes and fibroblasts, although it cannot be transferred across cell membranes.

INDICATIONS AND CLINICAL APPLICATIONS

Dexpanthenol has been recommended by the pharmaceutical industry for use in a variety of disorders including antioxidative treatment against UV-A radiation in skin aging, atopic dermatitis, contact dermatitis, corneal erosions, decubital ulcers, diaper rash, eczema, ichthyosis, inflammatory processes, irritated skin, pruritus, psoriasis, radiation dermatitis, scar treatment, skin lesions, and xerosis cutis.

Barrier Repair and Superficial Wounds

Dexpanthenol containing creams and ointments are most extensively used for the treatment of superficial lesions of the skin and mucous membranes. Dexpanthenol influences several steps in the healing of superficial wounds (10). Even minor disturbance of the stratum corneum leads to an inflammatory reaction of the epidermis involving cascades of cytokines TNF, interleukin-1, and interleukin-6 (11,12). Inflammatory reactions are needed for wound repair, but sustained inflammation such as redness, swelling, pain, and elevated temperature may delay complete healing (13,14). Therefore, reduction of the inflammation process is a goal in wound treatment. It has been shown that treatment with a dexpanthenol containing cream reduced redness as a sign of inflammation after superficial injury to the skin by tape-stripping (15). Also, in a double-blind comparison of dexpanthenol containing a cream and a dexpanthenol-free control, dexpanthenol demonstrated vasoconstriction and anti-inflammatory effects as shown by skin temperature measured with fluvography (16).

An increase in proliferation is also crucial for wound repair to compensate for the loss of cells. This is not only important for deep, but also for superficial wounds as has been shown after acetone or sodium lauryl sulfate treatment or after tape-stripping (17). Dexpanthenol and CoA must be increased for the replenishment of lost or damaged keratinocytes during wound healing and permeability barrier repair. We have shown that treatment with a dexpanthenol containing ointment, enhanced wound repair after superficial (15) and deep wounds, and this is probably related to enhanced proliferation (Proksch, unpublished data).

The last step in the wound healing process is epidermal differentiation represented by marker keratins and cornified envelope proteins (18). Disturbed epidermal differentiation leads to changes in the stratum corneum, histologically characterized by hyper- and parakeratosis and clinically noticed as scaling and roughness of the skin. We recently showed that treatment with a dexpanthenol containing ointment following sodium lauryl sulfate-induced skin injury, the roughness, as the result of excessive proliferation and disturbed differentiation, was reduced by both verum and vehicle, although the verum was more effective (15).

Lipids are essential components of the skin, cell membrane, and permeability layer in the stratum corneum. The stratum corneum consists of protein-rich corneocytes and a lipid-enriched intercellular space where lipids are organized into bilamellar layers. Without replenishment of corneocytes and generation of lipids, wound healing and repair of the skin's permeability barrier are not possible. If the skin's lipid composition is disturbed by water, detergents, or superficial injury, the permeability layer may also be disturbed, leading to excessive transepidermal water loss. Synthesis of cholesterol, free fatty acids, and ceramides are crucially involved in barrier repair. We recently showed that treatment with the dexpanthenol containing ointment enhanced permeability barrier repair as shown by an enhanced decrease of the elevated transepidermal water loss (15). Also, a barrier stabilizing effect in normal skin treated with dexpanthenol has been described (1).

Full Thickness Wounds

Full thickness wounds involve the epidermis and the dermis; for dermal repair activation of fibroblasts to replenish dermal cells is crucial. Fibroblast proliferation is activated by dexpanthenol (19). Notably, efficacy is inversely related to the

concentration (20), with the most significant effects at the lowest concentrations. We found that dexpanthenol in solution enhances repair of full thickness skin wounds compared to the vehicle in mice (Proksch, unpublished data).

Dexpanthenol in the Management of Heat Erythema, Sunburns, and Other Minor Burns

Recently, dexpanthenol containing foam sprays have been introduced, in particular, for the treatment of burns. Ninety-five percent of all burns are of the first degree, representing only superficial injury to the epidermis, characterized by redness, pain, swelling, dryness, itching, and tightness (21). The sprays are easily applied without a need for touching sensitive burned skin. A critical step in the repair of burns is the reduction in inflammation, but also the restoration of barrier function to prevent the entry of harmful agents into the skin. The sprays contain a high amount of water for cooling to reduce the inflammatory heat. In addition to this physical effect, dexpanthenol spray may have a biochemical function in reducing skin irritation after burns, as it has shown for sodium lauryl sulfate-induced skin irritation. In addition, a temporary artificial barrier aids in protecting the skin from external irritants and is provided by lipids within the spray preparation. The applied substances should not have a high fat content to avoid wound occlusion which would hinder heat emission and would worsen the underlying inflammation. For treatment of first degree burns, foam sprays may be superior to water-based gel products (22).

Radiation Dermatitis

In a recent double-blind study of patients receiving radiation therapy for breast malignancy, Schmuth et al. (23) compared topical treatments using dexpanthenol and methylprednisolone aceponate containing creams with a nontreated control group. Both preparations reduced radiation dermatitis compared to untreated, but the effect of corticosteroid was superior in comparison to dexpanthenol. Previously, however, skin treatment with a dexpanthenol cream versus no cream during radiotherapy in a randomized controlled trial did not indicate any clinically important benefits (24).

Dry and Irritated Skin

Xerosis cutis is very common especially in the winter months and increases with age. Dry skin may develop into eczema and is part of several diseases including irritant and allergic contact dermatitis, atopic dermatitis, seborrhoic dermatitis, ichthyosis, and psoriasis. Dexpanthenol containing creams and ointments have been widely used for the treatment of dry skin. The substance acts as a moisturizer thus improving stratum corneum hydration. We recently found that dexpanthenol increased stratum corneum hydration after sodium lauryl sulfate-induced irritation, and the verum showed superiority over both vehicle-treated and untreated skin (15,25). Gloor et al. (26) described that a combination of dexpanthenol and glycerine had an additive effect compared to the individual substances alone. Both substances show enhanced moisturizing capacity when combined, even over longer periods (27,28). Because of its sticky consistency at higher concentrations, glycerine is more accepted as a cosmetic ingredient when it is combined with a larger percentage of dexpanthenol (25).

Very recently the efficacy of dexpanthenol in skin protection against irritation has been investigated in a randomized placebo-controlled study and has shown that dexpanthenol exhibits protective effects (29). Therefore, dexpanthenol may be useful for the protection against contact dermatitis.

Dexpanthenol containing creams may be useful for the treatment of skin conditions showing reduced stratum corneum hydration, disturbed barrier function, and disturbed differentiation including irritant and allergic contact dermatitis, atopic dermatitis, seborrhoic dermatitis, diaper rash, ichthyosis, and psoriasis. However, placebo-controlled studies are lacking.

Antioxidative Effect and Skin Aging

Oxidative stress and formation of reactive oxygen radicals are critically involved in many biological processes including skin aging and UV irradiation. Dexpanthenol is easily oxidized to pantothenic acid, this means that dexpanthenol exhibits an antioxidative effect and could protect against various oxidative effects including UV irradiation, ozone oxidation, and radiation dermatitis and could protect against biological and UV induced skin aging. Therefore, dexpanthenol has a significant role in sun protection and after-sun preparations (30).

SIDE EFFECTS, CONTRA-INDICATIONS, AND PRODUCT SAFETY

Dexpanthenol is classified as nontoxic, and dexpanthenol containing creams and ointments is generally very well tolerated. However, in certain cases type IV contact allergy may occur. Contact allergies to dexpanthenol are extremely rare despite widespread use and seem to be clinically relevant predominantly for patients with stasis dermatitis and multiple allergies (31). Dexpanthenol is listed as a potential agent for type IV allergic reactions in ocular therapy, a finding confirmed by positive patch testing. Lymphocyte transformation tests using dexpanthenol modified microsomes also showed positive results (32). Sensitization through occupational exposure has been described only once, and contact allergies to sunscreens and cosmetics containing dexpanthenol have only been rarely reported (31,33,34). Urticaria involving hair care products containing panthenol has been reported (35). Therefore, dexpanthenol is recognized as a safe substance by national and international drug classification boards.

CONCLUSION

Treatment with dexpanthenol containing creams, ointments, and foam sprays significantly enhanced skin barrier repair and stratum corneum hydration, while reducing skin roughness and inflammation. It also exhibits antioxidative properties important for the aging process. Therefore, the use of dexpanthenol is recommended for the treatment of superficial wounds, barrier disrupted, dry, or inflamed skin. It is also suitable to reduce oxidation in UV irritated skin and to reduce chronological and sun-induced aging. Dexpanthenol is safe and easily applied in different preparations.

REFERENCES

1. Gehring W, Gloor M. Die Bedeutung von topisch appliziertem Dexpanthenol für die epidermale Barriere Funktion und die Hydration des Stratum corneum. Arzneimittelforschung 2000; 50:659–663.
2. Abiko Y, Tomikawa M, Shimizu M. Enzymatic conversion of pantothenyl alcohol to pantothenic acid. J Vitaminol (Kyoto) 1969; 10:59–69.
3. Stüttgen G, Krause H. Percutaneous absorption of tritium-labelled panthenol in man and animal. Arch Klin Exp Dermatol 1960; 209:578–582.
4. Förster T, Pittermann W, Schmitt M, Kietzmann J. Skin penetration properties of cosmetic formulations using a perfused bovine udder model. J Cosmet Sci 1999; 50:147–157.
5. Loftus EV Jr, Tremaine WJ, Nelson RA, Shoemaker JD, Sandborn WJ, Phillips SF, Hasan Y. Dexpanthenol enemas in ulcerative colitis: a pilot study. Mayo Clin Proc 1997; 72:616–620.
6. Esterbauer H, Gey F, Fuchs J, Clemens M, Sies H. Antioxidative Vitamine and degenerative Erkrankungen. Deutsches Ärzteblatt 1990; 47:A3735–A3741.
7. Tahiliani AG, Beinlich CJ. Pantothenic acid in health and disease. Vitam Horm 1991; 46:165–228.
8. Buddecke E. Über den Stoffwechsel der Fettsäuren und Lipid. In: Grundriss der Biochemie. 5th ed. Berlin: Walter de Gruyter, 1977:40, 196, 197.
9. Elias PM, Goerke J, Friend DS. Mammalian epidermal barrier layer lipids: composition and influence on structure. J Invest Dermatol 1977; 69:535–546.
10. Proksch E. Dexpanthenol: Neues zur Wirksamkeit eines dermatologischen Klassikers. DermoTopics 2002; 2:18–19.
11. Corsini E, Galli CL. Cytokines and irritant contact dermatitis. Toxicol Lett 1998; 102–103:277–282.
12. Wood LC, Jackson SM, Elias PM, Grunfeld C, Feingold KR. Cutaneous barrier perturbation stimulates cytokine production in the epidermis of mice. J Clin Invest 1992; 90:482–487.
13. Clark RAK. Wound repair. Overview and general consideration. In: RAF Clark: The Molecular and Cellular Biology of Wound Repair. 2nd ed. New York: Plenum Press, 1996:3–35.
14. Martin P. Wound healing-aiming for perfect skin regeneration. Science 1997; 276:75–81.
15. Proksch E, Nissen HP. Dexpanthenol enhances skin barrier repair and reduces inflammation after sodium lauryl sulphate-induced irritation. J Dermatol Treatment 2002; 13: 173–178.
16. Borelli S, Sorkin B. Über die Beeinflußbarkeit der Hautgefäße durch eine panthenolhaltige Salbe. Der informierte Arzt 1974; 2:2–3.
17. Proksch E, Feingold KR, Man MQ, Elias PM. Barrier function regulates epidermal DNA synthesis. J Clin Invest 1991; 87:1668–1673.
18. Ekanayake-Mudiyanselage S, Aschauer H, Schmook FP, Jensen JM, Meingassner JG, Proksch E. Expression of epidermal keratins and the cornified envelope protein involucrin is influenced by permeability barrier disruption. J Invest Dermatol 1998; 111: 517–523.
19. Hauptmann S, Schafer H, Fritz A, Hauptmann P. The growth modifying effect of wound ointments on cell cultures. Hautarzt 1992; 43:432–435.
20. Ebner F, Heller A, Rippke F, Tausch I. Topical use of dexpanthenol in skin disorders. Am J Clin Dermatol 2002; 3:427–433.
21. Schopf R. Thermische Schädigung der Haut—Formen and Therapiemanagement des Hitzeerythems. Press Conference on Bepanthen, Frankfurt am Main, Germany, 2003.
22. Grivet-Seyve M, Bellon C, Maares J, de Bony R, Welter Y, Girard F. Evaluation de l'effet hydratant de Bepanthen Burn Relief Foam Spray contre comparateurs, dans le traitement de la brûlure du premier degré. Real Therap Dermatol 2003; 25:38–47.

23. Schmuth M, Wimmer MA, Hofer S, Sztankay A, Weinlich G, Linder DM, Elias PM, Fritsch PO, Fritsch E. Topical corticosteroid therapy for acute radiation dermatitis: a prospective, randomized, double-blind study. Br J Dermatol 2002; 146:983–991.
24. Lokkevik E, Skovlund E, Reitan JB, Hannisdal E, Tanum G. Skin treatment with bepanthen cream versus no cream during radiotherapy—a randomized controlled trial. Acta Oncol 1996; 35:1021–1026.
25. Gehring W, Gloor M. Der Effekt von Dexpanthenol bei experimentell geschädigter Haut. Z Hautkr 2001; 76:212–218.
26. Gloor M, Senger B, Gehring W. Wirkt eine Kombination von Dexpanthenol und Glycerin stärker hydratisierend als die Einzelkomponenten allein? Akt Dermatol 2002; 28: 402–405.
27. Gloor M, Schermer S, Gehring W. Ist die Kombination von Harnstoff und Glycerin in Externagrundlagen sinnvoll? Z Hautkr 1997; 72:509–514.
28. Grunewald AM, Gloor M, Gehring W, Kleesz P. Barrier creams–commercially available barrier creams versus urea and glycerol containing oil in water emulsions. Dermatosen 1995; 43:69–74.
29. Biro K, Thaci D, Ochsendorf FR, Kaufmann R, Boehncke WH. Efficacy of dexpanthenol in skin protection against irritation: a double-blind, placebo-controlled study. Contact Dermatitis 2003; 49:80–84.
30. Wollina U. Zur klinischen Wirksamkeit von Dexpanthenol. Kosmetische Medizin 2001; 4:180–186.
31. Stables GI, Wilkinson SM. Allergic contact dermatitis due to panthenol. Contact Dermatitis 1998; 38:236–237.
32. Hahn C, Roseler S, Fritzsche R, Schneider R, Merk HF. Allergic contact reaction to dexpanthenol: lymphocyte transformation test and evidence for microsomal-dependent metabolism of the allergen. Contact Dermatitis 1993; 28:81–83.
33. Skudlik C, Schnuch A, Uter W, Schwanitz HJ. Berufsbedingte Kotaktekzem nach Anwendung einer Dexpanthenol-haltigen Salbe und Überblick über die IVDK-Daten zu Dexpanthenol. Akt Dermatol 2002; 28:398–401.
34. Jeanmougin M, Manciet JR, Moulin JP, Blanc F, Pons A, Civatte E. Contact allergy to dexpanthenol in sunscreens. Contact Dermatitis 1988; 18:240.
35. Schalock PC, Storrs FJ, Morrison L. Contact urticaria from panthenol in hair conditioner. Contact Dermatitis 2000; 43:223.

33
Hair Conditioners

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INTRODUCTION

Despite myriad claimed benefits, the primary purpose of a hair conditioner is to reduce the magnitude of the forces associated with combing or brushing hair (1), especially when wet (2,3). This is generally accomplished by the deposition of conditioning agents that lubricate the hair fiber, diminishing surface friction and, therefore, combing forces (4).

In general, deposition of a conditioning agent also causes the hair to feel softer and more moisturized. Another secondary benefit is the reduction or prevention of flyaway hair (5), especially, by cationic conditioners (6). Besides making the hair more manageable, increasing the ease-of-combing also improves the ability to align the hair fibers in a more parallel configuration, which can result in an increase in hair shine, even if the shine of the individual fiber is not increased (7).

A number of other benefits have sometimes been claimed or implied for conditioners including repair of damaged hair, strengthening of hair, repair of split ends, vitamin therapy, etc. Many of these are marketing hype or are based on laboratory conditions or concentrations not found under actual usage conditions. In this chapter, we will confine ourselves to a discussion of only the observable conditioner benefits presented above. The chapter will begin with a discussion of the relationship among hair damage, conditioning, and state of the hair surface. This will be followed by a discussion of the major classes of conditioning agents currently in use. Finally, we will end with a brief discussion of the auxiliary ingredients necessary for the production of a commercial conditioning product.

CONDITIONING AND THE HAIR FIBER SURFACE

Hair Damage

In previous chapters, it has been shown that hair fibers consist of a central cortex that comprises the major portion of the fiber, surrounded by eight to 10 layers of overlapping cells termed the cuticle. The cortex is responsible for the tensile properties of the hair (8,9), while the state of the cuticle affects a variety of consumer perceivable properties including hair feel, shine, combability, etc.

A major function of conditioners is to protect the hair's structural elements, especially the cuticle, from grooming damage. This type of stress, characterized by chipping, fragmenting, and wearing away of cuticle cells, is probably the single most important source of damage to the hair surface (10–12).

A rather extreme example of combing damage can be seen in Figure 1, which shows the results of an experiment in which a tress of virgin hair was washed with a cleaning shampoo and then combed 700 times while wet. Since hair is more fragile when wet (3) and combing forces are higher (2), combing under these conditions insures maximum damage. It can be seen that the damage to the cuticle was extensive with many cuticle cells lifted from the surface, while others were completely torn away by the combing process.

The ability of conditioning agents to protect the hair from the above type of damage can be seen in Figure 2 which shows the results of an experiment in which a tress was washed with a high conditioning 2-in-1 shampoo and then combed 700 times while wet. In this case, as the conditioning agents in the shampoo reduced combing forces, the hair surface is seen to be intact with evidence of only minor chipping and fragmenting of cuticle cells. This demonstrates the important role conditioners can play in maintaining the integrity of the hair fiber.

Another type of damage can be caused by the use of appliances. Many styles require the use of blow-dryers and/or curling irons, which can produce temperatures of 200°F to 400°F (14). Steam can be released from the hair fiber causing bubbling and buckling of the cuticles, especially if the hair is not completely dry while being curled. In addition to minimizing combing forces, to protect the hair from this type of damage, certain conditioning polymers can provide added protection in the presence of heat resulting in increased characteristic life of the hair fiber (13).

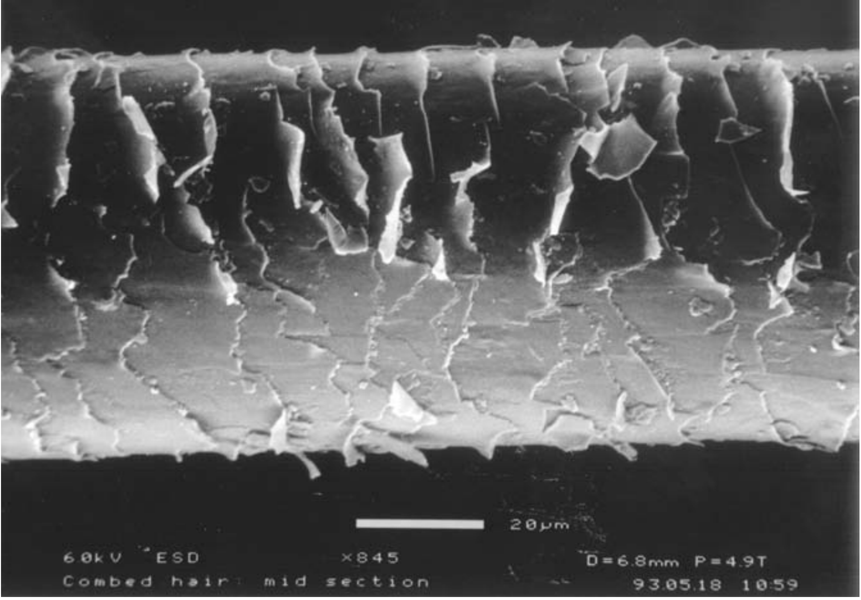

Figure 1 Typical scanning electron micrograph (SEM) of hair taken from a tress washed with a cleaning shampoo and then combed 700 times while wet. Note raised and chipped cuticle cells, and areas where cells have been completely torn away.

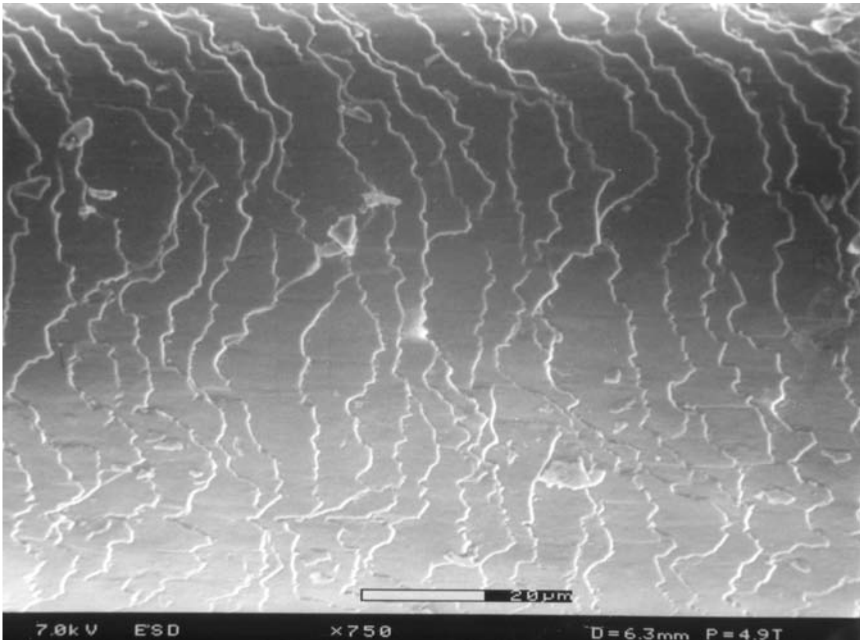

Figure 2 Typical SEM photo of hair taken from a tress washed with a high conditioning 2-in-1 shampoo and then combed 700 times while wet. Note the minimal damage compared to Figure 1.

Hair Damage and the Cuticle Surface

The susceptibility of a hair fiber to grooming damage and the type of conditioner most effective in preventing this damage is affected to a large degree by the nature and state of the hair surface. It is, therefore, helpful to precede a discussion of conditioning agents with a presentation on the hair surface and how it affects conditioner requirements and deposition.

Virgin Hair Surfaces

Hair that has not been chemically treated is termed "virgin hair." The cuticle surface of virgin hair in good condition is hydrophobic (15,16), in large part as a result of a layer of fatty acids covalently bound to the outermost surface of the cuticle (epicuticle) (17,18). As a result of its protein structure, however, the hair surface has an isoelectric point near 3.67 (19), which insures that the surface will contain negatively charged hydrophilic sites at the ordinary pH levels of hair care products. This mix of hydrophobicity and hydrophilicity affects, of course, the types of conditioning agents that will bind to the virgin hair surface.

The situation is further complicated by the fact that the negative charge density on virgin hair increases from root to tip. This is, primarily, a result of oxidation of cystine in the hair to cystine S-sulfonate and cysteic acid as a result of exposure to UV radiation in sunlight (20,21). The tip portions of the hair, being older than the root portions, will have been exposed to damaging (10) UV radiation for a longer period of time and will, therefore, be more hydrophilic, again affecting the nature of species that can bind to these sites.

In addition to greater UV damage, the hair tips are also subject to greater combing damage. One reason for this is simply that, being older, the tip portions will have been exposed to more combing. In addition, the surface friction of hair tips is higher (C. Reich, unpublished data) so that combing forces increase as one moves from root to tip. Finally, the ends of hair are subject to unusually high combing stress as a result of entangling during the combing process (2). This eventually results in destruction of the covalently bound lipid layer and a feeling of dryness at the tips. Because of this damage, the hair tips require more conditioning than the rest of the fiber. Without sufficient conditioning, the cuticle layer is eventually lost, resulting in a split end. An example is seen in Figure 3, which clearly shows the exposed cortical cells.

Chemically Treated Hair Surfaces

Chemical treatments, perming, bleaching, and permanent dyeing, can all cause significant damage to the hair fiber (3,10,22–24). In addition to causing tensile damage, all of these treatments, which include oxidative steps, modify the surface of the hair, introducing negative charges as a result of oxidation of cystine to cysteic acid (3,10,22,23,25). This can result in transformation of the entire fiber surface from a hydrophobic to a hydrophilic character.

All of the above treatments also increase surface friction considerably (3,4,26,27) resulting in a significant increase in combing forces. The result is hair that feels rough and dry, and is subject to extensive grooming damage. Because of this damage, treated hair generally requires significantly more conditioning than required by the virgin hair. By using a conditioner, one can prolong the health of the hair fiber. It has been found that the cuticle cells on damaged, chemically processed hair are in better condition when a conditioner is used as part of the grooming process (28).

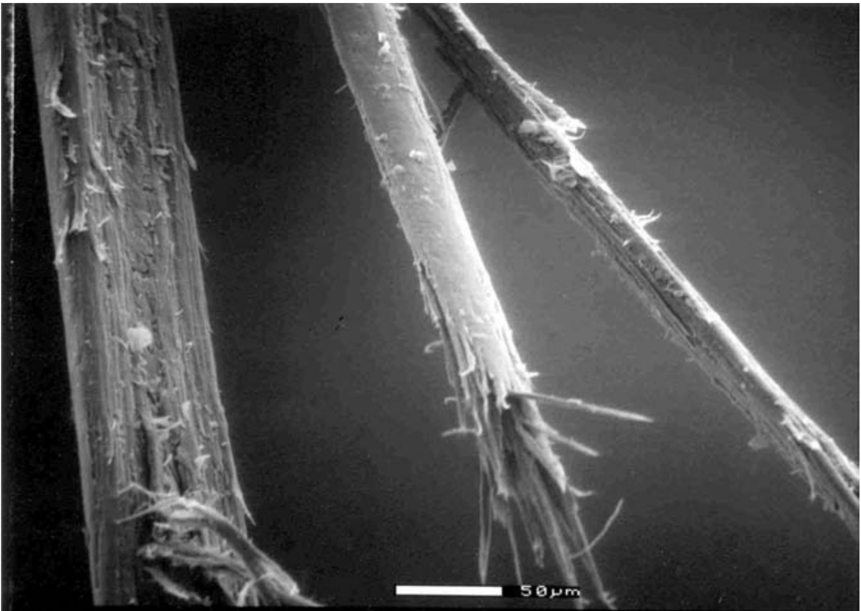

Figure 3 SEM photo of a split end. Note the exposed cortex and the complete loss of cuticle cells on the fiber surface.

COMMERCIAL CONDITIONERS

The commercial hair conditioners produced to deal with the above problems have appeared in almost every conceivable form, including thick vaseline pomades; thick, clear water soluble gels; spray mists of volatile substances; mousses; lotions; and creams. Conditioners have been marketed as leave-in or rinse-off products. They have also been positioned as preshampoo or postshampoo formulations.

Despite the wide variety of forms available, most commercial conditioners are oil-in-water emulsions in lotion form, having viscosities somewhere between 3000 and 12,000 centipoise. The great majority of these products are of the rinse-off type. In addition, despite different forms and positioning, most commercial conditioners contain the same general classes of conditioning agents with differences mainly in concentrations, numbers of different agents, and the particular members of a conditioning class employed.

The major classes of conditioning agents used in commercial products are surveyed in the following sections. Example formulae taken from the patent literature are listed below for some of the various forms of hair conditioners.

Ingredients	Weight %
Conditioning Spray (29)	
Trimethylolpropane triisostearate	1.00
Methyl myristate	1.00
Cetyl alcohol	1.00
Monoalkyl trimethyl ammonium chloride	0.2
Preservative	0.1
Perfume	0.1
Denatured ethyl alcohol	q.s. to 100
Conditioning Styling Gel (30)	
Sodium PCA (50% aqueous solution)	2.00
Glycerin	1.50
Hydrolyzed collagen	0.50
Carbomer 940	0.35
SD Alcohol 40	25.00
Nonionic surfactant	0.50
Fragrance	0.10
Water	q.s. to 100
Hair Conditioner (31)	
Water	q.s. to 100
Stearyl alcohol	2.50
Stearamidopropyl dimethylamine	1.00
Mineral oil	0.50
Cyclomethicone	0.25
Propylene glycol	0.50
Distearyl dimonium chloride	0.75
Hydroxyethylcellulose	1.00
Citric acid	0.20
Polyvinylpyrrolidone	0.10
Formalin (preservative)	0.10
Fragrance	0.20

Cationic Surfactants

Cationic surfactants in the form of quaternary ammonium compounds (quats) are the most widely used conditioning agents in commercial products (32–34). Among the reasons for their use are their effectiveness, versatility, availability, and low cost.

Important examples of these quats include stearalkonium chloride, cetrimonium chloride, and dicetyldimonium chloride.

$$C_6H_5CH_2 - N^+(CH_3)_2 - CH_2(CH_2)_{16}CH_3 \ \ Cl^-$$

Stearalkonium Chloride

$$CH_3 - N^+(CH_3)_2 - CH_2(CH_2)_{14}CH_3 \ \ Cl^-$$

Cetrimonium Chloride

$$CH_3 - N^+(CH_3)(CH_2(CH_2)_{14}CH_3) - CH_2(CH_2)_{14}CH_3 \ \ Cl^-$$

Dicetydimonium Chloride

Because of the positive charge of quaternary ammonium compounds such as the ones described above, they are substantive to hair and bind to negative sites on the hair surface. Treatment with these quats results, therefore, in a hydrophobic coating on the fiber that renders the hair softer and easier to comb (35). The buildup of static charge (flyaway) is also greatly reduced as a result of this surface modification (6).

Another consequence of the positive charge on quats is that deposition increases with increasing negative charge on the hair surface. This is seen in Table 1, which shows the results of an experiment in which hair tresses were treated with 1% stearalkonium chloride and then rinsed. Compared to the roots, 22% more quat

Table 1 Binding of Stearalkonium Chloride to Human Hair

	Quat deposition	
Type of hair	At roots (mg/g hair)	At tips (mg/g hair)
Virgin hair	0.649	0.789
Bleached hair	1.62	1.83

Source: From Ref. (36).

was found to bind to the tips of virgin hair, while deposition of stearalkonium chloride on bleached hair was found to be more than twice that on untreated fibers.

This result is important since, as was discussed above, damaged portions of the hair, which generally carry a greater amount of negative charge, require a greater amount of conditioning. The fact that cationic surfactants can supply this increased conditioning, makes them effective on a wide variety of hair surfaces. This is a major factor in the widespread use of these types of conditioning agents.

Conditioner Properties and Hydrophobicity

Many important properties of quaternary ammonium conditioners are related to the degree of hydrophobicity of the lipophilic portion of the surfactant. Thus, increasing the length of the alkyl chain of a monoalkyl quat and, therefore, making it more hydrophobic, leads to increased deposition (37–42) on hair. Cetrimonium chloride, as a result, deposits on hair to a greater extent than does laurtrimonium chloride. Increasing the number of alkyl chains also increases deposition so that tricetylmonium chloride exhibits greater deposition than does dicetyldimonium chloride, which, in turn, is more substantive than the monocetyl quat.

This dependence of deposition on degree of hydrophobicity indicates that van der Waals forces play an important role in deposition of quaternary ammonium conditioners (42). This conclusion is consistent with the entropy-driven deposition demonstrated by Ohbu et al. (43) and Stapleton (44) for a monoalkyl quat and a protonated long-chain amine.

Increased hydrophobicity also correlates with increased conditioning by quaternary ammonium compounds (37–40,45). Thus, cetrimonium chloride provides light to medium conditioning, while dicetyldimonium and tricetylmonium chlorides provide heavier conditioning. Detangling and wet combing, in particular, improve significantly from monocetyl to dicetyl to tricetyl quats; differences in dry combing and static charge among these compounds are not significant.

Increased conditioning with increased hydrophobicity is probably because, in part, of increased deposition of quat on hair. Data from Garcia and Diaz (46), however, indicate greater improvements in wet combing from heavier conditioning quats even when present on the hair in much lower amounts than less hydrophobic species. The degree of hydrophobicity of a quat must, therefore, play a direct role in the conditioning efficacy of these compounds (35).

Note that on some types of hair, the greater substantivity of higher conditioning quats can lead to a buildup with repeated use and result in limp, unmanageable hair. This is especially true, for example, for untreated, fine hair. Different quats or mixtures of conditioning agents are, therefore, suitable for different uses or different types of hair. A tricetyl quat might be used, for example, in an intensive conditioner meant only for occasional use.

The length and number of alkyl chains of quats also determines water solubility of these compounds. Monoalkyl quaternaries up to cetrimonium chloride are water soluble, for example, distearyldimonium chloride is water dispersible, while tricetylmonium chloride is insoluble in water (40).

Compatibility with Anionics

The quaternium compounds, normally used in commercial conditioners, are not generally found in shampoos because of incompatibility with common anionic

detergents (41). Introducing hydrophilic groups into the quat can increase compatibility with anionics. An example is the class of ethoxylated quaternaries, termed "ethoquats." Typical members of this class are PEG-2 cocomonium chloride, where x and y add up to 2 and R is a C12 alkyl chain, and PEG-15 stearmonium chloride where x and y add up to 15 and R is a C18 chain.

$$\begin{array}{c} (CH_2\,CH_2O)_xH \\ | \\ R—N^+—CH_3 \qquad Cl^- \\ | \\ (CH_2\,CH_2O)_yH \end{array}$$

ethoxylated quaternary

Both of these quats are compatible with typical anionic detergents. As would be expected from the above discussion, however, introducing hydrophilic groups decreases the conditioning efficacy of these materials (37,40). They are, therefore, suitable only in light conditioning formulations. Furthermore, conditioning shampoos based on ethoquats would not be expected to be very effective as a result of low deposition of the detergent-soluble ethoquat complex.

Other detergent-soluble quats have been produced. These include alkylamidopropyl dihydroxypropyl dimonium chlorides (48), lauryl methyl gluceth-10 hydroxypropyl dimonium chloride (49), and even a hydrolyzed ginseng-saponin quaternary derived from Korean ginseng saponin (50). Although certain advantages have been claimed for these surfactants, particularly low irritation, they all suffer from much the same conditioning limitations as the ethoquats.

Other Cationic Surfactants

In addition to the above examples, numerous other cationic surfactants are in use or have been proposed for commercial products. One example of a compound that has been receiving increased use recently is the behentrimonium (C22) quat. This quat exhibits significantly reduced eye and skin irritation compared to the corresponding C18 conditioner. In addition, superior conditioning and thickening properties have been claimed (51).

Another interesting example is hydrogenated tallow octyl dimonium chloride (52). This material is quite substantive and provides high conditioning as a result of its two hydrophobic chains. Unlike conventional dialkyl quats, however, this particular conditioner is soluble in water as a result of branching (2-ethylhexyl) in the octyl moiety. This solubility makes the compound much easier to formulate into a commercial product.

Stearamidopropyl dimethylamine is another conditioning agent that is found in many commercial conditioners. This material is cationic at the pHs normally employed in conditioning products and, therefore, acts as a cationic emulsifier and also as a secondary conditioning agent.

Concern for the environment has led to the synthesis of ester quats that exhibit increased biodegradability and environmental safety. One such example is

dipalmitoylethyl hydroxyethylmonium methosulfate, an ester quat based on a partially hydrogenated palm radical (53).

Other cationic surfactants used in conditioners include quats derived from Guerbet alcohols (45) (low to high conditioning depending on length of the main and side alkyl chains), distearyldimonium chloride (high conditioning), and the quaternized ammonium compounds of hydrolyzed milk protein, soy and wheat protein, and hydrolyzed keratin (varying conditioning efficacy depending on alkyl chain length).

Lipophilic Conditioners

Quaternary ammonium surfactants in commercial products are almost never used alone. Instead, they are employed in combination with long-chain fatty conditioners, especially cetyl and stearyl alcohols (34). These fatty materials are added to boost the conditioning effects of the quaternary compounds (49). In one study, for example, addition of cetyl alcohol to cetrimonium bromide nearly doubled the observed reduction in wet combing forces on hair (54). In another study, using a novel hydrodynamic technique, Fukuchi et al. (55) found that addition of cetyl alcohol to a behentrimonium chloride formulation resulted in significantly reduced surface friction.

Several workers have studied combinations of cationic surfactants and fatty alcohols. Under the right conditions, these mixtures have been found to form liquid crystal mesophases and gel networks (56–60) that can greatly increase viscosity and, at the same time, confer stability upon emulsions. As a result of reduced repulsion between cationic head groups when long chain alcohols are interposed, liquid crystal formation has been observed even at low concentrations (59,60). The ready formation of these extended structures between quats and cetyl and stearyl alcohols, along with the lower cost, stability, and compatibility with cosmetic ingredients of the latter are important reasons why these alcohols are so ubiquitous in conditioning formulations.

Care should be taken in manufacturing conditioners that the cooling rate is not too rapid to interfere with the proper formation of the liquid crystal. In addition, it has been claimed that improved freeze–thaw stability is conferred upon conditioners when using certain combinations of ethoxylated branched-chain fatty alcohol ethers or esters as stabilizers (61).

Other lipids found in commercial products include glycol distearate, triglycerides, fatty esters, waxes of triglycerides, liquid paraffin, etc.

Cationic Polymers

There are numerous cationic polymers that provide conditioning benefits, especially improved wet combing and reduced static charge. Important examples of these polymers are Polyquaternium-10 (PQ-10), a quaternized hydroxyethylcellulose polymer; Polyquaternium-7 (PQ-7), a copolymer of diallyldimethylammonium chloride and acrylamide; Polyquaternium-11, a copolymer of vinylpyrrolidone and dimethylaminoethyl methacrylate quaternized with dimethyl sulfate; Polyquaternium-16, a copolymer of vinylpyrrolidone and quaternized vinylimidazole; and Polyquaternium 6, a homopolymer of diallyldimethylammonium chloride.

By virtue of their cationic nature, the above polymers are substantive to hair. The particular conditioning effectiveness of any of these materials depends on the polymer structure. In one set of studies, deposition on hair was found to be inversely proportional, roughly, to cationic charge density (62,63). This has been explained by

the observation that the higher the charge density, the lower the weight of polymer needed to neutralize all the negative charge on the hair. Once deposited, however, multiple points of electrostatic attachment make these polymers harder to remove, especially, if charge density is high (36,65). Care must be taken, therefore, in formulating conditioners containing these materials to avoid over-conditioning as a result of a buildup with continued use.

As with the preceding monofunctional cationics, deposition of polyquaterniums increases on treated or damaged hair (36,64,65). Unlike common monofunctional quats, however, the first four of the above polymers are compatible in varying degrees with anionic surfactants (64–68). As a result, such polymers are used more often in shampoos than in stand-alone conditioners, although they find some use in leave-in conditioners.

PQ-10 and PQ-7 are two of the most frequently used polymers in commercial shampoos. Both of these polymers form negatively charged complexes (62,64) with excess anionic surfactant, resulting in reduced deposition because of repulsion by the negatively charged hair surface. The magnitude of this effect depends on the particular anionic employed and on the anionic surfactant/polymer ratio. In all cases, however, conditioning from shampoos is significantly less than from stand-alone conditioners.

Despite reduced deposition, Hannah (69) has reported that polyquaternium association complexes formed with sodium lauryl sulfate resist removal from hair. Buildup and a heavy, coated feel on the hair can, therefore, result from conditioning shampoos containing polyquats, unless they are carefully formulated.

Research conducted at TRI/Princeton has shown that the type of deposition and degree of penetration into the hair fiber depends on the size or molecular weight of the polymer. The interaction between the cationic conditioners and the hair fiber mainly occurs at the surface, however, low molecular weight polymers may penetrate into the interior via intercellular diffusion. Cetrimonium bromide (CETAB), for example, is capable of being absorbed into the cuticular sheath as well as the cortex. In addition to providing conditioning benefits, some polyquaternium materials have been shown to improve adhesion of the cuticle scales thereby increasing resistance to scale uplift when the hair is stressed. The same effects were observed for at least one quat—CETAB (70).

Silicones

The use of silicones in hair care products has increased considerably in the past two decades, although their first incorporation into commercial products dates back to the 1950s. Different types of silicones find use as conditioning agents in a wide variety of products including conditioners, shampoos, hair sprays, mousses, and gels (71). One of the most widely used silicones is dimethicone, which is a polydimethylsiloxane. Other important silicones are dimethiconol which is a dimethylsiloxane terminated with hydroxyl groups, dimethicone copolyol which is a dimethylsiloxane containing polyoxyethylene and/or polyoxypropylene side chains and amodimethicone, which is an amino-substituted silicone. In general, amodimethicones condition better than dimethicones which condition better than dimethicone copolyols (72). Presumably this is due to the differences in the substantivity from rinse-off products. Because of the increased substantivity, care should be taken with amodimethicones to make sure they do not build-up over time. Likewise, many dimethicone copolyols are soluble in water and, therefore, may not be as effective in rinse-off products.

$$
\begin{array}{ccccccc}
 & & CH_3 & & CH_3 & & CH_3 \\
 & & | & & | & & | \\
CH_3 & — & SiO & — & (SiO)_x & — & Si — CH_3 \\
 & & | & & | & & | \\
 & & CH_3 & & CH_3 & & CH_3
\end{array}
$$

Dimethicone

$$
\begin{array}{ccccccccc}
 & & CH_3 & & CH_3 & & CH_3 & & CH_3 \\
 & & | & & | & & | & & | \\
CH_3 & — & SiO & — & (SiO)_m & — & (SiO)_n & — & Si — CH_3 \\
 & & | & & | & & | & & | \\
 & & CH_3 & & CH_3 & & (CH_2)_3 & & CH_3 \\
 & & & & & & & & | \\
 & & & & & & & & O(C_2H_4O)_aH
\end{array}
$$

Dimethicone copolyol

$$
\begin{array}{ccccccc}
 & & CH_3 & & CH_3 & & CH_3 \\
 & & | & & | & & | \\
HO & — & SiO & — & (SiO)_x & — & (SiO)_y — H \\
 & & | & & | & & | \\
 & & CH_3 & & CH_3 & & (CH_2)_3 \\
 & & & & & & | \\
 & & & & & & NHCH_2CH_2NH_2
\end{array}
$$

Amodimethicone

Many silicones used in hair care products are insoluble and must, therefore, be emulsified. To increase ease of product manufacture, many suppliers offer silicones as preformed emulsions in addition to the pure material. Emulsions can vary in charge (anionic, cationic, or nonionic), size (microemulsion or macroemulsion), and how they are made (mechanical or emulsion polymerization). The factors

affecting deposition of silicones from such emulsions have been reported by Jachowicz and Berthiaume (73,74) and by Hoag et al. (75).

Silicone emulsions can also vary in particle size. Typically, the smaller the size of the silicone particles, the more stable is the product emulsion. Additionally, it has been claimed that reducing the particle size also improves conditioning performance (76). If a preformed silicone emulsion is not used, particle size of the silicone droplets can be controlled by combining the correct amount of heat and shear when making the product.

Conditioning Properties of Silicones

Silicones used in hair care products possess a range of unique properties including lubricity, low intermolecular forces, water insolubility, and low surface tension. These properties permit the silicones to spread easily on the hair surface, thereby forming a hydrophobic film that provides ease-of-combing and imparts a smooth, soft feel to the hair without greasiness.

The relative conditioning efficacy of silicones compared to other conditioners was demonstrated by Yahagi (77) who found that dimethicone lowered frictional coefficients and surface energy of virgin hair to a greater extent than did a series of cationic surfactants, including distearyldimonium chloride, a very effective conditioning agent. Dimethicones with molecular weights greater than 20,000 were found to be most effective in reducing surface tension.

Nanavati and Hami (78) measured conditioning on slightly bleached European hair treated with dimethicone fluids and dimethiconol gums. Both types of silicones were found to significantly reduce combing forces on hair. Ease of wet combing was roughly the same for the two silicone treatments, whereas dimethiconol was found to be more effective in reducing dry combing forces.

Interestingly, under the treatment conditions employed (exposure to silicone solutions for 30 seconds followed by drying without rinsing), deposition of all silicones studied was found to nearly double if tricetyldimonium chloride was present in the treatment solution. Reduction in combing forces was also roughly doubled when silicones were deposited in the presence of quat. This latter effect was found to be synergistic; i.e., it depended on deposition of both silicone and quat, and its magnitude was greater than the sum of the individual conditioner contributions.

Wendel et al. (79) used electron spectroscopy for chemical analysis (ESCA) to demonstrate that the presence of amino groups in silicones considerably increases substantivity of these materials. This is a result of the positive charge developed by these groups at the pHs commonly found in commercial products.

Comparison of conditioning effects of a series of silicone emulsions on bleached and virgin hair was carried out by Hoag et al. (75). Most of the silicones were dimethicones or amodimethicones, while emulsions were anionic, neutral, or cationic in nature. Diluted emulsions were applied directly to the hair and combing forces measured both before and after rinsing. Prior to rinsing, reduction of combing forces by most emulsions was greater than 80%. This number was decreased after rinsing as a result of partial removal of deposited silicone. Unsurprisingly, the least change in ease-of-combing was found for cationic emulsions, especially those containing amodimethicone. Combing forces on virgin hair increased less than on bleached hair, after rinsing, indicating that the silicones were more substantive to this type of hair. This is also unsurprising considering the hydrophobic nature of these conditioning agents.

Further effects of amodimethicones can be seen in work reported by Berthiaume et al. (80), who studied a series of amodimethicone emulsions in a prototype conditioner formulation. Deposition on hair from the conditioner was found to increase with increasing amine content in the silicone. This increased deposition was found, in half-head tests, to correlate with conditioning efficacy, including wet and dry combing, softness, and detangling. A microemulsion in the test series that provided high conditioning was also shown to significantly reduce the color fading caused by shampooing of temporarily dyed hair.

Other Silicones

One silicone that is widely used in conditioners to help improve wet combing is cyclomethicone, which refers to a class of cyclic dimethyl polysiloxanes ranging from trimer to hexamer. Cyclomethicone is volatile and will not remain on dry hair, especially after blow-drying. It helps other conditioning agents disperse, however, and form films on hair. It also helps improve wet combing and provides transient shine.

Newer silicones include dimethicone copolyol phosphates which are anionic functional silicones and fluorocarbon-modified organosilicones. The copolyol phosphates are able to form complexes with tertiary amines of cationic hair conditioners and form effective emulsifiers and conditioners (81). The fluorocarbon-modified silicones are very hydrophobic like dimethicone; however, they claimed to have a lighter more lubricious feel (81).

2-in-1 Shampoos

Silicones find important application as the primary conditioning agents in 2-in-1 conditioning shampoos. Upon their introduction in the latter part of the 1980s, these shampoos represented a major advance in hair care technology, providing a significantly higher degree of conditioning than was then the norm for conditioning shampoos and, at the same time, leaving a desirable soft, smooth feel on the hair.

Conditioning from 2-in-1 shampoos is expected to occur primarily at the rinsing stage during which time the shampoo emulsion breaks, releasing the silicone for deposition on hair. This separation of cleaning and conditioning stages permits the shampoo to perform both functions efficiently.

The conditioning agent used most frequently in 2-in-1 shampoos is dimethicone. This silicone can provide good performance in shampoo formulations without excessive buildup on the hair (82). With advances in technology, newer formulations are now employing easier to process silicones, such as dimethicone emulsions, amodimethicones, dimethiconols, and copolyols as well as combinations of these types to deliver the desired level of conditioning and the improved product aesthetics.

The level of conditioning from 2-in-1 shampoos is lower than that from standalone conditioners. This is especially true for treated hair since the greater the degree of negative charge on the hair surface, the lower the substantivity of a hydrophobic material like dimethicone. Many 2-in-1 products contain polyquats, which might be expected to increase conditioning on damaged hair. In shampoos with high levels of anionic detergent, however, polyquat performance on treated hair may be no better than that of dimethicone as a result of formation of the negatively charged polymer complexes discussed in the section "Cationic Polymers."

Yahagi (77) studied the performance of dimethicone, amodimethicone, and dimethicone copolyols in 2-in-1 shampoos. Ease-of-combing was found to be similar for hair treated with shampoos containing dimethicone or amodimethicone.

Unsurprisingly, soluble dimethicone copolyols did not perform well; insolubility, or at least dispersibility, was required for adequate silicone deposition. In the latter case, dimethicone copolyols were found to provide a slightly lower level of conditioning than the other two silicones studied, especially once blow-drying was begun. Yahagi also studied silicone effects on foam volume. In these studies, dimethicone was found to significantly reduce foam volume in a model shampoo formulation, while amodimethicone and dimethicone copolyol had a minimal effect on foam.

Auxiliary Ingredients

A number of ingredients besides conditioning actives are added to commercial conditioners for functional, esthetic, and marketing purposes (83). These include fragrances, dyes, preservatives, thickeners, emulsifying agents, pearlizers, herbal extracts, humectants, and vitamins. Some of these are discussed in the following sections. The literature also contains many examples of such additives (34,84–88).

Preservatives

Preservatives are necessary to insure the microbiological integrity of a conditioning product. If the product contains high concentrations of ethyl alcohol (generally 20% or above), additional preservatives are not needed and the product is described as self-preserving.

For other products, a wide variety of preservatives are available; in general, combinations of different preservatives provide the broadest possible protection. Every commercial product that is not self-preserving must be carefully tested over time for adequacy of preservation. Most of the preservatives used in personal care products are described in the Cosmetic Preservatives Encyclopedia (86).

Thickeners

"Lipophilic Conditioners" on page 415, described thickening as a result of liquid crystal formation in those products containing common quaternary ammonium compounds and fatty alcohols. Cationic conditioning polymers (section "Cationic Polymers") can also act as thickeners. Many formulations may require additional thickening agents. Hydroxyethylcellulose, a nonionic cellulose ether compatible with cationic surfactants and stable over a wide pH range, is the most common thickening agent added to conditioning products (34). In addition to providing increased viscosity, this material stabilizes viscosity over time.

Polyamides may also be used to thicken formulations. A commercial product, Sepigel® (which contains polyamide, laureth-7, and isoparaffin) can be used to emulsify and thicken lotion or cream conditioners. Other thickeners are described in Ref. (87).

Humectants

Many conditioners contain humectants whose purpose is to attract moisture. Examples are propylene glycol, glycerine, honey, chitosan, and hyaluronic acid. These materials are not expected to be very effective in rinse-off products.

Emulsifiers

As discussed on page 415 in "Lipophilic Conditioners" the fatty alcohol/quat combinations found in common conditioners confer stability on product emulsions. If necessary,

other emulsifiers may be added to improve stability. Information on emulsions and emulsifiers may be found in the literature (88,89), as well as from manufacturers' technical bulletins. Most emulsifiers utilized in conditioners are nonionic, including ethoxylated fatty alcohols, ethoxylated fatty esters, and ethoxylated sorbitan fatty esters.

CONCLUSION

The foregoing sections have surveyed the action and properties of a diverse assortment of commercially available conditioning agents. The availability of a large selection of conditioning materials enables the formulator to tailor products to a wide variety of people having differing conditioning needs and preferences. Thus, a person having short, straight hair in good condition might desire a conditioner primarily to control flyaway. Such a need could be satisfied by one of the ethoquats, which provide light conditioning benefits together with very good static control. A person having long, heavily bleached hair, on the other hand, would require improved hair feel, ease-of-combing, and manageability. These benefits could best be provided by a trialkyl quat.

Those people sensitive to hair feel might prefer a product containing a silicone as a secondary conditioner. Other people might prefer the convenience of a 2-in-1 shampoo. In many cases, both 2-in-1 shampoos and stand-alone conditioners are used to condition the hair.

There are a number of ways in which one might satisfy the conditioning needs of a target population. It is anticipated that the information in this chapter will help the formulator to quickly select the best conditioning system for a given purpose. It is also hoped that the material in this chapter will help the formulator to effectively evaluate new conditioning agents and even to work with synthetic chemists as well as suppliers to design new conditioning compounds to solve particular problems.

REFERENCES

1. Robbins CR. Chemical and Physical Behavior of Human Hair. 3rd ed. New York: Springer, 1994:343.
2. Kamath YK, Weigmann HD. Measurement of combing forces. J Soc Cosmet Chem 1986; 37:111–124.
3. Jachowicz J. Hair damage and attempts to its repair. J Soc Cosmet Chem 1987; 38: 263–286.
4. Scott GV, Robbins CR. Effects of surfactant solutions on hair fiber friction. J Soc Cosmet Chem 1980; 31:179–200.
5. Lunn AC, Evans RE. The electrostatic properties of human hair. J Soc Cosmet Chem 1977; 28:549–569.
6. Jachowicz J, Wis-Surel G, Garcia, ML. Relationship between turboelectric charging and surface modifications of human hair. J Soc Cosmet Chem 1985; 36:189–212.
7. Reich C, Robbins CR. Interactions of cationic and anionic surfactants on hair surfaces: Light-scattering and radiotracer studies. J Soc Cosmet Chem 1993; 44:263–278.
8. Robbins CR, Crawford RJ. Cuticle damage and the tensile properties of human hair. J Soc Cosmet Chem 1991; 42:59.
9. Robbins CR. Chemical and Physical Behavior of Human Hair. 3rd ed. New York: Springer, 1994:301.

10. Tate ML, Kamath YK, Ruetsch SB, Weigmann HD. Quantification and prevention of hair damage. J Soc Cosmet Chem 1993; 44:347–371.
11. Garcia ML, Epps JA, Yare RS. Normal cuticle-wear pattern in human hair. J Soc Cosmet Chem 1978; 29:155–175.
12. Kelley S, Robinson VNE. The effect of grooming on the hair surface. J Soc Cosmet Chem 1982; 33:203–215.
13. Ruetsch, SB, Kamath YK. Effects of thermal treatments with a curling iron on hair fiber. J Cosmet Sci 2004; 55:13–27.
14. Crudele J, et al. Heat-mediated conditioning from shampoo and conditioner hair care compositions containing silicone. US Patent 6,211,125 B1.
15. Kamath YK, Danziger CJ, Weigmann HD. Surface wettability of human hair. I. Effect of deposition of polymers and surfactants. J Appl Polym Sci 1984; 29:1011–1026.
16. Wolfram LJ, Lindemann MKO. Some observations on the hair cuticle. J Soc Cosmet Chem 1971; 22:839–850.
17. Negri AP, Cornell HJ, Rivett DE. A model for the surface of keratin fibers. Text Res J 1993; 63:109–115.
18. Shao J, Jones DC, Mitchell R, Vickerman JC, Carr CM. Time-of-flight secondary-ion-mass spectrometric (ToF-SIMS) and x-ray photoelectron spectroscopic (XPS) analyses of the surface lipids of wool. J Text Inst 1997; 88(Part 1,4):317–324.
19. Wilkerson VJ. The chemistry of human epidermis. II. The isoelectric points of the stratum corneum, hair, and nails as determined by electrophoresis. J Biol Chem 1936; 112:329–335.
20. Robbins CR, Bahl MK. Analysis of hair by electron spectroscopy for chemical analysis. J Soc Cosmet Chem 1984; 35:379–390.
21. Stranick MA. Determination of negative binding sites on hair surfaces using XPS and Ba^{2+} labeling. Surf Interface Anal 1996; 24:522–528.
22. Horiuchi T. Nature of damaged hair. Cosmet Toilet 1978; 93:65–77.
23. Kaplin IJ, Schwann A, Zahn H. Effects of cosmetic treatments on the ultrastructure of hair. Cosmet Toilet 1982; 97:22–26.
24. Sandhu SS, Ramachandran R, Robbins CR. A simple and sensitive method using protein loss measurements to evaluate damage to human hair during combing. J Soc Cosmet Chem 1995; 46:39–52.
25. Robbins CR. Chemical and Physical Behavior of Human Hair. 3rd ed. New York: Springer, 1994:120–126, 234–249.
26. Schwartz A, Knowles D. Frictional effects in human hair. J Soc Cosmet Chem 1963; 14:455–463.
27. Robbins CR. Chemical and Physical Behavior of Human Hair. 3rd ed. New York: Springer, 1994:341.
28. Feughelman M, Willis BK. Mechanical extension of human hair and the movement of the cuticle. J Cosmet Sci 2001; 52:185–193.
29. Mitsumatsu A. Hair Conditioning Compositions Comprising Water-Insoluble High Molecular Weight Oily Compound. Assigned to The Procter & Gamble Company. US Patent 6,368,582 B1 (2002).
30. Newell, Gerald P. Method of restoring normal moisture level to hair with severe moisture deficiency. Assigned to Helene Curtis Industries. US Patent 4,220166 (1980).
31. Patel A et al. Hair Rinse Conditioner. Assigned to Colgate-Palmolive Co. US Patent 4,726,945 (1988).
32. Quack JM. Quaternary ammonium compounds in cosmetics. Cosmet Toilet 1976; 91(2):35–52.
33. Gerstein T. An introduction to quaternary ammonium compounds. Cosmet Toilet 1979; 94(11):32–41.
34. Hunting ALL. Encyclopedia of Conditioning Rinse Ingredients. Cranford, New Jersey: Micelle Press, 1987.

35. Foerster T, Schwuger MJ. Correlation between adsorption and the effects of surfactants and polymers on hair. Progr Colloid Polym Sci 1990; 83:104–109.
36. Reich C. Hair cleansers. In: Rieger MM, Rhein LD, eds. Surfactants in Cosmetics. 2nd ed. Surfactant Science Series. Vol 68. New York: Marcel Dekker, 1997:373.
37. Jurczyk MF, Berger DR, Damaso GR. Quaternary ammonium salt. Applications in hair conditioners. Cosmet Toilet 1991; 106:63–68.
38. Finkelstein P, Laden K. The mechanism of conditioning of hair with alkyl quaternary ammonium compounds. Appl Polym Symp 1971; 18:673–680.
39. Jachowicz J. Fingerprinting of cosmetic formulations by dynamic electrokinetic and permeability analysis. II. Hair conditioners. J Soc Cosmet Chem 1995; 46:100–116.
40. Spiess E. The influence of chemical structure on performance in hair care preparations. Parfum Kosmetik 1991; 72(6):370–374.
41. Scott GV, Robbins, CR, Barnhurst JD. Sorption of quaternary ammonium surfactants by human hair. J Soc Cosmet Chem 1969; 20:135–152.
42. Robbins CR, Reich C, Patel A. Adsorption to keratin surfaces: A continuum between a charge-driven and a hydrophobically driven process. J Soc Cosmet Chem 1994; 45:85–94.
43. Ohbu K, Tamura T, Mizushima N, Fukuda M. Binding characteristics of ionic surfactants with human hair. Colloid Polym Sci 1986; 264:798–802.
44. Stapleton IW. The adsorption of long chain amines and diamines on keratin fibers. J Soc Cosmet Chem 1983; 34:285–300.
45. Yahagi K, Hoshino N, Hirota H. Solution behavior of new cationic surfactants derived from Guerbet alcohols and their use in hair conditioners. Int J Cosmet Sci 1991; 13:221–234.
46. Garcia ML, Diaz J. Combability measurements on human hair. J Soc Cosmet Chem 1976; 27:379–398.
47. Fox C. An introduction to the formulation of shampoos. Cosmet Toilet 1988; 103(3):25–58.
48. Smith L, Gesslein BW. Multi-functional cationics for hair and skin applications. Cosmet Toilet 1989; 104:41–47.
49. Polovsky SB. An alkoxylated methyl glucoside quaternary. Cosmet Toilet 1991; 106:59–65.
50. Kim YD, Kim CK, Lee CN, Ha BJ. Hydrolysed ginseng-saponin quaternary: a novel conditioning agent for hair care products. Int J Cosmet Chem 1989; 11:203–220.
51. Gallagher KF. Superior conditioning and thickening from long-chain surfactants. Cosmet Toilet 1994; 109:67–74.
52. Jurczyk MF. A new quaternary conditioner for damaged hair. Cosmet Toilet 1991; 106:91–95.
53. Shapiro I, Sajic B, Bezdicek R. Environmentally friendly ester quats. Cosmet Toilet 1994; 109:77–80.
54. Hunting ALL. Encyclopedia of Conditioning Rinse Ingredients. Cranford, New Jersey: Micelle Press, 1987:147.
55. Fukuchi Y, Okoshi M, Murotani I. Estimation of shampoo and rinse effects on the resistance to flow over human hair and hair softness using a newly developed hydrodynamic technique. J Soc Cosmet Chem 1989; 40:251–263.
56. Eccleston GM, Florence AT. Application of emulsion theory to complex and real systems. Int J Cosmet Chem 1985; 7:195–212.
57. Eccleston GM. The structure and rheology of pharmaceutical and cosmetic creams. Cetrimide creams: The influence of alcohol chain length and homolog composition. J Colloid Int Sci 1976; 57:66–74.
58. Barry BW, Saunders GM. Kinetics of structure build-up in self bodied emulsions stabilized by mixed emulsifiers. J Colloid Int Sci 1972; 41:331–342.
59. Barry BW, Saunders GM. The self-bodying action of the mixed emulsifier cetrimide/cetostearyl alcohol. J Colloid Int Sci 1970; 34:300–315.

60. Barry BW, Saunders GM. The influence of temperature on the rheology of systems containing alkyltrimethylammonium bromide/cetostearyl alcohol: Variation with quaternary chain length. J Colloid Int Sci 1971; 36:130–138.
61. Su, Dean Terng-Tzong. Hair conditioner compositions having improved freezing and freeze–thaw stability. Assigned to Colgate-Palmolive Company. US Patent 6,287.545 B1 (2001).
62. Hossel P, Pfrommer E. Test methods for hair conditioning polymers. In: In-Cosmet Exhib Conf Conf Proc Augsburg, Germany: Verlag fuer Chemishe Industrie H. Ziolkowsky, 1994:133–148.
63. Pfau A, Hossel P, Vogt S, Sander R, Schrepp W. The interaction of cationic polymers with human hair. Macromol Symp 1997; 126:241–252.
64. Sykes AR, Hammes PA. The use of Merquat polymers in cosmetics. Drug Cosmet Ind February, 1980; 62–66.
65. Ucare polymers: Conditioners for all conditions. Amerchol Corporation. Technical bulletin.
66. Faucher JA, Goddard ED. Influence of surfactants on the sorption of a cationic polymer by keratinous substrates. J Colloid Int Sci 1976; 55(2):313–319.
67. Goddard ED, Faucher JA, Scott RJ, Turney ME. Adsorption of Polymer JR on keratinous surfaces. Part II. J Soc Cosmet Chem 1975; 26:539–550.
68. Caelles J, Cornelles F, Leal JS, Parra JL, Anguera S. Anionic and cationic compounds in mixed systems. Cosmet Toilet 1991; 106(4):49–54.
69. Hannah RB et al. Desorption of a cationic polymer from human hair: Surfactant and salt effects. Text R J 1978; 48:57.
70. Ruetsch, Sigrid B, Kamath YK, Weigmann H-D. The role of cationic conditioning compounds in reinforcement of the cuticula. J Cosmet Sci 2003; 54:63–83.
71. Luoma A, Kara R. Silicones and the perm question. Society of Cosmetic Chemists 1988 Spring Conference on Hair Care, London, UK, April 21–23, 1998.
72. Abrutyn, Eric S. Organo-modified siloxane polymers. In: Schueller, Randy, Romanowski, Perry, eds. Conditioning Agents for Hair and Skin. Cosmetic Science and Technology Series. vol. 21. New York: Marcel Dekker, 1999:191.
73. Jachowicz J, Berthiaume MD. Heterocoagulation of silicon emulsions on keratin fibers. J Colloid Int Sci 1989; 133:118–134.
74. Berthiaume MD, Jachowicz J. The effect of emulsifiers on deposition of nonionic silicone oils from oil-in-water emulsions onto keratin fibers. J Colloid Int Sci 1991; 141:299–315.
75. Hoag CA, Rizwan BM, Quackenbush KM. Evaluating silicone emulsions for global hair care applications. Global Cosmet Ind April 1999:44–55.
76. Gallagher, P. et al. Shampoo compositions comprising and emulsified silicone an a microemulsified silicone. Assigned to Unilever Home & Personal Care USA. US Patent 6,706,258 B1 (2004).
77. Yahagi K. Silicones as conditioning agents in shampoos. J Soc Cosmet Chem 1992; 43:275–284.
78. Nanavati S, Hami A. A preliminary investigation of the interaction of a quat with silicones and its conditioning benefits on hair. J Soc Cosmet Chem 1994; 43:135–148.
79. Wendel SR, Disapio AJ. Organofunctional silicones for personal care applications. Cosmet Toilet 1983; 98:103–106.
80. Berthiaume MD, Merrifield JH, Riccio DA. Effects of silicone pretreatment on oxidative hair damage. J Soc Cosmet Chem 1995; 46:231–245.
81. Rosen, Meyer R. Silicone technologies for personal care. Global Cosmetic Industry May 2000:28–32.
82. Rushton H, Gummer CL, Flasch H. 2-in 1 shampoo technology: State of the art shampoo and conditioner in one. Skin Pharmacol 1994; 7:78.
83. Hoshowski MA. Conditioning of hair. In: Johnson DH, ed. Hair and Hair Care. Cosmetic Science and Technology Series. vol. New York: Marcel Dekker, 1997:65–104.
84. Wenninger JA, McEwen GN, eds. CTFA Cosmetic Ingredients Handbook. 3rd ed. Washington, DC: Cosmetic Toiletry and Fragrance Association, 1995.

85. Leung AY. Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics. New York: John Wiley & Sons, 1980.
86. Cosmetic Preservatives Encyclopedia—Antimicrobials. Cosmet Toilet 1990; 105(3);49–63.
87. Lochhead R. Encyclopedia of polymers and thickeners for cosmetics. Cosmet Toilet 1988; 103(12):99–129.
88. McCutcheon's Vol. 1: Emulsifiers and Detergents. North American ed. Glen Rock, New Jersey: MC Publishing, 1991.
89. Becher P, ed. Encyclopedia of Emulsion Technology. New York: Marcel Dekker, 1985.

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Skin Care Products

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AN OVERVIEW OF EMULSION-BASED SKINCARE PRODUCTS

A variety of skincare products exist in today's marketplace. They fulfill a variety of functions by either acting directly on the skin (e.g., moisturizers) or being a cosmetically elegant vehicle for the delivery of specific active ingredients (e.g., sunscreens or antipuretic or antiacne medicaments). In general, these products may be categorized into three functional groups, namely,

- Drugs: To prevent or ameliorate diseases by altering the structure and/or function of the body.
- Cosmetics: To beautify and improve the feeling or sensory aspects of normal and/or nondiseased skin; dry skin would be included in this category.
- Cosmeceuticals: An intermediate classification for cosmetic products that may enhance the function of the skin. Currently, the United States Food and Drug Administration (FDA) does not recognize this category (1).

There is a similar classification in the European Union.

The three product groups can also be classified by their physical properties. Most common forms of skincare products are emulsions. Emulsions are mixtures of two insoluble materials that are stabilized against separation. An example is oil-in-water, which will not mix unless an intermediate emulsifier is incorporated into the mixture.

Different Types of Emulsions

Emulsifiers can act as solubilizers, spreading or dispersing agents. Correct use of emulsifiers permits one to formulate homogeneous mixtures, dispersions or emulsions of oily, waxy substances with water. Solids may be dispersed in liquids or in insoluble liquids within other liquids. Greasy anhydrous ointments can be designed to be more washable. These types of properties may be achieved by appropriate selection of emulsifiers, active ingredients, and other compatible ingredients in the vehicle.

Emulsions may be water-in-oil (w/o), oil-in-water (o/w), aqueous gel, and silicone-in-water. Other products may be formulated as semisolids containing

oleaginous ingredients, absorption bases, and water-soluble types containing polyethylene glycol. Recently, there has been a growing interest in water-in-oil-in-water (w/o/w), also referred to as multiple emulsions.

O/W emulsions are the most commonly formulated. These types of emulsions tend to feel less greasy and have a lower formulation cost because of a higher water content. Water-in-oil (w/o) emulsions have historically been less popular because of a characteristic greasy, oily feel on application to skin. However, the development of newer emulsifiers has enabled a skilled formulator to develop w/o emulsions of a lighter texture. Silicone formulation aids may also be used to form stable water-in-silicone (w/Si) or w/o emulsions. These are polymeric surface-active agents with long bond lengths and wide bond angles. This provides for free rotation of functional groups permitting formulations of w/o and w/Si emulsions with exceptional elegance and good coverage when applied to skin (2). This enables formulation of stable emulsions with medium to low viscosity. These different chemical types of emulsions are commonly referred to as vehicles when "cosmetic" active or drug active ingredients are incorporated into them (Table 1).

Not all emulsifiers behave in the same way. Properties of the emulsifier will determine the emulsion type. Their compatibility with oils having different polarities is also a critical concern. Emulsifiers will impact the desired sensory properties of the product such as color, odor, and desired viscosity (e.g., lotion or cream consistency).

Different Types of Emulsifiers

Emulsifying agents, which are surface-active agents (surfactants), are available in a wide range of chemical types. These include nonionic, hydrophobic, lipophilic, ethoxylated, and nonethoxylated. A recent trend is to lower or even eliminate surfactants to minimize the prevailing low irritation potential of the formulation. It is possible to formulate emulsifier-free emulsions with crosslinked acrylic polymer derivatives. These materials are hydrophilic polymers that are hydrophobically modified by adding an alkylic chain. These molecules, known as polymeric emulsifiers, provide additional formulation options for new product development (4).

Table 1 Examples of Vehicle Types

Type of emulsion	Examples
W/O	Cold creams, cleansing or evening creams (overnight creams)
O/W	Common moisturizers hand and body lotions
Oleaginous	Petrolatum
Water-soluble	Polyethylene glycol based ointments
Aqueous gels	Lubricating jelly. Gelling agents such as Carbomers®, hydroxyethylcellulose, and magnesium aluminum silicate may be used in the formulation
Absorption bases	Hydrophilic petrolatum, these vehicles may contain raw materials able to function as water in oil emulsifiers permitting large quantities of water to be incorporated as emulsified droplets

Source: From Ref. 3.

FORMULATING HYDRATING CREAMS AND LOTIONS

The continuing development of biophysical instrumentation and test techniques has enabled formulation of highly effective skin care formulations. Formulators now have several options for formulating new products. When initiating formulation development, it is important to understand project/product requirements, type of product(s), performance and aesthetics needs, formulation cost constraints, packaging needs, product claims, and formulation safety. To what part of the body will the formulation be applied and at what time of day, morning or overnight? Will makeup be applied over the product? Will clothing come into contact with the product? Will the targeted consumer apply a fragrance to the body after application of the product, if so will the fragrances conflict? Once these requirements are defined, the formulator can consider active ingredients, emulsion systems, preservative systems color, and fragrance.

Emulsions allow the formulating chemist to combine otherwise incompatible ingredients into an effective commercially desirable cosmetic product. Key in product development is the technique employed to select appropriate raw materials. Commonly used emulsifying agents are either ionic (anionic or cationic) or nonionic. The function of the emulsifying agent depends on the unique chemical structure of the emulsifier. Each emulsifier has a hydrophilic (water-loving) and lipophilic (oil-loving) part. Examples of hydrophilic moieties are polyhydric alcohols and polyethylene chains. Lipophilic parts may be a long hydrocarbon chain, such as fatty acids, cyclic hydrocarbons, or combination of both. Nonionic agents may have hydrophilic action generated by hydroxyl groups and ether linkages, such as polyoxyethylene chains. Nonionic emulsifying agents can be neutral or acidic giving formulators greater flexibility regarding pH requirements for cosmetic actives. Nonionics can be used in formulating w/o or o/w type emulsions and will help to mitigate the characteristic oily feel of w/o emulsions.

Thousands of emulsifying agents are available in the world market today. Choosing the best agent is the key responsibility of the formulator. Many agents used in the cosmetic and drug industry are classified by a system known as hydrophilic–lipophilic balance (HLB) number. This system developed in the mid 1950s is a useful starting point in emulsifier selection. In this system, each surfactant having a specific HLB number is used to emulsify an oil phase having an HLB required for a stable emulsion. Using an emulsifier or combination of emulsifiers, matching the required HLB of the oil phase, will form a stable emulsion. Limitations to this method include incomplete data for required HLBs of many cosmetic ingredients. Combinations of or single emulsifying agents giving the appropriate theoretical HLB may not be the optimal combination for emulsion stability or product performance. Other emulsifying agents may work better, a provide a more elegant formulation with greater efficacy. In addition, theoretical HLB numbers of complex mixtures may not follow a linear additive rule specified in the calculation (2).

In this classification system, emulsifying agents with an HLB of 10 would indicate a more water-soluble agent compared to one having an HLB of 4.

For nonionic detergents of the ester type:

$$\mathrm{HLB} = 20(1 - s/a)$$

where s is the saponification number of the material, and a is the acid number of the fatty acid moiety of the product.

For ethoxylated esters and ethers when the saponification value is not known:

$$\mathrm{HLB} = E + P/5$$

where E is the percentage of ethylene oxide, and P the percentage of polyalcohol in the molecule.

When the hydrophobic portion contains phenols and monoalcohols without polyalcohols, the equation can be simplified to:

$\text{HLB} = E/5$

Most nonionics fall into this category, manufacturers who provide HLB values in their product specifications most frequently use the latter formula (Table 2).

Mixtures of anionic and nonionic agents obtain the best emulsion, whereas mixtures of cationic and nonionic emulsifiers may not be as elegant. Examples of nonionic emulsifiers include alcohol ethoxylates, alkylphenol ethoxylates, block polymers, ethoxylated fatty acids, sorbitan esters, ethoxylated sorbitan esters, and ethoxylated castor oil. The solubility of nonionic surfactants in water can often be used as a guide in approximating the HLB and usefulness.

OIL-IN-WATER EMULSIONS

Oil-in-water emulsions typically contain 10% to 35% oil phase, a lower viscosity emulsion may have an oil phase reduced to 5% to 15%. Water in the external phase of the emulsion helps hydrate the stratum corneum of the skin. This is desirable when one desires to incorporate water-soluble active ingredients in the vehicle. Oil droplets in emulsions have a lower density than the phase they are suspended in; to have a stable emulsion it is important to adjust the specific gravity of the oil and water phases as closely as possible. Viscosity of the water phase (external phase) may be increased to impede the upward migration of the oil particles. Addition of waxes to the oil phase will increase specific gravity, but may have a profound effect on the appearance, texture, and feel on application to skin of the product. Increasing water phase viscosity is one of the most common approaches. Natural thickeners (alginates, caragenates, xanthan) and cellulosic (carboxymethyl cellulose) gums are used for this purpose.

Table 2 Relationship Between HLB Range and Water Solubility

Water solubility	HLB range
No dispersibility in water	1–4
Poor dispersion	3–6
Milky dispersion after agitation	6–8
Stable milky dispersion	8–10
Translucent to clear dispersion	10–13
Clear solution	13+
HLB	*Application*
4–6	W/O emulsifier
7–9	Wetting agent
8–18	O/W emulsifier
13–15	Detergent
15–18	Solubilizer

Source: From Ref. 5.

Carbopol® resin is perhaps the most popular gum thickener for contributing towards emulsion stability, especially at higher temperatures. The addition of a fatty amine to a Carbopol resin will further enhance stability by strengthening the interface of the water and oil phases through partial solubilization into the oil droplets. Electrolytes and cationic materials will have a destabilizing effect on anionic sodium carboxymethyl cellulose and should not be used together. Veegum®, an inorganic aluminum silicate material is also commonly used to thicken emulsions. Carbopol and Veegum may be used together to modify the characteristic draggy feel of Carbopol when used at the higher levels.

Emulsifier blends, with HLBs ranging from 7 to 16, are used for forming o/w emulsions. In the blend, the hydrophilic emulsifier should be formulated as the predominate emulsifier to obtain the best emulsion. A popular emulsifier, glycerol monostearate and polyoxyethylene stearate is a self-emulsifying acid-stable blend. Emulsifiers are called self-emulsifying when an auxiliary anionic or nonionic emulsifier is added for easier emulsification of the formulation. Formulating with self-emulsifying materials containing nonionic emulsifiers permit a wide range of ingredient choice for the formulator, especially with acid systems. In alkaline formulations, polyoxyethylene ether–type emulsifiers are preferred for emulsion stability.

An alternative to glycerol monostearate self-emulsifying emulsifier is emulsifying wax, national formulary (NF). This emulsifier, when used with a fatty alcohol will form viscous liquids to creams depending on the other oil phase ingredients. Usage levels may vary from 2% to 15%, at lower levels a secondary emulsifier, such as oleths or PEG-glycerides, will give good stability. This system is good for stabilizing electrolyte emulsions or when other ionic materials are formulated into the vehicle. Polysorbates are o/w emulsifiers, wetting agents, and solubilizers, often used with cetyl or stearyl alcohol at 0.5% to 5.0% to produce o/w emulsions (6).

W/O Emulsions

Although less popular than o/w emulsions, these systems may be desirable when greater release of a medicating agent or the perception of greater emolliency is desired. Emulsifiers having an HLB range of 2.5 to 6 are frequently selected. When multiple emulsifiers are used, the predominant one is generally lipophilic with a smaller quantity of a hydrophilic emulsifier. These emulsions typically have a total of 45% to 80% oil phase.

During the last few years, formulators have become interested in more elegant w/o emulsions by formulating with new emulsifying agents, and emollients, such as esters, Guerbet alcohols, and silicones. Selection of a suitable emollient depends on ability of the material to spread on skin with low tack, dermal compatibility, and perceived elegance by the user. In achieving this elegance, some researchers suggest a correlation of emollient and molecular weight of the emollients. In these studies, viscosity of w/o creams has correlated with molecular weight of the emollients used in test formulations. High molecular weight co-emulsifiers formulated with high molecular weight emollients gave more stable w/o emulsions. The polarity of the emollients used was found to be important as well. Emollients or mixtures of emollients with medium polarity gave test lotions the most desirable stability results (7). Anionic emulsifiers are generally inefficient w/o emulsion stabilizers, because more surface-active agents are often needed to stabilize these emulsions. Sorbitan

stearates and oleates are effective emulsifiers when used at 0.5 to 5.0%; sorbitan isostearates, being branched chain materials, give a very uniform particle size for w/o emulsions.

Multiple Emulsions

Multiple emulsions are of interest to the skin care formulator because of the elegant appearance and less greasy feel of these formulation types. Two types of multiple emulsions are encountered in skin care, water-in-oil-in-water (w/o/w) where the internal and external water phases are separated by oil, and oil-in-water-in-oil (o/w/o), where the water phase separates the two oil phases. The method of preparation for each multiple emulsion type is similar.

Benefits of these types of formulations are the claimed sustained release of entrapped materials in the internal phase and separation of various incompatible ingredients in the same formulation.

A suggested technique for forming a w/o/w emulsion is to first create a w/o primary emulsion by combining water as one phase with oil and a lipophilic emulsifier as the second phase in the traditional method. Next, water and a hydrophilic emulsifier are combined with the w/o primary emulsion at room or warm (i.e., 40°C) temperature with mixing forming a w/o/w multiple emulsion. These emulsions typically contain about 18% to 23% oils and 3% to 8 % lipophilic emulsifier. The continuous oily phase is stabilized with about 0.5% to 0.8% magnesium sulfate. W/O emulsifiers have an HLB less than 6 and are frequently nonionic or polymeric. O/W emulsifiers have an HLB greater than 15 and are ionic with high interfacial activity. For o/w/o multiple emulsions, w/o emulsifiers have an HLB less than 6 with similar properties as w/o/w and w/o emulsifiers. O/W emulsifiers have an HLB greater than 15 and are nonionic with lower interfacial activity.

Water-In-Silicone Emulsions

Silicone compounds have evolved into a class of specialty materials used for replacement, substitutes, or enhancers for a variety of organic surface-active agents, resulting in the ability to formulate products with unique properties. Previously, silicone compounds were available as water-insoluble oily materials almost exclusively. Newer silicone compounds, such as polyethylene-oxide bases, grafted to polydimethylsiloxane hydrophobic polymers, known as dimethicone copolyol emulsifiers, have been developed. These types of emulsifiers permit formation of water-in-cyclomethicone emulsions. Further work in this field led to adding hydrocarbon chains to silicone polyether polymers. This resulted in improved aesthetics to o/s emulsions. Silicone copolyols exhibit high surface activity and function similarly to traditional emulsifiers. Unlike hydrocarbon emulsifiers with higher molecular weights, high molecular weight silicone emulsifiers can remain fluid. This gives very stable visco-elastic films at the water/oil interface. The ability to make silicones more formulator-friendly has led to development of several new silicone based surfactants. Both a water-soluble and an oil-soluble portion are needed to make a surface-active molecule. Silicone surfactants substitute or add on silicone-based hydrophobicity creating a distinctive skin feel and other attributes of typical silicones as well as attributes of fatty surfactants. These emulsions may be prepared in a traditional two phase method, for example, 2% to 3% w/w of laurylmethicone copolyol in 23% w/w oil phase can be mixed in a separate water phase with electrolyte to form a hydrating cream (8).

Water-Soluble Ointment Bases

Polyethylene glycol polymers are available in a variety of molecular weights. These materials are water-soluble, do not hydrolyze, or support mold growth. For these reasons, PEG's make good bases for washable ointments and can be formulated to have a soft to hard consistency. Polyethylene glycols dissolve in water to form clear solutions and they are also soluble in organic solvents. Polyethylene glycol ointment USP is a mixture of polyethylene glycol 3350 and polyethylene glycol 400 heated to 65°C, cooled and mixed until congealed. To formulate a water-soluble ointment base, water and stearyl alcohol may be incorporated into this base (Table 4).

Absorption Bases and Petrolatum

Absorption bases can serve as concentrates for w/o emollients, water may be added to anhydrous absorption bases to form a cream-like consistency. Petrolatum, a component of some absorption bases, has been shown to be absorbed into delipidized skin and to accelerate barrier recovery. Bases can be made washable by addition of a hydrophilic emulsifier. For example, formulation with polysorbate-type emulsifiers with polyoxypropylene fatty ethers will improve washability. These surfactants will form o/w emulsions with rubbing on skin. W/O petrolatum creams can be formulated by mixing 50% to 55% petrolatum with a sorbitan sesquioleate at 5% to 10%, having an HLB of about three to seven in one phase and water in a second phase. Both phases are blended at 67 to 70°C with mixing (Table 4).

OTHER INGREDIENTS

Consumer perceived benefits of a cream or lotion are often a result of ingredients remaining on the skin after water and other volatile materials have evaporated. Emollients and other skin conditioners are commonly used for this reason. The frequently used ingredients to modify the feel of the emulsion on skin are shown in Table 3.

Preservative Systems

Most formulations require preservative systems to control microbial growth. Microbial contamination with pathogenic microorganisms can pose a health risk to the consumer, especially from *Pseudomonas* infection in the eyes or from an existing illness. Microbial contamination may cause an emulsion to separate and/or form off-odors. Contaminated products are also subject to recall, which is undesirable from a commercial viewpoint.

Preservatives can be divided into two groups, namely, formaldehyde donors and those that cannot produce formaldehyde. The former group includes DMDM hydantoin, diazolidinyl urea, imidazolidinyl urea, Quaternium 15, and the parabens (esters of *p*-hydroxybenzoic acid), whereas preservatives such as Kathon GC, phenoxyethanol, and iodopropunyl butylcarbamate work by alternative mechanisms. The formulator is advised to consult appropriate preservative manufacturers to select the optimal preservative system for the emulsion.

Table 3 Examples of Moisturizer Ingredients and Their Functions

Ingredient	Use level (%)	Comments
Emollient esters	5–25	Modify the oily, greasy feel of mineral oil and petrolatum, light to moderate feel on skin
Triglyceride oils	5–30	Light to heavy feel, often used as spreading agents
Mineral oil/petrolatum	5–70	Heavy, oily feel, provides occlusion for appropriate vehicles
Silicone oils	0.1–15.0	Helps to prevent soaping of formulations, improves spread on skin, and improves water repellent and skin protective properties
Humectants (glycerin, propylene glycol, sorbitol, polyethylene glycol)	0.5–15.0	Moisture binding properties helps to retard evaporation of water from formulation, viscosity control, impacts body and feel of emulsion
Thickeners (Carbopol®, Veegum)	0.1–2.0	Help to obtain viscosity, enhance stability, bodying agents

Table 4 Examples of Emulsifiers

Nonionic	
Polyoxyethylene fatty alcohol ethers	Very hydrophobic to slightly hydrophobic
Polyglycol fatty acid esters	Very hydrophobic to slightly hydrophobic
Polyoxyethylene modified fatty acid esters	Very hydrophilic to slightly
Cholesterol and fatty acid esters	Slightly lipophilic to strong lipophilic
Glyceryl dilaurate	Secondary emulsifier
Glycol stearate	Secondary emulsifier
Anionic	
Disodium laureth sulfosuccinate	
Sodium dioctyl sulfosuccinate	
Alcohol ether sulfate	
Sodium alkylaryl sulfonate	
Cationic	
PEG-alkyl amines	
Quaternary ammonium salts	
Self-emulsifying bases (form o/w emulsions)	
PEG-20 stearate and cetearyl Alcohol	
Cetearyl alcohol and polysorbate 20	
Glyceryl stearate SE	
Absorption bases	
Lanolin alcohol and mineral oil and octyldodecanol	
Petrolatum and ozokerite and mineral oil	

SKIN CARE EMULSIONS FOR THE AGING POPULATION

Consumers frequently refer to young skin as having a healthy glow, radiance, or vitality that tends to diminish over time. These changes in appearance in part are related to the diminished ability of older skin to retain moisture. Cosmetic and cosmeceutical products that address the needs of the aging population by enhancing appearance are predicted to grow in product sales at twice the rate of the overall cosmetic market in the near future (9).

Early moisturizers were formulated primarily with lipids, based on the assumption that fats and oils make the skin soft and supple. In reality, it is difficult to specify exactly how much water content of skin is required for adequate moisturization. The water content of keratinocytes in the basal layer is about 70%. This decreases to about 15% to 20% as mature stratum corneum reaches the desquamating layers (10). Current moisturizing strategy is to:

- increase water-holding capacity of the stratum corneum by external application of hydroscopic ingredients, known as humectants; these ingredients act in the same way as natural moisturizing factor (NMF) in skin; some materials used in moisturizers, such as lactic acid and urea, are components of NMF;
- hold water in the stratum corneum by deposition of a water-insoluble oily material on the skin surface; these materials are known as occlusive agents; oily materials mimic the effect of the natural lipid bilayers of the skin to restrict evaporation from the surface, that is, petrolatum.

In general, required levels of occlusive agents are relatively high and will cause a formulation to become tacky when applied to skin. Emulsification of occlusive agents in combination with hydroscopic agents can reduce the ability of the agent to be effectively occlusive in the finished product. Humectants are used to improve moisturization of skin, but there are conditions when humectants may actually deprive the skin of water. Once a humectant has sorbed water, the activity coefficient of water is lowered. “If the water in skin tissue does not have a lower water activity compared to the surrounding humectant–water blend, water molecules will not be transferred to skin.” Consideration should be given in the selection of humectant to ensure that the formulation does not hamper the enzyme-controlled normal desquamatory process. Glycerin is frequently the humectant of choice for this reason. More recent formulations contain hydrophilic polymers (Table 5) that may function as humectants and help smooth skin as well (10) (Table 6).

Emulsion formulators are aware that the health of the epidermis may be affected by the following:

- the intracorneal lipid layer, its formation, hydrolysis, and oxidation,
- enzymatic dependency of synthesis of NMF,
- climatic changes.

Table 5 Hydrophilic Polymers Used in Skin Care Moisturizers

Hydrophilic Polymers
Alginic acid
Chitosan (and salts)
Collagen
Hyaluronic acid

Source: From Ref. 10.

A disadvantage of formulating with glycerin-based moisturizers is that they are poor solvents for cosmetic lipids (10). When it is desirable to have a lipophiloic "cosmetic active" in the formulation, the formulator must use skill and experience to optimize the formulation.

FORMULATING FOR IMMEDIATE IMPROVEMENT IN APPEARANCE AND TEXTURE OF SKIN

Various strategies are available to formulate emulsions that provide immediate cosmetic benefits to skin. Epidermis of young skin is translucent; it allows light to partially pass through it. Skin that appears translucent will exhibit a shine or glow. The layer between the epidermis and dermis has ridges known as rete pegs. In aging skin, this region becomes smaller and flatter, tending to reduce the translucent effect of skin. Further, keratinocytes at the surface of skin do not slough off as quickly. This results in skin that has a dull and uneven appearance. Other contributing factors to loss of "skin glow or radiance" are the irregular pattern of melanocytes that tends to develop in aging skin.

In normal daylight, one observes light that is both partially reflected from the surface of stratum corneum and partly reflected back from the dermis. Younger looking skin will reflect light from lower epidermis and blood vessels in the dermis with color contributed from melanin and hemoglobin. Incident light reflecting off dry skin will not penetrate as deeply and reflect back with a dull appearance.

Interference Pigments

One approach to alter the way light is reflected back from skin is to formulate with interference pigments. This approach initially used in facial products has recently found popularity in body moisturizers. Effect-enhancing pigments are used to "add natural, transparent luster to skin," they can improve the tactile qualities of skin by giving the emulsion a silky feel (12). The same enhancing effect pigments may be used to impart an elegant luster to the appearance of the product.

Effect pigments are composed of thin, translucent platelets that produce luster by partially reflecting and partially transmitting light. Pigments are available as natural pearl, mica, and bismuth oxychloride-based materials. Bismuth oxychloride

Table 6 Examples of Common Skin Care Moisturizing and Conditioning Agents

Emollients	Humectants	Occlusives
Acetylated lanolin	Acetamide MEA	Acetylated lanolin alcohol
C14–15 alcohols	Ammonium lactate	Caprylic/capric triglyceride
Dimethicone copolyol	Copper PCA	Cetyl ricinoleate
Hexyl laurate	Glucuronic acid	Dimethicone
Isopropyl myristate	Glycerin	Hydrogenated lanolin
Lanolin	PCA	Mineral oil
PPG-20 cetyl ether	Propylene glycol	Myristol myristate
Squalene	Sodium PCA	Petrolatum
Sucrose oleate	Sorbitol	Soybean lipid
Wheat germ glycerides	Urea	Squalane

Source: From Ref. 10.

crystals have a "brilliant" white pearlescense; some grades create metallic effects, whereas other grades provide a "subtle luster and smooth feel." Natural pearls can provide a "satiny luster" to emulsions. Metal oxide coated mica pigments with thin films of iron oxide or titanium dioxide are most commonly used. The colors in these materials will shift with the viewing angle to create complex iridescence on curved body surfaces. Smaller platelets provide a "satiny-smooth, silky luster, whereas larger ones provide sparkle, glitter, and a lively appearance (11)." Use of appropriate particle size and color combinations can give skin a "radiant glow."

Interference pigments are formulated in skin care products at levels of 0.1% to 2.0% by weight, depending on the qualities the formulator wishes to achieve. The selection of particle size can help diminish the appearance of age spots, fine lines, and uneven skin color. Interference effects are maximized when a variety of particle sizes are formulated.

Soft Focus Effects

Fine particles, such as microspheres, are used in emulsions and anhydrous formulations to enhance the feel and appearance of skin. The chemical composition of microspheres are diverse. Examples are polymethyl methacrylate, polyethylene, ethylene/acrylayes copolymer, nylon, polyurethane, silicone resins, and silica. Selection of the appropriate material can provide "optical blurring" effects to the formulation, minimizing the appearance of fine lines and uneven skin tone. Some skin care products can deposit a transparent layer on skin making fine lines more visible to the eye. Formulation with appropriate microspheres can help to minimize this effect and give skin an enhanced appearance (13). Formulating with varied particle size will further help to minimize the appearance of uneven skin (13).

When formulating with interference pigments and soft focus materials, a critical consideration is the refractive index (RI) of the primary vehicle and the material(s) to be incorporated into the vehicle. When the vehicle is applied to skin, the portion of the vehicle remaining on skin after evaporation is considered as the "primary vehicle." For example, an emulsion of oils and polymers applied to skin, the oil/polymer portion will be the primary vehicle after the water has evaporated from the skin's surface. In general, the RI of the light-diffusing particle must be greater than that of skin and the vehicle, to be effective (Table 7).

Table 7 Examples of Refractive Indexes (Various Sources)

Material	Refractive index
Air	1.00
Perspiration	1.33
Polyethylene	1.45
Titanium dioxide	2.51
PMMA	1.49
Silica	1.45
Skin	1.62
Microspheres (general)	1.41–1.53
Propylene glycol dibenzoate (ester)	1.54
Phenyl trimethicone (silicone)	1.46
PPG-3 benzyl ether myristate (ester)	1.465
Dimethicones, cyclomethicones (silicone)	1.375–1.403, 1.394–1.398

Emollient Esters

Chemically, esters are the covalent compounds formed between acids and alcohols. Esters can be formed from inorganic and carboxylic acids and any alcohol. Esters, when formulated in cosmetic emulsions, have diverse functions. They serve as emollients, skin conditioners, solvents, fragrance compounds, and preservatives (14).

More recently, emollient esters have been used in place of more expensive silicones to provide aesthetic benefits to cosmetic emulsions. Esters can be formulated with silicones to enhance stability and feel of the emulsion when applied to skin (15). Esters that function as co-emulsifiers provide improved skin adhesion of the formulation, reduced formulation tackiness, and can improve hydration properties of humectants.

Esters display properties that reflect their chain length and structural arrangement of their two starting materials. For this reason, different esters will have differing emollience. A simple monoester of a short-chain fatty alcohol or acid will possess a light feel. Branched esters will feel nongreasy; chemically more "complex" pentaerythrityl esters will have a "cushiony feel" (14). The structural composition of the ester will also affect its spreading behavior on skin. Branched esters typically have a higher spreading factor. Spreading will begin to decrease as the molecular weight increases. Emollient esters affect the viscosity of the emulsion, either improving texture and formulation aesthetics or detracting, if incorrectly formulated. When formulating with coated pigments, one must ensure that the selected ester is compatible with the coating (16).

Polymers

Polymers are small molecules that are chemically connected in long repeating units. Polymers are ubiquitous in nature. The DNA of all living cells, the protein and starches in our foods to the tires on our automobiles are composed of polymers. The use and function of polymers in cosmetic emulsions are equally diverse. Polymeric emulsifiers, such as those based on silicone or polyacrylic acids, are used as

Table 8 Examples of Polymers (Various Sources)

Polymer	Type	Potential Application
Acrylates/C10–30 alkyl acrylate crosspolymer	High molecular weight polyacrylate	Primary emulsification (o/w)
Carbomer	Acrylic acid	Synthetic thickener
Acrylates/steareth-20 methracylate copolymers	Acrylic polymer emulsion/ anionic	Thickener
PEG-150/decyl alcohol/SMDI copolymer	Hydrophobically modified nonionic polyol	Low pH formulations, cationic conditioners, o/w sunscreens, cationic silicone emulsions
Caprrylic/capric triglyceride sodium acrylates copolymer	Polyacrylic acid	W/O emulsions
PVP/eicosine copolymer	Copolymers of vinylpyrolidone	Oil soluble, rub resistance in sunscreen
Tricontanyl PVP	Copolymer of vinylpyrolidone	Oil soluble, rub resistance for pigments and sunscreens

Source: From Ref. 14.

emulsifiers. These polymers have cationic charges that are substantive to skin and impart a smooth, conditioning effect. Other polymers are formulated in emulsions to create the sensation of firming skin, to minimize interference pigments and other solid particles from rub off to clothing, and to provide water resistance to sunscreen containing emulsions. These polymers form a film on the skin's surface (Table 8).

FUTURE FORMULATION CHALLENGES

Cosmeceutical ingredients have been popular for many years, and new cosmetic active agents are continuously being identified. Many of these active ingredients have excellent in vitro data to support claims, but are lacking in vivo data. Further, formulators often formulate the active in an existing prototype rather than employing a strategy of formulation optimization. Consumers have come to expect functional cosmetic products. Products that fail to deliver on expectations are doomed to failure (17).

Future formulation challenges will be to:

- determine the optimal emulsion system to effectively deliver the desired ingredient to the viable epidermis via the stratum corneum (partition coefficients and penetrant polarity),
- understand the influence of formulation characteristics on skin delivery (influence of the emulsifier, solubility characteristics of the primary emollient or solvent, and influence of emollients in general),
- continuously advance regarding knowledge of skin molecular biology, specifically the intended region of product use on the body.

REFERENCES

1. Vermeer BJ, Gilchrest B. Cosmeceuticals. A proposal for rational definition, evaluation, and regulation. Arch Dermatol 1996; 132(3):340.
2. Silicone Emulsifiers Fast Look-up Guide, Dow Corning, Form no: 27-1063-01.
3. Block H. Medicated applications. In: Gennaro AR, ed. Remington's Pharmaceutical Sciences. 18th ed. Pennsylvania: Mack Publishing Company, 1980.
4. Konish PN, Gruber JV. J Soc Cosmet Sci 1998; 49:335–342.
5. The HLB System, ICI Americas, Inc., August 1984 Remington's Pharmaceutical Sciences. 18th ed. Pennsylvania: Mack Publishing Company, 1980.
6. Emulsification of Basic Cosmetic Ingredients, ICI United States, Inc., 102–6, 8/75.
7. Henkel Symposium, October 2–5, 1991.
8. Silicone Formulation Aids, Dow Corning, 1997.
9. Mouche C. Industry Watch: Consumer Products in www.chemicalprocessing.com. May 22, 2002.
10. Rieger MM, ed. Harry' Cosmeticology. 8th ed. New York: Chemical Publishing Comp., Inc., 2000.
11. Uzunian G. Formulating effect pigments in personal care products. Happi 1999; August.
12. Epstein H, Menzel T, Hu Z. US Patent 5,804,205 (1998).
13. Leon-Pekarek D. Kobo Products, Inc., July 2002 (discussions).
14. International Cosmetic Ingredient Dictionary and Handbook. 9th ed. Washington DC: The Cosmetic, Toiletry and Fragrance Ass, Inc., 2002.
15. Croda Bulletin DS-173 R-1, October 23, 2003.
16. Obukowho P, Woldin B. Selecting the right emollient ester. Cosmet Toilet 2001; 116(8): 61–72.
17. Wiechers JW, Kelly CL, Blease TG, Dederen JC. Formulating for efficacy. Cosmet Toilet 2004; 119(3):49–62.

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Antiwrinkle Products

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INTRODUCTION

Skincare products that affect wrinkles are a reality and are well established in consumer, practitioner, and corporate perspectives. In the broadest definition, "products" range from classic and simple cosmetic preparations through vitamins, antioxidants, topical and oral cosmeceutical and pharmaceutical preparations, and even to surgical and laser interventions. Substantiation of product effect ranges from user testimonials through rigorous consumer testing and claim substantiation to classical pharmaceutical trials. Methodologies vary from casual visual and tactile observations to elaborate scoring of specific clinical parameters, and may be enhanced and embellished by use of many sensitive, accurate, reproducible, and validated instrumental techniques. The topic is currently exceptionally rich and expansive.

BACKGROUND

Definition of Wrinkles

Although intuitively obvious, the strict scientific definition of a wrinkle has been somewhat elusive. The consumer easily observes the fine and coarse indented lines of the skin of the face and attributes them to "aging." Although many cultures of the past recognized the damaging effects of sun exposure, only recently, in fact, has science verified the exceptionally strong link between wrinkles and repetitive, chronic, even suberythrogenic ultraviolet irradiation (UVR). Difficult as it is to histologically identify or quantify individual wrinkles, there is much scientific evidence of distinct dermal structural alterations of collagen and elastin that correlate generally with wrinkled skin. Easily conceptualized, the underlying "weaknesses" caused by this damaged infrastructure of the skin allows various length and depth infoldings of the skin to occur as a result of repetitive and chronic contractions of the exceptionally varied superficial musculature of facial expression.

Causes of Wrinkles

All scientific evidence points to UVR as the primary cause of wrinkles and other stigmata of "photoaging," and plausible mechanisms of pathogenesis have been elucidated. The pleotropic effects of UVR on many different cellular and subcellular systems make it difficult however to establish a strictly linear sequence of events, and it is likely that as in most biological systems, interrelated damage and reparative pathways interplay to establish progression, regression, or equilibrium. It is most helpful in rationalizing the potential of various products for prevention or reversal of wrinkles to understand the underlying molecular events. UVR has long been thought to damage skin partly through its generation of reactive oxygen species and subsequent damage to membrane lipids, various cellular proteins, and DNA. It has recently been shown that, within minutes of suberythrogenic UVB exposure, there is induction in human skin of matrix-degrading metaloproteinase messenger RNAs, their translated proteins, and consequent activities, possibly through a complex process involving signal transduction, transcription factors, and cytokine release (1). Because the metalloproteinases are a large group of zinc-requiring enzymes that include collagenases, elastases, and several other proteinases, their induction, required cofactors, and potential inhibitors are logically of considerable interest in wrinkle causation, prevention, and treatment. Repetitive UVR radiation, presumably by chronic production of matrix damage attributable to this mechanism, would then, if inadequately repaired, lead to dermal "scars" and thus wrinkle formation (2). This theory logically leads to many diverse, possible therapeutic interventions to prevent, stabilize, or reverse photoaging, along with its characteristic and prominent stigmatum of wrinkles.

PREVENTION OF WRINKLES OF PHOTOAGING

Quite apart from specific products, elimination of UVR exposure essentially prevents wrinkles. The effect of lifelong UVR avoidance is easily shown by comparison of the never-exposed skin of the buttocks to even suberythrogenic exposed skin of the face in any individual of types I to III skin. Although wrinkles usually appear only after some years of exposure and are noticeable beginning in the second or third decade of life, other seemingly benign yet insidious signs of photoaging, such as freckling, can be shown even in young children, especially those with light skin and high solar exposure as in Australia (3). Complete avoidance of UVR is impractical, but avoidance during peak solar flux of midday is frequently possible. Protective hats and clothing are practical and highly desirable. Sunscreens of various types have definite utility in reducing UVR damage. Less well established is the potential role of a host of purported preventatives and treatments such as vitamins and antioxidants, many of which would appear to have a theoretical basis for consideration.

SUBSTANTIATION OF ANTIWRINKLE CLAIMS

Clinical Methodologies

Adequate methodologies of many and varied types now exist to accurately, precisely, reproducibly, and validly examine and quantitate the effects of products on wrinkles (4). Consumers can judge for themselves if a product meets their needs in wrinkle effacement and, even if objective proof of efficacy is lacking, this positive perception

is sometimes sufficient. There is a human tendency to estimate the age of other adults primarily by casual estimate of the degree of wrinkling of the skin of the face and, whether applied to others or the self, this quick estimate is fairly accurate (5). Consumer-panel testing of many types can be quite rigorous and can quantify effects surprisingly effectively. Global grading of overall appearance is performed by using photographically derived scales of severity, with 0 = none, 1 to 3 = mild, 4 to 6 = moderate, and 7 to 9 = severe photodamage (6). Specific grading of wrinkling and other parameters using visual analogue scales is simple and reproducible when used alone, and can be combined in very elegant clinical-panel testing (7). The scale may be continuous, rating from 0 to 100 the condition as absent to severe to balanced, with a score of 0 designating no change from baseline, improvement recorded to the right side of 0 (to a maximum of +50 mm), and worsening recorded to the left side of 0 (to a minimum of 50 mm). Pharmaceutically oriented trials have successfully used similar methodologies with good correlation between subject and investigator evaluations.

Instrumentation

The evolving "gold standard" is doubtlessly the area of bioengineering devices. For wrinkling, optical profilometry is the most useful technology and has been widely and successfully used even in large clinical trials (8). Most commonly, skin replicas of representative areas of wrinkling are evaluated by using image-analysis computer software that reflects wrinkle width and depth (9).

REPRESENTATIVE PRODUCTS FOR WRINKLES

Adequate sun avoidance and sunscreen use are partially prophylactic in the prevention of wrinkle formation. Purely cosmetic and emolliating products may substantially reduce the appearance of wrinkles without change in structure or function of the skin, whereas a number of cosmeceutical and pharmaceutical products fulfill both criteria.

Sunscreens

UVR, even in suberythrogenic doses, is damaging to skin. Prevention of wrinkles, especially in those most genetically predisposed, requires early initiation and lifelong minimization of exposure by sun avoidance and correct use of sunscreens. As multiple wavelengths of UVR are incriminated, it is prudent to use the most complete chemical block that the consumer and physical activity will permit. Substantial block of UVB and UVA is now available in many products, and with the addition of zinc oxide or titanium dioxide, nearly complete block of all damaging wavelengths is achieved.

Cosmetics

Innumerable cosmetic products exist, many of which claim to affect wrinkles and some of which may considerably minimize the appearance of wrinkles. Cosmetics of a simple, occlusive nature may essentially "fill in" the wrinkle valleys; others are of a color or substance that changes reflected light from the wrinkle sufficiently

to minimize its appearance. Some products currently regulated as cosmetic contain ingredients such as alpha-hydroxy acids or retinol with potential pharmacological actions, and could more logically be designated cosmeceutical. The effect of removing dead, loosely coherent surface keratinocytes, or of stimulating epidermal or dermal processes, may significantly improve the appearance of wrinkles. It is important to remember that, at least in the United States, if pharmaceutical claims are not stated, the product is legally cosmetic in nature, and thus its ingredients and marketing claims may vary considerably and creatively.

Moisturizers

Definite effects on skin appearance, and potentially on structure and function, can be achieved with moisturizers, especially those currently available, many of which are of sophisticated and elegant composition. Improvement in stratum-corneum structure and hydration and decrease in transepidermal water loss can be quickly achieved and may result in improvement in the appearance of wrinkles.

Alpha- and Beta-Hydroxy Acids

There is substantial evidence that meaningful improvement can be obtained in multiple signs and symptoms of photodamaged skin by the sustained topical application of alpha-hydroxy acids. Specifically, wrinkle effacement has been shown in multiple, well-designed and executed clinical trails using clinical and instrumental endpoints (10),(11). Fewer published trials are available that document a similar effect by use of alpha-hydroxy acids, but they nonetheless appear to have utility (12).

Retinoids

Incontrovertible evidence of wrinkle effacement by topical application of retinoids has been extensively shown in numerous, large, published clinical trials. Tretinoin (all transretinoic acid) has been the most studied (13,14), but results with topical isotretinoin (13 cisretinoic acid) appear comparable (15,16). Retinol, the parent compound, may require metabolism to the purported active transretinoic acid for pharmacological effect and is increasingly incorporated in cosmetic products claiming benefit in wrinkle appearance. Similarly, retinaldehyde has been shown to be active in wrinkle effacement (17). The most recently marketed retinoids, adapalene and tazorotene, will most likely be studied for similar effect.

Vitamins

Many vitamins, including vitamins A, C, D, and E, are vital in normal metabolic processes, and clinical skin changes resulting from their deficiencies were identified in many cases even in the 1800s. Some of these changes have been shown to be secondary to abnormal keratinization, altered differentiation, or impaired collagen synthesis. Nevertheless, it has been difficult to scientifically confirm cosmeceutical activity or utility of these vitamins under the conditions of normal nutritional status. Retinoids (vitamin A class), which were previously discussed, at pharmaceutical concentrations are the most thoroughly substantiated class in their general effect in photoaging and specific effect on wrinkles.

Vitamin E is an exhaustively studied antioxidant in many systems and could therefore logically be studied in photoaging (18). Some evidence for pharmaceutical

effect in treatment of wrinkles is available. A four-week study of 5% RRR alpha tocopherol naturally occurring oil-in-water (o/w) cream applied to the crows feet area showed, by optical profilometry, decreased skin roughness, length of facial lines, and depth of wrinkles compared with placebo (19).

An increasing number of vitamin C-containing topical products are being marketed with claims of improvement in skin wrinkling.

Vitamin D analogues have been highly successful in treatment of psoriasis and because of their modulating effect on keratinization, they should be studied in photoaging.

Hormones

Estrogens and their diminution at menopause have profound effects, especially on epithelium of the skin and vagina. Wrinkle effacement has been convincingly shown in at least one controlled clinical trail of topical application of 0.01% estradiol or 0.3% estriol-containing preparations (20). Other studies have shown beneficial changes in skin thickness and texture with topical estrogen application (21,22).

Minerals

That many minerals, such as sodium, potassium, calcium, magnesium, selenium, and zinc, are critical in normal mammalian physiology is well established. A potential cosmeceutical role in improvement of skin appearance has been suggested and requires confirmation (23).

Miscellaneous Agents

Hyaluronic acid is a normal component of epidermis and especially dermis. Stimulation of hyaluronic-acid production in skin by a device that produces a specific pulsed electromagnetic field (electrorydesis) produced improvement in appearance of wrinkles in a small study (24).

Natural cartilage polysaccharides as oral formulations derived from cartilage of marine fish have purported to improve dermal thickness and elasticity (25).

SUMMARY AND CONCLUSIONS

Skincare products now existing have various degrees of utility for preventing, minimizing the appearance of, or treating wrinkles caused by UVR. Conscientious use of sunscreens can minimize photoaging and wrinkle formation. Rigorous consumer-panel testing can show consistent improvement of the appearance of wrinkles with many products of a purely cosmetic nature. Application of well-established clinical methodologies and increasingly sophisticated instrumental techniques has conclusively shown pharmacologically mediated wrinkle improvement, especially with topical use of retinoids or alpha-hydroxy acids.

In conclusion, the substantial scientific progress that has driven the development of elegant cosmetic and pharmaceutically active products to ameliorate skin wrinkles warrants optimism for the future. Can the day be far in the future when present cosmetic and cosmeceutical treatments will be eclipsed by specific genetic manipulations to rejuvenate aging skin (26)?

REFERENCES

1. Fisher GJ, Datta SC, Talwar HS, Wang ZQ, Varani J, Kang S, Voorhees JJ. Molecular basis of sun-induced premature skin ageing and retinoid antagonism. Nature 1996; 379: 335–339.
2. Fisher GJ, Wang ZQ, Datta SC, Varani J, Kang S, Voorhees JJ. Pathophysiology of premature skin aging induced by ultraviolet light. N Engl J Med 1997; 337(20):1419–1428.
3. Fritschi L, Green A. Sun damage in teenagers' skin. Aust J Public Health 1995; 19(4): 383–386.
4. Cunningham WJ. Photoaging. In: Schwindt DA, Maibach HI, eds. Cutaneous Biometrics. New York: Kluwer Academic/Plenum Publishers, 2000.
5. Warren R, Gartstein V, Kligman AM, Montagna W, Allendorf RA, Ridder GM. Age, sunlight, and facial skin: a histologic and quantitative study. [Published erratum appears in J Am Acad Dermatol 1992; 26(4):558]. J Am Acad Dermatol 1991; 25(5 pt l):751–760.
6. Griffiths CE, Wang TS, Hamilton TA, Voorhees JJ, Ellis CN. A photonumeric scale for the assessment of cutaneous photodamage. Arch Dermatol 1992; 128(3):347–351.
7. Armstrong RB, Lesiewicz J, Harvey G, Lee LF, Spoehr KT, Zultak M. Clinical panel assessment of photodamaged skin treated with isotretinoin using photographs. Arch Dermatol 1992; 128(3):352–356.
8. Grove GL, Grove MJ, Leyden JJ, Lufrano L, Schwab B, Perry BH, Thorne EG. Skin replica analysis of photodamaged skin after therapy with tretinoin emollient cream. J Am Acad Dermatol 1991; 25(2 pt l):231–237.
9. Grove GL, Grove MJ. Effects of topical retinoids on photoaged skin as measured by optical profilometry. Methods Enzymol 1990; 190:360–371.
10. Ditre CM, Griffin TD, Murphy GF, Sueki H, Telegan B, Johnson WC, Yu RJ, Van Scott EJ. Effect of a-hydroxy acids on photoaged skin: a pilot clinical, histologic, and ultrastructural study. J Am Acad Dermatol 1996; 34(2 pt 1):187–195.
11. Stiller MJ, Bartolone J, Stern R, Smith S, Kollias N, Gillies R, Drake LA. Topical 8% glycolic acid and 8% L-lactic acid creams for the treatment of photoaged skin. Arch Dermatol 1996; 132:631–636.
12. Kligman AM. Salicylic acid: an alternative to alpha hydroxy acids. J Ger Dermatol 1997; 5(3):128–131.
13. Weiss JS, Ellis CN, Headington JT, Tincoff T, Hamilton TA, Voorhees JJ. Topical tretinoin improves photoaged skin: a double-blind vehicle-controlled study. JAMA 1988; 259:527–532.
14. Weinstein GD, Nigra TP, Pochi PE, Savin RC, Allan A, Benik K, Jeffes E, Lufrano L, Thorne EG. Topical tretinoin for treatment of photodamaged skin. Arch Dermatol 1991; 127:659–665.
15. Cunninghapi WJ, Bryce GF, Armstrong RA, Lesiewicz J, Kim HJ, Sendagorta E. Topical isotretinoin and photodamage. In: Saurat J-H, ed. Retinoids: 10 Years On. Basel: Karger, 1991:182–190.
16. Sendagorta E, Lesiewicz J, Armstrong RB. Topical isotretinoin for photodamaged skin. J Am Acad Dermatol 1992; 27(6 pt 2):S15–S18.
17. Creidi P, Vienne MP, Ochonisky S, Lauze C, Turlier V, Lagarde JM, Dupuy P. Profilometric evaluation of photodamage after topical retinaldehyde and retinoic acid treatment. J Am Acad Dermatol 1998; 39:960–965.
18. Nachbar F, Korting HC. The role of vitamin E in normal and damaged skin. J Mol Med 1995; 73:7–17.
19. Mayer P. The effects of vitamin E on the skin. Cosmet Toilet 1993; 108:99–109.
20. Schmidt JB, Binder M, Demschik G, Bieglmayer C, Reiner A. Treatment of skin aging with topical estrogens. Int J Dermatol 1996; 35(9):669–674.
21. Creidi P, Faivre B, Agache P, Richard E, Haudiquet V, Sauvanet JP. Effect of a conjugated oestrogen (Premarin) cream on aging facial skin. A comparative study with a placebo cream. Maturitas 1994; 19:211–223.

22. Callens A, Vaillant L, Lecomte P, Berson M, Gall Y, Lorette G. Does hormonal skin aging exist? A study of the influence of different hormone therapy regimens on the skin of postmenopausal women using non-invasive measurement techniques. Dermatology 1996; 193(4):289–294.
23. Ma'or Z, Magdassi S, Efron D, Yehuda S. Dead Sea mineral-based cosmetics—facts and illusions. Isr J Med Sci 1996; 32(suppl):S28–S35.
24. Ghersetich I, Teofoli P, Benci M, Lotti T. Ultrastructural study of hyaluronic acid before and after the use of a pulsed electromagnetic field, electrorydesis, in the treatment of wrinkles. Int J Dermatol 1994; 33(9):661–663.
25. Eskelinin A, Santalahti J. Natural cartilage polysaccharides for the treatment of sun-damaged skin in females: a double-blind comparison of Vivida and Imedeen. J Int Med Res 1992; 20(2):227–233.
26. Zhang L, Li L, Hoffmann GA, Hoffman RM. Depth-targeted efficient gene delivery and expression in the skin by pulsed electric fields: an approach to gene therapy of skin aging and other diseases. Biochem Biophys Res Commun 1996; 220(3):633–636.

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Skin Care Products: Artificial Tanning

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INTRODUCTION

The desire for a tanned appearance alongside increasing awareness of the hazards of ultraviolet (UV) light exposure has led to renewed interest in artificial tanning products. Better formulations of sunless or self-tanners with improved aesthetics are more widely available. As consumer experience with the newer products has grown this category has become more popular and has led to an increasing proportion of overall suncare sales.

Dihydroxyacetone (DHA) is the active ingredient in sunless or self-tanners, and is responsible for darkening the skin by staining. DHA is classified in the International Cosmetic Ingredient Dictionary and Handbook (1) as a colorant or a colorless dye. Other agents with the potential to enhance skin pigmentation, including tan accelerators containing tyrosine and other ingredients and tanning promoters containing psoralens which require UV exposure, will not be discussed here (2).

HISTORY

The first mention of DHA as an active ingredient in medicine appeared in the 1920s, when it was proposed as a substitute for glucose in diabetics. In the 1950s the oral administration of DHA was examined as a diagnostic procedure for glycogen storage disease when it was given in large doses orally (3). When the children spit up this sweet concentrated material, the skin became pigmented in areas splattered on the skin, without staining clothing. Aqueous solutions were then applied to the skin directly and the pigmentation reproduced (4). In the late 1950s, cosmetic tanning preparations first appeared in the marketplace. Cosmetic acceptance of these initial products was limited because of the uneven orange–brown color they imparted to the skin. With the availability of improved formulations in the 1990s, sales of sunless tanners grew exponentially as a total proportion of suncare product sales. In the last several years operator assisted spray tans using DHA have become popular in spas and salons.

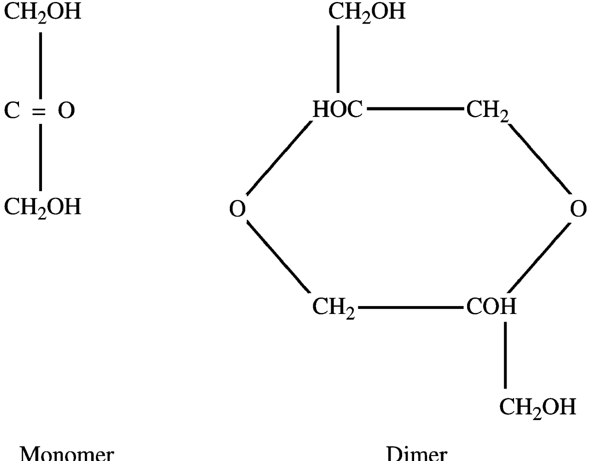

Figure 1 Chemical structure of DHA.

CHEMISTRY

DHA ($C_3H_6O_3$) is a white, crystalline hygroscopic powder. This 3-carbon sugar forms a dimer in freshly prepared aqueous solution (Fig. 1). With heating to effect a solution in alcohol, ether, or acetone it reverts to the monomer. The monomeric form is more important in the browning reaction which leads to the skin color change (5). DHA is stable between pH 4 and 6, but above pH 7 efficacy is lost with the formation of brown colored compounds. A buffered mixture at pH 5 is most stable. Heating above 38°C for long periods of time will also effect stability. DHA needs to be stored in a cool dry place. Glyceraldehyde, the isomer of DHA, is also present in solution (5). Glyceraldehyde may degrade into formaldehyde and formic acid. In acidic solution (pH 4), this isomerization and therefore these latter undesirable ingredients are minimized.

The Maillard or browning reaction has been defined as the reaction of an amino group of amino acids, peptides, or proteins with the glosidic hydroxyl group of sugars. DHA in the context of this reaction may be considered a 3-carbon sugar, reacting with free amino groups available as amino acids, peptides, and proteins supplied by the keratin to form products or chromophores referred to as melanoidins (6). Melanoidins have some physicochemical properties similar to naturally occurring melanin (7). Electron spin resonance has recently shown that free radicals are produced in vivo by the Maillard reaction (8).

FORMULATION

The concentration range of DHA in self-tanning products can range from 2.5% to 10%. Lower concentration products allow the consumer greater latitude with application because they tend to be more "forgiving" of uneven application or rough surfaces. Labeling products as light, medium, or dark can be particularly helpful with the depth of shade a function of DHA concentration.

DHA is predominantly formulated in oil-in-water emulsions. Formulating with silicones allows the formulator to obtain the spreadability of oils which potentially reduces streakiness with application to the skin. Minimizing particle size of the micelles in the emulsion chosen also improves uniformity of spreading on the skin surface. Based on the chemistry of DHA, formulations should be buffered to an acidic pH (4 to 5) and not heated to temperatures higher than 40°C.

After incorporation of DHA into a formulation, the pH may drop during storage, suggesting that stability may actually be increased when the pH is kept between 3 and 4 (9). The use of nonionic emulsifiers as opposed to ionic emulsifiers may also improve stability. Some thickeners such as carbomers, sodium carboxymethylcellulose, and magnesium aluminum silicate can cause rapid degradation of DHA. Hydroxyethylcellulose, methylcellulose, and silica, as well as xanthan gum and polyquaternium-10 for thickening DHA-containing emulsions, are better choices.

DHA can react with oxygen and nitrogen containing compounds, collagen, urea derivatives, amino acids, and proteins. They should be avoided in the formulation of the DHA-containing vehicle. Non-nitrogen containing sunscreen should be utilized if sun protection is desired. Attempts have been made to take advantage of this effect by adding amino acids to speed up the skin darkening process, but a less substantive color results. Methionine sulfoxide, a sulfur containing amino acid, has been used as an excipient applied before the application of the DHA-containing cream (10). Two compartment systems have been patented based on this reaction.

As with moisturizing products in general, lotions are more readily accepted by consumers than creams with ease of spreadability and aesthetics. Creams can produce a more intense tan owing to greater applied film thickness. Products may be formulated for dry skin types by the addition of emollients and humectants. Products formulated in gel or alcoholic vehicles may be more suitable for oily skin.

MECHANISM OF ACTION

The site of action of DHA is the stratum corneum (11). Tape stripping of the skin quickly removes the color (12), as does mechanical rubbing. Deeper staining in areas with thicker stratum corneum and no staining of mucous membranes without a stratum corneum are also consistent with this being the site of action. DHA may be used as a substitute for dansyl chloride as a measure of stratum corneum turnover time (13,14). Microscopic studies of stripped stratum corneum and hair reveal irregular pigment masses in the keratin layers (15) consistent with melanoidins. These melanoidins are formed via the Maillard reaction with DHA as a sugar reacting with the amino groups supplied by the keratin.

APPLICATION

Following application of a typical DHA-containing self-tanning lotion, color change may be observed within an hour (16). This color change may be seen under Wood's light (black light) within 20 minutes. Maximal darkening may take 8 to 24 hours to develop. Individuals can make several successive applications every few hours to achieve their desired color. Color may last as long as five to seven days with a single application. Depending on anatomical application, the same color can be maintained with repeated applications every one to four days. The face requires fewer

applications but more frequent reapplication to maintain the color than the extremities. Depth of color varies with the thickness and compactness of the stratum corneum. Palms and soles stain deepest necessitating washing of hands after application to avoid staining. Hair and nails will color but not mucous membranes lacking a stratum corneum or keratin layer. Rougher hyperkeratotic skin over the knees, elbows, and ankles will color more unevenly as will older skin with keratoses and mottled pigmentation. Color will also be maintained longer in these areas.

As in the formulation, the pH of the skin before application may have an effect on the tonality of the skin color (5). Alkaline residues from soaps or detergents may interfere with the reaction between DHA and the amino acids on the skin surface. Wiping the skin surface with a hydroalcoholic, acidic toner just prior to DHA application, may improve results. Ex-vitro epidermal studies suggest that skin hydration (17) and relative humidity (18) influence the development of coloration.

Careful directions provided with these products are therefore quite important in determining consumer satisfaction. The skin may be prepared with a mild form of exfoliation. Even application is required with lighter application around elbows, knees, and ankles to avoid excessive darkening in these areas. Care also needs to be taken around the hairline where lighter hair may darken. Hands need to be washed immediately after use to avoid darkening of the palms, fingers, and nails. Skill and experience are necessary with using these products resulting in greater user satisfaction.

ADDITIVES

As commonly occurs, growth in this category has compelled both formulators and marketers to seek points of differentiation between their product and that of their competitors. Besides formulating for specific skin types, active treatment ingredients may be incorporated into DHA-containing formulations. Vitamins, botanical extracts, antioxidants, anti-irritants, and even alpha hydroxy acids may be added to broaden the claims made with a given product. Addition of antioxidants can shift tonality to a more natural coloration (19). The addition of sunscreen ingredients to self-tanners warrants a more detailed discussion in the section that follows.

Some newer formulations have included colorants as used in bronzers to achieve an immediate make-up effect. Similarly tinting with iron oxides or titanium can provide immediate color and allow the user to more easily visualize the evenness of application. Metal oxides may however induce degradation of DHA (9).

SUNSCREEN ACTIVITY

In the United States the FDA Tentative Final Over-the-Counter Monograph on Sunscreens (Fed Reg. 1993) listed DHA as an approved sunscreen ingredient when used sequentially with lawsone (2-hydroxy-1,4-napthoquinone). The Final Monograph (Fed Reg. 1999) removed this combination from the approved list. The European Economic Community Directive does not list DHA as a permitted UV filter. DHA itself has at most a modest effect on SPF (20), providing perhaps SPF 3 or 4 protection. The brown color obtained on the skin does absorb in the low end of the visible spectrum overlapping onto a long UVA and may provide some UVA I protection (21). Melanoidins can act as free-radical scavengers as they demonstrate an

electron spin resonance signal (8). Superficial skin coloration induced by frequent topical application of DHA in high concentrations may delay skin cancer development in hairless mice irradiated with moderate UV doses (22).

Individuals using DHA-containing tanning products need to be cautioned that despite visible darkening of their skin, these products provide minimal sun protection. Confusion may be compounded by the addition of UV filters to the formulation providing significant sun protection. The stated SPF for the product is applicable for a few hours after application, but not for the days during which the skin color change may remain perceptible.

INDICATIONS

Even with recent improvement in DHA formulations, the color achieved remains dependent on skin type. Individuals of medium complexion with skin phototypes II or III (23), as opposed to those who are lighter or darker, will obtain a more pleasing color. Individuals with underlying golden skin tones will achieve better results than individuals with rosy, sallow, or olive complexion. Older consumers with roughened, hyperkeratotic skin or mottled pigmentation with freckling may be less pleased with their use.

Dermatologists regularly recommend these products for tanning as a safe alternative to UV exposure. They may be used to camouflage some skin irregularities such as leg spider veins. Light to medium complected patients with vitiligo, who show increased contrast with the vitiliginous areas with natural or unavoidable tanning in their normal skin, may also benefit (24,25). They may even provide some protection for individuals with certain photosensitivity disorders (26). Protection of uninvolved skin by DHA during psoralen-UVA treatment (PUVA) allows higher UVA exposures to be tolerated, with fewer treatments resulting in faster clearing known as Turbo-PUVA (27).

SAFETY

The visible color change associated with the use of artificial tanning products might suggest to some users that these products are hazardous. Based on the chemistry of DHA and its toxicological profile, it can be considered nontoxic. It reacts quickly in the stratum corneum minimizing systemic absorption. The acute toxicity of DHA was investigated for diabetics in the 1920s with oral intake well tolerated (15). The phosphate of DHA is found naturally as one of the intermediates in Kreb's cycle.

Contact dermatitis to DHA has only rarely been reported (28). As with other topical products with active ingredients, such as sunscreens, much of the reported sensitivity is secondary to other ingredients in the vehicle (29). Adverse reactions are more likely to occur on the basis of irritation and not true allergy. Ultimately all claims related to product safety are based on testing the final formulation.

ALTERNATIVE TANNING AGENTS

Lawsone found in the henna plant and juglone [5-hydroxy-1,4,-napthoquinone] derived from walnuts that they also stain hair, skin, and nails. They have been used

for centuries for hair coloring. Both substances lack skin substantivity and readily discolor clothing (30). The skin color they produce does not resemble a natural tan. Based on the underlying principle of the Maillard reaction, other molecules with a ketone function have been investigated (31). An alpha hydroxy group with electron withdrawing groups can also increase reactivity. Substances such as glyceraldehyde and glyoxal (32) have been described but found ineffective. Mucondialdehyde was described by Eichler (33) as an effective agent but associated toxicity mitigated against its use (31). Although several other aldehydes have been shown to have better color properties, stability issues limit their use (31).

CONCLUSION

Increasing consumer awareness as to the hazards of UV light should fuel ongoing interest in self-tanning products. The benign toxicological profile of DHA reinforces the notion that these products represent a safe alternative to a UV induced tan. The results obtained with these products are dependent on the final formulation, individual application technique, and the consumers' complexion type. Greater experience in formulation combined with increasing sophistication on the part of the consumer should lead to continuing growth and satisfaction with the use of these products.

Consumers need to be clearly informed that these products do not offer significant protection against UVB. If formulated with standard sunscreens, consumers should be cautioned that the duration of UV protection is more short-lived than the color change.

REFERENCES

1. Wenninger JA, McEwen GN Jr, eds. International Cosmetic Ingredient Dictionary and Handbook. 9th ed. Washington: The Cosmetic, Toiletry, and Fragrance Association, 2002.
2. Brown DA. Skin pigmentation enhancers. J Photochem Photobiol 2001; 63:148–161.
3. Guest GM, Cochrane W, Wittgenstein E. Dihydroxyacetone tolerance test for glycogen storage disease. Mod Prob Paediat 1959; 4:169–178.
4. Wittgenstein E, Berry HK. Staining of skin with dihydroxyacetone. Science 1960; 132:894–895.
5. Maes DH, Marenus KD. Self-tanning products. In: Baran R, Maibach NI, eds. Cosmetic Dermatology. London: Martin Dunitz, 1994:227–230.
6. Wittgenstein E, Berry HK. Reaction of dihydroxyacetone (DHA) with human skin callus and amino compounds. J Invest Dermatol 1961; 36:283–286.
7. Meybeck A. A spectroscopic study of the reaction products of dihydroxyacetone with aminoacids. J Soc Cosmet Chem 1977; 28:25–35.
8. Lloyd RV, Fong AJ, Sayre RM. In vivo formation of Maillard reaction free radicals in mouse skin. J Invest Dermatol 2001; 117:740–742.
9. Chaudhuri RK. Dihydroxyacetone: chemistry and applications in self-tanning products. In: Schlossman ML, ed. The Chemistry and Manufacture of Cosmetics. Vol. III. IL: Carol Stream, 2002:383–402.
10. Bobin MF, Martini MC, Cotte J. Effects of color adjuvants on the tanning effect of dihydroxyacetone. J Soc Cosmet Chem 1984; 35:265–272.
11. Purcetti G, Leblanc RM. A sunscreen tanning compromise: 3D visualization of the actions of titanium dioxide particles and dihydroxyacetone on human epidermis. Photochem Photobiol 2000; 71:426–430.

12. Maibach HI, Kligman AM. Dihydroxyacetone: a suntan-simulating agent. Arch Dermatol 1960; 82:505–507.
13. Pierard GE, Pierard-Franchimont C. Dihydroxyacetone test as a substitute for the dansyl chloride test. Dermatology 1993; 186(2):133–137.
14. Forest SE, Grothaus JT, Ertel KD, Rader C, Plante J. Fluorescence spectral imaging of dihydroxyacetone on skin in vivo. Photochem Photobiol 2003; 77:524–530.
15. Goldman L, Barkoff J, Blaney D, Nakai T, Suskind R. The skin coloring agent dihydroxyacetone. General Practitioner 1960; 12:96–98.
16. Levy SB. Dihydroxyacetone-containing sunless or self-tanning lotions. J Am Acad Dermatol 1992; 27:989–993.
17. Nguyen BC, Kochevar IE. Influence of hydration on dihydroxyacetone-induced pigmentation of stratum corneum. J Invest Dermatol 2003; 120:655–661.
18. Nguyen BC, Kochevar IE. Factors influencing sunless tanning with dihydroxyacetone. Br J Dermatol 2003; 149:332–340.
19. Muizzuddin N, Marenus KD, Maes DH. Tonality of suntan vs sunless tanning with dihydroxyacetone. Skin Res Technol 2000; 6:199–204.
20. Muizzuddin N. Marenus KD, Maes DH. UV-A and UV-B protective effect of melanoids formed with dihydroxyacetone and skin. Poster 360 presented at the 55th Annual Meeting of the American Academy of Dermatology, San Francisco, 1997.
21. Johnson JA, Fusaro RM. Protection against long ultraviolet radiation: topical browning agents and a new outlook. Dermatologica 1987; 175:53–57.
22. Petersen AB, Na R, Wulf HC. Sunless skin tanning with dihydroxyacetone delays broad spectrum ultraviolet photocarcinogenesis in hairless mice. Mutat Res 2003; 542:129–138.
23. Fitzpatrick TB. The validity and practicality of sunreactive skin types I through IV. Arch Dermatol 1988; 124:869–871.
24. Fesq H, Brockow K, Strom K, Mempel M, Ring J, Abeck D. Dihydroxyacetone in a new formulation—a powerful therapeutic option in vitiligo. Dermatology 2001; 203:241–243.
25. Suga Y, Ikejima A, Matsuba S, Ogawa H. Medical pearl DHA application for camouflaging segmental vitiligo and piebald lesions. J Am Acad Dermatol 2002; 47:436–438.
26. Fusaro RM, Johnson JA. Photoprotection of patients sensitive to short and/or long ultraviolet light with dihydroxyacetone/naphthoquinone. Dermatologica 1974; 148:224–227.
27. Taylor CR, Kwagsukstith C, Wimberly J, Kollias N, Anderson RR. Turbo-PUVA: dihydroxyacetone-enhanced photochemotherapy for psoriasis: a pilot study. Arch Dermatol 1999; 135:540–544.
28. Morren M, Dooms-Goossens A, Heidbuchel M, Sente F, Damas M. Contact allergy to dihydroxyacetone. Contact Dermatitis 1991; 25:326–327.
29. Foley P, Nixon R, Marks R, Frowen K, Thompson S. The frequency of reaction to sunscreens: results of a longitudinal population-based study on the regular use of sunscreens in Australia. Br J Dermatol 1993; 128:512–518.
30. Reiger MM. The chemistry of tanning. Cosmet Toilets 1983; 98:47–50.
31. Kurz T. Formulating effective self-tanners with DHA. Cosmet Toilets 1994; 109(11): 55–61.
32. Goldman L, Barkoff J, Blaney D, Nakai T, Suskind R. Investigative studies with the skin coloring agents dihydroxyacetone and glyoxal. J Invest Dermatol 1960; 35:161–164.
33. Eichler J. Prinzipien der Haptbraunung. Kontakte (Merck) 1981; 111:24–30.

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Skin-Whitening Products

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Skin-whitening products have been widely used in the cosmetic field and clinical therapy. They are supposed to either lighten skin (individuals who wish to change or modify their skin color) or depigment skin (treatment for abnormal-hyperpigmentation skin such as melasma, freckles, and senile lentigines). Whitening agents, such as hydroquinone, kojic acid, and ascorbic acid derivatives have shown efficacy in a variety of hyperpigmentary disorders (1–14) but with varying success (1,2,7–9). Their mechanism of action has been studied in vitro and in vivo (3,10–17). Recently, their safety of application have been extensively investigated (18–32). This chapter includes the most popular active ingredients of whitening agents and emphasizes their efficacy and safety.

HYDROQUINONE (1,4-DIHYDROXYBENZENE)

Hydroquinone is a nonvolatile chemical used in the photographic, rubber, chemical, and cosmetic industries. In the late 1930s, it was observed that a chemical used in rubber manufacture, monobenzyl ether of hydroquinone, caused depigmented skin in some workers (1). The efficacy of hydroquinone (1,4-dihydroxybenzene) as a skin-lightening agent has been established in both human and animal studies. The chemical structure of hydroquinone is shown in Figure 1. Clinically, hydroquinone is applied topically in the treatment of melasma, freckles, and senile lentigines, as well as postinflammatory hyperpigmentation. In the United States, hydroquinone is readily available in concentrations up to 2.0% as an over-the-counter (OTC) drug and by prescription at higher concentrations (1,2). Thus, hydroquinone is readily applied to the skin for medical and cosmetic reasons (Strauch et al., in preparation). Hydroquinone inhibits the conversion of dopa to melanin by inhibiting the tyrosinase enzyme (1–3). Other proposed mechanisms are inhibition of DNA and RNA synthesis, degradation of melanosomes, and destruction of melanocytes (2). Electron microscopic studies of black guinea-pig skin treated with hydroquinone show the anatomic consequences of this action: (*i*) the melanosome structure is disturbed, resulting in decreased production or increased degradation of these organelles, or both; (*ii*) hydroquinone exposure can ultimately lead to the degradation of the melanocyte; and (*iii*) keratinocytes are spared, showing no apparent injury (1).

Figure 1 Chemical structures of (**A**) hydroquinone, (**B**) arbutin, (**C**) kojic acid, and (**D**) L-ascorbic acid (vitamin C).

Arndt and Fitzpatrick (4), in a nonplacebo-controlled study, compared the efficacy of 2% and 5% hydroquinone cream for treatment of various pigmentary disorders in 56 patients. Results showed that hydroquinone was a moderately effective depigmenting agent in 80% of the cases and that there was no difference between the two concentrations in therapeutic efficacy. Two percent hydroquinone was less irritating than 5%. Fitzpatrick et al. (5), in a nonplacebo-controlled study, evaluated the efficacy of a 2% cream of stabilized hydroquinone in 93 patients. Sixty-four percent of them showed decreasing hypermelanosis without untoward effects. Sanchez and Vazquez (6) treated 46 patients with melasma using two versions of a 3% hydroalcoholic solution of hydroquinone. In this nonplacebo-controlled study, overall improvement was noted in 88% of the patients and moderate-to-marked improvement in 36%. Side effects were minimal. The usage of a sunscreen agent was necessary for therapeutic efficacy. The efficacy of hydroquinone may be improved when it is used in combination with other chemicals as well as tretinoin, salicylic acid, or corticosteroid (1,2). Kligman and Willis (7) noted an enhanced efficacy with 5% hydroquinone, 0.1% tretinion, and 0.1% dexamethasone in hydrophilic ointment for the treatment of melasma, ephelides, and postinflammatory hyperpigmentation in a nonplacebo-controlled study. In contrast, they experienced poor results with each of the aforementioned as monotherapies. However, senile lentigines were resistant to this therapy. Gano and Garcia (8) conducted a 10-week clinical trial in 20 women with melasma. Topical applications of 0.05% tretinoin, 0.1% betamethasone valerate, and 2% hydroquinone were used in a nonplacebo-controlled study. There was an objective improvement rate of 65% and a subjective improvement rate of 95%. Side effects were frequent but minimal. Caution is necessary when using potent fluorinated corticosteroids for prolonged periods on the face, because telangiectasia, atrophy, or acne rosacea can develop.

Pathak et al. (9) clinically tested the efficacy of hydroquinone in varying concentrations supplemented with corticosteroids or retinoic acid (tretinoin) in 300 Hispanic women with melasma in a nonplacebo-controlled study, and concluded that cream or lotion formulations of 2% hydroquinone and 0.05% to 0.1% retinoic acid provided the most favorable results. In addition, avoidance of sun exposure and constant use of broad-spectrum sunscreens are necessary for the best therapy effects. Recently, Clarys and Barel (33) tested the efficacy of an ascorbate–phytohydroquinone complex in 14 patients with lentigo senile lesions in a nonplacebo-controlled study. Objective skin-color changes were evaluated with a chromameter. After one month of treatment, a clear depigmentation of the macules was measured. None of the patients reported adverse effects.

Gellin et al. (34) established a reliable in vivo method to predict the depigmenting action of chemicals on mammalian melanocytes. They used black guinea pigs and black mice as animal models to screen the depigmenting capacity of several phenols, catechols, and organic antioxidants. Results showed that complete depigmentation on all test sites was achieved with monomethyl ether of hydroquinone and tertiary butyl catchall in the black guinea pig. Less-pronounced pigment loss was noted with these chemicals in black mice.

To treat some cases, higher concentrations of hydroquinone may be used. The formulations contain concentrations as high as 10% combined with nonfluorinated corticoid creams with or without the additional use of tretinoin or salicylic acid. Extemporaneously compounded preparations are often effective in patients that have failed to respond to lower concentrations of hydroquinone. With controlled use and monitoring, side effects from these preparations have proved minimal (2). Note, however, that hydroquinone may be quickly oxidized in such formulations.

Hydroquinone occurs in nature as the beta-glucopyranoside conjugate arbutin. Arbutin is a safe and mild agent for treating cutaneous hyperpigmentation disorders, including melasma and UV-induced ephelides (10). Arbutin is an active ingredient of the crude drug Uvae Ursi Folium—traditionally used in Japan and contained in the leaves of pear trees and certain herbs. The chemical structure of arbutin is shown in Figure 1. Maeda and Fukuda (10) determined the arbutin's inhibitory action on the melanin synthetic enzyme and its effects on melanin intermediates and melanin production in cultured human melanocytes. They indicated that the depigmentation effect of arbutin works through an inhibition of the melanosomal tyrosinase activity, rather than suppression of the expression and synthesis of tyrosinase in human melanocytes. Arbutin was much less cytotoxic than hydroquinone to cultured human melanocytes.

Adverse reactions associated with hydroquinone use include acute and chronic complications. Acute reactions include irritant dermatitis, nail discoloration, and postinflammatory hyperpigmentation (1). Although commonly assumed to be a common allergen, the documentation of hydroquinone allergic contact dermatitis is weak (1). Hydroquinone use can also induce hypopigmentation and, rarely, depigmentation of treated surrounding normal skin. However, these changes are temporary and resolve on cessation of hydroquinone treatment, in contrast to monobenzone use, which can cause permanent depigmentation (35). Hence, the only indication for monobenzone therapy is in the treatment of severe vitiligo.

A more recent concern regarding the use of hydroquinone is the occurrence of hydroquinone-induced ochronosis, a chronic disfiguring condition resulting, in general, from the prolonged use of strong concentrations of hydroquinone (35). Hydroquinone's acute and chronic toxicity toward higher terrestrial organisms appears to be minimal in humans (20,21). An epidemiologic investigation in 478 photographic processors has shown no significant excess mortality, sickness absence, or cancer incidence (20). The

reported nephropathy and cell proliferation, as evidence of carcinogenicity, observed in Fischer 344/N rats (22,23) appear to be strain- and sex-specific (23). Hydroquinone was negative in the Ames/Salmonella and Drosophila genotoxicity assays (24). Others suggest that carcinogenic and teratogenic potentials have been, at present inadequately studied (20,25), and that both hydroquinone and benzoquinone produce cytotoxic effects on human and mouse bone-marrow cells (26). Hydroquinone readily penetrates human forehead skin in vivo following a single topical exposure in an alcoholic vehicle of 24-hour duration. Elimination was complete within five days (19). Wester et al. (18) determined the topical bioavailability, metabolism, and disposition of hydroquinone on humans in vivo and in vitro; dose recovery in urine was 45.3%, of which the majority was excreted in the first 24 hours.

KOJIC ACID

Kojic acid, a fungal metabolic product, is increasingly being used as a skin-lightening agent in skincare products marketed in Japan since 1988. It was first isolated from Aspergillus in 1907 (31). The structure is shown in Figure 1. The mode of action of kojic acid is to suppress free tyrosinase, mainly attributable to chelation of its copper (11,16,31), and it has been shown to be responsible for therapy and prevention of pigmentation, both in vitro and in vivo (11,31).

In Japan, it is used in nonprescription skincare products up to a concentration of 1%. To increase percutaneous absorption and thus therapeutic activity, it is usually used at the highest concentration allowed (31). Because it is used intensively in foods (e.g., bean paste, soy, and sake) in some countries, particularly Japan, its oral safety has been studied. Shibuya et al. (28), investigating the mutagenicity of kojic acid by the Ames test, forward mutation test in cultured Chinese hamster cells, and dominant lethal test in mice, concluded that, although kojic acid is a weak mutagen in bacteria, it is nonmutagenic in eukaryotic systems either in vivo or in vitro. Abdel-Hafez and Shoreit (30) tested the mycotoxins using the dilution-plate method. Results showed that kojic acid may induce some toxins. Fujimoto et al. (32) examined the tumorigenicity of kojic acid in B6C3Fi mice. Three groups of animals were given 0, 1.5, and 3.0% kojic acid-containing food for six weeks; kojic acid groups significantly induced thyroid tumors in B6C3F, mice. But true adverse effects after human oral ingestion have not been shown. Nakagawa et al. (31) noted that there were no signs of relapse of dermatitis or any other adverse effects on sensitized patients upon ingestion of foods containing kojic acid. However, they reported that topical application of kojic acid may induce allergic contact dermatitis with sensitized patients. They postulated that kojic acid was considered to have a high sensitizing potential, because of the comparatively high frequency of contact sensitivity in patients using one or more kojic acid-containing products. Recently, Majmudar et al. (36) used an in vitro model to evaluate the efficacy, stability, and cytotoxicity of whitening agents. They also conducted a nonplacebo-controlled clinical study that indicated that kojic acid in an anhydrous base can induce more skin lightening than in the aqueous base.

ASCORBIC ACID (VITAMIN C) AND ITS DERIVATIVES

Ascorbic acid may inhibit melanin production by reducing *o*-quinones (12) so that melanin cannot be formed by the action of tyrosinase until all vitamin C is oxidized.

The chemical structure of vitamin C is shown in Figure 1. Although the lightening effect of vitamin C is considered, it is quickly oxidized and decomposes in an aqueous solution and is thus not generally useful as a depigmenting agent. Numerous stable derivatives of vitamin C have been synthesized to minimize this problem (12–14,17). Magnesium-L-ascorby-2-phosphate (VC-PMG) is a vitamin-C derivative that is stable in water, especially in neutral or alkaline solution containing boric acid or its salt (12). VC-PMG is hydrolyzed by phosphatases of liver or skin to vitamin C and thus, exhibits vitamin C-reducing activity (12). Kameyama et al. (12) investigated the effects of VC-PMG on melanogenesis in vitro and in vivo. Results from this nonplacebo-controlled study suggested the topical application of VC-PMG was significantly effective in lightening the skin in 19 of 34 patients with chloasma or senile freckles, and in three of 25 subjects with normally pigmented healthy skin.

OTHER AGENTS

Various systemic drugs and natural products may be used as protective agents, such as chloroquine, indomethacin, vitamin C and E, fish oil, and green tea, etc. Topical agents include azelaic acid and melawhite except where previously described (37). Recently, Kobayashi et al. (38) reported that neoagarobiose could be useful as a novel-whitening agent as it has shown moisturizing and whitening effects with low cytotoxicity. Ando et al. (39) evaluated the effects of unsaturated fatty acids on UV-induced hyperpigmentation of the skin in a placebo (vehicle)-controlled study. Skin hyperpigmentation was induced on the backs of guinea pigs by UVB exposure. Oleic acid, linoleic acid, and oc-linolenic acid (0.5% in ethanol), or ethanol alone as a control, were then topically applied daily five times per week for three successive weeks. Results suggest that the pigment-lightening effects of linoleic acid and a-linolenic acid are, at least in part, attributable to suppression of melanin production by active melanocytes as well as to enhanced desquamation of melanin pigment from the epidermis.

CONCLUSIONS

In general, skin-whitening products are considered modestly effective. High concentrations are not recommended except under a physician's supervision. The application as a combination with certain chemicals (retinoic acid and alpha-hydroxy acids) may enhance lightening. Optimal whitening agents remain a future goal.

REFERENCES

1. Engasser PG, Maibach HI. Cosmetics and dermatology: bleaching creams. J Am Acad Dermatol 1981; 5:143.
2. Grimes PE Melasma. Etiologic and therapeutic considerations. Arch Dermatol 1995; 131:1453.
3. Jimbow K, Obata H, Pathak MA, Fitzpatrick TB. Mechanism of depigmentation by hydroquinone. J Invest Dermatol 1974; 62:436.
4. Arndt KA, Fitzpatrick TB. Topical use of hydroquinone as a depigmenting agent. JAMA 1965; 194:965.

5. Fitzpatrick TB, Arndt KA, el-Mofty AM, Pathak MA. Hydroquinone and psoralens in the therapy of hypermelanosis and vitiligo. Arch Dermatol 1966; 93:589.
6. Sanchez JL, Vazquez MA. Hydroquinone solution in the treatment of melasma. Int J Dermatol 1982; 21:55.
7. Kligman AM, Willis I. A new formula for depigmenting human skin. Arch Dermatol 1975; 111:40.
8. Gano SE, Garcia RL. Topical tretinoin, hydroquinone, and betamethasone valerate in the therapy of melasma. Cutis 1979; 23:239.
9. Pathak MA, Fitzpatrick TB, Kraus EW. Usefulness of retinoic acid in the treatment of melasma. J Am Acad Dermatol 1986; 15:894.
10. Maeda K, Fukuda M. Arbutin: mechanism of its depigmenting action in human melanocyte culture. J Pharm Exp Ther 1996; 276:765.
11. Cabanes J, Chazarra S, Garcia-Carmona F. Kojic acid, a cosmetic skin-whitening agent, is a slow-binding inhibitor of catecholase activity of tyrosinase. J Pharm Pharmacol 1994; 46:982.
12. Kameyama K, Sakai C, Kondoh S, Yonemoto K, Nishiyama S, Tagawa M, Murata T, Ohnuma T, Quigley J, Dorsky A, Bucks D, Blanock K. Inhibitory effect of magnesium L-ascorbyl-2-phosphate (VC-PMG) on melanogenesis in vitro and in vivo. J Am Acad Dermatol 1996; 34:29.
13. Nomura H, Ishiguro T, Morimoto S. Studies on L-ascorbic acid derivatives. II. L-ascorbic acid 3-phosphate and 3-pyrophosphate. Chem Pharm Bull 1969; 17:381.
14. Nomura H, Ishiguro T, Morimoto S. Studies on L-ascorbic acid derivatives. III. Bis (L-ascorbic acid-3,3′)phosphate and L-ascorbic acid 2-phosphate. Chem Pharm Bull 1969; 17:387.
15. Nakajima M, Shinoda I, Fukuwatari Y, Hayasawa H. Arbutin increases the pigmentation of cultured human melanocytes through mechanisms other than the induction of tyrosinase activity. Pig Cell Res 1998; 11:12.
16. Kahn V. Effect of kojic acid on the oxidation of DL-DOPA, norepinephrine, and dopamine by mushroom tyrosinase. Pig Cell Res 1995; 8:234.
17. Morisaki K, Ozaki S. Design of novel hybrid vitamin C derivatives: thermal stability and biological activity. Chem Pharm Bull 1996; 44:1647.
18. Wester RC, Melendres J, Hui X, Cox R, Serranzana S, Zhai H, Quan D, Maibach HI. Human in vivo and in vitro hydroquinone topical bioavailability, metabolism, and disposition. J Toxicol Environ Health 1998; 54:301.
19. Bucks DAW, McMaster JR, Guy RH, Maibach HI. Percutaneous absorption of hydroquinone in humans: effect of l-dodecylazacycloheptan-2-one (azone) and the 2-ethylhexyl ester of 4-(dimethylamino)benzoic acid (escalol 507). J Toxicol Environ Health 1988; 24:279.
20. Friedlander BR, Hearne FT, Newman BJ. Mortality, cancer incidence, and sickness-absence in photographic processors: an epidemiologic study. J Occup Med 1982; 24:605.
21. Pifer JW, Hearne FT, Swanson FA, O’Donoghue JL. Mortality study of employees engaged in the manufacture and use of hydroquinone. Int Arch Occup Environ Health 1995; 67:267.
22. English JC, Hill T, O’Donoghue JL, Reddy MV. Measurement of nuclear DNA modification by 32P-postlabeling in the kidneys of male and female Fischer 344 rats after multiple gavage doses of hydroquinone. Fundam Appl Toxicol 1994; 23:391.
23. English JC, Perry LG, Vlaovic M, Moyer C, O’Donoghue JL. Measurement of cell proliferation in the kidneys of Fischer 344 and Sprague-Dawley rats after multiple gavage administration of hydroquinone. Fundam Appl Toxicol 1994; 23:397.
24. Gocke E, King MT, Eckhardt K, Wild D. Mutagenicity of cosmetics ingredients licensed by the European community. Mutat Res 1981; 90:91.
25. Whysner J, Verna L, English JC, William GM. Analysis of studies related to tumorigenicity induced by hydroquinone. Regul Toxicol Pharmacol 1995; 21:158.

26. Colinas RJ, Burkart PT, Lawrence DA. In vitro effects of hydroquinone, benzoquinone, and doxorubicin on mouse and human bone marrow cells at physiological oxygen partial pressure. Toxicol Appl Pharmacol 1994; 129:95.
27. Goffin V, Pierard GE, Henry F, Letawe C, Maibach HI. Sodium hypochlorite, bleaching agents, and the stratum corneum. Ecotoxicol Environ Safety 1997; 37:199.
28. Shibuya T, Murota T, Sakamoto K, Iwahara S, Ikeno M. Mutagenicity and dominant lethal test of kojic acid: Ames test, forward mutation test in cultured Chinese hamster cells and dominant lethal test in mice. J Toxicol Sci 1982; 7:255.
29. Wei CI, Huang TS, Fernando SY, Chung KT. Mutagenicity studies of kojic acid. Toxicol Letters 1991; 59:213.
30. Abdel-Hafez SI, Shoreit AA. Mycotoxins producing fungi and mycoflora of air-dust from Taif, Saudi Arabia. Mycopathologia 1985; 92:65.
31. Nakagawa M, Kawai K, Kawai K. Contact allergy to kojic acid in skin care products. Contact Dermatitis 1995; 32:9.
32. Fujimoto N, Watanabe H, Nakatani T, Roy G, Ito A. Induction of thyroid tumours in (C57BL/6N X C3H/N)F1 mice by oral administration of kojic acid. Food Chem Toxicol 1998; 36:697.
33. Clarys P, Barel A. Efficacy of topical treatment of pigmentation skin disorders with plant hydroquinone glucosides as assessed by quantitative color analysis. J Dermatol 1998; 25:412.
34. Gellin GA, Maibach HI, Mislaszek MH, Ring M. Detection of environmental depigmenting substances. Contact Dermatitis 1979; 5:201.
35. Grimes PE. Vitiligo. An overview of therapeutic approaches. Dermatol Clin 1993; 11:325.
36. Majmudar G, Jacob G, Laboy Y, Fisher L. An in vitro method for screening skin-whitening products. J Cosmet Sci 1998; 49:361.
37. Piamphongsant T. Treatment of melasma: a review with personal experience. Int J Dermatol 1998; 37:897.
38. Kobayashi R, Takisada M, Suzuki T, Kirimura K, Usami S. Neoagarobiose as a novel moisturizer with whitening effect. Biosci Biotechnol Biochem 1997; 61:162.
39. Ando H, Ryu A, Hashimoto A, Oka M, Ichihashi M. Linoleic acid and cc-linolenic acid lightens ultraviolet-induced hyperpigmentation of the skin. Arch Dermatol Res 1998; 290:375.

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Anticellulite Products and Treatments

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INTRODUCTION

Cellulite is a localized condition of subcutaneous fat and connective tissues with the typical visual appearance of the orange peel look of the skin. Cellulite, or more correctly local lipodystrophy, affects mostly women and rarely men and is considered as a common aesthetic problem for many women. Cellulite appears generally after puberty and worsens with age. There are preferential places of cellulite, namely, buttocks, thighs, upper part of the arms, knees and more rarely the lower parts of the legs, and the back of the neck (Fig. 1).

The aims of this chapter are to describe:

i. the histological, physiological, and biochemical characteristics of subcutaneous lipodystrophy,
ii. the different objective evaluation methods of lipodystrophy,
iii. the different anticellulite treatments available and their efficacy.

CLINICAL, VISUAL, AND TACTILE SYMPTOMS OF CELLULITE

Upon clinical examination of cellulite the following symptoms can be noticed (1–12):

- presence of the typical orange peel skin upon normal visual examination and after pinching of the skin,
- deep palpation of the skin reveals differences in the mobility of fat tissue: presence of micro- and macronodules and fibrosclerosis,
- irregularities in skin surface temperature: touching the skin reveals the presence of cold spots,
- sometimes presence of painful subcutaneous nodules through deep palpation.

There are different stages in the evolution of cellulite with age.

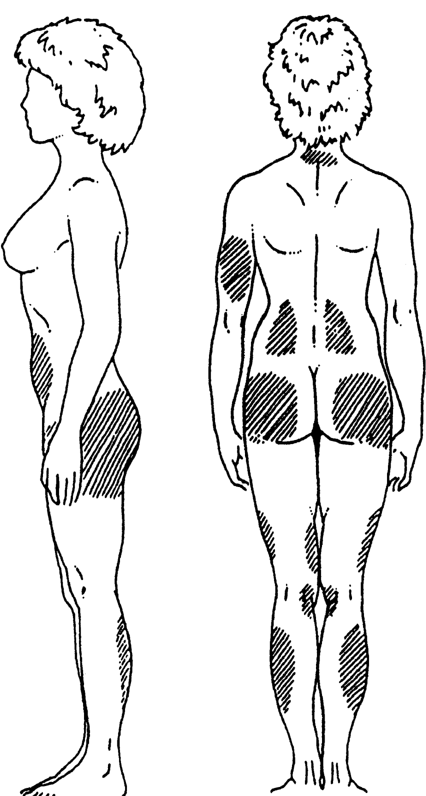

Figure 1 Preferential localizations of subcutaneous lipodystrophy in women.

It is difficult to detect cellulite by visual examination and by palpation at the initial stages, orange peel skin is not permanently present and is only visible after pinching the skin.

The clinical symptoms are clearly more visible at later stages of cellulite: permanent orange peel, colder skin areas, diminution in mobility of fat tissue upon palpation, and increased skin sensibility.

Because of this, there is a need for sensitive noninvasive bioengineering methods for the detection and the evaluation of the degree of cellulite at early stages and for the objective evaluation of the efficacy of various cosmetic treatments (13).

ETIOLOGY OF CELLULITE

Cellulite is probably a multicausal condition and many hypotheses have been proposed regarding the origin of fat lipodystrophy (1–13).

- Sexual differentiation in the histological distribution of subcutaneous fat lobules in women and in men. The differences between the sexes can be found in the structure of the septal connective fat tissue: the fat lobules

in women are larger and more rectangular, whereas men have diagonal septa and smaller lobules. Since cellulite is widely present in women, some authors consider cellulite as a secondary sexual characteristic.

- Alterations in the microvascular network (mostly venous blood circulation) in the fat tissue: venous stasis.
- Presence of plasmatic exudate in the subcutaneous connective tissue: noninflammatory oedema.
- Alterations in the reticular fibrillar network surrounding the blood vessels and adipocytes: fibrosclerosis. Stiffening and decrease in mobility of fibers.
- Alterations in the interstitial fundamental substance (proteoglycans).
- Modifications and hypertrophy of adipose tissues. Although cellulite is not always synonymous with overweight (some skinny persons could present symptoms of cellulite), there is a relation between cellulite and hypertrophy of fat tissues. Formation of first micronodules and later on macronodules in adipose tissues.

The combined effect of modifications and hypertrophy of adipose tissues, alterations in the both fibrillar connective tissue and microvascular venous network always lead to the presence of cellulite.

HISTOLOGICAL DESCRIPTION OF ADIPOSE TISSUES IN WOMEN

Nurnberg and Muller (14) were the first to discover differences in the general directions of the septae in fat tissues as a function of gender. In women one observes a higher percentage of septae perpendicular to the skin surface and a smaller percentage parallel to the surface is shown in men. Furthermore, in women with cellulite, deep indentations of adipose tissue into the skin were recorded. Using in vivo high frequency ultrasound imaging, Querleux et al. (15), Lucassen et al. (16), Nuijs et al. (17), and Rosenbaum et al. (11) confirmed an irregular dermo-hypodermal interface in women with cellulite. Querleux et al. (15) confirmed by magnetic resonance imaging (MRI) and spectroscopy the existence of indentations of adipose tissue into the dermis. An increase in the thickness of the inner fat layer, and a higher percentage of septae in the direction perpendicular to the skin surface are also observed in women with cellulite.

However, the microanatomical description of adipose tissue with cellulite has been critically revised by Piérard et al. (18). Curri and Ryan (6) suggested the hypothesis of an increase in water content of subcutaneous adipose tissue in case of cellulite. Lotti et al. (19) showed increasing concentration of glycosylaminoglycans, presumably leading to a rise in the amount of water retained in the skin. Querleux et al. did not confirm the hypothesis of increased water content in the adipose tissue of women with cellulite except if such water would be located in the connective septae.

DESCRIPTION OF THE DIFFERENT STAGES OF LIPODYSTROPHY OF FAT TISSUES

Skin surface contact thermographic pictures using thermographic foils give an indication of the degree of cellulite, as the skin surface temperature correlates

to some extent with the clinical symptoms of cellulite. Based on these thermographic patterns and clinical symptoms, Curri and coworkers proposed a classification of cellulite in four stages (4,5,20,21), which has been confirmed by others (13,22,23). In normal adipose tissues, a fine mesh of blood and lymph vessels supplies this adipose tissue with the necessary nutrients and oxygen and takes care of the removal of the metabolized products. In the early stage of cellulite (stage I), the walls of capillary blood vessels become more permeable, causing leakage of blood plasma from the vessels between the adipose tissues, which cause an oedema in the adipose tissues. In addition probably, problems with the lymph circulation hamper the removal of accumulating fluids. The aggregation of adipose cells and the amplification of the fibrillar network of collagen bundles interconnecting the adipose cells hamper blood circulation leading to hemostasis (stage II).

Adipose cells aggregates into "micronodules" surrounded by a less mobile collagen fibers (stage III). The size of these "micronodules" is in the order of millimeters. Finally, many of these "micronodules" aggregate into "macronodules" with larger sizes (2–20 mm) (stage IV). As nerves may be squeezed by these larger nodules, persons with severe cellulite often suffer from a sensitive to painful skin.

Stages 1, 2, and 3 of lipodystrophy are not considered clinically as pathological symptoms, but more as esthetic–cosmetic problems of the skin. Only in stage 4, some clinical symptoms, such as increased skin sensitivity, extensive fibrosclerosis of connective tissue, and very advanced oedema, are considered as light pathology symptoms. Furthermore, it is believed that the initial stages are more or less reversible, whereas the later stages are irreversible.

However, it must be said that the microscopic description of cellulite and the different stages in the evolution of lipodystrophy, as described by Curri, are not universally accepted (14,24).

OBJECTIVE EVALUATION OF THE SYMPTOMS OF LIPODYSTROPHY OF THE SKIN

In addition to the visual and tactile clinical evaluations of the symptoms of cellulite, various noninvasive bioengineering measurements may be used.

However, the clinical evaluation of cellulite remains important. The clinical evaluation of cellulite is based on either direct visual examination and palpation of the orange peel skin with a diminution of the mobility of the hypodermis (appearance of nodules of fat tissues and fibrosclerosis) or photograding of photographic pictures taken under well-standardized conditions. The visual evaluation is more closely related to the consumer's considerations and expectations. The appearance of differences in skin surface temperature and complaints of the patients of symptoms of hypersensitive skin and pain are also helpful clinical observations.

The different noninvasive bioengineering measurements are, respectively, the following:

- contact skin surface thermographic measurements using liquid crystals,
- noncontact skin surface thermography of skin surface using infrared video camera,
- microblood circulation using laser Doppler image analysis,

- ultrasonic skin analysis of skin density: Measurement of thickness of the hypodermis at 10 to 14 MHz and measurement of the surface of the interface between dermis and hypodermis at 20 MHz,
- hypodermis at 20 MHz,
- MRI,
- skin surface topographical imaging and fringe projection analysis,
- macroscopic normal and digitalized photographic pictures of the skin surface.

DESCRIPTION AND VALIDATION OF THE DIFFERENT BIOENGINEERING MEASUREMENTS USED FOR OBJECTIVE EVALUATION OF CELLULITE

Skin Surface Contact Thermography Using Encapsulated Liquid Crystals in the evaluation of Cellulite (13,20–23)

The principle of the encapsulated cholesteric liquid crystal contact thermography consists of different color plates presenting a pattern of different colors corresponding to a temperature range of about 3°C. Application of the color sheet with uniform pressure on the skin surface and photographic recording of the thermographic pattern using a polaroid camera. A qualitative global analysis of the thermographic pictures about the different stages of cellulite can be made. A cellulite free skin surface thermography shows a uniform color pattern without hypothermic and hyperthermic areas. A cellulite skin surface thermography shows a nonuniform color pattern with the presence of hypothermic (cold spots) and hyperthermic (warm spots) areas. Quantitative analysis of the thermographic pictures can also be carried out by image analysis. Computerized color image analysis gives the mean temperature of the thermogram and, respectively, the number and the percentage area of the hypo- and hyperthermic areas present on a well defined skin area. As experimentally observed, an anticellulite treatment will induce an increase of the mean temperature of the skin surface and a decrease of the percent hypothermic zones (with a concomitant increase of the percent hyperthermic zones).

This method is rapid, easy to use, and nonexpensive for screening subjects for cellulite and for confirmation of the clinical diagnosis.

However considering the low accuracy and reproducibility of the photographic pictures, quantitative image analysis of the thermograms is very difficult. One observes large interindividual variations in skin surface temperature (large number of subjects is necessary in a study) and long acclimatization time for temperature equilibrium of the skin (influence of external temperature). This method remains as a qualitative testing of cellulite at different stages.

Validation of Skin Surface Thermography Using Infrared Thermal Imaging System in the Evaluation of Cellulite (12,22,23)

Using an infrared video camera an infrared thermal image of the skin surface is obtained in a noninvasive manner. The thermographic picture can be quantitatively analyzed.

In the validation of this infrared video imaging technique, the same problems as with the contact thermography with liquid crystals are encountered such as large

interindividual variations in skin surface temperature, long acclimatization time for temperature equilibrium of the skin, and influence of external temperature.

Validation of Laser Doppler Imaging System in the Evaluation of Cellulite (12,22,23)

Using a laser Doppler perfusion imager, an image of the superficial blood circulation can be obtained. The He–Ne Laser light emitting at 633 nm has a penetration power in the skin of only about 300 μm.

This instrument measures the superficial blood flux of the skin (papillary dermis). The blood perfusion of the deeper layers of the skin, such as the hypodermis, cannot be measured with this technique. However a high correlation is obtained between the skin surface thermographic pictures and the laser Doppler imaging system when studying skin with cellulite. However, the measurements are delicate (long measuring times during which the volunteer must remain immobile).

Validation of the Ultrasonic Imaging of the Skin in the Evaluation of Cellulite

A promising method appears to be high-frequency ultrasound C-mode imaging (10–20 MHz). This noninvasive method has been frequently used both clinically and in research for studying the epidermis, dermis, and hypodermis (25–27).

Various authors have used the technique of the measurement of the thickness of the subcutaneous fatty layer using ultrasound imaging at 10 to 14 MHz (28–37); however, the determination of the echographic border line between subcutaneous fat and connective tissues/muscles is very delicate. As a consequence, the determination of the mean thickness of the hypodermis is not very accurate.

Measurement of the interface between the dermis and the subcutaneous fat using ultrasound imaging at 20 MHz (16,17).

The interface between the echogenic epidermis–dermis and the hypoechoic subcutaneous fat is clearly visible allowing measurements of skin thickness and of the surface of this border.

In normal cellulite-free skin, the interface between the dermis and the fat tissue is irregular but rather smooth. In skin with cellulite, this surface is not smooth and is very irregular. The surface of this interface is quantified and can used as a measure of the degree of cellulite. Quantification of the surface of the interface between the dermis and the hypodermis (fat tissue) is possible and can be considered as a measure of the extent of cellulite (16).

Measurement of Skin Surface Topography

Cellulite skin surface presents irregularities (orange peel skin) and, in principle, the classical skin surface roughness measurements, which are used in cosmetic research, could be applied for studying cellulite. It involves stylus profilometry, image analysis by shadow method and optical focus laser profilometry, topographical skin imaging techniques, and fringe projections analysis (30,35,38–44). Stylus profilometry measurements are carried out on soft or hard skin replicas of generally small size (2–3 cm^2 area) and have a limited vertical range of roughness capability (maximum 400–500 μm). These techniques are well suited for the determination of the microrelief of the skin surface (50–200 μm), but not for assessing

the skin surface with cellulite. Optical focus laser profilometry and fringe projections analysis can be carried directly on the skin surface. The macrorelief of the skin surface can also be evaluated using an optical triangular laser profilometry. This method involves measurements on large size soft replicas with an extended vertical range of skin irregularities (up to 8–10 mm). Quantification of the skin surface macrorelief involves a computerized correction for the curvature of the skin surface with cellulite.

Actually the skin surface topography of skin with cellulite could be more easily evaluated using 3D topographical skin imaging techniques and 3D map topopgraphy from fringe projections.

Normal and Digitalized Macroscopic Photographic Pictures of the Skin Surface

The macrorelief of the skin can be evaluated by taking photographic pictures (classical or digitalized) under standardized experimental conditions. These photographic pictures are then graded visually in double-blind manner by expert observers for the intensity of cellulite (photograding with numerical scales) (44,46–48). It is known since many years that the standardization of classical photographic pictures is not easy, considering the problems of reproducibility of the processing of the color film. The use of digitalized photographic pictures is aimed to overcome the standardization problems of classical processing of the color film. In addition, double-blind visual scoring of these photographic pictures remains subjective. Some authors have used photographic pictures to evaluate the efficacy of anticellulite treatments (35).

Macroscopic digitalized photographic pictures (with the use of a CCD camera) of the external part of the thighs were taken after application of a gripping system around the thigh to increase the orange peel look of the skin. The degree of cellulite was photograded by experts using a 0 to 7 scale of intensity of cellulite (47,48).

In Vivo MRI and Spectroscopy

Recently, high resolution MRI and localized spectroscopy data were published (15), allowing to investigate subcutaneous adipose tissue in men, and in women with and without cellulite. As previously mentioned in this chapter, MRI is very efficient for measuring the thickness, surface, and volume of the adipose tissue. In women with cellulite, an increase in skin thickness and the presence of deep indentations of adipose tissue into the skin were noticed. Unfortunately, because of the high cost and limited accessibility of this instrument, this promising technique will not be available for cellulite research for most laboratories and cosmetic companies.

TREATMENTS OF CELLULITE

Different anticellulite treatments are available (13), namely,

- manual and electromechanical deep massage (“pincer-rouler”),
- manual lymph drainage,
- sequential pneumatic compression (lymph drainage),
- electrolipolysis,
- mesotherapy,

Table 1 List of Dermato-Cosmetic Ingredients Most Frequently Used in Anticellulite Treatments

Caffeine
Barley (*Hordeum vulgare*)
Butcher's broom (*Ruscus aculeatus*)
Centella (*Centella asiatica*)
Cola (*Cola nitida*)
Gingko (*Gingko biloba*)
Green tea (*Thea sinensis*)
Horse Chesnut (*Aesculus hippocastanum*)
Horsetail (*Equisetum arvensis*)
Ivy (*Hedera helix*)
Thistle (*Cnicus benedictus*)
Witch Hazel (*Hamamelis virginiana*)
Algae
Fucus vesiculosus, *Garcinia combogia*, *Laminaria flexicaulis*, and *Ascophyllum nodosum*

- topical applications of dermato-cosmetic products with and without massage (13,49–51).

The physiotherapeutic treatments, such as deep massage, manual and pneumatic lymph drainage, stimulate the blood and the lymph microcirculation and increase the removal of the extra fluid in the adipose tissues. In addition, these massage techniques will retard the further development of fibrosclerosis and the aggregation of fat cells in nodules. These physiotherapeutic treatments are generally combined with the topical use of anticellulite dermato-cosmetic products (during massage or pre- or postmassage).

Electrolipolysis and mesotherapy are invasive medical treatments of cellulite; these techniques will not be described in this Chapter.

The use of various topical dermato-cosmetic products, generally applied with massage, in the treatment of cellulite and/or as slimming is known since many years (13,49–51).

A list of the "active" ingredients mostly used for this purpose is given in Table 1. The most widely used anticellulite ingredients are caffeine and its derivatives and plant extracts. As plant extracts the use of Butcher's broom (*Ruscues aculeatus*), Horse chesnut (*Aesculus hippocanthum*), Ivy (*Hedera helix*), Gingko Biloba, Witch hazel (*Hamamelis virginiana*), White oak, Green tea, etc. The "active" molecules of these plant extracts are probably flavonoids (rutins, rutinosides) or terpenes (Ginkgolides). Also retinol was used recently as ingredient in anticellulite/slimming products.

The main purpose of these topical slimming/anticellulite products is to influence the metabolism of the adipocytes. In vitro metabolism studies on fat cells have shown that it is possible to slow down the lipogenesis (uptake of glucose and free fatty acids to synthesize triglycerides) and to stimulate the lipolysis (degradation of triglycerides and release of the free fatty acids) in different ways (52–55). The degradation of triglycerides by different lipases is controlled by a c-AMP–activated enzymatic system (adenyl cyclase):

- diminution of the uptake of glucose by interfering with the membrane bound glucose transport proteins,
- diminution of the uptake of free fatty acids,

- stimulation of the hydrolysis of the triglycerides by blocking the enzyme (phosphodiesterase), which hydrolyses camp, and by activating the adenyl cyclase enzymatic system, which produces c-AMP through the membrane-bound beta receptors,
- inhibition of lipogenesis by binding with the alfa and NPY/PYY receptors.

In addition, some of these slimming/anticellulite plant extracts present properties of stimulation of the blood and lymph circulation and inhibit further the fibrosclerosis of the fat surrounding collagen matrix. A few examples of typical slimming ingredients include Witch hazel, Ivy, Gingko, Aloa Vera, and so on, they are also used for their supposed beneficial effects on venous circulation. Various algae such as *Fucus vesiculosus*, *Laminaria flexicaulis*, and *Ascophyllum nodosum* are incorporated in anticellulite cosmetic preparations for their hypothetical beneficial effect on the skin surface.

CRITICAL REVIEW OF RECENT CLINICAL ANTICELLULITE STUDIES

Very few anticellulite studies, which were performed under well-controlled experimental conditions (double-blind, vehicle controlled, etc.) and under medical and paramedical supervision are published in the scientific literature.

The results of other clinical studies carried out by cosmetic firms are accessible through internet or released press maps (these documents although interesting to consult are not considered by the author as real scientific publications).

A double-blind vehicle controlled clinical study on 15 female volunteers with moderate cellulite at the upper and middle thighs, involving a topical application of a commercial preparation containing mixture of algees (a 30 minutes topical application under plastic foil with a thermal electrical blanket). This typical balneotherapeutic treatment was carried out every three days during three consecutive weeks under the medical and physiotherapeutic control (56). A significant decrease in thigh perimeter was observed equally for the vehicle alone and for the vehicle with the "active" algees extract, probably because of the combined effect of plastic foil occlusion and heating with the blanket. No significant modifications were observed in skin surface color (Chromameter) and superficial blood flow (laser Doppler) after three weeks treatment with the vehicle and the algees extract.

A double-blind vehicle controlled clinical study was carried out on 15 female volunteers with cellulite at the upper and middle thighs, involving a manual massage with a cream containing various plant extracts, every three days during three consecutive weeks (massage carried out by a physiotherapist), showed after this period of treatment a significant diminution of the extent of cellulite as examined by skin surface thermography using liquid crystal sheets (57,58). However, no significant differences were obtained between massage treatment with the vehicle containing "active" plant extracts (ivy, thyme, centella, nettle, horse chesnut, bark, witch hazel, etc.) and with the placebo vehicle alone.

Recently, a clinical anticellulite study was published consisting of a massage treatment with the help of a hand-held electro-mechanical apparatus consisting of a low-pressure chamber (200 mbar) and two rollers (16,17,44). The duration of the treatment was three months, three times a week, during 15 minutes on each upper leg (thigh region) on 19 healthy female volunteers with moderate symptoms of

cellulite on the thighs. The efficacy of this treatment was evaluated using ultrasound measurements at 20 MHz.

This electromechanical treatment induces a significant smoothening of the dermis/hypodermis surface after 1, 2, and 3 months treatment, respectively. After the treatment was stopped, the dermis/hypodermis surface gradually increased again, which indicates that the effect of this massage on the skin is not permanent.

This modification of the interface structure (smoothening) after this mechanical treatment of the skin can be interpreted as the result of the diminution of the venous stasis (positive effect on the venous microcirculation) and an improvement also of the lymph circulation and prevention of further fibrosclerosis and of aggregation of fat micro and macronodules.

Similar positive improvements as measured by ultrasound echography, were obtained after comparable manual massage treatments and lymph drainage with pressotherapy of cellulite skin located at the thighs (58).

The intensity of cellulite was rated by visual skin surface roughness scoring, skin thickness using a caliper and the thickness of subcutaneous fat layer. Significant decreases were observed for these three experimental parameters after 28 days treatment.

In a double-blind, placebo-controlled clinical study Kligman et al. (54) have evaluated the anticellulite activity of retinol. The study involved 20 healthy women with cellulite, applying twice daily on both thighs during 6 months either the active product or the placebo. The results indicate on the retinol treated side, a visible improvement of the appearance of orange peel skin, improved resistance to skin stretching and increase in micro blood flow.

In a double-blind, placebo-controlled clinical study Perin et al. (48) have carried out a clinical study on 30 healthy female volunteers with cellulite. The thighs were twice daily treated during two months with a massage product containing various plant extracts. The intensity of lipodystrophy was rated using photographic digital pictures, and the thickness of subcutaneous fat tissue by echography. Significant decreases of the mean score of cellulite intensity (photogradation) and of the thickness of subcutaneous fat were observed only after two months treatment with the active product.

In a randomized, placebo-controlled study Piérard et al. (59) have evaluated the anticellulite property of a topical retinol treatment during six months on 15 healthy women with mild to moderate cellulite. After six months treatment, the mechanical properties of the skin treated with retinol were improved (increase of elasticity and decrease of visco-elastic properties).

In a double-blind, placebo-controlled study Bertin et al. (35) have studied the anticellulite activity on 46 healthy female volunteers with cellulite, of a product containing retinol, caffeine and ruscogenide (plant flavonoid). Using different non-invasive methods they demonstrated a significant activity of the anticellulite preparation (decrease of orange peel aspect of skin surface and increase in skin blood microcirculation).

In a clinical study (53), 53 healthy female volunteers applied during 28 days on one thigh the anticellulite cream and the other untreated thigh was considered as a control. Thigh perimeter and thickness of adipose tissues (ultrasonic measurement) were carried out, respectively, after 14 and 28 days. A small decrease in thigh perimeter and thickness of adipose tissue was reported. The anticellulite preparation contained caffeine, a keratolytic product (salicylate), and two plant extracts (apple and ruscus).

In a multicenter clinical study (52), 222 female healthy volunteers applied twice a day during 28 days on one thigh an anticellulite product containing a caffeine derivative and plant extracts and the other untreated thigh was considered as a control. The degree of cellulite was evaluated by visual evaluation (Curri scale), perimeter of thigh, and profilometry of skin surface. Small decreases in thigh perimeter, decrease of orange peel aspect, and diminution of macrorelief of skin surface were noted after 28 days treatment.

Finally in a double-blind, placebo-controlled, clinical study (55), 76 healthy female volunteers applied on both thighs twice a day during 2 months either an anticellulite product or a placebo preparation. The active product contained patented products obtained from microbial fermentation, and the degree of cellulite was rated using photographis pictures of the skin surface after pinching and profilometry. A decrease of orange peel aspect was noticed on the active side.

CONCLUSIONS

In vitro metabolism studies on adipocytes have shown that many different molecules (caffeine, plantextracts, etc.) can be considered as "active" ingredients to slow down the synthesis of triglycerides and to stimulate the degradation of triglycerides in the adipocyte. However, when using these "active" molecules in vivo as anticellulite ingredients one must take in account the following limitations.

Percutaneous penetration of the "active" molecules through the skin to reach the hypodermis. As an example, caffeine penetrates readily well in the skin, but scant information is published about the penetration of these plant extracts.

Concentration of the "active" products in commercial anticellulite products: It must be assumed that the concentrations of plant extracts are rather low considering the high cost of these extracts and the potential danger of these plant derivatives as allergens. Possibility of the problems of photoallergy and photoirritation must be considered.

In the critical analysis of the efficiency of the different clinical anticellulite treatments one must evaluate carefully the practical conditions of the clinical trial: number of volunteers, duration of treatment (going from 28 days to six months), number of applications of product daily, double-blind, placebo-controlled or application on one side and other side is left untouched as control.

For simple trials (one treated thigh and other thigh as control) improvements are always observed. One question remains, that is, whether the improvements are the result of the combined action of massage and the "active" ingredients or solely the result of the massage?

For more elaborated trials (double-blind and placebo-controlled), the results are variable.

Some clinical studies clearly show significant improvements of the degree of cellulite on the treated thigh compared to the placebo thigh. These improvements are significant, clearly visible (confirmed by the subjects themselves) but not very impressive.

Other clinical trials indicate that, similar if not identical, significant improvements of cellulite were observed with the inert massage product and the massage product with the "active ingredients." These trials substantiate the hypothesis that the cellulite improvements are because of physiotherapeutic treatments, such as massage, lymph drainage, or thermal occlusion of the skin, and not solely because of the so-called active anticellulite dermato-cosmetic ingredients.

REFERENCES

1. Bartoletti CA. La cellulite. J Médecine Esthétique 1975; 8:11–16.
2. Merleen JF, Curri SB, Sarteel AM. La cellulite, affection micro-vasculo-conjective. Phlebologie 1979; 32:279–280.
3. Curri SB. Lipödem and Zellulitis. In: Foldi M, Tischendorf F, eds. Ein Symposium, Munich, Medizischer Verlag Erdmann-Brenger, 1983:9–77.
4. Curri SB. Ödem, Lymphödem und perivaskuläee Grundsunstanz. In: Schriftenreihe Manuel Lymphdrainage nach Dr. Vodder, Band 2. Heidelberg: Karl F. Haug Verlag, 1988:7–101.
5. Gasbarro V, Zamboni P. Varicosités et cellulite: approche thérapeutique combinée. J Médecine Esthétique et de Chirurgie Dermatol 1988; 15:49–55.
6. Curri SB, Ryan TJ. Panniculopathy and fibrosclerosis of the female breast and thigh. In: Ryan TJ, Curry SB, eds. Cutaneous adipose tissue. Philadelphia: Lippincott, 1989: 107–119.
7. Ryan TJ, Curri SB. Blood vessels and lymphatics. Clin Dermatol 1989; 7:25–36.
8. Curri SB, Bombardelli E. Local liposystrophy and districtual microcirculation. Cosmet Toilet 1994; 109:51–65.
9. Di Salvo RM. Controlling the appearance of cellulite. Cosmet Toilet 1995; 110:50–59.
10. Smith WP. Cellulite treatments. Cosmet Toilet 1995; 110:61–70.
11. Rosenbaum M, Pietro V, Hellmer J, Boschmann M, Krueger J, Leibel J, Ship AG. An exploratory investigation of the morphology and biochemistry of cellulite. Plast Reconstr Surg 1998; 101:1934–1939.
12. Rossi AB, Vergnanini AL. Cellulite: a review. J Eur Acad Dermatol Venereol 2000; 14:251–262.
13. Barel AO. Etude objective de la lipodystrophie des tissus graisseux au moyen de méthodes de bioengineering non invasives. J Médecine Esthetique 1998; 25:181–189.
14. Nurnberger F, Muller G. So called cellulite; an invented disease. J Dermatol Surg Oncol 1978; 4:221–229.
15. Querleux B, Cornillon C, Jolivet O, Bittoun J. Anatomy and physiology of subcutaneous adipose tissue by in vivo magnetic resonance imaging and spectroscopy: relationships with sex and presence of cellulite. Skin Res Technol 2002; 8:118–124.
16. Lucassen G, Van der Sluys W, Van Herk J, Nuijs T, Wierenga P, Barel AO, Lambrecht R. The effectiveness of massage treatment on cellulite as monitored by ultrasound imaging. Skin Res Technol 1997; 3:154–160.
17. Nuijs AM, Van Herk J. Characterizing the texture of cellulite skin. Abstract of the 12th International Symposium on Bioengineering and the Skin, Boston, 1998.
18. Lotti T, Gheersetich I, Grappone C, Dini G. Proteoglycans in so-called cellulite. Int J Dermatol 1990; 29:272–274.
19. Piérard G, Nizet JL, Piérard-Franchimont C. From standing fat herniation to hypodermal Stretch marks. Am J Dermapatholgy 2000; 22:34–37.
20. Ippolito F, Di Carlo A. La thermographie: son utilité comme critère de diagnostic et d'efficacité dans le traitement de la cellulite. J Médecine Esthétique et de Chirurgie Dermatol 1984; 11:81–86.
21. Marzorati V, Curri SB. Contact thermography and cellulitis. Technical information IPS, Milan, 1990.
22. Barel AO, Noël G, Vandermeulen S, Goemare K, Clarys P. The use of contact thermography using liquid crystal in the objective evaluation of a topical anti-cellulitis treatment. Abstract of the 3rd Congress International Society for Ultrasound and the Skin, Elsinore, Denmark, 1993.
23. Barel AO. Study of subcutaneous fat tissue (normal and lipodystrophy, cellulite) using noninvasive bioengineering methods. Abstract of the 12th International Symposium on Bioengineering and the Skin, Boston, MA, 1998.

24. Kligman AM. The reality and mythology of cellulite. Abstract of the 12th International Symposium on Bioengineering and the skin, Boston, 1998.
25. Serup J. Ten years experience with high-frequency ultrasound examination of the skin: development and refinement of technique and equipment. In: Altmeyer P, ed. Ultrasound in Dermatology. Berlin: Springer-Verlag, 1992:41–54.
26. Serup J, Keiding J, Fullerton A, Gniadecka M, Gniadecka R, Fornage B. High frequency ultrasound examination of skin: introduction and guide. In: Serup J, Jemec GBE, eds. Non-invasive Methods and the Skin. Boca Raton: CRC Press, 1995:239–256.
27. Fornage B. Ultrasound examination of the skin and subcutaneous tissues at 7.5 to 10 MHz. In: Serup J, Jemec GBE, eds. Non-invasive Methods and the Skin. Boca Raton: CRC Press, 1995:279–288.
28. Pittet JC, Perrier C, Schnebert S, Perrier P, Tranquart F, Beau P. Variability of fatty tissue thighness measurements using ultrasonography. Abstract of the 5th meeting of the International Society for Skin Imaging, Vienna, 1997.
29. Perin F, Pittet JC, Perrier P, Schnebert S, Beau P. Ultrasound imaging assessment of adipose tissue thickness variations during the menstrual cycle. Abstract of the 5th meeting of the International Society for Skin Imaging, Vienna, 1997.
30. Bielfeldt S, Gassmüller J. Untersuchungen zu korrelation verschiedener messtechnischer parameter mit dem visuellen cellulite-befund weiblicher probanden. Parfümerie und kosmetiek 1997; 78:14–18.
31. Adenola J, Maibach H. Ultrasonography, thermography and the cutometer in the assessment of cellulite treatments. Abstract of the 12th International Symposium on Bioengineering and the Skin, Boston, MA, 1998.
32. Perin F, Pittet JC, Schnebert S, Perrier P, Tranquart F, Beazu P. Ultrasonic assessment of variations in thickness of subcutaneous fat during normal menstruam cycle. Eur J Ultrasound 1999.
33. Schnebert S, Perin F, Pittet JC, Beau P, Perrier P, Pourcelot L. Echographie, une technique accessible et fiable pour mesurer l'efficacité des produits amincissants. Cosmétologie 1999; 22:35–38.
34. Perin F, Pittet JC, Perrier C, Tranquart F, Beau P. Methological aspects of the ultrasonic measurement of subcutaneous adipous tissue thickness for the evaluation of the efficacy of slimming treatments. JEMU 1999; 20:318–325.
35. Bertin C, Zunino H, Pittet JC, Beazu P, Pineau P, Massoneau M, Robert C, Hopkins J. A double-blind evaluation of the activity of an anti-cellulite product containing retinol, caffeine and ruscogine by a combination of several non-invasive methods. J Cosmet Sci 2001; 52:199–210.
36. Diridollou S, Lehnisch A, Black D, Lagarde JM, Gall Y. Exploration of subcutaneous fat tissue using an in vivo ultrasound technique. Abstract of the International Congress of the ISBS, ISCS and SFIC, Paris, France, 27 June, 2002.
37. Pittet JC, Beau P. High resolution sonography and magnetic resonance microscopy. Abstract of the 14th International Congress of the International Society for Bioengineering and the Skin, Hamburg, Germany, 21 May, 2003. Skin Res Technol 2003; 9:218.
38. Gassmüller J, Kecskes A, Jah P. Stylus method for skin surface contour measurement. In: Serup J, Jemec GBE, eds. Handbook of Non-invasive Methods and the Skin. Bacon Raton, Florida: CRC Press, 1995:83–89.
39. Corcuff P, Lévêque JL. Skin surface replica image analysis of furrows and wrinkles. In: Serup J, Jemec GBE, eds. Handbook of Non-invasive Methods and the Skin. Bacon Raton, Florida: CRC Press, 1995:89–97.
40. Efsen J, Hansen HN, Christiansen S, Keiding J. Laser profilometry. In: Serup J, Jemec GBE, eds. Handbook of Non-invasive Methods and the Skin. Bacon Raton, Florida: CRC Press, 1995:97–107.
41. Mignot J. Three-dimensional evaluation of skin surface: micro- and macrorelief. In: Serup J, Jemec GBE, eds. Handbook of Non-invasive Methods and the Skin. Bacon Raton, Florida: CRC Press, 1995:97–107.

42. Tympanidou P, Tympanidou B. A non contact technique for the objective evaluation of cellulite and local mobilization. Abstract of the 12th International Symposium on Bioengineering and the Skin, Boston, 1998.
43. Lagarde JM, Vié K, Beau P, Zahouani H, Gall Y. Evaluation of a slimming product using multi-scale analysis of 3-D topographical skin imaging with continuous wavelet transformation. Abstract of the 12th International Symposium on Bioengineering and the Skin, Boston, 1998.
44. Nuijs AM, Van Herk JJ, Barel AO. The effect of massage treatment on cellulite. Abstract of the International Conference on Health and Beauty for women in the Arab world, October 30, 2002, London, U.K.
45. Endermology and Cellulitis, Technical Information LPG Systems, Valence, France.
46. Perrier C, Pittet JV, Schnebert S, Perrier P, Beau P. Photographic assessment of so-called cellulite. Abstract of the 5th Congress of the International Society for Skin Imaging, Vienna, Austria, 1997.
47. Perin F, Perrier C, Pittet JC, Schnebert S, Perrier P, Beau P. Assessment of anti-cellulite treatment efficacy using the photograding of mechanically-accentuated macrorelief of thigh skin, Spincontrol, Tours, France and Parfums Christian Dior, Saint-Jean-de Braye, France, 1999 (Unpublished results).
48. Perin F, Perrier C, Pittet JC, Beau P, Schnebert S, Perrier P. Assessment of skin improvement treatment efficacy using photograding of mechanically-accentuated macrorelief of thigh skin. Int J Cosmet Sci 2000; 22:147–156.
49. Collis N, Elliot LA, Sharpe C, Sharpe DT. Cellulite treatment: a myth or reality: a prospective randomized controlled trial of two therapies, endermologie and aminophylline creazm. Plast Reconstr Surg 1999; 104:1110–1114.
50. Lis-Balchin M. Parallel placebo controlled clinical study of a mixture of herbs sold as a remedy for cellulite. Phytother Res 1999; 13:627–629.
51. Sainio EL, Rantanen T, Kanerva L. Ingredients and safety of cellulite creams. Eur J Dermatol 2000; 10:596–603.
52. D-stock Slimming Gel, technical information and press map, Laboratoire Vichy, France, 2004, www.vichy.com.
53. Elancyl Liporeducing concentrate, technical information and press map, Laboratoires Galenic, Pierre Fabre, France, 2004, www.pierre-fabre.com.
54. Retinol Concentrate Body Contour, technical information and press map, Roc Jonhson&Johnson, France, 2004, www.
55. Lipofactor, technical information and press map, Sanofi-Synthelabo. France, www.Sanofi-Synthelabo.com.
56. Beelen I, Smeets K. Experimentele studie van het lokaal effect van een cosmetisch algenproduct op cellulitis door middel van niet invasieve metingen, delen I en II. Licentiate thesis, Bachelor in Physiotherapy, Vrije Universiteit Brussel, Brussels, Belgium, 1991.
57. Ghislain N, Vandermeulen S. Objectieve evaluatie van een lokale anti-cellulitis massage behandeling door middel van contact thermografie, delen I en II. Licentiate thesis, Bachelor in Physiotherapy, Vrije Universiteit Brussel, Brussels, Belgium, 1996.
58. Debremaeker N. Experimentele studie van een anti-cellulitis lymfedrainage behandeling. Licentiate thesis, Bachelor in Physiotherapy, Vrije Universiteit Brussel, Brussels, Belgium, 1996.
59. Piérard-Franchimont C, Piérard GE, Henry F, Vroome V, Cauwenbergh G. A randomized, placebo-controlled trial of topical retinol in the treatment of cellulite. Am J Clin dermatol 2000; 1:369–374.

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Skin Cleansing Bars

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INTRODUCTION

Although the origin of soap is not very clear, it is widely accepted that some form of primitive soap making methods existed several thousand years ago dating to as far back as 2000 BC. For many centuries, soaps were made by heating a mixture of animal fats (tallow) with lye, a basic solution obtained from wood ashes (1). Until the late 18th century, soap was considered a luxury item available only to the royalty and the social upper class. Today, soaps are produced using a variety of much more refined processes and different fats and oils, resulting in finished products that deliver consumer-relevant performance benefits with desirable aesthetics (1). In this section, the authors will discuss the chemical and physical properties of commercial soap bars with a focus on skin cleansing, the raw materials needed, the manufacturing and process requirements, and the final finished product performance evaluations.

WHAT IS SOAP?

Soap, in a general term, is defined as an alkali salt of a long-chain fatty acid. When a fat or oil is saponified, the sodium or potassium salt formed from the long-chain fatty acids is called a soap. The term "soap" refers to a group of neutralized long-chain carboxylic acids which result from two primary ingredients: an alkali and a triglyceride (fat or oil). The chain length of the aliphatic group is typically between 7 and 21 carbons with one carboxylate carbon, yielding a molecule containing 8 to 22 carbons. The cation associated with the carboxylate head group is generally comprised of sodium, potassium, or magnesium.

Soap cleans by altering the surface tension of the water and emulsifying and suspending soils that are to be rinsed away. The two ends of soap have different polarities where the long carbon chain end is nonpolar and hydrophobic whereas the carboxylate salt end is ionic and hydrophilic. When a soap is used to clean grease or dirt, the nonpolar ends of the soap molecules solubilize nonpolar fats and oils that accompany dirt. The water-loving (hydrophilic) salt ends of the soap molecules extend outside where they can be solubilized in water. The soap molecules coat the oil or grease, forming clusters called micelles. The hydrophilic ends of the soap

molecules provide polarity to the micelles, and thus emulsifying them in water. As a result, small globules of oil and fat coated with soap molecules are pulled into the water layer and can be rinsed away.

SOAP RAW MATERIALS

Fats and Oils

The naturally occurring fats and oils used in soap making are glycerides with three fatty acid groups randomly esterified with glycerol (trihydroxy alcohol). Fats and oils typically are comprised of both saturated and unsaturated fatty acid molecules containing between 7 and 21 carbons randomly distributed on the glycerol backbone. Overall, the reaction of caustic (lye) with triglycerides yields glycerin and soap in a reaction known as saponification. This is the most widely used soap making process. The second major soap making process is the neutralization of fatty acids with an alkali. Fats and oils are hydrolyzed (split) with a high-pressure steam to yield crude fatty acids and glycerin. The fatty acids are then purified by distillation and neutralized with an alkali to produce soap and water (neat soap) (2–7).

The selection of the appropriate starting fats and oils forming the base composition of soap is key to its quality and performance. The properties of the resulting soap are determined in large part by the quality and composition of the component fatty acids in the starting mixture. Fats and oils are treated as commodities in the open market, and the number of fats and oils suitable for commercial soap making is limited. Among the fats and oils used throughout the world, beef and sheep tallow are common fats, and oils from coconut, palm, soy, rice bran, and babassu are frequently used oils. Soap compositions containing fractions of oils such as palm stearin and other oils with hydrogenation or other upgrading techniques are also in the formulators' arsenal for selection. In the United States, most mass market toilet soaps are made from beef tallow and coconut oil or palm kernel oil. However, there has been an increase in the number of vegetable based soaps, particularly in specialty stores. Some of the common fats and oils used in commercial soap making are listed below (Table 1).

Tallow

Tallow, which is the principal animal fat in soap making, is obtained from the meat processing industry as a result of rendering the body fat from beef and in some cases sheep (8). The properties of these and other fats are dependent on the constituent fatty acids. Tallow from different sources may vary considerably in color (both initial and after bleaching), titer (solidification point of the fatty acids), free fatty acid content, saponification value (alkali required for saponification) and iodine value (measure of unsaturation), and odor. Tallow is composed of mostly long-chain saturated and unsaturated fatty acids—mostly C_{16} (palmitic, 28%), C_{18} (stearic, 18%), and $C_{18:1}$ (oleic, 44%)—providing hardness and thick and creamy long-lasting lather (Table 1).

Palm Oil

Palm oil, which often serves as a substitute for tallow, is obtained from the fruit of the palm tree. It is composed of mostly long-chain-length fatty acids—such as C_{16} (palmitic, 44%) and $C_{18:1}$ (oleic, 41%)—providing properties and compositions similar to tallow (Table 1).

Table 1 Fatty Acid Distribution and Characteristics of Soap Bases

Fatty acid distribution	Tallow	Coconut	Palm oil	Palm stearin	Palm kernel
Caprylic (C-8)		7.4			
Capric (C-10)		6.3			
Lauric (C-12)		47.8		49.7	
Myristic (C-14)	2.8	18.3	1.1	1.5	15.7
Palmitic (C-16)	27.8	9.0	43.5	56.5	8.0
Palmitoleic (C-16:1)	3.8			0.2	
Stearic (C-18)	17.9	2.8	4.2	4.8	2.4
Oleic (C-18:1)	43.9	6.3	40.8	29.6	15.2
Linoleic (C-18:2)	2.3	2.0	10.2	7.2	1.5
Linolenic (C-18:3)				0.1	
Characteristics					
IV	38–48	8–10	50–55	32–40	14–22
Titer, °C	40	26	40	49–51	25
SV	193–200	251–263	196–209	196–209	240–250
FA Ave mw	272	213	270	268	221

Abbreviations: IV, iodine value; SV: saponification value.

Palm Stearin

Like palm oil or tallow, palm stearin is composed of mostly long-chain-length fatty acids but with a lower degree of saturation. Palm stearin is produced by splitting palm oil into palm olein (which is used in foods) and palm stearin. Palm stearin provides properties similar to tallow.

Coconut Oil

Coconut oil is one of the most important vegetable oils used in soap making. Coconut oil is composed of mostly C_{12} (lauric, 48%) and C_{14} (myristic, 18%) fatty acids, reducing hardness and providing solubility and lather with large bubbles (Table 1). Coconut oil is obtained from the dried fruit, copra, of the coconut palm tree.

Palm Kernel Oil

Palm kernel oil is obtained from the center of the nuts of the palm tree and is composed of mostly shorter chain-length fatty acids—such as C_{12} (lauric, 50%) and C_{14} (myristic, 16%)—providing properties and composition similar to coconut oil (Table 1). Palm kernel oil is commonly used as a substitute to coconut oil in the soap making process.

While the five oils listed above are the most commonly used fats and oils in the soap making industry, other sources such as lard (hog fat), babassu oil, rice bran oil, palm kernel olein, and soybean oil are also used throughout the world.

SOAP PHASES

The physicochemical nature of soap has been shown to be critical for the in-use properties. It is generally accepted that four distinct sodium soap crystalline phases exist. These soap phases are referred to as the beta, delta, omega, and the liquid

crystalline phases. Today, X-ray diffraction (XRD) is considered the simplest and most reliable method for distinguishing the different phases. The phases designate the lattice spacing between the hydrocarbon chains and are predictive of the physical properties such as lather, slough, use-up rate, and even the degree of translucency of a soap bar (9). The large crystals of the omega phase with the liquid phase are formed when neat soap is cooled down (after the drying step). Beta phase conversion in soap bars depends on several factors, including temperature, type of surfactant, moisture level, number of millings, etc. Delta phase is formed by the recrystallization of saturated higher chain soaps under specific temperature conditions and moisture level. Ferguson et al. first linked XRD measurements to the physical properties and characteristics of soap bars as finished product. For instance, delta phase provides low slough and low wear rate, whereas beta phase has good lather, low wear rate, and high slough (9).

SOAP BASE COMPOSITION AND PERFORMANCE

Product performance profiles are critically dependent on the base composition selection. The selection of fats and oils used in soap making as well as their ratio is determined by the balance of product performance, cost, and manufacturability. For example, in terms of performance, the relatively less soluble tallow provides for bar hardness, a dense, stable, small bubbled lather while the more soluble palm kernel oil or coconut oil provides an easily generated lather consisting of large bubbles. In addition to bar hardness, color, odor, and lather considerations, the formulator must be concerned with the solubility of the soap as it impacts on the use-up and sloughing of the final product. A typical soap bar in the United States utilizes a tallow and coconut oil or palm kernel oil base, and the ratio of the two components determines lather attributes such as speed, quantity, and richness. An increase of all of these attributes occurs with the increasing proportions of the coconut oil but the higher proportion of coconut oil also results in an increasing degree of irritation to the skin due to the high short chain-length fatty acid composition and an increase in cost. Furthermore, the behavior of the base can be determined not only by the fatty acid chain but also by the cation by which it is neutralized. The cation can also have a significant influence on the solubility and mildness properties of the base. For example, a sodium soap would be harder than a potassium soap of the same carbon chain length (1).

ADDITIVES

Soap manufacturers have developed a variety of formulation approaches to deliver products that better meet the consumer needs of today. Even though the base soap composition has not changed, the consumer needs are met by the inclusion of various additives. As with any other product, the stability (physical and chemical state) of the soap base-additive or even additive-additive mix must be considered during the formulation. There are a variety of additives that are formulated into soap bars to provide additional consumer benefits and/or to modify the performance and esthetics of the final product. A complete list of functional additives can be found in the CTFA Cosmetic Ingredient Handbook (10).

Fragrance

Fragrance is by far the most important additive for the consumer acceptance of a personal cleansing product. Even though the primary purpose of the selection of a fragrance is to target a specific user group, it is used to mask the characteristic base odor associated with the fatty acids. Fragrances are compounded from several components including carboxylic acids, esters, aldehydes, ketones, and glycols where the selection of the components could adversely affect the stability and/or the processability of the final product. For instance, fragrances with solvents such as dipropylene glycol (glycol) and diethylphthlate (ester) tend to soften and cloud translucent soap bars (2). The raw-material manufacturer's ability to provide cleaner base with significantly less base odor has greatly improved in the past two decades and thus allowing the soap manufacturer to use less fragrance in the final product or even, in some cases, provide products that are fragrance free. Fragrances are also known to alter the mildness properties of soap bars. For example, a soap bar that targets consumers with sensitive skin has enough fragrance to mask the base odor of the fatty acid while providing some soft perfume that reinforces their mildness properties. The fragrance levels in the soap bar typically range from 0.3% (sensitive skin) to 1.7% (deodorant soaps). Long term aging studies are always necessary in order to assess the stability of the fragrance in the soap base and its continued ability to mask the base odor.

Free Fatty Acid or Superfatting

Traditional soap bars are alkaline in nature with a pH of around 10. A manufacturing process with excess fatty acid beyond what is needed by the reaction yields a final product with free fatty acid, also known as "superfatted" soap. Conversely, a process with caustic in excess of what is needed by the reaction yields a base soap with a slight excess of free caustic. Excess caustic can be neutralized by the addition of excess free fatty acids such as coconut, palm kernel, or stearic acid or by postaddition of weak acids such as citric or phosphoric acid. Superfatting enhances the lather profile of the soap bar, eliminates free alkali (lowers the pH) and can provide some improvement of skin mildness attributes (1).

Glycerin

Glycerin is a common ingredient formulated into soap bars which dates back to the ancient times. As discussed previously, it is the by-product of saponification and thus has always been present in soaps in varying levels (2). Glycerin is a well-known humectant and an attractive additive for skin care benefits. Glycerin, along with other humectants such as sorbitol, is commonly used in significant levels in translucent or transparent soap. The presence of humectants can alter the rinseability of the soap bar, thus modifying the consumer perception of the product as a clean rinsing product.

Colorants and Pigments

The visual appearance of a soap bar is known to influence the consumer acceptance of the product. Due to color differences of some of the base compositions, it is common for most manufacturers to alter the appearance of the final product. This is mostly accomplished by the addition of colorants and opacifying agents. Some of

the common additives utilized to alter the appearance of a soap bar include; food and/or cosmetic grade dyes and pigments, lakes, and opacifiers such as titanium dioxide and zinc oxide (10). Additionally, to enhance the aesthetic appeal of a bar, mica or colored micas may be added to produce a pearlized effect.

Preservatives

Soap bases with high proportions of unsaturated fatty acids (e.g., oleic, linoleic, and linolenic) (11,12) and the presence of certain soap additives, such as fragrance, tend to be susceptible to undesirable atmospheric oxidative changes. Therefore, preservatives (chelating agents and antioxidants) are necessary to prevent such oxidation from occurring. Some commonly used chelating agents (for trace metals present) in soap bars include; ethylenediaminetetraacetate (EDTA), diethylenetriamine pentaacetate (DTPA, also known as pentasodium pentate), sodium etidronate or ethane-1-hydroxy-1, 1-diphosphonic acid (EHDP) (13), and citric acid. The most commonly used antioxidants in conjunction with chelating agents in soap bars is butylatedhydroxytoluene (BHT) and recently the addition of tetradibutyl pentaerithrityl hydroxyhydrocinnamate (14). Both of these antioxidants are soluble in fragrances.

Skin Conditioners

As previously mentioned, consumer demand for products that not only cleanse the skin but also provide skin mildness and moisturization benefits is constantly changing. Therefore, it is common for manufacturers to add ingredients that are known to provide such benefits. We previously discussed two of the most commonly used additives, free fatty acid and glycerin. Other additives that are commonly used in soap bars include vitamin E, aloe, oils (jojoba oil, sunflower seed oil, olive oil, almond oil, etc.) lanolin, glyceryl stearate, isopropyl esters, sodium cetearyl sulfate, cetyl esters, petrolatum, silicones, beeswax, ceresin, cocoa butter, mineral oil, and polyethyleneoxideglycol-12 to name a few (2,10).

Antimicrobial Agents

Soap bars are very effective in removing microbial flora that are known to cause skin infections, pimples, and malodor during the washing/bathing process. The addition of antimicrobial actives to a soap bar extends the above benefit for a longer period of time, mainly between washing and bathing. Due to safety concerns of the different actives used in soap bars, the number of antimicrobial agents used in soap bars has decreased from several in the 1970s to only three today. Trichlorocarbanilide (TCC), trichlorodiphenylhydroxyether (triclosan), and *para*-chloro *m*-xylenol (PCMX) are commonly used in soap bars today. The selection of which active to use in different products is based on the claims or product positioning, efficacy, and cost of the final product. TCC is effective mostly against gram-positive bacteria whereas triclosan and PCMX have been shown to be effective against both gram-positive and gram-negative bacteria. The use level of these actives is dependent on the claims associated with the final products and government regulations. For instance, in the United States the maximum use level allowed for triclosan and TCC is 1.0% and 1.5%, respectively.

Synthetic Surfactants

The formulation of soap bars has become more complex because of the ever-increasing consumer demand of products that not only provide cleansing properties but also skin conditioning/moisturization benefits. Synthetic surfactants are often used to enhance the performance of soap bars resulting in improved skin feel, less irritation, and improved quality and quantity of lather. Synthetic surfactants are used at levels ranging from 5% (low-level combar) to 80% (Syndet) which will be discussed in detail in the later sections of this chapter. The selection of a good synthetic surfactant is critical for the performance of the final product. Some examples of commonly used synthetic surfactants in soap bars include sodium cocoyl isethionate, alkyl ether sulfonate, and cocomonoglyceride sulfates (15,16).

Other Additives

Several other additives not mentioned in the above sections are currently being used in soap bars. Some examples include processing aids, binders (gums and resins), fillers (dextrin, salt, talc, etc., for bar hardness), exfoliants, antiacne, and anti-irritants.

SOAP MAKING/MANUFACTURING PROCESS

The process of making soap begins with the receipt of fats and oils and ends with a soap bar pressed into a desired shape and packaged for sale. There are many unit operations involved in soap making, from distillation (glycerin recovery) to drying and pneumatic conveying. The soap making process involves the production of neat soap (wet soap) from fats and oils, followed by drying and finishing steps in order to complete the process. There are two basic routes of commercial soap making (17).

Neutral Fat/Oil Route or Saponification

In the saponification process, neutral fats and oils (tallow, palm oil, palm stearin, coconut oil, palm kernel oil) are first upgraded to remove particulate dirt, proteinaceious materials, and other odor and color bodies and then reacted with caustic (NaOH or KOH) yielding neat soap and free glycerin (Fig. 1A). Saponification can be done in either a batch (kettle) process or a continuous process (1,2).

Fat Splitting/Fatty Acid Route

In this method of soap production, the fats and oils (triglycerides) are hydrolyzed with high-pressure steam (fat splitting) to produce fatty acids and glycerin. The fatty acids are then purified by distillation and neutralized with an alkali to produce soap (neat soap) and water (Fig. 1B) (1,2).

Drying and Finishing

"Neat soap" produced by one of the processes outlined above contains over 30% moisture. The soap needs to be dried, typically by vacuum drying, to a final moisture level of 8% to 16% for the final finishing steps. Once the neat soap is dried to soap pellets (soap chips), it is transferred into mixers (amalgamators) and the minor

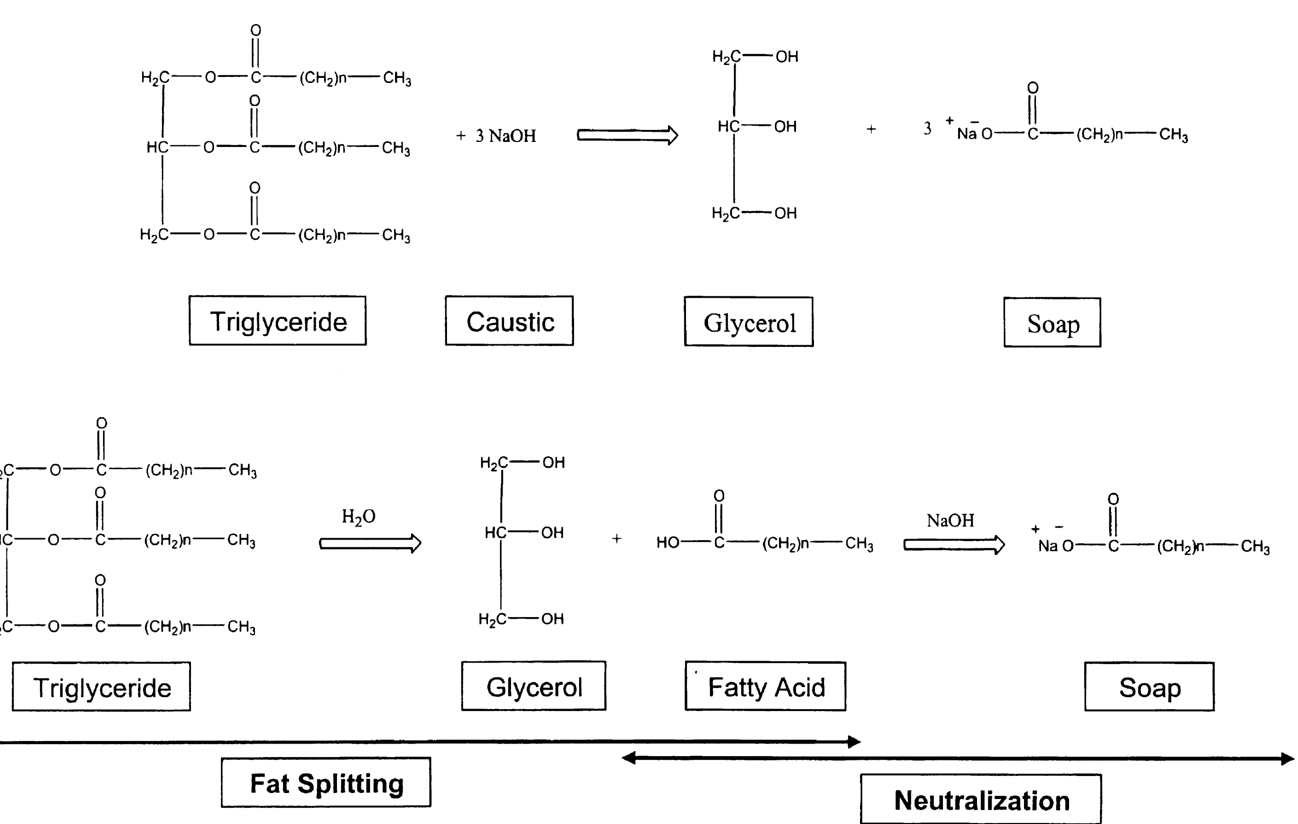

Figure 1 (**A**) Saponification of triglycerides. (**B**) Fat splitting (glycerol as a by-product) and fatty acid neutralization reaction.

additives such as fragrance, color, preservative, antibacterial agents, and other formula additives are added. These additives are mixed with the soap pellets, refined, and extruded into a long continuous billet. The billet is cut and pressed into the desired shape and packaged (Fig. 2). Some soaps are cast instead of cut into shapes. In this case the molten soap is poured into a mold of desired shape (1,2,18).

FORMULATIONS: REGULAR AND TRANSLUCENT SOAPS, COMBARS, SYNDETS, AND SPECIALTY SOAPS

Soap bars are formulated with a combination of longer carbon chain length fats (tallow, palm oil, palm stearin) and shorter carbon chain length oils (palm kernel oil, coconut oil). Common nomenclature for bar soaps is the ratio of the longer carbon chain length fat to the shorter carbon chain length oil. For example, a bar containing 80% tallow and 20% coconut oil as its soap base would be referred to as an "80/20" soap bar. Ratios used typically range from 90/10 to 60/40. The higher coconut or palm kernel oil levels in a soap bar not only leads to a higher lathering profile (1) but also to a higher use-up rate due, owing to the high portion of the shorter carbon chain length base. The lather profile can also be improved by the presence of unsaturates (C16-1 or C18-1) from tallow and palm oils. Regular soap bars generally contain approximately 75% to 85% soap. The remainder of the soap bar is made up of water, glycerin, salt, fragrance, and other additives that enhance its aesthetics and performance.

Soap bars frequently contain free fatty acids to ameliorate the harshness of the soap and improve the sensory profiles of the products (see section Soap Phases of this chapter). Superfat levels in soaps typically range between 1% and 7%.

Formulation of soap bars has become increasingly complex. As soaps have become more readily available to consumers, the demands on the product performance have increased. Consumer expectations have increased beyond basic cleaning to improved mildness, lathering, deodorant protection, antibacterial protection, and interesting product esthetics and packaging (2). Bars produced with synthetic surfactants have improved lathering and rinsing profiles especially in hard water. At higher levels of synthetic surfactants, the bars exhibit superior mildness versus traditional soap. Examples of synthetic bars (syndets) are Dove®, Caress®, and Oil of Olay®. The raw materials for syndets are more expensive than traditional soap, leading to a higher finished product cost versus soaps.

Combination bars, "combars," are designed to incorporate the most desirable properties of plain soap bars and synthetic cleansing bars (syndets) (Fig. 3). In general, their advantages over conventional soaps are superior rinseability and latherability in hard water. Examples of combars include Zest® and Lever 2000®. Another route to enhance product performance is the incorporation of high levels of fatty acids and skin conditioners to improve the mildness. An example of this is Irish Spring Aloe®.

The benefits of conventional soap bars are good lathering, thorough cleaning, and low cost compared to bars containing synthetic surfactants (Fig. 3). Some of the shortcomings are: (1) performance-dependence on water hardness conditions due owing to its reactions with calcium and magnesium salts in hard water causing difficulty to rinse "soap scum" and (2) lack of clinical and consumer-perceived mildness benefits. Some people can experience irritation and excessive dryness, especially during periods of low temperature and humidity such as in winter.

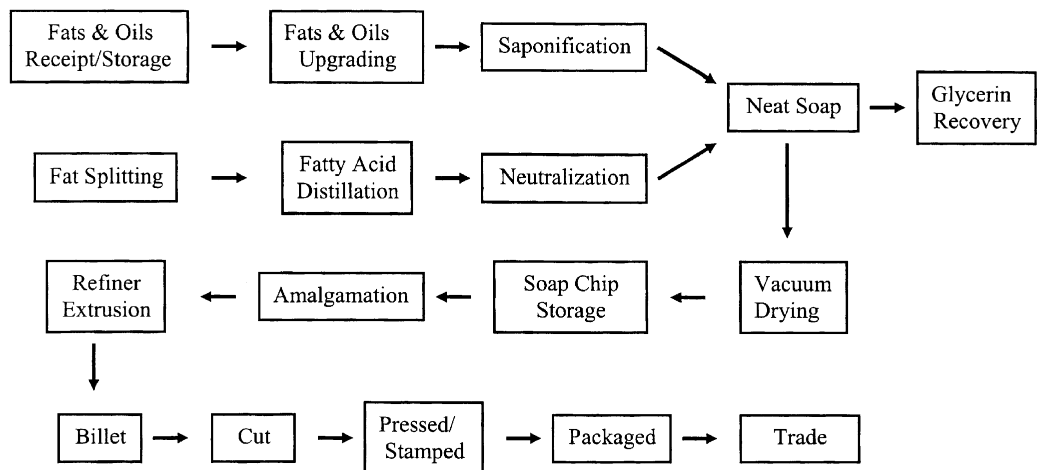

Figure 2 Flow chart of the soap manufacturing steps.

Soap	Soap Superfat	Combar	Syndet
Cleansing Lather	Cleansing Better Lather Potential Skin Feel Potential Mildness		Cleaning Higher Lather High Skin Feel Mildness
Hard Water- Soap Scum	Hard Water- Rinseable		Hard Water- Rinseable
Low Cost	Medium Cost		Very High Cost

Figure 3 Skin cleansing bar formulations and attributes.

Synthetic cleansing bars (syndets) generally contain only low levels or no soap. Instead, syndets are comprised of synthetic surfactants (between 20% and 80% of the total bar composition), high concentrations of emollients and conditioners, and some fillers and binders (19). They tend to cleanse and lather well in soft or hard water, and they are unaffected by calcium or magnesium salts which results in better rinsing properties from skin and hard surfaces. Also, because of the presence of high levels of skin moisturizers and conditioners, syndets impart a differentiated skin feel.

While all synthetic surfactants overcome the hard water deficiencies of soap, not all of them are suitable for use in cleansing bars because their effects on skin can be markedly different from soap. Selection criteria that one needs to follow choose a synthetic surfactant, and to use it in soap bars are quite strenuous. In addition to being mild, the surfactant must possess acceptable properties such as surface activity, physical and chemical stability, good odor and color, acceptable safety profile, processability into soap bars, quick lather, clean skin feel, etc. (1). Common anionic synthetic surfactants used in syndets and combars include sodium cocoylisethionate, alkylglycerylether sulfonate, and alkylsulfate. Amphoteric surfactants such as cocamidolpropylbetaine or nonionic surfactants are also sometimes used at low levels.

Translucent and transparent soaps incorporate high levels of solubilizers, which tend to control the crystal size and structure, thus allowing the transmittance of light through the product. Examples of solvents added to translucent and transparent soaps include glycerin, sorbitol, triethanolamine, and other sugars (20–22). These specialty soap products frequently have altered lathering, rinsing, and use-up rate characteristics due, owing to the high level of solubilizers in the finished product.

Other specialty soaps include the addition of unique aesthetics (marbleized and striated) as in Irish Spring, pearlized effects from mica, or the addition of specialty abrasives (pumice, seaweed, polyethylene beads, Lufa, etc,), and other botanical or natural ingredients.

BAR SOAP PERFORMANCE EVALUATIONS

Soap bars are evaluated for several characteristics to ensure that they meet consumer needs and expectations.

Lather

The amount of lather, how rapidly a product lathers, and the quality of lather can be judged by a trained panel. This trained panel rates the product on lather quantity, quality, and quickness by rating it on a numerical scale. Typically panelists are trained to rotate a soap bar a fixed number of times and evaluate it for attributes versus benchmark products. This is most useful in the analysis and comparison of formulation similarities and differences and also competitive products. Variables affecting lather performance of a product include water temperature, water hardness, bar shape and size, and method of washing. Trained panelists need to be trained and validated on a regular basis to ensure consistency of their evaluations.

Lab methods of lather evaluation include the Ross-Miles foam height test. In the Ross-Miles test, a dilute soap solution is poured from a fixed height onto the surface of the same soap solution. The foam height and stability are measured. However, results from this type of test can be misleading because the bar shape and solubility can affect the lather performance in use (1,2,25).

Wear Rate/Use-up

The measurement of how long a bar lasts under normal use conditions is an important attribute for consumer perceived value. The use-up rate is measured by first weighing the soap bar and then washing the bar for a set number and length of times (for example, 25 washings for 10 seconds each). The bar is then dried and weighed again and the use-up or wear rate is reported as the percent weight loss. Soap bar shape and size impact the reported use-up rate. The use-up rate measurement must be controlled for water hardness and temperature. For formulation comparison purposes, it is best to compare soap bars with similar sizes and shapes. Bars can be shaved to the same sizes and shapes in order for the measurement to reflect the true formula influence. To compare how bars will perform in the hands of consumers, actual commercial sizes and shapes should be used (1,2).

Slough/Mush

Slough or mush is the undesirable soft part of the bar that results from the hydration of a soap bar as it sits in a wet soap dish. Slough is measured by placing a preweighed bar in a high humidity chamber for a fixed period of time, then removing the soft part of the bar and allowing the soap bar to dry. The weights taken before and after determine the slough or mush measured as the percent weight loss. Syndet bars tend to have high slough relative to regular soaps. High humidity conditions exaggerate typical home usage conditions, but help differentiate products and formulations. Slough can also be run at room temperature. Commercial soap bar shapes can be selected by manufacturers to minimize the formation of slough or mush in use conditions (1).

Cracking

Cracking is the splitting of a bar along the side seams or at any part in the bar during use. Cracking of a soap bar in use conditions is a perceived as a negative by consumers. Cracking is evaluated by partially submerging bars in water of fixed hardness and temperature for a set period of time. The bars are then dried and evaluated for

cracking after one to two days. Ideally, there should be no cracks present in the soap bars (1).

Hardness

Bar hardness is a mechanical measure of how resistant the bar is to a physical pressure. Bar hardness can be mechanically measured in finishing trials for machineability as well as during routine lab evaluations. Bars that are too soft may be difficult to extrude on the finishing line and package without significant surface defects.

Bar Feel and Sandiness

Bar soaps are typically evaluated for dry specks and drag. Specks of dry soap (insoluble soap) can occur during the manufacture of the base soap or syndet or from the additives in the soap bar. These specks show up as distinct bumps on the surface of the bar. The bar is washed under controlled water conditions with cooler water bringing out more obvious dry specks. The bar is both evaluated during wash and after drying for feel and appearance and rated versus standard quality bars (1,24).

Stability

All bar soaps should complete studies to determine the stability of the color, and odor active materials. The stability should be completed in final packaging. For regulated products in the United States (those containing antibacterial claims), the FDA provides guidelines for product stability testing. Specific requirements for other regions are governed by local and regional regulatory authorities.

Sensory Skin Evaluations

Skin feel and lather are important attributes for consumers. Various skin feel attributes are evaluated by a trained panel of experts. These groups of panelists are trained to evaluate small (or large) differences in products focusing on a set of defined attributes. Products are usually compared with a reference product. Examples of attributes evaluated by a trained panel for skin feel include, time to rinse, skin slip, tightness of skin after drying, and smoothness of skin. It can be difficult to characterize small differences in skin feel from bar soap chemistry. Expert panel testing may be augmented by home use testing with consumers to evaluate product performance differences under normal use conditions. To differentiate product chemistries in home use testing, products must be matched in appearance and odor.

Clinical Evaluations

Clinical evaluations of soap products are used to determine how effective the products are on certain attributes, primarily mildness/irritation, skin dryness/tightness, antibacterial efficacy, and deodorancy. There are several methods of measuring the clinical attributes of a soap bar ranging from trained panels to biophysical instrumentation (1,2,23).

REFERENCES

1. Spitz L, ed. Soap Technology for the 1990s. Champaign, Illinois: American Oil Chemists Society, 1990.
2. Spitz L, ed. Soaps and Detergents: A Theoretical and Practical Review. Champaign, Illinois: American Oil Chemists Society, 1996.
3. Woolatt E. The Manufacture of Soaps, Other Detergents and Glycerin. New York: Halstead, 1985.
4. Thomsenn EG, Kemp CR. Modern Soap Making. New York: MacNair-Dorland, 1937.
5. Joshi D. U.S. Patent 4,493,786 (1985).
6. Jungerman E, Hassapis T, Scott R, Wortzman M. U.S. Patent 4,758,370 (1988).
7. Johnson RW, Fritz E, eds. Fatty Acids in Industry: Process, Properties, Derivatives, Applications. New York: Marcel Dekker, 1989.
8. Patterson HBW. Bleaching and Purifying Fats and Oils: Theory and Practice. Champaign, Illinois: American Oil Chemists Society, 1993.
9. Ferguson RH, Rosevear FB, Stillman RC. Ind Eng Chem 1943; 35:1005.
10. Wenninger JA, McEwen GN. CTFA International Cosmetic Ingredient Dictionary and Handbook. 7th ed. Washington, DC: The Cosmetic, Toiletry and Fragrance Association, 1997.
11. Zaidman B, Kisilev A, Sasson Y, Garti N. Ibid. 1988; 65:611.
12. Rojas-Romero AJ, Morton ID. J Sci Fd Agric 1977; 28:916.
13. U.S. Patent 3,511,783 (1970).
14. Payne R, Hwang A, Subramanyam R. U.S. Patent 5,843,876 (1998).
15. Blake-Haskins JC, Scala D, Rhein LD, Robbins CR. J Soc Cosmet Chem 1986; 37: 199–210.
16. Hollstein M, Spitz L. J Am Oil Chem Soc 1982:442.
17. Soaps and Detergents Handbook. 2nd ed. Soap and Detergent Association, 1994.
18. Krawczyk T. Soap bars. Inform, 1996; 478–486.
19. Milwidsky B. Syndet bars. Happi, 1985: 58–70.
20. U.S. Patent 2,970,116.
21. Toma K, Hassapis TJ. U.S. Patent 3,864,272 (1975).
22. Wood-Rethwill JC, Jawarski RJ, Myers EG, Marshal ML. U.S. Patent 4,879,063 (1989).
23. Kajs TM, Gartsein V. Review of the instrumental assessment of skin: effects of cleansing products. J Soc Cosmet Chem 1991; 42(4):249–279.
24. Whalley GR. Quality assurance for toilet soap bars. Happi, 1991; 48–54.
25. Surfactant Science Series. Handbook of Detergents. Part A: Properties. Vol. 82. Marcel Dekker, 1999:422.

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Skin Cleansing Liquids

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INTRODUCTION

Skin cleansing liquids are products that clean and refresh the skin by removing soil or dirty materials to help keep the skin's physiological condition normal. There are residual metabolites on the skin that are unstable and reactive with oxygen or deposited molecules. These reactions happen on exposure to the sun or skin microorganisms, and form harmful materials that lead to skin trouble. Thus, cleansing is a necessary daily skincare practice, even for normal skin. Furthermore, special care must be taken for sensitive skin or atopic skin, because of its vulnerability. In such troubled types of skin, cleanliness must be attained without contributing to their susceptibility (1). There are different types of cleansing products, developed and commonly used, depending on the types of materials to be removed from the skin, or the types of conditions in which it would be used. Typical types of commercial skin cleansing products are listed in Table 1 (2). Most of the common cleansing products contain a relatively high concentration of surfactants, and is applied with water to make foam before washing off thoroughly. Good lathering is the most important feature of these products, because sensory feeling of the rich and fine foam is the key factor of repeated use by the consumers, although the amount and quality of foam are not directly related to the detergency from a physicochemical viewpoint. On the other hand, fine and thick lather serves an important function in shaving foam preparations for smooth razor application. Ease of quick rinse and after-feeling are other factors that rule the quality of skin cleansing products. Refreshed and moist feelings are typical elements that fulfill the consumers' desires, and refreshing seems more important for body wash, especially for Japanese consumers.

In terms of formulations for surfactant-type skin cleansers, soap bars have been the most traditional skin cleansers, but there are liquid-, paste-, or aerosol-type cleansers getting more popular on the market. Facial cleansing powder—a rather new and niche trend in Japan—contains enzymes to help the cleaning of protein-type deposits, because of its anhydrous formula to preserve enzyme activities.

Solvent-type is mainly used to remove oily cosmetics applied to the skin. This type is further categorized to cleansing creams, lotions, liquids, or gels. The use of

Table 1 Types of Commercial Skin Cleansing Products

Product type	Form (formula type)	Features
Surfactant-based type	Solid (soap, transparent soap, neutral soap)	Main type of cleanser: easy to use and feels good, but skin feels tight afterwards.
	Cream • paste (cleansing foam)	Special face cleanser with excellent feeling and lather. It is easy to use. Bases may be selected in the range weakly acidic to alkaline depending on the purpose.
	Liquid or viscous liquid type (cleansing gel)	Weakly acidic to alkaline. The weakly acidic base produces a weak cleanser but the alkaline base produces a strong one. The main type of cleanser for hair and body.
	Granule/powder form (cleansing powder, face cleansing powder)	Easy to use. As they contain no water, papain or other enzymes may be incorporated.
	Aerosol type (shaving foam type, after-foaming type)	There are two types—one that comes out like a shaving foam and the other as a gel which becomes a foam on use (after-foaming type). A double container is used for the after-foaming type.
Solvent-based type	Cream • paste (cleansing cream)	The emulsion type uses mainly O/W emulsion. The type in which oils are made into a gel has high cleansing power. For heavy makeup.
	Milky lotion (cleansing milk); liquid form (cleansing lotion)	O/W emulsion milky lotion. Lighter feeling after use than with cleansing cream. Easy to use. Cleansing lotion. Contains large amounts of nonionic surfactants, alcohol, and humectants. There is also a physical cleansing effect as it is wiped off with cotton. For light makeup.
	Gel (cleansing gel)	The emulsion and liquid crystal types containing a lot of oils have high cleansing power and are rinsed off. They give a light feeling after rinsing off. The water-soluble polymer gel type has low cleansing power.
	Oil (cleansing oil)	Ingredients like surfactants and alcohol are added to the oil in small amounts. Rinsed off. When rinsed off forms O/W emulsion. Soft and moist feeling after use.
Others	Pack (cleansing mask)	Peel-off mask using water-soluble polymers. Skin has strong feeling of being stretched. Removes dirt from skin surface and pores when peeled off.

Source: From Ref. 2.

makeup products, such as waterproof or nonstaining and long-lasting lipsticks, require the use of special cleansers to remove them. Facial packs with cleansing gel that claim gentleness and sufficient cleansing power have been launched in Japan.

Table 2 Main Surfactants Used for Cleansing Products

Type	Ingredients
Anionic Surfactants	Soap
	Polyoxyethylene alkyl ether sulfate
	Acylglutamate
	Acylglycinate
	Acylmethyltaurate
	Acylsurcosinate
	Acylisethionate Ampoteric surfactants
	Alkyl dimethylaminoacetic acid betaine
	Alkyl amidopropyl dimethylaminoacetic acid betaine
Nonionic surfactants	POE alkyl ether
	POE glycerol fatty acid ester
	POE-POP block copolymer

SURFACTANT-TYPE SKIN CLEANSERS

Main surfactants used for surfactant-type skin cleansers are listed in Table 2. Soaps are used as a primary surfactant for solid bar cleansers and paste-type cleansers. Sodium soaps are commonly used for solid bars, and potassium soaps are mainly for paste-type cleansers or shaving foams. Opaque soft bar is made from triethanolamine soap, used as gentle facial cleanser. Soaps have excellent lathering properties and superior detergency, but some deposit in hard water and cause skin tightness. Additional surfactants are combined with soap, in order to improve tightness and give better mildness. Alkylethersulfate, acylisethionate, acylglutamate, acylmethyltaurate, and acylglycinate are commonly combined as a secondary or tertiary surfactant with soap. Acylglutamate has a unique feature as a weak acidic surfactant, pH similar to that of the skin, and is thus often used as a primary surfactant to give superb mildness for different formulation types.

As for their physicochemical nature, surfactants not only remove soils but also tend to strip useful substances from the skin. Thus, excessive solubilization and stripping of skin lipids and natural moisturizing factors (NMF) must be avoided, otherwise destruction of skin-barrier functions would happen. The composition of skin-surface lipids is listed in Table 3 (3) and composition of constitutive lipids in the stratum corneum is shown in Table 4 (4). Detergency of surfactant should be good enough

Table 3 Composition of Human Skin Surface Lipids

Lipid	Average amount (wt%)	Range (wt%)
Triglycerides	41.0	19.5–49.4
Diglycerides	2.2	2.3–4.3
Fatty acids	16.4	7.9–13.9
Squalene	12.0	10.1–13.9
Wax esters	25.0	22.6–29.5
Cholesterol	1.4	1.2–2.3
Cholesterol esters	2.1	1.5–2.6

Source: From Ref. 3.

Table 4 Composition of Constitutive Lipids in the Stratum Corneum

Lipid	Wt%
Cholesterol esters	1.7
Triglycerides	2.8
Fatty acids	13.1
Cholesterol	26.0
Ceramides	45.8
Glucosylceramides	1.0
Cholesteryl sulfate	3.9
Unidentified	5.7

Source: From Ref. 4.

to remote surface lipid, but not to strip minimally constitutive lipids, which are key components of skin-barrier function. Such selective detergency is found for several surfactants, and acylaminoacids such as acylglutamate or acylmethyltaurate are relatively better in this regard than soap (5,6). Composition of NMF is shown in the reference (7). Acylglutamate showed less stripping of NMF than soap (8). Changes of skin pH are dependent on the type of surfactant used too. As shown in Figures 1 and 2, the water-holding capacity and skin pH was not affected much by repeated wash with acylglutamate, while soap changed these two properties seriously.

Formulations are designed to fit a specific concept, which are the product's aim, along with the general requirement as a skin cleanser, such as the detergency, feeling, viscosity, stability, safety, and manageability or easiness of use, which are sometimes difficult to fulfill all at once. The consumers' desire for a natural product requires not only that the ingredients used are natural, but must also have a natural-looking appearance or look transparent. Such requirements cause further difficulties for the formulation work (9).

Liquid-type skin cleansers have been developed mainly for facial use, and diversified further to paste-type or gel-type formulations. Liquid-type body wash was developed first in Japan, and spread widely to western markets with a rapid growth that it even replaced a significant share of the soap bar market. This is because of their friendliness of use and added values such as natural and mildness concepts, among other reasons.

Following are the typical formulas of surfactant-type skin cleansers, with their characteristics described: Formula 1–7 .

Table 5 Analysis of Commercial Paste-Type Facial Cleansers

Sample	Distribution of fatty acid (wt%)				Total fatty acid (wt%)
	C12	C14	C16	C18	
Sample A	5.9	16.8	1.4	6.4	30.5
Sample B	10.9	4.7	9.6	8.5	33.7
Sample C	0.0	15.0	6.9	4.0	25.9
Sample D	5.8	6.4	2.2	3.6	18.0
Sample E	4.9	13.3	3.5	5.8	27.5
Sample F	1.2	23.1	3.9	5.6	33.8

Skin Cleansing Liquids

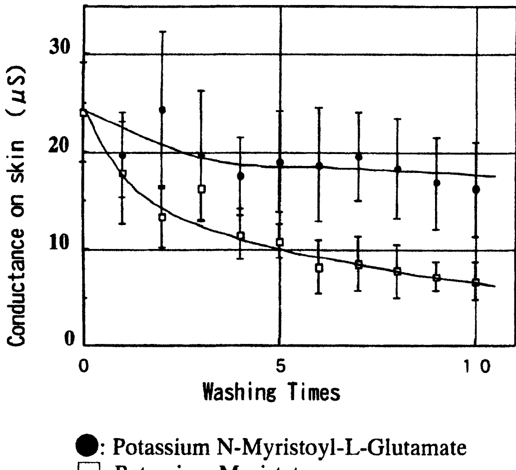

Figure 1 Effect of surfactant on the moisture content of the skin. Forearms were washed every 20 minutes with 5 mL of surfactant solution (10%) and skin surface conductance was measured by surface hygrometer (Skicon 200; IBS Japan, at 25°C, 40 RH%, $n = 6$) as indicator of the moisture of the skin.

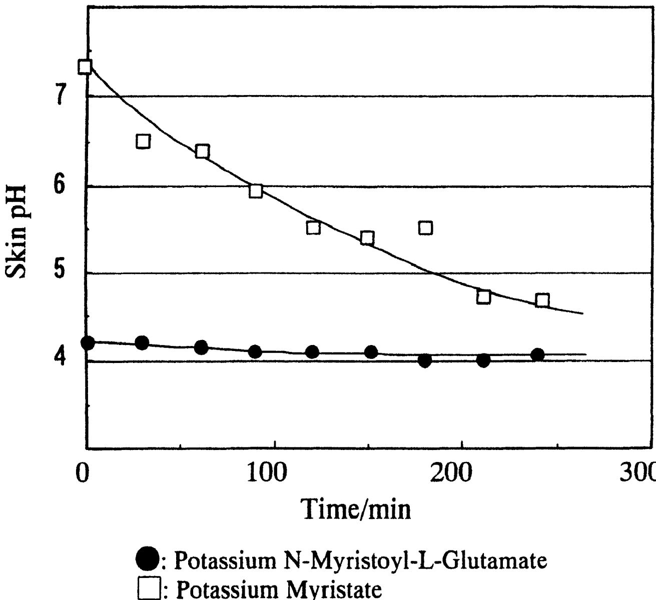

Figure 2 Effect of surfactants on the pH of human skin. Forearms were washed with 5 mL of surfactant solution (10%) and after that pH of the skin was measured every 20 minutes at 25°C, 40 RH%, $n = 6$.

Formula 1 Soap-Based Liquid Facial Cleanser (Excellent Lathering and Refreshing After-Feel)

Ingredients	%
Laurie acid	2.5
Myristic acid	7.5
Palmitic acid	2.5
Laurie acid diethanolamide	2.0
Propylene glycol	8.0
Potassium hydroxide	3.6
Water	q.s. to 100
Perfume	q.s.
Preservative	q.s.
Procedure: Add all the ingredients together and heat to dissolve with stirring. Cool down to room temperature.	

Abbreviation: q.s., quantum satis (in sufficient amount).

Formula 2 Laurylethersulfate (LES)-Based Liquid Facial Cleanser (Compatible with Hard Water)

Ingredients	%
Sodium polyoxyethylene(3)lauryl ether sulfate (30%)	40.0
Sodium *N*-lauroylmethyltaurate (30%)	10.0
Coconut acid diethanolamide	3.0
Glycerin	5.0
Sodium chloride	2.0
Water	q.s. to 100
Perfume	q.s.
Preservative	q.s.
Procedure: Add all the ingredients together and heat to dissolve with stirring. Cool down to room temperature.	

Abbreviations: q.s., quantum satis (in sufficient amount).

Formula 3 Acylglutamafe-Based Liquid Facial Cleanser Weakly Acidic, Leaves Skin Moist and Supple-Feeling

Ingredients	%
Triethanolamine *N*-cocoyl-L-glutamate (30%)	30.0
Cocoyl amide propyldimethyl glycine (30%)	30.0
1.3-butylene glycol	5.0
Sodium hydroxide	0.5
Water	q.s. to 100
Perfume	q.s.
Preservative	q.s.
Procedure: Add all the ingredients together and heat to dissolve with stirring. Cool down to room temperature.	

Abbreviations: q.s., quantum satis (in sufficient amount).

Formula 4 Acylglycinate-Based Liquid Facial Cleanser (Excellent Lather and Refreshed After-Feeling Without Tightness)

Ingredients	%
Potassium cocoyl glycinate (30%)	15.0
Potassium laurate	11.0
Potassium myristate	6.0
Glycerin	3.0
Sorbitol (70%)	2.0
Ethylene glycol distearate	2.0
Hydroxypropylcellulose	0.5
Water	q.s. to 100
Perfume	q.s.
Preservative	q.s.
Procedure: Add all the ingredients together and heat to dissolve with stirring. Cool down to room temperature.	

Abbreviation: q.s., quantum satis (in sufficient amount).

SOLVENT-TYPE SKIN CLEANSERS

Solvent-type cleansers are designed to remove oily residues from cosmetics. Normally, these cleansers are applied by hand to remove oily deposits of colors or pigments from the skin, and are then wiped out with tissue or cloth. Water-oil (W/O) emulsions or simple oils work satisfactorily for this purpose, but leave the skin oily. Thus surfactant-type cleansers are quite often applied after this treatment. The widespread trend of long-lasting cosmetics requires stronger and laborious

Formula 5 Soap-Based Paste-Type Skin Cleanser (Good Foaming and Cleansing Power)

Ingredients	%
Stearic acid	10.0
Palmitic acid	11.0
Myristic acid	12.0
Laurie acid	2.0
Squalane	2.0
Potassium hydroxide	6.0
PEG 1500	10.0
Glycerin	20.0
Glycerol monostearate	2.0
POE(30)glycerol monostearate ester	2.0
Water	q.s. to 100
Perfume	q.s.
Preservative	q.s.
Procedure: Heat fatty acids, emollient, humectants, and preservative together until melted and keep at 70°C (oil phase). Dissolve the alkali in the purified water and add this to the oil phase while stirring. Keep at 70°C until the neutralization reaction is completed. In Table 5, analytical results of the fatty acid compositions for the commercial soap-based paste-type facial cleanser are shown.	

Abbreviation: q.s., quantum satis (in sufficient amount).
Source: From Ref. 10.

Formula 6 Acylglutamate-Based Paste-Type Facial Cleanser (Weakly Acidic, Moist and Supple After-Feeling)

Ingredients	%
Sodium *N*-lauroyl-L-glutamate .	35.0
Potassium laurate	5.0
Coconut acid diethanolamide	2.0
1.3-butylene glycol	10.0
Dipropylene glycol	20.0
Polyvinyl pyrolidone	0.5
Water	q.s. to 100
Perfume	q.s.
Preservative	q.s.

Procedure: Mix polyols and surfactants completely. Add other ingredients and water, then heat to dissolve. Cool to room temperature under reduced pressure with stirring.

Abbreviation: q.s., quantum satis (in sufficient amount).

cleansing with solvent-type cleansers. In order to avoid excess burden to the skin and achieve effective cleansing of oily deposits, (1) solubilization and dispersibility, and (2) washability with water are the key properties of solvent-type cleansers, while mildness is a mandatory requirement for the product. For the former need, the product should be more lipophilic, and contrary to the latter purpose, it is rather better to be hydrophilic. To overcome these contradictory tasks, there are several different formulations developed that are W/O emulsions, gels, or liquid crystals, with special selections and combinations of the oil phase and aqueous phase. The principle of these formulas is to have potent oily phase, which can easily interact and solubilize liquid deposits when applied to the skin. Thereafter, by the application of an excess amount of water, a mixture will be formed between the cleanser and the oily deposit, which will easily turn into a hydrophilic mixture (such as a W/O emulsion) (11,12).

Following are typical formulas of solvent-type skin cleansers with their characteristics described: Formula 8–11.

Formula 7 Acylglycinate-Based Paste-Type Facial Cleanser (Neutral pH, Fresh After-Feeling)

Ingredients	%
Potassium cocoyl glycinate	32.0
Potassium myristate	1.5
Behenyl alcohol	0.5
Citric acid	2.5
1.3-butylene glycol	15.0
Glycerin	17.0
Ethylene glycol distearate	2.5
Water	q.s. to 100
Perfume	q.s.
Preservative	q.s.

Procedure: Mix polyols and surfactants completely. Add other ingredients and water then heat to dissolve. Cool to room temperature under reduced pressure with stirring.

Abbreviation: q.s., quantum satis (in sufficient amount).

Formula 8 Soap-Based Facial Cleansing Lotion (Soap Emulsion)

Ingredients	%
Stearyl alcohol	0.5
Hardened palm oil	3
Liquid paraffin	35
Cholesteryl/behenyl/octyldodecyl Lauroyl glutamate	2
Dipropylene glycol	6
PEG 400	4
Sorption sesquioleate	1.6
POE(20)oleyl alcohol ether	2.5
Carboxyvinyl polymer (1%)	15
Potassium Hydroxide	0.1
Water	q.s. to 100
Perfume	q.s.
Preservative	q.s.

Procedure: Add the humectants and chelating agent to the purified water and heat to 70°C (water phase). Heat the oil component ingredients together to make solution, add the surfactants, preservative, and perfume, and keep heating to 70°C. Add this mixture to the water phase.

Abbreviation: q.s., quantum satis (in sufficient amount).

Formula 9 Facial Cleansing Cream (with Arginine to Neutralize Carbomer)

Ingredients	%
Stearic acid	2
Cetyl alcohol	3
Petrolatum	10
Liquid paraffin	38
Isopropyl myristate	10
Propylene glycol	5
Glycerin monostearate	2.5
POE(20)sorbitan monostearate	2.5
Arginine	0.3
Water	q.s. to 100
Perfume	q.s.
Preservative	q.s.

Procedure: Add the humectant and alkali to the purified water phase. After heating the oil component ingredients together to make a solution, add the surfactants, preservatives, antioxidant, and perfume and keep heating to 70°C. Gradually add this to the water phase.

Abbreviation: q.s., quantum satis (in sufficient amount).

CONCLUSION

A hygiene consumer product must make skin clean and refreshed. There are industrial or heavy-duty cleansers available for skin, often with sufficient mildness but nothing especially elegant. Along with the skin cleansing bars, skin cleansing liquids

Formula 10 Gel-Type Makeup Remover

Ingredients	%
Glyceryl trictanoate	56.4
Cetyl octanoate	5.0
POE(25)octyldodecyl ether	16.0
Butyl paraben	0.2
POE(10)methyl glucoside	4.0
Glycerin	1.7
Sorbitol (70%)	9.0
Water	7.3
Methyl paraben	0.1
Perfume	0.3

Procedure: Mix (A) components at 80°C to dissolve completely. Mix (B) components separately and dissolve at 80°C completely. Add (A) to (B) with paddle stirring. Gradually cool down while stirring. Add perfume at 55°C; mixture turns to gel at 50 to 45°C.

Formula 11 Bod/ Wash Based on LES

Ingredients	%
Sodium laureth sulfate	40.0
Cocoamidopropylbetain	10.0
Sodium cocoyl glutamate	3.0
Laulamide DEA	3.0
Sodium PCA	2.0
Glycerin	3.0
PEG(150)distearate	0.1
Water	q.s. to 100
Perfume	q.s.
Preservative	q.s.

Procedure: Add all the ingredients together and heat to dissolve with stirring. Cool down to room temperature.

Abbreviation: q.s., quantum satis (in sufficient amount).

are the products categorized as cosmetics and personal care. Skin cleansing liquids are more and more chosen by consumers with highly perspective or emotional motives, which is why skin cleansing liquids must carry concepts that appeal to the consumers' trendy desires. Cosmetic scientists will continue to challenge such difficult tasks and would bring out innovative products, with the encouraging findings that the psychological effects of cosmetics use improves the quality of life.

REFERENCES

1. Sakamoto K. Surfactant and skin: surfactant suitable for sensitive or atopic skin. J Jpn Cosmet Sci Soc 1997; 21:125.
2. Naito N, Munakata A. Cleansing of sebum and skin treatment. Fragrance J 1988; 92:42.

3. Dawning DT, Strauss JS, Pochi PE. Variability in the chemical composition of human skin surface lipids. J Invest Dermatol 1969; 53:232.
4. Schwartzendruber DC, Wertz PW, Madison KC, Downing DT. Evidence that the corneocyte has a chemically bound lipid envelope. J Invest Dermatol 1978; 88:709.
5. Miyazawa K, Tamura U, Katsumura Y, Uchikawa K, Sakamoto T, Tomita K. Anionic surfactants as detergents for scalp and hair. Yukagaku 1989; 38:297.
6. Miyazawa K. Evaluation of haircare products: shampoo and rinse. J Soc Cosmet Chem Jpn 1995; 29:95.
7. Jacobi OK. About the mechanism of moisture regulation in the horny layer of the skin. Proc Sci Toilet Goods Assoc 1959; 31:22.
8. Nozaki T. Research and development of body cleanser. Fragrance J 1996; 8:24.
9. Fukuda T. Research and development of a face cleanser of liquid type. Fragrance J 1996; 7:24.
10. Mistui T, ed. New Cosmetic Science. Amsterdam: Elsevier, 1997.
11. Suzuki T, Takai H, Yamazaki S. Formation of fine three-phase emulsions by the liquid crystal emulsification method with arginine-branched monoalkylphosphate. J Colloid Interface Sci 1989; 129:491.
12. Sakai Y, Hashimoto F. Development of a high function make-up remover applying a new polar oil. The 40th Annual Meeting of The Society of Cosmetic Chemists of Japan, June 17, 1997 Osaka, Japan.

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Hair Cosmetics

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INTRODUCTION

Throughout history, hair has always been an important element of personal adornment. From the beautifully regular beard curls of the Assyrian kings to the elegant haircuts of Egyptian pharaohs to the carefully coiffured wigs of the European nobility, hair has been shown, admired, and envied. Over the years, what had been the privilege of the affluent few has become an almost consuming passion of many. The explosive growth of the haircare market since the middle of the 20th century is the result of deep, socioeconomic changes combined with an increasing focus on personal aesthetics, assisted by affordability of products. The attempt to satisfy the genuine needs of the consumer and the drive for competitive advantage among marketers has led to a variety of grooming aids and products, such as shampoos to cleanse the hair, hair conditioners to make it soft and combable, hair colorants and permanent waves to impart the hair the properties that it does not have, and hair sprays to keep hair in the desired style. Hair products are in the cosmetic category and, as such, are subject to all laws and regulations that control the labeling and claims of all cosmetic products.

THE STRUCTURE AND PROPERTIES OF HAIR

Hair follicles, which in tens of thousands are deeply invaginated in the scalp tissue, are the essential growth structures of hair. At the base of each follicle, the cells proliferate and, as they stream upwards, the complex and intertwined processes of protein synthesis, structural alignment, and keratinization transform the cytoplasm into the tough fibrous material known as hair. Hair is unique in that its structural and growth characteristics are different between races, sexes, individuals of the same race, areas in the same individual, and even within the same follicle. The development of hair is a dynamic, cyclical process in which duration of the growth cycle depends not only on the body site, but also on such variables as the individual's age, nutritional habits, and hormonal factors. In the scalp, each hair grows steadily (about 1 cm/month) and continuously for three to five years (anagen phase); growth then stops and is followed by a brief transient stage (catagen) and a 2- to 4-month

resting stage (telogen) during which the old hair is shed. With the onset of the anagen stage the new hair starts to grow from the same follicle. The growth process functions independently in each follicle, so hairs are not shed simultaneously as they are in most animals. At any given time, some hairs are growing, some are resting, and some are being shed. Normally, of about 150,000 scalp hairs, 90% are in the anagen phase and the remaining 10% are in the catagen and telogen phases, with 50 to 100 hairs being shed daily. Scalp hair is a fiber of 50 to 80 μm in diameter and its exterior consists of a layer of flat, imbricated cuticle cells pointing outward from root to tip. This ratched-like structure of the cuticle scales serves as an effective self-cleaning feature and, by interlocking with the scales of the inner root sheath of the follicle, helps to hold the hair firmly in place. The cuticles are thin (0.5 μm), 50 to 60 μm square sheets, attached at their approximal ends to the underlying cortex. Their longitudinal overlap is substantial resulting in an average separation of scale edges of approximately 5 μm. This overlap generates a multilayered shield three to 4 μm thick around the hair fiber. The structure of the cuticle fulfills well the role of a protective barrier for hair. A thin film of covalently bounded lipid on the exterior of the cuticle assures a low friction surface, together with water repellency. Just underneath, the highly crosslinked lamellae of the A-layer and exocuticle augment the mechanical stability of the scales, whereas the soft and water-absorbing endocuticle cushions effects the mechanical impact. The high water swellability of the endocuticle is the likely source of pronounced cuticle lifting on wetting of hair.

Enveloped by this formidable protective sheath of the cuticle layer is hair cortex, which constitutes the bulk of the fiber and is mainly responsible for the mechanical properties of hair. The spindle-shaped cortical cells are arranged parallel to the fiber axis, overlapping each other with frequent interdigitation. They have a unique arrangement of the constituent proteins, comprising intermediate filaments (IF), traditionally termed microfibrills, aligned in the direction of fiber growth and are surrounded by a matrix of IF associated proteins (IFAP). The filaments are composed of high-molecular weight protein chains of low sulfur (cystine) content and possess a high degree of molecular organization (α-helical), whereas the surrounding matrix of IFAP is made up of proteins more extensively crosslinked by cystine lacking definite structural pattern.

During the process of keratinization, the cell plasma membranes are modified to establish a strongly adhesive layer between the adjacent cells known as the cell membrane complex (CMC). This is the only continuous phase in the hair fiber providing adhesion between the cortical cells as well as the cuticle cells.

Dispersed throughout the structure of the cortex are melanin pigment particles. Their number, chemical characteristics, and distribution pattern determine the color of hair. In some hairs, coarse hairs in particular, vacuolated medulla cells are present in the central region of the fiber.

Although hair of different racial origin differs in shape, degree of curliness, and color, there is little difference in the underlying chemical properties and fiber structure. And, the amino acid composition of the constituent proteins and most physical properties are similar (1,2). The differences between hair of different ethnic groups are often smaller than the variation in the properties of hair taken from different individuals within one ethnic group.

Compared with Caucasian or Asian hair, African hair is more irregular in the shape of its cross-section. The sharp kinks seen in such hair are often associated with random unevenness of fiber diameter, resulting in weak spots along the fiber length. These are likely to cause problems during combing or chemical treatments.

SHAMPOOS: GENERAL COMMENTS

Cleansing is clearly a dominant element of personal hygiene and, when reinforced by the aspect of attractive appearance, translates into a powerful and highly marketable stimulus. Shampooing has become, thus, a *sine qua non* in maintaining the aesthetics of hair. The cleansing task is formidable. A mass of 100,000 to 150,000 flexible fibers has to be cleansed of oily deposits of sebum, sweat, entrapped desquamated scalp cells, along with the residues of mousses, gels, and hair sprays. All this has to be done within the span of a few minutes, leaving the individual hairs clean and free of tangles to which the ratched structure of hair cuticles makes it particularly vulnerable. It should also be kept in mind that although cleansing action is the fundamental assignment of a shampoo formulation, it is by no means the only goal. The promise of hair shine, softness, body, and manageability is inherently tied to product performance. Also, one must not ignore the process of shampooing itself. It is expected to provide a pleasurable experience in working up a rich and lubricous lather that seems almost to caress the hair and leave it, after rinsing, with a touch of refreshing fragrance.

Hair Soiling and Soil Removal

During its residence on the scalp, hair is exposed to a variety of events that contribute to its soiling. Among them are the innate processes of scalp desquamation, sweating, and sebum secretion, which are supplemented by deposition of extraneous substances arising either from environmental pollution (dust and other airborne contaminants) or from hair-grooming preparations, such as oils, waxes, hair spray, and mousse residues. Of all these, sebum, because of to its steady replenishment, greasy characteristics, high adhesiveness to hair, and ability to cement the other soil particulates together and to the hair surface, appears most insidious and thus it is not surprising that its efficacious removal is key in hair cleansing.

The sebaceous glands attached to each of the hair follicles provide a continuous supply (3) of this oily substance to the surface of hair. There are seasonal variations in the amount of sebum secreted (4), but more importantly its output is under hormonal control (3), reaching a maximum at puberty. Oily hair of adolescents is the obvious and often annoying consequence of the high activity of the sebaceous glands, and this at a time in one's life when personal adornment is particularly important. Sebum secreted from the sebaceous ducts spreads within the mass of hair primarily via physical contact between the fibers (5); brushing and combing (as well as contact with a pillow) further redistributes the sebum and partly assists in its removal. The quantity of sebum on hair at a particular moment thus reflects the relative efficacy of these two processes (sebum secretion and removal). The term "oily hair" often connotes a highly undesirable image of stringy and dull hair with little body and greasy feel. It is, however, worth bearing in mind that such a perception is not universal being strongly influenced by fiber texture and geometry. Thus, a visual appearance of curly African hair can visibly benefit from an increase in oiliness, a fact that is exploited in grooming products for such hair.

Because of the adhesiveness and sticky consistency of sebum-containing soil, its adequate removal by simple mechanical means is virtually impossible and satisfactory cleansing can only be attained by use of aqueous solutions of detergents. In the broadest sense, all materials used in cleansing such as water and other solvents, soaps and synthetic surfactants, salts, and abrasives may be considered as detergents. However, more specifically, the term "detergent" is limited to those surface-active

agents that, in addition to the property of lowering surface tension, are effective in deflocculating soil and dirt clumps and keeping them in suspension so that they can be washed away before redepositing on the surface that is being cleaned. This property is exhibited by compounds that contain both a hydrophilic group and a hydrophobic tail that serves as an emulsifying agent. In essence, the removal of soil from hair is governed by the same basic processes that had been previously identified as being involved in laundering of fabrics (6). Without elaborating on theories underlying the detergency, one should allude to the three fundamental mechanisms that have been proposed to account for the cleansing action of detergents.

1. The "roll-up" mechanism (6), particularly relevant to oily deposits in which the progressive wetting of the fiber surface leads to rapid detachment of oil droplets.
2. In the micellar solubilization mechanism (7), the soil is solubilized into micelles that come into contact with the soiled surface. The efficacy of this cleansing mode depends on the availability of sufficient quantity (concentration) of micelles, which does not usually present a problem with conventional shampoo formulations.
3. The third mechanism (8) invokes the dispersion and emulsification of soil particles penetrated by the diffusing detergent. The amphiphilic components of sebum might enhance cleansing by direct interaction with the molecules of the surfactant.

There is no precise information presently available as to which mechanism is dominant in hair cleansing. Quite possibly all three might be involved, depending on the characteristics of the soil. In any case, the vast majority of shampoo products are formulated to be operative under diverse conditions of detergent action, thus assuring their cleansing efficacy.

Shampoo Ingredients

Almost without exception, shampoos consist of an aqueous solution, emulsion, or dispersion of one or more surfactants together with some additives to enhance performance and aesthetic properties of the product. Additives are used to provide fragrance and color, thicken, opacify, and convey specific tactile attributes. They include stabilizers, foam modifiers, preservatives, conditioning, and antidandruff agents.

Surfactants

Surfactants are long-chain electrolytes and are usually classified according to the nature of their hydrophilic group, which may be anionic, nonionic, amphoteric, or cationic.

Anionic Surfactants

Soaps are salts of fatty acids and, not in the distant past, were the mainstay of shampoo products. In soft water, they lather copiously, cleanse well, and leave the hair in a well-conditioned style. Unfortunately, in hard water the lather is poor, and as the soap combines with calcium or magnesium salts present in hard water it deposits on hair a dulling film. The introduction of synthetic surfactants brought about the

end of soap-based shampoos, although some products still contain a small quantity of soap to exploit its conditioning property.

Alkyl sulfates are the most widely used anionics in shampoos, displaying excellent foaming and cleansing properties unaffected by hard water. Lauryl sulfate is the dominant ingredient being present in most shampoo formulations in the form of its ammonium or triethanol ammonium salt at a level of 6 to 18% w/w. Although very effective cleansers, the alkyl sulfates, particularly at high concentrations, have a tendency to irritate the scalp and remove some lipid constituents of hair cuticle. To make the alkyl sulfate-based shampoos milder, they are frequently modified by incorporation of less-irritating alkyl ether sulfates or amphoteric surfactants.

Alkyl ether sulfates are sulfated products of ethoxylated fatty alcohols. They are more water soluble than alkyl sulfates, are excellent solubilizers for fragrances and other oleophilic additives, and are particularly suitable for formulations of clear shampoos. As alluded to earlier, these surfactants are less irritating than the alkyl sulfates and are used, at a higher degree of ethoxylation, in baby shampoos.

Alpha-olefin sulfonates are complex mixtures resulting from sulfonation of alpha-olefins. These detergents exhibit excellent foaming in the presence of sebum, are effective over a wide range of pH, and compare favorably with other surfactants in dermal and eye irritation (9).

Other anionic surfactants worthy of note include alkyl monoglyceride sulfates and alkyl sulfosuccinates. Both are very mild to the skin and, although the former are good foamers and can be used in shampoo formulation in their own right, the latter are primarily used in combination with alkyl sulfates.

Nonionic Surfactants

They are considered to be the mildest of surfactants. Although poor foamers, owing to their good solubilizing and dispersing properties, they have been extensively utilized to supplement the action of the primary cleanser.

Alkanolamides are prepared by condensation of fatty acid (usually lauric) and primary or secondary alkanolamines. Their presence in a shampoo formulation can have a pronounced effect on stabilizing the foam level and improving lather consistency. *Amino oxides* are formed by oxidation of tertiary fatty amines and are used in shampoos primarily as foam modifiers and as antistatic agents to improve the overall manageability of hair.

Polyethoxylated surfactants represent the largest group of nonionics and include the ethoxylated derivatives of alkylphenols, fatty alcohols, fatty esters, and diglycerides. They exhibit excellent detersive power and cleansing properties, but because of poor foaming, their use has been restricted to solubilizing of shampoo fragrances and other oleophilic additives.

Amphoteric Surfactants

Often referred to as ampholytic, these surfactants contain both cationic and anionic groups in one molecule. Because the charge of these surfactants are pH dependent, their properties, such as foaming potential, solubility, and CMC, also vary with the change in pH. Most amphoterics are derivatives of imidazoline or betaine. They are quite compatible with anionic, nonionic, or cationic surfactants, and have been extensively used to formulate mild (baby) shampoos or as mollifying agents in the more irritating anionic compositions.

Shampoo Additives

These are materials incorporated into a shampoo formulation to enhance its aesthetics as well as to improve its performance.

Thickeners comprise a broad variety of compounds that are used to increase viscosity of the formulations, modifying their consistency from viscous liquids to thick gels. The most frequently used are electrolytes, such as sodium chloride, alkanolamides, and water-soluble cellulose derivatives, such as carboxymethylcellulose, hydroxyethylcellulose, carboxyvinyl polymers of the Carbopol type, polyvinyl alcohols, and natural gums, such as tragacanth. Magnesium aluminum silicates have found application as thickeners and suspending agents in antidandruff shampoos.

Opacifiers serve to impart to shampoo a pearlescent or opaque appearance. For this purpose, high-melting, wax-like materials are blended into formulations. Of particular utility in this respect are cetyl and stearyl alcohols and their esters as well as the latex emulsions of vinyl, styrene, and acrylic polymers.

The shampoo milieu offers itself as an ideal ground for microbial growth, particularly of the aerobic gram-negative Pseudomonas sp. This may have a deleterious effect on the shampoo properties, posing at the same time a health hazard to the consumer. The function of preservatives is to inhibit such bacterial development. Although formaldehyde has been one of the most popular and effective preservatives, its use has declined as other compounds have come to the fore. Examples include methyl and propyl parabenes, DMDM hydantoin, quaternium-15, imidazolidynyl urea, and others. The selection of a suitable preservative is made through a challenge test in which the product is subjected to the worst possible conditions anticipated during manufacture, shelf storage, and actual use.

Other additives: Fragrance is an essential ingredient, often deciding the market appeal and success of the product. Addition of alcohols (ethanol and isopropanol) or glycols may be required to maintain the clarity of clear shampoos, whereas the presence of sequestering agents, like ethylenediaminetetraacetic acid (EDTA), prevents the formation of insoluble calcium or magnesium soaps when the shampoo is rinsed off the hair. Food Dye & Coloring (FD&C) and Drug & Cosmetic (D&C) dyes are commonly added to enhance the aesthetics of shampoo formulations. "Squeaky" clean feel of shampooed hair is frequently accompanied by difficult combing and substantial "fly away." To overcome this, the shampoos contain "conditioning" additives that are substantive to hair, remaining adsorbed on the surface after rinsing. A plethora of materials has been used to this end. To these belong amine oxides, protein hydrolysates, cationic surfactants, cationic polymers, lanolin and its derivatives, as well as natural materials, such as beer, honey, and egg.

Shampoo Formula

It must have become clear from the foregoing that a shampoo product, although straightforward in its purpose, is a complex blend of ingredients carefully chosen and attuned to effectively address the needs of individual consumers. Table 1 shows the nature and relative concentration of materials contained in a typical shampoo formulation.

Specialty Shampoos

Baby shampoos place stringent requirements for nonirritancy of the scalp and eye. The majority of products are based on amphoteric detergent systems. Thus, derivatives of

Table 1 Typical Shampoo Formulation

Ingredient	Wt.%	Function
Ammonium lauryl sulfate	10–20	Primary cleanser
Lauramide DEA	3–5	Foam stabilizer
Methyl paraben	0.08	Preservative
Propyl paraben	0.05	Preservative
Sodium chloride	0.5–1.5	Thickener
Disodium EDTA	0.2	Sequesterant
Fragrance	0.5	Fragrance
FD&C Yellow No. 5	0.001	Colorant
D&C Orange No. 4	0.002	Colorant
Water	To 100.00	Dilutent

imidazoline, betaine, and sulfobetaine are usually combined with nonionic surfactants of the polyoxyethylated alcohol esters class to procure sting-free formulations.

Medicated dandruff shampoos are designed to lessen and alleviate the excessive desquamation of the scalp by inclusion of specific ingredients. These include antimicrobials, such as quaternary ammonium salts, keratolytic agents, for example, salicylic acid and sulfur, or antiseborrheic compounds like coal tar and resorcinol. Over the past 20 years, the shampoos containing selenium sulfide or zinc pyrithione as antidandruff actives have greatly risen in popularity, reflecting both the efficacy of the products and aesthetics of the formulations.

Although so-called conditioning shampoos, or *two-in-one shampoos,* have been on the market for a number of years, offering the feature of hair cleansing and conditioning in a single step, the early versions of such products did not perform to consumers' satisfaction leaving the hair often undercleansed and overconditioned. It was not until the mid1980s that significant improvements in performance were achieved by emulsifying silicones into an anionic shampoo base. Such products have proved to be efficacious cleansers, and the shampooed hair feels soft and silky and is easy to comb. In some recent renditions of two-in-one products, the silicones have been replaced by quaternized guar gums, cationic polymers, and guaternaries.

Product Forms

In general, the shampoo formulations are relatively simple aqueous systems and, as such, quite amenable to modulation of their physical forms. The latter are often the consequence of market considerations of consumer preferences. Thus, the clarity of clear liquid shampoos conveys the impression of superior cleansing, whereas opaque formulations of similar or slightly higher viscosity are suggestive of conditioning qualities. Clear gels are usually sold in compact flexible tubes that are convenient for storage and travel. A class apart is the aerosol dry shampoos that continue to occupy a small niche in the shampoo category. They consist of oil-absorbing powders, such as starch, talc, or clay, which are sprayed on to the hair and after a short while removed by brushing or combing.

Evaluation and Safety

As the work progresses at the formulator's bench, the efficacy of developed shampoo prototypes is being evaluated in the laboratory using established testing procedures.

Thus, foamability and lather characteristics are measured in the presence and absence of sebum, gaining some insight into the detersive aspects of the formulas. The properties of shampooed hair, for its luster, combability, body, and flyaway, are instrumentally assessed together with the subjective evaluation of hair appearance. The ultimate proof, however, of the potential success of the formulation is in the practical use. Thus, the consumer evaluation of the product either with outside panelists or an in-house testing facility is imperative. The preference of consumers for a particular fragrance is of vital importance and their comments as to the aesthetic characteristics of shampoo and the feel of shampooed hair when combined with the results of laboratory tests provide firm ground for potential product claims.

Shampoo ingredients do not pose a particular hazard for skin or eye safety. The contact time is short and a water rinse follows. The irritation potential of some surfactants has already been alluded to. It is a common practice for most of the manufacturers to make provisions to evaluate their product for skin and eye irritation.

HAIR CONDITIONERS

It is worth noting that the subject of hair "condition" appears to be restricted almost entirely to the domain of women's hair. Although, as a woman's "crowning glory" the hair evokes in her a particularly profound concern for its beauty, there are at work some more mundane factors. Women's hair, unlike men's, is subject to more frequent and diverse assaults that are injurious to its properties. It is perhaps ironic that except for environmental effects (weathering), most of these are associated with what we call the "haircare" practices. Thus, the handling of hair in the course of its daily shampooing, combing and brushing, and blow drying cause, even for intact hair, gradual abrasion of the hair cuticle signaling the onset of hair damage. This process of cuticle loss is particularly evident in longer hair leading often to the generation of split ends. Hair coloring, bleaching, waving, or straightening, although imparting to hair a much sought after different or novel appearance, impair the surface lipid layer of the cuticles, further aggravating the abrasive effects of daily hair regimens. Although gradual, these deleterious effects are additive and further exacerbated by sun exposure.

Clearly, by the use of conditioning shampoos, avoiding practices singularly injurious to the cuticle, such as teasing, and keeping the hair relatively short and shielded from sun, one might, for a considerable length of time, maintain the intact hair in satisfactory condition. Alternatively, one can go a step further and, by the use of products designed explicitly for conditioning, supplement the benefits obtained from a shampoo and significantly extend their range. A good conditioner eliminates tangling, makes the hair easy to comb and style, eliminates static charge, and, by fostering fiber alignment, enhances the luster and shine of hair. The soft feel of hair and improved manageability are additional important attributes of conditioned hair. It is important to stress that these effects are universal, that is, irrespective of cosmetic history of hair, whether the hair is intact, waved, colored, or bleached, the conditioner delivers its benefits.

Two general forms of conditioners are currently in use: (1) hair rinses and (2) leave-in products, often referred to as "deep" conditioners. Both are applied to freshly shampooed hair. True to their name, the rinse product is rinsed off after a few minutes, whereas the leave-in product is left on the hair for up to 30 minutes, after which it is rinsed off. The purpose of the longer time is to allow the product

to penetrate further (thus the name "deep") into the hair shaft thereby extending the conditioning effects.

The active ingredients in most conditioners are based on quaternary ammonium salts (cationic surfactants) such as steartrimonium chloride and, cetrimonium chloride, and the like. Because of their great affinity for hair, these compounds bind strongly to the cuticles, providing a low-friction surface, thus making the cuticles slick and less prone to abrasion. Other components present in the conditioning formulations, such as fatty amines, fatty alcohols, and amine oxides, supplement the action of cationic surfactants, adding primarily to the tactile benefits. The leave-in conditioners that are recommended for use on damaged hair frequently contain protein and lanolin derivatives.

Conditioning effects are usually lost in shampooing, and a reapplication is recommended to reinforce the protective effect. Conditioning formulations containing cationic polymers are somewhat longer lasting. The same is true for conditioners based on emulsions of polymeric silicones.

HAIRDRESSINGS

Hairdressing is a broad term describing products applied for final grooming. Including brilliantines, tonics, and gels, this category follows new fashions, hairstyle trends, and is attuned to progress in styling techniques. Hairdressings are applied by spreading it through the hair with the fingers and then combing through for an even distribution. As they are not rinsed off after application, care must be taken to avoid excessive buildup.

The primary purpose of brilliantines is to add sheen to hair. Thus, the main constituent of these products is oil—usually mineral oil—which is spread on fiber strands increasing their luster and providing grooming effects. Solid brilliantines (pomades) are based on petrolatum to which various waxes are added to attain the desired consistency and texture. Tonics might be viewed as lighter versions of brilliantines and usually consist of alcoholic solutions of various oils. The alcohol wets the hair and after evaporation leaves a thin film of oil. By using synthetic, rather than natural oils, much less greasy formulations can be obtained. Using a high concentration of ethoxylated emulsifiers, grooming oils can also be readily blended into clear gels. In contrast, setting gels based on hydroalcoholic solutions of carboxyvinyl polymers or methylcellulose ethers are oil free. They range in consistency from liquid to rigid gels and provide a good range of textures, volume, and hold.

Styling Products

Whereas most of the styling needs of short hair are satisfactorily met by a good haircut, those with longer hair require more effort which is, however, well rewarded by the diversity of styles that can be imparted. The underlying principle of all styling processes is hair setting, and a few comments on the subject seem appropriate. Hair fibers are flexible and elastic, and when dry bounce back immediately to their original configuration (straight or curly), when bent, extended, or twisted. On wetting, however, they become pliant and malleable and can be readily molded (set) to almost any desired form. On drying, they retain the new shape until exposed to water (moisture) again.

The primary function of all styling products is to assist in the setting process and/or to ensure the stability of the newly imparted configuration. Depending on the type of styling product, different mechanisms of action are operative.

Styling Aids

As the name implies, the role of these products are first to facilitate styling of the hair and second to keep it in a newly styled shape. Three general product forms represent this category: styling gels, mousses, and styling sprays. Most of the formulations are based on synthetic film-forming polymers and contain a variety of additives to improve film properties and performance. Thus, phtalates and glycols are used as plasticizers. Lanolin derivatives and silicones are added to improve feel and impart some resistance to moisture. The products are applied to wet hair, which is styled with fingers or a comb. Usually the more viscous the product, the easier it is to style the hair. As the hair dries and sets in the desired configuration, a polymeric film forms on the surface of hair, cementing adjacent fibers together and thus further stabilizing the newly imparted style.

Table 2 shows an example of typical styling formulations for a styling gel and a styling mousse.

Hairsprays

Also in this category, polymeric film formers are the backbone of the formulations, although both the intended use and the mode of action are somewhat different from those of styling aids.

These products are applied to dry and already styled (set) hair in the form of fine mist or spray. The spray droplets collide with and become deposited on hair fibers. As they spread on the hair surface, they tend to migrate and accumulate at

Table 2 Typical Formulas of Styling Aids

Ingredient	Wt.%	Function
Styling mousse		
Polyquaternium-11	1.4	Styling ease
Polyquaternium-4	0.6	Film former
Lauramide DEA	0.2	Foam stabilizer
Isosteareth-10	0.2	Foam stabilizer
Dimethicone copolyol	0.15	Styling ease
Fragrance	0.2	Fragrance
DMDH hydantoin	0.2	Preservative
Methyl paraben	0.1	Preservative
Isobutane/propane blend	7.0	Propellant
Water	To 100.00	Solvent
Styling spray		
Ethylester of PVM/MA copolymer	2.5	Film former
Dimethicone copolyol	0.3	Styling ease
Isopropyl alcohol	5.0	Solvent
Fragrance	0.3	Fragrance
Ethanol	45.0	Solvent
Water	To 100.00	Solvent

the points where adjacent fibers are very close or intersect with each other. This results in the formation of minute joints distributed throughout the hair mass. As the solvent evaporates, these joints become rigid bonds welding the fibers together and, thus, preventing the motion of individual hairs relative to each other. This cumulative restraining action of hundreds of such microscopic welds throughout the hair assembly accounts for the style-stabilizing performance of hair sprays, protecting the hair from mechanical deformation, wind, and humidity.

The strength of these hairspray bonds depends on a number of factors, of which the nature of the polymeric resin is of paramount importance. Most of the polymers used form adequately strong bonds at low relative humidity (RH). As the RH increases beyond 80%, however, most resins begin to absorb moisture from the environment, softening the welds. At the same time, water absorption by hair causes rapid relaxation of the set configuration of the fibers, and it is the tenacity of the hairspray welds alone that stabilizes the imparted style. Clearly, the polymers that are least sensitive to the plastizing effect of water are likely to be the better performers and are thus preferred for a hairspray product.

It should be stressed that in addition to the intrinsic strength of the resin, other factors may affect bond formation and/or bond toughness. For example, the characteristics of the solvent system used to deliver the resin to hair play an important role. Efficient weld formation depends on the wetting and spreading properties of the resin droplets on the hair surface. As mentioned earlier, the welds are formed by the accumulation of liquid spray at contact points between the fibers. Thus, an aerosol formulation with 30% alcohol and 70% highly volatile propellant will dry much faster than a solvent vehicle with 50% or more alcohol. As the solvent evaporates, the viscosity of droplets increases and mobility decreases. This reduced mobility results in relatively small bonds between adjacent or intersecting fiber, which might negatively affect the product performance. One might be led to a conclusion that the spray that stays "wetter" longer generates better performing welds. This may hold true for nonaqueous systems, as the organic solvents used in hair formulation do not have any adverse effect on the set of the styled hair. With the hydroalcoholic systems, however, and the water content of over 20% the long "residence" time of hairspray droplets on hair may lead to a significant loss of set caused by the selective water absorption by hair fibers.

Although a number of hairspray resins have been developed over the years and many of them have been in use, the combination of regulatory restrictions and increased demands on the aesthetics of product performance has narrowed the field somewhat. Thus the butyl and ethyl esters of poly(vinyl methyl ether/maleic anhydride) copolymers, which for years have been the most widely used polymers in hair sprays, have suffered a rapid decline, being surpassed by octylacrylamide/acrylates/butylaminoethyl methacrylate copolymer. The latter provides excellent holding properties at relatively low resin concentration. For the aerosol hairsprays, the resin of choice is vinyl acetate/crotonates/neodecanoate, which, by modulation of the extent of its neutralization, can substantially modify the film properties; essential as it is, the set holding is not the only attribute that has to be considered in formulating hair sprays. Clearly, the aesthetic aspect of sprayed hair cannot be neglected. Thus, the resin film should add shine (gloss) and not dull the hair and should not make the hair tacky in humid weather. It should resist flaking, but be readily removed by shampoo. By selection of appropriate additives and solvents, both the holding and aesthetic goals can be readily attained. Table 3 provides ingredient listings for typical aerosol and pump sprays.

Table 3 Typical Hair Spray Formulas

Ingredient	Wt. %	Function
Aerosol hairspray		
Poly(vinyl methyl ether)-maleic anhydride ethyl ester	5.0	Film former
Amino methyl propanol	0.2	Neutralizing agent
Dimethyl phthalate	0.4	Plasticizer
Fragrance	0.2	Fragrance
Ethanol	70.0	Solvent
Isobutane/propane	24.2	Propellant
Pump hairspray		
Octylacrylamine/acrylates/butyl amino ethyl methacrylate copolymer	3.5	Film former
Amino ethyl propanol	0.5	Neutralizing agent
Cetearyl octanoate	0.1	Plasticizer
Fragrance	0.15	Fragrance
Ethanol	80.00	Solvent
Deionized water	15.75	Solvent

Safety and Regulatory Issues

All aerosol hairsprays, whether containing hydrocarbon or carbon dioxide propellant, are classified as flammable by virtue of their flame propagative properties. The same is true of pump sprays on account of their high alcohol content. Appropriate warnings should be displayed on the package informing of potential eye irritancy of the product.

Federal regulation in 1978 that banned the use of chlorofluorocarbons in hairsprays brought about a drastic change in the technology of aerosol hairsprays. New propellants had to be evaluated and formulations developed to accommodate their different properties. The hydrocarbon gases, such as propane, butane, and isobutane have been found to generate the most consistent hairspray pattern, being at the same time compatible with alcohol and current hairspray resins. However, in 1990, both California and New York introduced the concept of volatile organic component (VOC) placing strict limits on allowable VOC content in hair sprays. As the VOC is defined as any organic compound having between 1 and 12 carbon atoms, the VOC restrictions also affect the nonaerosol hairsprays where the ethanol is both the resin solvent and the propellant. The decrease in VOC levels is primarily compensated for by the increase in water content of the hairspray, making it wetter, less efficacious, and sticky leaving aside the less aesthetic delivery characteristics. A search is underway to develop new resins that accommodate the high-water content formulas with performance standards equal or approaching those of current sprays.

Permanent Waving

It was perceived a long time ago that wavy hair not only surpasses straight hair in opportunities for more diverse styling, but because of its geometry, it appears more luxurious and, thus, highly desirable. Early records show that the ancient Assyrians wore a mass of curls falling over their shoulders and the beards of men displayed exquisite and highly uniform wave patterns. The earliest recorded methodology of

hair waving can be traced to Egyptians who curled their hair with mud and then dried it in the hot sun. The elaborate coiffures of Roman women relied on prototypes of the curling iron. Then, with the advances of the Middle Ages, hair virtually disappeared from view and did not make its re-emergence until the time of the renaissance. But then shortly it hid again—this time under wigs. The latter, made of human hair, were processed to desired configurations by techniques not greatly different from those developed by Egyptians and Romans. It was not until the early 20th century and the pioneering work of Nessler on hot waving that generated stimuli for affordable and simple waving procedures. Basic precepts of modern permanent waving were developed in the 1930s. Over the years, these principles have been further explored and creatively utilized to yield safe and efficacious products.

Hair-Waving Process

The immediate objective of waving is to impart to hair a durable configuration that is different from what the hair exhibits in its native form. Each hair has a geometry that is the result of processes of keratinization and follicular extrusion that transforms a viscous mixture of proteins into strong, resilient, and rigid keratin fiber. In principle, waving can be viewed as a combination of reversal and a stepwise restaging of these processes, as it entails softening of keratin, molding it to a desired shape, and annealing the newly imparted geometry. The underlying mechanism of waving is, thus, essentially molecular and involves manipulation of physicochemical interactions that stabilize the keratin structure.

It might be useful at this point to emphasize the essential difference between waving and setting of hair. Although both cases involve the impartation of new geometry to hair, only water-labile bonds are manipulated in setting, and thus the imparted geometry is moisture sensitive and lost on shampooing. In waving, both the covalent and secondary bonds are involved and the new geometry is stable to repeated washing cycles. The cleavage of covalent bonds (disulphide crosslinks of cystine) is conveniently attained by reducing agents that convert them to cysteine residues that can be relinked in the last phase (neutralization step) of the waving process.

In a typical waving procedure, freshly shampooed, damp (but not wet) hair is separated into 30 to 60 tresses. Each tress is wetted with the waving lotion and wound onto plastic rods or curlers with the help of a porous end paper or sponge. The size of the curler determines the character of the resulting wave; the smaller the curler, the tighter the wave. After 10 to 20 minutes, the hair is rinsed thoroughly and, while still on rods, wetted with the neutralizing lotion. The hair is then unwound, rinsed again, and either freely dried or set in the desired style. The waving procedure depends on the type of the waving product used and the desired end result. Thus, instead of wrapping with lotion, the hair can be wound wet and the lotion applied to curled hair. Sometimes a suggestion for a "creep" step is made to obtain a tighter and longer lasting curl. This involves an approximate 30 minute wait between rinsing off the lotion and application of the neutralizer.

The tight curl produced by permanent waving is frequently not the configuration desired for the final hairstyle. Often a water set of a larger curl configuration is superimposed on the wave. Then, as the temporary set begins to relax under the influence of moisture, the change of the hair form towards the tighter and waved configuration counterbalances the forces to straighten the hair with the net result of a greater set stability and more body than if the hair had not been waved.

Waving with Mercaptans as the Active Ingredients

European, American, and large segments of the Asian markets are dominated today by the formulations based on thioglycolic acid (TGA) and its derivatives. The popularity of TGA stems from a number of factors. The long history of use has built an impressive evidence of adequate medical safety. The incidence of injury has been extremely low and so has been the frequency of sensitization. High adaptability of TGA to various formulation types that provided markedly different end benefits coupled with performance reliability and a low price all contributed to its success. The unpleasant odor of TGA has remained it most perceptible drawback. Although some progress has been made in the fragrancing of TGA-based lotions, the results so far are at best mediocre.

Conventional waving lotions contain 0.5 to 0.8M TGA adjusted to pH 9.1 to 9.5. The neutralizing base can be ammonia, alkanol amines, sodium carbonate, or a mixture thereof. Ammonia appears to be more effective than the other bases in facilitating diffusion of TGA through hair. It is also preferred over nonvolatile amines, because it escapes during processing and the resultant drop in pH reduces the activity of the lotion with time and thus minimizes the danger of overprocessing.

Over the years, several TGA derivatives (primarily amides and esters) have been tried, but as of now, only one—glyceryl monothioglycollate (GMTG)—is of practical importance and used in so-called acid waves. In waving performance, GMTG works better than TGA at *low pH* under such conditions, however, the resulting wave lacks the crispness and durability of the conventional alkaline TGA wave. This is somewhat compensated for by less hair damage. To increase the efficacy of GMTG, the waving process is often carried out with the aid of heat.

Apart from the weaker waving performance of GMTG, when compared with TGA, there are several other disadvantages associated with the use of this mercaptan. Its low water solubility and propensity for hydrolysis necessitate a separate package (container), which represents inconvenience for the consumer and additional cost. Occasional reports of skin sensitization have limited the use of GMTGA to salon applications. Finally, its rather pungent odor has a tendency to stay on the hair even after the neutralization step. Perhaps because of its hydrophobic character, GMTGA may be tightly bound to the apolar domains of the keratin structure, and therefore be more resistant to rinsing.

There are on the market several types of TGA-based formulations that claim point of difference from the conventional lotions. One is called a "self-timing" wave, the other a "self-heating" or "exothermic" wave. Both use TGA under alkaline conditions. The self-timing wave contains, however, dithiodiglycollic acid (DTDGA), which is the oxidation product of TGA. The function of DTDGA is to prevent hair overprocessing without negatively affecting the waving performance. In the United States self-timing formulations command approximately 20% of the market share.

The exothermic wave product contains a small vial of aqueous H_2O_2 (separate from the neutralizer), which is to be added to the waving lotion just before its use. Oxidation of TGA (which in this case is in excess of concentration required for waving), generates some heat as well as small quantities of DTDGA. Although the warmth can be readily perceived on mixing, the heat dissipates quickly as the lotion is applied to hair and equilibrates itself with that of the environment.

The acid wave based on TGA is a conventional TGA formulation adjusted to a lower pH (6.8–8). Unlike the acid wave with its esters (GMTGA), these formulations perform poorly and often require heat to improve the result.

In the Far East, particularly in Japan, the use of cysteine as a waving agent is widespread. This amino acid is claimed to provide a "natural" and nonodorous alternative to TGA and to wave the hair without damage. Although some of these assertions are doubtlessly true, the waving efficacy of cysteine is mediocre. One can significantly increase its efficacy by the incorporation of a high concentration of urea (2–3M). Most of the Japanese formulations contain, apart from cysteine, hefty amounts of TGA as the effective ingredient.

Waving Formulations with Sulfite as the Active Ingredient

Sulfite, as a permanent setting agent, has found wide application in the wool industry (pleating, lustering, and flat setting) well ahead of TGA on account of its effectiveness and lack of odor. Sporadic attempts to use it as a waving agent had not been very successful until the late 1970s when it was successfully introduced. The rapid rise of sulfite products appeared initially to spell demise for conventional TGA formulations. Readily consumer-perceptible attributes, such as lack of odor and low hair damage, combined with the then preference for softer hairstyles greatly favored sulfite systems. A number of companies rushed to the market with offerings of formulations for tight curls, body waves, and hair straighteners. However, attractive as these formulations appeared to be, they could not match the waving efficacy or durability aspects of TGA systems. The TGA-based products regained their ubiquity, although the sulfite product held on to a stable, though small, market share.

It appears appropriate at this junction to re-emphasize that the current methodology of hair waving (ambient temperature, medically safe reagents, and short treatment time) relies heavily on the disulfide bond reactivity as the cornerstone of the process. The reductive cleavage of disulfide crosslinks is as essential to fiber softening as is their reformation to the stability of newly imparted configuration. Needless to say, throughout the waving process, secondary interactions (hydrogen bonds, salt links, and van der Waals interaction) participate therein, and their more or less intense contributions reflect themselves in the overall efficacy of the process. Nevertheless, so far it is the disulphide bonds that represent the *sine qua non* for waving.

Over the years, there have been numerous attempts to explore the ways of permanently altering the configuration of keratin fibers by exclusive manipulation of secondary bonds. Some success has been shown in fibers modified by inclusion of bulky apolar residues, high-temperature steam setting, or by blocking cysteine side chains with hydrophobic maleimides. Except for high-temperature steam setting of wool (in the crimping process), these approaches found little, if any, practical applications either because of the complexity and severity of treatment conditions or because of less-than-acceptable results.

Neutralizing Compositions

The principal active ingredient in most of the neutralizing formulation is acidic hydrogen peroxide at a concentration of 1% to 3%. Sodium bromate and sodium chlorite are occasionally used on account of their good stability and absence of bleaching power. H_2O_2-compatible conditioning agents, such as cationic surfactants or silicone emulsions, are often included to ensure easy combing, smooth texture, and control of flyaway of the waved hair.

Evaluation of Waving Efficacy

Although "permanent" is the defining adjective of the imparted wave, there are many other considerations that are important in the assessment of wave quality.

Among them are tightness of curl, its springiness, feel of the hair, its luster, and combability. Ultimately, the most reliable way of judging the characteristic of a wave is on the head of the consumer, and thus it is not surprising that this subjective approach has always been used as the final evaluative tool of product prototypes. The importance of using the consumer as the testing probe is of particular importance in assessing the wearing characteristics of the imparted wave. So far, no satisfactory laboratory procedures have been developed to accurately mimic this important aspect.

The objective laboratory measurements on both hair tresses and single fibers are the backbone of the development of new prototypes, screening processes, and further evaluation of competitive products.

Single-fiber technique is particularly useful in differentiating between different chemical systems (e.g., TGA vs. sulfite, alkaline vs. acid wave) providing rapid information as to the efficacy of the process. Some measure of the durability can be gained by submitting these microsprings to the action of hot water, detergents, and stress. Using calibrated fibers, the mechanical measurements can provide the first impression of process aggressiveness.

Clearly, hair tresses are required for evaluation of assembly characteristics—combability, flyaway, luster, and feel—as well as for porosity determination by liquid retention. The curl appearance, both wet and dry, can be both assessed and appropriate recordings (photographs) made. The tresses are also required for water-setting evaluations where the imparted wave is used as a background to the consequent hair-setting experiments. In this case, conventional techniques of set impartation and durability evaluation in the humidity chamber are used.

The cosmetic history of hair (before waving, straightening, color, bleaching, and weathering) influences not only the degree of damage that the waving lotion can inflict, but also the quality of wave it can impart. Both single-fiber techniques and tresses should be used in the manner previously described.

Prevention and/or Masking of Damage

Hair damage has become a constant companion and by-product of most of our hair care practices (e.g., combing, brushing, heat setting, coloring, and bleaching), with hair waving making its own contribution. Because the problem of damage is so widespread, there has been vigorous activity over the years to develop some general specific ways of damage repair. So far, none that are effective and reliable are available. A more promising route is that of damage prevention (the word "alleviation" would be more appropriate) or damage masking.

Taking a somewhat detached view, one should add that there is no evidence for the epidemic of hair damage with almost any of the cosmetic treatments of hair, and the damage reflected is usually well tolerated by the consumer for the benefits gained. But, even from the discussion presented, some measures can be taken to at least limit the damage inherent in the process. Thus, if a gentle wave is required, an acid type of a thiowave or one based on sulfite might be an alternative. With alkaline waving, the potential of acid-buffered salt solution before water rinsing should be considered, primarily for fine or weathered hair. Recovery of disrupted membrane structures can apparently be attained in the use of sulfite waves by using a cysteine after-treatment (a genuine harbinger of damage repair?). Consumers considering combined treatments (e.g., waving and bleaching or haircoloring) should wave the hair first, as the reduction step, irrespective of whether sulfite or TGA is used, is much more damaging to hair with an oxidative cosmetic history.

To mask and/or limit the damage after waving (and hair combing comes here to the fore), the use of both conditioning shampoos and conditioners is imperative. Clearly, the waving formulations containing effective cationic polymers are at an advantage, as every anionic detergent used in the shampoo (and the shampooing process can be quite abrasive to the wave-sensitized cuticle) forms a lubricating complex with the surface-adsorbed polymer.

Finally, as previously indicated hair undergoes faster weathering and sun lightening after waving than before it. Here, sunscreens would come in handy as long as they are delivered from an effective vehicle, such as a hairspray or mousse. The protection attained from sunscreen-containing shampoos or conditioners has been virtually nil up to now.

Hair Straightening

Although the molecular mechanism underlying hair-straightening parallels that of waving or setting of hair, there are some distinct differences in the composition of formulations and, naturally, in the mode of their application. There are essentially two different categories of straightening preparations: (1) those that aim at temporary straightening and (2) those designed to accomplish permanent effects.

Temporary Hair Straightening

The most frequently used technique in this category is hot combing. An oily material (pressing oil) is applied to hair, which is then combed under slight tension with a heated comb. The straightening effect is produced by the combined action of heat and the moisture present in hair. The function of the pressing oil is threefold: (1) to act as a protective heat-transfer agent between the comb and the hair, (2) to serve as a lubricant reducing the drag of the comb, and (3) to function as a barrier slowing diffusion into the hair of moisture from the scalp and environment, and thus delaying reversal of the straightening effect. Pressing oils are mostly based on petrolatum and mineral oil blended with some wax and perfume. Frequent combing dulls and damages the hair, leading ultimately to hair breakage.

Permanent Straightening

The most effective class of permanent straighteners (relaxers) is that based on alkali as an active ingredient. Sodium or potassium hydroxide or sodium carbonate in combination with guanidine is used at concentrations of 1.5% to 3% in a heavy cream base. Even though the recommended treatment time is only 5 to 20 minutes, the straightening effects in general, surpass those obtained with either thioglycollates or bisulfites because of the different chemistry of the process and the greater aggressiveness of alkaline relaxers. A 15 minute treatment irreversibly decreases the cystine content of hair to two-thirds of its initial value.

The damaging action of strong alkali on hair is not restricted to disulfide bonds alone. Apart from the potential of main-chain scission (peptide bond hydrolysis), the very nature of the base (high pH) leads to a build-up of negative charges in hair that results in increased swelling, which is intensified by a concurrent breakdown of the disulfide bonds. Great care must be exercised in the use of alkaline relaxers because even brief contact with skin can cause blistering. It should be pointed out that the chemistry underlying the hair-straightening process with alkaline relaxers is fundamentally different from the systems based on thioglycollates or sulfites. The alkalis

(irrespective of their nature, i.e., sodium hydroxide (lye), calcium hydroxide, or guanidine) cleave the disulfide bonds, and this cleavage is almost instantly followed by formation of new (monosulfide) crosslinks. The efficacy of this secondary process varies between 50% and 70%, and this, to a great extent, accounts for the observed alkali damage. If the crosslinking step is not accomplished at that time, there is no known way of crosslink reformation at a later stage of the process. The so-called neutralization step in alkaline relaxing should never be confused with that used in thio or sulfite processes, where its main function is bond rebuilding. For alkaline relaxing, the neutralization aims at removing the excess alkali from hair, which is accomplished by acid-containing (or acid-buffered) shampoo.

Alkaline thioglycollate has also been used as the active ingredient in relaxers, although in somewhat different form from that encountered in conventional waving lotions. The latter are always thin, promoting a fast lotion penetration into the tightly wrapped hair on the curler. Relaxers, in contrast, are formulated into thick (viscous) oil-and-water (o/w) emulsions or creams using a high concentration of cetyl and stearyl alcohols and high-molecular weight polyethylene glycols together with fatty alcohol sulfate as an emulsifier. The cream is worked into the hair while it is combed straight. The high viscosity of the formulation helps to maintain the extended configuration of the hair during processing, which may take from 30 minutes to 2 hours depending on the initial curliness of the hair. In the course of the treatment, the hair is often recombed to assure its straight configuration. Upon thorough rinsing, conventional oxidizing neutralizers (hydrogen peroxide, bromates, or perborates) are used as a final step of the process.

In recent years, hair-straightening compositions based on mixtures of ammonium bisulfite and urea have been introduced and found to be of some use, primarily in the Caucasian hair-straightening market. The re-crosslinking of bisulfite-treated hair is more effectively accomplished with an alkaline rinse (pH 8–10) than with oxidizing agents, although the latter can also be used to destroy the residual sulfite reductant.

Hair Coloring

Not belittling the importance of hair texture and its geometry, it is perhaps not surprising that the quintessence of hair beauty manifests itself in its color. This has been well recognized as much in the distant past as it is now. It is truly remarkable how nature, using the melanin pigment (a substance without an identifiable chromophore) as its primary colorant, has been able, via clever manipulation of physics and chemistry, to generate hundreds of shades ranging from the Scandinavian blondes through Scottish redheads to the intense black hair of Africans and Asians. Still, the need for color enhancement, or indeed its change, continues to exist and is clearly the driving force of the hair-coloring market as reflected by the variety of products available to the consumer.

Setting aside the diversity of claims and application techniques, hair-coloring products fall into two general categories: (1) those that are based on materials that are inherently colored, and (2) those that use colorless precursors and develop their hair-coloring characteristics only on interaction with an oxidant. Dyes of the first category are used in temporary (or shampoo-removable) products and semipermanent color formulations (color stable to several shampooings). The second category forms the mainstay of so-called permanent or oxidative hair colors. Their importance lies not only in the durability of the effect, but also in that the natural color

of hair can be modified, almost at will, to any desirable hue or shade, whether darker or lighter than the original. This is accomplished in one step through a combination of bleaching of the natural pigment present in the hair and simultaneous color development. Such shade manipulation is clearly not available in the temporary or semipermanent products, the function of which is primarily restricted to the build-up of color intensity. Although semipermanent colorants lack the versatility of oxidative dyes, they are recognized as being gentler to hair because no peroxide is required. In each hair-coloring category, a sizeable number of dyes (or precursors) are required to attain a viable palette of shades. These dyes differ not only in their chromophoric characteristics, but also in their affinity to hair, water solubility, and overall photostability. In color impartation, a delicate balance of constituent dyes is essential to obtain uniform and desirable results. However, subsequent exposure of dyed hair to shampooing, sunlight, perspiration, and simple wear and tear often highlights the differences in properties of dyes that can result in unpredictable color changes.

Temporary Hair Colorants

As the name itself implies, the dyes of this class are scheduled for only a fleeting residence on hair being removed at the first shampoo opportunity. Although the postulate of fast removing precludes the use of low-molecular weight colorants that could penetrate the hair shaft, it nevertheless extends the palette to almost any toxicologically acceptable dye that can be aesthetically formulated into a cosmetic vehicle. In general, food colors, cosmetic colors, pigments, or even textile dyes can be considered. To be avoided are strongly basic dyes that have a tendency toward intensive skin staining and a high affinity for chemically damaged and weathered hair. Table 4 lists some of the dyes currently used in temporary hair products.

Temporary color formulations are of the "leave-on" type, which means that they are applied to hair usually after shampooing and left there to dry. They can be simple solutions of dyes incorporated into a styling mousse or can be complexed with surfactants whereby more color can be deposited on hair. By the very nature of the application, the intensity of the coloring effect is low, but sufficient, to produce aesthetically pleasing effects. Exposing the colored hair to heat (whether from a blow dryer or bonnet) may bring about some increase in durability of the imparted color to shampooing.

Semipermanent Hair Colorants

This class of dyes, initially designed exclusively for gray-hair coverage, has progressively grown in importance as the formulation changes extended the color palette and improved the durability of the imparted color.

Table 4 Temporary Hair Colorants

Name	Type
FD&C Blue No. 1	Triphenyl methane
D&C Red No. 22	Xanthene
Ext. D&C Yellow No. 7	Nitro
D&C Brown No. 1	Disazo
D&C Green No. 5	Antraquinone
D&C Red No. 33	Azo

Table 5 Semipermanent Hair Colorants

Name	Color
4-nitro-*o*-phenylenediamine	Yellow
1,4,5,8-tetra amino anthraquinone	Blue
1,4-diamino anthraquinone	Violet
N′-(2-hydroxy ethyl)-2-nitro-*p*-phenylene diamine	Red

The majority of products' features a blend of low- and medium-molecular weight dyes that are capable of penetrating into the hair shaft, thus assuring a moderate degree of fastness. A blend is necessary to achieve the desired color and obtain a match between the roots and the more permeable ends. The dyes that are used are generally nitrophenyldiamines, nitroaminophenols, and, to a lesser extent, aminoantraquinones. Table 5 lists some of the dyes in use.

Several product forms are available: lotions, shampoo-in formulations, or mousses. In all cases, the dyes are dissolved or dispersed in a detergent base that contains a thickener so the product stays on the hair without running or dripping. Application time of 20 to 40 minutes is common, after which time the product is rinsed off and frequently followed by a conventional shampoo.

Recently, formulations providing more durable (color stability up to 20 shampoos) effects have become available. They consist of the conventional semipermanent dyes blended with oxidative dye precursors, which in conjunction with dilute hydrogen peroxide produces longer-lasting color moieties. Such products are occasionally referred to as "demipermanents." Unlike the conventional semipermanent products that are sold in single containers, they, in addition to the dye mixture, contain a separate package of the oxidant.

Often included in the semipermanent category is also the only vegetable dye that is permitted to be used in the United States: henna. Henna consists of the dried leaves of the plant *Lawsonia alba,* which grows in North Africa, the Middle East, and India. The active ingredient, lawsone (2-hydroxy-l-4-naphtoquinone), constitutes about 1% of the dried leaves (10). Using henna, only limited reddish shades can be achieved. In some products, henna is mixed with other dyes to obtain more variety in color. Such products are then subject to the label warnings used for coal-tar dyes.

A mention should also be made of metallic dyes, which are still popular with men. These products usually contain dissolved lead acetate and elemental sulfur. After application to hair and subsequent air exposure, the lead salt reacts to form a mixture of insoluble sulfides and oxides imparting to the hair a darker color, thus providing a gradual gray coverage.

Permanent Hair Colorants

Unmatched by other colorants in the shade palette, durability to shampooing, resistance to fading, and absence of skin staining, the permanent (oxidative) hair colorants have justifiably carved off the largest market share in hair dyes worldwide. Available in a variety of forms (e.g., lotions, gels, shampoos, and creams), these products deliver reliable results that last until the new hair grows out. Most often, the colorant is supplied as a two-component kit consisting of a mixture of colorless dye precursor and of a stabilized solution of hydrogen peroxide. Occasionally, the peroxide is provided in the form of a powder, such as urea peroxide or sodium

Table 6 Oxidation Dye Colors

Coupler	Colors on hair With	
	PPD	*P*-Aminophenol
Recorcinol	Greenish-brown	Yellow-brown
m-Phenylenediamine	Blue purple	Violet
m-Amino phenol	Reddish-brown	Light orange
1-Naphtrol	Blue violet	Reddish-violet
2-Methyl resorcinol	Yellow brown	Yellowish-beige
2-Amino pyridine	Dark grayish-blue	Light grayish-green

perborate. The two components are mixed immediately before use, applied to hair, and left for 20 to 40 minutes before being rinsed out with water.

The color formation commences upon mixing and involves complex reactions between precursors and the oxidant. The precursors consist of two classes of reactants: (1) primary intermediates, comprising *o*- and *p*-aminophenols and phenylenediamines, which upon oxidation by peroxide form colored quinone imines; (2) secondary intermediates (couplers). The latter condense with the imines to yield the final dye molecules. Whereas the color-forming reactions take place in the dye mixture, a significant fraction of the dye precursors diffuse rapidly into the hair together with the hydrogen peroxide forming the colorant moieties throughout the hair fiber. The process is carried out at alkaline pH, which also favors the bleaching of the melanin pigment by H_2O_2. Table 6 lists some of the primary and secondary intermediates and colors they produce.

Depending on product form, the formulation of the dye base varies. Ammonia and ethanol amines are preferred alkalizing agents, and a mixture of surfactants and solvents are used to solubilize the dyes and assure wetting of hair. A small quantity of reducing agents is added to prevent the auto-oxidation of the dyes during storage. It is important to realize that hydrogen peroxide, which so effectively assists in both the color development and lightening of hair pigment, also displays a less desirable role in causing oxidative hair damage. Although the damage associated with a single application is slight, the cumulative effect of subsequent treatments is quite perceivable.

Hair Repigmenting

The idea of dyeing the hair by melanin has always been alluring. The "natural" aspect of the colorant implied durability of the coloring effect and its insensitivity to haircare regimens, or shade fading—all these have been factors providing continuous incentive to use the potential of such process. Apart from intense patenting in this field, several papers have recently appeared (11,12) that describe such coloring systems as well as the characteristics of repigmented hair. Recently, products based on the principle of melanin repigmentation of hair have appeared on the market, but the information available to date is too scanty to offer a reliable judgment as to the market viability of these products.

Bleaching

The bleaching action of hydrogen peroxide has been already alluded to in the context of permanent hair coloring, and quite satisfactory levels of lightening can be obtained with such products.

To attain a significantly greater level of bleaching, hydrogen peroxide is combined with bleach accelerators or "boosters." The latter are mixtures of ammonium, potassium, or sodium persulfates. The salts are packaged as dry powders and mixed with hydrogen peroxide just before use. Thickeners and alkalizers (usually sodium silicates) are included in the booster package. Processing time depends primarily on the initial hair color and the desired level of lightening. The pH of these formulations is usually much higher than that of the permanent hair-color products and so is the concentration of H_2O_2. AH of these factors—high concentration of peroxide, presence of oxidizing salts, and high pH of the process—connote significant oxidative damage of hair. After thoroughly rinsing off the bleaching mixture, the hair should be given an acidic "bath" (lemon juice or solution of citric acid or diluted vinegar) followed by a 5- to 10-minute treatment with a "deep" conditioner.

Hair-Color Safety and Regulatory Issues

Because of the allergenic potential of some of the materials used in hair dyes [primarily *p*-phenylenediamine (PPD)], hair colorants in the United States display on the label as a legal requirement a warning, plus instructions for a 24-hour patch test with the precursors and hydrogen peroxide mixed in the same manner as in use. As required by Section 601 (a) of the Federal Food, Drug and Cosmetic Act, the warnings reads as follows:

> This product contains ingredients which may cause skin irritation on certain individuals and a preliminary test according to accompanying directions should be made. This product must not be used for dyeing the eyebrows or eyelashes; to do so may cause blindness.

It should be noted that allergic contact dermatitis to hair dyes appears to be far less common today than decades ago. It has been suggested that PPD, although a strong sensitizer, is not likely to produce skin sensitization because of the short contact time with skin and rapid reaction of PPD with the oxidizing agent and couplers (13).

Concerns as to the possible carcinogenicity of some hair ingredients arose in 1975 when these were reported to be mutagenic for bacteria in bioassays (14). Presently, it is not clear how significant a risk this poses to users of hair dyes. Because hair dyes have been in common use for over 50 years, epidemiological studies on cancer rates in occupationally exposed groups or the users of hair dyes are of particular value. So far, the results of most of these suggest that hair dyes do not pose a carcinogenic risk (13).

CONCLUDING REMARKS

This chapter on hair cosmetics is only a brief overview of what is used and practiced in this broad and important segment of personal-care products. Many aspects of hair chemistry and physics have only been fleetingly discussed, including properties of single hair fibers and their assemblies. The whole area of claim substantiation has been left out, together with the description of physicochemical techniques that are relevant to this subject. For a fuller account on these topics, the reader is referred to an excellent book by Zviak (15) and recent publications on haircare (16) and cosmetic-claim substantiation (17).

REFERENCES

1. Menkart J, Wolfram LJ, Mao I. J Soc Cosmet Chem 1966; 77:769.
2. Wolfram LJ. In: Orfanos, Montagna, Stiitgen, eds. Hair Research. Berlin: Springer-Verlag, 1981:479.
3. Kligman AM, Shelley WD. J Inv Dermatol 1958; 30:99.
4. Cunliffe WJ, Perera WD, Thackeray P, Williams M, Foster RA, Williams SM. Br J Dermatol 1975; 95:153.
5. Breuer MM. J Soc Cosmet Chem 1981; 52:437.
6. Schwartz AM, Perry JW, Belch J. Surface Active Agents and Detergents. Vol. 2. Huntington, New York: Robert E. Krieger, 1977.
7. Preston WC. J Phys Chem 1948; 52:84.
8. Stevenson DG. J Text Inst 1959; 50:T548.
9. Zviak C, Vanlerberghe G. In: Zviak C, ed. The Science of Hair Care. New York: Dekker, 1986:57.
10. Stamberg J, Werczberger R, Koltin Y. Mutat Res 1979; 62:383.
11. Brown K, Mayer A, Murphy B, Schultz T, Wolfram LJ. J Soc Cosmet Chem 1989; 40:65.
12. Brown K, Marlowe E, Prota G, Wenke G. J Soc Cosmet Chem 1997; 48:133.
13. Corbett JF. Rev Prog Coloration 1985; 75:53.
14. Ames BN, Kammen DH, Yannesaki E. Proc Nat Acad Sci USA 1975; 72:2423.
15. Zviak C, ed. The Science of Hair Care. New York: Marcel Dekker, 1986.
16. Johnson DH, ed. Hair and Hair Care. New York: Marcel Dekker, 1997.
17. Aust LB, ed. Cosmetic Claims Substantiations. New York: Marcel Dekker, 1998.

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Oral Care Products

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THE TEETH AND ORAL ENVIRONMENT

Like all mammals, each person generally has two sets of teeth during a lifetime. The first set, known as deciduous, primary, or "milk" teeth, begins to appear in infants between the age of five and nine months. All 20 of these "baby" teeth are generally in place by two and a half years of age.

The second set or permanent teeth forms within the gums during the period from infancy to puberty. These teeth, also known as succedaneous teeth, begin to erupt around age five, displacing the deciduous set as they appear. There are 32 permanent teeth. An individual will spend 91% of his or her lifetime chewing with these permanent teeth if they are properly cared for.

Of the 32 permanent teeth, 16 are located in the upper jaw or maxillary dental arch that is part of the cranium, or the skull, and is immovable. The other 16 are located in the mandibular dental arch that is part of the lower jaw and is the movable part of the skull. Each type of tooth is equally divided between these two dental arches.

The Parts of a Tooth

Each tooth consists of three parts: the part above the gum which is visible, the part below the gum which is not visible, and the constricted portion or neck between the other two parts.

The crown is the enamel-covered portion of the tooth. The root is the portion of the tooth, which by means of the periodontal ligament relates to the osseous (bony) structures of the jaw. The root makes up about two-thirds of the total length of a tooth.

The Tissues of a Tooth

A tooth is made up of five different tissues, each with a specific and important function. Serious disease in any of these tissues can affect the entire tooth and result in its decay and/or destruction. These tissues are:

- Enamel, a hard white outer covering surrounding the crown of the tooth, which protects it from wearing away due to the pressure of chewing.

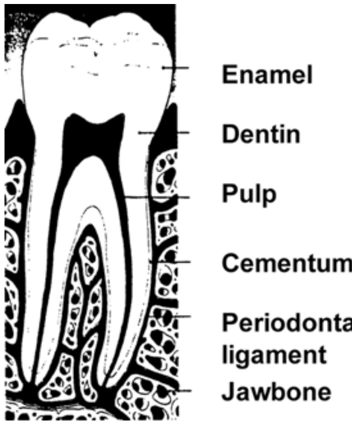

Figure 1 The five tissues of a tooth. *Source*: *Your Teeth And What They Do*, a publication of the American Dental Association, 1986.

It consists largely (96–98%) of inorganic substances, mainly calcium and phosphate.

- Dentin, a yellowish bone-like tissue under the enamel, which provides support and forms the bulk of the tooth structure, extending almost to its entire length. It is covered by the enamel on the crown and the cementum on the root. Chemically, dentin is composed of 20% organic and 75% inorganic matter, or collagen and calcium phosphate, respectively. The remaining 5% is mainly water and other mucosubstances.
- Pulp, a soft tissue within the center of the crown and root containing nerves, blood vessels, and lymph vessels which produce dentin and provide nourishment for the tooth throughout its life. Because of its rich supply of blood and nerves, the pulp also functions as a defense system against bacterial invasion and as a sensory signal of injury by causing toothache.
- Cementum, a thin bone-like tissue, which covers the root and serves as a means of attaching the tooth to the surrounding bone.
- Periodontal ligament, a layer of connective-tissue fibers that stretches between the cementum and the bone connecting the tooth root to the jawbone. It also cushions the tooth from the pressures exerted during the chewing (Fig. 1).

The Periodontium

The periodontium (from the Greek "peri" meaning "around" and "odous" for tooth) is a functional system consisting of several different tissues that surround and support the teeth. This system is also called the "attachment apparatus" or the "supporting tissues of the teeth." Anatomically, the term refers only to the connective tissue between the teeth and their bony sockets.

The tissues that make up the periodontium include the gingiva, the periodontal ligament, the cementum, and the alveolar bone or jawbone. Their good health is of great importance to the overall health of your mouth and the survival of your teeth.

The Gingiva

The gingiva, commonly called the "gums," is the most external part of the periodontium. It is composed of dense fibrous tissue which forms a close ring-like attachment around the neck of the teeth and connects with the epithelial covering (oral mucosa) that lines the mouth (Fig. 2).

The gingiva is firm in consistency and does not move from its underlying structures. It is covered by a smooth vascular mucous membrane, which is tender to touch and bleeds easily when penetrated or bruised. It also overlays the unerupted teeth, and the pain which occurs during the teething process is the result of the new tooth pushing through this sensitive tissue.

Clinically the gingiva is divided into:

- Free marginal gingiva that is about 1.5 mm wide and forms the skin-like soft-tissue fold around the teeth. The narrow shallow groove present between the tooth and the free gingiva is known as the gingival sulcus. It is approximately 0.5 mm deep and 0.15 mm wide, and surrounds the tooth on all sides. The bottom of the sulcus is made up of cells from the junctional epithelium. The size of this groove or "pocket" is of great importance when determining the health of the periodontium and the stability of the teeth.
- Attached gingiva that is firmly connected to the hard surface of the tooth by means of a ring of specialized tissue known as the junctional epithelial attachment. The attached gingiva becomes wider with age and may vary considerably among individuals and from tooth to tooth.
- The cells in the junctional epithelium are continuously being renewed during a lifetime and have a turnover rate of every four to six days. This results in a very permeable tissue that serves as a pathway for the metabolic products produced by the bacteria present in the mouth. This area plays a key role in the maintenance of periodontal health.
- Interdental gingiva that varies in depth and width, occupies the area between adjacent teeth.

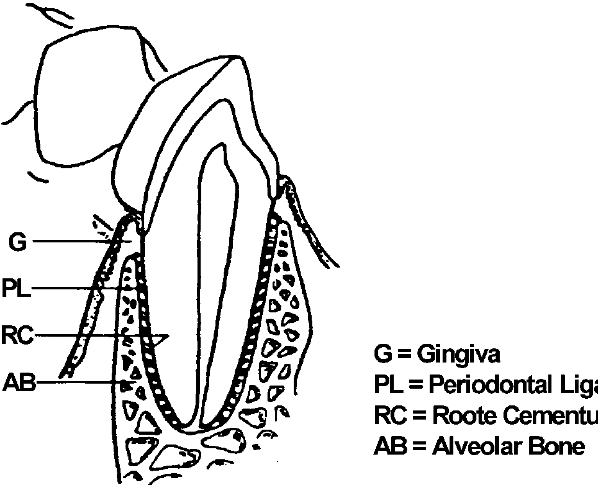

Figure 2 A healthy tooth with its periodontium. *Source*: Lindhe J. *A Textbook of Clinical Periodontology*. Copenhagen: Munksgaard, 1985:19.

The Periodontal Ligament and the Cementum

The periodontal ligament occupies the space between the root surface of the tooth and the alveolar bone or jawbone. It is composed of connective tissue fibers, blood vessels, nerves, and other cells. Its function is to provide the connection between the cementum layer of the tooth and the jawbone, the teeth and the gingiva, and between each tooth and its neighbor. Anatomically, the cementum is a part of the tooth, but functionally, which belongs to the tooth-supporting apparatus because the gingival and periodontal ligaments are anchored to it.

The Alveolar Bone

Alveolar bone, also referred to as the jawbone, develops along with the formation of the teeth throughout pregnancy and continues to grow during the eruption of the teeth in childhood. Three types of alveolar bone have been defined: compact bone, trabecular bone, and alveolar bone proper. The trabecular bone provides the major support structure of the teeth and is composed mainly of fatty marrow in adults.

Other Parts of the Mouth

There are several other areas in the mouth, which are important. These include the tongue, the palate, the salivary glands, and the oral mucosa or lining of the mouth or oral cavity itself.

Palate

The palate forms the roof of the mouth and consists of two portions: the hard palate in the front area behind the upper teeth and soft palate at the back, at the entrance to the pharynx or throat area.

The hard palate separates the mouth from the nasal cavity and serves as the roof of the mouth and the floor of the nose. The soft palate aids in swallowing and sucking functions.

Tongue

The tongue is the main organ of the sense of taste and an important organ of speech. It also assists the teeth in the chewing and swallowing of food.

The tongue is situated in the floor of the mouth and is connected to various muscles in the epiglottis and pharynx, or throat. It is covered by mucous membranes, and numerous mucous and serous glands as well as taste buds. Internally, it consists of fibrous tissue, muscles, blood vessels, and nerves.

Saliva and the Salivary Glands

Saliva is a fluid containing water, mucin, protein, salts, and enzymes. It is produced and secreted into the oral cavity by three pairs of salivary glands: the submaxillary, sublingual (or submandibular), and parotid glands.

The submaxillary glands are located beneath the floor of the mouth on the inner side of the jaw. Saliva secreted from these glands enters the mouth through a duct or opening beneath the tongue known as the duct of Wharton. The sublingual glands also are located below the floor of the mouth, but closer to the midline and pour their saliva into the mouth through a number of small ducts—the duct of

Bartholin and the duct of Rivinus. The parotid glands lie below the ears and along the sides of the jaws. The ducts from these glands enter from the inner cheek opposite the second upper molars.

The salivary glands contain both serous and mucous cells. The secretion from the serous glands is thin and watery while that from the mucous glands contains mucin and is, therefore, thicker and slimier.

These glands are controlled by the autonomic (or involuntary) nervous system and react by reflex to both direct and indirect stimulation. For example, saliva is automatically and directly produced when you take a mouthful of food, but it is also indirectly produced when you talk about or see some food you particularly like.

Saliva has several important functions:

- to assist in the digestion of food;
- to prepare food for swallowing by altering its consistency;
- to moisten and lubricate the mouth and lips;
- to cleanse the mouth and teeth from food debris and other foreign materials; and
- to excrete organic and inorganic substances from the body.

The latter function, especially, can result in serious inflammation of the oral mucosa (the lining of the mouth) and the gums.

Oral Mucosa—the Lining of the Mouth

The oral mucosa or "mucous membrane" lining of the mouth also has special functions that are important to oral health. This thin, freely movable lining is composed of several layers of epithelial cells. These are the same type of cells found on the outer layers of your skin and which serve as a protective covering. However, within the mouth, this covering lies on a thick layer of "mucous membranes" which secrete mucous.

As discussed earlier, mucous contains a protein material known as mucin, which is formed within the cytoplasm of these epithelial cells. As the mucin accumulates, the cells become distended until they finally burst, discharging their contents onto the surface of the mouth. The mucous coats the epithelial surface serving as a protection against injurious substances in the mouth or as a means to trap small foreign particles.

The production of mucous can be greatly increased by stimulation caused by infection, allergy or temperature. We are all familiar with the increased production of mucous caused by a cold or sore throat. Often, "cold sores" or "canker sores," which are small painful ulcerations on the oral mucosa, appear during these illnesses. Therefore, the oral mucosa can also be used as a mirror that reflects the general health of the body.

DENTAL DISEASES WORLDWIDE

Dental diseases including cavities (caries), tartar (calculus), sore gums (gingivitis), and periodontitis (loss of teeth supporting tissue) are global issues. The annual cost of dental care in the United States exceeds $37 billion, out of which roughly $6 billion is spent to repair the ravages of decay (1). However, the cost of dental disease cannot simply be measured in monetary terms. Other factors also need to be

considered; for example, the loss of teeth leading to impaired chewing ability, speech problems, and changes in facial aesthetics, which can cause embarrassment. The well-being of a person may also be compromised due to the associated dental pain, inability to chew properly, and potential of the infection spreading from the mouth to other parts of the body (2).

Currently, a tremendous amount of time is spent by dentists and hygienists to clean the teeth and the associated structures to prevent dental disease. Alternative methods to prevent dental diseases, which can be used by the general population are being developed to reduce the amount of time spent with the dental professional.

Factors Affecting Delivery of Actives in Mouth

Before discussing specific product technologies for the prevention and treatment of oral disease, we need to understand the general principles underlying the efficacy and delivery of therapeutic agents in the oral cavity (Fig. 3).

The effective use of active ingredients in oral products is dependent on several factors; some of the major ones are depicted schematically in Figure 8. Normally a therapeutic toothpaste or mouthrinse contains an active ingredient or drug that must be dissolved in the formulation. Mouthrinses currently on the market are aqueous-based formulations but contain numerous other ingredients, which must be compatible with the drug. The potential for undesirable interactions between ingredients is a major concern of formulators and manufacturers. Some interactions are specifically designed, such as the increased solubility of poorly water-soluble drugs (for example, triclosan) by adding surfactants and other ingredients to form a microemulsion. However, incompatible ingredients are sometimes unknowingly used, especially, in complex formulations where there is an incomplete understanding of the chemistry (3).

The packaging material can also be a source of compatibility problems. Any number of possible interactions can affect, either directly or indirectly, the availability of the drug in the formulation. This can usually be evaluated in the laboratory on new and aged samples of the product.

Drugs that are complexed with other materials, though soluble in the formulation, may exhibit reduced bioavailability in vivo. The term "bioavailability" is usually used to express a temporal relationship of free drug concentration at the target site. In this case, after mouthrinsing or toothpaste use, the bioavailability is the concentration of a free drug in the environment of the target site and the rapidity

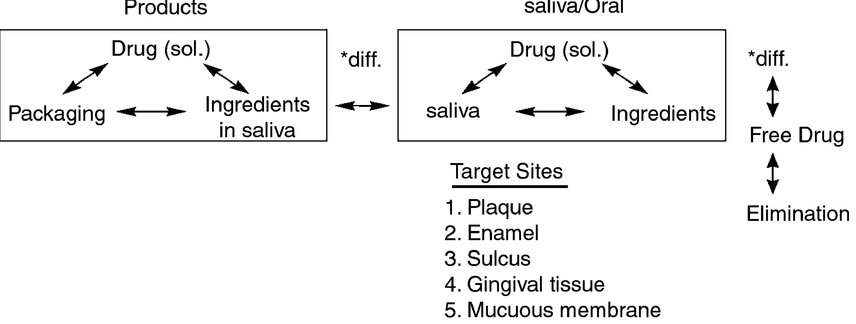

Figure 3 Factors affecting active agent delivery.

at which it disappears. This can be determined, provided the site can be sampled and the drug concentration measured in the medium contacting the target site (i.e., saliva, plaque fluid, and crevicular fluid).

The duration of exposure may be important. Since most of the dose in the oral product is expectorated, the time it stays in the mouth should be long enough for the optimal retention of the drug. This has been determined for some orally used antiseptics such as chlorhexidine and triclosan. In general, 30 to 45 seconds is usually sufficient.

Once introduced into the oral environment via a toothpaste/gel/mouthrinse, the residual drug must diffuse in saliva before it can reach its intended site of action. In saliva, the drug is then free to interact with salivary components before reaching oral surfaces. In theory, only a free available drug can interact optimally with target sites. Such sites include plaque, enamel, the gingival sulcus, gingival tissue, and the mucous membranes.

The amount of drug retained on oral surfaces after use is also considered to be important, as subsequent desorption of the drug into the microenvironment of the target site could provide a sustained effect. This will be determined mainly by the substantivity of the particular drug used. Because of the long dosage interval commonly practiced with the product (once or twice a day), highly substantive drugs may have a distinct advantage because of their longer presence in the oral cavity. Superimposed upon this is the normal clearance process by which materials are removed from oral surfaces by salivary flow. The longer a drug can be retained in the environment of the target site in active form, the better chance there is to exert a therapeutic effect.

Evolution of Technologies in Oral Products

Historically, dentifrices or toothpastes were developed to keep the teeth clean and free of stains. The essential ingredients of toothpaste are: a thickening agent, an abrasive cleaning agent, a surfactant, a humectant, flavor, and active therapeutic agents. One of the first dentifrices contained an abrasive (precipitated calcium carbonate) and a small amount of powdered soap. This toothpaste was irritating to the tissues of the mouth because the pH was relatively high due to its soap content (4). After the Second World War, many companies undertook scientific research to develop dentifrices, which were milder, gentler, and also had therapeutic properties. Instead of soap, a synthetic detergent—sodium lauryl sarcosinate—was introduced in toothpaste. Besides preventing irritation, the synthetic detergent improved the taste and was also shown to control plaque acids that cause cavities. Figure 4 provides an overview of the evolution of technologies in oral products. The category is driven by scientific advances and consumer benefits which have been broadly classified as a good smile (Fig. 4).

Stain Removal and Whitening Toothpastes

There are two types of stains on teeth: (a) stain on teeth (extrinsic stain) and (b) stain in the tooth (intrinsic stain). The extrinsic stain may originate from chromogenic materials in food or drink; whereas the intrinsic stain could be caused by therapeutic agents, such as tetracycline, or excessive fluoride exposure during teeth development (below the age of five). Several investigators have studied mechanisms of stain formation and developed methods to remove dental stain (Fig. 5) (5).

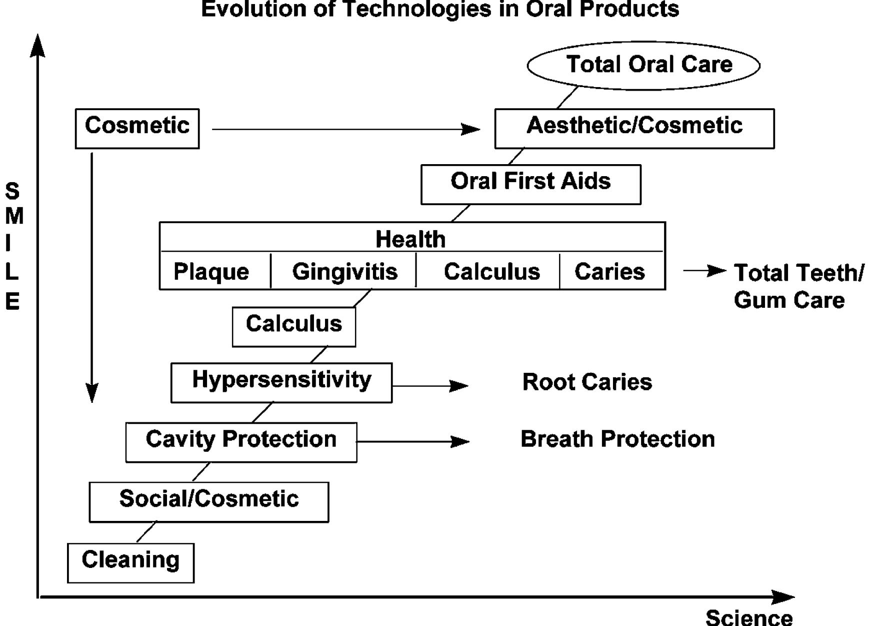

Figure 4 Evolution of technologies in oral products.

The evolution of whitening/cleaning technologies in toothpaste and gel is depicted in Figure 5. The most commonly used procedure for removing stains on teeth is the use of abrasives such as silicon dioxide, dicalcium phosphate dihydrate (DCPD), and aluminum salts such as calcined alumina. All these are used in combination with detergents to remove stains. In the early eighties, calcined alumina or enzymes with or without tartar-control ingredient, such as pyrophosphate, were added. Later on, fluoride preparations such as hydrogen peroxide, urea peroxide, or calcium peroxides were added to remove both intrinsic and extrinsic stains. To assess performance, several laboratory tests were developed but none of them correlate with in vivo stain removal on teeth. Therefore, in vivo clinicals are the best way to assess stain removal. Typical results from in vivo studies are depicted in Table 1.

It can be seen that the addition of calcined alumina with pyrophosphate gave good stain removal in vivo. Another procedure for stain removal in vivo is by

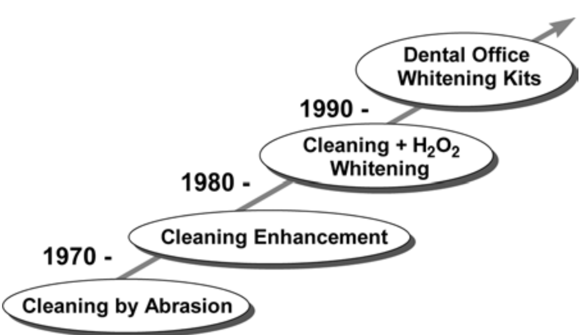

Figure 5 Evolution of cleaning/whitening technologies.

Table 1 In Vivo Stain Reduction Six Weeks Postbrushing

Dentifrice treatment	Stain reduction (%)
SiO_2/NaF toothpaste	No change
SiO_2/NaF/tripolyphosphate	14.0
SiO_2/Calcined Alumina/Pyrophosphate	49.0

reflective spectroscopy using a Minolta chromameter. The color change is measured by •E (difference in color). The higher the positive value, the whiter the teeth. Using •E in vivo, one would get •E of 2 to 4 with above technologies (in the taste). If one adds peroxide, the value could reach as high as 6. For the reference, an in-office treatment by a dentist would provide •E of 7 to 8 following two weeks procedure.

At-home Tooth Whitening Products

Tooth whitening is an important health and cosmetic benefit because stained teeth can cause embarrassment and affect the quality-of-life. One hallmark of a healthy appearance is a bright smile. Consequently, whitening by both professionally prescribed and monitored products and over-the-counter (OTC) products has become a growing business representing a $500 million market in the United States.

The basic workhorse for the whitening of teeth is peroxide, primarily hydrogen peroxide, carbamide peroxide, and percarbonate. The standard in-office treatment is conducted by covering teeth with gauze that has been saturated with 20–25% peroxide with or without heat lamp. Since higher concentration of peroxide is corrosive and can burn gingival tissues, the teeth must be isolated by rubber dam. At the end of bleaching time, teeth are thoroughly rinsed with water and polished. Multiple appointments may be required to achieve the desired tooth color.

More recently, at-home whitening using peroxide in paint-on varnishes or strips has become common. The varnish consists of a film-forming polymer, such as polyox and carbomer mixture with 6% peroxide or a strip consisting of polyethylene backing with 6% peroxide gel have been marketed for at-home whitening. These seem to give similar efficacy but not as good as in-office treatment.

Dentifrices to Reduce Offensive Bad Breath

Local mouth odor is caused by oral bacteria reacting with salivary proteins to form volatile sulfur compounds (VSC). Tonzetich has shown that hydrogen sulfide, methyl mercaptan, and dimethyl sulfide (H_2S, CH_3SH, and $(CH_3)_2S$, respectively) are the major components of mouth odor. A gas chromatographic method was developed to objectively measure VSC directly from orally exhaled air as an alternative to the organoleptic/sensory method. This instrumental method has, in turn, permitted investigators to carry out studies in a number of areas relevant to human malodor (6).

There are two methods currently available to assess the magnitude of oral malodor. The first is the organoleptic or sensory rating approach, and the second is the gas chromatograph instrumental method. A study was conducted to determine the correlation between these two methods in a controlled clinical study. An excellent correlation ($r = 0.78$) has been established between the instrumental method and the sensory evaluation. Using the analytical technique, the effect of dentifrices on oral odor has been evaluated in a variety of clinicals. A baseline reading is taken in the morning. The subjects then brushed with a placebo or an active dentifrice,

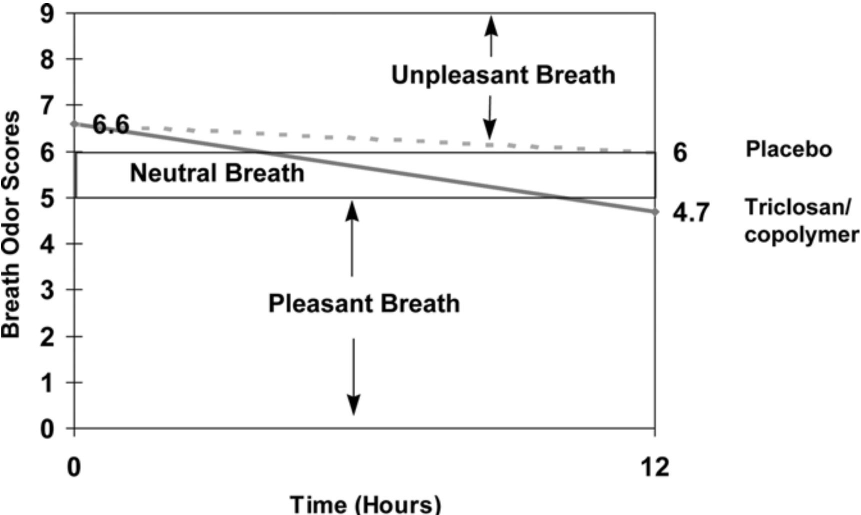

Figure 6 Plot of breath odor scores.

and then readings are taken three or 12 hours post-treatment to assess the effects. A dentifrice containing the antibacterial triclosan and a copolymer polyvinyl methyl ether–maleic acid (PVM/MA) has been developed. This provides sustained reduction in mouth odor. The typical clinical results are summarized in Figure 6 (7).

Oral Breath Film

A recent addition to the breath-freshening sector is breath film. It is a water-soluble thin film embedded with a high concentration of flavor and cooling agents and, sometimes, additional active ingredients. The film dissolves instantly on the tongue and releases an intense burst of flavor and active ingredients to freshen breath. The film usually appears as small pieces, each weighing under 100 mg and less than 100 μm thick. The films are often packed in a small, discrete, and convenient package. This unique delivery form has proven to be very appealing to consumers for its fast action, convenience of use, and novelty. It is particularly popular among youth and young adults. Since its introduction to the U.S. market in October 2001, the category has experienced an explosive growth. The total worldwide sales it generated in 2002 reached around United States $250 million, accounting for about half of the global mouth fresheners sector.

The basic compositions of the breath film are polymer film formers, flavors, cooling agents, plasticizers, emulsifying agents, and sweeteners. In addition, secondary film formers, structure modifying agents, fillers, and colorants are often incorporated into the film matrix to enhance the properties of/and to aid the processing of the film. The polymers used to form the film are usually natural polymers or their derivatives such as pullulan, hydroxyalkyl cellulose, alginates, gelatin, starches, and so on. The manufacturing of the film is relatively straightforward. Most films are produced by conventional solvent casting process. Typically, well-mixed aqueous slurry composed of all the ingredients is cast onto a moving substrate to form a wet film through a device that controls the film thickness. The substrate carries the wet film through a drying oven and a dry film comes out from the other end of the oven. During this process, the majority of the water and a large amount of flavor are lost.

The control of the drying condition can critically impact the final film properties. Three physical parameters of the film are probably the most important: dissolution, elastic modulus, and tensile strength. The dissolution of the film needs to be fast and clean so that it does not leave a slimy mouth feel. A film with a high elastic modulus tends to be stiffer and therefore, less prone to curling. The tensile strength of the film has to be high enough to undergo the downstream processing, which includes releasing the film from the substrate, converting and packaging the film. To meet these criteria, a suitable polymer film former has to be properly chosen. One example is the use of low molecular weight hydroxypropyl methyl cellulose as the film former.

The primary function of the breath films in the market today is to freshen breath. Most films achieve this function by using a high concentration of flavor to mask the mouth odor. However, this effect is usually superficial and transient. In some cases, mouth rinse ingredients, such as essential oils and metal ions were incorporated to deliver antibacterial effect and neutralize volatile sulfur compounds. With the increased acceptance of this delivery by consumers, it is likely that films with improved oral benefits are desired in the future. Further, the soluble films are also being explored to deliver benefits beyond oral care, such as medical and nutraceutical benefits.

Therapeutic Dentifrices

Dentifrices to Control Caries (Cavity)

It is well known that the formation of dental caries is a result of interactions among the tooth enamel, environment (saliva), plaque fluid, and ingestion of dietary carbohydrates. These interactions are also important in the formation of dental plaque on teeth. Dental plaque plays an important role in the formation of caries since it is the plaque bacteria that produce acids from sugars. However, the production of acids by plaque bacteria and subsequent dissolution of tooth enamel is not a constant process. Instead, it appears to be cyclical. At a given time, plaque acids attack the enamel surface and deplete it of minerals, creating a small microtrauma at the surface. These areas are actually called incipient caries or white spots and occur long before caries could be detected by dentists or hygienists. If left unchecked, the process eventually results in destruction of the teeth. Since caries is not a continuous process, early lesions can be repaired through interactions of various elements in the oral environment, that is, supersaturation with respect to calcium phosphate in saliva, fluoride, and pH of the plaque fluid (8).

Tooth enamel is not a smooth impervious surface, instead it is porous, and an apparent lack of activity on the surface may mask actual activity below. To create a caries lesion the acids must penetrate the enamel structure, which consists of hydroxyapatite (HA) crystals surrounded by an organic matrix consisting of water, protein, and lipid materials and this they do by removing some of the mineral from the crystalline rods below the surface of the teeth. This demineralization weakens the structure and, if unchecked, eventually results in a subsurface lesion often called a white spot, which will appear to be chalky and whiter than the normal surrounding tooth surface.

Continuation of the demineralization process results in the creation of cavities. This occurs when the surface enamel collapse as the underlying structure of mineral rods can no longer maintain the tooth structure. However, not all white spot lesions progress to cavities, one of the prime reasons being the process of remineralization which occurs when minerals are redeposited into the enamel that has

been weakened by bacterial acids. Remineralization can, therefore, only take place when there has been loss of tooth structure through demineralization. Thus, demineralization and remineralization are continuous processes with loss and replacement of minerals into the enamel within the oral environment. The most soluble mineral in the teeth is thereby replaced by the most insoluble calcium phosphate, such as DCPD. If the environment is rich in DCPD, the process of remineralization occurs. This process is greatly enhanced by fluoride ions, which convert DCPD into fluorohydroxyapatite, which forms on, and within, the tooth increasing the resistance to acid attack (9).

Fluoride increases remineralization by increasing the rate of crystal growth, but to restore tooth structure, a supersaturation of calcium phosphate in the environment is also necessary. The process of remineralization has been shown to be controlled by the presence of fluoride and a supersaturation of calcium and phosphate in plaque fluid. Thus, the tooth and environment are in a seesaw battle. Under healthy conditions when supersaturation is high and plaque acids are low, the ambient calcium phosphate (DCPD) in plaque fluid is sufficient to maintain healthy enamel. When the caries challenge is high and plaque is producing more acids, supersaturation with respect to DCPD decreases and demineralization occurs. Fluoride inhibits lesion formation by enhancing the process of remineralization, and this enhancement is greatly influenced by supersaturation of the plaque fluid with respect to HA.

Fluoride dentifrices are capable of adding minerals (remineralization) to early caries lesions. This process can be measured in vivo by using the model of intra-oral remineralization. A dose–response effect of fluoride is shown in Figure 12, which shows the percentage of mineral gains in either enamel or dentine following two weeks use of either 1100 ppm F from MFP (sodium monofluorophosphate) or sodium fluoride, NaF. Both fluoridating systems extend the same degree of mineralization as an equal concentration. Human clinical studies for caries (cavity) prevention require three years to document anticaries effect. In those studies, mean reduction in caries varies from 25% to 40% depending upon the population used in the study and whether or not the study area had water fluoridation. Current efforts are to enhance efficacy of 1000 to 1500 ppm of fluoride in dentifrices with additives such as xylitol, a nonfermentable sugar, or the antibacterial triclosan. These additives have been shown to boost the effectiveness of fluoride in toothpaste (Figs. 7, 8) (10).

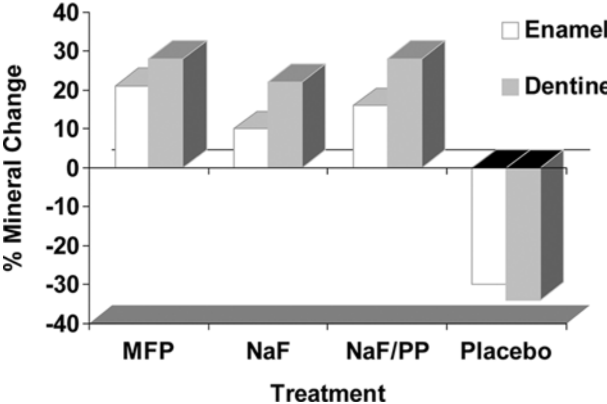

Figure 7 Average percent mineral changes for enamel and dentine.

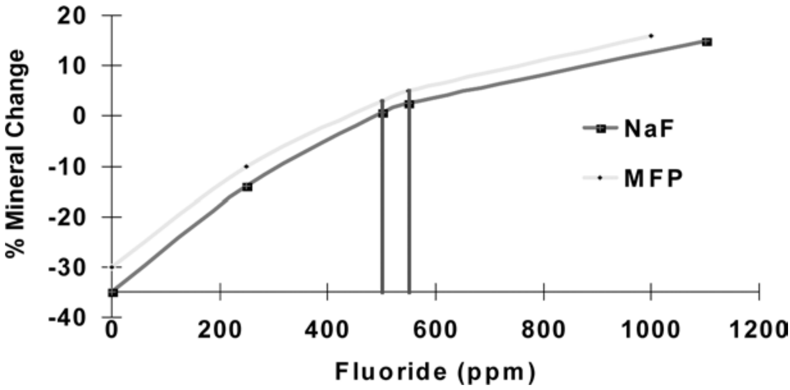

Figure 8 Fluoride dose response for MFP and NaF dentifrices.

Anticalculus and Anticavity Technologies

Calculus buildup on teeth is a worldwide problem. For nearly 5000 years since the time of the Sumerians, calculus has been considered an important factor in the etiology of periodontal diseases. Although it is not considered to be a principle cause of periodontal diseases today, calculus is an important contributor to the formation of dental plaque, which is implicated in periodontal disease. At a given time, hundreds, even thousands, of hygienists around the world are removing calculus buildup by mechanical cleaning. These procedures are very labor intensive and may cause a great deal of discomfort to the patient.

The extent and incidence of calculus, in the general U.S. population, has been shown in a comprehensive oral health survey by the National Institute of Dental Research (11). The data shown in Figures 14 and 15 indicate the incidence of calculus. Calculus was observed in 34% of school-aged children. In adults, 25% to 30% had calculus buildup above the gingival margin, but 60% to 65% had deposit below the gingival margin. Older adults showed an even higher incidence. The extent of calculus in the population indicates a need to develop an effective but safe chemical means to prevent calculus buildup on the teeth. This is especially important for the countries where the dentists and hygienists are not readily available. Therefore, the development of the technologies to prevent calculus is important around the world from a public health point of view (Figs. 9, 10).

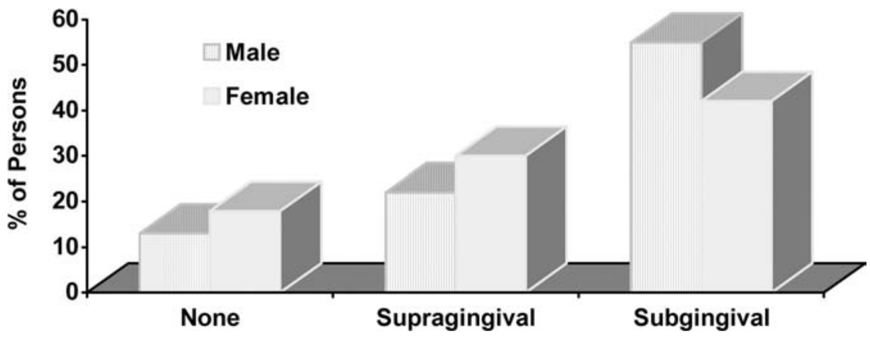

Figure 9 Data from "Oral Health of U.S. Adults," NIDR, 1985.

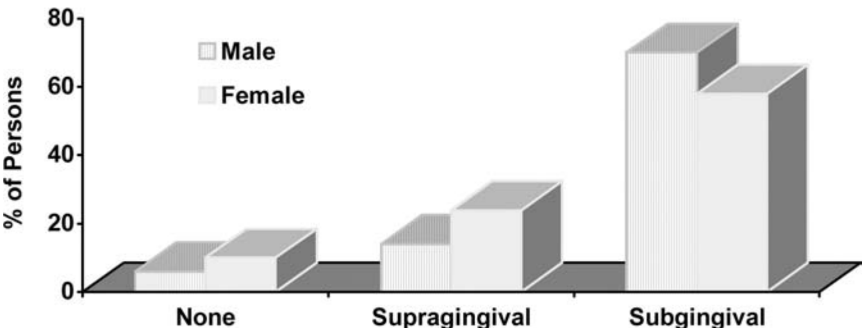

Figure 10 Calculus in U.S. population (seniors, "Oral Health of U.S. Adults," NIDR, 1985).

Chemical Composition of Dental Calculi on Teeth and Dental Materials

Dental calculus consists of both organic and inorganic components. The organic portion is a combination of epithelial cells, leukocytes, microorganisms, and polysaccharides. The inorganic part is primarily calcium phosphate salts, which include: carbonated hydroxyapatite (CHA), DCPD, and octacalcium phosphate (OCP). The X-ray diffraction patterns and infrared absorption spectra of human dental calculi and the samples obtained from the dentures and tooth surfaces show that the inorganic component of calculus from dentures is principally CHA, while material from tooth surfaces is a mixed calcium phosphate phase β-TCPC (Mg-substituted), CHA, and OCP. The deposits are primarily basic calcium and phosphate salts (12).

Technologies for the Prevention of Calculus Formation

A general method of removing calculus is by mechanical means. The mechanical methods are labor intensive and painful. Another approach is to develop a chemical method of preventing the formation of the basic phases of calcium phosphates. A large number of agents have been proposed to retard the formation of calculus on to surfaces. These agents are usually compounds, which inhibit the formation of calcium phosphate salts to the crystalline phases. Among the most effective inhibitors are pyrophosphate, pyrophosphate plus polymer, and zinc salts. In general, agents usually work via a surface effect. The inhibitors adsorb to the growing (calcium phosphate) crystals and they reduce the formation of crystalline phases allowing calcium phosphate to remain in an amorphous phase. In general, two types of tests have been used to evaluate the inhibitors. One test follows the spontaneous formation of HA (Fig. 11) using a supersaturation environment, which stimulates the plaque fluid. The second test is a seeded crystal growth for HA which uses the driving force equivalent to saliva environment (Fig. 12). Using these tests, the relative value of efficacy of these inhibitors is summarized, shown in Table 2. It shows that the most active inhibitor is pyrophosphate. Also a combination of pyrophosphate and the copolymer of pyrophosphate, and the copolymer (PVM/MA) provides an enhanced efficacy. Zinc salts, on the other hand, require a higher concentration for effectiveness. The relative clinical efficacy of these agents in various dentifrices is summarized in Table 3. Available data from the composite of several clinical studies indicate that calculus inhibition with the pyrophosphate and sodium fluoride combination is roughly in the range of 26%; with the copolymer/pyrophosphate (1.3% soluble pyrophosphate to 3.3%) the calculus reduction ranged as high as

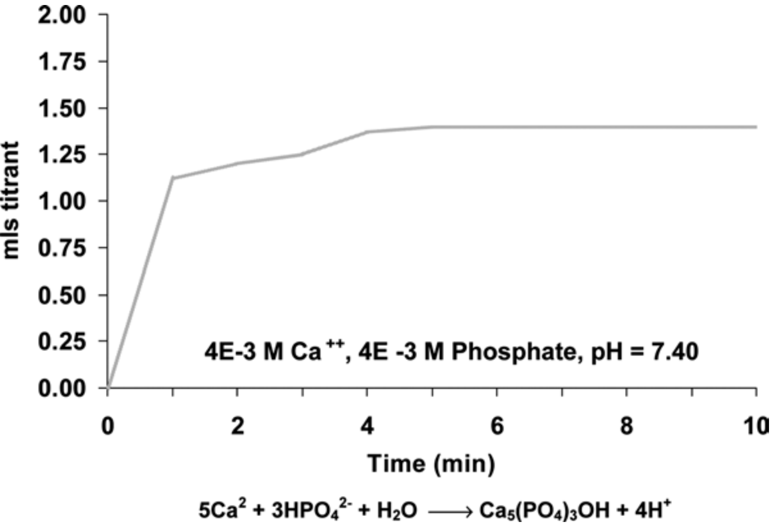

Figure 11 HAP formation.

50%; zinc salts require higher concentration for efficacy (2% or above). With a lower concentration (0.5%), the efficacy against supergingival calculus formation is very poor (13).

Mechanisms of Action of Anticalculus Agents

The mechanism for the inhibition of calculus formation by anticalculus agents are schematically illustrated. The calcium and phosphate from saliva or from plaque fluid precipitate form a precrystalline phase that matures to crystal phase in the absence of an inhibitor. In the presence of an inhibitor the amorphous phase is stabilized and the conversion of the crystalline phase is delayed. This is clearly evident from the electron micrographs of an calculus formed in the presence and the absence of inhibitor. In the absence of an inhibitor, the crystal size was very large and well defined; in the presence of an inhibitor, the deposit was very small and has morphology of amorphous calcium phosphate (Fig. 13).

The current technologies used for inhibiting calculus formation also contain fluoride. When the application of a potent inhibitor of calcium and phosphate crystal growth coexists with fluoride, a crystal growth promoter, we need to understand how they work together. The inhibitor prevents the formation of HA, but how do two agents coexist in the same system and exert the respective effect? Our early data indicated that crystal growth inhibitors work on tooth surfaces, whereas the fluoride ion works within the teeth. The effect can be explained by the fact that the calculus formation occurs on the teeth (above), whereas the demineralization occurs in the

Table 2 Calculus Control Technologies: Relative Efficacy

Compound	Inhibition (ppm)
Pyrophosphate	4.0
Pyrophosphate + Copolymer	3.0
Zinc	60.0

Table 3 Clinical Efficacy in Toothpastes in Humans

Toothpaste	Mean reduction in calculus vs. placebo (%)
3.3% pyrophosphate + NaF	26
3.3% pyrophosphate + 1% PVM/MA/NaF	50
1.3% pyrophosphate + 1.5% PVM/MA/NaF	47
0.5% zinc citrate + MFP	14
2% Zinc + sodium fluoride	38–50

Abbreviations: PVM/MA, copolymer polyvinymethyl maleic acid.
Source: From Ref. 13.

subsurface region of the enamel (under pellicle). The presence of pellicle on the tooth allows the selective transport of fluoride and the inhibitor. This mechanism has been elucidated by studies of natural inhibitors of crystal growth in saliva. The study indicated that the crystal growth inhibitory effect of the natural inhibitor could be overcome by the addition of the fluoride. This effect was because of neither the displacement of an adsorbed inhibitor by fluoride nor the activation of secondary growth sides. Rather, the effect was explained on the basis of the increased driving force of precipitation and the incomplete blockage of crystal growth sites on the basis of the steric effect. This has now been confirmed by in vivo studies.

Technologies to Reduce Tooth Sensitivity

The next evolution of toothpaste chemistry was developed as a means to prevent pain caused by sensitive teeth; i.e., hypersensitivity. Dentinal hypersensitivity is defined as an acute, localized tooth pain in response to thermal, tactile, or air blast stimulation to exposed dentine surfaces. Normally, the roots of teeth are covered by the gingival or gum tissue but when the gum recedes, the underlying tooth surface is exposed. Once exposed, with time, abrasion and erosion will remove the thin layer of cementum, thus exposing underlying porous dentine. Exposure of the dentine

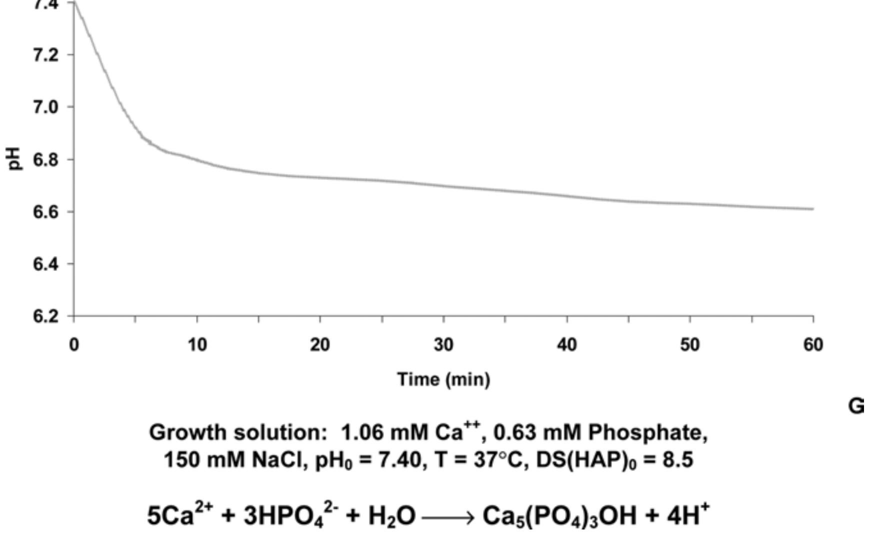

Figure 12 Crystal growth.

Figure 13 Mechanism of pyrophosphate/copolymer/NaF on tartar formation.

surface to dietary or bacterial acids can expose the dentine pores or tubules at the surface. It is well known that exposure and the presence of open tubules (Fig. 14A) on the surface is associated with increased dentinal hypersensitivity. The dentine tubes contain fluid.

Mechanistically, hot or cold stimuli can cause this fluid to expand or shrink, stimulating underlying pulpal nerve resulting in pain. Currently, salts of potassium are available as preventive therapies in OTC toothpaste. Various other agents such as potassium nitrate are believed to cause reduction in nerve activity by altering the threshold of pulpal nerve excitation. These approaches have been combined in a single toothpaste containing potassium nitrate and copolymer that adhere to tooth surfaces. Figure 14B shows occlusion that could result from in vitro treatment of dentine with such a toothpaste. Unfortunately, this therapy requires two to three weeks treatment before a reduction in sensitivity is observed. Therefore, there is currently a strong need for a fast reactive material in toothpaste which could rapidly reduce dentinal hypersensitivity (14) (Figs. 14A and B).

Multibenefit Technologies in Dentifrices

The next development in dentifrice technology was to incorporate antibacterial agents with fluoride and tartar reducing compounds.

Microbiota of Dental Plaque: Health Vs. Disease

The basic research within the past 30 years clearly established the role of dental plaque at the interfaces of tooth/gingiva as the main cause of gingival inflammation, which could lead eventually to periodontitis. The previous studies by Löe et al. (15) and subsequent studies by Syed and Loesche (16) indicated that there was a threshold level of bacteria, which was compatible with gingival health (Figs. 15 and 16). When that threshold level of bacteria increased by at least 2 or 3 orders of magnitude, then gingival inflammation was initiated. Therefore, the prime purpose of

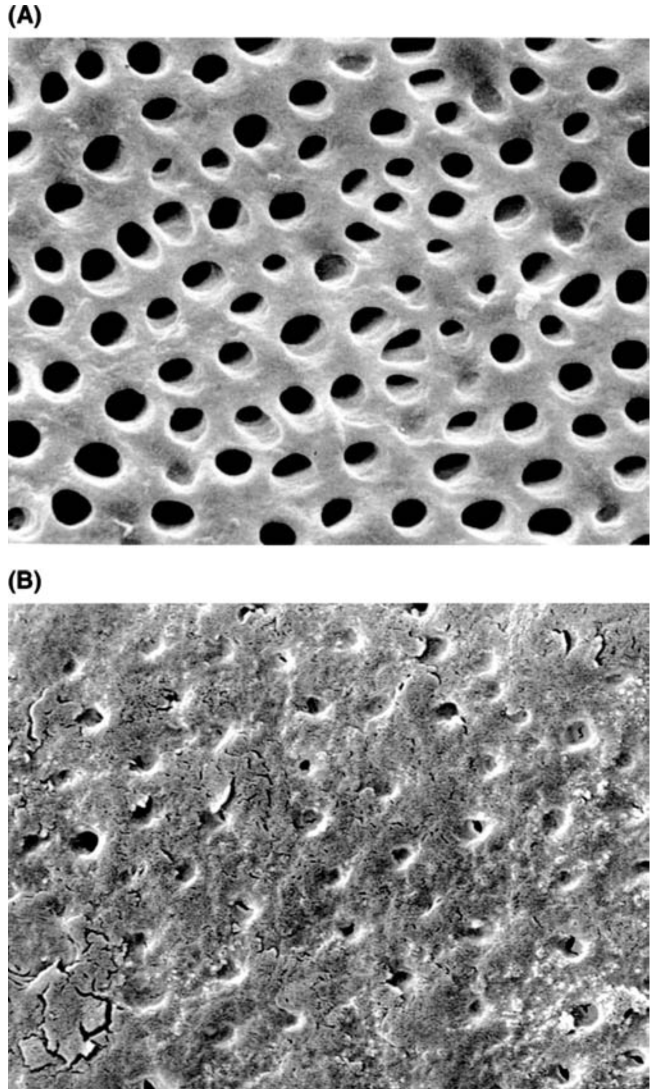

Figure 14 (**A**) Open dentinal tubules. (**B**) Occluded dentinal tubules.

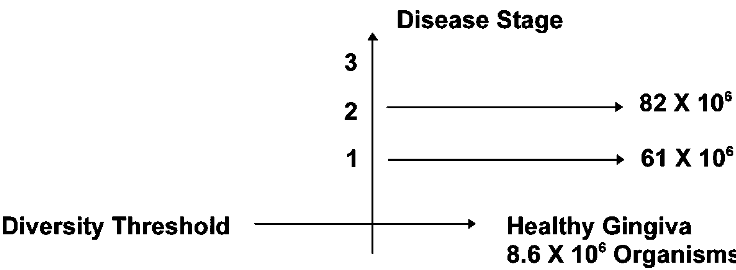

Figure 15 Microbiota (health vs. disease).

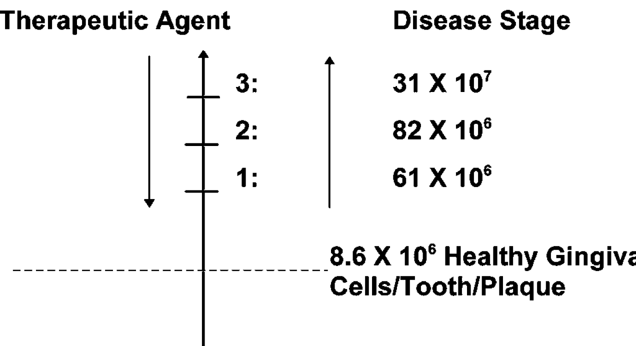

Figure 16 Therapeutic strategies for antiplaque agents.

chemical antiplaque agents is to bring the microflora to a healthy level at the gingival interfaces, primarily by reducing the total mass of microbiota at the surface, or by reducing the total number of pathogens at the surface (Figs. 17 and 18).

Since dental plaque is principally composed of microorganisms, it is logical to use antibacterials to reduce or prevent plaque formation. The rationale is that the antibacterials will either inactivate bacteria in the existing plaque or prevent colonization. However, early studies clearly showed that 99% of bacteria in the oral cavity must be killed to inhibit plaque formation for only six hours, provided teeth are brushed twice daily. As the oral cavity is an open system, the chance of continued reinfection is ever present. Based on recent studies, the general characteristics of antibacterial agents useful for an antiplaque effect can be summarized in Table 4. For an antibacterial antiplaque agent to be effective, a broad-spectrum activity against oral microflora is required, because the microbial composition of the plaque is complex. With cationic antibacterial agents, a minimum inhibitory concentration (MIC) in the range of 0.1–0.5 μg/ml against oral pathogens has been noted. However, the current understanding of the pharmacology of antibacterial antiplaque agents indicates that there are factors other than antibacterial activity in determining sustained antiplaque effect on teeth. These factors include the retention and release of antibacterials on oral surfaces, as well as their efficacy in the presence of the salivary environment. Furthermore, it is important that a given antibacterial does not affect taste, otherwise the patient's compliance would be very poor. Another consideration for use in oral products is compatibility with polishing agents and surfactants, as both of these ingredients are important for controlling stain on teeth as well as emulsifying flavor oils, which are incorporated in the oral hygiene products for compliance. Other important considerations are a low toxicity and a minimum potential to disturb the normal microbial oral ecology (17).

Cationic Antibacterial Agents

Among the widely studied agents are cationic antibacterials such as chlorhexidine digluconate (CHDG), benzethonium chloride (BTC), and cetyl pyridium chloride (CPC). CHDG is more effective than BTC or CPC and has higher retention in the

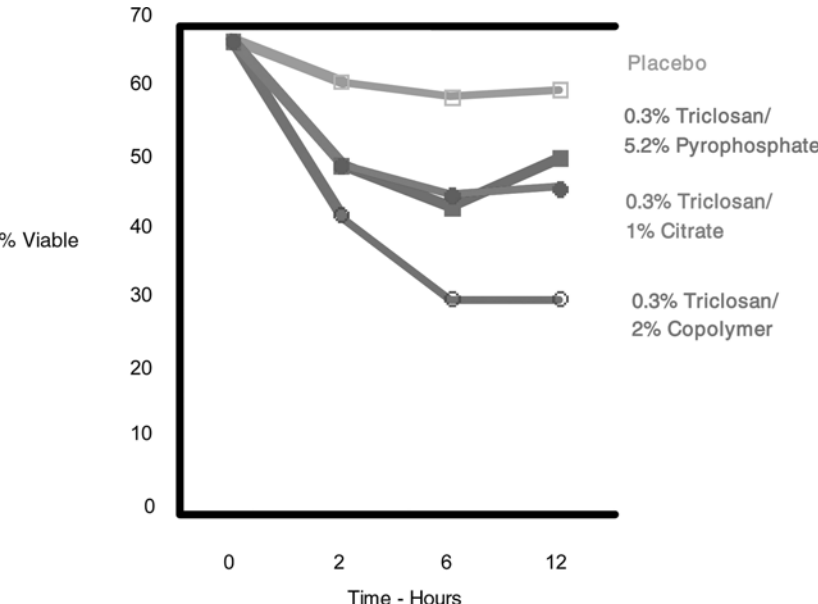

Figure 17 Plaque viability study determined via a fluorescent dye technique.

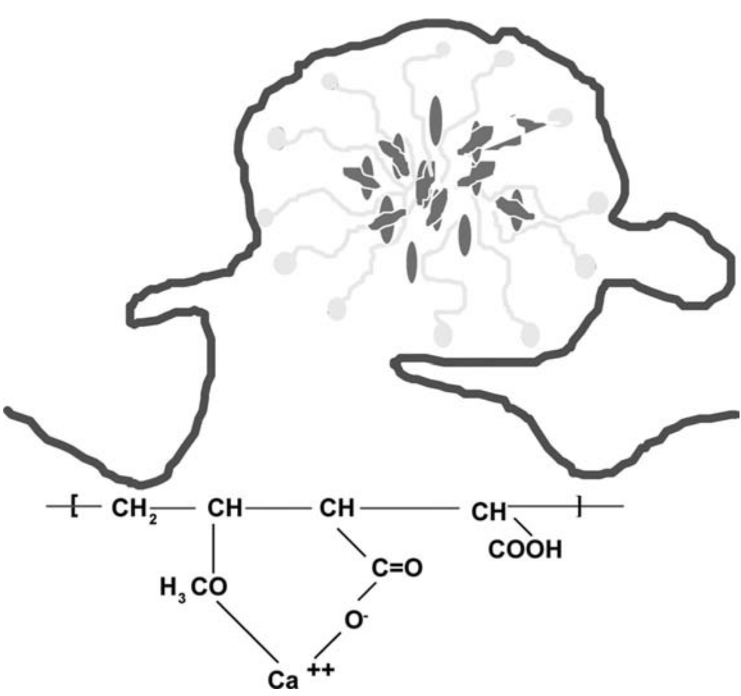

Figure 18 Mechanism of retention of triclosan on oral surfaces by the copolymer. The solubilizing group (methoxyether) traps triclosan/surfactant micelle while the attachment group (COOH) binds to calcium in an adherent liquid layer on tooth/enamel interface.

Table 4 Characteristics of Antibacterials for Plaque Effects

Broad spectrum antibacterial activity
Substantivity to oral surfaces
Good taste
Compatible with toothpaste ingredients
Low toxicity
No disturbance of oral ecology

oral environment. They also differ with respect to their reaction with salivary protein, which is an important parameter for the retention of cationic antibacterials on oral surfaces; increased retention provides a sustained release of concentrations active against oral pathogens.

Long-term clinical studies have demonstrated the efficacy of cationic antibacterials against plaque, gingivitis, and plaque microflora. However, these agents cause unacceptable staining of teeth and an increase in calculus formation. Therefore, their use in oral hygiene products clearly is limited (17).

Noncationic Antibacterial Agents

More recently (during the past 10 years), there has been tremendous interest in noncationic antibacterials, which provide multibenefits such as plaque, gingivitis, calculus, and caries reduction. This is primarily based on a nonionic antibacterial agent, triclosan, which has broad-spectrum antibacterial activity against gram-positive and gram-negative bacteria. For triclosan to be effective, a delivery system is required to increase its residence time in the oral cavity. A copolymer of polyvinyl methyl ether (PVM) and maleic acid (MA) has been shown to accomplish that. This copolymer was well suited for improving the delivery of triclosan, since PVM/MA has been shown to react with hard and soft surfaces in the oral cavity. In a four-day short-term study of de novo plaque formation, we evaluated a series of different antibacterial agents. We found that triclosan actually needs an improved delivery system, primarily a copolymer, to enhance its retention to both tooth and oral epithelial surfaces (18).

One of the important principles developed is that retention per se is not the only factor in antiplaque activity; the retained concentration has to be active biologically. To demonstrate this principle, we conducted a series of studies to understand how much triclosan was retained postbrushing. In one of the studies, we compared three triclosan formulations, each having a different enhancing system (Table 5). As can be seen (Table 6), even after 14 hours, a significant amount is retained in plaque, a concentration above the MIC of triclosan for oral bacteria (MIC being 0.3–4 μg/ml). The next important step was to determine whether this retained amount was active biologically. A plaque viability assay was used, in which we exposed the plaque to two fluorescent dyes to discriminate between live and dead bacteria by measuring the ratio of green to red fluorescence. In this study, one could quantitatively measure the ratio and ascertain whether the retained amount was active biologically. In one of the typical studies shown here, brushing with the placebo toothpaste gave some reduction of plaque viability; the triclosan copolymer system gave the highest reduction in viability, and the other systems, such as triclosan/pyrophosphate and triclosan/zinc citrate, were not significantly different

Table 5 Noncationic Antibacterials: Comparative Study for In Vivo Plaque Inhibition

Treatment	Mean P on all surfaces + S.D.	SNK Group
Placebo	1.46 ± 0.12	A
0.12% CHDG	0.53 ± 0.17	B
0.2% SnF_2 (Rinse)	1.10 ± 0.16	C
0.06 Triclosan	1.00 ± 0.14	C
S0.06 Triclosan + Gantrez	0.72 ± 0.17	B
0.06 Triclosan + PVPA	0.67 ± 0.16	B

Abbreviations: Gantrez: PVM/MA, PVPA, polyvinylphosphonic acid; SNK, Student Neuman Keuls test.

from the placebo (Fig. 18). These results have been corroborated by an independent six-month clinical study by Renvert and Birkhed (Table 6) (19).

The mechanism by which the copolymer enhances the delivery of triclosan has been elucidated (Fig. 23). The polymer has two groups: one is the attachment group and the other is the solubilizing group. The solubilizing group retains triclosan in surfactant micelles, and the attachment group reacts with the oral surfaces via calcium in the liquid adherent layer.

Triclosan is then slowly released via interactions with salivary environment. In long-term clinical trials, this technology has now been evaluated around the world in 12 six-month plaque/gingivitis studies, three calculus studies, three caries clinical trials, and five long-term studies monitoring the oral microbial population. The results of all these studies indicated that this technology was effective against plaque, gingivitis, calculus, and caries. No side effects of staining or calculus increase were seen. There was also no disturbance of the oral microbial ecology.

One of the most exciting aspects of triclosan is its "double-barrel" effect. This unique antibacterial not only kills bacteria, but also neutralizes the products of bacteria, which could provoke inflammation. We have shown that triclosan was a potent inhibitor of both cyclo-oxygenase and lipoxygenase pathways. It not only inhibited these enzymes in vitro but also inhibited the release of their products (prostaglandins and leukotrienes) in gingival fibroblasts that were stimulated by interleukin 1-β. These data were clinically confirmed in a study in which we blocked the antibacterial effect of triclosan but maintained its anti-inflammatory effect. Thus, triclosan has a "double-barrel" effect—both antibacterial and anti-inflammatory. This unique feature is not provided so far by other antibacterial, antiplaque agents (20). This terminology has been tested in long-term clinical studies to reduce onset and progression of periodontal diseases (21,22).

Table 6 Plaque Triclosan Levels After Brushing (μg/ml)

Postbrushing (hr)	0.3% Triclosan/ copolymer $n = 12$	0.3% Triclosan/ pyrophosphate $n = 12$	0.3% Triclosan/1% zinc $n = 12$
2	38.83 ± 18.28[a]	20.90 ± 14.14	30.60 ± 13.6
14	4.14 ± 1.72	2.74 ± 2.11	3.95 ± 1.79

[a] $P = 0.05$, compared to a placebo toothpaste.
Source: From Ref. 23.

Table 7 Therapeutic Mouthrinses

Mouthrinse	Active agents	Typical reduction in the diseases vs. placebo
Fluoride rinses	225 ppm F	50% reduction in caries children (3 yrs)
Tartar + calculus	1% pyrophosphate anion; 100 ppm F plus a copolymer of PVM/MA (0.5%)	30–35% reduction in tartar formation after six months use
Antiplaque/antigingi vitis	0.03–0.06 Triclosan + 1% copolymer PVM/MA + F	20–30% reduction in plaque/gingivitis after three months of use

MOUTHWASH

Mouth rinses currently on the markets are aqueous-based formulation where the therapeutic agents are at lower concentrations than toothpaste. For example, the general population uses toothpaste 1 g or 1 cc on the brush, but the rinses are used in 10 to 15 cc and some lower concentration of the actives are incorporated. Also, the rinses do not contain polishing agents or thickeners. A typical therapeutic rinse contains surfactants, flavor, active agent, and water. The general principles of active agent delivery, which were outlined above also apply for the active agent delivery in the mouthwash. Table 7 summarizes the typical clinical performance versus a placebo rinse of therapeutic rinse.

STRATEGY FOR CLINICAL STUDIES IN ORAL CARE PRODUCTS

Preclinical evaluation → Pilot studies → Controlled studies → Field trials

To document the effectiveness of oral products against dental diseases, the strategy for clinical studies is outlined in the above chart. The preclinical studies include laboratory and animal tests. For example, for fluoride efficacy a test would include fluoride uptake in teeth or HA, reduction in enamel solubility following fluoride treatment, and followed by an acid challenge. The effectiveness in rats includes the effects of topical application of fluoride solution in reducing caries. The pilot studies in humans are done to assess the effectiveness of fluoride to promote mineral deposition or prevent dissolution of artificially created lesions in enamel slabs implanted in partial dentures. Such studies are of two to four weeks duration and conducted in 20 to 30 subjects per group. If the pilot study significantly enhance remineralization of artificial lesions, control studies in 30 to 60 subjects for three to six months are conducted with the final formula for efficacy. The parameters could include promotion of mineralization, regression of early cavity lesions, and fluoride uptake in dental plaque and saliva. The field trials are conducted in children (1000 per group) for a period of three years to assess the effects on cavity development. For prevention of plaque and gingivitis formation, such trials are conducted for six months. The calculus reduction field trials are also conducted for a period of six months. Such field trials are of parallel/double-blind design.

FUTURE TRENDS

The global needs for prevention of dental diseases can be met by the development of knowledge resources in academia and industry and its subsequent applications. With a better understanding of processes occurring in the mouth, we will be able to design better actives and active agent delivery systems for the control of oral diseases, affecting general health. The technological trends are leading toward the goal of "healthy mouth/healthy body."

REFERENCES

1. Dental Spending Hits $37 Billion. Am Dent Assoc News 1992 (Jan 6).
2. Periodontal aspects of systemic health. Symposium Proceedings. Comp Cont Edu 1998 (Fall).
3. Gaffar A, Afflitto, J. General principles for delivery of active agents for mouthrinses. Int Dent J 1996; 42(4):251–256.
4. Fishman SL. Hare's teeth to fluorides, historical aspects of dentifrice use. In: Emery G, Rölla G, eds. Clinical and Biological Aspects of Dentifrice. Oxford University Press, 1972:1–7.
5. Nathoo SA, Gaffar A. Studies on dental stain induced by antibacterial agent and rational approaches for bleaching dental stains. Adv Dent Res 1995; 9(4):462–470.
6. Solis-Gaffar M, Niles HP. Instrumental evaluation of mouth odor in a human clinical study. J Dent Res 1977; 54:851–857.
7. Niles HP, Gaffar A. Relationship between sensory and instrumental evaluation of mouth odor. J Soc Cos Chem 1993; 44:101–107.
8. Gaffar A, Blake-Haskins J, Mellberg J. In vivo studies with dicalcium phosphate dihydate/MFP system for caries prevention. Int Dent J 1993; 63(1):81–90.
9. Sullivan RJ, Fletcher R, Barchman R, Legeros RZ. Intra-oral comparison and evaluation of the ability of dentifrices to promote remineralization of caries-like lesions in dentin and enamel. J Clin Dent 1995; 6:135–138.
10. Gaffar A, Blake-Haskins J, Sullivan RJ, Simone A, Saunders F. Cariostatic effects of a xylitol/NaF dentifrice in vivo. Int Dent J 1998; 48:32–39.
11. Mandel ID. Rinses for the control of supragingival calculus formation. Int Dent J 1992; 42:270–285.
12. Gaffar A, Legeros RZ, Gambogi RS, Afflitto J. Inhibition of formation of calcium phosphate deposits on teeth and dental materials: Recent advances. Adv Dent Res 1995; 9(4):419–426.
13. Volpe AR, Petrone M, Davies RM. A review of calculus clinical efficacy studies. J Clin Periodont 1993; 4(3):71–81.
14. Miller S, Gaffar A, Sullivan RJ, Truong T, Stranick M. Evaluation of new dentifrice for treatment of sensitive teeth. J Clin Dent 1994; 5:71–79.
15. Löe H, Theilde E, Jensen SB. Experimental gingivitis in man. J Periodont 1970; 36: 177–187.
16. Syed SA, Loesche WJ. Bacteriology of experimental gingivitis: effect of plaque. Info Immun 1978; 21:821–829.
17. Gaffar A, Volpe AR, Lindhe J. Recent advances in plaque/gingivitis control. In: Emery G, Rölla G, eds. Chemical Biological Aspects of Dentifrices. Oxford University Press, 1992:229–247.
18. Gaffar A, Afflitto J, Herles S, Nabi N. Recent advances in plaque, gingivitis, tartar and caries prevention. Int Dent J 1994; 44:63–70.
19. Renvert St, Birkhed D. Comparison of three triclosan dentifrices on plaque, gingivitis and salivary microflora. J Clin Periodont 1995; 23:63–70.

20. Gaffar A, Scherl D, Afflitto J, Coleman EJ. The effects of triclosan on the mediators of gingival inflammation. J Clin Periodont 1995; 22:280–284.
21. Rosling B, Wannfors B, Volpe AR, Furuichi Y, Ramberg P, Lindhe J. The use of a triclosan/copolymer dentifrice may retard progression of periodontitis. J Clin Periodont 1997; 24:873–880.
22. Elwood RP, Worthington HV, Blinkhorn AB, Volpe AR, Davies RM. Effect of triclosan/copolymer dentifrice on incidence of periodontal attachment loss in adolescence. J Clin Periodontol 1998; 25:363–367.
23. Mandel ID. Chemotherapeutic mouthrinses for control of oral disease. Int Dent J 1992; 42(4):251–285.

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Decorative Products

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INTRODUCTION

Decorative cosmetics are principally concerned with beautifying and decoration, rather than functionality. No discussion of decorative products can be complete without a full understanding of the importance of color, a prime component of every decorative cosmetic. Conventional pigments create color by absorption of certain wavelengths of incident light. The color perceived corresponds to that of the wavelengths reflected. Formulation of decorative cosmetics has been an exciting challenge for cosmetic chemists. Before formulating any color cosmetic product, one must check the current regulations in the country where the proposed product will be sold to make sure all the colors conform to those regulations. The following is a practical guide for the formulator and includes a maximum of technical and regulatory issues in an "easy-to-use" format.

Additionally, there has been an increasing demand for color cosmetics containing treatment "actives" or cosmeceuticals. Decorative products now contain UV filters, herbs, vitamins, and moisturizers to counteract the effects of aging and add moisture to the skin.

COLOR

Color Additive Regulation

In the past, colorants had been used in cosmetics without any consideration being given to their possible toxicity. Today, all countries have regulations that control the type and purity of colors that may be used in cosmetics.

USA: Food and Drug Administration (FDA)

21 CFR 73, 74: Positive list (1): Colors listed for general cosmetic use, including eye area only if stated specifically, or external only, meaning no contact with mucous membranes. Hair dyes and true soaps are exempted.

EUROPE (EU): European Commission (EC)

Directive 76/768

ANNEX IV (2): Positive list: Coloring agents allowed for use in cosmetic products.

ANNEX II: Negative list: Substances that must not be part of cosmetic products (not specific for colorants).

JAPAN: Ministry of Health and Welfare (MHW)

MHW Ordinance No. 30 (3):

Positive list: Coal-tar colors.

Premarket approval by MHW for all other cosmetic ingredients, including inorganic and natural colorants.

Color Additives—Definitions

Primary/straight color: A color that is pure, containing no extenders or dilutents.

Dye: A color that is soluble in the medium in which it is dispersed (e.g., Food Dye and Coloring (FD&C) Blue #1).

Pigment: A color that is insoluble in the medium in which it is dispersed (e.g., FD&C Blue #1 A1 lake, black iron oxide).

Lake[a]: A water-insoluble pigment composed of a water-soluble straight color strongly absorbed onto an insoluble substratum through the use of a precipitant (e.g., FD&C Blue #1 A1 LAKE). Generally, 10–40% color.

Toner: A pigment that is produced by precipitating a water-soluble dye as an insoluble metal salt (i.e., D&C Red #6 Barium salt, Drug and Cosmetic (D&C) Red #7 Calcium salt).

True pigment: A pigment that, based on its chemistry, precipitates as it is formed (e.g., D&C Red #36).

Extender: A pigment, diluted on a substrate

a. during manufacture by precipitation,
b. post-manufacture by intimate milling or mixing.

United States Regulations

21 CFR Part 73 (1)—Listing of color additives exempt from certification. Inorganic pigments, powdered metals, and naturally derived colorants approved for food, drug, and/or cosmetic use.

Listed permitted uses:

Food
Ingested/externally applied drugs
General cosmetic
Eye area only if mentioned
External (no mucous membrane) i.e., ultramarines, ferric ammonium ferrocyanide, not permitted in lip or bath products.

21 CFR Part 74 (1)—Listing of color additives subject to certification. Synthetic organic dyes and pigments. Each batch must be submitted by the manufacturer to the FDA for certification that specifications are met.

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[a] FDA has considered any certified colorant mixed with a diluent to be a lake:

D&C Red 30 plus Talc.
D&C Red #7 CA Lake on Calcium Carbonate.

Permitted uses as in Part 73
Four certified organic dyes and their lakes are now permitted for eye area use:

FD&C Blue #1
FD&C Red #40
FD&C Yellow #5
D&C Green #5

21 CFR Part 82 (1)—Listing of certified provisionally listed colors. Lakes:
FD&C: Aluminum or calcium salt on alumina.
D&C: Sodium, Potassium, Barium, Calcium, Strontium, or Zirconium Salt on Alumina, Blanc Fixe, Gloss White, Clay, Titanium Dioxide, Zinc Oxide, Talc, Rosin, Aluminum Benzoate, and Calcium Carbonate.

A salt prepared from straight color, i.e., D&C Red #6, by combining the color with a basic radical.

Proposed permanent listing of color additive lakes (FR Vol. 61 #43), March 4, 1996 (4).

- List substrate, i.e., D&C Red #27 Aluminum lake on alumina.
- Extenders of insoluble straight colors will no longer be called lakes, i.e., D&C Red #30.
- Permit blends of previously certified straight colors in a lake, i.e., FD&C Blue #1 and Yellow #5 Aluminum Lake.
- All lakes to be prepared from previously certified batches of straight color would necessitate process changes for D&C Reds #6, #7, and #34.
- Abbreviations permitted for cosmetic ingredient labeling, omitting FD&C, precipitate and substrate designation, i.e., Blue 1.

European Community

Directive 76/768, as amended (5).
Annex IV—List of coloring agents allowed in cosmetic products
List by color index number
Part 1: Permanently listed.
Part 2: Provisionally listed.
Four fields of application and restriction of use

i. All cosmetic products.
ii. All cosmetic products, except those intended to be applied in the vicinity of the eyes, in particular eye make-up and make-up remover.
iii. Allowed exclusively in cosmetic products intended not to come into contact with mucous membranes (including the eye area).
iv. Allowed exclusively in cosmetic products intended to come into contact only briefly with skin (not permitted in nail preparations).

Lakes and salts:

If a color index number is listed in Annex IV, then the pure color plus its salts and lakes are allowed, unless prohibited under Annex II (the list of substances that cosmetics may not contain). Exceptions are barium, strontium, and zirconium. Prohibited under Annex II, but where a "footnote 3" appears in Annex IV, "the insoluble barium, strontium, and zirconium lakes, salts, and pigments . . . shall also be permitted. They must pass the test for insolubility, which will be determined by the procedure in Article 8 (Insoluble in 0.1 NHC1).

Purity criteria:

Only colors designated by an “E,” those also permitted for food use, must meet the general specification for food colors.

< 5 ppm	As
< 20 ppm	Pb
< 100 ppm	Sb, Cu, Cr, Zn, $BaSO_4$ separately
< 200 ppm	Of those together
None detectable	Cd, Hg, Se, Te, Th, U Cr^{+6} or soluble Ba

Sixth amendment to the directive is currently adopted. An update of purity criteria is being considered, where test methods may be stipulated.

Japan

MHW ordinance No. 30 (1966) as amended by MHW ordinance No. 55 (1972) (6).

Positive List 83: Coal-Tar Colors

Must be declared on cosmetic product label.

Fields of application: oral, lip, eye area, external, rinse-off.

Inorganic/Natural Colorants

Listing, specifications, test methods.

Japan standards of cosmetic ingredients (JSCI).
Comprehensive licensing standards of cosmetics by category (CLS).
Japan cosmetic ingredient dictionary (CLS).

U.S. Colorants not Permitted/Restricted in Japan:

Pigments:

D&C RED #6 Ba Lake
D&C RED #21 A1 Lake
D&C RED #27 A1 Lake
D&C RED #33 Zr Lake
D&C ORANGE #5 A1 Lake

Substrates:

Aluminum Benzoate: 0.5% maximum in lipstick.
Rosin: 7.0% maximum in lipstick.
Calcium Carbonate: Not permitted.

COLOR CHEMISTRY AND MANUFACTURE

The property of a colorant that makes it absorb more in one part of the visible spectrum than in another is its chemical constitution. Molecules, like atoms, exist in different electronic states. As molecules contain two or more nuclei they also possess energies of rotation and vibration. This theory applies to both organic and inorganic colorants. With the inorganic colorants, colored compounds are obtained with the ions of the transition elements which have atomic numbers 22 to 29.

Organic Pigments

These are chiefly conjugated cyclic compounds based on a benzene ring structure, although some heterocyclic ones exist. There are three main types: lakes, toners, and true pigments. Organic pigments are seldom used without a diluent or substrate to maintain color consistency from batch to batch. A true pigment is an insoluble compound, which contains no metal ions, examples of which are D&C Red #30 and D&C Red #36. They are the most stable. A lake is essentially an insoluble colorant, produced by precipitating a permitted soluble dye to a permitted substrate. In cosmetics, most lakes are based on aluminum, although zirconium lakes are also found. Stability-wise, true aluminum lakes can be affected by extremes of pH, resulting in reforming of the soluble dye or "bleeding." They are fairly transparent and not particularly light-fast. Toners are colorants made with other approved metals besides aluminum, such as barium and calcium. Generally, they are more resistant to heat, light, and pH, although extremes of pH can result in shade changes. Generally, many organic colorants are unsuitable for certain cosmetics because of there chemical nature. D&C Red #36, a typical nonsoluble azo color, is not recommended for lipstick because of its very slight solubility in oils and waxes it tends to crystallize upon continual reheating of the lipstick mass. Soluble azo dyes such as FD&C Yellow #5, #6 and D&C Red #33 lakes are often used in lipstick and nail lacquer. Sparingly soluble type, such as D&C Red #6, is not highly soluble, but the Barium lake of Red #6 and the Calcium lake of Red #7 are the most popular colors for cosmetics. Colors in this group do not need a substrate to make them insoluble. The D&C Red #6 and #7 lakes are widely used in lipstick and nail lacquer because of high strength, bright hues, good light-fastness, chemical and heat stability. Nonazo soluble dyes such as D&C Red #21, Orange #5 and Red #27 all are fluoresceins and act as a pH indicator and will change accordingly. They all strain the skin and D&C Red #27 gives the strongest blue stain.

Organic pigments are characterized by

- transparency,
- variable chemical and physical stability, and
- "clean," bright colors.

Color is produced by chromophoric groups, generally electron donors.

–N=N–	–C=O
$–NO_{2s}$	–C=S
–NO	

Shade is modified or intensified by auxochromes, generally electron acceptors.

–NHR	–OH
$–NH_2$	$–OCH_3$
$–NR_2$	

Categories of Organic Colorants

AZO Colorants: –N=N–

Insoluble (unsulfonated): D&C Red #36; light stable
Soluble (sulfonated):

D&C Red #33, FD&C Red #40, FD&C Yellow #5, FD&C Yellow #6
Stable to acid, alkali, light, bleed in water

Slightly soluble (sulfonated/insoluble salt):

D&C Red #6; D&C Red #7, D&C Red #34
Color shift in acid and alkali; light-fast; resistant to oil bleed

Oil soluble (unsulfonated): D&C Red #17

Based on a Scientific Committee for Cosmetic Products and Non-Food Products (SCCNFP) opinion, certain European member states have proposed a ban on the azo dyes, which could split into aromatic amines classified as CMR 1 or 2, by the Dangerous Substances Directive. The SCCNFP has asked CTFA for data to demonstrate that there would be an acceptable risk in continued usage of azo dye in cosmetics. The issue is how the dyes can be broken down. The future of azo dyes in Europe is, thus, under active discussions and the reader is advised to get updated information on the regulatory status of azo dyes at the time of reading.

Xanthenes

D&C Orange #5, D&C Red, D&C Red #21, and D&C Red #27, are preferably "staining dyes" with structural changes with change in pH; they have poor light stability and bleed in solvents.

Triarylmethane

FD&C Blue #1 and FD&C Green #3 are water soluble and have poor light stability, whereas Anthraquinone and D&C Green #5 have good light stability.

Quinoline

D&C Yellow #10 and D&C Yellow #11 are oil soluble.

Indigoid

D&C Red #30, characterized by good chemical, light, and bleed resistance but are as an exception acetone soluble.

Stability of Organic Pigments

True pigments > Toners > True Lakes

Light: Anthraquinone > Quinone > Indigoid > Azo > Triarylmethane > Xanthene

Heat: True pigments are stable to heat.

Toners: D&C Red #7 Ca Lake changes reversibly.
Lakes: D&C Red #27 Al lake changes irreversibly.

pH: 4 to 9.
Metal ions: unstable.
Solubility: true lakes tend to bleed in water.

fluorescein lakes bleed in solvent.

Natural Dyes

Generally used in foods, there is no restriction in their use in cosmetics. For the most part, the resistance of the natural dyes to heat, light, and pH instability is much inferior when compared with their synthetic counterparts. A further disadvantage is that they often tend to exhibit strong odors (7).

Color	Description	Source
Yellow	Curcumim	Turmeric
Yellow	Crocin	Saffron
Orange	Capsanthin	Paprika
Orange	Annato	Annatto
Orange	Cartenoids	Carrots
Red	Cochineal	Coccus cactii
Red	Betanine	Beetroot
Red	Anthocyanins	Red berries
Green	Chlorophylls	Lucerne grass
Brown	Caramel	Sugars

All the above listed are of vegetable origin, with the exception of cochineal that is extracted from the crushed *Coccus cactii* insects. Natural pigments currently under study include sweet white lupine, alfalfa, grape, and uruku from the *bixa orellana* plant.

Inorganic Colors

In general, inorganic colors are more opaque, more light-fast, more solvent resistant but not as bright as organic colors. They may be affected by alkali and acid. Inorganic colorants are formed from compounds of the transition elements. Color is produced because the outer "d" electrons could absorb visible light and could be promoted to the next higher energy level.

Iron oxides	Red	Fe_2O_3
Good stability, opacity	Brown	
	Burgundy	Fe_2O_3
	Black	Fe_3O_4
	Yellow	FeOOH
Chromium oxide	Green	Cr_2O_3
Good stability, opacity		
Chromium hydroxide	Aqua	$Cr_2O_3\ xH_2O$
Good stability, lower tinting strength		
Ultramarines	Blue	
Good light stability; lower tinting strength; unstable to acid	Violet	$Na_x(AlSiO_4)_yS_z$ Pink
Manganese violet	Violet	$NH_4MnP_2O_7$
Good light stability; lower tinting strength; unstable to water		
Ferric ammonium	Deep blue	$FeNH_4Fe(CN)_6$
Ferrocyanide		
Lower light stability; high tinting strength; unstable to alkali, salts; difficult dispersion		
Ferric ferrocyanide	Deep blue	$Fe\ [Fe(CN)_6]_3\ xH_2O$
Physical/Chemical stability as above; precipitated on a substrate (i.e., Mica)		
Titanium dioxide	White	TiO_2
Medium light stability, good chemical stability, high opacity		Anastase Rutile

Inorganic Pigments

Titanium Dioxide

A brilliant white pigment that occurs in two crystal types: anatase and rutile. Two manufacturing processes are employed:

i. Sulfate process—either crystal may be produced.
ii. Chloride process—only rutile crystals are formed.

Properties: Crystals of both rutile and anatase are tetragonal, rutile having greater hiding power due to the closer packing of the atoms in the crystal. Refractive indices are 2.55 for anatase and 2.71 for rutile. Opacity is the result of the light scattering ability of titanium dioxide. Light, heat, and chemical stability are excellent. Additionally, in the United States, titanium dioxide is classified as a Category I sunscreen.

Zinc Oxide

Zinc ore is roasted and purified at 1000°C. Two methods of manufacture are utilized:

i. French (indirect)
ii. American (direct)

Properties: Zinc oxide forms transparent hexagonal crystals; the whiteness is due to the light scattering of the extremely fine particles. Refractive index is 2.0. Hiding power is less than titanium dioxide. Primary use is for antibacterial and fungicidal properties. Heat and light stability are good. It is soluble in acid and alkali. Zinc oxide in the United States is a Category I skin protectant and a Category III sunscreen.

Iron Oxide

These are used in all types of cosmetic products. By blending black, red, and yellow in certain properties—brown, tans, umbers, and sienna may be produced. Yellow iron oxide is hydrated iron II (ferrous) oxide, $Fe_2O_3\ xH_2O$. It is produced by the controlled oxidation of ferrous sulfate. Red iron oxide is Fe_2O_3 and is obtained by the controlled heating (at about 1000°C) of yellow iron oxide. Black iron oxide is Fe_2O_4 and is a mixture of ferrous and ferric oxide and is prepared by controlled oxidation of ferrous sulfate under alkaline conditions.

Ultramarines

Theoretically, they are polysulfide sodium/aluminum sulfosilicates. They range in color from blue to violet, pink, and even green. A mixture is calcined at 800–900°C for 4–5 days. Shades are determined by reaction time, formula variations, and particle size; ultramarine, violets, and pinks are obtained by treating ultramarine blue with HCl at 275°C, removing some amount of sodium and sulfur from the molecule.

Maganese Violet

Chemical formulation is $MnNH_4P_2O_7$ and is manufactured by heating maganese dioxide with ammonium dihydrogen phosphate and water. Phosphorous acid is added and the mixture is heated until the violet color develops.

Iron Blue

Chemically ferric ammonium ferrocyanide is $Fe[Fe(Cn)_6]_3$. Sodium ferrocyanide and ferrous sulfate are treated in the presence of ammonium sulfate. Pigments prepared with sodium or potassium salts are called ferric ferrocyanide.

Chromium Oxide (Cr_2O_3)

A dull yellow green pigment may be prepared by blending an alkali dichromate with sulfur or a carbonaceous material. Reduction to chrome (III) oxide is achieved in a kiln at 1000°C.

Chromium Hydroxide ($Cr_2O(OH)_4$)

A bright bluish green pigment prepared by the calcination of a bichromate with boric acid at 500°C. The mass, during cooling is hydrolyzed with water, yielding a hydrate.

Hydrated Alumina

Chemically Al_2O_3 xH_2O gives little opacity and is almost transparent.

Barium Sulfate

It is relatively translucent and may be used as a pigment extender.

Quality Control of Colorants

Establishment of Standards

- Insure that product development is performed with material representative of supplier's production.
- Prior to purchase, evaluate at least three lots; establish standard in consultation with the supplier.
- Supplier and end user should agree on specifications, standard, and test methods.

Test Methods

Shade evaluation: Methods should predict performance of the colorant under use conditions.

Light source for visual evaluations must be specified.

- Dyes: Visual or spectrophotometric evaluation of solutions.
- Pigments: Cannot be evaluated as received due to variable degree of agglomeration. Visual or instrumental evaluation is made of wet and dry dispersions prepared under defined conditions to a defined degree of dispersion.

Vehicles	Dispersion equipment
Talc	Osterizer
Nitrocellulose lacquer	Hoover Muller
Acrylic lacquer	Three-roll mill or
Castor oil	Ball mill

Heavy metals:

Wet chemical
Atomic absorption spectroscopy (AAS)
Inductive coupled plasma (ICP)

Particle size:

Wet/dry sieve analysis
Optical microscopy
Laser diffraction
Sedimentation

Bulk density:

Fischer–Scott volumeter
pH

Pearlescent Pigments and Other Specialty Pigments

Pearlescent Pigments

The most important requirement for a substance to be pearlescent is that its crystals should be plate-like and have a high refractive index. A thin, transparent, platy configuration allows light to be transmitted. A pearlescent material should have a smooth surface to allow specular reflection and must be nontoxic. Generally, when using pearlescent pigments one must use the most transparent formulation, avoiding grinding or milling the pearl pigments and blend pearls which complement one another.

Organic pearls. These pearls produce a bright silver effect and are obtainable from fish scales as platelets or needles which are highly reflective. The materials responsible for the pearl effect are crystals of a purine called guanine. Guanine is chiefly used in nail-enamel.

Inorganic pearls

Bismuth Oxychloride (BiOCl). Bismuth oxychloride produces a silvery-grey pearlescent effect and is synthesized as tetragonal crystals. Crystal sizes vary from approximately 8 μm, which gives a soft, opaque, and smooth luster, and 20 μm, which give a more brilliant sparkling effect. Its major disadvantage during use is poor light stability, which may cause darkening after prolonged exposure. UV absorbs in the finished products are used to overcome this defect. BiOCl is chiefly used to pearl nail-enamels, lipsticks, blushes, and eye shadows. BiOCl may be modified by deposition on mica, titanium dioxide and mica, or talc. Inorganic pigments may be bonded to BiOCl then deposited on mica. All these alter the final effect on the finished product.

Titanium dioxide coated micas. Titanium dioxide coated micas are extensively used in decorative cosmetics. They exist in several different forms: (i) Silver-titanium dioxide uniformly coats platelets of mica. Rutile crystals give a brilliant pearl effect because of a higher refractive index than the anatase grade. (ii) Interference pearlescent products can be made by altering the thickness of the film. At a certain thickness, interference of light can take place so that some wavelengths of the incident light are reflected and others transmitted. The colors created are complimentary to each other. As the layers become thicker the reflection goes from silvery white, to yellow–gold, red, blue, and finally green. Additionally, colorants such as iron oxides can be laminated with this interference film providing a two-color effect.

Pigment pearls. Colored pearls are produced by laminating a layer of iron oxides on titanium dioxides coated mica producing a color and luster effect.

Specialty pigments. In addition to BiOCl and the titanium dioxide coated mica systems, polyester foil cut into regular shapes which have been epoxy coated with light-fast pigments have been used for nail-enamels and body make-up. Finally, aluminum powder and copper/bronze powder have been used as reflective pigments, especially in eye shadows. For cosmetic use, such as aluminum powder, 100% of the particles must pass through a 200-mesh screen; 95% must pass through a 325-mesh (44 millimicron) screen.

Treated Pigments

Surface-treated colors and substrates allowed chemists to enhance the aesthetic and functional qualities of their formulations. The benefits of using these treatments may be divided into two categories: those evident in the finished cosmetic product, and the benefits derived from process improvements. Consumer benefits include hydrophobicity yielding greater wear, improved skin adhesion, smoother product feel, improved optical appearance, moisturization and ease of application. Processing benefits include ease of dispersion, pressability, less oil absorption, uniformity, and less moisture absorbtion.

The following surface treatments are commercially available:

- *Amino Acids*: N-lauroyl lysine, acyl amino acid (8)

  Natural
  Good skin adhesion
  pH balanced
  Heat sensitive

- *Fluorochemical*: Perfluoropolymethylisopropyl ether perfluoroalkyl phosphate

  Hydrophobic and lipophobic, greatly enhance wear
  Heat and shear resistance

- *Lecithin* (9):

  Natural
  Exceptionally smooth, silky skin feel, particularly in pressed products
  Heat sensitive, slightly soluble in water.

- *Metal Soaps*: (Zn, Mg Stearate)

  Good skin adhesion
  Enhanced compressibility

- *Natural Wax*:

  Natural
  Moisturizing skin feel
  Good skin adhesion
  Heat sensitive (low m.p.)

- *Nylon*: pure mechanical-coated

  Smooth skin feel

- *Polyacrylate*:

  Enhanced wetting in aqueous systems; feel is not very good, but is usually used in dispersion

- *Polyethylene*:

  Hydrophobic
  Waxy, smooth skin feel
  Enhanced compressibility
  Heat sensitive

- *Silicone*: (Polymethylhydrogensiloxane) Methicone will be chemically bonded and cannot be removed later.

  Hydrophobic
  Achieves full color development
  Main use is to improve wetting

- Other Silicones: No potential for hydrogen evolution

  Dimethiconol
  Absorbed dimethicone
  Silicone/lecithin

- Silane:

  Extremely hydrophobic, lipophilic
  No hydrogen potential

- Titanate ester: Isopropyl triisostearyl titanate (10)

  Enhances wetting in oil
  Smooth skin feel
  High pigment loading
  Lowers oil absorption of pigments

Microfine Pigments (Microfine/Ultrafine/Nanosized)

These pigments have a primary particle size below 100 nm; larger agglomerates/aggregates can be present. Properties such as surface area, bulk density, vehicle absorption, and UV absorption differ significantly from those of conventional pigment. Microfine titanium dioxide, zinc oxide, and iron oxides can be utilized in a range of color cosmetics to provide unique visual effects as well as UV protection. In pressed powders, anhydrous, and emulsified formulations, significant sun protection factor (SPF) values can be achieved in formulations having a translucent, natural looking finish. With microfine pigments, formulations for darker skin tones can be formulated which avoid the "ashy" or "made-up" appearance caused by conventional opaque pigments.

Light Diffusing Pigments

Some of the requirements for light diffusing pigments include a high refractive index, reflection to be diffused, translucency and its transmission must be primarily diffuse. Skin has a refractive index of 1.60. Examples of light diffusers include $BaSO_4$, silica, silica spheres coated on mica, $TiO_2/BaSO_4$ coated mica, Al_2OH_3/mica, ultrafine

TiO_2/mica, ultrafine TiO_2/polyethylene, ethylene acyrates copolymer, polymethyl methacrylate (PMMA), and there are many others. These products are chiefly used in powders to create illusions and hide wrinkles.

MAKE-UP TECHNOLOGY

Types of color cosmetics
Foundation
Blushers
Mascara
Eyeliner
Eye shadow
Lip color
Nail color
Purpose
Improve appearance
Impart color
Even out skin tones
Hide imperfections
Protection
Types of formulations
Suspensions
Aqueous
Anhydrous
Emulsions
Oil-in-water
Water-in-oil
Powder
Pressed
Loose
Anhydrous: Wax, solvent
Stick
Pan
Tube

Powder

Powdered cosmetics are generally used to describe face powders, eye shadows, and blushers. When the product is applied to the skin, the shade must not significantly change as when worn, should feel smooth during use making it easy to apply, and lastly, adhere well for a reasonable time without reapplication.

Face Powders

Some of the attributes of a satisfactory face powder is the following: (i) gives smoothness to overall texture; (ii) gives added skin translucency when excess is buffed (iii) makes the skin appear more refined and finer textured (iv) helps set the make-up base and adds longevity to the make-up overall (v) suppresses surface oil and shine. Generally, there is a wide range of raw materials used in powdered cosmetics and many of these carry over into the formulation of other decorative cosmetics.

Talc

Talc is the major component of most face powders, eye shadows, and blushers. Chemically it is a hydrated magnesium silicate. Cosmetic talcs are mined in Italy, France, Norway, India, Spain, China, Egypt, Japan, and the United States. Typically talcs are sterilized by gamma irradiation. Particle size should pass through a 200-mesh sieve. Cosmetic talc should be white, free of asbestos, have high spreadability or slip, with low covering power. Micronized talc is generally lighter and fluffier but less smooth on the skin than regular grades. Although talc is fairly hydrophobic, treated talcs have been used to enhance its texture. In some products talc is present in up to 70% of the formulation.

Kaolin

Kaolin or china clay is a naturally occurring, almost white, hydrated aluminum silicate. It does not exhibit a high degree of slip. Kaolin has good absorbency, is dense and sometime used to reduce bulk densities in loose powder products. It provides a matte surface effect, which can reduce the slight sheen left by some talc products.

Calcium Carbonate

Calcium carbonate or precipitated chalk has excellent absorption properties. It provides a matte finish and is a moderate covering powder. High levels should be avoided otherwise an undesirable, dry, and powdery feel could result.

Magnesium Carbonate

Magnesium carbonate is available in a very light, fluffy grade, which absorbs well and is often used to absorb perfume before mixing it into face powders.

Metallic Soap

Zinc and magnesium stearate are important materials for imparting adhesion to face powders. Usually incorporated at 3–10% of the formulation. Stearates add some water repellency to formulae while high levels give a blotchy effect on the skin. Zinc stearate, besides imparting adhesions, gives a smoothing quality to face powders. Aluminum stearate and lithium stearates have also been used. High levels can make pressed formulation too hard.

Starch

Starch is used in face powders to give a "peach-like" bloom and provides a smooth surface on the skin. One problem attributed to rice starch is that when moistened it tends to cake. Also, the wet product may provide an environment for bacterial growth.

Mica

Mica is chemically potassium aluminum silicate dihydrate. Cosmetic mica is refined and ground to particles of 150 microns or less. It imparts a natural translucence

when used up to 20% in formulations of face powder blushes. Mica is available as wet ground, which is creamy, or dry ground, which is matte. Sericite is a mineral, similar to white mica in shape and composition. It has a very fine grain size and a silky shine. It is soft and smooth and has a slippery feel on the skin. Sericite may be coated with silicone and other treatments for better water repellency and skin adhesion.

Polymers

Polymers are chiefly texture enhancers used at levels of 3% to 40% depending on whether they are to be included in a loose or pressed powder. Among these polymers, we find nylon-12 and nylon-6, lauroyl lysine, boron nitride (makes active ingredients spread more uniformly on inactive bases), polyethylene, polypropylene, ethylene acrylates copolymer (very sheer, will not effect binder in pressed powders, processing temperature less than 85–90°C), PMMA and silica beads (can carry oily ingredients into a system; increase wear on oily skin), polyurethane powders, silicone powders, borosilicate, microcrystalline cellulose, acrylate copolymers, Teflon® and Teflon® composites (effective at low concentrations, 1–5%), polyvinylidene copolymers (very light-ultra low density), and composite powders which are coated on inexpensive beads to reduce costs and increase effectiveness, like nylon/mica, silica/mica, lauryl lysine/mica, and boron nitride/mica. Many of these polymers are treated with silicones, titanates, lecithin, etc., for increased effectiveness.

Colorants

Titanium dioxide and zinc oxide, both pigmentary and ultrafine, organic, inorganic, carmine, and pearlescent pigments either predispersed or treated are found in all face powders because the textures of these colorants are not very satisfactory. Titanium dioxide and zinc oxide have anti-inflammatory properties and zinc is an antimicrobial.

Perfumes

The use of perfumes is important for face powder, which require them because most of the raw materials used are earthy smelling and should be masked. Perfumes should show stability and low volatility.

Preservatives

Preservation of Face powders is usually not a problem as they are used dry, but small amounts of Antibacterials are recommended. Powdered eye shadows should always contain antibacterials such as parabens, imidazolidinyl urea, and others.

Loose Face Powders

This type has declined in popularity in favor of pressed face powder products. The smoothness of loose face powder can be enhanced by use of the aforementioned texture enhancers. In the manufacturing process all ingredients except the pearls, if required, are combined in a stainless steel ribbon blender. Mixing time can be as long as one or two hours depending on the size of the batch and evenness of the color. The perfume, if required, is slowly sprayed into the batch and blended until homogenous.

The batch is then pulverized through a hammer mill and the color is checked. Color adjustments are made, if necessary in the ribbon blender and the batch is repulverized. Any pearl or mica is then added for a final mix. The batch is then stored and made ready for filling into appropriate containers.

Pressed Face Powders

Pressed face powders are more popular than loose powders because of their ease of application and portability. The basic raw materials are the same as loose powder except that one must use a binder to press the cake into a tin-plate godet. If water-based binders are used aluminum godets should be considered to prevent corrosion. The properties of a binder are as follows: provides creaminess to the powder, aids in compression and adhesion, develops colorants, enhances water-resistance, and pick-up and deposit. If the binder level is too high, it may be difficult to remove the powder with a puff. Also, high levels may lead to glazing of the powder surface making it waxy looking, with little or no payoff. Fatty soaps, kaolin, polyethylene, Teflon®, synthetic wax, and calcium silicate are some of the binder systems used. Usage levels of binder are between 3% and 10% depending on formulation variables. Silicone-treated pigments have given rise to pressed face powders, which may be used wet or dry. When used dry, they are usually smoother than regular pressed powders. When a wet sponge is applied to the cake, no water penetrates the cake; the water is repelled. These "two way" cakes can be used either as a foundation or as a face powder. When formulating pressed powders, one must be careful that the raw materials used do not corrode the godets or attack the plastic packaging materials. The manufacture of pressed powders including the mixing and color matching process is similar to loose powders. Sometimes the powder mix is pulverized without binder and then again after its addition. Pearls are usually added during the blending process and preferably without the milling operation, which can damage the pearl. If milling a batch containing pearls becomes necessary, it should be done with the mill screen removed. Powder pressing is several times more successful if the powder is kept for a few days to allow the binder system to fully spread, especially when pearls are present. The most commonly used press for face powder are the ALITE®-high speed hydraulic press and the KEM WALL®, CAVALLA®, or VETRACO® presses. The pressures used and the speed of pressing depends on the characteristics of the individual formulation and the size of the godet.

Powder Blushers

The attributes of blushers are as follows: (i) Adds color to the face; (ii) Can give more dimension to the cheekbones; (iii) harmonizes the face-balance between eye make-up and lipstick; and (iv) creates subtle changes in the foundation look when lightly dusted over the face. Pressed powder blushers are similar to face powder formulations, except that a greater range of color pigments are used. The three basic iron oxides and one or more of the lakes are used to achieve various blusher shades. Blushers are usually applied with a brush. Manufacture and pressing is similar to face powders. Care should be taken that only nonbleeding pigments be used to avoid skin staining. Total pigment concentration ranges from 2% to 10% excluding pearls. Pressed powder rouges were once popular and contained high levels of colorants 10–30%. Usually they are applied from the godet with the finger so that glazing may frequently occur if the rouge is improperly formulated.

Pressed Powder Eye Shadows

Eye shadows in general have the following functions: (i) Adds color to the face and improves personality; (ii) sharpens or softens the eye itself; (iii) creates the illusion of depth or brings out deep-set eyes; (iv) creates light and dark illusions for subtle character changes, and lastly, (v) can be used in wet or dry forms for different illusions. The technology is similar to other pressed powder products but the permitted color range is limited. In the United States, the only synthetic organic pigments, which may be used in eye products are FD&C Red No. 40, FD&C Blue #1, FD&C Yellow #5, and Green #5. Carmine N.F. is the only natural organic pigment allowed and all of the inorganic pigments and a wide range of pearls may be used. Preservation is very important in eye make-up products. Problems of poor adherence to the skin, color matching, and creasing in the eyelid is common when the binder formulation is ineffective with the type and level of pearls used. High binder levels may result in uneven pressing of the godets. In manufacture, formulas with high pearl content should be allowed to settle, in order to remove entrapped air, before pressing.

Quality Assurance on Powder Products

Color: The production batch and the standard are placed side-by-side on white paper and pressed flat with a palette knife. Shades are compared to one another. Shades of eye shadows and blushers are checked on the skin using a brush or wand.

Bulk density: Tests are carried out on loose powder to ensure that no entrapped air is present so that incorrect filling weights are minimized.

Penetration and drop tests: Tests are carried out on pressed godets. A penetrometer is used to determine the accuracy of the pressure used during filling. The drop test is designed to test the physical strength of the cake. Normally, the godet is dropped on to a wooden floor or rubber matte (1–3 times) from a height of two to three feet to note the damage to the cake.

Glazing and Payoff: The pressed cake is rubbed through to the base of the godet with a puff and any signs of glazing are noted. Payoff must be sufficient and the powder should spread evenly without losing adhesion to the skin.

Foundation

In general, foundation make-up's chief functions are to hide skin flaws, even out various color tones in the skin, act as a protectant from the environment, and makes the skin surface appear smoother. Requirements for an ideal make-up foundation's application are as follows: (i) should be moderately fast drying to allow for an even application; (ii) should be nonsettling, pour easily, be stable in storage; (iii) should not feel tacky, greasy, or too dry; (iv) it should improve appearance, not artificially; (v) it should have proper "play time" and slip. Depending on the formulations, several contain treated pigments and volatile silicones to add water resistance properties. There should be shade consistency between the bottle and the skin tone. Products should be uniform. Coverage or capacity will vary with skin types; finish on the skin may be matte, shiny, or "dewy." Wear is extremely important—product should not peel-off, go orangy on the skin, or rub-off on clothes.

Foundation make-up is available in the following forms:

- Emulsions: Oil-in-water—anionic, nonionic, and cationic; Water-in-oil—became more popular for waterproofness and contains volatile silicone, hydrocarbons, mineral oil, and light esters.

- Anhydrous: Cream powder and stick.
- Suspensions: Oil and aqueous.

Emulsified Foundations

Composition can vary widely depending on degree of coverage and emollient desired. Although nonionic (usually not stable), cationic (difficult to make, not in market), and water-in-oil systems have been marketed, most emulsified foundations are anionic oil-in-water emulsions, because of ease of formulation. Anionics possess the following properties:

- emulsion stability,
- pigment wetting and dispersion,
- easy spreading and blending,
- good skin feel,
- slippery (soap like) feeling.

Formulation Considerations

i. Prolonged skin contact. Minimize emulsifier levels to avoid irritation.
ii. Choose oils based on low *comedogenicity.*
iii. Preservation—foundations may be difficult to preserve containing water, gums, etc.

Make-Up Manufacturing Equipment

Emulsion make-up

- Pigment extenders: hammer mill and jet mill.
- Internal phase: propeller mixer/SS steam jacketed kettle.
- External phase: colloid mill, homogenizer/sidesweep and SS steam jacketed finishing kettle.
- Emulsification: sidesweep, homogenizer, and recirculating mill, i.e. colloid mill.
- With high viscosity systems, planetary mixer is needed.

Manufacturing

The coloration of the emulsion base may be handled in different ways: direct pigment, pigment dispersions, mixed pigment blender, and monochromatic color solutions (11). Each has its advantages and disadvantages. In the direct pigment method, the pigments are weighed directly into the aqueous phase and dispersed or colloid milled, then the emulsion is formed in the usual manner. The major problem is that there are too many color adjustments needed and accurate color matching is difficult. With the pigment dispersion method, the pigment is mixed with talc in a 50:50 dispersion ratio and pulverized to match a standard. This reduces the number of color corrections needed but storage may be a problem as well as the time taken to make these dispersions. During the mixed pigment blender method the pigments and the extenders are premixed, pulverized, and matched to a standard; it is then dispersed in the aqueous phase of the emulsion and the emulsion is formed in the normal way. The finished shade is color matched at the powder blender stage. Chances of error are reduced. The last method, the monochromatic color solutions, required one to make color concentrates of each pigment in a finished formula. It is

easy to color match by blending finished base but much storage space is needed and the possibility for contamination is increased.

Anhydrous Foundations

Generally are powdery, not fluid and easy to travel with.

Ingredients needed to be included:

Emollients: They are often texturally light and have low viscosity; include oils, esters and silicones.

Waxes:

Natural: beeswax, jojoba, orange, carnauba, candelilla, and castor.

Beeswax derivatives: Dimethicone copolyol beeswax, polyglyceryl-3 beeswax, butyl octanol, and hexanediol beeswax (nice texture, compatibility with silicone material).

Synthetic: paraffins, microcrystalline, polyethylene, and ''synthetic wax'' (highly branches olefin polymers).

Fatty alcohols and fatty alcohol ethoxylates: unithox and unilin.

Fatty esters: croda (syncrowaxes), koster keunen (kester waxes), Phoenix Chemical, Scher, Flora Tech and Residence time distribution.

Pigments (often surface treated):

TiO_2: pigmentary and ultrafine.

ZnO: pigmentary and ultrafine.

Iron oxides: pigmentary and ultrafine (enhances SPF value).

Texturizing agents often surface treated; include nylon, PMMA, sericite, talc, mica, boron nitride, Teflon®, borosilicates copolymer, polyvinylidene copolymer, spherical silica, starch (oats, rice, wheat, corn, dry flo-starch), BiOCl, Microcrystalline cellulose, Polyurethane powder and Silicone powder.

Wetting agents: A small amount to be used; include Low hydrophilic-lipophilic-balance (HLB) emulsifiers, polyglyceryl esters, e.g., polyglyceryl-3 diisostearate, hydrogenated lecithin, lanolin alcohols, polyhydroxy stearic acid and soya sterols.

Bioactives: The following ''actives'' have been included in foundations and liquid make-ups: algae extract (anti-inflammatory), hydrolyzed wheat protein (moisturizer and skin protectant) ginseng extract, green tea, linden extract, calcium pantothenate (antioxidants). Bisabolol (antiphlogistic), liposomes containing ceramide-2 cholesterol, linoleic acid and tocopheryl acetate. Titanium dioxide as a physical UV sunscreen and hydrolyzed soy protein and yeast for cell respiration. Vitamin C and E are antioxidants that help protect the skin from environmental damage. Urea and panthenol have been used for moisturizing and as anti-inflammatories.

Allantoin is used as an anti-irritant. Copper tripeptide-1 in concealers promises to firm and diminish dark circles under the eyes. There are many others as well such as AHAs, salicylic acid, and hyaluronic acid as a moisturizer.

Basic formulation:

Emollients (fluids, low melting point waxes, gel-like raws)	30–60%
Waxes	5–10%
Wetting agents	0.50–1.00%
Texturizing agents	30–60%

Surface treated raw materials are frequently utilized in these types of formulations for the following reasons:

- Improves dispersibility
- Enhances solids loading

  –provides drier texture
  –creates matte appearance
  –improves wear
  –overall improved aesthetics

Manufacturing Procedure

i. Emollients, waxes and wetting agent(s) are introduced into a jacketed kettle and heated until phase is clear and uniform.
ii. Pigments and texturizing agents are slowly introduced into the oil phase with higher shear mixing. High shear mixing is continued until dispersion is uniform and colorants are completely "extended."

Note:If surface treatments are temperature sensitive, care must be taken to prevent the displacement of that treatment from the surface of the powder into the oil phase itself.

Eye Make-Up

Mascara

(i) It brings out the contrast between the iris and the white of the eye, sharpens the white of the eye (ii) thickens the appearance of the lashes (iii) lengthens the appearance of the eye (iv) adds depth and character to the overall look (v) and sharpens the color of the eye shadow, when worn. Mascara's performance is usually judged by application, appearance, wear, and ease of removal. It is critical that proper brush is supplied for the chosen formulation. Generally, mascara and eyeliners consist of one or more film formers, pigment, and a vehicle that mostly evaporates to allow the film to set.

Three types of formulations are currently in use: (In the past, cake or block mascara was popular. This was basically a wax base with a soap or nonionic emulsifier present so that that color could be applied with a wetted brush).

- Anhydrous solvent based suspension: waterproof but not smudge-proof and difficult to remove.
- Water-in-oil emulsion: also waterproof but not smudge-proof and can be removed with soap and water.
- Oil-in-water emulsion: "water-based," if the film is sufficiently flexible, could be flake-proof and smudge-proof. Water resistance could be achieved with the addition of emulsion polymers, i.e., acrylics, polyvinyl acetates, or polyurethanes.

Oil-in-Water
Water phase
  Water
  Suspending agent: hydroxyethylcellulose
  Film former/dispersing agent: polyvinylpyrrolidone

(*Continued*)

Pigment
Hydrophilic emulsifier: alkali, high HLB nonionic
Wax phase
High melting point waxes
Lipophilic emulsifier: fatty acid, low HLB nonionic, coemulsifier
Plasticizer: lanolin or derivatives, liquid fatty alcohol
Petroleum solvent (optional) as extender for water phase
Preservative: propyl paraben
Additional film formers and actives
Solution polyacrylate (improves flake resistance)
Emulsion polyacrylate
Polyurethane
Polyvinyl acetate
Rosin derivatives
Dimethiconol
Proteins: wheat, soy, corn, keratin, oat, silk
Melanin and Tocopherol—antioxidant/anti-free radicals
Panthenol
Preservative
Formaldehyde donor (not for use in Japan)
Manufacturing
The procedure is the general oil-in-water emulsification procedure except that iron oxides are first soaked and milled in the water phase prior to emulsification and the final product goes through a colloid mill, roller mill, or homogenizer
Solvent based
Hard, high melting point waxes
Rosin derivative (optional)
Wetting agent
Pigment
Suspending agent (organoclay)
Volatile solvent (to achieve wax solubility)
Petroleum distillate
Cyclomethicone
Preservatives: parabens
Plasticizer: lanolin or derivative, liquid fatty alcohol
Water-in-Oil
Wax phase
High melting point waxes (carnauba, candellila, polyethylene)
Rosin derivative (optional)
Lipophilic emulsifier (lanolin acids, low HLB nonionic)
Pigment
Preservative: propyl paraben
Petroleum solvent, some cyclomethicone
Water phase:
Hydrophilic emulsifier (alkali, medium HLB nonionic)
Preservative: methyl paraben
Additives
Emulsion polymer (optional)
Preservative: Formaldehyde donor (not for use in Japan)

Anhydrous Mascara

Ingredients:

- *Solvents*: Branched chain hydrocarbons and petroleum distillates, isoparaffinic hydrocarbons, and volatile silicones.
- *Waxes*: Beeswax and its derivatives, candelilla, carnauba, paraffin, polyethylene, microcrystalline, castor, synthetic, ceresin, and ozokerite.
- *Resins*: (could be introduced, but do not have to be); include aromatic/aliphatic, hydrogenated aromatics, polyterpene, synthetic, rosin, acrylics, and silicones.
- *Gellants*: clays (stearalkonium hectorite, quaternium-18 bentonite, quaternium-18 hectorite), metal soaps (Al, Zn stearates).
- *Colorants*: Most often utilize a classic iron oxide without any surface treatment.
- *Functional fillers*: Spherical particles (PMMA, silica, nylon), boron nitride, starches, Teflon®.

Purpose:

- Provides body to film, to enhance thickening properties
- Improves transfer resistance
- Improves deposit on lashes

Basic formulation:

Solvent(s)	40–60%
Waxes10	10–20%
Resin(s)	3–10%
Gellant	3–7%
Colorant(s)	5–15%
Filler(s)	2–10%

Procedure

i. Heat waxes, solvents, and resins in a jacketed kettle until uniform and clear. Slowly add pigments under high shear and mill until dispersion is uniform.
ii. Under high shear, add gellant and mill until uniform. Activate gellant with polar additive-like propylene carbonate. Under high shear, add fillers and mill until uniform. Cool to desired temperature.

Mascara Componentry

Bottle:

Polyvinyl chloride (PVC) for solvent-based and H.D. polyethylene/polypropylene for water-based types.

Brush/Rod/Wiper:

Works complementary with each other to deliver required product attributes.

For a thickening mascara, the following is required:

Larger diameter rod
Larger diameter wiper
Larger brush with significant spacing between the bristles

For a defining mascara, the following are suggested:

A smaller diameter rod
Smaller diameter wiper
Brush with minimal spacing between the bristles

Brush materials, fiber diameter, brush shape, fiber shape, fiber length, wire diameter, and the number of turns in the wire, all affect performance.

Cream Eye Shadows

Generally, cream eye shadows are another form of eye shadows, which are not as popular as the pressed form. Care must be taken in formulation to avoid creasing and other wear problems. In the past, stick eye shadows were popular. They are similar to cream eye shadows but contain high melting point waxes to make them moldable. The ingredients utilized are as follows:

Ingredients:

- Volatile solvents: cyclomethicone, hydrocarbons, isoparaffins
- Waxes: similar to those utilized in the anhydrous waterproof mascaras although at lower concentrations
- Emollients: esters, oils, silicones
- Gellants: bentonite derivatives, hectorite derivatives
- Colorants and pearls: classical
- Fillers: mica, talc, sericite
- Functional fillers: boron nitride, PMMA, nylon, starches, silica, Teflon®, lauroyl lysine

For enhanced textural properties, higher solid loading, improved application, and coverage, surface-treated raw materials, whose coatings are neither temperature nor solvent sensitive, could be used. The absorption of fillers is balanced to maintain similar textures throughout the shade range.

Basic formulation

Solvent	35–55%
Gellants	1.50–3.50%
Waxes	7–12%
Emollients	3–8%
Colorants/pearls	5–20%
Fillers	10–20%
Functional fillers	5–15%

Manufacturing procedure: Identical to anhydrous mascaras

Eyeliners

Eyeliners frame the eye while adding shape or change the shape of the eye. They give the illusion of a larger or smaller eye bringing out the color contrast between the iris and white of the eye. Lastly, eyeliners assist in making the lashes appear thicker.

Generally, liquid eyeliners are the most popular and will be chiefly outlined. Cake eyeliner was popular in the past and was a wettable pressed cake applied with a wet brush. It contained powder fillers, waxes, resins, and a soap or nonionic. Liquid eyeliners include the following list of ingredients:

Ingredients:

- Solvent: Water
- Gellant: Gums (magnesium aluminum silicate and bentonite)
- Wetting agents: Water-soluble esters and high HLB emulsifiers
- Polyols: Propylene glycol, butylene glycol, and 2-methyl-1, 3 propanediol
- Colorants: Surface treatment is not essential but will enhance ease of dispersibility, maintain fluidity, improve adhesion, and may enhance water resistance. Chiefly, iron oxides and other inorganic colorants are utilized.
- Alcohol: solubilizes resins and improves dry time
- Film formers: PVP, PVA, acrylics, PVP/VA, PVP/urethanes

Basic formulations:

Water	50–70%
Gellant	0.50–1.50%
Wetting agent(s)	1–3%
Polyol	4–8%
Colorants	10–20%
Alcohol	5–10%
Film Former	3–8%

Manufacturing procedure: Gellants is premixed with the polyol and added to a heated water phase that also contains the wetting agent. Disperse with high shear until uniform. Add colorants and disperse until uniform. Cool and add alcohol and film former with low shear.

Pencils

Pencils are used in general for coloring the eyebrows and eyelids, although they are now popular as lipsticks, lip liner, and blushers depending on the hardness of the pencil and the color composition.

Products are nearly always manufactured by a handful of contract manufacturers.

Chemists' responsibility is to evaluate the finished product, rather than create one. Evaluation includes shade, texture, sharpenability, wear, application, stability (freeze–thaw and at 40°C to 45°C) and penetration.

Generally, extruded pencils are less stable than the molded ones.

Raw materials

- Oils, esters, silicones
- High melt point triglycerides
- Stearic acid, helps in the extrusion
- Synthetic waxes
- Japan wax
- Bright colorants and pearls in leads increase the variety available in cosmetic pencils
- Fillers, mica, talc, sericite
- Functional fillers, boron nitride, Teflon, PMMA, silicas

Product types: Eyeliner, Lipliner , Eyeshadow, Lipstick, Brow, Blush, Concealer

Manufacturing procedure

Molded and extruded; significant differences exist in how these products are evaluated initially after manufacturing.

Molded pencils set up within a few days.

Extruded pencil set up slowly over a few weeks. The molded or extruded lead is placed in a slat of wood grooved lengthwise. A second grooved slat, is glued onto the first slat and pressed together.

Lipstick

Lipsticks add color to the face for a healthier look, shapes the lips and sometimes its condition. It harmonizes the face with the eyes, hair, and clothes. It creates the illusion of smaller or larger lips depending on the color used. Certain pigments act to give an illusion of thicker lips. A list of lipstick actives may include herbal products and cholesterol derivatives as moisturizers. Pigments themselves tend to filter the sun, especially titanium dioxide and zinc oxide. Several lipsticks contain organic sunscreens.

There are two types of lipsticks—classical and volatile based.

The Ingredients in a Classical Lipstick

- Emollients: castor oil, esters, lanolin/lanolin oil, oily alcohols (octyl dodecanol), organically modified silicones (phenyltrimethicone and alkyl dimethicones), meadow-foam seed oil, jojoba oil, and esters and triglycerides.
- Waxes: candelilla, carnauba, beeswax and derivatives, microcrystalline, ozokerite/ceresein, alkyl silicone, castor, polyethylene, lanolin, paraffin, synthetic, and ester.
- Wax: modifiers (plasticizers): Work in conjunction with the waxes to improve texture, application and stability include cetyl acetate and acetylated lanolin, oleyl alcohol, synthetic lanolin, acetylated lanolin alcohol, and petroleum (white and yellow).
- Colorants widely used:

Colorants
D&Cs
Red #6 and Ba Lake
Red #7 and Ca Lake
Red #21 and Al Lake-(stains)
Red #27 and Al Lake-(stains)
Red #33 and Al Lake
Red #30
Red #36
Yellow #10
FD&C's
Yellow #5,6 Al Lake
Blue #1 Al Lake
Iron oxides
TiO_2
ZnO
Pearls
No Fe Blue, Ultramarines, and Mn Violet

- Actives: Raw materials are added for claims and moisturization; tocopheryl acetate, sodium hyaluronate, aloe extract, ascorbyl palmitate, silanols, ceramides, panthenol, amino acids, and beta carotene.
- Fillers (matting and texturizing agents): mica, silicas (classical and spherical), nylon, PMMA, Teflon®, boron nitride, BiOCl, starches, lauroyl lysine, composite powders, and acrylates copolymers.
- Antioxidants/preservatives: butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), rosemary extract, citric acid, propyl paraben, methyl paraben, and tocopherol.

Classical lipsticks

Classical lipsticks

Formula	Gloss	Matte
Emollients	50–70%	40–55%
Waxes	10–15	8–13
Plasticizers	2–5	2–4
Colorants	0.5–3.0	3.0–8.0
Pearl	1–4	3–6
Actives	0–2	0–2
Fillers	1–3	4–15
Fragrance	0.05–0.10	0.05–0.10
Preservatives/antioxidants	0.50	0.50

Procedure

(i) Pigments are premilled in either one of the emollients (e.g. castor oil) or the complete emollient phase by a three-roller mill, or a stone mill, or a type of ball mill, (ii) Ground phase is added to complete emollient phase and waxes, and heated and mixed until it is uniform (approximately 90°C to 105°C), (iii) Pearls and fillers are added to above phases and mixed with shear (if necessary) until it is homogenized, (iv) Actives, preservatives, fragrances, and antioxidants are added and mixed to obtain a uniform mixture, (v) Temperature is maintained just above the initial set point of the waxes and filled as required.

The Ingredients for Volatile Lipstick (nontransfer)

A proper balance of solvents and emollients prevent transfer and prevent lipstick from becoming too dry on the lips (12).

- Solvents: isododecane, alkyl silicones, and cyclomethicone
- Emollients: phenyl trimethicone, esters, alkyl silicones (fluids, pastes), and vegetable/plant oils
- Waxes: polyethylene, synthetic, ceresin, ozokerite, paraffin (not compatible with some silicones), beeswax, and alkyl silicones
- Fixatives: silicone resins (MQ type from GE) and silicone plus polymers (SA 70–5, VS 70-5)
- Colorants/pearls: identical to classical lipstick

- Fillers: identical to classical lipstick
- Actives: identical to classical lipstick
- Preservatives/antioxidants: identical to classical lipstick.

Solvent lipstick	
Formula	
Solvent	25–60%
Emollient	1–30%
Waxes	10–25%
Fixatives	1–10%
Fillers	1–15%
Colorants/pearls	1–15%
Fragrance	0.05–0.10%

Procedure

The process is identical to that of classical lipstick except that the product should be prepared in a closed vessel to prevent loss of volatile components. Two new lipsticks have recently appeared. A semi-permanent color which is marketed as two sticks: the first one is the color, the second a moisturizing topcoat (13). The wear is exceptional when compared to the previous developments. A different development uses interference pearlescent pigments to optically plump the lips (14).

Nail Color

Nail lacquers form the largest group of manicure preparations. They should be waterproof, glossy, adherent, dry quickly, and be resistant to chipping and abrasion. The main constituents include a film former, modifying resin, plasticizer, and solvents. Additionally, pigments, suspending agents and ultraviolet absorbers are usually included. Nitrocellulose is the chief film-forming ingredient. Nitrocellulose is derived from cellulose, a polymer made of several anhydroglucose units connected by ether linkages. Nitrocellulose by itself will produce a hard brittle film so it is necessary to modify it with resins and plasticizers to provide flexibility and gloss. The most commonly used modifying resin is para-toluene sulfonamide formaldehyde resin, which is contained at 5% to 10% levels. This resin provides gloss, adhesion and increases the hardness of the nitrocellulose film. The formaldehyde resin has caused allergies with a small number of consumers so that other modifiers such as sucrose benzoate, polyester resin, and toluene sulfonamide epoxy resin have been used in its place with varying results. The plasticizers used include camphor, glyceryl diesters (15), dibutyl phthalate, citrate esters, and castor oil. Other resins such as polyurethanes and acrylics have been used as auxiliary resins. Variations of plasticizers and resins will change the viscosity, dry time, and gloss of the lacquer. Colorants include titanium dioxide, iron oxides, most organics and pearlescent pigments. Soluble dyes are never used because of their staining effects on skin and nails. To reduce settling of the heavier pigments, treatment such as silicone (16) and oxidized polyethylene (17) have been utilized. Modified clays derived from bentonite and/or hectorite are used to suspend the pigments and make the nail enamel thixotropic and

brushable. Solvents which constitute approximately 70% of nail lacquers include n-butyl acetate, ethyl acetate, and toluene. Generally, those are cream and pearl nail lacquers. Cream shades may shear or provide full coverage, with titanium dioxide as the chief pigment. Pearlescent nail polish usually contains Bismuth oxychloride and/or titanium dioxide coated micas and may even contain guanine from natural fish scales. The manufacturing of nail lacquer is usually carried out by specialty manufacturing firms, which are familiar with the hazards of working with nitrocellulose and solvents. The manufacture consists of two separate operations (i) manufacture and compounding of the lacquer base, (ii) and the coloring and color matching of shades. Topcoats that are used to enhance gloss, extend wear, and reduce dry time are usually made with high solids and low boiling point solvents. Cellulose acetate butyrate (CAB) has been used as a substitute for nitrocellulose in nonyellowing topcoats but does not adhere well to the nail (18). Most topcoats are nitrocellulose based. Base coats function to create a nail surface to which nail lacquer will have better adhesion. Different auxiliary resins, such as polyvinyl butyral have been used in nitrocellulose systems. Fibers, polyamide resins, and other treatment items have been added to provide advertising claims and some may actually alter the effectiveness of the film. In the evaluation of nail enamels the following criteria are used: color, application, wear, dry-time, gloss, and hardness.

Most bioactives are found in nail care products such as cuticle massage creams and oils, cuticle removers and softeners, and nail hardeners. Vitamins, herbs such as aloe and seaweed extract, myrrh, milk and other proteins, keratin amino acids, and other botanical extracts may be present for moisturizing claims. Many new shades have been developed with higher levels of mica, and aluminum flakes to give a bright mirror-like appearance on the nail. Besides new and different color effects, a two-step acrylic color and sealer (19) have been developed which provides longer wear than most conventional nail enamels. The first component is the color, whereas the second one provides the sealer.

MAKE-UP FORMULARY

Face Products

Loose Face Powder (20)

Ingredients	W/W%
Zinc stearate	8.00
Magnesium carbonate	1.00
Iron oxides	q.s.
Bismuth oxychloride and mica	25.00
Fragrance	q.s.
Talc	100.00
Preservative	q.s.

Procedure

Ingredient three is mixed with a portion of ingredient six and pulverized. The other ingredients are added and mixed in a ribbon or double-cone blender until uniform powder is obtained.

Pressed Powder Foundation (21)

Ingredients	W/W%
PART A	
Talc	6.60
Titanium dioxide	19.20
Mica (and) titanium dioxide	4.80
Iron oxides	11.20
Zinc oxides	6.20
Barium sulfate	13.70
PART B	
Dimethicone	5.50
Lanolin	8.20
Petrolatum	1.40
Mineral oil	1.40
Isopropyl myristate	1.40
PART C	
Fragrance	q.s.
Preservative	q.s

Procedure

The pigments in Part A are mixed together, and Part B, Part C, and Part D elements are added with high shear mixing and pressed into a suitable container.

Two-Way Powder Foundation (Wet and Dry)

Ingredients	W/W%
Sericite	35.0
Talc	24.0
Mica	10.0
Nylon-12	10.0
Titanium dioxide	8.0
Zinc sterate	3.0
Iron oxide pigments, silicone treated	2.0
Cetyl octanoate	q.s.
Squalane	2.0
Octyldodecyl myristate	2.0
Mineral oil	2.0
Dimethicone	2.0
Propyl paraben	0.05
Butyl paraben	0.05
Perfume	q.s.

Procedure

All ingredients are mixed except for liquid-oils and perfumes in a blender, and liquid-oils and perfumes are sprayed or added. The resultant is mixed, pulverized, and pressed into pans.

Pressed Face Powder

Ingredients	W/W%
PART A	
Polymethyl methacrylate	12.00
Talc (and) polyethylene	q.s. to 100.0
Sericite	10.00
Mica (and) polyethylene	5.00
Magnesium stearate	3.00
Mica (and) Titanium dioxide	5.00
Kaolin	8.00
Color	q.s.
PART B	
Dimethicone	6.00
Glyceryl diisostearate	2.00
Tocopherol	0.10
Butyl paraben	0.05
Propyl paraben	0.05

Procedure

The ingredients of Part A are mixed well. The ingredients of Part B are heated to 80°C and mixed until a uniform powder is obtained. Thus, Part B is added to Part A, and blended until a uniform mixture is attained. It is pulverized and sieved, and the resulting powder is pressed into pans.

Liquid Compact Foundation

A hot-pour solid crème foundation that seems to "liquefy" when touched. It is easy to blend to a sheer finish.

Ingredients	W/W%
PART A:	
Titanium dioxide (and) isopropyl titanium triisostearate	12.99
Yellow iron oxide (and) isopropyl titanium triisostearate	0.33
Red iron oxide (and) isopropyl titanium triisostearate	0.33
Black iron oxide (and) isopropyl titanium triisostearate	0.10
Aluminum starch octenyl succinate (and) isopropyl titanium triisostearate	15.00
Sericite	6.25
Silica	2.00
PART B:	
Squalene	6.50
Dimethicone (5 centistoke)	11.00
Octyl palmitate	18.00
Polyglycerol-3 diisostearate	5.50
Mineral oil	3.00
Hydrogenated coco glycerines	2.00
Microcrystalline wax	4.00
Carnauba	1.00
PART C:	
Nylon-12	12.00
	100.00

Procedure

Part A is micronized until the color is fully developed. Part B is heated with stirring to 195°F to 200°F, and continuously stirred for 30 minutes. Part A and Part B are homogenized. The homogenate is cooled to 180°F and added to Part C and further homogenized. The resultant is poured into pans at 165–170°F.

Blusher (Pressed) (22)

Ingredients	W/W%
Talc	65.70
Zinc stearate	8.00
Titanium dioxide	3.50
Iron oxides (russet)	12.00
Iron oxides (black)	0.20
D&C Red No. 6 barum lake	0.30
Titanium dioxide (and) mica	6.00
Methyl paraben	0.10
Imidazolidinyl urea	0.10
Fragrance	0.10
Pentaerythritol tetraisostearate	4.00
	100.00

Procedure

The ingredients 1 to 9 are mixed well, pulverized, and placed in a ribbon blender. A batch of ingredients 10 and 11 is sprayed into it. The resultant is re-pulverized, sieved, and pressed into pans.

Eye Shadow (Pressed) (23)

Ingredients	W/W%
Mica (and) iron oxides (and) titanium dioxide	40.5
Talc	32.4
Cyclomethicone (and) Dimethicone	13.6
Oleyl Erucate	13.5
	100.00

Procedure

All the ingredients are mixed and milled through a 0.027″ herring-bone screen and pressed into a suitable container.

Eye Shadow (Pressed) (24)

Ingredients	W/W%
Talc	4.20
Bismuth oxychloride	10.00
Fumed silica	0.50

Ingredients	W/W%
Zinc stearate	5.00
Titanium dioxide (and) mica	65.00
Methyl paraben	0.10
Propyl paraben	0.10
Imidazolidinyl urea	0.10
Lanolin alcohol	3.75
Mineral oil	9.75
Isostearyl neopentanoate	1.50
	100.00

Procedure

The ingredients 1 to 8 are mixed in a ribbon blender, and binders 9 through 11 are mixed in a separate container. The resultant binder is sprayed into the blend of ingredients 1 through 8 and mixed, pulverized, if necessary without a screen, to obtain a uniform blend and pressed into pans.

Solvent Mascara (25)

Ingredients	W/W%
PART A	
Petroleum distillate	q.s. to 100.00
Beeswax	18.00
PEG-6 sorbitan beeswax	6.00
Ozokerite 170-D	4.00
Carnauba wax	6.00
Propylparaben	0.10
Glyceryl oleate (and) propylene glycol	1.50
PART B	
Iron oxides	15.00
PART C	
Petroleum distillate (and) quaternum-18 hectorite (and) propylene carbonate	12.50
PART D	
Deionized water	15.00
Methylparaben	0.30
Sodium borate	0.60
Quaternium-15	0.10

Procedure

Pigment (B) is milled into (A) which has been heated to 90°C. After (C) has been added slowly and heated with the milled (A), (D) is added at 90°C to (A), (B), and (C) and emulsified. The resultant emulsion is mixed until it is cooled.

Emulsion Resistant Mascara (25)

Ingredients	W/W%
PART A	
Deionized water	41.00
Hydroxyethyl cellulose	1.00
Methylparaben	0.30
Aqueous 0.10% phenyl mercuric acetate	4.00
Triethanolamine	1.00
Ammonium hydroxide, 28%	0.50
PART B	
Iron oxides	10.00
Ulltramarine blue	2.00
PART C	
Isostearic acid	2.00
Stearic acid	2.00
Glyceryl monostearate	1.00
Beeswax	9.00
Carnauba wax	6.00
Propylparaben	0.10
PART D	
Quaternium-15	0.10
PART E	
30% Acrylic/acrylate copolymer solution in ammonium hydroxide	20.00
	100.00

Procedure

The pigments of Part B are milled in the water phase (Part B) and heated to 80°C. The oil phase (Part C) is heated to 82°C. Emulsify the resultant and cool to 50°C. To this ingredient in Part D and then ingredients of Part E are added. Cool the resultant to 30°C.

Waterproof Eyeliner (26)

Ingredients	W/W%
Beeswax	16.50
PVP/eicosene copolymer	5.00
Petroleum distillate	35.00
Petroleum distillate (and) quaternium-18 hectorite (and) propylene carbonate	33.50
Preservative	0.20
Titanium dioxide (and) mica (and) ferric ferrocyanide	9.80
	100.00

Procedure

(i) Ingredient 1 is heated to 70°C and blended in ingredient 3 (n.b. flammable), (ii) The resultant is blended into ingredient 4 with low shear mixing, and (iii) it is cooled to 50°C while mixing, (iv) it is then blended in ingredients 2, 5, and 6, and mixed until a uniform resultant is obtained.

Aqueous Eyeliner (27)

Ingredients	W/W%
PART A	
Ammonium vinyl acetate/actylates copolymer	55.00
Polysorbate 80	1.00
Isopropyl myristate	4.00
PART B	
Propylene glycol USP	2.50
Methylparaben USP	0.25
Water, deionized	29.50
Hectorite (and) hydroxyethylcellulose	0.25
Iron oxides	7.50
	100.00

Make-Up Pencil (28)

Ingredients	W/W%
PART A	
a. Cyclomethicone	40.0
b. Bis Phenylhexamethicone	40.0
c. Diphenyl dimethicone	40.0
PART B	
Beeswax	15.0
Carnauba	7.0
Ozokerite	7.0
Paraffin	20.0
Mineral oil	q.s. to 100.0
Cetyl alcohol	1.0
PART C	
Pigments	q.s.
Titanium dioxide	q.s.

Procedure

The ingredients of Part B are melted and homogenized at 78°C to 82°C, and maintained by a thermostatic bath regulated to a temperature of 58°C to 62°C. The ingredients of Part C are dispersed in Part A; the mixture is placed in a thermostatic bath at 58°C to 62°C. The ingredients of Part C are then added. After homogenization, the obtained resultant is cooled in a silicone-treated mold (with dimethicone).

Classical Lipstick (29)

Ingredients	W/W%
Carnauba wax	2.50
Beeswax, white	20.00

Ingredients	W/W%
Ozokerite	10.00
Lanolin, anhydrous	5.00
Cetyl alcohol	2.00
Liquid paraffin	3.00
Isopropyl myristate	3.00
Propylene glycolricinoleate	4.00
Pigments	10.00
Bromo acids	2.50
Castor oil	q.s. to 100.00

Solvent Lipstick (30)

Ingredients	W/W%
Synthetic wax	6.00
Ceresin	4.00
Isododecane	10.00
Paraffin	3.00
Cetyl acetate/acetylated lanolin alcohol	5.00
Methylparaben	0.30
Propylparaben	0.10
BHA	0.10
D&C Red No. 7 calcium Lake	4.00
FD&C Yellow No. 5 aluminum Lake	3.00
Titanium dioxide/mica	5.00
Titanium dioxide/mica/iron oxides	3.00
Bismuth oxychloride	10.00
Cyclomethicone	41.50
Isostearyl trimetholpropane siloxy silicate	5.00
	100.00

Procedure

Dry ingredients are mixed with volatiles and silicone ester wax. The waxes and oils are added to it while heating and the powders are then added. The mixture is stirred before being poured into molds and allowed to cool.

Cream Nail Enamel (31)

Ingredients	W/W%
n-Butyl acetate: solvent	28.23
Toluene: diluent	24.54
Nitrocellulose 0.5 sec wet-film-former	12.00
Ethyl acetate: solvent	11.00
Toluene sulfonamide/formaldehyde resin: secondary resin	10.00
Acrylates Copolymer: resin	0.50
Dibutyl Phthalate: plasticizer	5.00

Ingredients	W/W%
Isopropyl alcohol, 99%: diluent	4.25
Stearalkonium hectorite: suspending agent	1.00
Camphor: plasticizer	1.50
D&C Red No. 6 Barium Lake: color	0.08
Titanium dioxide	0.75
Iron oxides	0.15
	100.00

Pearlescent Nail Enamel (32)

Ingredients	W/W%
n-Butyl acetate	34.04
Toluene	30.00
Nitrocellulose 0.5 sec. Wet	14.90
Toluene sulfonamide/formaldehyde resin	7.10
Dibutyl phthalate	4.80
Camphor	2.40
Stearalkonium hectorite	1.20
Benzophenone-1	0.20
D&C Red No. 7 calcium lake	0.08
D&C Red No. 34 calcium lake	0.05
FD&C Yellow No. 5 aluminum lake	0.08
Iron oxides	0.15
Bismuth oxychloride (25%)	5.00
	100.00

Acrylic Nail Hardener (32)

Ingredients	W/W%
Ethyl acetate	41.20
Butyl acetate	30.00
Nitocellulose 0.5 sec wet	14.00
Toluene sulfonamide/formaldehyde resin	10.00
Dibutyl Phthalate	4.00
Camphor	0.50
Acrylates Copolymer	0.20
Benzophenone-1	0.10
	100.00

REFERENCES

1. 21 CFR Parts 1–99, 1998(April 1).
2. EC Cosmetics Directive 76/768/EEC, Annex IV, Part 1, 1998(September 3).
3. MHW Ordinance No. 30, 1966(August 31).
4. 61 Federal Register 8372, 1996(March 6).
5. EC Cosmetics Directive 76/768/EEC, Annex IV, Part 1, 1998(September 3).
6. MHW Ordinance No. 30, 1966(August 31).
7. Knowlton JL, Pearce SEM. Decorative cosmetics. In: Handbook of Cosmetic Science and Technology. Oxford: Elsevier Advanced Technology, 1993:128.
8. Miyoshi R. U.S. Patent No. 4,606,914 (1986).
9. Miyoshi R, Isao Imai. U.S. Patent No. 4,622074 (1986).
10. Schlossman ML. U.S. Patent No. 4,877,604 (1989).
11. Dweck AC. Foundations—A Guide to Formulation and Manufacture. Cosmet Toilet 1986; 101(4):41–44.
12. Castrogiavanni A, Barone SJ, Krog A, McCulley ML, Callelo JF. U.S. Patent No. 5,505,937 (1996).
13. Drechsler LE, Rabe TE, Smith ED. US 6,340,466 (2000).
14. Cohen ID, Oko J. US 6,428,773 (2002).
15. Castrogiavanni A, Sandewicz RW, Amato SW, U.S. Patent No. 5,066,484 (1991).
16. Socci RL, Ismailer AA, Castrogiavanni, A, U.S. Patent No. 4, 832,944 (1989).
17. Weber RA, Frankfurt CC, Penicnak AJ, U.S. Patent No. 5,174,996 (1992).
18. Martin FL, Onofrio MV, U.S. Patent No. 5,130,125 (1992).
19. Armstrong G, Callelo J, Pabil A, Pagamo F, Sandewicz, R. US Patent Application Published US 2002 20018759 (April 26, 2001).
20. Hunting ALL. Face cosmetics. In: Decorative Cosmetics. Weymouth, Dorset, England: Micelle Press, 1991:3.
21. Personal Care Formulary, Waterford, NY, GE Silicones 1996:151.
22. Knowlton JL, Pearce SEM. Decorative products. In: Handbook of Cosmetic Science and Technology. Oxford, UK: Elsevier Advanced Technology, 1993:143.
23. Personal Care Formulary, Waterford, NY, GE Silicones (1996), p. 149.
24. Knowlton JL, Pearce SEM. Decorative cosmetics. In: Handbook of Cosmetic Science and Technology. Oxford, UK: Elsevier Advanced Technology, 1993:145.
25. Schlossman ML. Application of Color Cosmetics. Cosmet Toilet 1985; 100(5):36–40.
26. Hunting ALL. Eye cosmetics. In: Decorative Cosmetics. Weymouth, Dorset, England: Micelle Press, 1991:173.
27. Hunting, ALL. Eye cosmetics. In: Decorative Cosmetics. Weymouth, Dorset, England: Micelle Press, 1991:170.
28. Hunting ALL. Eye cosmetics. In: Decorative Cosmetics. Weymouth, Dorset, England: Micelle Press, 1991:174.
29. Bryce DM. Lipstick In: Poucher' Perfumes, cosmetic ans Soaps. London, UK: Chapman & Hall, 1992:234.
30. Castrogiavanni A, Barone SJ, Krog A, McCulley ML, Callelo JF. U.S. Patent No. 5,505,937 (1996).
31. Schlossman ML. Manicure preparations. In: Poucher' Perfumes, Cosmetic and Soaps. London, UK: Chapman & Hall, 1992:253,254.
32. Schlossman ML. Manicure preparations. In: Poucher' Perfumes, Cosmetic and Soaps. London, UK: Chapman & Hall, 1992:254.
33. Schlossman ML. Make-up formulary. Cosmet Toilet 1994; 109(4):104.

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Cosmetics for Nails

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The purpose of this chapter is to present the cosmetics used for the decoration of the nail among which the nail coating is of prime importance. Fingernail coatings consist of two types (1–3):

1. Coatings that harden upon evaporation: these products include nail polishes, topcoats, and base coats.
2. Coatings that polymerize: nail enhancements are a special type of coating used to create artificial fingernails.

EVAPORATION COATINGS

Base coat, top coat, and nail enamel have similar basic formulas. They consist of the following:

1. A *film former such as nitrocellulose.* This organic polymer creates a continuous coating over the nail-plate. Other non-nitrated cellulosic materials are also used with varying degrees of success, namely cellulose acetate and derivatives. Polyurethanes, polyamides, and polyesters have also been used. However, these cannot match the toughness and surface hardness of nitrocellulose. Being one of the most commonly used, nitrocellulose has several disadvantages: the surfaces produced by this polymer have low gloss and the films are brittle and adhere poorly to the nail-plate. Upon evaporation, nitrocellulose films shrink excessively which leads to poor adhesion. To overcome these drawbacks, additional film modifiers will offset some deficiencies of the primary film form.
2. *Film modifiers*. They are specifically used to improve adhesion and gloss. The most commonly used modifier is toluene sulfonamide/formaldehyde resin (TSFR), which is considered to be the heart of the product. This thermoplastic resin improves nail-plate adhesion while producing

water-resistant, glossy surfaces with improved flexibility. Unfortunately, this resin is the main culprit of the users' sensitization. Use of this resin imparts between 0.05% and 0.1% free formaldehyde (as impurity) into the formulation. Therefore, many alternate modifiers have been tried, including toluene/sulfonamide/expoxy resin, polyester sucrose benzoate, polyesters, acrylic ester oligomers, sucrose acetate isobutyrate, arylsulfonyl methanes, and glyceryl tribenzoate.

3. *Plasticizers.* Plasticizers are chemical flexibilizers for polymer films that improve their durability. They may also improve adhesion and gloss. Dibutyl phthalate and camphor are the most common examples of low-molecular weight, high boiling point plasticizers. Other examples of plasticizers are castor oil, glyceryl tribenzoate, acetyl tribenzoate citrate PPG-2 dibenzoate, glycerol, citrate esters, triacetin, and a polyether urethane.
4. *Solvents/diluents*. The solid film-forming polymers are deposited on the nail-plate upon evaporation. The most commonly used solvents are alkyl esters and glycol ethers. Coupling agents (aliphatic alcohols) are useful in varnishes to increase the overall solubility and flow of the system. Diluents are usually nonpolar compounds that will not dissolve nitrocellulose. Toluene was commonly used until the appearance of California Proposition 65. Most companies are now developing toluene-free formulas.
5. *Viscosity modifiers or thixotropic agents.* Ideally, a nail enamel should be gel-like when sitting on the shelf, but significantly thin when brushed on. Both consistencies are possible in one bottle by using thixotropic agents such as stearalkonium hectorite.
6. *Color additives.* Colorants should be nonsoluble pigments to prevent staining of the nail-plate. Guanine, derived from scales of Atlantic herring, produces pearlescent pigment. Bismuth oxychloride and mica coated with titanium dioxide are used to create iridescent shades.
7. *Base and top coats.* Base coats contain a high percentage of TSFR. They are applied to the nail before application of nail varnish. They are adhesion promoters that improve retention and coating toughness. Top coats use higher levels of film formers such as nitrocellulose to maximize surface gloss and hardness. Often the top coat contains UV-absorbing materials.

POLYMERIZING COATINGS

Sculptured Artificial Nails

Liquid-and-powder systems are based on methacrylates. They consist of a liquid monomer (ethyl methacrylate) mixed with a polymer powder (polyethyl and/or polymethyl methacrylate), the latter carrying only the heat-sensitive initiator (usually benzoyl peroxide) to the monomer. UV absorbers are polymer additives that prevent sunlight yellowing. Catalysts speed up polymerization.

Light-Curing Gels

UV or visible light-curing gels are made primarily of urethane acrylate and other acrylated oligomers. The catalyst and oligomers, being associated with an initiator,

are combined into a single product; they come premixed and ready to use. They may be considered a variant of sculptured artificial nails.

Preformed Artificial Nails

These are usually made of acrylonitrile-butadiene-styrene plastic, nylon, or acetate, and are adhered to the natural nail with cyanoacrylate monomer. For home-use, retail versions of these tips may be used as temporary natural overlays, worn not for more than 48 hours at a stretch. They are more often used as permanent nail-tip extensions. Professional nail technicians usually coat these tips with artificial nail products to create longer lasting nail extensions. Most nail technicians feel it is too time-consuming to sculpt nails and these tips speed up the process. The tip can be coated or overlaid with wraps, liquid-and-powder, or gel products.

Wraps

Wraps can be used to coat the nail-plate or add strength to thin, weak nails. The monomers used to create wraps are cyanoacrylates. In nail wrapping, the free edge of the nail should be long enough to be splinted by the various types of fabrics, thereby providing support and added strength to the coating. There are three fabrics in wide use: fiberglass, silk, and linen.

No-Light Gels

These products are wrap monomers that have been thickened to have a gel-like appearance. They should be used and handled as any other wrap product.

Removal of Fingernail Coatings

The most commonly used solvent for removal of nail products is acetone. Warming the solvent with great care can cut product removal time to half. However, most gels are difficult to remove because they are highly cross-linked and resistant to many solvents. Therefore, if gel enhancements have to be removed, slowly file (do not drill) the enhancement with a medium-grit file, leaving a very thin layer of product. Soak in the warm product remover and once softened, scrape the remaining product away with a wooden pusher stick (1).

Cuticle Removers

These are lotions or gels containing approximately 0.4% sodium or potassium hydroxide. The lotion is left in place for one to three minutes and then washed off. Creams containing 1% to 5% lactic acid (pH 3–3.7) are also used.

Nail Whitener

This is a pencil-like device with a white clay (kaolin) core used to deposit color on the undersurface of the free edge of the nail.

REFERENCES

1. Schoon DD. Nail Structure and Product Chemistry. Albany: Milady Publishing, 1996.
2. Baran R, Schoon DD. Cosmetology of normal nails. In: Baran R, Maibach HI, eds. Textbook of Cosmetic Dermatology. London: Martin Dunitz, 1998:213–231.
3. Baran R, Schoon DD. Cosmetics for abnormal and pathological nails. In: Baran R, Maibach HI, eds. Textbook of Cosmetic Dermatology. London: Martin Dunitz, 1998:233–244.

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Antiperspirants

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INTRODUCTION

This chapter presents an overview concerning the current knowledge of antiperspirant actives and their interactions with the human axilla. It is my intention to give the interested reader a short introduction about formulation work, drug delivery systems, and application forms developed for antiperspirant actives. The final section lists references that should be useful for anyone who wants to learn more about a specific topic of antiperspirant technology.

BIOLOGY OF SWEAT GLANDS IN THE HUMAN AXILLA

The axilla region of humans contains apocrine, eccrine, and sebaceous glands. Approximately 25,000 sweat glands/axilla can produce up to 12 g sweat/h (1). The current understanding concerning the structure and function of sweat glands is that thermoregulation is the only aspect of the body participating in immunological, metabolic, and hormonal aspects of human life (2).

Eccrine Glands

This is the gland responsible for the majority of sweat production. It has a sensory and an excretory function and can be stimulated by emotional and thermal stimuli (3). It produces a clear, colorless, and odorless liquid containing 98% to 99% water and 1% to 2% inorganic and organic compounds (4). Inorganic components include NaCl and traces of K^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, and Cu^{2+} ions. Organic components include lactic acid, citric acid, formic acid, propionic acid, butyric acid, urea, and ammonia. Underarm wetness comes mostly from the secretion of eccrine glands. Antiperspirants reduce the amount of sweat only from eccrine glands.

Apocrine Glands

Apocrine glands are apparently a relic from the phylogenetic development of man. These glands start to produce a milky, viscous fluid during puberty on special

locations of the body, especially the underarm pit (5). In contrast to eccrine glands, the openings of the glands are not at the skin surface but appear at the hair follicle. Decomposition of apocrine sweat by skin bacteria is responsible for the characteristic malodor of human sweat. Apocrine sweat consists, among water, of proteins, carbohydrates, and ammonium salts (6). Other investigators have reported that these glands secrete lipids, cholesterol, and steroids (7). Furthermore, it has been shown that androgen-converting enzymes in the apocrine glands are responsible for circulating androgens to dihydrotestosterone (5).

ANTIPERSPIRANTS

Antiperspirants are topically applied products designed to reduce underarm wetness by limiting eccrine sweat production. In the United States, these products are regulated by the FDA as over-the-counter (OTC) drugs, because they are intended to affect a "function of the body" (here, perspiration). Products containing antiperspirant actives have to reduce perspiration to minimum 20% in 50% of the test population under validated test conditions. Test protocols (in vivo clinical trials), to develop a safe and an effective product, have been designed to substantiate the desired claims (8–14).

Comparative quantitative determination of the activity of sweat glands on the forearm after application of aluminum chlorohydrate solutions is now possible by combining the classic starch iodine visualization technique with digital image analysis (15). A noninvasive optical technique that allows the analysis of the function of a number of glands, simultaneously, in vivo was recently reported (16). A new method for parallel testing of up to eight formulations on the backs of volunteers allows a very fast evaluation of product prototypes (1).

Sweat Reduction by Antiperspirants: Current Model/Theory

The reader should be aware that theories concerning the action of sweat-reducing agents depend strongly on the type of actives (aluminum salts, nonionics, or ionic agents). The efficacy of antiperspirants based on aluminum and/or aluminum zirconium salts can be understood by the formation of an occlusive plug of metal hydroxide in the eccrine duct (17). Tape-stripping experiments followed by analysis of transmission electron micrographs of an aluminum chlorohydrate (ACH) -treated eccrine sweat-gland duct show an obstructive amorphous material supporting the theory of a mechanical blockage of sweat glands from diffusion of the soluble ACH solution into the sweat gland and subsequent neutralization to a polymeric aluminum hydroxide gel (18,19). There seems to be no correlation concerning the efficacy of aluminum salts and the location of the plug in the duct, because it is known that, compared with ACH, the more effective Al–Zr compounds do not penetrate as deep as the, also highly effective, $A1C1_3$ solutions (17). The reader is referred to the literature concerning other theories of sweat reduction by aluminum salts (20).

Active Ingredients for Controlling Underarm Wetness—State of the Art

Buffered Aluminum Salts (ACH)

The first antiperspirant, Ever Dry, based on $A1C1_3$, was introduced to the market in 1903 (21). The first cream-containing aluminum sulfate was introduced during the

1930s. The acidic pH value (2.5–3.0) was a drawback of these products, leading to skin irritation in the underarm pit. History tells us that the development of actives with a higher pH value, so-called buffered aluminum chlorides (aluminum chlorohydrate, ACH, pH $=4.0$–4.2), was an appropriate step with the additional benefit of reduced destruction of fabric clothes. The formula of this buffering salt is $\{A1_2(OH)_5\}^+ + \{Cl^-\}$, or more conveniently $A1_2(OH)_3C1$.

The historical development from $A1C1_3$ to $A1_2(OH)_5C1$ can be easily understood by the following consideration:

$$\text{AlClj} = {}^{x}h\ A1_2C1_6 \text{ (substitute five } Cl^- \text{ ions against OH ions)} => A1_2(OH)_5C1$$

$A1_2(OH)_5C1$ is a 5/6 basic aluminumtrichloride. The accepted definition of ACH is the ratio of Al to Cl = 2.1 to 1.0. Lower levels lead to aluminum dichlorohydrate ($A1_2(OH)_4C1_2$) or to aluminum sesquichlorohydrate ($A1_2(OH)_{4.5}C1_{1.5}$—both actives are also generally regarded as safe (GRAS). ACH is supplied as a powder or a 50% solution in water. It can be formulated up to 25%, calculated on an anhydrous basis. The 20% aqueous solution reduces perspiration by 35% to 40% on average (22). Some dyes used in clothing may be acid sensitive and will change color when in contact with an antiperspirant.

The structure of the Lewis acid ACH is very complex, because ACH in water forms the so-called isopolyoxo-cations with chloride ions as couterions (23–25). There exists several polymer equilibria of the polycationic aluminum species in water-based systems. Short-chain polycationic species are more effective in reducing sweat.

Aluminum Zirconium Chlorohydrate–Glycine Complexes (AZG or ZAG)

Aluminum zirconium chlorohydrate is obtained by reaction of ACH with zirconylchloride. Reaction of the former ingredient in the presence of glycine leads to ZAG complexes. Glycine is used as a buffering agent. These antiperspirant actives form very complex polymeric structures in water. The actives are defined by the ratio of Al + Zr metal-to-chloride ratio and the Al to Zr atomic ratio. The interested reader is referred to the literature concerning available actives (26,27) and nomenclature of the Al–Zr complexes (21,22). These antiperspirant actives were developed especially for anhydrous formulations because they show, compared with ACH, enhanced sweat reduction (28–30). The maximal concentration of ZAG calculated on an anhydrous basis is 20%. They are not allowed to be formulated for use in aerosols.

New Concepts for Controlling Underarm Wetness

Titanium Metal Chelates

The understanding of the complex solution chemistry of aluminum-based antiperspirants gave input to the search for alternative antiperspirant salts. Titanium derivatives, like partially neutralized ammonium titanium lactate (ATL) salts, were shown to be effective in in vitro efficacy tests (31). The titanium metal chelates can be synthesized from the corresponding titanium alkoxides and organic acids allowed by neutralization with ammonia. Under acidic to neutral pH conditions the ATL active seems to be relatively stable to hydrolysis, and therefore probably a suitable antiperspirant active in water-based or anhydrous drug delivery systems.

Film-Forming Antiperspirant Polymers

The so-called polybarrier technology is another approach to reduce perspiration by using a polymer that forms an insoluble occlusive film barrier on the underarm skin (32). It was mentioned that the occlusive film is a barrier to the passage of moisture. The main advantages of this technology are reduced skin irritation, applicable after underarm shaving, and higher sweat reduction compared with today's classic antiperspirant salts. The preferred polymer is an olefinic acid amide/olefinic acid or ester copolymer–like octylacrylamide/acrylate copolymer (Versacryl′′′-40). This copolymer can be used alone or in combination with PVP/eicosene-copolymer in sticks, roll-ons, or alcohol-based products (33). The reduction of sweat depends on the choice of vehicle and exceeds in some formulations to 40%.

Lyotropic Liquid Crystals

Certain surfactant/cosurfactant combinations form in water depending on the variables of concentration/temperature instead of micelles' lamellar, hexagonal, inverted hexagonal, inverted micellar, or even cubic phases. The cubic phases can be of micellar or bicontinuous type (34). The water domains in lamellar or cubic phases can swell to a certain degree, while taking up water. The use of this swelling behavior is the basis of a patent where a surfactant/co-surfactant combination is applied to the underarm pit (35). Sweat (water) transfers the applied composition to a lyotropic liquid crystal of cubic structure, thus creating a sweat-absorbing system in the axilla. Oleic acid/glycerolmonolaurate is one of the surfactant combinations in the patent. Both components are also well known as deodorizers.

DRUG-DELIVERY SYSTEMS AND APPLICATION FORMS FOR ANTIPERSPIRANT ACTIVES

Antiperspirant actives can be formulated in a variety of delivery systems like anhydrous suspensions, water- or hydroalcoholic-based solutions, and emulsions. Typical application forms for antiperspirants are sticks, roll-ons, creams, pump sprays, aerosols, gels, and powders. A new technology for pump sprays is discussed in chapter 57. On a global basis, the three most important product forms are sticks, roll-ons, and aerosols.

Formulation Work

After the decision for the desired application form has been made, the formulator has to decide on the vehicle system for the antiperspirant active. It is the intent of this section to summarize some of the current knowledge concerning the influence of actives with the formula, efficacy of different delivery systems, and the function of the ingredients used in antiperspirants.

Antiperspirant actives, like ACH or ZAG complexes, are soluble in water. Application of a concentrated aqueous solution of an antiperspirant active gives a rather tacky feeling (36). Reduction of tackiness can be best achieved by silicone oils (cyclomethicones) or ester oils like di-(2 ethylhexyl) adipate (27). The acidic pH value (4.0–4.2) has to be taken into account by selecting additional components for the desired drug delivery system. Loss of viscosity and problems of a final formula with color stability are often a hint to change the gellant and/or perfume. Aluminum powders in anhydrous systems (aerosols and suspension sticks) often leave visible

white residues on skin or clothing. Liquid emollients, like PPG-14 butylether or the aforementioned adipate ester, minimize these residues. Another approach is to use the solid emollient isosorbide monolaurate (ICI, Arla-mol ISML) (37). In anhydrous aerosol formulations the ACH powder settles down and forms a hard-to-redisperse cake at the bottom of the aerosol can. Suspending aids, like Quaternium-18 hectorite or Quaternium-18 bentonite, prevents settling of the active and additionally thickens the cyclomethicone oil phase. Usage of fine powders of ACH is another approach to overcome nature's law of gravity.

The reader should be aware that hydrophobic ingredients, like emollients, have an influence on the effectiveness of an antiperspirant active, because a cosmetic oil phase or wax can cover the pores of the eccrine duct. The efficacy of an antiperspirant active, like ACH, is higher in water-containing systems compared with anhydrous formulations. The following rules concerning efficacy might be helpful:

1. Efficacy: aqueous solution > anhydrous suspension
2. As diffusion of an active in the vehicle and from the vehicle to the skin after application has to be considered, one can further differentiate the expected efficacy trends. Efficacy: aqueous solution > sprayable O/W emulsion > O/W-emulsion roll-on > O/W-emulsion cream
3. It is accepted that antiperspirant actives in the outer phase of an emulsion have a higher efficacy than in dispersed phase. Efficacy: O/W-emulsion > W/O-emulsion.
4. In water-free systems the viscosity of the drug delivery system might be of relevance. Suspended ACH in anhydrous vehicles needs to be solubilized after application to the axilla by sweat (water). The effectiveness of suspension sticks depends on the rapidity of active solubilization. The usage of ultrafine powders of ACH is expected to boost efficacy compared with fine powders. Efficacy: low viscous suspension > suspension stick.

The reader is referred to the literature concerning vehicle effects on antiperspirant activity (7,38,39).

Lipophilic ingredients might have an influence on the efficacy of a product, because it is known that the water-soluble propylene glycol can form complexes or hydrogen bonds with aluminum polycationic species thereby altering the efficacy of the salt (40). Also, propylene glycol in high concentrations may result in skin irritations (41). Successful formulation work aims at finding the right viscosity for the product in the desired application form, a lower viscosity during the flow into the underarm pit, and a higher viscosity after application so that the product stays where it was applied. Conventional shear shinning flow curves are characteristic for antiperspirant products. The reader is referred to the literature concerning rheology aspects of cosmetic products (42).

Deodorant/Antiperspirant Sticks

It is at present not easy to give the reader an overview about sticks, because nowadays there exists many technologies to develop this solid delivery system. In Figure 1, an attempt was made to summarize this area. In the following section only systems of major importance are discussed.

Sticks can be divided into different classes like suspension sticks, gel sticks, and emulsion sticks. Soft sticks have some properties of all three categories (Fig. 1).

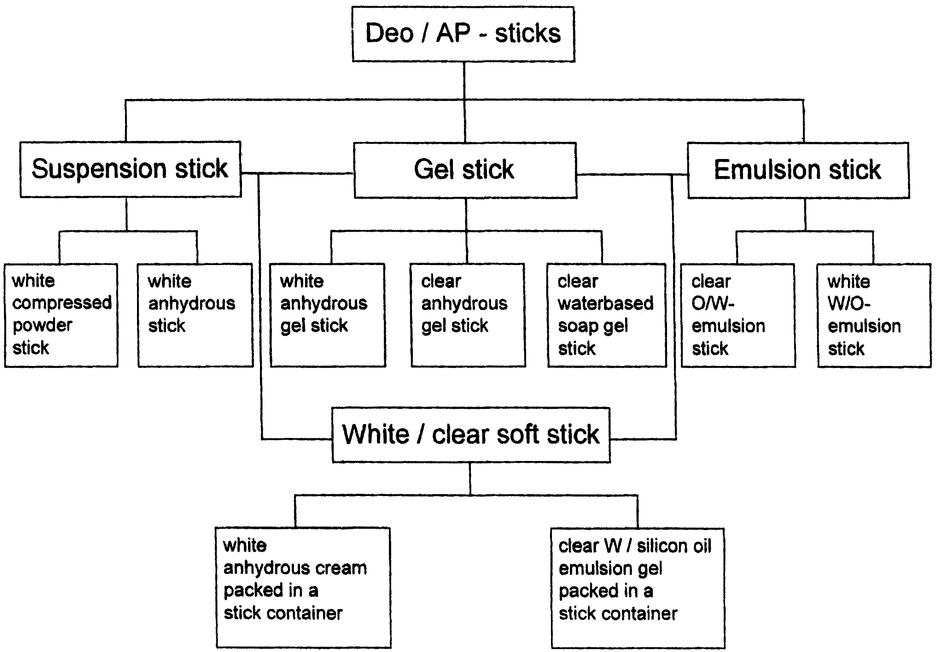

Figure 1 Overview of cosmetic Deo/AP-sticks.

Suspension Sticks

Dry deodorants, or antiperspirant solids, are synonyms for an application form where the active in the form of a powder is suspended in a silicone oil phase. Stearyl alcohol is usually used as the hardening agent. The molten mass crystallizes into a matrix of stearyl alcohol saturated with the silicone oil and suspended particles (43,44). Quaternium-18 hectorite can reduce the settling of the actives. Cyclomethicones give the stick a dry, silky feel; nonvolatile oils, like PPG-14 butylether, minimize white residues on skin (43). Low-residue sticks can be obtained by using a combination of high- and low-melting waxes and a volatile and nonvolatile silicone-oil combination (45).

Suspension stick	Wt.%
Stearyl alcohol	20.0
Cyclomethicone	54.0
PPG-14 butylether	2.0
Hydrogenated castor oil	1.0
Talc	2.0
Antiperspirant	20.0
Fragrance	1.0

Gel Sticks

This class can be subdivided into the following groups: white anhydrous gel sticks, clear anhydrous gel sticks, and clear water-based soap gel sticks. The last mentioned is discussed in the deodorant chapter.

White anhydrous gel sticks. Shear solids, or ultraclear solids, are synonyms for sticks with improved wash-out performance compared with the classic suspension sticks. They contain *N*-acyl amino acid amides (*N*-lauroyl-L-glutamic acid dibutylamide) and 12-hydroxyacid as gelling agents for an oil phase mixture (e.g., silicone oil/mineral oil). The wash-out agent is an ethoxylated solubilizer like Ceteareth-20. These white sticks turn clear after application to the skin (no-residue stick) (46).

Clear anhydrous gel sticks. They are quite popular in the United States, because clarity is associated by the consumer with a lack of white residue on skin, no dangerous ingredients, and high efficacy. A typical gelling agent is dibenzylidene sorbitol [dibenzylaldehyde monosorbitol acetal, (DBMS A)]. This acetal is not stable in an acidic aqueous environment (47). The sticks usually contain a high level of alcohol and/or polyols. At high polyol concentration, the active is regarded to be solubilized instead of suspended in the gel matrix (48). An alternative gelling agent is a polyamide (49).

White anhydrous gel sticks	Wt.%	Clear anhydrous gel sticks	Wt.%
N-lauroyl-L-glutamic acid dibutyl amide	5.0	Dibenzylidene sorbitol	2.0
12-Hydroxystearic acid	5.0	Dimethicone copolyol	2.0
Cyclomethicone	40.0	Di-isopropyl sebacate	2.0
Hydrogenated polyisobutene	15.0	Glycine	1.0
Di-isopropyl myristate	15.0	Dipropyleneglycol	10.0
Antiperspirant powder	20.0	Propyleneglycol	33.0
		Antiperspirant powder	50.0

Source: From Ref. 58.

Emulsion Sticks

They can be grouped into clear O/W emulsions, white W/O emulsions, and clear W/S: emulsion gels. The last mentioned is discussed below.

Clear O/W emulsions. They contain a high surfactant combination with the active solubilized in the external water phase. The high concentration of surfactants is a disadvantage; no products based on this technology are known to the author (47).

W/O emulsion sticks. The water phase containing the active is solubilized by a surfactant like polyglycerol-4 isostearate. A typical example for an oil/wax-phase combination is a mixture of silicone oil/stearyl alcohol (50).

W/O emulsion stick	Wt.%
Stearyl alcohol	19.0
Volatile silicone	26.0
Mineral oil	1.0
2-Methyl-2,4 pentandiol	2.0
Polyglycerol-4 isostearate	2.0
ACH solution (50%)	50.0

Source: From Ref. 50.

Soft Sticks (Soft Solids, Smooth-Ons)

These sticks can be differentiated into two subgroups, namely, white, anhydrous creams (suspensions) and clear water-in-silicone emulsion gels. Both delivery systems are packed in a container that gives the impression of a stick. The suspension or gel is extruded onto the skin from holes in the top of the stick container to a wide smooth area around the holes.

White, anhydrous creams. These creams contain an antiperspirant active, a volatile and nonvolatile silicone oil, and a thickener (*N*-acyl glutamic acid amide).

Clear water-in-silicone emulsion gels. These formulations can be achieved by adjusting the refractive index of the water and silicone–oil phase. Silicone formulation aids (Dow Corning 3225 C) are mixtures of cyclomethicone and dimethicone copolyol helping to solubilize the active (7,46,48,51). Low surface tension of cyclomethicones facilitates good spreading of a product on the skin and reduces the tackiness of antiperspirant actives.

Antiperspirant Roll-Ons

Roll-on products can be differentiated into several categories (Fig. 2). O/W emulsion-based delivery systems are quite popular in Europe, whereas anhydrous suspension roll-ons or transparent water-in-silicone emulsions are preferred in the United States. A new trend concerning the size of the roll-on applicator has been identified. Consumers prefer the big-ball format (3.0–3.5 cm), because of the ease of applying the product to the underarm pit (52). The popularity of roll-ons, in general, is because of the nongreasy and nonoily feel in the axilla and the good spreadability of the content on the underarm skin.

Clear Hydroalcoholic Roll-On

This delivery system contains a water/alcohol solution of the antiperspirant active thickened with a water-soluble polymer like hydroxyethylcellulose. The alcohol in the formula gives, compared with the clear aqueous solution-based roll-ons, a fresh sensation in the axilla and facilitates drying of the product. Excellent antiperspirant efficacy is another benefit of hydroalcoholic roll-ons.

O/W Emulsion Roll-On

This delivery system uses ethoxylated surfactants, like PEG-40 stearate, to solubilize an oil phase like mineral oil. The active is dissolved in the outer phase, allowing the

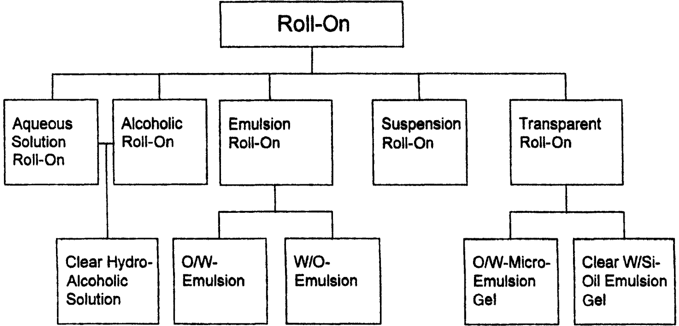

Figure 2 Overview of cosmetic Deo/AP-roll-on types.

formulation of a highly effective product. In alcohol-free formulated systems microbiological stability has to be checked.

O/W emulsion roll-on	Wt.%	Hydroalcoholic roll-on	Wt.%
PEG-40 stearate	5.0	Antiperspirant active	20.0
Cetyl alcohol	3.0	PPG-5 Ceteareth-20	2.0
Mineral oil	2.0	Water	35.4
Polysorbate-80	1.0	Ethanol	42.1
Glycerin	1.5	Hydroxyethylcellulose	0.5
Mg–aluminum silicate	0.8		
Antiperspirant active	20.0		
Water	66.7		

W/O Emulsion Roll-On

They are weaker in efficacy because the actives are encapsulated and the external oil phase often gives a sticky feeling.

W/Si Emulsion Roll-On

Silicone oils allow products to formulate based on a "W/O-technology," because the skin feeling is not comparable to traditional oily components like ester oils or triglycerides. The concentration of the thickener is reduced compared with sticks based on this type. The technology is discussed under soft sticks (see p. 604).

O/W Microemulsion Gel

An alternative approach to transparent products uses the phase inversion temperature (PIT) technology. A suitable mixture of surfactants, oils, and water is heated to 60 to 90°C to give a W/O emulsion above the PIT. During cooling, the mixture shows phase inversion to give white or transparent O/W emulsions; O/W microemulsion gels are obtained in the presence of hydrophobically modified water-soluble polymers (53). The technology is explained in more detail in the deodorant chapter.

Suspension Roll-On

The antiperspirant active in powder form is suspended in cylomethicone. The roll-on can be formulated with or without ethanol. Quaternium-18 hectorite is used as a thickener to prevent settling of the active. Consumers in the United States prefer this delivery system, as it does not give a wet feeling after application and because of the easy drying (39). Actives like ZAG-complexes give high efficacy to underarm products.

Suspension roll-on	Wt.%
Volatile silicone	65.0
Quaternium-18 hectorite	13.5
Silica	0.5
Antiperspirant powder	20.0
Fragrance	1.0

Antiperspirant Aerosols

Aerosols, in Europe and Asia, are popular delivery systems for consumers who prefer a hygienic and easy-to-use application form. Typical ingredients for aerosols include isopropylmyristate, isopropylpalmitate, volatile silicone, dimethicone, silica, clays, propylene carbonate, and ethanol. Propellants include propane, butane, and isobutane.

Antiperspirant aerosol	Wt.%
Volatile silicone	13.4
Quaternium-18 hectorite	0.8
Ethanol	0.8
Antiperspirant powder	10.0
Propellant (butane/propane)	75.0

As acidic aqueous ACH solutions lead to corrosion of the aerosol can, current aerosol antiperspirant products are formulated as water-free suspensions. The active is suspended as a powder in an oil phase like cyclomethicone or in a mixture of ester oils/cyclomethicone. Agglomeration of solid particles and settling of actives can be minimized by usage of suspending agents like fumed silica (amorphous silicon dioxide) or clays (bentonite and hectorite). The clays form a weak gel in the presence of an oil phase that can be destroyed by shaking the aerosol can before usage. The gel structure is reformed on standing, thereby holding the active in suspension. Because the organoclays are agglomerated, shear is needed to deagglomerate the platelets, and a polar activator like propylene carbonate or ethanol is used to disperse them and induce the gelation of the oil phase.

The steps involved to prepare an aerosol product can be summarized in the following sequence (7):

1. Preparing a bentonite or hectorite clay with the emollient in the presence of the polar activator and shearing the mixture.
2. Adding the antiperspirant active until a uniform agglomeration-free suspension is obtained.
3. Filling the concentrate into the aerosol can and adding the propellant (pressure filling).

Efficacy studies of aerosols, including comparison with other drug delivery systems, have been reported in the literature (30). ZAG-complexes are not allowed to be used in aerosols.

Environmental Issues

Aerosols contain volatile organic compounds (VOCs) usually in a weight ratio propellant/concentrate of 75/25 (54). The environmental impact of VOC, like the reaction with NO_X, in the presence of sunlight causes formation of unwanted ozone in the lower atmosphere. U.S. antiperspirant companies especially were forced to reduce VOC emissions by reformulating and/or exchanging of hydrocarbon propellants to the fluorohydrocarbons, 1,1 difluorethane (Propellant 152 a) or 1,1,2,2 tetrafluorethane (Propellant 134 a). The water-soluble dimethoxyethane (DME) is

another propellant that is thought to have no impact on the damage of the ozone layer (55).

The current trends in the aerosol market can be summarized as follows:

- higher ratio of concentrate/hydrocarbon propellant;
- higher amount of silicone oils;
- usage of 1,1 difluorethane (Propellant 152 a);
- formulations with lower vapor pressure;
- usage of smaller aerosol cans.

Aerosols containing 20% to 50% propellants with a concentrate/propellant ratio from 1.0–1.0 to 2.3–1.0 have been patented (56).

FUTURE TRENDS

Some new trends in the antiperspirant field concerning new actives and delivery systems have been described in this chapter. Improvements of current formulations and innovative concepts will need the ongoing investigation and better understanding of the interaction of active/vehicle and vehicle/skin. Improving efficacy and skin compatibility is another major trend in the antiperspirant field. New packaging concepts, like the extrudable gels, the big ball applicator for roll-ons, and reduced size aerosol cans with ozone-friendly propellants, are probably, in a few years, the state of the art. The influence of perfume components to the skin and the increasing rate of contact allergies attributable to fragrance ingredients have to be closely monitored (57).

REFERENCES

1. Bielfeldt S, Frase T, Gassmiiller J. New sensitive method for assessment of antiperspirants with intraindividual comparison of eight formulations. SOFW 1997; 1237:639–642.
2. Gebhardt W. Do cutaneous coryneform bacteria produce short-chain fatty acids in vitro? Dermatologica 1989; 178:121–122.
3. Sato K, Kang WH, Saga K, Sato KT. Biology of sweat glands and their disorders. I. Normal sweat gland function. J Am Acad Dermatol 1989; 20:537–563.
4. Anonymous. Deodorants and antitranspirants. In: Harry RG, ed. Harry's Cosmeticology. Aylesbury, England: Leonhard Hill Books, 1973:251–275.
5. Barth JH, Kealey T. Androgen metabolism by isolated human axillary apocrine glands in hidradenitis suppurativa. J Dermatol 1991; 125:304–308.
6. Klein RW. pH and perspiration. Cosmet Toilet 1980; 95:19–24.
7. Giovanniello R. Antiperspirants and deodorants. In: Williams DF, Schmitt WH, eds. Chemistry and Technology of the Cosmetics and Toiletries Industry. 2nd ed. London: Blackie Academic Professional, 1996:310–343.
8. Wooding WM, Finkelstein P. A critical comparison of two procedures for antiperspirant evaluation. J Soc Cosmet Chem 1975; 26:255–275.
9. Wooding WM, Finkelstein P. Procedures for evaluation of antiperspirant efficacy. Cosmet Toilet 1976; 91:28–32.
10. Majors PA, Wild JE. The evaluation of antiperspirant efficacy: influence of certain variables. J Soc Cosmet Chem 1974; 25:139–152.
11. Bakiewicz TA. A critical evaluation of the methods available for measurements of antiperspirants. J Soc Cosmet Chem 1973; 24:245–258.

12. Palanker AL. Substantiating the safety of antiperspirants. Cosmet Toilet 1985; 100: 43–45.
13. Murphy TD, Levine MJ. Analysis of antiperspirant efficacy test results. J Soc Cosmet Chem 1991; 42:167–197.
14. Wild JE, Bowman JP, Oddo LP, Aust LB. Methods for claim substantiation of antiperspirants and deodorants. Cosmet Sci Technol Ser 1998; 18:131–151.
15. Sauermann G, Hoppe U, Kligman M. The determination of the antiperspirant activity of aluminum chlorohydrate by digital image analysis. Int J Cosmet Sci 1992; 14:32–38.
16. Beck JS, Coulson HF, Hough GL, Mahers EG. Novel technique to investigate individual eccrine sweat gland function in vivo. 19th IFSCC Congress, Sydney, Australia, 1996; 3:95–98.
17. Quatrale RP. The mechanism of antiperspirant action. Cosmet Toilet 1985; 100:23–26.
18. Quatrale RP, Coble DW, Stoner KL, Felger CB. The mechanism of antiperspirant action on aluminum salts II. Historical observations of human eccrine sweat glands inhibited by aluminum chlorohydrate. J Soc Cosmet Chem 1981; 32:107–136.
19. Quatrale RP, Coble DW, Stoner KL, Felger CB. Mechanism of antiperspirant action on aluminum salts III. Historical observations of human sweat glands inhibited by aluminum zirconium chlorohydrate glycine complex. J Soc Cosmet Chem 1981; 32:195–221.
20. Laden K, Felger CB. Antiperspirants and Deodorants. New York: Marcel Dekker, 1988.
21. IFSCC Monograph No 6. Antiperspirants and Deodorants, Principles of Underarm Technology. Weymouth, MA: Micelle Press, 1998.
22. Cuzner B, Klepak P. Antiperspirants and deodorants. In: Butler H, ed. Poucher's Perfumes Cosmetics and Sops. Vol. 3. 9th ed. London: Chapman & Hall, 1993:3–26.
23. Teagarden DL, Kozlowski JF, White JL, Hem SL. Aluminum chlorohydrate I: structure studies. J Pharm Sci 1981; 70:758–761.
24. Teagarden DL, Radavich JF, Hem SL. Aluminum chlorohydrate II: physicochemical properties. J Pharm Sci 1981; 70:762–764.
25. Teagarden DL, White JL, Hem SL. Aluminum chlorohydrate III: conversion to aluminum hydroxide. J Pharm Sci 1981; 70:808–810.
26. Woodruff J. On the scent of deodorant trends. Manuf Chem 1994; 65:34–38.
27. Alexander P. Monograph antiperspirants and deodorants. SOFW 1994; 120:117–121.
28. Klepak P. In vitro killing time studies of antiperspirant salts. SOFW 1990; 116:478–481.
29. Rosenberg A. Enhanced efficacy antiperspirant actives. Soap Perfume Cosmet 1997; 7:27–30.
30. Fondots DC. Antiperspirants, a look across the Atlantic. Cosmetic Toilet Manuf Worldwide 1993; 108:181–185.
31. Hagan DB, Leng FJ, Smith PM, Snow M, Watson A. Antiperspirant compositions based on titanium salts. Int J Cosmet Sci 1997; 19:271–280.
32. Tranner F. Polybarrier: the future of antiperspirant technology? Soap Cosmet Chem Special 1998; 74:56–58.
33. Tranner F. Mineral salt-free topical antiperspirant compositions—comprises water insoluble, occlusive, film-forming polymers. US Patent No. 5508024.
34. Fontell K. Cubic phases in surfactant and surfactant-like lipid systems. Coll Polym Sci 1990; 268:264–285.
35. Leng FJ, Parrot DT. Antiperspirant materials and compositions. US Patent No. 5593663.
36. Abrutyn ES, Bahr BC. Formulation enhancements for underarm applications. Cosmet Toilet 1993; 108:51–54.
37. ICI Speciality Chemicals. A new emollient for antiperspirant sticks. HAPPI 1989; October:50–51.
38. Osborae GE, Lausier JM, Lawing WD, Smith M. Statistical evaluation of vehicle effect on antiperspirant activity with a limited number of subjects. J Soc Cosmet Chem 1982; 33:179–191.

39. Klepak P. Formulierungsbeispiele bei wasserhaltigen Antitranspirant Kompositionen. SOFW 1989; 115:415–418.
40. Abrutyn ES, Bahr BC, Fuson SM. Overview of the antiperspirant market. Technol Trends DCI 1992; 151:40–47.
41. Stephens TJ, Oresago C. Ethnic sensitive skin. Cosmet Toilet 1994; 109:75–80.
42. IFSCC Monograph No 3. An Introduction to Rheology. Weymouth, MA: Micelle Press, 1997.
43. Geria N. Formulation of stick antiperspirants and deodorants. Cosmet Toilet 1984; 99:55–66.
44. Geria N. Antiperspirant sticks. Cosmet Toilet 1996; 111:53–69.
45. Shevade M, Bianchini R, Lee R. Low residue antiperspirant solid stick composition. US Patent No. 5531986.
46. Fox C. OTC products. Cosmet Toilet 1996; 111:53–69.
47. Jungerman E. Clear antiperspirant stick technology. A review. Cosmet Toilet 1995; 110:49–56.
48. Smith J, Madore L, Fuson S. Attacking residue in antiperspirants. DCI 1995; 12:46–51.
49. Fox C. Technically speaking. Cosmet Toilet 1996; 111:23–26.
50. Hourihan JC, Krevald H. Water-in-oil emulsion antiperspirant sticks. US Patent No. 4704271.
51. Fox C. Cosmetic and pharmaceutical vehicles. Cosmet Toilet 1997; 112:31–48.
52. Anonymous. Does size matter? Soap Parf Cosmet 1998; 7:46–51.
53. Schreiber J, Klier M, Wolf F, Diec KH, Gers-Barlag H. Kosmetische oder dermatologische Gele auf der Basis von Mikroemulsionen. DE Patent No. 19509079.
54. Calagero AV. Antiperspirant and deodorant formulation. Cosmet Toilet 1992; 107: 63–69.
55. Romanowski R, Schueller R. Aerosols for apprentices. Cosmet Toilet 1996; 111:35–40.
56. Fox C. Technically speaking. Cosmet Toilet 1997; 112:21–25.
57. Johansen JD, Anderson TF, Kjoller M, Veien N, Avnstorp C, Andersen KE, Menne T. Identification of risk products for fragrance contact allergy: a case-referent study based on patient's histories. Am J Contact Derm 1998; 9:80–87.
58. Motley CB. Gel stick compositions comprising optically enriched gellants. US Patent 5552136.

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Deodorants

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INTRODUCTION

It is the intention of this chapter to give an overview on the current knowledge about the origin of underarm odor, the biology of the underarm microflora, and its interaction with deodorizing agents. The contents of this chapter have been arranged in particular sequence to facilitate the understanding of rational deodorant product development.

BIOLOGY OF THE UNDERARM MICROFLORA

The resident microflora of the human underarm skin consists of up to $10^6/cm^2$ organisms, e.g., aerobic cocci, lipophilic diphtheroids, and varying species of gram-negative bacteria (1). In the axillae two types of bacterial flora exist—coryneform bacteria and micrococcaceae such as *Staphylococcus epidermidis.* Coryneform- or *Staph. epidermidis-dominated* populations are characteristic for human beings. The resident microflora is a quite stable population not varying a lot between both axillae (2). The organisms are perfectly adapted to their ecological niche with its higher pH value and higher moisture content compared to other skin areas (3). Hair in the axilla according to the literature is not a good substrate for bacterial growth; the bacteria prefer to reside on the underarm skin (2). Moisture is required for bacterial proliferation and is secreted especially from the eccrine sweat glands (4). The origin of strong compared to low underarm odor is associated with a numerical dominance of coryneform bacteria (5). Components of apocrine secretion like, e.g., isovaleric acid and androstenone, were proposed to contribute to axillary odor. Hydrolytic exoenzymes of skin bacteria cleave the ester bonds of odorless water-soluble precursors of androstenol to the corresponding volatile steroid (6). Other studies proposed that the key odorants are branched, straight-chain and unsaturated C_6–C_n fatty acids (7). (E)-3-Methyl-2-hexenoic acid (E-3M2H) is the most abundant fatty acid compared to the rest of C_6–C_n fatty acids that contribute to the axillary odor bouquet. Apocrine sweat extracts have been analyzed and concentrations of 0.5 ng/^1 for androstenone and 357 ng/|iL for E-3M2H were detected (8). Volatile odor molecules of E-3M2H found in sweat secretions are transported according to the authors in a nonvolatile fashion

to the skin surface. Two apocrine secretion odor binding proteins (ASOB and $ASOB_2$) were identified, carrying 3M2H-molecules to the skin surface. Coryneform bacteria liberate the odor molecules from the protein precursor/odorant complex (8).

The reader should be aware that occurrence of these chemical compounds does not mean that all of us can smell them. Individual differences in odor perception for both isomers of 3M2H (9) and for the steroid androstenone are well known (8). Approximately 50% of the adult population is not able to smell androstenones; this anosmia to androstenone—or to 3-methyl-2-hexenoic acid—is genetically determined.

DEODORANTS

Deodorants are topically applied products designed to reduce underarm odor. They are considered in the United States as cosmetics while antiperspirants are treated by the FDA as drugs. Deodorants tend to be less irritating than antiperspirants. In continental Europe the consumer today prefer deodorants compared to antiperspirants. In the United States the trend is approximately reversed.

Concepts for Controlling Underarm Odor: State of the Art

The current knowledge of the biology of the underarm microflora and the origin of underarm odor is the basis for developing strategies against odor formation. Numerous patents and literature articles disclose the incorporation of chemical compounds for their deodorizing properties. It is the intention here to describe and exemplify major strategies, but not all deodorant actives that were developed in the past.

Strategies to reduce underarm odor include the following:

- antiperspirant active-containing deodorants,
- odor-masking deodorants,
- odor-neutralizing deodorants,
- odor-quenching deodorants,
- enterase inhibitors,
- antimicrobial active-containing deodorants.

Antiperspirant Active-Containing Deodorants

Antiperspirant actives like aluminum chlorohydrate or the Al–Zr complexes (see Chap. 56) reduce the secretion of eccrine sweat. Their excellent antimicrobial properties against *St. epidermidis* and coryneform bacteria have been published (10). The acidity of the aluminum salts may be a major factor in bacterial growth inhibition.

Odor-Masking Deodorants

Fragrance compositions (such as perfumes) have been used to mask odors since ancient times. It is conventional to incorporate 0.2% to 1.5% of a perfume in body deodorants (11). They are designed to blend with the underarm odor and thus act as a masking agent. The perception of a perfume may differ significantly between individuals because of different interactions with the skin, washing habits, and specific underarm odor. The fragrance materials are blended to achieve what is known as "top note," "middle note," and "bottom note" components. The first is the

refreshing note upon application while the last are the olfactoric components which stay on after application to the underarm skin.

Perfumes with antimicrobial properties have been described in patents and in the literature (12–14). An additional benefit, especially for emulsion-based products, is that they might also act as a preservative. The increasing rate of contact allergies against fragrance ingredients should be taken into account using this approach to combat underarm odor (15).

Odor-Neutralizing Deodorants

In chapter 56 it was mentioned that odorous C_6–C_n fatty acids contribute to underarm odor. Chemical neutralization with sodium bicarbonate ($NaHCO_3$) yields the corresponding odorless soaps (16). This active however is not stable for a long time in aqueous compositions. Patents for deodorant applications and usage of sodium bicarbonate in the presence of antiperspirant actives have been filed (17,18). Zinc carbonate containing deodorants are also content of a patent (19).

Odor-Quenching Deodorants

Zinc Ricinoleate

Zinc salts of ricinoleic acid have no bacteriostatic or antiperspirant effect (20). They strongly bind odorous fatty acids, amines, and mercaptanes. Ligand-exchange reactions of ricinoleic acid for odor molecules are probably the reason for the quenching properties of zinc ricinoleate (21). Interactions with perfume components in a deodorant formulation may weaken the desired quenching effect of the odor molecules after topical application to the underarm.

Metal Oxides

The oxides of calcium, magnesium, and zinc form in the presence of fatty acids in the corresponding metal soaps (22). Zinc oxide particles aggregate to form a massive lump. This leads to clogging of aerosol products (23). Hybrid powders were developed in which the metal oxide covers the surface of a spherical nylon powder (23). The advantage of this technology is the increased surface area of zinc oxide and thus enhanced odor-quenching efficacy and the reduced particle aggregation in aerosols.

Esterase Inhibitors

Zinc Glycinate

The inhibition of exoenzymes from the underarm bacteria (see discussion, p.) should also result in odor reduction. Zinc glycinate has been described as a suitable active (24). Antimicrobial tests showed no inhibitory effect against *St. epidermidis* or against the lipophilic diphtheroid bacteria supporting the suggested mechanism against microbial exoenzymes.

Triethylcitrate

The optimal pH value for development of underarm odor caused by coryneform bacteria is approximately about pH 6 in axillary extracts (25). Shifting the skin surface pH to the acidic side should decrease the activity of skin esterases, proposed to be

responsible for degradation of underarm secretions. Triethylcitrate was proposed to form citric acid by an enzymatic process on the underarm skin. In 1991 it was shown that this active has no pH-reducing effect after application to the underarm skin (26). Nevertheless deodorants containing this active are still in the market.

Antimicrobial Active-Containing Deodorants

This approach is currently the most commonly used strategy to prevent underarm odor. Ethanol is probably one of the best known actives for deodorization (27). Additional efficacy is normally required for a long-term deodorization, and this can be achieved by the additional usage of fragrance, an antiperspirant active, or other antimicrobial actives (famesol, phenoxyethanol, etc.).

Triclosan (2,4,4′-Trichloro-2′-Hydroxydiphenylether)

This active has a broad-spectrum antimicrobial activity against most gram-positive and gram-negative bacteria, molds, and yeasts. The presence of triclosan in antiperspirant sticks and roll-ons leads to a higher reduction of the bacterial microflora versus the triclosan-free antiperspirant composition (28). Triclosan is also used in skincare products, hand disinfectants, and household products (29).

Glyceryl Fatty Acid Ester

Mono- and oligoglyceryl fatty acid esters such as glyceryl monocaprylate, moncaprinate, monolaurate, and diglyceryl monocaprinate are effective deodorizers (30). Combinations of glyceryl monolaurate with farnesol and phenoxyethanol showed synergistic efficacy effects against coryneform bacteria (31). The advantage of this ingredient combination over the first generation deodorant actives such as triclosan is attributed to their higher biodegradability and their selective bacterial action. These actives are all naturally occurring in plants and animal species. In addition, it could be demonstrated that combinations of mono- and oligoglyceryl fatty acid esters with a variety of natural antimicrobials (e.g., wool wax acids) displayed a synergistic antimicrobial efficacy against underarm bacteria and serve as highly effective deodorant actives (32–35). Products containing such actives have been successfully marketed for a number of years.

Sucrose Fatty Acid Ester

The fatty acid esters of sucrose are well known as emulsifiers in food products (36). Sucrose can be substituted on eight hydroxyl groups with fatty acids. The antimicrobial potential depends strongly on the substitution degree of the sucrose. Sucrose monostearate and sucrose monolaurate have been described as deodorizers in the literature and in patents (37–39).

Glycerolether

2-Ethylhexyl glycerolether (octoxyglycerol) is a clear liquid with good solubility in cosmetic oils, polyols, and alcohol but only moderate solubility in water (0.2%). Synergistic antimicrobial activity with other ingredients has been described (40). This active has become popular recently in European deodorant formulations.

New Concepts for Controlling Underarm Odor

Ongoing research activities focusing on a better understanding of the interaction between underarm skin/skin microflora and skin microflora/odor formation, in combination with the discovery of highly selective actives, today allow more specific designs for deodorant products. In the next sections some of the new trends are discussed in detail. New concepts for controlling underarm odor include the following:

- chitosan,
- bacterial enzyme inhibitors,
- odor-inhibiting precursor mimics,
- product and skin-mediated perfume transformations,
- antiadhesives.

Chitosan

Chitin is a naturally occurring polysaccharide (e.g., in insects, lobster, crabs, or fungi) containing *N*-acetylated D-glucosamine units. Deacetylation of the amino group leads to the slightly water-soluble chitosan. The deodorizing properties of chitosan and the combination of this active with aluminum salts have been the subject of a patent (41).

Bacterial Enzyme Inhibitors

The enzyme amino acid (3-lyase) is, according to a patent filed in 1990, a catalyst for the formation of underarm odor (42). This enzyme is located in odor-releasing bacterial cells and cleaves the apocrine precursors of sweat components, such as amino acids with the structure unit COOH–CH–(NH_2)–CH_2–S–R, to the corresponding odorous sulfur products. Several classes of enzyme inhibitors such as derivatives of hydroxylamines, 3-substituted amino acids, cycloserine, and pyridoxal were identified

Odor-Inhibiting Precursor Mimics

Another approach to the inhibition of the above-mentioned enzyme f-lyase is to provide an alternative substrate for the bacteria that cleave the structure unit CH(NH_2) CH_2–O–C(O)–R instead of the sulfur-containing amino acid sequence (43). This approach leads to the corresponding nonodorous ingredients, like benzoic acid, or to pleasant odor generating substances, like phenylacetic acid.

Product- and Skin-Mediated Perfume Transformations

The physical and chemical interaction of a perfume with the underarm skin is a very complicated matter. Research activities in this area focused on the question which components of a perfume stay on and above the skin after topical application (44). Headspace analysis is one of the techniques to gain more information concerning skin/perfume interactions. It could be demonstrated that the long lastingness of a fragrance can be achieved by using a prodrug (ester, acetale) of a perfume ingredient (45). The esters or acetales of a fragrance composition hydrolyze on human skin due to the slightly acid pH value. The hydrolysis products (acids, alcohols, aldehydes) impart a pleasant smell to the underarm skin. These product- and skin-mediated perfume transformations are especially suitable for alkaline formulations like soap-based deodorant sticks. The advantage of the perfume precursor approach is attributed to a prolonged fragrance impression of a deodorant after topical application to the underarm skin.

Antiadhesives

An alternative concept to reduce the amount of skin bacteria in the underarm skin is the antiadhesion approach. The understanding of the adhesion mechanisms of the resident underarm microflora to the skin surface is the basis for developing strategies against bacterial adhesion. Numerous skin microorganisms adhere preferentially to specific sites on various body surfaces. For example, *S. aureus* and *P. aeruginosa* adhere to collected nasal epithelial cells (46). *C. xerosis* binds to epidermal cells whereas yeasts species like *Candida albicans* bind to corneocytes. Structures of the skin specifically involved in adherence to the underarm bacteria are thought to be proteins, oligosaccharide structures, lipids, and hydrophobic surfaces. Imitation of these adhesion motifs by saccharides, oligosaccharides, polysaccharides, and glycoproteins allows one to inhibit the bacterial adherence to the skin. Additionally it was discovered recently that among others, sucrose esters like sucrose myristate and sucrose laurate have antiadhesive properties to various microorganisms including the typical microflora of the underarm skin (47).

DRUG-DELIVERY SYSTEMS AND APPLICATION FORMS FOR DEODORANT ACTIVES

Products designed to reduce underarm odor can be formulated in a variety of delivery systems such as suspensions, water or hydroalcoholic solutions, and emulsions. Typical application forms are sticks, roll-ons, creams, pump sprays, aerosols, and gels. Sticks, roll-ons, and aerosols are discussed in detail in the antiperspirant chapter. Lowering the amount of an antiperspirant active, like aluminum chlorohydrate, in an antiperspirant is one option to formulate a deodorant. In this case the antiperspirant active has only deodorizing properties and nearly no impact on the eccrine sweat glands. Deodorants can be formulated in acidic, neutral, or alkaline environment. Designing a deodorant the formulator should have in mind the following points:

- long-term deodorization,
- no irritation potential,
- good solubility of the active in the delivery system,
- selection of a stable fragrance,
- viscosity control of the product,
- good skin feeling of the product.

Protocols for the in vitro and in vivo evaluation of deodorants have been designed. The reader is referred to the literature (48). A new method for in vivo evaluation of antimicrobial agents was recently developed where the underarm bacteria were translocated to the forearm allowing the simultaneous evaluation of multiple deodorizers in an individual (49).

Deodorant Sticks

Deodorant sticks are solidified by 6% to 8% of sodium stearate. The deodorizing agent and a fragrance are dissolved in a hydrophilic carrier. Two stick categories can be differentiated, the ethanol based and the propylene glycol based sticks (50).

Transparency is usually achieved by usage of a high polyol content. Clarifying agents for sticks like PPG-14 butylether, Cocamide DEA, Lauramide DEA, Steareth-100 have been patented (51,52). Ethanol based sticks are preferred if it is

the intent of the formulator to create a cooling sensation for the consumer. Shrinkage of the stick has to be taken into account because of evaporation of the alcohol. Propylene glycol based sticks tend to be more resistant to shrinkage, and solubilization of a fragrance is easier in some instances (53).

Deodorant stick	Wt%	Deodorant stick	Wt%
Water	16.0	Water	3.0
Ethanol	75.5	Propylene glycol	10.0
Deodorizer	1.0	Deodorizer	1.0
Sodium stearate	6.5	Sodium stearate	8.0
Fragrance	1.0	PPG-3 myristyl ether	77.0
		Fragrance	1.0

Deodorant Aerosols

Spray products containing a solution of an antimicrobial active in an ethanol and/or propylene glycol carrier blended with a liquefied propellant are typical for deodorant aerosols. The difference from an antiperspirant active containing aerosol is that the deodorizer is solubilized in an alcohol- or polyol-based formulation and not suspended. Deodorant sprays provide a dry skin feeling to the underarm skin because they are anhydrously formulated.

Typically, 20% to 60% of the sprayable contents of an aerosol reach the skin, because the liquefied hydrocarbon propellant vaporizes as it is sprayed (54). Propane, butane, and isobutane are the most commonly used propellants. They condense to form a clear, colorless, and odorless liquid with densities of 0.51 to 0.58 g/mL at 20°C (55). These propellants are inflammable in the presence of air or oxygen. Labeling of cosmetic aerosols concerning flammability risks of volatile organic compounds and volatile solvent abuse is discussed in detail in a recently published review (56). Aerosol containers can be fabricated from tin-coated steel, tin-free steel (chromium-coated steel), or aluminum. Numerous types of aerosol can cause corrosion and testing for it was recently discussed in the literature (57). The environmental issues of aerosols are explained in greater detail in the antiperspirant chapter.

Deodorant aerosol	Wt%
Alcohol	42.0
Laureth-4	0.5
Deodorizer	1.0
Fragrance	0.5
Isobutane	47.6
Propane	8.4

The formulator of an aerosol has to optimize the following parameters to get a dry deodorant product:

- spray rate,
- spray shape,
- particle size,

- concentrate/propellant ratio,
- fragrance/deodorizer concentration,
- pressure of the aerosol can.

Deodorant Pump Sprays

Hydroalcoholic Pump Sprays

An alternative to aerosols is pump sprays. This category is quite popular in Europe whereas it is of lower interest for the consumers in the United States, because they tend to prefer a dry application form, like the anhydrous sticks. Pump sprays allow a good dosage of the formulation to be delivered to the underarm skin in a hygienic way. They consist of low viscosity hydroalcoholic solutions of a deodorizer and a perfume. Usually a solubilizer, like PEG-40 hydrogenated castor oil, is incorporated into the formulation to maintain a clear and homogeneous solution.

Pump spray	Wt%
Water	35.6
Alcohol	60.0
PEG-40 hyd.	2.0
Castor oil	–
Deodorizer	2.0
Fragrance	0.4

PIT-Emulsion Pump Sprays

A disadvantage of hydroalcoholic pump sprays is the alcohol content in the formulation that may contribute to unwanted side reactions especially in the shaved axilla. Beiersdorf AG in Hamburg, Germany, introduced to the European market under the brand name "Nivea®" a new pump spray based on an emulsion in 1995. The sprayable low viscous deodorant is based on the phase inversion temperature (PIT) technology. Suitable mixtures of ethoxylated surfactants, oils, and water in the presence of antiperspirant and deodorizing actives are heated to 60°C to 90°C. Cooling the resulting W/O emulsion to room temperature yields via a PIT process a finely dispersed bluish-white O/W emulsion (58–60). The droplet size distribution of such PIT emulsions ranges from 80 nm to 250 nm. The above-mentioned pump spray contained a skin-friendly deodorizing combination of glyceryl monocaprinate and wool wax acids (see discussion p.) in an alcohol-free delivery system.

PIT-emulsion pump spray	Wt%
Glyceryl stearate, ceteareth-20, ceteareth-10, cetearyl alcohol, cetyl palmitate (Emulgade SE)	4.5
Ceteareth-20	1.0
Dioctyl cyclohexane	5.0
Dicaprylylether	5.0
Deodorizer	2.0
Aluminum chlorohydrate	5.0
Water	77.5

Source: From Ref. 60.

Microemulsion Pump Sprays

Hydroalcoholic pump sprays are usually transparent, whereas sprayable PIT emulsions are white or bluish-white products. Sprayable alcohol-free and additionally transparent pump sprays were recently introduced into the European market (e.g., Basis pH; Beiersdorf AG, Hamburg, Germany). Transparency of an emulsion is achieved when the size of the droplets is below 100 nm. This O/W microemulsion can be obtained with and without the PIT technology but needs careful selection of ingredients and considerable fine-tuning (61). The main advantage compared to classical microemulsions is the low surfactant concentration (<10%). Furthermore it could be demonstrated that, in the presence of hydrophobically modified water-soluble polymers, the above-mentioned technology allows the formulation of gels, sprayable gels, roll-ons, sticks, and aerosol products (62).

FUTURE TRENDS

The deodorant market has undergone some remarkable changes concerning the principles to reduce underarm odor in the last years. It is expected that the search for effective, skin-friendly actives with a highly selective action against the cutaneous underarm microflora will lead to long-lasting and safe deodorants. Improvements in understanding how microorganisms adhere to human skin should facilitate the development of new strategies to reduce underarm odor. Improvements of aerosols with no/low impact to the environment or aerosol alternatives, like sprayable emulsions, are probably in a few years in the portfolio of every deodorant-selling company.

REFERENCES

1. Korting HC, Lukacs A, Braun-Falco O. Mikrobielle Flora und Geruch der gesunden menschlichen Haut. Hautarzt 1988; 39:564–568.
2. Leyden JJ, Me Ginley KJ, Holzle E, Labow JN, Kligman AM. The microbiology of human axilla and its relationship to axillary odor. J Invest Dermatol 1981; 77:413–416.
3. Lukacs A, Korting HC, Lemke O, Ruckdeschel G, Ehret W, Braun-Falco O. The influence of pH value on the growth of *Brevibacterium epidermisin* continuous culture. Acta Derm Venerol 1995; 75:280–282.
4. Leyden JJ, Me Ginley KJ, Nordstrom KM, Webster GF. Skin microflora. J Invest Dermatol 1987; 88:65s–72s.
5. Rennie PJ, Gower DB, Holland KT. In vitro- and in vivo studies of human axillary odor and the cutaneous microflora. Br J Dermatol 1991; 124:596–602.
6. Froebe C, Simone A, Charig A, Eigen E. Axillary malodor production: a new mechanism. J Soc Cosmet Chem 1990; 41:173–185.
7. Zeng XN, Leyden JJ, Lawley HJ, Sawano K, Nohara I, Preti G. Analysis of characteristic odors from human axillae. J Chem Ecol 1991; 17:1469–1491.
8. Spielman AI, Zeng XN, Leyden JJ, Preti G. Proteinaceous precursors of human axillary odor: isolation of two novel odor binding proteins. Experientia 1995; 51:40–47.
9. Wysocki CJ, Zang XN, Preti G. Specifica anosmia and olfactory sensitivity to 3-methyl-2-hexenoic acid: a major component of human axillary odor. Chem Senses 1993; 18:652.
10. Klepak P. In vitro killing time studies of antiperspirant salts. SOFW 1990; 116:478–481.
11. Geria N. Fragrancing antiperspirants and deodorants. Cosmet Toilet 1990; 105:41–45.
12. Eggensberger H. Duftstoffe und Aromen als multifunktionelle Additive. SOFW 1996; 122:789–793.

13. Diehl KH, Oltmanns P, Ramsbotham J. Parfiiminhaltsstoffe-eine Alternative fur die konser-vierung von kosmetischen Produkten? SOFW 1992; 118:546–550.
14. Morris JA, Khettry J, Seitz EW. Antimicrobial activity of aroma chemicals and essential oils. J Am Oil Chem Soc 1979; 96:595–603.
15. Rastogi SC, Johansen JD, Frosch P, Menne T, Bruze M, Lepoitthevin JP, Dreier B, Andersen KE, White IR. Deodorants on the European market: quantitative chemical analysis of 21 fragrances. Contact Dermatol 1998; 38:29–35.
16. Lamp JH. Sodium bicarbonate. An excellent deodorant. J Invest Dermatol 1946; 7: 131–133.
17. Berschied JR. Antiperspirant-deodorant cosmetic stick products containing active agent particles in organic matrix, which matched densities for homogeneous products. Patent No. WO 9413256.
18. Winston AE. Microporous alkali metal carbonate powder—comprises particles of average particle size of 0.1–50 microns, surface area of 5–20 sq.m/f, average pore size of 10–500 nm and total pore volume of 0.1–2 cc/g and is useful as lightweight deodorant ingredient. Patent No. WO 9424996.
19. Park AC. Propellant free deodorant composition, for topical application—comprising sparingly water soluble salts or oxide (s) of zinc or magnesium, water absorbing cellulosic polymer and volatile silicone. Patent No. EP 471392 A.
20. Zekorn R. Deowirkstoff auf Basis Zinkricinoleat. Parf Kosmet 1996; 77:682–684.
21. Zekorn R. Zinc ricinoleate. Cosmet Toilet 1997; 112:37–40.
22. Kanda F, Yagi E, Fukuda M, Matsuoka M. Quenching short chain fatty acids responsible for human body odors. Cosmet Toilet 1993; 108:67–72.
23. Kanda F, Nakame T, Matsuoka M, Tomita K. Efficacy of novel hybrid powders to quench body malodors. J Soc Cosmet Chem 1990; 41:197–207.
24. Charig A, Froebe C, Simone A, Eigen E. Inhibitor of odor producing axillary bacterial exoenzymes. J Soc Cosmet Chem 1991; 42:133–145.
25. Rennie PJ, Gower DB, Holland KT, Mallet AI, Watkins WJ. The skin microflora and the formation of human axillary odor. Int J Cosmet Sci 1990; 12:197–207.
26. Lukacs A, Korting HC, Braun-Falco O, Stanzl K. Efficacy of a deodorant and its components. Triethylcitrate and perfume. J Soc Cosmet Chem 1991; 42:159–166.
27. Baxter PM, Reed JV. The evaluation of underarm deodorants. Int J Cosmet Sci 1983; 5:85–95.
28. Cox AR. Efficacy of the antimicrobial agent triclosan in topical deodorant products. J Soc Cosmet Chem 1987; 38:223–231.
29. Nissen HP, Ochs D. Triclosan. Cosmet Toilet 1998; 113:61–64.
30. Dillenburg H, Jakobson G, Klein W, Siemanowski W, Uhlig KH, Wolf F. Cosmetic deodorant preparations containing di- or triglycerin esters. Patent No. EP 666732 Al/Bl.
31. Haustein UF, Herrmann J, Hoppe U, Engel W. Growth inhibition of coryneforme bacteria by a mixture of three natural products. Farnesol, glyceryl monolaurate and phenoxyethanol: HGQ. J Soc Cosmet Chem 1993; 44:211–220.
32. Klier M, Schneider G, Traupe B, Voss I, Wolf F. Desodorierende Wirkstoffkombinationen auf der Basis von Wollwachssauren und Monocarbonsauren. DE 4305889.2.
33. Klier M, Rockl M, Schneider G, Siemanowski W, Traupe B, Uhlig KH, Voss I, Wolf F. Deodorant active substance combinations made from wool grease acids and partial glycerides. EP 689418 Al.
34. Klier M, Rockl M, Traupe B, Wolf W. Deodorizing combinations of agents based on *a*,co-alkane dicarboxylic acid and fatty acid partial glycerides. EP 729345 Al.
35. Klier M, Traupe B, Wolf F. Deodorant agent compositions containing cx,co-alcanoic diacids and mono-carboxylic esters of oligomer glycerols. EP 691125 Al.
36. Friberg SE, Larsson K. Food Emulsions. New York: Marcel Dekker, 1997.
37. Meyer PD, Vianen GM, Baal HCI. Sucrose fatty acid esters in deodorant formulations. Aerosol Spray Rep 1998; 37:18–22.

38. Meyer PD, Vianen GM, Baal HCI. Saccharose-Fettsaureester in deodorants. Parf Kosmet 1997; 78:22–24.
39. Vianen GM, Watraven BW, Meyer PD. Deodorant composition. EP 0750903 Al.
40. Beilfuss W. A multifunctional ingredient for deodorants. SOFW 1998; 124:360–366.
41. Wachter R, Lehmann R, Panzer C. Desodorierende Zubereitungen. DE 19540296.
42. Lyon S, O'Neal C, van der Lee H, Rogers B. Amino acid P-lyase enzyme inhibitors as deodorants. WO 9105541.
43. Laney J. O-Acyl serines as deodorants. WO 9507069.
44. Behan JM, Macmaster AP, Perring KD, Tuck KM. Insight how skin changes perfume. Int J Cosmet Sci 1996; 18:237–246.
45. Suffis R, Barr ML, Ishida K, Sawano K, van Loveren AG, Nakatsu T, Green CB, Reitz GA, Kang RKL, Sato T. Composition containing body activated fragrance for contacting the skin and method of use. US 5626852.
46. Carson RG, Schilling KM, Harichian B, Au V. Biospecific emulsions. US 5416075.
47. Biinger J, Schreiber J, Wolf F. Anti-adhesive active principles. EP 806935 A2.
48. IFSCC Monograph No 6. Antiperspirants and Deodorants. Weymouth, MA: Micelle Press, 1998.
49. Leyden JJ, McGinley K, Foglia AN, Wahrmann JE, Gropper CN, Vowels BR. A new method for in vivo evaluation of antimicrobial agents by translocation of complex dense populations of cutaneous bacteria. Skin Pharmacol 1996; 9:60–68.
50. Calogero AV. Antiperspirant and deodorant formulation. Cosmet Toilet 1992; 107: 63–69.
51. Dawn R, Morton B. Clear cosmetic stick composition. WO 9427567.
52. Kellner DM. Clear, stable deodorant compositions—containing soap, antimicrobial agent, water, polyhydric alcohol, pentadoxynol 200 and alcanolamide-alkoxylated alcohol mixture. US 5407668.
53. Geria N. Formulation of stick antiperspirants and deodorants. Cosmet Toilet 1984; 99:55–66.
54. Meyer G, Listro JA. Liquid deodorant compositions. WO 9301793.
55. Johnsen MA. The safety assessment of hydrocarbon aerosol propellants. Spray Technol Market March 1996:18–24.
56. Redbourn D. Cosmetic aerosol regulations. Living with labelling. Soap Perf Cosmet 1998:45–48.
57. Tait WS. Aerosol container corrosion and corrosion testing: what is state of the art? Spray Technol Market 1997:47–56.
58. Wadle A, Forster T, von Rybinski W. Influence of the microemulsion phase structure on the phase inversion temperature emulsification of polar oils. Colloids Surf A 1993; 76: 51–57.
59. Forster T, von Rybinski W, Tesman H, Wadle A. Calculation of optimum emulsifier mixtures for phase inversion emulsification. Int J Cosmet Sci 1994; 16:84–92.
60. Wadle A, Ansmann A, Jackwerth B, Tesmann H. PIT-Emulgiertechnologie in der Kosmetik Parf Kosmet 1996; 77:250–254.
61. Schreiber J, Eitrich A, Gohla S, Klier M, Wolf F. Cosmetic or pharmaceutical microemulsions. WO 9628131 A2/A3.
62. Schreiber J, Diec KH, Gers-Barlag H, Klier M, Wolf F. Cosmetic and pharmaceutical gels based on microemulsions. WO 9628132 A2/A3.

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Cosmetics for Men

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INTRODUCTION

It is the intention of this chapter to give an overview on cosmetics for men in general, history, developments, as well as current and future trends. In the past, for men grooming was all about shaving. Shaving is a duty for men and can be seen as a starting point for their care regime. Thus, this chapter will deal with shaving, aftershave, and face care for men. To understand the differences in female and male care, it is necessary to explain the physiological differences in female and male skin.

Physiological Differences in Female and Male Skin

Our skin is acting as an interface between the body and the external environment with many intricate structures and mechanisms that are connecting it to the entire body.

Women and men differ in their genetic code, which leads to a different hormonal distribution. These gender variations are the reason for special structural and anatomical characteristics of female and male skin. These variations are changing with age and are also influenced by exogenous factors (1), for example, sun radiation and dry or humid climate.

It was shown that male skin was thicker than female skin, whereas women had more subcutaneous fat. Men's skin showed gradual thinning with advancing age (12–93 years), whereas the thickness of women's skin remained constant until the fifth decade, after which it decreased with age. After the fifth decade the hormonal distribution is changing in women. This hormonal influence was shown when conjugated estrogenes were given to postmenopausal women. After 12 months of therapy the dermis was significantly thicker as at the beginning of the treatment.

The male skin density, which depends on the ratio of skin collagen to skin thickness—was higher at all ages. Women start with lower collagen content, therefore they seem to age earlier than men.

The sebaceous glands are hormone dependent. After puberty, sebum production is significantly greater throughout life in males than in females (2). Greater sebum production results in more severe and long-lasting acne in men. They present

dilated pores, sometimes with blackheads. Obviously, the distribution of hair over the body of men and women is different. These differences are also related to the effect of androgens, for example, the development of beard and chest hair after puberty.

History and Development in Skin Care for Men

Cosmetic products for men and women have traditionally been formulated differently. Products for men usually contained alcohol, which has rarely been used in cosmetics for women. Men treat their skin in response to a need, such as shaving, cleansing, and treating nicks and cuts. In the past, the men's market has been focused on fragrances for men and on shaving care. They are less prone to view skin care as an age prevention process. Analysts report that this attitude is changing. Some men are already using their partner's skin care products and do worry about ageing (ageing spots for example), probably to present an image compatible with their profession. Moreover, men ask for discreet products labeled "For Men," which appeal to their virility.

In the meantime, the following cosmetic products are formulated especially for men:

- alcoholic perfumery,
- shaving products (preshave, shave, and aftershave),
- hair care and shower products,
- antiperspirants and deodorants,
- skin care products (face and body).

This chapter will deal with pre/aftershave products, shaving products, and face care products.

SHAVING

General Introduction to Shaving

Shaving is considered to be the most masculine part in the daily routine of male grooming and has become a prerequisite for social acceptance in most modern societies. However, the removal of unwanted facial and body hair as a beauty and grooming attribute was established at least 2000 years ago. From today's perspective, methods and devices were rather crude and usually the whole process must have been rather painful and laborious. The hardware of those times, found as burial objects in Mesopotamia and Egypt, included shavers of flint and hardened bronze, which were attached to small sticks of wood. In more modern times, that is, 400 to 300 years ago, short steel blades attached to wood or ivory handles were used in Southern Europe and the Arab world (5).

Over 150 years ago the comfort of shaving improved significantly with the invention of shaving soaps, which were then prepared by saponification of cooking fats with potash and soda lye (6). At that time the strop or cutthroat type of blade was used solely, until the beginning of the twentieth-century, when in 1904 the safety razor, invented by King C. Gillette, was introduced.

During the following decades further major advances in shaving technology are recorded. In 1939, the blade surface reflectance meter to measure edge sharpness was invented, followed by the stainless steel blade from Wilkinson Sword in the 1950s.

Razor technology improved even further with the development of the polymer-coated blade in 1960 and the platinum- and chromium-coated blade in 1969. Two years later the twin blade razor was born; it took further 27 years, until in 1998, when the triple blade razor was invented (7).

Research reveals that in the beard area of adult males between 6000 and 25,000 coarse circular or oval hair fibers can be found. Two types of hair occur, very fine and poorly pigmented vellus hairs with diameter close to 0.01 mm and coarser hairs with diameter of about 0.10 mm. In these coarser hairs the soft-pigmented material (cortex) is surrounded by a scaly layer of hard material (cuticle) (7). The growth rate of beard hairs is about 0.4 mm/day and varies little from person to person. Thus, beard hair growth is similar to hair growth of the eyebrows, auricles and nasal vestibules, and body, with the exception of the pubic and axillary regions (8). The hairs are coated with an oily secretion of sebaceous glands, which are attached to most of them. This mixture of skin lipids is the skins own emollient called sebum. For men puberty does not only cause the growth of facial hair, but it also leads to an increase of sebum production. The ability to remove sebum from the hair to allow water to moisten it is one of the key properties of a good shaving aid. Hence, it does not come as a surprise that soap still plays an important role in the composition of shaving preparations.

Preshaving Preparations

The most important component in shaving is the preparation of the skin and beard (3). The aim of the treatment is to prepare the beard before cutting it with the blade. In wet shaving the beard is softened with water so that the hairs offer the least possible resistance to cutting, to avoid trauma to the skin. Wet shaving products contain soaps, syndets, and lubricants. Washing with hot water and soap before applying a shaving preparation makes wet shaving much easier, but this will be discussed in detail in the next section. In case of electric shaving the stiffness of the beard hair is preferable. Essential factors for dry shaving are drying and degreasing the skin. This can be achieved by a high amount of alcohol within the respective products. The alcohol also minimizes the risk of irritations and makes the beard hair stiff. Erecting the beard hair for a closer shave is another important prerequisite for preshave products. Alcohol and pilomotorica show this effect to hair follicles. Furthermore, reducing the friction between razor and skin is an important property of preshaving products. This allows the user to apply a greater pressure on the shaver, thus generating a closer shave. Reducing the friction can be achieved by the use of lubricants. But the amount and the chemical structure of lubricants are limited, because together with beard hair powder and clippings they can agglomerate and cause the razor mechanism to clog. This limitation may be overcome by using volatile silicones, which are also excellent lubricants. Now, preshaving products can hardly be found on the market, the cosmetic industry is focusing on shaving and aftershave products (Formulas 1 and 2).

Formula 1 Example for a Pre-Electric Shave Preparation by Inolex (4)

SD alcohol 40-B (%)	87.00
Propylene glycol dipelarogonate	10.00
Oleamidopropyl dihydroxypropyl diamonium chloride	3.00

Formula 2 Pre-Electric Shave Preparation by Goldschmidt (4)

SD alcohol 40 (%)	86.00
Phenyl trimethicone	2.00
Cyclomethicone	5.00
Isostearyl isononanoate	1.00
Water	6.00
Fragrance	q.s.

Wet Shaving Preparations

Introduction

It is said that dry hair is as hard to cut as copper wire of comparable thickness, thus softening the beard is one of the two most important functions of cosmetic preparations for wet shaving. As moistened hair is easier to cut, the decrease of cutting force results in a reduction of razor drag. Providing good gliding of the razor blade, that is, to act as a lubricant, is the other important function, further reducing the pull on the hair follicles.

Various factors are influencing the closeness of a shave. In an early study, Hollander and Casselman showed that beard hair was well prepared for shaving after being in contact with warm water (49°C) and a shaving cream for at least four minutes. Less preparation time resulted in shorter life spans of the razor blades. The ideal angle between the blades of the razor and the skin was reported to be between 28° and 32° to achieve the best compromise between closeness, discomfort, and irritation. Lower angles increased comfort but gave less efficient results. Interestingly, the growth rate of shaved beard hair was fairly fast directly after shaving, but then declined steadily, resulting overall in the normal growth rate of uncut hair (9).

Increased shaving irritation was observed, when sharp razors were used. But also large shaving angles, higher shaving pressure, repeated shaving over the same facial area, thin lathers, stretched skin, and shaving against the grain have resulted in increased skin trauma. In contrast, stiffer lathers with higher viscosity notably reduced skin irritation (8).

The most common pathological skin conditions related to shaving are bacterial infections of the beard area (barber's itch), most often following little injuries like nicks and cuts. An extremely unpleasant condition that is very widespread within the group of Negroid men who shave wet (50–75%) and to a lesser extent spread within the group of Caucasian men [3–5%] is pseudofolliculitis barbae (PFB), often known as "razor bumps." PFB results from ingrown beard hairs, commonly occurring when curly or tightly coiled hair is shaved too closely. When the tip of the freshly cut hair either penetrates the follicular wall or re-enters the skin, after growing out, it causes an inflammatory reaction of the follicle (3,10). Helpful in the prevention of PFB is to avoid shaving too closely and leaving the beard to rest, for example, at the weekend. Specially developed shaving aids containing anti-inflammatory or bacteriostatic ingredients are available.

Lather Shaving Creams

These types of formulations are typically based on a mixture of potassium and sodium soaps, normally amounting to 25% to 55% of the total formulation. Finding

the right balance of the soft potassium soaps and the hard sodium soaps is crucial for both texture of the cream and the resulting foam. Potassium soaps are more readily soluble in water and produce copious foam; sodium soaps produce less, more airy foam. An acceptable ratio of KOH to NaOH would be in the range of 4:1 to 6:1. Triethanolamine (TEA) is another alkali, which is mainly used in aerosol shave creams, but is also added to lather shaving creams in smaller portions. TEA-soaps show even better solubility in water and are very soft soaps producing copious foam very quickly. Another factor influencing foam and cream texture is the type and amount of fatty acid used in the formulation. Most commonly, blends of fatty acids are preferred to pure fatty acids, as the latter tend to give harder, less appealing foams. Still, as a general rule, at least 50% of the total fatty acid content of the formulation should consist of stearic acid. Short-chain fatty acids, like palmitic, myristic, lauric, or coconut fatty acids, contribute to the softness of the cream on the one hand and to the richness of the foam on the other hand. They can be added in varying ratios, but a high percentage can result in stability problems of the formulation at higher temperatures. Owing to the high amount of neutralized fatty acids, a typical pH of a shaving cream lies in the range of 10.0 to 11.0. However, the amount of alkali is best measured out to leave a level of at least 2% to 5% free fatty acids (FFAs) unneutralized. The FFAs are lubricating and refatting the skin and contribute to the texture of the lather as well as to the typical pearly appearance of the cream. They add structure and firmness to the product and are also responsible for the stability of the formulation at elevated temperatures. Most commonly, humectants like glycerin, sorbitol, or dipropylene glycol are added to the formula in the range of 10% to 15%. These ingredients make the cream softer and protect it from drying out. It usually takes a few days, if not weeks, for the cream to mature and to develop its final structure and appearance.

A typical soap-based shaving cream with well-balanced foaming properties is the following example by Henkel (4):

Shaving cream (%)	Wt.%
Coconut acid	10.00
Stearic acid	28.00
Glycerol (86%)	17.00
Distilled water	23.00
Potassium hydroxide, 45% soln.	15.00
Sodium hydroxide, 45% soln.	2.50
Stearic acid	3.50
Fragrance	1.00

Further ingredients, like emollients, can be very beneficial to shaving cream formulations as they act as lubricants, lowering the coefficient of friction between the razor and skin. Amongst the range of emollients, paraffin oil is the most popular one, probably for its low costs and high efficiency. Fatty alcohols and ester oils, together with lanolin and its derivatives, and silicon oils are on top of the list of valuable additives that improve the shaving performance. Attention must be paid to the quality of the lather, as many oils including perfumes are acting as antifoams. This effect can be at least partially compensated by the inclusion of surfactants of which a

wide range is available, like ether sulfates (e.g., sodium laureth sulfate), betaines, aminoxides, or alkylpolyglucosides. These surfactants boost and stabilize the foam and also improve its rinsability from skin, razor, and sink.

Ingredients like menthol, camphor, or eucalyptus are sometimes added to formulations often marketed as "medicated" products. They are not only added for their fragrance, but also, in the case of menthol, for the cooling sensation they leave on the skin, even some time after shaving. For mentholated products the choice of perfume is rather difficult and the best results are achieved, if the perfume is directly adapted to the respective formula base already containing menthol.

Lather Shaving Sticks

Lather shaving sticks could be regarded as solid concentrates of lather shaving creams. Although, the level of soap is higher than that used in creams (about 80% vs. 25–55%), the ratio of alkalies, that is, the sodium/potassium ratio and the proportion of the fatty acids is comparable. As the water phase is significantly smaller compared to creams, the portion of humectants like glycerol hardly exceeds 10%, the remainder being water. The mass is dried after saponification and milled afterwards, before it is molded. During the milling process, additives like perfume, dye, surfactants, or talc are being added.

Aerosol Shaving Foams

Aerosol shaving foams have become very popular shortly after they were introduced more than 40 years ago and are still the dominating shaving aid in many markets. Their economy and ease of use as well as their superior foam quality, which efficiently softens the beard and provides lubrication for a close and gentle shave, are the main reasons for their popularity. Although, many advances were made in the composition of aerosol shaving foams over the decades, the basic principles and systems as outlined in early patents still remain valid (11). These systems are oil-in-water (o/w) emulsions, using water-soluble soaps as emulsifier and liquefied chlorofluorocarbons (CFCs) gases as propellant, and are packed in pressure-resistant containers. Typical soaps employed at that time and still being used are TEA and potassium or ammonium soaps of stearic, palmitic, myristic, and lauric acid. For these systems the propellant builds a substantial part of the oil phase and is generally used at levels of 6% to 10% of the total composition. When CFCs were banned, because of their ability to deplete stratospheric ozone, they were replaced by blends of hydrocarbons like *n*-butane, isobutene, and *n*-propane. These are used at levels of 3% to 5% of the total composition. Only a small portion of the propellant is soluble in the soap emulsion with the remainder forming an undissolved layer on top of the mixture. By shaking, the whole amount of propellant becomes temporarily emulsified in the soap solution and expands upon discharge from the container into an instant foam.

Fatty acids account for 5% to 10% of the composition with 50% to 70% of that fraction usually being stearic acid. Foams solely based on stearic acid are stiff, also lacking body, hence their adherence to the wetted facial skin is not sufficient. To overcome these problems, portions of short-chain fatty acids are added making the foam softer and easier to dispense. The ability to soften the foam increases with decreasing chain length. Very often commercially available grades of long-chain fatty acids contain larger percentages of short-chain fatty acids. This has to be taken into account when calculating the right amount of base. The right quantity is

somewhat smaller than what stoichiometry would suggest. It is beneficial to the texture and stability of the foam to have at least 2% to 3% of fatty acids unneutralized. The same effect as with FFAs can be achieved by adding fatty alcohols to the formula. Usually stearyl, cetyl, and myristyl alcohols are used for this purpose.

Another key factor to foam texture is the right choice of *base*. TEA, potassium hydroxide, and mixtures thereof are typically used. Sodium hydroxide is not beneficial for this type of product, as sodium soaps have an inferior solubility, cause more airy foams, and have the tendency to gel at higher concentrations. TEA-soaps are readily soluble in water giving very soft and dense foams, with the little drawback, that they foam even under the pressurized conditions of the aerosol container. This problem can be partially reduced by adding potassium soaps to the system.

The following rich emollient foam was suggested by Goldschmidt (4):

Aerosol shaving foam (%)	Wt.%
Stearic acid—triple pressed	7.50
Cetyl alcohol	0.50
Glycerin	3.00
Cocamidopropylamine oxide	3.00
Cocamidopropyl betaine	1.00
Dimethicone copolyol	1.00
Laureth-23	0.50
Water	80.00
Triethanolamine, 98%	3.50
Fragrance, preservatives	q.s.

Fill and charge units with 96% concentrate and 4% isobutane.

Surfactants, available in a vast variety of anionic and nonionic types, can be very helpful in optimizing the properties of shaving foams. Depending on their chemical structure, they improve different aspects of the foam. Polyethoxylates of fatty alcohols, chosen from the group with high HLB values, and alkanolamides contribute to foam stability and lubricity. Anionic surfactants, like sodium dodecyl sulfate (SDS), improve the wetting ability and rinsability of the foam. Enhanced emolliency can be achieved by the incorporation of ethoxylated derivatives of natural plant oils, lanolin, or polysiloxanes. Classical emollients, like mineral oil, isopropyl myristate, or silicon fluids, can also be added, but caution is advised as higher amounts (>2%) tend to destroy the foam.

Humectants are added to foam formulations for their ability to bind water, thus preventing the foam from drying out during the shave. On top of the list are polyols, like glycerol, sorbitol, or propylene glycol, typically added at concentrations of 2% to 5%.

Polymers are valuable lubricants aiding good gliding of the razor over the skins' surface and also providing a slight conditioning effect. A wide variety of water-soluble polymers, like polyacrylic acid, sodium carboxymethyl cellulose, polyvinypyrrolidone, or xanthan gum, can be incorporated into shaving foam formulations. These polymers also increase stability of foam and aerosol emulsion, but have the tendency to thicken the concentrate so that it becomes difficult to dispense. Lowering the concentration of polymer can solve this problem.

Perfumes must be compatible with alkaline pH and should not interfere with the foam. Typically, they are added at levels of 0.25% to 1.2 %. Higher amounts are likely to have a negative impact on foam quality.

Preservatives are generally not needed for shaving foams, as the aerosol container perfectly protects the product from microbiological contamination. Still, owing to certain circumstances in the handling of the bulk prior to filling, preservation may be required. The choice of effective preservatives, however, is somewhat limited by the alkaline pH.

Active ingredients, like vitamins, skin soothing compounds, or anti-inflammatory agents, are often added to underline the marketing proposition of the respective product. Bisabolol, chamomile, and aloe vera are typical representatives of this group. Ingredients like allantoin or panthenol should be avoided, as they lack stability under alkaline conditions.

Finding the right type and amount of propellant is a challenge for the formulator of aerosol shaving foams. Too much propellant or pressure yields a stiff, dry, and unstable foam adhering badly to the skin. If the concentration or pressure is too low the foam becomes thin, wet, and runny.

Postfoaming Shaving Gels

Postfoaming shaving gels were first described in 1970 in a patent granted to S.C. Johnson & Son (12). They are the predominant product for wet shaving in markets, like the US and the UK, showing the highest growth rates amongst young consumers in many other markets. These formulations are soap-based systems discharged from an aerosol container. They are thickened by water-soluble polymers, like modified celluloses or polyacrylic acids, and contain blends of hydrocarbons, which are solubilized in the lipophilic phase of the formulation. Owing to the relatively high amount of polymer, a high degree of lubrication is achieved. The gel does not foam after discharge, but remains homogeneous and substantially free from bubbles for a couple of seconds. It instantly turns into a rich creamy lather when rubbed between the hands or massaged onto the face. For that reason propellants with a suitable boiling point, like *n*-butane, isobutane, *n*-pentane, or isopentane, are used solely or as blends. These products can only be produced using some kind of compartmentalized container. The gel including the foam-generating agent is filled into the inner chamber; the outer chamber is pressurized by addition of a small amount of hydrocarbon propellant or compressed nitrogen/air. Various packaging systems are available for tin or aluminum cans, either to be pressurized through the top, before the filling of the gel or afterwards, or through the bottom closed with a rubber seal. The inner compartment is built with a pouch from nylon or laminated multilayer foil for "bag-in-can" systems. In so-called "piston-can" systems a cup-shaped barrier typically made from polystyrene is separating the gel from the propellant that is needed to expel the product.

A typical formulation was suggested by Croda (13):

Postfoaming shaving gel (%)	Wt.%
Stearic acid	6.00
Myristic acid	2.00
Steareth-2	1.00
Water deionized	To 100

Postfoaming shaving gel (%)	Wt.%
Sorbitol 70%	10.00
Propylene glycol	3.50
Triethanolamine 99%	4.20
Hydroxypropyl cellulose [1% aqueous solution]	5.00
Carbomer 984 (2% aqueous solution)	10.00
Perfume, preservative, and color	q.s.

Aerosol pack: 97% concentrate, 3% isopentane.

Brushless Shaving Cream

Brushless shaving creams are o/w emulsions based on soap showing a pH in the range of 7.5 to 8.5. They are rich in emulsifying agents and oils, hence they are nonlathering creams showing many similarities to normal skin care creams. Owing to their high content of emollients and their relatively low pH, compared to pH 10 of lathering shaving creams, they are less irritating to the skin. The thick film of lubricants perfectly protects from nicks and cuts, but has the drawback that the beard-softening action is slower and less effective compared to aerosol foams. Also, the rinsability from the face and the razor is somehow difficult. Probably for these reasons the popularity of this product type is steadily declining.

The following example was suggested by Henkel Corp.(14):

Brushless shaving cream (%)	Wt.%
Stearic acid	18.00
Coconut fatty acid	2.00
Propylene glycol dipelargonate	3.50
Lanolin oil	0.50
Dow Corning F-157 wax	1.00
Water deionized	60.65
Propyl paraben	0.03
Glycerin	6.00
Borax, USP	0.50
Potassium hydroxide, pellets	0.20
Sodium hydroxide, pellets	0.15
Triethanolamine	0.40
Water deionized	7.00
Methyl paraben	0.07
Perfume and color	q.s.

Nonaerosol Shaving Foams

Nonaerosol shaving foams are generally solutions of surfactants with a very low, water-like viscosity. Typically, lubricants, like lanolin, and skin conditioners, like quaternary ammonium compounds—polyquaternium-7, are added. For this type of product, special packaging is needed as the foam is generated by a pumping action. The liquid is pumped through a kind of sieve entering a little chamber where it is mixed with air. As mechanically generated, surfactant foams are more airy and

therefore dry and less stable compared to aerosol foams, this type of product has not yet gained significant market share.

The following pump shaving mousse was suggested by BASF (15):

Nonaerosol shaving foam (%)	Wt.%
Sodium laureth sulfate (38%)	20.00
Cocamide DEA	1.00
Poloxamer 407	5.00
Bisabolol	0.20
Polyquaternium-16	1.00
PEG-40 hydrogenated castor oil	2.00
Lanolin oil	1.00
Perfume	0.30
Water deionized	69.30

Mix at room temperature; fill in supermatic foamer.

Shaving Oils

Shaving oils are a fairly recent member of the family of shaving products. They were first introduced in the United Kingdom market truly being something new. They are basically water-free mixtures of oils. The whole range of available oils from naturally occurring oils to purely synthetic silicon oils is used. These oils are either used alone or as blends. As oils do not cause a significantly fresh sensation on skin compared to water-based products, cooling agents like menthol or eucalyptus are often added to this type of product. They provide a lubricating effect leaving a very smooth skin feeling after shaving. The less effective beard-softening action and the weak rinsability from the razor and skin are drawbacks of this technology.

AFTERSHAVE PRODUCTS

Aftershave Lotions

Preparation for shaving is only one side of the process. After shaving has been completed by either wet or dry shaving the face has to be treated to complete the process. After the blade shaving process, the person rinses his face with water to remove residual materials from his face. Afterwards he uses an aftershave lotion, an aftershave balm, an aftershave cream, or nothing as the user deems fit. After an electric shaving process the water rinse may not be necessary as a residue may not be present. The aftershave product is employed to soothe and prevent razor burn. Historically, lotions such as shown in Formula 3 were no more than colognes with a reduced fragrance level (4).

Formula 3 Typical Cologne Aftershave

Deionized water (%)	5.00–20.00
SD alcohol	q.s.
Fragrance	1.00–5.00

These formulas have no skin care effect at all. They may close the pores of the skin that have been opened by hot water, relieve the burning sensation, stop bleeding from cuts, and subtly perfume the skin. Additional ingredients found in such after-shave lotions may include conditioning and cooling agents, moisturizers, colorants, and thickeners. Cooling agents, for example menthol, are used from 0.05% to 0.5%. If menthol is used, the perfume has to be adapted carefully, because of the strong influence of menthol to the scent of the formulation. Moisturizers that can be formulated in these alcoholic products are glycerine, butylene glycol, and propylene glycol. Actives such as panthenol, bisabolol, and Niacinamide are often incorporated from 0.1% to 2%. All these ingredients, especially the amount of thickener, have to be well balanced, to avoid a sticky skin feeling after application of the product (e.g., Formulas 4 and 5).

Formula 4 Nonstinging Moisturizing AfterShave Lotion (16)

Alcohol (%)	50.00
Water (%)	44.00
Fragrance (%)	3.00
Ethoxylated hydrogenated castor oil (%)	0.20
D-(+)-panthenol (%)	1.50
Sodium isostearoyl lactylate (%)	0.80
Hydrolyzed silk (%)	0.50

Formula 5 Aftershave Splash GAF (4)

Distilled water (%)	11.50
PVP/dimethylaminoethyl methacrylate copolymer (%)	1.50
Propylene glycol (%)	1.00
SD alcohol 40 (%)	86.00
Fragrance (%)	q.s.

The reduction of alcohol content in these aftershave products is one way of product improvement, because alcohol is responsible for the stinging sensation after the application of such products, and it also dries out the skin. It removes the sebum from the already stressed skin because of the shave. These sebum lipids are essential for the integrity and function of smooth skin, as they hold cells together, acting like pliable cement and providing water-holding capacity to the skin. Therefore, you can find watery liquid formulations with reduced alcohol content or even without alcohol on the men's care market. Often calming and anti-irritative ingredients, like bisabolol, are incorporated in these kinds of formulas. Attention must be paid to the solubility of the ingredients to avoid participation of ingredients. Solubility of ingredients can be achieved by the use of ethoxylated solubilizers, like ethoxylated hydrogenated castor oil, or the use of very mild surfactants. These products are for the aftershave lotion user, who is used to have these kinds of products with a water-like sensory. Formula 6 gives one example for an alcohol-free formulation.

Formula 6 Nonalcoholic Aftershave Lotion Croda (4)

Sucrose distearate (%)	3.00
Sucrose stearate	0.50
PPG-2 myristyl ether propionate	3.00
PPG-5 ceteth-20	1.00
Cetyl alcohol	3.00
Acteamide MEA and lactamide MEA	3.00
Allantoin	0.20
Preservatives	1.00
Distilled water	85.00
Ethyl-*para*-amino benzoate	0.05
Menthol	0.10
Lactic acid, 10% soln.	qs to pH 4.5

Aftershave Balms

Another way to reduce the loss of sebum lipids is to formulate emulsions with reduced alcohol content or even without alcohol. With the application of an emulsion containing lipids the loss of sebum lipids while shaving is compensated.

These products are reducing the stinging sensation after shaving, associated with the application of formulations containing alcohol. If the content of alcohol and replenishing ingredients is well balanced it may be possible to have active moisturization and skin care efficacy.

The emulsifier must be chosen very carefully, if the product contains alcohol. Alcohol is responsible for instabilities of emulsions and often makes the product thinner. It is possible to use normal ethoxylated emulsifiers or phosphoric acid esters. With the oil components and thickener, the viscosity can be adjusted. Balms have a very liquid consistency that the emulsion can be poured into the palms and applied like a lotion. These formulations cannot be found in female face care products. The lipid content has to be well balanced to achieve a light product sensory, without leaving a sticky skin feeling after application. These aftershave balms can also be formulated alcohol-free. Formula 7 gives one example for these kinds of products.

Formula 7 Aftershave Balm Henkel KGaA (4)

Ceteareth-20 (%)	0.50
Ceteareth-12 (%)	0.50
Glyceryl stearate (%)	3.00
Coco-caprylate/caprate (%)	5.00
Allantoin (%)	0.20
Glycerin (86% soln.) (%)	3.00
Distilled Water (%)	31.44
Ethyl alcohol (%)	20.00
Aqua hamamelis (%)	10.00
Menthol (%)	3.00
Carbomer 934 (%)	0.40
Sodium hydroxide (%)	0.16
Water (%)	25.00
Fragrance (%)	0.50

Aftershave Creams

Another category of aftershave products is aftershave creams. They have a thicker texture and a greater oil content. They can also be formulated with or without alcohol. These creams are definitely meant to be used for dry skin. They are comparable with a moisturizing cream that can be used after shaving. Actives like panthenol and conditioning agents can be incorporated. Formula 8 gives one example.

Formula 8 Aftershave Cream with Alcohol

Glycerylstearatecitrate (%)	4.00
Behenylalcohol (%)	2.00
Cetylstearylalcohol (%)	4.00
Paraffinum liquidum (%)	0.75
Octyldodecanol (%)	1.00
Cyclomethicone (%)	3.00
Dimethicone (%)	13.00
Hydroxypropyl Distarchphosphat (%)	7.5
Modified starch (%)	2.5
Alcohol Denat. (%)	6.00
Panthenol (%)	1.00
Preservatives (%)	0.50
Perfume (%)	q.s.
Glycerine (%)	5.00
Water (%)	ad. 100

Market Shares of the Different Aftershave Products

Currently, the market is dominated by alcoholic aftershave lotions. A trend can be seen towards products either with low alcohol contents or without alcohol.

The balm category has been existed for more than 10 years and has shown a very strong growth since introduction. Even in this product category, the trend goes to low levels of alcohol. Aftershave creams have a very small market share and can be regarded as special care aftershave products. The aftershave products have been a subtle fragrance for men,but now they are changing to a face care product for men.

FACE CARE FOR MEN

Face care for men is a very new product category. As mentioned earlier, men are treating themselves in response to a need. In the past, they did not view skin care as an ageing preventative or look-enhancing practice. In addition to that, in our society mature men with wrinkles are seen as still attractive. A reason for this may be based in our genes, as men with a lot of life experience are better able to care for a family. And women tend to look for such men that can protect them against the risks of life. But momentarily this tendency is changing: A well-groomed appearance is also becoming important for men; thus, the face care market for men is growing constantly. The starting point of this development was the selective market, but now even mass-market brands are entering the segment of face care for men. A company needs at least some face care products to have a complete product range for men.

Although, some men are using the face care products of their female partner, the face care products for men have to differ from face care products for women. Owing to physiological differences in female and male skin, important issues for the formulation of face care products for men are:

- greater sebum production—better water binding capacity,
- dilated pores—tendency to acne,
- greater thickness of skin with a better ratio of collagen to skin thickness—less or later ageing.

Face care products for men should fulfill the following characteristics:

- the products should be easily and fast absorbed,
- the products should show low tackiness,
- they should not leave a greasy residue on the skin,
- they should moisturize the skin,
- they should be refreshing, but at the same time soothing.

Another key difference between men and women is that men are expecting immediate sensory effects—razor burn relief and calming and soothing of inflamed skin. Women are more likely to expect key benefits to result from usage over time, such as improved skin elasticity and wrinkle reduction.

Instant effects in products for men can be achieved by the use of light and fast absorbing o/w emulsions with cooling ingredients. These cooling ingredients can be the traditional alcohol and menthol or new cooling actives. The cooling sensation coveys the feeling of an instant effect. The base formula is responsible for the caring job afterwards. Men's face care can be divided into three categories:

1. light moisturizers,
2. special face care creams,
3. special products (e.g., wipes, eye care, etc.).

The following section starts with the first product category, the light moisturizers.

Light Moisturizers

These products are often derived from an all-purpose cream and are sold in a tube. Adapted to men's needs they have a reduced oil content, are easily applied, and should be fast absorbed. Although these formulas have to be very light, in cosmetic product development one should not neglect the moisturizing effect, thus the use of moisturizers has to be considered. A basic UV-protection is often integrated in these products. With the incorporation of silicon oils a light skin feeling after application can be achieved.

Formula 9 gives an example for such a light-moisturizing face care product:

Formula 9 Protective Barrier Lotion Amerchol (15)

Oil phase	
Methyl gluceth-20 distearate (%)	1
Methyl glucose sesquistearate (%)	0.5

(*Continued*)

Isopropyl lanolate (%)	1.5
Stearic acid (%)	10
Dimethicone (%)	5.0
Water phase	
Deionized water (%)	76
Glycerin (%)	5
Triethanolamine (%)	1
Perfume and preservatives (%)	q.s.

Special Face Care Creams

This is the next product category. These products are comparable with special face care products for women. Women often have antiwrinkle, antiageing, and night/day care products. These special care products can also be found in face care for men. They however have to be adapted to the needs of male skin. Currently you will, for example, not find antiwrinkle products for men, because wrinkles are now not so important to men. As already mentioned, male skin ages later than female skin. But often the same actives are used in special face care products for men, as they are in the face care products for women. The active ingredients in antiageing products are in some cases UV-filters. In products for women these filters are protecting the skin from the UV-induced skin ageing. In a product for men these ingredients are protecting the skin against environmental influences during outside activities. Normally, special care products have a rich consistency. This can be achieved by a higher content of lipids and oils or by a higher content of thickener. These products are usually marketed in a glass jar to support the value of the product. Other ingredients used in special care products are antioxidants, radical scavengers, vitamins, and skin's components. But these products too have to fulfill the product characteristics for male skin care, namely, easily and fast absorbing, low tackiness, and no residue on the skin after application. A well-balanced oil phase with the right emulsifier and the incorporation of silicon oils can provide this skin feeling. It is important to have a spreading cascade of different oils in the product to reach this special skin feeling. The oil phase has to be adapted to the emulsifier system, which means you should not use the same oil composition for a stearic acid emulsifier as for a nonionic emulsifier. Formula 10 is an example for a special face care product for men.

Formula 10 Special Face Care

Stearic acid (%)	1.00
Palmic acid (%)	1.00
Behenyl alcohol (%)	3.00
Stearyl alcohol (%)	3.00
Cetylstearyl alcohol (%)	3.00
Hydrated polyisobutene (%)	0.5
Octyldodecanol (%)	0.5
Cyclomethicone (%)	2.5
Dimethicone (%)	5.00
Hydroxypropyl distarchphosphate (%)	5.00
Preservatives (%)	0.70

(*Continued*)

Panthenol (%)	0.50
Ubiquinone (%)	0.05
Perfume (%)	q.s.
Glycerine (%)	3.00
Water (%)	add. 100

Stearic acid emulsions often provide a good, silky skin feeling after application, because it improves the skin feeling. To achieve a nonsticky product performance, the incorporation of powder raw materials is very helpful.

Special Products

Special products such as eye care creams, face scrubs, or wipes are entirely new on the men's cosmetic market. The selective market is often the starting point for the development of these special products. Some of them find their way into the mass market afterwards. Examples for these developments are the face scrubs and wipe products for men. Special care products for women can often be used for men.

A face scrub for men, for example, only contains a different perfume and more or bigger scrubs. The exfoliating action can be adjusted with this scrubs variations. For men a stronger exfoliating effect is desired, because they have a tendency to oily skin and acne. With exfoliating products the development of pimples and blackheads can be prevented or slowed down. Formula 11 is an example for a face scrub.

Formula 11 Face Scrub

Water (%)	7.00
Preservatives (%)	0.8
Sodium laureth sulfate and lauryl glucoside (%)	2.00
Perfume (%)	q.s.
Carbomer (%)	0.70
Glycerine (%)	2.00
Water (%)	74.00 q.s.
Phantenol (%)	0.20
Sodium hydroxide in water (%)	add to pH 6–7
Polyethylene (%)	8.00

In these kinds of products the use of surfactants can be considered, because the product is rinsed off after application. Surfactants ease the rinsing off of product residues, but they should be very skin friendly as the scrubs peel of the first skin layer. A surfactant mixture is necessary to reach the right level of skin compatibility.

Wipe products for men often contain higher levels of alcohol to achieve the cooling action. In addition to that, alcohol also removes excess of sebum lipids. Wipes are relatively a new product category mostly pushed by convenient aspects. They are used for an in-between refreshing or cleansing during the day. Aftershave wipes are really new in the mass market and we will have to wait, to see if they are

accepted by the consumer. Usually, these aftershave wipes are soaked with alcoholic solutions comparable to after-shave lotions.

Other special products such as eye care until now only exist in the selective market. Time will show if they will find their way into the mass market.

CONCLUSION

The men's cosmetic market has changed dramatically during the last few years. Starting with shaving and aftershave products with a high content of alcohol, it has developed into a more sophisticated market. For shave and aftershave we do not only have job products any more. In the meantime, there is a great diversification even in this product category, such as shaving soaps, foams, gels, and oils. The consumer can choose products depending on personal preferences or skin needs. In the aftershave segment the consumer can choose between alcoholic lotions, nonalcoholic lotions, balms with or without alcohol, and creams. The first face care products for men have been developed and are on the market. The selective market is beginning to introduce special care products for men. Thus, cosmetic product development for men has to supply for the same product range as it has for women. We will have products for different skin types and we will also have special care products. All products for men have to be formulated to meet the needs of male skin. If all these issues are taken into consideration, the male skin care market will develop from daily basic grooming to a growing sophisticated cosmetic market.

REFERENCES

1. Ethel T. Physiology of the skin–differences between women and men. Clin Dermatol 1997; 15:5–16.
2. Cunliffe WJ, Shuster S. Pathogenesis of acne. Lancet 1969; i:685–687.
3. Baran R, Maibach HI. Textbook of Cosmetic Dermatology 7. 2nd ed. : Martin Dunitz Ltd., 1998:495–503.
4. Brooks GJ, Burmeister F. Preshave and aftershave products. Cosmet Toilet 1990; 105:67–87.
5. Johnsen MA. Aerosol depilatory foams and sprays. Spray Technol Market 1999; 6:24.
6. Schubert WR. Shaving preparations. In: Balsam MS, Sagarin E, eds. Cosmetics: Science and Techn. Chichester: John Wiley & Sons, 1974.
7. Brewster B. Closing in on comfort–shaving the male beard. Cosmet Toilet 2000; 115: 8–15.
8. Elden HR. Advances in understanding mechanisms of shaving. Cosmet Toilet 1985; 100:51–62.
9. Hollander L, Casselman EJ. Factors involved in satisfactory shaving. J Am Med Assoc 1937; 109:95.
10. Spencer TS. Pseudofolliculits barbae or razor bumps and shaving. Cosmet Toilet 1985; 100:47–49.
11. Spitzer JG, et al. US Patent 2,655,480.
12. Monson JA. US Patent 3,541,581.
13. Croda Formula C1456.Croda Formulary 4. Men's Toilet 1994; 9:6.
14. Happi.com. Formulary. August, 1999.
15. Dichter P. The Men's Product Explosion. Cosmet Toilet 1985; 100:79–89.
16. Simmons Maison, Lisboa Louis, Ferguson Elizabeth. Alcoholic moisturising after shave lotion. WO Patent 9601613.

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Baby Care

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INTRODUCTION

Skin undergoes an extraordinary development. It must grow rapidly and expand dramatically in size to cover the entire developing body. It is exposed to both internal and external environmental influences throughout the entire phase of its existence. However, despite the multitude of regionally specific influences that play a role in the development of skin, there is a remarkable similarity in its developmental pattern and in the ultimate end product of differentiation in every part of the body. The purpose of this chapter is to outline what is known about the development and physiology of baby skin and its implications on our daily care regimen of skin at this early stage of life.

THE DEVELOPMENT OF BABY SKIN

The development of skin usually begins seven to eight days after fertilization, during which an outer blastodermic layer, the ectoderm, is formed. During the embryonic phase of development, two layers evolve from the ectoderm, the underlying basal layer from which the uppermost skin layer—the epidermis—and the cutaneous appendages develop, along with the periderm, which faces the fetal cavity. When the epidermis is keratinized between week 22 and 24 of pregnancy, the periderm separates itself from most parts of the body. In the third trimenon all cell layers in the epidermis that are typical for mature skin are developed. However, until birth, the stratum corneum has still not developed a significant barrier function. This is made clear when premature babies are observed. One of the biggest problems in preemies is high transepidermal water loss (TEWL), although this decreases exponentially with increasing gestation age. High TEWL in turn may lead to hypothermia and difficulty in fluid balance (1–6).

The mesoderm, a middle layer, develops at day 18 or 19 after fertilization. The mesoderm, with its mesenchymal cells, forms the dermis (corium). Epidermis and dermis are connected by a membrane (basal lamina). Within the third trimenon this contact area (the dermo-epidermal junctional zone) between the dermis and epidermis can now be clearly identified by commencing undulations and by the development of epithelial crests and papillae. The development of the dermis also continues until the birth of the baby. In newborns it is about 60% as thick as in adults (1).

In the embryonic phase the dermis and the underlying subcutis cannot be differentiated from one another. In week 15 of gestation the subcutis can clearly be recognized. The lobuli network, in which the adipocytes (fat cells) spread, is formed. Within the third trimenon large fat lobuli develop in the subcutis, which protect the organism from heat loss in cold conditions. Today it is still not clear what exactly stimulates the adipocytes to produce fat. The subcutis does not become thicker until after birth, depending on the baby's nutritional condition (1,2).

The sweat glands begin forming on the palms of the hands and the soles of the feet between weeks 10 and 12 of gestation. A portion of the excretory glands, however, remains closed until the end of month 7 of gestation. This is the reason why babies born prematurely have developed, if at all, a limited ability to sweat (2). They also show a limited ability to regulate body temperature as well as an increased TEWL, both of which need to be considered when setting up a daily skincare regimen (7). In contrast, the skin of preterm and full-term infants usually shows no signs of a physiological deficit.

THE PHYSIOLOGY OF BABY SKIN

Protection Against Water Loss

The following two basic mechanisms account for fluid transport through the skin:

1. Perspiration: active process in which water is excreted through the openings of the sweat glands (Perspiration plays an important role in thermoregulation.) (8).
2. TEWL: passive diffusion of water through the skin (9,10).

Gestation age plays an essential role in the birth of a baby (11). The TEWL decreases with increasing gestation age. In a fully developed newborn, a TEWL of 6 to 8 g water/m^2 of skin/hr is low. However, the TEWL is considerably higher in prematurely born babies, especially those born before week 30 of pregnancy. In the first month of life, water loss in infants increases slightly. The explanation for this is that the babies begin to perspire slightly (12).

As the body temperature rises, the permeability of the skin also increases, leading to higher water loss. As environmental temperatures rise, water evaporates faster. This fact must be considered especially when caring for newborns. Creams and ointments with occluding effect can lower TEWL. The application of liquid paraffin on the skin can reduce TEWL by up to 50%.

Protection Against Percutaneous Absorption of Harmful Substances

In addition to providing protection against water loss, the skin barrier function ensures that chemical agents, which could harm the organism, cannot penetrate percutaneously (through the skin). The permeability rate in prematurely born babies is 5 to 50 times higher than in fully developed newborns. The ratio between body surface and body weight is almost 2.5 times higher in newborns than in adults. This surface volume ratio is one of the essential points that must be considered in the application of topically affective therapeutics. Particularly with treatment of large areas, for example, of dermatologicals containing corticoids, there is the danger of increased systemic absorption (13).

With increasing maturation, the epidermal cells develop increased metabolic activity. This means that the activation of enzymes can render potentially harmful substances harmless. They are modified through oxidation, hydrolysis, reduction,

deamination, or conjugation and thereby inactivated. This enzyme activity is very restricted, especially in prematurely born babies, so that potentially harmful substances can enter the blood stream in an unaltered state if absorbed percutaneously (13).

Protection Against Pathogenic Microorganisms

After birth, the body of the baby is exposed to numerous germs. The skin barrier not only protects mechanically against invading microorganisms, but also through the slightly acidic milieu of the hydrolipidic film on the surface of the skin. The surface of the skin is physiologically populated by specific germs (saprophytes), which are not pathogens but rather a vital microbial defense system on the skin's surface. For optimal living conditions, the saprophytes require an acidic milieu. Directly after birth, however, alkaline values prevail on the surface of the body of the newborn. It can be assumed that these alkaline values result from the vernix caseosa residue. Neither weight at birth nor gestation age seems to have an influence on the pH value. Within the first 24 hours after birth, the pH value drops noticeably. In the first month of life, the pH value then stabilizes, at a slightly acidic range (slightly below a pH value of 6) (14).

The natural acid mantle of the skin on the newborn is already developed in the first few days of life, so that pathogenic Microorganisms generally find the conditions unsuitable for their survival. However, the alkaline-neutralizing properties of the skin of newborns and small children are restricted. After contact with alkaline substances (e.g., alkaline soaps), the skin requires a longer time to restore its slightly acidic physiological pH value as compared with adult skin (15).

FREQUENT SKIN PROBLEMS IN NEWBORNS

Diaper Dermatitis

At the beginning of this century, in 1905, Jacquet, a French pediatrician, gave the peculiar frequently occurring skin rash in the diaper area the name, diaper dermatitis (16). The skin alterations subcategorized under the diagnosis diaper dermatitis can have a variety of causes. They can be directly related to the contact dermatitis, which is diaper dermatitis in a narrow sense. The occurrence can also be unrelated to the use of diapers. Today the factors that enhance this irritating contact dermatitis are known:

1. Diapers that have an occluding effect in an already moist environment, which results in an increased hydration of the stratum corneum.
2. The increased hydration facilitates penetration of xenobiotics.
3. The still very thin epidermis of the newborn reacts sensitively to mechanical stress and friction.
4. The skin barrier function is weakened, and the skin shows an increased irritability.

In addition, an increase of the pH value in the diaper area can also encourage an outbreak of diaper dermatitis. The alkaline urine activates enzymes (lipases and proteases) in the feces, which irritate the skin (17–19).

Boys and girls are equally afflicted with diaper dermatitis. It mainly occurs between 3 and 10 months of age, with a frequency peak between six and nine months. Typically, a skin erythema can be found on the inside of the thighs and on the baby's bottom. The skin is increasingly reddened, has a shiny, glassy appearance, and is wrinkled on the surface.

Corticosteroids are used occasionally in the treatment of diaper dermatitis. In the follow-up treatment, emollients containing zinc oxide are mainly used. Zinc oxide has an astringent, slightly disinfectant effect and offers the skin protection against urine and feces (17–21).

Protective creams containing zinc oxide are usually used to cover the skin of the diaper area with a highly viscous film, which inhibits the penetration of xenobiotics without fully occluding the skin. To accomplish this goal, usually water-in-oil (w/o) creams are used, which contain one or more of the following ingredients: petrolatum, lanolin, lanolin alcohol, paraffin oil, natural oils, waxes, zinc oxide, and possibly cod liver oil, vitamins, plant extracts, and titanium dioxide.

Diaper candidiasis is a fungal-infected diaper dermatitis. The most common causative agent is a yeast fungus called *Candida albicans.* It is a known fact that extensive use of antibiotics in newborns and small children increases that incidence of diaper candidiasis. Initially, diaper candidiasis can be treated with a specific antimycotic therapy (nystatin and clotrimazole), then followed up with the healing methods for basic diaper dermatitis as previously described (22).

Neurodermatitis

Neurodermatitis, also called atopic dermatitis, is a skin disease that may occur at a very early age. It can be identified by the so-called milk crust on the reddened, damp skin of the head and cheeks of the newborn. As the first indication of an outbreak of neurodermatitis, the milk crust often provides the starting point for other skin disorders. The skin becomes cracked and transparent, and the permeability increases. Once the skin is damaged, the risk of infection is higher. The skin becomes increasingly dry, transparent, and irritated, with intensified itchiness. The temptation to keep on scratching the skin is usually almost irresistable for small children. Atopic dermatitis is an immunological reaction that affects the skin to an especially large extent. More than 10% of children in industrialized countries are already afflicted, with a rising tendency. The combination of the genetic predisposition and environmental influences as well as psychological and neurovegetative factors can result in an outbreak of this disease (23–25).

Adequate skincare, which reinforces the skin's vital barrier, is a meaningful prophylaxis for avoiding the first outbreak of neurodermatitis in high-risk allergy children. The following measures can help:

- mild cleansing agents;
- moisturizing emulsions to support the skin's barrier function;
- skincare products with proven skin tolerability;
- skincare products and cleansers with few, carefully selected ingredients, to keep the risk of allergies as low as possible (23–25).

THE CARE OF BABY SKIN

The effects of baby-care products can usually be divided into the following categories: cleansing, caring, and protection. Currently, a multitude of product types can be found in the market. Although the shear number of products is overwhelming, there are features that they all have in common. The following three sections will deal with product characteristics and general usage advice in the various segments of baby care.

Cleansing

Bath Additives

As soon as the umbilical cord has fallen off, the baby can be bathed (26–29). However, daily bathing of the baby is not advisable, as this would dry out the skin too much. A bath every two to three days is sufficient. The bath temperature should lie between 36 and 37°C. Bath additives usually contain a mixture of various anionic (e.g., fatty alcohol ether sulfates, protein fatty acid condensates), nonionic (e.g., ethoxylated fatty alcohols, fatty acid glycerides), and amphoteric (e.g., betaines) surfactants. Numerous protein hydrolysates, superfatting agents, solubilizing agents, plant extracts, colorants, and perfumes are also found in this product category. In general, bath additives contain mild surfactant mixtures, which neither dry out the skin nor burn in the eyes.

Cleansers for the Diaper Area

Baby oils containing mineral oils as well as oil-impregnated towelets are widely used. (Towelets are usually supplied in dispenser boxes securing product hygiene up to the last towelet used.) Liquid petrolatum is a very desirable ingredient in view of its stability, touch, barrier function, and cost. Liquid petrolatum also has a remarkable occlusivity. Intertrigo areas should therefore be frequently cleansed (1–3 times daily) with oil or oil-containing towelets.

Soft towelets containing mild oil-in-water (o/w) cleansing milks or, alternatively, clear cleansing lotions are also frequently found. They normally contain anionic and/or nonionic surfactants in low concentrations as well as varying amounts of skincare ingredients like plant extracts and protein hydrolysates. These towelets are also offered in dispenser boxes.

Whereas the irritating effect of soaps mainly results from their alkalinity, the use of alkaline-free soaps has shown that all detergents induce a significant delipidizing effect, which can also contribute to skin irritation (26–29).

Liquid cleansers are usually used for cleansing of the face, armpits, and the genital area. Normally alkaline free, their composition resembles the composition of baby shampoos, whereas the concentration of surfactants is normally higher. The reasoning behind the higher surfactant level lies in the smaller product amount used for cleansing (26–29).

Shampoos

Baby shampoos are usually formulated to be nonirritating to the eyes. This guarantees extraordinary product safety and also ensures that babies do not object to shampooing. Although basically the ingredients used are comparable with the ingredients found in bath additives, the concentration of surfactants is normally lower. Viscosity is adjusted to about 1000 centipoise to make it hard for the shampoo to migrate into the eyes.

Care

Face and Body Creams/Body Lotions

Face creams are especially important for the protection against environmental influences like sunlight, wind, and cold temperatures, which may dry out baby skin. The composition resembles that of the body-care creams, although the moisturizer content is often higher. The ingredients used are often more compatible with the mucous membranes (especially in the area of the eyes) than for body creams. Body-care

creams are frequently used for their excellent superfatting properties. Both o/w and w/o emulsions are found in the market.

Body-care lotions are normally used for large-area body care, for example, after baby bath. Both o/w and w/o emulsions are found in the market. Classic ingredients used are lanolin, lanolin alcohol, paraffin oil, vaseline, natural and synthetic wax esters, natural oils, fatty alcohols, and emulsifiers (e.g., fatty acid glycerides, ethoxylated fatty alcohols). Many skin-caring, soothing, active ingredients are also found.

Protection

Sun Protection

Spending summer vacation at the seashore is a tradition of many families. Unfortunately, the beach is a high-risk environment for future skin cancer because it allows for maximum sunlight exposure. Heat, wind, and humidity are often present. These factors can enhance or intensify UV injury. With or without topical sun-protection measurements, babies and small children should be kept out of direct sunlight. As soon as children begin to explore their environment, it usually becomes impossible to confine them to the shade. In such cases, sunscreens need to be applied.

A wide variety of different o/w and w/o emulsions, hydrogels, and oleogels are found in the market using a variety of UV-filter systems. Many products contain broad-spectrum (UVA and UVB) sunscreens with a moderate sun protection factor (SPF). Products with a water-resistant SPF are favorable at the seashore (30–33).

Cold Protection

Mild facial creams are especially important in the winter for protection against the harsh effects of a dry, cold climate. At freezing temperatures, significant protection against frostbite is obviously helpful. Specific petrolatum-based water-free formulations, which optionally contain zinc oxide and skin-soothing agents like panthenol, can protect the skin at temperatures below freezing.

QUALITY MANAGEMENT IN BABY CARE

Despite careful research for the good skin tolerability of each individual ingredient in baby-care formulations, it should be made certain that this data will also apply to the final product after these ingredients are integrated into the formula. To rule out the possibility of contact allergies or sensitizing skin reactions, products are frequently tested using the repeated-insult patch test (RIPT). This test is a validated, recognized method for the testing of skin sensitization. The test preparations are repeatedly applied to the same localization for three weeks. After a 2-week break, the test materials are applied once again on another location and the skin is assessed for any allergic reaction that could possibly have been induced (34). Exposure to sunlight can cause certain ingredients to trigger photoallergic or phototoxic skin reactions. Photopatch or phototoxicity tests enable the detection of UV-induced irritant or allergic skin reactions.

In the elbow-wash test, the skin tolerability of cleansing formulas is tested in the sensitive crease of the elbow under controlled and extreme washing conditions,

and compared with a skin-friendly standard product. The evaluation of the skin reaction is performed after repeated washings over a period of five days, based on subjective and objective reports (35).

In a clinical application test, skin tolerability as well as the skincare properties of baby products can be tested. At the start, and again after four weeks of practical application of baby-care products, dermatological examinations are carried out. Parents are given diaries for the daily evaluation of product properties. Children known to have skin allergies to ingredients in the test products are excluded from the testing.

SUMMARY

Normal baby skin shows no natural inborn deficits that need special treatment. However, the elevated skin permeability in newborns needs to be considered when establishing a routine skincare regimen. The sensible use of skin-cleansing and caring products surely needs to be remembered.

However, there is a growing demand for specific dermatological treatments of newborns, as the number of skin disorders (e.g., neurodermatitis) in this age group is on the increase.

REFERENCES

1. Holbrook KA, Sybert VP. Basic science. In: Schachner LA, Hansen RC, eds. Pediatric Dermatology. New York: Churchill-Livingstone, 1996; 1–70.
2. Holbrook KA. Structure and function of the developing skin. In: Goldsmith LE, ed. Physiology, Biochemistry and Molecular Biology of the Skin. 1. 2nd ed. New York: Oxford University Press, , 1991; 63–110.
3. Hammerlund K, Sedin G, Stromberg B. Transepidermal water loss in newborn infants. VII. Relation to postnatal age in very pre-term and full-term appropriate for gestational age infants. Acta Paediatr Scand 1982; 71:369–374.
4. Doty SE, McCormack WB, Seagrave RC. Predicting insensible water loss in premature neonates. Biol Neonate 1994; 66:33–44.
5. Wilson DR, Maibach HI. Transepidermal water loss in vivo. Preterm and term infants. Biol Neonate 1980; 37:180–185.
6. Rutter N, Hull D. Water loss from the skin of term and preterm babies. Arch Dis Child 1979; 54:858–868.
7. Lane AT. Development and care of the premature infant's skin. Pediatr Derm 1987; 4:1–5.
8. Hey EN, Katz G. Optimum thermal environment for naked bodies. Arch Dis Child 1970; 45:328–334.
9. Hammerlund K, Nilsson GE, Oberg PA, Sedin G. Transepidermal water loss in newborn infants. II. Acta Paediatr Scand 1978; 68:371–376.
10. Fanaroff AA, Wald M, Gruber HS, Klaus MH. Insensible water loss in low birth weight infants. J Pediatrics 1972; 50:236–245.
11. Dubowitz LMS, Dubowitz V, Golberg C. Clinical assessment of gestational age in the newborn infant. J Pediatr 1970; 77:1–10.
12. Harpin VA, Rutter N. Barrier properties of the newborn infant's skin. J Pediatr 1983; 102:419–425.
13. Barrett DA, Rutter N. Transdermal delivery and the premature neonate. Crit Rev Therapeutic Drug Carrier Systems 1994; 11(1):1–30.

14. Tunnessen WW. Practical aspects of bacterial skin infections in children. Pediatr Derm 1985; 2(suppl):255–265.
15. Braun F, Lachmann D, Zweymiiller E. Der Einfluss eines synthetischen Detergens auf den pH der Haut von Sauglingen. Der Hautarzt 1986; 37:329–334.
16. Jacquet L. Traite de Maladie de l'Enfance. Paris: Grauncher & Comby, 1905.
17. Lane AT. Diaper rash: causes and cures. Patient Care 1988:167–173.
18. Honig PJ. Diaper dermatitis. Postgrad Med 1983; 74(6):79–88.
19. Rasmussen JE. Classification of diaper dermatitis: an overview. Pediatr 1987; 14(suppl 1): 6–10.
20. Agren MS. Percutaneous absorption of zinc and zinc oxide applied topically to intact skin in man. Dermatol 1990; 180:36–39.
21. Derry JE, McLean WM, Freeman JB. A study of the percutaneous adsorption from topically applied zinc oxide ointment. J Parenteral Enteral Nutr 1983; 7:131–135.
22. Stogmann W. Empfehlungen zur Lokaltherapie banaler Dermatosen im Kindealter. WMW 1984; 1:19–24.
23. Stogmann W. Empfehlungen zur Behandlung und Prophylaxe der Atopischen Dermatitis im Kindesalter. WMW 1989; 18:414–421.
24. Saurat JH. Atopische Dermatitis beim Kind. Annales Nestle 1987; 45:10–28.
25. Queille-Roussel C, Raynaud F, Saurat JH. A prospective computerized study of 500 cases of atopic dermatitis in childhood. Acta Derm Venerol (Stockholm) 1985; 114: 87–92.
26. Schneider W. Nutzen und Schaden von Seifen und Syndets. Kosmetologie H 1971; 2: 54–56.
27. Debsi S, Jonte G. Skin cleansing and skin care in infants. Arztl Kosmet 1987; 17(1): 65–69.
28. Vergesslich KA, Zweymiiller E. Sind die neuen Waschmittel in der Pediatrie von Vorteil? Wiener klinische Wochenzeitschrift 1982; Jg 94, Heft 12; 4:321–359.
29. Cowan ME, Frost MR. A comparison between a detergent baby additive and baby soap on the skin flora of neonates. J Hosp Infect 1986; 7:91–95.
30. Stern RS, Weinstein MC, Baker SG. Risk reduction for nonmelanoma skin cancer with childhood sunscreen use. Arch Dermatol 1986; 122:537–545.
31. Owens DW, Knox JM, Hudson HT, Troll D. Influence of humidity on ultraviolet injury. J Invest Dermatol 1975; 64:250–252.
32. Freeman RG, Knox JM. The influence of temperature on ultraviolet injury. Arch Derm 1967; 89:858–864.
33. Owens DW, Knox JM, Hudson HT, Troll D. Influence of wind on ultraviolet injury. Arch Derm 1974; 109:200–201.
34. Schelanski H, Schelanski M. A new technique of human patch test. Proc Sci Section 1953; 19:46–49.
35. Lukacovic MF, Dunlpa FE, Michaels SE, Visscher MD, Watson DD. Forearm wash test to evaluate the clinical mildness of cleansing products. J Soc Cosmet Chem 1988; 39: 355–366.

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Cosmetics for the Elderly

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INTRODUCTION

Aging is a basic biological process common to all living organisms. Its biochemical mechanisms have yet to be elucidated in detail. Aging is usually understood as an irreversible, progressive loss of homeostatic capacity. By definition, aging affects everyone, but at a variable rate. At present, aging is widely assumed to result partly from a genetically determined program and partly from endogenous and exogenous insults. Both processes occur at the level of individual cells.

At the organ level, generally, and in the skin, specifically, aging is manifested by a loss of maximum metabolic activity and increasing sensitivity or susceptibility to certain diseases and environmental factors. The purpose of this chapter is to outline what is known about morphological and physiological aging of the skin and its implications for a tailored skincare of the elderly.

AGE-ASSOCIATED CHANGES IN HUMAN SKIN: MORPHOLOGICAL AND HISTOLOGICAL CHANGES

The major aging changes in the morphology of the skin include dryness (roughness and scaliness), wrinkling, and laxity (1). The most striking and consistent change is a flattening of the dermal–epidermal junction (2). This results in a considerably smaller surface between the two compartments. This presumably leads to less nutrient transfer and may cause a relatively smaller proliferative compartment in the epidermis. It is also responsible for the lower resistance to shear forces (1). However, most of the apparent clinical changes associated with advanced age are attributable to chronic sun damage (3,4).

Physiological Changes

An age-associated decrease in the epidermal turnover rate of approximately 30% to 50% between the third and the eighth decade has been determined by a study of desquamation rates at selected body sites (5). The thymidine-labeling index of the epidermis in vivo has been reported to decline nearly 50% during the human life span (6).

Recent studies using highly sensitive techniques for the measurement of sebum secretion rates have documented a decline of approximately 23% per decade (7). The physiological consequences of decreased sebum production in old age, if any, are unknown (1).

Clinical studies showed that eccrine sweating is markedly impaired with age. Spontaneous sweating in response to dry heat, measured on digital pads, was reduced by more than 70% in healthy old subjects (8), primarily attributable to a decreased output per gland.

The decreased vascular responsiveness in elderly skin has been documented by clinically assessing vasodilation and transduction after application of standard irritants like histamine (9). The intensity of erythema after UV exposure also decreases with age (10).

An age-associated decrease in delayed hypersensitivity reactions in human skin is manifested by a relative inability of healthy elderly subjects to develop sensitivity to dinitrochlorobenzene (DNCB) and by their lower rate of patch-test reactions to standard recall antigens. The cutaneous manifestations of immediate hypersensitivity similarly decline with age (1).

Langerhans cells are the epidermal cell population, which is largely responsible for recognition of foreign antigens. An approximately 25% to 50% reduction in the number of epidermal Langerhans cells occurs between early and late adulthood (11) and substantially contributes to the age-associated decrease in cutaneous immune responsiveness. The amount of dermal mast cells likewise decreases with age. The resulting consequences beyond the reduced rate of immediate hypersensitivity reactions, such as a positive "prick-test" (12) or acute urticaria, are unknown.

Photoaging

Photoaging is a term used to describe the array of clinical and histological findings in the chronically sun-exposed skin of middle-aged and elderly adults. It has also been called dermatoheliosis (13) and heliodermatitis (14), the latter term reflecting the low-grade inflammatory nature of the process.

Clinical features of actinically damaged skin include coarseness, wrinkling, irregular pigmentation, telangiectasia, and scaliness as well as a variety of premalignant and malignant neoplasms. The relative severity of these changes varies considerably among individuals. This undoubtedly reflects strong differences in past sun exposure and marked individual differences in vulnerabilities and repair capacities for solar insults. Photoaging usually involves most severely the face, neck, or extensor surface of the upper extremities (15).

THE COSMETIC CARE OF ELDERLY SKIN

Cosmetics for elderly skin can usually be divided into the categories of face care and body care. Currently, a multitude of product types can be found. Although the number of products is overwhelming, there are common features to be mentioned. The following two sections will deal with product characteristics in various segments for the cosmetic care of elderly skin.

Face Care

Skincare

Concepts for cosmetics suited for elderly people are often based on the dry skin conditions typical for elderly skin. Many skincare formulations contain humectants, which enable excellent transient hydrational/moisturizing effects. When lessening the prominence of undesirable surface defects, these formulations have only minor influence on dermal losses. However, evidence is accruing that the following groups of topically applied actives do seem to reverse the degenerative skin changes seen with aging.

By far the most exciting discovery in cutaneous gerontology during the past decade is the effect of tretinoin (*all-trans* retinoic acid) on the clinical and histological appearance of photoaged skin. Kligman first realized that topical tretinoin improved the appearance of middle-aged women using the drug to control facial acne. Support for the concept was provided by a double-blind vehicle-controlled trial documenting tretinoin's effectiveness on human photoaging. After four months of daily application, 0.1% tretinoin cream produced statistically significant improvement in fine and coarse wrinkling, sallowness, and roughness of sun-damaged facial and arm skin (16).

Tretinoin was the first agent shown to reverse age-associated changes in any tissue. This statement must be qualified in that it is unclear whether tretinoin truly reverses aging changes or simply produces new changes that mimic a reversal. It is unclear whether tretinoin affects exclusively sunlight-induced pathologies or a combination of sun damage and intrinsic aging changes (1).

In the past years, estrogen supplementation of climacteric women has opened new aspects on the wide variability of estrogen effects in various tissues. In skin, estrogens increase vascularization and show effects at various levels of dermal tissue (17,18).

Several attempts have been made to check the skincare efficacy of estriol (0.3%) or estradiol (0.01%) in perimenopausal women. Daily application of a cream over a period of seven months resulted in a significant increase of skin parameters like skin firmness, wrinkles, and skin moisture content. Hormonal levels showed a slight increase in the prolactin level, whereas the estradiol level was unchanged. Side effects were not found (19,20).

Many further topical actives with excellent antiaging potential are currently used in marketed formulations, the number of which is increasing each year (21–23).

Skin Cleansing

Active detergent substances contained in cleansing agents for human skin inevitably result in a degreasing of the keratinous layer, so that natural, moisture-retaining substances are also rinsed out in the process. It is, however, possible, by selecting the proper cleansing agents and reducing the frequency and intensity of their application, to reduce the unfavorable influence of various washing procedures on the skin of such elderly persons to a considerable extent.

Facial skin cleansers for elderly skin are usually particularly mild and superfatting. Both surfactant-based and oil-in-water (o/w) emulsion-based formulations are currently found. In the surfactant-based formulations, surfactants like ampholytes, betaines, sulfo-succinates, and various types of alkylpolyglucose are frequently used, whereas o/w emulsion-based formulas frequently contain superfatting agents and various humectants, which secure good cleansing efficacy without drying out the skin.

Body care

Skincare

For active care by humidity and lipid substitution, mainly o/w and water-in-oil (w/o) emulsions are used, which combine occlusive effects and moisturizing action. In addition to pyrrolidone, carboxylic acid salt, and urea, other humectant substances such as alpha-hydroxy acid and hyaluronic acid, a highly efficient moisturizer, are frequently found. It is self-evident that such formulation ingredients as glycerine, propylene glycol, and other glycols also contribute to their humectancy.

Polar and nonpolar lipids are frequently used in body care formulations. They act as emollients, as protective lipids, and as structure formers of the liquid crystalline bilayers between the corneocytes. These three functions are usually performed by fatty alcohols, fatty acids, and short- and long-chain esters, along with triglycerides and waxes. Special effects are frequently delivered by liposomes, containing phospholipids, sphingolipids, and ceramides, and lead to the desired long-term effects. This is attributable to special binding mechanisms in the skin, an anchor capacity of transported and/or encapsulated active ingredients, and their slow release.

Skin Cleansing

Bath additives usually contain a mixture of various anionic, nonionic, and amphoteric surfactants. Numerous superfatting agents, solubilizing agents, plant extracts, and perfumes are also found in products within this category. However, only an oil bath for elderly skin may provide skin cleansing and conditioning at the same time. For serious dry-skin conditions, oil baths are indispensable.

A variety of shower products, meanwhile, also contain high amounts of superfatting agents, thus securing their good skin compatibility and low drying-out potential.

CONCLUSIONS

There is an increasing demand for face- and body-care formulations tailor-made for the cosmetic treatment of elderly skin. Modern topical formulations not only deliver excellent moisturizing and superfatting capabilities, but also many products, especially face care products, contain one or more actives counteracting the signs of intrinsic and/or photoaging. However, it is still not clear whether these actives reverse the signs of aging or induce other effects on the skin that mimic a reversal of skin aging.

REFERENCES

1. Gilchrest BA. Physiology and pathophysiology of aging skin. In: Goldsmith LA, ed. Physiology, Biochemistry, and Molecular Biology of the Skin. . Vol. 2. 2nd ed. New York: Oxford Press, , 1991:1425–1444.
2. Hull MT, Warfel KA. Age-related changes in the cutaneous basal lamina: scanning electron microscopic study. J Invest Dermatol 1983; 81:378–380.
3. Tindall JP, Smith JG. Skin lesions of the aged and their association with internal changes. J Am Med Assoc 1963; 186:73–76.
4. Beauregard SB, Gilchrest BA. A survey of skin problems and skin care regimes in the elderly. Arch Derm 1987; 123:1638–1643.

5. Tan CY, Statham B, Marks R, Payne PA. Skin thickness measurement by pulsed ultrasound: its reproducibility, validation and variability. Br J Dermatol 1982; 106:657–662.
6. Kligman AM. Perspectives and problems in cutaneous gerontology. J Invest Dermatol 1979; 73:39–46.
7. Jacobsen E, Billings JK, Frantz RA. Age-related changes in sebum secretion rate in men and women. J Invest Dermatol 1985; 85:483–485.
8. Silver AF, Montagna W, Karacan I. The effect of age on human eccrine sweating. In: Montagna W, ed. Advances in Biology of Skin. Oxford: Pergamon Press, 1965:129–137.
9. Grove GL, Lavker RM, Holzle E, Kligman AM. Use of nonintrusive tests to monitor age-associated changes in human skin. J Soc Cosmet Chem 1981; 32:15–19.
10. Gilchrest BA, Stoff JS, Boter NA. Chronologic aging alters the response to UV-induced inflammation in human skin. J Invest Dermatol 1982; 79:11–15.
11. Thiers BH, Maize JC, Spicer SS, Cantor AB. The effect of aging and chronic sun exposure on human Langerhans cell populations. J Invest Dermatol 1984; 82:223–226.
12. Barbee RA, Levowitz MD, Thompson HC, Burrows B. Immediate skin-test reactivity in a general population sample. Ann Intern Med 1976; 84:129–133.
13. Gilchrest BA. Skin and Aging Processes. Boca Raton: CRC Press, 1984.
14. Lavker RA, Kligman AM. Chronic heliodermatitis: a morphologic evaluation of chronic actinic dermal damage with emphasis on the role of mast cells. J Invest Dermatol 1988; 90:325–330.
15. Knox JM, Cockcrall EG, Freeman RB. Etiological factors and premature aging. J Am Med Assoc 1962; 179:630–634.
16. Weiss JS, Ellis CN, Headington JT, Voorhees JJ. Topical tretinoin in the treatment of aging skin. J Am Acad Dermatol 1988; 19:169–175.
17. Schmidt JB. Externe Ostrogenapplikation bei Hautalterung im Klimakterium—Ein Ther-paieansatz-. H + G 1993; Band 68; Heft 2:84–87.
18. Artner J, Gitsch E. Uber Lokalwirkungen von Ostriol. Geburtshilfe und Frauenheilkunde 1959; 19:812–819.
19. Punnonnen R, Vaajalahti P, Teisala K. Local oestriol treatment improves the structure of elastic fibres in the skin of postmenopausal women. Ann Chir Gynaecol 1987; 202(suppl):39–41.
20. Schmidt JB, Binder M, Demschick G, Biegelmayer C, Reiner A. Treatment of skin aging with topical estrogens. Int J Derm 1996; 35:669–674.
21. Smith WP. Hydroxy acids and skin aging. Cosmet Toilet 1994; 109:41–48.
22. Pierrefriche G, Laborit H. Oxygen free radicals, melatonin, and aging. Exp Gerontol 1995; 30:213–227.
23. Coles LS, Harris SB. Coenzyme Q10 and lifespan extension. In: Klatz RM, ed. Advances in Anti-Aging Medicine. Larchmont, New York: Mary Ann Liebert, Inc., 1996:205–216.

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Stability Testing of Cosmetic Products

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INTRODUCTION

Products formulated by cosmetic chemists are intended to perform a variety of "miracle" functions such as reshaping hair, delivering fragrance, smoothing and softening the skin, imparting color to the face, and cleansing the entire body. Chemists can deliver many of these miracles by using a variety of technologies, which have been described in this book. In using these technologies to develop products, chemists must be aware of the formulation issues that might prevent the product from performing optimally. Assessing the product stability is a critical part of this formulation process. This chapter discusses the basic principles of stability testing of cosmetic delivery systems. We will begin with a general definition of stability testing and move on to problems encountered by specific formula types. We will conclude this section with a discussion of stability issues that are not necessarily, directly related to the formulation, such as processing and packaging.

PRACTICAL DEFINITION OF STABILITY TESTING

Stability testing may be defined as the process of evaluating a product to ensure that the key attributes stay within acceptable guidelines. In order to make this testing meaningful, it is important to accurately establish the nature of these critical product attributes, to measure how they change over time, and to define the degree of change that is considered acceptable. Defining the parameters that are crucial requires a combination of the chemical knowledge about the formula and a common sense about the product usage. The chemist should be aware that cosmetic products must not only continue to function over time, but must also look, feel, and smell the same, each time the consumer uses them. Therefore, testing must evaluate aesthetic characteristics in addition to the functional properties. This is an important consideration because cosmetic products can change in a number of different ways that may affect consumer perception. For example, fragrances become distorted, colors may fade or darken, and consistency may change, which might result in a thicker or a thinner product. Chemists must determine which of these product characteristics will

change over time and design appropriate testing methods to measure the extent of changes. Nacht cites several technical issues to be considered, including the compatibility between the delivery system and active ingredient, compatibility with the overall formula, appropriate mechanism of release for the particular application, the rate of release of the active ingredient, and the overall safety for the user (1). This chapter discusses some of the key tests that the chemist can use to measure the changes in these characteristics. An important fact to remember is that no product remains unchanged forever. Depending on the intended use of the product and its anticipated shelf life, a small change over time may be inconsequential or devastatingly detrimental. In general, if a change is consumer-perceptible, the product may not be considered stable.

USEFUL INFORMATION PROVIDED BY STABILITY TESTING

Stability data are useful as an "early warning system" that can alert the chemist to potential formulation/package-related problems. Such advance information can be helpful in many ways.

Guiding the Chemist During Product Development

While you are formulating a product, preliminary testing of its stability can guide you in making modifications to ensure that it is stable. If you determine, for example, that an emulsion shows separation after exposure to freeze/thaw conditions, you may choose to modify the surfactant system to correct the problem, and then repeat the test on the modified formula to determine whether it performs better or worse. Preliminary stability test data are an important part of the trial and error development process.

Ensuring That the Product Will Continue to be Aesthetically Acceptable to the Consumer

More than other products, cosmetics are intended to be aesthetically pleasing to the consumer. For this reason, consumers are likely to notice subtle changes in the odor or appearance of their favorite products. Since no product remains 100% unchanged as it ages, it is critical that the chemist anticipates the changes that may occur and ensures that they stay within limits that are not consumer-perceptible. Stability testing allows you to see how the product will behave over time.

Determining That the Product Will Perform as Intended and Remains Safe to Use

Studying the performance of samples that are exposed to accelerated aging lets you assess how the product will function over time. This is particularly important for those cosmetic products that use technologies, which are described in this book, to deliver "active" ingredients. If the formula is not stable, the delivery of the active ingredient may be impaired. Take, for example, the case of an antiperspirant stick with an encapsulated fragrance that is released upon exposure to moisture and heat; if the delivery system is poorly designed, the fragrance may be released too soon or not at all. Properly designed stability testing can reveal such problems, so that corrective actions can be taken.

Forewarning the Company About Problems That Might Occur After Consumer Purchase

Testing can show, for example, that the product may thicken somewhat over time, and may be difficult to dispense it from the package. Realizing this beforehand is important to the company because it will allow the company to anticipate the consumer reaction.

Even though stability testing provides many useful informations, it is not an exact science and will not guarantee a trouble-free product; but, it can give an idea of the risks involved and help provide a solid scientific foundation for the evaluation of future problems.

STABILITY TEST DESIGN

When faced with a situation where testing might be appropriate, ask some basic questions about the task ahead.

Why Is Testing Being Done?

Why is testing necessary? Are you concerned with the product's appearance or do you want to determine if specific performance characteristics change over time? The reasons for doing the tests will determine the kind of tests that are required. Therefore, it is critically important to approach this testing with a scientific mindset, and to have a clearly defined hypothesis to be tested. Take, for example, the case of a skin lotion formula that develops an unpleasant odor. The reason for the test will be to determine what is causing the odor. Your hypothesis may be that the fragrance you have selected is reacting with the formula ingredients to cause this problem. To test this hypothesis appropriately, you will need to assess the odor of the unfragranced base to determine how the fragrance affects the overall smell of the product. In this example, the unfragranced samples are the controls because the fragrance, which is the scientific variable, has been removed. Evaluation of appropriate control samples can prove or disprove the hypothesis, i.e., that the fragrance is causing the problem.

Another example illustrating the importance of conducting a properly controlled study, is the case of an emulsion that separates after prolonged storage in its plastic bottle. In this case, the reason for conducting the test will be to determine the cause of separation. One of the hypotheses may be that the package is allowing water vapor to escape, thus leading to emulsion instability. To test this hypothesis, you will need to screen out the variable of concern: the packaging material. Therefore, the control samples could be packaged in glass to eliminate the possibility of moisture loss. If the control samples do not show the same instability that the packaged samples show, you have demonstrated that the packaging material is indeed having a negative effect on the product.

Finally, consider a case where the variable of interest is the viscosity of the product. If you are concerned that the product may become too thick over time and will not dispense properly, you could design a study to track product batches with varying initial viscosity. Suppose the target viscosity is 20,000 cps, you could monitor the viscosity of a series of batches, with viscosities ranging from low to high. You may make a first, second, third, and fourth batch, which are initially at 5000, 10,000,

15,000, and 20,000 cps, respectively. You would then monitor the viscosity of these batches, as a function of time and temperature. You may learn that viscosity does not change significantly from the initial value, which means that a very narrow specification will be required. In other words, the product must be very close to its final viscosity when it is produced. On the other hand, you may discover that as long as the initial viscosity is between 5000 and 15,000 cps, the product will build to 20,000 cps within two weeks, and stay at that level for two to three years. In this case your specification can be rather broad, since—regardless of the initial value—the consumer will only be exposed to product that is 20,000 cps. In all these cases, understanding why the test needs to be done helps you establish the appropriate controls, which are essential if meaningful test results are to be obtained.

What Is Being Tested?

Another important factor to understand is the status of the formula being tested. Is it a developmental prototype or the final production material? Consider a situation, as in the example provided above, where you are primarily concerned with the change in product viscosity. Furthermore, consider that the final color and fragrance of the product have not yet been firmly established, although there are several candidates under evaluation. You could prepare samples with every possible color-fragrance combination and measure their viscosity over time. This could involve thousands of samples and tens of thousands of measurements, which are both costly and time-consuming. So, bearing in mind that you are testing a prototype and not a finished product, you may instead opt to test the uncolored, unfragranced base formulation first. In this way you can expeditiously get data on the parameter of interest—in this case viscosity. By evaluating the prototypes early on, you have given yourself more time to react to the problems. Of course, the testing may have to be repeated once the final formula is established, because the fragrance may affect viscosity. Similarly, if the final production package is not yet available, you may choose to evaluate the formula stability in a packaging material that approximates the characteristics of the final container. Here too, the final formula and packaging combination must eventually be tested together, because the formula may interact unfavorably with the packaging material. Asking the "what" question will help make your testing meaningful, without forcing you to go to excessive lengths.

Where Will Test Samples be Stored and How Many Are Necessary?

Ideally, you could gain information on formula stability by performing exhaustive tests on every variable involved in every formulation you work with; but this is not always feasible because proper testing requires a significant commitment of time and resources. Therefore, most companies have standardized test procedures for the storage of stability samples, which depend on the objective of the study. Such procedures involve evaluations of the samples stored at a variety of conditions and include enough samples to be statistically significant. Usually, sample storage is done at elevated temperatures, under freeze and/or freeze–thaw conditions, and with exposure to various types of light. Elevated temperature storage is critical, since the rate of chemical reactions becomes roughly doubled for every 10°C increase in temperature. Storage at higher temperatures allows you to accelerate the aging process and to see certain problems much sooner than they would appear at room temperature. Of course, the potential drawback is that at high temperatures

you may be forcing reactions to occur that would not happen at all at lower temperatures. Cold storage evaluates conditions that may negatively affect the solubility of ingredients or the stability of emulsions. Sunlight and ultraviolet (UV) light exposure can reveal problems of the ingredients that are reactive to the respective wavelengths; fragrances and colors are particularly sensitive in this regard. The most common storage conditions used in this industry are 54°C or 50°C, 45°C, 37°C or 35°C, room temperature (25°C), and 4°C, freeze/thaw, and exposure to fluorescent and natural light.

Since many of the tests that must be conducted to evaluate product performance will affect the sample physically (e.g., spraying an aerosol can), multiple samples are required at each storage condition to ensure that there will be enough samples left for evaluation at the end of the test period. Depending on the protocol set by your organization, as many as one hundred or more samples may be required for a complete study. Again, you should follow your corporate guidelines to make sure that sample quantities will be enough for a thorough evaluation of all necessary conditions.

How Samples Are Evaluated and What to Look for—Identification of Instability

How samples are evaluated depends entirely on the type of product, and the nature of the problems that might occur. Instability is typically identified by evaluating various product characteristics, either by the subjective observation of properties such as color, odor, and appearance, or by the objective instrumental evaluation of pH, viscosity, particle size, and electrical conductivity. For instance, simply looking at a sample that has been stored at accelerated temperatures can often reveal significant changes such as color changes, emulsion separation, or rheological changes. Similarly, a quick olfactory evaluation can uncover major flaws in fragrance stability. More rigorous characterization of product attributes can be obtained instrumentally, for example, with a viscometer or pH meter. These instruments are highly sensitive and can distinguish small changes in products. Such changes are important to note since, as in the case of a change in pH, they may represent chemical reactions that are occurring in the formula.

Other specialized testings can be performed to quantify specific changes in formulated systems. For example, microscopic evaluation and the light scattering technique are used to appraise changes in particle size and the distribution of emulsions. A Coulter counter is also used for these determinations (2), as are conductivity measurements (3). Nuclear magnetic resonance (NMR) and X-ray crystallography can also be used to reveal additional information regarding emulsion structure. In certain systems, specific assays are performed to measure the activity of functional ingredients. These types of tests are tailored for the compound in question. For instance, the bactericidal efficacy of preservatives or other antimicrobial compounds may be measured over the course of a stability test. In addition to this, chromatographic tests, spectroscopic measurements, titrametric evaluations, and other wet chemical methods can be used to detect signs of instability. Other indications of instability include incompatibility of a product and its package, which can lead to weight loss and package degradation [such as softening or cracking of container walls, clogging of orifices, corrosion of metal parts, etc. (4)]. But perhaps, the most important question to ask in assessing instability is to determine how much change is acceptable. Knowlton and Pearce have stated that a useful rule of thumb is to

consider product rejection, if the attributes being measured deviate by more than 20% of their original value (4). This value is an interesting reference point. However, for some formulations, much smaller deviations may be critical. The impact of such changes must be assessed on a case-by-case basis.

SITUATIONS THAT REQUIRE STABILITY TESTING

A good chemist should have an understanding of factors that are critical to product stability, so that appropriate testing can be conducted when necessary. Situations in which stability testing is generally necessary include, but are not limited to, the following situations: consideration of a new formulation, qualification of new raw materials, evaluation of new manufacturing processes, and testing of different packaging components. As you will see, stability testing is not a finite onetime task, instead it is an ongoing dynamic process that begins when the product is being developed, and continues to evolve as the formula, packaging, or manufacturing processes change.

FORMULA-RELATED REASONS FOR STABILITY TESTING

Specific Considerations Related to the Development of Particular Formula Types

The process of stability testing a product is closely tied to the process of creating the formulation. As you develop formulations, you should always screen stability samples early in the process to make sure that your efforts are headed in the direction and will lead to a stable product. Every formula will have slightly different stability testing requirements, but for the sake of this discussion, we will give primary consideration to the types of cosmetic delivery systems detailed in this book.

Emulsions

Emulsions are among the most common types of delivery systems used for cosmetic products. They enable a wide variety of ingredients to be quickly and conveniently delivered to the hair and skin. While many definitions of emulsions have been proposed, we will define them as heterogeneous systems in which, at least one immiscible or barely miscible liquid is dispersed in another liquid in the form of tiny droplets of various sizes (5). Consequently, these systems are inherently unstable, and eventually, given enough time or energy, will separate into their respective phases.

Emulsions used for cosmetic products are typically semisolid materials, composed of an oil (hydrophobic) phase and a water (hydrophilic) phase. These phases are characterized as either the internal-phase or external-phase, depending on the overall composition of the emulsion. The internal-phase is that which is contained inside separate discrete particles surrounded by surfactants; these particles are known as micelles. The external-phase is the "solvent" or diluent, which surrounds the micelles. Usually, the external-phase is the more abundant one. Depending on the composition of each phase, simple emulsions can be either oil-in-water (o/w) or water-in-oil; the type depends specifically on what emulsifier is used.

Although the internal-phase particles of an emulsion are polydisperse (meaning they have various sizes), their average size is often used for emulsion classification (6). When the average diameter of internal particles is less than 100 Å, the system

is called a *micellar emulsion*. A particle diameter of 2000 to 100 Å is called a *microemulsion*. Larger particles produce macroemulsions, which are the most common types found in cosmetic formulations. More complex emulsions can have multiple internal-phases. These emulsions, called *multiple emulsions* can be oil-in-water-in-oil or some combination. For cosmetic applications, they are formed by first making a water-in-oil emulsion, and then mixing that emulsion with a water phase. These types are particularly useful for encapsulating materials, giving prolonged release when applied to a surface such as skin (7).

Stability Considerations. Since emulsions represent a mixture of two or more materials that are not miscible with each other, they are, according to the second law of thermodynamics, inherently unstable. This means that, eventually, the two phases will separate. The degree and speed of instability are quite variable. For example, a mixture of mineral oil and water, when shaken, will form a macroemulsion that immediately separates upon standing. Other emulsions can remain stable for years, but eventually all emulsions will separate. While the second law of thermodynamics suggests that emulsions will separate over time, it does not provide a mechanism of this destabilization. Investigation into how emulsions destabilize has revealed three primary processes leading to instability: flocculation, creaming, and coalescence (8).

Flocculation. This process is characterized by a weak, reversible association between droplets of the emulsion's internal-phase. Each individual droplet maintains its own identity and thus, there is no change in the basic droplet size (8). Flocculation represents a less serious sign of instability, which can be reversed by shaking the system (9).

Creaming. When particles of an emulsion aggregate, there is a tendency for upward sedimentation. This causes a partial separation of the emulsion into two emulsions, one of which is richer in the internal-phase and the other richer in the external-phase (9). As in the case of flocculation, this stability problem can be reversed by agitation.

Coalescence. An aggregation between two particles can, if the two particles combine, lead to the formation of a single larger particle. This process, known as coalescence, represents a more serious stability problem. A related phenomenon is that of Ostwald ripening, in which all the particles tend to have the same size. Both of these processes are irreversible and can eventually lead to complete separation of the internal and external-phases of the emulsion (10). An alternative consequence of these forms of instability is phase inversion in which the internal-phase becomes the external-phase, and vice versa (9). For stability considerations, this change is typically undesirable since it will change the physical properties of the product.

All emulsions are potentially subject to all of these destabilizing processes simultaneously and the resulting effects on any given emulsion will vary. For example, microemulsions and micellar emulsions are initially transparent. The size of the internal-phase particles may increase over time and they will develop a translucent appearance. Since macroemulsions are opaque, a similar change in appearance will not be notable; however, there may be changes in viscosity and measurable separation. Multiple emulsions are typically less stable than monoemulsions. Over a short period of time, the number of multiple emulsion particles tend to be reduced. This results in the "leaking out" of some of the encapsulated material and reduces the duration of prolonged release.

In addition to the inherent processes that destabilize emulsions, other factors may be involved. Storage temperature has been shown to affect emulsion product stability. Generally, elevated temperatures result in destabilization, while reduced temperatures improve emulsion stability. Aqueous-phase evaporation may also contribute to the instability over the life of a product. Microbial contamination can also cause a breakdown of emulsion stability. Finally, chemical reactions within the emulsion can lead to a change in the stability of the emulsion. While these types of reactions can be initiated by temperature increases, they can also be prompted by UV light or other types of electromagnetic radiation.

Vesicular Systems—Liposomes and Niosomes

Definition/Description. Vesicular systems encompass a number of delivery technologies including liposomes and niosomes. Both of these systems employ a "vessel" to contain active ingredients within a formula and to provide a controlled delivery of these ingredients. Nacht defines controlled delivery as a "system that would result in a predictable rate of delivery of its active ingredients to the skin" (1). Liposomes are a classic example of this technology in which phospholipids are used to create lipid "capsules" that can be loaded with various ingredients. Although liposomes are enjoying tremendous popularity in cosmetics today, they have their roots back in the early 1960s. At that time, Professor Bangham at the Institute for Animal Physiology in Cambridge, United Kingdom, was one of the first to speculate that lipids such as phosphatidyl choline could be used to create sealed vesicles with bilayer membranes similar to cell membranes (1). Niosomes are another delivery technology related to liposomes, the difference is that, unlike liposomes, niosomes are based on nonionic surfactants. L'Oreal pioneered the development of nonionic liposomes using nonionic surfactants such as polyoxyethylene alkyl ethers combined with fatty alcohols or fatty acids (1).

Stability Considerations. Liposome and niosome stability may be referred to in terms of leakage of contents, presence of oxidation products, or changing particle size due to aggregation formation and fusion. They are rather fragile capsules and certain precautions must be taken to make sure that they remain intact and are able to deliver their contents. Leakage can be caused by mechanical forces like high-shear processing, which should be avoided. Similarly, excessive heat, which may destabilize the lipid bilayers, should be avoided. Perhaps, most notably, the liposomes may be solubilized by surfactants that may be present and therefore are not suitable for use in detergent systems. This is particularly true of systems such as shampoos and body washes which contain strong anionic surfactants that can dissolve the lipid walls. In fact, even though liposomes are often used in creams and lotions, the emulsifiers used in these formulas may also be enough to disrupt the fragile walls. For these reasons, many formulators believe that gels are the ideal vehicle for liposomes because they lack the high hydrophilic lipophilic balance (HLB) surfactants present in many conventional emulsions, which might disrupt the lipid bilayers (10). There is hope for using liposomes in emulsion. Uji et al. report that stable liposome suspensions can be prepared by using a cross-linked acrylic acid/alkyl acrylate copolymer at very low concentrations, because it can effectively stabilize lecithin liposomes in o/w emulsions (11). Furthermore, there is some evidence in the patent literature that the addition of collagen, albumin, or gamma globulin to the liposomes can decrease the harmful effects of detergents (10).

In addition to leakage, the vesicle systems may fuse together and no longer be available as discrete units for the delivery of active agents. According to Weiner, such fusion can occur for several reasons, including the preparation below their transition temperature, the presence of contaminants such as fatty acids and divalent cations, changes in pH, or the addition of nonelectrolyte hydrophobic molecules (12). Furthermore, phase separation of bilayer components can occur upon extended storage. In an excellent review on the subject, Fox refers to an article by Crommelin et al., that reports on preserving the long-term stability of liposomes. Crommelin discusses the chemical pathways by which phospholipids can degrade: by hydrolysis of the ester groups, or oxidation of the unsaturated acyl chains. This research points to an optimal pH for liposome stability. For phosphotidylcholine liposomes, the pH for the lowest hydrolysis rate was found to be 6.5. The stability of liposomes was further enhanced by using phospholipids with fully saturated acyl chains (like those made from hydrogenated soybeans, so the opportunity for oxidation is reduced) (10). Similarly, liposomes may be stabilized by sugar esters, for example, maltopentose monopalmitate have been used to improve stability of cosmetic systems (13).

For a more detailed discussion of the morphology of liposomal bilayers, the readers are asked to refer to "Liposomes: From Biophysics to Therapeutics" (12). The author provides an excellent discussion of the elastic properties and tensile strength of liposomes, as well as the effect of solvents and osmotic effects on liposomal structures.

Molecular Carriers

Definition/Description. Molecular carriers represent a delivery system in which one compound is used for binding another compound to a substrate, thereby changing the former's characteristics. This allows the bound material to be delivered to a surface, and released when conditions are appropriate. One example of this type of technology is cyclodextrin chemistry. Cyclodextrins are created from starch-derived glucopyranose units and are classified as cyclic oligosaccharides. When formed, they contain a hydrophobic cavity, capable of entrapping molecules of different sizes, shapes, and polarities. Molecules entrapped as such are found to be more resistant to environmental stresses, and therefore more stable (14). They can be used to entrap various types of compounds such as fragrances, vitamins, pigments, and dyes. Cyclodextrins have been used in cosmetic products for a variety of reasons, such as, to reduce odor in mercaptan-containing systems (15), improve the stability of hair dyes (16), and as an active ingredient to treat acne (17).

Stability Issues. The complex of the cyclodextrin, with a guest molecule, is typically quite stable under ambient temperatures and dry conditions. However, in the presence of certain materials, the guest molecule can be prematurely displaced; thereby, reducing the effectiveness of the delivery system (18). This factor is of major concern when developing, and particularly while assessing the stability of a formula.

Particulate Systems—Microcapsules, Beads, and Microspheres

Definition/Description. Microcapsules are one of the oldest controlled release technologies. They were developed to produce carbonless carbon paper, and are composed of a core, with the active ingredient surrounded by a shell, analogous to an egg. Microcapsules may have a multilayer construction, with multiple cores containing the active ingredients. The active ingredients are released either by rupture of the capsule walls, or by diffusion/permeation of the contents (1). Fairhurst

and Mitchnick list a range of materials that are typically used in this regard including adhesives, drugs, colors, fragrances, flavors, agricultural chemicals, solvents, and oils. Classic shell materials include gelatin or gum arabic, cellulosic polymers, or synthetic polymers (19). Starch based capsules are often used to deliver fragrance and cosmetic ingredients.

Beads and microspheres are small solid particles onto which other ingredients can be adsorbed for later delivery. Nylon particles, for example, are useful for delivery of certain active ingredients. Antiperspirant salts are said to be more efficacious when delivered via nylon spheres, and the aesthetics of the product are said to be improved; coloring agents may be delivered in this manner as well. Schlossman discloses a patented method (U.S. patent 5,314,683) of coupling cosmetic pigments to microspheres to provide uniform reflectivity, improved dispersion, and superior viscosity characteristics (10). Tokubo et al. describe a process for preparing spherical hectorite particles, with a diameter of about 100 Å, which can be used to deliver glycerin and solid pigments such as titanium dioxide, zinc oxide, and ferric oxide.

Stability Considerations. Microcapsules are somewhat fragile physically, therefore care must be taken to avoid premature rupture and release of the contents. Excessive temperature should be avoided by adding microencapsulated ingredients late in the manufacturing process. Likewise, refrain from formulating with materials that may act as solvents on the capsules walls. Finally, avoid high-shear processing, such as milling and homogenizing, which can physically disrupt the capsules. Additional techniques for enhancing the stability of microcapsules can be found in the technical literature. Fox refers to an interesting Shiseido patent for improving the stability of gelatin microcapsules, i.e., by coating the surface of the capsule with a basic amino acid or its polymer (10). In general, microcapsules are a stable, efficacious method of delivering chemicals in cosmetics. In fact, when properly formulated, microcapsules can actually enhance the stability of systems, by protecting the ingredients they carry, against external forces. For instance, in an example provided by the Mono-Cosmetic Company, ascorbic acid particles are coated with silicone or a polymer, e.g., ethyl cellulose, to protect the ascorbic acid against oxidation (10). Similarly, in delivering cosmetic materials via beads and microspheres, care must be taken not to disturb the matrices physically. As with microcapsules, excessive shear can be a problem, for, if the capsules are broken, their ability to retain the ingredient to be delivered will be impaired.

GENERAL CONSIDERATIONS RELATED TO FORMULA MODIFICATION

Regardless of which delivery technology you choose to utilize in a formulation, there are certain fundamental stability considerations that you must deal with. For each of the technologies discussed above, factors such as raw material sources, manufacturing process, and packaging composition all play a role in product stability.

Raw Material Substitution

Often, it becomes necessary to substitute one raw material for another similar material. This frequently occurs because a supplier discontinues one of the raw materials used in your formula. In exchange, a different, yet supposedly "identical," material may be offered. Depending on the chemistry of the materials involved, there is no way to anticipate if such a change will affect formula stability. Therefore, in such

situations you must conduct a testing, to ensure that your formula will remain stable. Similarly, you may wish to substitute another material that is cheaper but is not anticipated to function differently. For example, in a shampoo formula, you may substitute sodium lauryl sulfate for ammonium lauryl sulfate. Given the functional similarities between the two, you would not anticipate significant problems; nonetheless, some degree of stability testing would be prudent.

Alternate Vendor Qualification

You may also choose to qualify alternate raw material suppliers for the ingredients of the formula. It is desirable to have secondary sources for most raw materials, to ensure a steady supply and competitive pricing. Unfortunately, even though raw materials from different suppliers may have the same Cosmetics, Toiletries, and Fragrance Association (CTFA) designation, they may not be chemically identical, because chemical feedstocks and processing conditions vary between suppliers. Therefore, a raw material from one supplier cannot always be automatically inserted into a formula, which has been developed with a different supplier's raw material. The impact of even seemingly inconsequential change in raw materials must be established by stability testing.*

NONFORMULA-RELATED REASONS

Processing Issues

In addition to the formulation and raw material issues described above, there are processing issues that can affect product stability. For example, stability testing is typically required the first time a new formulation is made on a large scale. This is because, the way in which the product is made on a large scale can have a dramatic effect on its stability. This is particularly true of emulsions, because the energy used in processing determines the particle size and distribution, which helps determine product stability. The only way to fully assess the impact of the chosen manufacturing method on product stability is to evaluate samples made under actual production conditions. This may require a trial production-batch to be made, prior to commercialization of the formula. At the very least, stability testing should be done on the first production-batch of any new product, so that the impact of actual production processing conditions may be evaluated.

Once a manufacturing process has been shown to be successful, any changes to that process may require additional testing. Alterations, in the order of raw material addition, may be necessary to reduce processing time. Changes in heating and cooling rates may occur, due to the differences in heat transfer in large batches; and different mixing conditions will affect the amount of shear the product experiences. Any one of these changes will cause stability problems.

Packaging Issues

Even with the formulation and manufacturing processes held constant, variations in packaging material can cause problems that require stability testing. Not all packages are created equal: glass and plastic behave differently, and different kinds of plastic vary in properties such as oxygen permeability, color fastness, and thermal resistance. Certainly, a new combination of formula and package should be tested,

and even a change in an existing packaging material, or the supplier of that material, merits evaluation. The stability of aerosol systems, for example, is extremely package-dependent, since the package composition will help to determine how resistant the final product is to corrosion. The overall objective is to be alert for changes in the formulation/manufacturing/packaging system that may necessitate additional testing, so that you can be confident that your product will remain stable. Of course, your observations should not be limited to the formula itself. Changes that result from formulation and packaging interaction, may be critical for the total product integrity. For this purpose, the weight loss, changes in plastic color and odor, and other package-related observations are important. The objective is to gain as much knowledge as possible regarding the behavior of the product, over time.

CONCLUSION

This chapter is intended to provide insight into the issues associated with the stability testing of cosmetic products. For the new chemist who is just beginning, we stress the importance of careful, methodical observations, to ensure that as many stability problems as possible are identified. For the veteran formulator, we urge a periodic review of the latest technical literature, so that it will be possible to keep pace with new developments in stabilizing the specific delivery systems, discussed in this book. Hopefully, the references we have provided will be helpful in this regard.

REFERENCES

1. Nacht S. Encapsulation and other topical delivery systems. Cosmet Toilet 1995; 110(9):25–30.
2. Rieger M. Stability testing of macroemulsions. Cosmet Toilet 1991; 106(5):59–66.
3. Jayakrishnan A. Microemulsions: evolving technology for cosmetic applications. J Soc Cosmet Chem 1983; 34:343.
4. Knowlton J, Pearce S. The Handbook of Cosmetic Science and Technology. Oxford, England: Elsevier Advanced Technology, 1993:436–439.
5. Becher P. Emulsions: Theory and Practice. New York: Reinhold, 1965:2.
6. Prince L. Microemulsions: Theory and Practice. New York: Academic Press, 1977:1–2.
7. Fox C. An introduction to multiple emulsions. Cosmet Toilet 1986; 101(11):101–102.
8. Becher P. Encyclopedia of Emulsion Technology. New York: Marcel Dekker, 1983:133–134.
9. Eccleston GM. Application of emulsion stability theories to mobile and semisolid O/W emulsions. Cosmet Toilet 1986; 101(11):73–135.
10. Fox C. Advances in cosmetic science and technology: IV. Cosmetic vehicles. Cosmet Toilet 1995; 110(9):59–68.
11. Uji K et al. J Soc Cosmet Chem Jpn 1993; 27:206–215.
12. Ostro MJ, ed. Liposomes: From Biophysics to Therapeutics. New York: Marcel Dekker, 1987:343.
13. Fox C. Cosmetic raw materials literature and patent review. Cosmet Toilet 1991; 106(8):78.
14. Dalbe B. Use of cyclodextrins in cosmetics. 16th IFSCC Meeting, New York, 1991: 635–639.
15. Kubo S, Fumiaki N. US patent 4,548,811. Shiseido Company Ltd.
16. Oishi T et al. US patent 4,808,189, Hoyu Co.
17. Koch J. US patent 4,352,749.
18. Duchene D. New Trends in Cyclodextrins and Derivatives. Dermal Uses of Cyclodextrins and Derivatives. Paris 1991:473–474.
19. Fairhurst D, Mitchnik M. Submicron encapsulation of organic sunscreens. Cosmet Toilet 1995; 110(9):47.

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Stability Control: Microbiological Tests

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MICROBIOLOGICAL CONTROL OF RAW MATERIALS

Microbial Health Hazards by Contaminated Products

The microbial spoilage of cosmetics has been reported in the literature for many years (1–3). One of the first reported incidents (4) was the death of four babies due to tetanus in New Zealand in the year 1946, caused by using a contaminated talcum powder. The same vector was the source of two other cases of tetanus in an English hospital (5). Since the 1960s, cases of cosmetic-induced infections were described in parallel with the awareness of the same problem for topical drugs (6–12). The isolated organisms were Gram-negative bacteria from the genus *Klebsiella*, *Enterobacter*, *Serratia*, and *Pseudomonas* (13,14). The organism *Pseudomonas aeruginosa*, a particularly virulent hospital pathogen transmitted by eye cosmetics, led to cases of infections and even blindness (15–20), and also folliculitis from sponges (21). Studies were then conducted to evaluate the importance of the problem (22–29), and to investigate the primary contaminating sources such as raw materials, the personnel involved in the process manufacturing water, and packaging, and also the secondary sources such as the consumer (30).

Sources of Contamination

These can be divided into three groups (11,28,31,32):

1. The microbiological quality of raw materials, including water.
2. The manufacturing process.
3. The galenical form (which is made with vegetable and/or animal extracts) of the product.

Microbiological Quality of Raw Materials, Including Water

Their quality depends upon their origin. Raw materials from animal or vegetable origin can be heavily contaminated with 10^6 or even more organisms per gram or milliliter (33–35). Fecal bacteria are regularly identified. In contrast to this, synthetic

raw materials are relatively free from contamination, with an exception for some that have steps in their manufacture, such as kaolin, some sugars and vitamins, some synthetic surfactants [e.g., sodium lauryl ether sulfate (SLES)], or partially hydrated salts. A stubby in our laboratory (Boussard et al., unpublished data) showed that out of 188 different synthetic tested raw materials, only 48, i.e., 25.5% gave results higher than 100 organisms/g or mL. The recovered organisms were bacilli or Gram-positive cocci. A microbiological testing program of the raw materials must be set up.

Water remains one of the most important contamination factors of a product. Species like *Pseudomonas*, *Achromobacter*, *Aeromonas*, or *Flavobacterium* are recovered from natural waters (36). Softening or deionization treatments frequently alter the microbiological water quality. These systems must be well maintained and the water microbiologically treated, using, e.g., ultraviolet (UV) lamps or/and bacterial filtration to ensure optimal quality. Microbiological control of production water should be made at least each working day, and a validation program of the water quality setup.

Manufacturing Process

During the manufacturing process, contamination can occur through contact by the operators, the manufacturing equipment, and air. The microorganisms capable of contaminating a cosmetic from human sources are part of the rhinopharyngeal, buccal skin, hair, skin on the hand, and, in some circumstances, intestinal floras. Among these, fecal *Streptococci*, *Staphylococci*, *Enterobacteria*, and *Pseudomonas* have sufficient vitality to survive, and even to multiply in a product.

The manufacturing equipment is also an important source of contamination, coming from maintenance materials (oils, greases), from poor cleaning and/or disinfection on a regular basis, and from product changeover. The design of the equipment is also participating in this process: a piece of equipment that cannot be totally emptied is a critical factor; the equipment storage conditions must also be optimized to avoid product residues stagnant in the system. The design of cleaning in place (CIP) systems must be carefully evaluated: a CIP that leaves a small quantity of stagnant water along with the diluted product will have a negative effect instead of a beneficial one.

Attention must be paid to the air quality of the manufacturing rooms. The number of workers, and the importance of their movements, contribute to 80% of air contamination (37). Air conditioning contributes to 15% of this contamination, and the room structure (materials used) contributes to 5% of the same. It is thus necessary to fix acceptable levels for the biocontamination of air, and to control the air quality. According to the European Good Manufacturing Practices (GMPs) (38), the limits of the class D rooms should be used (200 organisms/m^3).

Galenical Form of the Product

A parameter of crucial importance in the microbiological stability of a formulation is its water availability, or a_w. This aspect will be discussed at the end of this chapter. Some processes, such as manufacturing at high temperature (e.g., lipsticks), can help to reduce or avoid bacterial contamination. Thus high-risk products are aqueous-based products, such as lotions, suspensions, creams, gels, and emulsions, which contain raw materials of biological origin, and especially when they are manufactured at room temperature.

Establishment of Microbial Limits

For many years there have been discussions on whether the total count of microorganisms would be sufficient to guarantee the microbiological quality of a cosmetic, or if the exclusion of specified microorganisms, pathogens, or potential pathogens would also be required. The current trend is to require quantitative and qualitative microbial limits. Acceptance criteria for cosmetics and control methods would have been issued in the Seventh Amendment of the European Cosmetic Directive. Nevertheless, the acceptance criteria will be the minimal criteria requred to fulfill the public health expectations, such as:

1. *Microbial limits for finished products*. Maximum 1000 organisms/g or mL, and the absence of *Staphylococcus aureus*, *Candida albicans*, enterobacteria, and *Pseudomonas aeruginosa* in one gram or milliliter of the product. Exceptions are baby-care products, eye products, and products for intimate hygiene—maximum 100 organisms/g or mL, and absence of *Staphylococus aureus*, *Candida albicans*, enterobacteria, and *Pseudomonas aeruginosa* in one gram or milliliter of the product.
2. *Microbial limits for raw materials*. Maximum 100 organisms/g or mL, and absence of *Staphylococcus aureus*, *Candida albicans*, enterobacteria, and *Pseudomonas aeruginosa* in one gram or milliliter. Limits for water as raw material could be fixed at maximum 100 organisms/mL and the absence of coliforms and *Pseudomonas aeruginosa* in 100 mL.

However, what must be the attitude of a manufacturer, if one of the following germs is identified in a product: Gram-negative bacilli other than enterobacteria and *Pseudomonas aeruginosa*, *Staphylococci*, other than *Staphylococcus aureus*, or fecal *Streptococci*? What is the significance of this regarding manufacturing hygiene? Are these organisms harmless? Furthermore, in addition to the human safety, it must be emphasized that contamination of products with nonharmful organisms can partially or totally destroy the product aesthetic (e.g., perfume, color), and can alter the product performance. The rise of these questions emphasizes the need of internal quantitative and qualitative microbiological safety margins and of a quality-assurance system.

Use of Validated Methods to Control Products and Water

Microbiological Control of Finished Products and Raw Materials

The method described here is based on the method for microbiological analysis of nonsterile pharmaceuticals in the 3rd edition of the European Pharmacopeia (39,40), and from a publication of a working party of the "Federation Internationale Pharmaceutique" (41).

Sample Preparation. A 10% homogeneous solution or suspension of the product is prepared with a sterile neutralizing solution or a sterile buffered peptone saline solution at pH 7. The neutralizing solution is used, in case of the presence of known or suspected antimicrobial substances in the product. The pH 7 solution is used in case of preservative-free raw materials. For nonsoluble products, 0.1% of tween 80 or heating, at a temperature not higher than 40°C for half an hour maximum, can help in the homogenization. The neutralizing solution is basically letheen broth (Difco) supplemented with various inhibitors of the preservatives or disinfectants. The 10% homogenate is then used to perform the bacterial and fungal counts, and to investigate the presence of specified microorganisms. If, for technical reasons,

the use of 10 g sample is not possible, 5, 2.5, or even 1 g can be mixed for a total suspension of 100 mL.

Validation of the Preservative's Inactivation. The efficacy of the neutralizing solution must be validated, in order to avoid false-negative results. For this purpose, 1 mL of the preserved sample or 1 mL sterile normal saline is added to 9 mL neutralizing solution. The two tubes are mixed well and let to rest for 10 minutes. Next, 0.1 mL of a mixed suspension of *Staphylococcus aureus* ATCC 6538 and *Pseudomonas aeruginosa* ATCC 9027 at 10^4 bacteria/mL are then added to the tubes, which are mixed again. The colony-forming units in each tube are estimated. The difference in the results must be lower than the 1/2 log between the tubes.

Bacterial and Fungal Counts. From the 10% homogenate, an appropriate number of successive tenfold dilutions in the sterile buffered peptone saline at pH 7 are carried out. A plate-count is then made by transferring duplicates of 1 mL of the dilutions in sterile Petri dishes, followed by the addition of 15 mL melted agar. Tryptic soy agar (TSA) is used for the bacteria and Sabouraud Chloramphenicol agar for yeast and moulds. For the bacterial counts the dishes are incubated at 30°C to 35°C for five days, and for the yeast and moulds, 20°C to 25°C for five to seven days. The Petri dishes used for the fungal counts are also used, to check the presence of *Candida albicans*.

Investigations for the Presence of Specific Microorganisms.

1. Enterobacteria and other gram-negative organisms. One milliliter or 1 g of the 10% homogenate is mixed with 100 mL enterobacteria enrichment broth (EEB), and incubated at 35°C to 37°C for 24 hours to 48 hours. Subcultures are then carried out on violet red bile dextrose (VRBG) agar dishes and incubated at 35°C to 37°C for 18 hours to 24 hours. The colonies of presumptive Gram-negative organisms are then identified.
2. *Escherichia coli*. One milliliter or 1g of the 10% homogenate is mixed with 100 mL of Mac Conkey broth and incubated at 43°C to 45°C for 18 hours to 24 hours. Subcultures are carried out on Mac Conkey agar dishes incubated at 43°C to 45°C for 18 hours to 24 hours. The colonies of lactose-fermenting gram-negative organisms are then identified.
3. *Pseudomonas aeruginosa* and other gram-negative organisms growing on Cetrimide agar. One milliliter or 1g of the 10% homogenate is mixed with 100 mL tryptic soy broth (TSB) and incubated at 35°C to 37°C for 24 hours to 48 hours. Subcultures are carried out on Cetrimide agar dishes incubated at 35°C to 37°C for 18 hours to 24 hours. The colonies are then identified.
4. *Staphylococcus aureus*. One milliliter or 1g of the 10% homogenate is mixed with 100 mL TSB and incubated at 35°C to 37°C for 24 hours to 48 hours. Subcultures are carried out on Baird Parker agar dishes, incubated at 35°C to 37°C for 18 hours to 24 hours. The black colonies are then identified.

Validation of the Sterility of the Media. Sterility of all the media must be checked. For example, sterile saline is used instead of the sample, and the bacterial counts and the appropriate investigations for specific organisms are performed. No microbial growth must be recorded in this assay.

Validation of the Growth-Promoting Properties of the Selective Media. The following reference strains are incubated separately in TSB at 30°C to 35°C for 18 hours to 24 hours: *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, and *Escherichia coli* ATCC 8739. Each bacterial suspension is diluted

to obtain around 1000 organisms/mL. The three suspensions are equally mixed together, and 0.3 mL of the mixture (containing about 100 organisms of each strain) are used as the inoculum to perform the investigations for the specific microorganisms. The organisms must be detected in the media used for this assay.

Microbiological Control of Water

The microbiological quality of water is of particular importance and can be checked quantitatively and qualitatively. For the quantitative determinations of a potential water contamination, 100 mL or 10 mL of water are filtered through bacteriological filters (porosity of 0.45 μm). After filtration, the filters are deposited on the surface of TSA Petri dishes. An amount of 1 mL and 0.1 mL of water are also incorporated in two melted TSA, for a plate-count in duplicate. All the dishes are incubated at 30°C to 35°C for three days to five days. For the qualitative determinations, 100 mL of water are filtered through 0.45 μm sterile filters. The filters are laid down on sterile Mac Conkey Petri dishes for the coliform bacteria, and on Cetrimide agar Petri dishes for *Pseudomonas*. These are incubated at 30°C to 35°C for three to five days. Questionable colonies are identified.

CHALLENGE TEST FOR THE EFFICACY OF PRESERVATION

Aim of Preservation

It is generally accepted that adequate preservation of a finished product, with preservatives or on the basis of active preservation of a formulation, implies that the product remains stable and safe during storage (shelf-life) and consumer use (1,42–46). From a public-health point of view, preservation must avoid infection of the consumer, and for product-quality reasons, it must prevent the deterioration of a preparation. It is especially important to point out that the use of preservatives must not mask a lack of hygiene during manufacture. It is thus imperious to manufacture any cosmetic product according to the GMPs (34), as imposed by the 6th Amendment of the European Directive 76/768/CEE (47), and to reach, at the end of the manufacture, the microbiological quality level discussed earlier in this chapter. Furthermore, the challenge test to evaluate the efficacy of preservation must not be simply performed on a lot-per-lot basis. The test has to be essentially connected with each development phase of the preparation (48). It must be as simplified as possible for routine use, easy to standardize, and reproducible. Moreover, the test method must be able to show the potential intrinsic antibacterial efficacy of a formulation, and should thus be performed on each finished product in its intact original container as well. Indeed, changes in the composition of a preparation have a tremendous influence on preservation (49,50). Even minor changes in perfumes or dyes can affect the global behavior of the product (2,51,52). Moreover, the material of the container and its type of closure influences the efficacy of the preservation and the protection of the product during use (45,53–55). Rubber closures are, for example, known to absorb some amount of preservative from a solution (56,57). Shave foams are often presented in containers under pressure, with a propeller gaze such as butane. These storage conditions can widely influence the survival of some aerobic contaminants. Moreover, refrigeration can alter the preservative's efficacy (58). The preservatives may be inactivated by the components of the product (59).

Activity Spectrum of a Preservative

The use of the word ‘‘antimicrobial’’ preservative raises the need to define exactly what kind of activity is needed for a preservative. What are the organisms of concern: bacteria, fungi, viruses, or even spores? The scale of the activity spectrum is based on almost three parameters: (1) the survival, or even multiplication, of particular organisms in a wide range of products; (2) the pathogenicity of these organisms by the route of administration; and (3) the possibility to find effective chemicals at nontoxic concentrations.

Sporicidal action must not be considered because sporicidal chemicals are very rare (e.g., aldehydes are too toxic to be used in a cosmetic product at effective concentrations). Moreover, infectious problems induced by spore formers are very seldom, as seen in the previously discussed talcum powder case in this chapter. Even if aerobic spore formers are often found in raw materials and finished products, according to Davis (13), they should not be a hazard to human health.

Virucidal action is not considered for cosmetics. These facts restrict the spectrum of a cosmetic preservative to bacteria and fungi. According to the most widespread opinion, a bactericidal and a fungicidal effect is needed so that the contaminating organisms accidentally introduced in the preparation will be killed. A bacteriostatic or fungistatic action could eventually be accepted to stabilize a preparation during the shelf-life of a unidose, nonsterile product. For the fungicidal and bactericidal actions, the concentration of the preservative must be toxicologically acceptable.

Test Organisms

As previously discussed, the range of organisms must contain bacteria and fungi. Within these, we must find Gram-positive and Gram-negative bacteria because the structure of the bacterial wall influences the penetration, and thus the efficacy of the preservating agent. For the fungi, representatives of the two fungal forms, namely the vegetative yeast cell and the mould spore, must be used. The choice of species is directed by their skin and mucosal pathogenicity. Product degradation capabilities are also taken into account to choose the species. So among the Gram-positive species, *Staphylococcus aureus* is an important skin pathogen, as is *Pseudomonas aeruginosa* among the Gram-negative bacteria. The latter organism is also able to use many compounds, such as preservatives or even disinfectants, as a carbon source and is very adaptative in adverse environmental conditions, even in pure water (60,61). For yeast, *Candida albicans* is a skin pathogen, and *Aspergillus niger* is a representative of the degradation flora. The choice of strains for a standardized assay must be guided by the need to compare results obtained in different laboratories, and in this way culture-collection strains are chosen, from the American Type Culture Collection (ATCC). The strains normally used are as follows: (1) *Staphylococcus aureus* ATCC 6538, (2) *Pseudomonas aeruginosa* ATCC 9027, (3) *Candida albicans* ATCC 10231, and (4) *Aspergillus niger* ATCC 16404. These strains are to some extent resistant to the antimicrobials, and some are also used for testing disinfectants or antibiotics. For a representative preservation-efficacy test, it is also recommended to add strains isolated from the environment, water, or contaminated products. These strains living in the vicinity of, or even inside the product, are well adapted to adverse conditions, and are often resistant to preservatives or even disinfectants (62–64). Nevertheless, after a few passages in culture media, this particular resistance can disappear. Precautions must be taken to avoid this by immediate storage in appropriate medium by deep freezing or in liquid nitrogen.

Test Conditions and Validations

The challenge test consists of an artificial contamination of the tested sample and counting of the survivors during a period of four weeks, maximum. Even if several preservative efficacy tests exist as described in the USP23 (65), the Japanese Pharmacopeia (66), or the CTFA test (67–74), the general conditions of the test described here are those of the European Pharmacopeia (43), adapted from a Federation Internationale Pharmaceutique (FIP) working party publication (44). Several points, such as validations and strain maintenance, are described here in more detail.

Maintenance of Microbial Strains

The cultures can be maintained as described in the CEN 216 PrEN 12353 document (75). Stock cultures are maintained at a temperature below −18°C. To prepare the working culture, subcultures are originated from the culture stock by streaking onto adequate agar medium slopes. The second and/or third subcultures can be used as the working cultures.

Preparation of the Inoculum

The subcultures to be used in the test are plated on Petri dishes of suitable media, e.g., TSA for the bacteria and sabouraud dextrose agar (SDA) for the fungi. After adequate incubation—18 hours to 24 hours for the bacteria, 48 hours for the yeast, and three to five days for the mould—the cultures are collected with sterile, normal saline. The suspensions are then calibrated against a Mac Farland scale or by using any suitable calibration system. This calibrated suspension, homogenized at a maximum ratio of 1:100 (0.2 mL in 20 μg or mL, for example) of the tested sample must give between 5.10^5 and 5.10^6 organisms/mL or g. Such a high inoculum density is imposed, not only by the counting technique of the survivors or the "plate-count," but also by the importance of the logarithmic reduction asked for the products.

Test Conditions

The first day of the challenge test, the product and two controls—one comprising the tested product without preservatives, and one having normal saline with 1% peptone—are inoculated with each microbial strain. A microbial count is immediately performed after homogenization of this group of three vials. Counts are performed after the dilution of 1 g or mL of the sample, with 9 mL of neutralizer. The neutralizing solution used is the same as in the first part of this chapter. Further dilutions are made in normal saline, in order to perform a plate-count technique, according to the estimate contamination. Sampling is performed in the same way for the preserved samples, after 2, 7, 14, and 28 days of storage of the inoculated product, kept at room temperature in the dark or in its normal storage conditions. To estimate the 100% starting value, the product effect must be evaluated on the inoculum. So, the inoculum level is estimated in a nonpreserved test product, if available, and compared with the level measured into normal saline containing 1% of peptone. If the following occur: (1) data obtained in the nonpreserved product are equivalent to those obtained in saline, and this value is chosen as the starting level (100%); (2) the data obtained in the product is less than or equal to 1 log from the saline data, the value obtained in the saline control is chosen as the starting value; and (3) if the product data are less than 1 log from the saline control, this is an indication of

product contamination and the test is invalid. The results of the test are expressed as logarithmic reduction versus time of the value taken as 100%.

Validation of the Contamination of the Sample

The contamination of the sample consists of a homogeneous incorporation of a single strain at a maximum ratio of 1% of calibrated suspension into the sample. Most of the time, the inoculum is aqueous and dispersed in an aqueous phase; for some products, addition of tween 80 ou isopropylic myristate could be useful to homogenize the inoculum. In some cases, a dried inoculum suspended in isododecane is used to contaminate fatty products. It is indispensable to ascertain that the inoculum can homogeneously be dispersed through the product. This is nearly immediate for liquids, but much more difficult for oily products such as creams or mascaras. A validation is thus performed using a nonpreserved product that is inoculated with the calibrated suspension and homogenized. At least three different samples are taken from the product, and the results of the counts obtained for these samples are compared. The difference between samples must be less than 1 log.

Validation of the Neutralizing Solution

Since a neutralizing solution is used as first dilutant when counting the survivors, the efficacy of the neutralizing solution must be validated in order to avoid false-negative results. For this purpose, 1 mL of the preserved sample or 1 mL sterile normal saline are added to 9 mL of the neutralizing solution. The two tubes are well mixed and let at rest for 10 minutes. The calibrated suspension of about 0.1 mL with a dilution of 10^{-3} is then added and mixed to both tubes. The colony-forming units in each tube are estimated, and the difference in results between the tubes must be less than 1 log.

Interpretation of the Results

The criteria taken by the European Pharmacopeia for the topically applied product are a good base of discussion (43). For bacteria, the recommended criteria (level A) are a 2 log reduction after two days, 3 log after seven days, and no increase in the recovered bacteria after 28 days. For fungi, a 2 log reduction is requested after 14 days with no increase of the counts after 28 days. This requirement of "no increase" of the counts at the end of the test period is of particular importance. Indeed, even if the logarithmic reduction attained by a product is greater than the requirement, a regrowth of the organisms during the examination period is unacceptable. This would indicate that the microorganisms are able to adapt their metabolic capacities to use the product and its preservative, in particular, as the carbon source. In the European Pharmacopeia, it is also stated that, in justified cases, e.g., when adverse reactions could occur, level B criteria can be used to interpret the results. These are: for bacteria, a 3 log reduction after 14 days and no increase of the counts after 28 days; and for fungi, 1 log reduction after 14 days and no increase of the counts after 28 days.

DETERMINATION OF WATER AVAILABILITY OR A_W

Water availability (a_w) is defined as the water available for bacterial metabolism and is evaluated by measurement of the water vapor pressure at the surface of a product

or a raw material. It can be defined as the following ratio:

$$A_w = \text{water vapor pressure over substance at } t^\circ/\text{water vapor pressure over pure water at } t^\circ$$

It depends on the temperature and formulations. It is not correlated with the total water content of a formula, but depends on the quantity of water "trapped" into the formula chemicals. Ingredients such as humectants, gums, or others use the water to swell; therefore, this water is no longer available for bacterial growth. As water is a critical growth factor for microorganisms, the means to preserve a formula is to decrease the level of water availability, or optimizing a formula by the inclusion of ingredients that fix the water. Most *Pseudomonas* cannot grow if the a_w is less than 90%; and under 70%, the probability of microorganism growth in the product is lowered (76).

The a_w of a product is evaluated through the use of a moisture-sensing device that measures the head space relative humidity above the product's surface, contained in a closed jar or dish, after equilibration. This device must first be calibrated, using calibration standards. The standards are selected to represent low, medium, and high value operation or to bracket the area of interest. In general, the standards are saturated salt solutions such as NaCl ($a_w = 0.75$), $BaCl_2$ ($a_w = 0.90$), and LiCl ($a_w = 0.11$). As the a_w measurement is temperature dependent, it is recommended to perform the calibrations and measures at controlled room temperature. Table of commonly used standards and their temperature variations can be found in the reference (77).

CULTURE MEDIA, NEUTRALIZING SOLUTION, AND BUFFERS

Table 1–12

Table 1 Sterile Neutralizing Solution

Lecithin	4.0 g
Polysorbate 80	30.0 g
Peptamin	10.0 g
Beef extract	5.0 g
Histidine	1.0 g
Sodium laurylsulfate	4.0 g
Sodium chloride	5.0 g
Distilled water	1000 mL

Table 2 Sterile Buffered Peptone Saline at pH 7

Monopotassium phosphate	3.56 g
Dihydrated disodium phosphate	7.23 g (equivalent to 0.067 M)
Sodium chloride	4.30 g
Meat or casein peptone	1.0 g
Purified water	1000 mL

Note: 1 g/L or 10 g/L of polysorbate 20 or 80 can be added to the solution. Sterilize in the autoclave at 121°C for 15 minutes.

Table 3 Tryptic Soy Agar

Tryptone	15.0 g
Soya peptone	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Purified water	1000 mL

Note: Sterilize in the autoclave at 121°C for 15 minutes. pH must be 7.3 ± 0.2.

Table 4 Sabouraud Chloramphenicol Agar

Meat and casein peptone	10 g
Dextrose	40 g
Chloramphenicol	0.05 g
Agar	15 g
Purified water	1000 mL

Note: Sterilize in the autoclave at 121°C for 15 minutes.

Table 5 Enterobacteria Enrichment Broth (EEB Mossel)

Tryptose	10.0 g
Dextrose	5.0 g
Disodium phosphate	8.0 g
Monopotassium phosphate	2.0 g
Oxgall	20.0 g
Brilliant green	0.0135 g
Purified water	1000 mL

Note: Heat to 100°C for 30 minutes, cool immediately. pH 7.2 ± 0.2.

Table 6 Agar with Crystal Violet, Neutral Red, Bile Salts, and VRBG Agar with Glucose

Yeast extract	3.0 g
Peptone	7.0 g
Bile salts	1.5 g
Lactose	10.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Neutral red	0.03 g
Crystal violet	0.002 g
Dextrose	10.0 g
Purified water	1000 mL

Note: Heat to boil, do not autoclave. pH 7.4 ± 0.2.

Table 7 Mac Conkey Broth

Peptone	20.0 g
Lactose	10.0 g
Oxgall	5.0 g
Brom cresol purple	0.01 g
Purified water	1000 mL

Note: Sterilize by autoclave at 121°C for 15 minutes. pH 7.3 ± 0.2

Table 8 Mac Conkey Agar

Casein peptone	17.0 g
Meat peptone	3.0 g
Lactose	10.0 g
Sodium chloride	5.0 g
Bile salts	1.5 g
Agar	13.5 g
Neutral red	0.03 g
Crystal violet	0.001 g
Purified water	1000 mL

Note: Sterilize by autoclave at 121°C for 15 minutes. pH 7.1 ± 0.2.

Table 9 Tryptic Soy Broth

Casein peptone	17.0 g
Soja peptone	3.0 g
Sodium chloride	5.0 g
Dipotassium phosphate	2.5 g
Dextrose	2.5 g
Purified water	1000 mL

Note: Sterilize by autoclave at 121°C for 15 minutes. pH 7.3 ± 0.2.

Table 10 Cetrimide Agar

Peptone	20.0 g
Magnesium chloride	1.4 g
Dipotassium sulfate	10.0 g
Cetrimide	0.3 g
Agar	13.6 g
Glycerol	10.0 mL
Purified water	1000 mL

Note: Sterilize by autoclave at 121°C for 15 minutes. pH 7.2 ± 0.2.

Table 11 Baird Parker Agar

Peptone	20.0 g
Beef meat extract	5.0 g
Yeast extract	1.0 g
Lithium chloride	5.0 g
Agar	20.0 g
Glycine	12.0 g
Sodium pyruvate	10.0 g

Note: Sterilize by autoclave at 121°C for 15 minutes. Cool to 45–50°C and add 10 mL of a sterile potassium tellurite solution at 10 g/L and 50 mL of an egg yolk emulsion.

Table 12 Sabouraud Dextrose Agar

Peptone	10.0 g
Dextrose	40.0 g
Agar	15.0 g

Note: Sterilize by autoclave at 121°C for 15 minutes.

REFERENCES

1. Hugo WB. Antimicrobial agents as preservatives in pharmaceutical and cosmetic products. Introduction: the scope of the problem. J Appl Bacteriol 1978; 44:Siii–Sv.
2. Orth DS, Lutes CM. Adaptation of bacteria to cosmetic preservatives. Cosm Toil 1985; 100(2):57–59.
3. Anelich LE, Korsten L. Survey of micro-organism associated with spoilage of cosmetic creams manufactured in South Africa. Int J Cosm Science 1996; 18:25–40.
4. Tremewan HC. *Tetanus neonatorium* in New Zealand. N Z Med J 1946; 45:312–313.
5. Sevitt S. Source of two hospital infected case of tetanus. Lancet 1949; 2:1075–1077.
6. Noble WC, Savin JA. Steroid cream contaminated with *Pseudomonas aeruginosa*. Lancet 1966; 1:347–349.
7. Morse LJ, Williams HL, Grenn FP Jr, Eldridge EE, Rptta JR. Septicemia due to *Klebsiella pneumoniae* originating from a hand cream dispenser. New Engl J Med 1967; 277:472–473.
8. Morse LJ, Schonbeck LE. Hand lotions a potential nosocomial hazard. New Engl J Med 1968; 278:364–369.
9. Wilson LA, Keuhne JW, Hall SW, Ahearn DGH. Microbial contamination in ocular cosmetics. Am J Ophtalmol 1971; 71:1298–1302.
10. Smart R, Spooner DF. Microbiological spoilage in pharmaceuticals and cosmetics. J Soc Cosmet Chem 1972; 23:721–737.
11. Dony J. Problemes microbiologiques poses par les cosmetiques. J Pharm Belg 1975; 30:223–238.
12. Wilson LA, Julian AJ, Ahearn DG. The survival and growth of microorganisms in mascara during use. Am J Ophthalmol 1975; 79:596–601.
13. Davis JG. The microbial stability of cosmetics and toilet preparations. Soap Perfum Cosmet 1973; 46:409–418.
14. Goldman CL. Microorganisms isolated from cosmetics. D&CI July 1975:40–41.

15. Marzulli FN, Evans JR, Yoder PD. Induced pseudomonas keratitis as related to cosmetics. J Soc Cosmet Chem 1972; 23:89–97.
16. Chowchuvech E, Sawicki L, Tenenbaum S, Galin MA. Effect of various microorganisms found in cosmetics on the normal and injured eye of the rabbit. Am J Ophthalmol 1973; 75:1004–1009.
17. Wilson LA, Ahearn DG. *Pseudomonas* induced corneal ulcers associated with contaminated eye mascaras. Am J Ophthalmol 1977; 54:112–119.
18. Ahearn DG, Sanghvi J, Haller GJ, Wilson LA. Mascara contamination: in use laboratory studies. J Soc Cosmet Chem 1978; 29:127–131.
19. Reid FR, Wood TO. Pseudomonas corneal ulcer: the causative role of eye cosmetics. Arch Ophthalmol 1979; 97:1640–1641.
20. Brannan DK. Cosmetic preservation. Cosmet Toiletries 1996; 111:69–83.
21. Frenkel LM. *Pseudomonas* folliculitis from sponges promoted as beauty aids. J Clin Microbiol 1993; 31:2838.
22. Jarvis B, Reynolds AJ, Rhodes AC, Armstrong M. A survey of microbiological contamination in cosmetics and toiletries in the UK (1971). J Soc Cosmet Chem 1974; 25: 563–575.
23. Dawson NL, Reinhardt DJ. Microbial flora of in-use, display eye shadow testers and bacterial challenges of unused eye shadows. Appl Environ Microbiol 1981; 42:297–302.
24. Economou-Stamatelopoulou C, Chitiroglou-Lada A, Papavassiliou J. Contamination microbienne de savons. Pharm Acta Helv 1982; 57:298–300.
25. Baird RM. Bacteriological contamination of products used for skin care in babies. Int J Cosmet Sci 1984; 6:85–90.
26. Ashour MSE, Hefani H, El-Tayeb OM, Abdelaziz AA. Microbial contamination of cosmetics and personal care items in Egypt 1. Cosmet Toiletries 1987; 102:61–68.
27. Abdelaziz AA, Alkofahi A. Microbiological profile of selected samples of (Al-Kohl) eye cosmetics in northern Jordanian provinces before and after use. Zentralbl Bakteriol Mikrobiol Hyg B 1989; 187:244–253.
28. Dony J, Devleeschouwer MJ. Contamination microbienne des produits bruts d'origine vegetale: incidence pour les preparations cosmetiques. J Pharm Belg 1989; 44:411–419.
29. Misclivec PB, Bandler R, Allen G. Incidence of fungi in shared-use cosmetics available in the public. J AOAC Int 1993; 76:430–436.
30. Hingst V. The importance of contaminated dental care commodities results of field research. Zentralbl Bakteriol Mikrobiol Hyg B 1989; 187:337–364.
31. FIP 1972. Purete microbiologique des formes pharmaceutiques non obligatoirement steriles. Rapport commun du Comite des laboratoireset des services officiels de controle des medicaments et de la section des pharmaciens de l'industrie de la FIP. J Mond Pharm 1972; 15:88–100.
32. Devleeschouwer MJ. Flore Microbienne Des Medicaments. Especes Opportunistes Et Antibioresistance. Ph.D. thesis, Universite Libre de Bruxelles, Bruxelles, Belgium, 1980.
33. Schiller I, Kuntscher H, Wolff A, Nekola M. Microbial content of nonsterile therapeutic agents containing natural or seminatural active ingredients. Appl Microbiol 1968; 16:1924–1928.
34. Pedersen EA, Ulrich K. Microbial contents in nonsterile pharmaceuticals III raw materials. Dansk Tideskr Farm 1968; 42:71–83.
35. Steinberg D. Botanical extracts and preservation issues. Cosmet Toiletries 1991; 106: 73–74.
36. Wallhausser KH. Sterilisation-Desinfektion-Konservierung-Keimidentifizierung-Betriebshygiene. Stuttgart: Georg Thieme Verlag, 1978.
37. Agnew B. The Laminar Flow Clean Room Handbook. 3rd. California: Agnews Higgins, 1968.
38. European Commission, Directorate General III, working party on (control of medicines and inspections). Revision of the annex 1 of the EU guide to Good Manufacturing Practice. Manufacturing of sterile medicinal products, 1 January 1997.

39. Controle de la contamination microbienne dans des produits non obligatoirement steriles, denombrement des germes viables totaux; 2.6.12. Pharmacopee europeenne 3d ed., Conseil de l'Europe Strasbourg, 1997:83–87.
40. Controle de la contamination microbienne dans des produits non obligatoirement steriles, recherche des microorganismes specifies. Pharmacopee europeenne 3d ed., Conseil de l'Europe, Strasbourg, 1997:87–89.
41. FIP 1975. Purete microbiologique des formes pharmaceutiques non obligatoirement steriles: methodes d'examen. 2eme rapport commun du Comite des laboratoires et Services Officiels—de controle des medicaments et de la section des Pharmaciens de rindustrie. Pharm Acta Helv 1976; 51:33–40.
42. Baird RM. The occurrence of pathogens in cosmetics and toiletries. J Soc Cosm Chem 1977; 28:17–20.
43. Efficacite de la conservation antimicrobienne 5.1.3. Pharmacopee europeenne 3d ed., Conseil de l'Europe Strasbourg, 1997:296–298.
44. FIP 1980. Essai d'efficacite de la conservation antimicrobienne des preparations pharmaceutiques. 3eme rapport commun du Comite des Laboratories et Services offciels de cobntroledes medicaments et de la section des pharmaciens de l'industrie de la FIP. Pharm Acta Helv 1980; 55:40–49.
45. FIP 1984. The test for efficacy of antimicrobial preservatives of Pharmaceuticals. In: Kabara JJ, ed. 3rd joint report of the Committee of Official laboratories and drug control services and the section of industrial pharmacists. FIP. New York: Marcel Dekker, 1984:423–440.
46. Lorenzoitti OJ. Kabara JJ, ed. A Preservative Evaluation Program For Dermatological And Cosmetic Preparation. New York: Marcel Dekker, 1984:441–463.
47. 6th amendment (93/35/EEC) of the Council Directive of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products (76/768/EEC). Official Journal n°L 151, June 23, 1993.
48. Moore KE. Evaluating preservative efficacy by challenge testing during the development stage of pharmaceutical products. J Appl Bacteriol 1978; 44:SXLIII–SLV.
49. Wan LS, Kurup TRR, Chan LW. Partition of preservatives in oil/water systems. Pharm Acta Helv 1986; 61:308–313.
50. Kurup TR, Wan LSC, Chan LW. Availability and activity of preservatives in emulsified systems. Pharm Acta Helv 1991; 66:76–82.
51. Sakamoto T, Yanagi M, Fukushima S, Mitsui T. Effect of some cosmetic pigments on the bactericidal activities of preservatives. J Soc Cosm Chem 1987; 38:83–98.
52. Steinberg DC. Preserving foundations. Cosmet Toiletries 1995; 110:71–74.
53. McCarthy TJ. Interaction between aqueous preservative solutions and their plastic containers. Pharm Weekbld 1970; 105:557–563, 1139–1146.
54. Melichar M, Podstatova H, Pokorny J, Hybasek P, Pokorna M. Mikrobiologische reinheit der arzneizubereitungen Teil 1: Externa: der einfluss von cremetyp, behalter, aufbewahrung, temperatur und applikation. Pharmazie 1980; 35:484–488.
55. Brannan DK, Dille JC. Type of closure prevents microbial contamination of cosmetics during consumer use. Appl Environ Microbiol 1990; 56:1476–1479.
56. Lachamn L, Weinstein S, Hopkins G, Slack S, Eisman P, Cooper J. Stability of antibacterial preservatives in parenteral solutions I. Factors influencing the loss of antimicrobial agents from solutions in rubber-stopped containers. J Pharm Sci 1962; 51:224–232.
57. Lachman L, Urbanyl T, Weinstein S. Stability of antibacterial preservatives in parenteral solutions IV. Contribution of rubber closure composition on preservative loss. J Pharm Sci 1963; 52:244–249.
58. Lehmann CR. Effect on refrigeration on bactericidal activity of four preserved multiple-dose injectable drug products. Am J Hosp Pharm 1977; 34:1196–1200.
59. Grigo J. Microorganisms in drugs and cosmetics—occurrence, harms and consequences in hygienic manufacturing. Zentralbl Bakteriol [Orig B] 1976; 162:233–287.

60. Yanagi M, Onishi G. Assimilation of selected cosmetic ingredients by microorganisms. J Soc Cosmet Chem 1971; 22:851–865.
61. Levy E. Insights into microbial adaptation to cosmetic and pharmaceutical products. Cosmet Toiletries 1987; 102:69–74.
62. Decicco BT, Lee EC, Sorrentino JV. Factors affecting survival of *Pseudomonas cepacia* in decongestant nasal sprays containing thimerosal as preservative. J Pharm Sci 1982; 71:1231–1234.
63. Bosi C, Davin-Regli A, Charrel R, Rocca B, Monnet D, Bollet C. *Serratia marcescens* nosocomial outbreak due to contaminated hexetidine solution. J Hosp Infect 1996; 33:217–224.
64. Zani F, Minutello A, Maggi L, Santi P, Mazza P. Evaluation of preservative efficacy in pharmaceutical products: the use of a wild strain of *Pseudomonas cepacia*. J Appl Microbiol 1997; 83:322–326.
65. Antimicrobial preservatives efficacy. (51), United States Pharmacopeia 23, United States Pharmacopeial Convention, Rockwell, MD, 1994: 1681.
66. Preservatives—efficacy test. Japanese Pharmacopeial Forums, 1995; 4:664–668.
67. McEwen GN, Curry AS. Determination of the adequacy of preservation testing of aqueous liquid and semi-liquid eye cosmetics (1975). Cosmetic Toiletry and Fragance Association Guidelines, Washington, D.C.: CFFA, 1983.
68. Brannan DK, Dille JC, Kaufman DJ. Correlation of in vitro challenge testing with consumer use testing for cosmetic products. Appl Environ Microbiol 1987; 53:1827–1832.
69. Connolly P, Bloomfield SF, Benyer SP. A study of the use of rapid methods for preservative efficacy testing of pharmaceuticals and cosmetics. J Appl Bacteriol 1993; 75:456–462.
70. Connolly P, Bloomfield SF, Denyer SP. The use of impedance for preservative efficacy testing of pharmaceuticals and cosmetic products. J Appl Bacteriol 1994; 76:68–74.
71. Farrington JK, Martz EL, Wells SJ, Ennis CC, Holder J, Levchuk JW, Avis KE, Hoffman PS, Hitchins AD, Madden JM. Ability of laboratory methods to predict in-use efficacy of antimicrobial preservatives in an experimental cosmetic. Appl Environ Microbiol 1994; 60:4553–4558.
72. Hodges NA, Denyer SP, Hanlon GW, Reynolds JP. Preservative efficacy tests in formulated nasal products: reproducibility and factors affecting preservative activity. J Pharm Pharmacol 1996; 48:1237–1242.
73. Lenczewski ME, McGavin ST, Vandyke K. Comparison of automated and traditional minimum inhibitory concentration procedures for microbiological cosmetic preservatives. J AOAC Int 1996; 79:1294–1299.
74. Lenczewski ME, Kananen LL. Automated screening method for determining optimum preservative systems for personal and home care products. J AOAC Int 1998; 81: 534–539.
75. PrEN12353. Chemical disinfectants and antiseptics. Preservation of microbial strains used for the determination of bactericidal and fungicidal activity. CEN/TC 216 HWG N114, 18/02/1998.
76. Legenhausen R. Water activity measurements. Microbiological quality of water-based product. Center for Professional Advancement Course, Amsterdam, 1989.
77. Greenspan M. Humidity fixed points of binary saturated aqueous solutions. J Res Nat Bureau Standards 1977; 81a:89–96.

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In Vitro Tests for Skin Irritation

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INTRODUCTION

The manufacture, transport, and marketing of chemicals and finished products require the prior toxicological evaluation and assessment of skin corrosion and skin irritation that might result from intended or accidental skin exposure. Traditionally, animal testing procedures have provided the data needed to assess the more severe forms of skin toxicity, an assessment requiring extrapolation of the data from the animals to humans (1). Various regulatory authorities have generally required animal test data before permission would be granted for the manufacture, transport, or marketing of chemicals (2) or the formulations that contain them (3).

In recent years, animal testing for dermatotoxic effects has come under increasing scrutiny and criticism from various sources as inhumane and unnecessary. The recently approved seventh amendment to the European Union's Cosmetic Directive bans animal testing after 2009 and the sale of products tested on animals or containing ingredients tested on animals, if such testing is conducted after the initiation of the testing ban (4). Provisions for delaying implementation of this ban are included, if suitable alternative methods are not available. The often conflicting needs to protect worker and consumer safety, comply with regulatory statutes, and reduce animal testing procedures have led to a significant effort within industry, government, and academia to develop alternative testing methods for assessing the skin corrosion and irritation hazard of chemicals and product formulations without reliance on animal test procedures (5).

A recent example for which regulatory requirements have been coupled to the pressing need for alternative methods development is in the evaluation of skin corrosion. United States and international regulations require that chemicals be properly classified, labeled, packaged, and transported on the basis of their potential to damage or destroy tissue, including the speed with which such tissue destructive reactions occur (6). The most common animal testing methods used over the years for the evaluation of chemical corrosion potential are all based on the original method of Draize et al. (7). Various laboratories have been active in the development of alternative procedures for skin corrosion testing (8–11). Several years ago, multiple test methods were evaluated in an international validation program (12). Certain

of these methods provided short-term and cost-effective alternatives to the Draize procedure and also provided experimental systems for developing a better mechanistic understanding of the process of skin corrosion (10).

Skin irritation, by definition, is a less severe response than corrosion, but can span a range of responses from near corrosive to weak cumulative or neurosensory responses. The development of alternatives for skin irritation testing has lagged behind that of skin corrosion testing, likely because of both the greater urgency of developing alternatives for the testing of more severe skin responses and the range of responses encompassed within the "skin irritation" umbrella. Currently, the irritation hazard potential of chemicals is often determined through use of the same Draize procedure used for corrosion testing, the difference being mainly in the duration of chemical exposure, with results used to determine labeling requirements for chemicals and products according to EC directives (2,3). For noncorrosive chemicals, there has been a recent effort to develop and promote the use of clinical patch testing methods for a more relevant assessment of chemical skin irritation potential than that provided by the rabbit test (13–16). This approach has also been successfully applied to the assessment of soap and detergent containing products (17). For cosmetic products, the European cosmetics trade association (COLIPA) has issued guidelines for skin compatibility testing of cosmetic formulations in man (18).

The major problem regarding human testing for skin irritation or compatibility is the extended duration and relatively high cost of this clinical testing. In vitro skin irritation test methods could be used to rank chemicals or formulations for skin irritation potential, even at the low end of the irritation spectrum (19). These and other methods might provide for short-term, cost-effective approaches for screening chemicals and product formulations of interest, so that only those with satisfactory skin irritation profiles would be progressed into longer and more costly clinical evaluations. Prediction of acute irritation and/or skin compatibility, without the need to rely on additional clinical testing, would be a distinct advantage.

This chapter will provide a brief summary of the developmental status of in vitro skin irritation test methods. It includes a brief description of the assay systems and current validation status of skin corrosion tests. It then summarizes efforts in various laboratories, towards development of a battery of skin irritation tests that might predict varying degrees of skin irritation potential of chemicals and formulations, including many with relatively mild clinical skin irritation properties. No detailed descriptions of specific test methods are provided. However, a recent review provides details for many of the validated and developmental test methods (20).

SKIN CORROSION TESTING

Assay Systems

Screening of chemicals for skin corrosion properties in vitro has followed three general formats. These include changes in electrical conductance across an intact skin (rat or human), breaching of noncellular biobarriers, and cellular cytotoxicity in skin or epidermal-equivalent cell culture systems. Each of these systems has been subject to intra- and interlaboratory development, evaluation, and validation.

Skin corrosivity has been distinguished from skin irritation in two important ways. Firstly, corrosive skin reactions generally occur quickly after chemical exposure and are irreversible. Secondly, it is thought that the major processes leading to chemical corrosivity are more commonly physicochemical in nature rather than

the result of inflammatory biological events (9), although inflammation is a common consequence of skin corrosion.

Initial efforts to develop a screening test for skin corrosivity were built on this hypothesis by examining effects of chemical exposure on barrier function of skin through assessment of changes in the resistance of the exposed skin to transmission of electric current (11,21). This test method, called transcutaneous electrical resistance (TER), was based on early studies of the electrical resistance properties of skin (22) and has been developed as a corrosivity assay over the past 15 years using either rat or human skin (9,11,21,23–28). In the TER assay, full-thickness skin is stretched over a hollow tube opening with the stratum corneum side exposed to the lumen. Test materials are applied to the skin surface for varying periods of time, while the skin is immersed in buffer. After chemical exposure, the electrical resistance of the skin is measured. TER values empirically established as corrosion thresholds have been set at 4 kΩ for rat skin and 11 kΩ for human skin (11,29). The current validation status of this assay is described below.

The biobarrier destruction assay approach for corrosivity testing is exemplified by the commercial Corrositex® assay system manufactured by In Vitro International (Irvine, California). Like the TER assay, the premise here is physicochemical destruction of a barrier by direct chemical action of a test material. Instead of intact stratum corneum, the Corrositex assay relies on a macromolecular protein matrix as the barrier. Chemicals that breach this barrier come into contact with an underlying chemical detection system (CDS). A color change indicates penetration of the test material into the CDS. The speed with which the color change occurs after application of the chemical to the biobarrier is proportional to the severity of corrosive action. A summary of results on 75 chemicals and detergent-based formulations has been published (8), as well as a study on the corrosivity of organosilicon compounds (30). An update of the current validation status of this assay is provided below.

A variety of cell-based biological assay systems have been developed over the past 10 years to investigate the dermatotoxic effects of chemicals and product formulations on the skin. These have included simple submerged cell cultures, submerged cell co-cultures incorporating more than a single cell type, and, more recently, the development of full-thickness skin and epidermal-equivalent systems. The latter are characterized by stratified epidermal cell layers and a multilayered stratum corneum. The full-thickness culture systems also have different types of cellular and macromolecular matrices serving as a dermal element. These systems have undergone extensive development and evaluation in various academic and commercial laboratories (28,31–41). We have reviewed features of many of the submerged and skin/epidermal-equivalent cell systems (20,42,43). A few of these systems have been used to develop skin corrosion screening assays (10,30). A review of the current validation status of those assays is also presented below.

Validation Status

In the early 1990s, a program was initiated under the auspices of ECVAM (European Center for the Validation of Alternative Methods) to develop and validate alternative methods for the assessment of skin corrosion. This program focused on three assay systems, the TER, Corrositex®, and Skin^{2}™ systems. The Skin^2 system was a commercial "skin equivalent" culture system, manufactured at the time by Advanced Tissue Sciences (San Diego, California) and comprised of human neonatal foreskin-derived dermal fibroblasts in a collagen matrix grown on nylon mesh and

seeded with human neonatal foreskin-derived epidermal keratinocytes to form a stratified and cornified epidermal component. A prevalidation study was completed with these three assay systems in seven different laboratories to assess intra- and interlaboratory consistency as well as overall sensitivity and specificity of the assays in identifying known corrosive and noncorrosive chemicals. The results of the prevalidation study were published in 1995 (44). All the three tests performed well, and no firm conclusions could be drawn as to the superiority or inferiority of one test versus the others. Individual tests had specific problems that needed further study to address. These problems included relatively low specificity (TER), a high number of incompatible chemicals (Corrositex), and an inferior interlaboratory consistency profile (Skin^2). It was recommended that effort be made to address these individual deficiencies and that each assay be further evaluated in a follow-up validation study.

The formal ECVAM sponsored skin corrosivity validation study began in early 1995 and was completed in October 1997 with the submission of the study findings (12). In addition to the assays included in the prevalidation work (TER, Corrositex, and Skin^2), the validation study included a second commercially available skin equivalent culture construct, Episkin™, an epidermal-equivalent culture system manufactured by Episkin SNC (Lyon, France). Each assay was evaluated by three independent test laboratories and each laboratory evaluated only one of the four assays. Hence, 12 laboratories participated in the validation study. A total of 60 corrosive and noncorrosive chemicals from a variety of chemical classes (including organic and inorganic acids and bases, neutral organics, phenols, inorganic salts, electrophiles, and soaps/surfactants) were tested (45).

All of the four assay systems showed acceptable intra- and interlaboratory reproducibility and all except Corrositex were applicable to the testing of all the selected chemicals. Two of the assays, TER and Episkin met the first of two major objectives of the validation study. They were capable of distinguishing corrosive from noncorrosive chemicals with acceptable rates of under or over prediction. Only the Episkin assay system met the second major objective of the study, the ability to distinguish between known R35 (UN packing group I) and R34 (UN packing group II/III) chemicals across all of the chemical classes. Only 60% of the test chemicals could be adequately evaluated by the Corrositex assay. Therefore, it did not meet the criteria for a validated replacement test, although it might be valid for certain chemical classes. The Skin^2 assay system showed high specificity (100% of noncorrosive chemicals were properly identified) but low sensitivity (only 43% of corrosive chemicals were correctly identified). It also performed poorly in distinguishing known R35 and R34 chemicals. Only 35% of the assays conducted on these chemicals resulted in proper classification. Previously, both the Skin^2 and Corrositex assays had received exemptions from the Department of Transportation as valid alternatives to assess skin corrosivity, based on more limited evaluation; however, the Skin^2 assay is no longer commercially available.

After this validation study, another commercial epidermal-equivalent construct, EpiDerm™ (Mattek Corp., Ashland, Massachusetts) completed a successful "catch-up" validation process (46,47) using a partial set of the validation chemicals. It was endorsed by ECVAM as a suitable alternative corrosion test method (48). At this same time, the United States Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) qualified the Corositex™ assay as a suitable test method for predicting corrosivity and noncorrosivity for specified chemical classes (acids and bases) (49), a position endorsed by ECVAM in 2001 (50). In 2000, the European Union incorporated new in vitro test methods for skin

corrosion into Annex V of the Dangerous Substances Directive (51). These included the rat skin TER assay and human skin model assays. The latter were not specified by name so as to avoid tacit endorsement of the commercial suppliers of these culture systems. In 2002, ICCVAM reviewed the data on the EVCAM validation studies for Episkin, EpiDerm, and the rat skin TER assays (52). It concluded that positive assay results were sufficient for labeling chemicals as corrosive, but that negative results would not be sufficient for labeling as noncorrosive. Negative results would require confirmation in an animal test.

Although there has been some obvious success in developing and validating different in vitro methods for skin corrosion and obtaining endorsement and regulatory test guideline inclusion, work has continued to improve the test methods for specific needs (53). Also, in spite of this trend away from animal testing over the past decade, one can still find efforts to develop new predictive animal test methods (54).

SKIN IRRITATION TESTING

Assay Systems

As indicated previously, development of in vitro methods to assess skin irritation is complicated by the fact that skin irritation encompasses a range of clinical responses from near corrosive to very mild (perhaps sensory only) skin responses. Hence, it is likely that test methods and prediction models will need to be optimized for different categories of test materials or formulations and for anticipated ranges of irritation severity. Research studies over the past 10 to 15 years have focused on skin culture methods that are similar to those used for corrosion testing; the main difference being in the duration of exposure needed to quantify responses that can be correlated with existing in vivo (generally clinical) test results. As detailed below, there has been success in developing methods that correlate well with both acute as well as cumulative/chronic skin irritation responses. Also as detailed below, early test method validation efforts have focused mainly on prediction of acute skin irritation (38).

The EpiDerm culture system was used for development of a model of acute skin irritation induced by surfactants or surfactant-containing products (55). Using cytotoxicity as the endpoint, they used time– and dose–response profiles to compare their in vitro results with acute irritation test data in human subjects. At the same time, Roguet and co-workers used the Episkin model for acute irritation testing of surfactants, using both cytotoxicity and interleukin-1α (IL-1α) measurement as an early indicator of inflammation. They observed a good correlation ($r > 0.8$) between both in vitro measures and historical human data (56–58). Ponec and co-workers used a noncommercial full-thickness skin construct called RE-DED (reconstituted epidermis on de-epidermized dermis) or Pruniéras model (59,60). These cultures were exposed to different concentrations of acute irritants [e.g., sodium dodecyl sulfate (SDS), oleic acid] for 24 hours. Toxicity was assessed by morphological changes, cytotoxicity, enzyme release, and production of inflammatory mediators (37,38,61).

Recently, another commercial epidermal-equivalent construct, SkinEthic™ (SkinEthic, Nice, France), has been proposed for acute skin irritation testing. Toxicity was assessed in this culture system following one-day exposure to different surfactants, retinoic acid, or calcipotriol cream, using enzyme release, cytokine release, or tissue expression of cytokine mRNA (41,62). Very recently, this construct was used to try and distinguish acute skin irritants from contact allergens using differential cytotoxicity and cytokine release responses (63).

In vitro skin equivalent culture systems developed originally for screening for corrosion or acute irritation, have also been adapted for screening of milder chemicals and product formulations. It might appear to be a stretch to be able to detect sufficient response in a relatively short-term cell culture system to correlate with clinical irritation that may take days or weeks of continuous or repetitive exposure to become manifest. However, the reduced barrier properties inherent to many of these culture systems (61), along with extended durations (24 hours) of the exposures to skin irritants and the use of more sensitive endpoints of irritation (e.g., cytokine production), has facilitated success in correlating in vitro irritation profiles with varying degrees of cumulative or chronic skin irritation in man.

Augustin and co-workers used skin and dermal equivalent culture systems to examine the irritation potential of cosmetic product formulations. Testing cosmetic formulations of various types (creams, lotions, oils, mascaras) they observed a good correlation between in vitro indices of irritation (enzyme and cytokine release) and previously known Draize irritation indices (64,65). An interlaboratory comparison study used the Episkin™ construct to assess 38 cosmetic formulations of different types (gels, oils, mascaras, emulsions). An 18-hour exposure protocol using cytotoxicity and cytokine release as endpoints was found to provide a good correlation with human 48-hour patch test results (66). Novartis Pharma scientists examined two skin constructs, SkinEthic™ and Apligraf™ (Organogenesis, Canton, Massachusetts), to assess both acute (mentioned earlier) and cumulative/chronic irritation to topical products (41,67). They evaluated innocuous (Vaseline®) and differentially irritant test materials (0.005% calcipotriol ointment, 0.025% *trans*-retinoic acid cream, and 0.2–1% SDS in water), using one-day (Apligraf™) or three-day (SkinEthic™) cultures to assess cumulative irritation. They measured enzyme release and cytokine production as well as tissue expression of cytokine mRNA. Human skin responses were assessed using 21-day cumulative irritation patch test protocols. In both in vitro systems, they found that the responses obtained correlated well with the severity of response obtained in the clinical patch tests. The acute (24-hour culture) and chronic (three-day) culture systems have been incorporated into an internal testing strategy for screening of the acute and chronic skin irritation potential of topical drug products (20).

In our laboratory, the EpiDerm™ construct was evaluated in a series of studies comparing in vitro irritation data with different types of clinical test data in the assessment of surfactants, antiperspirants/deodorants, and cosmetics. Test material exposures were up to 24 hours, and the cultures were assayed for cytotoxicity, enzyme release, and cytokine release (19). Surfactants (anionic, nonionic, and amphoteric) were tested in vivo using three repeat 24-hour exposures under occluded patch, and cumulative erythema grades were determined for each material. The in vitro irritancy was assessed using the cytotoxicity assay. With the exception of one nonionic surfactant, the rank ordering of irritation was the same for the in vivo and in vitro tests. For antiperspirants/deodorants, the clinical irritation data were derived from home use study diaries. The in vitro data included cytotoxicity, enzyme release, and IL-1α assays. All showed good correlation with the human data ($r^2 = 0.75$–0.94), but the IL-1α assay showed the greatest ability to discriminate mid-range irritation. For cosmetics, the clinical data were derived from cumulative irritation tests where benchmark materials (0.05% and 0.1% SDS) were included as high irritant controls. The cumulative irritation indices for different cosmetic formulations were compared to the in vitro test data. Again, all in vitro endpoints showed good correlation with the human data ($r^2 = 0.74$–0.91), with the IL-1α assay again showing the best ability to discriminate mid-range irritation.

Validation Status

In contrast to the in vitro tests for skin corrosion, to date no in vitro tests for evaluating the skin irritation potential of chemicals have been validated and accepted into regulatory testing requirements. Several in vitro methods for identifying acute skin irritants were evaluated in an independently managed prevalidation study funded by ECVAM (68–70). The main overall objective of the validation activities was to identify those in vitro tests capable of discriminating skin irritants from non irritants, as defined according to the EU risk phrases (R38; no classification) and the harmonized Organization for Economic Cooperation and Development (OECD) criteria (Irritant; no label). The prevalidation study involved three phases: (I) refinement of the test protocols, (II) an initial assessment of the transferability of the test protocols between laboratories, and (III) an initial assessment of protocol performance (i.e., the reproducibility and predictive ability of the in vitro tests). Five test systems comprised the prevalidation study:

- EpiDerm
- Episkin
- Prediskin™ (Biopredic, Rennes, France)
- Pig Ear Test (TNO-PML, Rijkswijk, The Netherlands)
- Mouse skin integrity function test (SIFT) (developed by Syngenta, Macclesfield, the U.K.)

Specifics of these test methods and their respective prediction models are provided elsewhere (20,70). In ECVAM's prevalidation study, none of these test methods performed well enough through all three phases to be recommended for a full validation study (70). EpiDerm and Episkin progressed to phase III, but did not meet ECVAM predictive criteria. Both required improvements to the test protocol and prediction models. Recent reports indicate that this has been achieved and that both human skin models are now ready for full validation (70a,70b). Neither Prediskin nor the pig ear test advanced beyond phase II. In repeats of phase I (protocol refinement) the predictiveness of the Prediskin method improved, whereas the pig ear test showed no improvement in reproducibility. The mouse SIFT method completed phases I and II through the ECVAM prevalidation study, but had not been tested for protocol performance. This was done subsequently and the results reported very recently (71). Against the initial protocol prediction method, overall accuracy of the method was insufficient. However, refinement of the prediction method and a retrospective analysis of the data enabled adequate precision for correct irritation classification. This method will now move into full validation.

DISCUSSION

From the mid 1940s to the end of the 20th century, the focus for skin corrosion and irritation testing and risk assessment was largely confined to animal procedures adapted from the original method of Draize (7). At the start of the new millennium, it was clear that this landscape was changing. Protection of workers and consumers from toxic skin responses owing to chemical or product exposures could be insured through use of in vitro and clinical test methods and appropriate risk assessment based thereon. Schemes for skin irritation testing and risk assessment have been devised (20,72–74) that incorporate pretest evaluation of chemical and formulation information (75–77), anticipated worker and consumer exposures, in vitro corrosion

testing (using validated methods), and (if needed) the clinical assessment of skin irritation (16). In vitro tests for acute and/or cumulative skin irritation (though not yet validated and accepted) are included in these schemes for additional data generation that might be necessary (or desired) for screening purposes or to further position risk prior to human exposures (72,73).

The dilemma once faced by the dermatotoxicologist; that is, to protect the skin health of the worker and consumer, comply with regulatory requirements, and reduce or eliminate animal testing has been eased by the advent of these various testing methods and risk assessment practices. In the future, we can expect additional in vitro test method validation and regulatory acceptance (particularly for acute and cumulative skin irritation), improvements in the sensitivity and specificity of existing methods, and refinements in skin culture constructs (e.g., introduction of melanocytes, Langerhans cells, etc.) to provide functional enhancements. Ethical clinical testing for acute, chronic, and cumulative skin irritation can be conducted using a variety of methods. Various instrumental methods can be included in these clinical studies to assess effects of chemicals/formulas on biophysical parameters (78), and noninvasive skin sampling can also be used to analyze inflammatory skin changes (79–81), including subclinical (sensory) responses (82). Capabilities such as these will only improve with further research and test method development.

REFERENCES

1. OECD. OECD Guideline for Testing of Chemicals 1992; 404:1–6.
2. EEC. Annex I to Commision Directive 91/325/EEC of 1st March 1991 adapting to technical progress for the twelth time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provision relating to the classification, packaging and labeling of dangerous substances. Off J Eur Commun 1991; L180:34.
3. EEC. Council Directive of 7 June 1988 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the classification, packaging and labeling of dangerous preparations. Off J Eur Comm 1988; L18:14.
4. EEC. Directive 2003/15/EC of the European Parliament and of the Council of 27 February 2003 amending Council Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products. Off J Eur Comm 2003; L066:26–35.
5. In Vitro Skin Toxicology. New York: Mary Ann Liebert, Inc., 1994.
6. CFR. Department of Transportation. Method of testing corrosion to the skin. Title 49, Appendix A, 1991. Code of Federal Regulations.
7. Draize JH, Woodard G, Calvery HO. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharm Exp Ther 1944; 82:377–390.
8. Gordon VC, Harvell JD, Maibach HI. Dermal corrosion, the CORROSITEX® system: a DOT accepted method to predict corrosivity potential of test materials. In: Rougier A, Goldberg AM, Maibach HI, eds. In Vitro Skin Toxicology. New York: Mary Ann Liebert, 1994:37–45.
9. Lewis RW, Botham PA. Measurement of transcutaneous elecrical resistance to assess the skin corrosivity potential of chemicals. In: Rougier A, Goldberg AM, Maibach HI, eds. In Vitro Skin Toxicology. New York: Mary Ann Liebert, 1994:161–169.
10. Perkins MA, Osborne R, Johnson GR. Development of an in vitro method for skin corrosion testing. Fundam Appl Toxicol 1996; 31:9–18.

11. Whittle E, Barratt MD, Carter JA, Basketter DA, Chamberlain M. Skin corrosivity potential of fatty acids: in vitro rat and human skin testing and QSAR studies. Toxicol In Vitro 1996; 10:95–100.
12. Fentem JH, Archer GEB, Balls M, Botham PA, Curren RD, Earl LK, Esdaile DJ, Holzhütter HG, Liebsch M. The ECVAM international validation study on in vitro tests for skin corrosivity. 2. Results and evaluation by the management team. Toxicol In Vitro 1998; 12:483–524.
13. Basketter DA, Whittle E, Griffiths HA, York M. The identification and classification of skin irritation hazard by a human patch test. Food Chem Toxicol 1994; 32:769–775.
14. York M, Griffiths HA, Whittle E, Basketter DA. Evaluation of a human patch test for the identification and classification of skin irritation potential. Contact Dermatitis 1996; 34:204–212.
15. Griffiths HA, Wilhelm KP, Robinson MK, Wang XM, McFadden J, York M, Basketter DA. Interlaboratory evaluation of a human patch test for the identification of skin irritation potential/hazard. Food Chem Toxicol 1997; 35(2):255–260.
16. Robinson MK, McFadden JP, Basketter DA. Validity and ethics of the human 4-h patch test as an alternative method to assess acute skin irritation potential. Contact Dermatitis 2001; 45:1–12.
17. Robinson MK, Kruszewski FH, Al-Atrash J, Blazka ME, Gingell R, Heitfeld FA, Mallon D, Snyder NK, Swanson JE, Casterton PL. Comparative assessment of the acute skin irritation potential of detergent formulations using a novel human 4-hour patch test method. Food Chem Toxicol 2005. (In press).
18. Walker AP, Basketter DA, Baverel M, Diembeck W, Matthies W, Mougin D, Paye M, Rothlisberger R, Dupuis J. Test guidelines for assessment of skin compatibility of cosmetic finished products in man. Food Chem Toxicol 1996; 34:651–660.
19. Perkins MA, Osborne R, Rana FR, Ghassemi A, Robinson MK. Comparison of in vitro and in vivo human skin responses to consumer products and ingredients with a range of irritancy potential. Toxicol Sci 1999; 48:218–229.
20. Robinson MK, Cohen C, de Fraissinette AD, Ponec M, Whittle E, Fentem JH. Non-animal testing strategies for assessment of the skin corrosion and skin irritation potential of ingredients and finished products. Food Chem Toxicol 2002; 40:573–592.
21. Oliver GJA, Pemberton MA, Rhodes C. An in vitro skin corrosivity test — modifications and validation. Food Chem Toxicol 1986; 24:507–512.
22. Blank IH, Finesinger JE. Electrical resistance of the skin. Arch Nerurol Phychiat 1964; 56:544–557.
23. Oliver GJA, Pemberton MA. An in vitro epidermal slice technique for identifying chemicals with potential for severe cutaneous effects. Food Chem Toxicol 1985; 23:229–232.
24. Oliver GJA, Pemberton MA, Rhodes C. An in vitro model for identifying skin-corrosive chemicals: I. Initial validation. Toxicol In Vitro 1988; 2:7–17.
25. Barlow A, Hirst R, Pemberton MA, Rigden A, Hall TJ, Oliver GJA, Botham PA. Refinement of an in vitro test for the identification of skin corrosive chemicals. Toxicol Meth 1991; 1:106–115.
26. Botham PA, Hall TJ, Dennett R, McCall JC, Basketter DA, Whittle E, Cheeseman M, Esdaile DJ, Gardner J. The skin corrosivity test in vitro: results of an inter-laboratory trial. Toxicol In Vitro 1992; 6:191–194.
27. Basketter DA, Whittle E, Chamberlain M. Identification of irritation and corrosion hazards to skin: an alternative strategy to animal testing. Food Chem Toxicol 1994; 32:539–542.
28. Harvell J, Maibach HI. In vitro dermal toxicity tests: validation aspects. Cosmet Toilet 1992; 107:31–34.
29. Basketter D, Reynolds F, Rowson M, Talbot C, Whittle E. Visual assessment of human skin irritation: a sensitive and reproducible tool. Contact Dermatitis 1997; 37(5):218–220.
30. Cassidy SL, Stanton ES. In vitro skin irritation and corrosivity studies on organosilicon compounds. J Toxicol Cutan Ocul Toxicol 1996; 15:355–367.

31. Harvell J, Bason MM, Maibach HI. In vitro skin irritation assays: relevance to human skin. J Toxicol Clin Toxicol 1992; 30:359–369.
32. Harvell JD, Maibach HI. Validation of in vitro skin irritation assays using human in vivo data. In Vitro Toxicol 1992; 5:235–239.
33. Harvell JD, Tsai YC, Maibach HI, Gay R, Gordon VC, Miller K, Mun GC. An in vivo correlation with three in vitro assays to assess skin irritation potential. J Toxicol Cutan Ocul Toxicol 1994; 13:171–183.
34. Osborne R, Perkins MA. An approach for development of alternative test methods based on mechanisms of skin irritation. Food Chem Toxicol 1994; 32:133–142.
35. Rheins LA, Edwards SM, Miao O, Donnelly TA. Skin$^{2(TM)}$: an in vitro model to assess cutaneous immunotoxicity. Toxicol In Vitro 1994; 8:1007–1014.
36. Ponec M. The use of in vitro skin recombinants to evaluate cutaneous toxicity. In: Rougier A, Goldberg AM, Maibach HI, eds. In Vitro Skin Toxicology. New York: Mary Ann Liebert, Inc., 1994:107–116.
37. Ponec M, Kempenaar J. Use of human skin recombinants as an in vitro model for testing the irritation potential of cutaneous irritants. Skin Pharmacol 1995; 8:49–59.
38. Boelsma E, Tanojo H, Bodde HE, Ponec M. Assessment of the potential irritancy of oleic acid on human skin: evaluation in vitro and in vivo. Toxicol In Vitro 1996; 10: 729–742.
39. Lawrence JN. Application of in vitro human skin models to dermal irritancy: a brief overview and future prospects. Toxicol In Vitro 1997; 11:305–312.
40. Rosdy M, Bertino B, Butet V, Gibbs S, Ponec M, Darmon M. Retinoic acid inhibits epidermal differentiation when applied topically on the stratum corneum of epidermis formed in vitro by human keratinocytes grown on defined medium. In Vitro Toxicol 1997; 10:39–47.
41. de Brugerolle de Fraissinette A, Picarles V, Chibout S, Kolopp M, Medina J, Burtin P, Ebelin ME, Osborne S, Mayer FK, Spake A, Rosdy M, De Wever B, Ettlin RA, Cordier A. Predictivity of an in vitro model for acute and chronic skin irritation (SkinEthic) applied to the testing of topical vehicles. Cell Biol Toxicol 1999; 15:121–135.
42. Robinson MK, Perkins MA, Osborne R. Comparative studies on cultured human skin models for irritation testing. In: van Zutphen LFM, Balls M, eds. Animal Alternatives, Welfare and Ethics. Amsterdam: Elsevier, 1997:1123–1134.
43. Perkins MA, Robinson MK, Osborne R. Alternative methods in dermatotoxicology. In: Marzulli FN, Maibach HI, eds. Dermatotoxicology Methods. Washington, D.C.: Taylor & Francis, 1998:319–336.
44. Botham PA, Chamberlain M, Barratt MD, Curren RD, Esdaile DJ, Gardner JR, Gordon VC, Hildebrand B, Lewis RW, et al. A prevalidation study on in vitro skin corrosivity tesing. The report and recommendations of ECVAM Workshop 6. ATLA-Altern Lab Anim 1995; 23:219–255.
45. Barratt MD, Brantom PG, Fentem JH, Gerner I, Walker AP, Worth AP. The ECVAM international validation study on in vitro tests for skin corrosivity. 1. Selection and distribution of the test chemicals. Toxicol In Vitro 1998; 12:471–482.
46. Balls M, Fentem JH. The validation and acceptance of alternatives to animal testing. Toxicol In Vitro 1999; 13:837–846.
47. Liebsch M, Traue D, Barrabas C, Spielmann H, Uphill P, Wilkins S, Wiemann C, Kaufmann T, Remmele M, Holzhütter HG. The ECVAM prevalidation study on the use of EpiDerm for skin corrosivity testing. ATLA-Altern Lab Anim 2000; 28:371–401.
48. ECVAM. Statement on the application of the Epiderm™ human skin model for skin corrosivity testing. ATLA-Altern Lab Anim 2000; 28:365–366.
49. Scala R, Fentem JH, Chen J, Derelanko MJ, Green S, Harbell J, Kohrman KA, Sauder DN, Stegeman J. Corrositex®: An In Vitro Test Method for Assessing DermalCorrosivity Potential of Chemicals. Internet URL, 1999.
50. ECVAM. ECVAM News and Views. ATLA-Altern Lab Anim 2001; 29:96–97.

51. EEC. Annex I to Commission Directive 2000/33/EC adapting to technical progress for the 27th time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labeling of dangerous substances. Off J Eur Commun 2000; L136:91–97.
52. ICCVAM. ICCVAM Evaluation of EPISKIN™, EpiDerm™ (EPI-200), and the Rat Skin Transcutaneous Electrical Resistance (TER) Assay – In Vitro Test Methods for Assessing the Dermal Corrosivity Potential of Chemicals, 2002.
53. Rogers JV, McDougal JN. Improved method for in vitro assessment of dermal toxicity for volatile organic chemicals. Toxicol Lett 2002; 135:125–135.
54. Rausch L, Bisinger EC Jr, Sharma A, Rose R. Use of the domestic Swine as an alternative animal model for conducting dermal irritation/corrosion studies on fatty amine ethoxylates. Int J Toxicol 2003; 22:317–323.
55. Cannon CL, Neal PJ, Southee JA, Kubilus J, Klausner M. New epidermal model for dermal irritancy testing. Toxicol In Vitro 1994; 8:889–891.
56. Roguet R, Cohen C, Rougier A. A reconstituted human epidermis to assess cutaneous irritation, photoirritation and photoprotection in vitro. In: Rougier A, Goldberg A, Maibach HI, eds. In Vitro Skin Toxicology. New York: Mary Ann Liebert, Inc., 1994:141–149.
57. Roguet R, Cohen C, Dossou KG, Rougier A. Episkin, a reconstituted human epidermis for assessing in vitro the irritancy of topically applied compounds. Toxicol In Vitro 1994; 8:283–291.
58. Roguet R, Cohen C, Rougier A, Leclaire J. Measurement of proinflammatory mediator production by cultured keratinocytes. Curr Probl Dermatol 1995; 23:230–242.
59. Regnier M, Pruniéras M, Woodley D. Growth and differentiation of adult human epidermal cells on dermal substrates. Front Matrix Biol 1981; 9:4–35.
60. Pruniéras M, Regnier M, Woodley D. Methods for cultivation of keratinocytes with an air–liquid interface. J Invest Dermatol 1983; 81:28s–33s.
61. Boelsma E, Gibbs S, Faller C, Ponec M. Characterization and comparison of reconstructed skin models: morphological and immunohistochemical evaluation. Acta Derm Venereol 2000; 80:82–88.
62. Coquette A, Berna N, Vandenbosch A, Rosdy M, Poumay Y. Differential expression and release of cytokines by an in vitro reconstructed human epidermis following exposure to skin irritant and sensitizing chemicals. Toxicol In Vitro 1999; 13:867–877.
63. Coquette A, Berna N, Vandenbosch A, Rosdy M, De Wever B, Poumay Y. Analysis of interleukin-1 alpha (IL-1 alpha) and interleukin-8 (IL-8) expression and release in in vitro reconstructed human epidermis for the prediction of in vivo skin irritation and/or sensitization. Toxicol In Vitro 2003; 17:311–321.
64. Augustin C, Collombel C, Damour O. Use of dermal equivalent and skin equivalent models for identifying phototoxic compounds in vitro. Photodermatol Photoimmunol Photomed 1997; 13:27–36.
65. Augustin C, Collombel C, Damour O. Use of dermal equivalent and skin equivalent models for in vitro cutaneous irritation testing of cosmetic products: comparison with in vivo human data. J Toxicol Cutan Ocul Toxicol 1998; 17:5–17.
66. Roguet R, Cohen C, Robles C, Courtellemont P, Tolle M, Guillot JP, Pouradier Duteil X. An interlaboratory study of the reproducibility and relevance of Episkin, reconstructed human epidermis, in the assessment of cosmetics irritancy. Toxicol In Vitro 1998; 12:295–304.
67. Medina J, de Brugerolle de Fraissinette A, Chibout SD, Kolopp M, Kammermann R, Burtin P, Ebelin ME, Cordier A. Use of human skin equivalent Apligraf for in vitro assessment of cumulative skin irritation potential of topical products. Toxicol Appl Pharmacol 2000; 164:38–45.
68. Fentem JH. Skin irritation. In: Clark DG, Lisansky SG, Macmillan R, eds. Alternatives to Animal Testing. II. Proceedings of the Second International Scientific Conference

Organised by the European cosmetic Industry, Brussels, Belgium. Newbury, U.K.: CPL Press, 1999:30–37.
69. Fentem JH, Botham PA, Earl LK, Roguet R, van de Sandt JJM. Prevalidation of in vitro tests for acute skin irritation. In: Clark DG, Lisansky SG, Macmillan R, eds. Alternatives to Animal Testing. II. Proceedings of the Second International Scientific Conference Organised by the European Cosmetic Industry. Newbury, U.K.: CPL Press, 1999: 228–231.
70. Fentem JH, Briggs D, Chesne C, Elliott GR, Harbell JW, Heylings JR, Portes P, Roguet R, van de Sandt JJM, Botham PA. A prevalidation study on in vitro tests for acute skin irritation: results and evaluation by the Management Team. Toxicol In Vitro 2001; 15:57–93.
70a. Portes P, Grandidier MH, Cohen C, Roguet R. Refinement of the Episkin protocol for the assessment of acute skin irritation of chemicals: follow-up to the ECVAM prevalidation study. Toxicol In Vitro 2002; 16:765–770.
70b. Kandarova H, Liebsch M, Genschow E, Gerner I, Traue D, Slawik B, Spielmann H. Optimisation of the EpiDerm test protocol for the upcoming ECVAM validation study on in vitro skin irritation tests. ALTEX 2004; 21:107–114.
71. Heylings JR, Diot S, Esdaile DJ, Fasano WJ, Manning LA, Owen HM. A prevalidation study on the in vitro skin irritation function test (SIFT) for prediction of acute skin irritation in vivo: results and evaluation of ECVAM Phase III. Toxicol In Vitro 2003; 17: 123–138.
72. Robinson MK, Osborne R, Perkins MA. Strategies for the assessment of acute skin irritation potential. J Pharmacol Toxicol Meth 1999; 42:1–9.
73. Robinson MK, Osborne R, Perkins MA. In vitro and human testing strategies for skin irritation. Ann NY Acad Sci 2000; 919:192–204.
74. Robinson MK, Perkins MA. A strategy for skin irritation testing. Am J Contact Dermat 2002; 13:21–29.
75. Young PC, How T. Product classification as corrosive or irritant by measuring pH and acid/alkali reserve. In: Rougier A, Goldberg AM, Maibach HI, eds. In Vitro Skin Toxicology. New York: Mary Ann Liebert, Inc., 1994:23–27.
76. Bagley DM, Gardner JR, Holland G, Lewis RW, Regnier JF, Stringer DA, Walker AP. Skin irritation: reference chemicals data bank. Toxicol In Vitro 1996; 10:1–6.
77. Kodithala K, Hopfinger AJ, Thompson ED, Robinson MK. Prediction of skin irritation from organic chemicals using membrane-interaction QSAR analysis. Toxicol Sci 2002; 66:336–346.
78. Serup J, Jemec GBE. Handbook of Noninvasive Methods and the Skin. Boca Raton: CRC Press, 1995.
79. Perkins MA, Osterhues MA, Farage MA, Robinson MK. A noninvasive method to assess skin irritation and compromised skin conditions using simple tape adsorption of molecular markers of inflammation. Skin Res Technol 2001; 7:227–237.
80. Perkins MA, Cardin CW, Osterhues MA, Robinson MK. A non-invasive tape absorption method for recovery of inflammatory mediators to differentiate normal from compromised scalp conditions. Skin Res Technol 2002; 8:187–193.
81. Robinson MK, Schwartz JF, Perkins MA. Application of a novel and noninvasive skin sampling technique for analyzing cytokine-mediated inflammation in rosacea. J Toxicol Cutan Ocul Toxicol 2003; 22:13–22.
82. Perkins MA, Osterhues MA, Vogelpohl S, Robinson MK. A clinical skin sampling approach to asses sensory skin irritation. Toxicol Sci 2000; 54:146.

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Reconstructed Corneal and Skin Models

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During the last decade, tissue engineering became a progressing field in biotechnological research. The vision of medical treatment of burns patients, the treatment of ulcers, and the idea of reconstructing damaged organs revealed very rapidly further possibilities. Tissues not only resembled morphologically the situation in vivo but also revealed comparable physiology. This made artificial tissues interesting for testing efficacy of pharmaceutical and cosmetics products. The development of the tissue models was paralleled by an increasing demand in using alternative methods for the identification of toxicological hazards inherent to raw material, with the vision to replace animal testing for human safety assessment in toto. Increasing efforts were made to validate such alternatives against the existing animal tests. Some of those are already successful, others though promising, need further refinement. As the major field of applications for the cosmetic industry is doubtlessly the surface epithelial lining of humans, i.e., skin and the mucous membranes of mouth and eye, progress in reconstructing such models is followed thoroughly, and applications for these alternatives are evaluated extensively.

This chapter deals with the comparability of the reconstructed human corneal and skin models with the in vivo situation, and gives an insight on some areas of application in cosmetic science.

RECONSTRUCTED CORNEAL MODELS

Corneal Tissues

The epithelium of the eye surface distinguishes three regions: centrally the cornea, the limbus as transitional zone, and the peripheral conjunctiva. As a mucous membrane, it is a squamous epithelium, not keratinized but stratified. In contrast to the conjunctiva, the central cornea and the limbus are devoid of other cells such as Langerhans cells, melanocytes, and endothelial capillary cells.

The cornea itself is formed by three layers: an epithelium, a stroma, and an endothelial lining. The epithelium consists of four to five cell layers with changing morphology. The cells of the basal layer are polygonal in shape. While proliferating, they produce lateral extensions to form so-called wing cells. Apically, they become flattened

and compose a superficial cell lining. The outermost cells are strongly interlocked building tight junctions to build a nonkeratinized barrier. All cells contain a nucleus.

The stroma below the basal cells constitutes the majority of the corneal thickness. Directly below the basal cells of the epithelium, there is an acellular region called the Bowman's membrane, going over into the stroma. The stroma itself is a highly organized tissue made up by paralleled lamellae of collagen fibrils. Elongated fibroblast-like keratocytes are found throughout the stroma, running in parallel to the collagen lamellae. Posteriorly, a single layer of endothelial cells lines the stroma separated by the Descemet's membrane, a true basement membrane.

Damage to the Cornea

Irritation of the eye is a local and mostly reversible response to external stimuli. Corneal and conjunctival cells are often involved in this response. When evaluating the damaging effects in animal testing like the rabbit Draize test the majority derives from damage to the cornea (1). Studies on pathological changes after application of surfactants in standard animal irritation assays revealed that, at time points of three hours and 1 day after application, the potency of the compounds could be differentiated. Innocuous and slight irritants affected superficial cells, mild to moderate compounds affected the epithelium and the superficial stroma, whereas severely irritating substances deteriorated deep through the stroma down to the endothelium. Studies using nonsurfactant compounds widened the insight into irritation indicating that compounds may differentially injure cornea and conjunctivae. Furthermore these studies indicated that time points of evaluation played a critical role as well since effects observed at three hour time points were not as predictive for irritating effects at one hour time points. Examples were given that, though in many cases damage by compounds is a progressive event, firstly, affecting the epithelium, some compounds have stronger effects on the stroma without involving the epithelium (2).

Thus, aspects of penetration, cytotoxicity, as well as time-related effects have to be considered when addressing hazard assessment by alternative methods. Therefore, as injury is a three-dimensional process, alternatives should focus on three-dimensional models.

Reconstructed Cornea Models

Corneal tissues produced by methods of tissue engineering increasingly resemble the in vivo situation with respect to morphology, physiology, and biochemistry.

Current cornea constructs exist of cells of different animal origin, i.e., human, rabbit, or bovine. They differ in their complexity. While some models are simply made up by epithelial cells (3), others comprise an outer epithelium grown on a stromal equivalent (4). The most complex equivalents even contain a posterior endothelial lining (5,6).

In all these models the in vivo situation is closely mimicked. Depending on the origin of corneal cells, the epithelium is composed of five to six or 9 to 10 cell layers. Basal cells and wing cells might not be differentiated as clearly as in vivo, but stratification is obvious by flattened superficial cells being tightly packed, interlocked and joined by tight junctions forming a barrier. Slight differences in the morphology of this superficial cell lining are discussed to be due to the absence of lacrimal fluid and eyelid blinking (6). Basal layers express hemidesmosomes. Their internal placodes are connected to the cytoskeleton (3).

A functional expression of extracellular matrix plays an important role in the integrity and function of a tissue. As a major component in the basement membrane Laminin is detectable in the basal layers of the reconstructed skin model. Fibronectin localized in the basement membrane promoting corneal migration and re-epithelialization is mostly detected at the epithelial–stromal junction as well as the collagen matrix making up the stromal equivalent (4,6). Integrins are expressed differentially throughout the epithelium according to their location of action. Their expression resembles the situation in vivo (4).

Permeation studies with pharmacologically active compounds underline the close resemblance and functionality of the described models to the in vivo organ, e.g., penetration coefficients from appropriate studies with organotypic cornea models differed form the in vivo situation in factors only smaller than two, indicating the functionality of the epithelial barrier (6).

Use of Reconstructed Corneal Epithelia in Safety Assessment

In contrast to these complex models, reconstructed epithelial models are preferred for the in vitro assessment of eye irritation. These are commercially available. The use comprises testing of neat chemicals as well as formulations.

Main endpoint evaluated in such prediction models is the cell viability, respectively, the cytotoxicity demonstrated as the conversion of dimethylthiazoldiphenyl tetrazolium bromide (MTT) in the vital layers of the cornea (7,8). Applying cosmetic formulations for different times to the corneal equivalents, T50 values are calculated estimating a time at which 50% of the tissues lost their vitality. Based on internal benchmarks such models are used for product development (7). In a study with 68 tested products, irritant effects were overestimated by 10% compared to Draize modified maximum average score (MMAS) data, while there was only an underestimation of 1.5% (8). Therefore, it could be argued that they have a predictive ability to distinguish nonirritant from irritant products.

Testing pure compounds showed that prediction models, from formulation testing with cytotoxicity as the only endpoint, could not simply be transferred in this situation and needed further adaptation (8). Cytotoxicity testing focuses on the conversion of MTT in the viable layers of the equivalent and not on the necrotic effects in the suprabasal layers. Additional parameters for evaluation of irritation, such as histological parameters should be taken into account as well. Further, endpoints often used in other epithelial models, as cytokine and chemokines, are not yet subject to prediction models for eye irritation. Although they are released after the damage of the cornea (9), they are detected in tears (10,11) and play a vital role in its regeneration (12). Studies have shown the capability to produce cytokines after stimulation of corneal epithelial rat cell lines (13) and immortalized human cell lines (14). The evaluation of these parameters together with the already established multiendpoint analyses might give us the chance to have a validated and internationally accepted alternative method to the Draize test.

Skin Equivalents

It is twenty years since epidermal equivalents have been produced successfully for clinical applications (15). Since then, several equivalents of different complexities have been produced for scientific investigations as well as for commercial use. Today, the most common equivalents are made up by keratinocytes. Biopsies from

clinical surgery of adults and foreskin of young boys are sources for keratinocytes. In the simplest models, these cells are grown in culture under submerged condition for approximately 14 days until a multilayered tissue equivalent is formed. This equivalent is lifted to the so-called air–liquid interface in culture dishes to become stratified and cornified. Thus, such models distinguish several layers resembling in morphological characteristics to native skin: a stratum basale, a stratum spinosum, a stratum granulosum, and a stratum corneum.

Skin equivalents consist of 5 to 14 layers of viable cells depending on the model. Their thickness varies between 23 and 100 μm. Overall they seem not to be as thick as native skin with 80 to 90 μm.

The cells of the stratum corneum are columnar to round in shape. In contrast to native skin, they regularly contain intracellular lipid droplets. Keratinocytes of this layer are involved in the formation of the basement membrane. A lamina densa and lucida is produced intermittently rather than continuously by all equivalents. Hemidesmosomes as anchoring structures between cells and basement membrane, indicate the functionality of the stratum basale.

The stratum spinosum is characterized in the upper layers in vivo as in vitro by flattened cells. Unlike in native skin few intracellular lipid droplets are recognized in cells of this layer.

The stratum granulosum produces numerous lamellar bodies of normal appearance thus correlating with the in vivo situation. Depending on the skin equivalent they are more or less rapidly extruded at the interface with the stratum corneum. The unique organization of the alternating electron-dense and electron-lucent lipid lamellar sheets in the intercellular space between the corneocytes indicates a physiological processing of extracellular lipids of the stratum corneum.

The stratum corneum is made up by 14 to 25 cell layers compared with 15 to 20 in native skin. Its thickness ranges from 12 to 37 μm instead of 10 to 12 μm. In equivalents used for penetration studies, this number increases to 100 μm due to 100 layers of cells. A detailed comparison of the characteristics of the different epidermal equivalents is given in Ponec et al. (16,17).

In concordance with the morphological resemblance to native skin, expression and localization of differentiation markers correlates to the in vivo situation. Keratin 1 and 10 as indicators for early differentiation are present in all suprabasal layers of nearly all skin equivalents. The same holds true for Loricrin and SPRR2, markers only present in the stratum granulosum. The localization of other differentiation markers like involucrin and transglutaminase do not resemble native skin as they are not restricted only to the stratum granulosum but also found in all suprabasal layers. SKALP and SPRR3 are expressed in some epidermal tissue equivalents though absent in vivo.

Studies on comparative gene expression uncovered similarities between equivalents and native skin, and showed up differences to monolayer cultures.

Monolayer cultures lack differentiation markers expressed in the upper layers of the epidermis, such as filaggrin, loricrin and involucrin, and keratins K1 and K10. Furthermore, they over express actin-associated cytoskeletal proteins and different integrins, reflecting their motility and adherence to the culture dishes, respectively. Proteins related to cell cycle and DNA replication are expressed in cultured keratinocyte monolayers while repressed in native skin. Together with a high expression of nucleoskeletal proteins they reflect requirements in rapidly proliferating cells. The expression of corresponding genes identifies skin equivalents as metabolic active tissues, somewhere in between monolayer cultures and native skin.

Similarities to native skin are found in the expression of cell-to-cell signaling molecules, as secreted proteins and cell surface receptors. This difference to monolayer cultures might indicate that cell–cell communication is important in the organization and maintenance of a stratified epidermis (18).

Barrier Function and Penetration

One of the major functions of the human skin is the protection of the body against the loss of water. This function is fulfilled by a barrier in the upper layers of the stratum corneum being produced during the process of keratinization. In epidermal equivalents this terminal differentiation is induced by culturing tissue equivalents at the air–liquid interface. Its development can be followed by the cutaneous permeability of caffeine in epidermal equivalents, which decreases with time at the air–liquid interface finally reaching a plateau. After approximately 16 days in culture no further improvement of the penetration characteristics can be observed (19).

The barrier is made up by three major components: the multiple lipid lamellae filling the extracellular space between the corneocytes, an impermeable cornified envelope made of proteins produced during terminal differentiation and coating corneocytes internally, and a corneocyte lipid envelope of omega-OH-ceramides, omega-OH-hydroxyacids, and free fatty acids situated externally to the cornified envelope. A proper composition and a structural organization of the lipids in the stratum corneum are required for a functional barrier (16,17,20). Though the skin equivalents contain all major lipid classes, differences are noticed in content and profile either between the models or the native skin. None of the models resembled native skin in terms of lipid composition and ceramide profiling. With respect to ceramides, content of ceramide two is much higher in the epidermal equivalents, while polar ceramides are under-represented or even missing.

Penetration studies with compounds of different lipophilicity revealed great differences with respect to flux across the membranes between excised human skin and epidermal equivalents. Permeability towards hydrophilic compounds such as salicylic acid and caffeine showed increased fluxes by a factor of 20 for the epidermal equivalents. Mannitol, another hydrophilic compound, revealed an increased flux by a factor of 20 to 50 depending on the equivalents tested (21). Hydrophobic substances penetrated epidermal equivalents 900-fold faster. Reproducibility of penetration data between different batches of equivalents is regarded as an indicator for reproducible barrier function in skin equivalents (19,22). Depending on the compounds tested, one can conclude that reproducibility between batches is dependent on its lipophilicity. Gysler et al. (23,24) reported a variability of 14% between various batches with regard to penetration of prednilosone, being better than that of native skin. Garcia demonstrated satisfactory coefficient of variation (CV) at approximately 20% regarding penetration of caffeine, confirming data published by Lotte et al. (22). Reproducibility of penetration of strongly hydrophilic mannitol was poor between batches in all the different models tested, while best for lipophilic lauric acid.

Thus, the barrier of commercially available epidermal equivalents cannot be regarded as an effective one. This is discussed to be an intrinsic property of all epidermal equivalents (25). The only model resembling native skin in terms of composition and ceramide profile is the re-epithelialized–de-epidermized dermis by Ponec (16,17). With respect to penetration, this model shows best penetration characteristic for caffeine only differing by a factor of two, in terms of flux from native skin (Ponec, personal communications).

Irritation Testing

The close resemblance of the epidermal equivalents to native human epidermis favors its use for the prediction of skin irritation that is still assessed on animals. As keratinocytes are the first cells coming into contact with external compounds they play an important role in the initiation and modulation of skin irritation (26). Markers produced and released by these cells are the initial signals for visible clinical signs of irritation such as edema and erythema, due to responses of the deeper tissues in human skin.

Early effects of irritation are mostly studied with models consisting only of keratinocytes, pure epidermis models. They are mostly commercially available: Epiderm®, Episkin®, Apligraf®, and Skinethic®. Further models are in house developments (27) or used for scientific purposes (16,17,28,29).

The most common parameters used to assess toxic effect in vitro are measurement of vitality (e.g., MTT conversion) and membrane integrity (e.g., neutral red uptake, lactate dehydrogenase (LDH) release). A prevalidation study at the European Center for the Validation of Alternative Methods (ECVAM), investigated cytotoxicity as the only parameter for predicting skin irritation. The two skin equivalents being part of this study, EPISKIN® and EpiDerm®, showed acceptable intra- and interlaboratory reproducibilities. When testing 20 chemicals (10 irritants, and 10 nonirritants) it turned out that measurement of cytotoxicity alone does not allow distinguishing between irritating compounds and nonirritating compounds. Both models showed an overall accuracy of 58% in prediction. While Epiderm overestimates irritation to 37% and underestimates to 47%, EPISKIN reacted to 23% and 60%, respectively (30). Currently protocols have been refined (31) increasing sensitivity, specificity, and accuracy to 70%, 80%, and 75%, respectively, so that a new validation trial to replace the Draize test for skin irritation is under way.

Further parameters should be taken into account when assessing skin irritation, which is evaluated in vivo by visible effects related to secondary inflammatory responses. In this respect, measurement of the release of inflammatory mediators and the subsequent induction of secondary markers of inflammation as well as the monitoring of inflammation related gene expression might be a promising approach.

Several studies dealt with the detection of inflammatory mediators, such as cytokines, chemokines, and prostaglandins. Interleukin 1 alpha (IL1α), constitutively expressed, is one of the most important cytokines as it is released from keratinocytes immediately after membrane damage. Its release resembles to data obtained by LDH release. Further studies looked at Interleukin 6. Though not released by pure epidermal equivalents, it is produced after irritation in skin model consisting of an additional dermal part (reviewed in 32).

Interleukin 8 has strong chemotactic effects. It is induced by (IL1α) and produced by keratinocytes and fibroblasts, therefore, transducing effects to deeper part of the skin. Prostaglandin E2 is the best investigated prostalandin with respect to the application of irritants to skin equivalents. Though some models produce PGE3 in a dose-dependent manner (29), other models fail to show such a relationship (32).

A detailed description of investigate inflammatory mediator in different skin equivalents is given in Welss et al. (32).

Predicting potential irritation of ingredients is only one of the points that interests cosmetic industry. Another point is at least as important as the knowledge about hazardous effects is the predictivity of the irritating effects of formulations.

Measuring the turnover of MTT definitely faces the fact that the epidermal equivalents metabolize it only in the (supra) basal layers. Toxic effects not affecting

the lower parts of the epidermal equivalent but the apical layers, as SLS, therefore, cannot be predicted and evaluated (32). At least the evaluation of histological sections has to be taken into account. Several studies, therefore, include additionally the determination of inflammatory mediators and enzyme release. Perkins et al. (34) compared data concerning vitality (MTT), the release of enzymes as LDH and aspartate–amino transferase (AST), and the release of IL 1α after treatment of skin equivalents for definite times towards a human 14 day repeated patch test, assessing the irritating potency of cosmetic formulation. The results revealed that for the prediction of irritating effects due to cosmetic formulations, endpoints like vitality (MTT) was useful for rank-ordering skin irritancy levels of surfactants. In addition with enzyme release (LDH and AST), these parameters distinguished lower and higher irritancy products. IL1α was able to distinguish and rank-order the compounds of irritancy between these two extreme points. Another study (27) compared the irritation effects of 22 cosmetic formulations. Endpoints evaluated in vitro were (i) the effective time (ET50) after application, when 50% of the tissues lost their viability, (ii) the percentage of viability left 16 hours after application, determined by conversion of MTT, (iii) the release of IL1α and (iv) the release of LDH. In vivo irritation was assessed under occlusive conditions by the modified Frosch–Kligman soap chamber patch test. In this test, material is applied repeatedly: the first time for 24 hours, followed by three applications of six hours on each of the following three days. Skin reactions are scored on each day until day 5. In addition, skin reddening was measured with a chromameter and barrier interference was assessed by transepidermal water loss at the beginning and at the end of the study. The best rank correlation in the in vivo and in vitro data was achieved for ET50 followed by MTT at 16 hours and the IL1α release, while for LDH release correlation was generally low.

Comparing the mean total score of the in vivo evaluation at day 5 with ET50, linear regression analysis gave coefficients of correlation of r=0.84 to R=0.94 depending on the model. Further analysis of the data by contingency tables, taking into account a visual score of two as a cut off value between irritancy and nonirritancy and MTT50 values as discriminator, revealed equivalent results in all models tested: sensitivity=92%, specificity=100%, and concordance of 95%.

Protocols for the prediction of irritating effects of formulations are mainly established within companies; therefore, it is limited and direct comparisons are hardly possible. Some of the protocols concentrate on the scoring and grading on biochemical parameters (Chatelain, personal communication) others use statistical methods to work out correlation between in vivo and in vitro data.

One may conclude that in vitro assessment of irritation induced by formulations can distinguish between nonirritating and irritating formulations when data are compared to objective endpoint, clinical signs of irritation such as edema, erythema, and fissures. Other more subjective effects like stinging, itching, and pain are hardly to be predicted with the existing in vitro approaches.

Pharmacotoxicology

The reaction to topically applied irritants with the release and production of inflammatory mediators indicates that epidermal equivalents resemble native skin not only in terms of morphology but also in terms of physiology and biochemistry. Different studies deal with the biochemical characterization and metabolic competence of these models in order to identify them as alternatives for pharmacotoxicological studies.

Gysler et al. (23,24) demonstrated the metabolic activity of reconstructed skin models by application of glucocorticoids. The double ester prednicarbamate (PC) esterified at position 17 and 21 was hydrolyzed by esterases during its passage through the skin equivalent into the monoester P17EC, and later on after passage nonenzymatically to P21EC. No PC itself was detected after passage, as it was totally metabolized. These results were in analogy to those obtained from experiments with native skin. A fluorinated monoester betamethasone-17-valerate (BM17V), not affected by esterases due to a missing ester bond at position 21, passed the skin unconverted. The only metabolites found were BM17V and the nonenzymatically converted BM21V, after permeation of skin equivalents as well as native skin. Thus, the metabolism of PC and BM17V was well reflected by the skin equivalents (23,24).

Another study showed the applicability for screening skin targeted androgen modulators since skin equivalents express type 1 5alpha reductase activity. Reverse transcription-polymerase chain reaction (RT-PCR) experiments revealed the expression of a unique 5alpha R1cDNA fragment, while there were no traces of 5alpha R2. This reflects the situation in native skin where 5alpha R1 is highly predominant and regarded as the important enzyme for testosterone turnover. Topically applied testosterone is metabolized during permeation by 5alpha reductase mainly to dihydroxytestosterone. When inhibiting the enzyme by finasteride, this metabolic pathway is blocked dose-dependently in skin equivalents. As main metabolite 4-androstene-3,17-dione is produced (35).

In this respect, enzymes of the xenobiotic metabolism are of comparable importance. Activities of phase I enzyme Cytochrome P 450 IA1 (CYP IA1) concerning its 7-ethoxyresorufin-O-deethylase (EROD) and 7-ethoxycoumarin-O-deethylase (ECOD) activities, and of these phase II enzyme glutathione S-transferase (GST) by CNDB conversion were examined in several skin equivalents (36). Furthermore, NAD(P) H:Quinone reductase (NQR) activities were tested.

EROD activities were below detection levels in all tested models, but could be induced by 3-methylcholanthrene. This induction was strongly batch dependent in all the models, since some batches were not inducible at all. With respect to ECOD activity there was a basal activity in all models. ECOD activity was only inducible in those batches that were inducible for EROD as well. Both activities could be inhibited by clotrimazole.

General GST activity against the standard substrate 1,4-dinitrochlorobenzene was detected in all equivalents. Variation within and between batches of all models did not exceed 20%. Activities were higher than in normal skin except for Episkin (37).

NQR is an enzyme that catalyzes the reduction of quinone, compounds present in the environment naturally or anthropogenic. Activities were tested against menadione. All tested models were competent concerning NQR, with Skinethic showing highest and Epiderm showing lowest activities. Activities in Epiderm resembled those of native skin best. Inhibition by dicumarol could be induced in all the tested equivalents.

These studies reveal the metabolic competence of skin equivalents and their use for pharmaco-toxicological studies.

Studies with Melanocytes

For examination of mechanisms of skin tanning and the identification of ingredients influencing this process, the addition of melanocytes to epidermal equivalents is of great advantage. Cocultures of melanocytes and keratinocytes resulted in an enhanced survival of these cells and promoted melanin synthesis (38). Melanocytes and keratinocytes form together a so-called epidermal melanin unit. It typically

consists of one melanocyte that is in contact with approximately 35 keratinocytes. Dendrites formed by melanocytes interdigitate into the intercellular spaces. Melanosomes produced within these dendrites are transported into the keratinocytes. Here they orient themselves toward the nucleus and are organized to form an apical cap protecting the nucleus against irradiation. Thus, functionality of the melanin unit can be monitored in the in vitro system. A recent study by Yoon et al. (39) shows the applicability of these in vitro systems to screen for melanogenesis affecting compounds. Reconstructed epidermal equivalents contained melanocytes of different origin: of African Americans, Asians, and Caucasians. Two compounds, melanin stimulating hormone (MSH) and dihydroxyphenylalanine (DOPA), known as stimulators of melanogenesis, were examined for their ability to induce melanin content and tyrosinase activity. MSH increased tyrosinase activity in all three types of equivalent. This resulted in an increased content of melanin. In histological sections, Fontana–Masson staining of the melanin revealed an extended pigmentation in the upper layers of the skin.

DOPA increased melanin content, but decreased tyrosinase activity due to competition with the substrate used. Effects were more obvious in models with melanocytes from African Americans and Asians than from Caucasians.

Key enzyme in the melanogenesis is the tyrosinase regulating the hydroxylation of tyrosine. Therefore, known inhibitors affect the activity of this enzyme. Four inhibitors, hydroquinone, arbutin, kojic acid, and niacinamid, were tested. All compounds inhibited tyrosinase more or less dose-dependently in all tissues, with hydroquinone having the strongest effects. Melanin content was decreased in all tissues accordingly, whereas Fontana-Masson-stained section revealed a decreased melanin content only in the hydroquinone and arbutin-treated equivalents.

Though some properties need further improvement, many characteristics resemble the in-vivo situation. Besides a comparable morphology, tissue equivalents show similar reactions with respect to physiology; whether this is a biochemical answer to irritating compounds, the conversion of pharmacological active compounds by a comparable enzyme system, a resembling xenobiotic metabolism or a coculture of skin relevant cell types.

Therefore, tissue equivalents are used as a reliable tool supporting product development. Protocols set up for this purpose are accepted and validated within companies either for questions of compatibility or efficacy.

Further efforts are on the way to promote the acceptance of the use of these models not only internally but also for regulatory acceptance.

REFERENCES

1. Xu KP, Li XF, Yu FS. Corneal organ culture model for assessing epithelial responses to surfactants. Toxicol Sci 2000; 58(2):306–314.
2. Maurer JK, Parker RD, Jester JV. Extent of initial corneal injury as the mechanistic basis for ocular irritation: key findings and recommendations for the development of alternative assays. Regul Toxicol Pharmacol 2002; 36(1):106–117.
3. Nguyen DH, Beuerman RW, De Wever B, Rosdy M. Three-dimensional construct of the human corneal epithelium for in vitro toxicology. In: Harry Salem, Sidney Katz, eds. Alternative Toxicological Methods. : CRC Press, 2003:147–159, .
4. Germain L, Auger FA, Grandbois E, Guignard R, Giasson M, Boisjoly H, Guerin SL. Reconstructed human cornea produced in vitro by tissue engineering. Pathobiology 1999; 67(3):140–147.

5. Griffith M, Osborne R, Munger R, Xiong X, Doillon CJ, Laycock NL, Hakim M, Song Y, Watsky MA. Functional human corneal equivalents constructed from cell lines. Science 1999; 286(5447):2169–2172.
6. Reichl S, Bednarz J, Muller-Goymann CC. Human corneal equivalent as cell culture model for in vitro drug permeation studies. Br J Ophthalmol 2004; 88(4):560–565.
7. Courtellement P. The use if in vitro reconstituted Human Corneal epithelium (HCE) in ocular risk assessment. First International Workshop on the Use of Human Epidermal and Epithelial Tissue Models Reconstituted in Chemically Defined Medium for Toxicology & Pharmacology, Nice, France, 2002.
8. Lanvin M, Doucet O. In vitro assessment of the eye irritating potential of chemicals & formulated propducts by sing 3D-epithelial models. Second International Skin Ethic Workshop 'In vitro Reconstituted Human Tissue Models in Applied Pharmacology and Toxicology Testing,' Nice, France, 2003.
9. Sotozono C, He J, Matsumoto Y, Kita M, Imanishi J, Kinoshita S. Cytokine expression in the alkali-burned cornea. Curr Eye Res 1997; 16(7):670–676.
10. Nakamura Y, Sotozono C, Kinoshita S. Inflammatory cytokines in normal human tears. Curr Eye Res 1998; 17(6):673–676.
11. Cook EB, Stahl JL, Lowe L, Chen R, Morgan E, Wilson J, Varro R, Chan A, Graziano FM, Barney NP. Simultaneous measurement of six cytokines in a single sample of human tears using microparticle-based flow cytometry: allergics vs. non-allergics. J Immunol Methods 2001; 254(1&2):109–118.
12. Imanishi J, Kamiyama K, Iguchi I, Kita M, Sotozono C, Kinoshita S. Growth factors: importance in wound healing and maintenance of transparency of the cornea. Prog Retin Eye Res 2000; 19(1):113–129.
13. Jozwiak J, Skopinski P, Grzela T, Malejczyk J. Potential application of cytokine level measurement in corneal epithelium. Int J Mol Med 2001; 7(6):665–667.
14. Offord EA, Sharif NA, Mace K, Tromvoukis Y, Spillare EA, Avanti O, Howe WE, Pfeifer AM. Immortalized human corneal epithelial cells for ocular toxicity and inflammation studies. Invest Ophthalmol Vis Sci 1999; 40(6):1091–1101.
15. Gallico GG III, O'Connor NE, Compton CC, Kehinde O, Green H. Permanent coverage of large burn wounds with autologous cultured human epithelium. N Engl J Med 1984; 311(7):448–451.
16. Ponec M, Boelsma E, Gibbs S, Mommaas M. Characterization of reconstructed skin models. Skin Pharmacol Appl Skin Physiol 2002; 15(suppl 1):4–17.
17. Ponec M. Skin constructs for replacement of skin tissues for in vitro testing. Adv Drug Deliv Rev 2002; 54(Suppl 1):S19–30.
18. Gazel A, Ramphal P, Rosdy M, De Wever B, Tornier C, Hosein N, Lee B, Tomic-Canic M, Blumenberg M. Transcriptional profiling of epidermal keratinocytes: comparison of genes expressed in skin, cultured keratinocytes, and reconstituted epidermis, using large DNA microarrays. J Invest Dermatol 200; 121(6):1459–1468.
19. Garcia N, Doucet O, Bayer M, Fouchard D, Zastrow L, Marty JP. Characterization of the barrier function in a reconstructed human epidermis cultivated in chemically defined medium. Int J Cosm Sci 2002; 24:25–34.
20. Feingold KR. The regulation of epidermal lipid synthesis by permeability barrier requirements. Crit Rev Ther Drug Carrier Syst 1991; 8(3):193–210.
21. Dreher F, Patouillet C, Fouchard F, Zanini M, Messager A, Roguet R, Cottin M, Leclaire J, Benech-Kieffer F. Improvement of the experimental setup to assess cutaneous bioavailability on human skin models: dynamic protocol. Skin Pharmacol Appl Skin Physiol. 2002; 15(suppl 1):31–39.
22. Lotte C, Patouillet C, Zanini M, Messager A, Roguet R. Permeation and skin absorption: reproducibility of various industrial reconstructed human skin models. Skin Pharmacol Appl Skin Physiol 2002; 2002(suppl 1):18–30.

23. Gysler A, Kleuser B, Sippl W, Lange K, Korting HC, Holtje HD, Korting HC. Skin penetration and metabolism of topical glucocorticoids in reconstructed epidermis and in excised human skin. Pharm Res 1999; 16(9):1386–1391.
24. Gysler A, Königsmann U, Schäfer-Korting M. Tridimensional skin models recording percutaneous absorption. Altex 1999; 2:67–72.
25. Schmook FP, Meingassner JG, Billich A. Comparison of human skin or epidermis models with human and animal skin in in-vitro percutaneous absorption. Int J Pharm 2001; 215(1&2):51–56.
26. Coquette A, Berna N, Poumay Y, Pittelkow MR. The keratinocyte in cutaneuos irritation and sensitization. In: Kydonieus AF, Wille JJ, eds. Biochemical Modulation of Skin Reactions, CRC Press, 2000:125–143.
27. Faller C, Bracher M, Dami N, Roguet R. Predictive ability of reconstructed human epidermis equivalents for the assessment of skin irritation of cosmetics. Toxicol In Vitro 2002; 16(5):557–572.
28. Ponec M, Gibbs S, Pilgram G, Boelsma E, Koerten H, Bouwstra J, Mommaas M. Barrier function in reconstructed epidermis and its resemblance to native human skin. Skin Pharmacol Appl Skin Physiol 2001; 14(suppl 1):63–71.
29. Ponec M, Kempenaar J. Use of human skin recombinants as an in vitro model for testing the irritation potential of cutaneous irritants. Skin Pharmacol 1995; 8(1&2):49–59.
30. Fentem JH, Briggs D, Chesne C, Elliott GR, Harbell JW, Heylings JR, Portes P, Roguet R, van de Sandt JJ, Botham PA. A prevalidation study on in vitro tests for acute skin irritation results and evaluation by the Management Team. Toxicol In Vitro 200; 15(1):57–93.
31. Portes P, Grandidier MH, Cohen C, Roguet R. Refinement of the Episkin protocol for the assessment of acute skin irritation of chemicals: follow-up to the ECVAM prevalidation study. Toxicol In Vitro 2002; 16(6):765–770.
32. Welss T, Basketter DA, Schröder KR. In vitro skin irritation: facts and future. State of the art review of mechanisms and models. Toxicol In Vitro 2004; 18:231–243.
33. De Wever B, Charbonnier V. Using tissue engineered skin to evaluate the irritation potential of skin care products. Cosmet Toilet 2002; 117(10):28–38.
34. Perkins MA, Osborne R, Rana FR, Ghassemi A, Robinson MK. Comparison of in vitro and in vivo human skin responses to consumer products and ingredients with a range of irritancy potential. Toxicol Sci. 1999; 48(2):218–229.
35. Bernard FX, Barrault C, Deguercy A, De Wever B, Rosdy M. Development of a highly sensitive in vitro phototoxicity assay using the SkinEthic reconstructed human epidermis. Cell Biol Toxicol 2000; 16(6):391–400.
36. Harris IR, Siefken W, Beck-Oldach K, Brandt M, Wittern KP, Pollet D. Comparison of activities dependent on glutathione S-transferase and cytochrome P-450 IA1 in cultured keratinocytes and reconstructed epidermal models. Skin Pharmacol Appl Skin Physiol 2002; 15(suppl 1):59–67.
37. Harris IR, Siefken W, Beck-Oldach K, Wittern KP, Pollet D. NAD(P)H:quinone reductase activity in human epidermal keratinocytes and reconstructed epidermal models. Skin Pharmacol Appl Skin Physiol 2002; 15(suppl 1):68–73.
38. Archambault M, Yaar M, Gilchrest BA. Keratinocytes and fibroblasts in a human skin equivalent model enhance melanocyte survival and melanin synthesis after ultraviolet irradiation. J Invest Dermatol 1995; 104(5):859–867.
39. Yoon TJ, Lei TC, Yamaguchi Y, Batzer J, Wolber R, Hearing VJ. Reconstituted 3-dimensional human skin of various ethnic origins as an in vitro model for studies of pigmentation. Anal Biochem 2003; 318(2):260–269.
40. Germain L, Carrier P, Auger FA, Salesse C, Guerin SL. Can we produce a human corneal equivalent by tissue engineering? Prog Retin Eye Res 2000; 19(5):497–527.
41. Van Goethem F. Pre-validation of a new in vitro human corneal model as alternative to the in vivo rabbit eye Draize test. First International Workshop on the Use of Human Epidermal and Epithelial Tissue Models Reconstituted in Chemically Defined Medium for Toxicology & Pharmacology, Nice, France, 2002.

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In Vitro Reconstructed Human Skin and Skin Organ Culture Models Used in Cosmetic Efficacy Testing

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INTRODUCTION

The use of animals for research purposes and for cosmetic efficacy testing is a sensitive matter. Regulatory agencies and political and scientific communities are pressing to ban the use of animals for such purposes. In Europe, this has led the Council of the European Union to approve legislation for banning the use of animals in the testing of cosmetic products, effective in 2009. This implies that alternative methods have been validated by the ECVAM (European Center for the Validation of Alternative Methods). At this time, only a few alternative testing methods have been validated for toxicological testing (previous chapter) and some of these methods use reconstructed human skin models. Nevertheless, human skin equivalents and skin organ culture models have already invaded cosmetic laboratories as alternatives to animal experimentation. They offer not only a way to comply with the demands of regulatory authorities, animal welfare organizations, and consumers, but also a means to improve and extend our knowledge on the biological processes in the skin. Moreover, they appear to be reliable, efficient, informative, and predictive tools for the screening, bioavailability, and efficacy testing of active ingredients.

One of the key reasons for the huge development of three-dimensional skin models is the presence of a stratum corneum (SC), allowing topical application of both aqueous and oily solutions as well as final formulations. Three-dimensional skin models are composed of either the epidermal compartment or both the epidermal and dermal compartments. Various types of cells can be incorporated within each compartment, including melanocytes and Langerhans cells in the epidermal compartments, and fibroblasts and endothelial cells in the dermal compartments. As the use of reconstructed models requires a certain amount of investment and expertise, and as these models present some limitations, especially a low barrier function, skin organ culture models provide alternatives for cosmetic efficacy testing.

In this chapter, we will first present an overview of commercially available skin models, used for in-house testing, as well as homemade models developed in research laboratories; that is, either reconstructed skin equivalents or skin organ culture models. This overview will be illustrated by a selection of recent work done on cosmetic efficacy testing using these different models. Then, we will report on and discuss detailed results of two sets of tests carried out using reconstituted human epidermis (RHE) and skin organ culture models. The first experiment studies the response of RHE to UV stimulation, and the use of RHE in the efficacy testing of sunblock formulations. The second experiment deals with two aspects of a vitamin E prodrug, namely its diffusion and metabolism in RHE and viable human skin, and its efficacy in the prevention of UV-induced lipid peroxidation in RHE.

The publications referred to in this overview and the two detailed studies on cosmetic efficacy testing confirm that reconstructed human epidermis (RHE) and skin organ culture models represent a genuine added value for the cosmetic industry.

SKIN EQUIVALENTS USED IN COSMETIC EFFICACY TESTING

Basic Research on Reconstructed Human Skin

New and stricter regulations have led numerous researchers and companies to develop in vitro tissue models for use in the study of cutaneous biochemistry and physiology. An excellent guideline enumerates the processes used in developing these cell culture models (1). Briefly, the first in vitro reconstructed model, developed in 1979, was a dermal equivalent, Bell's model (2), composed of fibroblasts in a collagen lattice. Later, a major breakthrough was the cell culture of keratinocytes at the air–liquid interface (3), leading to the formation of a multilayered and differentiated epidermis in all types of three-dimensional skin models. This provided the crucial SC layer, which constitutes a barrier function for reconstructed models. As cell culture undergoes constant evolution, various models were designed by growing differentiated keratinocyte cultures on fibroblast-populated dermal substrates (4), such as a de-epidermized dermis (DED) (5), collagen matrices (6,7), inert filters (8,9), and

Table 1 Commercially Available Models of Human Reconstituted Epidermis and Skin Equivalents

Type of model	Company/laboratory	Reference	Website
Reconstructed human epidermis	SkinEthic, Nice, France	8,13,15,16,20,21,29	www.skinethic.com
Reconstructed human epidermis	Episkin, Lyon, France	7,16,17,18,23,24	www.loreal.com
Reconstructed human epidermis	MatTek Corp. Ashland, U.S.A.	9,16,17,19	www.mattek.com
Reconstructed human epidermis and skin equivalent	CellSystems, Germany	4	www.cellsystems.de
Reconstructed pigmented epidermis	SkinEthic, France	33	www.skinethic.com
Pigmented skin equivalent (artificial dermal matrix)	MatTek Corp. Ashland, U.S.A.	34	www.mattek.com

lyophilized collagen-GAG membranes (10,11). Some of the above are commercially available (Table 1).

Morphological studies (12) have shown that reconstructed skin models form a multilayered epithelium, displaying characteristic epidermal structure and expressing markers of epidermal differentiation. Ultrastructurally, keratohyalin granules, lamellar bodies, and lamellar structures filled with epidermal lipids are present in the stratum granulosum and SC. The SC composed of multiple lipid lamellae located in the intercellular spaces between keratinized cells, a corneocyte lipid envelope, and desmosomal structures, constitutes a mature barrier for reconstructed skin models. This barrier is associated with an epidermis calcium gradient, similar to that found in native human skin (13). However, it has been established that there is a difference in the lipid composition of reconstructed skin as opposed to that of native skin, that is, in reconstructed skin the packing of SC lipids is hexagonal, whereas in native skin it is orthorhombic (14). This difference (12) may account for the 5- to 50-fold higher penetration rate observed in reconstructed skin models for most of the substances tested (15).

Applications of Skin Equivalents in Cosmetic Efficacy Testing

In spite of these differences in barrier function between native human skin and reconstructed models (15–17), the presence of the SC on both native human skin and skin equivalents makes it possible to apply a great variety of active ingredients topically. In addition, skin equivalents have similar enzymatic activities, such as phase I and II enzymes (18), which make them efficient models for toxicological assessment. Other enzymatic activities, such as esterase, β-glucocerebrosidase, or 5α-reductase are also found in skin equivalents. Accordingly, reconstructed models are used in the study of both the controlled release of provitamin E into free tocopherol (19,20; skin absorption and metabolism of vitamin E prodrugs and antioxidant efficiency), and the testosterone metabolism with androgen modulators (21).

Table 1 shows the commercially available skin models used for pharmacotoxicological trials. The first three reconstructed epidermis models are mainly used for irritation testing, and have already been validated or are in the process of being

Table 2 Research and Homemade Skin Equivalents

Type of model	Company/laboratory	Reference
Skin equivalent (dermal matrix)	University Medical Center, Leiden, The Netherlands	5,12,14
Skin equivalent (collagen-GAG matrix)	Laboratory of Cutaneous Substitutes, Lyon, France	10,28
Skin equivalent (dermal matrix)	L'Oréal, Paris, France	30
Reconstructed skin, Mimeskin®; reconstructed dermis, Mimederm®	Coletica, Lyon, France	11
Reconstructed pigmented epidermis	University Medical Center, Leiden The Netherlands	32
Reconstructed pigmented epidermis	L'Oréal, Paris, France	36
Reconstructed pigmented epidermis	Department of Dermatology, Bordeaux, France	35
Reconstructed pigmented epidermis with Langerhans cells	L'Oréal, Paris, France	30

Table 3 Skin Organ Culture Models

Type of model	Company/laboratory	Reference
Natskin® Kit (human skin)	Bioprédic, Rennes, France	20
Viable human skin	Hopital Pitié-Salpetriere, Paris, France	27,37
Viable human skin	Department of Genetics and Pathology, Uppsala, Sweden	52
Viable domestic pig skin	Center for Drug Research, Leiden, The Netherlands	25,26

The Natskin® kit is a commercially available model, validated for 72-hour viability

validated. So, a good deal of data is available: range of possible applications, inter-batch variations, and many others (details can be found on websites shown in Table1). The research models shown in Table 2 all have particular characteristics, owing to the specificity of their production processes. Table 3 lists the skin organ culture models commonly used. As the validation process is extremely long (prevalidation plus validation including intra- and inter-laboratory reproducibility, transferability, and determination of criteria such as specificity, sensitivity, error, etc.), none of the above-mentioned models has yet been validated for cosmetic efficacy testing. The use of these models requires great care and must include controls and an analysis of reliable and reproducible endpoints. Accordingly, multiple endpoint analysis (MEA) is used to determine the efficacy of a cosmetic. One of these endpoints is the MTT test (22), which measures either the cellular viability or the cytotoxic effects of external stress. The MTT test also measures the cytotoxic effects of an ingredient in a cosmetic formulation.

Below are examples of experiments carried out to determine the effects of oxidative stress from UV irradiation and the effects of UVA irradiation on photoaging. Further examples of experiments carried out to measure the protection afforded by a photoprotector and to monitor the pigmentation process on a pigmented skin model are presented. Finally, borderline models of skin equivalents are presented, especially those developed for wound healing.

Oxidative Stress and Photoprotection

The skin is directly exposed to external oxidative stress, and in vitro skin equivalents have been used in the evaluation of ozone- (23) or UVA- (24) induced lipid hydroperoxides. Findings have determined that these skin equivalents are susceptible to oxidative stress and to antioxidants, and that ion chelators are efficient in the prevention of cellular damage. An ex vivo pig skin model has been developed (25) as an inexpensive tool for use in investigating short-term UV-induced damage. Topical application of various antioxidants on this viable pig skin reduces oxidative stress caused by UVB radiation and thereby reduces apoptotic response (26). In addition, dermal alterations have been induced by UVA irradiation in an ex vivo viable human skin model. Topical application of retinoids (27) led to the formation of newly synthesized collagen and to the confirmation of dermal repair on this ‘‘photoaged’’ skin model.

Although in vivo tests are essential for sunblock testing [determination of sun protection factor (SPF) and protection factor A (PFA)], in vitro techniques based on skin equivalents are being developed and are also useful for these types of tests. Using a 3D skin equivalent, Augustin et al. (28) showed the deleterious effects of

both UVA and UVB irradiation by measuring viability and inflammatory response (Il-1α release assay), which disappeared following the application of sunblocks. The apoptotic response (p53 expression and sunburn cells), histological changes, and viability were assessed to determine the biological response of RHE (29) following solar and UVA irradiation with and without photoprotection (details in *biological effects of solar and UVA irradiation and the efficiency of photoprotection*). In reconstructed human skin equivalents, sunburn cells and pyrimidine dimers were found after UVB exposure, and alterations of dermal fibroblasts were found after UVA exposure (30,31). These types of damage enabled the authors to discriminate between UVB and UVA absorbers after topical application of photoprotection. This illustrates the fact that the photoprotection afforded by two sunblock formulations having similar SPF values cannot be identical for dermal damage related to photoaging.

In addition, an immunoreactive epidermis model (30) comprising keratinocytes, melanocytes, and Langerhans cells was developed to assess UV-induced immunosuppression. In this model, irradiation provoked morphological changes and a reduction in the number of Langerhans cells. These examples indicate that skin equivalents can be used to test the efficiency of sunblocks in the prevention of the damage described earlier.

Pigmentation

Pigmented reconstructed three-dimensional human skin equivalents are co-cultures of keratinocytes and melanocytes. Gibbs et al. have shown (32) that following UVB irradiation and supplementation with 3-isobutyl-1-methyl-xanthine, a complete program of melanogenesis occurs: melanosome synthesis, melanosome transport to keratinocytes, supranuclear capping of keratinocyte nuclei, and tanning of the epidermis. Using these co-cultured epidermis models, it has been demonstrated that the PAR-2 pathway (33) regulates pigmentation via melonasome transfer, but only when keratinocytes and melanocytes are in contact with each other. Accordingly, this pigmented skin tissue provides a useful tool in the comparison of the inhibitors (kojic acid, arbutin, and hydroquinone) and activators (α-MSH and dihydroxyphenylalanine) of melanogenesis (34), after repeated topical application or by systemic delivery (35). Pigmented skin tissue is also useful in the assessment of the antipigmentation effects of sunblocks. The integration of melanocytes from different ethnic regions results in a pigmented epidermis (36) or skin equivalent (34) reflecting Caucasian, Asian, and African American skin phenotypes. These models provide an interesting alternative to animal testing for evaluating the regulation of mammalian pigmentation by melanogenic factors and for elucidating the mechanisms of action of these factors.

Dermal Testing and Wound Healing

Skin equivalents comprising either differentiated epidermis and living dermis or reconstructed dermis alone, are used to imitate and investigate the mechanisms of aging and wound healing. Using these models, active ingredients were tested as a way to modulate the synthesis of extracellular matrix molecules, collagen-GAG, hyaluronic acid (11), and collagen (27). Also assessed was the influence of UV irradiation (30) on the apoptosis of fibroblasts, and the efficacy of photoprotectors (30,31) was confirmed. As human skin organ culture contains vessels, a vasoactive intestinal polypeptide (VIP)-induced inflammation was used for testing the anti-inflammatory efficacy of a plant extract (37) by measuring the variation in dilatation of the vessels.

In addition to these "cosmetic use" models, so-called "borderline" reconstructed skin models developed for wound healing should be briefly mentioned. By testing different wound dressings in a stressed collagen lattice (38), it has been shown that a reconstructed skin model can mimic the conditions of wound repair (39). Skin equivalents were used for testing the efficacy of products on burn wounds (40) and in studying the role of metalloproteinases (41) in basal membrane reconstruction, showing the importance of the dermal matrix in the epidermis. More complex reconstructed models, comprising endothelial cells, are now being developed for testing potentially angiogenic molecules (42,43) as well as in the treatment of recalcitrant leg ulcers, through split-thickness skin autografting (44).

New Types of Skin Equivalents

Tissue engineering reconstructs complete tissue, integrating several different cellular types. Most of the previously mentioned skin models comprise keratinocytes, fibroblast melanocytes, and endothelial cells. However, the skin is an immune organ and a very sensitive one. Immunoreactive reconstructed skin containing Langerhans cells has been developed. The immune response of this reconstructed skin was demonstrated by a reduction in the number of Langerhans cells and by a modification in their dendritic morphology (31,45) after exposure to sensitizers or UV irradiation.

A "neuronal" epidermal model, a co-culture of neurons and keratinocytes (46), has been used to screen molecules with anti-inflammatory properties through the release of neuropeptides. Another similar model was developed to evaluate the regeneration of sensory neurons on injured skin (47). Studies have shown that the regeneration was influenced by the extracellular matrix molecules, matrix-binding growth factors, and trophic factors.

Aging occurs when cells permit cumulative damage by altering their gene expression. Using telomerase, it is possible to offset this damage and grow histologically young reconstructed human skin using old human skin cells (48). The development of this type of model opens up one of the most exciting perspectives of the next decade: the treatment of aging and age-related disease.

EXAMPLES OF COSMETIC EFFICACY TESTING USING IN VITRO SKIN MODELS

Since 2000, we have been using one of the few models available in the market, RHE (SkinEthic®). Although this three-dimensional model has a differentiated epidermis (Fig. 1) with an SC and has an epidermal lipid composition similar to that of native skin (12), it is known to have, like all other reconstructed models, a lower barrier function (15) than human skin. Moreover, owing to its cost, compared to human skin coming from plastic surgery, RHE is not the best model for use in the study of the percutaneous absorption of cosmetic ingredients. However, this differentiated viable epidermis is able to biochemically respond to external stress and to metabolize active ingredients applied topically to the SC. Accordingly, in compliance with regulations, RHE is a valuable model for use in cosmetic efficacy testing.

The following example (29) shows the biological response to UV irradiation and the determination of the efficacy afforded by photoprotection. The second example studies the skin diffusion and metabolism of two vitamin E prodrugs on

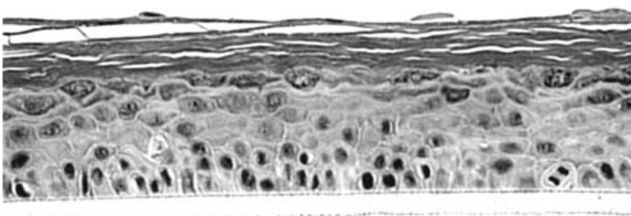

Figure 1 Histological section of a reconstructed human epidermis (SkinEthic®). A polycarbonate membrane supports a well-stratified epidermis, with a SC composed of approximately 5 to 10 layers (18 days differentiation; hemalun–eosin coloration). *Abbreviation*: SC, stratum corneum.

RHE and viable human skin models (20) and the antioxidant protection afforded against UV-induced lipid peroxidation on RHE.

Biological Effects of Solar and UVA Irradiation and the Efficiency of Photoprotection

Solar irradiation plays a causal role in photoaging, photodermatoses (49), and skin cancer (50). One means of protection against UVA and UVB irradiation is to use sunblocks. However, tests to determine the efficacy of sunblocks are usually carried out on human volunteers, and therefore present some disadvantages, because of their potential hazards for humans, and more data are required at the cellular level concerning potential photo-induced damage. RHE has been used as an alternative to human volunteers in the evaluation of the biochemical effects of UV radiation. In our study (29), a multi-endpoint analysis (MEA) was carried out, using histology, MTT assay, and apoptotic response assessed by sunburn cell (SBC) counting and p53 expression.

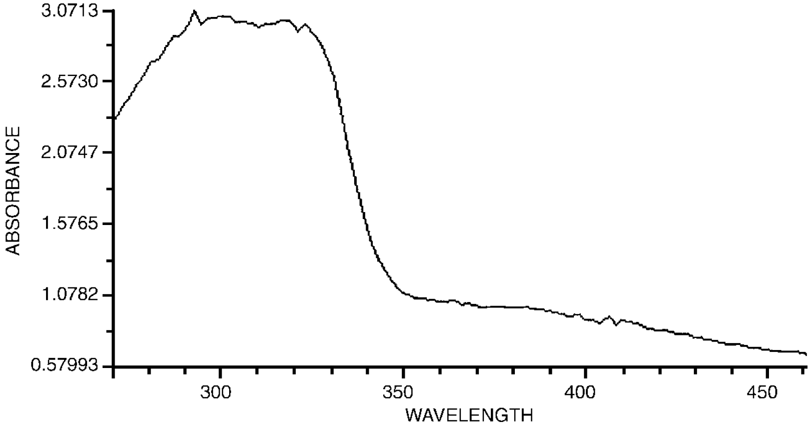

Figure 2 Absorbance curve of the mineral and organo-mineral sunblock preparation. The absorption spectra of the sunblock preparation were recorded within a range of 280 to 460 nm after 2 mg/cm^2 of product was spread on a quartz slide.

The RHE was irradiated with a 420 mJ/cm^2 solar dose and a 20 J/cm^2 UVA dose, with and without photoprotection. The photoprotection used was a broad-spectrum sunblock formulation (Fig. 2) composed of octyl-methoxy-cinnamate, Tinosorb M, titanium dioxide, and zinc oxide, applied at a dose of 2 mg/cm^2 (29).

Under solar irradiation at 420 mJ/cm^2, the number of SBC/cm increased from 4 to 41 and was associated with a loss of viability (Table 4). A response of p53 overexpression was first observed, and subsequently a cleavage appeared, generating a 40 kDa fragment (Table 4). These results confirmed the fact that RHE, because of its thinness and most likely a low barrier function, is very sensitive to UV irradiation. This is a key point in the evaluation of the efficiency of photoprotection. When the RHE was protected from irradiation, viability increased and the number of SBC/cm decreased to 12. In addition, the p53 expression decreased and proteolysis disappeared (Table 4). After UVA irradiation (20 J/cm^2), the number of SBC/cm increased to 22, but was associated with a less significant loss in viability (70%). With photoprotection, viability increased to 85% and only nine SBC/cm were counted.

These data revealed that this broad-spectrum sunblock formulation can prevent solar and UVA damage. These results indicate that the UV absorber may protect the skin against UVB-induced DNA alterations, and are in accordance with those previously found (51) with a sunblock containing TiO_2 and ZnO. This mineral sunblock formulation protected cells from DNA damage, such as UV-induced DNA single-strand breaks, with a better efficiency in the UVB than in the UVA range. Moreover, in vivo data confirms excellent photoprotection in the overall solar spectrum, especially owing to the broad-spectrum sunfilter formulation (Fig. 2).

The RHE model used here showed good reactivity to UV irradiation and enabled us to identify the regulation of the p53 protein by protease. The accumulation of p53 and its reduction when the skin was photoprotected have been reported to be similar in tests using skin organ culture models and human skin in vivo (52). Our data obtained on this RHE are similar to the previous data, especially in vivo. This confirms that this RHE model is a relevant tool in the study of external stress and cellular regulation and in the efficacy testing of photoprotective products.

Table 4 Influence of a 420 mJ/cm^2 Solar Irradiation on the Viability, the Epidermal Contraction, the Number of SBC, and the Expression of p53 with and Without OMS Protection. The western blot shows a cleavage of p53 in a 40 kDa fragment, which disappears when the sunblock is applied on the RHE.

Mean and SD (n = 6)	Non irradiated	Irradiated without photoprotection	Irradiated with photoprotection
Viability	95 ± 2%	12 ± 3%	40 ± 3%
Contraction	−	+++	+
Vacuoles	+	+++	+
SBC/cm	4 ± 0.5	41 ± 6	12 ± 7
p53 expression	+	+++++	++
p53 expression	24 h	24 h	24 h
p53 (53kDa)			
40 kDa			

Table 5 Compartmental Analysis (Surface, *Stratum Corneum* (10 Tape Strippings), Epidermis, and Dermis) of Two Prodrugs (δ-Tocopherol-Glucoside and α-Tocopherol Acetate), Their Conversion into Free Tocopherol and Percentage of Metabolism after 18 Hours Incubation in Viable Human Skin [Mean (SE)] ($n = 6$)

Analyte	Surface (μg/cm^2)	Stratum corneum (μg/cm^2)	Epidermis (μg/cm^2)	Dermis (μg/cm^2)
α-TAc	0.17 (0.04)	2.98 (0.18)	0.80 (0.27)	0.79 (0.45)
α-Toc	Not detected	Not detected	Not detected	Not detected
% of metabolite	–	–	–	–
δ-TG	3.44 (0.45)	0.78 (0.27)	0.35 (0.24)	0.16 (0.09)
δ-Toc	0.04 (0.04)	0.12 (0.07)	0.10 (0.04)	0.02 (0.02)
% of metabolite	1.1	15.3	29.2	13.1

Abbreviations: α-TAc, α-tocopherol acetate; α-Toc, α-tocopherol; δ-TG, δ-tocopherol glucoside; δ-Toc, δ-tocopherol.

Skin Absorption and Metabolism of Vitamin E Prodrugs and Antioxidant Efficiency

The development of prodrug technology is a way to avoid certain undesirable properties of topically applied drugs, such as instability and pro-oxidant effects. Accordingly, cutaneous metabolism may be a critical determinant of the therapeutic efficacy of these topically applied molecules, for the delivery of a pharmacologically active molecule through the skin. The most common vitamin E prodrug, α-tocopherol acetate (α-TAc), has been bioconverted in reconstructed human skin and in skin organ culture models through esterase activity (19,53). One of the major enzymes of the SC is β-glucocerebrosidase, which hydrolyzes amphiphilic β-glucocerebrosides into ceramides. A gluco-vitamin E conjugate, δ-tocopherol glucoside (δ-TG), has been synthesized by making use of β-glucocerebrosidase activity. The resulting glycosidic bond cleavage allows the release of active vitamin E into the SC.

The skin penetration and metabolism of δ-TG were evaluated in 18-hour studies in both RHE and viable human skin, a model closer to in vivo human skin (20). In RHE, the efficacy of delivery of free tocopherol in a 0.5% δ-TG solution was tested by lipid hydroperoxide (LPO) assay prior to solar irradiation (105 mJ/cm^2) on the extracted lipids. All these experiments were assessed in comparison with α-TAc.

With an infinite dose of a 0.1% prodrug solution applied on RHE, better diffusion was observed for α-TAc. However, no metabolism was detected with α-TAc. In contrast, 20% and 50% of δ-TG was bioconverted into free tocopherol in the SC and in the epidermis, respectively. A kinetic study with a 0.05% finite dose of δ-TG solution applied on RHE showed that 45, 70, and 90% of the prodrug was converted at 2, 6, and 18 hours (20). A similar experiment on viable human skin confirmed that no free tocopherol was detected from α-TAc, although the amount diffused was four times higher than the amount of δ-TG (Table 5). A compartmental distribution study (20) confirmed a delivery of free tocopherol from δ-TG. The highest amount was found in the SC, then in the epidermis and also in the dermis (Table 5). Antioxidant efficacy is obtained only if the prodrug is metabolized into free tocopherol, and this was confirmed by a 70% decrease in LPO concentration after treatment with δ-TG, in contrast to treatment with α-TAc (Fig. 3) or the positive control (irradiated lipids).

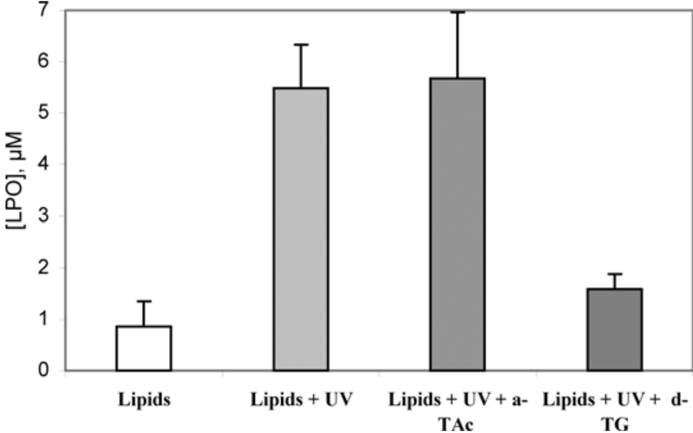

Figure 3 Concentration of LPO in lipids extracted from RHE, after an 18-hour topical application of a 0.5% solution of the prodrugs and UV irradiation ($n = 4$). *Abbreviation*: LPO, lipid hydroperoxide.

After topical application, δ-TG had a considerable reservoir effect, associated with gradual delivery of free tocopherol. This experiment enabled us to demonstrate that reconstructed epidermis and skin organ culture models are complementary. RHE can be used to confirm the metabolism and the efficacy of an active ingredient, thanks to its low barrier function, offering better bioavailability of the molecule being tested. Skin organ culture models possess a more efficient barrier function and can be used either as preclinical models or in the optimization of an active ingredient to be included in the formulation of a cosmetic product.

CONCLUSION

A great deal of effort is being put into the development and validation of reconstructed skin equivalents and skin organ culture models. Even if these skin equivalents require time for validation, they are already being used to prove product effectiveness, and they represent a genuine added value for the cosmetics industry. In spite of some limitations such as low barrier function, a lack of desquamation (11), and the inaccessibility of certain research models, the increasingly sophisticated reconstructed models offer a wide range of possibilities for use in evaluating the efficacy of cosmetic ingredients. The skin organ culture models, which may suffer from a lack of desquamation, are less expensive than the reconstructed models and are easy to obtain and possess good barrier function.

More studies are needed to correlate in vitro with in vivo data. However, the combination of RHE and skin organ culture models currently provides a complementary approach and represents an essential step in the development of cosmetic products, between screening on cellular models and final validation in clinical trials.

REFERENCES

1. Boelsma E, Ponec M. Basics (guidelines) on cell cultures testing for topical/dermatological drug/products and cosmetics with regard to efficacy and safety of the preparations. In: Gabard B, Elsner P, Surber C, Treffel P, eds. Dermatopathology of topical preparations: a product development-oriented approach. Heidelberg: Springer-Verlag, 2000:37–57.
2. Bell E, Ivarsson B, Merrill C. Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential *in vitro*. Cell Biol 1979; 76:1274–1278.
3. Regnier M, Prunieras M, Woodley DT. Growth and differentiation of adult epidermal cells on dermal substrates. Front Matrix 1981; 9:4–35.
4. Hoffman JJ, Peters P, Frost P, Fuchs HW. Advanced skin test 2000: reconstructed human skin designed for dermatological and pharmaceutical research. 4th World Congress on alternatives and animal use in the life sciences, New Orleans, USA, August 11–15, 2002.
5. Ponec M, Weerheim A, Kempanaar J, Mulder A, Gooris GS, Bouwstra J, Mommaas AM. The formation of competent barrier lipids in reconstructed epidermis requires the presence of vitamin C. J Invest Dermatol 1997; 109:348–355.
6. Wha KS, Lee IW, Cho HJ, Cho KH, Han KK, Chung JH, Song PI, Chan PK. Fibroblasts and ascorbate regulate epidermalization in reconstructed human epidermis. J Dermatol Sci 2002; 30(3):215–223.
7. Tinois E, Tillier J, Gaucherand M, Dumas H, Tardy M, Thivolet J. *In vitro* and post transplantation differentiation of keratinocytes growth on the human type IV collagen film of a bilayered dermal substitutes. Exp Cell Res 1991; 193:310–319.
8. Rosdy M, Clauss MC. Terminal differentiation of human keratinocytes grown in chemically defined medium on inert filter substrates at the air–liquid interface. J Invest Dermatol 1990; 96:409–414.
9. Cannon CL, Neal PJ, Southee JA, Kubilus J, Klausner M. New epidermal model for dermal irritancy testing. Toxicol In Vitro 1994; 8:889–891.
10. Damour O, Augustin C, Black AF. Applications of reconstructed skin models in pharmaco-toxicological trials. Med Biol Eng Comput 1998; 36:825–832.
11. Augustin C, Frei V, Perrier E, Huc A, Damour O. A skin equivalent model for cosmetological trials and *in vitro* efficacy study of a new peptide. Skin Pharmacol 1997; 10(2):63–70.
12. Ponec M. Skin constructs for replacement of skin tissues for *in vitro* testing. Adv Drug Deliv Rev 2002; 54(suppl 1):S19–S30.
13. Mavon A, de Wever B, Moretto A. Micro-PIXE analysis of reconstituted human epidermis. 2nd International SkinEthic Workshop, Nice France, October 16–17, 2003.
14. Ponec M, Boelsma E, Weerheim A, Kempanaar J, Mulder A, Bouwstra J, Mommaas AM. Lipid and ultrastructural characterization of reconstructed skin models. Int J Pharm 2000; 203:211–225.
15. Garcia N, Doucet O, Bayer M, Fouchard D, Zastrow L, Marty JP. Characterization of the barrier function in a reconstructed human epidermis cultivated in a chemically defined medium. Int J Cosmet Sci 2002; 24:25–34.
16. Lotte C, Patouillet C, Zanini M, Messager A, Roguet R. Permeation and skin absorption: reproducibility of various industrial reconstructed human skin models. Skin Pharmacol Appl Skin Physiol 2002; 15(Suppl 1):18–30.
17. Dreher F, Patouillet C, Fouchard F, Zanini M, Messager A, Roguet R, Cottin M, Leclaire J, Benech-Kieffer F. Improvement of the experimental setup to assess cutaneous bioavailability on human skin models: dynamic protocol. Skin Pharmacol Appl Skin Physiol 2002; 15(suppl 1):31–39.
18. Harris IR, Siefken W, Beck-Oldach K, Brandt M, Wittern KP, Pollet D. Comparison of activities dependent on glutathione S-transferase and cytochrome P-450 IA1 in cultured

keratinocytes and reconstructed epidermal models. Skin Pharmacol Appl Skin Physiol 2002; 15(suppl 1):59–67.
19. Nabi Z, Tavakkol A, Dobke M, Polefka TG. Bioconversion of vitamin E acetate in human skin. In: Thiele J, Elsner P, eds. Oxidants and Antioxidants in Cutaneous Biology. 29. Basel Karger, , 2001:175–186.
20. Mavon A, Raufast V, Redoulès D. Skin absorption and metabolism of a new vitamin E prodrug, tocopherol-glucoside: *in vitro* evaluation in human skin models. J Contr Release 2004; 100:221–231.
21. Bernard FX, Barrault C, Deguercy A, De Wever B, Rosdy M. Expression of type 1 5α-reductase and metabolism of testosterone in reconstructed human epidermis (SkinEthic): a new model for screening skin-targeted androgen modulators. Int J Cosmet Sci 2000; 22:397–407.
22. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Meth 1983; 65:55–63.
23. Cotovio J, Onno L, Justine P, Lamure S, Catroux P. Generation of oxidative stress in human cutaneous models following *in vitro* ozone exposure. Toxicol In Vitro 2001; 15(4–5):357–362.
24. Seite S, Popovic E, Verdier MP, Roguet R, Portes P, Cohen C, Fourtanier A, Galey JB. Iron chelation can modulate UVA-induced lipid peroxidation and ferritin expression in human reconstructed epidermis. Photodermatol Photoimmunol Photomed 2004; 20(1):47–52.
25. Rijnkels JM, Witheley LO, Van Henegouwen GMJ. Time- and dose-related UVB damage in viable pig skin explants held in a newly developed organ culture system. Photochem Photobiol 2001; 73(5):499–504.
26. Rijnkels JM, Moison RM, Podda E, Van Henegouwen GMJ. Photoprotection by antioxidants against UVB radiation-induced damage in pig skin organ culture. Radiat Res 2003; 159(2):210–217.
27. Boisnic S, Branchet-Gumila MC, Le Charpentier Y, Segard C. Repair of UVA-induced elastic fiber and collagen damage by 0.05% retinaldehyde cream in an *ex vivo* human skin model. Dermatology 1999; 199(suppl 1):43–48.
28. Augustin C, Collombel C, Damour O. Measurements of the protective effect of topically applied sunscreens using in vitro three-dimensional dermal and skin equivalents. Photochem Photobiol 1997; 66(6):853–859.
29. Gelis C, Girard S, Mavon A, Delverdier M, Paillous N, Vicendo P. Assessment of the photoprotective capacities of an organo-mineral broad-spectrum sunblock on two *ex vivo* skin models. Photodermatol Photoimmunol Photomed 2003; 19(5):242–253.
30. Bernerd F, Vioux C, Lejeune F, Asselineau D. The sun protection factor (SPF) inadequately defines broad spectrum photoprotection: demonstration using skin reconstructed *in vitro* exposed to UVA, UVB or UV-solar simulated radiation. Eur J Dermatol 2003; 13(3):242–249.
31. Duval C, Schmidt R, Regnier M, Facy V, Asselineau D, Bernerd F. The use of reconstructed human skin to evaluate UV-induced modifications and sunscreen efficacy. Exp Dermatol 2003; 12(suppl 2):64–70.
32. Gibbs S, Murli S, De Boer G, Mulder A, Mommaas AM, Ponec M. Melanosome capping of keratinocytes in pigmented reconstructed epidermis – effect of ultraviolet radiation and 3-isobutyl-1-methyl-xanthine on melanogenesis. Pigment Cell Res 2000; 13(6):458–466.
33. Seiberg M. The PAR-2 pathway regulates pigmentation via melanosome transfer. 2nd International SkinEthic Workshop, Nice, France, October 16–17, 2003.
34. Yoon TJ, Lei TC, Yamaguchi Y, Batzer J, Wolber R, Hearing VJ. Reconstituted 3-dimensional human skin of various ethnic origins as an *in vitro* model for studies of pigmentation. Anal Biochem 2003; 318(2):260–9.30.

35. Cario-Andre M, Briganti S, Picardo M, Nikaido O, Gall Y, Ginestar J, Taieb A. Epidermal reconstructs: a new tool to study topical and systemic photoprotective molecules. J Photochem Photobiol B 2002; 68:79–87.
36. Regnier M, Duval C, Galey JB, Philippe M, Lagrange A, Tuloup R, Schmidt R. Keratinocyte–melanocyte co-cultures and pigmented reconstructed human epidermis: models to study modulation of melanogenesis. Cell Mol Biol 1999; 45(7):969–980.
37. Boisnic S, Branchet-Gumila MC, Coutanceau C. Inhibitory effect of oatmeal extract oligomer on vasoactive intestinal peptide-induced inflammation in surviving human skin. Int J Tissue React 2003; 25(2):41–46.
38. Viennet C, Bride J, Gabiot AC, Humbert P. Comparison of different wound dressings on cultured human fibroblasts and collagen lattices. J Wound Care 2003; 12(10):385–390.
39. Bride J, Viennet C, Lucarz-Bietry A, Humbert P. Indication of fibroblast apoptosis during the maturation of disc-shaped mechanically stressed collagen lattices. Arch Dermatol Res 2004; 295(8–9):312–317.
40. Wang TW, Huang YC, Sun JS, Lin FH. Organotypic keratinocyte–fibroblast cocultures on a bilayer gelatin scaffold as a model of skin equivalent. Biomed Sci Instrum 2003; 39:523–528.
41. Nova D, Le Griel C, Holvoet S, Gentilhomme E, Vincent C, Staquet MJ, Schmitt D, Serres M. Comparative studies on the secretion and activation of MMPs in two reconstructed human skin models using HaCaT- and HaCaT-ras-transfected cell lines. Clin Exp Metastasis 2003; 20(8):675–683.
42. Hudon V, Berthod F, Black AF, Damour O, Germain L, Auger FA. A tissue-engineered endothelialized dermis to study the modulation of angiogenic and angiostatic molecules on capillary-like tube formation *in vitro*. Br J Dermatol 2003; 148(6):1094–1104.
43. Sahota PS, Burn JL, Heaton M, Freedlander E, Suvarna SK, Brown NJ, Mac Neil S. Development of a reconstructed human skin model for angiogenesis. Wound Repair Regen 2003; 11(4):275–284.
44. Tausche AK, Skaria M, Bohlen L, Liebold K, Hafner J, Friedlein H, Meurer M, Goedkoop RJ, Wollina U, Salomon D, Hunziker T. An autologous epidermal equivalent tissue-engineered from follicular outer root sheath keratinocytes is as effective as split-thickness skin autograft in recalcitrant vascular leg ulcers. Wound Repair Regen 2003; 11(4):248–252.
45. Facy V, Flouret V, Regnier M, Schmidt R. Langerhans cells integrated into human reconstructed epidermis respond to known sensitizers and ultraviolet exposure. J Invest Dermatol 2004; 122(2):553–554.
46. Steinschneider R, Quelven E, Grousson J, Laurent C. Screening of molecules with anti-inflammatory properties and for sensitive skin applications using rat sensory neurons cultured or not with human keratinocytes. Cosm'ing 2001 Saint Malo, France, May 17–18, 2001.
47. Taherzadeh O, Otto WR, Anand U, Nanchahal J, Anand P. Influence of human skin injury on regeneration of sensory neurons. Cell Tissue Res 2003; 312(3):275–280.
48. Fossel M. Cell senescence in human aging and disease. Ann NY Acad Sci 2002; 959: 14–23.
49. Gilchrest BA. A review of skin ageing and its medical therapy. Br J Dermatol 1996; 135:867–875.
50. De Gruijl FR. Skin cancer and solar UV radiation. Eur J Cancer 1999; 35(4):2003–2009.
51. Cayrol C, Sarraute J, Tarroux R, Redoulès D, Charveron M, Gall Y. A mineral sunscreen affords genomic protection against ultraviolet UVB and UVA radiation: *in vitro* and *in situ* assays. Br J Dermatol 1999; 141:250–258.
52. Bachvall H, Wassberg C, Berne B, Ponten F. Similar UV responses are seen in a skin organ culture as in human skin *in vivo*. Exp Dermatol 2002; 11(4):349–356.
53. Baschong W, Artmann C, Hueglin D, Roeding J. Direct evidence of bioconversion of vitamin E acetate into vitamin E: an *ex vivo* study in viable human skin. J Cosmet Sci 2001; 52:155–161.

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Squamometry: A Sensitive Testing Approach

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INTRODUCTION

Consumers are not typically exposed to surfactants under the occlusive application conditions that are found in patch tests and frequently used to determine the skin compatibility or irritation potential of surfactant-based products (1,2). Consumer contact with surfactants is generally repetitive and for brief periods such as for hand washing or personal hygiene. In this type of interaction, the part of the skin that is in contact with the surfactants is essentially the stratum corneum (SC).

Human skin irritation is classically evaluated by visual or palpatory scoring. For more objective evaluations or for skin functions that are not possible to evaluate by the above assessments, bioengineering techniques have been developed and are widely used such as colorimetric, capacitance, transepidermal water loss, skin surface pH, or topographic assessments (3–7). However, for very mild products and short contact time with the skin, these methods often reach a limit of sensitivity that does not permit comparing product effect.

An extremely sensitive method, squamometry (SQM) (Fig. 1), focuses only on the alteration of the first layers of the SC by surfactants and proves to have a higher sensitivity than all other compared techniques.

SQM involves harvesting the superficial layer of the SC with a sticky tape and staining the adhered cells with a mixture of cationic dyes. The drier or more irritated the surface of the skin is, the more dye staining is present. SQM allows for the classification of surfactants/cleansing products in many different test procedures to be consistent with their known skin irritation/compatibility potential, while remaining at a subclinical irritation level.

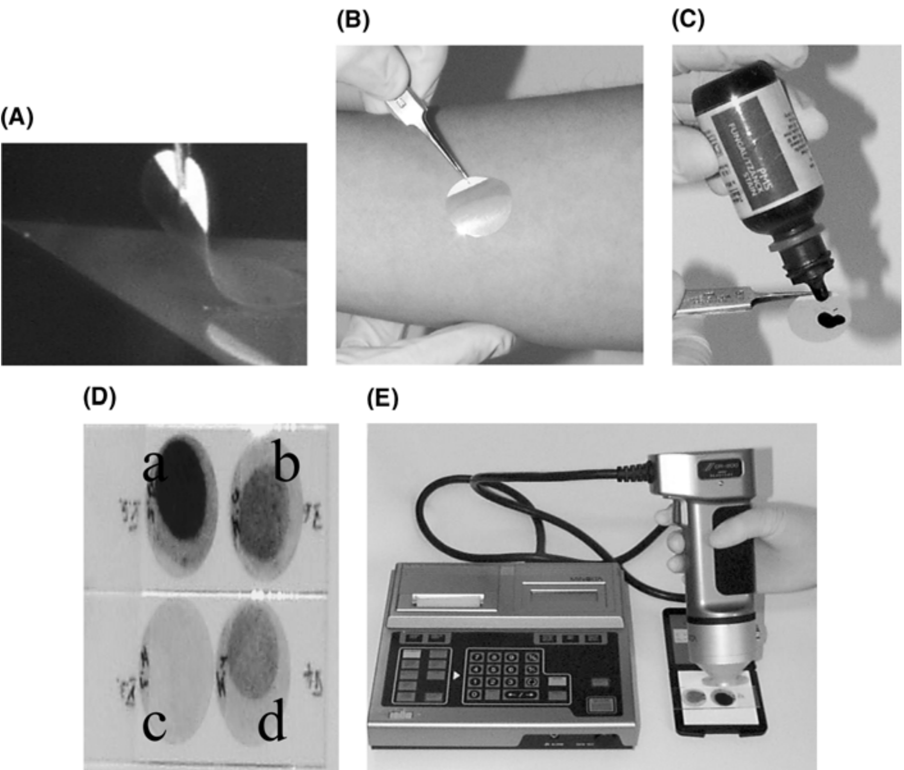

Figure 1 The different steps in squamometry are illustrated. The sticky tape is removed from its support (**A**) and firmly pressed to treated skin for 15–30 seconds. It is removed (**B**) and stained with 2 drops of polychrome multiple stain® (**C**) for 30 seconds. After rinsing, it is applied on a microscope slide. In the example (**D**), product "c" induced no staining (very mild product), product "a" caused intense staining (highly irritant) while products "b" and "d" caused intermediate staining (moderately mild). The intensity of staining is quantified by measuring the chroma C* value with the Minolta Chroma Meter (**E**).

SQM was first developed to assess skin xerosis (8) and further adapted to investigate the interaction of surfactants with the SC (9,10). The method is based on the fact that irritant surfactants denature proteins. Many in vitro skin irritation predictive tests are based on this principle (11–14). In SQM, when skin proteins are denatured, more sites that are binding become available for cationic dyes and staining is more intense. As SQM investigates the effect of the surfactants with the SC at a suberythematous level, the technique does not require long application times of the product on the skin of the volunteers, as usually needed in most in vivo skin irritation tests (1,2,15,16).

SQM: PRINCIPLE

SC harvested by tape stripping is currently used to investigate superficial alteration of the skin; different assessment modes of the strippings have been described and reviewed (17) including gravimetry, reduction in light transmission, microscopy with or without image analysis, chromametry, protein and enzyme assay, or others. One

Table 1 List of Potential Applications for Squamometry

Test procedure	Test samples	References
Suberythematous irritation testing		
Single short-term patch test—for 15 min	Surfactants	10,18
Single short-term patch test—for 30 min	Shampoos	
Repeated short-term patch tests	Surfactants Dishwashing liquid	9,19
Exaggerated hand wash test	Surfactants	20,21
Hand immersion test in laboratory-controlled, realistic, conditions	Dishwashing liquid	22
Nonexaggerated open use assay in the laboratory by washing	Surfactants	23
Combined laboratory-controlled wash test and home use test	Surfactants	24
Combined laboratory-controlled soaking test and home use test	Dishwashing liquid	25
Other applications		
Kligman regression test	Emollients	26
Assessment of dry skin		8,26
Assessment of sensitive hands		27
Assessment of softened vs. nonsoftened fabrics	Fabrics	28
Potential in safety assessment of cosmetics	Cosmetic product	29
Effect of a barrier protectant	Leave-on product	30
Screening of stratum corneum hydration	Leave-on product	31
Assessment of inter-corneocytes cohesion	Squamolytic agents	32
Effect of bleaching agents on stratum corneum	Bleaching product	33
Effect of UV-radiation on skin	UV light	34,35
Investigation of stratum corneum renewal		35
Review papers		26,36

of them, called Squamometry, has proved to be fast, easy, sensitive, reproducible, and applicable to many situations.

SQM has been described in detail elsewhere (9,10). Briefly, after the product has been used/applied, and rinsed, on the skin of the volunteer, a stratum corneum tape stripping is collected by using a D-squame® sticky tape (CuDerm Corp, Dallas, Texas) firmly pressed to the skin. The stripping is then stained for 30 (or 60 in the original paper) seconds with 1 or 2 drop(s) of PMS (Polychrome Multiple Stain, Councill Bluff, Delasco), a cationic dye composed of toluidin blue and basic fuschin, and rinsed. Staining is quantified by measurement of the chroma C^* value by using the Minolta Chroma Meter®. The more irritant the applied product is, the higher the staining and the higher the chroma C^* value.

SQM has been used for testing the interaction of cosmetics and household products with the SC according to various test procedures and several teams (Table 1).

SQM AND SKIN DRYNESS

The SC continually renews its structure, by eliminating nonvisible single corneocytes from its surface through a protease-mediated breakdown of corneosomes (37,38).

Failure to correctly degrade corneosomes leads to an abnormal corneocyte shedding process and is a fundamental factor in many dry skin conditions where flaking is present.

Scaliness and flakiness can be evaluated by clinical examination based on visual or tactile assessments (39). However, clinical grading remains subjective and may sometimes vary from one evaluator to another one, or because of the influence of external environmental conditions. SQM was developed by Pierard et al. (8) to provide means for objective and more comparable measurement of xerotic states.

Its application to follow the effect of squamolytic agents and emollients on xerosis has been documented in a clinical trial (26) using the regression method of Kligman (40).

SQM AND SKIN HYDRATION

Several publications report a negative correlation between SC hydration and the amount of scales estimated by image analysis of SC tape stripping (41,42) or by light transmission measurement through the tape-stripped SC (43,44). As the intensity of staining in SQM normally reflects the amounts of corneocytes and scales collected on the adhesive discs, it seems obvious that SQM should also be negatively related to the level of hydration of the SC.

Starting from very dry skin patients treated with a moisturizer, Pierard et al. (35) observed a decrease in the SQM value and concluded on the potential of the technique to assess the effect of hydrating products. The method was further used in Kligman regression tests on forelegs for a similar purpose (26).

However, De Paepe et al. (31) were somewhat more restrictive on the use of SQM to determine the effect of moisturizers on the scaling pattern and hydration level of the SC. In their study, they also showed a decreased overall SQM value associated with a better skin hydration and lower scaliness after treatment. Nevertheless, no correlation was observed between the individual hydration (skin surface capacitance measurement) and the chroma C^* values after the application of a cream. In their test, all subjects reported improved hydration values while a few of them did not lower the intensity of staining by SQM. The test population was different from the other studies and more narrowed (all women aged 20–29) to start with subjects having "not too dry" skin. This may explain why all subjects did not show improvement by SQM with moisturizer treatment, whereas they did in the studies starting from much drier skin. The authors concluded from their experience that SQM could be proposed only as a screening technique for SC hydration estimation, but not as a quantitative method to appreciate skin moisturization.

In our opinion, although SQM may not be as sensitive as other bioengineering methods to assess small changes in skin hydration, it remains that it is also a very helpful and predictive technique for estimating or comparing the effect of moisturizers when starting from dry skin condition. Regarding the absence of good mathematical correlation between SQM and skin hydration, it is not as surprising as comparisons were made essentially with nonlinear indirect methods (through scaling or skin surface electrical measurement) to evaluate the water content of the SC.

SQM AND SKIN COMPATIBILITY TO SURFACTANT-BASED SOLUTIONS

Applied to many different test procedures, SQM has demonstrated its capability to detect, at a very early stage, any irritation potential of surfactant-based products. This characteristic of the methodology enabled the users to progressively modify the application conditions in the tests to make them closer to the normal usage conditions or, at least, much less exaggerated and more respectful for the volunteers involved in the tests (10). The following examples show the advantages brought by SQM over other assessment procedures to investigate the irritation potential of surfactant products.

Single Short-Term Patch Tests

Two successive studies were performed (10) to compare the interaction with SC of surfactants solutions or shampoos in short-term skin application tests.

In the first study, surfactant solutions (1% w/v) or binary mixtures of surfactants (1+1% w/v) were applied for a period of 15 minutes before being rinsed-off and the skin surface dried. SC was harvested by tape stripping and processed for SQM assessment.

Surfactant solutions were classified in four distinct groups corresponding to their known irritation potential. Lauryl sulfate surfactants (sodium, ammonium and magnesium salts) induced the highest staining of the SC, followed by the group of alkyl benzene sulfonates (ammonium and magnesium salts). Alkyl betaine caused less staining than the two previous groups, while nonionic surfactants did not induce more staining than water. Ethoxylated lauryl sulfates (laureth sulfates) were classified in the groups based on their overall level of ethoxylation with higher the overall ethoxylation levels indicating lesser staining of the stripping (Fig. 2).

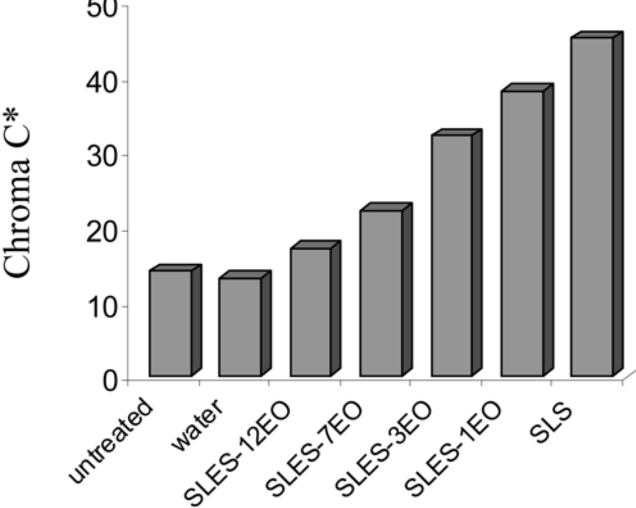

Figure 2 Effect of ethoxylation of alkyl sulfate on SC staining by SQM. The mean chroma C* staining value for an untreated site (baseline value) and for sites treated with water, sodium lauryl ether sulfate at various levels of ethoxylation, and sodium lauryl sulfate.

Table 2 Effect of Secondary Surfactants on the Staining of SC by SQM

Tested solutions	Chroma C* value: mean ± SD
Water	12.1 ± 2.2
SLS (1%)	39.2 ± 1.4
SLS (1%) + CAPB (1%)	27.1 ± 2.1
SLS (1%) + alcohol ethoxylate C9-11,5EO (1%)	25.4 ± 1.9
Na-LAS (1%)	24.5 ± 2.3
Na-LAS (1%) + CAPB (1%)	17.8 ± 2.9
Na-LAS (1%) + alcohol ethoxylate C9-11,5EO (1%)	17.3 ± 2.0

Where amphoteric (alkyl betaine) or nonionic (alcohol ethoxylate) surfactants were added to the solution of SLS or Na–LAS, the staining measured by SQM on the tape strippings was significantly weaker than from the skin sites where SLS and Na–LAS were applied alone, as expected from literature (Table 2).

Similarly, four solutions of shampoos (10% v/v) were tested by short-term application and SQM. The application time was increased to 30 minutes instead of 15, expecting less response than with the pure surfactant solutions. For comparative purposes, the same shampoos were tested in a soap chamber test (SCT) according to Simion et al. (2) to define their global (erythema + dryness) irritation score.

The ranking of the shampoos in terms of skin compatibility was exactly the same in the two tests, with similar statistical differentiation between them.

Repeated Short-Term Patch Tests

Ten dishwashing liquids (3.0% v/v, 2.5 ml) and water were applied to the forearms of the volunteers for three periods of 30 min on the same day, using glass chambers. One hour after the last application and rinsing, tape strippings were collected and processed for SQM. No visible sign of irritation was observed in the test. The same solutions were compared in a Frosch–Kligman SCT (1) to determine an overall skin irritation score defined as the sum of the erythema score at day 5 and of the dryness score at day 8. A good correlation ($r^2 = 0.87$) was obtained between the two procedures for comparing the irritation potential of the dishwashing products (25).

Realistic Hand Immersion Tests

Two dishwashing liquids, well differentiated in a Frosch–Kligman SCT (1) in terms of erythema and dryness induction, were included in a hand immersion test representing a usual consumer dishwashing liquid usage situation with minimal exaggeration. Volunteers soaked their hands in two product solutions (1 per hand, 0.2% v/v, 40°C) twice for 10 min a day (cycles in solution for 10 seconds, out of solution for 20 seconds), for four consecutive days. One hour and a half after the last soaking, hands condition was assessed by a trained evaluator and measured with sensitive bioengineering methods (evaporimetry, electrical capacitance). SC tape strippings were also collected and processed for SQM. In that specific study, no erythema and only minute dryness were induced without any difference between the two products. Only SQM clearly differentiated the two products, at a subclinical level

and without causing undue irritation, similarly to what had been predicted in the Frosch–Kligman SCT.

Laboratory-Controlled Hand Wash Test

In a laboratory-controlled hand wash test (21,24), the dorsal hands of volunteers were washed by a technician, for a minute, five times daily for four consecutive days to compare two diluted solutions of SLS (0.1 and 0.75% w/v). Owing to a small panel size ($n = 9$), methods as sensitive as self-perception of dryness/tightness by the volunteers, transepidermal water loss and skin surface capacitance measurements failed to differentiate the two solutions at a statistically significant level. Only SQM showed significant ($p < 0.05$) difference between the two solutions. Furthermore, this difference was observed as soon as from day 3 in this open application model.

SQM not only allows prediction of the skin irritation potential of surfactant-based products before any other method can do but also permits decreasing the number of volunteers in tests without decreasing the power of the statistical analysis.

Combined Home-Use and Laboratory-Controlled Hand Immersion Test (10)

Four dishwashing liquids were successively used at home for one week each for the usual dishwashing task. Simultaneously, subjects ($n = 45$) were asked to soak their hands once daily from Monday to Friday in a solution of the same product (at 0.25% v/v, 37°C, 5 minutes soaking), under laboratory-controlled conditions. Between two test weeks, panelists followed a one-week rest period with a given ultra-mild dish product to be used at home.

One hour after the last weekly soaking, a SC tape stripping was collected on the back of the dominant hand for SQM analysis. The irritation potential of the 4 test products was known from a Frosch–Kligman SCT and is expressed as the cumulative redness (day 5) and dryness (day 8) scores in Figure 3.

After a one-week at home usage combined to a 5 minutes realistic soak per day, the 4 dishwashing liquids were ranked similarly to the Frosch–Kligman SCT ($A < B = C < D$) without causing clinical signs of irritation.

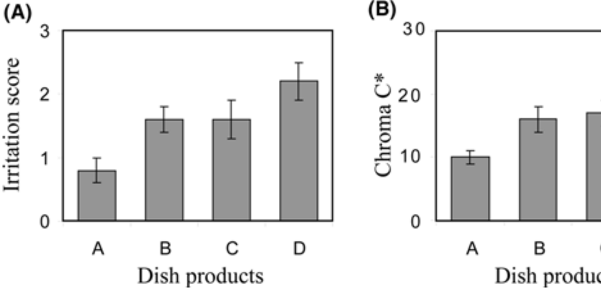

Figure 3 Application of SQM in a combined home-use and laboratory-controlled hand immersion test. (**A**) shows the mean (± SEM) cumulative irritation score (redness + dryness) for four dishwashing liquids (A–D) in a Frosch–Kligman Soap Chamber Test. (**B**) shows the mean chroma C* staining value (± SEM) for the four same dishwashing liquids tested by the combined home-use and laboratory-controlled hand immersion test.

Combined Home-Use and Laboratory-Controlled Hand Wash Test

In this test, subjects ($n = 20$) used successively two solutions of surfactants (SLS and SLES at 5% w/v) at home for personal cleansing (one week each with a one-week rest period between the two), and came twice daily to the laboratory to have their hands washed by a technician, for 1 minute, with the same solution (on days 0, 1, 2, 3, 4, and 7). On day 7, measurements were performed one hour after the last wash. Tape stripping was collected on day 7, before the first washing and one hour after the second washing.

In this combined-controlled wash at-home use test, no erythema developed on hands of the volunteers; dryness slightly developed and showed higher scores with SLS than with SLES. However, most instrumental measurements (capacitance, transepidermal water loss, and chromametry) did not differentiate between the two treatments. Only SQM objectively confirmed the significant difference between the two solutions. Furthermore, SQM showed differences between the solutions even after the volunteers were self-dosed (no laboratory washing) over the weekend (day 7 before washing), in a real open application assay.

SQM and Skin Compatibility of Bleaching Products

Sodium hypochlorite (NaOCl) is present in many household bleaching products. The use of such products may sometimes elicit discrete sensory irritation signs such as stinging sensations, with rare clinical symptoms. A study was run where bleaching products were applied for 15 to 90 minutes to human skin under patch test conditions. No clinical irritation was observed. Results revealed that conductance and SQM assessments were more sensitive than evaporimetry measurements to detect the effect of sodium hypochlorite on the SC (33).

SQM and Friction to the Skin

In this series of two studies, the frictional effect of softened and unsoftened fabrics was compared on normal and previously irritated skin of self-perceived sensitive skin individuals (28) and on infant skin (45). In all cases, skin condition was evaluated by visual assessment, instrumental measurements (capacitance, evaporimetry, and chromametry) and SQM. Only SQM demonstrated a slight, but significant, beneficial effect on skin with softened versus unsoftened fabrics in the cases of preirritated skin and infant skin. None of the other methods provided differences between the two types of tissues.

SQM and Hand Skin Sensitivity

A survey performed on a panel of 150 women differentiated between those claiming to have "sensitive hands" and those claiming to have "non sensitive hands" (27). Hand skin condition was determined in the two groups by means of a self-evaluation questionnaire, clinical examination (erythema, dryness), instrumental measurements (reflectance spectrometry, evaporimetry, skin surface capacitance, and biomechanic properties measurements) and analysis of SC tape stripping (SQM).

"Sensitive hands" claiming subjects mainly perceived their hands as being slightly dry and partly irritated. Such subjective perceptions were confirmed by visual assessment of erythema (mostly localized on finger joints), lower capacitance

measurement and higher staining in SQM (on the back of hands). Other assessment methods did not reveal differences between the two groups.

SQM and Effect of UV Light

SQM offers a convenient, rapid, and sensitive method to evaluate the early physical changes occurring in the SC after UVB irradiation (34,35). Forty subjects were irradiated on their forearms with 1.5 times their minimal erythematous dose (MED), and alteration in the SC assessed by SQM. Subjects with normal or moderately dry skin showed an increase in SQM values within the two first days after irradiation, while subjects with severe xerosis showed a decrease in that value. The method is proposed to assess the effect of sun-protective or after-sun products.

SQM and Skin Barrier Protection

In a study on eight volunteers, skin irritation was induced on the volar forearms by using semiopen patches with SLS at different concentrations (30). For each SLS concentration, one site was pretreated with tannic acid as potential skin protectant and the second one with water as the control. SQM was used to quantify skin irritation and more especially skin barrier alteration in this test with minimally induced damages to the skin. SQM proved to be a suitable method to evaluate the barrier protective effect of tannic acid in such "soft" test conditions.

CONCLUSION

SQM, as described here, was introduced in 1992 (8) to allow a quantitative follow-up of patients' skin treated for xerosis. Rapidly, it appeared that the method could be extremely useful to study the interaction of surfactant-based products with the SC (9) and compare their skin irritation potential. Since then, numerous applications of SQM have been investigated, often with great success.

SQM may be used to compare the skin hydrating potential of moisturizers, the squamolytic effect of products on SC scaliness or the skin barrier protective action of specific ingredients.

However, one of the major advantages of SQM over conventional techniques is its unique sensitivity to detect alterations of the SC by surfactant-based products. This property allowed scientists to design test procedures that are much less exaggerated and that approach normal use conditions for comparing surfactant-based products interaction with the skin. In all investigated procedures, SQM proved to be sensitive, accurate and reproducible and allowed testing products at a suberythematous level, without causing undue irritation.

Furthermore, SQM seems not to be limited for studying the irritation potential of surfactant-based products and was found to work as well as with household bleaching products.

More applications of SQM may be found in the literature. However, the objective of this review was to illustrate that SQM is an emerging technology that has to be taken into consideration in all skin clinical testing strategies where minimal alteration of the SC is induced while searching for a maximal discrimination power between test products.

REFERENCES

1. Frosch PJ, Kligman AM. The soap chamber test. A new method for assessing the irritancy of soaps. J Am Acad Dermatol 1979; 1:35–41.
2. Simion FA, Rhein LD, Grove GL, Wojtkowski JM, Cagan RH, Scala DD. Sequential order of skin responses to surfactants during a soap chamber test. Contact Dermat 1991; 25:242–249.
3. Piérard GE. EEMCO guidance for the assessment of skin colour. J Eur Acad Dermatol Venereol 1998; 10:1–11.
4. Berardesca E. EEMCO guidance for the assessment of stratum corneum hydration: electrical methods. Skin Res Technol 1997; 3:126–132.
5. Rogiers V and the EEMCO group. EEMCO guidance for the assessment of the transepidermal water loss in cosmetic sciences. Skin Pharmacol Appl Skin Physiol 2001; 14:117–128.
6. Parra JL, Paye M. EEMCO guidance for the in vivo assessment of skin surface pH. Skin Pharmacol Appl Skin Physiol 2003; 16:188–202.
7. Lévêque JL. EEMCO guidance for the assessment of skin topography. J Eur Acad Dermatol Venereol, 1999; 12:103–114.
8. Piérard GE, Piérard-Franchimont C, Saint-Léger D, Kligman AM. Squamometry: The assessment of xerosis by colorimetry of D-squame adhesive discs. J Soc Cosmet Chem 1992; 40:297–305.
9. Piérard GE, Goffin V, Piérard-Franchimont C. Squamometry and corneosurfametry for rating interactions of cleansing products with stratum corneum. J Soc Cosmet Chem 1994; 45:269–277.
10. Paye M, Cartiaux Y. Squamometry: A tool to move from exaggerated to more and more realistic application conditions for comparing the skin compatibility of surfactant-based products. Intl J Cosmet Sci 1999; 21:59–68.
11. Goffin V, Paye M, Piérard GE. Comparison of in vitro predictive tests for irritation induced by anionic surfactants. Contact dermat 1995; 33:38–41.
12. Götte E Van. Synthetische tenside in medizinisch-kosmetischen badern. Aesthet Medizin 1966; 10:313–319.
13. Blake-Haskins JC, Scala D, Rhein LD, Robbins CR. Predicting surfactant irritation from the swelling response of a collagen film. J Soc Cosmet Chem 1986; 37:199–210.
14. Tavss EA, Eigen E, Kligman AM. Anionic detergent-induced skin irritation and anionic detergent-induced pH rise of bovine albumin. J Soc Cosmet Chem 1988; 39:267–272.
15. Sharko PT, Murahata RI, Leyden JJ, Grove GL. Arm wash evaluation with instrumental evaluation—A sensitive technique for differentiating the irritation potential of personal washing products. J Derm Clin Eval Soc 1991; 2:19–27.
16. Clarys P, Manou I, Barel AO. Influence of temperature on irritation in the hand/forearm immersion test. Contact Dermat 1997; 36:240–243.
17. Piérard GE. EEMCO guidance for the assessment of dry skin (xerosis) and ichthyosis: evaluation by stratum corneum strippings. Skin Res Technol. 1996; 2:3–11.
18. Paye, M, Morrison BM Jr. Non visible skin irritation. Proceedings of the CESIO, Barcelona, Spain, 1996; 3:42–51.
19. Goffin V, Piérard-Franchimont C, Piérard GE. Anti-dandruff shampoos and the stratum corneum. J Dermatol Treat 1996; 7:215–218.
20. Morrison Jr BM, Cartiaux Y, Paye M, Charbonnier V, Maibach HI. Demonstrating invisible (subclinical) sodium lauryl sulfate irritation with squamometry. 56th Annual Meeting of the American Academy of Dermatology, Orlando, Florida, Feb 27–Mar 4, 1998.
21. Charbonnier V, Morrison BM Jr, Paye M, Maibach HI. Open application assay in investigation of subclinical irritant dermatitis induced by sodium lauryl sulfate (SLS) in man: advantage of squamometry. Skin Res Technol 1998; 4:244–250.

22. Paye M, Gomes G, Zerweck CR, Piérard GE, Grove GL. A hand immersion test under laboratory-controlled usage conditions: the need for sensitive and controlled assessment methods. Contact Dermat 1999; 40:133–138.
23. Morrison BM Jr, Paye M, Charbonnier V, Maibach HI. The effect of surfactants on skin as measured by squamometry: a novel way to observe sub-clinical irritant dermatitis. Proceedings of the IFSCC meeting, Orlando, 24–27 October 2004.
24. Charbonnier V, Morrison B M Jr, Paye M, Maibach HI. An open assay model to induce subclinical non-erythematous irritation. Contact Dermat 2000; 42:207–211.(2000).
25. Paye M, Charbonnier V, Morrison BM Jr, Maibach HI. Skin compatibility testing on human volunteers using squamometry. In: Clark D, Lisanski S, MacMillan R, eds. Alternatives to Animal Testing II. Proceedings of the COLIPA symposium on alternatives to animal testing. Newbury, UK: CPL Press, 1999:238–244.
26. Pierard GE, Pierard-Franchimont C. Drug and cosmetic evaluations with skin strippings. In: Wilhelm KP, Elsner P, Berardesca E, Maibach HI, eds. Dermatology Research Technique. Boca Raton: CRC Press, 1996:133–150.
27. Paye M, Dalimier C, Cartiaux Y, Chabassol C. Consumer perception of sensitive hands: what is behind?. Skin Res Technol 1999; 5:28–32.
28. Pierard GE, Estrada J, Rodriguez C, Daskaleros PA. Effects of softened and unsoftened fabrics on sensitive skin. Contact Dermat 1994; 30:286–291.
29. Rogiers V, Balls M, Basketter D, Berardesca E, Edwards C, Elsner P, Ennen J, Leveque JL, Loden M, Masson P, Parra J, Paye M, Pierard G, Rodrigues L, Schaeffer H, et al. Potential use of non-invasive methods in safety assessment of cosmetics. Report of ECVAM Workshop. ATLA, 1999; 27:515–537.
30. Shimizu T, Maibach HI. Squamometry: an evaluation method for a barrier protectant (tannic acid). Contact Dermat 1999; 40():189–191.
31. De Paepe K, Janssens K, Hachem JP, Roseeuw D, Rogiers V. Squamometry as a screening method for the evaluation of hydrating products. Skin Res Technol 2001; 7:184–192.
32. Pierard-Franchimont C, Pierard GE. Modulation of human stratum corneum properties by slicylic acid and all-*trans*-retinoic acid. Skin Pharmacol Appl Ski Physiol 1998; 11:266–272.
33. Goffin V, Pierard GE, Henry F, Letawe C, Maibach HI. Sodium hypochlorite, bleaching agents, and the stratum corneum. Ecotoxicol Environ Safety 1997; 37:199–202.
34. Pierard GE, Pierard-Franchimont C. Squamometry in acute photodamage. Skin Res Technol 1995; 1:137–139.
35. Pierard-Franchimont C, Henry F, Pierard GE. The SACD method and the XLRS squamometry tests revisited. Intl J Cosmet Sci 2000; 22:437–446.
36. Charbonnier V, Morrison BM Jr, Paye M, Maibach HI. Quantification of non-erythematous irritant dermatitis. In: Maibach HI, ed. Toxicology of Skin. Ann Arbor, Michigan: Taylor and Francis, 2000:31–38.
37. Rawling AV. Trends in stratum corneum research and the management of dry skin conditions. Intl J Cosmet Sci 2003; 25:63–95.
38. Horikoshi T, Igarashi S, Uchiwa H, Brysk H, Brysk MM. Role of endogenous cathepsin D-like and chymotrypsin-like proteolysis in human epidermal desquamation. Br J Dermatol 1999; 141:453–459.
39. J. Serup, EEMCO guidance for the assessment of dry skin (xerosis) and ichtyosis: clinical scoring systems, Skin Res. Technol. 1995; 1:109.
40. Kligman AM. Regression method for assessing the efficacy of moisturizers. Cosmet Toilet 1978; 93:27–32.
41. el Gammal C, Kligman AM, el Gammal S. Anatomy of the skin surface. In: Wilhelm KP, Elsner P, Berardesca E, Maibach HI, eds. Bioengineering of the skin: skin surface imaging and analysis. Boca Raton: CRC Press, 1996:3–19.
42. Manuskiatti Y, Schwindt A, Maibach HI. Influence of age, anatomic site and race on skin roughness and scaliness. Dermatology 1998; 196:401–407.

43. Serup J, Winther A, Blichmann C. A simple method for the study of scale pattern and effects of a moisturizer-qualitative and quantitative evaluation by D-squame tape compared with parameters of epidermal hydration. Clin Exp Dermatol 1989; 14:277–282.
44. Serup J. A three-hour test for rapid comparison of effects of moisturizers and active constituents (urea): measurements of hydration, scaling and skin surface lipidisation by non-invasive techniques. Acta Derm Venereol 1992; 177:29–33.
45. Pierard GE, Arrese JE, Dowlati A, Daskaleros PA, Rodriguez C. Effects of softened and unsoftened fabrics on infant skin. Intl J Dermatol 1994; 34:138–141.

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Tests for Sensitive Skin

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INTRODUCTION

Sensitive skin is a condition of subjective cutaneous hyperreactivity to environmental factors. Subjects experiencing this condition report exaggerated reactions when their skin is in contact with cosmetics, soaps, and sun screens, and they often report worsening after exposure to dry and cold climate.

Though, no sign of irritation is commonly detected, itching, burning, stinging, and a tight sensation are constantly present. Generally substances that are not commonly considered irritants are involved in this abnormal response. They include many ingredients of cosmetics such as dimethylsulfoxide (DMSO), benzoyl peroxide preparations, salicylic acid, propylene glycol, amyldimethylaminobenzoic acid, and 2-ethoxyethyl methoxycinnamate (1).

Sensitive skin and subjective irritation are widespread but still far from being completely defined and understood.

On the one hand, Burckhardt (2) hypothesized a correlation between sensitive skin and constitutional anomalies and/or other triggering factors such as occupational skin diseases or chronic exposure to irritants. On the other hand, Bjornberg (3) supported that no constitutional factors play a role in the pathogenesis of sensitive skin, though the presence of dermatitis demonstrates a general increase in skin reactivity to primary irritants lasting months.

EPIDEMIOLOGIC STUDIES

Recent findings suggest that higher sensitivity can be because of different mechanisms.

Hyperreactors may have a thinner stratum corneum with a reduced corneocyte area causing a higher transcutaneous penetration of water soluble chemicals (4).

Until 1977 Frosch and Kligman (5), by testing different irritants, showed a 14% incidence of sensitive skin in the normal population, likely correlated to a thin permeable stratum corneum which makes these subjects more susceptible to chemical irritation.

Many epidemiologic studies have been carried out to assess whether or not a correlation with sex, age, skin type, or race could be found (6).

Contradictory findings have been reported. Some authors (7–9) documented a higher reactivity to irritants mostly in females; some others noted that male subjects were directionally or significantly more reactive than female (6). Other experimental studies did not confirm this observation. Bjornberg (10), using six different irritants by patch test application, found no sex-related differences. Moreover Lammintausta (11), studying the response to open and patch test application of SLS, found mild interindividual variations in transepidermal water loss (TEWL) and dielectric water content values but no sex-related differences in the reaction pattern.

In 1982 Frosch (12), using DMSO, demonstrated a correlation between the minimal erythema dose (MED) and the response to irritants: the higher the inflammation, the lower the MED. Subsequently, a correlation between skin reactivity and skin type was reported: higher reactions were detected in subjects with skin type I (13). However in a total of 110 subjects covering all six skin types, the SLS dose response, generated applying the substance under four-hour occlusion, demonstrated that there was no significant difference between the groups. Even for type VI skin, the dose–response curve fell within the general pattern (14). In fact conflicting findings have been reported on incidence of allergic contact dermatitis in different races (15–18). While there is a clinical consensus that blacks are less reactive and Asians are more reactive than Caucasians, the data supporting this hypothesis rarely reach statistical significance (19). Conflicting data have also been found on subjective (sensory) irritation. Frosch reported that most common "stingers" were light-complexioned persons of Celtic ancestry who sunburned easily and tanned poorly (20). Grove found no skin-type propensity to stinging; he noted that increased stinging was related mainly to the person's history of sensitivity to soaps, cosmetics, and drugs (21). Arakami instead, found no significant differences after SLS testing, but significant subjective sensory differences between Japanese and German women. So Arakami concluded that Japanese women may complain about stronger sensations reflecting a different cultural behavior rather than measurable differences in skin physiology; however, a faster penetration of SLS in Japanese women cannot be excluded (18).

Moreover in eczema skin reactivity is enhanced (22). Studies performed on animal models demonstrated that strong irritant reactions in guinea pigs significantly reduced the threshold of skin irritation (23). On the other hand, hyporeactive states may be induced by skin treatment. Subclinical dermatitis, after repeated cutaneous irritation by open application, may induce skin hyporeactivity (24). This can also be one of the mechanisms of false negative patch test.

Skin reactivity seems also to change depending on age. The literature is contradictory. For example, Nilzen and Voss Lagerlung (25) reported higher reactivity patch test reactions to soaps and detergents in the elderly, while Bettley and Donoghue (26) reported a lower reactivity in the same group. Coenraads (27) demonstrated a higher skin reactivity to croton oil in the older patient group, but no differences by testing thimochinone or croton aldehyde. In 1993, Grove (28), by testing croton oil, cationic and anionic surfactants, weak acids and solvents, reported a lower susceptibility in older subjects in terms of less severe skin reactions. Recently Robinson (6) confirmed this less reactivity. In fact in his study the oldest age cluster of subjects (56–74 years of age) was directionally or significantly less reactive than younger age clusters. Moreover Wohrl noted that while the rate of positive reactions to nickel and thimerosal decreased with age, fragrance mix and metallic mercury stayed at the same level through all ages (9). The overall sensitization rate was highest in children less than 10 years and decreased steadily, to be lowest among patients more than 70 years.

Aged skin seems to have a reduced inflammatory response either to irritants or to irritation induced by UV light (29). The UVB-induced increase in both TEWL and DNA synthesis was significantly diminished, with decreased epidermal hyperplasia evident in intrinsically aged versus young mouse epidermis (30).

On the other hand, after irritating the skin, increased TEWL values were recorded in the older subjects compared to the young. This finding could be related to a deficient "early warning detection system" in the elderly.

The lack of any visible response can lead to a continued exposure to external irritants and to a higher risk of damage to skin barrier function.

CLINICAL PARAMETERS

Sensitive skin can be defined in both subjective and objective terms. Subjective perceptions of sensitive skin are derived from patient observations regarding stinging, burning, pruritus, and tightness following various environmental stimuli. Because of the lack of clinical signs the phenomenon of sensitive skin is difficult to document. Attempts to identify clinical parameters in subjects with subjective irritation indicate that these individuals tend to have a less hydrated, less supple, more erythematous, and more teleangiectatic skin, compared to the normal population. In particular, significant differences were found for erythema and hydration/dryness (31).

TESTS FOR SENSITIVE SKIN

Approximately 50% of patients with sensitive skin demonstrate their uncomfortable symptoms without accompanying visible signs of inflammation (32). For this reason, new methods of sensory testing have been increasingly utilized to provide definite information.

Quantification of Cutaneous Thermal Sensation

The superficial skin layer includes sensory nerve fibers connected to specialized receptors such as corpuscles or naked nerve endings. Three types of fibers are generally recognized in the sensory subclass of fibers:

- A-beta fibers, myelinated (conduction velocity of 2–30 m/sec), the largest fibers, mediate the touch, vibration, and pressure sensation.
- A-delta fibers, smaller and myelinated (conduction velocity of >30 m/sec), mediate cold and pain sensation.
- C fibers, slowest, smaller, and nonmyelinated (conduction velocity of <2 m/sec), mediate warm and itching sensation. C fibers subserve most of the autonomic peripheral functions.

Quantitative sensory testing (QST) methods have been utilized mainly to study the impairment of somatosensory function in neurological diseases; particularly in dermatology, thermal sensation testing analysis is becoming the most utilized QST technique (33). It assesses function in free nerve endings and their associated small myelinated and nonmyelinated fibers. Thermal somatosensory testing allows the clinician to test small nerve fibers. In this technique, thresholds for warmth, cold, as well as hot and cold pain are quantitatively measured and then compared to

age-matched normal population values. A deviation from the normal range can indicate the existence of peripheral nerve disease. A small device, called a Thermode, is attached to the patient's skin. The device is capable of heating or cooling the skin, as needed. Technically, the thermode is based on Peltier elements. It consists of semiconductor junctions which produce a temperature gradient between the upper and lower stimulator surfaces produced by the passage of an electric current.

In the center of the thermode a thermocouple records the temperature.

TSA-II (Medoc Company, Ramat Yshai, Israel) is considered one of the most advanced portable thermal sensory testing devices. It operates between 0 and 54°C.

The TSA-II measures thresholds for four sensory submodalities:

- Warm sensation, usually at 1 to 2°C above adaptation temperature. This is a C fiber mediated sensation.
- Cold sensation, at a similar range below adaptation, mediated by A-delta fibers.
- Heat induced pain, threshold around 45°C, a mostly C fiber mediated sensation, with some involvement of A-delta fibers.
- Cold induced pain, the most variable and difficult to assess of all previous modalities, at about 10°C; mediated by a combination of both C and A-delta fibers.

Basically it measures the hot or cold threshold and the suprathreshold pain magnitude. The thermode in contact with the skin produces a stimulus whose intensity increases or decreases until the subject feel the sensation. As the sensation is felt the subject is asked to press a button. The test is then repeated two more times to get a mean value. Using this method, artifacts can occur owing to the lag time the stimulus needs to reach the brain. This inconvenience can be avoided by using relatively slow rates of increasing stimuli.

The stimulus can also be increased stepwise and the subject is told to say whether or not the sensation is felt. When a positive answer is given, the stimulus is decreased by one half the initial step and so on, until no sensation is felt. The subject's response determines the intensity of the next stimulus. The limitation of this second method is that a longer performance time is required.

Stinging Test

Stinging seems to be a variant of pain that develops rapidly and fades quickly any time the appropriate sensory nerve is stimulated. Though this method lacks objective criteria, it is widely accepted as a marker of sensitivity and has often been utilized in skin irritation studies (5,31). It provides information to establish those subjects experiencing invisible cutaneous irritation.

It is performed by applying to the skin hydrosoluble substances such as lactic acid or capsaicin. The test is usually carried out on the nasolabial fold, a site richly innervated with sensory fibers. This may be performed utilizing two methods (34):

1. Subjects first undergo a facial sauna for 5 to 15 minutes or are conditioned to a state of profuse sweating in an environmental chamber at 110°F and 80% relative humidity; then an aqueous 5% lactic acid solution is applied with a cotton swab on the test site.
2. A 10% aqueous solution of lactic acid at room temperature is rubbed with a cotton swab on the test site.

To have a more reliable response, it is recommended to apply an inert control substance, such as saline solution, to the controlateral test site. After application, within a few minutes, a moderate to severe stinging sensation occurs for the "stingers group." These subjects are then asked to describe the intensity of the sensation using a point scale. Hyperreactors, particularly those with a positive dermatologic history, have higher scores. An alternative test involves the application of 2 ml of 90% aqueous DMSO in a small glass cup on the cheek for five minutes. This procedure causes intense burning in stingers and, after application, tender wheal and persistent erythema often occur. By contrast, lactic acid produces no visible changes.

Using this screening procedure, 20% of the subjects exposed to 5% lactic acid in a hot, humid environment were found to develop a stinging response (5). Lammintausta et al. confirmed these observations (35). In this study 18% of subjects were identified as stingers. In addition, stingers were found to develop stronger reactions to materials causing nonimmunologic contact urticaria, to have increased values of TEWL and increased blood flow velocimetry values after application of an irritant under patch test.

Nicotinate Test and Erythema Following Sodium Lauryl Sulfate Occlusion Test

A different approach to identifying sensitive skin patients relies on vasodilatation of the skin as opposed to cutaneous stinging. Many investigators prefer this approach because objective changes can be visually and biomechanically assessed. These two tests are the nicotinate test (36) and erythema assessment following sodium lauryl sulfate exposure (37). In the first test, methyl nicotinate, a potent vasodilator, is applied to the upper third of the ventral forearm in concentrations varying between 1.4% and 13.7% for a period of 15 seconds. The vasodilatory effect is assessed by observing the erythema and employed laser doppler velocimetry (LDV). Similar analysis can be performed following application of various concentrations of sodium lauryl sulfate to the forearm.

Evaluation of Itching Response

Recent studies show that a new class of C fibers with an exceptionally lower conduction velocity and insensitivity to mechanical stimuli can be likely considered as afferent units which mediate the itchy sensation (38).

Indeed this subjective feeling has been extensively investigated but no explanation of the individual susceptibility to the itching sensation, without any sign of coexisting dermatitis, has been found. Laboratory investigation of the itch response has also been limited.

An itch response can be experimentally induced by topical or intradermal injections of various substances such as proteolytic enzymes, mast cell degranulators, and vasoactive agents.

Histamine injection is one of the more common procedures: histamine dihydrochloride (100 μg in 1 ml of normal saline) is injected intradermally in one forearm. Then, after different time intervals, the subject is asked to indicate the intensity of the sensation using a predetermined scale and the duration of itch is recorded. Information is always gained by the subject's self-assessment.

A correlation between whealing and itching response produced by applying a topical 4% histamine base in a group of healthy young females has been investigated by Grove. The itching response was graded by the subjects using the following

scale: none, slight, moderate, and intense. The data showed that, despite the fact that 90% of the wheals were greater than 8 mm in diameter, only 50% of the subjects experienced pruritus; patients with large wheals often had no complaints of itching, suggesting that the dimensions of the wheals do not correlate well with pruritus.

Also itch and sting perceptions seem to be poorly correlated.

Grove (28) compared the cumulative lactic acid sting scores with the histamine itch scores in 32 young subjects; all the subjects who were stingers were also moderate to intense itchers, while 50% of the moderate itchers showed little or no stinging response. Yosipovitch (39), studying the effects of drugs on C fibers during experimentally induced itch, demonstrated that topically applied aspirin rapidly decreases histamine-induced itch. This result can be attributed to the role that prostaglandins play in pain and itch sensation (40).

Localized itching, burning, and stinging can be also a feature of nonimmunologic contact urticaria. This condition, still not completely defined, is characterized by a local wheal and flare after exposure of the skin to certain agents. Different combinations of mediators such as nonantibody-mediated release of histamine, prostaglandins, leukotriens, substance P, and other inflammatory mediators may likely be involved in the pathogenesis of this disorder (41). The fact that prostaglandins and leukotriens may play a role in the inflammatory response is supported by the inhibition of the common urticants by both oral acetylsalicylic acid and indomethacin and by topical diclofenac and naproxen gel (1). Several substances such as benzoic acid, cinnamic acid, cinnamic aldehyde, and nicotinic acid esters are capable of producing contact nonimmunologic urticaria, eliciting local edema and erythematous reactions in half of the individuals. Provocative tests are usually utilized to identify subjects experiencing this condition: benzoic acid, sorbic acid, or sodium benzoate in open application will reproduce the typical symptoms in subjects suspected of contact nonimmunologic urticaria.

Washing and Exaggerated Immersion Tests

The aim of these tests is to identify a subpopulation with an increased tendency to produce a skin response.

In the washing test (42), subjects are asked to wash their face with a specific soap or detergent. After washing, individual sensation for tightness, burning, itching, and stinging, is evaluated using a point scale previously determined.

The exaggerated immersion test is based on soaking the hands and forearms of the subjects in a solution of anionic surfactants (such as 0.35% paraffin sulfonate, 0.05% sodium lauryl sulfate-2EO) at 40°C, for 20 minutes.

After soaking, hands and forearms are rinsed under tap water and patted dry with a paper towel. This procedure is repeated two more times, with a two-hour period between each soaking, for two consecutive days. Prior to the procedure, baseline skin parameters are evaluated. The other evaluations are taken two hours after the third and sixth soakings and 18 hours after the last soaking (recovery assessment). All of the skin parameters are performed after the subjects have rested at least 30 minutes at 21 ± 1°C.

Bioengineering Tests

Recently there is a great interest in the development of noninvasive mechanistic skin assessment allowing presumably more accurate evaluation of small cutaneous

changes. Bioengineering tests should be able to measure preclinical disease without altering the underlying skin condition. So, the physiological changes indicative of sensitive skin can be detected at low levels prior to clinical disease presentation (43). The most useful tests are TEWL, corneometry, colorimetry, and LDV.

TEWL is used to evaluate water loss that is not attributed to active sweating from the body, through the epidermis, to the environment. So, it is widely used to characterize the stratum corneum barrier function, both in physiological and pathological conditions, to perform predictive irritancy tests, and to evaluate the efficacy of therapeutic treatments on diseased skin (44,45). TEWL assessment can be performed using different techniques (closed chamber method, ventilated chamber method, and open chamber method) (46,47). The measurements of TEWL is based on the estimation of water pressure gradient above the skin surface. The evaporative TEWL is approximately proportional to the difference between the vapor pressures measured at two different fixed heights situated perpendicularly above the skin surface and within the zone of diffusion. These open chamber instruments consists of a detachable measuring probe connected by a cable to a portable main signal processing unit. The Teflon® capsule of the probe head has a cylindrical measuring chamber, open at both ends where relative humidity sensors (hygrosensors) are paired with temperature sensors (thermistors). From this gradient, the evaporative TEWL value, in $g/m^2/hr$, is calculated by the signal processing units in the probe handle and main unit, and digitally displayed. The instrument is extremely sensitive to any disturbances in the microclimate, whether owing to environment, instrument, or individual related variables.

By the use of a closed chamber instrument the disturbance related to external or body-induced airflows on the measurement can be avoided. The instrument consists of a closed cylindrical chamber with an air volume of $2.0\,cm^3$ and an open contact area of $1.0\,cm^2$. The chamber contains sensors for relative humidity and temperature. The humidity sensor is based on a thin-film capacitive sensor with features of rapid response time, insensitive to temperature changes in the chamber between 15 and 40°C. The sensor was directly integrated to a hand-held microprocessor-controlled electronic unit provided with a digital readout for the TEWL value.

Corneometry is a method for measuring stratum corneum water content (electrical measurement) (48,49). The importance of water to the proper functioning of the SC is well recognized. The reliable quantification of water in the corneum and its interaction with topically applied products is, in fact, essential for understanding skin physiology and developing efficient skin care formulation. This instrument is described as being a "capacitance" measuring device operating at low frequency (40–75 MHz), which is sensitive to the relative dielectric constant (or permittivity) of material placed in contact with the electrode surface. Because increasing the water content of the stratum corneum will in general increase its relative permittivity (although by a very complex and variable relationship), the device can therefore estimate in about 20 milliseconds the stratum corneum water content in arbitrary (relative) units. However, it should be noted that this result is based on the assumption of ceteris paribus, which may not always be valid. The probe exerts a constant pressure on the skin surface of 3.5 N, and covers an area of $49\,mm^2$. It estimates water content in the epidermis to an approximate depth ranging between 60 and 100 μm. The presence of salts or ions on the surface of the skin tends to affect the readings. The instrument consists of a probe that should be placed normally to a hair-free skin surface with slight pressure just sufficient to start the measurement process. It is advisable to measure at least three times, once at each of three different but nearby sites, and calculate the median, to have more reproducible data (50).

LDV. A monochromatic light from a helium–neon laser is transmitted through optical fibers to the skin. The light is reflected with Doppler shifted frequencies from the moving blood cells in the upper dermis at the depth of ~1 mm. The LDV extracts the frequency-shifted signal and derives an output proportional to the flux of erythrocytes in the blood flow. The shift increases with the increasing velocity. In a mechanical model simulating the microvascular pattern of the skin, a linear relationship between LDV and blood flow was detected for low and moderate flow rates. For higher flow, photo multiple scattering and increased light absorption owing to higher erythrocyte volume fraction cause a slight underestimation of the blood flow (51). LDV seems useful in discriminating between negative and positive reactions but fails to quantify strongly positive reactions (52,53). LDV is useful in evaluating the degree of skin irritation (54). The degree of the experimentally induced irritant contact dermatitis usually correlates well with blood flow detected by LDV; however, dithranol and sodium hydroxide may give discordant results. Although LDV can be used to quantify the strength of allergic and irritant skin reactions, the technique cannot discriminate between these two types of reaction (53). LDV is one of the most important parameters to predict early signs of skin irritation (55).

Colorimetry. Surface color may be quantified using the Commission Internationale de L'Eclairage (CIE) system of tristimulus values. Commercially available devices utilizing high sensitivity silicon photocells assure good reproducibility and accuracy. The measuring head of these units contains a high power pulsed xenon arc lamp which provides two CIE illuminant standards. The color is expressed in a three dimensional space. The coordinates of such space are expressed as L (brightness, i.e., integrated reflection of light from the surface), a^* value (color range from green to red) and b^* value (color range between blue and yellow). Natural skin tones can be stored in the colormeter memory for direct comparison. The colormeter allows for quantitative comparison of erythema in individuals and between individuals comparable with visual assessment: the a^* value, related to skin erythema, shows an increase in relation to irritation and skin damage (56).

Corneosurfametry. This method investigates the interaction of surfactants with the human stratum corneum, using the reflectance colorimetry (57). It is performed as follows: cyanoacrylate skin surface stripping is taken from the volar aspect of the forearm and sprayed with the surfactant to be tested. After two hours the sample is rinsed with tap water and stained with basic fuchsin and toluidine blue dyes for three minutes. After rinsing and drying the sample is placed on a white reference plate and measured by reflectance colorimetry (Chroma Meter® CR200, Minolta, Osaka, Japan).

The index of redness [colorimetric index of mildness (CIM) = luminance L^* − chroma C^*] is taken as a parameter of the irritation caused by the surfactant. This index has a value of 68 ± 4 when water alone is sprayed on the sample and decreases when the surfactant is tested, with stronger surfactants lowering the values.

Piérard et al. (58), testing different shampoo formulations in volunteers with sensitive skin, demonstrated that corneosurfametry correlates well with in vivo testing. A significant negative correlation ($p < 0.001$) was found between values of CIM and the skin compatibility parameters that include a global evaluation of the colorimetric erythemal index and the TEWL differential, both expressed in the same order of magnitude.

In the same study corneosurfametry showed less interindividual variability than in vivo testing, allowing a better discrimination among mild products.

An interesting finding showed that sensitive skin is not a single condition. Goffin (59) hypothesized that the response of the stratum corneum to an environmental threat might be impaired in different groups of subjects experiencing sensitive skin. Data of the corneosurfametry performed after testing eight different house cleaning products showed that the overall stratum corneum reactivity, as calculated by the average values of the corneosurfametry index and the CIM, is significantly different ($p < 0.01$) between detergent-sensitive skin and both nonsensitive and climate/fabric sensitive skin, as well.

CONCLUSIONS

Sensitive skin represents a widespread condition of susceptibility to exogenous factors. The reason why some subjects react with subjective symptoms like itching, burning, stinging, prickling, or tingling, is unclear. However, a correlation of increased reactivity in subjects with a history of dermatitis and the association of increased reactivity with skin type I has been reported. Noninvasive evaluation of sensitive skin may successfully predict individual susceptibility to cosmetic-related adverse reaction. All of the efforts in this direction appear undoubtedly important to improve tolerance to the majority of cosmetic products.

REFERENCES

1. Amin S, Engasser PG, Maibach HI. Side-effects and social aspect of cosmetology. In: Baran R, Maibach HI, eds. Textbook of Cosmetic Dermatology.
2. Burckhardt W. Praktische und theoretische bedeutung der alkalineutralisation und alkaliresistenzproben. Arch Klin Exp Derm 1964; 219:600–603.
3. Bjornberg A. Skin reactions to primary irritants in patients with hand eczema. Goteborg: Isaccsons, 1968.
4. Berardesca E, Cespa M, Farinelli N, Rabbiosi G, Maibach HI. In vivo transcutaneous penetration of nicotinates and sensitive skin. Contact Dermatitis 1991; 25:35–38.
5. Frosch PJ, Kligman AM. A method for appraising the stinging capacity of topically applied substances. J Soc Cosmet Chem 1977; 28:197–209.
6. Robinson MK. Population differences in acute skin irritation responses. Contact Dermatitis 2002; 46(2):86–93.
7. Agrup G. Hand eczema and other hand dermatoses in South Sweden. Academic dissertation. Acta-Dermato-Venereol 1969; 49(suppl):161.
8. Fregert S. Occupational dermatitis in 10 years material. Contact Dermatitis 1975; 1:96–107.
9. Wohrl S, Hemmer W, Focke M, et al. Patch testing in children, adults, and the elderly: influence of age and sex on sensitization patterns. Pediatr Dermatol 2003; 20(2):119–123.
10. Bjornberg A. Skin reactions to primary irritants in men and women. Acta Dermato-Venereol 1975; 55:191–194.
11. Lammintausta K, Maibach HI, Wilson D. Irritant reactivity in males and females. Contact Dermatitis 1987; 17:276–280.
12. Frosch K, Wissing C. Cutaneous sensitivity to ultraviolet light and chemical irritants. Arch Dermatol Res 1982; 272:269–278.
13. Lammintausta K, Maibach HI, Wilson D. Susceptibility to cumulative and acute irritant dermatitis. An experimental approach in human volunteers. Contact Dermatitis 1988; 19:84–90.
14. McFadden JP, Wakelin SH, Basketter DA. Acute irritation thresholds in subjects with type I-type skin. Contact Dermatitis 1998; 38(3):147–149.

15. Berardesca E, Maibach H. Ethnic skin: overview of structure and function. J Am Acad Dermatol 2003; 48:S139–S142.
16. Berardesca E, Maibach HI. Contact dermatitis in blacks. Dermatol Clin 1998; 6(3):363–368.
17. Robinson MK. Racial differences in acute and cumulative skin irritation responses between Caucasian and Asian populations. Contact Dermatitis 2000; 42(3):134–143.
18. Arakami J, Kawana S, Effendy I, et al. Differences of skin irritation between Japanese and European women. Br J Dermatol 2002; 146(6):1052–1056.
19. Modjtahedi SP, Maibach HI. Ethnicity as a possible endogenous factor in irritant contact dermatitis: comparing the irritant response among Caucasian, blacks and Asians. Contact Dermatitis 2002; 47(5):272–278.
20. Frosch P, Kligman AM. A method for appraising the stinging capacity of topically applied substances. J Soc Cosmet Chem 1981; 28:197.
21. Grove GL, Soschin DM, Kligman AM. Adverse subjective reactions to topical agents. In: Drill VA, Lazar P, eds. Cutaneous Toxicology. New York: Raven Press, 1984: 200–210.
22. Bettley FR. Non specific irritant reactions in eczematous subjects. Br J Dermatol 1964; 76:116–121.
23. Roper SS, Jones EH. An animal model for altering the irritability threshold of normal skin. Contact Dermatitis 1985; 13:91–97.
24. Lammintausta K, Maibach HI, Wilson D. Human cutaneous irritation: induced hyporeactivity. Contact Dermatitis 1987; 17:193–198.
25. Nilzen A, Voss Lagerlund K. Epicutaneous tests with detergents and a number of other common allergens. Dermatologica 1962; 124:42–52.
26. Bettley FR, Donoghue E. The irritant effect of soap upon the normal skin. Br J Dermatol 1960; 72:67–76.
27. Coenraads PJ, Bleumink E, Nofer JP. Susceptibility to primary irritants. Age dependence. Contact Dermatitis 1975; 1:377–381.
28. Grove GL. Age-associated changes in intertegumental reactivity. In: Léveque JL, Agache PG, eds. Aging Skin. Properties and Functional Changes. New York, Basel, Hong Kong, 1993.
29. Haratake A, Uchida Y, Mimura K, et al. Intrinsically aged epidermis displays diminished UVB-induced alterations in barrier function associated with decreased proliferation. J Invest Dermatol 1997; 108(3):319–323.
30. Gilchrest BA, Stoff JS, Soter NA. Chronologic aging alters the response to ultraviolet-induced inflammation in human skin. J Invest Dermatol 1982; 79:11–15.
31. Seidenari S, Francomano M, Mantovani L. Baseline biophysical parameters in subjects with sensitive skin. Contact Dermatitis 1998; 38:311–315.
32. Simion FA, Rau AH. Sensitive skin. Cosmet Toilet 1994; 109:43–50.
33. Yosipovitch G, Yarnitsky D. Quantitative sensory testing. In: Maibach HI, Marzulli FN, eds. Dermotoxicology Methods: The Laboratory Worker's Vade Mecum. New York: Taylor & Francis, 1997.
34. Facial Sting Task Group, ASTM Committee, E-18.03.01.
35. Lammintausta K, Maibach HI, Wilson D. Mechanisms of subjective (sensory) irritation: propensity of non immunologic contact urticaria and objective irritation in stingers. Dermatosen in Beruf und Umwelt 1988; 36(2):45–49.
36. Guy RH, Maibach HI. Rapid radial transport of methyl nicotinate in the dermis. Arch Dermatol Res 1982; 273:91–95.
37. Agner T, Serup J. Skin reaction to irritants assessed by non-invasive bioengineering methods. Contact Dermatitis 1989; 20:352–359.
38. Schmelz M, Schmidt R, Bickel A, Handwerker HO, Torebjörk HE. Specific C-receptors for itch in human skin. J Neurosci 1997; 17(20):8003–8008.
39. Yosipovitch G, Ademola J, Ping Lui, Amin S, Maibach HI. Topically applied aspirin rapidly decreases histamine-induced itch. Acta Dermato-Venereol (Stockh) 1977; 77:46–48.

40. Lovell CR, Burton PA, Duncan EH, Burton JL. Prostaglandins and pruritus. Br J Dermatol 1976; 94:273–275.
41. Lahti A, Maibach HI. Species specificity of nonimmunologic contact urticaria: guinea pig, rat and mouse. J Am Acad 1985; 13:66–69.
42. Hannuksela A, Hannuksela M. Irritant effects of a detergent in wash and chamber tests. Contact Dermatitis 1995; 32:163–166.
43. Andreassi L. Bioengineering in dermatology: general aspects and perspectives. Clin Dermatol 1995; 13(4):289–292.
44. Pinnagoda J, Tupker RA, et al. Guidelines for transepidermal water loss (TEWL) measurement. A report from the Standardization Group of the European Society of Contact Dermatitis. Contact Dermatitis 1990; 22(3):164–178.
45. Berardesca E, Vignoli GP, et al. Effects of water temperature on surfactant-induced skin irritation. Contact Dermatitis 1995; 32(2):83–87.
46. Wilson DR, Maibach H. Transepidermal water loss: a review. In: Lévêque JL, ed. Cutaneous Investigation in Health and Disease. Noninvasive Methods and Instrumentation. New York: Marcel Dekker Inc., 1989:113–133.
47. Lévêque JL. Measurement of transepidermal water loss. In: Lévêque JL, ed. Cutaneous Investigation in Health and Disease. Noninvasive Methods and Instrumentation. New York: Marcel Dekker Inc., 1989:135–153.
48. Fluhr J, Gloor M, Lazzerini S, et al. Comparative study of five instruments measuring stratum corneum hydration (corneometer CM 820 and CM 825, Skicon 200, Nova DPM 9003, DermaLab). Part I. In vitro. Skin Res Technol 1999; 5:161–170.
49. Barel AO, Clarys P. In vitro calibration of the capacitance method (Corneometer CM 825) and conductance method (Skicon-200) for the evaluation of the hydration state of the skin. Skin Res Technol 1997; 3:107–113.
50. Berardesca E. EEMCO guidance for the assessment of the stratum corneum hydration: electrical methods. Skin Res Technol 1997; 3:126–132.
51. Andersen KE, Staberg B. Quantization of contact allergy in guinea pigs by measuring changes in skin blood flow and skin fold thickness. Acta Dermato-Venereol 1985; 65(1):37–42.
52. Serup J, Staberg B. Quantification of weal reactions with laser Doppler flowmetry. Comparative blood flow measurements of the oedematous centre and the perilesional flare of skin-prick histamine weals. Allergy 1985; 40(4):233–237.
53. Staberg B, Serup J. Allergic and irritant skin reactions evaluated by laser Doppler flowmetry. Contact Dermatitis 1988; 18(1):40–45.
54. Bircher A, De Boer EM, et al. Guidelines for measurement of cutaneous blood flow by laser Doppler flowmetry. A report from the Standardization Group of the European Society of Contact Dermatitis. Contact Dermatitis 1994; 30(2):65–72.
55. Zuang VR, Archer G, Berardesca E. Detection of skin irritation potential of cosmetics by non invasive measurements. Skin Pharmacol Appl Skin Physiol 2000; 13:358–371.
56. Agner T, Serup J. Sodium lauryl sulphate for irritant patch testing a dose response study using bioengineering methods for determination of skin irritation. J Invest Dermatol 1990; 95(5):543–547.
57. Piérard GE, Goffin V, Piérard-Franchimont C. Corneosulfametry: a predictive assessment of the interaction of personal care cleansing products with human stratum corneum. Dermatology 1994; 189:152–156.
58. Piérard GE, Goffin V, Hermanns-Le T, Arrese JE, Piérard-Franchimont C. Surfactant-induced dermatitis: comparison of corneosurfametry with predictive testing on human and reconstructed skin. J Am Acad Dermatol 1995; 33:462–469.
59. Goffin V, Piérard-Franchimont C, Piérard GE. Sensitive skin and stratum corneum reactivity to household cleaning products. Contact Dermatitis 1996; 34:81–85.

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Tests for Skin Hydration

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INTRODUCTION

Writing about skin hydration means simultaneously writing about dry skin and its treatment by moisturizers. Dry skin has never really been defined in a repeatable way. In fact, this expression prejudices into believing that the skin does have reduced water content, although this was never confirmed or denied. Generally speaking, dry skin signifies that the skin surface looks as though it is lacking in water, this being reinforced by the pharmacological effect of hydrating the skin surface by appropriate treatments.

Experimental models used for measuring skin hydration are basically clinical models with or without noninvasive bioengineering measurements. To ensure meaningful results, the outlines of the intended studies should be of modern design such as blinding, randomization, and a suitable statistical control (particularly if different products are to be compared). This last point means including a predetermined adequate number of subjects in the study. The general ethical and legal frames of such clinical studies required for claim support are well defined in corresponding monographs or publications covering extensively the general procedures to be followed and the prerequisite information needed about the products to be tested (1–3).

Regardless of the method used, a further important point concerns standardization of the experimental conditions. To obtain acceptable and reproducible results, measurements should be performed with relaxed patients and/or volunteers already acclimatized for at least 30 minutes to controlled ambient temperature and relative humidity conditions. Both factors mainly affect sweat-gland activity, but other parameters should equally be considered with attention to, e.g., anatomical skin site, test products remaining or not on the skin, and correct handling of the measuring equipment if any. All these possible influences on measurement outcome have been discussed in detail in recent guidelines and in pertinent reviews (4–7).

A CLINICAL EVALUATION: THE REGRESSION METHOD

The dermatologist is perfectly able to clinically grade a given state of skin dryness (e.g., surface roughness, squames, and fissures). Clinical evaluation and grading of skin hydration is based on visual and tactile evaluation of clinical signs. There are

numerous possibilities of testing, but basically they rely on the regression method, published in 1978 by Kligman (8), which is still used as an industry standard. Briefly, female subjects with moderate to severe xerosis of the legs are selected following strict criteria. The test products are applied under controlled conditions by trained employees twice daily five days a week for three weeks. Three days after treatment ends, the follow-up period begins. Scoring is also completed three and seven days later. Treatment period may be shortened to two weeks if necessary. A recent guideline ensures that clinical scoring of the hydration state of the skin surface will be conducted based on the same definitions (4). Caution is given upon scoring by the subjects themselves, as their perception of their skin condition may not be the same as the dermatologist's (4,9).

INCORPORATING BIOENGINEERING METHODS

A large number of bioengineering methods are now available to evaluate hydration (or dryness) of the skin directly or indirectly. Inclusion of these methods in the study protocol opens many possibilities for getting meaningful results such as design variations, optimization of the claim support, and also, most importantly, improvement of cost effectiveness by shortening the duration of the experiment, using a lesser number of subjects, and strengthening the statistical evaluation.

Concerning the numerous techniques available for the evaluation of skin hydration, the reader is referred to recent monographs describing these methods in a detailed fashion (9–15). They mainly include measurements of electrical properties, spectroscopic methods such as infrared absorption spectroscopy and emission, evaluation of the barrier function of the stratum corneum (SC), measurement of mechanical properties, nuclear magnetic resonance imaging, skin-surface topography, and scaling evaluation. However, in this short review, examples of possible designs will be given that use bioengineering techniques based only on the electrical properties of the SC or on measurement of transepidermal water loss (TEWL; for a review of modem suitable measuring equipment see Refs. 9,13–15).

Static Measurements

Short-Term Tests: Single Application

The tests are conducted, e.g., on the forearm of healthy subjects and allow a randomized side-to-side comparison of test products with a placebo or vehicle, a known active product, and untreated control skin. Four to six products may be simultaneously tested. The products are applied at the rate of $2\,mg/cm^2$. Two different experimental designs may be used:

1. The test products are left in place for one hour [or another suitable duration, e.g., three hours (16)]. Measurements are conducted at different times thereafter. Removal of excess or nonpenetrated product is preferable before measuring, especially if the preparation contains a high proportion of lipids. Most moisturizers show a rapid increase of measured hydration values (Fig. 1).
2. The test products may be applied on similar areas at the same rate but under occlusion with a standard occluding patch overnight for 16 hours. The next morning, measurements are conducted in the same way as in

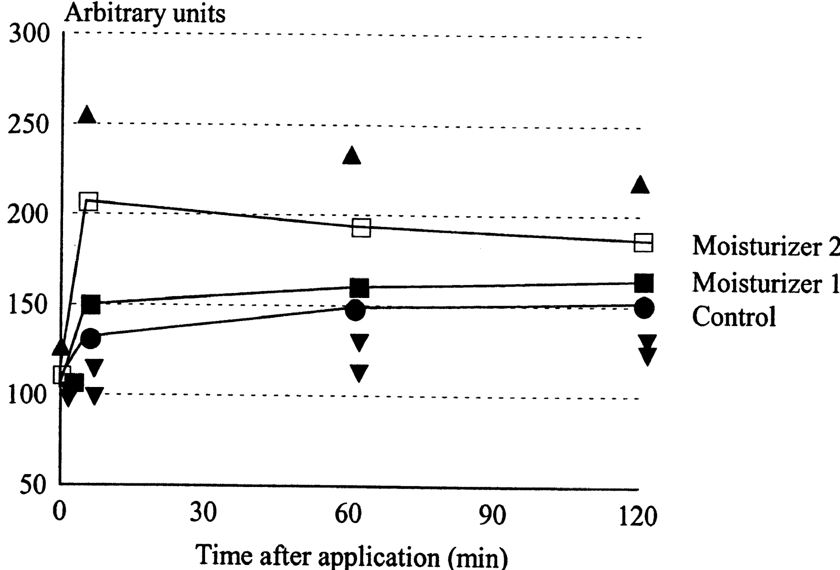

Figure 1 Example of hydration changes over time after one-hour application of two different O/W moisturizers containing both 2% urea as humectant (measurements conducted with the NOVA DPM 2003; means $\pm \frac{1}{2}$ SD: ▲ ▼). ■: moisturizer 1; □: moisturizer 2; •: control (untreated skin). Start values (time = 0) measured before application of the products.

part 1 beginning one hour after removal of the occlusion patch (Fig. 2). This last procedure picks up the activity of a humectant contained in the test preparation better, whereas the vehicle effect is strongly attenuated by the uniform conditions encountered under the occlusion patch.

Long-Term Tests: Multiple Applications

The design of these tests and selection of subjects are similar to the regression method previously described but with a modified and shortened regression protocol (17). The treatment period extends over one week only, and the regression phase takes place over the following week. Bioengineering measurements are conducted 12 to 16 hours after the treatment or moisturizer application, and for the last time on the Monday following the regression week. Inclusion of these noninvasive measurements allowed rapid and reliable product-performance evaluation.

Dynamic Measurements

These tests, in addition to the classic evaluation of skin hydration, provide information on dynamic properties of the SC (18–20). These properties are likely to be modified by the humectants (e.g., glycerol, urea, alpha-hydroxy acids, etc.) incorporated in the moisturizers used for treatment. Generally speaking, dynamic function tests are characterized by the assessment of the skin response to a given external stimulus that can be of physical (e.g., water, occlusion, stretch, heat) or chemical (e.g., drugs, irritants) nature. These dynamic tests may be used either during short-term or long-term product testing, and will usually be performed before and at different time points after treatment.

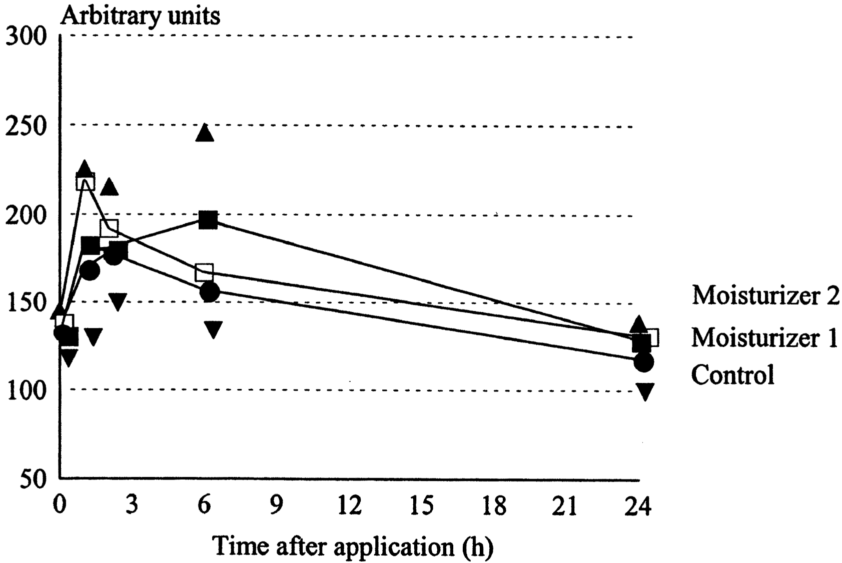

Figure 2 Example of hydration changes over time after 16-hour application of two different O/W moisturizers containing both 2% urea as humectant (same products as in Fig. 1; measurements conducted with the NOVA DPM 2003; means $\pm \frac{1}{2}$ SD: ▲ ▼). ■: moisturizer 1; □: moisturizer 2; •: control (untreated skin). Start values (time = 0) measured before application of the products.

The Sorption-Desorption Test

This test gives information about the water-binding capacity of the uppermost layers of the SC (18,20,22). It is best conducted using measurement devices that are able to measure hydration on a wet surface and that give instantaneous readings on contact with the skin. This first value represents the hydration state of the SC. Then 50 µl of distilled water are pipetted onto the skin, left in place for exactly 10 seconds, wiped with a soft paper towel, and then hydration is immediately measured. This value represents the hygroscopic capacity of the superficial SC. Further measurements are taken at 0.5, 1, 1.5, and 2 minutes. The area under the curve from 0.5 minutes onwards represents the water-holding capacity of the superficial SC (Fig. 3).

The Moisture-Accumulation Test

This test gives information about the quantity of moisture the SC may accumulate during a given time (19,20,22). This test is conducted with a device able to measure continuously after bringing the probe in contact with the skin surface. The probe then remains on the skin for three minutes, thereby creating occlusive conditions. The Moisture-accumulation test measures the accumulation of water under the probe every 0.5 minutes. Water accumulation is evaluated by calculating the area under the time curve until three minutes (Fig. 4).

The Plastic Occlusion Stress Test

The plastic occlusion stress test may also be considered a dynamic test and gives information about SC hydration, integrity of the barrier function, and SC water-holding capacity (21,22). It consists of occluding the skin with a plastic chamber (e.g., Hilltop

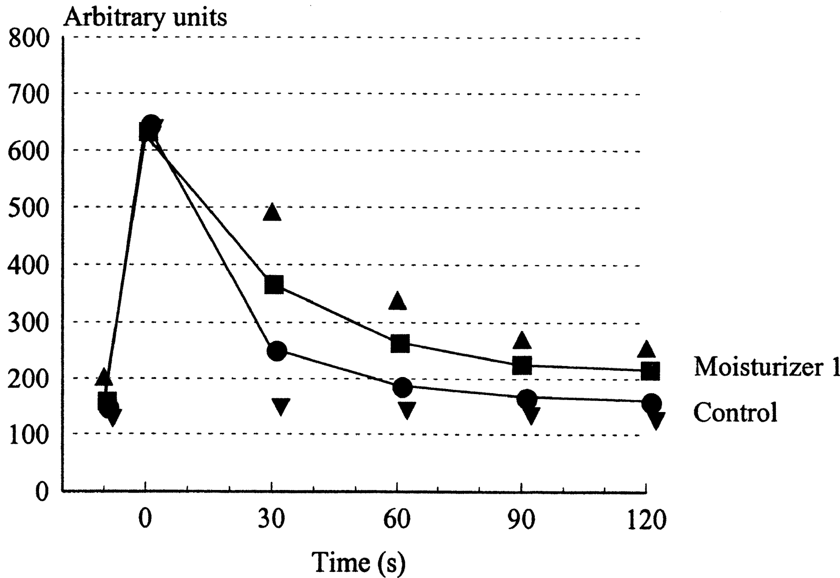

Figure 3 Time course of hydration changes during a SDT performed 60 minutes after a single one-hour application of a moisturizer (moisturizer 1 from Figs. 1 and 2; measurements conducted with the NOVA DPM 2003; means $\pm \frac{1}{2}$ SD: ▲). ■: moisturizer 1; •: control (untreated skin). *Abbreviations*: SDT, sorption–desorption test.

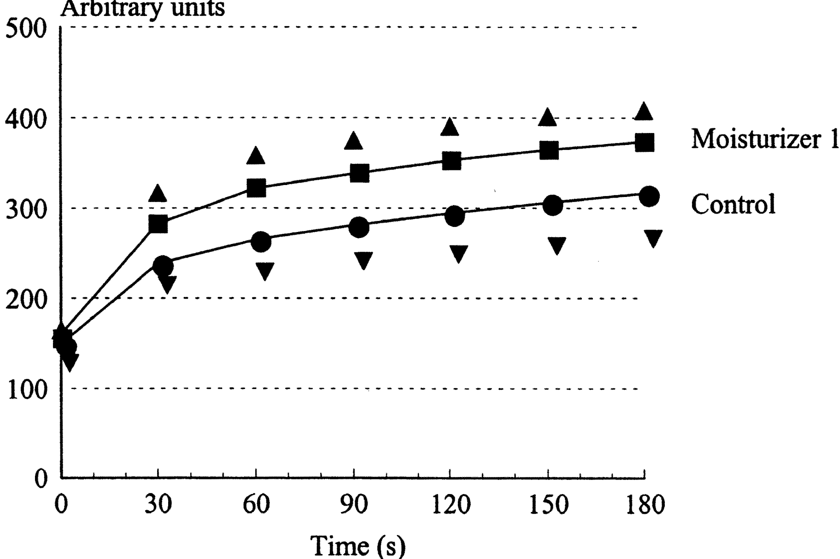

Figure 4 Time course of hydration changes during a MAT performed 60 minutes after a single one-hour application of a moisturizer (moisturizer 1 from Figs. 1 and 2; measurements conducted with the NOVA DPM 2003; means $\pm \frac{1}{2}$ SD: ▲ ▼). ■: moisturizer 1; •: control (untreated skin). *Abbreviations*: MAT, moisture accumulation test.

chamber or a similar occlusive device) for 24 hours. Then the occlusion is removed and the evaporation of the accumulated water is measured each minute for 30 minutes as TEWL. This technique has been thoroughly described in recent guidelines (23,24). The measured values represent skin-surface water loss (SSWL) and not the true TEWL. SSWL illustrates rather the sum of the TEWL and of the evaporation of water trapped within and over the SC under the occlusive equipment, at least at the beginning of the measurement period. During these first minutes of evaporation, the SSWL is proportional to SC hydration. At the end of the dehydration time, SSWL is greatly reduced and mainly TEWL is measured. Therefore, changes induced in the last part of the curve reflect the barrier function of SC.

Other Suitable Tests

Some well-defined properties of the skin are more or less dependent on SC hydration and may be evaluated with the following bioengineering methods:

- Mechanical or viscoelastic properties (elasticity, extensibility, firmness) (25)
- Skin-surface roughness (26)
- Skin-surface scaling (5,27,28)

Some other techniques are also indicated for evaluating SC hydration, but they are not available for routine experimentation at the present moment. They have been critically reviewed and evaluated in a publication to which the reader is referred (9). A very recent monograph describes the last developments and application possibilities of these particular techniques (15).

CONCLUSION

During the evaluation of SC hydration in vivo, it must be kept in mind that absolute determination of water content or concentration is not possible. This holds for clinical evaluation and for bioengineering measurements as well, which provide the investigator with numbers we think or believe to be related in an unknown manner to the water content of the SC. Therefore, as we do not really know what is measured, it is not possible to express measured hydration changes in percent, as this is unfortunately the case very often, particularly in the cosmetic field. Recently, the "dryness" of the skin was tentatively classified using one of the most popular hydration measuring devices (28). This may constitute a working basis for a better appreciation of the effectiveness of moisturizers.

Nevertheless, still several measurement techniques should be used simultaneously during a study. Not only is the information gained from these different experimental approaches complementary, and of great benefit if integrated in a clinical evaluation, but one should remember that moisturizers may influence skin hydration in different ways. Thus, different aspects of hydration changes need to be investigated, such as water binding, water retention, or emolliency, which is also a further part of a moisturizer's action. Lastly, it should be remembered that, to obtain meaningful results, proper design of the study, inclusion of a suitable number of subjects, strict standardization of measurement conditions, and other relevant factors need to be tightly controlled. Only by assuring the best quality level results will be obtained that will help to design and use optimal moisturizers.

REFERENCES

1. Seidenschnur EK. FDA and EEC regulations related to skin: documentation and measuring devices. In: Serup J, Jemec GBE, eds. Handbook of Non-Invasive Methods and the Skin. Boca Raton: CRC Press, 1995:653–665.
2. COLIPA (The European Cosmetic, Toiletry and Perfumery Association). Guidelines for the evaluation of the efficacy of cosmetic products, 2nd ed., 2001.
3. Davis JB, McNamara SH. Regulatory aspects of cosmetic claims substantiation. In: Aust LB, ed. Cosmetic Claims Substantiation. New York: Marcel Dekker, 1998:1–20.
4. Serup J. EEMCO guidance for the assessment of dry skin (xerosis) and ichthyosis: clinical scoring systems. Skin Res Technol 1995; 1:109–114.
5. Piérard GE. EEMCO guidance for the assessment of dry skin (xerosis) and ichthyosis: evaluation by stratum corneum strippings. Skin Res Technol 1995; 2:3–11.
6. Berardesca E. EEMCO guidance for the assessment of stratum corneum hydration: electrical methods. Skin Res Technol 1997; 3:126–132.
7. Wilhelm KP. Possible pitfalls in hydration measurements. In: Elsner P, Barel AO, Berardesea E, Gabard B, Serup J, eds. Skin Bioengineering: Techniques and Applications in Dermatology and Cosmetology. Current Problems in Dermatology. . Vol. 26. Basel Karger , 1998:223–234.
8. Kligman AM. Regression method for assessing the efficacy of moisturizers. Cosmet Toilet 1978; 93:27–35.
9. Barel AO, Clarys P, Gabard B. In vivo evaluation of the hydration state of the skin: measurements and methods for claim support. In: Elsner P, Merk HF, Maibach HI, eds. Cosmetics: Controlled Efficacy Studies and Regulations. Berlin: Springer, 1999:57–80.
10. Tagami H, Ohi M, lwatsuki K, Kanamaru Y, Yamada M, lchijo B. Evaluation of the skin surface hydration in vivo by electrical measurements. J Invest Dermatol 1980; 75:500–507.
11. Levêque JL, De Rigal J. Impedance methods for studying skin moisturization. J Soc Cosmet Chem 1983; 34:419–428.
12. Levêque JL. Cutaneous Investigation in Health and Disease: Noninvasive Methods and Instrumentation. New York: Marcel Dekker, 1989.
13. Fluhr J, Elsner P, Berardesea E, Maibach HI. Bioengineering of the Skin: Water and the Stratum Corneum. Boca Raton: CRC Press, 2nd ed., 2005.
14. Serup J, Jemec GBE. Handbook of Non-Invasive Methods and the Skin. Boca Raton: CRC Press, 1995:159–170.
15. Agache P, Humbert P. Measuring the Skin: Non-invasive Investigations, Physiology, Normal Constants. Berlin: Springer, 2004.
16. Serup J. A three-hour test for rapid comparison of effects of moisturizers and active constituents (urea). Acta Dermato-Venereol (Stockh) 1992 (suppl 177):29–33.
17. Grove G. Skin surface hydration changes during a mini-regression test as measured in vivo by electrical conductivity. Curr Therap Res 1992; 52:1–6.
18. Tagami H, Kanamaru Y, Inoue K, Suehisa S, Inoue F, lwatsuki K, Yoshikuni K, Yamada M. Water sorption–desorption test of the skin in vivo for functional assessment of the stratum corneum. J Invest Dermatol 1982; 78:425–428.
19. Van Neste D. In vivo evaluation of unbound water accumulation in stratum corneum. Dermatologica 1990; 181:197–201.
20. Treffel P, Gabard B. Stratum corneum dynamic function measurements after moisturizer or irritant application. Arch Dermatol Res 1995; 287:474–479.
21. Berardesca E, Maibach HI. Effect of nonvisible damage on the water-holding capacity of the stratum corneum, utilizing the plastic occlusion stress test (POST). In: Frosch PJ, Doom-Goossens A, Lachapelle JM, Rycroft RIG, Scheper RJ, eds. Current Topics in Contact Dermatitis. Berlin: Springer, 1989:554–559.
22. Primavera G, Berardesca E. Dynamic measurements: the plastic occlusion stress test, the moisture accumulation test, and sorption-desorption test. In: Fluhr J, Elsner P,

Berardesca E, Maibach HI, eds. Bioengineering of the Skin: Water and the Stratum Corneum. Boca Raton: CRC Press, 2nd ed., 2005:237–245.

23. Pinnagoda J. Hardware and measuring principles: evaporimeter. In: Elsner P, Berardesca E, Maibach HI, eds. Bioengineering of the Skin: Water and the Stratum Corneum. Boca Raton: CRC Press, 1994:51–58.
24. Pinnagoda J, Tupker RA, Agner T, Serup J. Guidelines for transepidermal water loss measurement: a report from the Standardization Group of the European Society of Contact Dermatitis. Contact Dermatitis 1990; 22:164–168.
25. Barel AO, Lambrecht R, Clarys P. Mechanical function of the skin: state of the art. In: Elsner P, Barel AO, Berardesca E, Gabard B, Serup J, eds. Skin Bioengineering: Techniques and Applications in Dermatology and Cosmetology. Current Problems in Dermatology. Vol. 26. Basel: Karger, 1998:69–83.
26. Marks R. How to measure the effects of emollients. J Dermatol Treat 1997; 8:S15–S18.
27. Schatz H, Altmeyer PJ. Dry skin and scaling evaluated by D-squames and image analysis. In: Serup J, Jemec GBE, eds. Handbook of Non-Invasive Methods and the Skin. Boca Raton: CRC Press, 1995:153–157.
28. Heinrich U, Koop U, Leneveu-Duchemin MC, Osterrieder K, Bielfeldt S, Chkarnat C, Degwert J, Häntschel D, Jaspers S, Nissen HP, Rohr M, Schneider G, Tronnier H. Multicentre comparison of skin hydration in terms of physical-, physiological- and product-dependent parameters by the capacitive method (Corneometer CM 825). Int J Cosmet Sci 2003; 25:45–53.

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Tests for Skin Protection: Barrier Effect

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Barrier creams (BC) may play an important role in the prevention of contact dermatitis (CD) (1–6), and various in vitro and in vivo methods have been developed to evaluate their efficacy. In practice, their use remains the subject of lively debate; some reports suggest that inappropriate BC application may exacerbate rather than prevent irritation (1–3,6–9). The accuracy of measurements depends on the use of proper methodology. This chapter reviews the investigative details of pertinent scientific literature, and summarizes the methodology and efficacy of BC.

IN VITRO METHODS

In 1946, Sadler and Marriott (10) introduced some facile tests to evaluate the efficiency of BC. One method used the fluorescence of a dyestuff and eosin as an indicator to measure the penetration and the rates of penetration of water through BC; this is rapid and simple, but provides only a qualitative estimate.

Suskind (11) used a simple method to measure the relative efficacy or repellency of several formulations with film-immersion tests in a specific exposure. Results showed that both the formulations (containing 52.5% silicone in bentonite and 30% silicone in petrolatum) were effective against a range of aqueous irritants or sensitizers.

Langford (12) conducted in vitro studies to determine the efficacy of the formulated fluorochemical resin complex included against solvent penetration through treated filter paper, solvent repellency on treated pigskin, and penetration of radiotagged sodium lauryl sulfate through treated hairless mouse skin. He also conducted an in vivo study on 75 persons who had all previously experienced irritation on their hands, caused by continued contact with solvents. Eighty-three percent of the panelists stated that the cream was effective in protecting their hands.

Reiner et al. (13) examined the protective effect of ointments, both on guinea pig skin in vitro and on guinea pigs in vivo. The permeation values of a toxic agent through unprotected and protected skin, within 10 hours as a function of time, were determined radiologically and enzymatically. Permeation of the toxic agent was markedly reduced by polyethyleneglycol ointment base and the ointments containing

active substances. In in vivo experiments on guinea pigs, mortality was greater after applying the toxic agent to the unprotected skin. All formulations with nucleophilic substances markedly reduced the mortality rate.

Loden (14) evaluated the effect of BC on the absorption of (^{3}H)-water, (^{14}C)-benzene, and (^{14}C) -formaldehyde into the excised human skin. The control and BC-treated skins were exposed to the test substance for 0.5 hours, whereupon the absorption was determined. The experimental cream "water barrier," reduced the absorption of water and benzene but not that of formaldehyde. One cream slightly reduced benzene and formaldehyde absorption. The other two creams did not affect the absorption of any of the substances studied.

Treffel et al. (15) measured the effectiveness of BC against three dyes (eosin, methylviolet, and oil red O) with varying *n*-octanol/water partition coefficients (0.19, 29.8, and 165, respectively). The experiment was done in vitro on the human skin. BC efficacy was assayed by the measurement of dyes in the epidermis of protected skin samples after 30 minutes of application. The efficacy of BC against the three dyes showed data that was contrary to the manufacturer's information, in several cases. There was no correlation between the galenic parameters of the assayed products and the protection level, indicating that neither the water content nor the consistency of the formulations influenced the protection effectiveness.

Fullerton and Menne (16) tested the protective effect of various ethylenediaminetetraacetate barrier gels against nickel contact allergy, using in vitro and in vivo methods. In an in vitro study, about 30 mg of barrier gel was applied on the epidermal side of the skin and a nickel disc was applied above the gel. After 24 hours of application, the nickel disc was removed and the epidermis separated from the dermis. Nickel content in epidermis and dermis was quantified by absorption differential pulse voltammetry. The amount of nickel in the epidermal skin layer on the barrier gel–treated skin was significantly reduced, when compared with the untreated control. In vivo patch testing of nickel-sensitive patients was performed using nickel discs, with and without barrier gels. Test preparations and nickel discs were removed one day after the application, and the test sites were evaluated. Reduction in positive test reactions was significantly high on the barrier gel-treated sites.

Zhai et al. (17) used an in vitro diffusion system to measure the protective efficacy of Quaternium-18 bentonite (Q18B) gels to prevent 1% concentration of [^{35}S] sodium lauryl sulfate (SLS) penetration on human cadaver skin. The accumulated amount of [^{35}S]-SLS in receptor-cell fluid were counted to evaluate the efficacy of the Q-18B gels over a 24-hour period. These test gels significantly decreased SLS absorption when compared with the unprotected-skin control samples. The percent protection effect of three test gels against SLS percutaneous absorption was 88%, 81%, and 65%, respectively.

IN VIVO METHODS

In 1940, Schwartz et al. (18) introduced an in vivo method to evaluate the efficacy of a vanishing cream against poison ivy extract, by using the visual assessment of erythema on human skin. The test cream was an effective prophylaxis against poison ivy dermatitis when compared with unprotected skin.

Lupulescu and Birmingham (19) observed the ultrastructural and relief changes of human epidermis, after exposure to a protective gel, acetone, and kerosene on humans. In the unprotected skin, the cell damage and a disorganized pattern of

the upper layers of epidermis were seen. Application of a protective agent before the solvent exposure substantially reduced the ultrastructural and relief changes of the epidermal cells.

Lachapelle and coworkers (3,20–23) used a guinea pig model to evaluate the protective value of BC and/or gels by laser doppler flowmetry and histological assessment. The histopathological damage after 10 minutes of contact to toluene was mostly confined to the epidermis, whereas the dermis was almost normal. The dermal blood-flow changes were relatively high on the control site compared with the gel-pretreated sites.

Frosch et al. (1,8,9,24,25) developed the repetitive irritation test in guinea pigs and in humans, to evaluate the efficacy of BC by using a series of bioengineering techniques. The cream-pretreated and untreated test skin (guinea pig or humans) was exposed daily to the irritants for two weeks. The resulting irritation was scored on a clinical scale and assessed by biophysical techniques' parameters. Some test creams suppressed irritation with all test parameters; some failed to show such an effect, and some even exacerbated the irritation (9).

Zhai (2) used an in vivo human model, to measure the effectiveness of BC against the dye-indicator solutions: methylene blue in water and oil red O in ethanol, which are representative of model hydrophilic and lipophilic compounds. Solutions of 5% methylene blue and 5% oil red O were applied to an untreated and BC-pretreated skin, with the aid of aluminum occlusive chambers for zero and four hours. At the end of the application time, the materials were removed and consecutive skin-surface biopsies were obtained. The amount of dye penetrating into each strip was determined by colorimetry. Two creams exhibited effectiveness, but one cream enhanced the cumulative amount of dye.

Zhai et al. (5) introduced a facile approach to screening protectants in humans, in vivo. Two acute irritants and one allergen were selected: (1) sodium lauryl sulfate (SLS), representative of irritant, household, and occupational contact dermatitis, (2) the combination of ammonium hydroxide (NH_4OH) and urea, to simulate diaper dermatitis, and (3) Rhus, to evaluate the effect of model protective materials. Test materials were spread onto test area, massaged, allowed to dry for 30 minutes, and reapplied with another 30-minute drying period. The model irritants and allergen were applied with an occlusive patch for 24 hours. Inflammation was scored with an expanded 10-point scale at 72 hours after application. Most test materials, according to the statistics, suppressed the SLS irritation and Rhus allergic reaction rather than NH_4OH and urea-induced irritation.

Wigger-Alberti et al. (26) determined the areas of the hands that were likely to be skipped on self-application of BC when applied at the workplace, using fluorescence technique. Results showed the application of BC was incomplete, especially on the dorsal aspects of the hands. Brief data of the recent experiments done to study BC are summarized in Table 1.

CONCLUSIONS

Some BC reduce CD under experimental conditions. But, inappropriate BC application may enhance irritation rather than causing beneficial effects. To achieve the optimal protective effects, BC should be used with careful consideration based on specific exposure conditions; and the proper use of BC should also be instructed.

Table 1 Brief Data from Recent Experiments of Barrier Creams

Models						
In vitro	In vivo animals or humans	Irritants or allergens	Barrier creams	Evaluations by	Efficacy	Authors and references.
Human skin		Dyes (eosin, methylviolet, oil red O)	16 Barrier creams	Amount of dyes in the epidermis	Various % protection effects	Treffel et al. (15)
Human skin	Nickel-sensitive patients	Nickel disc	Ethylenediaminete-tra-acetate gels	Nickel content	Significantly reduced the amount of nickel in the epidermis in vitro, and significantly reduced positive reactions in vivo	Fullerton and Menne (16)
Human skin		[^{35}S]-SLS	Three quaternium-18 bentonite (Q-18B) gels	Amount of [^{35}S]-SLS	Percentage of protection effect was 88%, 81%, and 65%, respectively	Zhai et al. (17)
	Guinea pigs	n-Hexane, trichlorethylene, toluene	Three water-miscible creams	Morphological assessment	Limited protective effects	Lachapelle et al. (26)

Guinea pigs and humans	SLS, sodium hydroxide, toluene, lactic acid	Several barrier creams	Various bioengineering techniques	Some of them suppressed irritation, some failed	Frosch, et al (1,8,24,25)
Humans	Dyes (methylene blue and oil red O)	Three barrier creams	Amount of dye penetrating into strips	Two of them exhibited effectiveness, one enhanced the cumulative amount of dye	Zhai and Maibach (2)
Humans	SLS, ammonium hydroxide ($NH^{4}0H$) and urea, Rhus	Several protectants	Clinical scores	Most suppressed the SLS irritation and Rhus allergic reaction, failed to $NH_{4}0H$ and urea irritation	Zhai et al. (5)
Humans	Self-application of BC	An oil-in-water emulsion	Fluorescence technique	Self-application of BC was incomplete	Wigger-Alberti et al. (26)

In vitro methods are simple, rapid, safe, and are recommended in the screening procedure for BC candidates. With radiolabeled methods, we may determine the accurate protective and penetration results even in the lower levels of chemicals, because of the sensitive radiolabeled counting when BCs are to be evaluated. Animal experiments may be used to generate kinetic data because of a greater similarity between humans and some animals (e.g., pigs and monkeys), on regarding the percutaneous absorption and penetration of some compounds. But no one animal, with its complex anatomy and biology, will simulate the penetration in humans for all compounds. Therefore, the best estimate of human percutaneous absorption is determined by in vivo studies in humans. The histological assessments may define what layers of skin are damaged or protected, and may provide the insight mechanism of BC. Noninvasive bioengineering techniques may provide an accurate, highly reproducible, and objective observation in quantifying the inflammation response to various irritants and allergens when BC are to be evaluated, which could assess the subtle differences to supplement the traditional clinical studies.

To validate these models, well-controlled field trials are required in defining the relationship of the model to the occupational setting. Finally, the clinical efficacy of BC should be assessed in the workplace rather than in experimental circumstances.

REFERENCES

1. Frosch PJ, Schulze-Dirks A, Hoffmann M, Axthelm I, Kurte A. Efficacy of skin barrier creams (I). The repetitive irritation test (RIT) in the guinea pig. Contact Dermat 1996; 28:94.
2. Zhai H, Maibach HI. Effect of barrier creams: human skin in vivo. Contact Dermat 1996; 35:92.
3. Lachapelle JM. Efficacy of protective creams and/or gels. In: Eisner P, Lachapelle JM, Wahl-berg JE, Maibach HI, eds. Prevention of Contact Dermatitis, Current Problems in Dermatology. Basel: Karger, 1996:182.
4. Zhai H, Maibach HI. Percutaneous penetration (Dermatopharmacokinetics) in evaluating barrier creams. In: Eisner P, Lachapelle JM, Wahlberg JE, Maibach HI, eds. Prevention of Contact Dermatitis, Current Problems in Dermatology. Basel: Karger, 1996:193.
5. Zhai H, Willard P, Maibach HI. Evaluating skin-protective materials against contact irritants and allergens. An in vivo screening human model. Contact Dermat 1998; 38:155.
6. Wigger-Alberti W, Eisner P. Do barrier creams and gloves prevent or provoke contact dermatitis? Am J Contact Dermat 1998; 9:100.
7. Goh CL. Cutting oil dermatitis on guinea pig skin (I). Cutting oil dermatitis and barrier cream. Contact Dermat 1991; 24:16.
8. Frosch PJ, Schulze-Dirks A, Hoffmann M, Axthelm I. Efficacy of skin barrier creams (II). Ineffectiveness of a popular "skin protector" against various irritants in the repetitive irritation test in the guinea pig. Contact Dermat 1993; 29:1A.
9. Frosch PJ, Kurte A, Pilz B. Biophysical techniques for the evaluation of skin protective creams. In: Frosch PJ, Kligman AM, eds. Noninvasive Methods for the Quantification of Skin Functions. Berlin: Springer-Verlag, 1993:214.
10. Sadler CGA, Marriott RH. The evaluation of barrier creams. Br Med J 1946; 23:169.
11. Suskind RR. The present status of silicone protective creams. Ind Med Surg 1955; 24:413.
12. Langford NP. Fluorochemical resin complexes for use in solvent repellent hand creams. Am Ind Hyg Assoc J 1978; 39:33.
13. Reiner R, Rossmann K, Hooidonk CV, Ceulen BI, Bock J. Ointments for the protection against organophosphate poisoning. Arzneim-Forsch/Drug Res 1982; 32:630.
14. Loden M. The effect of 4 barrier creams on the absorption of water, benzene, and formaldehyde into excised human skin. Contact Dermat 1986; 14:292.

15. Treffel P, Gabard B, Juch R. Evaluation of barrier creams: an in vitro technique on human skin. Ada Derm Venereol 1994; 74:1.
16. Fullerton A, Menne T. In vitro and in vivo evaluation of the effect of barrier gels in nickel contact allergy. Contact Dermat 1995; 32:100.
17. Zhai H, Buddrus DJ, Schulz AA, Wester RC, Hartway T, Serranzana S, Maibach HI. In vitro percutaneous absorption of sodium lauryl sulfate (SLS) in human skin decreased by Quaternium-18 bentonite gels. In vitro Molecular Toxicol 1999; 12:11.
18. Schwartz L, Warren LH, Goldman FH. Protective ointment for the prevention of poison ivy dermatitis. Public Health Rep 1940; 55:1327.
19. Lupulescu AP, Birmingham DJ. Effect of protective agent against lipid-solvent-induced damages. Ultrastructural and scanning electron microscopical study of human epidermis. Arch Environ Health 1976; 31:29.
20. Mahmoud G, Lachapelle JM, Neste DV. Histological assessment of skin damage by irritants: its possible use in the evaluation of a ‘barrier cream’. Contact Dermat 1984; 11:119.
21. Mahmoud G, Lachapelle JM. Evaluation of the protective value of an antisolvent gel by laser Doppler flowmetry and histology. Contact Dermat 1985; 75:14.
22. Mahmoud G, Lachapelle J. Uses of a guinea pig model to evaluate the protective value of barrier creams and/or gels. In: Maibach HI, Lowe NJ, eds. Models of Dermatology. Basel: Karger, 1987:112.
23. Lachapelle JM, Nouaigui H, Marot L. Experimental study of the effects of a new protective cream against skin irritation provoked by the organic solvents n-hexane, trichlorethylene and toluene. Dermatosen 1990; 58:19.
24. Frosch PJ, Kurte A, Pilz B. Efficacy of skin barrier creams (III). The repetitive irritation test (RIT) in humans. Contact Dermat 1993; 29:113.
25. Frosch PJ, Kurte A. Efficacy of skin barrier creams (IV). The repetitive irritation test (RIT) with a set of 4 standard irritants. Contact Dermat 1994; 57:161.
26. Wigger-Alberti W, Maraffio B, Wernli M, Eisner P. Self-application of a protective cream. Pitfalls of occupational skin protection. Arch Dermatol 1997; 755:861.

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Tribological Studies on Skin: Measurement of the Coefficient of Friction

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INTRODUCTION

Studying the friction of skin, supplements other mechanical tests. Friction studies can be conducted with noninvasive methods and give a measure of the skin's health—skin hydration for example: Naylor (1) showed that moistened skin has an elevated friction response and El-Shimi (2) demonstrated that drier skin has a lowered friction response. Friction provides a quantitative measurement to assess skin.

The friction parameter generally measured is the coefficient of friction. To measure the friction coefficient, a surface is brought into contact with another and moved relative to it. When the two surfaces are brought into contact, the perpendicular force is defined as the normal force (*N*). The friction force (*F*) is that force which opposes relative movement between the two surfaces. From Amonton's law, the coefficient of friction (μ) is defined as the ratio of the friction force to the normal force:

$$\mu = F/N$$

The friction coefficient can be measured in two ways: the static friction coefficient (μ_s) and the dynamic or kinetic friction coefficient (μ_k). The static friction coefficient is defined as the ratio of the force required to initiate relative movement and the normal force between the surfaces; the dynamic or kinetic friction coefficient is defined as the ratio of the friction force to the normal force when the two surfaces are moving relative to each other. For simplicity, much of the research has focused on the dynamic friction coefficients wherein the two surfaces move at a relative constant velocity. Most of the friction studies on skin have dealt with the dynamic

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friction coefficient and the subscript "k" is usually dropped. This overview references the dynamic coefficient of friction, unless otherwise noted.

According to Amonton's Law, the dynamic friction coefficient remains unchanged regardless of the probe velocity or applied normal load in making the measurement. Amonton's laws hold true in the case of solids with limited elastic properties. Although Naylor (1) concluded Amonton's Law to be true, later studies by El-Shimi (2), Comaish and Bottoms (3), and Koudine et al. (4) have found that skin deviates from Amonton's Law as the friction coefficient increased when the normal load was decreased. El-Shimi (2) and Comaish and Bottoms (3) reasoned that the rise in friction coefficient resulted from the viscoelastic nature of the skin allowing for a nonlinear deformation of the skin with increasing load.

Experimental Designs

Various experimental designs have been devised to measure the friction on skin. They focus on measuring friction by pressing a probe onto the skin with a known normal force, and then detecting the skin's frictional resistance to movement of the probe. The designs are classified into two categories:

i. A probe moved across the skin in a linear fashion.
ii. A rotating probe in contact with the skin surface.

In the linear designs, the probe movement is accomplished in several ways. Comaish and Bottoms (3) utilized one of the simplest linear designs: they moved the probe across the skin by attaching it to a pan of weights by means of a pulley. Weights are placed in the pan such that the probe slides over the skin at a constant velocity. This allows for the calculation of the dynamic friction coefficient by dividing the total weight in the pan by the normal load on the probe.

More sophisticated linear designs, followed the simple design used by Comaish and Bottoms (3), but provide motorized unidirectional movement of the probe or the use of a reciprocating motor to move the probe back and forth. In both designs, the motorization affords greater control in maintaining the velocity of the probe. Strain gauges measure the friction force as the probe moves along the skin surface. A Biomedical Tribometer, a friction measurement device where the normal load and the probe speed are computer controlled, can also be used.

The second design category measures friction with a rotating wheel pressed onto the surface of the skin with a known normal force. Highley et al. (5) measured the frictional resistance by determining the angular recoil of the instrument as the wheel contacted the skin. They measured this angular recoil by recording the proportion of light that hit a dual element photocell. An electrical signal was then generated in proportion to the frictional resistance. Comaish et al. (6) developed a portable, hand-held device (Newcastle Friction Meter) that relied on a torsion spring to measure the skin's frictional resistance.

An important aspect of designing a friction measurement apparatus is choosing the probe size, shape, and material. Because friction is an interaction between two surfaces, the probe geometry and the material will affect the values calculated for the friction coefficient of the other surface. Also, results will be more accurate when the probe's normal force is maintained at a constant value or continuously monitored; previous methods used to maintain the normal force include spring mechanisms or static weights to weigh down the probe. These parameters are revisited critically later in this article.

Much effort has been spent in understanding how skin friction changes with differing biological conditions and upon the application of various products to the skin surface. These studies have been of interest to various industries that manufacture products meant as skin topical agents because friction measurements can provide clues regarding the effectiveness of their products.

Hydration

Hydration is a complex phenomenon influenced by intrinsic (i.e., age and anatomical site) and extrinsic (i.e., ambient humidity and chemical exposure) factors. These factors can affect the mechanical properties of the skin, and research has been performed to correlate hydration levels with the skin's friction coefficient (24). Hydration studies have investigated how increases and decreases in skin hydration correlated with the friction coefficient. In past studies, researchers generally induced increases in skin hydration through water exposure. However, decreases in skin hydration were not experimentally induced and dehydration studies were performed between subjects with "normal" skin and subjects that had clinically "dry" skin (2,12).

Lubricants/Emollients/Moisturizers

Much of the reviewed research has been devoted to ascertaining how the application of certain ingredients influences the skin surface, of interest to the cosmetic/moisturizer and lubricant industries. The studies focused on the effects of talcum powder (2,3), oils (2,3,5,14), and skin creams/moisturizers (1,1). Hills et al. (15) analyzed how changes in the friction coefficient, following emollient application, differed with temperature.

Probes

Probe geometry and material influence the measured value of the friction coefficient because friction is a probe–skin interaction phenomenon. Few studies have examined probe effects: El-Shimi (2) studied probe roughness and Comaish and Bottoms (3) studied probe roughness and material composition.

Normal Load

Friction measurements can offer quantitative insight into changes on the skin surface, and the BioMT (BioMicroTribometer, Center for Tribology Inc, Campbell, California, U.S.) offers technical advances over existing friction measurements. The control of the probe speed and the real-time monitoring of the normal load allow for real-time calculation of the friction coefficient. As seen in Figure 1, the control of the load is important because the friction coefficient does not adhere to Amonton's Law. Wolfram (18) theoretically deduced that the friction coefficient would relate to the normal load as follows:

$$\mu \propto N^{-1/3}$$

where N is the applied normal load to the skin. Sivamani et al. (17) found that the friction coefficient related to the normal load as follows:

$$\mu \propto N^{-0.32}$$

and Koudine et al. (4) found the dependence on the applied normal load to be:

$$\mu \propto N^{-0.28}$$

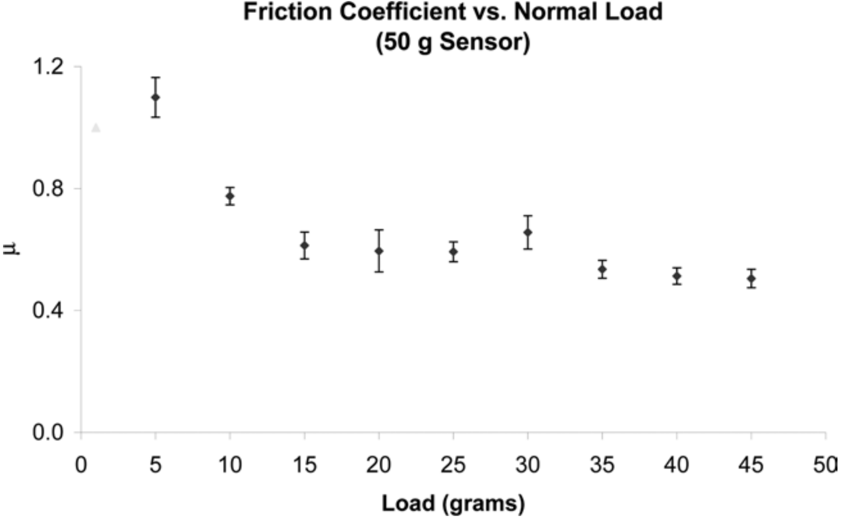

Figure 1 Friction coefficient versus normal load. The friction coefficient increased as the normal load was decreased suggesting that the skin does not follow Amonton's Law. The probe was moved at 5 mm min^{-1} ($n = 4$). *Source*: From Ref. 24.

SKIN FRICTION COEFFICIENT VALUES

Friction is an important characteristic of skin because it allows us to execute many of our daily activities. In addition, friction studies offer insight into how skin and the skin surface change across age, gender, race, anatomical site, and chemical applications. This can provide better information about the expected skin variations in the population and as to why certain topical applications are effective. Comparative studies are particularly useful in following how the skin mechanically changes under different conditions.

Previous studies have reported various values for the skin's friction coefficient. Dynamic friction coefficient measurements (Table 1) fall in the range 0.12–0.7; however, most fall in a narrower range of 0.2–0.5 (Fig. 2). Besides natural variations in skin, the wide range in results may be because of differences in probe movement, geometry, a controlled monitoring of the normal force, and material chosen to make the friction measurement. In designing the friction measurement apparatuses, the two types of probe movement utilized were rotational and linear. As a result, the linear probe constantly moves over "untested" skin and the rotational probe spins over "tested" skin. This can lead to discrepancies in reported values for the skin friction coefficient. Another important source of variation may be the ability to control the normal force while the probe is testing the skin surface. The skin friction instruments are designed to measure the fictional resistance of the skin and it is assumed that the normal force is constant. During a test, the normal force may not stay constant as a result of an uneven skin surface, inaccurate spring, and/or a nonuniform distribution of static weights placed above the probe head. Therefore, the assumption of a constant normal force may be incorrect and can lead to a variation in the calculated friction coefficient. A third source for variation is the choice of the probe material. As friction is a surface phenomenon between two materials, the choice of the probe influences the numerical value obtained for the friction coefficient.

Table 1 Reported Values of the Dynamic Friction Coefficient (μ) for Untreated "Normal" Skin In Vivo

Author	μ
Naylor (1)	0.5–0.6
El-Shimi (2)	0.2–0.4 (stainless steel rough)
	0.3–0.6 (stainless steel smooth)
Comaish and Bottoms (3)	0.2 (teflon)
	0.45 (nylon)
	0.3 (polyethylene)
	0.4 (wool)
Koudine et al. (4)	0.24 (dorsal forearm)
	0.64 (volar forearm)
Highley et al. (5)	0.2–0.3
Prall (7)	0.4
Cua et al. (8)	0.34 (forehead)
	0.26 (volar forearm)
	0.21 (palm)
	0.12 (abdomen)
	0.25 (upper back)
Johnson et al. (9)	0.3–0.4
Asserin et al. (10)	0.7
Elsner et al. (11)	0.48 (forearm)
	0.66 (vulva)

Hydration

Hydration studies reveal that drier skin had lowered friction while hydrated skin had an increased amount of friction (Table 2). However, the skin response is more complex, because very wet skin also has a lowered friction coefficient much like the characteristics of dry skin (16). Most studies focus on an intermediate zone of hydration where the skin has been moistened without an appreciable "slippery" layer of water

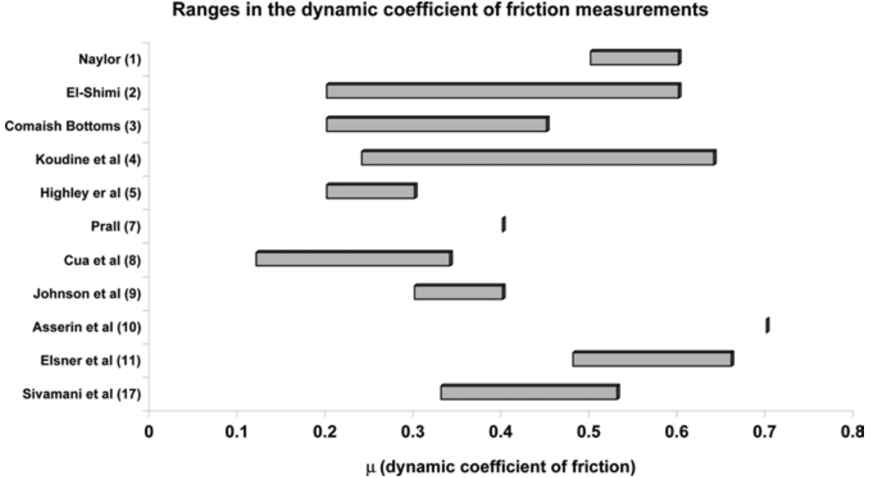

Figure 2 Outline of the ranges in the dynamic coefficient of friction. These ranges reflect measurement of untreated "normal" skin friction in vivo. *Source*: From Ref. 25.

Table 2 Comparative Studies of the Changes in Dynamic Friction Coefficient (μ) with Increasing Hydration (Hydration) and Decreasing Hydration (Dryness)

Author	Probe material	% Increase due to hydration $[(\mu_{moist}-\mu_{normal}/\mu_{normal})\times 100]$	% Decrease due to dryness $[(\mu_{moist}-\mu_{normal}/\mu_{normal})\times 100]$
Naylor (1)	Polyethylene	80	–
El-Shimi (2)	Stainless steel (rough); stainless steel (smooth)	100–200 (stainless steel rough)	28 (stainless steel rough); 41 (Stainless Steel Smooth)
Comaish and Bottoms (3)	Wool; Teflon	40[a] (Wool); 400[a] (Teflon)	–
Highley et al. (5)	Nylon	500	–
Prall (7)	Glass	200	–
Johnson et al. (9)	Glass	100–233	
Lodén et al. (12)	Stainless Steel	–	33 (hand) 41 (back) 14 (arm)
Nacht et al. (14)	Teflon	45	–
Sivamani et al. (17)	Stainless steel	55 (in vitro)	10 (in vivo)

[a]Comaish and Bottoms (3) studied the change in the static friction coefficient in their hydration study.

on the skin. Results in Table 2 show that the increases in friction were varied and this possibly results from the various probes used. Although the addition of water increases the friction coefficient, this effect lasts for a period of minutes before the skin returns to its "normal" state (2,5,14,17). The water has an effect of softening the skin and this in turn allows for greater contact area between the probe and the skin. Also, water results in adhesive forces between the water and the probe. Thus, there is more frictional resistance between the skin and the probe, and results in a higher friction coefficient (18). As the water evaporates in minutes, the skin returns to its "normal" state in the same time frame. For dry skin, the skin becomes less supple and the probe does not achieve as much contact area and this allows the probe to glide more easily over the skin surface. This results in a lowered friction coefficient, as seen in the isopropyl study (17) and in prior studies involving subjects with clinically dry skin (2,12). The agreement between the experimentally induced dry skin and clinical dry skin is expected (18).

Lubricants/Emollients/Moisturizers

The studies on lubricants, emollients, and moisturizers are important for cosmetics and products developed to make the skin look and feel healthier. The literature reports that the important qualitative characteristics in skin topical agents are skin smoothness, greasiness, and moisturization (17,19). Previous research has tried to describe these subjective, qualitative descriptions in a quantitative fashion, by correlating them against the friction coefficient. Prall (7) tried to find a quantitative correlation for skin smoothness but was unable to make a direct correlation to the friction coefficient until he added the skin topography and hardness into the analysis. Nacht et al. (14) found a linear correlation between perceived greasiness and the friction coefficient (Fig. 3).

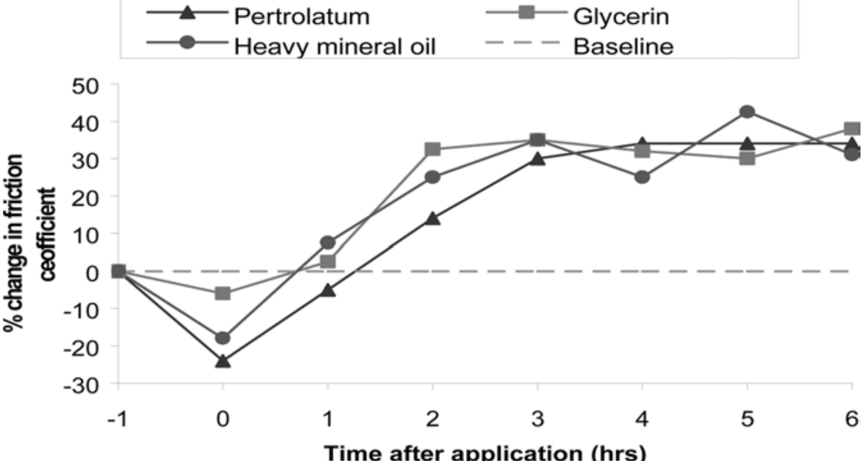

Figure 3 Effect of lubricant cosmetic ingredient on skin friction coefficient. Amount applied of each material: approximately $2\,mg/cm^2$. *Source*: From Ref. 14. (mean of five subjects but *P* value was not published). Time = –1 is immediately prior to application; Time = 0 is immediately after application.

Talcum Powder

El-Shimi (2) and Comaish and Bottoms (3) showed that the friction coefficient decreased after the application of powder. El-Shimi (2) found the friction coefficient to decrease by 50% after application; Comaish and Bottoms (3), in analyzing the static friction coefficient, observed an insignificant change for a wool probe and a 30% decrease in friction with a polyethylene probe. However, they also found that wetting the talc powder caused an increase in the measured friction.

Lubricant Oils

A lowering in the friction coefficient is the initial effect after the application of oils and oil-based lubricants (2,5,14). Nacht et al. (14) and Highley et al. (5) also showed that after the initial decrease in friction, the oils eventually raised the skin's friction coefficient. The results of the lubricant cosmetic studies by Nacht et al. (14) are shown in Figure 4.

Emollients and Moisturizers

Prall (7) and Nacht et al. (14) found that the friction coefficient rises with the addition of emollients and creams in a similar fashion to water. However, the effects of the creams lasted for hours, whereas the water effects lasted for about 5 to 20 minutes (7,14,17). Sivamani et al. (17) quantified the friction, greasiness, and "stickiness" of the skin following application of creams and treatments (Fig. 5). Hills et al. (15) also studied emollients, but they examined how different emollients compared with one another and how changes in temperature changed the friction coefficient. At a higher temperature (45°C), most emollients lowered the friction coefficient to a greater degree than at a lower temperature (18°C).
When lubricant/moisturizers are applied to the skin, the skin friction is affected in three general ways (14,18).

i. A large, immediate increase in the friction coefficient, similar to water application, that follows with a slow decrease in the friction coefficient.

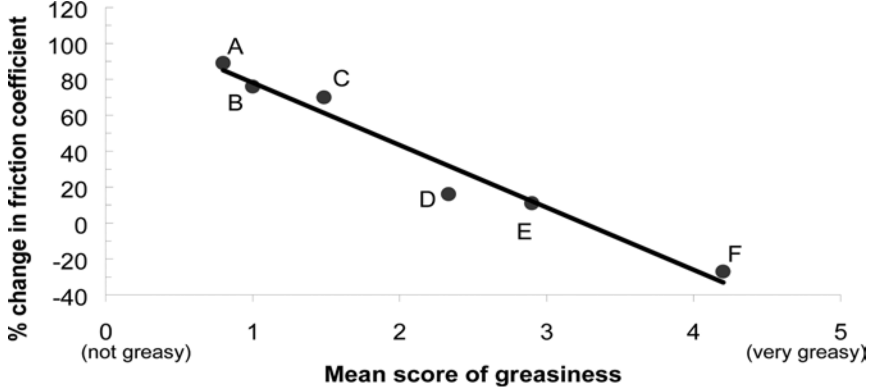

Figure 4 Correlation between changes in the friction coefficient and the sensory perception of greasiness. A, B, C, D, E, and F represent different creams that were applied to the skin. The reported percent change in the friction coefficient is immediately after application and the greasiness scores were subjective evaluations. *Source*: From Ref. 14.

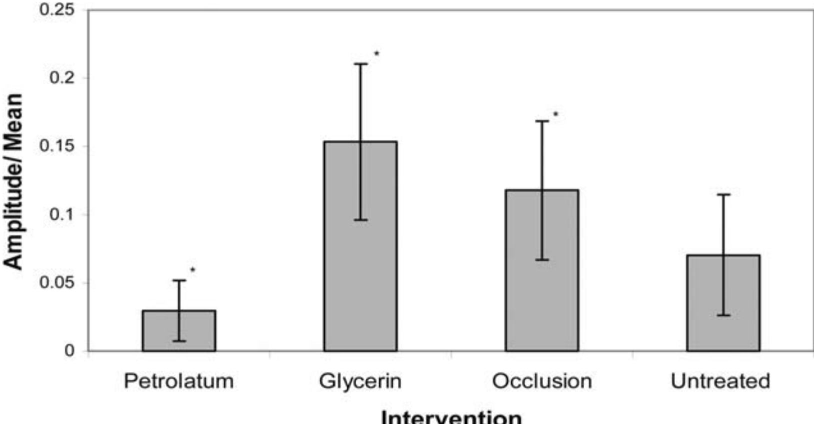

Figure 5 Amplitude/mean measurements for interventions. The application of glycerin and the PVDC occlusion increased the amplitude/mean of the volar forearm. Also, the addition of glycerin raised the amplitude/mean significantly more than the PVDC occlusion. Petrolatum significantly decreased the amplitude/mean and this is a quantitative evidence of petrolatum's greasiness ($P < 0.001$). *Source*: From Ref. 24.

These agents can be interpreted to act by immediate hydration of the skin through some aqueous means to give the immediate increase in friction.

ii. An initial decrease in the friction coefficient that is followed by an overall increase in the friction coefficient over time. These agents are fairly greasy products (Fig. 4) and this greasiness causes the immediate decrease in the friction coefficient. The eventual rise in the friction coefficient is probably because of the occlusive effects of these agents (21). In other words, these products and ingredients act to prevent water loss from the skin, thereby increasing the hydration of the skin.

iii. A small, immediate increase in the friction coefficient that later increases slowly with time. These agents are interpreted to act as a combination of effects seen in the two previous cases. These lubricants/moisturizers have ingredients and agents that serve to both hydrate the skin through some aqueous method, and prevent water loss through some occlusive mechanism. Because of the presence of the these occlusive agents, which tend to be more slippery, the immediate rise in the friction coefficient is lower than in products that fall into the first category listed above.

Probes

El-Shimi (2) and Comaish and Bottoms (3) compared probes (Tables 1 and 2) and found that smoother probes gave higher friction coefficient measurements. El-Shimi (2) noted that higher friction coefficient measurements were made with a smoother stainless steel probe as opposed to a roughened stainless steel probe. Comaish and Bottoms (3) found a similar result with two types of nylon probes: a sheet probe and a knitted probe. The sheet probe (the smoother of the two) gave a higher friction coefficient measurement. El-Shimi (2) postulates that the smoother probe forms more contact points with the skin and has a greater skin contact area than the rougher probe, resulting in more resistance from the skin and a larger measurement for the friction coefficient.

Anatomic Region, Age, Gender, and Race

Few studies address the effects of anatomic region, age, gender, or race as they pertain to the friction coefficient. To date, no significant differences have been found with regard to gender (8,22,24) or race (23,24).

The friction coefficient varies with anatomical site: Cua et al. (8,22) found that friction coefficients varied from 0.12 on the abdomen to 0.34 on the forehead. Elsner et al. (11) measured the vulvar friction coefficient at 0.66, whereas the forearm friction coefficient was 0.48. Sivamani et al. (24) found that the proximal volar forearm had a higher friction coefficient than the distal volar forearm. Manuskiatti et al. (23) studied skin roughness and found significant differences in skin roughness at various anatomical sites. Differences in environmental influences (i.e., sun exposure) and hydration may account for this. Elsner et al. (11) showed that the more-hydrated vulvar skin had a 35% higher friction coefficient than the forearm, and this is in agreement with hydration studies that contend that skin has an increased friction coefficient under increased hydration.

Age-related studies have been contradictory where some authors found no difference (8,22,24) and others found differences (Fig. 6) (10,11). Cua et al. (22) showed no differences in friction with respect to age, except for friction measurements on the

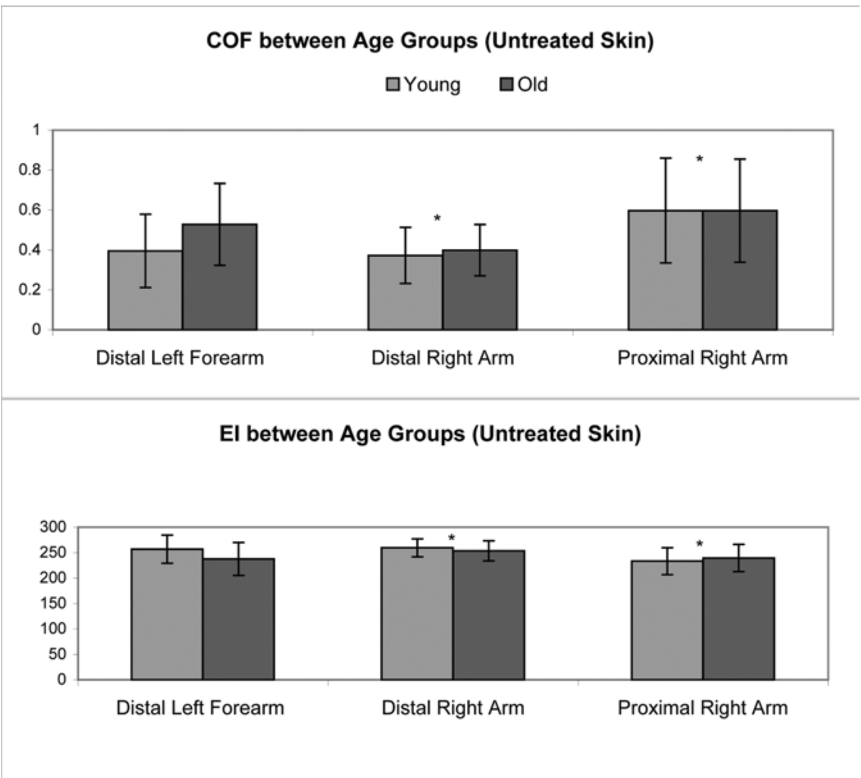

Figure 6 Age-related comparisons of friction and electrical impedance. No significant differences were apparent between old and young skin on the volar forearm. Within each category, the proximal right arm friction and electrical impedance measurements were different from the distal right arm ($P < 0.001$). *Source*: From Ref. 24.

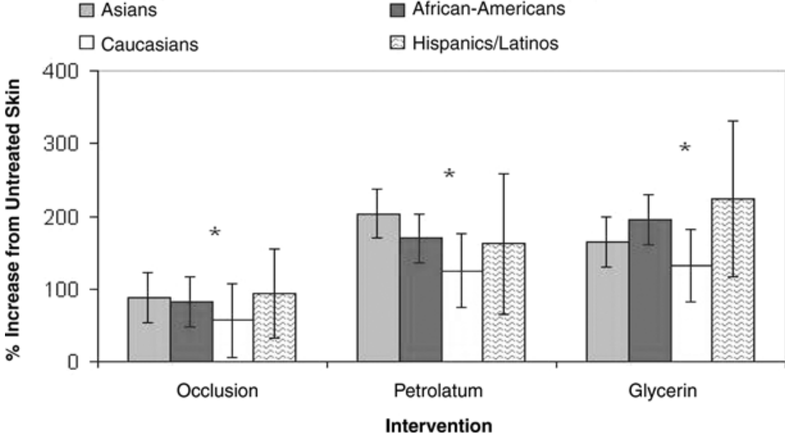

Figure 7 Coefficient of friction across ethnicity. Data represents increase in friction when compared with untreated skin of the volar forearm. No significant differences were found between the different ethnic groups. Petrolatum and glycerin increased the friction coefficient significantly more than PVDC occlusion ($P < 0.01$). The increase in the friction coefficient because of petrolatum was not significantly different from the effect of glycerin. *Source*: From Ref. 24.

ankle. Elsner et al. (11) also performed age-related tests and found no differences in the vulvar friction coefficient, but observed a higher forearm friction coefficient in younger subjects. They postulate that the skin on parts of the body that become exposed to sunlight can undergo photoaging and thus, forearm skin shows evidence of age-related differences while the light-protected vulvar skin does not (11). Asserin et al. (10) concluded that younger subjects had a higher forearm friction coefficient than older subjects.

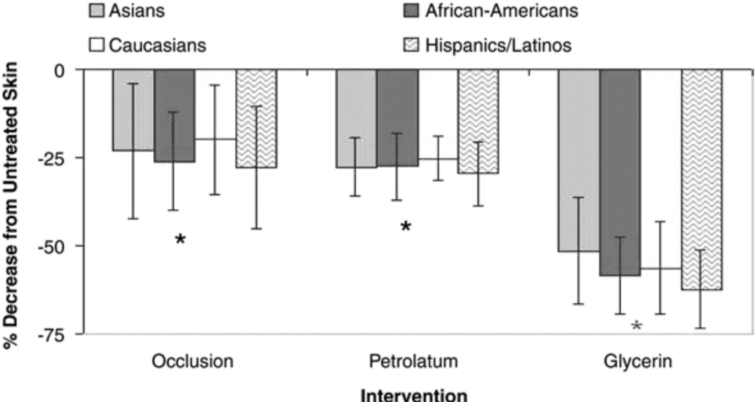

Figure 8 Change in electrical impedance across ethnicity. Data represents decrease in electrical impedance when compared with untreated skin of the volar forearm. No significant differences were found between the different ethnic groups. Glycerin lowered the electrical impedance significantly more than PVDC occlusion or petrolatum ($P < 0.01$). The decrease in the electrical impedance because of PVDC occlusion was not significantly different from the effect of petrolatum. *Source*: From Ref. 24.

There are few gender-related and racial friction studies; Cua et al. (8,22) and Sivamani et al. (24) found no significant friction differences between the genders. Manuskiatti et al. (23) found no significant racial (black and white skin) differences in skin roughness and scaliness. Sivamani et al. (24) found no differences in volar forearm friction among different ethnicities before and after chemical treatments (Fig. 7,8).

CONCLUSION

Although there have been limited studies dealing with the measurement of the skin friction coefficient, past studies and our study (17) show that differences in skin, because of various factors such as age and hydration, can be correlated with the friction coefficient. Friction coefficient studies can serve as a quantitative method to investigate how skin differs on various parts of the body and how it differs between different people. It is also a useful method for tracking the changes resulting from environmental treatments such as sunlight, and when various chemicals are applied to the skin such as soaps, lubricants, and skin creams. The reviewed studies also indicate that the design of the test apparatus is an extremely important factor, because test design parameters can also have an influence on friction measurements. A better appreciation of the importance of the friction coefficient will become clearer as measurement methods improve and allow for greater accuracy.

REFERENCES

1. Naylor PFD. The skin surface and friction. Br J Dermatol 1955; 67:239–248.
2. El-Shimi AF. In vivo skin friction measurements. J Soc Cosmet Chem 1977; 28:37–51.
3. Comaish S, Bottoms E. The skin and friction: deviations from Amonton’s laws, and the effects if hydration and lubrication. Br J Dermatol 1971; 84:37–43.
4. Koudine AA, Barquins M, Anthoine PH, Auberst L, Leveque J-L. Frictional properties of skin: proposal of a new approach. Int J Cosmet Sci 2000; 22:11–20.
5. Highley DR, Coomey M, DenBeste M, Wolfram LJ. Frictional properties of skin. J Invest Dermatol 1977; 69:303–305.
6. Comaish JS, Harborow PRH, Hofman DA. A hand-held friction meter. Br J Dermatol 1973; 89:33–35.
7. Prall JK. Instrumental evaluation of the effects of cosmetic products on skin surfaces with particular reference to smoothness. J Soc Cosmet Chem 1973; 24:693–707.
8. Cua A, Wilheim KP, Maibach HI. Frictional properties of human skin: relation to age, sex and anatomical region, stratum corneum hydration and transepidermal water loss. Br J Dermatol 1990; 123:473–479.
9. Johnson SA, Gorman DM, Adams MJ, Briscoe BJ. The friction and lubrication of human stratum corneum. In: Dowson D et al., eds. Thin Films in Tribology. Proceedings of the 19th Leeds–Lyon Symposium on Tribology. Elsevier Science Publishers, 1993: 663–672.
10. Asserin J, Zahouani H, Humbert PH, Couturaud V, Mougin D. Measurement of the friction coefficient of the human skin *in vivo*. Quantification of the cutaneous smoothness. Coll Surf B Biointerfaces 2000; 19:1–12.
11. Elsner P, Wilhelm D, Maibach HI. Frictional properties of human forearm and vulvar skin: influence of age and correlation with transepidermal water loss and capacitance. Dermatologica 1990; 181:88–91.

12. Lodén M, Olsson H, Axéll T, Linde YW. Friction, capacitance and transepidermal water loss (TEWL) in dry atopic and normal skin. Br J Dermatol 1992; 126:137–141.
13. Sulzberger MB, Cortese TA Jr, Fishman L, Wiley H. Studies on blisters produced by friction. J Invest Dermatol 1966; 47:456–465.
14. Nacht S, Close J, Yeung D, Gans EH. Skin friction coefficient: changes induced by skin hydration and emollient application and correlation with perceived skin feel. J Soc Cosmet Chem 1981; 32:55–65.
15. Hills RJ, Unsworth A, Ive FA. A comparative study of the frictional properties of emollient bath additives using porcine skin. Br J Dermatol 1994; 130:37–41.
16. Dawson D. In: Wilhelm K-P, Elsner P, Berardesca E, Maibach H, eds. Bioengineering of the Skin: Skin Surface Imaging and Analysis. Boca Raton: CRC Press, 1997:159–179.
17. Sivamani RK, Goodman J, Gitis NV, Maibach HI. Friction coefficient of skin in real-time. Skin Res Tech 2003; 9:235–239.
18. Wolfram LJ. Friction of skin. J Soc Cosmet Chem 1983; 34:465–476.
19. Denda M. In: Lodén M, Maibach H, eds. Dry Skin and Moisturizers: Chemistry and Function. Boca Raton: CRC Press, 2000:147–153.
20. Wolfram LJ. In: Leveque J-L, ed. Cutaneous Investigation in Health and Disease: Noninvasive Methods and Instrumentation. New York, NY: Marcel Dekker, 1989 (Chapter 3).
21. Zhai H, Maibach HI. Effects of skin occlusion of percutaneous absorption: an overview. Shkin Pharmacol Apl Skin Physiol 2001; 14:1–10.
22. Cua AB, Wilhelm K-P, Maibach HI. Skin surface lipid and skin friction: relation to age, sex, and anatomical region. Skin Pharm 1995; 8:246–251.
23. Manuskiatti W, Schwindt DA, Maibach HI. Influence of age, anatomic site and race on skin roughness and scaliness. Dermatology 1998; 196:401–407.
24. Sivamani RK, Wu GC, Gitis NV, Maibach HI. Tribological testing of skin products: gender, age, and ethnicity on the volar forearm. Skin Res Tech 2003; 9:299–305.
25. Sivamani RK, Goodman J, Gitis NV, Maibach HI. Coefficient of friction tribological studies in man—an overview. Skin Res Tech 2003; 9:227–234.

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Anti-Itch Testing (Antipruritics)

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INTRODUCTION

Itching, or pruritus, is an unpleasant sensation that provokes a desire to scratch. Various factors such as chemical, mechanical, thermal, and electrical stimuli can elicit itch (1–5). Mediators of itch, presumably, directly act on nerve fibers or lead to a nerve stimulation cascade whose final common pathway is interpreted in the central nervous system as itching (2–6). Putative receptors for itching are C-fibers with exceptionally low conduction velocities and insensitivity to mechanical stimuli (4–6). Histamine, the prototypical chemical mediator of itch, which is released during mast cell degranulation and mediates its effects in the skin via H_1 receptor (3,5), is the best-known experimental pruritogen (2,3,5,7).

Antipruritics may alleviate or diminish itching sensation. Topical antipruritics such as antihistamines, anesthetics, capsaicin, corticosteroids, and cooling agents are extensively used (8–10). To define the antipruritic effects, various testing methodologies have been developed (11–13). However, the clinical effects of anti-itch may vary and sometimes it is difficult to compare efficacy between antipruritics. One reason may be inadequate biometrics, as itch is a subjective symptom; its magnitude (intensity) can be only estimated from reports of patients or volunteers. Therefore, a standardized method may aid future development of anti-itch products.

This chapter reviews the literature that focuses on the evaluation of topical antipruritics, and describes the most commonly used methodologies.

METHODOLOGIES

Histamine-Induced-Itch Human Model

Rhoades et al. (14) examined the inhibition of histamine-induced pruritus by three antihistaminic drugs using a double-blind crossover study on 28 human subjects. These included diphenhydramine HCl, cyproheptadine, hydroxyzine HCl, and a lactose placebo in identical capsules. All subjects were given intradermal injections of increasing doses of aqueous histamine phosphate in the volar aspect of the forearm

to establish their individual threshold levels at which itching occurred. Following the establishment of a baseline, the subjects received two doses of one of the three antihistamines or placebo on four test periods with a one-week interval between test days. Results revealed a fivefold increase above baseline of the histamine dose was required to produce pruritus following both cyproheptadine and placebo. This is compared to a 10-fold increase following diphenhydramine and a 750-fold increase following hydroxyzine HCl.

Yosipovitch et al. (15–18) performed human studies to evaluate the antipruritics with this histamine-injection–induced itch model in man. They also utilized the visual analogue scale (VAS) to measure the itch magnitude (intensity). One study compared the effect of antipruritics of a high-potency corticosteroid, clobetasol propionate (CP), ointment versus its placebo in a double-blind manner on 16 healthy volunteers. Also, they evaluated the effect of CP and its placebo to thermal sensation and pain (15). They demonstrated that the CP had rapidly decreased histamine-induced itch but did not alter warmth sensation and thermal pain thresholds. Another study determined the effect of menthol and its vehicle (alcohol) on thermal sensations, pain, and histamine-induced experimental itch with 18 human subjects (16). Menthol showed a subjective cooling effect lasting up to 70 minutes in 12:18 subjects; however, it did not affect or did affect the cold and heat pain threshold. Alcohol produced an immediate cold sensation lasting up to five minutes in 4:18 subjects and lowered the sensitivity of cold sensation threshold ($p < 0.05$). Histamine injection did not change thermal and pain thresholds. Menthol did not alleviate histamine-induced–itch magnitude or its duration. They suggested that menthol fulfills the definition of a counterirritant, but does not affect histamine-induced itch or does affect pain sensation.

Later, they examined the effect of topical aspirin and its model vehicle dichloromethane on histamine-induced itch in 16 human subjects (17). Aspirin significantly reduced itch duration ($p = 0.001$) and decreased itch magnitude ($p < 0.04$). Aspirin and vehicle application did not affect thermal and pain thresholds during histamine-induced itch. Further, they tested the antipruritics effect and thermal sensation of a local anesthetic, 1% pramoxine, and its vehicle control in 15 human subjects (18); pramoxine significantly reduced both the magnitude and duration of histamine-induced itch. The pramoxine also reduced the cold pain threshold, but did not affect warm sensation or heat pain threshold.

Weisshaar et al. (19) evaluated the effect of topical capsaicin on the cutaneous reactions and itching to histamine in atopic eczema (AE) patients and healthy human subjects. Capsaicin 0.05% was applied three times daily over a five-day period to the same infrascapular region. The effects of pretreatment on the pruritogenic and wheal and flare reactions to subsequent histamine ionotophoresis were evaluated on the following day. In control subjects, but not in AE patients, capsaicin pretreatment significantly reduced the flare area. Compared with control subjects, AE patients showed a lack of alloknesis (itchy skin) or significantly smaller areas of alloknesis in pretreated and nonpretreated skin. In control subjects, capsaicin pretreatment significantly reduced itch sensations compared with nonpretreated skin, whereas in AE patients no differences were seen. Itch sensations in capsaicin-pretreated skin were significantly lower in control subjects than in AE patients. They concluded that capsaicin effectively suppresses histamine-induced itching in healthy skin but has less effect in AE. The diminished itch sensations and the absence of alloknesis in atopic individuals indicate that histamine is not the key factor in itching in AE.

Thomsen et al. (20) conducted a randomized, double-blind, and placebo-controlled human study to determine the antipruritic ability of topical aspirin in inflamed skin. In 24 nonatopic volunteers, an inflammatory skin reaction was induced in forearm skin at five sites by sodium lauryl sulfate (SLS) contained in Finn Chambers. Aspirin 10% and 1%, mepyramine 5%, and vehicle were applied to the inflamed and corresponding noninflamed areas 20 minutes before itch induction with intradermal histamine injection. No difference in itch intensities was found after application of aspirin, mepyramine, and vehicle, but more itch was induced in aspirin and mepyramine pretreated sites in inflamed skin compared to normal skin ($p < 0.05$). In normal skin, flare areas were smaller after pretreatment with aspirin 10% ($p < 0.05$) and mepyramine ($p < 0.001$), as were wheal areas after mepyramine ($p < 0.01$), compared to vehicle pretreatments. In inflamed skin, flare areas were smaller after pretreatment with aspirin 10% ($p < 0.01$) and mepyramine ($p < 0.001$), as were wheal areas after aspirin 10% ($p < 0.01$), aspirin 1% ($p < 0.05$), and mepyramine ($p < 0.001$). They concluded that despite a significant skin penetration, as measured by the influence on wheal and flare reactions, topically applied aspirin did not decrease histamine-induced itch in the model used.

Zhai et al. (21) evaluated the antipruritic effect of hydrocortisone (1% and 2.5%) and its vehicle control on histamine-induced itch and sensory effects in 18 human subjects. In comparison to placebo, 2.5% hydrocortisone significantly ($p = 0.03$) reduced itch duration from 12.6 ± 11.0 to 8.6 ± 8.2 minutes (the reducing rate was 32%) as well as itch magnitude (at minutes 3, 6, 7, and overall). Placebo, 1% and 2.5% hydrocortisone significantly altered ($p < 0.05$) the cold sensation threshold. No treatment altered cold or heat pain thresholds. They suggested that topical application of 2.5% hydrocortisone might be significantly beneficial for the treatment of histamine-induced itch.

They ascertained the antipruritic effects of topical strontium salts with the histamine-induced–itch model on eight human subjects (22). Strontium nitrate, compared to its vehicle control, significantly shortened itch duration from 28.1 ± 5.4 to 18.5 ± 4.2 minutes ($p < 0.01$) and reduced itch magnitude at time points 12 to 20 minutes and overall ($pp < 0.05$). They concluded that strontium nitrate may act as a topical antipruritic agent in reducing histamine-mediated itch. Furthermore, they utilized this histamine-induced–itch human model to screen and to compare the efficacy of a group of topical antipruritics in 10 individuals who were responsive to histamine-induced–itch sensation (23). The pramoxine-containing cream (formulation D) significantly ($p < 0.05$) decreased itch magnitude (within a 20-minute test period), from 2.6 ± 2.1 to 2.2 ± 2.1 cm when compared to its vehicle control; it also significantly ($p < 0.05$) shortened itch duration (15.0 ± 7.4 minutes) in comparison with its vehicle control (20.3 ± 7.0 minutes). Of all the formulations tested, pramoxine-containing cream was the most effective antipruritic in decreasing histamine-induced itch.

Scratch Behavior Measurement

Tohda et al. (24) studied the effect of byakko-ka-ninjin-to (BN), which is composed of gypsum, the root of anemarrhena, ginseng, licorice, and rice, on the inhibition of itch using naive/challenged (NC) mouse model of atopic dermatitis. BN (200 mg/kg, p.o.) significantly inhibited the scratching frequency in NC mice and decreased the skin temperature by 1.97°C.

Table 1 Summary Data of Models and Efficacy of Antipruritics

Models	Efficacy	References
Intradermal histamine injection-induced itch	Fivefold increased above baseline of the histamine dose required producing pruritus following both cyproheptadine and placebo. A tenfold increased following diphenhydramine and a 750-fold increase following hydroxyzine HCL	(14)
Intradermal histamine injection-induced itch	Clobetasol propionate ointment rapidly decreased itch but did not alter warmth sensation and thermal pain thresholds	(15)
Intradermal histamine injection-induced itch	Menthol failed to show the effect of antipruritics	(16)
Intradermal histamine injection-induced itch	Aspirin significantly reduced itch duration and decreased itch magnitude	(17)
Intradermal histamine injection-induced itch	Pramoxine significant reduced both the magnitude and duration of itch	(18)
Histamine iontophoresis-induced itch	Capsaicin significantly reduced itch sensations	(19)
Intradermal histamine injection-induced itch	Aspirin did not decrease histamine-induced itch	(20)
Intradermal histamine injection-induced itch	2.5% hydrocortisone significantly reduced histamine-induced itch	(21)
Intradermal histamine injection-induced itch	Strontium nitrate showed a good antipruritic effect in reducing histamine-mediated itch	(22)
Intradermal histamine injection-induced itch	Pramoxine-containing cream was the most effective antipruritic in decreasing histamine-induced itch	(23)
Scratch behavior measurement	Byakko-ka-ninjin-to significantly inhibited the scratching frequency in NC mice	(24)

Abbreviation: NC, naive/challenged.

Others

These include contact allergic dermatitis model (poison ivy), contact irritant dermatitis induced by sodium lauryl sulfate, etc. (11–13).

Table 1 summaries data of models and efficacy of antipruritics.

CONCLUSION

When measuring itch, several factors must be taken into account: severity, duration, variation between individuals, and subjective differences in determination of itching threshold. To better quantify this subjective response, several approaches have been employed (11–25). The measurement of scratch behavior is problematic and has been addressed in a variety of ways such as lack of validation and unlikeliness to be reproducible, etc. (12). As human's verbalization may be more accurate in describing itch sensation, the VAS may be superior to other methods (11,12,24,25). To evaluate antipruritic drugs, clinical methods may rely on either naturally occurring or experimentally induced pruritus. Methods and judgments based on naturally occurring pruritus better reflects the actual clinical setting (11). However, they have several disadvantages including: (1) pruritus intensity may fluctuate on its own if the study is conducted over several days, as the naturally occurring pruritus may not be stable over time; (2) comparing the pruritic intensity of specific lesions in different patients is often difficult and not always relevant; (3) adequate controls are difficult to achieve (11).

Histamine-induced–itch model was utilized because acute itching is most commonly evoked by chemical stimuli (e.g., histamines) (2). Some individuals do not itch after histamine injection (15–17); therefore, to diminish the variation of responses, we suggest that only subjects with histamine-induced–itch sensation should be enrolled. This will improve discrimination—an obvious advantage in a screening assay. However, the histamine injection model may induce pain sensation; it may partially interrupt the itch sensation. We note that a 1 ml injection appears high and undoubtedly spreads; this large volume has added reproducibly to previous studies (14–18,21–23). The VAS score was comparatively low; however, this level (3 cm) was adequate for the discrimination noted. Higher concentrations might be considered in the future. Furthermore, it is essential that studies of topical antipruritics are well designed and double-blind so that resulting data are valid and can distinguish between effective and noneffective treatments.

REFERENCES

1. McMahon SB, Koltzenburg M. Itching for an explanation. Trends Neurosci 1992; 15:497.
2. Tuckett RP. Neurophysiology and neuroanatomy of pruritus. In: Bernhard JD, ed. Itch Mechanisms and Management of Pruritus. New York: McGraw-Hill, 1994:1.
3. Lerner EA. Chemical mediators of itching. In: Bernhard JD, ed. Itch Mechanisms and Management of Pruritus. New York: McGraw-Hill, 1994:23.
4. Heyer GR, Hornstein OP. Recent studies of cutaneous nociception in atopic and non-atopic subjects. J Dermatol 1999; 26:77.
5. Fleischer AB. Science of itching. In: Fleischer AB, ed. The Clinical Management of Itching. New York: The Parthenon Publishing Group, 2000:1.

6. Schmelz M, Schmidt R, Bickel A, Handwerker HO, Torebjörk HE. Specific C-receptors for itch in human skin. J Neurosci 1997; 17:8003.
7. Heyer G, Ulmer FJ, Schmitz J, Handwerker HO. Histamine-induced itch and alloknesis (itchy skin) in atopic eczema patients and controls. Acta Derm Venereol 1995; 75:348.
8. Bernhard JD. General principles, overview, and miscellaneous treatments of itching. In: Bernhard JD, ed. Itch Mechanisms and Management of Pruritus. New York: McGraw-Hill, 1994:367.
9. Litt JZ. Topical treatments of itching without corticosteroids. In: Bernhard JD, ed. Itch Mechanisms and Management of Pruritus. New York: McGraw-Hill, 1994:383.
10. Fleischer AB. Treatment. In: Fleischer AB, ed. The Clinical Management of Itching. New York: The Parthenon Publishing Group, 2000:159.
11. Spilker B. Clinical evaluation of topical antipruritics and antihistamines. In: Maibach HI, Lowe NJ, eds. Models in Dermatology. Basel: Karger, 1987:55.
12. Fleischer AB. Measuring itching and scratching. In: Fleischer AB, ed. The Clinical Management of Itching. New York: The Parthenon Publishing Group, 2000:13.
13. Fleischer AB. Evaluation of the itching patient. In: Fleischer AB, ed. The Clinical Management of Itching. New York: The Parthenon Publishing Group, 2000:21.
14. Rhoades RB, Leifer KN, Cohan R, Wittig HJ. Suppression of histamine-induced pruritus by three antihistaminic drugs. J Allergy Clin Immunol 1975; 55:180.
15. Yosipovitch G, Szolar C, Hui XY, Maibach HI. High-potency topical corticosteroid rapidly decreases histamine-induced itch but not thermal sensation and pain in human beings. J Am Acad Dermatol 1996; 35:118.
16. Yosipovitch G, Szolar C, Hui XY, Maibach HI. Effect of topically applied menthol on thermal, pain and itch sensations and biophysical properties of the skin. Arch Dermatol Res 1996; 288:245.
17. Yosipovitch G, Ademola J, Lui P, Amin S, Maibach HI. Topically applied aspirin rapidly decreases histamine-induced itch. Acta Derm Venereol 1997; 77:46.
18. Yosipovitch G, Maibach HI. Effects of topical pramoxine on experimentally induced itch in man. J Am Acad Dermatol 1997; 37:278.
19. Weisshaar E, Heyer G, Forster C, Handwerker HO. Effect of topical capsaicin on the cutaneous reactions and itching to histamine in atopic eczema compared to healthy skin. Arch Dermatol Res 1998; 290:306.
20. Thomsen JS, Benfeldt E, Jensen SB, Serup J, Menne T. Topically applied aspirin decreases histamine-induced wheal and flare reactions in normal and SLS-inflamed skin, but does not decrease itch. A randomized, double-blind and placebo-controlled human study. Acta Derm Venereol 2002; 82:30.
21. Zhai H, Hannon W, Hahn GS, Harper RA, Pelosi A, Maibach HI. Strontium nitrate decreased histamine-induced itch magnitude and duration in man. Dermatol 2000; 200:244.
22. Zhai H, Frisch S, Pelosi A, Neibart S, Maibach HI. Antipruritic and thermal sensation effects of hydrocortisone creams in human skin. Skin Pharmacol Appl Skin Physiol 2000; 13:352.
23. Zhai H, Simion FA, Abrutyn E, Koehler AM, Maibach HI. Screening topical antipruritics: a histamine-induced itch human model. Skin Pharmacol Appl Skin Physiol 2002; 15:213.
24. Tohda C, Sugahara H, Kuraishi Y, Komatsu K. Inhibitory effect of Byakko-ka-ninjin-to on itch in a mouse model of atopic dermatitis. Phytother Res 2000; 14:192.
25. Ebata T, Aizawa H, Kamide R, Niimura M. The characteristics of nocturnal scratching in adults with atopic dermatitis. Br J Dermatol 1999; 141:82.

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Evaluation Methods for Hair Removal Efficacy

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INTRODUCTION

The removal of facial and body hair for cosmetic reasons is widespread and has been around for ages. Subjects generally seek a long lasting hair removal result, with no pain or side effects, and an inexpensive and easy to perform treatment. This chapter concentrates on evaluating the hair removal efficacy of treatments.

To be able to analyze and interpret the regrowth of hairs after a treatment, it is important to recognize the growth cycle of hair follicles. Three phases can roughly be distinguished: the anagen phase, during which the follicle produces a hair, the catagen phase, which is seen as a transition phase, and the telogen phase, during which the follicle is in rest and no hair is formed (1–4).

Various hair removal methods will be introduced below, and special attention will be paid to the general working principle of each of these methods. Then, the perceptive and instrumental experimental methods to determine the efficacy of hair removal treatments will be discussed. Finally, a computer model that provides an insight to various aspects of hair regrowth after hair removal will be explained.

METHODS FOR HAIR REMOVAL

Hair removal methods can be classified according to the region of the hair or follicle they work on. Some methods only remove the hair above or somewhat below the skin surface and do not affect the hair follicle. If the hair is long enough to be grabbed, various forms of plucking can be applied. These methods affect the hair above and considerably below the skin surface and are capable of mildly damaging the follicle. Finally, electrolysis and photo hair removal can affect the hair (re-) generating cells in the bulge and bulb of the follicle, which makes them, in principle, capable of inducing a permanent hair reduction effect (1,5,6).

Removal of Hair Above and Somewhat Below the Skin Surface

Shavers, abrasives, and chemical depilatories represent the hair removal methods that are capable of removing hairs above the skin surface. All types of shavers (7), either blade (8) or electric (9), use a knife to cut the hairs that protrude through the skin surface. During the last two decades, electric and blade shavers that can lift the hair from the follicle and thereby cut it extra short have been introduced. Shavers can be used for both facial and body hair, although women generally prefer to use other methods to remove their facial hair.

Abrasives, similar to sanding paper, use friction and wear to remove the hairs. The abrasive is moved over the skin and removes the hairs above the skin, while at the same time scrubbing the skin. This method is only used for thin hair, such as leg hair.

Chemical depilatories use thioglycolates to hydrolize the disulfide bonds in hair fibers, thus decreasing the hair strength (10). The hair can than be wiped off easily. As chemical depilatories cannot deeply penetrate the hair follicle, they are only effective on the part of the hair shaft that is exposed to the chemicals above the skin surface. Chemical depilatories are, generally speaking, only used by women for the removal of body hair.

Generally, the above mentioned hair removal methods leave the part of the hair shaft that is still deeper in the follicle unaffected. As a result of this, the hair from the anagen follicles will reappear above the skin surface within a short time (typically hours to days) after the treatment.

Removal of Hair Above and Considerably Below the Skin Surface

If the hair that protrudes through the skin surface is long enough to be grabbed, various methods of plucking can be applied. Plucking is generally done only for female hair removal, although in some cases men also apply this technique, especially for the removal of back or chest hair.

Tweezers and electric epilators (11) are the most straightforward devices for plucking hair. The latter is a type of device that has a number of mechanically driven tweezers in the form of rotating discs or a rotating spring. Threading (12) is an ancient, but still frequently used technique. Two twined strings are moved over the skin surface to grab and pluck hair. Finally, waxing and sugaring (13) use a sticky substance that is spread over the skin, covered with a piece of paper or cloth, and then manually removed along with the hair.

To what extent the hair itself is removed from the follicle and to what extent the follicle remnants also are pulled out during the plucking treatment depends, amongst others factors, on the pulling speed (14–17). However, it is clear that the depth of the hair follicle and the growth speed of the hair, at least partially, determine the time it will take for a hair to grow back after being plucked. Various investigators have measured these two parameters for various body areas, but large differences have been found (18–20). Additionally, it has been proposed that the follicle depth and the hair growth speed are not the only factors that determine the hair regrowth term after plucking (6). It has been established that plucking will induce telogen to at least a part of the anagen follicles. This implies that typically only half of the hair that are plucked from anagen follicles will grow back within the first few days to weeks after the treatment, and the remainder will reappear only after several weeks to months (depending on the telogen duration on a specific body area) (1,6,21).

Damage to the Follicle: (Semi-) Permanent

The third category of hair removal methods consists of methods that are capable of inducing damage to the follicle, thus preventing any future hair regrowth. The oldest method in this category is electrolysis. In this method a needle is inserted parallel to the hair shaft into the anagen follicle, where an electric current is applied to the follicle. This current can be a DC current ("galvanic electrolysis"), high frequency current ("thermolysis" or "diathermy"), or a combination of both ("blend"). This current may induce damage to the hair matrix and to the hair bulge, which will prevent future hair regrowth. Note that during electrolysis the hair is not physically removed (20,22–26). In principle, electrolysis can be applied on any skin area. However, the treatment is very time-consuming and can be painful.

The most recent hair removal method is photo hair reduction, sometimes called photo-epilation. Intense pulsed light—either from a laser or flash lamp system—induces damage to the follicle via the principle of selective photothermolysis (27–29). According to this principle, light of a specific wavelength or wavelength band is selectively absorbed by chromophores in the skin, resulting in heating of the specific target structures. Melanin, in the anagen follicle and hair shaft, acts as the chromophore for photo hair removal. Absorption of light results in heating of the bulb and bulge area of the anagen follicle. This thermal damage induces telogen effluvium, resulting in shedding of the hair, some time after the treatment (typically 2 weeks). Furthermore, hair regrowth may be permanently reduced. To what extent permanent hair reduction is achieved depends on the chosen optical parameters, and on the hair and skin pigmentation of the subject. Note that photo hair reduction depends on the presence of melanin or another chromophore. Both electrolysis and photo hair removal treatments are performed only by trained medical professionals or by trained cosmeticians.

Finally, the so-called "electronic tweezers" or "tweezers electrolysis" devices have been marketed. Their manufacturers claim that this type of device permanently removes the hair, but so far none of them have presented any scientific evidence to support this claim (20,30).

EXPERIMENTAL TESTING OF HAIR REMOVAL EFFICACY

The efficacy of a hair removal treatment can be evaluated perceptually or instrumentally. Perceptive evaluation generally uses some sort of personal opinion, of a judge or of the subject, with respect to the smoothness of the treated body area. Instrumental evaluation methods are aimed at mimicking perceptive evaluation (feeling and looking) in a standardized and reproducible way. The most suitable evaluation moment depends on the expected efficacy of the hair removal method involved: days for shaving, weeks to months for plucking, and one month to years for (semi-) permanent hair reduction.

Perceptive Evaluation

The basis of perceptual evaluations are judgments by persons, which they make having a certain criterion in mind. The more background knowledge a person has (e.g., a beautician or a dermatologist), the more likely it is that his/her criterion is based on clinical experience and therefore very stable. It is also possible to train a certain

criterion to other people, the lay observers, which will take a number of separate sessions (31). An advantage of employing untrained lay observers is that their criterion will reflect what is considered important in everyday life. For instance, a dermatologist might assess mainly the number of hairs, independent of their color; whereas, a lay observer might be reacting to their conspicuity, possibly even in relation to the skin color. Nevertheless, untrained observers also receive a slight form of guidance by means of the task they are given in the assessments. Though we are interested in the assessment of hair *removal*, the task given to the observers will usually be something like "judge the *hairiness*." The reason is that hair removal involves two or more moments in time and perceptually will amount to a difference in the hairiness.

Evaluation of Photographs

An important basis for perceptual evaluations is photographs (32). They are meant to bridge time, and they can be used in two ways—both of them assessing the *visual* aspects of hair removal. One possible way of using them is by compiling a set of photos, which covers all levels of hairiness that are appropriate for the body part under study. They are ranked according to the increase in hairiness and considered as distinct hairiness categories (numbered 1–*N*). Thus, this set of photographs can be used as a tool (a scale) to assess the hairiness of each subject at each possible moment in time, after the start of the treatment. Of course, there is also an assessment done before the treatment. Usually, the assessments are done by a small team of professional observers or skin professionals (dermatologists, beauticians) who can use their expertise or training to correctly classify the subjects according to preset criteria. The scale of photographs helps them to consistently classify various treated and untreated areas, even if they have a considerable period of time in between.

A second way of using photographs in the assessment of hair removal is by taking pictures of the treated areas. Usually, one photo is taken before treatment as well, and it is compared to the photos taken at time *t* after the start of the treatment. These photographs can be used in various procedures. As in the above case, a panel of trained professionals can use a predefined category range of hairiness classifications as a basis to assess all individual photographs taken in the experiment. Again they will use their preset criteria for these classifications.

Another possibility is that lay, untrained observers could do the assessments of these photographs. Two assessment methods are useful for lay observers. The first is the well-known two-alternative forced choice (TAFC) method (33), in which the photographs are presented in pairs. The observer is asked to indicate which of the two photos is least hairy, and a "don't know" answer is not allowed. In case there is no visible difference, 50% of the answers will be in favor of any one of the two photos. A significant, though maybe small, difference between the photos is found when the percentage of choice for one of the photos in the pair differs significantly from 50%. This method is well suited to assess small differences (34), for instance, whether a treatment has some effect at all. Care has to be taken, so that only the photos of exactly the same part of the body of the same person are compared in the presented pairs.

A second method by which the effects of hair removal can be perceptually assessed is scaling: Observers (lay observers) judge all photos individually on a categorical scale. These scales usually range from one to 10 or from one to seven, and are mostly anchored on both sides. The lower anchor (e.g., "no hairs" or "not hairy at all") and the high-end anchor ("very hairy" or "extremely hairy") help the observers in applying the scale to the photographs at hand. It should not be forgotten that the

probable variation of the judgment criteria among lay observers might bring extra variation in the data, which can be compensated to some extent by involving higher numbers of observers.

Data from scaling assessments, whether by professionals or by lay observers, can be treated in a similar way. Average scores can be calculated for each moment of time after the treatment starts, and also for the photo taken before treatment. This gives an impression of the course of perceived hairiness over time. This is usually done for the group of subjects who underwent the same treatment as a whole. If the number of observers is sufficiently high, it can be done separately for each of the individuals from that group, which will give an impression of the differences between subjects. When interested in the effect of a certain treatment over a certain period, it is best to take the difference in hairiness scores, since this quantifies the perceived hair removal (or growth).

Thus, photographs taken at different moments of time are compared either explicitly or implicitly (e.g., by means of TAFC and scaling, respectively). In these comparisons, we do not want our observers to be misguided by differences that are not related to hair growth. As a consequence, it is important to take great care in keeping constant illumination conditions during photographing. By the same token it is advised, if possible, to prevent the changes of skin and hair color due to sun exposure during the time-period of the experiment.

Tactile Evaluation

Apart from the visual aspects of hair removal covered by photographs, differences in hair growth can also be reflected tactually. Since it is difficult to make replicas of the hair present on skin such that they can be felt, tactual assessment have to be done instantaneously, and there is no way to delay the assessments until later because this changes the nature of the assessments. In principle, it is possible to ask a panel of assessors to feel the hairy skin and categorize it according to a predefined range of skin-and-hairiness examples (much like the tool of photo categories described above). Category scaling of hairiness without such a tool is cumbersome, since it is difficult for the assessors to keep their criterion and scale use stable over a period of at least several days without any help to bridge time.

Therefore, the most likely use of tactual assessments is instantaneous judgments of the presence of hair. A panel of assessors feel the treated area and judge whether any hair can be felt (35). This is done at several points of time after the start of the treatment. It allows determination of that point of time at which the last hair has been removed, at which the first hair reappears, and consequently, the length of any hair-free period in between (36). These tactual assessments can, of course, also be done by the persons undergoing treatment themselves, which is easier to incorporate in any clinical test. A disadvantage, however, is the lower number of judgments and the introduction of a wider range of criteria, which will raise the variance in the data.

Other Evaluations

Finally, Liew and Gault have proposed the *subjective hair-free* interval as a perceptive measure for hair removal efficacy (36). This subjective hair-free interval is defined as the time between treatments, during which the subject perceives the skin as hair-free. Therefore, this method is a combination of both tactile and visual evaluations. It is clear that the subject is generally a lay untrained observer and may be biased.

Instrumental Evaluation

Visual Evaluation

Generally, the number of hairs that is visible on an area of skin at a point of time after the treatment is taken as a measure of efficacy. To compensate for pretreatment inter- or intrapersonal differences in the number of hairs, the number of hairs is expressed as a regrowth percentage, i.e., the number of hairs that (re-) appear above the skin surface after the treatment as a percentage of the number of hairs before treatment:

$$\text{regrowth} = \frac{\text{number of hairs visible at certain time post-treatment}}{\text{number of hairs visible before treatment}} \times 100\% \qquad (1)$$

A measure for the portion of hair that has been removed is the hair clearance:

$$\text{clearance} = 100\% - \text{regrowth} \qquad (2)$$

Evidently, lower the regrowth or higher the clearance of hair at a certain point of time after treatment, greater is the efficacy of treatment. The first method to obtain hair counts is manual counting, done by trained observers (37). The reason we classify this method as instrumental rather than perceptive is that the observers only count the visible hairs on an area of skin (usually from a photograph), which is a very concrete task where no opinion is involved. The second method is automated image analysis (38). In this method, digitized images from areas of skin are used as input for an algorithm that automatically counts the number of hairs. An example of a digitized image of a male cheek, 24 hours after shaving is shown in Figure 1.

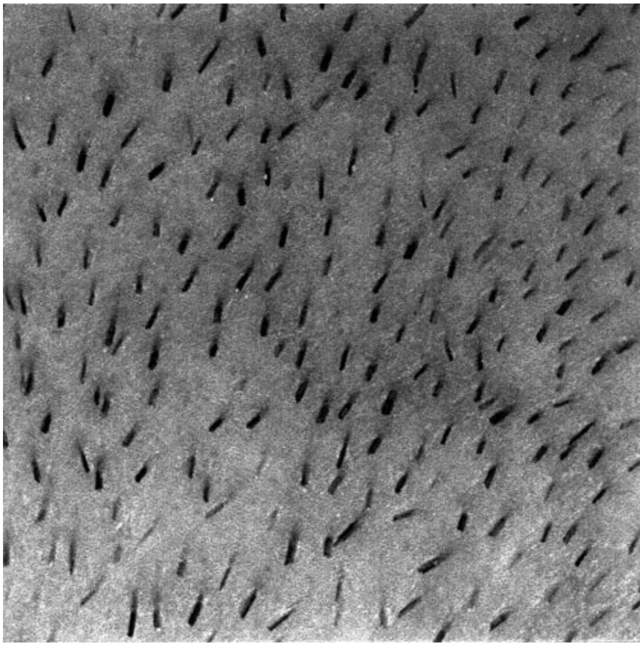

Figure 1 Example of a digital image of the skin on the male cheek taken 24 hours after shaving. Area is circa 1.8×1.8 cm.

Tactile Evaluation

Drozdenko et al. (39) have described an instrument that measures the friction of a standard object over the skin. The principle behind this instrument is very similar to the feeling by the subject themself, and can also be used as an objective evaluation of skin smoothness after hair removal.

COMPUTER SIMULATION OF HAIR REMOVAL EFFICACY

Hair growth and the effects of hair removal on follicles have successfully been modeled using the so-called Monte Carlo method (6,21,40). Such a model can predict the effect of hair removal treatments and treatment schemes, without the need for time-consuming experiments.

The Monte Carlo method is a statistical algorithm in which all relevant events in a process are described using probabilities and draws from Gaussian or uniform distributions. The relevant events for hair follicles are changes from one growth phase to another, hair growth rate, and hair removal. This can be illustrated using the flow chart for a day of a follicle, as depicted in Figure 2. At the start of the day t, it is checked in which phase the follicle was at the previous day. Then, it is checked if the growth phase of the follicle must be changed from anagen to telogen or vice versa. The timing of these changes is determined by a Gaussian distribution with mean value μ and standard deviation σ. based on literature data. The catagen phase and the various subphases of the hair growth cycle have been omitted from the model for reasons of simplicity. Subsequently, the hair length is increased for anagen follicles from the length on the previous day to a new length. The growth speed is also determined by a Gaussian distribution. Finally, if the hair is shaved, the hair length is reduced to 0. Then, the next day starts.

If the described algorithm is applied to a large number of follicles that all have a different starting point in the hair growth cycle, the Monte Carlo method delivers, for example, the number of anagen and telogen follicles on each day in the simulation, the number and length of the visible hairs on each day, etc. Figure 3 shows the number of hairs that is visible on the skin (as a percentage of the number of hairs on an untreated leg) for the two hair removal methods, and illustrates the principle of this computer simulation. Two simulations have been done: one in which the hairs on an untreated leg was mechanically epilated at $t = 0$, 4 weeks, and 8 weeks (solid line), and one in which the hairs on an untreated leg were shaved and photo-epilated at the same point of time (dashed line). The parameters in the algorithm were taken from (6).

Two important differences between the two curves are observed. Initially, during the interval between the treatments, the regrowth of hair is higher in the epilated situation than in the shaved and photo-epilated area. This is explained by the fact that mechanical epilation only induces telogen to part of the anagen follicles, while the remaining anagen follicles show only a relatively short, temporary growth delay. Photo-epilation on the other hand induces telogen to a larger number of anagen follicles (telogen effluvium). Moreover, photo-epilation is able to induce permanent damage to follicles. The percentages of telogen effluvium and permanent damage, which as a result of photo-epilation vary with the skin and hair pigmentation and with optical settings and per body area (21), were chosen as 30% and 70%, respectively, for this illustration. The second difference between the two curves is the level

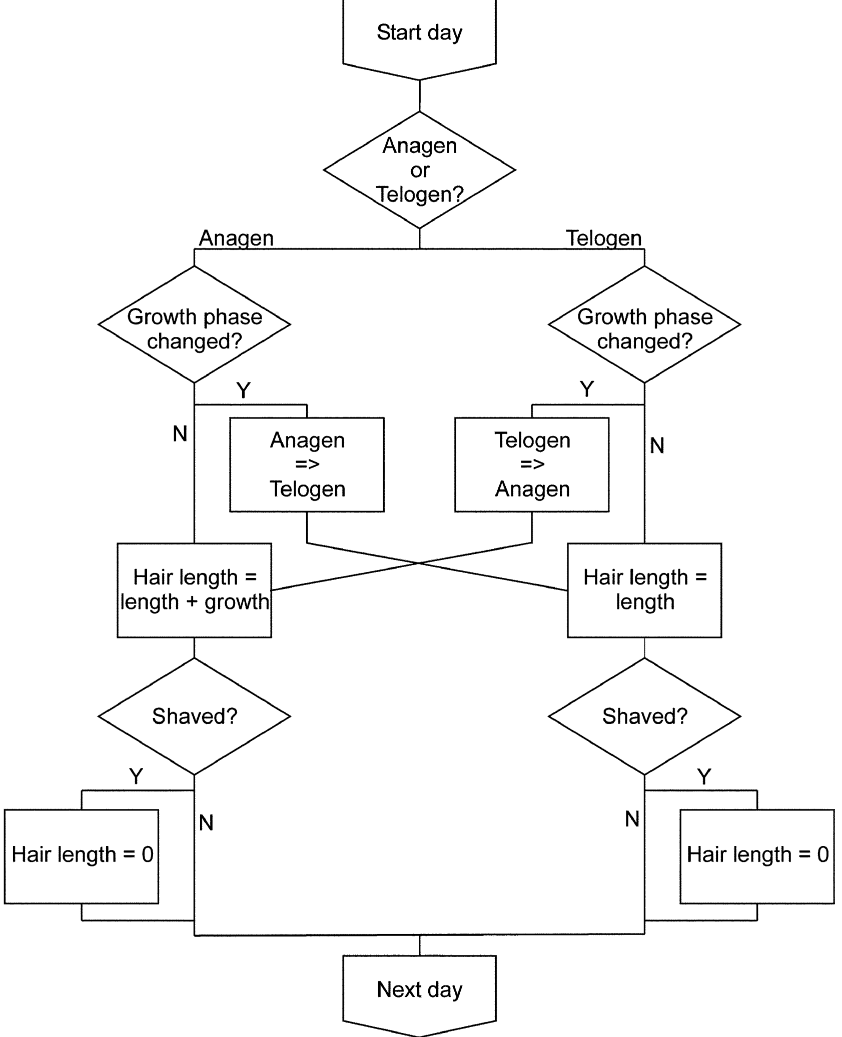

Figure 2 Flow chart for 1 day in a Monte Carlo simulation of hair growth after shaving.

of the maximum that is reached after the third treatment. The maximal percentage of hairs that is visible is nearly 100% in case of mechanical epilation, whereas it is around 40% after photo-epilation. This difference is obviously due to the permanent damage that is induced to a portion of the anagen follicles in case of photo-epilation.

Some more observations can be made from Figure 3. As stated above, the maximal percentage of hairs that is visible after three mechanical epilation treatments is more than 100%. This means that more hair is visible at this point of time than before the first treatment. The reason this high level is reached is the synchronization of follicles: a large portion of the telogen follicles enters the telogen phase at once during the treatments. As a result, these follicles all enter the anagen phase after the same telogen period. Finally, it can be seen that the difference between follicles that undergo telogen effluvium and follicles that are permanently damaged as a result of a hair removal treatment can only be seen after a long waiting period.

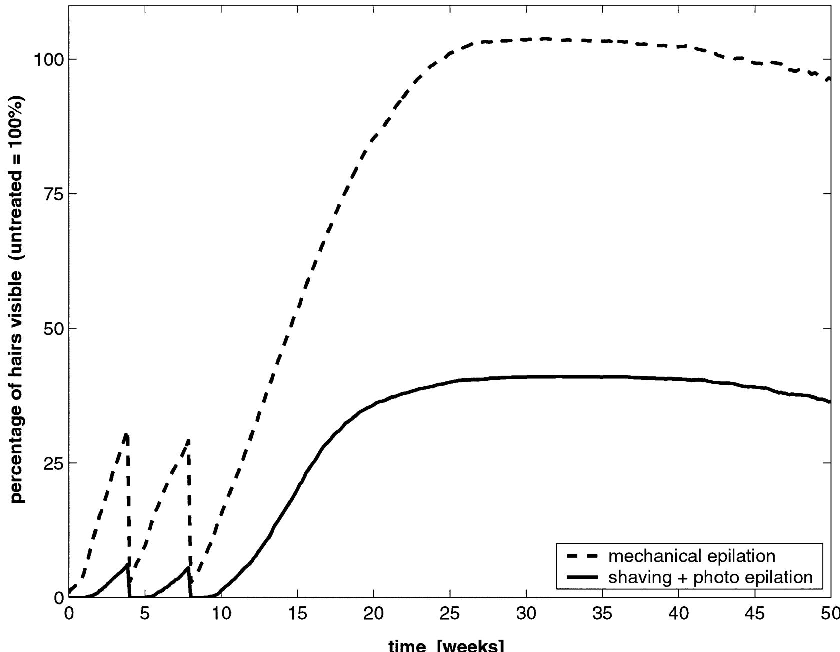

Figure 3 Typical result of a Monte Carlo simulation of hair regrowth after a number of subsequent hair removal treatments. The percentage of visible hairs is given as a function of time. 100% visibility is reached if no hair removal is done. The dashed line gives the result for 3 mechanical epilation treatments at $t = 0$, 4, and 8 weeks. The solid line gives the result for shaving immeditely followed by photo-epilation at $t = 0$, 4, and 8 weeks.

The minimal waiting time is determined by the average telogen duration plus two times the standard deviation on the telogen duration plus the time it takes for a new anagen hair to appear above the skin surface. According to the parameters for the leg in this simulation, this waiting time equals $13 + 2 \times 2.5 + 1.5 = 19.5$ weeks, but this fully depends on the exact body area.

CONCLUDING REMARKS

The efficacy of different hair removal methods depends on the exact principle of the method involved. It can be measured using various perceptive and instrumental evaluation methods. Also, the regrowth of hair after hair removal can be estimated using computer simulations.

REFERENCES

1. Olsen EA. Methods of hair removal. J Am Acad Dermatol 1999; 40:143–155.
2. Saitoh M, Uzuka M, Sakamoto M. Human hair cycle. J Invest Dermatol 1970; 54:65–81.
3. Paus R, Muller-Rover S, Botchkarev VA. Chronobiology of the hair follicle: hunting the “hair cycle clock”. J Investig Dermatol Symp Proc 1999; 4:338–345.
4. Seago SV, Ebling FJ. The hair cycle on the human thigh and upper arm. Brit J Dermatol 1985; 113:9–16.

5. Liew SH. Unwanted body hair and its removal: a review. Dermatol Surg 1999; 25: 431–439.
6. Roersma ME, Veldhuis GJ. Proposal and evaluation of a Monte Carlo model for hair regrowth following plucking. Skin Res Technol 2001; 7:176–183.
7. Klein AW, Rish DC. Depilatory and shaving products. Clin Dermatol 1988; 6:68–70.
8. Tseng MM, Kwiecien MJ. Shaving system. US6442839, 3-9-2002.
9. De Vries T, Aitink AJ, Adriaansen M, Voorhorst FR, Kadijk SE. Shaving apparatus. US6502309, 7-1-2003.
10. Natow AJ. Chemical removal of hair. Cutis 1986; 38:91–92.
11. Waldner R, Schumi C, Jacobs N. Depiation system with a depilation device and a cooling device. US6416521, 9-7-2002.
12. Scott MJ Jr, Scott MJ III, Scott AM. Epilation. Cutis 1990; 46:216–217.
13. Tannir D, Leshin B. Sugaring: an ancient method of hair removal. Dermatol Surg 2001; 27:309–311.
14. Chapman DM. The bare: ensheathed anagen root ratio as a function of the speed of epilation. Clin Exp Dermatol 1992; 17:99–101.
15. Roersma ME, Douven LF, Lefki K, Oomens CW. The failure behavior of the anchorage of hairs during slow extraction. J Biomech 2001; 34:319–325.
16. Ludwig E. Removal of intact hair papilla and connective tissue sheath by plucking anagen hairs. J Invest Dermatol 1967; 48:595–597.
17. Bassukas ID, Hornstein OP. Effects of plucking on the anatomy of the anagen hair bulb. A light microscopic study. Arch Dermatol Res 1989; 281:188–192.
18. Barth JH. Measurement of hair growth. Clin Exp Dermatol 1986; 11:127–138.
19. Randall VA, Ebling FJ. Seasonal changes in human hair growth. Br J Dermatol 1991; 124:146–151.
20. Richards RN, Meharg G. Cosmetic and Medical Electrolysis and Temporary Hair Removal. Vol. 39. Medric Ltd., Toronto, 1991:39.
21. Kolinko V, Littler CM. Mathematical modeling for the prediction and optimization of laser hair removal. Lasers Surg Med 2000; 26:164–176.
22. Peereboom-Wynia JD, Stolz E, van Joost T, Kleiman H. A comparative study of the effects of electrical epilation of beard hairs in women with hirsutism by diathermy and by the blend method. Arch Dermatol Res 1985; 278:84–86.
23. Richards RN. Point-counterpoint: electrolysis for permanent hair removal. J Cutan Med Surg 1999; 3:239–240.
24. Richards RN, McKenzie MA, Meharg GE. Electroepilation (electrolysis) in hirsutism. 35,000 hours' experience on the face and neck. J Am Acad Dermatol 1986; 15:693–697.
25. Richards RN, Uy M, Meharg G. Temporary hair removal in patients with hirsutism: a clinical study. Cutis 1990; 45:199–202.
26. Urushibata O, Kase K. A comparative study of axillar hair removal in women: plucking versus the blend method. J Dermatol 1995; 22:738–742.
27. Anderson RR, Parrish JA. Selective photothermolysis: precise microsurgery by selective absorption of pulsed radiation. Science 1983; 220:524–527.
28. Altshuler GB, Anderson RR, Manstein D, Zenzie HH, Smirnov MZ. Extended theory of selective photothermolysis. Lasers Surg Med 2001; 29:416–432.
29. Dierickx CC. Hair removal by lasers and intense pulsed light sources. Dermatol Clin 2002; 20:135–146.
30. Verdich J. A critical evaluation of a method for treatment of facial hypertrichosis in women. Dermatologica 1984; 168:87–89.
31. IFSCC monograph Principles of product evaluation: Objective sensory methods, 2003.
32. Alster TS, Bryan H, Williams CM. Long-pulsed Nd: YAG laser-assisted hair removal in pigmented skin: a clinical and histological evaluation. Arch Dermatol 2001; 137:885–889.
33. Matlin MW, Foley HJ. Sensation and Perception. 4th ed. Allyn & Bacon, 1997.
34. Westerink JHDM. Assessment of visibility of facial wrinkle reduction by variuous types of observers. Proceedings 2003 IFSCC Conference, Seoul, Korea, 22-9-2003, 2003.

35. Breuer M, Sneath R, Ackerman CS, Pozzi SJ. Perceptual evaluation of shaving closeness. J Soc Cosmet Chem 1989:141–149.
36. Liew SH, Gault DT. Laser hair removal: the subjective hair-free interval as a simple outcome measure. Br J Plast Surg 1999; 52:322–323.
37. Bjerring P, Egekwist H, Blake T. Comparison of the efficacy and safety of three different depilatory methods. Skin Res Technol 1998; 4:196–199.
38. Gruber DM, Berger UE, Sator MO, Horak F, Huber JC. Computerized assessment of facial hair growth. Fertil Steril 1999; 72:737–739.
39. Drozdenko R, Weinstein C, Mitchell J, Weistein S. Product testing measurement methods: applications to shaving. Cosmetics & Toiletries 1994; 109:67–72.
40. Halloy J, Bernard BA, Loussouarn G, Goldbeter A. The follicular automaton model: effect of stochasticity and of synchronization of hair cycles. J Theor Biol 2002; 214: 469–479.

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Skin Lipid Structure Measured by Electron Paramagnetic Resonance

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INTRODUCTION

The skin barrier against chemicals, surfactants, and UV irradiation is its outermost layer, the stratum corneum (SC). SC has a heterogeneous structure, composed of corneocytes embedded in the intercellar lipid lamellae. Ordering of the lipid bilayer is associated with the main epidermal barrier. Knowledge of the lipid structure is important in understanding the mechanism of irritant dermatitis and other SC diseases. The ordering (or fluidity) change of the lipid bilayer is measured by the analyses of aliphatic spin probes incorporated into intercellar lamella lipids using electron paramagnetic resonance (EPR) (1–5). The EPR spin probe method measures nondestructively the ordering of the lipid bilayer of SC.

EPR [or electron spin resonance (ESR)] utilizes spectroscopy, which measures the freedom of an unpaired electron in an atom or molecule. The principles behind magnetic resonance are common to both EPR and nuclear magnetic resonance (NMR), but there are differences in the magnitudes and signs of the magnetic interactions involved. EPR probes an unpaired electron spin, whereas NMR probes a nuclear spin.

EPR in conjunction with the spin probe (or label) method has considerable advantages in the study of lipid structures as well as behaviors. The macroscopic and local viscosity of the environment profoundly influences the rate of lipid molecular reorientation. The physicochemical properties of intercellar lipids of SC as a function of various surfactants (2,3), water contents (4), and various kinds of spin probes (5) were investigated. These studies provided the fluidity-related behaviors of SC at the different conditions by measuring EPR signal intensities and hyperfine coupling values. However, the conventional method of calculating the chain ordering using hyperfine values cannot differentiate subtle EPR spectral changes. Changes in the probe behavior are reflected in the EPR linewidth as well as the lineshape, besides hyperfine values. Careful analysis of EPR spectra can be performed by a spectral simulation to extract quantitative ordering regarding the lipid structures (6,7). To have detailed evaluations of complex biological samples, EPR in conjunction with a simulation method can be utilized to verify skin lipid structures. Thus, a

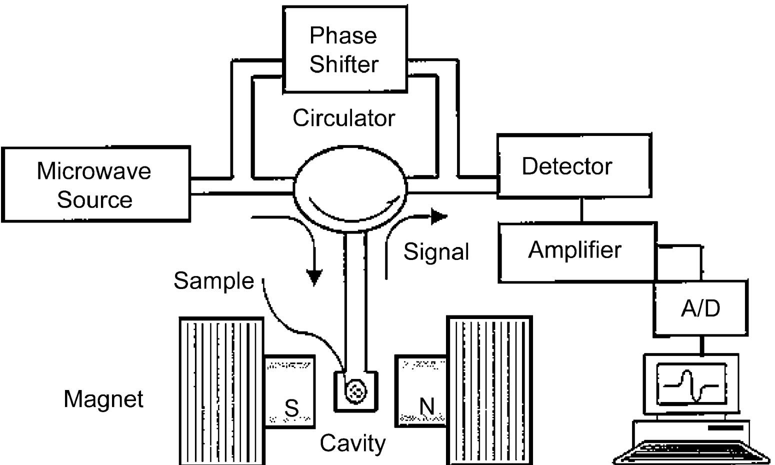

Figure 1 Block diagram of EPR spectrometer.

computational EPR method provides greater detailed structure of the lipid bilayer than that of a conventional method.

EPR APPARATUS

EPR apparatus consists of a klystron to generate microwaves, electromagnet, resonant cavity, microwave detector, amplifier, A/D converter, and PC as shown in Figure 1. The microwaves from the klystron have a constant frequency, and those microwaves reflected from the resonant cavity are detected, changed to an electronic signal, amplified, and then recorded. In contrast to NMR, substances which contain unpaired spin can be observed by EPR. Paramagnetic substances including transition metal complexes, free radicals, and photochemical intermediates are observed. Approximately 10^{-13} mole of a substance gives an observable signal, indicating the great sensitivity of EPR.

EPR OF NITROXIDE SPIN PROBE

Momentum of electron spin in a magnetic field orients only two quantum states: $m_s = 1/2$ and –1/2 (Fig. 2). Application of an oscillating field perpendicular to a steady magnetic field (H) induces transitions between the two states provided the frequency (ν) of the oscillating field satisfies the resonance condition:

$$\Delta E = h\nu = g\beta H, \tag{1}$$

where h is a Planck's constant, g is a dimensionless constant called the g-value, and β the electron Bohr magneton, and H the applied magnetic filed.

The interaction of an electron spin in resonance with a neighboring nuclear spin in a molecule is called hyperfine coupling. For nitroxide spin probe, ^{14}N of the probe has three quantum states: +1, 0, and –1. Each quantum state interacts with an electron spin and further splits into two sets of energy states as shown in Figure 2. The selection rules for transitions in hyperfine coupling are $\Delta m_s = 1$ and $\Delta m_I = 0$.

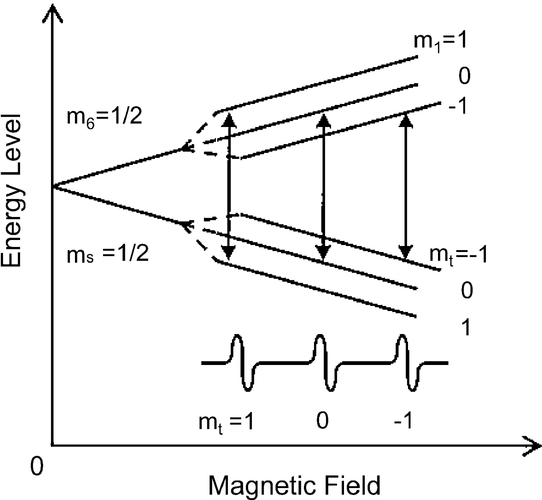

Figure 2 Hyperfine levels and transitions for a single nucleus (^{14}N) of $I = 1$ with positive coupling constant.

Thus, one can observe three transition lines for the freely tumbling nitroxide spin probe. The EPR spectra are usually recorded as the first derivative of the absorption spectrum as shown in the lower part of Figure 2.

SPIN PROBE LINESHAPES OWING TO MOLECULAR MOTIONS

The linewidths can vary under certain spin probe environments. When line broadening arises from incomplete averaging of the g-value and the hyperfine coupling interactions within the limit of rapid tumbling in a medium, EPR lineshape starts changing from the triplet pattern. EPR spectra of nitroxide radicals for different tumbling times and order parameters are presented in Figure 3. If a spin probe is oriented (immobilized) in a membrane, EPR spectrum shows an anisotropic pattern, which clearly shows parallel and perpendicular hyperfine coupling structures (the top spectrum in Fig. 3). The order parameter is approximately 0.7 or higher. If a spin probe tumbles relatively fast (weakly immobilized) in a membrane, EPR spectrum shows a triplet pattern with unequal intensities. The order parameter is usually very small.

SPIN PROBES (OR SPIN LABELS)

The fluidity of the lipid bilayer is obtained with doxylstearic acid (DSA), which is most commonly used. The chemical structures of DSAs are depicted in Figure 4. Changes of lipid chain ordering are able to be monitored using the probes. The orientation of spin probe reflects the local molecular environment and should serve as an indicator of conformational changes in lipid bilayers.

The fluidity at different position of the lipid bilayer is obtained with 5-, 7-, 12-, and 16-DSA. The 5-DSA is usually used for extraction of information near the head group or surface region in a membrane. The 16-DSA is for near the end of the lipid chain.

Description of spectra	Approx. tumbling time (ns)	Approx. order parameter
Immobilized	0.5	0.7
Moderately Immobilized	2.5	0.3
Weakly Immobilized	5.0	0.1

Figure 3 Nitroxide EPR lineshape as a function of tumbling time and order parameter. Parallel and perpendicular hyperfine couplings are also indicated for an anisotropic EPR spectrum.

CONVENTIONAL ORDER PARAMETER (*S*)

The order parameter indicates the lipid fluidity and microenvironment of the medium in which the spin probe is incorporated. The conventional order parameter (S) is determined by the hyperfine coupling of the EPR spectrum according to the following relations (8):

$$S = \frac{A_{\|} - A_{\perp}}{A_{zz} - \frac{1}{2}(A_{XX} + A_{YY})} \frac{a}{a'}, \tag{2}$$

$$a' = \frac{A_{\|} + 2A_{\perp}}{3}, \tag{3}$$

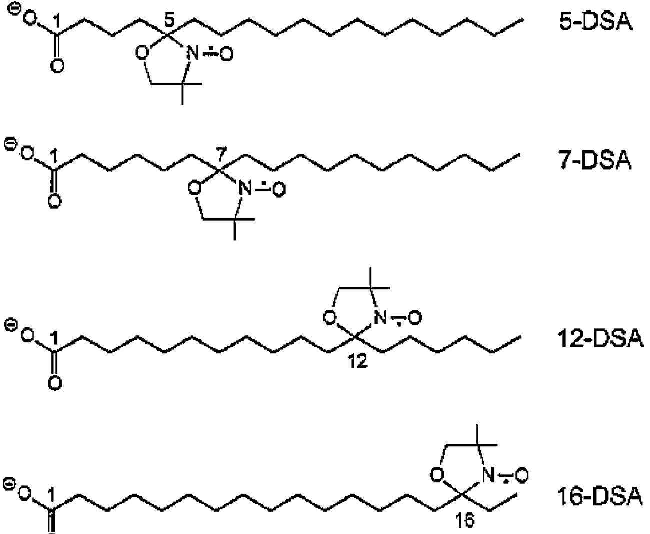

Figure 4 Structures of various DSA spin probes. *Abbreviation*: DSA, doxylstearic acid.

where a is the isotropic hyperfine coupling value $(A_{XX}+A_{YY}+A_{ZZ})/3$; A_{XX}, A_{YY}, and A_{ZZ} are the principal values of the spin probe. In a calculation of the experimental spectra, the following principal components were used for 5-DSA (9):

$$A_{XX}, A_{YY}, A_{ZZ} = (6.6, 5.5, 34.5)\,G,$$

$$g_{XX}, g_{YY}, g_{ZZ} = (2.0086, 2.0063, 2.0025).$$

The experimental hyperfine couplings of $2A_{||}$ and $2A_{\perp}$ are obtained from the EPR spectrum. The large S value indicates the anisotropy of the probe site in the lipid as shown in Figure 3. For example, the spin probe is incorporated in the highly oriented intercellular lipid bilayer in normal skin, the probe cannot move freely because of the rigidity of the lipid structure (large $2A_{||}$ value), and its EPR spectrum represents the broad profile. Once the normal lipid structure is completely destroyed by chemical and/or physical stress, the EPR spectral profiles become sharp three-lines because nothing inhibits probe mobility. Therefore, the EPR spectral profile represents the rigidity of the probe moiety. Note that the conventional analysis measuring $2A_{||}$ and $2A_{\perp}$ gives limited information concerning the probe moiety in the lipid. In some cases S values do not reveal the subtle difference in overall EPR spectral changes related to the lipid chain ordering (6).

ORDER PARAMETER (S_0) BY THE EPR SIMULATION

Calculation of Slow-Motional EPR Spectra

The slow-tumbling motions of the aliphatic spin probes can be calculated using a computer program. The nonlinear least-squares fitting program called NLLS analyzes the EPR spectra based on the stochastic Liouville equation (10–12). The simulation of the EPR spectra for spin probes incorporated into multilamella lipids is carried out using a microscopically ordered but macroscopically disordered (MOMD) model introduced by Meirovitch et al. (13). This model is based on the characteristics of the dynamic structure of lipid dispersions.

The order parameter, S_0, is defined as (14,15):

$$S_0 = \left\langle D^2{}_{00} \right\rangle = \left\langle \frac{1}{2}\left(3\cos^2\gamma - 1\right) \right\rangle = \frac{\int \mathrm{d}\Omega \exp(-U/kT) D^2{}_{00}}{\int \mathrm{d}\Omega \exp(-U/kT)}, \tag{4}$$

which measures the angular extent of the rotational diffusion of the nitroxide moiety. Gamma (γ) is the angle between the rotational diffusion symmetry axis and the z-axis of the nitroxide axis system; z is the axis of the nitrogen $2p_z$ atomic orbital, and x-axis is along the N–O bond as shown in Figure 5. The local or microscopic ordering of the nitroxide spin probe in the membrane is characterized by the S_0 value. A larger S_0 value indicates very restricted motion. Therefore, S_0 reflects the local ordering of bilayer molecules in the membrane.

CONVENTIONAL ORDER PARAMETER AND ORDER PARAMETER BY SIMULATION METHOD

Stripped SC from a patch test was examined to characterize the lipid chain ordering using two methods: conventional order parameter and spectral simulated order

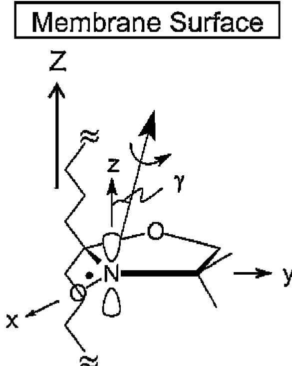

Figure 5 A schematic representation of a configuration of DSA spin probe in the membrane, where Z-axis of the acyl chain is parallel to z-axis.

parameter (16). One piece of stripped SC ($\sim$0.5 cm^2) was incubated in 1.0 mg/dl 5-DSA aqueous solution for 1 hour at 37°C. After rinsing with deionized water to remove excess spin probe, the SC sample was mounted on an EPR cell. The detailed sample preparations are described elsewhere (1). Commercially available X-band (9 GHz) EPR spectrometer was used to measure the fluidity of the SC sample. The typical spectrometer settings were the following: microwave power, 25 mW; time constant, 200 milliseconds; sweep time, 120 seconds; modulation, 1 G; sweep width, 125 G.

Figure 6 presents the EPR spectra obtained from 5-DSA in stripped SC and in a membrane. The EPR spectrum of the stripped SC is a complicated pattern. If the spin probe incorporates in a lipid, EPR spectrum appears to be the dotted lineshape as shown in Figure 6. The sharp signals marked with the asterisks are originated from the second species which does not incorporate in the lipid. The sharp peaks are because of the relatively faster probe motion. Thus, the EPR pattern is composed of two signals. The conventional S value, 0.80, was obtained by using Eq. (2) measuring the hyperfine values from the observed spectrum.

To quantitatively obtain the order parameter of the complex EPR spectrum, the spectrum was simulated. Figure 7 shows the experimental and simulated EPR

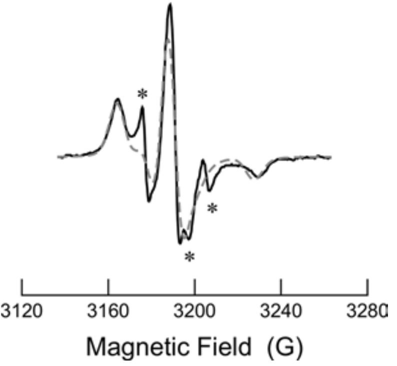

Figure 6 EPR spectra obtained from 5-DSA in stripped SC (*solid line*) and in a membrane (*dotted line*). The asterisks indicate the second species. *Abbreviation*: SC, stratum corneum.

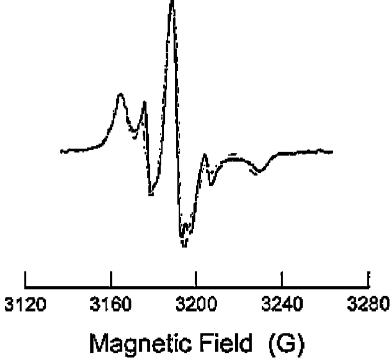

Figure 7 Experimental (solid line) and simulated (dotted line) EPR spectra of 5-DSA in stripped SC. *Abbreviations*: EPR, electron paramagnetic resonance; SC, stratum corneum.

spectra of 5-DSA in stripped SC. The simulated S_0 value was 0.73. Order parameter (S_0) obtained by the simulation differs from the conventional method (S).

OTHER APPLICATIONS OF EPR METHOD

Effects of Surfactants

Different types as well as mixtures of surfactants change the fluid structure of lipid bilayer differently. Kawasaki et al. examined the influence of anionic surfactants, sodium lauryl sulfate (SLS), and sodium lauroyl glutamate (SLG), on human SC by the EPR spin label method (2). The order parameter obtained by 1.0 wt.% SLS-treated cadaver SC (C-SC) was 0.52. In contrast, the high S value of 0.73 for 1.0 wt.% SLG was obtained. The results suggest clear surfactant effects on the structure of lipid bilayer. In addition, a reasonable correlation between order parameters and human clinical data (visual scores and transepidermal water loss values) was shown.

Effect of Skin Penetration Enhancers

Interaction of skin penetration enhancers correlates with the fluidity of the intercellular lipid bilayers. Quan and Maibach investigated the effects on a cadaver (C-SC) at three concentrations of laurocapram (1-dodecylazacyclo-heptan-2-one), utilizing the EPR spin probe method (17). The EPR spectra of laurocapram-treated human SC were totally different from those of untreated C-SC. The results suggest that laurocapram causes an increase in the flexibility and polarity of local bilayers surrounding 5-DSA.

SUMMARY AND FUTURE PROSPECTS

EPR along with a modern computational analysis provides quantitative insight into the lipid structure. EPR spectral pattern contains useful information for probe moiety as well as the lipid structure. Satisfactory agreement between the experimental and calculated spectrum can provide a quantitative S_0, which gives the microscopic lipid chain ordering in the lipid bilayer. The spectral simulation offers a reliable value of the chain ordering where conventional order parameters cannot reveal the lipid chain ordering (6). In addition, the dynamics of the sulfhydryl group region of SC

protein were studied by EPR and EPR simulation (18). Thus, EPR together with a computational analysis can extract important information about the detailed skin lipid structure.

REFERENCES

1. Mizushima J, Kawasaki Y, Sakamoto K, Kawashima M, Cook R, Maibach HI. Electron paramagnetic resonance: a new technique in skin research. Skin Res Technol 2000; 6:100.
2. Kawasaki Y, Quan D, Sakamoto K, Cook R, Maibach HI. Influence of surfactant mixtures on intercellular lipid fluidity and skin barrier function. Skin Res Technol 1999; 5:96.
3. Mizushima J, Kawasaki Y, Tabohashi T, Kitano T, Sakamoto K, Kawashima M, Cook R, Maibach HI. Effect of surfactants on human stratum corneum: electron paramagnetic resonance. Int J Pharm 2000; 197:193.
4. Alonso A, Meirelles NC, Yushmanov VE, Tabak M. Water increases the fluidity of intercellar membranes of stratum corneum: correlation with water permeability, elastic and electrical resistance properties. J Invest Dormatol 1996; 106:1058.
5. Kitagawa S, Ikarashi A. Analysis of electron spin resonance spectra of alkyl spin labels in excised guinea pig dorsal skin, its stratum corneum, delipidized skin and stratum corneum model lipid liposomes. Chem Pharm Bull 2001; 49:165.
6. Nakagawa K. ESR investigation of chain ordering of a trigylcerol membrane. Bull Chem Soc Jpn 2004; 77:269.
7. Nakagawa K. Diffusion coefficient and relaxation time of aliphatic spin probes in a unique triglyceride membrane. Langmuir 2003; 19:5078.
8. Hubbell WL, McConnell HM. Molecular motion in spin-labeled phospholipids and membrane. J Am Chem Soc 1971; 93:314.
9. Ge M, Rananavare SB, Freed JH. ESR studies of stearic acid binding to bovine serum albumin. Biochim Biophys Acta 1990; 1036:228.
10. Meirovitch E, Igner D, Igner E, Moro G, Freed JH. Electron-spin relaxation and ordering in smectic and supercooled nematic liquid crystals. J Chem Phys 1982; 77:3915.
11. Schneider DJ, Freed JH. Calculating slow motional magnetic resonance spectra. In: Berliner LJ and Reuben J, eds. Biological Magnetic Resonance. Vol. 8. New York: Plenum Press, 1989 (chapter 1).
12. Budil DE, Lee S, Saxena S, Freed JH. Nonlinear-least-squares analysis of slow-motion EPR spectra in one and two dimensions using a modified Levenberg–Marquardt algorithm. J Magn Reson Ser A 1996; 120:155.
13. (a) Meirovitch E, Nayeem A, Freed JH. Analysis of protein–lipid interactions based on model simulations of electron spin resonance spectra. J Phys Chem 1984; 88:3451. (b) Meirovitch E, Freed JH. Analysis of slow-motional electron spin resonance spectra in smectic phases in terms of molecular configuration, intermolecular interactions, and dynamics. J Phys Chem 1984; 88:4995.
14. Crepeau RH, Saxena S, Lee S, Patyal BR, Freed JH. Studies on lipid membranes by two-dimensional Fourier transform ESR: enhancement of resolution to ordering and dynamics. Biophys J 1994; 66:1489.
15. (a) Ge M, Field KA, Aneja R, Holowka D, Baird BA, Freed JH. Electron spin resonance characterization of liquid ordered phase of detergent-resistant membranes from RBL-2H3 cells. Biophys J 1999; 77:925. (b) Ge M, Freed JH. Polarity profiles in oriented and dispersed phosphatidylcholine bilayers are different. An ESR study. Biophys J 1998; 74:910.
16. Mizushima J, Kawashima M, Kawasaki Y, Nakagawa K, Takino Y, Sakamoto K. Utilization of ESR spectral simulation analysis with oil soluble spin probe for the determination of lipid structures in the stratum corneum. The 28th Ann Meeting of Jpn Cosmet Sci Soc 2003; 50, Tokyo, Japan.

17. Quan D, Maibach HI. An electron paramagnetic resonance study. I. Effect of azone on 5-doxyl stearic acid-labeled human stratum corneum. Int J Pharm 1994; 104:61.
18. Alonso A, dos Santos WP, Leonor SJ, dos Santos JG, Tabak M. Stratum corneum protein dynamics as evaluated by a spin-label maleimide derivative: effect of urea. Biophys J 2001; 81:3566.

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Effects of Occlusion on Human Skin

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BACKGROUND

Occlusion occurs on a regular basis from diapers and feminine hygiene products to clothes, gloves, and bandages. Originally felt to be a neutral intervention, occlusion per se was found to be active on the skin when studies evaluated the effect of occlusion per se. Our aim is to broadly capture research in medical disciplines related to the effect of occlusion on human skin, both healthy and diseased, and to illustrate the current knowledge regarding occlusion as an active agent.

RESULTS

Skin Physiology and Chemistry

Aly et al. (1) performed five days of occlusion with vinylidene polymer plastic film (Saran Wrap®) on human forearm skin: pH became more basic under occlusion from a preocclusion value of 4.3 to 7.0 postocclusion. Transepidermal water loss (TEWL) increased from 0.56 to 1.87 mg/cm^2/hr postocclusion and carbon dioxide emission increased from 25 to 118 nl/cm^2/min. Though *p*-values are not provided in the tables, the commentary indicates that these data are statistically significant.

King et al. (2) studied the effect of occlusion with plastic versus paper tape on carbon dioxide emission from human back skin. They found a significant increase in the rate of CO_2 emission when skin was occluded with Blenderm™ occlusive plastic tape while occlusion with Micropore™ semiocclusive paper tape yielded no significant difference in CO_2 emission when compared with nonoccluded skin.

Hartmann (3) performed a three-day continuous occlusion on the forearms of 26 subjects with an impermeable and nonadhesive polyethylene foil: relative skin moisture content measured using an evaporimeter increased significantly from 20% to 75% after three days of occlusion. Twenty-four hour postocclusion, relative skin moisture decreased to 5%, 15% below the baseline value, indicating an overdrying effect following occlusion. Skin pH increased significantly ($p = 0.00001$) after occlusion from an average baseline of 4.9 to an average of 7.2. This value fell to 5.2 24 hours following removal of the occlusive device.

Faergemann et al. (4) confirmed the findings of aforementioned studies with statistical analysis. After eight days of occlusion with vinylidene polymer plastic film (Saran Wrap) the $p\mathrm{CO_2}$, pH, TEWL, and skin water content (WC) were all significantly increased. $p\mathrm{CO_2}$, pH, and WC remained elevated throughout the eight days of occlusion while TEWL peaked at three days and decreased thereafter. TEWL was significantly lower after eight days of occlusion than it was at its three-day peak. Significance of the increase for $p\mathrm{CO_2}$ and WC was seen after 24 hours.

Baxter and Stoughton (5) originally studied the mitotic index of keratinocytes in psoriatic plaques when treated with Blenderm (3M™, St. Paul, Minnesota, U.S.) plastic tape occlusion alone, flurandrenolide (Cordran) tape, and anthralin. The mitotic index was lowered most by coricoid tape followed by anthralin, then occlusion. All treatments significantly lowered the mitotic index compared with untreated plaque, and flurandrenolide tape caused a significant lowering of the mitotic index when compared to treatment with plastic tape occlusion and anthralin.

Gottlieb et al. (6) studying the effect of an occlusive hydrocolloid dressing (Actiderm) on clinical assessment and keratinocyte growth fraction in psoriatic plaques found a significant decrease in scale and skin thickness after two weeks of treatment with occlusion when compared with nonocclusion, and the keratinocyte growth fraction, the population of keratinocytes actively dividing, was significantly decreased after treatment with occlusion when compared with nonoccluded plaques. This study was conducted while patients were receiving systemic therapy; each patient served as their own control.

Fisher and Maibach (7) studying the differences in cell kinetics in tape-stripped skin dressed with either Micropore (3M, St. Paul, Minnesota, U.S.) nonocclusive tape, Blenderm occlusive tape, vinylidene plastic film (Saran Wrap), or the adhesive used in Blenderm without the occlusive backing found that the rate of mitosis and epidermal thickness was decreased with all four agents thus questioning the belief that occlusivity of a dressing is the measure of how active a dressing is.

Agren and Wijesinghe (8) examined the effects of 48 hours of occlusion on human forearm skin with an occlusive absorptive hydrocolloid dressing (Duoderm®) versus an occlusive nonabsorptive zinc-medicated dressing (Mezinc®), a dressing which does not absorb physiologic water loss from the skin surface. Measuring with an evaporimeter, they found that both dressings caused a significant increase in skin hydration as they decreased the TEWL from a normal value of $8.5\,\mathrm{g/m^2/hr}$ to 2.0 to $2.5\,\mathrm{g/m^2/hr}$. Thus these two barriers, Duoderm and Mezinc, are slightly more water vapor permeable than vinylidene polymer plastic film (Saran Wrap), a vapor impermeable barrier with a vapor transmission rate of only $0.5\,\mathrm{g/m^2/hr}$ (9). After dressing removal, TEWL was increased in both cases. Skin surface temperature did not change significantly with either dressing. Interestingly, the skin pH under the nonabsorptive dressing increased significantly to 5.6, consistent with earlier findings, but the pH under the absorptive hydrocolloid dressing did not. The authors did not offer an explanation for this finding.

Ramsing and Agner (10) compared latex gloved human hands for a minimum of six hours per day for 14 days with nonoccluded hands and use of a rubber glove over a cotton glove. A significant increase in TEWL occurred after 14 days of occlusion when compared to nonocclusion. TEWL increase was prevented with the use of a cotton glove below the occlusive rubber glove. This phenomenon could possibly be explained by an irritant effect of the latex itself rather than an effect of occlusion (11).

Hwang et al. (12) performed seven days of occlusion in psoriatic plaques and unaffected skin on the back of five patients. The occlusion was water vapor impermeable latex covered with Tegaderm™, 7 g/m^2/hr vapor permeability, and Micropore™ (3M, St. Paul, Minnesota, U.S.) tape. Occlusion by vapor impermeable devices caused a nearly complete correction of the epidermal calcium gradient. The normal epidermal calcium gradient allows proper keratinocytes differentiation. Occlusion of unaffected skin, however, did not alter the gradient. Earlier studies of occlusion following tape stripping as a means of disrupting the epidermal permeability barrier found that occlusion did not lead to restoration of the calcium gradient. They hypothesized that their results differ from those of prior studies because psoriasis is a chronic disease resulting in long-standing disruption of the permeability barrier while tape stripping is a more acute perturbation of otherwise normal skin.

Zhai et al. (13) studied the effect of occlusion of forearm skin with a wet occlusive patch for three hours and occlusion with wet diapers for three and eight hours. Skin hyperhydration was measured by measuring the water evaporation rate, creating a decay curve with this data and then measuring the area under the water evaporation rate curve. Skin hydration was significantly higher with both patch and diaper occlusion when compared with nonoccluded skin.

Schafer et al. (14) occluded forearm skin with vapor permeable and vapor impermeable films over an absorbent feminine hygiene product. Each participant had both forms of occlusion applied, one to each forearm with 3 mL of normal saline used to premoisten the absorbent pads before occlusion to simulate physiologic discharge. After six hours of occlusion there was no significant difference between baseline and occlusion with a vapor permeable film whereas occlusion with a vapor impermeable film caused a significant increase in stratum corneum (SC) hydration measured with an evaporimeter. When ambient temperature and humidity are held relatively constant, skin surface water loss and relative humidity below the dressing were significantly lower with the vapor permeable dressing than with the vapor impermeable dressing. The relative humidity below the dressing and the skin surface water loss were elevated under the impermeable dressing as compared with the permeable dressing at all levels of ambient temperature and humidity studied except when temperature was raised to 30°C with 75% ambient humidity. The difference in skin surface water loss between the two dressings lost significance—indicating that ambient humidity and temperature can negate the effects of a vapor permeable dressing. Thus increased skin surface temperature may be the mechanism by which occlusion acts on the skin and may indicate that thermal treatments may be effective in occlusion-responsive dermatoses.

Friebe et al. (15) compared the effect of occlusion with sodium lauryl sulfate (SLS), water, or nothing in an aluminum test chamber on TEWL. Occluding the skin with water in the test chamber or with an empty test chamber resulted in increased TEWL that lasted up to 180 and 120 minutes, respectively, after removal of the test chamber from the skin. This illustrates the ability of simple occlusion without an irritant to disturb the SC permeability barrier.

Berardesca (16) studying the plastic occlusion stress test (POST) showed that normal skin has increased SSWL after only 24 hours of occlusion with an occlusive

plastic (Hill Top, Cincinnati, Ohio, U.S.) chamber which returned to normal values, approximately 8 g/m^2/hr, within 25 minutes of postocclusion. Skin pretreated with 7% SLS, however, had significantly higher SSWL both immediately after chamber removal and 25 minutes after chamber removal thus showing that the POST is a sensitive method for detecting skin irritation even in skin which is not visibly irritated.

Histology

Lindberg and Forslind (17) examined the effect of simple occlusion with an aluminum chamber on normal human forearm skin at the electron microscopic level after 48 hours of occlusion. After three to six hours the intercellular spaces in the basal layer were widened with perinuclear vacuolization of the keratinocytes. They also noted a change in the appearance of the Langerhans cells (LC) after occlusion with fewer organelles, a more electron dense cytoplasm and increased concentration of filaments. LC were in apposition with mononuclear cells in the epidermis after occlusion, a feature not seen in nonoccluded skin.

Lindberg et al. (18) studied with electron microscopy the morphological changes caused by simple occlusion using aluminum chambers on healthy human forearms. They noted a time-dependent change with increased size of intracellular spaces in the basal layer and invasion of mononuclear cells within three hours of occlusion. Stratum spinosum intracellular spaces were widened by six hours with vacuoles forming in the cytoplasm and perinuclear zone of keratinocytes as well as dermal edema visible after 24 hours of occlusion. Intracellular debris was seen after 48 hours of occlusion.

Mikulowska (19) studied the effect of occlusion with SLS versus water on LC in healthy subjects. Aluminum (Finn®, Epitest, Helsinki) chambers were used to occlude forearm skin with either SLS or water. Mikulowska found that after 48 hours of occlusion with water followed by 24 hours of nonocclusion, the LC population showed a pronounced increase in reactivity evidenced by an increase in dermal LC, a decrease in epidermal LC, a penetration of LC dendrites into the granular layer, and ultrastructural signs of LC activation. This indicated that occlusion with water alone caused activation of the epidermal immune system.

Warner et al. (20) occluded forearm skin with distilled water inside plastic occlusive (Hill Top) chambers for 4 and 24 hours followed by shave biopsy of the site and examination with electron microscopy. Water pools were visible in the intercorneocyte lipid layers, the thickness of the SC increased due to water retention, and swelling of the corneocytes occurred.

Matsumura et al. (21) examined the effect of occlusion on microscopic skin surface morphology of upper arm skin in healthy volunteers. Occlusion was accomplished using the following films: polypropylene: 0.9 g/m^2/hr; polyester: 1.9 g/m^2/hr; nylon: 12 g/m^2/hr, 16.6 g/m^2/hr, and 17.7 g/m^2/hr; polyurethane: 24.9 g/m^2/hr, 30.7 g/m^2/hr, and 32.1 g/m^2/hr; microporous polyethylene: 80.9 g/m^2/hr and nonwoven polypropylene: 145.6 g/m^2/hr. After 24 hours of occlusion by these films of varying permeability, skin occluded by film with a permeability of 30 g/m^2/hr or less had deepening of skin surface furrows.

Hwang et al. (12) in their aforementioned study of occlusion in psoriatic plaque and unaffected skin also did light and electron microscopy analyses following occlusion. Light microscopy revealed decreased inflammation as evidenced by reduced hyperkeratosis, parakeratosis, and neutrophilic infiltration, but the epidermis remained hyperplastic with elongated rete ridges. Decreased thickness of the SC, normalization of

the intercorneocyte lipid layers, and a disappearance of the calcium precipitates normally found in the SC of psoriatic plaque were seen by electron microscopy.

Note for all histologic studies microscopic observations are a subjective and qualitative assessment and thus are subject to more errors and interpretations than quantitative information.

Microbiology

Bibel et al. (22) examined the changes in microbial flora produced by 48 hours of occlusion on healthy human back skin with sterile gauze moistened with sterile water over which a Teflon® (DuPont™) sheet was secured. The density of bacterial colonization increased four orders of magnitude after 48 hours of uninterrupted occlusion. The bacterial isolates also changed with *Klebsiella pneumoniae* appearing after occlusion in one of the three subjects. Previously Bibel and Lebrun (23) also detected the appearance of the enterobacteriaceae on skin after occlusion. The remainder of the increased bacterial population found after occlusion was primarily composed of *S. epidermidis* and diphtheroids.

Aly et al. (1) studying the effects of occlusion for five days with vinylidene polymer plastic film (Saran Wrap) on human forearm skin found that both the density and composition of the cutaneous bacterial flora changed after five days of continuous occlusion. Bacterial counts increased significantly from a preocclusion value of 1.8×10^2 organisms/cm^2 to 1.4×10^6 after only one day of occlusion and up to a maximum count of 9.8×10^7 on day 4 of occlusion. After five days of occlusion the count decreased to 7.5×10^6 organisms/cm^2. During occlusion coagulate-negative staphylococci remained the predominant bacterial isolate, but the percent composition of lipophilic diphtheroids, micrococci, and gram-negative rods increased while nonlipophilic diphtheroids and bacillus decreased.

Allen and King (24) cite King et al. (2) in their hypothesis stating that the raised CO_2 levels found under occlusive dressings lead to activation of dermatophytes to form their infective arthrospores that then may cause fungal skin infection. No studies showing an association between occlusion and development of a clinical infection with dermatophytes were found.

Hartman (3) observed clinically that there was a foul odor on removal of the dressing following three days of occlusion. Hartman also demonstrated a significant increase in the number of bacteria per square centimeter: Staphylococci increased by five logs and Cornynebacterium increased by four. Hartman did not detect an increase in gram-negative bacteria as found earlier by Bibel in 1976 and Aly in 1978.

Faergemann et al. (4) confirmed findings of earlier studies of the effect of occlusion on bacterial counts in addition to examining the effect of occlusion on *Pityrosporum orbiculare* population and morphology. After eight days of occlusion with vinylidene polymer plastic film (Saran Wrap) bacterial counts increased significantly. With respect to *P. orbiculare*, the etiologic agent in tinea versicolor, the density increased significantly by a factor of 10 after three days of occlusion and remained significantly elevated through eight days of occlusion.

Percutaneous Absorption

Bucks et al. (25) proved in an in vivo model that occlusion facilitated the penetration of the more lipophilic steroids such as estradiol, testosterone, and progesterone while not improving the penetration of hydrocortisone, a more water-soluble steroid.

Lipophilicity and absorption were not directly related, as progesterone, the most lipophilic of the steroids tested, did not have the highest percent absorption. This study was performed with daily washing and reapplication and occlusion of the different steroids was studied over a 14-day period.

Bucks (26) provides explanation for the varying penetration of topical corticosteroids under occlusion. Under nonoccluded conditions, there is normally a large contrast between the hydrophobic SC layer and the more hydrophilic remainder of the epidermis. Under occlusion, the SC is hydrated thus making the SC and the viable epidermis more similar in hydrophilicity thus increasing the driving force for hydrophobic molecules from the SC into the lower layers of the epidermis. The limit to the increased penetration of increasingly hydrophobic molecules lies in the fact that highly hydrophobic molecules would be repulsed from the hydrated SC and would not travel to the SC—viable epidermis partition layer.

Feldmann (27) studying the effect of 96 hours of continuous occlusion of a single application of ^{14}C-labeled 0.5% hydrocortisone acetonide solution with vinylidene plastic wrap (Saran Wrap) found that penetration of labeled hydrocortisone was significantly increased 10-fold as measured by urine excretion of radiolabel when compared to daily application of identical hydrocortisone solution over 96 hours.

Ladenheim et al. (28) performed an in-vitro study of penetration of triamcinolone acetonide (TACA) through abdominal and breast skin samples either under occlusion with carmellose sodium hydrocolloid or pectin hydrocolloid dressings for 96 hours or without occlusion. Absorption of radiolabeled TACA was elevated threefold under either type of occlusion when compared to nonoccluded skin; there was no significant difference between the two different hydrocolloids in terms of TACA penetration. The fact that neither the composition of the hydrocolloid nor its ability to absorb TEWL has an effect on the penetration of TACA was confirmed in vivo by Martin et al. (29).

Reviewing the effect of occlusion on the percutaneous absorption of chemicals, Zhai (30) concluded that, though occlusion facilitates the penetration of some chemicals through the SC, it does not do so for all chemicals. They cite Ref. 31, which states that drug permeability in transdermal delivery systems is dependent on the physicochemical properties, such as volatility, molecular weight, solubility in water, and partition coefficient, of the chemical in question. They also mention that the topical vehicle used in drug delivery may itself act as a form of occlusion if it is composed of fat or certain oils.

Treatment of Disease

Friedman (32) compared the effect of weekly hydrocolloid occlusion on psoriatic plaques against fluocinolone cream twice daily and daily erythmogenic UVB treatment. This was a bilateral comparison study, and patients served as their own controls. Both fluocinolone cream twice daily and weekly occlusion significantly improved psoriatic plaques, but after 10 weeks of treatment, occlusion alone was significantly more effective than fluocinolone alone. In addition, 40.9% of plaques treated with occlusion alone completely resolved in 10 weeks with remissions of the plaques lasting up to one year in many cases. When occlusion alone was compared with five times per week erythmogenic UVB no significant difference was found between the two treatments. Two forms of hydrocolloid were used, Duoderm® (ConvaTec, Princeton, New Jersey, U.S.) and Surgihesive, and no difference in efficacy was detected between these dressings. The only difference between these dressings was their thickness, with

Surgihesive being thinner, and patients preferred the thinner Surgihesive barrier because it was more comfortable. Adverse reactions related to occlusive dressing treatment included Koebner phenomenon from the tape-stripping-like effect of dressing removal leading to worsening of four plaques, Auspitz sign after removal of the dressing in two plaques, and hyperpigmentation conforming to the region of the dressing in some patients. Importantly, no infections or purulence was associated with weeklong occlusive therapy over the course of 10 weeks.

Volden (33) studied the once weekly application of clobetasol proprionate under occlusion with Duoderm hydrocolloid dressing in steroid responsive dermatologic diseases over the course of many months. Volden reported that 93% of the 141 patients so treated with this method had a complete resolution of the treated lesions with the remaining 10% of patients experiencing a partial remission defined as 50% clearance of lesions. Patients with palmoplantar psoriasis and pustulosis experienced great benefit from this treatment according to Volden because the hydrocolloid dressing immediately relieved the pain associated with walking or use of the hands. Volden did not conduct statistical analysis, nor did he use a control arm in this study, so it is difficult to draw conclusions from his data.

Bourke et al. (34) demonstrated that calcipotriol under an occlusive dressing is significantly better than topical calcipotriol without occlusion in treating psoriatic plaques and did not result in systemic changes in calcium metabolism.

Griffiths et al. (35) studied the effects of three weeks of treatment of psoriatic plaques with weekly occlusion alone versus fluocinonide ointment versus the combination of weekly occlusion over fluocinonide ointment. Topiclude® (Ferndale Medical Products, Canada), an adhesive waterproof occlusive barrier, was the method of occlusion used. When psoriatic plaques were treated with either occlusion alone or fluocinonide alone, the plaques resolved significantly when compared to baseline, but the treatments did not differ from each other significantly. Further, the length of remission of the psoriatic plaque after treatment lasted significantly longer after occlusion alone than that induced by treatment with fluocinonide alone. Fluocinonide under occlusion with weekly dressing changes was significantly superior to either modality in terms of both plaque resolution and remission length. This study also searched for changes in immunohistologic and proliferation markers after one week of treatment but found no changes from baseline.

Focht et al. (36) demonstrated that treatment of verruca vulgaris with repeated six-day-long occlusion with duct tape over two months was significantly more effective than liquid nitrogen cryotherapy every two to three weeks for a maximum of six treatments per lesion.

Because testing of topical corticosteroids for FDA approval is often done in patients with psoriasis and dermatitis to satisfy two treatable diagnoses for class approval, most studies of occlusive therapy have also been done in these diseases as the most frequently occluded topical medications are corticosteroids.

Healing and Repair

Winter (37) originally studied the effect of polythene film occlusion versus nonocclusion on wound healing and showed significantly increased speed of healing of split-thickness skin donor sites on pigs when occlusion was employed.

Hinman (38) showed that occlusion of superficial human skin wounds led to significantly more rapid healing than in wounds, which were exposed to air and allowed to dry.

Welzel et al. (39) studied the effect of occlusion on healing after SC perturbation with either SLS exposure or tape stripping. TEWL measured with an evaporimeter increased from a normal value of $7\,g/m^2/hr$ to up to $40\,g/m^2/hr$ after tape stripping or SLS exposure. Occlusion was accomplished with four different membranes with the following water vapor permeabilities: self-adhesive OpSite® (Smith & Nephew, London): $10\,g/m^2/hr$; self-adhesive Tegaderm™ (3M, St. Paul, Minnesota, U.S.): $7\,g/m^2/hr$; nonadhesive Gore-Tex® (WL Gore and Assoc, Newark, Delaware, U.S.): $26\,g/m^2/hr$ and nonadhesive polyethylene foil: $2\,g/m^2/hr$. Occlusion was neither beneficial nor detrimental to the speed of restoration of the SC permeability barrier as measured by the TEWL despite the variability in water vapor permeability of the various dressings used. There was no statistically significant difference between any of the occlusive barriers used and the control nonoccluded skin in the SLS irritation arm of the study. In the tape-stripping arm of the study, TEWL returned to normal values by day 4 with polyethylene foil and Gore-Tex, but not with OpSite. Tegaderm was not studied in this arm of the study. They hypothesized that any benefit that OpSite may have had by maintaining skin hydration in the absence of the permeability barrier was negated by the additional tape-stripping-like effect of the daily dressing changes.

Collawn (40) examined the effect of occlusion on wound healing following laser skin resurfacing (LSR) of the face in a study where each patient served as his or her own control. The occlusive device used was Flexzan™ (Mylan, Canonsburg, Pennsylvania, U.S.). Biopsies were taken at 2, 3, and 4 days after the procedure and examined with light microscopy and immunohistochemical staining for keratin 17, laminin-1, and vimentin to examine for keratinocyte migration and dermal injury. Occlusion led to faster keratinocyte migration and restoration of a multilayered epidermis though no analysis of statistical significance was done. No eschar formed under any of the occluded areas while all nonoccluded areas did form eschar.

Disa et al. (41) evaluated the use of a calcium sodium alginate dressing applied below a Tegaderm occlusive film in the treatment of split-thickness skin graft donor sites. They used calcium sodium alginate because of its ability to absorb excess physiologic fluid from the wound bed and incorporate it into a gel that allowed for a moist wound bed without excess fluid accumulation. All 57 patients who were enrolled in the study had re-epithelialized their wounds after seven days of occlusion with a single dressing. There was no incidence of hematoma, seroma, or wound infection. In addition there was no pain associated with the wound except that encountered when the dressing was removed.

Batra et al. (42) studied the effect of silicone occlusive dressings on wound care following LSR of the face by comparing prospective use with matched historical controls using open wound care. They found that the severity and time until complete resolution of erythema were significantly lower with silicone occlusive dressing than with open wound management. The time to complete resolution of swelling and time to resolution of crusting were significantly lower as well.

Zhai (43) proposed that the optimal dressing for wound healing is a semiocclusive dressing which absorbs physiologic fluid loss, prevents formation of eschar and has a composition which does not allow proliferation of bacteria.

DISCUSSION

Occlusion by completely or almost completely vapor impermeable devices has been clearly shown to disturb the SC permeability barrier as measured by increases in

TEWL after varying lengths of occlusion (1,4,8,10,15). TEWL is transiently increased after removal of the occlusive dressing because the occlusion prevents normal physiologic loss of water vapor, which then accumulates under the dressing leading to increases in SC hydration (3,4,6). Histologically, the effects of increased skin hydration after occlusion are widening of intercellular spaces from the SC down to the basal layer and a deepening of skin surface furrows (17,18,21). Dermal edema is visible after 24 hours of occlusion (18). While these changes likely facilitate penetration of medications into the skin, they also may increase the penetration of allergens or irritants, and excessive skin hydration itself can act as a primary skin irritant (44). This is why there is interest in developing a dressing that avoids overhydration of the SC—it has been shown that when excess water is absorbed from the surface of the skin by the dressing or allowed to escape through a vapor permeable dressing, the increases in TEWL and SC hydration can be avoided (10,14).

The disturbance of the skin's permeability layer caused by occlusion appears to cause some immune activation as is evidenced by histological studies which found that occlusion leads to activation of LC, infiltration of mononuclear cells into the epidermis, and interaction between Langerhans and mononuclear cells (17,19). This may account for at least part of the mechanism by which verruca vulgaris is effectively treated by occlusion with duct tape (36).

Vapor impermeable nonabsorbent occlusion also leads to significant increases in the pH below the dressing while absorbent occlusive devices cause no alteration in skin pH (1,3,4). The increase in pH provided by impermeable nonabsorbent occlusive dressings coupled with the knowledge that calcipotriene is deactivated when exposed to acidic conditions may possibly account for the increased efficacy of treatment of psoriatic plaques with calcipotriene under occlusion versus nonoccluded use of calcipotriene (34).

Occlusion modifies the amount and types of bacteria on the skin surface and increases the density of bacteria per cm^2 by four to five orders of magnitude while also leading to the appearance of gram-negative bacteria, lipophilic diphtheroids, an increase in the amount of *P. orbiculare*, and the reduction of Bacillus and nonlipophilic diphtheroids (1,3,4,22). Despite this increase in Pityrosporum and bacteria, an association between occlusion and skin infection has yet to be demonstrated (4,32,45).

The increase in penetration of some steroids because of occlusion is well proven. However, the magnitude of this enhanced penetration appears to depend on whether the steroid is hydrophilic or hydrophobic—with hydrophobic steroids generally showing greater enhancement of penetration (25). Penetration of TACA was shown in vitro to have threefold higher penetration under occlusion than without occlusion (28,29). Different drugs will have different responses to occlusion depending on their physicochemical properties (30). Penetration studies have not yet been conducted under occlusion with other classes of topical agents used in dermatology such as tacrolimus, pimecrolimus, and retinoids.

Occlusion alone can lead to complete resolution of psoriatic plaques and possibly lasting remissions of individual plaques (24). Occlusion alone was shown to have an equal efficacy to fluocinonide ointment and efficacy superior to fluocinolone cream in treating psoriatic plaques, and a combination of fluocinonide ointment and occlusion gives significantly better results than either of the treatments (32,35). The increased efficacy of topical corticoid therapy under occlusion is thus not simply due to enhanced penetration, but also to a therapeutic effect of the occlusive device itself. Weekly occlusion with hydrocolloid dressings is not significantly different from

erythmogenic UVB in its ability to treat individual psoriatic plaques (32). Simple occlusion in psoriasis decreases the keratinocyte growth fraction (6) and correction of the calcium gradient in the epidermis necessary for normal keratinocyte differentiation, a process that is disturbed in psoriasis (12). Despite its somewhat proinflammatory properties in normal human skin (17,19,36), occlusion of psoriatic plaques has been shown to decrease hyperkeratosis, parakeratosis, and neutrophilic invasion while thinning the SC and normalizing the appearance of the intercorneocyte lipid layers (12). Occlusion alone has thus been shown to affect both clinical and histologic changes in psoriasis, and can thus serve as yet another adjunct in the dermatologist's armamentarium when approaching difficulty in treating psoriasis. Occlusion, either alone or with topical corticoids, may be used to supplement outpatient UVB therapy, or any other light or systemic therapy, for slowly responding or recalcitrant plaques that are commonly found on the shins.

Forms of occlusive dressings have also been studied in wound healing. A decrease in pain associated with the wound, faster healing time, faster keratinocyte migration, reduction of eschar, and faster resolution of erythema and swelling have all been associated with the use of occlusive wound dressings (33,42,44). Healing can be expedited and pain and morbidity reduced with occlusive dressing of superficial wounds such as after laser resurfacing or split-thickness skin graft harvesting.

CONCLUSIONS

Occlusive dressings are not simply inert devices used to cover the skin; they are biologically active just as is the medicine with which they are often used in conjunction. Occlusive dressings hold significant promise as adjunctive therapy or monotherapy for psoriasis, verruca vulgaris, and other skin diseases where the SC permeability barrier or normal epidermal differentiation is disrupted. Occlusion can be used alone in mild psoriasis, or used in addition to systemic treatment for stubborn plaques. They enhance the efficacy of many corticosteroids as well as calcipotriene, and it has been shown that they increase the penetration of lipophilic steroids into the skin by hydrating the lipid-filled intercellular spaces in the SC. They are not commonly associated with increased incidence of skin infection, even when used for up to one week without a dressing change, though they do increase the amount of bacteria present on the skin. Occlusive dressings speed up wound repair after superficial damage, and reduce pain and morbidity following these procedures. The only notable side effect of the treatment is the possibility of irritation of normal skin that is enhanced if irritants or allergens are knowingly or unknowingly occluded between the dressing and the skin. Much has been learned about the complex skin effects of occlusion; more remains to be resolved.

REFERENCES

1. Aly R, Shirley C, Cunico B, Maibach H. Effect of prolonged occlusion on the microbial flora, pH, carbon dioxide and transepidermal water loss on human skin. J Invest Dermatol 1978; 71(6):378–381.
2. King RD, Cunico RL, Maibach HI, Greenberg JH, West ML, Jeppsen JC. The effect of occlusion on carbon dioxide emission from human skin. Acta Derm Venereol 1978; 58(2):135–138.

3. Hartmann AA. Effect of occlusion on resident flora, skin-moisture and skin-pH. Arch Dermatol Res 1983; 275:251–254.
4. Faergemann J, Aly R, Wilson DR, Maibach HI. Skin occlusion: effect on Pityrosporum orbiculare, skin PCO_2, pH, transepidermal water loss and water content. Arch Dermatol Res 1983; 275:383–387.
5. Baxter DL, Stoughton RB. Mitotic index of psoriatic lesions treated with anthralin, glucocorticosteroid and occlusion only. J Invest Dermatol 1970; 54:410–412.
6. Gottlieb AB, Staiano-Coico L, Cohen SR, Varghese M, Carter DM. Occlusive hydrocolloid dressings decrease keratinocyte population growth fraction and clinical scale and skin thickness in active psoriatic plaques. J Dermatol Sci 1990; 1:93–96.
7. Fisher LB, Maibach HI. The effect of occlusive and semipermeable dressings on the cell kinetics of normal and wounded human epidermis. In: Epidermal Wound Healing. Chicago: Year Book, 1972:113–122.
8. Agren MS, Wijesinghe C. Occlusivity and effects of two occlusive dressings on normal human skin. Acta Derm Venereol 1994; 74(1):12–14.
9. Visscher M, Hoath SB, Conroy E, Wickett RR. Effect of semipermeable membranes on skin barrier repair following tape stripping. Arch Dermatol Res 2001; 293:491–499.
10. Ramsing DW, Agner T. Effect of glove occlusion on human skin (II) long term experimental exposure. Contact Dermatitis 1996; 34:258–262.
11. Chowdhury MMU, Maibach HI. Latex Intolerance: Basic Science, Epidemiology and Clinical Management. New York: CRC Press, 2005.
12. Hwang SM, Ahn SK, et al. Basis of occlusive therapy in psoriasis: correcting defects in permeability barrier and calcium gradient. Int J Dermatol 2001; 40:223–231.
13. Zhai H, Ebel JP, Chatterjee R, Stone KJ, Gartstein V, Juhlin KD, Pelosi A, Maibach HI. Hydration versus skin permeability to nicotinates in man. Skin Res Technol 2002; 8: 13–18.
14. Schafer P, Bewick-Sonntag C, Capri MG, Berardesca E. Physiological changes in skin barrier function in relation to occlusion level, exposure time and climactic conditions. Skin Pharmacol Appl Skin Physiol 2002; 15:7–19.
15. Friebe K, Effendy I, Loffler H. Effects of skin occlusion in patch testing with sodium lauryl sulfate. Br J Dermatol 2003; 148:65–69.
16. Berardesca E, Maibach HI. Monitoring the water-holding capacity in visually non-irritated skin by plastic occlusion stress test (POST). Clin Exp Dermatol 1990; 15: 107–110.
17. Lindberg M, Forslind B. The effects of occlusion of the skin on the Langerhans' cell and the epidermal mononuclear cells. Acta Derm Venerol 1981; 61:201–205.
18. Lindberg M, Johannesson A, Forslind B. The effect of occlusive treatment on human skin: an electron microscopic study on epidermal morphology as affected by occlusion and dansyl chloride. Acta Derm Venerol 1982; 62:1–5.
19. Mikulowska A. Reactive changes in the Langerhans' cells of human skin caused by occlusion with water and sodium lauryl sulphate. Acta Derm Venerol 1990; 70:468–473.
20. Warner RR, Stone KJ, Boissy YL. Hydration disrupts human stratum corneum ultrastructure. J Invest Dermatol 2003; 120:275–284.
21. Matsumura H, Oka K, Umekage K, Akita H, Kawai J, et al. Effect of occlusion on human skin. Contact Dermatitis 1995; 33(4):231–235.
22. Bibel DJ, Lovell DJ, Smiljanic RJ. Effects of occlusion upon population dynamics of skin bacteria. Br J Dermatol 1976; 95:607–612.
23. Bibel DJ, Lebrun JR. Changes in cutaneous flora after wet occlusion. Can J Microbiol 1975; 21:496.
24. Allen AM, King RD. Occlusion, carbon dioxide and fungal skin infections. Lancet 1978; 1:360–362.
25. Bucks DA, McMaster JR, Maibach HI, Guy RH. Bioavailability of topically administered steroids: a 'mass balance' technique. J Invest Dermatol 1988; 91:29–33.

26. Bucks D, Maibach HI. Occlusion does not uniformly enhance penetration in vivo. In: Bronaugh RL, Maibach HI, eds. Percutaneous Absorption Drugs-Cosmetics-Mechanisms-Methodology. 3rd ed. New York: Marcel Dekker, 1999:81.
27. Feldmann RJ, Maibach HI. Penetration of ^{14}C hydrocortisone through normal skin. Arch Dermatol 1965; 91:661–666.
28. Ladenheim D, Martin GP, Marriott C, Hollingsbee DA, Brown MB. An in-vitro study of the effect of hydrocolloid patch occlusion on the penetration of triamcinolone acetonide through skin in man. J Pharm Pharmacol 1996; 48:806–811.
29. Martin GP, Ladenheim D, Marriott C, Hollingsbee DA, Brown MB. The influence of hydrocolloid patch composition on the bioavailability of triamcinolone acetonide in humans. Drug Dev Ind Pharm 2000; 26:35–43.
30. Zhai H, Maibach HI. Effects of skin occlusion on percutaneous absorption: an overview. Skin Pharmacol Appl Skin Physiol 2001; 14:1–10.
31. Wiechers JW. The barrier function of the skin in relation to percutaneous absorption of drugs. Pharm Weekbl Sci 1989; 11:185–198.
32. Friedman SJ. Management of psoriasis vulgaris with a hydrocolloid occlusive dressing. Arch Dermatol 1987; 123:1046–1052.
33. Volden G. Successful treatment of chronic skin diseases with clobetasol proprionate and a hydrocolloid occlusive dressing. Acta Derm Venereol 1992; 72:68–69.
34. Bourke JF, Berth-Jones J, Hutchinson PE. Occlusion enhances the efficacy of topical calcipotriol in the treatment of psoriasis vulgaris. Clin Exp Dermatol 1993; 18:504–506.
35. Griffiths CEM, Tranfaglia MG, Kang S. Prolonged occlusion in the treatment of psoriasis: a clinical and immunohistologic study. J Am Acad Dermatol 1995; 32:618–622.
36. Focht DR, Spicer C, Fairchok MP. The efficacy of duct tape vs cryotherapy in the treatment of verruca vulgaris (the common wart). Arch Pediadtr Adolesc Med 2002; 156: 971–974.
37. Winter GD. Formation of the scab and the rate of epithelialization of superficial wounds in the skin of the young domestic pig. Nature 1962; 193:293.
38. Hinman CD, Maibach HI. Effect of air exposure and occlusion on experimental human skin wounds. Nature 1963; 200:377–378.
39. Welzel J, Wilhelm KP, Wolff HH. Skin permeability barrier and occlusion: no delay of repair in irritated human skin. Contact Dermatitis 1996; 35:163–168.
40. Collawn SS. Occlusion following laser resurfacing promotes reepithelialization and wound healing. Plast Reconstr Surg 2000; 105(6):2180–2189.
41. Disa JJ, Alizadeh K, Smith JW, Hu Q, Cordeiro PG. Evaluation of a combined calcium sodium alginate and bio-occlusive membrane dressing in the management of split-thickness skin graft donor sites. Ann Plast Surg 2001; 46:405–408.
42. Batra RS, Ort RJ, Jacob C, Hobbs L, Arndt KA, Dover JS. Evaluation of a silicone occlusive dressing after laser skin resurfacing. Arch Dermatol 2001; 137(10):1317–1321.
43. Zhai H, Maibach HI. Occlusive and semipermeable membranes. In: Rovee DT, Maibach HI, eds. The Epidermis in Wound Healing. New York: CRC Press, 2004:103.
44. Zhai H, Maibach HI. Occlusion versus. skin barrier function. Skin Res Technol 2002; 8:1–6.
45. Hutchinson JJ, Lawrence JC. Wound infection under occlusive dressings. J Hosp Infect 1991; 17:83–94.

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Definition of Cosmetics

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INTRODUCTION

Cosmetics are a category of consumer products marketed worldwide; their purpose and functions are universal to people of all cultures. The 2003 global cosmetics and toiletries market had a retail value of \$ 201.45 billion (1), representing 4.8% growth as compared to 2002 figures. Product categories tabulated included adult and baby toiletries, skincare, fragrance, haircare, depilatories, personal and oral hygiene, suncare, makeup products, and men's grooming products. In the United States alone, there are over 1400 domestic manufacturing and repacking establishments, which in the aggregate use more than 12,500 different cosmetic ingredients (2), and a corresponding number of fragrance ingredients that make over 25,000 product formulations (3). Once considered luxuries by consumers of modest economic means, cosmetics and toiletries are seen today as necessities by a growing number of consumers, regardless of their relative states of affluence (4). Cosmetics are regarded not as mere pampered indulgences, but as key aids to maintaining and promoting better standards of personal hygiene and health. Yet, what are these products that we call "cosmetics"?

COSMETICS IN HISTORY

The word "cosmetic" is derived from the Greek *Kosm tikos* meaning "having the power to arrange, skilled in decorating," giving *kosmein*, "to adorn," and *kosmos*, "order, harmony" (5). However, the true origins of cosmetics probably lies further still in antiquity, since early cave paintings that were 30,000 years old depict the use of body adornment (rudimentary cosmetics) in the rituals of mating and hunting (5).

Throughout the recorded history of man, cosmetics have been employed with essentially the same three goals in mind; namely: *(i) to enhance personal appeal through decoration of the body; (ii) to camouflage flaws in the integument;* and, *(iii) to alter or improve upon Nature* (6). Consider several historical vignettes demonstrating the role of cosmetics down through the ages (4–6). Vases of alabaster and

obsidian for cosmetics, discovered by Flinders Petrie in 1914, illustrate that the ancient Egyptians were well versed in the use of eye and face paints, body oils, and ointments. Theophrastus (363–278 B.C.), a student of Aristotle, demonstrated a considerable knowledge of the compounding of perfumes; and the Roman physician, Galen of Pergamon (130–200 A.D.), is said to have innovated that time-honored toiletry, cold cream (Cera Alba). People throughout the Middle East and the Orient too were reported to have made extensive use of cosmetics. The Babylonians were said [by Herodotus (490–420 B.C.)] to be well-practiced in the use of depilatories and the eye adornment, Kohl, while Alexander the Great (356–323 B.C.) reported the use of unguents, incense and other cosmetics by the countries of the Indo-Sumerian civilization. In Tudor England of the 1500's, sycophants of the virgin queen Elizabeth I adopted whatever cosmetic artifice and whimsy she chose to champion—whether it was powdering their faces with the toxic lead paint, ceruse, to simulate the Queen's pale complexion, rouging their cheeks with red ochre, or dyeing their hair orange to simulate the Queen's once-abundant wavy red-gold hair, which she had inherited from her father, King Henry VIII. In the 17th Century, the phrase "makeup" was first used to connote "cosmetics" by the poet Richard Cranshaw (1612–1649), while the author and playwright Ben Johnson satirized women who "put on their faces" upon rising each morning, before facing the world.

STATUTORY DEFINITION OF COSMETICS

Consumers possess a reasonable operational understanding of what a "cosmetic" *does* (i.e., its so-called 'function'). The average consumer envisions a "cosmetic" to be products, such as lipstick, cold cream, facial foundation powder, nail polish, and the other so-called "decorative" personal care items of makeup, designed to enhance the superficial appearance and to beautify the body. Frequently, the consumer will also equate the term "cosmetic" with "toiletry." In such cases, the topical preparations intended to cleanse and perfume the body are also included in the lay-person's operational definition of the term.

Despite the increasingly systematic and objective science associated with the art, formulation, and manufacture of cosmetics, our operational definition of cosmetics has not produced a harmonized international statutory agreement concerning what a "cosmetic" *is*, and what the legitimate functions of such products ought to be. In the United States, the statutory definition of "cosmetic," enacted in the 1938 Federal Food, Drug, and Cosmetic Act, i.e., the FD&C Act (hereinafter, the "Act"), is more far-reaching than the lay person's definition, and implicitly addresses the intended use as much as it does the beauty-enhancing attributes of a "cosmetic" (7).

The term "cosmetic" is defined in Section 201 (i) of the 1938 FD&C Act as:

" . . . 1) articles intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to the human body or any part thereof for cleansing, beautifying, promoting attractiveness, or altering the appearance, and 2) articles intended for use as a component of any such articles; except that such term shall not include soap . . . "

The Act thus views cosmetics as articles intended to be applied on the human body for cleansing, beautifying, promoting attractiveness, or altering the appearance. No mention has been explicitly made in this definition as to "how" such cosmetic endpoints are to be achieved, and whether changes in a body's "structure" or "function" are legitimate mechanisms for arriving at such endpoints.

The 13 subdivided "cosmetic" product categories, currently recognized by the U.S. Food & Drug Administration (FDA) for the voluntary filing of cosmetic product ingredient composition statements, are enumerated in Title 21 of the Code of Federal Regulations (c.f., 21 CFR 720.4); these are presented in Table 1. Here one can find all of the product categories that the consumer usually connotes with the terms "cosmetics & toiletries." The products included in the definition of "cosmetics" are those intended to cleanse the body in the bath or shower, mask the various malodors of the oral, perigenital, and axillary regions of the human anatomy, adorn the face, eyes, hair, and extremities in fashionable topical "decorative" colors, alter the color and style of the scalp hair, and conditioning of the integument against losses of moisture due to changes in environmental conditions (i.e., sun, wind, and relative humidity) (8). Note that the Act includes, in the definition of "cosmetic", any material intended for use as a component of a cosmetic product, so that an ingredient intended to be used in a cosmetic is also considered to be a cosmetic.

Soap products, consisting primarily of an alkali metal salt of free fatty acids, making no label claims other than cleansing of the human body, and are labeled, sold, and represented only as a soap, are not considered cosmetics under the law (c.f., 21 CFR 701.20). However, detergent-based "beauty or body bars," the so-called combination or "Combo-bars" that are based upon mixtures of soap and detergent(s), and those products containing other functional cosmetic ingredients (i.e., emollients, moisturizers, or botanical ingredients) which make product performance claims other than cleansing of the human body, are considered as "cosmetics." However, soaps that contain antimicrobial active ingredients and which make antibacterial or "germ-killing" efficacy claims are regulated under the FD&C Act as "over-the-counter" (OTC) drug products. If they make cosmetic claims as well, they may also be regulated as cosmetics (8) (see below).

Other authoritative treatises in cosmetic science, such as those of Jellinek (9), Poucher (5), De Navarre (10), Balsam & Sagarin (11), and Harry (12), discuss cosmetic product formulations in categories similar to those which have been adopted by regulation under authority of the Act in the United States. Jackson (13) also presents an excellent and up-to-date tabulation of the product types that could reasonably be considered, wholly or in part, as "cosmetics." Some topical OTC drug products are included among his 77 bonafide "cosmetic" product types.

The Act also contains statutory provisions to regulate cosmetics, in order to ensure that only products deemed safe for their intended use and properly labeled are legally offered for sale in the United States. Thus, various prohibited actions are defined in Section 301 of the Act that relate to the conditions under which cosmetics are deemed to be "adulterated" (Section 601), or "misbranded" (Section 602) under the Act. These regulatory provisions will be discussed in another chapter of this volume.

COSMETICS THAT ARE ALSO DRUGS: THE INTENDED USE DOCTRINE

Not all topical products are cosmetics. Dermatologics, for example, are topical products that are generally regulated as "drug" products, which are marketed by making claims of their therapeutic or medicinal purpose. The term applies also for those products having a formulation which includes one or more pharmacologically "active ingredients." Section 201 (g)(1) of the FD&C Act defines the term "drug" as:

Table 1 Cosmetic Product Categories (21 CFR 720.4)

Baby products
Baby shampoos
Lotions, oils, powders, and creams
Other baby products
Bath preparations
Bath oils, tablets, and salts
Bubble baths
Bath capsules
Other bath preparations
Eye makeup preparations
Eyebrow pencil
Eyeliner
Eye shadow
Eye lotion
Eye makeup remover
Mascara
Other eye makeup preparations
Fragrance preparations
Colognes and toilet waters
Perfumes
Powders (dusting and talcum) (excluding aftershave talc)
Sachets
Other fragrance preparations
Hair preparations (noncoloring)
Hair conditioners
Hair sprays (aerosol fixatives)
Hair straighteners
Permanent waves
Rinses (noncoloring)
Shampoos (noncoloring)
Tonics, dressings, and other hair grooming aids
Wave sets
Other hair preparations
Hair preparations (coloring)
Hair dyes and colors[a]
Hair tints
Hair rinses (coloring)
Hair shampoos (coloring)
Hair color sprays (aerosol)
Hair lighteners with color
Hair bleaches
Other hair coloring preparations
Makeup preparations (not eye)
Blushers (all types)
Face powders
Foundations
Leg and body paints
Lipstick
Makeup bases
Rouges

(*Continued*)

Table 1 Cosmetic Product Categories (21 CFR 720.4) (*Continued*)

Category
Makeup fixatives
Other makeup preparations
Manicuring preparations
Basecoats and undercoats
Cuticle softeners
Nail creams and lotions
Nail extenders
Nail polish and enamel
Nail polish and enamel removers
Other manicuring preparations
Oral hygiene products
Dentifrices (aerosol, liquid, pastes, and powders)
Mouthwashes and breath fresheners (liquids and sprays)
Other oral hygiene products
Personal cleanliness
Bath soaps and detergents
Deodorants (underarm)
Douches
Feminine hygiene deodorants
Other personal cleanliness products
Shaving preparations
Aftershave lotions
Beard softeners
Men's talcum
Preshave lotions (all types)
Shaving cream (aerosol, brushless, and lather)
Shaving soap (cakes, sticks, etc.)
Other shaving preparation products
Skin care preparations, (creams, lotions, powder, and sprays)
Cleansing (cold creams, cleansing lotions, liquids, and pads)
Depilatories
Face and neck (excluding shaving preparations)
Body and hand (excluding shaving preparations)
Foot powders and sprays
Moisturizing
Night
Paste masks (mud packs)
Skin fresheners
Other skin care preparations
Suntan preparations
Suntan gels, creams, and liquids
Indoor tanning preparations
Other suntan preparations

Note: Disclaimer: The views expressed herein are those of the authors and do not necessarily represent those of the FDA.
[a]All types requiring caution statement and patch test.

". . . (A) articles recognized in the official United States Pharmacopoeia, official Homeopathic Pharmacopeia of the United States, or official National Formulary, or any supplement to any of them; and (B) articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals; and (C) articles (other than food) intended to affect the structure or any function of the body of man or other animals; and (D) articles specified in clause (A),(B), or (C); but does not include devices or their components, parts, or accessories."

The so-called Doctrine of Intended Use of an FDA-regulated product generally will govern how it is to be regulated (14); the maxim frequently cited here, and which embodies this doctrine is *"You are what you claim."* In its 1996 attempt to regulate tobacco products (15 a–d) the FDA asserted that nontherapeutic but pharmacologically significant efficacy could also confer drug status on a product, even if no such explicit claims were made for the product; and the product's intended use need not be derived solely from the manufacturer's promotional claims for the product but could be inferred from the natural and foreseeable consequences of a manufacturer's action, based on a "reasonable person" test. A comprehensive discussion of the FDA's views in the mid-1990s of "intended use," now superceded by a preponderance of more recent judicial opinions and regulatory policy issued in accordance with such opinions, may be found in Section II.E. of the August 1996 Annex to the "Nicotine in Cigarettes and Smokeless Tobacco Jurisdictional Determination" document issued by the FDA (15e), as well as in the more recent, excellent discussion by Hutt (15f).

Prior to enactment of the 1938 Act, a 1935 Senate report foreshadowed the direction that the Congress would later take, in providing that the manufacturer's "intended use" of the product should determine if it is to be regulated as a "drug," "cosmetic," or some other regulatory category (14):

". . . The use to which the product is to be put will determine the category into which it will fall. If it is to be used only as a food it will come within the definition of food and none other. If it contains nutritive ingredients but is sold for drug use only, as clearly shown by the labeling and advertising, it will come within the definition of drug, but not that of food. If it is sold to be used both as a food and for the prevention or treatment of disease it would satisfy both definitions and be subject to the substantive requirements for both. The manufacturer of the article, through his representations in connection with its sale, can determine the use to which the article is put . . ."

Thus, the definitions of drug and cosmetic are not mutually exclusive. A product may legally be a drug or a cosmetic, or both a drug and a cosmetic. Products that are cosmetics but are also intended to treat or prevent disease, or otherwise intended to affect the structure or any functions of the human body, are also considered drugs under the Act and must comply with both the drug and cosmetic provisions of the law (8).

Examples of products which are drugs as well as cosmetics (if they make cosmetic claims) are anticaries ("fluoride") toothpastes, hormone creams, suntanning preparations containing a sunscreen active ingredient and intended either to protect against sunburn, or which make tanning claims (16), antiperspirant-deodorants, antibacterial detergent bars or soaps, and antidandruff shampoos. Most currently marketed cosmetics which are also drugs are OTC drugs. Several of them are "new drugs," for which safety and effectiveness had to be proven to the FDA (i.e., in a New Drug Application or NDA) before they could be marketed (8). A "new drug" is defined in Section 201 (p) of the Act as a drug which is not "generally recognized as safe and effective" (GRAS/E) by experts under the conditions of intended use, or which has become so recognized but has not been used to a material extent or for a material time under such conditions.

It is relatively easy to market a cosmetic. Cosmetic products can be brought to market very quickly—a fact that is clearly reflected in the rapid pace with which innovations and changes occur in the cosmetic marketplace. No premarket approval (or mandatory manufacturing establishment, product, or ingredient registration) is required. No delays are thereby incurred by the marketer while waiting for FDA approval. Nor does the FDA have a statutory mandate to monitor and regulate cosmetic performance advertising claims; the Agency's oversight responsibility in this area extends only to ensure that cosmetic product package labeling is not in violation of the Act with respect to "misbranding" (i.e., that the product performance claims are not false or misleading) (8). More about United States cosmetic regulations will be said in a later chapter in this volume.

The regulatory requirements for drugs (which are beyond the scope of this volume) are more extensive than the requirements applicable to cosmetics. For example, the Act requires that drug manufacturers register every year with the FDA and update their lists of all manufactured drugs twice, annually (c.f., 21 CFR 207). Additionally, FDA drug labeling requirements and regulatory oversight of prescription drug advertising [Federal Trade Commission has regulatory oversight for OTC drug advertising (17,18)] are more stringent than for cosmetics. Finally, drugs must be manufactured in accordance with Current Good Manufacturing Practice (CGMP) regulations (c.f., 21 CFR 210–211) (8).

THE COSMETIC/DRUG DISTINCTION: THE ROLE OF THE INTENDED USE DOCTRINE IN FDA ASSIGNMENT OF REGULATORY CATEGORY (TRADE CORRESPONDENCE)

The regulatory category occupied by a product clearly has a great impact on the marketing of that product. Since the drug approval process required by the Act (see above) is rigorous, expensive, and time-consuming, marketers of personal care products would market their products rather as cosmetics than as drugs. Some topical personal care products are formulated in a nearly identical manner, and it is the manufacturer of the topical product that frequently determines what the "intended use" of the product is, and whether it should be marketed as a "drug" or as a "cosmetic" based on the statements and other representations or performance claims made on product package labeling, collateral promotional literature, and advertising. In other circumstances, whether this is done intentionally for marketing reasons or is otherwise unintentional, the manufacturer's intentions of the product's usage may not be easy to discern, and it is not nearly as straightforward for the FDA to determine the most appropriate regulatory category for the product. How then, is the FDA to determine whether such a product is a "drug" or a "cosmetic"?

It is the interpretation of what "intended use" means that has helped the FDA to clarify how cosmetic products are distinguished from drugs. Needless to say, it has also caused uncertainty, as topical cosmetic formulations have become more sophisticated and capable of delivering enhanced performance benefits to the consumer, or when viewed from the other end of the drug–cosmetic continuum as dermatologic drug products have been formulated with ever increasing degrees of cosmetic elegance. FDA's interpretation of the "cosmetic" versus "drug" status for the various products that it regulates in the years since the enactment of the 1938 Act has been guided by several sources of information:

Labeling

"Intended use" is determined principally, but not solely, by the claims that are made on product "labeling" (i.e., all labels and other written, printed, or graphic matter, either on or accompanying the product). "Puffery" claims (19) may draw upon the stylized artful imagery and "hope-in-a-bottle", which has traditionally sold cosmetics from the dawn of the cosmetic marketing era, when the formulation of cosmetics was more an art than science, to the present day. "Objective" and "subjective" claims (20) are those which can and should be substantiated, usually by controlled-use, medically-supervised clinical studies, with or without the use of accompanying bioengineering instrument assessments of various skin, hair, eye, or nail condition parameters, focus-group panel interviews, home-placement tests employing follow-up questionnaires, and phone interviews. The Agency has even, on occasion, determined the "intended use" of a product based, in part, upon statements made on behalf of a product by its manufacturer's sales associates at the point of sale, or on the training and guidance provided to salespersons at the cosmetic counter.

Recent court decisions involving the laws enforced by the FDA have resulted in greater First Amendment protections being accorded to commercial speech; regulatory policy development by the Agency has followed these trends. FDA's Office of the Chief Counsel has defended the Agency's continued regulation of commercial speech and has stated that the First Amendment does not protect speech which is literally true but misleading to consumers (58). Nevertheless, the use of "intended use" doctrine has recently been more strictly interpreted and the demonstration of explicit drug claims on cosmetic product package labeling has been necessary for legally-supported regulatory enforcement actions to be brought out by the agency under the misbranding provisions of the FD&C Act.

The recent *University Medical Products USA, Inc.,* case (59), which resulted in the issuance of a warning letter on January 22, 2004, illustrates an example of FDA action when a product's labeling claims establish its intended use to affect the structure of the body and thereby cause the product to be drugs, as defined in Section 201 (g) (1)(C) of the FD&C Act. In this case, wrinkle-reduction and anticellulite "structure–function" claims were made on the company's topical skincare product package labeling, as well as in the firm's Web site, which, the agency decided, would fall under the 'drug' definition of the Act. *The University Medical Products USA, Inc.,* case was the first example of the implementation of a renewed 3-year Memorandum of Understanding (MOU) between the FDA's Center for Food Safety and Applied Nutrition (CFSAN) and the Center for Drug Evaluation and Research, which took effect from June 1, 2003. The MOU is intended to assist the FDA in implementing the drug and cosmetic provisions of the FD&C Act for products that purport to be cosmetics but meet the statutory definition of a drug.

Trade Correspondence

Early FDA guidance with respect to the "intended use" commenced soon after passage of the 1938 Act, when the Agency issued a series of informal opinions known as Trade Correspondence (TC). This applied the statute to specific questions and situations, and some of the TC's are still relied upon as support for FDA regulatory policy (21). Such TC's were the basis for decisions setting the Agency policy with respect to a cosmetic's intended use. TC-10, for example, notified marketers of cosmetic

claims considered by the Agency to be "misbrandings" in that they are "false and misleading" (22), while TC-229 stated that the word "healthful" contained in the labeling of a tooth powder would trigger the drug provisions of the Act (23). TC-26 held that a product's mechanism of action could be the basis of a cosmetic versus drug "intended use" determination, in that a deodorant powder inhibiting the normal physiological process of perspiration would be a drug (i.e., an "antiperspirant-deodorant"), but the same product merely serving as a "reodorant-deodorant" by absorbing the perspiration or masking the malodor would probably be a cosmetic (24). TC-42 provided further clarification of the "affect the body" clause of Section 201 (g) of the Act, by stating that a topical product containing emollient ingredients, where its claims to efficacy were through temporary improvements in skin condition parameters such as "Softening" (or, by extrapolation, by "smoothing" or "moisturizing") would not necessarily be considered drugs (25). TC-61 , recently revoked in the light of new science (16), served for many years as the "line in the sand" for distinguishing between products that referred to "sunburn protection" as drugs and those represented exclusively for the production of an "even tan" as cosmetics (26). Other TC's have established that ordinary facial tissue for wiping purposes is not a cosmetic (27), that other appliances used as adjuncts to, or in combination with bonafide cosmetic products, such as manicuring instruments (28), razors and razor blades (28), shaving brushes (29), toothbrushes (29), and toilet brushes (29) are not considered devices, and cuticle removers (30) are considered to be cosmetics rather than drugs.

FDA Case Law

The most direct guidance has been provided by the agency enforcement actions involving cosmetics that were determined to be drugs. For example, case law from the 1960's established that promotional claims for the "Bovine Serum Albumin" antiwrinkle products, *Sudden Change*® *(Hazel Bishop)* and *Line Away*™ *(Coty)*, taken in the overall context of product labeling, caused these products to be classified as drugs (31,32). The court held that advertising claims for these products, which included claims such as: "Not a face lift, not a treatment ... "; "Contains ... no hormones"; "You'll feel a tingling sensation ... "; "Nourishes the skin ... "; "... tightens and goes to work on wrinkles ... "; "... made in a pharmaceutical laboratory"; "... packaged under biologically aseptic conditions ... "; "... a face lift without surgery"; "... it lifts puffs under the eyes ...," among others, established the respective vendor's intent that the article had physiological and therapeutic effects. It is important to note in these cases that, apart from the claims, there was no evidence that they exerted any real effects on the structure or function of the body. In a third instance, a court case in the early 1970's claims, that the "Bovine Serum Albumin" containing product, *Magic Secret (Helene Curtis)*, is "pure protein" and "causes an astringent sensation" were alone considered appropriate for a cosmetic (33).

1980's Regulatory Letters

The next actions taken by FDA that served to "define" labeling claims that may cause a product to be classified as a drug, occurred in the late 1980's. In the Spring of 1987, the FDA sent 23 Regulatory Letters (34) to companies that were again marketing antiwrinkle and antiaging topical skincare products with aggressive marketing claims, which were deemed by the Agency to be "... daring ... " (35). These products made

claims such as: "...revitalizes by accelerating the rate of cellular renewal..."; "...revitalizes skin cells and promotes the skin's natural repair process..."; "...helps stimulate the natural production of structural proteins..."; "increases the proper uptake of oxygen and blood supply to the cells..."; "...reverses facial aging..."; "...restructures the deepest epidermal layers..."; "...increases collagen production..."; "...provides vital nourishing supplements...," among others. All of these claims, taken in the context of individual product labeling, were sufficient in the view of the Agency to establish "intended use" as a drug; indeed, it would be very difficult to use these terms and not trigger the structure or function definition of a drug. Again, in all of the products covered in this action, there was little expectation that they actually exerted an effect on the body outside of that which normally occurs from topical application of any conventional moisturizer. The Regulatory Letters issued by the Agency served as useful precedents of the legal rationale regarding product classification, and also provided very clear guidance to the Industry, as had been requested in a Citizen Petition (36), concerning what label claims could get a product into regulatory difficulty.

OTC Drug Monographs. Cosmetics That Contain Active Ingredients

The FDA has clearly stated that determination of "intended use" goes beyond direct label statements. The history of the use of the ingredient, its functionality in the product, and the consumer's perception, all play a role in product classification. This is the case with products that contain drug "active ingredients" in their formulations but that do not make explicitly stated claims about the drug effects of the "active ingredient." While there is no case law that addresses product classification based on presence of active ingredients alone, this issue has been addressed over the years in regulations for OTC drug products and other actions by the FDA.

The FDA acknowledged in the Tentative Final Monograph for First Aid Antiseptic Drug Products, published on August 16, 1991 (56 FR 33644) that antimicrobial soap products making cosmetic claims only are not subject to regulation as OTC drugs, and should not be considered in a review of drug effectiveness. The Agency further established the policy that the presence of an antimicrobial ingredient does not, in and of itself, make a product a drug, provided that no drug claim (i.e., "kills germs", "antibacterial") is made. However, the level of antimicrobial ingredient in a cosmetic product, when such ingredient is intended only as part of a cosmetic preservative system, may not exceed the concentration provided for in the OTC Monograph. The Agency also noted in this rulemaking that the "intended use" of a product may be inferred from labeling, promotional material, advertising, and any other relevant factor, arguing that, based on case law, a manufacturer's subjective claims of intent may be pierced to find its actual intent on the basis of objective evidence.

Analogously, the Agency acknowledged, in the Final Monograph for Topical Acne Drug Products, published in August, 1991 (56 FR 41008) that the final rule covers only the drug uses of the active ingredients and does not apply to the use of the same ingredients for nondrug effects in products, intended solely as cosmetics.

The FDA noted in the May 12, 1993 Tentative Final Monograph for OTC Sunscreen Drug Products (58 FR 28194) that a product may contain a sunscreen ingredient and be a cosmetic, if it is not intended to protect against the sun and no claims are made about the ingredient. In these cases, the term sunscreen is not used, no sun protection factor value is given, and the sunscreen ingredient is only

mentioned in the product's labeling by its cosmetic name in the ingredient list, in accordance with Agency regulations at 21 CFR 701.3. However, the presence of a sunscreen active ingredient in a product *intended* to protect from sun exposure makes the product a "drug." Again, FDA noted that it is not bound by the manufacturer's subjective claims, but can find actual therapeutic intent on the basis of objective evidence. Such intent may be derived from labeling, promotional material, advertising, and any other relevant source, where "relevant source" can even include the consumer's intent in using the product. The Agency reaffirmed these views in the May 21, 1999 Final Monograph for OTC Sunscreen Drug Products (64 FR 27666) and codified them at 21 CFR 700.35, adding only the caveat that when a cosmetic product contains a sunscreen ingredient not intended to be used for therapeutic or physiologic efficacy and uses the term "sunscreen" or similar sun protection terminology anywhere in its labeling, the term must be qualified by describing the cosmetic benefit provided by the sunscreen ingredient, and this statement must appear prominently and conspicuously at least once in the labeling, contiguous with the term "sunscreen" or other similar sun protection terminology used in the labeling.

The FDA provided clear guidance in the February 3, 1994 Withdrawal of Advance Notice of Proposed Rulemaking for OTC Vaginal Drug Products (59 FR 5226) that the mere presence of a pharmacologically active ingredient in therapeutically active concentrations could make a product a drug, even in the absence of explicit drug claims, if the "intended use" would be implied because of the known or recognized drug effects of the ingredient (i.e., fluoride in a dentifrice or zinc pyrithione in a shampoo). Thus, although explicitly stated "intended use" is the primary factor in determining "cosmetic" versus "drug" product category, the type and amount of ingredient(s) present in a product must be considered in determining its regulatory status, even if that product does not make explicit drug claims.

The FDA noted in a "Notice of Proposed Rulemaking" concerning "Cosmetic Products Containing Certain Hormone Ingredients" that was published on September 9, 1993 (58 FR 47611), along with a final rule on the topically applied hormone-containing drug products for over-the-counter use (58 FR 47608) that: *"... certain hormone-containing products not bearing drug claims could be cosmetics depending on the levels of hormones used and whether that level of use affects the structure or any function of the body ...."* It was noted that only those hormone ingredients present at a level below that which exerts an effect on the structure or function of the body would be acceptable for use in products marketed as cosmetics. However, if the hormone ingredient was present at physiologically active levels, the product would be classified as a drug for regulatory purposes. This proposal has recently been placed on a list of suggested rulemakings, targeted for withdrawal (68 FR 19766, April 22, 2003), in part, in order to improve focus limited agency resources on public health. Although public comments to this nomination are still being evaluated, a final decision by the agency is in abeyance (Docket No. 02N-0434).

With the recent publication on June 4, 2003 of its Final Monograph for Skin Protectant Drugs for Human Use (68 FR 33362 @ 3336–33364, Comments 6,7). The FDA has reiterated its position that, with the exception of the ingredient declarations of OTC drug–cosmetics (which must follow cosmetic ingredient labeling regulations, see Chapter 61), cosmetic claims are not allowed within the "Drug Facts" box nor are the ingredient's 'advertising claims' in ingredient declarations, for example, "tocopherol (Vitamin E)." The agency reaffirmed that "soothes" and "relieving the symptoms of dryness" are cosmetic claims, while "temporarily protects" or "helps relieve" are drug claims.

Recent discussion in a May 29, 2003 Proposed Rule of "dental plaque" and "tartar" claims for Oral Health Care Drug Products for Over-the-Counter Human Use (68 FR 32232–32287 @ 32239) has also led to the reaffirmation that the "intended use" of the product, which is largely dependent on the claims made for the product and the accompanying labeling, is a significant consideration in classifying the product as either a "drug" or a "cosmetic," when the product does not clearly fall under one statutory definition or the other. The Dental Plaque Subcommittee proposed that any reference to "supragingival tartar (calculus)" be considered a cosmetic claim, whereas any qualified or unqualified reference to the control of "dental plaque" (or its equivalent) should be interpreted as a drug claim. The Subcommittee also proposed than antiplaque claims should not stand alone, in the absence of clinically significant demonstrations of efficacy against gingivitis; no conclusions were stated in the proposal with reference to "subgingival tartar" claims.

THE ALPHA HYDROXY ACID (AHA) SITUATION

The Alpha Hydroxy Acids (AHAs), hailed by some in the industry as the first examples of the new "cosmeceuticals," since their first appearance in the marketplace several years ago (37), immediately provoked controversy, as there is no regulatory status accorded for the term under the Act (see Section Cosmeceuticals, below). Through their promotional claims, AHAs promised skincare benefits that far exceed the more traditional humectant and moisturization attributes once associated with AHA salts such as sodium lactate, said to be a component of the skin's so-called "natural moisturizing factor" (NMF) in the cosmetics of the 1970's (38). The scientific, clinical , and patent literature demonstrate that AHAs, as used today, probably function under at least certain conditions of formulation, not only as traditional cosmetic moisturizers but also as epidermal exfoliants and modulators of epidermal and dermal structure & function (39–42). They are promoted in mass-marketed and salon-treatment products; similarly, for the treatment of a number of cosmetic (i.e., severe dry skin, tone/texture) and more significant dermatologic (i.e., acne, photoaging, age spots) conditions (43,44). Manufacturers of these products have sought to market them directly to consumers as "cosmetics," or through physician offices, salons, and professional aestheticians (37,45–47). Although most marketers have artfully avoided making direct efficacy claims that might invite triggering the "drug" provisions of the Act (48), the FDA is also cognizant that the addition of chemical exfoliants to cosmetics on such a wide scale is unprecedented (43), and 10 years of marketing history with such products may prove an inadequate and unreliable predictor of future adverse impacts on the public health. Therefore, despite prior evaluations of AHA safety by the Cosmetic Ingredient Review (CIR) (49) and some more recent ones conducted by the FDA (50), as well, the FDA has also reserved its judgement concerning the appropriate regulatory category designation(s) for AHA skincare products and remains vigilant concerning the adequacy of the safety substantiation for AHAs, particularly with respect to potential chronic effects of AHAs on the sun sensitivity and photocarcinogenic responses of the skin (51).

The FDA recently published a document entitled as the "Draft Guidance for Industry on Labeling for Topically Applied Cosmetic Products Containing Alpha Hydroxy Acids (AHA) as Ingredients" in the *Federal Register* (67 FR 71577–71579, December 2, 2002) partly in response to a citizen petition from the Cosmetic Toiletry and Fragrance Association (CTFA). The petition was supported by a review

of the scientific literature cited therein (experimental and epidemiological), as well as by more recent human clinical studies, some sponsored by the industry and evaluated by the CIR and some by the FDA. These studies demonstrated that topically applied AHAs increase sun sensitivity to UV radiation during the period of regular topical application of such products and that this increased skin sensitivity to ultraviolet radiation is reversible, diminishing after discontinuing application for a week. The draft guidance is intended to provide the cosmetic industry with suggested wording for a label statement that would alert consumers regarding the possibility of increased skin sensitivity, particularly sunburn, associated with the use of such products during periods of concurrent sun exposure, as well as the protective measures to be taken. The "exclusivity language" recommended by the agency for use on product package labeling is:

"Sunburn Alert: This product contains an alpha hydroxy acid (AHA) that may increase your skin's sensitivity to the sun and particularly the possibility of sunburn. Use a sunscreen and limit sun exposure while using this product and for a week afterwards."

The FDA received public comments to the docket concerning this draft guidance document and is expected to issue a final guidance document following the completion of its review of these comments.

COSMECEUTICALS, COSMETIC THERAPEUTICS, AND OTHER PROPOSED DEFINITIONS

Topical products marketed in the United States are regulated under the Act, in various ways, as cosmetics, drugs, or OTC drug–cosmetics. There is no intermediate category that corresponds, for example, to the "quasi-drugs," defined under the Japanese Pharmaceutical Affairs Law (52). Neither are there any provisions under the United States statute that would accomodate classes of topical skincare products with levels of efficacy that exceed those of traditional cosmetics but whose safety has not been as rigorously substantiated as traditional drugs. Reed (53) and Kligman (54)a proposed that such high performance cosmetics be classified as *"cosmeceuticals,"* despite the lack of legal standing of such a product category. Even Kligman (54)f, however, concedes that claims for such alleged high performance cosmetic range from the credible to the fantastic, and that the field is ripe for potential exploitation by marketers not constrained by ethical standards, and that "*... this open-door policy has led to situations in which some regulatory action within the industry or by federal authorities is indicated ....*" Vermeer (54e) envisions "*cosmeceuticals*" as a subclass within the cosmetic–drug continuum of a cosmetic or drug, evaluated within the legal rubric of particular jurisdictions according to their pharmaceutical activity, intended effect in skin disease or mild skin disorder, and any side effects. In Europe and Japan, "*cosmeceuticals*" would thus be regarded as a subclass of cosmetics. However, in the United States, it would be a subclass of drugs. Piacquadio (55) favors the term "*cosmetic therapeutics*" when referring to drugs and devices, having known the risk/benefit profiles and established efficacy for a cosmetic indication, pending or with FDA approval. Privat (56) suggests the categories "*decorative and/or protective cosmetics*" for those products which embellish by modifying (appearance, color, feel), or by protecting the integument from external insults (i.e., UVR or bacteria), while reserving the term "*remedial and/or active cosmetics*" for those products that modify or correct the physiological state of the integument

[i.e., Stratum Corneum (SC), epidermis, melanocytes, intercellular lipid layer, sudoral glands, hypodermis, etc.]. Morganti (57) coined the term "*Cosmetognosy*" to denote the science that deals with the biological effects of cosmetics. Although these proposals each have varying degrees of merit, they too have no regulatory standing in the United States, under provisions of the 1938 FD&C Act.

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REFERENCES

1. Briney C. State of the Industry. In: GCI (Global Cosmetic Industry), 2004, page 26–30.
2. In: Canterbery Pepe R, Wenninger JA, McEwen GN Jr, JD, eds. CTFA International Cosmetic Ingredient Dictionary 9th ed. 2001.
3. FDA Compliance Program Guidance Manual 7329.001, pt. 1 at 1, August 1993.
4. McDonaugh EG, Truth About Cosmetics, Drug Markets Inc., 1937:vii.
5. Butler H. Historical background. In: Butler H, ed. Poucher's Perfumes, Cosmetics and Soaps. Vol. 3 (Cosmetics). 9th ed. London: Chapman & Hall, 1993, Chapter 24.
6. Romm S. The Changing Face of Beauty. St. Louis, MO: Mosby-Yearbook Inc., 1992, Chapter 8.
7. Yingling GL, Onel S. Cosmetic regulation revisited. In: Brady RP, Cooper RM, Silverman RS, eds. Fundamentals of Law and Regulation, Vol. 1. Washington, DC: , 1997:321.
8. FDA's Cosmetics Handbook. Washington, D.C.: FDLI, U.S. Government Printing Office, 1993:1–3.
9. Jellinek JS. Formulation and Function of Cosmetics. New York: Wiley-Interscience, 1970.
10. DeNavarre MG. The Chemistry and Manufacture of Cosmetics. Vol. I–IV. 2nd ed. Princeton, NJ: D. Van Nostrand Company, Inc., 1969.
11. Balsam MS, Sagarin E. In: Cosmetics: Science and Technology. Vol. 1–3. New York: John Wiley and Sons Inc., 1972.
12. Wilkinson JB, Moore RJ. Harry's Cosmeticology. 7th ed. New York: Chemical Publishing Co. Inc., 1982.
13. Jackson EM. Consumer products: Cosmetics and topical over-the-counter drug products. In Chengelis CP, Holson JF, Gad SC, eds. Regulatory Toxicology. New York: Raven Press, 1995, Chapter 5.
14. Yingling GL, Swit MA. Cosmetic regulation. In: Cooper RM. Food and Drug Law. Washington, D.C.: FDLI, 1991:362.
15. a) 60 FR 41314 and 41453 (August 11, 1995); b) 60 FR 41467–70; c) 61 FR 44396 (August 28, 1996); d) 61 FR @ 44667; e) The 'Intended Use' of a Product is Not Determined Only on the Basis of Promotional Claims. Nicotine in Cigarettes and Smokeless Tobacco is a Drug and These Products Are Nicotine Delivery Devices Under the Federal

Food, Drug, and Cosmetic Act: Jurisdictional Determination, U.S. Food & Drug Administration, Department of Health and Human Services, August 1996, Annex, Section II E; f) Hutt PB. The Legal Distinction in the United States Between a Cosmetic and a Drug. In: Elsner P, Maibach HI., eds. Cosmeceuticals: Drugs vs. Cosmetics. New York: Marcel Dekker Inc., 2000: 234–236, Chapter 15.

16. Final Rule for Over-the-Counter (OTC) Sunscreen Products for Human Use, 64 FR 27666 @ 27668, May 21, 1999.
17. Hobbs CO. The FDA and the Federal Trade Commission. In Cooper RM. Food and Drug Law. Washington, D.C.: FDLI, 1991:429–430, 452–456.
18. Memorandum of Understanding Between FTC and FDA, 36 FR 18539 (1971).
19. a) McNamara SH. FDA Regulation of Cosmeceuticals. Cosmet. Toiletries 1997; 112 (3), 41–45; b) FTC Deception Policy Statement, October 14, 1983 (Letter to The Honorable John D. Dingell, Chairman, Committee on Energy and Commerce, U.S. House of Representatives, n42); c) Feldman JP. Puffery in Advertising, Arent Fox Advertising Law (http://www.arentfox.com), June 1995; d) Hobbs CO. Advertising for Foods, Veterinary Products, and Cosmetics, Ref. 7, *op.cit.*, Chapter 12, p. 350; e) Legal Aspects of Promotion Strategy: Advertising. In: Stern LW, Eovaldi TL. Legal Aspects of Marketing Strategy: Antitrust and Consumer Protection Issues, Englewood Cliffs, NJ: Prentice-Hall Inc. 1984: 375–377, Chapter 7.
20. (a) McNamara SH. Performance Claims for Skin Care Cosmetics. Drug & Cosmetic Industry. October 1985:34; (b)Weinstein S, Weinstein C, Drozdenko R. A Current and comprehensive skin-evaluation program. Cosmet. Technol. 1982; April:36; (c) Grove GL. Noninvasive methods for assessing moisturizers. In: Waggoner WC, ed. Clinical Safety and Efficacy Testing of Cosmetics. New York: Marcel Dekker, 1990:121–148; (d) Smithies RH. Substantiating Performance Claims., Cosmet. Toiletries 1984, 99 (3):79–81,84.
21. Kleinfeld VA, Dunn CW. Trade correspondence. Federal Food, Drug, and Cosmetic Act. Judicial and Administrative Record (1938–1949). New York: Commerce Clearing House Inc. 1949:561.
22. TC-10, August 2, 1939.
23. TC-229, April 11, 1940.
24. TC-26, February 9, 1940.
25. TC-42, February 12, 1940.
26. TC-61, February 15, 1940.
27. TC-39, February 9, 1940.
28. TC-112, February 29, 1940.
29. TC-109, February 29, 1940.
30. TC-245, April 25, 1940.
31. United States v. An Article ... Line Away. 284 F. Suppl 107 (D. Del. 1968); affirmed, 415 F. 2d 369 (3d Cir. 1969).
32. United States v. An Article ... Sudden Change, 288 F. Suppl 29 (E.D.N.Y. 1968); reviewed 409 F. 2d 734 (2d Cir 1969).
33. United States v. An Article ... Magic Secret, 331 F. Supp. 912 (D. MD 1971).
34. FDA Regulatory Letters No. 87-HFN 312–08 to 87-HFN 312–29 (April 17, 1987 to June 23, 1987).
35. McNamara SH. Performance claims for skin care cosmetics or how far may you go in claiming to provide eternal youthfulness. Food Drug Law J 1986; 41:151–152.
36. Citizen Petition of McCutcheon, Doyle, Brown & Emerson Re: Bio Advance, FDA Docket No. 87P-0006, (January 6, 1987).
37. (a) Godfrey-June J. The AHA Phenomenon. Longevity 1993; September:36–39; (b) Jackson EM. AHA-type products proliferate in 1993. Cosmet. Dermatol. 1993; 6(12):22, 24–26; (c) Kintish L. AHAs: Today's Fountain of Youth ? Soap/Cosmetics/ Chemical Specialties 1994, 26–31.

38. (a) Harding CR, Bartolone J, Rawlings AV. Effects of natural moisturizing factor and lactic acid isomers on skin function. In: Loden M, Maibach HI, eds. Dry Skin and Moisturizers: Chemistry and Function. Boca Raton, FL: CRC Press, 2000, Chapter 19; (b) Middleton JD. Sodium Lactate as a Moisturizer, Cosmet. Toiletries 1978, 93:85–86.
39. (a) Leyden JJ, Lavker RM, Grove G, Kaidbey K. Alpha hydroxy acids are more than moisturizers, J. Geriatr. Dermatol. 1995; 3(Suppl A):33A–37A; (b) Van Scott EJ, Yu RJ, Actions of alpha hydroxy acids on skin compartments, J. Geriatr. Dermatol. 1995; 3(Suppl A):19A–25A.
40. Smith WP. Hydroxy acids and skin aging. Soap/Cosmetics/Chemical Specialties 1993, 93(9):54,56, 57–58,76.
41. Smith WP. Hydroxy acids and skin aging. Cosmet. Toiletries 1994; 109:41–48.
42. Smith WP. Epidermal and dermal effects of topical lactic acid. J. Am. Acad. Dermatol. 1996; 35:388–391.
43. Kurtzweil P.. Alpha hydroxy acids for skin care. FDA Consumer, March-April. 1998: 30–35.
44. Anon. Alpha hydroxy acids in cosmetics. FDA backgrounder, BG 97–4, February 19. 1997.
45. Brody HJ. Chemical peeling and resurfacing. , ed. St. Louis, MO: Mosby-Year Book Inc., 1997:3–17.
46. Draelos ZD. New developments in cosmetics and skin care products. In: In Advances in Dermatology.. Vol. 12. St. Louis, MO: Mosby-Year Book Inc., 1997:3–17.
47. (a) AHA ‘95 preview: new developments in alpha hydroxy acids. Symposium and Live Patient Workshop, Jointly Sponsored by Cosmetic Peel Workshop and Medical Education Resources, Inc., Orlando, FL, December 3–4, 1994; (b) AHA ‘96 preview: new advances in ahas and skin rejuvenation techniques. Symposium and Live Patient Workshop, Jointly Sponsored by Medical Education Resources, Inc. and Herald Education & Research Foundation, San Diego, CA, December 2–3, 1995.
48. Yingling GL, Onel S. Cosmetic regulation revisited. In: Brady RP, Cooper RM, Silverman RS, eds. Fundamentals of Law and Regulation, Vol. 1. Washington, DC: FDLI, 1997:341–342.
49. (a) Final Report: Safety Assessment of Glycolic Acid; Ammonium, Calcium, Potassium and Sodium Glycolate; Methyl, Ethyl, Propyl, and Butyl Glycolate; Lactic Acid; Ammonium, Calcium, Potassium, Sodium, and TEA-Lactate; Methyl, Ethyl, Propyl, and Butyl Lactate; and Lauryl, Myristyl, and Cetyl Lactate. Washington, DC: Cosmetic Ingredient Review, 1997; (b) Jackson EM. CIR expert panel releases AHA report, Cosmet Dermatol 1997, 10 (7).
50. Effects of alpha hydroxy acids on skin. Report Submitted by KRA Corporation (Silver Spring, MD) to the Office of Cosmetics and Colors, CFSAN, FDA, DHHS under Contract No. 223–94–2276, February 22, 1996;.
51. (a) Kaidbey K. An Investigation of the Effects of Topical Treatment with an Alpha-Hydroxy Acid (AHA) on the Sensitivity of Human Skin to UV-Induced Damage (FDA Sponsored Study # 1), Ivy Laboratories (KGL, Inc.), Philadelphia, PA, June 22, 1999; (b) Kaidbey K. An Investigation of the Effects of Topical Treatment with Alpha-Hydroxy Acid (AHA) on UVB-Induced Pyrimidine Dimers in Human Skin (FDA Sponsored Study #2). Ivy Laboratories (KGL, Inc.). Philadelphia, PA, June 22, 1999.
52. Santucci LG, Rempe JM. Legislation and Safety Regulations for Cosmetics in the United States, Europe, and Japan, FDA Compliance Program Guidance Manual 7329.001, pt. 1 at 1, August 1993, Chapter 20;.
53. Reed RE. The Definition of ‘Cosmeceutical’. J Soc Cosmet Chemist 1962; 13:103–106.
54. (a) Comment of Kligman AM. (“I invented the term “cosmeceutical” . . . ”), Panel Discussion “Skin: The Hot Topics”, Vogue 1988, October:417 < Author, please confirm if the volume given here is correct. > ; (b) HAPPI 1996, May:61; (c) Kligman AM. Why

Cosmeceuticals?, Cosmet. Toiletries 1993,108 (8):37–38; (d) Waleski M. Reed Coined 'Cosmeceutical', Letter to the Editor, HAPPI 1996, August:12; e) Vermeer BJ. Definition. In: Elsner P, Maibach HI, eds. Cosmeceuticals: Drugs vs. Cosmetics, New York: Marcel Dekker Inc., 2000:10–11, Chapter 2; (f) Kligman AM. Cosmetics. A Dermatologist Looks to the Future: Promises and Problems. In Draelos ZD, MD, eds. Dermatologic Clinics, 18 (4), W.B. Saunders Co., Philadelphia, 2000 (October), p. 699–709.

55. Piacquadio D. Cosmetic therapeutic vs. Cosmeceutical: Which Is It And Why ?, AHA '95 preview: new developments in alpha hydroxy acids. Symposium and Live Patient Workshop, Jointly Sponsored by Cosmetic Peel Workshop and Medical Education Resources, Inc., Orlando, FL, December 3–4, 1994.
56. Privat Y. A new definition of cosmetology. In: Baran R, Maibach HI, eds. Cosmet Dermatol. London, UK: Martin Dunitz Ltd., 1994:xiv–xv.
57. Morganti PF. The cosmetic patch. A new frontier in cosmetic dermatology. Soap Cosmetics Chem Special 1996; 96(2):48–50.
58. Grimaldi JV. With Public Notice on First Amendment, FDA Appears on the Verge of Major Policy Shift. Hearsay: The Lawyer's Column, The Washington Post, July 1, 2002, Section E, page 3.
59. (a) Cruse AE (Director, FDA Los Angeles District) to Francis RJ, (President, University Medical Products USA, Inc), Warning Letter, January 22, 2004; (b) Warning Letter Addresses Claims Made for Topical Skincare Preparations, CFSAN'Office of Cosmetics and Colors, February 18, 2004; (c) http://www.cfsan.fda.gov/~dms/cos-skin.html; (d) FDA draws the line on wrinkle reduction claims in warning letter, The Rose Sheet, 25 (12), p.7, March 22, 2004.

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Regulatory Requirements for the Marketing of Cosmetics in the United States

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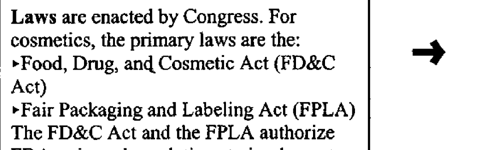
SCOPE

This chapter discusses the Federal regulatory requirements for the marketing of cosmetics in the United States, under the laws administered by the U.S. Food and Drug Administration (FDA). Federal control of cosmetics is a complex and shared responsibility, and, although this chapter focuses on the FDA's regulation of cosmetic products and their labeling, the overlapping jurisdictions of the U.S. Federal Trade Commission (FTC), the U.S. Consumer Product Safety Commission (CPSC), and the U.S. Environmental Protection Agency (EPA) should be noted. It is clearly beyond the scope of this chapter to discuss the legitimate consumer advocacy role played by the State Legislatures and by the State Attorney-General, but such discussions are readily available to the interested reader elsewhere (1). The role of "self-regulation" in the joint oversight responsibility for cosmetics by FDA and its stakeholders in the Industry is also discussed. Finally, the chapter concludes with a brief discussion of international harmonization and its potential influence on the future course of cosmetic regulation in the U.S.

BASIC U.S. LEGAL STRUCTURE FOR COSMETICS

The FDA is the principal regulatory agency charged with the enforcement of the *Laws* governing the marketing of cosmetics in the United States. The *Laws* are the basic enabling authority enacted by Congress. For cosmetics, the agency is given the mandate for enforcing the statutory requirements of the 1938 Federal Food Drug and Cosmetics Act (FD&C Act, also referred to as the "Act"), the 1960 Color Additive Amendments to the Act, and the 1966 Federal Fair Packaging and Labeling Act (FPLA). Under the authority of these statutes, FDA has promulgated *Regulations* (or *Rules*) to implement the mandate conferred by the *Laws*. *Guidance Documents*, which include *Policy Statements* (and those documents formerly termed *Advisory*



Regulations are issued to implement the provisions of the law based on the authority provided by the law.

The development of regulations must follow specific procedures that allow public notice and comment

Guidance Documents - Policy Statements

▸These documents serve to provide the agency's interpretation of the law and applicable regulations.

▸Guidance documents and policy statements are not legally binding on the public or the agency.

Figure 1 An example of Guidance Documents.

Opinions) have also been issued by the agency. Although not legally binding on the public or on the agency, *Guidance Documents* nonetheless serve to provide FDA's interpretation of the laws and applicable regulations (Fig. 1).

Federal regulation of cosmetics involves oversight of print, radio, television, and multimedia advertising (including the Internet) as well as of product package labeling. The jurisdiction of the FTC to regulate the advertising of cosmetic and "over-the-counter" (OTC) Cosmetic-Drug products overlaps that of the FDA, and is largely based upon the portion of Section 5 of the 1914 Federal Trade Commission Act (FTCA) and subsequent amendments and legislation to the FTCA that prohibits "unfair" and "deceptive" acts or practices (2). FDA has signed a memorandum of understanding (MOU) with the FTC establishing the parameters and boundaries of this relationship (3).

The FDA also shares its regulatory responsibilities for the regulation of cosmetics and topical personal care products with other Federal agencies. The U.S. Consumer Product Safety Commission (CPSC) exercises regulatory oversight over "soap" products not making cosmetic or drug performance claims under the 1960 Federal Hazardous Substances Act (FHSA; 16 CFR 1500); more about the regulation of soap will be discussed later in this chapter. The CPSC also is delegated the authority under the 1970 Poison Prevention Packaging Act (PPPA) for promulgating "child-resistant" (CR) packaging regulations for cosmetic products and soap products (4a); these regulations are codified at 16 CFR 1700. In recent years, final rules have been promulgated, requiring CR packaging for nail care products (e.g., primers) containing 5% methacrylic acid (4b) and household (artificial nail) glue removers containing acetonitrile and home cold wave permanent neutralizers containing sodium bromate or potassium bromate (4d). Proposed rules have also been published in the *Federal Register*, which would require CR packaging for fluid cosmetic products (among other categories of household substances) formulated with 10% of low viscosity hydrocarbons (100 SUS @ 100°F) (4c). Finally, the Environmental Protection Agency (EPA) is yet a fourth Federal agency that has become a significant presence in the regulation of multifunctional, "2-in-1" or "3-in-1" personal care products, such as the OTC sunscreen/insect-repellant/lotions; EPA's authority to regulate such products is derived from the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (5).

Table 1 U.S. Federal Statutes for Personal Care Products

1. Cosmetics and OTC Drug-Cosmetics
a. Products, Ingredients, Packaging and Labeling (FDA, CPSC, BATP[a], EPA[b]
Federal Food, Drug, and Cosmetic Act (FC&C Act), 1938
Color Additive Amendment to the FD&C Act, 1960
Federal Fair Packaging and Labeling Act (FPLA), 1966
Federal Hazardous Substances Act (FHSA), 1960
Federal Poisoning Prevention Packaging Act (PPPA), 1970
Federal Insecticide, Rodenticide, and Fungicide Act (FIFRA)[b], 1947
b. Print and Media Advertising (FTC)
Federal Trade Commission Act (FTCA), 1914
Wheeler-Lea Act, 1938
Magnuson-Moss Warranty-Federal Trade Commission Improvement Act, 1975
2. Soap Products
Soap (Saponification), FHSA, CPSA
Soap (Detergent, "Syndet"[c]), FD&C Act
Soap (Combination Saponification + "Syndet"), FD&C Act
Soap (with Active Drug Ingredient), FD&C Act
Soap (Saponification or "Syndet" making cosmetic claims), FD&C Act, FPLA

[a]BATF = Bureau of Alcohol, Tobacco, and Firearms (U.S. Dept. of the Treasury), are Specially Denatured Alcohol formulations (see 27 CFR 21).
[b]Containing pesticide or claiming insect-repellant efficacy.
[c]"Syndet" = Synthetic Detergent.

Table 1 summarizes the complex federal agency interrelationships involved in the regulation of cosmetics in the U.S.

BASIC U.S. REGULATORY STRUCTURE FOR COSMETICS

Definitions: Cosmetics, Soaps, and Drugs

The statutory definition of "cosmetic" is given at Section 201 (i) of the FD&C Act as:

"... (1) articles intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to the human body or any part thereof for cleansing, beautifying, promoting attractiveness, or altering the appearance, and (2) articles intended for use as a component of any such articles, except that such term shall not include soap."

For reasons discussed earlier in this volume (chapter 2), the use of the term "cosmetics" in the current chapter refers not only to finished cosmetic products marketed to consumers but also to constituent ingredients and other components of such finished products (e.g., packaging). Under current legal standards, topical products functioning as cosmetics may cleanse, beautify, promote attractiveness, or alter the appearance but without affecting the body's structure or functions. The FDA Voluntary Cosmetic Registration Program (VCRP) currently lists 13 subdivided cosmetic product categories, which appear in the codified regulations at *21 CFR 720.4* (chapter 2, Table 1).

"Soap" products are generally exempt from the cosmetic provisions of the FD&C Act, and, indeed, from the definition of "cosmetic" given in the statute. The FDA interprets the term "soap" at *21 CFR 701.20* to apply to products:

- intended for cleansing the human body;
- labeled, sold, and represented solely as soap;
- consisting primarily of alkali metal salts of free fatty acids (i.e., the bulk of its nonvolatile matter that serves as the detergent);
- detergent properties of which articles are due to the alkali metal salts of free fatty acids.

Liquid and solid product formulations consisting of synthetic detergents ("syndets"), combinations of soap and synthetic detergents ("combo" bars) intended not only for cleansing but also for claiming other cosmetic product performance attributes (e.g., "beauty bars" or "body bars" claiming to beautify, moisturize, soften, smooth, or firm the skin) must comply with the regulatory requirements applicable to cosmetics (e.g., bear ingredient declarations required at *21 CFR 701.3*). Even if such detergent or combination soap-detergent products are intended solely for cleansing of the human body, possess the characteristics consumers generally ascribe to "soap," and are identified in labeling as "soap" or some fanciful adaptation of this descriptor (e.g., "sope," "jabon," "liquid soap," etc.), these products are still regulated as cosmetics.

The statutory definition of the term "drug" is given at Section 201 (g) (1) of the FD&C Act, in pertinent part, as:

"(B) articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention in man ... and (C) articles (other than food) intended to affect the structure or any function of the body of man ... and (D) articles intended for use as a component of any [such] articles."

Regardless of their respective legal standings as "cosmetics" regulated under the FD&C Act or "soaps" regulated under the FHSA, personal care products that are also intended to treat or prevent disease or otherwise affect the structure or functions of the human body are considered "drugs" and must comply with these provisions of the law as well as any other provisions such as cosmetics or soaps, respectively, to which they may be subject. Most currently marketed cosmetics, which are also drugs, are OTC drugs (e.g., "fluoride" anticaries toothpastes, antiperspirant deodorants, antidandruff shampoos, and sunscreen lotions). However, several drug-cosmetics are "new drugs" (6), for which safety and effectiveness had to be proven to the agency before they could be marketed.

Analogously, soap products formulated to contain "active ingredients," if intended to cure, treat, or prevent disease, or if intended to affect the structure or any function of the human body, may also be regulated as drugs. This would include, for example, "medicated" antiacne soaps, the "antibacterial" bar and liquid soaps first introduced into the market in the late 1980s (7), and the alcohol-based liquid "hand sanitizers" of the late 1990s (8).

Statutory Controls on Cosmetics

The FD&C Act not only defines the term "cosmetic" (8) but also sets forth the basic requirement that cosmetic products introduced into interstate commerce within the United States must be safe for their intended use and properly labeled. The Act accomplishes this by explicitly prohibiting the adulteration or misbranding of cosmetics, and the introduction into, or receipt in, interstate commerce of "adulterated" or "misbranded" cosmetics (FD&C Act, Section 601 and 602, respectively). The delivery or proffered delivery of an adulterated or misbranded cosmetic for pay or otherwise is also a "prohibited act" (FD&C Act, Sec. 301).

Adulterated Cosmetics

A cosmetic is "adulterated" according to the FD&C Act, Sec. 601 (a)–(e) if:

- it bears or contains any poisonous or deleterious substance, which may render it injurious to users under the conditions of use prescribed in the labeling or under "customary or usual" uses;
- it consists wholly or in part of any filthy, putrid, or decomposed substance;
- it has been prepared, packed, or held under insanitary conditions whereby it may have become contaminated with filth, or whereby it may have been rendered injurious to health;
- its container is composed, wholly or in part, of any poisonous or deleterious substance which may render the contents injurious to health; or,
- it is not a hair dye and it is, or bears or contains, a color additive, which is unsafe within the meaning of the Act.

Coal-Tar Hair-dye Exemption. The FD&C Act exempts so-called "coal-tar" hair-dyes from the adulteration provision at Section 601 (a), if they bear the cautionary statement prescribed by law on the label and give "patch test" instructions, even if they are irritating to the skin or are otherwise harmful to the human body. The "coal-tar hair-dye exemption," named for the synthetic organic colors originally derived from the coal tar derivative, aniline, to which the exemption was initially applied (9), does not include eyelash and eyebrow dyes; other than "coal-tar" hair-dyes, all color additives used to impart color to the human body, including the hair, must be specifically approved by the FDA for such use.

Sources of Adulteration. Cosmetic adulterations may be associated with unintentional trace level contaminants (e.g., *N*-Nitrosamines, 1,4-Dioxane, or Diethanolamine) of the ingredients (also referred to as "raw materials") employed in finished cosmetic products (10–12) or to the manner of product formulation. Quality control problems (e.g., pH) or failure to follow good manufacturing practices guidelines (13) can also result in deviations of particular product batches from master formula specifications. In the past four (4) fiscal years (FY96–FY99), the FDA has found that approximately 88% of cosmetic product adulterations subject to voluntary recall actions (see "*Voluntary recalls*," below) were most frequently related to problems of microbiological contamination (Table 2) (14).

Table 2 Cosmetic Product Voluntary Recalls

	FY '00	FY '01	FY '02	FY '03
Total Recalls	8	6	4	15
Microbiology Recalls	7	6	4	12
Misbranding Recalls	0	0	0	1
Other Recalls	1[a]	0	0	2[b]

[a]1 Class II Eye Injury.
[b]2 Class II Colors.
Abbreviation: FY, Fiscal Year.

Misbranded Cosmetics

A cosmetic is "misbranded" according to the FD&C Act, Sec. 602 (a)–(f) if:

- its labeling is false or misleading in any particular way;
- its package label fails to contain the name and place of business of the manufacturer, packer, or distributor, as well as an accurate statement of the quantity of the contents in terms of weight, measure, or numerical count;
- any word, statement, or other information required to appear on the label is not prominently and conspicuously placed and in terms likely to be read and understood by the ordinary consumer under customary conditions of purchase and use;
- its container is made, formed, or filled in a manner likely to be misleading;
- it is a color additive, unless its packaging and labeling are in conformity with requirements in the regulations; or,
- its packaging or labeling are in violation of an applicable regulation issued under the *1970 PPPA*.

A cosmetic is misbranded as a consumer commodity according to the FPLA, Sec. 7, if it is introduced or delivered for introduction into commerce in violation of any of the provisions of the law or its implementing regulations, including the requirements contained in Sections 4 and 5 of the FPLA, which provide that the label of a commodity must state:

- the identity of the commodity
- the name and place of business of the manufacturer, packer, or distributor;
- the net quantity of contents (in terms of weight, measure, or numerical count) separately and accurately stated in a uniform location upon the principal display panel (PDP);
- the net quantity of contents "per serving," if the label bears a statement indicating the "number of servings" provided by the package;
- the "common or usual name" of the commodity and, if it contains 2 or more ingredients, the "common or usual name" of each ingredient listed in order of decreasing predominance, with the exception of such ingredients deemed to constitute a "trade secret."

Law Enforcement of FD&C Act Violations

Violations of the adulteration and misbranding provisions of the Act may subject the violator to various enforcement tools available to the FDA; these include:

- "*Warning letters*," which are subject to public disclosure under the Freedom of Information Act (FOIA), may be posted on the Internet FDA Website and are regularly publicized in the trade press and industry newsletters such as *The Rose Sheet*;
- *Targeted establishment inspections* and sampling programs;
- "*Seizure*" and "*detention*" of cosmetics offered for entry into U.S. interstate commerce that appear to be in violation of the law (for example, FD&C Act, Section 801(a);
- "*Injunction*" against present and future commercial operations;
- "*Criminal prosecution*" of responsible persons within violator cosmetic firms;
- "*Voluntary recalls*." Recalls of cosmetic products can either be "firm-initiated" or be "FDA-requested." The FDA has no authority under the

FD&C Act to order the recall of a defective or possibly harmful consumer product, although it can request a firm to recall a product. Resistance to an FDA request for voluntary recall can, however, trigger other enforcement actions, which have recently been reviewed by Calogero (15). The FDA has defined policies concerning such "voluntary" cosmetic (as well as food, drug, and medical device) product recalls; these are codified at *21 CFR 7.45–7.59*, and additional guidance can be found at the FDA internet website (http://www.fda.gov). FDA's guidelines divide voluntary recalls into three (3) categories:

- *Category I*—Products that are clearly dangerous or defective that pose clear or irreversible hazards to the public health;
- *Category II*—Products that are intermediate in their potential for adverse public health consequences, but may cause a temporary or reversible health problem;
- *Category III*—Products that are unlikely to cause any adverse health reaction but which violate FDA regulations.

Regulatory Controls on Cosmetics

Cosmetics marketed in the United States, whether manufactured domestically or imported from abroad, must be in compliance with the provisions of the FD&C Act, the FPLA, and the regulations published under the authority of these laws. Yet, cosmetics are arguably the least regulated category of articles subject to the jurisdiction of the FD&C Act (16). There is no premarket approval requirement for cosmetic products or their constituent ingredients under the law. Other than color additives and those few ingredients restricted or prohibited by regulation from use in cosmetics, no mandatory regulatory controls exist on the chemistry and structure substantiation of the ingredients, themselves, conditions of manufacture of the finished cosmetic products, or safety testing that the ingredients and products must undergo prior to marketing; no premarket test results need be submitted to FDA.

Charged through statutory mandates in the FD&C Act and the FPLA to ensure that only cosmetics deemed safe for their intended use and which are neither "adulterated" nor "misbranded" enter interstate commerce, yet lacking premarket approval authority to require proof of adequate safety substantiation, manufacturing controls, and truthful accurate, and informative labeling, FDA has found it necessary to promulgate regulations and guidance documents to supplement the laws it is charged with enforcing. These regulatory documents address the following issues:

Cosmetic Safety

Cosmetics are not currently subject to the same FDA safety and effectiveness standards as are drugs, biologics, and medical devices. The FD&C Act does not require that cosmetic manufacturers or marketers test their products for safety, nor does the FDA specify particular test batteries of preclinical (i.e., animal or in vitro alternative tests) and human clinical safety tests by cosmetic product category that marketers must use to substantiate cosmetic product safety. Manufacturers or marketers of cosmetic products are not required to submit the results of such safety substantiation tests to the agency on a premarket approval basis. Nonetheless, the FDA strongly urges cosmetic manufacturers and/or raw material suppliers to conduct safety substantiation assessments and whatever toxicological or other tests are appropriate to substantiate the safety

Table 3 Cosmetic Ingredients Prohibited or Restricted in the U.S.[a]

A. By Regulation (21 CFR 700, 21 CFR 250.250)
- bithionol
- mercury compounds
- vinyl chloride
- halogenated salicylanilides
- zirconium complexes (aerosol cosmetics)
- chloroform
- methylene chloride
- chlorofluorocarbon propellants
- hexachlorophene[b]

B. Miscellaneous Ingredients of Regulatory Concern[a]
- 100% liquid methyl methancrylate monomer (in nail products)[c]
- >5% formaldehyde (in nail products)
- acetylmethyltetramethyltetralin (AETT) (in fragrances)
- musk ambrette (MA) (in fragrances)
- 6-methylcoumarin (6-MC) (in fragrances)

[a]See FDA's Cosmetics Handbook, 1994 Edition, p. 8.
[b]21 CFR 250.250.
[c]A.R. Halper to J. Nordstrom (President Nail Manufacturers Council), personal communication. September 20, 1996.

of their cosmetic products and the ingredients formulated therein prior to marketing them. If the safety of a cosmetic is not "*adequately substantiated*," the product may be considered misbranded and may be subject to regulatory action unless the label bears the following statement, using the exclusivity language found at *21 CFR 740.10 (a)*:

"*Warning*—The safety of this product has not been determined."

Cosmetic Ingredients

The FD&C Act provides no statutory authority for the premarket approval of cosmetic ingredients. This is reflected in FDA's regulations, which are generally silent on the subject of permitted or "positive listed" cosmetic ingredients. With the sole exception of color additives (*21 CFR 70–82*), which are subject to premarket approval, and a few "negative listed" or prohibited/restricted ingredients at *21 CFR 700* [Table 3, a cosmetic manufacturer may use essentially any raw material as a cosmetic ingredient (regardless of whether it was specifically designed for use in cosmetic end-use applications) and market the finished cosmetic product without premarket approval (18)]. The marketer of the finished cosmetic product bears legal responsibility for any adverse reactions experienced by consumers or public health consequences that may result from this action. The number of ingredients used in cosmetics has grown exponentially since the early 1970s (Fig. 2). The *Ninth* (9th) *Edition* of the CTFA *International Cosmetic Ingredient Dictionary* (19), one of the most authoritative tabulations of cosmetic ingredients, contains monographs for approximately 12,500 such raw materials.

Color Additives

The term "color additive" is defined in the FD&C Act at Section 201 (t) and by regulation at *21 CFR 70.3 (f)*. The 1960 Color Additive Amendments to the FD&C Act

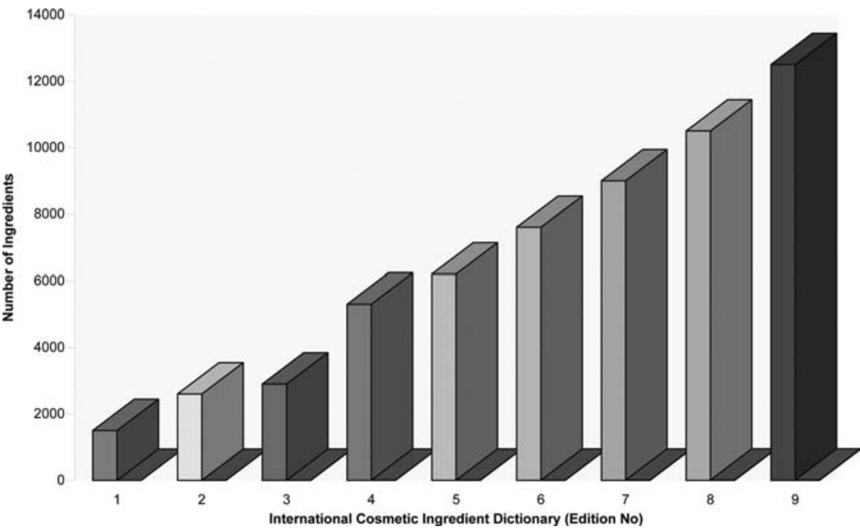


Figure 2 Growth in number of ingredients used in cosmetics since 1970.

requires that color additives used in food, drugs, medical devices, and cosmetics be approved by the FDA for their intended use, a process that requires both chemistry and safety reviews of the color additive by color chemistry and toxicology staff experts at the FDA. A cosmetic containing an unlisted color additive (i.e., a color additive that has not been approved by the FDA for its intended use) is considered adulterated and subject to regulatory action. Color additives listed at *21 CFR 73* are predominantly of inorganic (mineral) or botanical origins and are considered exempt from FDA's "batch certification" requirements (see *21 CFR 80*). Color additives listed at *21 CFR 74* are largely synthetic organic dyes and pigments (i.e., so-called "coal tar" colors) and are subject to the FDA's "batch certification" requirements at *21 CFR 80*; provisionally listed color additives, including color additive lakes, are listed at *21 CFR 82*. The FDA recently published in the Federal Register a proposal to permanently list color additive lakes (20); proposed simplifications in nomenclature for declaring straight colors and their lakes were also included as part of this proposal. It is important to note that all batches of certifiable color additives must actually be tested and certified in the laboratories of the FDA's Color Certification Branch for compliance with the identity and specifications established by regulation for that color additive before they may be represented and sold as an FDA-certified color additive.

The FDA listing regulations for color additives specify permitted end-use applications, which may be general or specific in nature, sometimes with particular caveats or restrictions in permitted uses or allowed concentrations. Cosmetic color additives, for example, may be listed for general use in imparting color to product formulations, for use in decorative cosmetics intended for external application to the hair and other appendages of the human body (other than the area of the eye), or may be specifically listed, solely or together with other cosmetic product applications, for eye area use (21). Only one color additive, dihydroxyacetone (DHA), is specifically listed for an intended use in externally applied cosmetics "to impart a color to the human body"; this finds widespread application in today's "sunless or self-tanning" cosmetic products (22). No color additives are currently

approved for use in injectable cosmetic tattoos (23). Further details about the color additives currently listed (approved) by regulation for use in cosmetics in the United States may be found on the Internet at the FDA's website (e.g., http://www.cfsan.fda.gov/~dms/col-toc.html).

Although the term "colorant" (or "colourant") is used interchangeably in the European Union (EU) in referring to color additives, the term "colorant" has a very specific regulatory definition in the U.S., distinct from that of "color additive" at 21 CFR 70.3(t), discussed earlier (23). A colorant is a "food contact substance (FCS)" (formerly, an "indirect food additive") for product packaging. The term is defined at 21 CFR 178.3297(a) to mean:

"...a dye, pigment, or other substance [including optical brighteners and fluorescent whiteners] (parentheticals added) that is used to impart color to or alter the color of a food-contact material, but that does not migrate to food in amounts that will contribute to that food any color apparent to the naked eye..."

Cosmetic Labeling

Cosmetic products distributed in the United States must comply with the labeling regulations published by the FDA under the authority of the FD&C Act and the FPLA (24a). The FPLA gives the FDA authority to require labeling of products considered "consumer commodities"; that is, products regulated under the FD&C Act, which are "*customarily produced or distributed for sale through retail sales...for consumption by individuals, or use by individuals for purposes of personal care or in the performance of services ordinarily rendered within the household*" (24b).

The statute requires that products be honestly and informatively labeled so that consumers can conduct "value comparisons" at the point of purchase; this is usually interpreted to include comparisons of ingredients, product attributes claimed, and net contents in order that the consumer may conclude which product among several alternatives being considered for purchase is the best value. This determination includes medical considerations, since the FDA has previously concluded (25) that a cosmetic product or ingredient to which a consumer is allergic (and which the consumer, therefore, cannot use) has no value to such a consumer.

"Labeling" means actual product package labels as well as other written, printed, or graphic material on or accompanying a product (e.g., hang-tags, promotional fliers, package inserts). Label statements required under the FD&C Act must appear on both the inside as well as an outside container or wrapper, if any; FPLA requirements need only appear on the label of the outer container or wrapper.

Cosmetic product package labeling regulations enacted under authority of the FD&C Act require that cosmetic labels bear certain fields of information that provide the consumer with proper identification and other data that will enhance the consumer's understanding of the product being purchased and facilitate the ability of the consumer to contact the manufacturer or distributor of the product, should there be a need to do so. Although the cosmetic labeling regulations at 21 CFR 701 generally require all labeling information to be written in the English language commonly understood by most American consumers, 21 CFR 701.2 (b) also provides certain accommodations in the case of articles distributed in Puerto Rico or other territories in which the predominant language is other than English. The required fields of information include the following:

- *Statement of Identity*, (i.e., common name) rendered in bold type on the cosmetic product "principal display panel");

- *Name and Address of Manufacturer* [(or) packer (or) distributor];
- Net Quantity of Contents [net weight (or) count (or) measure, as customary or as required]. English units are mandatory in the U.S., but a technical amendment to the FPLA under the 1991 American Technology Preeminence Act (ATPA), as revised in 1992 (26a,b), and more recent regulatory proposals to implement the ATPA provisions for FDA-regulated products (26c), now advocate the use of the most appropriate units of the metric international system (SI) of weights or measures, wherever practicable. This proposal includes the dual declaration of net quantity of contents in terms of both English units and the international metric (SI) system of weights or measures;
- *Cosmetic Ingredient Label Declarations* (see below);
- *Warning Statements* (or Cautionary Statements) concerning safe use, as required at *21 CFR 740* (see below).

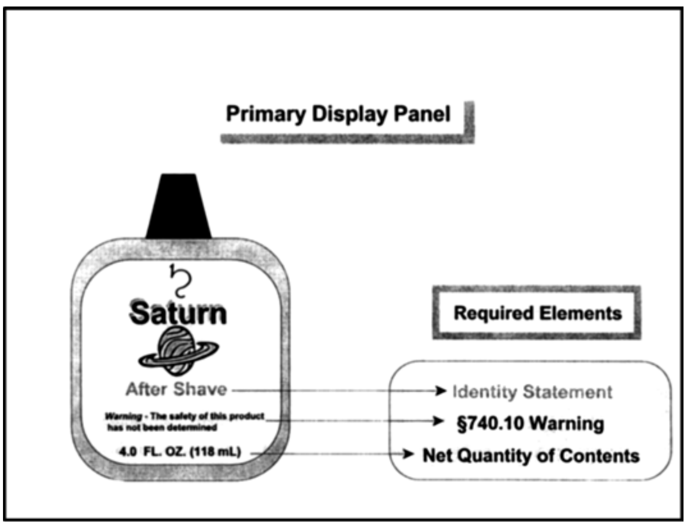
A typical cosmetic product package label exemplifying these features is shown in Figure 3.

Cosmetic Ingredient Label Declarations. The FPLA specifically authorizes the FDA to promulgate regulations requiring the declaration of all cosmetic ingredients on product package labels of cosmetics produced or distributed for retail sale to consumers for their personal care, such as home use; these regulations are codified at *21 CFR 701.3*. However, cosmetics not customarily distributed for retail sale, such as hair preparations or makeup products used by cosmetologists, beauticians, or aestheticians on clients at their salons or spas, and labeled "for professional use only," skin cleansers made available to persons at occupational settings, such as construction sites, hospitals, clinics, etc., "free" samples, or gifts are exempt from this requirement, provided that these products are not also sold to consumers at professional establishments or workplaces for their consumption at home.

Ingredient declarations must be "conspicuous" and "prominent" in placement on any information panel of the outer container, and not less than certain size specifications in relationship to the size and shape of the product package, to insure that the declaration is likely to be read at the time of purchase by the consumer.

FPLA labeling requirements specify that cosmetic ingredients must be declared, in qualitative descending order of predominance (see 21 CFR 701.3 (a)), utilizing ingredient names derived in hierarchical order of precedence from the nomenclature sources specified by regulation (see *21 CFR 701.3 (c)* and *701.30*); alternatively, the ingredients may be grouped and the groups declared according to *21 CFR 701.3 (f)*. The "common or usual" names specified by regulation in the U.S. are generally required to be stated in the language understood by American consumers, namely English. Cosmetic ingredients present at one percent or less (1%) may be declared after ingredients present at higher levels without regard to order of predominance, and fragrance and flavor, if any, being complex compositions of matter in themselves, may be declared for purposes of product package labeling as "flavor" and "fragrance," respectively; the term "aroma" utilized in the EU under the Cosmetic Directive (76/768/EEC) has no direct counterpart in the U.S. "Incidental Ingredients" (see *21 CFR 701.3 (l)*) need not be declared, and those ingredients accepted by the FDA the as exempt from public disclosure and granted "confidentiality" or "trade secret" status may be declared as "*and other ingredients*" (see *21 CFR 720.8*).

"Soap" is exempt from the FPLA requirement for mandatory label ingredient declarations applicable to cosmetics.



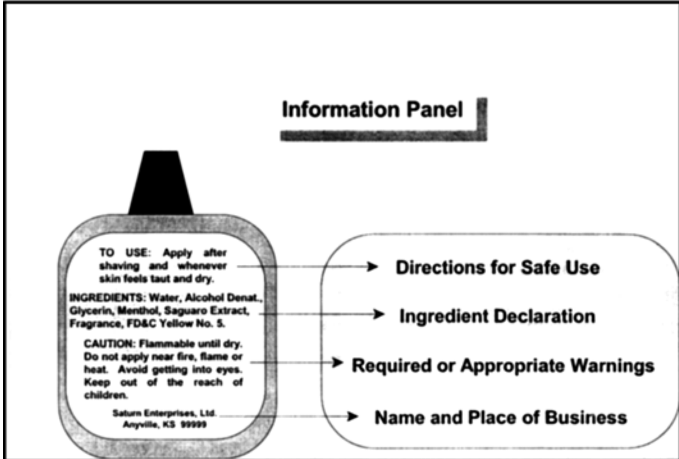


Figure 3 Typical cosmetic product package label.

Cosmetics containing sunscreen ingredients for nontherapeutic, nonphysiological uses (i.e., as an ultraviolet (UV) absorber to protect the color, flavor, or fragrance integrity or aesthetics of the formulation) must qualify the use of the term ''sunscreen'' or similar descriptor by describing the cosmetic benefit provided by the sunscreen ingredient and by including this qualification at least once in the labeling in conjunction with the term ''sunscreen'' or similar descriptor; for example, ''*Contains a sunscreen–to protect product colo*'') (see *21 CFR 700.35*);

The manner of declaration of ingredients in OTC drug-cosmetic products is specified at 21 CFR 701.3 (d). Drug ''active ingredients'' present in OTC drug-cosmetic product formulations are declared first, as required at 21 CFR 201.66(c)(2) and (d) of this chapter, followed by any ''inactive (cosmetic) ingredients,'' which are declared in descending order of predominance or grouped, in accordance with the provisions of 21 CFR 701.3 (a) and (f), respectively. An exception is provided for, if there is a difference in the labeling provisions in 21 CFR 201.66 and Sections 701.3 or 720.8; under these circumstances, the labeling provisions at 21 CFR 201.66 are controlling (see 21 CFR 201.66(c) (8) and (d) of this chapter).

Recent efforts to achieve "international harmonization" with cosmetic ingredient nomenclature standards required by the 1976 EU Cosmetic Directive (27) and its more recent amendments (28) have resulted in the FDA agreeing to exercise regulatory discretion towards the interim use of parenthetical "dual declarations," employing systematic Linne (Latin) taxonomic genus/species nomenclature for certain categories of ingredients (i.e., botanicals and/or "trivial" ingredients) (29). Color additives are named using the monograph titles in their respective listing regulations (see *21 CFR 73, 74, 82*), although, here, too, the impact of "international harmonization" efforts has resulted in the FDA agreeing to exercise regulatory discretion towards the interim use of parenthetical "color index (CI) numbers" in a dual declaration (29). Examples of the new interim "harmonized" ingredient declarations are given in Table 4.

Cosmetic Label Warnings. Cosmetics that may be hazardous to consumers when misused must bear appropriate label warnings and adequate directions for safe use. Manufacturers and marketers of cosmetics have a general responsibility to ensure that the labels of their finished cosmetic products bear a warning statement whenever necessary or appropriate to prevent a health hazard that may be associated with the product (21 CFR 740.1(a)). These warning statements must be prominent and conspicuous (21 CFR 740.2). Some cosmetics must also bear more specific label warnings or cautions prescribed by regulation. Specific cosmetic product categories requiring such statements currently include:

- Cosmetic Products for which adequate substantiation of safety has not been obtained (*21 CFR 740.10*)
- Cosmetics in self-pressurized containers (*21 CFR 740.11*)
- Feminine deodorant sprays (*21 CFR 740.12*)
- Foaming detergent bath products (*21 CFR 740.17*)
- "Coal tar" hair-dyes posing a risk of cancer (*21 CFR 740.18*) [*Effective date stayed at 47 FR 7829, February 23, 1982*]
- Cosmetic suntanning preparations not containing a sunscreen (*21 CFR 740.19) [Effective Date: May 22, 2000*]

Tamper-Resistant Packaging.

The FDA is given the authority under Sections 601 (a) and (c) and 701 (a) of the FD&C Act to issue package security requirements for cosmetics. Requirements for tamper-resistant packaging for *Liquid Oral Hygiene Products* (e.g., mouthwashes and breath fresheners) and all *Cosmetic Vaginal Products* (e.g., douches and tablets) cosmetic products were promulgated at *21 CFR 700.25*. Details about such packaging are found in *FDA's Cosmetics Handbook* (30) and at the FDA Internet Website (http://www.fda.gov).

Cosmetic Good Manufacturing Practices Guidelines

The FDA has never published current Good Manufacturing Practice (cGMP) regulations for cosmetics, although the Agency has actively promoted good manufacturing practices by firms marketing cosmetics in the United States and is aware of the international harmonization draft standard on cosmetic GMPs currently being developed by a Working Group of ISO/TC-217. The FDA has published *Cosmetic Good Manufacturing Practice Guidelines*, patterned in pertinent part after the food cGMP regulations

Table 4 Selected Examples of U.S. Cosmetic Labeling Names, EU Cosmetic Labeling Names, and Proposed Interim Harmonized Cosmetic Labeling Names

U.S. Cosmetic Ingredient (U.S. INCI Labeling Name)	EU Cosmetic Ingredient (EU INCI Labeling Name)	Proposed Interim Harmonization (EU/U.S. Dual Declaration)
A. Color Additives		
FD&C Green No. 3	Cl 42053	Green 3 (Cl 42053)
D&C Orange No. 4	Cl 15510	Orange 4 (Cl 15510)
D&C Blue No. 1 Aluminum Lake	Cl 42090	Blue 1 Lake (Cl 42090)[a]
Ext. D&C Violet No.2	Cl 60730	Ext. Violet 2 (Cl 60730)
B. Botanicals		
Peach Leaf Extract	Prunus Persica	Peach (Prunus Persica) Leaf Extract
Sambucus Nigra Extract[b]	Sambucus Nigra Extract	Sambucus Nigra Extract
Sweet Cherry Pit Oil	Prunus Avium Pit Oil	Sweet Cherry (Prunus Avium) Pit Oil
Oat Flour	Avena Sativa Flour	Oat (Avena Sativa) Flour
C. Denatured Alcohols		
SD Alcohol 38B[c]	Alcohol Denat.	Alcohol Denat.
D. "Trivial" Ingredients		
Water	Aqua	Water (Aqua)
Fragrance	Parfum	Fragrabce (Parfum)
Tallow	Adeps Bovis	Tallow (Adeps Bovis)
Yeast Extract	Faex	Yeast (Faex) Extract
Goat Milk	Caprae Lac	Goat Milk (Caprae Lac)
Beeswax	Cera Alba	Beeswax (Cera Alba)
Honey	Mel	Honey (Mel)
Sea Salt	Maris Sal	Sea Salt (Maris Sal)
Egg Oil	Ovum	Egg (Ovum) Oil
Silk Powder	Serica	Silk (Serica) Powder
Mineral Oil	Paraffinum Liquidum	Mineral Oil (Paraffinum Liquidum)
Coal Tar	Pix Ex Carbone	Coal Tar (Pix Ex Carbone)
Fish Extract	Pisces	Fish (Pisces) Extract
Pigskin Extract	Sus	Pigskin (Sus) Extract
Mink Oil	Mustela	Mink (Mustela) Oil

[a]Annex IV of the EEC Cosmetic Directive 76/768/EEC provides that, for those color additives allowed for use in cosmetic products, the Lakes or salts of these coloring agents using substances not prohibited under Annex II or not excluded under Annex V from the scope of teh Directive are equally allowed and may also be declared under the same Color Index Number as for the corresponding straight color additive.
[b]Certain botanical (plant) ingredients may have LInne System (Latin genus/species) naames that have no English language 'common or usual name' equivalents.
[c]27 CFR 21.

(13a) but applicable to the cosmetic manufacturing environment, in *FDA's Cosmetics Handbook (13b)*; the latter document references the *FDA Investigation Operations Manual (IOM)* (31). The *Cosmetic Good Manufacturing Practice Guidelines* is a guidance document reflecting FDA policy, but it is not legally binding, either on the cosmetics industry or on the FDA. The FDA has also published drug cGMP regulations (32), which apply to prescription drugs and cosmetic-drugs (i.e., OTC drug products making cosmetic claims).

The Voluntary Cosmetic Registration Program (VCRP)

The FD&C Act does not require cosmetic firms to register manufacturing establishments or formulations with the FDA, nor does it mandate that companies submit product adverse reaction report data. Nevertheless, the FDA has encouraged the voluntary registration of such data as being in the public interest and consistent with the spirit of responsible "self-regulation" advocated by the cosmetic industry, itself. In the early 1970's, the FDA developed a three-part system of regulations, under which manufacturers or distributors of cosmetics may submit this information to the agency on a voluntary basis (33). The three-parts of the *Voluntary Cosmetic Registration Program (VCRP)* originally comprised the following:

- Part I—*Cosmetic Establishment Registration Program (CERP)*, requests that cosmetic manufacturing sites be registered with FDA (see *21 CFR 710*).
- Part II—*Cosmetic Product Ingredient Statements (CPIS)*, requests that cosmetic formulations and cosmetic raw material composition statements must be registered with FDA (*21 CFR 720*). This regulation also set forth the 13 product category codes (PCC) at *21 CFR 720.4* recognized by FDA as legitimate cosmetic functions. Semiquantitative raw material disclosures were abandoned and purged from the VCRP database in the early 1990s (34).
- Part III—"*Product Experience Reports (PER)*," requests the annual filing of "reportable" adverse reactions (*21 CFR 700.3 (q)*) to the use of cosmetic products by manufacturers with the FDA (euphemistically called "product experiences" (*21 CFR 730*). The use of optional "screening" protocols to be filed with FDA, designed by individual manufacturers, for use in determining the "reportability" of experiences, was also provided for in the PER Program (*21 CFR 700.3 (p), 730.4 (d) (2)*). This data was collected, tabulated, and analyzed for statistical deviations of individual products from industry-wide adverse reaction trends by product category, until the PER program was discontinued in 1996.

Despite its voluntary nature, the VCRP has never enjoyed full industry participation. Table 5 illustrates the VCRP registration statistics for the years 1992–1996, the last five (5) fiscal years during which all parts of the VCRP were in operation. *Part III (PER)* annual filings by firms considered by the FDA to be eligible to participate in the program have historically been the lowest of the three parts of the VCRP. *Part III (PER)* of the VCRP was discontinued in 1996 (35) and the VCRP, itself, was temporarily put into operational abeyance in 1998 due to resource reallocations within the FDA (36). With partial funding restoration by Congress "earmarked" specifically for the FDA's Cosmetics Program, Parts I and II of the VCRP were restarted in 1999 (37), and a new, streamlined electronic World Wide Web-based system to facilitate industry participation has been developed and beta-tested by the FDA and the industry (38).

Self-Regulation

The regulatory paradigm for cosmetics operative in the United States has evolved from a system notable in the lack of Federal premarket approval of cosmetics in the *1938 FD&C Act* into an exercise in "self-regulation," with significant partnership

Table 5 FDA Voluntary Cosmetic Registration Program (VCRP), FY '93–96, FY '04

	FY '93	FY '94	FY '95	FY '96	FY '04[a]
Establishments Registered	969	954	757	773	930
Companies Filing Formulations	782	810	806	684	697
Formulations Registered	18,369	16,929	18,558	15,982	18,740
Companies Filing Product Experience Reports	116	113	97	75	–

[a]FY '04 data are partial year, current through June 25, 2004.
FY 93-FY 96 are the last years for which complete data on VCRP, Parts I-III is currently available
Abbreviation: FY, Fiscal Year.

roles played by the FDA's other stakeholders, particularly the cosmetic industry trade associations and consumer advocacy groups. Self-regulatory programs currently in effect have been initiated both by government and private industry; they include:

- The *FDA Voluntary Cosmetic Registration Program (VCRP)* (28);
- The *Cosmetic Ingredient Review* (CIR). Originated in the 1970s as a cosmetic industry initiative (39), CIR is a semiautonomous Program affiliated with the CTFA, in which the FDA as well as its industry and consumer advocate stakeholders play significant liaison roles in assessing the safety of cosmetic ingredients. The CIR does not generally assess the safety profiles of ingredients that are reviewed by the FDA as "active ingredients" of drugs (OTC or prescription) nor does it conduct safety assessments of fragrance materials.
- The *Research Institute for Fragrance Materials* (RIFM) evaluates the safety profile and publishes monographs concerning fragrance materials, while the *International Fragrance Association* (IFRA), a trade association of national fragrance trade associations, establishes usage guidelines for fragrance materials by industry fragrance houses (40).

FDA's *VCRP* and the industry-sponsored *CIR* and *RIFM/IFRA* programs might well be called the "cornerstones" of the government–industry partnered experiment in self-regulation. Other strategic approaches in the toolbox of self-regulation include:

- *Federal Statutes*. The *Lanham Act (1946)* empowers companies to seek judicial redress in the Federal District Courts for unfair business practices resulting in negative impact on market share (41). The *Robinson-Patman Act (1936)* enables companies to seek to recoup lost sales and profits ascribed to anti-competitive, predatory pricing tactics (42).
- *Advertising Self-Regulation. NAD/CBBB*. Excesses in product performance advertising claims are more frequently addressed through competitor/ peer-review challenges brought through the self-regulatory protocols of

the *National Advertising Division (NAD)*, an arm of the *Council of Better Business Bureaus (CBBB)* (43), and its appeals panel, the *National Advertising Review Board (NARB)*. Failure to resolve advertising controversies through these self-regulatory processes can result in an ultimate referral by the *NARB* to the FTC. Scrutiny of proposed story-boards prior to being accepted for mass-media air-time is also undertaken by advertising agency legal departments and television/radio network "Standards and Practices Boards" (e.g., network censors) (44).

The cosmetic industry is characterized by highly competitive marketing strategies and depends on the freedom to rapidly introduce new, innovative cosmetic products to the marketplace without lengthy regulatory delays. It is hardly surprising, therefore, that the industry has sought to portray itself as responsible enough to self-police its own manufacturing and marketing practices, or that it has argued (45) that existing laws and FDA regulatory programs concerning cosmetics, together with the industry's commitment to self-regulation and product safety, provide ample consumer protection, given the low potential for risk inherent in cosmetics relative to other categories of products regulated by the FDA. Steinberg (46a) advocates compliance within a self-regulatory environment as being in the industry's own self-interest. He notes that regulatory compliance can be a "win-win" end result for the industry, consumers, and regulators, alike, but cautions that trying to "*beat the system may succeed in the short term, but it results in significant long-term losses.*" Steinberg envisions lost sales, public reputation, and market share as consequences to noncompliant firms; far more Draconian measures, involving the termination of self-regulation and the imposition of mandatory pre-market approval of cosmetics, were advocated in 1990 by Wyden (46b).

International Harmonization and Future Regulatory Challenges

The United States regulatory scheme for cosmetics presumes the innocuous character of cosmetics and the improbability of a major public health risk arising from the use of a cosmetic product (47); it is easy to infer such a presumption from the lack of premarket approval authority for cosmetics granted to the FDA under the 1938 FD&C Act.

Although many of the regulatory systems of other countries have similar goals to those of the United States, such as protecting public health and safety and promoting trade (48), the means by which these goals are achieved may be quite different from the United States system. These differences are often based upon political and philosophical views deep-seated in the culture of the particular country and may be trivial or profound. They can influence not only specific regulatory requirements, such as labeling, but also the very definition of just what constitutes a cosmetic or a drug. Several categories of topical products regulated as OTC drugs or OTC drug-cosmetics in the United States, such as sunscreens, skin bleaches, antiperspirants, and antidandruff shampoos (49) are regulated as cosmetics under the 1976 EU Cosmetics Directive (27). Japan, which currently regulates cosmetics according to a system of premarket approval and licensure rather than the postmarket surveillance system used by the U.S. or the notification system used by the EU, allows cosmetics to have some effect on the structure and function of the skin and hair, provided that the effect is "mild" and provides for a third "quasi-drug" category of product accommodating "mild," borderline physiological effects, such as hair-growth promoters (50a). However, initiatives currently underway in Japan promise to result

in deregulation of cosmetics and a shift to a postmarketing surveillance regulatory system more nearly aligned with those in effect in the U.S. and EU by a target date of March 31, 2001 (50b). Some regulatory systems currently reflect features of both the U.S. and EU systems; this is true, for example, of the system operative in Canada (50c). In some cases, the EU Model of a Member State consortium is being employed to facilitate international cooperation (such as the Andean Pact and Mercosur groups of nations in South America) (50d,e). Still other Third-World national regulatory systems are relatively rudimentary by comparison with those of the United States, EU, or Japan, and these are currently being updated to afford their citizens increased levels of protection.

The unprecedented growth experienced by the cosmetic industry in the 1980s and 1990s has also had its impact on international cosmetic regulation. Corporate consolidations and acquisitions of well-known homegrown American companies and domestic product brands by foreign-based corporations have refashioned the concept of multinational corporations. The economic imperatives of these new "world-class" companies... to expand market penetration and market share in global overseas markets... have resulted in formidable regulatory challenges to be overcome, as the firms attempt to achieve compliance with multiple sets of regulatory requirements in the international marketplace. International efforts have been directed towards modifying existing legislation that may serve as arbitrary impediments to international trade, and toward achieving better alignment and harmonization of national laws and cosmetic regulations. Hendrick and Horton (51) observe that:

"*Precisely because the regulatory requirements of different countries vary considerably, harmonization of regulations among countries is a worthy goal. As we move toward a global economy with more countries placing an emphasis on imports and exports, harmonization would assist in the reduction of barriers to trade.*"

The United States, a member of the World Trade Organization (WTO) since its formation in 1995, is a signatory to two principal international trade agreements that are relevant to the marketing of cosmetics and other FDA-regulated products: the General Agreement on Tariffs and Trade (GATT) and the North American Free Trade Agreement (NAFTA). Both the GATT and NAFTA agreements contain separate agreements on Technical Barriers to Trade (TBT) and Sanitary and Phytosanitary Measures (SBS), whose provisions seek to eliminate arbitrary, nonscientifically defensible regulations, product standards, and procedures that constitute artificial technical barriers to trade. Both, however, also reserve to sovereign signatory states the right to determine whatever level of public health protection they believe necessary for the benefit of their citizens, agriculture, and environment.

The FDA's policy on the international harmonization of regulatory requirements and guidelines was published in the *Federal Register* in 1995 (52); its stated goals are to simultaneously facilitate international trade and promote mutual understanding, while protecting national interests and establishing a rational paradigm for resolving issues on the basis of sound scientific evidence in an objective atmosphere. The agency is committed to working towards facilitating the exchange of scientific and regulatory information and knowledge with foreign government officials, and accepting the equivalent standards, compliance activities, and enforcement programs of other countries, provided that the FDA is satisfied such standards, activities, and programs meet the FDA's level of public health protection. However, the FDA is equally committed to the thesis that harmonization activities must not result in a lowering of the gate to furtherance of public health protections afforded by U.S. law (e.g., "downward harmonization").

The FDA Office of Cosmetics and Colors (OCAC), which is responsible for administering the cosmetics provisions of the FD&C Act, is committed to seeking implementation of the United States Government policies on international harmonization. Outreach conferences with regulatory authorities in Israel, the Andean Pact Nations, the EU, Canada, Japan, China, and others have sought to achieve international harmonization through identifying areas of accommodation, equivalence, and commonality among the regulatory schemes in the various administrations, rather than hoping to arrive at a single global regulatory structure. In particular, two (2) quadrilateral *Cosmetic Harmonization and International Cooperation* (C.H.I.C.) conferences between the U.S., EU, Canada, and Japan, held in 1999 and 2000, have identified a number of areas of mutual interest, listed below. These areas, and others of current relevance, are being considered as possible agenda items for future C.H.I.C. meetings:

- Memoranda of cooperation (MOC)
- Regulatory reform
- Animal testing
- Cosmetic ingredient nomenclature
- Approved color additives
- Sunscreens
- Drug-cosmetics and quasi-drugs
- Safety substantiation
- Fragrance allergenicity
- International adverse event safety "alert system"

Further details about the 2nd C.H.I.C. Meeting are posted on FDA's Website at the Cosmetics Program Homepage (http://www.cfsan.fda.gov/cosmetics.html).

Bioterrorism

No discussion of regulatory challenges facing the agencies of the United States in the context of international relations today should fail to mention one of the single most traumatic events of our times; namely, the terrorist attacks at the World Trade Center (WTC) and Pentagon on September 11, 2001 (9/11). The Congress responded to the highlighted need to enhance the security of many elements of our economic infrastructure, including the U.S. food supply and other consumer product streams, by passing *"The Public Health Security and Bioterrorism Preparedness and Response Act of 2002" (PL 107–188, BT Act)*; President Bush signed this act into law on June 12, 2002. Title III of the BT Act Title III {"*Protecting Safety and Security of the Food and Drug Supply*"} contained provisions that called upon the FDA to initiate Rulemaking concerning *Registration of Food Facilities* (Section 305), *Establishment and Maintenance of Records* (Section 306), *Prior Notice of Imported Food Shipments* (Section 307), and *Administrative Detention* (Section 303), most sections with statutory timelines and effective dates of December 12, 2003. However, the BT Act did not explicitly cover cosmetics.

FDA's Center for Food Safety and Applied Nutrition (CFSAN) published a separate draft guidance document dealing with cosmetics security issues on March 21, 2003 for the cosmetics industry, under the authority of *Operation Liberty Shield*, Titled, *"Guidance for Industry. Cosmetics Processors and Transporters Cosmetics Security Preventative Measures Guidance"* (53b), this voluntary and non-binding document attempts to be comprehensive in its presentation of the FDA's current

thinking on appropriate measures the cosmetics establishments may take to minimize risks regarding cosmetics under their control becoming subject to tampering or other malicious, criminal, or terrorist actions. The Guidance for Industry breaks its recommendations into five categories of actions:

- Management
- Human element—staff
- Human element—the public
- Facilities
- Operations

In announcing the availability of this guidance, CFSAN noted (53a) that there are voluntary guidelines that the cosmetic industry, itself, has already published on its own initiative, such as that recently issued by the CTFA (54). The FDA encouraged cosmetic trade associations to evaluate the preventative measures contained in its guidance document and adapt them to specific products and operations of the industry and to supplement this guidance with additional preventative measures when appropriate. The FDA guidance became final in November, 2003.

Bovine Spongiform Encephalopathy (BSE) and Cosmetics

The need of U.S. regulatory authorities to deal with the international epidemic of Bovine Spongiform Encephalopathy (BSE), sometimes called "Mad Cow Disease," has also had profound overtones for the FDA Cosmetics Program as well as for all of the other product categories regulated by the FDA . . . food, drug, medical device, and biologic. BSE is a slow, progressive, degenerative, fatal disease affecting the central nervous system (and other tissues) of adult cattle. It is generally accepted by the scientific community (55) that abnormal isoforms of a protein normally found in animals, known as "prions," are associated with the onset of disease, following a relatively long latent period of several years after infection. The exact cause of BSE is not known, although consumption of infected feed by cattle and other ruminants, particularly as "meat and bone meal (MBM)" starter rations for calves, has been associated with the disease. The FDA prohibited the use of ruminant protein in the manufacture of animal feed intended for cows and other ruminants in 1997 and extended the prohibition in 2001 to forbid the use of all mammalian protein in ruminant feed (56). A variant form of Creutzfeldt–Jacob Disease (v-CJD), a disease in humans first identified in the United Kingdom in 1996, is believed to be associated with consumption of contaminated beef products from BSE-infected cattle.

Cosmetics have never been implicated to date in reported or known cases of v-CJD, although passage of "prion" infectivity to humans through topical application of cosmetics formulated with animal-derived ingredients (principally bovine), remains a theoretical but nonzero risk for several reasons:

Compromised skin (cuts, abraded, or diseased) or other peripheral routes of administration, such as oral ingestion, inhalation, eye-area applications, or introduction to the body through the mucosal membranes are vulnerable to infection, even though the remarkably robust barrier to infection constituted by native, intact human skin makes the integument a much less likely target for prion infection.

Although, the CTFA has advocated "safe-sourcing" practices to its members through policy statements (57), a subset of the more than 12,500 ingredients in use today in retail cosmetic products are derived from animal sources, some of which

are ruminant or other animal species subject to BSE or other transmissible spongiform encephalopathies (TSE), such as sheep (*Ovidae*), deer or elk (*Cervidae*), or mink (*Mustela*). A number of these ingredients are monographed in the *CTFA International Cosmetic Ingredient Dictionary and Handbook* (although they are not necessarily actively marketed by current suppliers).

Potential use of "high risk" animal tissues as source materials for cosmetic ingredients, whether due to country of origin (i.e., BSE-country), organ source (i.e., so-called "specified risk materials (SRMs)" such as spine, spinal cord, and eye tissue), or animal health status (i.e., nonambulatory, disabled or "downer" cattle) continues to be of concern.

Other ingredients, such as gelatin and its respective derivatives, can be of much lower BSE-risk if they are "safe-sourced" from low-risk animal species (i.e., porcine, equine, or fish), low-risk animal tissues (i.e., skin, hair, and hides), harvested from animals that originate or have resided in non-BSE countries and or can be traced to BSE-free herds, if they have been passed as "edible" (fit for human consumption) by the U.S. Department of Agriculture (USDA), if heads, spines, and spinal cords of animals from BSE-countries and/or BSE status-indeterminate countries are removed directly after slaughter, if they are rigorously processed by validated procedures known to inactivate prion infectivity, and if special good manufacturing practices are instituted to ensure that no cross-contamination or comingling occurs of product streams possibly bearing BSE-infectivity with those presumed BSE-free.

Agencies of the United States such as the FDA and USDA have taken several specific measures over the past decade to ensure an effective "firewall" against the establishment of epidemic BSE in the United States, from domestic or foreign sources, and these interventions have obvious relevance to both cosmetic raw materials and finished products, as well as to other manufactured consumer goods and products of agriculture:

- Since 1990, the U.S. Department of Agriculture (USDA) has conducted aggressive surveillance of the highest risk cattle going to slaughter in the United States, in which 10,000–20,000 animals per year have historically been tested; the USDA has announced that this rate of surveillance was dramatically increased as of 2004 (59). To date, the only cow that has been found to be affected with BSE in the United States was the one diagnosed in Washington State in December, 2003; subsequent trace-back investigations conducted by the USDA and FDA determined that this animal was not native born in the U.S. (59).
- Guidance to the industry concerning "safe-sourcing" of cosmetic ingredients was provided in the form of "Letters to Manufacturers of Cosmetics and Dietary Supplements" in 1994 and 1996 (62), and recommendations for gelatin processed for use in FDA-regulated products were published in 1997 (60). Additional guidance provided to the FDA by its TSE Advisory Committee in 1998 and 2003 with respect to the use of gelatin and tallow and their specific derivatives in FDA-regulated products resulted in further recommendations to the industry (61).
- The FDA's "firewall" protections for cosmetics, contained in the FDA Cosmetics Compliance Program (62) and a companion Import Alert (63), incorporate by reference requirements published in 1998 by the USDA Animal and Plant Health Inspection Service (APHIS) designed to enhance safeguards against BSE by preventing entry into the U.S. of imported cosmetic

(and dietary supplement) ingredients containing SRMs from animals originating in BSE-countries (64); future regulatory initiatives by the FDA will also take cognizance of the recent regulations implemented in early 2004 by the USDA Food Safety and Inspection Service (FSIS) (65). Details on these programs and import alerts may be found at the FDA and USDA internet websites, respectively (66).

- Most recently, the FDA published an "interim final rule (IFR)" (67) that prohibits the use of the bovine-derived materials that carry the highest risk of harboring BSE infectivity in human food, including dietary supplements, and in cosmetics. The FDA's rule considers SRMs, cattle material from nonambulatory disabled cattle, cattle material from organs of cattle $\geq$30 months of age, the tonsils, and small intestines of cattle of all ages, cattle material from mechanically separated (MS) beef, and cattle material from cattle that are not inspected and passed for human consumption as "prohibited cattle materials"; significantly, it does not include tallow that contains $\leq$0.15% hexane-insoluble impurities (presumed to be protein) and tallow derivatives. Recordkeeping requirements sufficient to demonstrate compliance with the IFR have also been proposed by the FDA.

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DISCLAIMER

The views expressed herein are those of the authors and do not necessarily represent those of the FDA.

REFERENCES

1. Jackson EM. Consumer products: cosmetics and topical over-the-counter drug products. In: Chengelis CP, Holson JF, Gad SC, eds. Regulatory Toxicology. New york: Raven Press Ltd., 1995:117–119.

2. Hobbs CO. Advertising for foods, veterinary products, and cosmetics. In: Brady RP, Cooper RM, Silverman RS, eds. Fundamentals of Law and Regulation. Vol. 1, Washington, D.C.: Food and Drug Law Institute (FDLI), 1997:347–379 (chapter 12).
3. (a) Working Agreement Between FTC and FDA, FTC Press Release, Federal Trade Commission, Washington, DC, June 9, 1954; (b) Memorandum of Understanding (MOU) Between the Federal Trade Commission and the Food and Drug Administration Concerning Exchange of Information (FDA-225–71–8003), FDA Compliance Policy Guide 7155m.01, April 27, 1971 (FDA); Approved and Accepted for the FTC May 14, 1971.
4. (a) Poison Prevention Packaging Act of 1970 (15 U.S.C. 1471 n, Public Law 91–601, 84 Stat. 1670, December 30, 1970, as amended); (b) Household Products Containing Petroleum Distillates and Other Hydrocarbons; Advance Notice of Proposed Rulemaking, 62 FR 8659, February 26, 1997; (c) Requirements for Child-Resistant Packaging; Household Products Containing Methacrylic Acid; Proposed Rule, 63 FR 71800, December 30, 1998; (d) Requirements for Child-Resistant Packaging; Requirements for Household Glue Removers Containing Acetonitrile and Home Cold Wave Permanent Neutralizers Containing Sodium Bromate or Potassium Bromate, 55 FR 51897, December 18, 1990.
5. Federal Insecticide, Fungicide, and Rodenticide Act of 1972 (FIFRA, 7 U.S.C. Sec.136–136 y); codified regulations at 40 CFR 162–180.
6. FD&C Act, Section 201p.
7. (a) Liquid Soap Category Will Reach $250 Million by 1985, The Rose Sheet (June 29, 1981:3; (b) SoftSoap Expected to Add $65 Million to Colgate-Palmolive's, The Rose Sheet, August 17, 1987:2–3.
8. (a) Antiseptic Wash Monograph Directions With Manufacturer Reference Suggested, The Rose Sheet, February 10, 1997:6–7; (b) Fischler G, Shaffer M, Healthcare Continuum: A Model for the Classification and Regulation of Topical Antimicrobial Wash Products, The Healthcare Continuum Model Symposium, Washington, DC, June 2–3, 1997.
9. (a) FD&C Act, Section 601 (a); (b) Hair-dye products. In: FDA's Cosmetics Handbook, Washington, DC: U.S. Government Printing Office, October 15, 1992:11–12..
10. (a) Nitrosamine-Contaminated Cosmetics; Call for Industry Action; Request for Data; Notice, 44 FR 21365–21367, April 10, 1979; (b) FDA's Cosmetics Handbook, Washington, DC: U.S. Government Printing Office, October 15, 1992:8–9; (c) Greif M, Wenninger JA, Yess N. Cosmetic regulation: an overview of FDA'S role Cosmetic Technol April 1980:43–44; (d) Chou, HJ. Determination of Diethanolamine and *N*-Nitrosodiethanolamine in Fatty Acid Diethanolamines, J AOAC Int 1998; 81(5):943–947; (e) Havery, DC, Chou, HJ. *N*-Nitrosamines in cosmetic products. An overview. Cosmet Toilet 1994; 109(5):53–58,61–62..
11. FDA's Cosmetics Handbook, U.S. Government Printing Office, Washington, DC, October 15, 1992:8–9.
12. (a) Havery, DC, Chou, HJ. *N*-Nitrosamines in cosmetic products. An overview. Cosmet Toilet 1994; 109(5):53–58,61–62; (b) Schoenberg T. Formulating Without Diethanolamides. HAPPI, July 1998:76,78,80,82; (c) Diethanolamine in Cosmetic Products, Office of Cosmetics Fact Sheet, FDA/CFSAN Website address http://vm.cfsan.fda.gov/~dms/cos-dea.html; (d) VCRP Reporting incentives to boost industry participation considered, The Rose Sheet, November 15, 1999:8–9; (e) CFSAN Program Priorities. FY 2003 Report Card, Part II: Assuring Food & Cosmetic Safety & Improving Nutrition. Specific Program Areas. Cosmetics. Goal 101. Voluntary Cosmetic Registration Program (VCRP), November 2003.
13. (a) *21 CFR 110.3*–110.93; (b) Cosmetic Good Manufacturing Practice Guidelines. In: FDA's Cosmetics Handbook, U.S. Government Printing Office, Washington, DC, October 15, 1992, 4–6.
14. (a) Halper AR, Milstein SR, personal communication, February 1, 2000; (b) Food and Drug Administration Recall Policies (informational flier), U.S. Department of Health

and Human Services, Public Health Service, Food and Drug Administration, Center for Food Safety and Applied Nutrition, Washington, DC, 20204.
15. Calogero C. Regulatory review. Cosmet Toilet 2000; 115(7):26.
16. (a) Diane T Duffy Esq, Classification and Regulation of Cosmetics and Drugs: A Legal Overview and Alternatives for Legislative Change. American Law Division, Congressional Research Service, The Library of Congress, Washington, DC 20540, May 4, 1990: CRS-16; (b) Yingling GL, Onel S, Cosmetic regulation revisited. In: Brady RP, Cooper RM, Silverman RS, eds. Fundamentals of Law and Regulation Vol. 1. Washington, DC: Food and Drug Law Institute (FDLI), 1997:315–346 @ 346: (Chapter 11); (c) Murphy EG, Wilson PJ, Regulation of cosmetic products. In: Williams DF, Schmitt WH, eds. Chemistry and Technology of the Cosmetics and Toiletries Industry, 2nd edn., London, UK: Blackie Academic & Professional, (an Imprint of Chapman & Hall), 1996:344–361 @ 355.
17. Rumore MM, Strauss S, Kothari AB. Regulatory Aspects of Color Additives, Pharmaceut Technol, March 1992: 68,70,72,74,76,78,80,82.
18. FDA's Cosmetics Handbook. Washington, DC: U.S. Government Printing Office, October 15, 1992:2.
19. Canterbery RC, Wenninger JA, McEwen GN Jr. International Cosmetic Ingredient Dictionary and Handbook. 9th edn., Washington, DC: The Cosmetic, Toiletry, and Fragrance Association, Inc. (CTFA), 2001.
20. Permanent Listing of Color Additive Lakes; Proposed Rule, 61 FR 8372–8417, March 4, 1996.
21. 21 CFR 70.5 (a).
22. 21 CFR 73.1150; 21 CFR 73.2150.
23. 21 CFR 70.5 (b).
24. (a) The Federal Fair Packaging and Labeling Act, 15 U.S.C. Sec. 1451 et. seq.; (b) 15 U.S.C. Sec. 1459 a (Definitions)..
25. Cosmetic Ingredient Labeling and Voluntary Filing of Cosmetic Product Experiences. Regulations for the Enforcement of the Federal Food, Drug and Cosmetic Act and the Fair Packaging Labeling Act. Cosmetic Ingredient Labeling, 38 FR 28912–28917 @ 28912, October 17, 1973.
26. (a) The American Technology Preeminence Act of 1991 (Pub. L. 102–245, Section 107), February 14, 1992; (b) Pub. L. 102–329, August 3, 1992; (c) Metric Labeling; Quantity of Contents Labeling Requirements for Foods, Human and Animal Drugs, Animal Foods, Cosmetics, and Medical Devices; Proposed Rule, 58 FR 67444–67464, December 21, 1993.
27. Council Directive 76/768/EEC on the Approximation of the Member States Relating to Cosmetic Products, OJECNI, July 27, 1976; 169:262 (hereinafter referred to as the 'Cosmetic Directive').
28. Council Directive 93/35/EEC June 14, 1993 (hereinafter, referred to as the Sixth Amendment to the Cosmetic Directive).
29. (a) Bailey JE,. McEwen GN Jr, Personal communication (Docket No. 96P-0347), June 1, 1995; (b) Bailey JE,. McEwen GN Jr, Personal communication (Docket No. 96P-0347), January 17, 1996.
30. Tamper-Resistant Packaging Requirements; Certain Over-the-Counter Human Drugs and Cosmetic Products; Contact Lens Solutions and Tablets; Final Rules 47 FR 50442–50456 @ 50447 November 5, 1982.
31. (a) FDA Investigations Operations Manual, FDA Office of Regulatory Affairs, Washington, DC, January 2000, Chapter 10—Reference Materials, Subchapter 1020—Guidelines and Other Guidance Materials, Section 1023—Cosmetics; (b) Guide to Inspections of Cosmetic Product Manufacturers, FDA/ORA website address: (http://www.fda.gpv/ora/inspect_ref/igs/cosmet.html).
32. (a) Beyond Approval: Drug Manufacturer Regulatory Responsibilities. In: Mathieu M, ed. New Drug Development: A Regulatory Overview, 4th ed. Waltham, MA: Parexel

International Corporation, 1997:272–279 (Chapter 12). Current Good Manufacturing Practice; 21 CFR., 211 (Current Good Manufacturing Practice for Finished Pharmaceuticals), April 1, 2000.
33. (a) Subchapter G—Cosmetics. Reorganization and Republication, March 15, 1974; 39 FR 10054–10064 @ 1059–10062; (b) Modification in Voluntary Registration of Cosmetic Industry Data. Final Rule, 46 FR 38073–38074, July 24, 1981; (c) Modification of Voluntary Filing of Cosmetic Product Experiences. Final Rule, 51 FR 25687, July 16, 1986.
34. Modification in voluntary filing of cosmetic product ingredient and cosmetic raw material composition statements. Final Rule 57 FR 3128–3130, January 28, 1992.
35. Food and cosmetic labeling; revocation of certain regulations. Final Rule 62 Fr 43071–43075 @ 43073, August 12, 1997.
36. (a) Voluntary Cosmetics Registration Program: Suspension of Activity—March 30, 1998, (Letter to Industry Participants, Department of Health and Human Services, Public Health Service, Food and Drug Administration); (b) FDA Cosmetics office registration program suspended. The Rose Sheet, April 6, 1998:1.
37. Voluntary Cosmetics Registration Program Reinstated With No Changes, The Rose Sheet, January 11, 1999:3.
38. (a) VCRP Reporting Incentives to Boost Industry Participation Considered, The Rose Sheet, November 15, 1999:8–9; (b) CFSAN Program Priorities. FY 2003 Report Card, Part II: Assuring Food & Cosmetic Safety & Improving Nutrition. Specific Program Areas. Cosmetics. Goal 101. Voluntary Cosmetic Registration Program (VCRP), November 2003.
39. Bergfeld WF, Elder RL, Schroeter AL. The cosmetic Ingredient Review Self-Regulatory Safety Program, Dermatol Clin 1991; 9(1):105–122.
40. Ford RA. The toxicology and safety of fragrances. In: Muller PM, Lamparsky D, eds. Perfumes. Art, Science, and Technology, London and New York: Elsevier Applied Science, 1991:441–463 (Chapter 16).
41. MorrisonT. Using the lanham act to achieve truth in advertising, Drug Cosmet Ind, April 1989; 24,26,28,30,32,81–83; (b) Donegan TJ, Section 43 (a) of the Lanham Trademark Act as a private remedy for false advertising. Food Drug Cosmet Law J, 1982; 37:264–288.
42. (a) Government regulation of competition and pricing. In: Anderson RA, Fox I, Twomey DP, Business Law & The Legal Environment. Comprehensive Volume 16th edn. Cincinnati, Ohio: South-Western College Publishing, 1996:60–68 (Chapter 4); (b) Antitrust issues and pricing strategy (discriminatory pricing). In: Stern LW, Eovaldi TL, Legal Aspects of Marketing Strategy: Antitrust and Consumer Protection Issues, Englewood Cliffs, New Jersey: Prentice-Hall, Inc. 1984: 263–279 (Chapter 5).
43. (a) National Advertising Division, Children's Advertising Review Unit, & National Advertising Review Board Procedures June 10, 1993, Council of Better Business Bureaus, Inc. New York, NY (1996); (b) Smithies RH, Substantiating Performance Claims, Cosmet Toilet, 1984; 99(3):79–81,84.
44. (a) The Social and Legal Impact of Advertising, In: Bovee CL, Arens WF eds. Contemporary Advertising, Homewood, Illinois: Richard D. Irwin, Inc., 1982:60–86; (b) Handler J, The self-regulatory system—an advertiser's viewpoint, Food Drug Cosmet Law J 1982; 37:257–263.
45. McNamara SH. The 'C' in the FDC Act. FDA Consumer 1981; 15(5):62–63.
46. (a) Steinberg DC. Compliance with self-regulation. Cosmet and Toilet, 115 (4), 37–40, April 2000; (b) Wyden R, (D-Ore). Tighten Safety Regulation of Cosmetics, USA TODAY, April 12, 1990.
47. Hendrick BS, Horton LR. International Harmonization of Cosmetic Regulation, In: Brady RP, Cooper RM, Silverman RS, eds. Fundamentals of Law and Regulation, Vol. 1, Washington, DC: Food, Drug, and Law Institute (FDLI), 1997:485–505 @ 503 (Chapter 14).

48. Hendrick BS, Horton LR. International Harmonization of Cosmetic Regulation, In: Brady RP, Cooper RM, Silverman RS, eds. Fundamentals of Law and Regulation, Vol. 1, Washington, DC: Food, Drug, and Law Institute (FDLI), 1997:488.
49. (a) Sunscreen drug products for over-the-counter human use. Final Monograph. Final Rule 64 FR 27666–27693, May 21, 1999; (b) Skin Bleaching Drug Products for Over-the-Counter Human Use; Tentative Final Monograph; Notice of Proposed Rulemaking, 47 FR 39108–39117, September 3, 1982; (c) Antiperspirant Drug Products for Over-the-Counter Human Use; Tentative Final Monograph; Proposed Rule, 47 FR 36492–36505, August 20, 1982; (d) Dandruff, Sebborheic Dermatitis, and Psoriasis Drug Products for Over-the-Counter Human Use; Final Rule, 56 FR 63554–63569, December 4, 1991 (as amended at 59 FR 4000, January 28, 1994).
50. (a) Santucci LG, Rempe JM. Legislation and Safety Regulations for Cosmetics in the United States, Europe, and Japan. In: Butler H, ed. Poucher's Perfumes, Cosmetics, and Soaps, Vol. 3, 9th edn. London, UK: Chapman & Hall, 1993:566–571 (Chapter 20); (b) Steinberg DC, Global Understanding 2000. Toward Global Harmonization of Cosmetic Regulation, Cosmet Toilet 2000; 115(8):27; (c) Hendrick BS, Horton LR. International Harmonization of Cosmetic Regulation, In: Brady RP, Cooper RM, Silverman RS, eds. Fundamentals of Law and Regulation. Vol. 1. Washington, DC: Food, Drug, and Law Institute (FDLI), 1997:496–498; (d) Anon, Minutes of the Third Summit of the Public Health Authorities of the Americas, June 15–16, 2000, Lima, Peru; (e) Hendrick BS, Horton LR. International Harmonization of Cosmetic Regulation, In: Brady RP, Cooper RM, Silverman RS, eds. Fundamentals of Law and Regulation, Vol. 1, Washington, DC: Food, Drug, and Law Institute (FDLI), 1997:498–501.
51. Hendrick BS, Horton LR. International Harmonization of Cosmetic Regulation, In: Brady RP, Cooper RM, Silverman RS, eds. Fundamentals of Law and Regulation, Vol. 1, Washington, DC: Food, Drug, and Law Institute (FDLI), 1997:504.
52. (a) International Harmonization; Draft Policy on Standards; Availability; Notice, 59 FR 60870–60874 (November 28, 1994); (b) International Harmonization; Policy on Standards; Notice, 60 FR 53078–53084 (October 11, 1995).
53. (a) Statement of Joseph Levitt, Esq., March 19, 2003; (b) Guidance for Industry. Cosmetics Processors and Transporters. Cosmetics Security Preventive Measures Guidance. Draft Guidance, DHHS/FDA/CFSAN (March 21, 2003).
54. CTFA Product Security and Tampering Incident Guidelines, December 2001.
55. Brown P. Mad-Cow Disease in Cattle and Human Beings. Am Sci 2004; 92(4):334–341.
56. 21 CFR 589. 2000 Animal Proteins Prohibited in Ruminant Feed..
57. CTFA Board Policy on Mad Cow Disease, Approved April 20, 2001 (see also, Introduction – Cosmetic Ingredients and BSE, in Ref. (19, p.ix))..
58. Joint Statement by Dr. Ron DeHaven (APHIS) and Dr. Barbara Masters (FSIS), USDA–FSIS News Release, May 3, 2004, www.fsis.usda.gov/News_&_Events/NR_050304_01/index.asps.
59. (a) Case of BSE in the United States. Chronology of Events http://www.usda.gov (click on link to BSE Information and Resources); (b) Statement of Probable Case of BSE in Washington State, FDA Statement, December 24, 2003, http://www.fda.gov (click on link to Hot Topics. BSE (Mad Cow Disease)).
60. Guidance for industry. The Sourcing and Processing of Gelatin to Reduce the Potential Risk Posed by Bovine Spongiform Encephalopathy (BSE) in FDA-Regulated Products for Human Use, DHHS/FDA, Docket No. 97D-0411, September 1997.
61. FDA TSE Advisory Committee Meetings (April 1998, July 2003), http://www.fda.gov (click on links to Advisory Committees → Biologics (Blood, Vaccines) → Transmissible Spongiform Encephalopathies).
62. FDA Cosmetics Compliance Program Guides CPG 7329.001 (Domestic Cosmetics Program, Issued July 31, 2000)/7329.002 (Imported Cosmetics Program, Issued December 8, 2000).

63. Import Alert # 17–04, Detention without physical examination of bulk shipments of high-risk bovine tissue from BSE-countries—Bovine Spongiform Encaphalopathy, Revision, May 20, 2003.
64. (a) 9 CFR 94.18; (b) 9 CFR 95.4.
65. USDA Issues New Regulations To Address BSE, FSIS News Release, January 8, 2004 (Washington, DC) http://www.fsis.usda.gov/oa/news/2004/bseregs.htm.
66. Expanded mad cow safeguards announced to strengthen existing firewalls against TSE transmission, DHHS News Release, January 26, 2004 (Washington, DC), http://www.hhs.gov/news/press/2004pres/200f40126.html.
67. (a) Use of materials derived from cattle in human food and cosmetics; and recordkeeping requirements for human food and cosmetics manufactured from, processed with, or otherwise containing, material from cattle. Final Rule and Proposed Rule, 69 FR 42255–42279, July 14, 2004; (b) Commonly Asked Questions About BSE in Products Regulated by FDA's Center for Food Safety and Applied Nutrition (CFSAN), January 14, 2004 (Updated July 9, 2004).

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Legislation in Japan

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REGULATORY ENVIRONMENT

The cosmetic regulations in Japan are extensive and complex (1). The legal classification of topically applied products is different from the United State and Europe, where they are divided into only two categories, drugs and cosmetics. In Japan, there are additional regulations covering cosmetic products with pharmacological action, called quasidrugs, which are ranked between cosmetics and drugs (2). The definitions of drugs, cosmetics, and, quasidrugs in the regulations (3,4) are as follows:

Drugs are defined as

1. articles recognized in the official Japanese Pharmacopoeia;
2. articles (other than quasidrugs) that are intended for use in the diagnosis, cure, or prevention of disease in humans or animals, and that are not equipment of instruments (including dental materials, medical supplies, and sanitary materials);
3. articles (other than quasidrugs and cosmetics) that are intended to affect the structure or any function of the body of humans or animals, and that are not equipment or instruments (paragraph 1, article 2 of the law).

Quasidrugs are articles that have the purposes given below and exert mild actions on the human body, or similar articles designated by the Minister of Health, Labour, and Welfare, (MHLW). They exclude not equipment and instruments but also any article intended, in addition to the following purposes, for the use of drugs described in (b) and (c) above.

1. Quasidrugs prescribed by the law:
    a. prevention of nausea or other discomfort, or prevention of foul breath or body odor;
    b. prevention of prickly heat, sores, and the like;
    c. prevention of falling hair, or hair restoration or depilation;
    d. killing or prevention of rats, flies, mosquitoes, fleas, etc., for maintaining the health of humans or animals.

2. Quasidrugs designated by MHLW (MHW Notification No. 14, 1961, MHLW Notification No. 202, 1995, and MHLW Notification No. 31, 1999):
   a. cotton products intended for sanitary purpose (including paper cotton);
   b. the following products with a mild action on the human body:

      i. disinfecting solutions for soft contact lenses,
      ii. products used to disinfect or protect abrasions, cuts, puncture wounds, scratches, and wound surfaces,
      iii. products that combine the purposes of use as stipulated in paragraph 3, article 2 of the law (on cosmetics), with the purpose of prevention of acne, chapping, itchy skin rash, chilblain, etc., as well as disinfection of the skin and mouth,
      iv. products used to improve such symptoms as chapped skin, prickly heat, sores, corns, calluses, and dry skin,
      v. hair dyes,
      vi. agents for permanent waving,
      vii. bath preparations,
      viii. products used to alleviate discomfort of the throat,
      ix. products used to alleviate discomfort of the stomach,
      x. products intended to supply vitamins or calcium to the fatigued or middle-aged body,
      xi. products used to nourish the body or improve a weak body (diet supplements).

Among the products just described, the category (2)-(iii) comprises the so-called medicated cosmetics. On April 1, 1999, after much deliberation, a total of 15, described in (2)-(viii)–(xi), were shifted from nonprescription medicines to quasidrugs (5) as they had relatively mild pharmacological actions and could be sold without need for health professionals to provide information to the general public at the point of purchase. For differentiating the new entry from those "conventional" quasidrugs, they are specifically called "newly designated quasidrugs" (5).

The term "cosmetic" means any article intended to be used by means of rubbing, sprinkling, or by similar application to the human body for cleaning, beautifying, promoting attractiveness, altering the appearance of the human body, and for keeping the skin and hair healthy, provided that the action of the article on the human body is mild. Such articles exclude the articles intended, besides the above purposes, for the use of drugs described in 2 or 3 above, and quasidrugs (paragraph 3, article 2 of the law).

The scopes of the efficacy of cosmetics (6) are specified in Table 1.

COSMETICS

At each stage of development, manufacture/import, distribution, and use, the prescribed regulations are put into practice, including systems of the examination for approval, manufacture/importation, distribution control, and postmarketing surveillance, respectively (3,4).

Table 1 The Scopes of Efficacy of Cosmetics

(1) Clean the scalp and hair
(2) Mask unpleasant scalp and hair odors with perfume
(3) Keep the scalp and hair healthy
(4) Make hair firm and resilient
(5) Moisten the scalp and hair
(6) Keep moisture in the scalp and hair
(7) Make hair supple
(8) Make hair easier to comb
(9) Keep the luster of hair
(10) Make hair lustrous
(11) Treat dandruff and itching
(12) Suppress dandruff and itching
(13) Supplement and maintain hair moisture and oil
(14) Prevent hair from breaking, severing, or splitting
(15) Set and keep hairstyle
(16) Prevent electrical charge of hair
(17) Clean the skin by removing dirt
(18) Prevent pimples and heat rash by cleaning (face cleanser)
(19) Condition the skin
(20) Condition skin texture
(21) Keep the skin healthy
(22) Prevent skin roughness
(23) Tighten the skin
(24) Moisten the skin
(25) Supplement and maintain skin moisture and oil
(26) Keep skin soft and elastic
(27) Protect the skin
(28) Prevent dry skin
(29) Make the skin soft
(30) Make the skin strong
(31) Give the skin luster
(32) Make the skin smooth
(33) Make shaving easier
(34) Condition the skin after shaving
(35) Prevent heat rash (powders)
(36) Prevent sunburn
(37) Prevent spots and freckles due to sunburn
(38) Give a pleasant fragrance
(39) Protect nails
(40) Keep nails healthy
(41) Moisten nails
(42) Prevent lip roughness
(43) Condition lip texture
(44) Moisten the lips
(45) Make the lips healthy
(46) Protect the lips; prevent dry lips
(47) Prevent chapped lips due to dryness
(48) Make the lips smooth
(49) Prevent cavities (toothpastes used in brushing)
(50) Whiten teeth (toothpastes used in brushing)

(*Continued*)

Table 1 The Scopes of Efficacy of Cosmetics (*Continued*)

(51) Remove dental plaque (toothpastes used in brushing)
(52) Clean the oral cavity (toothpastes)
(53) Prevent foul breath (toothpastes)
(54) Remove teeth stains (toothpastes used in brushing)
(55) Prevent formation of dental calculus (toothpastes used in brushing)

Notes: For example, efficacies with the phrase "supplement and maintain" can be divided into "supplement" or "keep." A products in parentheses is not included in the efficacy, but the efficacy is limited for the product based on the usage. *Source*: From Ref. 6.

As for manufacture or import licenses of cosmetics, the Ministry of Health and Welfare (presently MHLW) had reviewed each formulation on a case-by-case basis before granting them (7). In 1986, the "Comprehensive Licensing System (licensing by cosmetic category)" was introduced for simplification and rationalization of the prior evaluation. The deregulation was further carried out based on the government's policy to review licensing systems and ingredient labeling controls (8). The new regulations, effective as of April 1, 2001, superceded the Quality Standards of Cosmetics of 1967, eliminated the premarket licensing requirement for cosmetics, and established lists of prohibited and restricted ingredients, approved sunscreens, preservatives, and coal tar colors, as well as new labeling requirements (7). This deregulation indicated the shift of the regulatory system to one based on the manufacturers' self-responsibility. The responsibility to ensure that any cosmetic product placed on the market is safe falls on the manufacturer (7). Similar to the requirements in the United States and Europe, the health authorities may require a manufacturer to substantiate product safety (7).

Full ingredient labeling must be provided for cosmetics using International Nomenclature Cosmetic Ingredients names translated or transliterated into Japanese (7) to give consumers sufficient information to help them evaluate and select the cosmetics.

The above-mentioned specific ingredient groups (preservatives, sunscreens, coal tar colors) require cautious handling under appropriate safety evaluation. For the amendment of positive lists, such as adding a new raw material or increasing permitted concentration, the procedure for introducing them shall be as indicated on the flow chart shown in Fig. 1 (9,10). The following data must be attached to the application wherever appropriate.

- origin and background of discovery,
- previous use in foreign countries,
- characteristics and comparison with other cosmetics,
- determination of chemical structure,
- physicochemical properties,
- safety.

QUASIDRUGS

In the Pharmaceutical Affairs Law, "quasidrugs" are defined as articles having "fixed purpose of use" and "mild action on the body" or similar articles designated by MHWL. Most of the products in this category are what we call "pseudodrugs" or

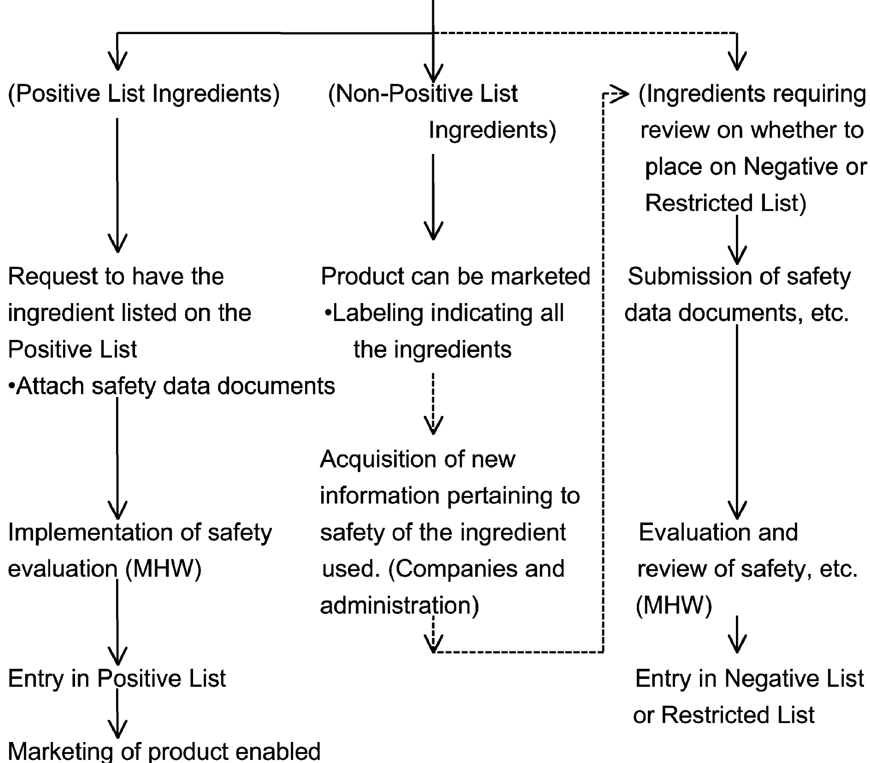

Figure 1 Flow chart of the procedure for treating new ingredients for cosmetics. *Source*: From Refs. 9, 10.

"cosmeceuticals", a current definition of which would be "those products that will achieve cosmetic results by means of some degree of physiological action" (11). The defined quasidrug products are mouth refreshers, body deodorants, talcum powders, hair growers, depilatories, hair dyes, permanent waving products, bath preparations, medical cosmetics (including medical soaps), medicated dentifrices, etc. (3,4).

At each stage of development, manufacture/import, distribution, and use, the prescribed regulations are enforced (3,4). Manufacturers of quasidrug are required to obtain government approval before marketing. Approval of a product under an

application for manufacturing/importing is the responsibility of the MHLW. Is it adequate as a quasidrug in view of its efficacy, safety, etc.? Therefore, the examination procedures for approval as well as the data and documentation required to be submitted for filing an application differ with indications and effects of each product (3,4). The following data must be attached according to the kind of ingredients employed, etc.:

- origin, background of discovery, use in foreign countries employed, etc.,
- physicochemical properties, specifications, testing methods, etc.,
- stability,
- safety,
- indications or effects.

The scope of the data to be attached to the application depends on the type of quasidrug: (a) new quasidrugs which obviously differ from any previously approved products with respect to active ingredients, usage and dosage, and/or indications or effects, (b) quasidrugs identical with previously approved quasidrug(s), or (c) other quasidrugs which are other than those specified in (a) and (b) above (3,4).

All products for approval as a quasidrug must be within the scope stipulated by the Pharmaceutical Affairs Law. Thus, approval of a product as a quasidrug is determined by an integrated judgment of various factors such as its ingredients, quantity (composition), indications and effects, usage and dosage, and dosage form. For example, those products whose effects are not mild, hence coming under the category of poisons or deleterious drugs, are not approved even if their indications and effects and dosage forms are within the scope of the quasidrugs legislation. Likewise, products for which the intended use deviates from the scope of quasidrug are also not approved even if their effects are mild (3,4).

COSMETICS IN THE FUTURE

The Law Concerning the Evaluation of Chemical Substances and Regulation of their manufacture, etc. (hereinafter "Chemical Substance Control Law") requires notification and assessment of chemical substances. With the aim of preventing damage to human health caused by environmental pollution from chemical substances, Japan's 1973 Chemical Substances Control Law requires prior evaluation of certain hazardous properties of new chemical substances intended for industrial use, and also regulates the manufacture, import, and use of chemical substances that persist in the environment (i.e., persistent) and are toxic to human health if taken in continuously (i.e., having long-term toxicity to humans), such as polychlorinated biphenyls and trichloroethylene, in ways that reflect their hazardous properties (12). Cosmetics, quasidrugs, and drugs are exempt as they are covered by the Pharmaceutical Affairs Law (7). Meanwhile, chemical evaluations and regulations in many industrialized countries pay attention not only to effects on human health but also to those on living organisms in the environment, and take into account the potential for environmental releases of chemical substances, in evaluating new chemical substances (12). In January 2002, the OECD recommended that Japan should further improve effectiveness and efficiency of chemical management, and further extended the scope of regulation to include ecosystem protection (12). Under these circumstances, three governmental councils of MHLW, Ministry of Economy, Trade and Industry, and Ministry of the Environment reviewed chemical evaluation and regulation systems in Japan and concluded in their joint report that the government should amend

the Chemical Substances Control Law to introduce evaluation and regulation that could take into account the adverse effects of chemical substances on living organisms in the environment (12). Based on the joint report, the government submitted the Bill for Partial Amendment to the Chemical Substances Control Law to the Diet on March 7th, 2003 (12). The Diet passed the Bill on May 22nd, and the amended law was promulgated on May 28th, 2003 (12).

The MHLW is now preparing to amend the Cosmetics Standard to introduce evaluation and regulation that could take into account the adverse effects of chemical substances on living organisms in the environment as well as the Chemical Substances Control Law. A specific description of the partial revision will be provided hereafter.

QUASIDRUGS IN THE FUTURE

There has been a great demand by consumers for innovative cosmetic products with pharmacological action, i.e., pseudodrugs or cosmeceuticals such as skin antiaging products. To satisfy their demands, research on the skin has been undertaken to develop new active ingredients for skin antiaging products. How should these products be legally categorized? Quasidrugs would seem to be suitable for such products to be categorized. However, all of the products have not always been approved as quasidrugs to date. Taking antiwrinkle products, for example, no new products have been approved under the existing quasidrug specifications.

Generally, topically applied quasidrugs are intended to mollify unwanted aspects of the skin and have a mild action on the human body, while medical drugs are intended to treat specific diseases. Therefore, hair growth products with a mild action on male-pattern baldness, which is not a disease (2), are quasidrugs. On the other hand, products intended for alopecia areata, which is a disease, are regarded as drugs. The natural aging of skin, like wrinkling, is not a disease, for example. We should also keep in mind that "high efficacy" should not always involve "strong action." There will be many pseudodrugs or cosmeceutical products with mild actions showing good efficacy.

Legally, MHLW can add new, novel types of product to the current list of types of quasidrug (13). Therefore, we hope that before long the above-mentioned new products will be listed as quasidrugs.

REFERENCES

1. Schmitt WH, Murphy EG. An overview of worldwide regulatory programs. In: Estrin NF, ed. The Cosmetic Industry: Scientific and Regulatory Foundations. New York and Basel: Marcel Dekker, 1984:133–159.
2. Vermeer BJ, Gilchrest BA. Cosmeceuticals: a proposal for rational definition, evaluation, and regulation. Arch Dermatol 1996; 132:337–340.
3. Editorial supervision by Pharmaceuticals and Cosmetics Division, Pharmaceutical Affairs Bureau, Ministry of Health and Welfare. Guide to Quasidrug and Cosmetic Regulations in Japan. Tokyo: Yakuji Nippo, 1992.
4. Editorial supervision by Pharmaceuticals Affairs Assessing Group. Guide to Quasidrug and Cosmetic Regulations in Japan. 4th ed. Tokyo: Yakuji Nippo, 2001.
5. Newly designated quasi-drugs. In: The Proprietary Association of Japan, ed. OTC Guide Book 2002. Tokyo: JIHO, 2002.

6. Director General of the Pharmaceutical and Medical Safety Bureau, Ministry of Health and Welfare of Japan. Revisions to the Scope of Efficacy of Cosmetics. Iyakuhinhatsu Notification No. 1339, 2000.
7. CTFA International Regulatory Resource Manual. The Cosmetic, Toiletry, and Fragrance Association. 5th ed. 2001.
8. Arimoto T. The Current State of Japan's Cosmetic Regulatory System Liberalization. International Regulatory Congress, Florence, Italy, Apr. 22–23, 1998.
9. Final report on how cosmetic regulations should be in the future. Committee on Cosmetic Regulations, Tokyo, Japan, July 23, 1998.
10. Uemura N. Final report on how cosmetic regulations should be in the future (review). Fragrance J 1998; 9:79–85.
11. Stimson N. Cosmeceuticals: Realising the Reality of the 21st Century. SÖFW-J 1994; 120:631–641.
12. Outline of the 2003 Partial Amendment to the Chemical Substances Control Law, June 2003. Ministry of Economy, Trade and Industry, Ministry of Health, Labour and Welfare, Ministry of the Environment, Government of Japan. http://www.meti.go.jp/policy/chemical_management/kasinhou/kaiseihou/eng.pdf.
13. Komiya H. Regulatory frame and problems related to quasidrug. J Jpn Cosmet Sci Soc 1991; 15:37–40.

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EEC Cosmetic Directive and Legislation in Europe

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THE LAWS OF THE MEMBER STATES RELATING TO COSMETIC PRODUCTS AND THE 6TH AMENDMENT

The Council of the European Communities in regard to the Treaty establishing the European Economic Community (today European Union, E.U.), and in particular Article 100 thereof has decided to harmonize the legislations in the E.U. (1,2). The Directive gives a clear definition of the cosmetic products. "Any substance or preparation intended to be placed in contact with the various external parts of the human body or with the teeth and the mucous membranes of the oral cavity, with a view exclusively or mainly to clean them, perfuming them, changing their appearance, and/or correcting body odors, and/or protecting them or keeping them in good condition." The philosophy of the Directive is that all products should have equal and immediate access to the market throughout the E.U., provided that they are proven safe for human use. The Directive has been adapted, completed, and modified several times between 1976 and 2003. The important 6th Amendment has made mandatory that by January 01, 1997, the cosmetic products shall be marketed only if the labeling bear specific information in a legible and visible lettering (Article 6) as follows: the name and address of the registered office of the manufacturer or the responsible person for marketing in the Union, the nominal content at the time of packaging, the date of minimum durability and the conditions of storage, if appropriate, the conditions of use and warnings, the batch number, the function, and the list of ingredients in descending order of weight. The Article 7a requires that for control purposes the following information shall be readily accessible to the competent authorities of the Member State: the qualitative and quantitative composition of the product (perfumes may still be coded, but the composition shall be available for the Health Authorities at the Perfume Manufacturer's) [(GLP) Good Laboratory Procedures: O.J. EU n° L 15, 17/01/87, p.29], the physico-chemical and microbiological specifications of the raw materials and the finished product, the purity and the microbiological control criteria of the cosmetic product, the method of manufacture (GMP, good manufacturing procedures), the person responsible for the manufacturing or first importation into the Union (who shall possess an appropriate level

of qualification), the assessment of the safety (GLP, Council Directive 87/18/EEC of 18 December 1986), the name and address of the responsible person (who must hold a diploma in cosmetics according to Article 1 of Directive 89/48/EEC), undesirable effects if existing, proof of effect by the nature of effect. The competent authority of the Member State shall be notified of the place of manufacture of the cosmetic products or its initial import into the Union. Before the product is placed in the market, the Poison Information Centers shall be informed about the formula, and the European cosmetic and perfumery association (COLIPA) has negotiated that only major deviations from basic formulas shall be indicated, (the basic formulas have been given by COLIPA).

A committee was set up to monitor the adaptation to technical progress of the directives, and for the removal of technical barriers in the trade of cosmetic products. This Committee is located in Brussels at the European Commission, DG (ex III), Industry, 200 rue de la Loi, B-1029 Brussels, Belgium, tel. 32 2 299 1111. Article 12 that deals with products may represent a risk to human health, in spite of complying with the Directive.

The Directive includes eight annexes:

Annex I

Illustrative list by category of cosmetic products.

Annex II

List of substances, which must not form a part of the composition of cosmetic products. More than 451 substances are listed and regularly updated, and new substances are included in the list on a time-to-time basis. The list includes cosmetics in the market containing a newly forbidden substance or an authorized substance revised for a lower concentration, those cosmetics that are regulated in the sense that they are "authorized for a short defined period of time, the manufacturing of the cosmetic in question becoming often forbidden," hormones, anesthetics, chloroform, drug type molecules, crude and refined coal tar, and recently, the products of animal origin carrying the risk such as transmissible spongious encephalopathy (TSE).

Annex III

List of substances which cosmetic products must not contain, except subject to restrictions and conditions. For instance: in hydrogen peroxide containing or releasing cosmetics for hair care, 12% H_2O_2 is authorized, but for oral hygiene 0.1% only is authorized; fluorides for oral hygiene products are limited to 0.15% as F.

Annex IV

List of coloring agents allowed for use in cosmetic products. Four classes are given: (1) all purposes, (2) not for use around the eye, (3) exclusively for products not meant to have contact with mucous membranes, and (4) products that might come briefly in contact with the skin.

Annex V

List of substances excluded from the scope of the Directive.

Annex VI

List of preservatives which may be present in the cosmetic products. For instance: Hexetidine, 0.1% as preservative for the product but may be present at higher concentration (justify), as deodorant in soap or antidandruff shampoos [note of the author: deodorants and antiperspirants in a nonscientific press have been suspected of some relation with breast cancer. But according to the Belgian Association of Dermato-Cosmetic Sciences (BADECOS), during the last meeting in spring 2004, it has to be considered as a nonscientifically justified statement].

Annex VII

List of UV filterswhich cosmetic products may contain.

Annex VIII

A pictogram calling the attention of the customer to the information for use.

In summary, the Directive covers every cosmetic (see definition), imported or manufactured within the E.U. Cosmetics not allowed for children for safety reasons must carry the warning "not for children" or "not below some year of age". Samples and testers are handled under the same Directive. National language for the labels is often required, ingredients may be given in International Nomenclature for Cosmetic Ingredients(INCI). Manufacturing date is still not required, but batch number can be decoded; expiry date is required for less than 30 months shelf life. In case of damage, and in order to deal with emergency situations, a channel of information is built between the member states, through the "Poison Information Centers". Some member states are disseminating adverse effects encountered by Cosmetics via a "Cosmeto-vigilance" system. Such a system is currently being developed after a pilot phase in several member states (France, Germany, and Austria for the E.U. and in Norway). It is evident that the system will be implemented rather rapidly (2–5 years) in the 25 member states. Cosmetics are controlled regularly on a random base by the competent authorities. The checking could be done at the manufacturing site in the E.U., at the distribution centers, or at the selling points.

7th Amendment of the European Cosmetic Directive

Summary

- Important dates
  - 27th February 2003: publication in the Official Journal of E.U.
  - 11th March 2003: entry into force.
  - 11th September 2004: must be transposed in member states' regulations.
  - 11th September 2004: contents of Annex IX (scientifically accepted alternative methods for specific products/chemicals) must be established.

  - 11th March 2005: All cosmetic products placed on the market or imported in the E.U. must comply with (→ date of min. durability, ingredients listed, . . .).
  - 11th March 2009: maximum[a] deadline for most alternative methods validation and animal tests for those parameters.
  - 11th March 2013: maximum[a] deadline for alternative methods validation for repeated-dose toxicity, reproductive toxicity, and toxico-kinetics and animal tests for those parameters.

- Animal testing (10)

  - Marketing a cosmetic product is banned if the finished product, one of its prototypes, ingredients, or combination of ingredients have been animal tested after validation of an alternative method (OECD level).
  - Testing a finished cosmetic product is banned.
  - Testing ban of ingredients or combinations at the latest, when a test is validated, is in Annex IX or at the deadlines for alternative methods (11.03.2009 or 11.03.2013).
  - A derogation for animal testing may be granted (SCCNFP consultation), if the ingredient is in wide use, cannot be replaced, and the test is needed.

- Period after opening (PAO)

If minimum durability of more than 30 months: indicate a symbol (Annex VIIIa) + duration after opening (in months or years).

- CMR (Carcinogenic, Mutagenic, Toxic for Reproduction)

  - CMR 1, 2, 3 is prohibited.
  - CMR 3 may be allowed after evaluation and approval of SCCNFP, if it can be demonstrated that their levels do not cause threat to consumer's health.

- List of ingredients to be labeled

  - Should be preceded by "Ingredients"
  - Items that are not regarded as ingredients: impurities, technical material, solvents, or carriers for perfumes.
  - INCI list as before
  - +26 fragrance allergens (list in Annex III, part I) if at a concentration >0.01% (rinse-off) or 0.001% (leave-on)

- Safety test

  - They must take into account, the specific exposure characteristics of the intended use area.
  - Specific assessment is required for products intended for children under 3. Specific assessment is required for products intended for external intimate hygiene.

[a] Deadlines may be shortened tests (Commission, SCCNFP, ECVAM consultation); new deadlines must then be made publicly available no later than 11 September 2004. They will also be reviewed after each yearly progress report.

- Product information easily accessible to the public
  - Quantitative data on "Dangerous Substances" of Directive 67/548/EEC Qualitative data on product composition
  - Adverse effects among consumers
- New information to be included in the cosmetic dossier
  - Animal tests data (number and type) performed on the finished product or one of its ingredients for the purpose of the development of the safety assessment of the product or its ingredients (including tests to meet regulatory or legislative requirements, even if tests are run outside EEC).

Some other critical *modifications* of the basic Directive 76/768/EC are listed below.

1. The Commission Directive 2003/83/EC of 24 September 2003 (9) has adapted the technical progress of the basic Directive in Annexes II (Substances forbidden), III (Substances authorized under defined limits and uses), and VI (List of preservatives authorized).
2. The Commission Directive 2003/16/EC of 19 February 2003 (11) following the consultation with the Scientific committee on cosmetic products and nonfood products intended for consumers (SCCNFP), which is the Advisory Body of the Commission, has modified the use of Musk xylene and Musk ketone.
3. Commission Directive 2003/1/EC (12), deals with the risks of TSE.
4. Twenty six Commission Directive 2002/34EC of 15 April 2002 (13), based on recommendations of the SCCNFP, has included 28 substances forbidden in Annex I, and modified Annex III in indicating use allowed until 30/09/2004 for 60 substances.

In 1998 and 2003, the European Parliament and The Council decided about the *Biocides*. The Directive 98/8/EC (15) and the Commission Regulation 2032/2003 EC (16) have excluded (Art 1,1,p) the cosmetics (Directive 76/768). The documents require licensing for biocides. However, the understanding of the Biocides Directive varies according to the member states. Products may fall under the Biocides Directive if they fall under Annex V: human hygiene biocidal products and veterinary hygiene biocidal products.

The difference between a cosmetic product containing a preservative, and a product containing the same preservative at a level (biocidal efficacy) above the authorized level in the Directive 76/768, makes the difference (as well as the intention or primary purpose of the project, provided that no therapeutic indication is mentioned. For instance, cleaning or disinfecting *intact* skin is acceptable but not damaged skin. The Directive on Biocides (98/8/EC) includes annexes. Annex I lists the active substances with requirements agreed at Community level. Annex II lists the common core data set for active substances. Annex III lists additional data set. Annex IV lists data set for specific agents such as viruses, fungi, and microorganisms. Annex V lists biocidal product types.

The Commission Regulation 2032/2003 EC lays down detailed rules for the implementation of the second phase of the program of work for the systematic examination of all active substances already on the market. Annex I lists existing substances. Annex II lists existing active substances and product types included in the review program. Annex III lists existing active substances already identified with

no notification to the member states. Annex IV lists *the requirements for the complete dossier and the summary dossier.*

The methodology for *adding new cosmetic ingredients* to the existing positive list or modifying the restrictions is as follows: prepare a full dossier from the analytical Cosmetic GMP, good manufacturing procedures (14), safety and efficacy points of view and submit it to the COLIPA. After evaluation, the dossier is sent by the COLIPA ad hoc working party, to the European Commission. At the Commission level, the dossier is discussed in the scientific advisory body, the Committee for cosmetics, and will be published as an amendment in the O.J. E.U.

The application may as well be submitted directly by the applicant to the DG (exIII) Industry, Cosmetic Division in Brussels.

In November 1995, COLIPA (4,5) published two important documents related to the safety information and provision for cosmetics and raw materials, in order to prepare the dossiers required by the 6th amendment. For the provision of safety information for finished products, a process is recommended, to be followed by the safety assessor in arriving at the safety assessment. First, a toxicological profile of ingredients must be identified and second, for the finished products. For finished products the assessment may take in consideration the formulas that can be compared by composition, and a general statement including several products is often acceptable.

The information for raw materials must often be required at the supplier level. One expects the supplier to consolidate: identity, safety data sheet, toxicology, and human experience (if available). The past chairman of the Scientific Committee on Cosmetology Products and non-Food Products intended for Customers (17) of the Commission of the E.U., Loprieno published in 1992, the views of the Committee (5). Categories of cosmetic products and exposure levels in use, physico-chemical specifications, safety studies in vitro and in vivo and observation on human subjects are examined in his article, together with toxico-kinetics, and long-term studies.

The microbiological information on raw materials and finished product is an important part of the dossier (6). The microbiological quality is identified by validated methods for quantitative limits to be 10^3 g or ml and 10^2 g or ml for eye products, baby care, and intimate hygiene, and for qualitative limits, the absence of *Pseudomonas aeruginosa*, other Gram negative organisms (enterobacteria), *Staphylococcus aureus*, *Candida albicans*.

IMPLEMENTATION OF THE EUROPEAN DIRECTIVE ON COSMETIC PRODUCTS IN THE DIFFERENT MEMBER STATES OF THE EUROPEAN UNION

The Directive must "normally" be implemented in the 15 member states, within 18 months after the publication in 1993 (6th amendment). This is the case, despite the nationalistic protection and political reasons. The Council of Europe called the attention of the "slow" member states and even called the Justice Court of Luxembourg for nonimplementation.

A very brief summary of the situation in the 15 member states and Norway is given below. The information hereafter has been modified recently, will be continuously modified, but remains a good way to locate centers and authorities. For the enlarged Union, reference 19 gives access to the health authorities through the sites of Bulgaria, Czech Republic, Estonia, Hungary, Latvia, Lithuania, Poland, Romania, Slovakia, Slovenia, and Cyprus plus Malta.

Note: the Health Authorities in the E.U. have sites and e-mails, and precise and updated information is available on the site (20).

Austria

Competent authority: Bundesministerium fur Gesundheit und Konsumentenschutz, Abteilung II–C–16, Radetzkystrasse 2, A-1030 Wien, tel.: 43 1 71172–4668.

Belgium

Poison Information Center: Centre Antipoison, rue Joseph Stallaert 1, B-1050 Bruxelles Belgium, tel: 32 2 345 4545. Competent authority: Ministère des Affaires Sociales, de la Santé Publique et de l'Environnement, Inspection Générale des Produits Cosmétiques, Cité Administrative de l'Etat, Quartier Vésale, B-1010 Bruxelles, Belgium, tél.: 32 2 210 4869.

Denmark

Poison information to: Sundhedsstyrelsen, Fredreikssundsvej 378, DK-2700 Bronshoj, Denmark, tel.: 54 44 889111, Competent Authority: Danish Environmental Protection Agency, Strangade 29, DK-1401 Köbenhaven, Denmark.

Finland

Poisoning Information Center: Central University Hospital in Helsinki, competent authority: Finnish Consumer Administration Apnasgatan 4, PB 5 FIN-00531 Helsinki (National Agency: 358 9 473341).

France

The arrêté du 27 janvier 1978 (Journal Officiel- N.C. du 7 février 1978) gives the list of the 16 Poison Information Centers. In Paris, the Centre Antipoisons de Paris is located in the Hospital Fernand Vidal, 200 rue du Faubourg-Saint-Denis, F-75010 Paris, France. Competent authority: Directions Departementales des Affaires Sanitaires et Sociales (DDASS), Ministère de l'Emploi et de la Solidarité, Administration Sanitaire et Sociale, Service de l'Information et de la Communication (SICOM), Bureau de la Communication Interne, 1, Place Fontenoy, F-75007 Paris, France tél.: 33 1 40 567009.

Germany

The Poison Information Center: IKW, Karistrasse 21, Frankfurt am Main, D-60329, tel.: 49 692556 1323. Competent Authority: BgW, Tielallee 8892, Berlin, D-14195.

Greece

Poison Information Center address, via the competent authority: National Drug Organization (EOF), 284 Mesogion avenue, GR-155 62 Holargos, Greece, tel.: 301 654 1964.

Ireland

Competent Authority: Irish Department of Health, The Earlsfort Center, Earlsfort Terrace, IRL-Dublin 2, Ireland, tel.: 353 1676 8490, Poison Information Centers not yet identified.

Italy

Poison Information Center location to obtain from the competent authority: Ministero di Sanita, Istituto Superiore di Sanita, Via Regina Helena, 299, I-00161 Roma, Italia, tel.: 39 6 493 87114.

Luxembourg

Poison Information Center via competent authority: Ministère de la Santé, rue Auguste Lumière 1, L-2546 Luxembourg, tel.: 352 491191.

Netherlands

Poison Information Center via competent authority: Inspectie Gezondheidbescherming, Keuringdienst van Waren, Postbus 777, NL-7500 AT Entschede, tel.: 31 53471111.

Portugal

Poison Information Center via competent authority: Instituto da Farmacia e do Medicamento, Parque de Saude de Lisboa, av. do Brazil 53, P-1700 Lisboa, Portugal, tel.: 351 1 790 8500.

Spain

Competent authority: Ministerio de Sanidad y Consumo, Direccion General de Farmacia y Productos Sanitarios, Paseo del Prado 18–20, E-28014 Madrid, Espana, tel.: 34 1 596 4070. (fax preferred for language problems: 34 1 596 1547)

Sweden

Fees 200€ per product, maximum 415000 € per Company. Poison Information Center: Giftinformationcentralen, Karolinska Sjukuset, Box 60500, S-10401 Stockholm 80, Sweden, competent authority Makamedelsverket (Medical Products Agency) Box 26, Husargatan 8, S-75103, Uppsala, tel.: 46 18174687.

United Kingdom

Poison Information Center via competent authorities: Consumer Safety Unit, Department of Trade and Industry, 1, Victoria street London SW1H OET, fax preferred: 44 171 215 0357.

Norway (Not a Member State)

The Royal Norwegian Ministry of Health, P.O. Box 8011 Dep, N 0030 Oslo, Norway.

Other European Countries

The Directive 78–768 and the 6th amendment are applied, sometimes more restrictive in the forbidden molecules. The applicant for importation or local manufacturing is "recommended" to follow the Directive. A hearing with the competent authority, the Ministry of Health is hardly recommended (10).

REFERENCES

1. Council Directive of 27 July 1976 on the Approximation of the Laws of the Member States Relating to Cosmetic Products.(Dir.76/768/EEC) O.J. EEC 27.09.76, n° L 262.
2. 6th Amendment to the Directive 76/768, 93/35, 14.06.93. O.J. EEC 23.06.93, n° L 151.
3. COLIPA: Cosmetic Product Information Requirement in the European Union. Information Required for the Safety Evaluation of Cosmetic Raw Materials 95–242-mc. November 1995.
4. COLIPA: Cosmetic product information requirement in the European Union. The Provision of Safety Information for a Cosmetic Product 95–200-mc. November 1995.
5. Loprieno N. Guidelines for safety evaluation of cosmetic ingredients in the EC countries. Food Chem Toxic 1992; 30(9):809–815.
6. Devleeschouwer MJ. Incidence for the cosmetic industry of the 6th amendment of the European Directive concerning the Cosmetics. Presentation. Colgate 04-OCT-1994. Laboratory of Microbiology and Hygiene, Free University of Brussels.
7. Poppe K, Van Essche R, et al. Guide Pratique de la mise en oeuvre de la directive européenne sur les produits cosmétiques, Free University of Brussels, Technopol, 1998.
8. Commission Directive 2003/80/EC Official Journal of the European Union L 224/27 (2003).
9. Commission Directive 2003/83/EC Official Journal of the European Union L 238/23 (2003).
10. Commission Directive 2003/15/EC Official Journal of the European Union L 66/26 (2003).
11. Commission Directive 2003/16/EC Official Journal of the European Union L 46/24 (2003).
12. Commission Directive 2003/16/EC Official Journal of the European Union L 46/24 (2003).
13. Twenty Six Commission Directive 2002/34/EC, Official J E.U. L 102/19 (2002).
14. Council of Europe Guidelines for Good Manufacturing Procedures for Cosmetic Products, Health Protection of the Consumer, ISBN 92–871–2848–0, © Council of Europe.
15. Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998, Official J E.U. L 123/1 (1998).
16. Commission Regulation (EC) 2032/2003 of 4 November 2003, Official J E.U. L 307/1 (2003).
17. http://europa.eu.int/comm/health/ph_risk/committees/sccp/sccp_members_en.htm.
18. http://europa.eu.int/comm/health/ph_enlargement/candidates_en.htm.
19. http://globepharm.org /resource_agency2.html.

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Introduction to the "Proof of Claims"

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With the continuous increase in the variety of cosmetic products proposed to consumers over the last decades, it has become often difficult for them to decide what are the most appropriate products for their needs. Aware of such difficulties, cosmetic manufacturers have understood that the success of a product today is not only a question of performance, but also a question of how it is promoted to the potential buyer. Progressively, product promotion took more importance and the advertising claims became more aggressive and closer to the limit of what could be scientifically demonstrated and consumer perceived.

To monitor the claims made about cosmetic products and protect the consumer against misleading advertisement, several national/federal agencies have issued rules under the form of laws or directives to make sure that proper substantiation of the claims exists. Also, relying on such rules, competitors always remain ready to challenge unfair or doubtful claims. And last, but not least, the consumers themselves have become more critical and, when they feel that their product does not provide the properties that it claims, do not hesitate anymore to stop buying this product and often also the other products of the same brand. Even more than for the ethical aspect, it has become a real priority for the cosmetic chemist to be able to demonstrate and substantiate the properties, which are claimed for his product.

The objectives of this introduction to the proof of claims are as follows:

- to briefly describe the regional requirements related to the proof of claims;
- to explain the different existing categories of claims;
- to review the types of support which might be made.

REGIONAL REQUIREMENTS

Although all over the world the same motivation exists, which is to protect the consumer against misleading claims, the current situation is quite different among Europe, the United States, and Japan regarding claim substantiation requirements

and the limit of definition of a cosmetic product. This latter point has been discussed in previous sections (Part 6, chaps. 64 to 67 of this book). Specific regulations are summarized hereafter.

The United States

The US federal law does not require premarketing proof of claims, but prohibits false advertisement. In the case of a challenge (by a competitor, a consumer association, a government agency), the manufacturer must be prepared to defend the claims made on the product. However, the ‘‘challenger’’ has to first provide arguments questioning the validity of the claim. It is quite frequent in the United States for claims to be challenged and most companies preferably develop scientifically valid claim support strategies and dossiers before marketing their new product.

Several federal authorities controlling cosmetic claims exist. The U.S. Food and Drug Administration (FDA), through the Federal Food, Drug and Cosmetic Act (FD&C Act) and the Fair Packaging and Labeling Act (FPL Act), has the main jurisdiction and responsibility on claims made on the labeling of the products. The Federal Trade Commission (FTC) monitors product advertising (e.g., television, radio, and magazine). When a claim is related to both advertising and labeling, the two agencies usually collaborate with each other.

However, even if both agencies condemn in their respective acts consumer misleading, none of them clearly defines the legal standard for illegality. The manufacturer will thus rely upon a ‘‘reasonable’’ basis to support its claims, and most challenges will be treated on a case-by-case basis.

Another significant control of advertising is performed by the National Advertising Division (NAD) of the Council of Better Business Bureaus, which is a self-regulatory, nongovernmental body evaluating the truth and accuracy of challenged advertising. NAD is usually the first body receiving the complaints about claims from competing companies or consumer associations. Through several control and communication steps between the two challengers, NAD may decide to involve the appropriate government Agency (FDA or FTC).

Several examples of challenged claims have been summarized by Davis and McNamara (1) and Friedel (2) that can help in understanding the U.S. situation.

The European Union

In the European Union, cosmetic claims substantiation is regulated by the 6th Amendment to the Cosmetic Directive, effective since January 1, 1997. In that Amendment, it is stated that cosmetics and toiletries manufacturers making claims for their products have to demonstrate the proof of their claims. The dossier containing these proofs has to be readily available if requested by the competent authority. The dossier may be written in English or in the language of the country where it is presented. More details on the Cosmetic Directive can be obtained in chapter 67.

As in the United States, no clear definition about the meaning of ‘‘proof of claims’’ has been given, so manufacturers have to define themselves what they consider to be a ‘‘reasonable’’ and ‘‘acceptable’’ proof for their claim. Such a consideration will often depend on the type and originality of the claim, the type of product and the market in which it competes, the consequences and benefits that the consumer can expect from the claimed effect, and the image, scientific honesty and competency of the manufacturer.

Table 1 Categories of Claims

Categories of claims	Examples
Claims related to physical and chemical properties[a]	Contain x% of an active
	Neutral pH
	20% more in the bottle
	More concentrated
Claims related to the test procedure or to an endorsement[b]	Dermatologist and dermatologically tested
	Tested under the good clinical practices principles
	Tested and approved by an institute
Safety related claims[c]	Mild, gentle for your skin
	For sensitive skin
	Skin repair properties
	Hypoallergenic
Objectivable efficacy claims[d]	Moisturizing and hydrating
	Improves elasticity, firmness of skin
	Skin whitening effect
	Sunscreen effect
	Deodorant
Subjective claims[e]	Your skin will feel softer and more hydrated
	With a pleasant feel, texture, etc.
	Smells fresher, etc.
Cultural claims[f]	Contains 100% natural ingredients
	Nontested on animals (comments are given in the text)
Juxtaposition claims[g]	Contains an ingredient known for such a property
Borderline cosmetic claims[h]	Antibacterial soap and cosmeceuticals

[a]Claims related to physical and chemical properties of the product can be substantiated by measuring directly the claimed characteristic in the product by an analytical method. The measurement methodology has however to be well established and validated.

[b]Claims related to the test procedure or to an endorsement by an outside authority simply describing the way, the person/title, or the place where the product has been tested. They are usually perceived by the consumer as a proof of well-tested, quality product. It is essential for such claims to demonstrate the property that the consumer can expect from the product even if it is not directly advertised. For example, the claim "dermatologist tested" means that the product has been tested by a dermatologist, but also implies that the results of the test were good and that the product is mild for the skin or has a skin beneficial property shown by the dermatologist.

[c]Safety-related claims make the consumer confident about the innocuousness of the product and the best respect of their body. These claims usually require clinical tests on human volunteers according to protocols published in the scientific literature and performed under high quality standards. In some cases, in vitro tests can also be accepted if it can be shown that they are able to prove the claimed property for the type of product in test.

[d]These are probably the most frequent, and those inducing the highest expectation from the consumers. This is why they require solid efficacy data dossiers. Many biometric methodologies currently allow to get a direct measurement of the skin properties (3) which are expected to be respected or modified by the cosmetic product. In vivo tests with human volunteers are often recommended or even the only possibility offered to the cosmetic chemist, but other types of tests can also be used in some cases, such as cell culture tests (4–6a).

[e]Subjective claims are related to a property or function of the product which is perceived by the consumer. The property has not necessarily to be objectivated by direct measurement. Only tests on human volunteers can be performed such as sensory tests (6b) or well-designed consumer tests.

(*Continued*)

Table 1 Categories of Claims (*Continued*)

[f]Cultural claims are usually related to the composition of the product and take advantage of the current trends. Their value for the consumer is often dependent on the education, country, or environment. They link the composition of the product to ecological, ethical, or moral considerations (e.g., naturality of ingredients, absence of tests on animals, etc.). It should be noticed that in Europe, with the implementation of the 7th Amendment of the cosmetic directive in the member states, claims related to the absence of animal testing should become better regulated. Indeed, the European Union Commission is, in co-operation with the COLIPA (The European Cosmetic, Toiletry and Perfumery Association), developing guidelines for the interpretation of the text of Directive 2003/15/EC (7) that states that "... the manufacturer ... may take advantage ... of the fact that no animal tests have been carried out only if the manufacturer and his suppliers have not carried out or commissioned any animal tests on the finished product, or its prototype, or any of the ingredients contained in it... or used any ingredients that have been tested on animals by others for the purpose of developing new cosmetic products." Such guidelines should be legally binding. They are not available at the time of editing this chapter.

[g]Juxtaposition claims refer to the presence of an ingredient in a product and to the known property of the ingredient, without claiming that the complete product has the property. This type of claim can be supported by proving the presence of the ingredient in the product (analytical methods) and relating the claimed property to that ingredient through literature data or any type of appropriate test on the pure ingredient.

[h]Borderline claims are those that place a product at the boundary between two different regulations (e.g., cosmetics or drug, cosmetics or biocide). As the Cosmetic Directive is an exclusive directive that does not allow a cosmetic to belong at the same time to another product category, for borderline claims it has to be clearly decided if the product is a cosmetic or not. Such an ambiguous situation may be encountered for cosmetic-like products with health-related claims, with claims implying skin functional changes, or with antibacterial or insect-repellent claims. In these two latter cases, the European Union Commission is developing guidelines to clarify the limits between cosmetic and biocidal products. At the time of printing those guidelines are not available. They should be accessible through the Commission website on biocides (8). However, from a legal point of view, a product cannot simultaneously be a cosmetic and a biocidal. Decision should be taken on a case-by-case situation depending on the primary claim of the product, its presentation, positioning, and ingredients. A manual of decision has been prepared for helping in positioning borderline products (9). An example is given with antibacterial hand soap that may be considered as cosmetics if they are sold for the general consumer (first intent being to clean the hands) or as biocides if sold for butchers, nurses, or surgeons (first intent being then to disinfect the hands). This question of borderline claims for cosmetics is still largely debated.

Although the 6th Amendment to the Directive aimed at uniforming the differences between countries, big differences still exist regarding how to monitor the proof of claims dossiers, which is basically subject to the interpretation of the Directive within the individual state laws. In most countries such monitoring will essentially be postmarketing for a challenge, but in some countries a premarketing review of the claims can be requested by a National Review Board (e.g., Greece). Some types of claims are also not uniformly accepted for cosmetic products by all the European Union members; this is, for example, the case of claims that can be overlooked as "medically oriented" such as "dermatologically tested" or "hypoallergenic" (e.g., not allowed in Denmark for cosmetics).

The decision for acceptable claims and reasonable supporting dossier should thus always be reviewed in line with the individual national laws, if any, of the country where marketing is intended.

Japan

In Japan, the situation is different in the sense that the claims are reviewed prior to the marketing of the cosmetic product. The Ministry of Health and Welfare (MHW) has to provide a license to the product to allow its marketing. The limit of definition

of purely cosmetic products is also different in Japan than in the European Union and the United States with the existence of "quasi-drugs" classified between cosmetics and drugs (chap. 66).

CATEGORIES OF CLAIMS

In whatever way they are used (e.g., label, television, or magazine advertising), claims related to cosmetic products can be subdivided into several categories. Table 1 summarizes these categories and provides some examples for each of them. As explained earlier, all claims are not applicable everywhere in the world for cosmetic products and can fall under other regulations in some places.

Several of these categories can be further subdivided in absolute or comparative claims. The four following subcategories can be described:

a. Noncomparative claims. They simply refer to a property of the product without any direct comparison to another product. However, it is obvious that even if not classified as such, all claims contain a comparative connotation. For example, claiming that a product is mild means that this is not the case for all other products. Similarly, claiming that a product is hydrating for the skin refers to the hypothesis that some other products are not.
   Examples include claims that a product is mild, hydrating, protects the skin, and softens the skin.
b. Claims comparing a new product to the one it replaces in the market place. In the proof of claim dossier, a direct comparison between the two products will be required. The kind of test depends on the claim.
   Examples include x% more efficacy and milder than ever or even milder than before.
c. Purely comparative claims comparing the new product to competitive ones for the claimed property. This kind of claim is likely to be challenged by competitors and requires a solid supporting dossier where direct comparison between the products is made. The test methodology has to be well justified and validated for the objective. Such comparative claims are quite usual in the United States; in Europe they are allowed under restricted conditions and rarely used.
   Examples include milder than product x and y or more hydrating than product z.
d. Absolute claims. The comparison is not limited to a few mentioned products as previously discussed but the product claims to be the best in the market for a given property or to completely fulfill a specific function. Such claims require a very solid dossier and can be invalidated if even one competitive product can be shown superior on this property.
   Examples include the mildest, nothing more hydrating, total protection, or complete diet for the skin.

TYPE OF SUPPORT

Whenever the nature of the effect or the product justifies it, the claims on cosmetic products must be shown. However, the type of support has never been clearly and

officially defined, so that any kind of support could be acceptable if it can be scientifically and reasonably justified. Different ways to support cosmetic claims (10) are reviewed hereafter; some of them have already been briefly discussed earlier in this chapter.

Comparison to a Similar Formula

If the product is derived from another formula by a minor modification, it is not always necessary to repeat the claim-supporting test for the new product. In such a case, it has however to be clearly justified that the change is not to affect the claimed property. Depending on the claims, certain modifications can be considered as minor or not. Similarly, for a line of products with minor differences between individual products, some claims can often be substantiated on only a few products of the line and then extended to the other products.

Literature Search

For some types of claims, literature data can be considered as effective claim support dossier. This is, for example, the case of claims on ingredients entering into the composition of the cosmetic product; often, the proof of the ingredient property can be found into the scientific literature. It should be noticed, however, that peer-reviewed literature usually has more credit than supplier literature for a challenge, although this latter can also be used if supported by well-controlled tests.

In Vitro Tests

In vitro tests never have the same value as in vivo data obtained from clinical tests run on human volunteers. This is why they are mostly used in combination with other types of data. However, in some cases (depending on the claim or on the availability of alternative tests) in vitro tests can be used on their own to support claims, provided that the test is proven to be scientifically valid for the intended objective. From the most promising and usual in vitro tests, 3D-cell culture methodologies probably receive the most credit for investigating many cosmetic product properties from skin mildness to more specific properties like sun protection (4–6a).

For special dermato-cosmetic claims, such as fat reduction or anticellulitis effects, in vitro data are mostly presented as direct support. However, in such cases, scientifically valid in vivo testing about the efficacy of these treatments is not always available, and often cumbersome, difficult, and of long duration. Extrapolation of in vitro data or even of supplier literature on the efficacy of the actives is often used, without really proving the claims.

In Vivo Tests on Human Volunteers: Clinical Studies

The most direct proof of a claim is to show the product effect directly on the human volunteers using the product. Many test protocols may be used depending on the objective. Most protocols have been published in scientific literature and are well-established tests. They go from very exaggerated application conditions (11–14) up to a normal usage of the product by the subjects in the laboratory or at home (15,16). It is obvious that the more realistic the application condition, the more powerful the demonstration of the effect.

Besides the application procedure, these protocols can also differ by the following assessment technique of the claimed effect.

a. Assessment by an expert evaluator
This type of evaluation concerns a cosmetic effect, which can be determined by visual, tactile, or olfactive assessment. Examples of scoring scales for the assessment of dry skin have been given by Serup (17). The evaluator is trained to make such an assessment, reliable and fully independent of the product manufacturer. In some cases the evaluator will be a dermatologist, an ophthalmologist, or a dentist, but this is not mandatory provided that the evaluator can justify his/her qualification. When the test protocol is appropriate, expert evaluations are frequently combined with other assessment methods. Examples of claims easily supported by expert evaluation are: skin whitening, antiwrinkle, hair shine, and deodorancy. Safety claims are also appropriate for such an assessment to check the absence of erythema or dryness after product application.

b. Measurement by biometric methodologies
A huge amount of biometric methods have developed over the two last decades, which now allow objective and quantitative measurement of most skin properties such as elasticity, firmness, color, barrier properties, moisture content, relief, or blood flow (3,18,19). This kind of evaluation is highly valuable, thanks to its objectivity and sensitivity, and can identify small differences between products to support comparative claims. Those biometric measurements must however take into account several key rules: (i) many external factors can affect the measurements that have to be made according to specific guidelines (20–23); (ii) the interpretation of the data has to be done by an expert in the field, able to relate the collected data to physiological parameters; (iii) the instruments must be highly reliable.
Under such conditions, instrumental measurements are highly valuable and have revolutionized the way of supporting cosmetic claims. However, instrumental methodologies have nowadays become so sensitive that questions can sometimes be raised about the relevancy of such small differences between products for consumers who are not always able to detect them.

c. Self-assessment of the effect by the volunteers
When used in clinical tests, this type of evaluation is usually combined with other assessments. It is, however, not applicable to all test procedures and requires that the product has been placed in contact with a sufficiently large area of the body to provide an effect that can be self-perceived. When confirming objective measurements of the property, this self-evaluation is extremely powerful because it expresses that the measured effect is really meaningful to the consumer.

Sensory Tests with Human Volunteers

The self-perception of the product effect by the volunteers can be done independently of a clinical test; in such a case a specific test procedure has to be designed. Sensory tests are limited to the so-called "sensory claims" which clearly state that the product modifies the perception of a property of the skin or hair (e.g., "you can feel your skin softer or more hydrated"). When the sensory effect of the product

is obvious and can be easily perceived by a large majority of people, the test can be performed on a panel of regular (so-called "naïve") volunteers, without any specific selection criteria regarding their capability to feel differences. However, the self-perception of stimuli or of a skin feel is very variable between subjects; often, differences between products are not so obvious for a "naïve" user; it is then necessary to run the sensory test either on a very large panel (sometimes several hundreds of volunteers) or to use a panel of volunteers specifically selected and trained to perceive small differences for the kind of product in test.

For more details on sensory tests, the reader is referred to Oddo et al. (6b).

Consumer Tests

These tests are performed at the end of the development phase of the product and consist in providing consumers with the product to use at home for a certain period of time, according to their usual habits and practice. Expected sensory/efficacy properties of the product can be checked from these tests by a questionnaire filled out by the users.

The information collected from the consumer tests are very helpful in supporting cosmetic claims, as it will reassure the manufacturer that its product is not misleading the user about the claimed property. Consumer tests, like sensory tests, are mostly used to support claims such as those related to odor perception, skin sensation, tactile or visible properties of skin or hairs, and taste of oral care products. However, because of the subjectivity of the data, such tests, to be valuable, must be performed very carefully. The questionnaire has to be prepared by a specialist on this kind of test and cannot be oriented towards the answers of the users. For more details, the reader is referred to specific guidelines for consumer testing (24).

Multiapproach for Claim Support

As previously shown, all the approaches described for supporting the claims on cosmetic products have their own advantages as well as some weaknesses. To combine the strengths of several approaches, a multi-test or multi-evaluation approach, combining expert assessments, instrumental measurements, and subjective data from the user, is often considered as an ideal support for a claim. However, depending on the type of claim, for cost and time reasons, it is not always necessary to go so far in the dossier if one test obviously and undoubtedly provides the proof of the claimed effect.

CONCLUSION

Claims on cosmetic products are extremely varied and often depend on the product, the market, and the current trends. However, several claims have been used on different product types for many years. Testing strategy for some is described in the following chapters. They cover some safety-related claims (e.g., mildness, sensitive skin designed products, and noncomedogenicity claims), some efficacy claims (e.g., skin hydration effect, smoothing, and antiwrinkles effect) and sensory claims. The proposed tests especially aim at guiding the skin scientists to design their own protocols based on reasonable scientific considerations and do not intend to impose strict testing procedures.

REFERENCES

1. Davis JB, McNamara SH. Regulatory aspects of cosmetic claims substantiation. In: Aust LB, ed. Cosmetic Science and Technology Series: Cosmetic Claims Substantiation. Vol. 18. New York: Marcel Dekker, Inc. Publ., 1998:1–20.
2. Friedel SL. Technical support for advertising claims. J Toxicol Cut Ocul Toxicol 1992; 11:199–204.
3. Willoughby M, Maibach HI. Cutaneous biometrics and claims support. In: Aust LB, ed. Cosmetic Science and Technology Series: Cosmetic Claims Substantiation. Vol. 18. New York: Marcel Dekker, Inc. Publ., 1998:69–86.
4. Jackson EM. Supporting advertising claims. Reviewing a three-dimensional in vitro human cell test. Cosmet Toilet 1993; 108:41–42.
5. Majmudar G, Smith M. In vitro screening techniques in dermatology. A review of the tests, models and markers. Cosmet Toilet 1998; 113:69–76.

6a. Roguet R. Intérêt des modèles de peaux reconstruites en cosmétologie. Cosmétologie 1997; 13:38–43

6b. Oddo LP, Shannon K. Sensory testing. In: Barel AO, Paye M, Maibach HI eds. Handbook of Cosmetic Science and Technology 1st ed. New York: Marcel Dekker, 2001:845–857.

7. Directive 2003/15/EC of the European Parliament and of the Council of 27 Feb 2003 amending Council Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products. Off J EU 2003; L66/26.
8. Biocide Products Directive (98/8/EC). http://europa.eu.int/comm/environment/biocides/index.htm.
9. Manual of decisions for implementation of Directive 98/8/EC concerning the placing on the market of biocidal products (revised on 13.11.2003). http://europa.eu.int/comm/environment/biocides/manualofdecisions030618.pdf.
10. DGCCRF-D'UMA Commission 30. Evaluation de l'efficacité des produits cosmétiques. Les recommandations de la DGCCRF. Cosmétologie 1997; 15:44–46.
11. Frosch PJ, Kligman AM. The soap chamber test: a new method for assessing the irritancy potential of soaps. J Am Acad Dermatol 1979; 1:35–41.
12. Sharko PT, Murahata RI, Leyden JJ, Grove GL. Arm wash with instrumental evaluation–a sensitive technique for differentiating the irritation potential of personal washing products. J Derm Clin Eval Soc 1991; 2:19–27.
13. Clarys P, Manou I, Barel AO. Influence of temperature on irritation of the hand/forearm immersion test. Contact Dermatitis 1997; 36:240–243.
14. Charbonnier V, Morrison BM Jr, Paye M, Maibach HI. Open application assay in investigation of subclinical irritant dermatitis induced by sodium lauryl sulfate (SLS) in man: advantage of squamometry. Skin Res Technol 1998; 4:244–250.
15. Jackson EM, Robillard NF. The controlled use test in a cosmetic product safety substantiation program. J Toxicol Cutan Ocul Toxicol 1982; 1:117–132.
16. Paye M, Gomes G, Zerweg Ch, Piérard GE, Grove GG. A hand immersion test under laboratory-controlled usage conditions: the need for sensitive and controlled assessment methods. Contact Dermatitis 1999; 40:133–138.
17. Serup J. EEMCO guidance for the assessment of dry skin (xerosis) and ichtyosis: clinical scoring systems. Skin Res Technol 1995; 1:109–114.
18. Wiechers JW, Barlow T. Skin bioengineering techniques for substantiating cosmetics claims. Cosmet Toilet 1998; 113:81–83.
19. Kajs TM, Gartstein V. Review of the instrumental assessment of skin: effects of cleansing products. J Soc Cosmet Chem 1991; 42:249–271.
20. Rogiers V, Derde MP, Verleye G, Roseeuw D. Standardized conditions needed for skin surface hydration measurements. Cosmet Toilet 1990; 105:73–82.
21. Berardesca E. EEMCO guidance for the assessment of stratum corneum hydration: electrical methods. Skin Res Technol 1997; 3:126–132.

22. Piérard GE. EEMCO guidance for the assessment of skin colour. J Am Acad Derm Venereol 1998; 10:1–11.
23. Morrison BM Jr. ServoMed evaporimeter: precautions when evaluating the effect of skin care products on barrier function. J Soc Cosmet Chem 1992; 43:161–167.
24. Guidelines for The Public Use of Market and Opinion Research, The Advertising Research Foundation, New York, 1981.

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Safety Terminology

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INTRODUCTION

One of the skin's primary physiological functions is to act as the body's first line of defense against exogenous agents. However, the skin should not be viewed as a flawless physicochemical barrier. Many low-molecular weight compounds are capable of penetrating this barrier. When toxic agents (such as irritants or allergens in cosmetic products) permeate it, the resulting adverse effects may cause considerable discomfort to the consumer. Even minor disturbances of the skin surface can produce discomfort, especially in the facial area, which has an extensive network of sensory nerves. Moreover, because most cosmetics are applied to the highly permeable facial skin, the majority of reported cosmetic reactions occur in the face. Therefore, safety with regard to cosmetic products is a vital issue.

This chapter provides a brief summary of the safety terminology pertaining to cosmetic reactions, as well as an overture to the succeeding chapters. The reader is directed toward some in-depth reviews of each topic in the bibliography.

CONTACT DERMATITIS

This is a nonspecific term used to describe any inflammatory skin disease resulting from contact with an irritant or allergenic substance. Whatever the causative agent, the clinical features are similar: itching, redness, and skin lesions. It is also often used (inaccurately) as a synonym for allergic contact dermatitis (ACD).

IRRITANT CONTACT DERMATITIS (IRRITATION)

"Irritant contact dermatitis (ICD)" is a term given to a complex group of localized inflammatory reactions that follow nonimmunological damage to the skin. The inflammation may be the result of an acute toxic (usually chemical) insult to the skin, or of repeated and cumulative damage from weaker irritants (chemical or physical). There is no definite laboratory test for ICD—diagnosis is by clinical morphology, and appropriate negative patch-test results.

Irritant

An irritant is any agent, physical or chemical, that is capable of producing cell damage, if applied for sufficient time and in sufficient concentrations. Irritants can produce a reaction in anyone, although individual susceptibility varies. The clinical reaction produced by irritants varies considerably.

Acute Irritant Contact Dermatitis

Acute ICD is the result of a single overwhelming exposure to a strong irritant or a series of brief physical or chemical contacts, leading to acute inflammation of the skin. The resultant clinical appearance is that of erythema, edema, pain, and sometimes vesiculation at the site of contact, usually associated with burning or stinging sensations.

Irritant Reaction

An irritant reaction is a transient noneczematous dermatitis characterized by erythema, chapping, or dryness, and resulting from exposure to less potent irritants. Repeated irritant reactions may lead to contact dermatitis.

Cumulative Irritant Contact Dermatitis

Cumulative irritant contact dermatitis or chronic ICD develops as a result of a series of repeated and damaging insults to the skin. The insults may be chemical or physical.

Delayed Acute Irritant Contact Dermatitis

Some chemicals produce acute irritation in a delayed manner so that the signs and symptoms of acute irritant dermatitis appear 12 to 24 hours or more after the original insult.

Subjective (Sensory) Irritation

This refers to sensations of burning, stinging, and itching that are experienced by certain susceptible individuals after contact with certain chemicals, although no visible inflammatory pathology can be seen. Examples of sensory irritants in cosmetics are lactic acid, salicylic acid, propylene glycol, and some benzoyl peroxide preparations.

ALLERGIC CONTACT DERMATITIS

ACD occurs when a substance comes into contact with skin that has undergone an acquired specific alteration in its reactivity as a result of prior exposure of the skin to the substance eliciting the dermatitis. The skin response of ACD is delayed, immunologically mediated (Type IV), and consists of varying degrees of erythema, edema, papules, and papulovesicles. Patch testing is the gold standard; it is imperative for proving ACD, determining the actual allergen, predictive testing, i.e., determining "safe" materials for the consumer, and exclusion of other diagnoses.

Allergen

Allergens are low-molecular-weight ($<$500–1000 Da) molecules capable of penetrating the skin and binding to skin proteins to form a number of different antigens that

may stimulate an allergic response in an individual. Common allergens in cosmetic products are fragrances (e.g., cinnamic aldehyde) and preservatives (e.g., formaldehyde and formaldehyde donors).

PHOTOIRRITANT CONTACT DERMATITIS (PHOTOIRRITATION/PHOTOTOXICITY)

Photoirritant contact dermatitis (PICD) is a chemically induced nonimmunological skin irritation requiring light. This reaction will occur in all individuals exposed to the chemical-light combination. The clinical picture is that of erythema, edema, or vesiculation in sun-exposed areas, resembling an exaggerated sunburn. This may be followed by hyper-pigmentation, or if the exposure is repeated, scaling and lichenification may occur. Bergapten, a component of bergamot oil, which used to be a popular ingredient in perfume, is a potent photoirritant that causes berloque dermatitis.

PHOTOALLERGIC CONTACT DERMATITIS

Photoallergic contact dermatitis (PACD) is an immunological response to a substance that requires the presence of light. The substance in the skin absorbs photons and is converted to a stable or unstable photoproduct, which binds to skin proteins to form an antigen, which then elicits a delayed hypersensitivity response. Examples of photoallergens present in cosmetics are musk ambrette and 6-methylcoumarin, which are present in fragrances. Photopatch testing is the diagnostic procedure for photoallergy.

CONTACT URTICARIA SYNDROME

Contact urticaria syndrome (CUS) represents a heterogeneous group of inflammatory reactions that appear, usually within a few minutes to an hour, after contact with the eliciting substance. Clinically, erythematous wheal-and-flare reactions are seen, and sensations of burning, stinging, or itching are experienced. These are transient, usually disappearing within a few hours. In its more severe forms, generalized urticaria or extracutaneous manifestations, such as asthma, nausea, abdominal cramps, and even anaphylactic shock, may occur. Diagnosis may be achieved by a variety of skin tests—the open test is the simplest of these and is the "first-line" test.

CUS may be divided into two categories on the basis of pathophysiological mechanisms: nonimmunologic and immunologic. There are also urticariogens that act by an uncertain mechanism.

Nonimmunological Contact Urticaria

Nonimmunological contact urticaria (NICU), which occurs without prior sensitization, is the most common class of CUS. The reaction usually remains localized. Examples of cosmetic substances known to produce NICU are preservatives (e.g., benzoic acid and sorbic acid) and fragrances (e.g., cinnamic aldehyde).

Immunological Contact Urticaria

Immunological contact urticaria (ICU) are immediate (Type I) allergic reactions in people who have previously been sensitized to the causative agent. ICU is IgE mediated and is more common in atopic individuals. Food substances are common causes of ICU.

ACNEGENICITY

This refers to the capacity of some agents to cause acne or aggravate existing acne lesions. This term may be subdivided to include comedogenicity and pustulogenicity.

Comedogenicity

This is the capability of an agent to cause hyperkeratinous impactions in the sebaceous follicle or the formation of microcomedones, usually in a relatively short period of time.

Pustulogenicity

This refers to the capability of an agent to cause inflammatory papules and pustules, usually in a relatively short period of time.

SENSITIVE SKIN

This term is a neologism for consumers' feelings about their intolerance to a variety of topical agents, be it topical medicaments or cosmetics and toiletries. Individuals present with very similar complaints, such as burning, stinging, or itching sensations, on contact with certain cosmetic products that most people do not seem to react to, sometimes accompanied by slight erythema or edema. They frequently complain of a "tight feeling" in their skin, secondary to associated dry skin. Sensitive skin describes the phenotype noted by the consumer; mechanisms include sensory irritation, suberythematous irritation, acute and cumulative irritation, contact urticaria, ACD, as well as photoallergic and phototoxic contact dermatitis. Sensory irritation and suberythematous irritation are believed to be far more common than the remaining mechanisms.

Cosmetic Intolerance Syndrome

The term "cosmetic intolerance syndrome" (CIS) is applied to the multifactorial syndrome in which certain susceptible individuals are intolerant of a wide range of cosmetic products. CIS is thought to be caused by one or more underlying occult dermatological conditions, such as subjective irritation, objective irritation, ACD, and contact urticaria, or subtle manifestations of endogenous dermatological diseases, such as atopic eczema, psoriasis, and rosacea.

Status Cosmeticus

Status cosmeticus is a condition in which every cosmetic product applied to the face reproduces itching, burning or stinging, rendering the sufferer incapable of using any cosmetic product. The patient's history usually includes "sensitivity" to a wide range of products. This diagnosis is only declared after a full battery of tests has proved negative, and may be considered the extreme end of the spectrum of sensitive skin.

BIBLIOGRAPHY

Irritant Contact Dermatitis

Eisner P, Maibach HI, eds. Irritant dermatitis: new clinical and experimental aspects. Current Problems in Dermatology Series. Vol. 23. Basel: Karger, 1995.

Lammintausta K, Maibach HI. Irritant contact dermatitis. In: Moschella SL, Hurley HJ, eds. Dermatology. 3rd ed. Philadelphia: W.B. Saunders Company, 1992: 425–432.

Van Der Valk PGM, Maibach HI. The Irritant Contact Dermatitis Syndrome. Boca Raton: CRC Press, 1996.

Wilkinson JD, Rycroft RJG. Contact dermatitis. In: Champion RH, Burton JL, Ebling FJG, eds. Rook/Wilkinson/Ebling Textbook of Dermatology, 5th ed. Oxford: Blackwell Scientific Publications, 1992:611.

Allergic Contact Dermatitis

Cronin E. Contact Dermatitis. Edinburgh: Churchill Livingstone, 1980.

Larsen WG, Maibach HI. Allergic contact dermatitis. In: Moschella SL, Hurley HJ, eds. Dermatology, Vol. 17, 3rd ed. Philadelphia: W.B. Saunders Company, 1992:391–424.

Rietschel RL, Fowler JF Jr, eds. Fisher's Contact Dermatitis, 4th ed. Williams & Baltimore: Williams and Wilkins, 1995.

Phototoxic/Photoallergic Contact Dermatitis

DeLeo VA, Maso MJ. In: Moschella SL, Hurley HJ, eds. Dermatology, 3rd ed. Philadelphia: W.B. Saunders Company, 1992:507.

Harber LC, Bickers DR, eds. Photosensitivity Diseases: Principles of Diagnosis and Treatment. 2nd ed. Ontario; BC: Decker Inc., 1989.

Marzulli FN, Maibach HI. Photoirritation (phototoxicity, phototoxic dermatitis). Dermatotoxicology, 5th ed. Washington, DC: Taylor & Francis, 1996:231–237.

Contact Urticaria Syndrome

Amin S, Lahti A, Maibach HI. Contact Urticaria Syndrome. Boca Raton: CRC Press, 1997.

Lahti A, Maibach HI. Contact urticaria syndrome. In: Moschella SL, Hurley HJ, eds. Dermatology. Vol. 19, 3rd ed. Philadelphia: W.B. Saunders Company, 1992:433.

Acnegenicity

Mills OH Jr, Berger RS. Defining the susceptibility of acne-prone and sensitive skin populations to extrinsic factors. Dermatol Clin 1991; 9(l):93–98.

Sensitive Skin

Amin S, Engasser P, Maibach HI. Sensitive skin: what is it? In: Baran R, Maibach HI. Textbook of Cosmetic Dermatology, 2nd ed. London: Martin Dunitz Ltd, 1998:343–349.

Fisher AA. Cosmetic actions and reactions: therapeutic, irritant and allergic. Cutis 1980; 26:22–29.

Maibach HI, Engasser P. Management of cosmetic intolerance syndrome. Clin Dermatol 1988; 6(3):102–107.

REFERENCES

1. Eisner P, Maibach HI. Irritant dermatitis: new clinical and experimental aspects. In: Current Problems in Dermatology Series. Basel: Karger, 1995:23.
2. Lammintausta K, Maibach HI. Irritant contact dermatitis. In: Moschella SL, Hurley HJ, eds. Dermatology. 3rd ed. Philadelphia: W.B. Saunders Company, 1992:425–432.
3. Van Der Valk PGM, Maibach HI. The Irritant Contact Dermatitis Syndrome. Boca Raton: CRC Press, 1996.
4. Wilkinson JD, Rycroft RJG. Contact dermatitis. In: Champion RH, Burton JL, Ebling FJG, eds. Rook/Wilkinson/Ebling Textbook of Dermatology. 5th ed. Oxford: Blackwell Scientific Publications, 1992:611.
5. Cronin E. Contact Dermatitis. Edinburgh: Churchill Livingstone, 1980.
6. Larsen WG, Maibach HI. Allergic contact dermatitis. In: Moschella SL, Hurley HJ, eds. Dermatology. Philadelphia: W.B. Saunders Company, 1992:391–424.
7. Rietschel RL, Fowler JF Jr, eds. Fisher's Contact Dermatitis, 4th ed. Williams & Baltimore: Williams and Wilkins, 1995.
8. DeLeo VA, Maso MJ, Moschella SL, Hurley HJ, eds. Dermatology. Philadelphia: W.B. Saunders Company, 1992:507.
9. Harber LC, Bickers DR, eds. Photosensitivity Diseases: Principles of Diagnosis and Treatment. 2nd ed. Ontario, BC: Decker Inc., 1989.
10. Marzulli FN, Maibach HI. Photoirritation (phototoxicity, phototoxic dermatitis). In: Dermatotoxicology. 5th ed. Washington, DC: Taylor & Francis, 1996:231–237.
11. Amin S, Lahti A, Maibach HI. Contact Urticaria Syndrome. Boca Raton: CRC Press, 1997.
12. Lahti A, Maibach HI. Contact urticaria syndrome. In: Moschella SL, Hurley HJ, eds. Dermatology. 3rd ed. Philadelphia: W.B. Saunders Company, 1992:433.
13. Mills OH Jr, Berger RS. Defining the susceptibility of acne-prone and sensitive skin populations to extrinsic factors. Dermatol Clin 1991; 9(l):93–98.
14. Amin S, Engasser P, Maibach HI. Sensitive skin: what is it? In: Baran R, Maibach HI, eds. Textbook of Cosmetic Dermatology. London: Martin Dunitz Ltd, 1998:343–349.
15. Fisher AA. Cosmetic actions and reactions: therapeutic, irritant and allergic. Cutis 1980; 26:22–29.
16. Maibach HI, Engasser P. Management of cosmetic intolerance syndrome. Clin Dermatol 1988; 6(3):102–107.

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Principles and Mechanisms of Skin Irritation

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INTRODUCTION

In contrast to allergic contact dermatitis (ACD), irritant contact dermatitis (ICD) is the result of unspecified damage attributable to contact with chemical substances that cause an inflammatory reaction of the skin (1). The clinical appearance of ICD is extremely variable. It is determined by the type of irritant and a dose–effect relationship (2). The clinical morphology of acute ICD as one side of the spectrum is characterized by erythema, edema, vesicles that may coalesce, bullae, and ooze. Necrosis and ulceration can be seen with corrosive materials. Clinical appearance of chronic ICD is dominated by redness, lichenification, excoriations, scaling, and hyperkeratosis.

Any site of the skin may be affected. Most frequently the hands as human "tools" come into extensive contact with irritants, whereas most adverse reactions to cosmetics occur in the face because of the particular sensitivity of this skin region. Airborne ICD develops in uncovered skin areas, mostly in the face and especially the periorbital region after exposure to volatile irritants or vapor (3,4).

Despite their different pathogenesis, ACD and ICD, particularly chronic conditions, show a remarkable similarity with respect to clinical appearance, histopathology (5,6), and immunohistology (7,8). Therefore, ICD can be regarded as an exclusion diagnosis after negative patch testing. The histological pattern of chronic ICD is characterized by hyper- and parakeratosis, spongiosis, exocytosis, moderate to marked acanthosis, and mononuclear perivascular infiltrates with increased mitotic activity (9,10).

MOLECULAR MECHANISMS OF SKIN IRRITANCY

As mentioned, striking clinical similarities exist between ICD and ACD, and even extensive immunostaining of biopsies does not allow discrimination between the two types of dermatitis (8).

In contrast to ACD, ICD lacks hapten-specific T-lymphocytes. The pathogenic pathway in the acute phases of ICD starts with the penetration of the irritant into the

barrier, either activation or mild damage of keratinocytes, and release of mediators of inflammation with unspecific T-cell activation (11). Epidermal keratinocytes play the crucial role in the inflammation of ICD; they can be induced to produce several cytokines and provoke a dose-dependent leukocyte attraction (12). The upregulation of certain adhesion molecules like a6 integrin or CD 36 is independent of the stimulus and not cytokine induced (13,14). A number of agents and cytokines themselves are capable of mediating cytokine production in keratinocytes. IL-1 and TNF-oc play a role as inflammatory cytokines, IL-8 and IP-10 are known to act as chemotaxins, and IL-6, IL-7, IL-15, granulocyte-macrophage colony-stimulating factor (GM-CSF), and TGF-alpha can promote growth. Other cytokines, such as IL-10, IL-12, and IL-18, are known to regulate humoral versus cellular immunity (15). It is controversial whether the cytokine profile induced by irritants differs from that induced by allergens (16). In irritant reactions, TNF-alpha, IL-6, IL-ip, and IL-2 have been reported to be increased (17,18).

In subliminal contact to irritants, barrier function of the stratum corneum and not the keratinocyte is the main target of the insulting stimulus. Damage of the lipid barrier of the stratum corneum is associated with loss of cohesion of corneocytes and desquamation with increase of transepidermal water loss (TEWL). This is one triggering stimulus for lipid synthesis and it promotes barrier restoration (19). Nevertheless, recent studies show that the concept of TEWL increase after sodium lauryl sulfate (SLS) being directly related to a delipidizing effect of surfactants on the stratum corneum cannot be kept up without limitation. Fartasch et al. showed that SLS exposure for 24 hours causes damage in the deeper nucleated cells of the epidermis, leaving the lamellar arrangements of lipids intact. This means that the hypothetical model of SLS-induced irritation is mainly modulated by keratinocytes rather than the stratum corneum (20).

The stratum corneum influences epidermal proliferation after contact to irritants by increasing the mitotic activity of basal keratinocytes and in this way enhancing the epidermal turnover (21,22). Disruption of the stratum corneum can even stimulate cytokine production itself and in this way promote the inflammatory skin reaction, as shown by Wood et al. (23). They found an increase of TNF-α, various interleukins, and GM-CSF.

Recently it has been shown that chemically different irritants induce differences in the response in the epidermis during the first 24 hours with respect to cytokine expression, indicating different “starting points” for the inflammatory response that results in the same irritant response clinically after 48 hours. Nonanionic acid, but not SLS, induced an increase in m-RNA expression for IL-6, whereas m-RNA expression for GM-CSF was increased after SLS (24). Forsey et al. saw a proliferation of keratinocytes after 48 hours of exposure, and apoptosis of keratinocytes after 24 and 48 hours of exposure to SLS. In contrast, nonanionic acid decreased keratinocyte proliferation after 24 hours of exposure and epidermal cell apoptosis after only six hours of exposure (25). In conclusion, it becomes clear that the concept of skin irritation is complicated and we are only beginning to understand the underlying molecular mechanisms.

FACTORS PREDISPOSING TO CUTANEOUS IRRITATION

The skin of different individuals differs in susceptibility to irritation in a remarkable manner, and a number of individual factors influencing development of irritant

dermatitis that have been identified include age, genetic background, anatomical region exposed, and pre-existing skin disease.

Although experimental studies did not support sex differences of irritant reactivity (26,27), females turned out to be at risk in some epidemiological studies (28,29). It is probable that increased exposure to irritants at home, caring for children under the age of 4 years, lack of a dishwashing machine (30), and preference for high-risk occupations contribute to the higher incidence of ICD in females (27). The most established individual risk factor, out of several studies about occupational hand eczema, is probably atopic dermatitis (28,31–33). On the other hand, experimental studies concerning the reactivity of atopies and nonatopics to standard irritants have given contradictory results (34,35) and, as shown in a Swedish study, about 25% of the atopies in extreme-risk occupations, such as hairdressers and nursing assistants, did not develop hand eczema (36). Age is as well related to irritant susceptibility insofar as irritant reactivity declines with increasing age. This is true not only for acute but also for cumulative irritant dermatitis (37,38). Fair skin, especially skin type I, is supposed to be the most reactive to all types of irritants, and black skin is the most resistant (39,40).

Clinical manifestation of ICD is also influenced by type and concentration of irritant, solubility, vehicle, and length of exposure (41), as well as temperature and mechanical stress. During the winter months, low humidity and low temperature decrease the water content of the stratum corneum and increase irritant reactivity (42,43).

EPIDEMIOLOGY

Population-based data on the incidence and prevalence of ICD are rare, but there is an agreement that incidence of ICD is higher than that of ACD in general. The figures on the incidence of ICD vary considerably, depending on the study population. Most data stem from studies about occupational hand dermatoses, and in this an overview is given about the important findings of these studies. In general, it can be assumed that nonoccupational contact dermatitis attributable to all causes is more frequent in comparison to occupational contact dermatitis (29).

Coenraads and Smit reviewed international prevalence studies for eczema attributable to all causes conducted with general populations in different countries (England, The Netherlands, Norway, Sweden, the United States) and found point prevalence rates of 1.7% to 6.3%, and 1- to 3-year period prevalence rates of 6.2% to 10.6% (44).

An extensive study of Meding on hand eczema in Gothenburg, Sweden, included 20,000 individuals randomly selected from the population register (28). Meding estimated a 1-year period prevalence of hand eczema of 11% attributable to all causes, and a point prevalence of 5.4%. ICD contributed to 35% of the cases, whereas 22% were diagnosed as atopic hand dermatitis and 19% as ACD. In a multicenter epidemiological study on contact dermatitis in Italy by Gruppo Italiano Ricerca Dermatiti da Contatto e Ambientali (GIRDCA) 42,839 patients with contact dermatitis underwent patch testing. In accordance with the findings of Meding, nonoccupational as well as occupational ICD affected women in a higher percentage compared with males (28,29). In Heidelberg, Germany, a retrospective study of 190 cases of hand dermatitis revealed 27% as ICD, 15.8% as ACD, and the majority (40%) as being of atopic origin with 10% as various other diseases (45).

Shenefelt studied the frequency of visits by university students to campus' prepaid-health-plan dermatologists for ICD and ACD compared with other types of dermatitis and skin problems. In contrast to other studies, he found slightly more allergic cases (3.1% of all first visits) than ICD (2.3%) (46).

Reports on adverse reactions to cosmetics, including those with only subjective perceptions without morphological signs, are more frequent than assumed. In a questionnaire carried out in Thuringia, eastern Germany, even 36% of 208 persons reported adverse cutaneous reactions against cosmetics, 75% of them being female (47). Nevertheless, it must be emphasized that this includes, in addition to ACD, dermatoses as seborrheic dermatitis, perioral dermatitis, rosacea, and psoriasis, which cannot be separated by the inexperienced. Higher incidence in females was confirmed by several studies (48). Most untoward reactions caused by cosmetics occur on the face, including the periorbital area (49).

In a study by Broeckx et al., 5.9% of a test population of 5202 patients with possible contact dermatitis had adverse reactions to cosmetics. Patch testing classified only 1.46% as irritant reactions whereas 3.0% could be classified as ACD. More than 50% of the cases of irritation were attributable to soaps and shampoos (50). In Sweden, the top-ranking products causing adverse effects, as reported by the Swedish Medical Products Agency, were moisturizers, haircare products, and nail products (48).

In other studies, the incidence of cosmetic intolerance varied between 2% and 8.3%, depending on the test population (49,51,52). In a large multicenter prospective study on reactions caused by cosmetics, Eiermann et al. found irritancy to account for only 16% of 487 cases of contact dermatitis caused by cosmetics. Of 8093 patients tested, 487 cases (6%) were diagnosed as contact dermatitis caused by cosmetics (53). Because most consumers just stop using cosmetics when experiencing mild irritant or adverse reactions and seldom consult a physician, it can be assumed that mild irritant reactions to cosmetic products are underestimated (54).

CLINICAL TYPES OF ICD

According to the highly variable clinical picture, several different forms of ICD have been defined. The following types of irritation have been described (55,56):

- acute ICD
- delayed acute ICD
- irritant reaction
- cumulative ICD
- traumiterative ICD
- exsiccation eczematid
- traumatic ICD
- pustular and acneiform ICD
- nonerythematous
- sensory irritation

Acute ICD

Acute ICD is caused by a contact to a potent irritant. Substances that cause necrosis are called corrosive and include acids and alkaline solutions. Contact is often

accidental at the workplace. Cosmetics are unlikely to cause this type of ICD because they do not contain primary irritants in sufficient concentrations.

Symptoms and clinical signs of acute ICD develop with a short delay of minutes to hours after exposure, depending on the type of irritant, concentration, and intensity of contact. Characteristically the reaction quickly reaches its peak and then starts to heal; this is called "decrescendo phenomenon." Symptoms include burning rather than itching, stinging, and soreness of the skin, and are accompanied by clinical signs such as erythema, edema, bullae, and even necrosis. Lesions are usually restricted to the area that comes into contact, and sharply demarcated borders are important signs of acute ICD. Nevertheless, clinical appearance of acute ICD can be highly variable and sometimes may even be indistinguishable from the allergic type. In particular, combination of ICD and ACD can be troublesome. Prognosis of acute ICD is good if irritant contact is avoided.

Delayed Acute ICD

For some chemicals, such as anthralin, it is typical to produce a delayed acute ICD. Visible inflammation is not seen until 8 to 24 hours or more after exposure (57). Clinical picture and symptoms are similar to acute ICD. Other substances that cause delayed acute ICD include dithranol, tretinoin, and benzalkonium chloride. Irritation to tretinoin can develop after a few days and results in a mild to fiery redness followed by desquamation, or large flakes of stratum corneum accompanied by burning rather than itching. Irritant patch-test reactions to benzalkonium chloride may be papular and increase with time, thus resembling allergic patch-test reactions (58). Tetraethylene glycol diacrylate caused delayed skin irritation after 12 to 36 hours in several workers in a plant manufacturing acrylated chemicals (59).

Irritant Reaction

Irritants may produce cutaneous reactions that do not meet the clinical definition of a "dermatitis." Irritant reaction is therefore a subclinical form of irritant dermatitis and is characterized by a monomorphic rather than polymorphic picture. This may include one or more of the following clinical signs: dryness, scaling, redness, vesicles, pustules, and erosions (60). Irritant reactions often occur after intense water contact and in individuals exposed to wet work, such as hairdressers or metal workers, particularly during their first months of training. It often starts under rings worn on the finger or in the interdigital area, and may spread over the dorsum of the fingers and to the hands and forearms. Frequently, the condition heals spontaneously, resulting in hardening of the skin, but it can progress to cumulative ICD in some cases.

Cumulative ICD

Cumulative ICD is the most common type of ICD (55). In contrast to acute ICD that can be caused by single contact to a potent irritant, cumulative ICD is the result of multiple subthreshold damage to the skin when time is too short for restoration of skin-barrier function (61). Clinical symptoms develop after the damage has exceeded a certain manifestation threshold, which is individually determined and can vary within one individual at different times. Typically, cumulative ICD is linked to exposure of several weak irritants and water contact rather than to repeated exposure to a single potent irritant. Because the link between exposure and disease is often not

obvious to the patient, diagnosis may be considerably delayed, and it is important to rule out an allergic cause. Symptoms include itching and pain caused by cracking of the hyperkeratotic skin. The clinical picture is dominated by dryness, erythema, lichenification, hyperkeratosis, and chapping. Xerotic dermatitis is the most frequent type of cumulative toxic dermatitis (62). Vesicles are less frequent in comparison to allergic and atopic types (28); however, diagnosis is often complicated by the combination of irritation and atopy, irritation and allergy, or even all three. Lesions are less sharply demarcated in contrast to acute ICD.

Prognosis of chronic cumulative ICD is rather doubtful (63,64). Some investigators suggest that the repair capacity of the skin may enter a self-perpetuating cycle (61).

Traumiterative ICD

This term is often used similarly to cumulative ICD (55,60). Clinically, the two types are very similar as well. According to Malten and den Arend, traumiterative ICD is a result of too-early repetition of just one type of load, whereas cumulative ICD results from too-early repetition of different types of exposures (2).

Exsiccation Eczematid

Exsiccation eczematid is a subtype of ICD that mainly develops on the extremities. It is often attributable to frequent bathing and showering as well as extensive use of soaps and cleansing products. It often affects elderly people with low sebum levels of the stratum corneum. Low humidity during the winter months and failure to remoisturize the skin contribute to the condition. The clinical picture is typical, with dryness, ichthyosiform scaling, and fissuring. Patients often suffer from intense itching.

Traumatic ICD

Traumatic ICD may develop after acute skin traumas such as burns, lacerations, and acute ICD. The skin does not heal as expected, but ICD with erythema, vesicles and/or papulovesicles, and scaling appears. The clinical course resembles that of nummular dermatitis (55).

Pustular and Acneiform ICD

Pustular and acneiform ICD may result from contact to irritants such as mineral oils, tars, greases, some metals, croton oil, and naphthalenes. Pustules are sterile and transient. The syndrome must be considered in conditions in which acneiform lesions develop outside typical acne age. Patients with seborrhea, macroporous skin, and prior acne vulgaris are predisposed along with atopies.

Nonerythematous ICD

Nonerythematous ICD is an early stage of skin irritation that lacks visible inflammation but is characterized by changes in the function of the stratum corneum that can be measured by noninvasive bioengineering techniques (55,65).

Sensory Irritation

Sensory irritation is characterized by subjective symptoms without morphological changes. Predisposed individuals complain of stinging, burning, tightness, itching, or even painful sensations that occur immediately or after contact. Those individuals with hyperirritable skin often report adverse reactions to cosmetic products with most reactions occurring on the face. Fisher defined the term "status cosmeticus," which describes a condition in patients who try a lot of cosmetics and complain of being unable to tolerate any of them (66). Lactic acid serves as a model irritant for diagnosis of so-called "stingers" when it is applied in a 5% aqueous solution on the nasolabial fold after induction of sweating in a sauna (67). Other chemicals that cause immediate-type stinging after seconds or minutes include chloroform and methanol (1:1) and 95% ethanol. A number of substances that have been systematically studied by Frosch and Kligman may also cause delayed-type stinging (67,68). Several investigators tried to determine parameters that characterize those individuals with sensitive skin, a term that still lacks a unique definition (69,70). It could be shown that individuals who were identified as having sensitive skin by their own assessment have altered baseline biophysical parameters, showing decreased capacitance values, increased TEWL, and higher pH values accompanied by lower sebum levels (70). Possible explanations for hyperirritability (other than diminished barrier function) that have been discussed are heightened neurosensory input attributable to altered nerve endings, more neurotransmitter release, unique central information processing or slower neurotransmitter removal, and enhanced immune responsiveness (69,71). It is not clear whether having sensitive skin is an acquired or inherited condition; most probably it can be both. As in other forms of ICD, seasopal variability in stinging with a tendency to more intense responses during winter has been observed (72). Detailed recommendations for formulation of skincare products for sensitive skin have been given by Draelos (69).

REFERENCES

1. Mathias CGT, Maibach HI. Dermatotoxicology monographs I. Cutaneous irritation: factors influencing the response to irritants. Clin Toxicol 1978; 13:333–346.
2. Malten KE, den Arend JA. Irritant contact dermatitis. Traumiterative and cumulative impairment by cosmetics, climate, and other daily loads. Derm Beruf Umwelt 1985; 4:125–132.
3. Dooms-Goossens AE, Debusschere KM, Gevers DM, Dupre KM, Degref HJ, Loncke JP, Snauwaert JE. Contact dermatitis caused by airborne agents. A review and case reports. J Am Acad Dermatol 1986; 15:1–10.
4. Lachapelle JM. Industrial airborne irritant or allergic contact dermatitis. Contact Dermatitis 1986; 14:137–145.
5. Brand CU, Hunziker T, Braathen LR. Studies on human skin lymph containing Langerhans cells from sodium lauryl sulphate contact dermatitis. J Invest Dermatol 1992; 5:109s–110s.
6. Brand CU, Hunziker T, Limat A, et al. Large increase of Langerhans cells in human skin lymph derived from irritant contact dermatitis. Br J Dermatol 1993; 2:184–188.
7. Medenica M, Rostenberg A Jr. A comparative light and electron microscopic study of primary irritant contact dermatitis and allergic contact dermatitis. J Invest Dermatol 1971; 4:259–271.
8. Brasch J, Burgard J, Sterry W. Common pathogenetic pathways in allergic and irritant contact dermatitis. J Invest Dermatol 1992; 2:166–170.

9. Cohen LM, Skopicki DK, Harrist DJ, Clark WH. Noninfectious vesiculobullous and vesiculopustular diseases. In: Elder D, Elenitsas R, Jaworsky C, Johnson B, eds. Lever's Histopathology of the Skin. 8th ed. Philadelphia: Lippincott-Raven, 1997:209–252.
10. Le TK, Schalkwijk J, van de Kerkhof PC, van Haelst U, van der Valk PG. A histological and immunohistochemical study on chronic irritant contact dermatitis. Am J Contact Dermat 1998; 9:23–28.
11. Berardesca E, Distante F. Mechanisms of skin irritation. In: Eisner P, Maibach HI, eds. Irritant Dermatitis: New Clinical and Experimental Aspects. Current Problems in Dermatology. Basel: Karger, 1995:1–8.
12. Nickoloff BJ, Naidu Y. Perturbation of epidermal barrier function correlates with initiation of cytokine cascade in human skin. J Am Acad Dermatol 1994; 30:535–546.
13. Willis CM, Stephens CJ, Wilkinson JD. Epidermal damage induced by irritants in man: a light and electron microscopic study. J Invest Dermatol 1989; 93:695–699.
14. Jung K, Imhof BA, Linse R, Wollina U, Neumann C. Adhesion molecules in atopic dermatitis: upregulation of a6 integrin expression in spontaneous lesional skin as well as in atopen, antigen and irritative induced patch test reactions. Int Arch Allergy Immunol 1997; 113:495–504.
15. Corsini E, Galli CL. Cytokines and irritant contact dermatitis. Toxicol Lett 1998; 28:277–282.
16. Kalish RS. T cells and other leukocytes as mediators of irritant contact dermatitis. In: Beltrani VS, ed. Immunology and Allergy Clinics of North America. Contact Dermatitis. Irritant and Allergic. Philadelphia: W.B. Saunders Company, 1997:407–415.
17. Larsen CG, Ternowitz T, Larsen FG, Zachariae CO, Thestrup-Pedersen K. ETAF/interleukin-1 and epidermal lymphocyte chemotactic factor in epidermis overlying an irritant patch test. Contact Dermatitis 1989; 20:335–340.
18. Hunziker T, Brand CU, Kapp A, Waelti ER, Braathen LR. Increased levels of inflammatory cytokines in human skin lymph derived from sodium lauryl sulphate-induced contact dermatitis. Br J Dermatol 1992; 127:254–257.
19. Grubauer G, Elias PM, Feingold KR. Transepidermal water loss: the signal for recovery of barrier structure function. J Lipid Res 1989; 30:323–333.
20. Fartasch M, Schnetz E, Diepgen TL. Characterization of detergent-induced barrier alterations—effect of barrier cream on irritation. J Invest Dermatol Symp Proc 1998; 3:121–127.
21. Fisher LB, Maibach HI. Effects of some irritants on human epidermal mitosis. Contact Dermatitis 1975; 1:273–276.
22. Wilhelm KP, Saunders JC, Maibach HI. Increased stratum corneum turnover induced by sub-clinical irritant dermatitis. Br J Dermatol 1990; 122:793–798.
23. Wood LC, Jackson SM, Elias PM, Grunfeld C, Feingold KR. Cutaneous barrier perturbation stimulates cytokine production in the epidermis of mice. J Clin Invest 1992; 90:482–487.
24. Grangsjo A, Leijon-Kuligowski A, Torma H, Roomans GM, Lindberg M. Different pathways in irritant contact eczema? Early differences in the epidermal elemental content and expression of cytokines after application of 2 different irritants. Contact Dermatitis 1996; 35:355–360.
25. Forsey RJ, Shahidullah H, Sands C, McVittie E, Aldridge RD, Hunter JA, Howie SE. Epidermal Langerhans cell apoptosis is induced in vivo by nonanionic acid but not by sodium lauryl sulphate. Br J Dermatol 1998; 139:453–461.
26. Bjornberg A. Skin reactions to primary irritants in men and women. Acta Derm Venereol (Stockh) 1975; 55:191–194.
27. Hogan DJ, Dannaker CJ, Maibach HI. The prognosis of contact dermatitis. J Am Acad Dermatol 1990; 23:300–307.
28. Meding B. Epidemiology of hand eczema in an industrial city. Acta Derm Venereol (Stockh) (Suppl) 1990; 153:1–43.

29. Sertoli A, Francalanci S, Acciai MC, Gola M. Epidemiological survey of contact dermatitis in Italy (1984–1993) by GIRDCA (Gruppo Italiano Ricera Dermatiti da Contatto e Ambientali). Am J Contact Dermat 1999; 10:18–30.
30. Nilsson E. Individual and environmental risk factors for hand eczema in hospital workers. Acta Derm Venereol (Stockh) (Suppl) 1986; 128:1–63.
31. Wilhelm KP, Maibach HI. Factors predisposing to cutaneous irritation. Dermatol Clin 1990; 8:17–22.
32. Coenraads PJ, Diepgen TL. Risk for hand eczema in employees with past or present atopic dermatitis. Int Arch Occup Environ Health 1998; 71:7–13.
33. Berndt U, Hinnen U, Iliev D, Eisner P. Role of the atopy score and of single atopic features as risk factors for development of hand eczema in trainee metal workers. Br J Dermatol 1999; 140:922–924.
34. Gallacher G, Maibach HI. Is atopic dermatitis a predisposing factor for experimental acute irritant contact dermatitis? Contact Dermatitis 1998; 38:1–4.
35. Basketter DA, Miettinen J, Lahti A. Acute irritant reactivity to sodium lauryl sulfate in atopies and non-atopies. Contact Dermatitis 1998; 38:253–257.
36. Rysted I. Work-related hand eczema in atopies. Contact Dermatitis 1985; 12:164–171.
37. Suter-Widmer J, Eisner P. Age and irritation. In: van der Valk PGM, Maibach HI, eds. The Irritant Contact Dermatitis Syndrome. Boca Raton: CRC Press, 1994:257–261.
38. Schwindt DA, Wilhelm KP, Miller DL, Maibach HI. Cumulative irritation in older and younger skin: a comparison. Acta Derm Venereol 1998; 78:279–283.
39. Lammintausta K, Maibach HI, Wilson D. Susceptibility to cumulative and acute contact dermatitis. Contact Dermatitis 1988; 19:84–90.
40. Maibach HI, Berardesca E. Racial and skin color differences in skin sensitivity: implications for skin care products. Cosmet Toilet 1990; 105:35–36.
41. Dahl MV. Chronic, irritant contact dermatitis: mechanisms, variables, and differentiation from other forms of contact dermatitis. Adv Dermatol 1988; 3:261–275.
42. Mozzanica N. Pathogenetic aspects of allergic and irritant contact dermatitis. Clin Dermatol 1992; 10:115–121.
43. Uter W, Gefeller O, Schwanitz HJ. An epidemiological study of the influence of season (cold and dry air) on the occurrence of irritant skin changes of the hands. Br J Dermatol 1998; 138:266–272.
44. Coenraads PJ, Smit J. Epidemiology. In: Rycroft RJG, Menne T, Frosch PJ, eds. Textbook of Contact Dermatitis. 2nd ed. Berlin: Springer, 133–150.
45. Kiihner-Piplack B. Klinik und Differentialdiagnose des Handekzems. Eine retrospektive Studie am Krankengut der Universitats-Hautklinik Heidelberg 1982–1985. Thesis, Ruprecht-Karls-University, Heidelberg, Germany.
46. Shenefelt PD. Descriptive epidemiology of contact dermatitis in a university student population. Am J Contact Dermat 1996; 7:88–93.
47. Ropcke F. Auswertung zur Umfrage "Epidemiologie von Kosmetika-Unvertraglichkeiteneine bevolkerungsbasierte Studie," 1999, unpublished data.
48. Berne B, Bostrom A, Grahnen AF, Tammela M. Adverse effects of cosmetics and toiletries reported to the Swedish Medical Products Agency 1989–1994. Contact Dermatitis 1996; 34:359–362.
49. Adams RM, Maibach HI. A 5-year study of cosmetic reactions. J Am Acad Dermatol 1985; 13:1062–1069.
50. Broeckx W, Blondeel A, Dooms-Goossens A, Achten G. Cosmetic intolerance. Contact Dermatitis 1987; 16:189–194.
51. Skog E. Incidence of cosmetic dermatitis. Contact Dermatitis 1980; 6:449–451.
52. Romaguera C, Camarasa JMG, Alomar A, Grimalt F. Patch tests with allergens related to cosmetics. Contact Dermatitis 1983; 9:167–168.
53. Eiermann HJ, Larsen W, Maibach HI, Taylor JS. Prospective study of cosmetic reactions: 1977–1980. J Am Acad Dermatol 1982; 6:909–917.

54. Amin S, Engasser PG, Maibach HI. Adverse cosmetic reactions. In: Baran R, Maibach HI, eds. Textbook of Cosmetic Dermatology. 2nd ed. London: Martin Dunitz Ltd, 1998:709–746.
55. Lammintausta K, Maibach HI. Contact dermatitis due to irritation: general principles, etiology, and histology. In: Adams RM, ed. Occupational Skin Disease. Philadelphia: W.B. Saunders Company, 1990:1–15.
56. Berardesca E, Distante F. Mechanisms of skin irritation. In: Eisner P, Maibach HI, eds. Irritant Dermatitis. New Clinical and Experimental Aspects. Basel: Karger, 1995:1–8.
57. Malten KE, den Arend JA, Wiggers RE. Delayed irritation: hexanediol diacrylate and butanediol diacrylate. Contact Dermatitis 1979; 3:178–184.
58. Bruynzeel DP, van Ketel WG, Scheper RJ, von Blomberg-van der Flier BME. Delayed time course of irritation by sodium lauryl sulfate: observations on threshold reactions. Contact Dermatitis 1982; 8:236–239.
59. Nethercott JR, Gupta S, Rosen C, Enders LJ, Pilger CW. Tetraethylene glycol diacrylate. A cause of delayed cutaneous irritant reaction and allergic contact dermatitis. J Occup Med 1984; 26:513–516.
60. Frosch PJ. Cutaneous irritation. In: Rycroft RJG, Menne T, Frosch PJ, eds. Textbook of Contact Dermatitis. 2nd ed. Berlin: Springer, 1995:28–61.
61. Malten KE. Thoughts on irritant contact dermatitis. Contact Dermatitis 1981; 7:238–247.
62. Eichmann A, Amgwerd D. Toxische Kontaktdermatitis. Schweiz Rundsch Med Prax 1992; 19:615–617.
63. Keczkes K, Bhate SM, Wyatt EH. The outcome of primary irritant hand dermatitis. Br J Dermatol 1983; 109:665–668.
64. Eisner P, Baxmann F, Liehr HM. Metal working fluid dermatitis: a comparative follow-up study in patients with irritant and non-irritant dermatitis. In: Eisner P, Maibach HI, eds. Irritant Dermatitis: New Clinical and Experimental Aspects. Basel: Karger, 1995: 77–86.
65. Van der Valk PGM, Maibach HI. Do topical corticosteroids modulate skin irritation in human beings? Assessment by transepidermal water loss and visual scoring. J Am Acad Dermatol 1989; 21:519–522.
66. Fisher AA. Cosmetic actions and reactions: therapeutic, irritant and allergic. Cutis 1980; 26:22–29.
67. Frosch PJ, Kligman AM. A method for appraising the stinging capacity of topically applied substances. J Soc Cosmet Chem 1977; 28:197–209.
68. Parrish JA, Pathak MA, Fitzpatrick TB. Facial irritation due to sunscreen products. Letter to the editor. Arch Dermatol 1975; 111:525.
69. Draelos ZD. Sensitive skin: perceptions, evaluation, and treatment. Am J Contact Dermat 1997; 8:67–78.
70. Seidenari S, Francomano M, Mantovani L. Baseline biophysical parameters in subjects with sensitive skin. Contact Dermatitis 1999; 38:311–315.
71. Muizzudin N, Marenus KD, Maes DH. Factors defining sensitive skin and its treatment. Am J Contact Dermat 1998; 9:170–175.
72. Leyden JJ. Risk assessment of products used on skin. Am J Contact Dermat 1993; 4:158–162.

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In Vivo Irritation

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INTRODUCTION

Irritant Dermatitis

Skin irritation is a localized nonimmunologically mediated inflammatory process. It may manifest objectively with skin changes such as erythema, edema, and vesiculation, or subjectively with the complaints of burning, stinging, or itching, with no detectable visible or microscopic changes. Several forms of objective irritation exist (Table 1). Acute irritant dermatitis may follow a single, usually accidental, exposure to a potent irritant and generally heals soon after exposure. An irritant reaction may be seen in individuals such as hairdressers and wet-work performing employees, who are more extensively and regularly exposed to irritants. Repeated irritant reactions may develop into a contact dermatitis, which generally has a good prognosis. Other forms of irritant dermatitis include delayed acute irritant contact dermatitis, which occurs when there is a delay between exposure and inflammation, and cumulative irritant dermatitis, which is the most common form of irritant contact dermatitis. After exposure, an acute irritant dermatitis is not seen but invisible skin changes occur, which eventually lead to an irritant dermatitis when exposure reaches a threshold point. This may follow days, weeks, or years of exposure (1). These various forms require specialized models to predict their occurrence after exposure to specific products.

Need for Models

Prevention of skin irritation is important for both the consumer who will suffer from it and for the industry, which needs a licensable and marketable product. Accurate prediction of the irritation potential of industrial, pharmaceutical, and cosmetic materials is therefore necessary for the consumer health and safety and for product development. Presently, animal models fulfill licensing criteria for regulatory bodies. In the European Union, animal testing for cosmetics was to be banned in 1998; however, the deadline was extended to June 30, 2000 because scientifically validated models were not available. Until alternative models can be substituted, in vivo models provide a means by which a cosmetic can be tested on living skin, at various sites, and under conditions that should closely mimic the intended human use.

Table 1 Classification of Irritant Dermatitis

Classification	Features	Clinical picture
Acute irritant dermatitis	Single exposure	Reaction usually restricted to exposed area, appears within minutes
	Strong irritant	Erythema, edema, blisters, bullae, pustules, later eschar formation
	Individual prodisposition considered generally unimportant	Symptoms include burning, stinging, and pain
		Possible secondary infection
		Good prognosis
Irritant reaction	Follows repeated acute skin irritation	Repeated irritant reactions may develop into contact dermatitis
	Oftern occupational; hairdressers, wet workers	Good prognosis
Cumulative irritant dermatitis	Repeated exposures required	Initially subject may experience stinging
	Initial exposures cause invisible damage	Eventually erythema, edema, or scaling appears
	Exposure may be weeks, months, or years until dermatitis develops	Variable prognosis
	Individual variation is seen	
Delayed acute irritant contact dermatitis	Latent period of 12–24 hours between exposure and dermatitis	Clinically similar to acute irritant dermatitis
		Good prognosis
Subclinical irritation	Irritation detectable by bioengineering methods prior to development of irritant dermatitis	
Subjective irritation	Subject complains of irritant symptoms with no clinically visible irritation	Perceived burning, stinging, or itching
Traumatic irritant dermatitis	Follows acute skin trauma, e.g., burn or laceration	Incomplete healing, followed by erythema, vesicles, vesicopapules, and scaling; may later resemble nummular (coin-shaped) dermatitis
Pusstular and acneiform dermatitis	Caused by metals, oils, greases, tar, asphalt, chlorinated napthalenes, polyhalogenated naphthalenes, cosmetics	Develops over weeks to months
		Variable prognosis
Friction dermatitis	Caused by friction trauma	Sometimes seen on hands and knees

Table 2 Draize–FHSA Model

Number of animals	Six albino rabbits (clipped)
Test sites	2×1 inch^2 sites on dorsum
	One site intact, the other abraded, e.g., with hypodermic needle
Test materials	Applied undiluted to both test sites
	Liquids: 0.5 mL
	Solids/semisolids: 0.5 g
Occlusion	1 inch^2 surgical gauze over each test site
	Rubberized cloth over entire trunk
Occlusion	24 hr period
Assessment	24 and 72 hr
	Visual scoring system

Abbreviations: FHSA, Federal Hazardous Substance Act.

Many aspects of irritation have been described, ranging from the visible erythema and edema to molecular mediators such as interleukins and prostaglandins. Therefore, a variety of in vivo and in vitro approaches to experimental assay are possible. However, no model assays inflammation in its entirety. Each model is limited by our ability to interpret and extrapolate the features of inflammation to the desired context. Therefore, predicting human responses based on data from nonhuman models requires particular care.

Various human experimental models have been proposed, providing irritant data for the relevant species. Human models allow the substance to be tested in the manner that the general public will use it, e.g., wash testing (see the following section) attempts to mimic the consumer's use of soaps and other surfactants. Also, humans are able to provide subjective data on the degree of irritation caused by the product. However, human studies are also limited by pitfalls in interpretation, and by the fear of applying new substances to human skin before their irritant potential has been evaluated.

ANIMAL MODELS

Draize Rabbit Models

The Draize model (2) and its modifications are commonly used to assay skin irritation using albino rabbits. Various governmental agencies have adopted these methods as standard test procedure. The procedure adopted in the U.S. Federal Hazardous Substance Act is described in Tables and (3–5). Table 4 compares this method with some other modifications of the Draize model.

Draize used this scoring system to calculate the primary irritation index (PII). This is calculated by averaging the erythema scores and the edema scores of all sites (abraded and nonabraded). These two averages are then added together to give the PII value. A value of less than 2 was considered nonirritating, 2 to 5 mildly irritating, and greater than 5 severely irritating. A value of 5 defines an irritant by Consumer Product Safety Commission standards. Subsequent laboratory and clinical experience that has shown the value judgments (i.e., nonirritating, mildly irritating, and severely irritating) proposed in 1944 requires clinical judgment and perspective,

Table 3 Draize–FHSA Scoring System

	Source
Erythema and eschar formation	0
No erythema	
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Edema formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised >1 mm)	3
Severe edema (raised >1 mm and extending beyond the area of exposure)	4

Source: From Ref. 4.

and should not be viewed in an absolute sense. Many materials irritating to the rabbit may be well tolerated by human skin.

Although the Draize scoring system does not include vesiculation, ulceration, and severe eschar formation, all of the Draize-type tests are used to evaluate corrosion as well as irritation. When severe and potentially irreversible reactions occur, the test sites are further observed on days 7 and 14, or later if necessary.

Table 4 Examples of Modified Draize Irritation Method

	Draize	FHSA	DOT	FIFRA	OECD
Number of animals	3	6	6	6	6
Abrasion/intact	Both	Both	Intact	2 of each	Intact
Dose liquids	0.5 mL undiluted	0.5 mL undiluted	0.5 mL	0.5 mL undiluted	0.5 mL
Dose solids in solvent	0.5 g	0.5 g moistened	0.5 g moistened	0.5 g	0.5 g
Exposure period (hr)	24	24	4	4	4
Examination (hr)	24, 72	24, 72	4, 48	0.5, 1, 24, 48, 72	0.5, 1, 24, 48, 72
Removal of test materials	Not specified	Not specified	Skin washed	Skin wiped	Skin washed
Excluded from testing	—	—	—	Toxic materials pH S2 or >11.5	Toxic materials pH S2 or >11.5

Abbreviations: FHSA, Federal Hazardous Substance Act; DOT, Department of Transportation; FIFRA, Federal Insecticide, Fungicide and Rodenticide Act; OECD, Organization for Economic Cooperation and Development.
Source: From Ref. 4.

Modifications to the Draize assay have attempted to improve its prediction of human experience. The model is criticized for inadequately differentiating between mild and moderate irritants. However, it serves well in hazard identification, often overpredicting the severity of human skin reactions (5). Therefore, Draize assays continue to be recommended by regulatory bodies for drugs and industrial chemicals.

Cumulative Irritation Assays

Several assays study the effects of cumulative exposure to a potential irritant. Justice et al. (6) administered seven applications of surfactant solutions at 10-minute intervals to the clipped dorsum of albino mice. The test site was occluded with a rubber dam to prevent evaporation, and the skin was examined microscopically for epidermal erosion.

Frosch et al. (7) described the guinea pig repeat irritation test (RIT) to evaluate protective creams against the chemical irritants, sodium lauryl sulfate (SLS), sodium hydroxide (NaOH), and toluene. The irritants were applied daily for two weeks to shaved back skin of young guinea pigs. Barrier creams were applied to the test animals two hours before and immediately after exposure to the irritant. Control animals were treated with the irritant only. Erythema was measured visually, and by bioengineering methods: laser Doppler flowmetry and transepidermal water loss. One barrier cream was effective against SLS and toluene, whereas the other tested was not. In a follow-up study, another allegedly protective cream failed to inhibit irritation caused by SLS and toluene and exaggerated irritation to NaOH, contrary to its recommended use (8). The RIT is proposed as an animal model to test the efficacy of barrier creams, and a human version, described below, has also been proposed.

Repeat application patch tests have been developed to rank the irritant potential of products. Putative irritants are applied to the same site for 3 to 21 days, under occlusion. The degree of occlusion influences percutaneous penetration, which may in turn influence the sensitivity of the test. Patches used vary from Draize-type gauze dressings to metal chambers. Therefore, a reference irritant material is often included in the test to facilitate interpretation of the results. Various animal species have also been used, such as the guinea pig and the rabbit (9,10). Wahlberg measured skinfold thickness with Harpenden calipers to assess the edema-producing capacity of chemicals in guinea pigs. This model showed clear dose–response relationships and discriminating power, except for acids and alkalis where no change in skinfold thickness was found.

Open application assays are also used for repeat irritation testing. Marzulli and Maibach (11) described a cumulative irritation assay in rabbits that uses open applications and control reference compounds. The test substances are applied 16 times over a three-week period, and the results are measured with a visual score for erythema and skin thickness measurements. These two parameters correlated highly. A significant correlation was also shown between the scores of 60 test substances in the rabbit and in man, suggesting that the rabbit assay is a powerful predictive model.

Anderson et al. (12) used an open application procedure in guinea pigs to rank weak irritants. A baseline response to SLS solution was obtained after three applications per day for three days to a 1 cm^2 test area. This baseline is used to compare other irritants, of which trichloroethane was the most irritant, similar to 2% SLS. Histology showed a mononuclear dermal inflammatory response.

Immersion Assay

The guinea pig immersion assay was developed to assess the irritant potential of aqueous surfactant-based solutions, but might be extended to other occupational settings such as aqueous cutting fluids. Restrained guinea pigs are immersed in the test solution while maintaining their head above water. The possibility of systemic absorption of a lethal dose restricts the study to products of limited toxic potential. Therefore, the test concentration is usually limited to 10%.

Ten guinea pigs are immersed in a 40°C solution for four hours daily for three days. A comparison group is immersed in a reference solution. Twenty-four hours after the final immersion, the animals' flanks are shaved and evaluated for erythema, edema, and fissures (13–16). Gupta et al. (17) concomitantly tested the dermatotoxic effects of detergents in guinea pigs and humans, using the immersion test and the patch test, respectively. Epidermal erosion and a 40% to 60% increase in the histamine content of the guinea pig skin were found, in addition to a positive patch test reaction in seven of eight subjects.

Mouse Ear Model

Uttley and Van Abbe (18) applied undiluted shampoos to one ear of mice daily for four days, visually quantifying the degree of inflammation as vessel dilatation, erythema, and edema. Patrick and Maibach (19) measured ear thickness to quantify the inflammatory response to surfactant-based products and other chemicals. This allowed quantification of dose–response relationships and comparison of chemicals. Inoue et al. (20) used this model to compare the mechanism of mustard oil-induced skin inflammation to the mechanism of capsaicin-induced inflammation. Mice were pretreated with various receptor antagonists, such as 5-HT_2, Hi, and tachykinin antagonists, showing that the tachykinin NK1 receptor was an important mediator of inflammation induced by mustard oil. The mouse models provide simplicity and objective measurements. Relevance for man requires elucidation.

Other Methods

Several other assays of skin irritation have been suggested. Humphrey (21) quantified the amount of Evans blue dye recovered from rat skin after exposure to skin irritants. Trush et al. (22) used myeloperoxidase in polymorphonuclear leukocytes as a biomarker for cutaneous inflammation.

HUMAN MODELS

Human models for skin irritation testing are species relevant, thereby eliminating the precarious extrapolation of animal and in vitro data to the human setting. As the required test area is small, several products or concentrations can be tested simultaneously and compared. Inclusion of a reference irritant substance facilitates interpretation of the irritant potential of the test substances. Prior animal or in vitro studies, depending on model relevance and regulatory issue, can be used to exclude particularly toxic substances or concentrations before human exposure.

Single-Application Patch Testing

The National Academy of Sciences (NAS) (23) outlined a single-application patch test procedure determining skin irritation in humans. Occlusive patches may be applied to the intrascapular region of the back or the volar surface of the forearms, using a relatively nonocclusive tape for new or volatile materials. More occlusive tapes or chambers generally increase the severity of the responses. A reference material is included in each battery of patches.

The exposure time may vary to suit the study. NAS suggests a four-hour exposure period, although it may be desirable to test new or volatile materials for 30 minutes to 1 hour. Studies longer than 24 hours have been performed. Skin responses are evaluated 30 minutes to 1 hour after removal of the patch, using the animal Draize scale (Table 2) or similar. Kligman and Wooding (24) described statistical analysis on test data to calculate the IT50 (time to produce imitation in 50% of the subjects) and the ID50 (dose required to produce irritation in 50% of the subjects after a 24-hour exposure).

Robinson et al. (25) suggested a four-hour patch test as an alternative to animal testing. Assessing erythema by visual scoring, they tested a variety of irritants on Caucasians and Asians. A relative ranking of irritancy was obtained using 20% SLS as a benchmark. Taking this model further, McFadden et al. (26) investigated the threshold of skin irritation in the six different skin types. Again using SLS as a benchmark, they defined the skin irritant threshold as the lowest concentration of SLS that would produce skin irritation under the four-hour occluded patch conditions. They found no significant difference in irritation between the skin types.

Cumulative Irritation Testing

Lanman et al. (27) and Phillips et al. (9) described a cumulative irritation assay, which has become known as the "21-day" cumulative irritation assay. The purpose of the test was to screen new formulas before marketing. A one-inch square of Webril was saturated with a liquid of 0.5 g of viscous substances and applied to the surface of the pad to be applied to the skin. The patch was applied to the upper back and sealed with occlusive tape. The patch was removed after 24 hours, and then reapplied after examination of the test site. This was repeated for 21 days and the IT50 could then be calculated. Note that the interpretation of the data is best done by comparing the data to an internal standard for which human clinical experience exists.

Modifications have been made to this method. The chamber scarification test (see the following) was developed to predict the effect of repeated applications of a potential irritant to damaged skin, rather than healthy skin. The cumulative patch test described above had failed to predict adverse reactions to skin damaged by acne or shaving, or sensitive areas such as the face (28).

Wigger-Alberti et al. (29) compared two cumulative models by testing skin reaction to metalworking fluids (MWF). Irritation was assessed by visual scoring, transepidermal water loss, and chromametry. In the first method, MWF were applied with Finn Chambers® on the volunteers' midback, removed after one day of exposure, and reapplied further for two days. In the second method, cumulative irritant contact dermatitis was induced using a repetitive irritation test for two weeks (omitting weekends) for six hours per day. The three-day model was preferred because of its shorter duration and better discrimination of irritancy.

For low-irritancy materials in which discrimination is not defined with visual and palpatory scores, bioengineering methods (i.e., transepidermal water loss) may be helpful.

The Chamber Scarification Test

This test was developed (30,31) to test the irritant potential of products on damaged skin. Six to eight 1-mm sites on the volar forearm were scratched eight times with a 30-gauge needle without causing bleeding. Four scratches were parallel and the other four are perpendicular to these. Duhring chambers, containing 0.1 g of test materials (ointments, creams, or powders), were then placed over the test sites. For liquids, a saturated fitted pad (0.1 mL) may be used. Chambers containing fresh materials are reapplied daily for three days; the sites are evaluated by visual scoring 30 minutes after removal of the final set of chambers. A scarification index may be calculated if both normal and scarified skins are tested to reflect the relative degree of irritation between compromised and intact skins; this is the score of scarified sites divided by the score of intact sites. However, the relationship of this assay to routine use of substances on damaged skin remains to be established. Another compromised skin model, the arm immersion model of compromised skin, is described in the following immersion tests section.

The Soap Chamber Test

Frosch and Kligman (32) proposed a model to compare the potential of bar soaps to cause "chapping." Standard patch testing was able to predict erythema, but unable to predict the dryness, flaking, and fissuring seen clinically. In this method, Duhring chambers fitted with Webril® pads were used to apply 0.1 mL of an 8% soap solution to the human forearm. The chambers were secured with porous tape, and applied for 24 hours on day 1. On days 2 to 5, fresh patches were applied for six hours. The skin is examined daily before patch application and on day 8, the final study day. No patches are applied after day 5. Applications were discontinued if severe erythema was noted at any point. Reactions were scored on a visual scale of erythema, scaling, and fissures. This test correlated well with skin-washing procedures, but tended to overpredict the irritancy of some substances (33).

Immersion Tests

These tests of soaps and detergents were developed to improve irritancy prediction by mimicking consumer use. Kooyman and Snyder (34) describe a method in which soap solutions of up to 3% are prepared in troughs. The temperature was maintained at 105 °F while subjects immersed one hand and forearm in each trough, comparing different products (or concentrations). The exposure period ranged from 10 to 15 minutes, three times each day for five days, or until irritation was observed in both arms. The antecubital fossa was the first site to show irritation, followed by the hands (6,34). Therefore, antecubital wash tests (see the following) and hand immersion assays were developed (5).

Clarys et al. (35) used a 30-minute/four-day immersion protocol to investigate the effects of temperature as well as anionic character on the degree of irritation caused by detergents. The irritation was quantified by assessing the stratum corneum barrier function (transepidermal water loss), skin redness (a^* color parameter), and

skin dryness (capacitance method). Although both detergents tested significantly affected the integrity of the skin, higher anionic content and temperature increased the irritant response.

Allenby et al. (36) describe the arm immersion model of compromised skin, which is designed to test the irritant or allergic potential of substances on damaged skin. Such skin may show an increased response, which may be negligible or undetectable in normal skin. The test subject immersed one forearm in a solution of 0.5% sodium dodecyl sulfate for 10 minutes, twice daily until the degree of erythema reached 1 to 1+ on visual scale. This degree of damage corresponded to a morning's wet domestic work. Patch tests of various irritants were applied to the dorsal and volar aspects of both the pretreated and untreated forearms, and also to the back. Each irritant produced a greater degree of reaction on the compromised skin.

Wash Tests

Hannuksela and Hannuksela (37) compared the irritant effects of a detergent in use testing and patch testing. In this study of atopic and nonatopic medical students, each subject washed the outer aspect of the one forearm with liquid detergent for one minute, twice daily for one week. Concurrently, a 48-hour chamber patch test of five concentrations of the same detergent was performed on the upper back. The irritant response was quantified by bioengineering techniques: transepidermal water loss, electrical capacitance, and skin blood flow. In the wash test, atopies and nonatopics developed irritant contact dermatitis equally, whereas atopies reacted more readily to the detergent in chamber tests. The disadvantage of the chamber test is that, under occlusion, the detergent can cause stronger irritation than it would in normal use (38). Although the wash test simulates normal use of the product being tested, its drawback is a lack of standard guidelines for performing the test. Charbonnier et al. (39) included squamometry in their analysis of a hand-washing model of subclinical irritant dermatitis with SLS solutions. Squamometry showed a significant difference between 0.1% and 0.75% SLS solutions whereas visual, subjective, capacitance, transepidermal water loss, and chromametry methods were unable to make the distinction. Charbonnier suggests squamometry as an adjunct to the other bioengineering methods. Frosch (33) describes an antecubital washing test to evaluate toilet soaps, using two washing procedures per day. Simple visual scoring of the reaction (erythema and edema) allows products to be compared. This comparison can be in terms of average score, or number of washes required to produce an effect.

Assessing Protective Barriers

Zhai et al. (40) proposed a model to evaluate skin protective materials. Ten subjects were exposed to the irritants, SLS and ammonium hydroxide (in urea), and Rhus allergen. The occluded test sites were on each forearm, with one control site on each. The irritant response was assessed visually using a 10-point scale, which included vesiculation and maceration unlike standard Draize scales. The scores were statistically analyzed for nonparametric data. Of the barrier creams studied, paraffin wax in cetyl alcohol was found to be the most effective in preventing irritation.

Wigger-Alberti and Eisner (41) investigated the potential of petrolatum to prevent epidermal barrier disruption induced by various irritants in a repetitive irritation test. White petrolatum was applied to the backs of 20 human subjects who

were exposed to SLS, NaOH, toluene, and lactic acid. Irritation was assessed by transepidermal water loss and colorimetry in addition to visual scoring. It was concluded that petrolatum was an effective barrier cream against SLS, NaOH, and lactic acid, and moderately effective against toluene.

Frosch et al. (42) adapted the guinea pig RIT previously described for use in humans. Two barrier creams were evaluated for their ability to prevent irritation to SLS. In this repetitive model, the irritant was applied to the ventral forearm, using a glass cup, for 30 minutes daily for two weeks. One arm of each subject was pretreated with a barrier cream. As in the animal model, erythema was assessed by visual scoring, laser Doppler flow, and transepidermal water loss. Skin color was also measured by colorimetry (La^* value). The barrier cream decreased skin irritation to SLS, the most differentiating parameter being transepidermal water loss and the least differentiating being colorimetry.

Bioengineering Methods in Model Development

Many of the models previously described do not use the modern bioengineering techniques available, and therefore data based on these models may be imprecise. Despite the skill in investigations, subjective assessment of erythema, edema, and other visual parameters may lead to confusion by inter- and intraobserver variation. Although the eye may be more sensitive than current spectroscopy and chromametric techniques, the reproducibility and increased statistical power of such data may provide greater benefit. A combination of techniques, such as transepidermal water loss, capacitance, ultrasound, laser Doppler flowmetry, spectroscopy, and chromametric analysis, in addition to skilled observation may increase the precision of the test. Andersen and Maibach (43) compared various bioengineering techniques, finding that clinically indistinguishable reactions induced significantly different changes in barrier function and vascular status. An outline of many of these techniques is provided by Patil et al. (5).

REFERENCES

1. Weltfriend S, Bason M, Lammintausta K, Maibach HI. Irritant dermatitis (irritation). In: Marzulli FN, Maibach HI, eds. Dermatotoxicology. 5th ed. Washington, D.C.: Taylor Francis, 1996.
2. Draize TH, Woodland G, Calvery HO. Methods for the study of irritation and toxicity of substances applied to the skin and mucous membranes. J Pharmacol Exp Ther 1944; 82:377–390.
3. Code of Federal Regulations. Office of the Federal Registrar, National Archive of Records, General Services Administration, 1985, title 16, parts 1500.40–1500.42.
4. Patrick E, Maibach HI. Comparison of the time course, dose response and mediators of chemically induced skin irritation in three species. In: Frosch PJ et al., eds. Current Topics in Contact Dermatitis. Springer-VerlagNew York1989399–402.
5. Patil SM, Patrick E, Maibach HI. Animal, human and in vitro test methods for predicting skin irritation. In: Marzulli FN, Maibach HI, eds. Dermatotoxicology Methods: The Laboratory Worker's Vade Mecum. Washington, D.C.: Taylor & Francis, 1998:89–104.
6. Justice JD, Travers JJ, Vinson LJ. The correlation between animal tests and human tests in assessing product mildness. Proc Sci Sec Toilet Goods Assoc 1961; 35:12–17.
7. Frosch PJ, Schulze-Dirks A, Hoffmann M, Axthelm I, Kurte A. Efficacy of skin barrier creams (I) The repetitive irritation test (RIT) in the guinea pig. Contact Dermatitis 1993; 28(2):94–100.

8. Frosch PJ, Schulze-Dirks A, Hoffmann M, Axthelm I. Efficacy of skin barrier creams (II). Ineffectiveness of a popular "skin protector" against various irritants in the repetitive irritation test in the guinea pig. Contact Dermatitis 1993; 29(2):74–77.
9. Phillips L, Steinberg M, Maibach HI, Akers WA. A comparison of rabbit and human skin responses to certain irritants. Toxicol Appl Pharmacol 1972; 21:369–382.
10. Wahlberg JE. Measurement of skin fold thickness in the guinea pig. Assessment of edema-inducing capacity of cutting fluids acids, alkalis, formalin and dimethyl sulfoxide. Contact Dermatitis 1993; 28:141–145.
11. Marzulli FN, Maibach HI. The rabbit as a model for evaluating skin irritants: a comparison of results obtained on animals and man using repeated skin exposure. Food Cosmet Toxicol 1975; 13:533–540.
12. Anderson C, Sundberg K, Groth O. Animal model for assessment of skin irritancy. Contact Dermatitis 1986; 15:143–151.
13. Opdyke DL, Burnett CM. Practical problems in the evaluation of the safety of cosmetics. Proc Sci Sec Toilet Goods Assoc 1965; 44:3–4.
14. Calandra J. Comments on the guinea pig immersion test. CTFA Cosmet J 1971; 3(3):47.
15. Opdyke DL. The guinea pig immersion test—a 20 year appraisal. CTFA Cosmet J 1971; 3(3):46–47.
16. MacMillan FSK, Ram RR, Elvers WB. A comparison of the skin irritation produced by cosmetic ingredients and formulations in the rabbit, guinea pig, beagle dog to that observed in the human. In: Maibach HI, ed. Animal Models in Dermatology. Edinburgh: Churchill Livingstone, 1975:12–22.
17. Gupta BN, Mathur AK, Srivastava AK, Singh S, Singh A, Chandra SV. Dermal exposure to detergents. Veterinary Human Toxicol 1992; 34(5):405–407.
18. Uttley M, Van Abbe NJ. Primary irritation of the skin: mouse ear test and human patch test procedures. J Soc Cosmet Chem 1973; 24:217–227.
19. Patrick E, Maibach HI. A novel predictive assay in mice. Toxicologist 1987; 7:84.
20. Inoue H, Asaka T, Nagata N, Koshihara Y. Mechanism of mustard oil-induced skin inflammation in mice. Eur J Pharmacol 1997; 333(2,3):231–240.
21. Humphrey DM. Measurement of cutaneous microvascular exudates using Evans blue. Biotech Histochem 1993; 68(6):342–349.
22. Trush MA, Egner PA, Kensler TW. Myeloperoxidase as a biomarker of skin irritation and inflammation. Food Chem Toxicol 1994; 32(2):143–147.
23. National Academy of Sciences. Committee for the Revision of NAS Publication 1138. Principles and Procedures for Evaluating the Toxicity of Household Substances. Washington, D.C.: National Academy of Sciences, 1977:23–59.
24. Kligman AM, Wooding WM. A method for the measurement and evaluation of irritants on human skin. J Invest Dermatol 1967; 49:78–94.
25. Robinson MK, Perkins MA, Basketter DA. Application of a 4-h human patch test method for comparative and investigative assessment of skin irritation. Contact Dermatitis 1998; 38(4):194–202.
26. McFadden JP, Wakelin SH, Basketter DA. Acute irritation thresholds in subjects with type I-type VI skin. Contact Dermatitis 1998; 38(3):147–149.
27. Lanman BM, Elvers WB, Howard CS. The role of human patch testing in a product development program. In: Proc. Joint Conf Cosmetic Sciences. Washington, D.C.: Toilet Goods Association, 1968:135–145.
28. Battista CW, Rieger MM. Some problems of predictive testing. J Soc Cosmet Chem 1971; 22:349–359.
29. Wigger-Alberti W, Hinnen U, Eisner P. Predictive testing of metalworking fluids: a comparison of 2 cumulative human irritation models and correlation with epidemiological data. Contact Dermatitis 1997; 36(l):14–20.
30. Frosch PJ, Kligman AM. The chamber scarification test for irritancy. Contact Dermatitis 1976; 2:314–324.

31. Frosch PJ, Kligman AM. The chamber scarification test for testing the irritancy of topically applied substances. In: Drill VA, Lazar P, eds. Cutaneous Toxicity. New York: Academic Press, 1977:150.
32. Frosch PJ, Kligman AM. The soap chamber test. A new method for assessing the irritancy of soaps. J Am Acad Dermatol 1979; 1(1):35–41.
33. Frosch PJ. The irritancy of soap and detergent bars. In: Frost P, Howitz SN, eds. Principles of Cosmetics for the Dermatologist. St. Louis: C.V. Mosby, 1982:1–12.
34. Kooyman DJ, Snyder FH. The test for mildness of soaps. Arch Dermatol Syphilol 1942; 46:846–855.
35. Clarys P, Manou I, Barel AO. Influence of temperature on irritation in the hand/forearm immersion test. Contact Dermatitis 1997; 36(5):240–243.
36. Allenby CF, Basketter DA, Dickens A, Barnes EG, Brough HC. An arm immersion model of compromised skin (I). Influence on irritation reactions. Contact Dermatitis 1993; 28(2):84–88.
37. Hannuksela A, Hannuksela M. Irritant effects of a detergent in wash, chamber and repeated open application tests. Contact Dermatitis 1996; 34(2):134–137.
38. Van der Valk PG, Maibach HI. Post-application occlusion substantially increases the irritant response of the skin to repeated short-term sodium lauryl sulfate (SLS) exposure. Contact Dermatitis 1989; 21(5):335–338.
39. Charbonnier V, Morrison BM Jr, Paye M, Maibach HI. Open application assay in investigation of subclinical dermatitis induced by sodium lauryl sulfate (SLS) in man: advantage of squamometry. Skin Res Technol 1998; 4:244–250.
40. Zhai H, Willard P, Maibach HI. Evaluating skin-protective materials against contact irritants and allergens. An in vivo screening human model. Contact Dermatitis 1998; 38(3):155–158.
41. Wigger-Alberti W, Eisner P. Petrolatum prevents irritation in a human cumulative exposure model in vivo. Dermatology 1997; 194(3):247–250.
42. Frosch PJ, Schulze-Dirks A, Hoffmann M, Axthelm I, Kurte A. Efficacy of skin barrier creams (I). The repetitive irritation test (RIT) in the guinea pig. Contact Dermatitis 1993; 28(2):94–100.
43. Andersen PH, Maibach HI. Skin irritation in man: a comparative bioengineering study using improved reflectance spectroscopy. Contact Dermatitis 1995; 33(5):315–322.

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General Concepts of Skin Irritancy and Anti-Irritant Products

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INTRODUCTION

Serious adverse reactions to cosmetic ingredients and preparations are actually infrequent. However, side effects do occur and are by no means rare. The unwanted effects of cosmetics can be classified into the following different categories (1–4):

1. Irritation and contact urticaria
2. Contact allergy
3. Photosensitive reaction (photoallergy and photoirritation)
4. Acnegenesis and comedogenesis
5. Color changes of the skin and appendages
6. Systemic side effects
7. Other local side effects

When considering skin-irritation symptoms, we are dealing with nonimmunological mediated inflammation of the skin induced by external agents. Chemicals irritants are the major cause, but mechanical, thermal, climatic, and UV and IR light are also important factors or cofactors of irritancy (5). This nonimmunological skin irritancy reaction comprises two forms: the acute irritant reaction with a monofactorial cause (detergent, acid, oxidant, etc.) and the chronic multifactorial form. The symptoms of skin-irritation are well known: erythema, dryness, scaling, itching, burning, and tingling. These clinical symptoms are described by some authors as objective irritation (1–4). Because these symptoms are clearly perceptible, in vivo testing in humans can easily and reliably detect strong and moderate irritant ingredients and eliminate these potential hazards. However most cosmetic-use ingredients do not produce acute irritation from a single exposure because they are mild or very mild and consequently difficult to detect. Nevertheless, they may produce inflammation after repeated application on the same area of the skin, which is referred to as cumulative irritation.

On the application of a cosmetic, when symptoms such as burning, stinging, or itching, without detectable visible or microscopic changes appear, it is designated as

a subjective irritation or subclinical irritation, (2–4). This reaction is common in certain sensitive individuals (a majority being women), occuring most frequently on the face. These persons can be considered as presenting a "sensitive skin." Attempts to identify these very reactive subjects by association with other skin problems such as atopy, or with phototype or extreme skin dryness have not been very fruitful (6).

Among the potential adverse reactions of cosmetic ingredients and products, such as irritant contact dermatitis, immediate contact reaction (urticaria), allergic contact dermatitis, and acnegenesis and comedogenesis, we will consider the adverse reactions of irritancy in particular.

It is the purpose of this chapter to:

1. describe shortly the different symptoms of irritancy and how to evaluate skin irritants by clinical, visual, and tactile assessments, by noninvasive bioengineering measurements, and by self-perception of skin-irritation;
2. give a short overview of the different chemical ingredients, which are potential cosmetic and occupational skin irritants;
3. give an overview of the different possibilities of conceiving anti-irritant cosmetics and treatments.

IRRITANCY AND SKIN IRRITANT EVALUATION AND SYMPTOMS

Methods to evaluate skin alteration induced by topical products can be classified into three categories (7), which are as follows:

1. Clinical, visual, and tactile assessments.
2. Instrumental noninvasive bioengineering measurements.
3. Self-perception by the subjects themselves.

Clinical, Visual, and Tactile Assessments

Several skin modifications induced by irritants can be easily evaluated visually and tactilely, e.g., by skin redness (erythema), skin dryness with increased desquamation, scaliness and flakiness, and skin roughness or edema.

Moderate–to–very intense signs of skin redness/erythema are the visual manifestation of a skin inflammatory process with vasodilatation of the capillary system and increase of the blood flow.

After contact with an irritant (particularly with soaps and detergents), the skin shows symptoms of dryness after a certain time with a whitish appearance, flakiness, scaliness, and roughness. In the most severe cases of irritation, fissuring and cracking can also appear.

Edema is the result of an accumulation of fluid from the blood vessels in the upper dermis. It appears only in very severe cases of irritancy, which happens very rarely except in experimental conditions.

The visual and tactile assessments of irritancy are made by dermatologists or trained evaluators. These observations remain always subjective in nature, even with trained evaluators and well-standardized clinical, experimental, and well-established scoring grades. However, the clinical assessments are precise and very reproducible.

Instrumental Noninvasive Bioengineering Measurements

Many changes in skin properties induced by irritant cosmetic ingredients can be evaluated quantitatively in a noninvasive manner by using instrumental techniques.

In this section, the following techniques will be described: (i) skin redness by reflectance skin colorimetry and by Laser Doppler flowmetry; (ii) alterations in the integrity of the barrier function by measuring transepidermal water loss; (iii) skin hydration measurements using electrical impedance and squamometry; and (iv) other bioengineering methods that are based on the skin's elasticity and microrelief.

Skin Redness/Erythema by Measuring Skin Color

Most color measurements of the skin surface are based on reflectance colorimetry instruments, such as tristumulus color analysis, Chromameter Minolta, erythema index, Erythemameter Diastron, Mexameter® Courage-Khazaka and Dermaspectrometer® Cortex (8–10).

The Minolta Chromameter CR-200 and CR-300, considered by many as a sort of reference instrument, quantifies skin surface color using the three-dimensional CIE color representation with the $L^*a^*b^*$ system. Skin redness is readily evaluated by means of the a^* values; erythema is always characterized by an increase in the a^* skin color parameter.

Other simpler reflectance meters (Erythemameter Diastron, Mexameter Courage-Khazaka, and Dermaspectrometer Cortex) are also used (9,11). These instruments are based on the same optical principle, namely measurements of light absorption and reflection by the melanin and hemoglobin components of the skin. The specific absorption of melanin and hemoglobin in the visible (green and red) and in the near-infrared region is determined, and these instruments quantify redness by a relative erythema index. The erythema index is proportional to the hemoglobin content of the upper layers of the dermis. Excellent correlations have been shown between visual clinical scoring and erythema, and the chromameter measurements of the a^* color parameter (12). Furthermore, reasonably good correlations were noticed between the a^* chromameter parameter and the erythema index of the simple reflectance meters (9,13).

Measurement of Superficial Blood Flux by Laser Doppler Flowmetry

The hemoglobin of the red blood cells of the upper-dermis microcirculation system partially absorb the light of a helium laser beam. The laser Doppler method measures the shift in frequency of the reflected light of this laser beam. This small frequency shift is proportional to the number and the speed of red blood cells present in the superficial blood microcirculation system. An inflammatory reaction with vasodilatation of the capillaries will produce a marked increase in blood flow (14). There are two types of laser Doppler instruments: the first generation flowmeters, which measure the blood flux of a small spot area of the skin (2–3 mm^2), (Servomed, Sweden, Lisca, Sweden, and Moor, United Kingdom); and more recently the development of the latest generation of Doppler instruments, namely the laser Doppler imaging instruments, which has enabled the two-dimensional quantitative measurement of blood microcirculation of a much larger skin area (maximum 10 cm^2) (15). Good correlations were found between the clinical assessments of irritancy such as the skin color and the respective noninvasive bioengineering methods such as laser Doppler flowmetry (16).

Alterations in the Integrity of the Barrier Function

When some irritant cosmetic ingredient comes in contact with the skin, the earliest modification in the skin structure is an alteration of the lipidic barrier structure of

the stratum corneum (17). The physiological function of this barrier is to protect the skin from the penetration of irritants, and to assure low insensible perspiration of the skin [transepidermal water loss (TEWL)]. When the barrier function of the skin is altered by an irritant, the amount of water vapor passing through the stratum corneum is increased, which is characterized by an increase in TEWL. Modern TEWL instruments are very sensitive; the slightest alterations of the barrier function can be measured with this technique: skin-irritation almost not visible ("nonvisible" subclinical irritation). This happens mostly when extremely mild cosmetic ingredients are tested or when normal use applications protocols are considered (18).

Alterations in the Skin Surface Hydration

The assessment of the hydration status of the superficial layers of the epidermis is important for characterizing the skin. The hydration level of the stratum corneum is maintained more or less constant, which is due to the following mechanisms: (1) hydration coming from the deeper layers of the fully hydrated viable epidermis, and retarded in the stratum corneum by the lipids from the hydrolipidic barrier; (2) hydration due to equilibrium with the external ambient humidity; and (3) the presence of entrapped water bound to the natural moisturing factors (NMF) present in the layers of the stratum corneum. When an irritant cosmetic ingredient, such as a surfactant, interacts with the skin surface, it partially or completely removes the lipidic film coating the surface of these and extracts some NMF components, altering the equilibrium mechanism of the hydration of the skin surface. Such a dehydration of the horny layer will have many different consequences, such as (1) an increase in the desquamation rate of corneocytes, giving the skin a scaly aspect, (2) a modification of the relief of the skin, giving it a rough and wrinkled appearance, and (3) modifications in the visco-elastic properties of the stratum corneum. The modifications in the hydration level of the stratum corneum have been extensively investigated using bioengineering methods based on the electrical impedance of the skin to an alternating current (19). Many commercial instruments are used to measure the electrical properties of the skin.These instruments work based on the principles such as capacitance, impedance, and conductance. The measured electrical properties of the superficial layers of the epidermis (impedance–capacitance units or arbitrary electrical units) are indirectly related to the amount of water present in the horny layer. When used under standard conditions and in thermostatized experimental rooms, all the instruments are able to provide highly accurate and reproducible hydration values. Excellent correlations were obtained between the visual scoring of skin dryness induced by surfactants in a soap chamber test and instrumental readings (20).

Skin-Surface Stripping Tests

The investigation of skin-surface alterations has made great progress owing to the development and use of skin-surface stripping systems. The superficial layers of the stratum corneum can be easily collected, without any damage to the viable epidermis, simply by pressing a sticky tape on the skin (D-Squames®). A few seconds after removing the sticky tape, several layers of corneocytes can be collected and analyzed. The level of desquamation can be quantified by squamometry, which is staining of the corneocytes and measuring the amount of color (21). The degree of cohesion between the corneocytes can be measured by visual scoring using a microscope, and by image analysis. With some surfactants, no clinical irritation could be observed; however, they induce significant changes at the surface of the stratum

corneum as shown by an increase of the amount of harvested corneocytes and a deorganization/loss of the intercorneocyte cohesion (21,22).

Other Noninvasive Bioengineering Methods

Other methods are available to measure some symptoms of skin irritancy, but will not be described in this chapter. Skin dryness and roughness as induced by some irritants can be evaluated by the following techniques: (i) measurement of the viscoelastic properties of the upper layers of the epidermis (23), and (ii) studying the skin-surface microrelief (24–26).

Self-Perception of Skin-Irritation

Generally, when a finished cosmetic product comes into contact with the skin of potential consumers, it is very unlikely that observable signs of irritation are noticed in normal use. However, the overall perception of the finished product by the consumer is an important criterion for accepting its cosmetic use. In this global perception, many different parameters may play a role, some independent of the potential irritancy of ingredients, such as feeling of aesthetic nature, ease of spreading on the skin, viscosity, perfume, and color. However, the subjective perception of skin-feel is closely related to the composition of the cosmetic product. Skin-feel attributes of dryness (feels tight, rough, and dry), irritation (itching and burning), softness, and smoothness are easily perceived by the subjects. In most cases, the subjects are able to perceive very early on the effects of some cosmetics on the skin—well before they become clinically observable or measurable by bioengineering techniques.

The assessment of the self-perception of the interaction between some cosmetic ingredients with the stratum corneum is performed by means of questionnaires, where several skin attributes are evaluated. Some questionnaires are designed to receive a "YES or NO" answer to each of the attributes, or the subject will have to rate each of the attributes on a 0 to 10 point scale.

FACTORS THAT INFLUENCE SKIN RESPONSIVENESS TO IRRITANTS

Many factors can influence the responsiveness of a consumer's skin to a potential irritant. Some factors are intrinsic, inherent to the subjects themselves (e.g., sensitive skin, atopic skin), the body site, and previous traumas to the considered skin area. Other factors are external, such as the composition of product, conditions of exposure, occupation of the subject, and climatic factors (4,5,7). The reason why these factors are covered in this chapter is obvious. Some cosmetics with anti-irritant ingredients are designed for some specific skin sites, such as the face; or considered as seasonal products, such as cosmetics against winter dryness of the skin.

Factors inherent to the constitution of the skin of the subjects that may influence skin responsiveness are numerous. A marked interindividual variability in response to irritants has been reported and ascribed to host-related factors. Regarding the interindividual variability of subjects to skin irritants, one must mention here the concept of "sensitive skin." The term "sensitive skin" has clearly a different meaning for consumers than cosmetic scientists and dermatologists (4,6). Consumers use the term "sensitive skin" to indicate that their skin readily experiences adverse reactions to or unwanted changes due to external factors, such as the use of personal

care products. Subjects with "sensitive skin" tend to more readily develop skin reactions to cosmetics and other topical drugs than do normal persons. Many attempts have been made by cosmetic scientists and dermatologists to describe and demonstrate in a scientific way what "sensitive skin" is. Visible effects, such as erythema and skin dryness, are noticed. However, half of the adverse reactions are purely sensory perceptions, subjective symptoms of stinging, itching, burning, and feeling of dryness with lack of visible effects.

Regional Differences in the Sensitivity of Normal Skin

It has been clearly demonstrated that when measuring the potential irritancy of cosmetic ingredients, great regional differences in the sensitivity of normal skin are observed (27,28). Several factors must be considered in order to explain the observed regional differences in skin sensitivity, such as differences in total skin thickness, skin permeability, the amount and composition of epidermal and sebaceous lipids, blood microcirculation, hydration level of the horny layer, thickness of the horny layer and desquamation rate, and local daily exposure to irritants products. Most skin-irritation phenomena are noticed in the face.

Influence of Gender, Age, and Ethnic Group

Contradictory data are presented in the scientific literature about the influence of ethnic group on skin sensitivity (29). It has been demonstrated that the irritant response may be higher in babies and children, and may decrease with age (30). Regarding the gender related variations in the skin's sensitivity to irritants, many studies appear to show that women are more reactive than men (31,32). However, this difference could be due to the fact that women are more exposed to household chemicals and more frequently use face care cosmetics rather than the real physiological differences.

Other factors are external to the subject, such as composition of their usual products, conditions of exposure, occupation of the subject, and climatic factors.

Mode of Exposure of the Product on the Skin

Acute skin exposures of a very irritant chemical cosmetic ingredient are very rare and attributable to accidents, inappropriate use, or problems in the manufacturing of the cosmetic product. The list of very irritant products are known and must be totally avoided, or used at very low concentrations. We will be dealing mostly with subacute and chronic exposure of the skin. Subacute exposures will provoke an immediate impairment of the skin barrier. Repeated exposures to certain cosmetic products with very limited impairment of the skin barrier can induce cutaneous reactions after a certain time.

Climatic Factors

There is clearly a seasonal or climatic effect on the amplitude of the skin-irritation reaction. Generally, much higher irritation reactions are observed in winter than in summer. This difference is related to a dehydration factor: a situation of dryness of the horny layer, provoked by ambient air with very low relative humidity. This situation is particularly present on the lower legs more and frequent in older subjects: typical symptoms of winter xerosis, i.e., extreme dryness, scaling, and rough skin surface. Furthermore, in winter, the epidermis is intensely affected by extreme

temperature changes between the inside and outside world. In summer, the upper layers of the epidermis are well hydrated, the skin is smooth, unless it is excessively exposed to sun damage. Actinic aging of the skin is characterized by various clinical symptoms, including dryness of the skin.

COSMETIC AND OCCUPATIONAL IRRITANTS

Occupational Skin Irritants

A broad definition of occupational contact irritant dermatitis is contact dermatitis caused wholly or partially by the occupation of the subject. Occupational irritants may cause an acute response that may take from 1 hour to 1 day to appear, and is usually traceable to a single factor. Chronic irritant contact dermatitis may take months or years to appear and is often multifactorial (33). Hands are involved in 80% to 90 % of all cases of occupational contact dermatitis, and in a minority of cases the wrists, forearms, lower legs, or face is the primary site.

The clinical features are described as follows. Many cases of occupational irritant contact dermatitis start as erythema and scaling on the back of the joints and adjacent parts of the back of the fingers, as well as in the web spaces between the fingers. A generalized, rather shiny, superficially fissured, scaly fingertip dermatitis is also characteristic of certain forms of irritancy. Exclusive or more severe involvement of the thumb, index finger, and/or the middle finger of the dominant hand (or of their nails) is generally an indication of possible occupational causation (33).

The principal occupational irritants are listed in Table 1.

Table 1 List of Common Irritants Which are Important for Occupational Dermatitis

Skin cleansers	Soaps, detergents, specific cleansers
Industrial cleaning agents	Detergents, emulsifiers, solubilizers, wetting agents, enzymes
Organic solvents	Alkanes, alkenes, halogenalkanes and alkenes, alcohols, ketones, aldehydes, esthers, ethers, toluene, carbon sulfide, petroleum derivates, silicones, etc.
Oils	Cutting oils, metal working fluids, lubricating oils, braking oils, etc.
Acids	Severe irritants are sulfuric, chromic, nitric, chlorhydric, hyperchloric, fluorhydric, and trichloroacetic acids. Milder irritants are formic, acetic, proprionic, oxalic, and salycilic acids
Alkaline substances	Soaps, soda, ammonia, sodium, potassium, and calcium hydroxides, various amines
Oxidizing agents	Hydrogen peroxide and peroxides, benzoyl peroxide, sodium (hypo)chlorate, and bromate
Reducing agents	Phenols, aldehydes (formaldehyde), thioglycolates, hydrazines
Plants	Various plants are potentially irritant but particularly the Euphorbiaceae, Brassicaceae, and Ranunculeae families
Products of animal, plant, and bacterial origin	Food proteins, proteolytic enzymes such as pepsine, papaine, trypsine, subtilisine, etc.
Physical factors	

Source: Adapted from J.P. Frosch, 1995 (5).

Table 2 List of Common Potential Cosmetic Irritant Ingredients

Conservatives/antimicrobials
Antioxidants
Fragrance
Colors
UV filters
Lipids/Emollients
Emulgators, surfactants, and rheological agents
Humectants
Specific cosmetic ingredients such as keratolytic agents, tanning and whitening agents, etc.

Cosmetic Skin Irritants

Cosmetics are complex mixtures of chemical compounds. The abundance of commercially available ingredients has created an endless variety in cosmetic formulation. The cosmetic substances used in cosmetic products may be arbitrarily divided in great categories of product and/or function. The principal categories of cosmetic irritants are listed in Table 2.

Intolerance to some ingredients is related to symptoms of contact dermatitis and allergic dermatitis. A clear distinction between these problems is not always present. Some cosmetic ingredients present both an irritant character with in the additional possibility of allergic reaction. (e.g., cinnamic acid derivatives). An overview of cosmetic categories causing irritant side effects, in the order of their descending importance, has been given by De Groot AC, Weyland JW, and Nater JP (1–3), and are summarized briefly in Table 3. It has clearly been shown that certain categories of cosmetics, taking into account their composition, frequency of use, mode of application on the skin, and the skin area to be treated, are more specific candidates for causing symptoms of skin-irritation.

A short overview of the potential irritant character of each category of cosmetic ingredients will be given. Some chemicals are used in industries (occupational irritants), as well as in the cosmetic world (cosmetic irritants). Chapter 29 describes the irritancy of the most frequent emulgators and detergents, used primarily in cleansing products.

Preservatives/antimicrobials, antioxidants, fragrances, colors, and UV filters are potentially irritant components. However, these components are often present in cosmetic preparations at low concentrations, and thereby do not affect the overall irritation potential of the final product. These substances are more often incriminated for their allergic reactions.

Table 3 Cosmetic Categories Causing Irritant Side Effects[a]

Soap
Deodorant/antiperspirant
Aftershave
Shampoo
Lipstick
Hair dye
Perfume

[a]In descending importance.
Source: Refs. (2,3).

Lipids/Emollients

Most oils and fats are relatively mild. However, some oils from plant origin are incriminated for their allergic reactions. Emulgators, surfactants, and rheological factors are also potentially irritant components. Some surfactants are rather known as irritants. These substances are classified as follows, going from the most irritating to the mildest:

cationics > anionics > amphoterics > nonionics.

In shampoos and body and shower gels or creams, anionic detergents are rarely used alone, but are always used in combination with amphoterics and nonionic surfactants. In creams and milks, nonionic and amphoteric emulgators are essentially used for their mildness.

Humectants

The classical humectants such as NMF are nonirritant. The other humectants such as proteins, hyaluronic acid, chitosan, proteoglycans, and polysaccharides are very rarely the irritant components.

Specific cosmetic ingredients such as keratolytic agents, tanning and whitening agents, etc., can be more irritant.

In the use of alpha hydroxy acid (AHA), irritancy increases with the concentration and with a decrease of pH, which is controlled by the proportion of free acid to AHA salts. Classic alkaline soaps were potential irritants, perhaps because of the rise in the skin pH and induction of skin dryness. Modern synthetic soaps are actually very mild because they are buffered to a neutral or slightly acidic pH, and contain lipids such as emollients and humectants.

Solvents in Aftershave Products and Lotions

The irritancy of these products is easily related to the very high alcohol content (usually more than 50 %) of this category of cosmetics. Alcohol dehydrates the skin, and especially the skin that has been predamaged by the wet or dry shaving products. Modern preparations are much milder, either due to the fact that alcohol is absent or due to lower alcohol concentrations.

STRATEGY OF MAKING ANTI-IRRITANT COSMETICS

Strictly by definition, an "anti-irritant" is an agent which by its presence minimizes the irritating effect of a cosmetic preparation on the skin.

The anti-irritant could reflect all the mechanisms which have an opposed effect to an irritant insult. Hence, the term could reflect actions such as skin calming, soothing and healing, and assisting in the recovery of the skin from an irritation provoked by, e.g., contact with soaps and household cleaning products. As has been demonstrated earlier, irritant reactions are very often associated with inflammation; the so-called anti-irritant effect could eventually also mean alleviation from the inflammatory symptoms that arise shortly after the impairment of the skin barrier. The concept of anti-irritant activity also includes skin protection with barrier creams that decrease the irritant potential of some harmful substances encountered in cases of occupational dermatitis (33). Despite the numerous claims of skincare products for anti-irritant or protective activity, there is some lack of scientific data to substantiate these claims. There is also a lack of suitable standardized clinical protocols to quantify these anti-irritant properties.

The basic principle of development of general anti-irritant cosmetics or cosmetics for sensitive skin is to avoid, as much as possible, any risk of irritation. The safest way is to use well-tolerated chemical compounds for the vehicle, and to use active ingredients without any history of "skin problems." Allergic reactions and skin irritancy are generally provoked by known specific ingredients, mostly fragrances, colors, and preservatives. The easy task is to remove fragrances and coloring agents; hypoallergenic cosmetics minimize the use of or do not contain these ingredients. Actually, a modern trend in cosmetics is to develop specific cosmetics without preservatives. This challenge can be partially answered in cosmetic preparations with none or low water content: oils, fats, water/oil emulsions, and lipogels using some synthetic lipids and/or essential oils with bactericidal properties as preservatives. With aqueous solutions, hydrogels, and oil/water emulsions, this goal is very difficult to achieve and presently not realized, and consequently these types of cosmetics still contain preservatives.

In order to elaborate an anti-irritant cosmetic preparation or cosmetic preparation for sensitive skin, we have the choice of the following possibilities:

1. The vehicle must have a pH close to the natural, slightly acidic pH of the skin (pH around 5.3) or be neutral, therefore avoiding highly alkaline preparations.
2. Strenghten or restore the hydrolipidic barrier function of the skin. As described earlier in this chapter, irritancy reactions are often accompanied by modifications of the structure of the intercellular lipids and water binding capacity, resulting in an increase of TEWL, and consequently a higher penetration rate of irritants. Therefore, anti-irritant preparations should restore the disturbed barrier function, or maintain the barrier function by providing the appropriate lipids to the lipidic film. Modern skin care products contain endogeneous components of the epidermal lipids, such as ceramides and gamma linoleic acid. In a general way, lipids are emollients with soothing capacities.
3. Soothing effect by filmogen compounds. The skin surface is anionic in character. Quaternized derivatives of plant proteins or emollients, which are positively charged, will smooth out the skin surface by a filmogen effect.
4. Irritated skin is very often a partially dehydrated skin. In order to alluviate the symptoms of dehydration, water is brought back to the horny layer by humectants (NMF), or by the occlusive effect of water/oil emulsions, lipogels, or silicone oils.
5. Use of very mild surfactants and emulgators in cosmetic preparations.
   General use of amphoteric and nonionic emulgators in creams/milks and cleansing products. In the preparation of shampoos and shower gels, use of anionic emulsifiers with an adequate carbon chain length, and sufficient degree of ethoxylation in order to reduce irritancy. Another possibilty is to use an adequate mixture of several surfactants. A strong antagonism effect occurs when combining the potential irritant anionic surfactants with amphoterics, nonionic, or even other anionic surfactants, with resultant decreased skin-irritation (7).
6. Use of specific anti-irritant ingredients.
   There are a lot of soothing ingredients in dermatological treatments, which have their origin mainly from plants such as hamamelis, algae, chamomile,

aloe vera, and alpha-bisabolol. Allantoin is a known soothing ingredient. Polysaccharides, proteoglycans, and glycoproteins with filmogen and hydrating properties can provide a feel of less- or nonirritated skin. Polymers, when used at high concentration, have also been demonstrated as reducing the irritation potential of anionic surfactants, essentially by entrapping high quantities of surfactants into micelles in solution (see chapter 23).

7. Sun exposure without UV filters can induce or increase irritant reactions of the skin and accelerate actinic ageing. The cosmetic industry has developed suncare products with very high protection factors which are waterproof, and have a reasonably good cosmetic acceptance. There are sun protection products with active UV filters having the lowest allergenic potential, developed especially for a sensitive skin by using a minimum amount of emulgators and are fragrance free. Modern skincare products for the face contain UV-A and UV-B filters in order to protect the skin against actinic ageing.

IN VIVO STUDIES OF THE ANTI-IRRITATION PROPERTIES OF SOME COSMETIC INGREDIENTS

In vivo evaluation of the anti-irritant and/or anti-inflammatory effect of dermatocosmetic formulations on human skin is usually based on the quantification of the inhibition presented by these products, against an artificially induced contact dermatitis (42). The model irritant for this purpose can be selected out of a wide range of skin-aggravating factors. Irritation of the skin can be provoked after topical application of Peru Balsam (43), solutions of anionic surfactants (44,45), and nicotinates (46,47), after exposure to UV-B radiation (48,49), skin abrasion (50), or tape stripping (51,52). There is clearly a difficulty in identifying the conditions under which these various irritants can be used for inducing a "suitable" irritation. The induced irritation should be great enough to be measurable with good reproducibility and to allow the quantification of its inhibition by the tested products. The anionic surfactant sodium lauryl sulphate (SLS) has lately become the model irritant of choice, used widely for inducing experimental contact dermatitis in anti-irritation protocols (45,53–55), or as a reference irritant in safety tests ranking the skin-irritation potential of soaps and detergents (56–58). The irritant character of SLS is due to different factors such as:

1. Modification of the protein and lipid structure of the stratum corneum [Impairment of the highly ordered bilayers and changes in the fluidity of the lipids (59), swelling of the horny layer that occurs because of protein denaturation and exposure of new water-binding sites of the keratins (54).]

2. Alterations in skin permeability (60) [This surfactant is often used as a pretreatment, in order to enhance the penetration of topically applied products (45).]

3. Vascular inflammatory response (61,62).

SLS is not a sensitizer or carcinogenic agent; it causes no systemic toxicity or permanent cosmetic inconvenience to the skin (45). The great sensitivity of TEWL parameter in quantifying the impairment of the barrier, caused by SLS (63), and the property as a primary irritant has led to the large use of this surfactant in studies of experimental irritant contact dermatitis. However, as for other irritants, the induced cutaneous irritation is not completely reproducible. A marked interindividual

variability in response has been reported for this irritant, and is ascribed to several host-related factors (42,45,64). Furthermore, intraindividual variability within anatomical regions of the skin site have been reported (65). In the experimental study of the anti-irritant properties of a cosmetic ingredient, three different types of clinical protocol are generally used: postirritation treatment protocols, pretreatment protocols, and treatment with the combined introduction of the anti-irritant product.

In the postirritation treatment protocol, the considered skin regions are irritated by treatment with SLS during a certain time and with a certain frequency. After the SLS irritation challenge, the skin areas are treated with the anti-irritant ingredient or finished product during a certain time and frequency. One irritated area remains untreated and serves as a control, while the remaining irritated areas are treated with the vehicle alone and with the vehicle containing an active anti-irritant ingredient. The last site should heal significantly quicker than the site treated with the vehicle alone. In the pretreatment protocol, the considered skin areas are pretreated during a certain time and frequency, with either the vehicle alone or the vehicle with an anti-irritant component. A nonpretreated skin area serves as a control. Following this pretreatment, the different skin areas are irritated using an SLS solution.

The typical clinical signs of skin irritancy (redness and dryness) are visually assessed by trained evaluators. Furthermore, redness is quantified by the skin color (reflectance colorimetry) and microcirculation of the blood flux by Laser Doppler flowmetry. Alterations in the barrier function are measured by TEWL, and hydration is measured by electrical impedance of the skin. In order to obtain a significant measurable irritancy, the SLS challenge is carried under occlusive dressing. It can also be treated by repetitive open applications with the SLS solution. Different anti-irritant experimental protocols are described in the scientific literature (42).

As found in the literature, these studies are often concerned with the anti-irritant properties of plant extracts. A short overview of the anti-inflammatory/anti-irritant studies described in the literature has been given in the following list:

- Anti-inflammatory properties of the active ingredients—alpha-bisabolol and guaiazulene of Chamomile oil (66–70)
- Anti-inflammatory and healing effect of a cream containing glycolic extract of six plants (Calendula, Roman and German chamomile, linden, cornflower, and millepertuis) (71)
- Anti-inflammatory effect of the active ingredient namely esculoside extracted from horse chesnut (72)
- Anti-inflammatory properties of the active ingredient namely ursolic acid extracted from rosemary (73)
- Anti-irritant properties of a preparation containing licorice and chamomile against a wide range of daily-life skin-irritations (aftershave, depilation, solar eryhthema, and insect stings) (74)

All these studies differ with respect to the irritation challenge and the anti-irritant treatment. In both type of protocols, namely postirritation treatment and pretreatment with the anti-irritant cosmetic ingredients, significant anti-irritant effects were observed between the treated skin sites and the untreated skin sites used as reference. With more discriminative protocols (double-blind vehicle-controlled), where the anti-irritancy efficiency of an anti-irritant ingredient solubilized or dispersed in suitable vehicles (water/oil or oil/water) is compared with the efficiency of the vehicle alone, one generally expects that the specific effect of the anti-irritant

alone will be very small, and not very often significantly different from that of the vehicle alone. To illustrate this statement we refer to a recent work on plant anti-irritants (42).

Manou (42) has studied, in a double-blind vehicle-controlled way, the potential anti-irritant properties of essential oils and glycolic extracts obtained from different plants such as chamomile, sage, clary sage, peppermint, and hyssop. The essential oils were solubilized at a concentration of 3% to 5 % in oil/water and water/oil vehicles. The anti-irritant properties were examined according to the postirritation treatment protocols and the pretreatment protocols, using visual clinical assessments of redness and dryness (skin color, TEWL, and hydration) and bioengineering methods (laser Doppler flowmetry). The results do not support the existence of a significant anti-irritant effect of the essential oils tested under these very strict conditions. In general, the treated skin was found to have benefited from the treatment with the vehicle, along with or without the essential oils, when compared with the irritated but untreated skin. These results could be explained, taking into account the following points. First, the concentration range of the active anti-irritant ingredients used in these experiments is rather low (3% to 5 %) and are concentrations that can be founded in the commercial cosmetic preparations. Probably at higher concentrations (5% to 10%), a significant specific anti-irritant effect will be observed. But, because of the problems of high cost of these plant extracts and the possibility of increasing the risk for allergic contact dermatitis, such high concentrations are rarely used in commercial cosmetic preparations. Secondly, there is always a significant anti-irritant, anti-inflammatory effect on the skin, brought about by the lipids and emollients present in the vehicle.

REFERENCES

1. Cosmetics: Introduction. In: De Groot AC, Weyland JW, Nater JP, eds. Unwanted Effects of Cosmetics and Drugs Used in Dermatology. Amsterdam: Elsevier, 1994:422.
2. The spectrum of side effects of cosmetics. In: de Groot AC, Weyland JW, Nater JP, eds. Unwanted Effects of Cosmetics and Drugs Used in Dermatology. Amsterdam: Elsevier, 1994:437.
3. The frequency of adverse reactions to cosmetics and the products involved. In: De Groot AC, Weyland JP eds. Unwanted Effects of Cosmetics and Drugs Used in Dermatology. Amsterdam: Elsevier, 1994:442.
4. Simion FA, Rau AH. Sensitive skin: what it is and how to formulate for it. Cosmet Toiletries 1994; 109:43.
5. Frosch PJ. Cutaneoaus irritation. In: Rycroft RJG, Menné T, Frosch PJ, eds. Textbook of Contact Dermatitis. Berlin: Springer-Verlag, 1995:28.
6. Amin S, Engasser PG, Maibach HI. Adverse cosmetic reactions. In: Baran R, Maibach HI, eds. Texbook of Cosmetic Dermatolgy. 2nd ed. London, UK: Martin Dunitz, 1998:709.
7. Paye M. Models for studying surfactant interactions with the skin. In: Broze G, ed. Handbook of Detergent Properties. Part A: Properties. Surf Sci Series d Vol. 82. 82. New York: Marcel Dekker, 1999:469.
8. Bjerring P. Spectrophotometric characterization of skin pigments and skin color. In: Serup J, Jemec CBE, eds. Handbook of Non-invasive Methods and the Skin. Boca Raton: CRC Press, 1995:385.
9. Takiwaki H, Serup J. Measurement of erythema and melanin indices. In: Serup J, Jemec CBE, eds. Handbook of Non-invasive Methods and the Skin. Boca Raton: CRC Press, 1995:373.
10. Westerhof W. CIE colorimetry. In: Serup J, Jemec CBE, eds. Handbook of Non-invasive Methods and the Skin. Boca Raton: CRC Press, 1995:385.

11. Diffey BL, Oliver RJ, Farr PM. A portable instrument for quantifying erythema induced by ultraviolet radiation. Br J Dermatol 1984; 111:663.
12. Babulak SE, Rein LD, Scala DD, Simion FA, Grove GG. Quantification of erythema in a soap chamber test using the Minolta Chroma (Reflectance) Meter: Comparison of instrumental results with visual assessments. J Cosmet Chem 1986; 37:475.
13. Clarys P, Alewaeters K, Barel AO. Comparative study of skin colour using different bioengineering methods[abstr]. 6th Congress of the International Society for Skin Imaging, London, United Kingdom, 1999.
14. Oberg PA, Tenland T, Nilsson GE. Laser Doppler flowmetry: a non invasive and continuous method for blood flow evaluation in microvascular studies. Acta Med Scand Suppl 1984; 687:17.
15. Wärdell K, Nilsson G. Laser Doppler imaging of skin. In: Serup J, Jemec CBE, eds. Handbook of Non-invasive Methods and the Skin. Boca Raton: CRC Press, 1995:421.
16. Anderson PH, Abrams K, Bjerring P, Maibach HI. A time correlation study of ultraviolet B-induced erythema measured by reflectance spectroscopy and Laser Doppler flowmetry. Photodermatol Photoimmunol Photomed 1991; 8:123.
17. Imokawa G. In vitro and invivo models. In: Elsner P, Maibach HI, eds. Bioengineering of the Skin: Water and the Stratum Corneum. Boca Raton: CRC Press, 1994:23.
18. Simion FA, Rhein LD, Grove GG, Wojtkowski JJ, Cagan RH, Scala DS. Sequential order of skin responses to surfactants during a soap chamber test. Contact Derm 1991; 27:174.
19. Barel AO, Clarys P, Gabard B. In vivo evaluation of the hydration state of the skin. In: Elsner P, Merck HF, Maibach HI, eds. Cosmetics Controlled Efficacy Studies and Regulation. Berlin: Springer, 1999:57.
20. Paye M, Van de Gaer D, Morrison BM Jr. Corneometry measurements to evaluate skin dryness in the modified soap chamber test. Skin Res Technol 1995; 1:123.
21. Piérard GE, Piérard-Franchimont C, Saint Leger D, Kligman AM. Squamometry: the assessment of xerosis by colorimetry of D-Squame adhesive discs. J Cosmet Chem 1992; 47:297.
22. Paye M, Goffin V, Cartiaux Y, Morrison BM Jr, Piérard GE. D-Squame strippings in the assessment of intercorneocyte cohesion. Allergologie 1995; 18:462.
23. Barel AO, Lambrecht R, Clarys P. Mechanical function of the skin: state of the art. In: Elsner P, Barel AO, Berardesca E, Gabard B, Serup J, eds. Skin Bioengineering: Techniques and Applications in Dermatology and Cosmetology. Basel: Karger, 1998:69.
24. Gasmüller J, Keckes A, Jahn P. Stylus method for skin surface contour measurements. In: Serup J, Jemec CBE, eds. Handbook of Non-invasive Methods and the Skin. Boca Raton: CRC Press, 1995:83.
25. Corcuff P, Lévêque JL. Skin surface replica image analysis of furrows and wrinkles. In: Serup J, Jemec CBE, eds. Handbook of Non-invasive Methods and the Skin. Boca Raton: CRC Press, 1995:89.
26. Efsen J, Hansen HN, Christiansen S, Keiding J. Laser profilometry. In: Serup J, Jemec CBE, eds. Handbook of Non-invasive Methods and the Skin. Boca Raton: CRC Press, 1995:97.
27. Hannuksela M. Sensitivity of various skin sites in the repeated open application test. Am J Contact Derm 1991; 2:102.
28. Van der Valk PGM, Maibach HI. Potential for irritation increases from the wrist to the cubital fossa. Br J Dermatol 1989; 121:709.
29. Berardesca E, Maibach HI. Racial differences in sodium lauryl sulphate induced cutaneous irritation: black and white. Contact Derm 1988; 18:65.
30. Coenraads PJ, Bleumink E, Nater JP. Susceptibility to primary irritants. Contact Derm 1975; 1:377.
31. Rystedt I. Factors influencing the occurence of hand eczema in adults with a history of atopic dermatitis in childhood. Contact Derm 1985; 12:247.

32. Lantinga H, Nater JP, Coenraads J. Prevalence, incidence and course of eczema on the hand and forearm in a sample of the general population. Contact Derm 1984; 10:135.
33. Rycroft RJG. Occupational contact dermatitis. In: Rycroft RJG, Menné T, Frosch PJ, eds. Textbook of Contact Dermatitis. Berlin: Springer-Verlag, 1995:343.
34. Hannuksela M, Salo H. The repeated open application test (ROAT). Contact Derm 1986; 14:221.
35. Tronnier H, Heinrich U. Prüfung der hautvertraglichkeit am menschen zur sicherheitsbewertung von kosmetika. Parf Kosmet 1995; 76:314.
36. Tausch I, Bielfeldt S, Hildebrand A, Gasmüller J. Validation of a modified Duhring Chamber Test (DCT) as a repeated patch test for the assessment of the irritant potential of topical preparations. Parf Kosmet 1996; 76:28.
37. Frosch PJ, Kligman AM. The soap chamber test: a new method for assessing the irritancy of soaps. J Am Acad Dermatol 1979; 1:35.
38. York M, Griffiths HA, White E, Basketter DA. Evaluation of a human patch test for the identification and classification of skin irritation potential. Contact Derm 1996; 34:204.
39. Lukakovic MF, Dunlap FE, Michaels SE, Visscher MO, Watson DD. Forearm wash test to evaluate the clinical mildness of cleansing products. J Cosmet Chem 1988; 39:355.
40. Strubbe DD, Koontz SW, Murahata RI, Theiler RF. The flex wash test: a method for evaluating the mildness of personal washing products. J Cosm Chem 1989; 40:297.
41. Clarys P, Van de Straat R, Boon A, Barel AO. The use of the hand/forearm test for evaluating skin irritation by various detergent solutions. Proc Eur Soc Contact Derm 1992, Brussels, Belgium. p 130.
42. Manou I. Evaluation of the dermatocosmetic propêrties of essential oils from aromatic plants by means of skin bioengineering methods. Ph.D. Thesis, Free University of Brussels (VUB), Brussels, Belgium, 1998.
43. Muizzudin N, Marenus K, Maes D, Smith WS. Use of a Chromameter in assessing the efficacy of anti-irritants and tanning accelarators. J Soc Cosmet Chem 1990; 41:369.
44. Mahmoud G, Lachapelle JM, Van Neste D, Smith WS. Histological assessments of skin damage by irritants: its possible use in the evaluation of a barrier cream. Contact Derm 1984; 11:179.
45. Lee CH, Maibach HI. The sodium lauryl sulfate model: an overview. Contact Derm 1995; 33:1.
46. Poelman PC, Piot B, Guyon F, Deroni M, Lévêque JL. Assessment of topical non-steroidal anti-inflammatory drugs. J Pharm Pharmacol 1989; 41:720.
47. Smith WP, Maes D, Marenus K, Calvo L. Natural cosmetic ingredients:enhanced function. Cosmet Toiletries 1991; 106:65.
48. Bjerring P. Inhibition of UV-B induced inflammation monitored by laser Doppler blood flowmetry. 1993; 6:187.
49. Woodbury RA, Kligman LH, Woodbury MJ, Kligman AM. Rapid assay of the inflammatory activity of topical corticosteroids by inhibition of UV-A induced neutrophil infiltration in hairless mouse skin. I. The assay and its sensitivity. Acta Derm Venereol (Stockhilm) 1994; 74:15.
50. Fleischner AM. Plant extracts: to accelerate healing and reduce inflammation. Cosmet Toiletries 1985; 100:45.
51. Albring M, Albrecht H, Alcorn G, Lücker PW. The measuring of the anti-inflammatory effect of a compound on the skin of volunteers. Meth Find Exp Clin Pharmacol 1983; 5:575.
52. Mao-Quang M, Brown B, Wu-Pong S, Feingold KR, Elias PM. Exogenous nonphysiologic versus physiologic lipids. Divergent mechanism for correction of permeability barrier dysfunction. Arch Dermatol 1995; 131:809.
53. Frosch PJ, Pilz B. Irritant patch test techniques. In: Serup J, Jemec GBE, eds. Handbook of Non-invasive Methods and the Skin. Boca Raton: CRC Press, 1995:587.
54. Effendy I, Maibach HI. Surfactants and experimental irritant contact dermatitis. Contact Derm 1995; 33:217.

55. Gabard B, Elsner P, Treffel P. Barrier function of the skin in a repetitive irritation model and influence of 2 different treatments. Skin Res Technol 1996; 2:78.
56. Berardesca E, Fideli D, Gabba P, Cespa M, Rabiosi G, Maibach HI. Ranking of surfactant skin irritancy in vivo in man using the plastic occlusion stress test. Contact Derm 1990; 23:1.
57. Basketter DA, White E, Griffith HA, York M. The identification and classification of skin irritation hazard by human patch test. Second International Symposium on Irritant Contact Dermatitis, Zurich, Zwitserland. Allergologie 1994; 17:131.
58. Morrison BM Jr, Paye M. A comparison of three in vitro screening tests with an in vivo clinical test to evaluate the irritation potential of antibacterial soaps. J Soc Cosmet Chem 1995; 46:291.
59. Forslind B. A domain mosaic model of the skin barrier. Acta Derm Venereol (Stockholm) 1994; 74:1.
60. Di Nardo A, Sugino K, Wertz P, Adenola J, Maibach HI. Sodium lauryl sulfate induced irritant contact dermatitis: a correlation study between ceramides and in vivo parameters of irritation. Contact Derm 1996; 35:86.
61. Bruynzeel DP, Van Ketel WG, Scheper RJ, Blomberg R, Van Der Flier BME. Delayed time course of irritation by sodium lauryl sulfate: observation on threshold reactions. Contact Derm 1982; 8:236.
62. Novak E, Francom SF. Inflammatory response to sodium lauryl sulfate in aqueous solutions applied to the skin of normal human volunteers. Contact Derm 1984; 10:101.
63. Van Der Valk PGM, Kruis-DeVries MH, Nater JP, Bleumink E, De Jong MC. Eczematous (irritant and allergic) reactions of the skin and barrier function as determined by water vapour loss. Clin Exp Dermatol 1985; 10:185.
64. Judge MR, Griffiths HA, Basketter DA, White IR, Rycroft RJG, McFadden JP. Variations in response of human skin to irritant challenge. Contact Derm 1996; 34:115.
65. Van Der Valk PGM, Maibach HI. Potential for irritation increases from the wrist to the cubital fossa. Br J Dermatol 1989; 121:709.
66. Isaac O. Pharmacological investigations with compounds of chamomile: on the pharmacology of alpha-bisabolol and bisabolol oxides. Planta Med 1979; 35:118.
67. Jellinek J. Alpha-bisabolol un agent anti-inflammatoire pour produits cosmétiques. Parfums Cosmétique Arômes 1984; 57:55.
68. Jakovlev V, Isaac O, Flaskamp E. Pharmacological investigations with compounds of chamomile: investigation of the anti-phlohistic effects of chamazulene and matricine. Planta Med 1983; 49:67.
69. Mann C, Staba EJ. The chemistry, pharmacology and commercial formulations of chamomile. In: Cracker L, Simon JE, eds. Herbs, Spices and Medicinal Plants Vol. 1. 1. Phoenix: Oryx Press, 1986:235.
70. Stanzl K, Vollhardt J. Anti-irritants: the case of alpha-bisabolol. In: Barel AO, Paye M, Maibach HI, eds. Handbook of Cosmetic Science and Technology. New York: Marcel Dekker, 2001:277.
71. Fleischner AM. Plant extracts: to accelerate healing and reduce inflammation. Cosmet Toiletries 1985; 100:45.
72. Esculoside, Veinotonic molecule, treatment of the red blotches of the skin and rosacea, Technical information, Laboratoires Phybiotex, France, 1997.
73. Ursolic acid, a multifunctional anti-inflammatory principle, Technical information, Laboratoires Phybiotex, France, 1997.
74. Cher S. Botanical: myth and reality. Cosmet Toiletries 1991; 106:65.

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Ethnicity as a Possible Endogenous Factor in Irritant Contact Dermatitis: Comparing the Irritant Response Among Caucasians, Blacks, and Asians

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INTRODUCTION

Irritant contact dermatitis (ICD) is a common and potentially serious dermatological disorder (1–3). It is also the second most common occupational illness (4). Because contact dermatitis can develop into chronic skin disease, understanding the underlying factors of its etiology is clinically important.

This condition is divided into several forms depending on the nature of exposure and the resulting clinical presentation. Two common entities are acute and cumulative dermatitis. Acute contact dermatitis presents the classic symptoms of irritation such as localized and superficial erythema, edema, and chemosis. It occurs as a result of single exposure to an acute irritant (5). Cumulative irritant dermatitis presents similar symptoms, but occurs when exposure to a less potent irritant is persistent or repeated until signs and symptoms develop over weeks, years, or decades.

The ability of the offending irritant to cause dermatitis depends on both the nature of the irritant agent and the initial skin condition. The severity of symptoms depends on exogenous and endogenous factors (6–8). Exogenous factors include the irritant's chemical and physical properties, and the vehicle and frequency of application as well as environmental factors. Endogenous factors have been speculated to be age, sex, pre-existing skin diseases, skin sensitivity, genetic background, and—the subject of this review—race (6), or, in today's parlance, ethnicity.

Ethnic differences in skin physiology and pathophysiology exist (9–11), and so whether ethnicity, in fact, an endogenous factor, affects ICD is an important question in dermatotoxicology. Ethnic predisposition to ICD has been studied by comparing the irritant responses of blacks and Asians to those of Caucasians. We review these studies to evaluate whether ethnic differences in susceptibility to ICD do exist.

The answer to the question of ethnicity as a factor in ICD has clinical and practical research consequences. Premarket testing of topical products (soaps, detergents, perfumes, and cosmetics), risk assessment for occupational hazards, and subject-inclusion requirements for product safety studies require knowledge about ethnic differences in irritation (12).

BLACK VS. CAUCASIAN IRRITATION RESPONSE

Using erythema as the parameter to quantify irritation, early studies note that blacks display less redness than Caucasians. In a hallmark paper Marshall et al. (13) showed that while 59% of Caucasians exhibit acute irritant dermatitis as defined by erythema from 1% dichlorethylsulfide, only 15% of blacks do. Later, Weigand and Mershon (14) performed a 24-hour patch test using orthochlorobenzylidene malononitrile as an irritant, which confirmed that blacks are less susceptible than Caucasians to ICD as defined by erythema. Further studies, also using erythema as a measure of irritation, showed that blacks are less reactive than Caucasians to irritants (160 and 1280 mM/L methacoline) (15,16).

Weigand and Gaylor (17) showed that if the stratum corneum of black and Caucasian subjects is removed, there is no significant difference in irritation as measured by erythema between the two groups. They conclude that there might be structural differences in the stratum corneum that provides more protection from chemical irritation to black skin than Caucasian skin. Indeed, while the stratum corneum thickness is the same in both races (18), the stratum corneum of black skin has more cellular layers (12), more casual lipids (19), increased desquamation (20), decreased ceramides (21), and higher electrical resistance (22) than Caucasian skin. Some of these anatomical and physiological differences of the stratum corneum could be used to explain the observed reduced irritation in black skin as measured by erythema (3).

It is difficult, however, to conclude that blacks are less susceptible to cutaneous irritation based only on studies using visual scoring. Erythema is notoriously difficult to measure in darker skin. Perhaps the difference in skin irritation between the two test groups is simply a result of the difficulty of assessing erythema in black subjects.

To better understand this issue it is necessary to analyze studies that use alternative accurate detection methods (23) to assess the level of induced cutaneous irritation. Berardesca et al. (24) performed such a study to determine the difference in irritation between young Caucasian ($n = 9$) and young black ($n = 10$) skin. They applied 0.5% and 2.0% of sodium lauryl sulfate (SLS) to untreated, preoccluded, and pre-delipidized skin. Then they quantified the resulting level of irritation using objective techniques: laser Doppler velocimetry (LDV), transepidermal water loss (TEWL), and water content of the stratum corneum (WC). They found no statistical difference in irritation between the two groups as measured by LDV and WC, but they did find a statistical difference in the TEWL results of the preoccluded test with 0.5% of SLS. In that test, blacks had higher TEWL levels than Caucasians, suggesting that in the preoccluded state blacks are more susceptible to irritation than Caucasians. The finding of this study contradicts the hypothesis that blacks are less reactive than Caucasians.

Similarly Gean et al. (25) found no statistically significant difference in the maximum LDV response between black ($n = 45$) and Caucasian ($n = 45$) subject groups when they challenged skin with topical methyl nicotinate (0.1, 0.3, and

Table 1 Findings That Show a Statistically Significant Difference in the Irritation Response Between Blacks and Caucasians

Interference	End-point	Comment	Direction of difference	Reference
1% DCES	Erythema	Untreated	Caucasians had a greater response	Marshall et al. (13)
Orthochloro-benzylidene	Erythema	Untreated	Caucasians had a greater response	Weigand et al. (14)
100 mM methyl nicotinate	PPG	Untreated	Caucasians had a greater response	Guy et al. (26)
0.05% Clobetasol	LDV	Preoccluded	Caucasians had a greater response	Berardesca et al. (27)
0.5–2.0% SLS	TEWL	Preoccluded	Blacks had a greater response	Berardesca et al. (24)

Abbreviations: DCES, dichlorethylsulfide; SLS, sodium lauryl sulfate; LDV, laser Doppler velocimetry; TEWL, transepidermal water loss; PPG, photoplethysmography.

1.0 M). Further, unlike the earlier studies, they found no difference in the blood flow and erythema responses between the two groups.

Guy et al. (26) support the results finding that LDV measurements of induced blood flow after application of 100-mM methyl nicotinate reveal no significant differences between black ($n = 6$) and Caucasian ($n = 6$) subject groups; however, a significant difference was found using photoplethysmography (PPG). Caucasians had a greater PPG value than blacks, suggesting that Caucasians may be more susceptible to irritation. The authors did not explain why blood flow measurements using PPG showed a statistically significant difference between the groups when LDV did not.

Berardesca et al. (27) also found decreased reactivity in blood vessels in the black test group ($n = 6$) than the Caucasian test group ($n = 8$). They measured the postocclusive cutaneous reactive hyperemia—temporary increase in blood flow after vascular occlusion—after an application of a potent corticoid, and measured vasoconstriction using LDV; the black subject group had several significantly different parameters of the hyperemic reaction. They found a decreased area under the LDV curve response, a decreased LDV peak response, and a decreased decay slope after peak blood flow, showing that blacks have a decreased level of irritation-induced reactivity of blood vessels. These results are consistent with their previous work.

In conclusion, older studies using erythema as the only indicator for irritation show that blacks have less-irritable skin than Caucasians, but more recent studies using objective bioengineering techniques suggest that the eye may have misled us to an incorrect interpretation. Findings that do and do not show statistically significant differences in the irritation response between blacks and Caucasians are summarized in Tables 1 and 2.

ASIAN VS. CAUCASIAN IRRITATION RESPONSE

An early paper comparing Caucasian and Japanese susceptibility to cutaneous irritation was done by Rapaport (28). He performed a standard 21-day patch test

Table 2 Findings That Do Not Show a Statistically Significant Difference in the Irritation Response Between Blacks and Caucasians

Interference	End-point	Comment	Reference
0.5–2.0% SLS	LDV and WC	Untreated, preoccluded, and pre-delipidized	Berardesca et al. (24)
100 mM methyl nicotinate	LDV	Untreated	Guy et al. (26)
0.1, 0.3, and 1.0 M methyl nicotinate	LDV and erythema	Untreated	Gean et al. (25)

Abbreviation: SLS, sodium lauryl sulfate.

protocol on Caucasian ($n = 20$) and Japanese ($n = 20$) females in the Los Angeles area in which 15 irritants (different types or concentrations of cleansers, sunscreen, and SLS) were tested. The results were reported according to the cumulative readings of all subjects in an ethnicity group for each irritant. Japanese women had higher cumulative irritation scores for 13 of the 15 irritants tested; Rapaport interpreted these findings to confirm the common impression that Japanese are more sensitive to irritants than Caucasians. Also, this sensitivity was independent of the concentration or exact chemical formulation of the substance tested, suggesting that Japanese are in general more sensitive than Caucasians.

While these findings are important, it is difficult to interpret these data. First, as also noted by Robinson (12), Rapaport provides little experimental detail and data. For example, while the study required 21 separate days of irritation readings, only the end cumulative irritation scores are reported. If he had reported daily irritation readings, we would have been able to note the time pattern of response. Further, no statistical tests were conducted to ascertain whether the differences between the Japanese and Caucasian subjects were statistically significant. Note, too, that the cumulative irritation test score does not distinguish between the intensity of a subject's response and the number of subjects responding. Thus it is possible, for example, for a few extremely sensitive Japanese subjects to inflate the overall irritation score. Therefore, at the minimum, it would be helpful to provide standard deviations to rule out such problems. Rapaport provides no information on the different phototypes within an ethnic study group, but notes that this may influence the results, and therefore concludes that more studies need to be conducted to draw any definitive conclusions.

What at first seems surprising is the study, by Basketter et al. (29), that Germans are more sensitive than Chinese subjects. Subjects in Germany, P.R. China, and the United Kingdom ($n = 100$ for each group) were exposed to varying concentrations (1–20%) of sodium dodecyl sulfate (SDS) for four hours on the upper outer arm, and the resulting dose–response irritation was measured based on erythema. They concluded that Germans tend to be more sensitive than Chinese subjects, and the Chinese subjects slightly more sensitive than the British subjects. This conclusion is the opposite of popular belief and of the Rapaport study, which indicated that Asians are more likely to develop ICD than Caucasians.

There are, however, inherent flaws in this study, some of which the authors acknowledged. First and foremost, this study does not control the variables of time and location. The German and Chinese studies were performed in three to six weeks in the winter, while the U.K. study was spread over 15 months. Also, in particular,

German winters are colder and drier than Chinese winters, and Chinese winters tend to be colder than English winters. These variables will distort the results in a predictable way if we assume that an individual becomes more sensitive to ICD in colder and drier climates (2). We would then expect, based on climatic conditions, that the German subjects would be more reactive than the Chinese subjects, and the Chinese subjects more reactive than those from the United Kingdom. As these are the actual results, we cannot necessarily contribute the differences in irritant response to ethnicity, as it is possible that the differences are possibly due to weather conditions. Also, they mention that 15% of the U.K. volunteers were black. While they account for this by showing that the black irritant response was similar to the overall U.K. group response, it is scientifically problematic to mix racial groups in a study testing for racial differences. Furthermore, they supplied no statistical tests for their conclusion that Germans are slightly more sensitive than the other ethnic groups. To shed more light on the results, we conducted simple binomial tests of the differences in the percentage response of the subject groups. Using the resulting statistics, we found a larger statistically significant difference between the two predominately Caucasian groups than between each of the Caucasian and the Chinese groups (Table 3). These results indicate that race may not be the predominant factor affecting susceptibility to ICD in this study; other uncontrolled variables may dominate the results.

Variables such as time and location were eliminated by the Goh and Chia (30) study that tested the susceptibility to acute irritant dermatitis in Chinese ($n = 15$), Malaysian ($n = 12$), and Indian ($n = 11$) subjects. These subjects were exposed to

Table 3 Statistical Analysis of the Basketter et al. (29), Study

	0.1% SDS	0.25% SDS	0.5% SDS	1.0% SDS	2.5% SDS	5.0% SDS	10% SDS	20% SDS
Germany	0.03	0.09	0.23	0.50	0.65	0.72	0.76	ND
P.R. China	0	0	0.01	0.21	0.45	0.61	0.79	0.90
U.K.	0.01	0.01	0.06	0.15	0.33	0.41	0.49	0.76
N	100	100	100	100	100	100	100	100
Z (Germany–P.R. China)	1.75	3.07*	4.79*	4.29*	2.84*	1.65	−0.51	NA
Z (U.K.–P.R. China)	1.00	1.00	1.92	−1.10	−1.74	−2.83*	−4.42*	−2.64*
Z (U.K.–Germany)	−1.01	−2.60*	−3.41*	−5.28*	−4.53*	−4.42*	−3.94*	NA

The numbers in the first three rows are the decimal value of the % of the group that developed a positive irritant reaction at a specific SDS concentration. The numbers in the last three rows are the *Z*-values. We applied the binomial test to ascertain the differences in the percentage response of the subject groups:

$$Z = (r_1 - r_2)/[2r(1-r)/100]^{50},$$

where r_1 and r_2 are the ratios for the two ethnic groups and r is the weighted average. Because the sample sizes for different groups are equal, r becomes the simple average. An asterisk indicates that the ratios are significant at the 5% level.

Note that all the U.K.–Germany differences, except one, are statistically significant; however, more than half of the U.K.–P.R. China and almost half of Germany–P.R. China differences are not statistically significant. This indicates a larger statistically significant difference between the two Caucasian groups than between the Caucasian and Asian groups.

Abbreviation: SDS, sodium dodecyl sulfate.

2% of SLS in the right scapular region, and resulting irritation measured using TEWL. This technique is an objective way to indirectly quantify irritation—the higher the TEWL value, the greater the implicit irritation. There was no significant difference in the TEWL level of irritant skin in a three-way statistical test of the three racial groups. There was a significant difference, however, between the TEWL values of Chinese and Malaysian subjects such that Chinese subjects were more susceptible to contact dermatitis. While this test does not contribute to the discussion of the difference in predisposition of irritation in Caucasian versus Asian skins, it does add to the overall question of whether race can be a predisposition to irritant dermatitis.

Foy et al. (31) clearly added the difference in the acute and cumulative irritation response in Japanese ($n = 22$) and Caucasian ($n = 22$) female skin to our knowledge. They reduced some variables that compromised other studies: location, time, season, and scorer were the same for both study populations. Eleven different materials were tested in the acute test; they were applied to the upper arms for 24 hours, and irritation was measured based on erythema. The cumulative test consisted of testing five irritants using a 4-exposure cumulative patch protocol.

In the acute test, while there is a slight tendency to greater susceptibility to irritation among Japanese subjects, only four out of the 11 irritants caused a significant difference in reactivity between the two groups—these were the most-concentrated irritants used. This shows that perhaps for more-concentrated irritants there is indeed a statistical difference in the acute contact dermatitis response; of course, this study needs to be interpreted in context with others to follow. For the cumulative study the skin irritation scores between the two test groups are close—but the Japanese tended to have slightly higher numbers. The differences, however, only reached statistical significance in two instances. And as the authors noted, it is difficult to interpret the importance of those two instances, as the statistical significant differences are not maintained at later points in the timeline. It is safe to conclude, therefore, that while the acute irritant response to highly concentrated irritants was significantly different between the Japanese and Caucasian subjects, the cumulative irritant response rarely reaches a statistical difference.

Studies that include both acute and cumulative irritant tests, like the one above, are more informative than single tests because they give a more complete view of differences in skin irritation between groups. Robinson (32) conducted a series of studies that tested racial differences in acute and cumulative skin irritation responses between Caucasian and Asian populations. In the first acute test, Caucasian ($n = 28$) and Japanese ($n = 20$) groups were exposed on the upper outer arm to five irritants under occlusion for up to four hours. The resulting erythema was scored on an arbitrary visual scale. The results were represented as the cumulative percent incidence of positive test reactions to the different irritants.

It is curious to note that while Japanese subjects tend to be more susceptible to acute irritation than Caucasians, no one irritant nor one tests time caused a significant response difference between the two groups; rather, the significant differences were scattered across five different test materials and time points. The acute irritation response data was then reanalyzed in terms of possible differences in temporal response. It was shown that Japanese subjects generally react faster than their Caucasian counterparts, as indicated by their shorter TR50 values (the time taken for the cumulative irritation score to reach 50%). While this result is interesting, and adds the new dimension of temporal differences in reactivity between the two groups, hard data were not given and statistical analysis was not presented to show whether this temporal pattern difference is indeed statistically significant.

The cumulative irritation test was conducted concurrently and on the same Japanese and Caucasian subjects. Four concentrations of SDS (0.025%, 0.05%, 0.1%, and 0.3%) were applied on the subjects' upper backs for 24 hours for a total of 14 days. The resulting skin grades were summed for all subjects for all test days. For the two lower SDS concentrations the Japanese subjects reacted only slightly more than the Caucasian subjects—but only the difference in skin grades for 0.025% SDS reached statistical significance. When these data were analyzed in terms of temporal response, the Japanese reacted only slightly faster than their Caucasian counterparts for the two lowest concentrations. Whether the difference in reaction time is statistically significant is not known.

In the same study, Robinson then applied both the acute and cumulative irritation protocols to compare three new subject groups—Chinese ($n = 15$), Japanese ($n = 17$), and Caucasian ($n = 18$)—with each other. The cumulative irritation study found no statistically significant differences between the different groups. In the acute test, Robinson found that, in most cases, the Chinese subjects were more reactive to irritants than Caucasians, but that in only one case was this difference significant, and further states that most likely this was an anomaly. There was no discernable difference between the Japanese and Chinese groups. And surprisingly, when the Japanese subjects were again compared to the Caucasian subjects as they were in the beginning of the study, the results showed no significant difference between the two groups.

While Robinson's first two-way irritation response comparison test between Japanese and Caucasian subjects did show some statistical differences, the fact that they could not be confirmed in the second half of the study emphasizes the difficulty in obtaining repeatable results in this type of study. For one thing, in the statistical sense Robinson's sample sizes were small, combined with the variability between human skins within an ethnic group; this makes it difficult to make concrete conclusions. Robinson's study showed, however, that there were essentially no significant differences between the Asian and Caucasian groups—at least none that could be repeated.

Robinson et al. (33) had similar results by using the four-hour occlusion patch method. They compared the relative acute skin reactivity of Asian and Caucasian subjects using the irritation temporal response to measure the difference in reactivity between the test groups. They tested five chemicals, including 20% SDS and 100% decanol. Unlike the previously described study, they failed to find a statistical difference between the reactivity to multiple irritants between the two groups—even at the four-hour mark. Then they divided the racial subpopulations into "sensitive" and "normal" groups to test any differences in percent cumulative scores and temporal responses within these new groups but across race (i.e., they compared sensitive Asians to sensitive Caucasians). There were no statistically significant differences between subjects of the same skin type in different racial groups. This further contradicts the hypothesis that Asians are more reactive to irritants than Caucasians.

Recently Robinson (34) compiled five years of his previous data and compared the acute reactivity differences between Caucasian and Asian (combined Japanese and Chinese) subgroups using the four-hour human patch method. The data were represented in terms of the time taken by the subjects to have a positive response to the irritant chemical. Again, as in most experiments, Asians displayed a greater irritation response score than Caucasians. Note that while the results of this study are probably more representative of the population at large because of the relatively large sample size (200 plus), the data from this study were compiled from three

Table 4 Potent Factors that Might Influence Refinement of Interpretation in Future Investigations

Experimental design
Baseline versus "stress" test differences
Anatomic site
Open versus occluded irritant stresses
Ethnic groups in the same versus varying geography
Comparable climatic conditions
Presentation of hard data and statistical analysis
Sufficient population size
Use of bioengineering assessment method

different testing centers over five years. This could have potentially added uncontrolled and unaccounted-for variables.

In support of the long-held belief that Asians are more susceptible to ICD, several studies do indeed demonstrate this tendency (31,33,34). Rarely, however, this trend is statistically significant—and even more rarely the statistical significance can be repeated in another study. Therefore, it can be concluded from these studies that there is no fundamental difference between Asian and Caucasian cutaneous irritant reactivity—the overall irritant response and the time to reach that response are similar in both subgroups.

But the lack of comparable studies, small sample sizes, external variability, and intravariability within the subgroups make it difficult to completely dismiss Rapaport's original findings that Asians are more reactive than Caucasians. For example, different studies apply the irritant test material on different parts of the body, which might have different reaction responses. This makes it difficult to compare the results of one study to another, and therefore raises the question of whether a more solid trend among studies would exist if the irritants were applied to the same anatomical site. Some potent factors that might influence refinement of interpretation in future investigations are listed in Table 4. For the time being in terms of topical product safety, risk assessment for occupational hazards, and global product marketing it would be practical to assume that few statistically significant differences between Asian and Caucasian cutaneous reactivity exist, that may be less than the variability of response to the same irritant between different subgroups of the same ethnicity.

CONCLUSION

The studies reviewed demonstrate that there is little evidence in favor of statistically significant differences in the irritant response between Caucasian and Black or Asian groups. We can see no consensus on whether race is indeed an endogenous factor in ICD. Intuitively, we suspect that ethnic differences exist in skin function and may have evolved as have hair and other differences. Basically, the studies suggesting differences in skin (24,26) are "stress" in nature (preoccluded). Most of the comparative data found in the literature between races were obtained in experimental conditions where the test substances were occluded to the skin or applied for a relatively long period. In such conditions, substances are artificially forced to cross over the stratum corneum, and the models do minimally consider ethnic differences at the stratum

corneum level or in the barrier function. Presumably new insights into physiology, pharmacology, and toxicology may clarify this situation.

Also it is possible that the well-known divergent response to irritants is due to intraindividual variations in the skin irritation response (35–37). This is a relatively new idea, and therefore further studies need to be conducted in this area before a definitive statement can be made linking intraindividual variation to ethnic differences in the intensity of an irritation response.

REFERENCES

1. Hjorth N, Fregert. Contact dermatitis. In: Rook A, Wilkinson DS, Ebling FJG, eds. Textbook of Dermatology. Oxford: Blackwell, 1968 (chapter 4).
2. Malten KE. Thoughts on irritant dermatitis. Contact Dermatitis 1981; 7:238–247.
3. Lammintausta K, Maibach HI. Irritant contact dermatitis. In: Samuel LM, Harry JH, eds. Dermatology. I. 3rd. Philadelphia: WB Saunders Co, Harcourt Brace Jovanovinch Inc, 1992:425–432.
4. NORA. Allergic & Irritant Dermatitis, 11 June 1999. Center for Disease Control, April 9, 2002. http://www.cdc.goc/niosh/nrderm.html.
5. Wilkinson JD, Rycroft RJG. Contact Dermatitis. In: Rook A, Wilkinson DS, Ebling FJG, eds. Textbook of Dermatology. 1. 4th. Oxford: Blackwell, 1986:435–532.
6. Lammintausta K, Maibach HI. Exogenous and endogenous factors in skin irritation. Int J Dermatol 1988; 27:213–222.
7. Mathias CGT, Maibach HI. Dermatoxicology monographs I. Cutaneous irritation: factors influencing the response to irritants. Clin Toxicol 1978; 13:333–346.
8. Wilhelm KP, Maibach H. Factors predisposing cutaneous irritation. Dermatol Clin 1990; 8:17–22.
9. Berardesca E, Maibach H. Racial differences in skin pathophysiology. J Am Acad Dermatol 1996; 34:667–672.
10. Berardesca E, de Rigal J, Leveque JL, Maibach HI. In vivo biophysical characterization of skin physiological differences in races. Dermatologica 1991; 182:89–93.
11. Berardesca E, Maibach HI. Contact dermatitis in Blacks. Dermatol Clin 1988; 6(3): 363–368.
12. Robinson MK. Population differences in skin structure and physiology and the susceptibility to irritant and allergic contact dermatitis: implications for skin safety testing and risk assessment. Contact Dermatitis 1999; 41:65–79.
13. Marshal EK, Lynch V, Smith HW. On dichlorethylsulphide (mustard gas) II. Variations in susceptibility of the skin to dichlorethylsulphide. J Pharm Exp Therap 1919; 12: 291–301.
14. Weigand DA, Mershon M. The cutaneous irritant reaction to agent O-chlorobenzylidene (CS). Edgewood Arsenal Technical Report 4332, February 1970.
15. Anderson KE, Maibach HI. Black and white human skin differences. J Am Acad Dermatol 1976; 1:276–282.
16. Buckley CE III, Lee KL, Burdick DS. Methacoline induced cutaneous flare response: bivariate analysis of responsiveness and sensitivity. J Allergy Clin Immunol 1982; 69:25–34.
17. Weigand DA, Gaylor JR. Irritant reaction in Negro and Caucasian skin. South Med J 1974; 67:548–551.
18. Thomson ML. Relative efficiency of pigment and horny layer thickness in protecting the skin of Europeans and Africans against solar ultraviolet radiation. J Physiol (Lond) 1955; 127:236–238.
19. Rienerston RP, Wheatley VR. Studies on the chemical composition of human epidermal lipids. J Invest Dermatol 1959; 32:49–51.

20. Corcuff P, Lotte C, Rougier A, et al. Racial differences in corneocytes. Acta Derm Venereol (Stockh) 1991; 71:146–148.
21. Sugino K, Imokawa G, Maibach H. Ethnic difference of stratum corneum lipid in relation to stratum corneum function [Abstract]. J Invest Dermatol 1993; 100:597.
22. Johnson LC, Corah NL. Racial differences in skin resistance. Science 1963; 139:766–769.
23. Flusher JW, Kuss O, Diepgen T, Lasserini S, Pelosi A, Gloor M, Berardesca E. Testing for irritation with a multifactorial approach: comparison of eight non-invasive measuring techniques of five different irritation types. Br J Dermatol 2001; 145:696–703.
24. Berardesca E, Maibach HI. Racial difference in sodium lauryl sulphate induced cutaneous irritation: Black and White. Contact Dermatitis 1988; 18:65–70.
25. Gean CJ, Tur E, Maibach HI, RH Guy. Cutaneous responses to topical methyl nicotinate in Black, Oriental, and Caucasian subjects. Arch Dermatol Res 1989; 281:95–98.
26. Guy RH, Tur E, Bjerke S, Maibach HI. Are there age and racial differences to methyl nicotinate-induced vasodilation in human skin?. J Am Acad Dermatol 1985; 12:1001–1006.
27. Berardesca E, Maibach HI. Cutaneous reactive hyperaemia: racial differences induced by corticoid application. Br J Dermatol 1989; 120:787–794.
28. Rapaport M. Patch testing in Japanese subjects. Contact Dermatitis 1984; 11:93–97.
29. Basketter DA, Griffith HA, Wang XA, Wilhelm KP, McFadden J. Individual, ethnic and seasonal variability in irritant susceptibility of skin: the implications for a predictive human patch test. Contact Dermatitis 1996; 35:208–213.
30. Goh CL, Chia SE. Skin irritability to sodium lauryl sulphate—as measured by skin water loss—by sex and race. Clin Exp Dermatol 1988; 13:16–19.
31. Foy V, Weinkauf R, Whittle E, Basketter DA. Ethnic variation in the skin irritation response. Contact Dermatitis 2001; 45(6):346–349.
32. Robinson MK. Racial differences in acute and cumulative skin irritation responses between Caucasian and Asian populations. Contact Dermatitis 2000; 42:134–143.
33. Robinson MK, Perkins MA, Basketter DA. Application of a 4-h human test patch method for comparative and investigative assessment of skin irritation. Contact Dermatitis 1998; 38:194–202.
34. Robinson MK. Population differences in acute skin irritation responses. Contact Dermatitis 2002; 46(2):86–92.
35. Robinson MK. Intra-individual variations in acute and cumulative skin irritation responses. Contact Dermatitis 2001; 45:75–83.
36. Judge MR, Griffith HA, Basketter DA, White IR, Rycroft RJG, McFadden JP. Variations in response of human skin to irritant challenge. Contact Dermatitis 1996; 34:115–117.
37. McFadden JP, Wakelin SH, Basketter DA. Acute irritation thresholds in subjects with Type I-Type V1 skin. Contact Dermatitis 1998; 38:147–149.

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Principles and Practice of Percutaneous Absorption

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INTRODUCTION

Percutaneous absorption is a complex biological process. The skin is a multilayered biomembrane that has certain absorption characteristics. If the skin were a simple membrane, absorption parameters could easily be measured, and these would be fairly constant provided there was no change in the chemistry of the membrane. However, skin is a dynamic tissue and as such its absorption parameters are susceptible to constant change. Many factors and skin conditions can rapidly change the absorption parameters. Additionally, skin is a living tissue and it will change through its own growth patterns, and this change will also be influenced by many factors. This chapter reviews some of the principles and technologies of percutaneous absorption for developers and users of cosmetics.

STEPS TO PERCUTANEOUS ABSORPTION

A cosmetic that comes in contact with human skin will be absorbed into and through the skin. The components of the cosmetic will respond to the chemical and physical laws of nature, which direct the absorption process. Examples of this are solubility, partition coefficients, and molecular weight. The skin presents a barrier, both physical structure and chemical composition. A cosmetic component will transverse from a lipophilic stratum corneum to a more progressively hydrophilic epidermis, dermis, and blood microcirculation.

Percutaneous absorption has been defined as a series of steps (1). Table 1 lists our current knowledge of these steps. Step 1 is the vehicle containing the chemical(s) of interest. There is a partitioning of the chemical from the vehicle to the skin. This initiates a series of absorption and excretion kinetics that are influenced by a variety of factors, such as regional and individual variation. These factors moderate the absorption and excretion kinetics (2).

Once a chemical has been absorbed through the skin, it enters the systemic circulation of the body. Here, the pharmacokinetics of the chemical define body

Table 1 Steps to Percutaneous Absorption

Vehicle
Absorption kinetics
Skin site of application
Individual variation
Skin condition
Occlusion
Drug concentration and surface area
Multiple-dose application
Time
Excretion kinetics
Effective cellular and tissue distribution
Substantivity (nonpenetrating surface adsorption)
Wash and rub resistance/decontamination
Volatility
Binding
Anatomical pathways
Cutaneous metabolism
Quantitative structure activity relationships
Decontamination
Dose accountability
Models

interactions. This is illustrated for [^{14}C]hydroquinone in vivo in man, where plasma radioactivity was measured ipsilaterally (next to the dose site) and contralaterally (in the opposite arm) after a topical dose. Thirty minutes after the dose, the hydroquinone has been absorbed through the skin and has reached a near-peak plasma concentration (Fig. 1) (3). Figure 2 shows hydroquinone disappearance from the surface of the skin (decreased wash recovery) and concurrent appearance in the stratum corneum (obtained from skin tape strips) (3). As the cosmetic component transverses the skin, the chemical can be exposed to skin enzymes, which are capable of altering the chemical structure through metabolism (3).

METHODS FOR PERCUTANEOUS ABSORPTION

Ideally, information on the dermal absorption of a particular compound in humans is best obtained through studies performed on humans. However, because many compounds are potentially toxic, or it is not convenient to test them in humans, studies can be performed using other techniques. Percutaneous absorption has been measured by two major methods: (1) in vitro diffusion cell techniques, and (2) in vivo determinations, both of which generally use radiolabeled compounds. To ensure their applicability to the clinical situation, the relevance of studies using these techniques must constantly be challenged (4).

In vitro techniques involve placing a piece of human skin in a diffusion chamber containing a physiological receptor fluid. The compound under investigation is applied to one side of the skin. The compound is then assayed at regular intervals on the other side of the skin. The skin may be intact, dermatomed, or separated into epidermis and dermis; however, separating skin with heat will destroy skin viability.

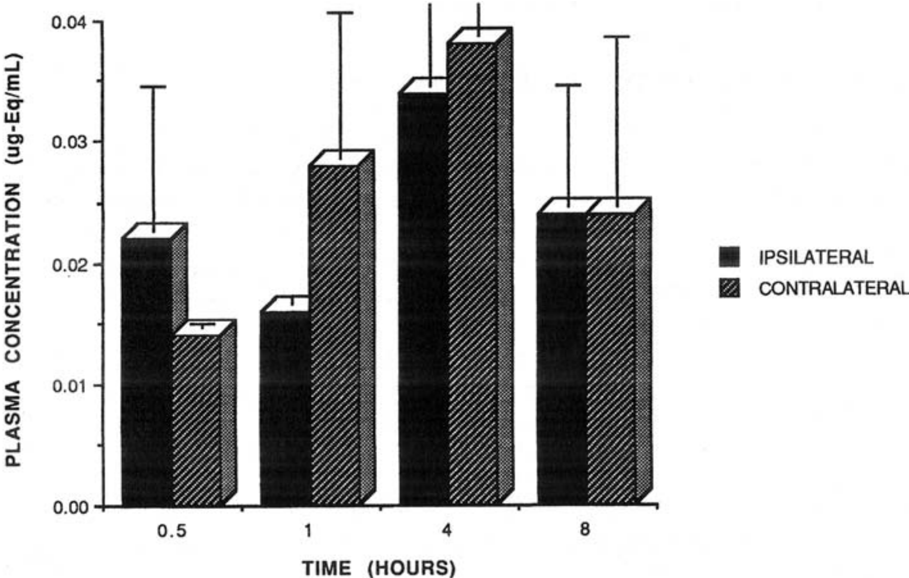

Figure 1 Plasma radioactivity is detected in human volunteers 30 minutes after [^{14}C] hydroquinone is applied to skin. Ipsilateral is the blood taken near the site of dosing, and contralateral is from the other arm. Hydroquinone is rapidly absorbed into and through human skin.

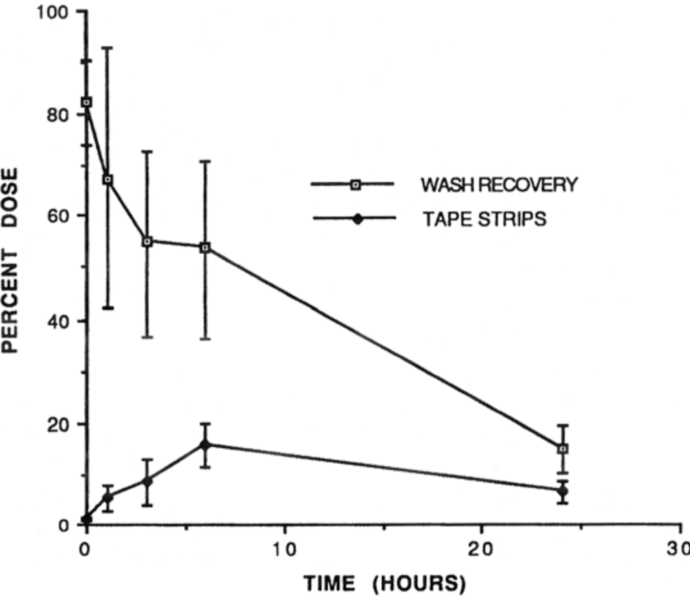

Figure 2 Hydroquinone is applied to human skin. Wash recovery with time decreases because hydroquinone is being absorbed into and through human skin. At the same time, tape strips of the skin surface show a rise in stratum corneum content of hydroquinone. It is a dynamic process; hydroquinone disappears from the skin surface, appears and increases in the stratum corneum, and then appears in the blood.

The advantages of the in vitro techniques are that they are easy to use and results are obtained quickly. Their major disadvantage is the limited relevance of the conditions present in the in vitro system to those found in humans.

Percutaneous absorption in vivo is usually determined by the indirect method of measuring radioactivity in excreta after the topical application of a labeled compound. In human studies, the plasma level of a topically applied compound is usually extremely low—often below assay detection. For this reason, tracer methodology is used. After the topical application of the radiolabeled compound, the total amount of radioactivity excreted in urine or in urine plus feces is determined. The amount of radioactivity retained in the body or excreted by a route not assayed (CO_2) is corrected by determining the amount of radioactivity excreted after parenteral administration. Absorption represents the amount of radioactivity excreted, expressed as percentage of the applied dose. Percutaneous absorption can also be assessed by the ratio of the areas under the concentration-versus-time curves after the topical and intravenous administration of a radiolabeled component. The metabolism of a compound by the skin as it is absorbed will not be detected by this method. A biological response, such as vasoconstriction after the topical application of steroids, has also been used to assess dermal absorption in vivo (4). An emerging method is that of skin tape stripping. After washing, consecutive stratum corneum tape strips exhibit a profile, such as that for estradiol (Fig. 3) in human stratum corneum. The first few strips have higher estradiol content because they contain residual surface estradiol. Tape stripping can show a profile of a cosmetic within skin over a time course. In addition, the chemical content of the tape stoppings can be used to compare bioavailability of competing products. Proof can be obtained by using this technique to observe which products penetrate skin faster and deeper.

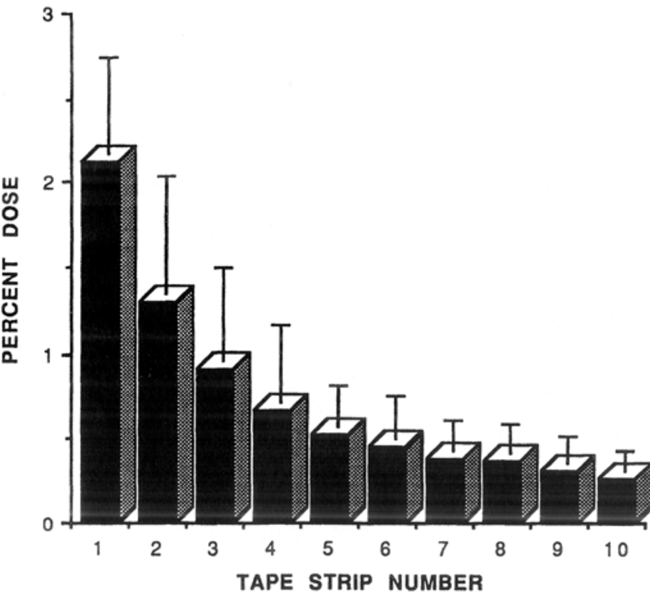

Figure 3 Estradiol is applied to human skin, then washed 24 hours after dosing. Tape strips (consecutive 1–10 in some areas) show a concentration pattern of estradiol through the stratum corneum.

INDIVIDUAL AND REGIONAL VARIATION

In vivo and in vitro percutaneous absorption studies give data as mean absorption $\pm$ some standard deviation. Some of this variability is attributable to conduct of the study and is called *experimental error.* However, when viewing a set of absorption values it is quite clear that some people (as well as some rhesus monkeys) are low absorbers and some are high absorbers. This becomes evident with repeat studies. This is *individual variation.*

The first occupational disease in recorded history was scrotal cancer in chimney sweeps. The historical picture of a male worker holding a chimney brush and covered from head to toe with black soot is vivid. But why the scrotum? Percutaneous absorption in humans and animals varies depending on the area of the body on which the chemical resides. This is called *regional variation.* When a certain skin area is exposed, any effect of the chemical will be determined by the quantity absorbed through the skin. Feldmann and Maibach (5–7) were the first to systemically explore the potential for regional variation in percutaneous absorption. The first absorption studies were performed on the ventral forearm because this site is convenient to use. However, skin exposure to chemicals exists over the entire body. The scrotum was the highest-absorbing skin site (scrotal cancer in chimney sweeps is the key). Skin absorption was lowest for the foot area, and highest around the head and face (Fig. 4). There are two major points. First, regional variation was confirmed with different chemicals. Second, those skin areas that would be exposed to cosmetics—the head and face—were among the higher absorbing sites.

VEHICLE INFLUENCE ON PERCUTANEOUS ABSORPTION

A cosmetic can be a single ingredient or a mixture of chemicals in a vehicle. The vehicle can have a great effect on skin absorption of the chemical(s). Lidocaine

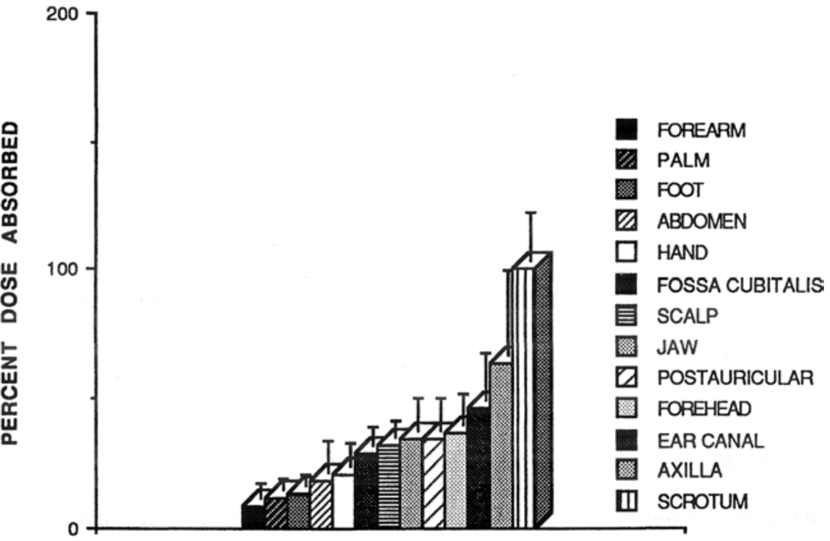

Figure 4 Percutaneous absorption of parathion from various parts of the body varies with region of the body.

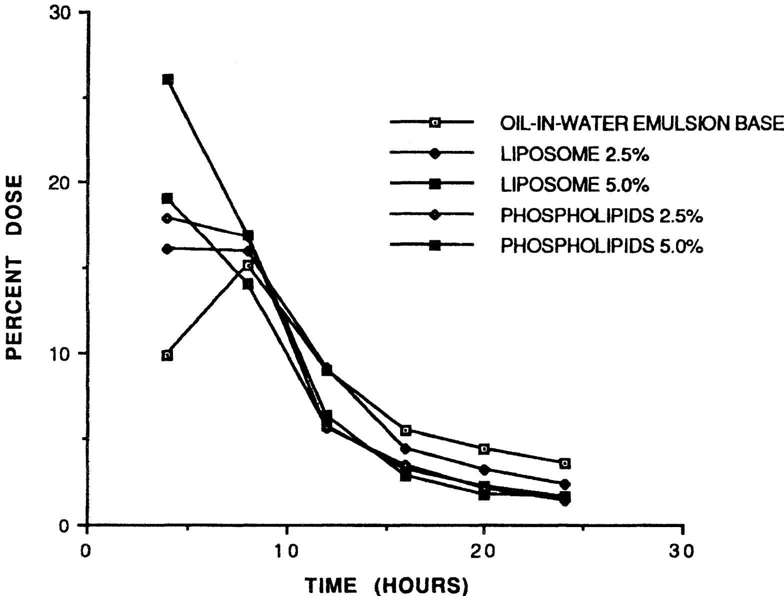

Figure 5 Lidocaine percutaneous absorption through human skin. Formulation determines the initial absorption.

was applied to human skin in an in vitro absorption study. Figure 5 shows receptor-fluid (circulating under the skin to collect absorbed lidocaine) accumulation with time. Initially the vehicle had a great influence on the partitioning of lidocaine into the skin. With time, the influence of the vehicle decreased and lidocaine absorption was constant for all vehicles. Interestingly, when the lidocaine content of epidermis and dermis was determined, there was more lidocaine retained by the oil-in-water (o/w) emulsion (Fig. 6). Vehicles can direct chemical distribution within skin and this can be validated with the proper experiment. There is also an interesting vehicle effect for multiple dosing on skin. A multiple dose exceeds the one that is predicted by absorption from single-dose administration (Fig. 7). The hypothesis is that the second and subsequent dosed vehicles "reactivate/solubilize" the initial chemical from skin binding and push the chemical further down into and through the skin (8).

SKIN CLEANSING AND DECONTAMINATION

Although decontamination of a chemical from the skin is commonly performed by washing with soap-and-water (because it is largely assumed that washing will remove the chemical), recent evidence suggests that the skin and the body are often unknowingly subjected to enhanced penetration and systemic absorption/toxicity because the decontamination procedure does not work or may actually enhance absorption (9).

Figure 8 (alachlor) shows skin decontamination with soap-and-water or water only over a 24-hour dosing period, using the grid methodology. A series of 1 cm^2 areas are marked on the skin and each individual area is washed at a different time. Certain observations are made. First, the amount recovered decreased over time. This is because this is an in vivo system, and percutaneous absorption is taking place,

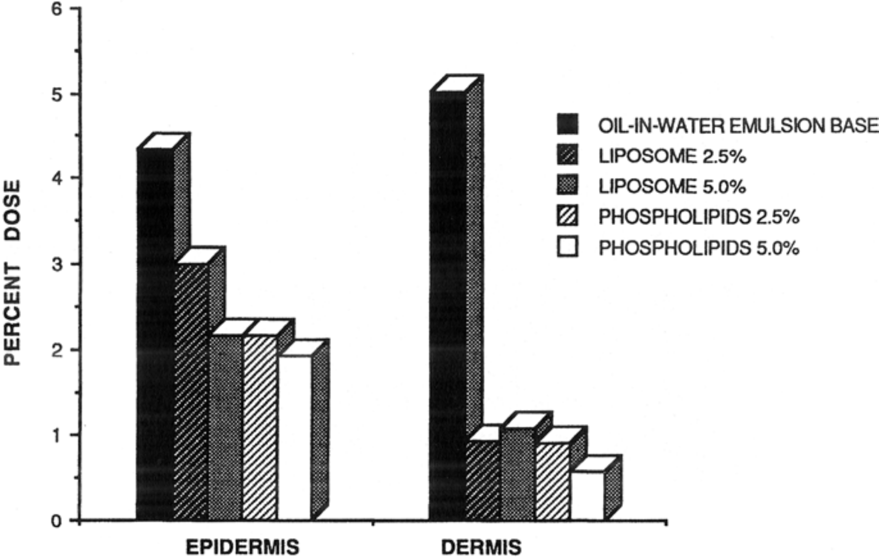

Figure 6 Distribution of lidocaine in human epidermis and dermis. Formulation determines the concentration within the skin component.

decreasing the amount of chemical on the skin surface. There also may be some loss attributable to skin desquamation. The second observation is that alachlor is more readily removed with soap-and-water wash than with water only. Alachlor is lipid soluble and needs the surfactant system for more successful decontamination (10).

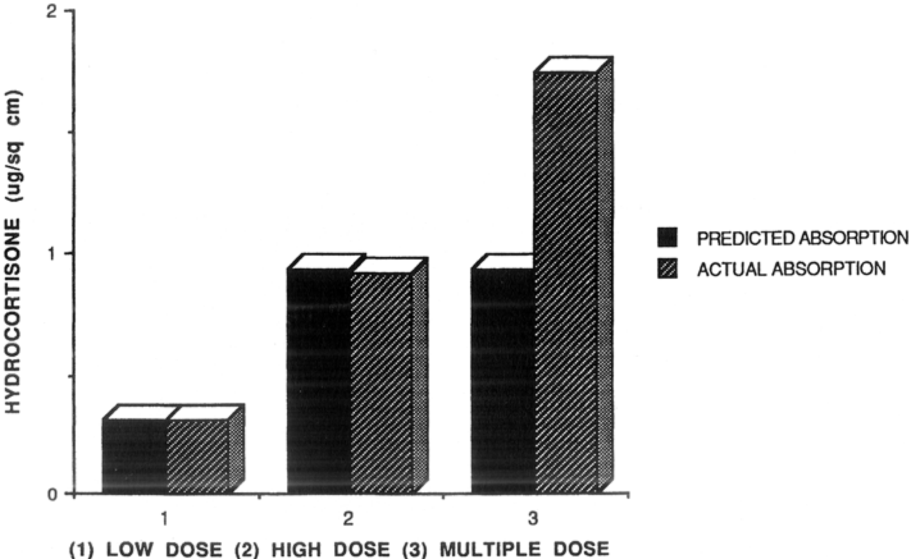

Figure 7 Hydrocortisone in cream base was dosed on human skin as a low dose (x) and a high dose ($3x$). When the low dose (x) was dosed three consecutive times (9 A.M., 1 P.M., and 9 P.M.) totaling the high dose ($3x$), the absorption exceeded the one that predicted from the single high dose.

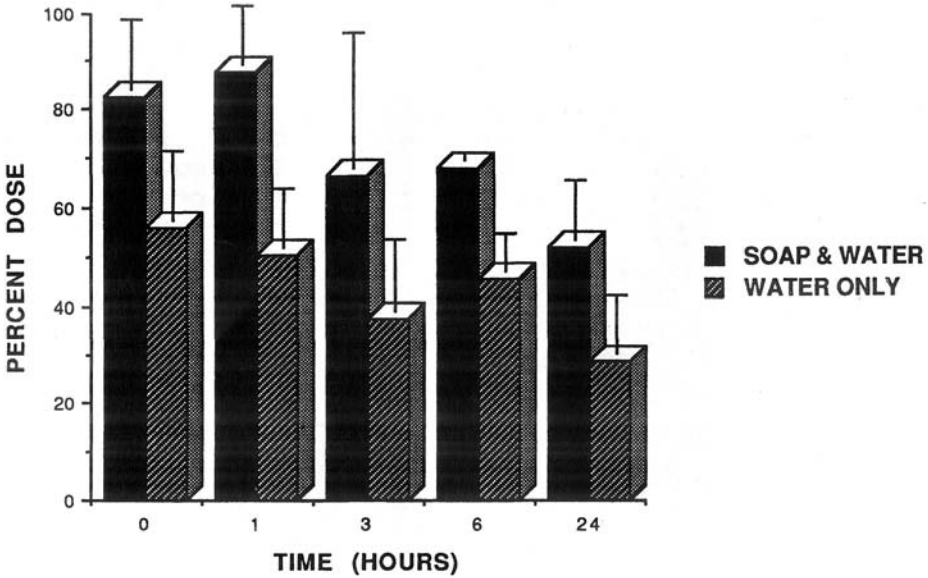

Figure 8 Skin decontamination of alachlor (lipophilic chemical) requires some soap to exceed removal by water only.

Soap-and-water wash may not be the best method to cleanse skin. Soap-and-water will remove visible dirt and odor, but may not be a good skin cleanser. Figure 9 shows methylene bisphenyl isocyanate (an industrial chemical) decontamination

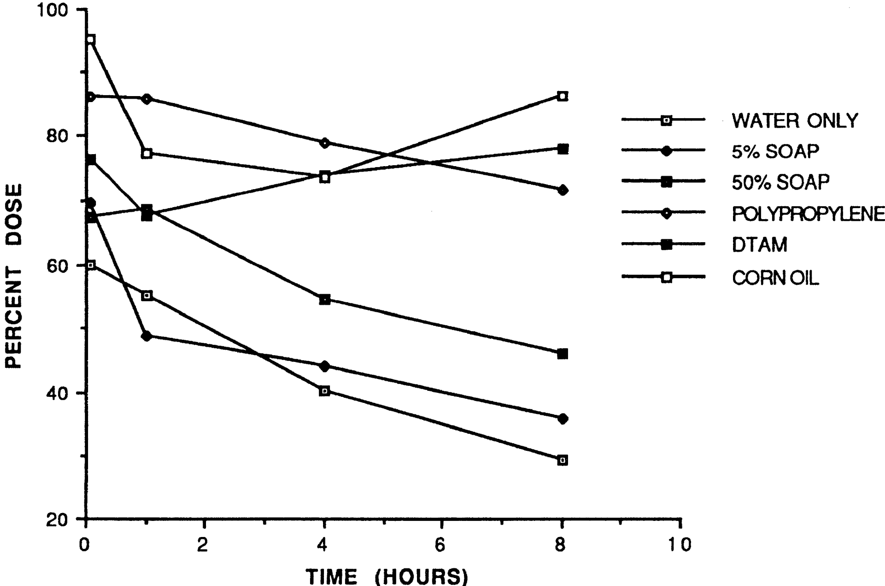

Figure 9 MDI skin decontamination. Water alone and soap-and-water were relatively ineffective in removing MDI compared with the polypropylene-based decontaminants and corn oil. *Abbreviations*: MDI, methylene bisphenyl isocyanate.

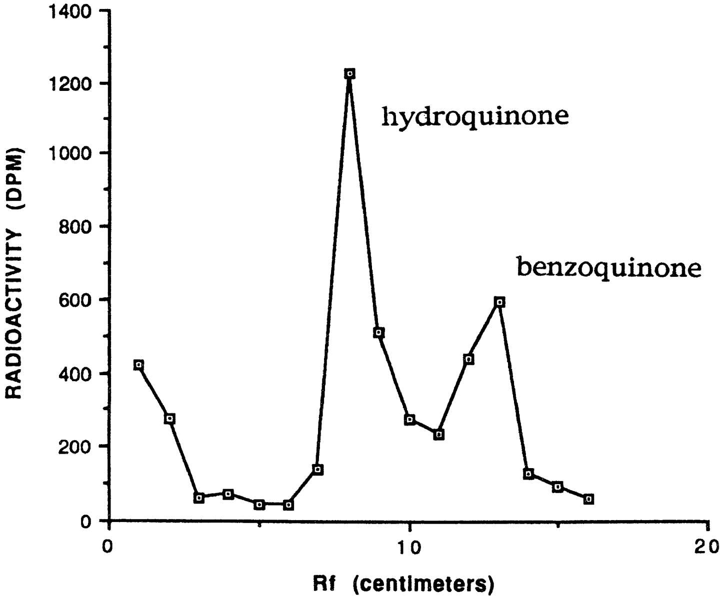

Figure 10 Hydroquinone dosed on viable skin was metabolically converted into the potential carcinogen benzoquinone within the human skin. The fate of a chemical within skin is more important than what is on the surface of skin.

with water, soap-and-water, and some polyglycol and oil-based cleansers. Water and soap-and-water did not work well but the polyglycol and oil-based cleansers did the job. The unknown question that remains is whether soap-and-water would then remove the polyglycol and oil-based cleansers (11).

COSMETIC PERCUTANEOUS ABSORPTION AND TOXICITY

The potential toxicity of cosmetics has in the past been dismissed as an event unlikely to occur. The argument was put forth that cosmetics did not contain ingredients that could prove harmful to the body. The argument went further to say that, because cosmetics were applied to skin with its barrier properties, the likelihood that a chemical would become systemically available was remote. The argument was proven false when carcinogens were shown to be present in cosmetics, and subsequent studies showed that these carcinogenic chemicals could be percutaneously absorbed (12).

Table 2 shows the relationship between percutaneous absorption and erythema for several oils used in cosmetics. The investigators attempted to correlate absorbability with erythema. The most-absorbed oil, isopropyl myristate, produced the most erythema. The lowest-absorbing oil, 2-hexyldecanoxyoctane, produced the least erythema. Absorbability and erythema for the other oils did not correlate (13). The lesson to remember with percutaneous toxicity is that a toxic response requires both an inherent toxicity in the chemical and percutaneous absorption of the chemical. The degree of toxicity will depend on the contributions of both criteria.

In the rhesus monkey, the percutaneous absorption of safrole, a hepatocarcinogen, was 6.3% of applied dose. When the site of application was occluded, the

Table 2 Relationship of Percutaneous Absorption and Erythema for Several Oils Used in Cosmetics

Absorbability (greatest to least)	Erythema
Isopropyl myristate	+ +
Glycol tri(oleate)	–
n-Octadecane	±
Decanoxydecane	+
2-Hexyldecanoxyoctane	–

percutaneous absorption doubled to 13.3%. Occlusion is a covering of the application site, either intentionally, as with a piece of plastic taped over the dosing site during experimentation, or unintentionally, as by putting on clothing after applying a cosmetic. The percutaneous absorption of cinnamic anthranilate was 26.1% of the applied dose, and this increased to 39.0% when the site of application was occluded. The percutaneous absorption of cinnamic alcohol with occlusion was 62.7%, and that of cinnamic acid with occlusion was 83.9% of the applied dose. Cinnamic acid and cinnamic aldehyde are agents that elicit contact urticaria (14), and cinnamic aldehyde is positive for both Draize and maximization methods (15,16).

In vivo human skin has the ability to metabolize chemicals. Figure 10 shows the metabolic profile of extracted human skin after pure hydroquinone had been dosed on the skin for 24 hours. The metabolic profile shows unchanged hydroquinone and its metabolite benzoquinone (3).

We have thus learned that common cosmetic ingredients can readily penetrate skin and become systemically available. If the cosmetic chemical has inherent toxicity, then that chemical will get into the body of a user and exert a toxic effect. Metabolically, the skin can also produce a more toxic compound.

The development of topical drug products requires testing for skin toxicology reactions. A variety of patch-test systems are available with which chemicals are applied to skin. A study was performed to determine the skin absorption of 7-phenylenediamine (PPDA) from a variety of such systems. [^{14}C]PPDA (1% petrolatum UDP) was placed in a variety of patch-test systems at a concentration normalized to equal surface area (2 mg/mm^2).

Skin absorption was determined in the guinea pig by urinary excretion of ^{14}C. There was a 6-fold difference in the range of skin absorption ($p < 0.02$). In decreasing order, the percentage of skin absorption from the systems were 53.4 ± 20.6 (Hill Top chamber), 48.6 ± 9.3 (Teflon control patch), $23.1 + 7.3$ (small Finn chamber), and $8.0 + 0.8$ (AL-test chamber). Thus, the choice of patch system could produce a false-negative error if the system inhibits skin absorption, with a subsequent toxicology reaction (Table 3) (17).

COSMECEUTICS

The early concept of cosmetics was one of inert ingredients used as coloring or cover agents to enhance visual appearance. There was no concern with systemic toxicity because skin had barrier properties and it was assumed nothing would permeate across the skin. The line between cosmetics and pharmaceutics has become a gray area as more active agents are incorporated into cosmetics. These active agents

Table 3 Percutaneous Absorption of PPDA from Patch-Test Systems

	Total load in chamber (mg)	Concentrationin chamber (mg/mm^2)	Absorption	
			Percent[a]	Total (mg)
Hill Top chamber	40	2	53.4 ± 20.6	21.4
Teflon (control)	16	2	48.6 ± 9.3	7.8
Small Finn chamber	16	2	29.8 ± 9.0	4.8
Large Finn chamber	24	2	23.1 ± 7.3	5.5
AL-test chamber	20	2	8.0 ± 0.8	1.6
Small Finn chamber with paper disc insert	16	2	34.1 + 19.8	5.5

[a]Each value is the mean + standard deviation for three guinea pigs.
Abbreviation: PPDA, *p*-phenylenediamine.

are referred to as *cosmeceutics.* Hydroquinone when prescribed by a physician is a drug. Hydroquinone in a cosmetic as a lightening agent is not a drug. The only differentiation between the two preparations is the hydroquinone concentration in the preparation. However, applied concentration does not matter; what matters is how much of the hydroquinone gets into and through the skin. For hydroquinone, percutaneous absorption is 45% of the applied dose for a 24-hour application to in vivo human skin (3). That is a lot of drug—or is it cosmetic, or cosmeceutic? The important point is that for active chemicals the bioavailability needs to be known to assess risk assessment.

Another example is octocopherol, or vitamin E (18). The biological activities of vitamin E in cosmetics are supported by several studies of its percutaneous absorption. In data obtained in vitro on rat skin six hours after application of a 5% vitamin E alcohol solution, 38.6% of the applied dose was recovered in the viable epidermis

Table 4 In Vitro Percutaneous Absorption of Vitamin E Acetate into and Through Human Skin

Treatment	Receptor fluid	Percent dose absorbed: Skin content	Percent dose absorbed: Surface wash
Formula A			
Skin source 1	0.34	0.55	74.9
Skin source 2	0.39	0.66	75.6
Skin source 3	0.47	4.08	89.1
Skin source 4	1.30	0.96	110.0
Mean + standard deviation	0.63 + 0.45[a]	1.56 + 1.69[b]	87.4 + 16.4
Formula B			
Skin source 1	0.24	0.38	—
Skin source 2	0.40	0.64	107.1
Skin source 3	0.41	4.80	98.1
Skin source 4	2.09	1.16	106.2
Mean + standard deviation	0.78 + 0.87[a]	1.74 + 2.06f	103.8 + 5.0

[a]$p = 0.53$ (nonsignificant; paired f-test).
[b]$p = 0.42$ (nonsignificant; paired f-test).

and dermis. The amount detected in the horny layer was 7.12%, and the residual fraction persisting on the surface on the integument represented 54.3% of the applied dose. Both the alcohol and acetate forms of vitamin E are readily absorbed through the human scalp, and within 6 to 24 hours after treatment they concentrate in the dermis. These results substantiate the claim that vitamin E can be used as an active ingredient in cosmetology with the possibility of efficacy in the deeper structures of the skin. Table 4 summarizes the in vitro percutaneous absorption of vitamin E acetate into and through human skin. Each formulation was tested in four different human skin sources. The percent dose absorbed for a 24-hour dosing period is given for receptor-fluid accumulation (absorbed), skin content, and surface wash (soap-and-water wash recovery after the 24-hour dosing period). Table 4 also contains what is referred to as *material balance.* All of the applied doses are accounted for in the receptor fluid, skin content, and skin-surface wash. Total absorbed dose would be the sum of that in the receptor fluid plus that in the skin (content). This is an example of a complete in vitro percutaneous absorption study.

DISCUSSION

The concepts of cosmetics and of the skin have undergone changes in the last few decades. Cosmetics have evolved from being formulations of inert ingredients to containing ingredients that have some biological activity directed to living skin. This is sometimes referred to as cosmeceutics. The concept of skin has evolved from an impenetrable barrier to one where percutaneous absorption does occur. Risk assessment requires a knowledge of percutaneous absorption so that health is not jeopardized. This applies to any topically applied chemical, be it cosmetic, pharmaceutic, industrial, or environmental.

REFERENCES

1. Wester RC, Maibach HI. Cutaneous pharmacokinetics: 10 steps to percutaneous absorption. Drug Metab Rev 1983; 14:169–205.
2. Wester RC, Maibach HI. Percutaneous absorption of drugs. Clin Pharmacokin 1992; 23:253–266.
3. Wester RC, Melendres J, Hui X, Wester RM, Serranzana S, Zhai H, Quan D, Maibach HI. Human in vivo and in vitro hydroquinone topical bioavailability. J Toxicol Environ Health 1998; 54:301–317.
4. Wester RC, Maibach HI. Toxicokinetics: dermal exposure and absorption of toxicants. In: Bond J, ed. Comparative Toxicology. Vol. 1 General Principles. . New York: Elsevier Sciences, , 1997:99–114.
5. Feldmann RJ, Maibach HI. Percutaneous penetration of steroids in man. J Invest Dermatol 1969; 542:89–94.
6. Feldmann RJ, Maibach HI. Absorption of some organic compounds through the skin in man. J Invest Dermatol 1970; 54:399–404.
7. Feldmann RJ, Maibach HI. Percutaneous penetration of some pesticides and herbicides in man. Toxicol Appl Pharmacol 1974; 28:126–132.
8. Wester RC, Melendres J, Logan F, Maibach HI. Triple therapy: multiple dosing enhances hydrocortisone percutaneous absorption in vivo in humans. In: Smith E, Maibach HI, eds. Percutaneous Penetration Enhancers. Boca Raton: CRC Press, 1995:343–349.

9. Feldmann RJ, Maibach HI. Systemic absorption of pesticides through the skin of man. In: Occupational Exposure to Pesticides: Report to the Federal Working Group on Pest Management from the Task Group on Occupational Exposure to Pesticides. Appendix B, 120–127.
10. Wester RC, Melendres J, Maibach HI. In vivo percutaneous absorption of alachlor in rhesus monkey. J Toxicol Environ Health 1992; 36:1–12.
11. Wester RC, Hui X, Landry T, Maibach HI. In vivo skin decontamination of methylene bisphenyl isocyanate (MDI): soap-and-water ineffective compared to polypropylene glycol, polyglycol-based cleanser, and corn oil. Toxicol Sci 1999; 48:1–4.
12. Wester RC, Maibach HI. Comparative percutaneous absorption. In: Maibach HI, Boisits EK, eds. Neonatal Skin: Structure and Function. New York: Marcel Dekker, 1982:137–147.
13. Suzuki M, Asaba K, Komatsu H, Mockizuki M. Autoradiographic study on percutaneous absorption of several oils useful for cosmetics. J Soc Cosmet Chem 1978; 29:265–271.
14. von Krogh G, Maibach HI. The contact urticaria syndrome. In: Marzulli FN, Maibach HI, eds. Dermatotoxicology. Washington, DC: Hemisphere, 1983:301–322.
15. Marzulli FN, Maibach HI. Contact allergy: predictive testing in humans. In: Marzulli FN, Maibach HI, eds. Dermatotoxicology. Washington, DC: Hemisphere, 1983:279–299.
16. Marzulli FN, Maibach HI. Allergic contact dermatitis. In: Marzulli FN, Maibach HI, eds. Dermatotoxicology. Washington, DC: Taylor and Francis, 1996:143–146.
17. Kim HO, Wester RC, McMaster JA, Bucks DAW, Maibach HI. Skin absorption from patch test systems. Contact Dermatitis 1987; 17:178–180.
18. Wester RC, Maibach HI. Cosmetic percutaneous absorption. In: Baran R, Maibach HI, eds. Textbook of Cosmetic Dermatology. London: Martin Dunitz, 1998:75–83.

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The Correlation Between Transepidermal Water Loss and Percutaneous Absorption: An Overview

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INTRODUCTION

Transepidermal water loss (TEWL) is the outward diffusion of water through skin (1). TEWL measurements are used to gauge skin water barrier function. An increase in TEWL reflects impairment of the water barrier (2). TEWL measurements allow parametric evaluation of the effect of barrier creams against irritants and characterization of skin functionality in clinical dermatitis and in irritant and allergic patch test reactions (3). An evaporimeter determines TEWL by measuring the pressure gradient of the boundary layer, resulting from the water gradient between the skin surface and ambient air. TEWL measurements can be affected by the anatomical site, sweating, skin surface temperature, inter- and intraindividual variation, air convection, ambient air temperature and humidity, and instrument-related variables, to name a few. Although, TEWL is influenced by many variables, experiments show that evaporimeter measurements are reproducible in vitro and in vivo (3,4).

Percutaneous Absorption

Percutaneous absorption refers to the rate of absorption of a topically applied chemical through the skin. A compound's absorption rate is important for determining the effectiveness and/or potential toxicity of topically applied compounds. Since many topical formulations are used on diseased skin, where the integrity of the permeability barrier is in doubt, the dose absorbed into the body could vary greatly (5). The rate of absorption in vivo through the stratum corneum (SC) cannot be described by a zero- or first-order mathematical rate equation, because the SC is a complex system variable in its penetration properties. Many factors contribute to the percutaneous absorption of a given chemical. One rate limiting step of

a compound's absorption through the skin is the rate of diffusion through the SC. This review discusses three main categories that give rise to percutaneous absorption rate variation, namely, methodology (including the effects of application time, method of measurement, and physicochemical properties of the topical compound), interindividual variation (including the effects of skin condition, age of individual, and blood flow), and intraindividual variation (including the differences between anatomic sites) (6,7).

Why Do We Want to Correlate TEWL and Percutaneous Absorption?

The extensive procedure required to measure percutaneous absorption versus TEWL enhances the desire to find a correlation between the two measurements to more easily assess skin barrier function. Experimentation of the correlation between TEWL and percutaneous absorption has resulted in studies concluding significant quantitative correlation and a few concluding no quantitative correlation.

The majority of studies investigating TEWL and percutaneous absorption correlation observe a quantitative correlation. It is our hypothesis that the papers which did not observe a quantitative correlation (17b,18) or observed a weak correlation (1,9) do so because of assumptions made in the experiment's design.

Many of the experiments investigating TEWL and percutaneous absorption make large assumptions, which could affect the results of experimentation, and hence be the source of the controversy. For example, Tsai et al. (16) and Chilcott et al. (18) assume that an in vitro measurement of TEWL and percutaneous absorption are equivalent to in vivo measurements, whereas Lamaud et al. (14) assume that animal skin may serve as a permeability model for human skin. Great sources of error and variation can also be induced depending on the measurement method and type of absorption compound used in obtaining percutaneous absorption rates. As we do not completely understand the qualitative relationship between TEWL and percutaneous absorption, it is hard to determine which assumptions made during the experiment could be affecting the correlation results. This section investigates the probable causes that could influence the results of the correlation experiments. Provided in Table 2 is a summary of the major assumptions made by the studies discussed.

In this section we review some major studies defining the correlation between TEWL and percutaneous absorption and discuss major assumptions made in these experiments which could significantly affect those studies that did not conclude a quantitative correlation. Provided in Table 1 is a summary of the major assumptions made by the studies discussed.

Main Review Correlation Studies

Oestmann et al. investigated the correlations between TEWL and hexyl nicotinate (HN) penetration parameters in man. HN penetration was indirectly measured by laser–Doppler flowmeter (LDF), which quantifies the increase in cutaneous blood flow (CBF) caused by penetration of HN, a vasoactive substance. Lipophilic HN was chosen over hydrophilic methyl nicotinate because HN is a slower penetrant, hence, making it easier to distinguish an intact barrier from an impaired barrier.

LDF parameters t_0 and t_{max} were compared with corresponding TEWL values, and a weak quantitative negative correlation was made ($r = -0.31$ and -0.32). This

Table 1 A Summary of the Major Assumptions Made by the Studies Discussed in This Review

Ref.	In vivo versus in vitro (precabs)[c]	Skin type	Percutaneous absorption measurement method	Type of absorption compound[b]	Healthy skin versus damaged skin	Correlation results
(1)	Vivo	Human	LDF	Lipophilic	Healthy	Yes
(8)	Vivo	Human	Urinary	Lipophilic	Healthy	Yes
(13)	Vivo	Human	Urinary	Hydrophilic and lipophilic	Healthy	Yes
(15)	Vivo	Human	Plasma cortisol level	Lipophilic	Damaged	Yes
(16a)[a]	Vitro	Animal	Diffusion cell	Hydrophilic and lipophilic	Damaged	Yes
(16b)[a]	Vitro	Animal	Diffusion cell	Highly lipophilic	Damaged	No
(17)	Vivo	Human	LDF	Lipophilic	Damaged	Yes
(18)	Vivo	Animal	Urinary	Lipophilic	Both	Yes
(18)	Vitro	Both	Diffusion cell	Hydrophilic and lipophilic	Both	No

[a]Reference Tsai et al. (16) was divided into two experiments in this table, as the study found a correlation between TEWL and percutaneous absorption with some compounds and no correlation with others.

[b]Type of absorption compound was determined by their octanol–water partition coefficient, $K_{o/w}$ (Table 2). Values less than 1 are hydrophilic and more than 3 is very lipophilic.

[c]As TEWL in vivo and in vitro measurements are considered equivalent. The authors are only concerned with how percutaneous absorption measurements were taken.

Table 2 Summary of the Permeability and Lipophilicity of all the Compounds Tested on the Barrier Disrupted Hairless Mouse.

Compound	Partition coefficient ($K_{o/w}$)	Correlation coefficient (r)
Sucrose	–3.7	0.82
Caffeine	–0.02	0.86
Hydrocortisone	1.5	0.82
Estradiol	2.7	0.72
Progesterone	3.9	0.01

Source: From Ref. 16.

correlation suggests that when an individual's response time, t_0, was fast, the skin barrier was impaired. The weak negative correlation found may be because of the percutaneous absorption method used. The LDF method has some negative attributes and is not as reproducible as other methods. Further research should investigate this weak correlation between TEWL and penetration of HN.

Lamaud et al. (14) investigated whether permeability changes of hydrophilic compounds (TEWL) are correlated to those of lipophilic compounds (hydrocortisone). In the first part of the experiment, penetration of 1% hydrocortisone and TEWL rates were recorded for the hairless rats in vivo before and after UV irradiation (660 J/cm^2). Both the before and after UV irradiation results correlated well with the TEWL values for application periods up to 1 hour.

In the second part, drug penetration was evaluated by urinary excretion 5 days after a single 24-hour application on normal, stripped, or UV-irradiated skin of hairless rats. The quantity of drug eliminated correlated with the level of TEWL for up to two days.

These results suggest that TEWL can predict the changes of skin permeability to lipophilic drugs in normal and some damaged skin.

Lavrijsen et al. (17) characterized the SC barrier function in patients with various keratinization disorders using two noninvasive methods, namely, measuring outward transport of water through skin by evaporimetry (TEWL) and the vascular response to HN penetration into the skin determined by LDF. Three of the five types of keratinization disorders studied, autosomal dominant ichthyosis vulgaris (ADI), X-linked recessive ichthyosis (XRI), and autosomal recessive congenital ichthyosis (CI), have impaired barrier function and are a type of ichthyosis, whereas the other two keratinization disorders studied, dyskeratosis follicularis (DD) and erythrokeratoderma variabilis (EKV), have no prior information available on barrier impairment. In this experiment, the two methods of barrier function assessment, TEWL and LDF, were correlated.

TEWL measurements and the LDF parameter, t_0, showed a high negative correlation in the patient group ($r = –0.64$) and a weaker negative correlation among the control group ($r = –0.39$). As TEWL reflects the SS-flux of a compound across SC, and parameter t_0 is a function of the duration of the lag phase (non-SS), this study suggests that these two methods should not be considered as exchangeable alternatives but rather as complementary tests. Each method reflects a different aspect of the barrier function.

This paper concludes that TEWL and HN penetration injunction are suitable methods to monitor skin barrier function in keratinization disorders and are helpful in discriminating between some of these disorders.

Rougier et al. (8) attempted to establish the relationship between the barrier properties of the horny layer (percutaneous absorption and TEWL) and the surface area of the corneocytes according to anatomic site, age, and sex in man. The penetration of benzoic acid (BA) was measured in vivo at seven anatomic sites and compared with its TEWL measurement taken on the contralateral site. The amount of BA penetrated was measured through urinary extraction up to 24 hours after application. It was discovered that irrespective of anatomic site and gender a linear relationship ($r = 0.92$, $p < 0.001$) exists between total penetration of BA and TEWL.

Comparing corneocyte surface area to permeability, the study found a general correlation of increasing permeability for both H_2O and BA with decreasing corneocyte size. The smaller the volume of the corneocyte, the greater the intercellular space available to act as a reservoir for topically applied molecules (9). This thinking is because of other studies which have shown that the smaller the capacity of the reservoir, the less the molecule is absorbed (9–12). However for certain anatomic sites where corneocyte size was similar (980–1000 μm^2), there were large differences in permeability. Therefore, showing that, when percutaneous absorption and TEWL are quantitatively correlated, corneocyte size only partially explains the difference in permeability between the different anatomic sites and age of the skin.

Lotte et al. (13) examined the relationship between the percutaneous penetration of four chemicals (acetyl-salicylic acid, BA, caffeine, and sodium salt of BA) and TEWL in man as a function of anatomic site. The amount of chemical penetrated was measured by urinary excretion for up to 24 hours after application. For a given anatomic site the permeability varies widely about the nature of the molecule administered because of the physicochemical interactions which occur between the molecule, vehicle, and SC. For all anatomic sites investigated, irrespective of physicochemical properties of the molecules administered, there was a linear relationship between TEWL and percutaneous absorption.

Aalto-Korte and Turpeinen (15) attempted to find the precise relationship between TEWL and percutaneous absorption of hydrocortisone in patients with active dermatitis. Percutaneous absorption of hydrocortisone and TEWL were studied in three children and six adults with dermatitis. All the subjects had widespread dermatitis covering at least 60% of the total skin area. Plasma cortisol concentrations were measured before and two and four hours after hydrocortisone application by radioimmunoassay. TEWL was measured in six standard skin areas immediately before application of the hydrocortisone cream. Each individual TEWL value was calculated as a mean of these six measurements.

The concordance between the postapplication increment in plasma cortisol and the mean TEWL was highly significant resulting in a correlation coefficient of $r = 0.991$ ($p < 0.001$). In conclusion, this study found a highly significant correlation between TEWL and percutaneous absorption of hydrocortisone.

Tsai et al. (16) investigated the relationship between permeability barrier disruption and the percutaneous absorption of various compounds with different lipophilicity values. Acetone treatment was used in vivo on hairless mice to disrupt the normal permeability barrier, and in vivo TEWL measurements were used to gauge barrier disruption. The hairless mouse skin was then excised and placed in diffusion cells for the in vitro percutaneous absorption measurements of five model

compounds. The permeability and the lipophilicity of all the compounds tested on the barrier disrupted hairless mouse are summarized in Table 2.

The permeability barrier disruption by acetone treatment and TEWL measurements significantly correlated with the percutaneous absorption of the hydrophilic and lipophilic drugs, sucrose, caffeine, and hydrocortisone. However, acetone treatment did not alter the percutaneous penetration of the highly lipophilic compounds, estradiol and progesterone, hence suggesting that there is no correlation between TEWL and the percutaneous absorption of highly lipophilic compounds. The results imply the need to use both TEWL and drug lipophilicity to predict alterations in skin permeability.

Chilcott et al. (8) investigated the relationship between TEWL and skin permeability to tritiated water (^3H_2O) and the lipophilic sulfur mustard (^{35}SM) in vitro. No correlation was found between basal TEWL rates and the permeability of human epidermal membrane to ^3H_2O ($p = 0.72$) or ^{35}SM ($p = 0.74$). Similarly, there was no correlation between TEWL rates and the ^3H_2O permeability of full thickness pig skin ($p = 0.68$). There was no correlation between TEWL rates and ^3H_2O permeability following up to 15 tape strips ($p = 0.64$) or up to four needle stick punctures ($p = 0.13$). These data indicate that under these experimental circumstances TEWL cannot be used as a measure of skin's permeability to topically applied compounds.

More on Assumptions

It is no question that the best experimental conditions are those that are closest to reality; in our case TEWL and percutaneous absorption measured in vivo, on human skin, and using the most reliable percutaneous absorption method of measurement available. It is not coincidence that the studies which used these ideal experimental conditions all came up with the same result; that TEWL and percutaneous absorption are quantitatively correlated. It is only the studies which veered from these most ideal conditions by measuring in vivo or using animal skin to model human skin or using alternate and less reliable methods or percutaneous measurement that found no significant quantitative correlation between the two skin barrier indicators. Below we discuss the possible repercussions of varying experimental conditions form the ideal.

Using In Vitro Methods to Model In Vivo Experiments

Skin permeation can be measured in human or in vitro by using excised skin in diffusion cells. In theory, studies using excised skin are feasible models for in vivo experiments, because passage through the skin is a passive diffusion process and the stratum corneum is composed of non-living tissue. Many studies comparing in vivo and in vitro TEWL and percutaneous absorption measurements have been conducted and the results from those experiments support the contention that reliable measurements can be obtained from in vitro methodology (6,19–25).

Although the consensus is that in vitro experiments are reasonable models for in vivo human experiments, some experiments note significant differences between these methods for measuring skin permeation. The most significant study by Bronaugh and Stewart (23) found that the effects of UV irradiation could not be duplicated using an in vitro experimentation model, hence suggesting that in vitro experiments examining the TEWL and percutaneous absorption after barrier damage may not be an acceptable model for in vivo experimentation. In vitro damage to the SC barrier may not be an accurate model to in vivo SC damage,

because in vivo exposure to skin irritants results in a cascade of reactions that do not occur in human cadaver skin (19).

Chilcott et al. (18) investigated TEWL and percutaneous absorption correlation in vitro after inducing different types of barrier damage. This was also one of the only studies reviewed which did not observe a correlation between TEWL and percutaneous absorption. Perhaps using in vitro methodology in the experimental design may be responsible for the lack of correlation to skin damage reported in this study.

Using Animal Skin to Model Human Skin

Comparing the skin morphology and chemical absorption of human versus animal skin, it is clear that human skin is unique in both aspects and should be used for the most meaningful results (26). Yet an experiment by Bronaugh et al. (27) found that depending on the compound of interest and the vehicle used, permeability values obtained using animal skin can be well within an order of magnitude of the permeability values for human skin.

Independently, in vitro methods and animal skin models prove to be reliable models for human in vivo absorption. Therefore, it seems logical to assume that in vitro and animal methods may be used in unison to accurately model in vivo human absorption. However Rougier et al. (28) document a distinct difference between animal studies done in vivo versus in vitro when compared to human absorption. This experiment compares the skin permeability of humans to the hairless rat (29) and the hairless mouse (22) using molecules of widely different physicochemical properties. The results show that, in vivo, for whatever the molecule tested the permeability ratios remained relatively constant, whereas in vitro they do not. Therefore when application conditions are strictly identical in humans and in animals it may be possible to model human in vivo absorption by measuring in vivo animal absorption but not using in vitro animal absorption. The inaccurate results obtained when conducting experiments in vitro using animal skin may have affected the results studied by Tsai et al. (16) and Chilcott et al. (18) which were the only two papers to conclude no correlation between TEWL and percutaneous absorption and these were the only two papers using in vitro animal methodology.

Percutaneous Absorption Measurement Methods

A major factor affecting percutaneous absorption measurements is methodology (30,31). All methods for percutaneous absorption measurements are not equal and hence can give different results. Table 1, column 4 summarizes the percutaneous absorption methods used in these correlation studies.

The most common method for determining percutaneous absorption in vivo is measuring the radioactivity of excreta following topical application of a labeled compound. Determination of percutaneous absorption from urinary radioactivity does not account for metabolism by skin, but has been proven to be a reliable method for absorption measurement and is widely accepted as the "gold standard" when available.

The most commonly used in vitro technique involves placing a piece of excised skin in a diffusion chamber, applying radioactive compound to one side of the skin, and then assaying for radioactivity in the collection vessel on the other side (32). The advantages of using this in vitro technique are that the method is easy to use and

that the results are obtained quickly. The disadvantage is that the fluid in the collection bath, which bathes the skin is saline, which may be appropriate for studying hydrophilic compounds is not suitable for hydrophobic compounds. If the parent compound is not adequately soluble in water then determining in vitro permeability into a water receptor fluid will be self-limiting.

When conducting in vitro experiments animal skin is often substituted for human skin. Because animal skin has different permeability characteristics than human skin, one should be careful which type of animal skin is used (refer section on animal vs. human skin). In addition, proper care should be taken in skin preparation of excised skin to make sure not to damage skin barrier integrity. Anatomical site is also important as well as using many different skin samples.

The only two experiments which did not find a correlation between TEWL and percutaneous absorption, Tsai et al. (16) and Chilcott et al. (18), were that measured percutaneous absorption in vitro. Perhaps using a diffusion cell to measure percutaneous absorption is the reason for not finding a correlation.

Oestmann et al. (1) and Lavrijsen et al. (17) used LDF to measure HN penetration. LDF measures the increase in CBF caused by the penetration of HN, a vasoactive substance. One problem with this method is that LDF measurements are not only dependant on the amount of HN absorbed, but also on the individual's vasoreactivity, gender, and age. This may be the reason that Oestmann et al. (1) and Lavrijsen et al. (17) obtained only a weak correlation between TEWL and percutaneous absorption of HN. Another disadvantage of this method is that LDF measurements have many sources of variation which make it difficult to compare interlaboratory results. If an attempt should be made, note that LDF parameters t_0 and t_{max} are the function of HN concentration, the vehicle used, and the application time; the LDF parameters LDF_{base} and LDF_{max} are relative values depending on the type of LDF used.

Type of Compound Used to Measure Percutaneous Absorption

The percutaneous absorption rate and/or total absorption of a compound varies greatly depending on the compound and its lipophilicity. Yet, many of the papers reviewed did not consider how lipophilicity of the test compound would affect percutaneous absorption and hence affect correlation results. Feldmann and Maibach (20) measured both the total absorption and maximum absorption rate for 20 different compounds of different lipophilicities. The range for total absorption for the 20 compounds tested was >250 times, whereas the difference in maximum absorption rate was >1000-fold (20). Because of the extreme range of absorption for topically applied compounds it seems reasonable to assume that the correlation between TEWL and percutaneous absorption may not be independent of the physicochemical properties of the compound applied. Namely, can TEWL measurements predict the skin barrier's permeability changes to both hydrophilic and very lipophilic compounds?

Correlation results from many studies, Oestmann et al. (1), Lamaud et al. (14), Lavrijsen et al. (17), Lotte et al. (13), Aalto-Korte et al. (15), and Tsai et al. (16a), suggest that TEWL can predict the changes in skin permeability to hydrophilic and slightly lipophilic topical drugs. Tsai et al. (16b) also discovered that the percutaneous absorption of highly lipophilic compounds does not correlate with TEWL.

The highly lipophilic compounds are the compounds that did not show evidence of a correlation between percutaneous absorption and TEWL, whereas the

moderately lipophilic compounds, such as hydrocortisone and BA, did. This should be further investigated. In the future, it may be necessary to use both TEWL and drug lipophilicity to predict alterations in skin permeability.

EXPLORING THE QUALITATIVE REASONING FOR THE CORRELATION BETWEEN PERCUTANEOUS ABSORPTION AND TEWL

Yet, despite the significant quantitative correlation demonstrated in some experiments, the precise qualitative relationship between percutaneous absorption and TEWL remains unsettled. Is the quantitative correlation just a coincidence or have we not discovered the link between the two indicators?

Experiments investigating the correlation between TEWL and percutaneous absorption have found a quantitative correlation between the two skin barrier indicators, yet have failed to find their precise qualitative relationship. Most experiments looking for an explanation of skin permeability examine and compare trends in physical aspects of the skin such as SC membrane thickness, corneocyte size, area of the horny layer, transcorneal routes, sebum lipid film, intercellular volume, to name a few. Yet we remain clueless about the structure function relationship of the SC, because there is no morphology aspect that explains the permeability of the SC. Skin has particular features which combine together in varying degree to produce different experimental values of TEWL and percutaneous absorption (13). Further investigation needs to be done investigating the relationship between TEWL and percutaneous absorption in skin structure and morphology.

CONCLUSION

Although, it is not certain why studies by Tsai et al. (16) and Chilcott et al. (18) showed no quantitative correlation we can postulate some estimations.

The study by Tsai et al. (16) is the only paper demonstrating a clear distinction between highly lipophilic compounds and slightly lipophilic compounds when correlating percutaneous absorption and TEWL. Acetone treatment could affect a certain aspect of the skin barrier that mostly affects and interacts with hydrophilic compounds hence having no affect on the highly lipophilic compounds such as estradiol and progesterone. It would be interesting to ascertain if the same results were obtained when selecting a different form of barrier damage such as physical tape stripping. Or it could be the fact that the lipophilic compounds chosen were even more hydrophobic than those used in other experiments and indeed TEWL and percutaneous absorption of highly lipophilic compounds are not correlated.

It is difficult to understand why Chilcott et al. (18) found no correlation between TEWL and percutaneous absorption. The results could have been affected, because the experiment was done in vitro, partly on animal skin, using an extremely lipophilic compound, ^{35}SM. It would be interesting to ascertain if TEWL and percutaneous absorption of ^{35}SM correlated with the results up to 1 hour after application.

Taken together, the weight of evidence confirms a relationship between TEWL (water transport) to percutaneous penetration, yet much remains before this can fully be generalized and the mechanism understood. Future experiments should take into consideration the effects of modeling realistic situations using alternative methods to the ideal.

REFERENCES

1. Oestmann E, Lavrijsen A, Hermans J, Ponec M. Skin barrier function in healthy volunteers as assessed by transepidermal water loss and vascular response to hexyl nicotinate: intra- and inter-individual variability. Br J Dermatol 1993; 128:130–6.2.
2. Nilsson J. Measurement of water exchange through skin. Med Biol Eng Comput 1997; 15:209–218.
3. Pinnagoda J, Tupker R, Agner T, Serup J. Guidelines for transepidermal water loss (TEWL) measurement. Contact Derm 1990; 22:164–178.
4. Pinnagoda J, Tupker P, Coenraads P, Nater J. Comparability and reproducibility of the results of water loss measurements: a study of 4 evaporimeters. Contact Derm 1989; 20:241–246.
5. Bronaugh R, Weingarten D, Lowe N. Differential rates of percutaneous absorption through the eczematous and normal skin of a monkey. J Invest Dermatol 1986; 87:451–453.
6. Noonan P, Gonzalez M. Pharmacokinetics and the variability of percutaneous absorption. J Toxicol 1990; 9(2):511–516.
7. Wester R, Maibach H. Chair's summary: percutaneous absorption — in vitro and in vivo correlations. In: Dermatology: Progress and Perspectives. 18th World Congress of Dermatology, New York, June 12–18. New York: The Parthenon Publishing Group., 1993: 1149–1151.
8. Rougier A, Lotte C, Corcuff P, Maibach H. Relationship between skin permeability and corneocyte size according to anatomic site, age and sex in man. J Soc Cosmet Chem 1988; 39:15–26.
9. Dupuis C, Rougier A, Roguet R, Lotte C, Kalopissis K. In vivo relationship between horny layer reservoir effect and percutaneous absorption in human and rat. J Invest Dermatol 1984; 82:353–356.
10. Rougier R, Dupuis D, Lotte C, Roguet R, Schaefer H. In vivo correlation between stranum corneum reservoir function and percutaneous absorption. J Invest Dermatol 1983; 81:275–278.
11. Rougier A, Lotte C, Maibach H. In vivo percutaneous penetration of some organic compounds related to anatomic site in man: predictive assessment by the stripping method. J Pharm Sci 1987; 76:451–454.
12. Rougier A, Dupuis D, Lotte C, Roguet R. The measurement of the stratum corneum reservoir. A predictive method for in vivo percutaneous absorption studies: influence of application time. J Invest Dermatol 1985; 84:66–68.
13. Lotte C, Rougier A, Wilson D, Maibach H. In vivo relationship between transepidermal water loss and percutaneous penetration of some organic compounds in man: effect of anatomic site. Arch Dermatol Res 1987; 279:351–356.
14. Lamaud E, Lambrey B, Schalla W, Schaefer H. Correlation between transepidermal water loss and penetration of drugs. J Invest Dermatol 1984; 82:556.
15. Aalto-Korte K, Turpeinen M. Transepidermal water loss and absorption of hydrocortisone in widespread dermatitis. Br J Dermatol 1993; 128:663–635.
16. Tsai J, Sheu H, Hung P, Cheng C. Effect of barrier disruption by acetone treatment on the permeability of compounds with various lipophilicities: implications for the permeability of compromised skin. J Pharm Sci 2001; 90:1242–1254.
17. Lavrijsen A, Oestmann E, Hermans J, Bodde H, Vermeer B, Ponec M. Barrier function parameters in various keratinization disorders: transepidermal water loss and vascular response to hexyl nicotinate. Br J Dermatol 1993; 129:547–554.
18. Chilcott R, Dalton C, Emmanuel A, Allen C, Bradley S. Transepidermal water loss does not correlate with skin barrier function in vitro. J Invest Dermatol 2002; 118(5):871–875.
19. Nangia A, Camel E, Berner B, Maibach H. Influence of skin irritants in percutaneous absorption. Pharm Res 1993; 10:1756–1759.

20. Feldmann R, Maibach H. Absorption of some organic compounds through the skin in man. J Invest Dermatol 1970; 54:399–404.
21. Franz T. The finite dose technique as a valid in vitro model for the study of percutaneous absorption in man. Curr Probl Dermatol 1978; 7:58–68.
22. Bronaugh R, Stewart S. Methods for in vitro percutaneous absorption studies VI: preparation of the barrier layer. J Pharm Sci 1986; 75:487–491.
23. Bronaugh R, Stewart R. Methods for in vitro percutaneous absorption studies V: permeation through damaged skin. J Pharm Sci 1985; 74:1062–1066.
24. Bronaugh R, Stewart R. Methods for in vitro percutaneous absorption studies III: hydrophobic compounds. J Pharm Sci 1983; 73:1255–1258.
25. Bronaugh R, Stewart R, Congdon E, Giles A. Methods for in vitro percutaneous absorption studies I. Comparison with the in vivo results. Toxicol Appl Pharm 1982; 62:474–480.
26. Bronaugh R, Franz T. Vehicle effects on percutaneous absorption: in vivo and in vitro comparisons with human skin. Br J Dermatol 1986; 115:1–11.
27. Bronaugh R, Stewart R, Congdon E. Methods for in vitro percutaneous absorption studies II. Animal models for human skin. Toxicol Appl Pharm 1982; 62:481–488.
28. Rougier A, Lotte C, Maibach H. The hairless rat: a relevant model to predict in vivo percutaneous absorption in humans? J Invest Dermatol 1987; 88:5/77–581.
29. Walker J, Dugard D, Scoot T. In vitro percutaneous absorption studies: a comparison of human and laboratory species. Hum Toxicol 1983; 2:561–565.
30. Bronaugh R, Maibach H. Percutaneous Absorption. 2nd ed. New York: Marcel Dekker, 1989.
31. Wester R, Maibach H. Percutaneous absorption in diseased skin. In: Maibach, Surber, eds. Topical Corticosteriods. Basel: Karger, 1992:128–141.
32. Bronaugh R, Maibach H. In Vitro Percutaneous Absorption. Boca Raton: CRC Press, 1991.

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Allergy and Hypoallergenic Products

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INTRODUCTION

The assessment and detection of the number of contact allergic reactions to cosmetics are not simple. Generally, a consumer who has a problem with cosmetics will consult a doctor, only if he or she does not recognize the cause to be a particular cosmetic product, or if the dermatitis persists when the suspect product has been replaced by another, determined by trial and error. Consequently, only a small proportion of the population with cosmetic intolerance problems is ever seen by a dermatologist. Moreover, cosmetic reactions may present in unusual clinical forms, which may evoke an erroneous diagnosis (1–3).

In general, adverse effects are under-reported (4), certainly to the cosmetics industry, which obtains its most reliable information in this regard mainly from the relatively few dermatologists who concentrate on cosmetic-intolerance problems and from reports in the literature which are, almost by definition, out of date. Sometimes beauticians and consumers report adverse reactions, but in most cases this kind of information is difficult to objectify unless it is verified by a dermatologist.

Application of cosmetic products to the skin may cause irritant, photo-toxic, contact, and photo-contact allergic reactions, as well as contact urticaria. It is generally agreed that most skin-adverse reactions to cosmetic products are irritant in nature and that people with "sensitive skin," as indicated by conditions like atopic dermatitis, rosacea, or seborrheic dermatitis, are particularly liable to develop such reactions. However, contact allergic reactions attract much more attention, and thus tend to be overestimated (4). Indeed, the identification of cosmetic allergen is by no means a simple task. It demands special skills and interest on the part of the dermatologist, although labeling of all cosmetic ingredients is facilitating that task. Moreover, there are many factors involved in the sensitization to a specific cosmetic product, all of which have to be taken into account when one seeks an allergen (1,2) (see the following section).

FACTORS CONTRIBUTING TO CONTACT ALLERGIC REACTIONS TO A COSMETIC PRODUCT

Frequency of Use

One may expect the frequently used products to cause more skin reactions than the more exclusive products, simply because more people are exposed to the former. This alone does not imply anything about the quality of these products (the same thing may be said about individual cosmetic ingredients).

Composition

The complexity of a formula can be either positive or negative, as far as its allergenicity is concerned. One of the principles of creating "hypoallergenic" cosmetics and perfumes is the simplicity of formula. The fewer the constituents, the easier it is to identify the offending substance should difficulties arise, and there would be a lesser danger of synergism. The presence of more ingredients leads to an increase in the chance of the skin's sensitization to one of them. However, some investigators recommend placing upper limits on concentrations, rather than advising against the use of any particular ingredient. They may also suggest more complex formulas (5). Preservatives, needed for water-based or other easily contaminated products, are the common cosmetic allergens. It seems that it is very difficult to combine potent antimicrobial and antifungal properties with low allergenicity. Indeed, it is very difficult to restrict the biological activity of a substance to a single domain.

Concentration of Ingredients

Although the use of low concentrations does not assure complete safety, the incidence of sensitization induction is, indeed, a function of the concentration of the allergen, at least to some extent. Cases of allergy to the preservative agent methyl (chloro)isothiazolinone illustrate this problem very well. At first, a 50 ppm concentration of this agent was allowed for use in cosmetic products in the European Community, following which this concentration was actually used in some products, there were "epidemics" of contact allergic reactions to it (6). Of late, the frequency of positive reactions has been diminishing considerably, not only because its use is declining and primarily limited to "rinse-off" products (3), but also because its usage concentration has been reduced to about 15 to 7.5 ppm (as the manufacturers recommended). Of course, once a patient has become sensitized, even low concentrations can trigger a reaction.

Purity of Ingredients

It is impossible to refine raw materials to absolute purity. A more or less strict quality control of raw materials and finished products has long been the general practice in modern cosmetic manufacturing. However, one can never rule out the sensitizing potential of impurities in these materials (5).

The Common Use of Cosmetic Ingredients in Pharmaceuticals

Patients easily become sensitized to topical pharmaceutical products which, unlike cosmetics, are most often used on a diseased skin. However, once sensitization has occurred, they may react also to the cosmetics containing the same ingredients (5).

The Role of Cross-Sensitivity

Chemically related substances are likely to induce cross-reactions, and contact eczematous lesions may be maintained in this way. This is especially the case with perfume ingredients, which often cross-react with each other, and applies to all other cosmetic ingredients as well.

Penetration-Enhancing Substances

The chemical environment can substantially affect a person's sensitizing potential of individual chemicals. For example, emulsifiers and solvents enhance skin penetration, and thereby contact sensitization. Penetration-enhancing agents can also be the root of false-negative patch-test reactions; the cosmetic product itself may be clearly allergenic (or irritant), although the individual ingredients, abstracted from the environment of the product and tested separately, may not cause a reaction.

Application Site

Some areas of the skin, like the eyelids, are particularly prone to contact dermatitis reactions. A cream applied to the entire face, such as a face care product, along with hair products may cause an allergic reaction only on the eyelids. Moreover, "ectopic dermatitis" [caused by the transfer of an allergen by hand, as often occurs with tosylamide/formaldehyde (i.e., para-toluenesulfonamideformaldehyde) resin, the allergen in nail polish], "airborne" contact dermatitis (e.g., caused by perfumes) (7), as well as "connubial" dermatitis (caused by products shared between partners) (8), often occur only on "sensitive" skin areas such as the eyelids, lips, and the neck.

Moreover, the penetration potential of cosmetics is heightened in certain "occluded" areas, such as the body folds (axillary, inguinal) and the anogenital region, due to which the risk of contact sensitization is increased. In the body folds, the allergenic reactions tend to persist for weeks after the initial contact with the allergen. This may be partly attributable to the residual contamination of clothing as well as the increased penetration of the allergen, which is certainly assisted by occlusion and friction (9). Indeed, a reservoir may be formed from which the allergen is subsequently released.

Condition of the Skin

Application on a damaged skin, where the skin barrier is impaired, enhances the penetration of substances, and thus increases the risk of an allergic reaction. This is the case with body-care products used to alleviate dry, atopic skin, and with barrier creams used for protecting the hands which often suffer from irritancy problems (e.g., dryness, cracking). Sometimes, the allergic reaction may be limited to certain areas of the skin (areas already affected react more readily to another application of the same allergen), and may even present an unusual clinical picture that does not immediately suggest contact dermatitis. Indeed, contact allergic reactions to preservative agents on the face may present as a lymphocytic infiltrate, or even have a lupus erythematous-like picture (3,10).

Contact Time

In the world of cosmetics, a distinction is now being made between leave-on products, which remain on the skin for several hours (e.g., face- and body-care products and makeup), and rinse-off products, which are removed almost immediately.

The division between these two kinds of products is not always relevant to the sensitization process, because a thin film can remain on the skin and be sufficient to allow ingredients to penetrate. This occurs, for example, with moist toilet paper (with mainly preservatives as the allergens) and makeup removers.

Frequency of Application and Cumulative Effects

Daily use or several-times-a-day usage of cosmetics may cause ingredients to accumulate in the skin and thus, increase the risk of adverse reactions. In fact, the concentration of an ingredient may be too low to induce sensitivity in a single product, but may reach critical levels in the skin if several products containing it are used consecutively. This may be the case for people who are loyal to the same brand of products, e.g., day and night creams, foundations, and cleansing products; because a manufacturer will often use the same preservative system for all his products. This should be taken into consideration by companies that use biologically active ingredients such as preservative agents, emulsifiers, antioxidants, and perfumes, because it might well account for many of the adverse reactions to these particular substances. In our experience, intense users of cosmetics are more prone to cosmetic dermatitis than others.

CORRELATIONS WITH THE LOCATION OF THE LESIONS

Like many other contact allergens, cosmetics can reach the skin in several different ways (1,2): by direct application; by airborne exposure to vapors, droplets, or particles that are released into the atmosphere and then settle on the skin (7); by contact with people (partners, friends, coworkers) who transmit allergens to cause "connubial" or "consort" dermatitis (8); by transfer from other sites on the body, often the hands, to more sensitive areas such as the mouth or the eyelids (ectopic dermatitis); and by exposure to the sun with photo-allergens.

The most common sources of cosmetic allergens applied directly to the body are listed in Table 1 .

THE NATURE OF COSMETIC ALLERGENS

Fragrance Ingredients

Fragrance ingredients are the most frequent culprits in cosmetic allergies (11–15). Katsarar et al., who investigated the results of patch testing over a 12-year period, found an increasing trend in the sensitivity to fragrance compounds, which reflects the effectiveness of the advertising of perfumed products (16). Common features of a fragrance contact dermatitis are localization in the axillae, localization on the face (including the eyelids) and neck, well-circumscribed patches that appears in areas where dabbing-on perfumes are used (wrists, behind the ears), and hand eczema or its aggravation. Airborne or connubial contact dermatitis should be

Table 1 Cosmetic and Cosmetic-Related Dermatitis Caused by Direct Application of the Allergen

Area of dermatitis	Cosmetics that may contain allergens
Face in general	Facial skincare products (creams, lotions, masks), sunscreen products, makeup (foundations, blushes, powders), cleansers (lotions, emulsions), and cosmetic appliances (sponges), perfumed products (aftershave lotion)
Forehead	Hair-care products (dyes, shampoos)
Eyebrows	Eyebrow pencil, depilatory tweezers
Upper eyelids	Eye makeup (eye shadow, eye pencils, mascara), eyelash curlers
Lower eyelids	Eye makeup
Nostrils	Perfumed handkerchiefs
Lips, mouth, and perioral area	Lipstick, lip pencils, dental products (toothpaste, mouthwash), depilatories
Neck and retroauricular area	Perfumes, toilet waters, hair-care products
Head	Hair-care products (hair dyes, permanent-wave solutions, bleaches, shampoo ingredients), cosmetic appliances (metal combs, hairpins)
Ears	Hair-care products, perfume
Trunk/upper chest, arms, wrists	Body-care products, sunscreens, and self-tanning products, (elbow flexures) cleansers, depilatories
Axillae	Deodorants, antiperspirants, depilatories
Anogenital areas	Deodorants, moist toilet paper, perfumed pads, depilatories
Hands	Hand-care products, barrier creams, all cosmetic products that come in contact with the hands
Feet	Foot-care products, antiperspirants

considered as well. Other less frequent adverse reactions to fragrances are photocontact dermatitis, contact urticaria, irritation, and pigmentation disorders (17).

Sensitization is most often induced by highly perfumed products, such as toilet waters, aftershave lotions, and deodorants, the last of which have been shown to contain well-known allergens such as cinnamal (cinnamic aldehyde) and isoeugenol (18).

As reported in the literature, the fragrance mix remains the best screening agent for contact allergy caused by perfumes, because it would detect some 70% to 80 % of all perfume allergies (19,20). However, it depicts also the need to test with additional perfume allergens. Indeed, testing with additional markers, for example, the individual components such as hydroxyisohexyl 3-cyclohexene carboxaldehyde (Lyral®), farnesol, and citral, as well as with the complex natural mixtures (21–25), increases the sensitivity of the testing. Because of the increasing importance of fragrance allergy, and to ensure that sensitized consumers are adequately informed, 26 fragrance components will have to be labeled as cosmetic ingredients on the package [Annex 3 (Table 2 of the Cosmetic Directive (26)]. With fragrance, allergy associated

Table 2 Twenty-six Substances to be Labelled Regardless of Function and Origin

References in Annex III of the cosmetics directive	Name in the cosmetics directive	INCI name
(67)	Amyl cinnamal	Amyl cinnamal
(68)	Benzyl alcohol	Benzyl alcohol
(69)	Cinnamyl alcohol	Cinnamyl alcohol
(70)	Citral	Citral
(71)	Eugenol	Eugenol
(72)	Hydroxy-citronellal	Hydroxycitronellal
(73)	Isoeugenol	Isoeugenol
(74)	Amyl cinnamyl alcohol	Amylcinnamyl alcohol
(75)	Benzyl salicylate	Benzyl salicylate
(76)	Cinnamal	Cinnamal
(77)	Coumarin	Coumarin
(78)	Geraniol	Geraniol
(79)	Hydroxymethylpentyl-cyclohexenecarboxaldehyde	Hydroxyisohexyl 3-Cyclohexene Carboxaldehyde
(80)	Anisyl alcohol	Anise alcohol
(81)	Benzyl cinnamate	Benzyl cinnamate
(82)	Farnesol	Farnesol
(83)	2-(4-tert-Butylbenzyl) propionaldehyde	Butylphenyl methylpropional
(84)	Linalool	Linalool
(85)	Benzyl benzoate	Benzyl benzoate
(85)	Hexyl cinnamaldehyde	Hexyl cinnamal
(86)	Citronellol	Citronellol
(88)	d-Limonene	Limonene
(89)	Methyl heptin carbonate	Methyl 2-Octynoate
(90)	3-Metyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one	Alpha-Isomethyl Ionone
(91)	Oak moss extract	EU: Evernia Prunastri *USA: Evernia Prunastri (Oak Moss) Extract*
(92)	Treemoss extract	EU: Evernia Furfuracea *USA: Evernia Furfuracea (Tree Moss) Extract*

positive patch-test reactions frequently occur and often indicate the presence of common or cross-reacting ingredients in natural products, the occurrence of cross-reactions between simple fragrance chemicals, or concomitant sensitivity. Moreover, oxidation products of fragrance ingredients, such as limonene (27) or resin acids (being the main allergens in colophony), found as contaminants in tree moss (a widely used substitute for oak moss) as well as in oak moss itself (28), play an important role in the allergenic potential of these substances (29).

Preservatives

Among the allergy causing agents, preservatives are second in frequency to fragrance ingredients; they are important allergens in cleansers, skincare products, and makeup

(2,30). However, within this class, important shifts have occurred over the years (30,31).

The methyl(chloro)isothiazolinone mixture was commonly used in the 1980s and was then a frequent cause of contact allergies. This frequency has declined considerably in recent years (3,12). Since then, formaldehyde and its releasers, particularly methyldibromo glutaronitrile—as used in a mixture with phenoxyethanol, better known as Euxyl K400—did gain importance in this regard (12,30–34), although the frequency of positive reactions observed seems to be influenced by the patch-test concentration (33,34).

The spectrum of the allergenic preservatives also varies from country to country. For example, in contrast to continental Europe where reactions to the methyl-(chloro) -isothiazolinone mixture and more recently methyldibromo glutaronitrile have been the most frequent (12,13,30,31,35), in the United Kingdom, formaldehyde and its releasers have always been much more important, particularly as concerns quaternium-15 (30), although its incidence seems to have slightly decreased of late (36). Parabens are rare causes of cosmetic dermatitis. When a paraben allergy does occur, the sensitization source is most often a topical pharmaceutical product, although its presence in other products can be sensitizing as well (37); this is often the case for other ingredients also. For instance, a young lady, after having previously been sensitized to mefenesin in a rubefacient, presented with an acute contact dermatitis on the face at the first application of a new cosmetic cream containing chlorphenesin, which was used as a preservative agent (data on file). Apparently, it is a potential sensitizing agent (38), and cross-reacts with mefenesin which is used in pharmaceuticals.

Antioxidants

Antioxidants form only a minor group of cosmetic allergens. Examples are propyl gallate, which may cross-react with other gallates and are also used as food additives, and t-butyl hydroquinone, a well-known allergen in the United Kingdom, but not in continental Europe (30). Sodium metabisulfite, present in oxidative hair dyes (data on file), may cause allergic contact dermatitis both to the clients and to the hairdressers.

"Active" or Category-Specific Ingredients

With regard to "active" or category-specific ingredients, in contrast to de Groot (3), we found an increase in the number of reactions to oxidative hair dyes (para-phenylenediamine or PPD and related compounds) during the period of 1991–1996, as compared with the period 1985–1990 (12,13). According to one cosmetic manufacturer (personal communication, L'Oréal, 1997), the use of such hair dyes has more than doubled in recent years. However, the replacement since 1987 of PPD-hydrochloride by a PPD-base—a more appropriate screening agent for PPD-allergy—may also have influenced the incidence (39). They are important causes of professional dermatitis in hairdressers, who also often react to allergens in bleaches (persulfates, also causes of contact urticaria) and permanent-wave solutions (primarily glyceryl monothioglycolate, which may provoke cross-sensitivity to ammonium thioglycolate) (40,41). Tosylamide/formaldehyde (= toluenesulfonamide/formaldehyde) resin is considered an important allergen (4) and is the cause of "ectopic" dermatitis attributable to nail lacquer, which may also contain epoxy

and (meth) acrylate compounds (3). It often gives rise to confusing clinical pictures and may mimic professional dermatitis (42). Acrylates (Methacrylates) are also causes of reactions to artificial nail preparations, more recently to gel formulations, with both manicurists and their clients (43).

Moreover, “natural” ingredients may induce contact allergic reactions as well. Some examples are butcher broom (*Ruscus aculateus*), which is also a potential allergen in topical pharmaceutical products (44), hydrocotyl (asiaticoside) (45), and panthenol (46). Farnesol, a well-known perfume ingredient and cross-reacting agent to balsam of Peru, has become a potential allergen in deodorants, in which it is used for its bacteriostatic properties (47).

Some sunscreen agents such as benzophenone-3, which may also cause contact urticaria, and dibenzoylmethane derivatives have been recognized in the past as being important allergens (3,30,48–50). Indeed, isopropyl dibenzoylmethane was even withdrawn for this reason (3). 4-Methylbenzylidene camphor, cinnamates, and phenylbenzimidazole sulfonic acid are only occasional, sometimes even rare, causes of cosmetic reactions. The use of para-aminobenzoic acid and its derivatives has decreased considerably. Contact allergic reactions to them were generally related to their chemical relationship to para-amino compounds (51), although they were also important photo-sensitizers (48).

In our experience (12,13,30), the contribution of sunscreens to cosmetic allergy is relatively small, despite the increase in their use as a result of the media attention given to the carcinogenic and accelerated skin-aging effects of sunlight. The low rate of allergic reactions observed may well be because a contact allergy or a photo-allergy to sunscreen products is often not recognized, since a differential diagnosis with a primary sun intolerance is not always obvious. Furthermore, the patch-test concentrations generally used might be too low (52), in part, because of the risk of irritancy.

Excipients and Emulsifiers

Many excipients and emulsifiers are common ingredients to topical pharmaceutical and cosmetic products, the former being likely to induce sensitization. Typical examples are wool alcohols, fatty alcohols (e.g., cetyl alcohol), and propylene glycol (13). Emulsifiers in particular have long been regarded as irritants, but their sensitization capacities should not be overlooked. It is imperative, of course, that patch testing be properly performed to avoid irritancy, and that the relevance of the positive reactions be determined. This is certainly the case for cocamidopropyl betaine, an amphoteric tenside mainly present in hair- and skin-cleansing products. Whether the compound itself or cocamidopropyl dimethylamine, an amidoamine, or dimethylaminopropylamine (both intermediates from the synthesis) are the actual sensitizers, is still a matter of discussion (53,54). It is also not clear whether cocamidopropyl-PG-dimonium chloride phosphate (phospholipid FTC (55), an allergen in skin-care products, can cross-react with cocamidopropyl betaine. Other emulsifiers and vehicle components that were more recently found to be contact allergens in cosmetics are maleated soybean oil (56), butylene glycol and pentylene glycol (aliphatic alcohols with similar uses to propylene glycol that is considered to have more irritant and allergenic effects) (57,58), ethylhexylglycerin (syn.: octoxyglycerin) (59), methoxy PEG-17 and PEG-22/dodecyl glycol copolymers (alkoxylated alcohols and synthetic polymers) (60), and alkylglucosides (condensation products of fatty alcohols with glucose) (61,62).

Coloring Agents

Coloring agents, other than hair dyes, have rarely been reported as cosmetic allergens. However, with the increased use of cosmetic tattoos (e.g., eye and lip makeup), more treatment-resistant skin lesions might develop in the future (63).

DIAGNOSING COSMETIC ALLERGY

Taking the history of the patient and noting the clinical symptoms and localization of the lesions are critical. Allergen identification for a patient with a possible contact allergy to cosmetics is performed by means of patch testing with the standard series, specific cosmetic-test series, the product itself, and with all its ingredients. We can only find the allergens we look for. There are several guidelines for skin tests with cosmetic products that the patients supply themselves (64). Not only the patch and photo-patch tests, but also semi-open tests, usage tests, or repeated open application tests may be needed to be performed to obtain a correct diagnosis.

HYPOALLERGENIC PRODUCTS

Most of the cosmetic industry is making a great effort to commercialize products that are the safest possible. Some manufacturers market cosmetics containing raw materials that have a "low" sensitization index or a high degree of purity, or from which certain components have been eliminated (5,65) (generally perfume ingredients). Sometimes "active" preservative agents are also omitted, and in sunscreens immunologically inert physical agents are being used more often than chemical ultraviolet absorbers.

Statements such as "recommended by dermatologists," "allergy-tested," or "hypoallergenic" have been put on the packaging material by manufacturers, to distinguish their products from those of their competitors. Although there are several ways to reduce allergenicity (3), there are no governmentally mandated standards or industry requirements (66).

The latest trend is target marketing to people with a "hypersensitive" or "intolerant" skin, term often used for the shadowy zone between normal and pathological skin. These would be the people with increased neurosensitivity (e.g., atopics), heightened immune responsiveness (e.g., atopic and contact allergic individuals), or a defective skin barrier, i.e., people with irritable skin such as atopics, those suffering from seborrheic dermatitis (67), or rosacea. This means that part of the cosmetic industry is moving more into the area of pathological skin, and that certain products are in fact becoming drugs, often called cosmeceuticals. This has caused a great deal of regulatory concern (68,69), both in the United States and the European Union because; it suggests some middle category between cosmetics and drugs that does not yet legally exist. In Japan, however, these products fall in the category of "quasi-drugs."

The meaning of most such claims used nowadays is unclear, both for the dermatologist (65–67) and the consumer, the latter being convinced that the hypersensitive skin is the allergic skin. It is the dermatologist's task to diagnose the skin condition, and to provide specific advice about the products that can safely be used. All such problems must be approached individually, at least the contact allergic types, because people sensitive to specific ingredients must avoid products containing them.

Therefore, ingredient labeling can be of tremendous help. Providing the allergic patient with a limited list of cosmetics that can be used is practical and effective (70).

CONCLUSION

The identification of cosmetic allergens is challenging because of the extreme complexity of the problem. This applies not only for the dermatologist who is trying to identify the culprit and advise his patient, but also certainly for cosmetic manufacturers, who are extremely concerned about assuring the innocuousness of their products. Precise, current, and rapid information about the adverse reactions to cosmetic products is critical in a product design. Apparently, premarketing studies are unable to identify all the pitfalls. Therefore, fruitful communication that is developing between dermatologists and cosmetic manufacturers must be encouraged. Sensitivity to cosmetics can never be totally avoided, but its incidence can be substantially reduced.

REFERENCES

1. Dooms-Goossens A. Contact allergy to cosmetics. Cosmet Toiletries 1993; 108:43–46.
2. Dooms-Goossens A. Cosmetics as causes of allergic contact dermatitis. Cutis 1993; 52:316–320.
3. de Groot AC. Fatal attractiveness: the shady side of cosmetics. Clin Dermatol 1998; 16:167–179.
4. Berne B, Boström A, Grahnén AF, Tammela M. Adverse effects of cosmetics and toiletries reported to the Swedish medical products agency 1989–1994. Contact Dermatitis 1996; 34:359–362.
5. Dooms-Goossens A. Reducing sensitizing potential by pharmaceutical and cosmetic design. J Am Acad Dermatol 1984; 10:547–553.
6. Pasche B, Hunziker N. Sensitization to Kathon CG in Geneva and Switzerland. Contact Dermatitis 1989; 20:115–119.
7. Dooms-Goossens AE, Debusschere KM, Gevers DM, Dupré KM, Degreef H, Loncke JP, Snauwaert JE. Contact dermatitis caused by airborne agents. J Am Acad Dermatol 1989; 15:1–10.
8. Morren MA, Rodrigues R, Dooms-Goossens A, Degreef H. Connubial contact dermatitis. Eur J Dermatol 1992; 2:219–223.
9. Dooms-Goossens A, Dupré K, Borghijs A, Swinnen C, Dooms M, Degreef H. Zinc ricinoleate: sensitizer in deodorants. Contact Dermatitis 1987; 16:292–293.
10. Morren MA, Dooms-Goossens A, Delabie J, Dewolf-Peeters C, Mariën K, Degreef H. Contact allergy to isothiazolinone derivatives. Dermatologica 1992; 198:260–264.
11. Adams RM, Maibach HI. A five-year study of cosmetic reactions. J Am Acad Dermatol 1985; 13:1062–1069.
12. Goossens A, Merckx L. I'Allergie de contact aux cosmétiques. Allergie et Immunologie 1997; 29:300–303.
13. Dooms-Goossens A, Kerre S, Drieghe J, Bossuyt L, Degreef H. Cosmetic products and their allergens. Eur J Dermatol 1992; 2:465–468.
14. Berne B, Lundin A, Enander Malmros P. Side effects of cosmetics and toiletries in relation to use: a retrospective study in a Swedish population. Eur J Dermatol 1994; 4: 189–193.
15. de Groot AC, Nater JP, van der Lende R, Rijcken B. Adverse effects of cosmetics: a retrospective study in the general population. Int J Cosm Science 1987; 9:255–259.

16. Katsarar A, Kalogeromitros D, Armenaka M, Koufou V, Davou E, Koumantaki E. Trends in the results of patch testing to standard allergens over the period 1984–1995. Contact Dermatitis 1997; 37:245–246.
17. de Groot AC, Frosch PJ. Adverse reaction to fragrances. Contact Dermatitis 1997; 36:57–86.
18. Rastogi SC, Johansen JD, Frosch P, Menné T, Bruze M, Lepoittevin JP, Dreier B, Andersen KE, White IR. Deodorants on the European market: quantitative chemical analysis of 21 fragrances. Contact Dermatitis 1998; 38:29–35.
19. Johansen JD, Menné T. The fragrance mix and its constituents: a 14-year material. Contact Dermatitis 1995; 32:18–23.
20. Frosch PJ, Pilz B, Andersen KE, Burrows D, Camarasa JG, Dooms-Goossens A, Ducombs G, Fuchs T, Hannuksela M, Lachapelle JM, Lahti A, Maibach HI, Menné T, Rycroft RJG, Shaw S, Wahlberg JE, White IR, Wilkinson JD. Patch testing with fragrances: results of a multi-center study of the European Environmental and Contact Dermatitis Research Group with 48 frequently used constituents of perfumes. Contact Dermatitis 1995; 33:333–342.
21. Larsen W, Nakayama H, Fischer T, Elsner P, Frosch P, Burrows D, Jordan W, Shaw S, Wilkinson J, Marks J, Sugawara M, Nethercott M, Nethercott J. A study of new fragrance mixtures. Am J Contact Dermatitis 1998; 9:202–206.
22. Frosch PJ, Johansen JD, Menné T, Rastogi SC, Bruze M, Andersen KE, Lepoittevin JP, Gimenez Arnau E, Pirker C, Goossens A, White IR. Lyral is an important sensitizer in patients sensitive to fragrances. Br J Dermatol 1999; 141:1076–1083.
23. Frosch PJ, Johansen JD, Menné T, Pirker C, Rastogi SC, Andersen KE, Bruze M, Goossens A, Lepoittevin J-P, White IR. Further important sensitizers in patients sensitive to fragrances. I. Reactivity to 14 frequently used chemicals. Contact Dermatitis 2002; 47:78–85.
24. Frosch PJ, Johansen JD, Menné T, Rastogi SC, Andersen KE, Bruze M, Goossens A, Lepoittevin J-P, White IR. Further important sensitizers in patients sensitive to fragrances. II. Reactivity to essential oils. Contact Dermatitis 2002; 47:279–287.
25. Bordalo O, Pereira F, Ferreira L, Picoto A. Patch testing with commercial perfumes (abstract). Contact Dermatitis 2000; 42(suppl 2):15.
26. Directive 2003/15/EC of the European Parliament and of the Council of 27 February 2003 amending Councel Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products. TGD-D7- Annex 3. Off J Eur Union 11.3.2003.
27. Karlberg A-T, Dooms-Goossens A. Contact allergy to oxidized d-limonene among dermatitis patients. Contact Dermatitis 1997; 36:201–206.
28. Lepoittevin JP, Meschkat E, Huygens S, Goossens A. Presence of resin acids in "oakmoss" patch test material: a source of misdiagnosis. J Invest Dermatol 2000; 115:129–130.
29. Goossens A, Lepoittevin J-P. Allergie de contact aux cosmétiques et aux composants de parfums: aspects cliniques, chimiques et diagnostiques nouveaux. Contact allergy to cosmetics and their perfume components: new clinical, chemical and diagnostic aspects. Revue Française d'Allergologie et d'Immunologie Clinique 2003; 43:294–300.
30. Goossens A, Beck M, Haneke E, McFadden J, Nolting S, Dumpt G, Ries G. Cutaneous reactions to cosmetic allergens. Contact Dermatitis 1999; 40:112–113.
31. Wilkinson JD, Shaw S, Andersen KE, Brandao FM, Bruynzeel DP, Bruze M, Camarasa JMG, Diepgen TL, Ducombs G, Frosch PJ, Goossens A, Lachapelle J-M, Lahti A, Menné T, Seidenari S, Tosti A, Wahleberg JE. Monitoring levels of preservative sensitivity in Europe. A 10-year overview. (1991–2000). Contact Dermatitis 2002; 46:207–210.
32. de Groot AC, de Cock PAJJM, Coenraads PJ, van Ginkel CJW, Jagtman BA, van Joost T, van der Kley AMJ, Meinardi MMHM, Smeenk G, van der Valk PGM, van der WalIe HB, Weyland JW. Methyldibromoglutaronitrile is an important contact allergen in the Netherlands. Contact Dermatitis 1996, 34:118–120.

33. Corazza M, Mantovani L, Roveggio C, Virgili A. Frequency of sensitization to Euxyl K400 in 889 cases. Contact Dermatitis 1993; 28:298–299.
34. Tosti A, Vincenzi C, Trevisi F, Guerra L. Euxyl K400: incidence of sensitization, patch test concentration and vehicle. Contact Dermatitis 1995; 33:193–195.
35. Perrenoud D, Birchner A, Hunziker T, Suter H, Bruckner-Tuderman L, Stäger J, Thürlimann W, Schmid P. Suard A, Hunziker N. Frequency of sensitization to 13 common preservatives in Switzerland. Contact Dermatitis 1994; 30:276–279.
36. Jacobs MC, White IR, Rycroft RJG, Taub N. Patch testing with preservatives at St. John's from 1982–1993. Contact Dermatitis 1995; 33:247–254.
37. Verhaeghe I, Dooms-Goossens A. Multiple sources of allergic contact dermatitis from parabens. Contact Dermatitis 1997; 36:269–270.
38. Wakelin SH, White IR. Dermatitis from chlorphenesin in a facial cosmetic. Contact Dermatitis 1997; 37:138–139.
39. Dooms-Goossens A, Scheper RJ, Andersen KE, Burrows D, Camarasa JG, Frosch PJ, Lahti A, Wilkinson J. Comparative patch testing with PPD-base and PPD-dihydrochloride: human and animal data compiled by the European Environmental Contact Dermatitis Research Group. In: Frosch PJ, Dooms-Goossens A, Lachapelle JM, Rycroft RJG, eds. Current Topics in Contact Dermatitis. Berlin, Heidelberg: Springer-Verlag, 1989:281–285.
40. Frosch PJ, Burrows D, Camarasa JG, Dooms-Goossens A, Ducombs G, Lahti A, Menné T, Rycroft RJG, Shaw S, White IR, Wilkinson JD. Allergic reactions to a hairdressers' series: results from 9 European centers. Contact Dermatitis 1993; 28:180–183.
41. Holness DL, Nethercott JR. Epicutaneous testing results in hairdressers. Am J Contact Dermatitis 1990; 1:224–234.
42. Liden C, Berg M, Färm G, Wrangsjö K. Nail varnish allergy with far-reaching consequences. Br J Derm 1993; 128:57–62.
43. Kanerva L, Lauerma A, Estlander T, Alanko K, Henriks-Eckerman ML, Jolanki R. Occupational allergic contact dermatitis caused by photo-bonded sculptured nail and a review of (meth)acrylates in nail cosmetics. Am J Contact Dermatitis 1996; 7:109–115.
44. Landa N, Aguirre A, Goday J, Ratón JA, Díaz-Pérez IL. Allergic contact dermatitis from a vasoconstrictor cream. Contact Dermatitis 1990; 22:290–291.
45. Santucci B, Picardo M, Cristando A. Contact dermatitis to Centelase®. Contact Dermatitis 1985; 13:39.
46. Stables CI, Wilkinson SM. Allergic contact dermatitis to panthenol. Contact Dermatitis 1998; 38:236–237.
47. Goossens A, Merckx L. Allergic contact dermatitis from farnesol in a deodorant. Contact Dermatitis 1997; 37:179–180.
48. Gonçalo M, Ruas E, Figueiredo A, Gonçalo S. Contact and photo-contact sensitivity to sunscreens. Contact Dermatitis 1995; 33:278–280.
49. Berne B, Ros AM. 7 years experience of photo-patch testing with sunscreen allergens in Sweden. Contact Dermatitis 1998; 38:61–64.
50. Schauder S, Ippen H. Photoallergische und allergisches Kontaktekzem durch dibenzoylmethanverbindungen und andere lichtschutzfilter. Hautarzt 1988; 39:435–440.
51. Theeuwes M, Degreef H, Dooms-Goossens A. Para-aminobenzoic acid (PABA) and sunscreen allergy. Am J Contact Dermatitis 1992; 3:206–207.
52. Ricci C, Vaccari S, Cavalli M, Vincenzi C. Contact sensitization to sunscreens. Am J Contact Dermatitis 1997; 8:165–166.
53. Pigatto PD, Bigardi AS, Cusano F. Contact dermatitis to cocamidopropyl betaine is caused by residual amines: relevance, clinical characteristics and review of the literature. Am J Contact Dermatitis 1995; 6:13–16.
54. Fowler JE, Fowler LM, Hunter JE. Allergy to cocamidopropyl betaine may be due amidoamine: a patch and product use test study. Contact Dermatitis 1997; 37:276–281.
55. Lorenzi S, Placucci F, Vincenzi C, Tosti A. Contact sensitisation to cocamidopropyl-PG-dimonium chloride phosphate in a cosmetic cream. Contact Dermatitis 1996; 34:149–150.

56. Dooms-Goossens A, Buyse L, Stals H. Maleated soybean oil, a new cosmetic allergen. Contact Dermatitis 1995; 32:49–51.
57. Diegenant C, Constandt L, Goossens A. Allergic contact dermatitis due to 1,3-butylene glycol. Contact Dermatitis 2000; 43:234–235.
58. Gallo R, Viglizzo G, Vecchio F, Parodi A. Allergic contact dermatitis from pentylene glycol in an emollient cream, with possible co-sensitization to resveratrol. Contact Dermatitis 2003; 48:176–177.
59. Linsen G, Goossens A. Allergic contact dermatitis from ethylhexylglycerin. Contact Dermatitis 2002; 47:169.
60. Goossens A, Armingaud P, Avenel-Audran M, Begon-Bagdassarian I, Constandt L, Giordano-Labadie F, Girardin P, Le Coz CJ, Milpied-Homsi B, Nootens C, Pecquet C, Tennstedt D, Vanhecke E. An epidemic of allergic contact dermatitis due to epilating products. Contact Dermatitis 2002; 47:67–70.
61. Goossens A, Decraene T, Platteaux N, Nardelli A, Rasschaert V. Glucosides as unexpected allergens in cosmetics?. Contact Dermatitis 2003; 48:164–166.
62. Le Coz CJ, Meyer M-T. Contact allergy to decyl glucoside in antiseptic after body piercing. Contact Dermatitis 2003; 48:279–280.
63. Duke D, Urioste SS, Dover JS, Andersen RR. A reaction to a red lip cosmetic tattoo. J Am Acad Dermatol 1998; 39:488–490.
64. Dooms-Goossens A. Testing without a kit. In: Gum JD, ed. Handbook of Contact Dermatitis. New York: McGraw-Hill, 1995:63–74.
65. Dooms-Goossens A. Hypo-allergenic products. J Appl Cosmetol 1985; 3:153–172.
66. Draelos ZD, Rietschel RL. Hypoallergenicity and the dermatologist's perception. J Am Acad Dermatol 1996; 35:248–251.
67. Draelos ZD. Sensitive skin: perceptions, evaluation, and treatment. Am J Contact Dermatitis 1997; 8:67–78.
68. Barker MO. Cosmetic industry. If the regulators don't get you, your competitors will. Am J Contact Dermatitis 1997; 8:49–51.
69. Jackson EM. Science of cosmetics. Lawyers, regulations, and cosmetic claims. Am J Contact Dermatitis 1997; 8:243–246.
70. Goossens A, Drieghe J. Computer applications in contact allergy. Contact Dermatitis 1998; 38:51–52.

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about the book…

Ranging from studies on the structure and function of the skin to research on a wide array of cosmetic compounds, this ***Second Edition*** updates readers on the latest regulatory guidelines, new cosmetic ingredients, state-of-the-art safety assessment technologies, and anticipated trends in the market—keeping pace with rapid advancements in chemistry, physics, biology, cosmetology, and toxicology to stand alone as the foremost guide to the subject.

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