9 | Mechanisms of Skin Hydration

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INTRODUCTION

One of the main functions of the skin is to maintain a competent barrier to water loss (Table 1). Water is continuously lost from the outermost skin layers to the atmosphere (evaporative water loss); and to control the rate of water loss, the barrier integrity must be preserved. Maintaining the barrier to water loss is important since hydration affects the skin's appearance, mechanical properties, and cell signaling processes (1–9). The barrier integrity can be compromised by chemical insult (e.g., the use of surfactant-containing cleansing products or harsh chemicals), mechanical insult, dry relative humidity conditions, and sun exposure (10–16).

There are several excellent review articles discussing stratum corneum structure, biochemical processes, and the importance of maintaining well-hydrated skin (17–25). In this chapter, we will build on these reviews with data pertaining to the importance of cleansing with mild products, adaptability of the skin to changing environments, effect of excess water exposure, and influence of diet on skin hydration. This chapter begins by examining the skin's intrinsic mechanisms for maintaining adequate hydration and concludes by discussing the external influences that affect the skin's water content (i.e., the environment, cleansing products, moisturizing systems, and dietary practices).

STRATUM CORNEUM

The skin is divided into three main components: the epidermis, dermis, and subcutaneous fat tissue. The stratum corneum is the uppermost layer of the epidermis. It is, in most body sites, 10 to 20 μm in depth and is composed of intercellular lipids and dead cells known as corneocytes (26). Corneocytes are flat, hexagonal-shaped keratin-containing structures surrounded by a protein-strengthened envelope. The protein envelope is made up of a variety of proteins including involucrin, loricrin, filaggrin, proline-rich proteins, and keratolinin (27,28). Corneocytes originate from proliferative epidermal cells known as keratinocytes. As the keratinocytes divide and migrate up toward the outermost skin layers, by a process known as differentiation, they change their morphology and cell content. By the time they reach the stratum corneum, they become flattened, protein-rich sacs. The corneocytes have no nucleus or any other cell organelles. Although the stratum corneum is sometimes referred to as the nonviable epidermis, perturbation of this tissue initiates a cascade of events occurring in the stratum corneum as well as in the viable epidermis [e.g., changes in protease activity, lipid biosynthesis, aquaporin (AQP), and filaggrin expression, etc.] (29).

The epidermis is divided into four main continuous layers: the stratum corneum, stratum granulosum, stratum spinosum, and stratum basale. As Figure 1 illustrates, the character of the cells within each of these layers is quite distinct. The basal, keratinocyte cells are columnar in shape, are found in the deepest layer of the viable epidermis, and divide and migrate upward to eventually replace the corneocyte cells. The entire process from cell birth to the "desquamation" of the corneocyte cells takes three to four weeks. The cells in the stratum spinosum are more polygonal shaped and have spinelike projections that cross intercellular spaces and form desmosomes and tight junctions. It is within these cells that keratin synthesis is initiated. In the stratum granulosum, the cells begin to flatten and the major organelles (including the mitochondria and nucleus) begin to degenerate. The stratum corneum represents the skin's uppermost "horny layer" that consists of dead, keratin-filled corneocytes.

Elias and Friend DS (31–35) have proposed a model for the stratum corneum, known as the "brick and mortar" model. The rigid, keratin-filled corneocytes are the bricks, and the intercellular lipids are the mortar. The intercellular lipids, along with lectins, desmosomes, and

Table 1 Main Functions of the Skin

| Functions of the skin	Activity
Protective shield	Protects body from mechanical insult, chemical penetration, germ invasion, and UV radiation
Barrier to water loss and foreign body penetration	Prevents the evaporation of excess water and thwarts the penetration of chemicals and pathogens
Temperature regulator	Contains sweat ducts that modulate body temperature
Detoxification system	Because skin continuously desquamates, it provides an avenue for the body to eliminate toxins
Early defense system	Langerhan cells capture and transfer foreign material (e.g., viruses and bacteria) to the lymph nodes for their safe removal from the body
Sensory organ	The presence of nerve endings and Merkel cells enables the sense of touch
Appearance	The skin defines a person's physical appearance
Wound repair	Natural restorative response to repairing tissue damage

Abbreviation: UV, ultraviolet.
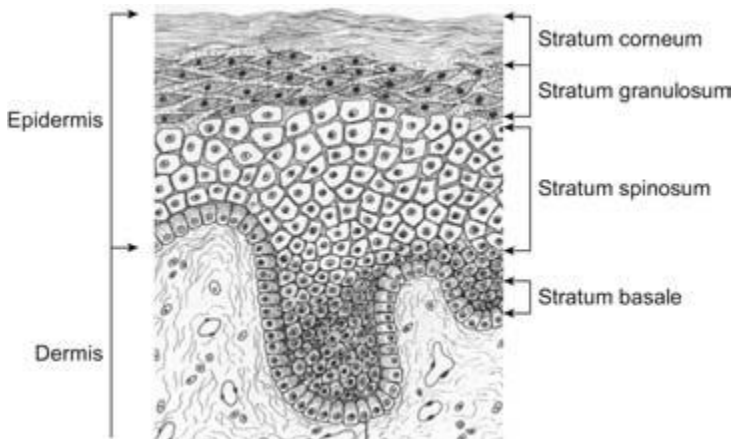
Figure 1 Schematic diagram of the skin's major epidermal layers. *Source*: From Refs. 10 and 30.

corneodesmosomes, bind to corneocytes that help to hold them in place (36). It is the physical arrangement of corneocytes and lipids, which enables the skin to resist high transepidermal water loss (TEWL) and prevent foreign microbial and chemical entities from gaining entry into the body.

Natural Moisturizing Factor

In addition to keratin, which can bind a substantial amount of water, the stratum corneum contains a number of other hydrophilic agents listed in Table 2. These materials are called natural moisturizing factors (NMF) (37–39). The NMF constitute about 20% to 30% dry weight of the stratum corneum (40) and are found intracellularly as well as extracellularly

Table 2 Composition of Natural Moisturizing Factor

Components	Mole percent (%)
Amino acids	40.0
Sodium pyrrolidone carboxylic acid	12.0
Lactate	12.0
Urea	7.0
Ions (e.g., Cl^-, Na^+, K^+, Ca^{2+}, Mg^{2+}, PO_4^{3-})	18.5
Sugars	8.5
Ammonia, uric acid, glucosamine, creatine	1.5
Citrate and formate	0.5

Source: From Refs. 18 and 25.

[e.g., sugars, hyaluronic acid (HA), urea, and lactate] (41). The major contributors to the intracellular NMF are basic amino acids and their derivatives, such as pyrrolidone carboxylic and urocanic acid, comprising up to 50% weight of the total NMF. The NMF concentration varies as a function of age and skin depth (20). Harding et al. report that for healthy skin, not exposed to surfactant damage, the NMF content is independent of depth until one approaches the filaggrin-containing levels of the skin (20). In the deeper stratum corneum layers of older individuals (50–65 years), the NMF concentration is low. This observation is a reflection of the skin's diminished ability to degrade filaggrin.

Because the NMF are effective humectants, they have a positive impact on the biochemical and mechanical properties of the stratum corneum. I.H. Blank communicated the importance of maintaining effective concentrations of water in the stratum corneum to prevent or reduce skin tightness, cracking, scaling, and flaking (12,14). In addition to enhancing the skin's water content, the NMF improve skin plasticity due to specific interactions with keratin. The NMF reduce the mobility of water as well as intermolecular forces between the keratin fibers (42). Neutral and basic amino acids appear to be the major contributors to the plasticization process. Removal of the soluble NMF can occur during water rinsing and cleansing (43). Mild cleansing systems should thus be used to minimize the NMF removal.

Most amino acid–based NMF (and their derivatives, pyrrolidone carboxylic and urocanic acid) are derived by the enzymatic hydrolysis (proteolysis) of the protein, filaggrin, and to a lesser extent by the hydrolysis of corneodesmosomes (17,44–46). Filaggrin is a protein found in the stratum granulosum layer. It is derived from the 500 kDa, highly basic profilaggrin protein found in the keratohyalin granules of the epidermis. Profilaggrin is degraded to filaggrin (via a dephosphorylation process) in the uppermost layers of the viable epidermis. Because profilaggrin is osmotically inactive, the skin has engineered a process to protect the water-rich epidermal cells from osmotic pressure–induced lysis (17). Conversely, the ability of filaggrin to degrade into the components of the NMF in the stratum corneum makes it possible for the outermost skin layers to maintain an adequate water supply when exposed to dry environments. The breakdown of filaggrin is strictly controlled by the water activity (1,18,47). On the basis of in vitro experiments, the degradation of filaggrin only occurs when the water activities are between 0.7 and 0.95. At higher activities, no breakdown occurs (48). At lower activities, the proteolytic enzymes are inactivated, and the desquamation process ceases. Consequently, when the skin is occluded (or when the relative humidity is high), there is minimal breakdown of filaggrin. Drier conditions lead to an increase in proteolytic activity, resulting in the production of more NMF. A mechanism is thus present that ensures adequate water content in the skin layer most influenced by changes in environmental conditions or chemical insult.

Using tape-stripping methods (49,50) and confocal Raman spectroscopy (43), investigators have shown that the concentration of NMF declines substantially as one approaches the stratum granulosum. This is consistent with the fact that filaggrin degradation begins in the stratum compactum, the lowest region of the stratum corneum. Given the higher water content, one expects that low amounts of NMF would be formed near the stratum granulosum/stratum corneum border. As the concentration of water decreases in the upper stratum corneum, an enhanced degradation of filaggrin occurs. Surprisingly, Egawa and Tagami reported no changes in the concentration of NMF (other than lactic acid and urea, which could have been produced via sweating) as a function of season (51). The only correlation was the panelist's subjective feeling of "not feeling dry" and higher amounts of NMF. In this same report, younger Japanese individuals (mean age: 32 years) had a lower amount of NMF versus older individuals (mean age: 67 years). This result was attributed to the faster stratum corneum turnover of the younger age group. Unlike what was reported previously (20), these authors showed a high amount of NMF at the skin surface that decreased as a function of depth. Typically, the uppermost layer of the stratum corneum has a lower NMF content than the mid–stratum corneum presumably because cleansers remove the surface material.

Some NMF behave as simple humectants and have other functions. Lactate and potassium, for example, affect the pH and stiffness of the stratum corneum (52). The L-isomer of lactic acid also stimulates ceramide biosynthesis and improves barrier function (53).

Two additional NMF, HA (54) and glycerol, have also been found in the stratum corneum. HA, a nonsulfated glycosaminoglycan, is a hygroscopic polymer of repeating disaccharide units of *N*-acetylglucosamine and glucuronic acid. It is a well-known component of the dermis, maintaining its hydrated state and providing structural integrity. In the stratum

corneum, it not only functions as a humectant but also interacts with the intercellular lipids and regulates the mechanical properties of the stratum corneum.

Glycerol may be derived from the breakdown of sebaceous triglycerides or originate from the conversion of phospholipids to free fatty acids. The importance of glycerol was revealed in a study completed by Fluhr et al. (55). These authors employed mice models where sebaceous glands (which produce triglycerides that degrade to glycerol) were largely absent and showed that although the permeability barrier responded to mechanical abrasion similar to the control, skin hydration was only enhanced by the addition of glycerol. Like HA, glycerol also influences the skin's pliability by interacting with skin lipids. Froebe et al. (56) and Mattai et al. (57) showed how glycerol could modulate the phase behavior of intercellular lipids favoring a more pliable, liquid crystalline structure at low relative humidities.

Stratum Corneum Lipids

Stratum corneum lipids play a major role in maintaining skin hydration. These intercellular lipids comprise approximately 40% to 50% ceramides, 20% to 25% cholesterol, 15% to 25% fatty acids (that have chain lengths between 16 and 30 carbons, C24:0–C28:0 being the most abundant), and 5% to 10% cholesterol sulfate; the approximate molar ratios of these lipids are 1:1:1 (ceramide: fatty acid: cholesterol) (58–60). They represent about 15% of the dry weight of the stratum corneum (61). These intercellular lipids are arranged in a highly organized lamellar arrangement (or bilayer) with only very small amounts of water present, presumably interacting with the lipid polar head groups (62). This compact lamellar structure is a very effective barrier to the TEWL. When the skin is exposed to solvents such as toluene, n-hexane, or carbon tetrachloride, which remove barrier lipids, the TEWL is increased (63). The ceramides are major components of the intercellular lipids, and this is reflected in their contribution to the structural organization of the lamellar bilayer. There are about nine major ceramides, which are synthesized from glucosylceramides, epidermosides (acylglucosylceramides), and sphingomyelin (64). These ceramides have complex structures varying in both their polar head groups and dual hydrophobic chains (Fig. 2) (65). Each ceramide contributes in specific ways to stratum corneum organization and cohesion and thus to the integrity of the barrier. In particular, the ω-hydroxyacyl portion of ceramide EOS (Fig. 2) completely spans a lamellar bilayer and the linoleate tail is believed to intercalate between a closely apposed bilayer, essentially linking two bilayers together (60,66). In fact, when any of the acylceramides is extracted, the periodicity of the lamellar bilayer structure is eliminated (67).

Figure 2 lists the structure and names of the nine identified ceramides. The ceramide (CER)-naming nomenclature was proposed by Motta et al. (68). Ceramides are designated: CER FB, where F is the type of fatty acid and B is the type of base. N represents normal fatty acids; A stands for α-hydroxy fatty acids; O represents ω-hydroxy fatty acids; and E represents ester linked linoleic acid. S, P, and H represent sphingosines, phytosphingosines, and 6-hydroxysphingosine, respectively.

Lipid Organization and Structural Models

Electron diffraction studies (69) have shown that as corneocytes migrate from the lower regions of the stratum corneum to the outer layers, there is a corresponding change in lipid packing from a more ordered, orthorhombic packing to a more fluid hexagonal phase. This observation is consistent with the known weakening of the barrier and complete loss of lamellar ordering in the topmost layers of the stratum corneum (70–72). Changes in the composition of the stratum corneum lipids in the upper stratum corneum (i.e., increased concentration of cholesterol sulfate, hydrolysis of CER EOS, increased concentration of short–chain length fatty acids, crystallization of cholesterol, and decreased levels of ceramides) presumably influence the loss of lamellar order (71). Indeed, factors that can affect lipid composition, such as washing with harsh cleansers, perturb the lamellar structure and adversely change the condition of the skin (72).

There are several models that have been proposed to describe the structural phases of the lipid bilayer (Table 3). The domain mosaic model suggests that lipids coexist as a mixture of liquid crystalline and gel phases (73). The more ordered gel phase allows for greater packing of the lipids and hence a more effective barrier.

X-ray diffraction studies of hydrated stratum corneum have shown two types of lamellar structures, having repeat distances of 13.2 to 13.4 nm (long periodicity phase) and 6.0 to 6.4 nm (short periodicity phase) (62,74). Bouwstra et al. (75) have proposed a molecular arrangement
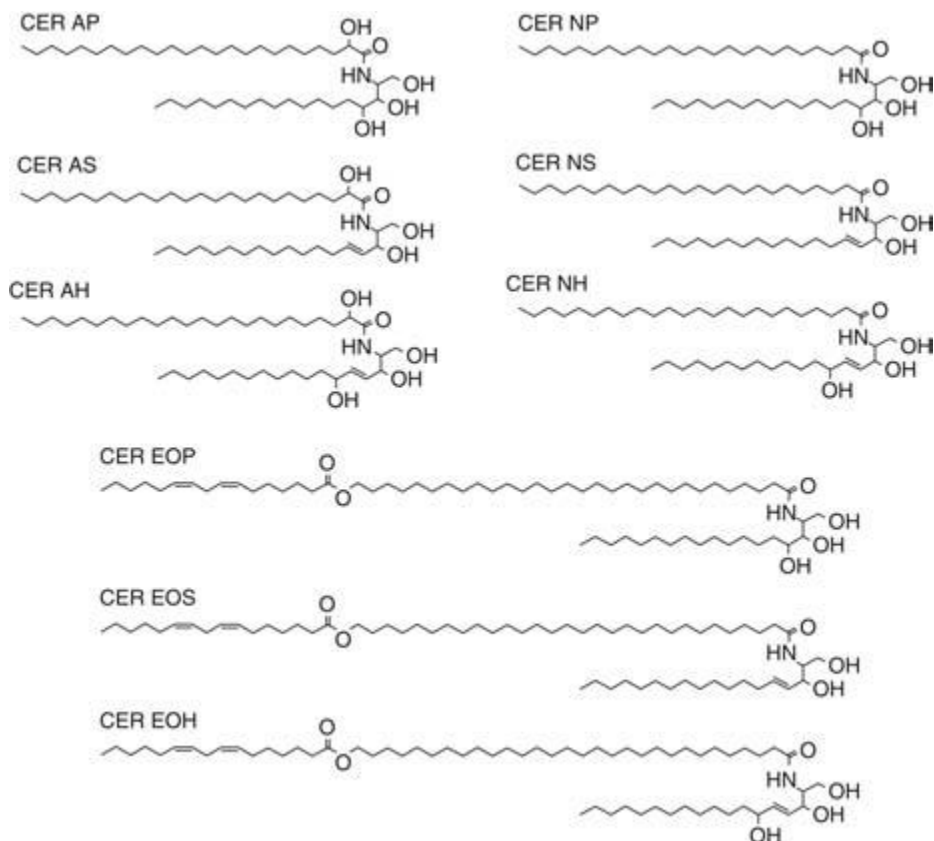
Figure 2 Chemical structure of stratum corneum ceramide lipids. The depicted ceramide naming, CER FB, was proposed by Motta et al. (68). F refers to the type of fatty acid and B to the type of base. N represents normal fatty acids; A stands for α-hydroxy fatty acids; O represents ω-hydroxy fatty acids; and E represents ester linked linoleic acid. S, P, and H represent sphingosines, phytosphingosines and 6-hydroxysphingosine, respectively.

Table 3 Proposed Models Describing How Barrier Lipids Structure Within Stratum Corneum

Skin barrier model	Description
Domain mosaic	Stratum corneum barrier lipids coexist in liquid crystalline (water permeable) and highly ordered gel phase (water impermeable) domains. Water is expected to be most permeable at the phase boundaries. The more fluid crystalline phase allows for the permeation of water.
Sandwich model	Proposes a more structured arrangement of liquid crystalline and gel domains. A narrow central lipid layer with fluid domains (3 nm wide) lies between two broad, crystalline lipid layers (6.4 nm wide).
Single–gel phase model	Skin barrier lipids exist as a single lamellar gel phase with no phase boundaries.

of the long periodicity phase, called the sandwich model, consisting of two broad lipid layers of about 5 nm each, with a crystalline structure separated by a narrow central lipid layer of about 3 nm with fluid domains. Cholesterol and ceramides are important for the formation of the lamellar phase, while fatty acids mostly impact the lateral packing of the lipids.

L. Norlen has proposed yet a third skin barrier model (76). This model suggests that the lipid matrix has a homogeneous lamellar gel phase with a low degree of lipid fluidity. Stratum corneum epidermal lipid heterogeneity, the long length of the fatty acids chains, and the presence of cholesterol are used to support this model since these factors have been shown to stabilize gel phases (77,78). This model does not require the presence of water or any bilayer conformation.

There are also different models describing the mechanism of skin barrier formation. The Landmann model suggests that "lamellar bodies" or stacked monobilayer vesicles separate from the trans-Golgi network, extrude into the intercellular space at the stratum granulosum/stratum corneum border, fuse with the cell plasma membrane of the stratum granulosum, and discharge the lipids into continuous multilamellar membrane sheets in the intercellular space (79). The membrane-folding model (80–82) argues against abrupt changes in lipid phase transitions that would result from the disruption, diffusion, and fusion of the lamellar bodies. On the basis of this model, the skin barrier formation takes place as a direct, continuous unfolding of a three-dimensional membrane into a flat, multilayered two-dimensional lipid structure (having only hexagonal hydrocarbon chain packing and no abrupt phase transitions) (80,81). By evaluating vitreous sections of non-pretreated, non-stained, full-thickness, hydrated, skin, using cryo-transmission electron microscopy, cubic-like membrane structures were observed. This organizational pathway was proposed to be more thermodynamically preferred to that previously described by Landmann (79). The cryo-transmission electron microscopy preparation was also reported to be improved over conventional electron microscopy methods because it does not require dehydration and chemical fixation of the sample. With additional innovations in instruments and instrumental techniques, active research will be sure to continue in this area.

Lamellar Lipid Arrangement and Water Permeability

The lamellar or bilayer arrangement, independent of the nature of the lipids from which it is derived, is a natural barrier to water permeability (83). In the skin, there is a relatively large gradient in water chemical potential between the viable epidermis, where the water content is about 70% by weight, and the stratum granulosum/stratum corneum junction, where the water content drops to 15% to 30% (84). Under this large water gradient, the stacked bilayer arrangement of lipids, which is a continuous region in the stratum corneum, provides an optimal way to reduce water loss through the skin. Water escaping from the stratum corneum would have to traverse the tortuous pathway of the bilayer (73,85). In addition, fully matured corneocytes would also increase the tortuosity and hence the diffusional path length of water (19). The combination of a lamellar arrangement of lipids and increased diffusional path length due to corneocytes reduce water diffusion to the atmosphere.

AQUAPORINS AND TIGHT JUNCTIONS

Another mechanism by which the skin maintains its hydrated state is the use of AQPs. These transmembrane proteins form water channels across cell membranes, facilitating the transport of small polar molecules across the cell membrane. Specific AQPs also have the ability to facilitate the transport of glycerol and urea. AQP3 is most relevant to skin hydration (19). AQP3 is localized in the basal and suprabasal layers of the epidermis, and is not expressed in the stratum corneum. In AQP3-deficient mouse skin, the skin is less hydrated, less elastic, the permeability of water and glycerol within the skin is reduced, and there is a delayed barrier recovery (86–88). Only by adding glycerol does the condition of the skin improve (89). Skin diseases associated with impaired barriers and low skin hydration also tend to have reduced expression of AQP3. Boury-Jamot et al. found that AQP3 expression was inversely correlated to the severity of patients with eczema and spongiosis (90).

Tight junctions consist of more than 40 transmembrane [i.e., claudins, occludin, and junctional adhesion molecules (JAMs)] and plaque proteins (zonula occludens) (91). This protein combination forms a semipermeable barrier between aligning cell membranes, making it very difficult for water to pass through the space between the epidermal cells. Ions or fluids must actually diffuse or be actively transported through the cell to pass through the tissue. Claudins, occludins, and JAMs are principally responsible for controlling water permeability. Claudin 1–deficient mice die within one day of birth because of excessive TEWL (92). The presence of organized tight junctions and an intact stratum corneum barrier ensures low values of TEWL. For those diseases due to which patients experience dry skin and a compromised barrier (e.g., psoriasis vulgaris and ichthyosis vulgaris), the location of tight-junction proteins may also be altered. Proteins that may be expressed homogeneously throughout the epidermis may be preferentially expressed in the upper or lower layers.

DESQUAMATION

So far, the above discussion has centered on natural ways in which the human skin has evolved to retain water. In addition to hydrating the skin, water also plays a crucial role in the exfoliation or desquamation of corneocytes. Corneocytes are linked in the lower stratum corneum by corneodesmosomes, which are macromolecular glycoprotein complexes. As the corneocytes move from the lower to the outer region of the stratum corneum, the corneodesmosomes are progressively degraded by hydrolytic enzymes. This leads to desquamation in the outer stratum corneum. These enzymes include serine proteases such as stratum corneum chymotryptic enzyme (SCCE) and stratum corneum tryptic-like enzyme (SCTE), which are more effective at neutral pHs and are most active on the outermost layers of the stratum corneum (19,47,93–95). The cathepsin family of proteases is more active under lower pH conditions and are present throughout the stratum corneum. Other proteases include cysteine proteases, sulfatases, and glycosidases. Many of these enzymes are localized in the intercellular space, and their activity is affected by both the lipid organization and water content (20,96). Clearly, low water content within the stratum corneum affects the activities of stratum corneum proteases, which leads to dry, flaky skin. Recently, these changes have been studied as a function of season, anatomical site, and skin depth (97). To maintain these processes, in vitro results suggest that optimally hydrated skin requires water content between 10% and 20% (13).

ENVIRONMENTAL IMPACT ON SKIN HYDRATION

Changes in lipid biosynthesis (71,98), epidermal DNA synthesis (9), barrier function (99), and skin thickness (100) are all influenced by the skin's water content. There are many studies showing that biochemical processes are also altered as a function of changes in the environmental relative humidity (101,102). Rawlings et al. demonstrated that dry conditions inhibit corneodesmosomal degradation, while increasing humidity increases corneodesmosomal degradation (103). Moreover, when the human skin was exposed to low humidity conditions (10%) even for short exposure periods (3 and 6 hours), a significant decrease in water content of the stratum corneum and increase in skin roughness was observed (3).

Even in humid conditions, the skin is still subject to a number of environmental insults that can negatively affect skin hydration. Excess UV radiation, for example, causes UV-induced erythema leading to a compromised barrier (104). Several animal studies have demonstrated that abrupt changes in the environment, such as going from humid (80% relative humidity) to dry (less than 10% relative humidity) conditions, increases the time required for barrier function to return to normal (99). In this situation, the skin does not have enough time to adapt to the new climatic conditions. Declercq et al. have further demonstrated that skin can adapt to dry climatic conditions (5). They found that the panelists living in a hot, dry climate such as Arizona had a better barrier function and less dry skin compared with the panelists living in New York, which had a more humid climate (5).

While prolonged exposure to conditions of low relative humidity (<20%) enhance barrier function, sustained exposure to high-humidity conditions leads to a gradual deterioration in the barrier (1). A relative humidity greater than 80% is associated with a decrease in NMF and corneocyte hydration in the epidermis of hairless mice (1). It has also been shown that when normal skin is exposed to a moist environment, the kinetics of barrier recovery is delayed because of a reduction in the number of epidermal lamellar bodies and lipid content, in direct contrast with what is observed at low humidities (102). Therefore, when the skin adapts to a high-humidity environment, its capacity to respond to external changes is decreased, partially because of a reduction in the reservoir of stratum corneum lipids.

It is remarkable that a human fetus has a mechanism to protect the outermost skin barrier to the damaging effects of amniotic fluid, an environment that would result in a loss of barrier function in adults (105). During the third trimester of gestation, a biofilm known as vernix caseosa forms and coats the prenatal skin. This film acts as a barrier and facilitates the formation of the acid mantle, which provides an optimal environment for inhibiting bacterial colonization (106,107). Vernix caseosa consists of ∼80% water, 10% protein (corneocytes with no desmosomal attachments), and 10% lipids by weight (consisting of barrier and sebaceous

lipids not arranged in any lamellar structure). This material has been shown to have multiple functions, besides being an efficient moisturizer and osmoregulator (108). On the basis of transmission electron microscope images, the limited structure of vernix caseosa is very similar to that of the topmost layers of the stratum corneum. The body appears to have retained this structural feature of vernix caseosa during the course of stratum corneum maturation.

PERSONAL CARE PRODUCTS AND SKIN HYDRATION

The Effect of Cleansing Systems

Cleansers are designed to remove unwanted materials from the skin such as dirt, oils, and sebum. However, the use of harsh surfactants damages the skin barrier; increases the skin's susceptibility to environmental sources of irritation and sensitization; and reduces skin moisture and smoothness (109). Charged surfactants, such as anionic and cationic, are the most aggressive. Sodium lauryl sulfate (SLS) is a harsh surfactant that, given its small hydro-dynamic radius, is the only surfactant that can extract the intercellular lipids and disrupt the lipid bilayer (110). It, along with most of the charged surfactants, adsorb skin proteins, causing them to denature and swell. Rhein et al. reported that the extent of protein denaturation is dependent on the surfactant monomer concentration and exposure time (111). As surfactants denature skin proteins, enzymatic reactions that control desquamation, inflammation, and oxidation processes are negatively impacted (112,113). The resulting enhanced barrier permeability leads to skin dryness, roughness, cracking, and inflammation (10,47,114).

Fortunately, there are a number of surfactants used commercially that are mild to the skin. These include mostly nonionic and amphoteric variants and the anionic variants: highly ethoxylated (at least 5-EO) alkyl sulfates, sulfosuccinates, isethionates, sarcosinates, taurates, alkyl phosphates, and alkyl glutamates. The aggressiveness of charged surfactants can be mitigated by reducing the concentration of the surfactant's monomer species, reducing the charge by incorporating various counterions and/or cosurfactants to form mixed micelles, and introducing ethoxylation (10). The improved mildness reduces the incidence of barrier damage, which aids in the maintenance of hydrated skin (i.e., nondrying cleansers).

Surfactants also negatively impact the skin hydration properties by removing NMF. Blank and Shappirio (14) showed that when isolated human stratum corneum was exposed to 1% solutions of soap, alkyl sulfate or alkyl benzylsulfonate, all surfactants reduced the ability of the tissue to absorb water from the atmosphere, relative to water. This water-holding capacity is correlated with the loss of NMF. A similar correlation has been found between natural saponified soaps and mild synthetic surfactants using confocal Raman spectroscopy (115) (Fig. 3).

There has been a great deal of research focused on delivering enhanced skin moisturization using cleansers (109). Emollient-containing cleansers have been found to alleviate the dry skin condition of people having rosecea, sensitive skin, and/or atopic dermatitis (116,117). Emulsion-based liquid body washes are commonly employed to mildly
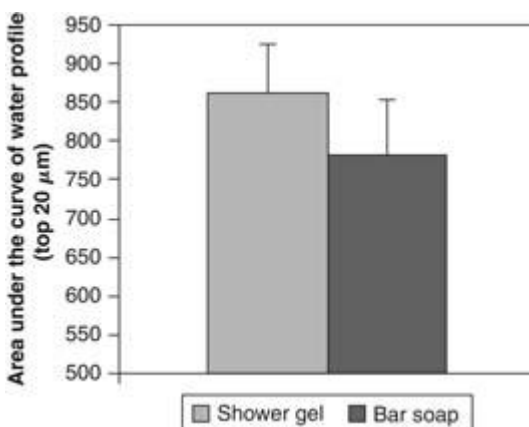

Figure 3 Water content as a function of cleanser type determined using confocal Raman spectroscopy. *Source*: From Ref. 115.

cleanse and moisturize the skin. Although delivering a moisturization benefit using lipophilic agents is difficult to achieve in a rinse-off system, clinical studies have confirmed that enhanced moisturization can be achieved in formulas containing a large quantity of oils and/ or humectants (118). The patent literature is replete (but will not be further discussed in this chapter) with examples of approaches to improve the delivery of actives from cleansing systems. Invariably, it has been demonstrated that cleansing systems are able to remove dirt and bacteria while simultaneously depositing oils on the skin to improve skin feel, smooth desquamating corneocytes, and improve barrier function.

Research has demonstrated that oatmeal is a good choice for gentle cleansing and moisturizing dry, sensitive skin (119). Oatmeal has been used for centuries as a soothing agent to relieve itch and irritation associated with various xerotic dermatoses. Many clinical properties of colloidal oatmeal are derived from its chemical polymorphism. Its high-concentration of starches and β-glucan is responsible for the protective and water-holding functions of oat. The presence of different types of phenols confers antioxidant and anti-inflammatory activity. Some of the oat phenols are also strong UV absorbers. The cleansing activity of oat is mostly due to saponins. Many of its functional properties make colloidal oatmeal a good cleanser, moisturizer, buffer, as well as a soothing and protective anti-inflammatory agent (120).

Although cleansers have been formulated to successfully deliver oils to the skin, delivering humectants has been more challenging. Humectants are highly water soluble and, consequently, harder to deposit onto the skin during the washing process. Special delivery systems have yet to be developed to improve the competency in this area.

Moisturizing the stratum corneum using lotions and creams is typically the best way to hydrate the skin. This is typically accomplished by using emulsion formulas, which contain humectants, emollients, and/or occlusive agents (121). Humectants attract and hold on to water. Occlusive agents form a barrier across the skin, reducing the TEWL. "Emollient" comes from a Latin derivation meaning a material designed to soften and soothe the skin (122). Emollients can be occlusive or semiocclusive meaning they may not be very effective at preventing evaporative water loss, but are effective in smoothing skin.

Glycerol and urea are well-known humectants (123–125). Glycerol also prevents the crystallization of stratum corneum lipids at low relative humidity, which leads to less TEWL and higher water content of the skin. Previous studies evaluated the influence of glycerol on the recovery of damaged stratum corneum induced by repeated washings with SLS. The authors found that glycerol created a stimulus for barrier repair and improved stratum corneum hydration (126).

Petrolatum is a common occlusive agent. Application of hydrophobic materials such as petrolatum to prevent skin dryness may be as old as mankind itself. In recent times, however, manufacturers are incorporating lipids that can form lamellar bilayers in their formulations to enhance the barrier properties of the skin (127,128). They typically use ceramides or ceramide-like molecules to accomplish this goal and have found even greater benefit when they combine the lipid technology with glycerol (129). Niacinamide has also been shown to enhance lipid biosynthesis, which again improves barrier function (130). As in the above situation, the addition of glycerol further improves the clinical dry-skin condition.

Water in Excess

Skin exposure to extrinsic water is usually considered to be harmless. Often times it is used as the "control" site in experiments that investigate the way compounds interact with the skin. However, there is evidence that prolonged contact with water can negatively affect SC barrier function, similar to surfactants (131). In addition to eliciting erythema, inflammation, and intense dermatitis, excess water exposure can increase SC swelling and suppleness, weaken SC corneocyte cohesion, and increase the permeability of all substances, especially water. Warner et al. (114,131) showed that overexposure of skin to water causes a disruption of the SC intercellular lamellar bilayer ultrastructure in vitro as well as in vivo. Similar to surfactant exposure, the swelling response was time dependent, and wide intracellular clefts between corneocytes were observed. These studies as well as others show that prolonged hydration of the SC can directly disrupt the barrier lipids, leading to compromised skin (114,131,132).

DIETARY IMPACT ON SKIN CONDITION

It is generally stated that topically applied cosmetic products can be helpful in restoring normal hydration to dry skin. However, less recognized is the positive influence that drinking plenty of water can have on the skin's appearance. Approximately 45% to 70% of human body weight consists of water. One-third of the total body water is extracellular, and two-thirds are within the intracellular compartment (133). Water is free to move between the cell membranes with any net movement controlled by the effective osmotic and hydrostatic pressures. This balance of body fluid is dependent on the intake of water through drinking, food, and metabolism and the loss of water through natural processes. The three components of the skin, the epidermis, dermis, and subcutaneous fat tissue, play a major role in water regulation, with the SC water content helping to maintain many of the skin's biophysical properties (134). Soft, smooth skin has an optimally hydrated SC with a water content of approximately 20% to 30%, and a water content of less than 10% to 20%, resulting in abnormally dry skin (133,134). While the environment can play a role in TEWL, a good balance between water intake and loss is vastly important in helping to maintain healthy water content in the SC, which has a positive influence on skin hydration.

An increased intake of pure, healthy water helps to enhance nutrient absorption, skin hydration, detoxification, and virtually every aspect of better health. However, studies have also shown that drinking dietary natural mineral water or taking a food supplement containing pro-hydrating actives maintains adequate skin hydration as well. Mac-Mary et al. (135) showed that the magnitude of change in a Corneometer® measurement on the forearm of healthy subjects increased by 14% when 1 L of mineral water was consumed per day for 42 days, which was clinically significant and similar to the observed modifications with moisturizing cosmetic products (10–30%). Primavera and Berardesca (133) investigated how a capsule containing an active product based on vegetable ceramides, amino acids, sea fish cartilage, antioxidants, and essential fatty acids improved skin hydration after oral use. Significant improvement in Corneometer readings were seen in the active-treated groups (+30%), in addition to a decrease in skin roughness and improved skin smoothness after 40 days, as measured using a VisioScan®. Self- and clinical-assessment data confirmed the results of the biophysical measurements. These studies demonstrate that a proper diet with adequate water and mineral intake is just as important in the management of skin hydration as a complementary cosmetic approach. Puch et al. further showed that ingesting a probiotic-containing dairy product enriched in γ-linolenic acid (an ω-6-polyunsaturated fatty acid that has been shown to enhance the rate of barrier recovery when applied topically and when taken orally), vitamin E, and catechins improved barrier function after six weeks of taking twice a day dosage. The average improvement was 13% (136). The reduction in TEWL was observed throughout the six-month study, despite the changes in season.

SUMMARY

Maintaining hydration of the stratum corneum can be accomplished using a number of different mechanisms. From using mild surfactants that minimally compromise the skin barrier to delivering moisturizers (humectants, occlusive oils, and lipid modulating agents), these materials offer a means of adding moisture back to the skin or, alternatively, reducing water loss (137,138). The skin itself, in fact, has a natural process to minimize excess water loss. Through the water-dependent production of intercellular skin lipids and NMF, an intricate mechanism is in place to function optimally in an often arid, external environment. The skin is a remarkable organ, producing vernix caseosa to protect (as a barrier, anti-infective and antioxidant) the fetus while it is immersed in amniotic fluid, a potential damaging environment, and following birth enhancing the acid mantle development, which facilitates skin maturation during the postnatal period. The production of urocanic acid and free fatty acids in the stratum corneum further contributes to the regulation of stratum corneum pH (139,140). As for those living in dry climates, the skin is adaptable and can generate an improved barrier function and increased water content. The development of the confocal Raman spectrometer has allowed researchers to noninvasively monitor the skin's water content and composition changes as a function of the environment and product use

(43,141–143). The identification of AQPs and tight junctions provides increasing evidence for internal mechanisms that the skin is using to improve the opportunities for corneocyte hydration. More importantly, there is increasing data confirming the importance of maintaining an optimal skin's water content to insure the activity of processes that occur in the epidermis. Protecting and maintaining an adequate water content and barrier function of the skin are proving to be essential to achieving healthy, youthful-looking skin.

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10 | 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).

Dry hair and dry skin are the target areas in the body for treatment with humectants. Sometimes mucous membranes also benefit from application of humectants. Dry hair is brittle and 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 (SC) is a controlling factor in maintaining skin flexibility and desquamation (2,3). Hydration plays an important role in maintaining the metabolism, enzyme activity, mechanical properties, appearance, and finally, barrier function of the skin.

The special blend of humectants found in the SC is called natural moisturizing factor (NMF) (4). NMF can make up about 10% of the dry weight of the SC cells (4). Substances belonging to this group are amino acids, pyrrolidone carboxylic acid (PCA), lactates, urea, and inorganic ions (Tables 1 and 2) (4). Furthermore, glycerol is found naturally in SC, and the mean amounts are found to be about $0.7 \ \mu g \ cm^{-2}$ on the cheek and $0.2 \ \mu g \ cm^{-2}$ on the forearm and sole (8). The proportion of the inorganic ions and lactate in the SC differs from that in sweat and also changes between winter and summer (9). The level of lactate and potassium in the SC appears to correlate with each other as well as with the physical properties of the SC (9). The levels of lactate have been found to be approximately 100 times higher than that of glycerol (8,9).

NMF is formed from the protein filaggrin, whose formation is regulated by the moisture content in the SC (2). In skin diseases such as ichthyosis vulgaris (10,11) and psoriasis (12), there is a virtual absence of NMF. In ichthyosis vulgaris, the stratum granulosum is thin or missing because of a defect in the processing of profilaggrin, which is also noticed as tiny and crumbly keratohyalin granules (13).

Glycerin is another humectant suggested to be important for the SC 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 (14). This type of dryness may also be applicable to clinical situations where sebaceous glands are absent or involuted, 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 (14).

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) (15,16). 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 (20). Urea also has strong osmotic activity (21,22). 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.

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 B5	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	$5 \times 10^4 – 8 \times 10^6$	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, 5–7.
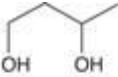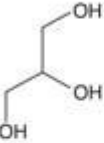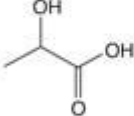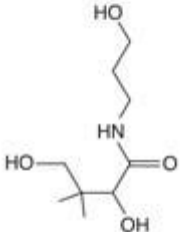
Table 2 Chemical Formulas of Humectants
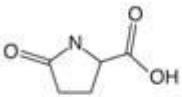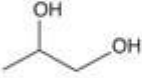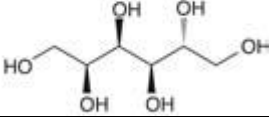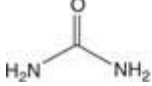
Humectant	Formula
Butylene glycol	
Glycerin	
Lactic acid	
Panthenol	
PCA	
Propylene glycol	
Sorbitol	
Urea	

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]From Ref. 15.
[b]From Ref. 16.
[c]From Ref. 17.
[d]From Ref. 18.
[e]From Ref. 5.
[f]From Ref. 19.

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?	

Hence, the in vitro humecancy should be distinguished from the in vivo moisturizing effect (23). 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 flavor and bitter aftertaste (5). It is soluble in water, acetone, and castor oil, but practically insoluble in aliphatic hydrocarbon (5).

General Use
Butylene glycol is used as humectant for cellophane and tobacco (5). 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 (24). The alcohol also retards loss of aromas and preserves cosmetics against spoilage by microorganisms (24).

Safety
Butylene glycol is considered safe by the Cosmetic Ingredient Review (CIR) Expert Panel (25). Human skin patch test on undiluted butylene glycol produced a very low order of primary skin irritation, and a repeated patch test produced no evidence of skin sensitization (25). The substance is reported to be less irritating than propylene glycol (26,27). Few reports of contact allergy exist, but the substance does not seem to cross-react with propylene glycol (26).

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) (5,12), approximately 0.6 times as sweet as cane sugar (5,12). It is miscible with water and alcohol, slightly soluble in acetone, and practically insoluble in chloroform and ether (12,13).

General Use

Glycerin is used as a solvent, plasticizer, sweetener, lubricant, and preservative (5). The substance has also been given intravenously or by mouth in a variety of clinical conditions in order to benefit from its osmotic dehydrating properties (6). This effect can also be used topically for the short-term reduction of vitreous volume and intraocular pressure of the eye (6). Moreover, concentrated solutions of glycerin are used to soften earwax (6) and suppositories with glycerin (dose 1–3 g) promote fecal evacuation (6).

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 (28). Glycerin not only attracts water but has also been suggested to modulate the phase behavior of SC lipids and to prevent crystallization of their lamellar structures in vitro at low relative humidity (29). Incorporation of glycerin into an SC model lipid mixture enables the lipids to maintain the liquid crystal state at low humidity (29). 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 (30). Thereby, the rate of corneocyte loss from the superficial surface of human skin increases, probably because of an enhanced desmosome degradation (2,30).

The mode of action of glycerol both on SC hydration and epidermal barrier function seems to be related to the aquaporin 3 channel. The aquaporins are a family of small, integral membrane proteins that function as plasma membrane transporters of water and in some cases small polar solutes [reviewed in (31)]. Glycerol is transported very slowly into the epidermis, and thus, its transport rate is sensitive to the intrinsic glycerol permeability of the basal keratinocyte layer. Repeated tape stripping taken from skin treated with 15% glycerin cream indicates that glycerin diffuses into the SC to form a reservoir (32). During some hours after application, a decrease in transepidermal water loss (TEWL) has been noted (32–35) followed by increased values after some hours in animal skin (35). No evidence of deterioration of the skin barrier function has been noted after long-term treatment of normal and atopic skin with 20% glycerin (36,37). Instead, glycerin has been found to accelerate barrier recovery after acute external perturbations (38). 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 (33). Glycerin is also suggested to induce a shrinking of superficial corneocytes, which was independent from osmotic effects (39). This contraction might give a more compact SC and reduce the risk for irritant contact dermatitis (39).

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 (6). Glycerin dropped on the human eye causes a strong stinging and burning sensation, with tearing and dilatation of the conjunctival vessels (40). There is no obvious injury, but studies have indicated that glycerin can damage the endothelial cells of the cornea (6,40). Glycerin has been shown to have excellent skin tolerability, and treatment with 20% glycerin did not show any signs of adverse effects on atopic dry skin (28).

HYALURONAN (HYALURONIC ACID)

Description
The earliest work on skin was devoted predominantly to the cells that make up the layers of skin: epidermis, dermis, and underlying subcutis. Now it is beginning to be appreciated that the materials that lie between cells, the matrix components, have major instructive roles for cellular activities. This extracellular matrix endows skin with its hydration properties. The components of the extracellular matrix appear amorphous by light microscopy, but form a highly organized structure of glycosaminoglycans (GAGs), proteoglycans, glycoproteins, peptide growth factors, and structural proteins such as collagen and, to a lesser extent, elastin. The predominant component of the extracellular matrix, however, is hyaluronan; one of the first extracellular matrix component to be elaborated in the developing embryo [reviewed in (41)]. The term "hyaluronan" is used to cover both hyaluronic acid and sodium hyaluronate. Hyaluronan is a member of the class of amino sugars containing polysaccharides known as the GAGs 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. Molecular weight is within the range of 50,000 to 8×10^6, depending on source, methods of preparation, and determination (5). 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 (42).

The skin is the largest reservoir of hyaluronic acid, containing more than 50% of the total body. The papillary dermis has the most prominent levels of hyaluronic acid than the reticular dermis. Hyaluronic acid is extracted from cock's comb or obtained from streptococci (Lancefield Groups A and C) (6). During manufacturing, the large, unbranched, noncross-linked, water-containing molecule is easily broken by shear forces (42). The carbohydrate chain is also very sensitive to breakdown by free radicals, UV radiation, and oxidative agents (42).

General Use
A viscous solution of sodium hyaluronate is used during surgical procedures on the eye and is also given by intra-articular injection in the treatment of osteoarthritis of the knee (6). Hyaluronic acid is also applied topically to promote wound healing. Topical application of 0.1% solution in patients with dry eye has been suggested to alleviate symptoms of irritation and grittiness (6).

Effects on Skin
High molecular weight hyaluronic acid solutions form hydrated viscoelastic films on the skin (42). 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 (42). 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 (6).

LACTIC ACID

Description
Lactic acid is colorless to yellowish crystal or syrupy liquid, miscible with water, alcohol, and glycerol, but insoluble in chloroform (6). 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 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 SC 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 (6,20). 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 SC, such as dandruff, callus, keratosis, and verrucae (viral warts) (6). Moreover, lactic acid has been suggested to be effective for adjuvant therapy of mild acne (43). 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 (44).

Investigators have also reported increases in the thickness of viable epidermis (45,46) as well as improvement in photoaging changes (45,47). 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 (48).

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 (20). Potassium lactate has been suggested to restore SC hydration more effectively than sodium lactate, suggesting that potassium ion itself may play certain roles in maintaining the physical properties of the SC (9). With increasing pH, the adsorption of lactic acid decreases, because of the ionization of the acid (20). In another study on strips of SC from human abdominal skin, the uptake of water by sodium lactate was greater than that by lactic acid, but the SC was plasticized by lactic acid and not by sodium lactate (15). Lactic acid also reduces the cohesion between the corneocytes and interferes with the bonding between the cells, which causes an increased cell turnover, especially at pH around 3 (49–51).

The concentrations used for treatment of ichthyosis and dry skin have ranged up to 12% (52). After treatment with 5% lactic acid combined with 20% propylene glycol, increased TEWL has been noted in patients with lamellar ichthyosis (53). However, lactic acid has been suggested to stimulate the ceramide synthesis and improve skin barrier function (54,55).

Safety

Lactic acid is caustic to the skin, eyes, and mucous membranes in a concentrated form (40). Compared to other acids, lactic acid has no unusual capacity to penetrate the cornea, so its injurious effect is presumably attributable to its acidity (40).

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 (50,51,56). The emulsion type has been reported to influence the degree of stinging, where water-in-oil emulsions induced less stinging than ordinary oil-in-water ones (57). In normal skin, irritation and scaling may be induced when the acids are applied in high concentrations and at low pH (58). 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 (51). Increased sensitivity to UV-light has also been detected, which raises concerns over long-term use (59). Due to insufficient safety data, the FDA recommends that lactic acid should be used up to a maximum level of 2.5% and a pH $\geq$ 5 (59).

PANTHENOL

Description

D-Panthenol is a clear, almost colorless, odorless, and viscous hygroscopic liquid, which may crystallize on prolonged storage (Tables 1 and 2) (6). 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) (60). Panthenol is very soluble in water, freely soluble in alcohol and glycerol, but insoluble in fats and oils (18). 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 (18). The rate of hydrolysis is lowest at pH 4 to 6 (18).

General Use
Panthenol is widely used in the pharmaceutical and cosmetic industry for its moisturizing, soothing, and sedative properties (60,61). It is also found in topical treatments for rhinitis, conjunctivitis, sunburn, and wound healing (ulcers, burns, bed sores, and excoriations); usually 2% is used (6,60). The mechanisms of action are only partly known. The hygroscopic alcohol can further be used to prevent crystallization at the spray nozzles of aerosols (18).

Effects on Skin and Hair
Topically applied panthenol is reported to penetrate the skin and hairs and to be transformed into pantothenic acid (60,62). Treatment of sodium lauryl sulfate (SLS)-induced irritated skin with panthenol accelerates skin barrier repair and SC hydration (61). Moreover, skin redness decreased more rapidly by panthenol treatment (61). Pantothenic acid can be found in normal hair (18). Soaking of hair in 2% aqueous solution of panthenol has been reported to increase the hair diameter up to 10% (63).

Safety
Panthenol has very low toxicity and is considered safe to be used in cosmetics (62). 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 (62). Low concentrations have also been tested on humans, and those formulations did not induce sensitization or significant skin irritation (62). 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 (60,64).

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 SC at levels about 12% of the NMF (4) corresponding to about 2% by weight in the SC (17). The sodium salts of PCA are among the most powerful humectants (Table 3). PCA is also combined with a variety of other substances, such as, arginine, lysine, chitosan, and triethanolamine (1).

Effects on Skin
A significant relationship has been found between the moisture-binding ability and the PCA content of samples of SC (17). Treatment with a cream containing 5% sodium-PCA also increased the water-holding capacity of isolated corneum compared with the cream base (65). 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 (65).

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 (66). Minimal, transient ocular irritation has been produced by 50% PCA (66). Immediate visible contact reactions in back skin have also been noted after application of 6.25% to 50% aqueous solutions of sodium PCA (67). 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 (66).

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) (7). Under ordinary conditions it is stable in well-closed containers, and it is also chemically stable when mixed with glycerin, water, or alcohol (7).

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 (7) (5,60). It is also often used in foods as antifreeze and emulsifier (5,7). Propylene glycol is also used as an inhibitor of fermentation and mold growth (5).

Effects on Skin
Propylene glycol has been tried in the treatment of a number of skin disorders, including ichthyosis (53,68,69), tinea versicolor (70), and seborrheic dermatitis (71), because of its humectant, keratolytic, antibacterial, and antifungal properties (7,72).

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 (7,73). Poisoning has been found after oral doses of around 100 to 200 mg/kg to children (74–76) and after topical treatment with high concentrations in burn patients (77), but the alcohol is considered safe for use in cosmetic products (78).

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% (27,78). The nature of the cutaneous response remains obscure and, therefore, the skin reactions have been classified into four mechanisms: (*i*) irritant contact dermatitis, (ii) allergic contact dermatitis, (*iii*) nonimmunologic contact urticaria, and (*iv*) subjective or sensory irritation (79). This concept allows a partial explanation of effects observed by different authors (79).

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 grown in importance during recent years 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 (80) and wheat flour and wheat starch (81) are considered safe as cosmetic ingredients by CIR. The most frequent clinical presentation of protein contact dermatitis is a chronic or recurrent dermatitis (82). Sometimes an urticarial or vesicular exacerbation has been noted a few minutes

after contact with the causative substance (82,83). Hair conditioners containing quaternary hydrolyzed protein or hydrolyzed bovine collagen have induced contact urticaria and respiratory symptoms (83). Atopic constitution seems to be a predisposing factor in the development of protein contact dermatitis (83).

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) (6). It occurs naturally in fruit and vegetables and is prepared commercially by the reduction of glucose. Sorbitol is most commonly available as 70% aqueous solution, which is clear, colorless, and viscous. It 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 (7).

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 (6). Its hygroscopic properties are reported to be inferior to that of glycerin (Table 3) (15,84).

Safety
When ingested in large amounts (>20 g/day), it often produces a laxative effect (6,7).

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 (85).

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 (6). Urea in solution hydrolyzes slowly to ammonia and carbon dioxide, which may cause swelling of the packaging (6).

General Use
Urea is used as a 10% cream for the treatment of ichthyosis and hyperkeratotic skin disorders (85,86) 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 (87).

Effects on Skin
An increased water-holding capacity of scales from psoriatic and ichthyotic patients has been observed after treatment with urea-containing creams (86,88).

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 (21). The increase in 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 (89–91). Not all studies, however, support the belief that urea is an effective penetration promoter (92,93), 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 (94,95). One moisturizer with urea also reduced TEWL in atopic patients (36,96) and made skin less susceptible against irritation to SLS (97). Improvement in skin barrier function has also been shown in dry skin (98) and in ichthyotic patients (86).

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 (6). 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 (98–100).

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 (83).

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 SC 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 SC 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.

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11 | Skin Care Products

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AN OVERVIEW OF EMULSION-BASED SKIN CARE PRODUCTS

A variety of skin care 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 are categorized in the United States into three functional groups:

- 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 Food and Drug Administration (FDA) does not recognize this category (1).

The three product groups can also be classified by their physical properties. Most common forms of skin care products are emulsions. Emulsions are mixtures of two insoluble materials that are stabilized against separation. An example is mixture of oil and water, which will not mix unless an intermediate emulsifier is incorporated into the mixture.

Different Types of Emulsions

Emulsifiers can act as solubilizers and 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 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 formulated of 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 (PEG). Recently, there has been a growing interest in water-in-oil-in-water (w/o/w) emulsions, 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 cost formulation because of a higher water content. 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-type 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 of 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).

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 w/o emulsifiers permitting large quantities of water to be incorporated as emulsified droplets.

Abbreviations: W/O, water in oil; O/W, oil in water.
Source: From Ref. 3.

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 in an effort to minimize the already low irritation potential of the formulation. It is possible to formulate emulsifier-free emulsions with cross-linked 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).

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 with respect to 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 the 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, and 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. The key to product development is the technique employed to select appropriate raw materials. Commonly used emulsifying agents are ionic (anionic or cationic) or nonionic. The function of the emulsifying agent is dependent upon 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 on 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 HLB number or hydrophilic-lipophilic balance 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 and 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 with one having an HLB of 4.

For nonionic detergents of the ester type:

$$HLB = 20\left(1 - \frac{s}{a}\right)$$

s = saponification number of the material
a = acid number of the fatty acid moiety of the product

For ethoxylated esters and ethers, when the saponification value is not known:

$$HLB = E + \frac{P}{5}$$

E = percentage of ethylene oxide
P = percentage of polyalcohol in the molecule

When the hydrophobic portion contains phenols and mono-alcohols without poly-alcohols, the equation can be simplified to:

$$HLB = \frac{E}{5}$$

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; mixtures of cationic and nonionic emulsifiers may not be as elegant. Examples of nonionic emulsifiers are 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

O/W 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.

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

Abbreviations: HLB, hydrophilic-lipophilic balance; W/O, water in oil; O/W, oil in water.
Source: From Ref. 5.

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.

Carbopol® resin is perhaps the most popular gum thickener for contributing toward 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 blend 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 permits a wide range of ingredient choice for the formulator, especially with acid systems. In alkaline formulations, polyoxyethylene ether type emulsifiers are preferred with respect to 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. Use levels may vary from 2% to 15%; at lower levels, a secondary emulsifier such as the 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 that are often used with cetyl or stearyl alcohol at 0.5% to 5.0% to produce o/w emulsions (6).

Water-in-Oil 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, e.g., emollient 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 coemulsifiers 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, 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 is 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% oil 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 a w/o/w w/o emulsifier. 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 oil in silicone emulsions as well. 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 viscoelastic 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, e.g., 2% to 3% weight/weight (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

PEG polymers are available in a variety of molecular weights. These materials are water-soluble and do not hydrolyze or support mold growth. For these reasons, PEGs make good bases for washable ointments and can be formulated to have a soft-to-hard consistency. PEGs dissolve in water to form clear solutions; they are also soluble in organic solvents. PEG ointment Unites States Pharmacopeia (USP) is a mixture of PEG 3350 and PEG 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.

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 3 to 7 in one phase and water in a second phase. Both phases are blended at 67°C to 70°C with mixing.

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. Following are frequently used ingredients to modify the feel of the emulsion on skin (Table 3).

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	5–0	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, is water repellent, and has skin-protective properties.
Humectants (Glycerin, Propylene Glycol, Sorbitol, Polyethylene glycol)	0.5–15.0	Moisture-binding properties help retard evaporation of water from formulation, control viscosity, and impact body and feel of emulsion.
Thickeners (Carbopol®, Veegum)	0.1–2.0	Help obtain viscosity, enhance stability, bodying agents.

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: 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 4).

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 hydrophilic
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	

Abbreviation: PEG, polyethylene glycol.

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 on the basis of 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, i.e., 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 the skin, but there are conditions when humectants may actually deprive the skin of water. Once a humectant has absorbed 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).

Table 5 Hydrophilic Polymers Used in Skin Care Moisturizers

Alginic Acid
Chitosan (and salts)
Collagen
Hyaluronic Acid

Source: From Ref. 10.

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.

Emulsion formulators are aware that the health of the epidermis may be affected by

- the intracorneal lipid layer, its formation, hydrolysis, and oxidation;
- enzymatic dependency of synthesis of NMF; and
- climatic changes.

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 the 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 partially reflected from the surface of stratum corneum and light that is 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 altering 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 the skin by giving the emulsion a silky feel. The same effect-enhancing pigments may be used to impart an elegant luster to the appearance of the product (11).

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 crystals have a "brilliant" white pearlescense; some grades create metallic effects while 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, while larger ones provide sparkle, glitter, and a lively appearance (11)." Use of appropriate particle size and color combinations can give the skin a "radiant glow."

Interference pigments are formulated in skin care products at levels of 0.1% to 2.0% by weight, depending upon 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 compositions 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 the skin, making fine lines more visible to the eye. Formulation with appropriate microspheres can help

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

to minimize this effect and give the skin an enhanced appearance (12). Formulating with varied particle size will further help 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 the skin after evaporation is considered 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 the skin and the vehicle to be effective (Table 7).

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 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. Another consideration is the pH of the finished product. Below a pH of 3.4, esters tend to hydrolyze, resulting in a product that may develop an undesirable odor (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 and the protein and starches in our foods as well as the tires of our automobiles are all 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 emulsifiers. These polymers have cationic charges that are substantive to skin and impart a smooth, conditioning effect. Others polymers are formulated in emulsions to create the sensation of firming skin, minimize interference pigments and other solid particles from rub off to clothing, and provide water resistance to sunscreen containing emulsions. These polymers form a film on the skin's surface (Table 8).

Table 8 Examples of Polymers (Various Sources)

Polymer	Type	Potential application
Acrylates/C10-30 alkyl acrylate cross-polymer	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 vinylpyrrolidone	Oil soluble, rub resistance in sunscreen
Tricontanyl PVP	Copolymer of vinylpyrolidone	Oil soluble, rub resistance for pigments and sunscreens

Abbreviations: W/O, water in oil; O/W, oil in water.
Source: From Ref. 14.

FORMULATING EFFECTIVE COSMECEUTICALS FOR AN AGING POPULATION

An aging consumer population seeks products to address fine lines and wrinkles, improve the appearance of an uneven skin tone, smoothen rough-textured skin, and reduce skin dispigmentation referred to as "age spots." Advances in molecular biology have enabled research investigators to develop numerous in vitro screening protocols demonstrating the potential of various cosmeceutical ingredients to help improve the appearance of aging skin.

Peptides, Vitamin Derivatives, Botanicals

In vitro data may produce very dramatic results supporting efficacy of cosmeceutical agents. Many of these agents must be properly formulated and should be properly tested in vivo to confirm they will function as desired to meet consumer expectations. Cosmeceutical agents must be compatible and stable in the vehicle they are formulated in to be effective. For example, peptides are available with variations in the number of amino acids and sequence. The peptide must be designed to have the ability to penetrate skin in order to be effective. One approach is to add a lipohilic chain, such as a palmitate (Table 9), to the peptide. A copper peptide is commercially available; to be effective, it must be formulated at significantly higher levels compared with the palmitoyl pentapeptide (17–19).

Published literature supports claims that retinoic acid improves the appearance of wrinkles, promotes collagen formation, and evens skin tone. Retinoic acid has limited stability, and consumers frequently experience dry, irritated skin during product use. To promote stability, formulation exposure to oxygen and light should be minimal. Formulating with an antioxidant and encapsulation of the retinoic acid are other options. The primary package should be designed to be oxygen and light impermeable. Irritation potential may be reduced by formulating with an appropriate retinoic acid derivative. Retinol is better tolerated by skin than *trans*-retinoic acid (20). Incorporation of anti-inflammatory agents may further mitigate irritation. Sugar amines such as glucosamine and *N*-acetyl-glucosamine can help hydrate skin and reduce fine lines/wrinkles and facial hyperpigmentation. Glucosamine tends to be unstable in formulations formulating with antioxidants, and at an acidic pH it may help overcome this problem (21). Formulations with glycolic acid are associated with an increase in sensitivity to solar exposure and sunburn cell formation in skin. For this reason, products with glycolic acids should also contain sunscreens.

Medicinal and cosmetic use of botanicals has a long history spanning many centuries. Selection of a botanical is influenced by experience passed on from generation to generation. Despite this long history of use, traditional medicine has not been officially recognized by many countries (22). In recent years, interest has increased regarding use of botanicals in skin care. Data addressing safety, quality, efficacy, and guidelines for formulating with botanicals to achieve optimal benefits are lacking. The formulator of botanical-based products

Table 9 Cosmeceutical Ingredients

Ingredient	Claimed benefit	Mechanism	Formulation consideration
Botanicals: soy, green tea, pomegranate, red clover, curcumin, resveratrol (in skin and seeds of grapes)	Soy: skin tone evening, improvement in dyspigmentation	Inhibition of PAR-2 activation by protease inhibitors	Source of soy important.
	Green tea: UVB-induced formation of thymine dimers (a marker for DNA damage) inhibited a 5% pretreatment prior to UVB exposure inhibited keratinocyte damage	Quenching of reactive oxygenating species (ROS); also, modulation of NF-$\kappa\beta$ pathway, a signal transduction pathway responsive to UV radiation	Green tea extract tested in vivo, applied topically to skin in a solution of ethanol or water. A 5% GTP solution was effective, 10% was optimal (1–10% solutions demonstrated a dose-dependent response) (23).
Milk thistle (silibinin)	Protect skin from UVR	Antioxidant, free radical scavenger, downregulates chemically induced lipoxygenase, TNFα, and IL-1α in mouse skin.	
Hydroxyacids, e.g., Alpha, beta, poly, and bionic acids	Photoaging and hyperkeratosis (age spots and hyperkeratotic lesions)	Antioxidant	pH of final product is low, ≤ 4.0 hydrolysis of esters in formulation will occur causing an off-odor.
	Increased dermal thickness	Bionic acid inhibits matrix metalloproteinase enzyme activity responsible for degradation of skin's matrix and structural integrity (wrinkle formation, skin laxity, and telangiectasia) Increased production of collagen and fibroblast proliferation	Sun sensitivity with α and β Acids.
Peptides, e.g., palmitoyl pentapeptide	Improve appearance of fine lines and wrinkles of the eye area	Stimulation of type I and type III collagens and fibronectin production	Peptide lipidated to penetrate skin.

(Continued)

Table 9 Cosmeceutical Ingredients (*Continued*)

Ingredient	Claimed benefit	Mechanism	Formulation consideration
Other peptides, e.g., acetyl hexapeptide-3	Wrinkle reduction (limited data available)	Inhibits calcium-dependent catecholamine release from and assembly of SNARE protein complex	Short amino acid sequence to facilitate cell membrane permeability.
Miscellaneous vitamins:			
Vitamin C (ascorbic acid, ascorbyl phosphate)	Wrinkle reduction, improvement in skin tone evening and texture	Improvement in skin collagen, reduced pigment transfer from melanocyte to keratinocyte	Stability, proper formulation pH, penetration into skin.
Vitamin B3 (niacinamide and its esters)	Improved skin tone, reduced dyspigmentation	Antioxidant	Formulate at appropriate pH to avoid hydrolysis.
Vitamin E (tocopherol and tocopherol acetate)	Protection against UV-induced effects to skin, reduced inflammation of skin	Antioxidant	Stability against oxidation, oil-soluble forms are less elegant, acetate form is subject to hydrolysis in formulation.
Retinoic acid (functional form of vitamin A)	Wrinkle reduction via thickened skin. Reduce appearance of dyspigmentation	Increased epidermal thickness and ground substance inhibit collagenase production. Reduce expression of tyrosinase	Oxygen and light render material unstable. Antioxidants may improve stability.
Sugar amine, e.g., N-acetyl-glucosamine	Moisturization, reduce fine lines/wrinkles Improve skin tone	Precursor of hyaluronic acid, a water-binding component of skin Inhibits tyrosinase, thus inhibiting melanin production	Tends to be unstable, creating a brown colored product.

in addition to information supplied by the manufacturer is advised to consult other resources such as the World Health Organization (22), Journal of Nutrition (24), and other reliable published data.

Two promising groups of botanically derived agents appear to hold promise, as chemotherapeutic treatments for aging skin are polyphenolic antioxidants (catechins and flavonols) and isoflavones. Green tea contains epigallocatein-3-gallate (EGCG), and grape seed contains polyphenolic antioxidants. Silymarin found in milk thistle and genistein found in soybean extract are other examples of useful ingredients for photoaging (25).

Many botanical extracts are available to the formulator. Plant constituents of extracts vary with respect to chemical compounds. Variations in solubility and stability have potential to cause shelf life and stability challenges of the finished product over time. Many extracts have a dark color or an odor that may create aesthetic concerns. Extraction methods intended to lighten color or mitigate odor may remove a compound with the desired activity. To minimize aesthetic and stability concerns, formulators should consult with the extract manufacturer regarding availability of technical information addressing polarity of plant-derived oils and optimal formulation pH range for extracts containing alkaloids. Botanically derived lipids are often not hydrogenated and are subject to oxidation promoting product rancidity (26). Pharmaceutical grade extracts are typically 5 to 10 times stronger than cosmetic grade extracts. Cosmetic extracts may be aesthetically acceptable in emulsions. They may lack key desirable chemical constituents. Alternatively, pharmaceutical grade extracts are very resinous, dark in color, and not soluble in many cosmetic formulations (27).

Notes from a Herbalist: Formulating with Botanical Extracts
A tincture is a solution of soluble plant constituents in a solvent known as the menstrum. Poor filtration, exposure to light, temperature changes from warm to cold, or chemical degradation of extractives can cause precipitation to occur. The precipitate may contain active constituents or inert proteins. Precipitation can be minimized by storage at constant temperature and avoidance of exposure to light. Massive precipitation, development of a marked color change, and "off" odor indicate that the tincture should be discarded. Alkaloids in extracts have diverse medicinal benefits. Acidification of the extraction solvent may increase potency, but efficacy may be neutralized by mixing with tannins. Glycerine is commonly used as an extraction solvent when it is undesirable to use alcohol. This type of extract is referred to as a glycerite. Glycerites tend to be less potent than alcoholic extracts and have a shorter shelf life.

Vegetable oils are good extraction solvents for many plant constituents. Herbalists are concerned that they are also good solvents for pesticides and herbicides. For this reason it is advisable to formulate with organic certified organic vegetable oils, ideally cold-pressed oils (28).

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 consumer expectations are unlikely to succeed long term in the marketplace (29).

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, 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.

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12 | Tests for Skin Hydration

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INTRODUCTION

Writing about skin hydration means writing simultaneously about dry skin and its treatment by moisturizers (1). 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. Hopefully, the recent availability of near-InfraRed (IR)-based water measurement will now allow to resolve this issue (2).

Experimental models used for measuring skin hydration are basically clinical models using or not using noninvasive bioengineering measurements. To ensure meaningful results, the outlines of the intended studies should be of modern design, incorporating 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 (3,4).

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 20 minutes to controlled ambient temperature and relative humidity conditions. Both factors mainly affect activity of the sweat gland, 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 (5–8).

A CLINICAL EVALUATION: THE REGRESSION METHOD

The dermatologist can perfectly clinically grade a given state of skin dryness (e.g., surface roughness, squames, and fissures). Clinical evaluation and grading of skin hydration are based on visual and tactile evaluation of clinical signs. There are numerous possibilities of testing, but basically these rely on the regression method, published in 1978 by Kligman (9), 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 the 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. Following a published guideline ensures that clinical scoring of the hydration state of the skin surface will be conducted on the basis of the same definitions (5). Caution is given upon scoring by the subjects themselves, as their perception of their skin condition may not be the same as that of the dermatologist's perception (5,10).

USING BIOENGINEERING MEASUREMENT 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 experiment, using a lower 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 (10–15). They mainly include measurements of electrical properties, spectroscopic methods such as IR absorption spectroscopy and emission, evaluation of the barrier function of the stratum corneum (SC), measurement of mechanical properties, transient thermal transfer, nuclear magnetic resonance imaging, skin surface topography, and scaling evaluation. Most frequently, bioengineering techniques based only on the electrical properties of the SC together with measurement of transepidermal water loss (TEWL) are used. Other methods remain confined to research laboratories. However, as stated in the introduction, recent availability of near-IR-based water measurement will now allow to improve hydration measurements and to better define product efficacy (2,16,17).

Static Measurements

Short-Term Tests/Single Application

The tests are conducted most of the time on the inner side of 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 an 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., 3 hours) (18). Measurements are conducted at different times thereafter. Removal of excess or non-penetrated 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 part 1 beginning one hour after removal of the occlusion patch (Fig. 2). This last procedure better picks up the activity of a humectant contained in the test preparation, whereas the vehicle effect is strongly attenuated by the uniform conditions encountered under the occlusion patch.
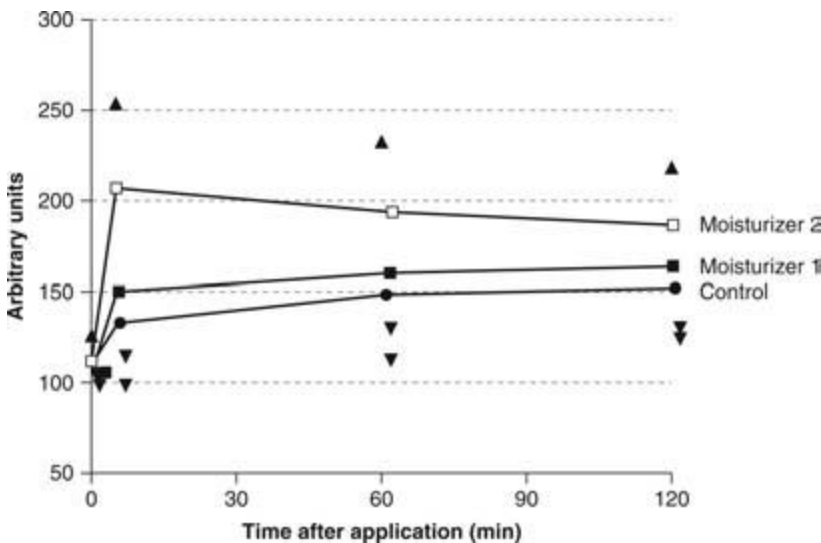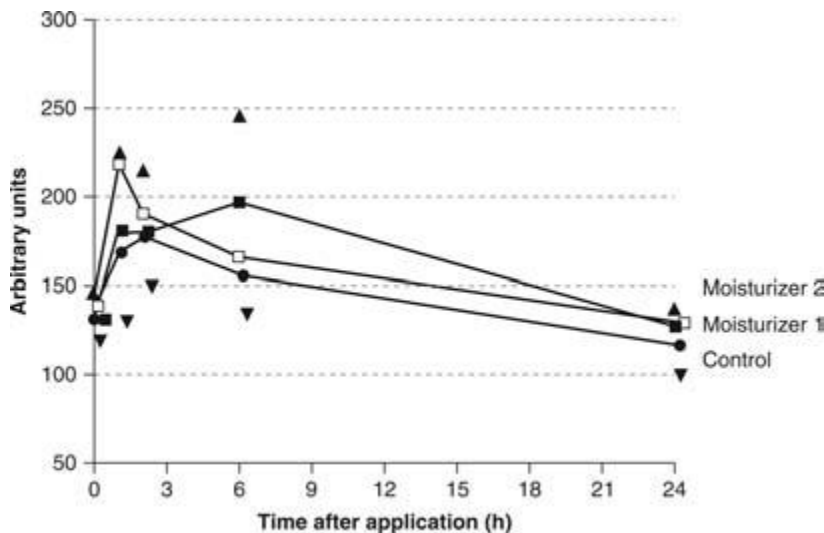

Figure 1 Example of hydration changes over time after one-hour application of two different O/W moisturizers containing both 2% urea as humectant (measurements conducted with the NOVA DPM 2003; means ± ½SD, ▲▼). ■, moisturizer 1; □, moisturizer 2; ●, control (untreated skin). Start values (time = 0) measured before application of the products.

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 ± ½SD, ▲ ▼). ■, moisturizer 1; □, moisturizer 2; ●, control (untreated skin). Start values (time = 0) measured before application of the products.

Long-Term Tests/Multiple Applications
The design of these tests and selection of subjects is similar to the regression method previously described but with a modified and shortened regression protocol (19). 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 (20,21). These properties are likely to be modified by the humectants (e.g., glycerol, urea, α-hydroxy acids) incorporated in the moisturizers used for treatment. Generally speaking, dynamic function tests are characterized by the assessment of the skin's response to a given external stimulus that can be physical (e.g., water, occlusion, stretch, and heat) or chemical (e.g., drugs and irritants) in 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.

The Sorption-Desorption Test
This test gives information about the water-binding capacity of the uppermost layers of the SC (20,21). 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.

The first value represents the hydration state of the SC. Then 50 μL of distilled water is pipetted onto the skin, left in place for exactly 10 seconds, and wiped with a soft paper towel. Then hydration is immediately measured. Further measurements are taken at 0.5, 1, 1.5, and 2 minutes. Parameters such as hygroscopicity, water sorption capacity, water-holding capacity, and accumulated water decay may be calculated from the measurement curve and used to characterize the state of the SC and/or different properties of the tested products (Refs. 20 and 21, Fig. 3).

The Moisture Accumulation Test
This test gives information about the quantity of moisture the SC may accumulate during a given time (20,21). It is conducted with a device that can measure continuously after bringing the probe in contact with the skin surface. The probe then remains on the skin for three
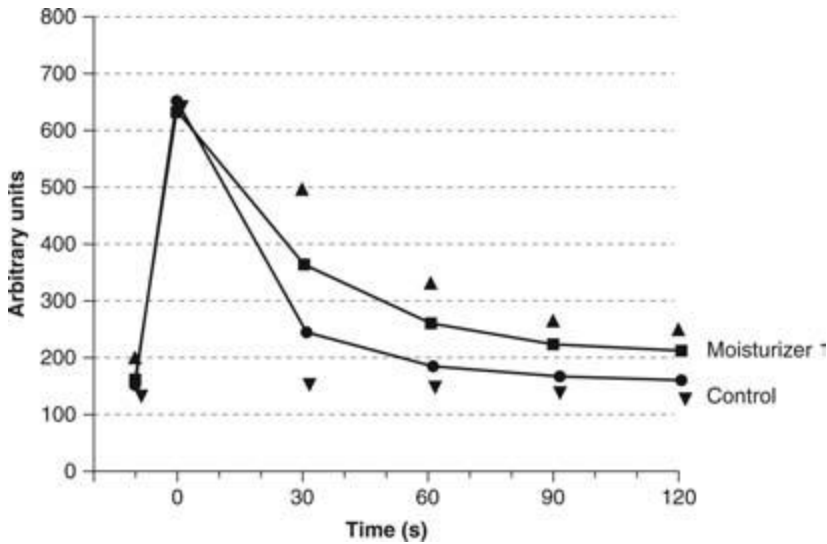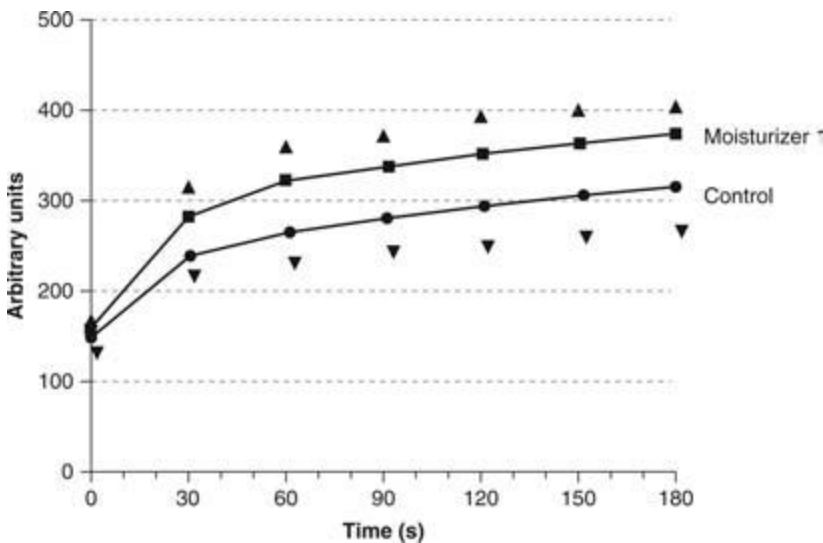
Figure 3 Time course of hydration changes during a SDT performed 60 minutes after a single one-hour application of a moisturizer (moisturizer 1 from Figs. 1 and 2; measurements conducted with the NOVA DPM 2003; means ± ½SD, ▲). ■, moisturizer 1; ●, control (untreated skin). *Abbreviation*: SDT, sorption-desorption 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 ± ½SD, ▲ ▼). ■, moisturizer 1; ●, control (untreated skin). *Abbreviation*: MAT, moisture accumulation test.

minutes, thereby creating occlusive conditions. The moisture accumulation test (MAT) 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 (POST) may also be considered a dynamic test. It gives information about SC hydration, integrity of the barrier function, and SC water-holding capacity (20,21). It consists of occluding the skin with a plastic chamber (e.g., Hilltop 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. The TEWL-technique has been thoroughly described in recent reviews and guidelines (8,13–15,22,23). The measurement is called skin surface water loss (SSWL) and not TEWL, because it does not

Table 1 Moisture-Related Skin Types and Corresponding Corneometer CM 825 Units

Clinical grade	Arbitrary units (Corneometer CM 825)
Very dry	< 30
Dry	30–40
Normal	> 40

represent the true TEWL but the sum of the TEWL and the evaporation of water trapped within and over the SC under the occlusive equipment, at least at the beginning of the measurement period. The SSWL decay curve appears biexponential. During the 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.

Near-IR-Based Spectroscopic Measurements

Methods using near-IR spectroscopy for evaluating the water content of the SC have been used for several years. They are very sensitive to changes in the water content of the tissue, and they allow fast determination, thus avoiding occlusive conditions that would change the water content (2,16,17,24). However, a major inconvenience has been the uncertainty related to the variations in skin penetration of the different wavelengths in the skin.

This has now been eliminated through the recent introduction of Raman spectroscopy. For the first time, in vivo measurements of the water content of the SC at different levels of depth are possible. The applications of this technique are numerous, and the development potential for skin hydration testing appears huge (2,16,17).

CLINICAL RELEVANCE OF BIOENGINEERING MEASUREMENTS

A recent study, including several research centers but featuring the same experimental conditions, has investigated the relation between measurements of very dry, dry, and normal skin using one of the most popular device, the Corneometer CM 825, and clinical grading of dry skin following stringent criteria (25). Categories that could be defined are shown in Table 1.

This allows for the first time relating in a reasonable manner a clinical score of skin dryness to a bioengineering measurement.

CONCLUSION

During the evaluation of SC hydration in vivo, it must be kept in mind that no absolute determination of a water content or concentration is possible if measurement methods other than the near-IR-based spectrophotometric determination are used. This holds for clinical evaluation and for bioengineering measurements as well. For this reason, 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 they are integrated in a clinical evaluation, but one should also 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 all other relevant factors need to be tightly controlled. Only by assuring the best quality level will results be obtained that will help to design and use optimal moisturizers.

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13 | Skin Capacitance Imaging

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INTRODUCTION

Recently, a special type of non-optical skin surface imaging was designed under the heading of skin capacitance imaging (SCI). This method is an application of the silicon image sensor (SIS) technology, which was primarily developed for recording fingerprints for security reasons (1)]. The sensor is composed of 92160 microcapacitors dispersed on a 18×12.8-mm sensor plate measuring skin capacitance every 50 μm. These microcapacitors are protected by a thin silicon oxide layer. SkinChip® (ST Microelectronics, Geneva and L'Oréal, Paris, France) is not yet commercially available. It represents a dedicated device for computer recordings of the skin's surface hydration and microrelief (1–3). The device must be closely applied to the skin surface for five seconds at the most not to interfere with the water flux and content inside the stratum corneum (SC). SCI images are acquired and displayed in real time on a computer screen where the capacitance values are transformed into a range of 256 gray levels to form a non-optical image. On a flat surface, the darker pixels represent high capacitance spots, and the clear ones, the lower capacitance values. Besides the regular software providing images, other softwares were developed for routinely characterizing some other specific skin parameters (3). The mean gray level (MGL) of the image represents the average skin surface hydration. The so-called corner density (CD) parameter corresponds to the number of crossings between the primary lines per centimeter square (4). The main orientations of the primary lines of the skin microrelief can also be assessed.

SKIN SURFACE PATTERNS

SCI scrutinizes the skin surface texture. Indeed, most of the features defining the skin microrelief (lines, pores, furrows, and wrinkles) appear as whitish objects because their deeper portions are not in close contact with the measuring probe (1,3,5–7). The gray levels of the skin surface in close contact with the measuring sensor correspond to the capacitance, i.e., the water content of the outer SC (Fig. 1). The primary and secondary lines of the microrelief network are well identified using SCI. In young subjects, the method shows two main, almost perpendicular, orientations of the skin microrelief and their rotation when the skin is stressed. According to age, CD varies from about $250/\text{cm}^2$ to $400/\text{cm}^2$ on the forearms. The lower lip exhibits a distinctive SCI map. Fine transversal furrows are present, and a whitish and drier area is located at the most internal part of the lip (6)] (Fig. 2).

Skin aging is in part characterized by changes in the main orientations of the microrelief lines (5,6) (Fig. 3). Indeed, the skin of elderly subjects shows microrelief lines mostly oriented along one single direction. In addition, wrinkles are visible as larger whitish lines.

DERMATOGLYPHICS

"Dermatoglyphics" is a term applied to the configurations of ridged skin. Dermatoglyphics not only have characteristic patterns, but the ridges are interrupted and branched in a way, which is unique for any individual. In the human hand, the distal segment of each digit has one of three configurations, namely a whorl, a loop, or an arch (Fig. 4). The systematic classification of ridge patterns, as a means of personal identification or for use in studies of inheritance, requires numerical procedures, such as counting the ridges between specified points or measuring angles. These aspects are conveniently highlighted using SCI.

Of particular interest, however, is the fact that distortions of the dermatoglyphic patterns occur in relation to chromosomal aberrations. For example, various alterations have been
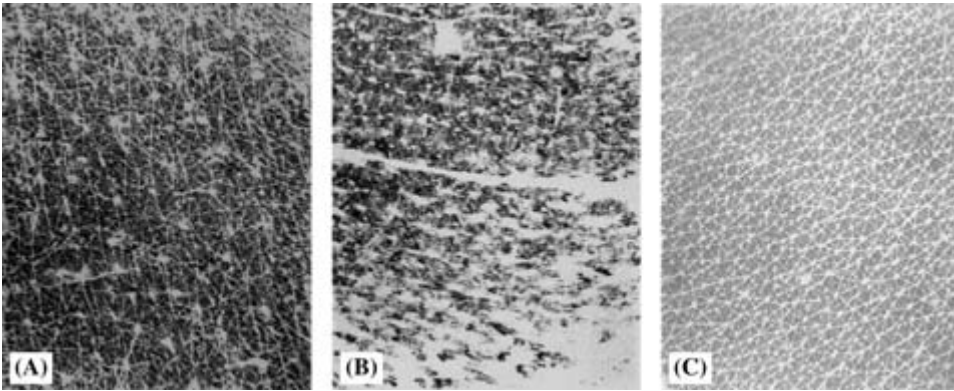
Figure 1 SCI at different anatomical sites. (**A**) Lateral side of the neck with numerous pore openings, (**B**) Wrinkles of the face (crowfeet), (**C**) Inner aspect of the arm with a dense criss-cross network of the microrelief lines. *Abbreviation*: SCI, skin capacitance imaging.

Figure 2 SCI of the lower lip: the inner portion appears drier than the outer part. *Abbreviation*: SCI, skin capacitance imaging.
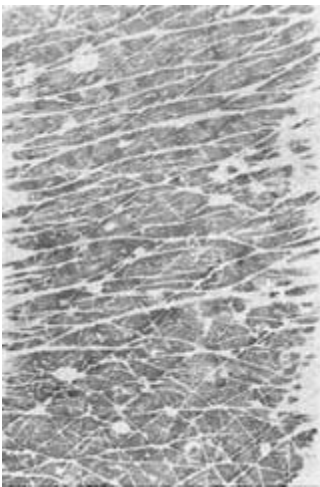

Figure 3 Heterogeneous aspects of the skin of the inner aspect of the forearm of an elderly men. The microrelief lines are mainly oriented along one direction, and CD is decreased. *Abbreviation*: CD, corner density.

described in Down's syndrome, Klinefelter's syndrome, and Turner's syndrome. Deficient ridge formation has also been reported in some dermatoses including Darier disease, alopecia areata, and psoriasis (8).

SCI is a rapid inkless procedure useful for recording dermatoglyphics. The observed features may bring an aid to diagnosis in some medical conditions.

Figure 4 SCI of dermatoglyphics. *Abbreviation*: SCI, skin capacitance imaging.

SKIN SURFACE HYDRATION

The SCI-derived MGL correlates with the average capacitance values given by the Corneometer® (C+K electronic, Cologne, Germany) (1,3). Both methods establish a partial contact with the skin surface because of its microrelief. The Corneometer® gives the average capacitance of the whole contact area with the probe, while SkinChip® displays a more detailed distribution histogram of the capacitance values.

Any prolongation over five seconds of the contact time with the SkinChip® probe may increase the density in darker pixels owing to accumulation of sweat, transepidermal water loss (TEWL), and water saturation of the superficial SC. Similarly, SCI aspects are modified by the application of moisturizers. Images become darker with increased hydration, and sometimes the texture of the skin can be improved after treatment (Fig. 5).

On chronically photo-exposed skin, SCI presentation usually appears heterogeneous. Some areas look quite dry in close vicinity with other areas looking unremarkable. Such a patchy heterogeneity in hydration of the skin surface could be related to focal variations in the
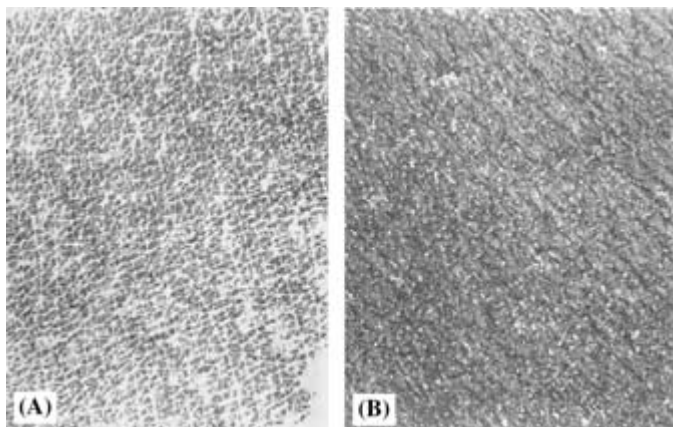

Figure 5 Skin of the neck of a young adult before (**A**) and immediately after (**B**) application of a moisturizer. The moisturizer dramatically darkens the SCI aspect. *Abbreviation*: SCI, skin capacitance imaging.

epidermal differentiation of photo-exposed skin (Fig. 4). Whether or not these aspects are in part related to field cancerogenesis is yet unsettled (9,10).

SKIN PORES

"Skin pore" is a dermocosmetic term, which does not encompass one single defined structure. It is replaced to the best advantage by the acroinfundibulum and the acrosyringium for distinguishing the openings of the folliculo-sebaceous ducts and the sweat gland apparatus, respectively (7).

  Measurement of the TEWL is often used as a convenient assessment of the SC barrier function. A number of variables affect TEWL measurements, including person-linked factors as well as environmental and instrumental variables. Among them, it is acknowledged that physical, thermal, and emotional sweating need to be controlled. Therefore, a premeasurement of 15- to 30-minute rest without any physical activity in a temperature-controlled room of 20°C to 22°C is taken into consideration in most studies. The same considerations apply to the electrometric measurements performed under occlusion (11–13), including SCI. In these different technical approaches, it is impossible to control the so-called imperceptible perspiration. The contribution of this physiological parameter in the TEWL values has never been thoroughly evaluated and is neglected in the interpretation of TEWL data.

  The clinically imperceptible perspiration is easily observed using SCI. Tiny black dots mark the active sweat gland openings (Fig. 6). In our experience, this aspect has no effect on the casual TEWL determinations. When sweating is more active, SCI black dots become larger, and some merge to form irregular black areas. Because sweat appears as black dots, it is possible to measure its contribution to the SCI-derived MGL by thresholding the histogram values. The activity of antiperspirants can be conveniently assessed by this method.

  Pilosebaceous openings at the skin surface appear as whitish dots (Fig. 6). The open comedones and the keratin-filled funnel-like acroinfundibular structures are highlighted (7,14). These structures are revealed as whitish low capacitance spots. This aspect is in part due to the absence of contact between the probe and the epithelial lining of each empty infundibulum, or to the low hydration of the constitutive cornified cells of the microcomedo.

  SCI of acne highlights a peculiar heterogeneous patchwork of electrical properties of the skin. Among the typical whitish pinpoint pattern of normal-looking acroinfundibula, microcomedones and open comedones appear as larger low-capacitance objects (Fig. 7). Inflammatory papules appear as targetoid structures centered by a whitish comedo surrounded by a darker rim. The latter structure reveals a weakened skin barrier function and the presence of a discrete serosity exudate (15) (Fig. 7). These electrometric features are not perceived clinically, but may be important for antiacne drug delivery according to their hydrophilic or hydrophobic characteristics.
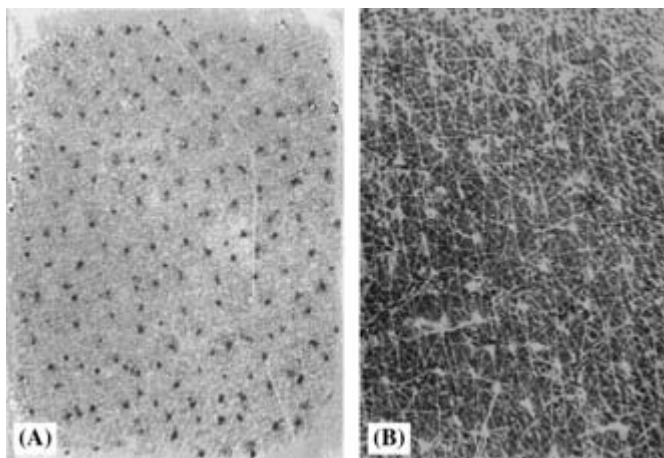

Figure 6 Skin pores. (**A**) Imperceptible perspiration. Tiny black dots mark the active sweat gland opening. (**B**) Pilosebaceous openings at the skin surface appear as whitish dots.
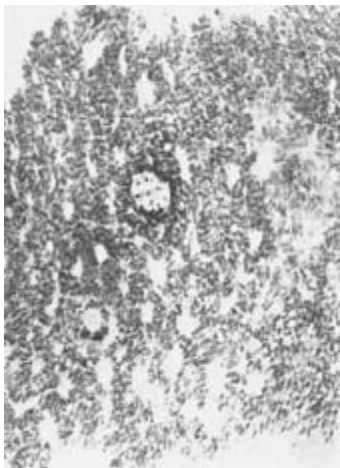
Figure 7 SCI of acne. The larger white spots (low capacitance) correspond to comedones, and inflammatory papules are identified as targoid structures centered by a whitish comedo surrounded by a well-circumscribed darker rim. *Abbreviation*: SCI, skin capacitance imaging.

SURFACTANT-INDUCED REACTION

The dynamics of SC reactivity to surfactants is quite complex. Surfactants present in hygiene and skin care products are in part adsorbed at the skin surface, and they also permeate the SC where they interact with proteins and lipids. A number of physicochemical interactions exist between corneocytes and surfactants (16). One of the earliest events following surfactant-induced protein denaturation is perceived as corneocyte swelling (17). This condition leads to a paradoxical and transient SC hydration, following surfactant challenge in vivo (18). The structure and physical properties of the SC are further altered following prolonged contact with anionic surfactants (17,19,20). As a result, minimal to severe irritation usually develop. Full-blown lesions show erythema, increased TEWL, altered cutaneous microrelief, increased skin surface roughness, and impaired desquamation. The SC water content can be assessed in vivo using devices measuring changes in electrical properties of skin at different frequencies and at different depths inside the SC (12,21,22). SCI has an added value to the conventional assessment methods because its sensitivity discloses focal and minute changes that are blurred by the methods averaging data on a relatively large area corresponding to the size of the sensor probe.

Two discrete effects of mild surfactants on human SC were assessed using SCI. The short-term patch-testing procedure (23) and the open method close to the in-use conditions were used (24). Both experimental procedures disclosed the early step of corneocyte swelling induced by surfactants. Delayed assessments after a couple of hours as well as repeated surfactant insults were responsible for a second event corresponding to a skin surface–drying effect. The earliest change in the irritation zone was revealed by darker pixels, corresponding to water-enriched corneocytes in contact with the probe. This aspect probably resulted from the transient intracellular accumulation of unbound water. In a second step, this hydration state was replaced by the opposite dehydrated condition pictured as white pixels (Fig. 8). As a result, SCI reveals the surfactant-induced irritation kinetic with high sensitivity. A correlation was also found between SCI and data gained by the corneosurfametry bioassay (24).

HYPERKERATOTIC DERMATOSES

Hyperkeratosis is a typical feature of pityriasis (tinea) versicolor corresponding to a *Malassezia* spp infection. The condition is conveniently highlighted using SCI because the skin surface is dryer than the surrounding skin. The method allows to detect small lesions of pityriasis (tinea) versicolor almost invisible to the naked eye (Fig. 9). Interestingly enough, lesional skin appears anhidrotic, perhaps due to the occlusion of each acrosyringium (25).

Psoriasis is the paradigm of inflammatory hyperkeratotic dermatoses. SCI reveals a patchwork of different electrical properties on lesional skin (26). Whitish low capacitance is typical for stable hyperkeratotic plaques. More inflammatory and evolving plaques show
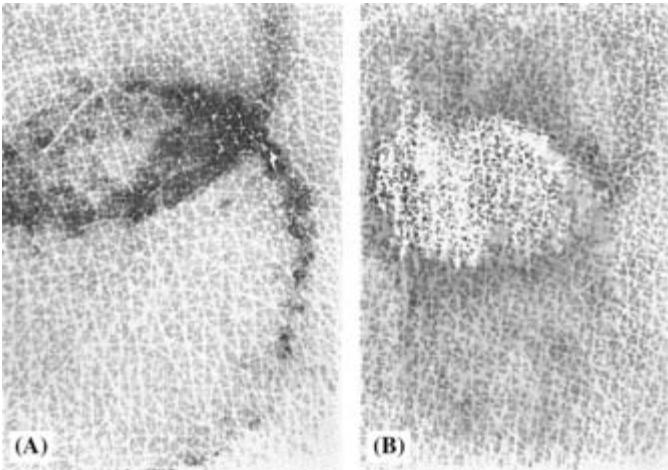
Figure 8 Corneocyte reactivity to anionic surfactant. (**A**) Skin surface imaging of the partial overlap between two successive patch tests performed with a diluted anionic surfactant. (**B**) Skin area examined two days after the condition depicted in (**A**). The overlap region shows a white appearance, indicating a decreased water content in corneocytes. *Source*: From Ref. 23.
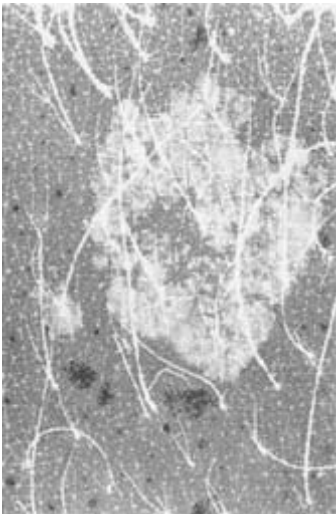

Figure 9 SCI of small lesions of pityriasis (tinea) versicolor. The aspect in bunch characteristic of this kind of lesion is well highlighted. The lesion appears anhidrotic. *Abbreviation*: SCI, skin capacitance imaging.

darker high-capacitance spots (Fig. 10). This aspect is likely related to sites exhibiting increased TEWL (27). SCI can thus provide clues of disease activity in the plaque stage of psoriasis and can be used to monitor therapy.

KERATOTIC OR PIGMENTED TUMORS

Viral warts are easily identified using SCI. They exhibit a dry hyperkeratotic aspect of their surface (25,28,29). No difference in capacitance reduction was found between different types of warts (Fig. 11).

Melanocytic nevi and pigmented seborrheic keratoses may be difficult to distinguish during the clinical inspection. SCI shows variable aspects irrespective of the nature of these lesions. Low capacitance is commonly yielded, but increased capacitance is also possible, particularly on minimally inflamed lesions (30). Inflammation in the superficial dermis produces edema and discrete transudate through the epidermis. Such a water flux ultimately steeps the SC. Inflamed lesions of seborrheic keratoses and melanocytic nevi exhibit a capacitance map, which is not uniform. Spotty areas of decreased capacitance are dispersed in a buckshot pattern over the background. The lesions are commonly rimmed by a thin border of lower capacitance (Fig. 12). This situation was also observed in inflammatory lesions of acne and acute psoriasis (Figs. 7 and 10).

Figure 10 SCI of a psoriatic lesion, combining white hyperkeratotic areas and darker inflammatory sites. *Abbreviation*: SCI, skin capacitance imaging.
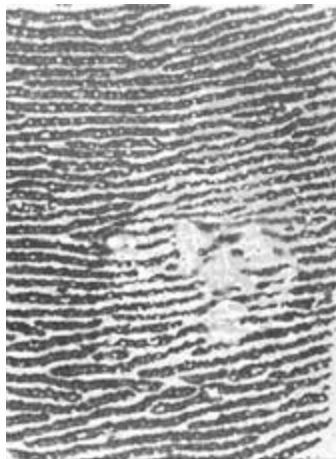

Figure 11 SCI of plantar warts. *Abbreviation*: SCI, skin capacitance imaging.
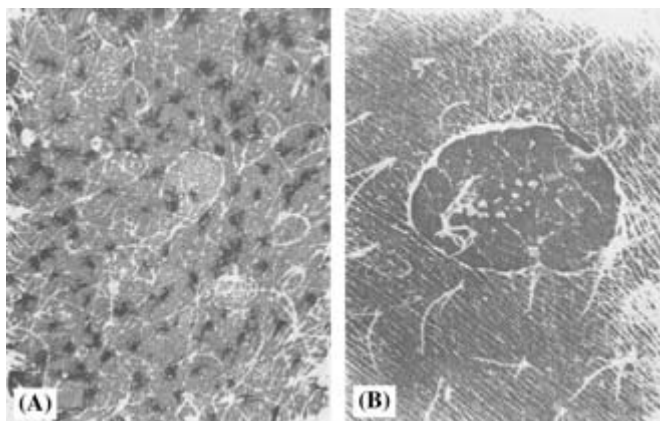

Figure 12 SCI of keratotic pigmented tumors. (**A**) Small lesions of seborrheic keratosis with white low-capacitance dots corresponding to horny plugs. (**B**) Moderately inflamed melanocytic naevus. *Abbreviation*: SCI, skin capacitance imaging. *Source*: From Ref. 29.

HAIR SHAFT MOISTURIZATION

Similar to the SC, the cuticle of hair shafts can show variations in hydration. The kinetics of water sorption and desorption is possibly altered following some hair weathering and damage. It is also influenced by the application of some hair care products. SCI determinations can be used for assessing these modifications in hair shaft moisture.

CONCLUSION

In conclusion, SCI provides non-optical pictures showing aspects invisible to the naked eye. It represents a procedure allowing both visualization and quantification of the skin microrelief, SC and hair shaft hydration, acneiform follicular cornification, imperceptible perspiration, and active sweating. The method brings unique and sound information in dermocosmetology, also giving insights in the physiopathology of skin disorders.

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14 | Confocal Raman Spectroscopy for In Vivo Skin Hydration Measurement

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INTRODUCTION

Confocal Raman microspectroscopy of skin in vivo is nowadays routinely being applied in human panelist studies. Ever since the first Raman spectra of skin were presented, it is known that these spectra contain unique information on the chemical composition of the skin. The ability to measure the chemical composition of living biological tissues nondestructively is a valuable tool in the skin sciences. Raman spectroscopy has qualities that make it unusually attractive for such measurements. Especially the ability to measure the chemical composition of tissues noninvasively at defined depths, using confocal optics (optical sectioning), is unique. Because of the complexity of biological tissues, Raman spectroscopy has only in the last decade begun to make significant contributions in skin science. A very recent review discusses the in vivo applications of Raman spectroscopy in the measurement of the composition of skin, including topically applied compounds and their effects on skin composition, in the context of pharmaceutical applications (such as transdermal drug delivery) (1).

Skin research increasingly depends on more detailed knowledge of the molecular composition of skin and the spatial distribution of skin constituents. On a microscopic scale (the scale of the confocal Raman measurement), the skin is highly heterogeneous. Its molecular composition and structure vary tremendously over different body sites and at different depths below the surface of the skin. In the stratum corneum (SC) especially, concentration gradients (e.g., water gradients, pH gradients, diffusion kinetics) play a role in biochemical or skin physiological processes. The composition of the skin is also affected by skin disorders, environmental factors such as sun exposure, seasonal variation, diets, and bathing habits, and cosmetic or medical treatments. Skin treatments may also bring about changes in dimension, such as an increase in SC thickness due to swelling. The spatially complex skin tissue can be excellently studied using Raman spectroscopy with a confocal approach, where spatial resolution can be achieved that is consistent with the size of many features of interest (~ 5 μm in depth and ~ 1 μm horizontally).

Noninvasive methods are particularly welcome. This is partly because they cause less discomfort for the patient or volunteer subject, but also because noninvasive methods enable investigation of the skin in its natural state without affecting its integrity, morphology, or molecular composition. Noninvasive measurements can be performed repeatedly on the same skin area in vivo and can thus be used to monitor time-dependent changes in the skin brought about by skin treatments.

Caspers et al. presented the first in vivo confocal Raman spectra of human skin (2) and were able to clearly show compositional differences at different depths below the skin surface. For example, large changes in composition were observed near the SC–stratum granulosum interface, from which the SC thickness could be derived in vivo. This was confirmed shortly after by combined confocal microscopy and Raman spectroscopy (3), (see discussion below).

Whereas the aforementioned, more general review, discusses the measurement of the main chemical composition of the SC (1), this chapter focuses on how measurements of water concentration gradients can be used to study the moisturization process of the skin in its outer layer.

ALTERNATIVE METHODS TO MEASURE SKIN HYDRATION

It has been known for centuries that humans continuously lose water through the skin, for instance, from experiments in which human subjects and their ingested and excreted liquids are carefully weighted. The detailed hydration process of the skin and the dynamic transport

of water through the skin have been subject to investigations for decades. Modern methods for studying the moisturization of the skin can be coarsely classified, using the following criteria:

1. the relationship of the measured parameters to the hydration state of the skin (direct or indirect, straightforward, or complex);
2. spatial resolution, parallel and perpendicular to the skin surface;
3. the extent to which the measurement influences the skin state (invasiveness and the ability to resample the probed site); and
4. the ease of use (both the measurement procedure and the data processing and interpretation).

It is beyond the purpose of this chapter to discuss the features of the various methods in use. However, a short and simplified classification, using the aforementioned criteria, along with the newest method of confocal Raman spectroscopy, will help clarifying the quite remarkable position of the latter. The methods included in the comparison are Karl Fischer titration, light microscopy, electron microscopy, electrical methods (capacitance and conductance), transepidermal water loss (TEWL), magnetic resonance imaging (MRI), near infrared spectroscopy (NIRS), Fourier transform infrared spectroscopy using attenuated total reflection sampling (ATR-FTIR), opto-thermal transient emission radiometry (OTTER), and of course Raman spectroscopy. In Table 1, these methods are ranked according to their favorability toward the four criteria (from $--$ to $++$). The table also contains a short comment. In the right column, some key reference papers are suggested for further reading. The selection of key references is a personal selection by the authors. It will provide the reader with more details, helpful in gaining a better understanding of the different ways in which skin hydration may be measured. The authors by no means claim the selection to be complete or to effectively represent the whole field of science.

Since the hydration of the skin is so closely related to water gradients that reside within the very thin SC, confocal Raman seems unusually suitable, especially when one takes its in vivo applicability into account. Despite its apparent complexity, of which we believe may be mainly due to relative unawareness of Raman spectroscopic technologies, the measurement routine is not difficult to master. It is the experience of the authors that an instrument operator (with no prior knowledge of spectroscopy) can be trained in one to two days, enabling him to carry out the in vivo measurements of water depth concentration profiles on human volunteers.

A method that appears to potentially possess comparable features as confocal Raman microspectroscopy is MRI. An impressive spatial resolution of 4 μm (in all directions) has already been demonstrated on very small (single biological cell) isolated samples (22). However, the extremely difficult challenges to overcome for large samples, such as human volunteers, will be the size and stability of the magnet and foremost the definition and stability of the magnetic field gradient. Whereas the Raman methodology already allows for measurement of the overall chemical composition (1), this is not yet possible for in vivo MRI of large samples. Finally, the cost of ownership of MRI equipment can become very high.

REQUIREMENTS FOR IN VIVO RAMAN METHODOLOGY

Qualitative Description of the Raman Effect

In Raman spectroscopy, a sample of interest (this can be gaseous, liquid, or solid) is illuminated by a laser beam. The light in the laser beam is of single (or very narrow) wavelength nature. The electric component of the electromagnetic fields within the laser beam drives the electronic cloud of the molecules present in the sample. The driven (and oscillating) electronic clouds reemit most of the collided laser light without energy loss (by the physical laws of induction, an oscillating electronic charge emits electromagnetic radiation at the oscillating frequency); the only difference may be the direction in which the photons are ejected out of the molecules. This process is referred to as elastic scattering (or Rayleigh scattering). A very small amount of the laser light, however, scatters inelastically; the ejected photons have a different energy than the injected laser photons. The difference in energy is taken up or released by the molecules and is used to promote or demote, respectively, the

Table 1 Brief Comparison of Some Features of Current Methods to Assess Skin Hydration

Method	Water direct/indirect	Spatial resolution	Invasiveness	Ease of use	Key references
Karl-Fisher	++ Direct and absolute	--	-- Destructive	-- Long preparation	(4)
Light microscopy	+ Swelling can be observed	+ 0.5 µm	-- Sections required	- Long preparation	(5,6)
Electron microscopy (SEM and STEM)	+ Indirect	++ <0.01 µm	-- Cryo-sections	-- Long prep, complex instrument	(7–9)
Capacitance and impedance	- Influenced by products	- Probes top 30 µm	+ Contact probe	++ Push button	(10–12)
Conductance	- Influenced by products	- Probes top 1–10 µm	+ Contact probe	++ Push button	(13–15)
TEWL	+ Measures flux	- cm	+ Contact probe	++ Push button	(16,17)
MRI	++ Direct	- 70 µm in vivo	++	-- Complex instrument	(18)
NIR	+ Difficult to quantify	- Probes top 1–2 mm	++ Noncontact	+ Moderate complex	(19)
ATR-FTIR	+ Difficult to quantify	+/- Probes top 1–2 µm	+ Contact probe	+ Moderate complex	(20)
OTTER	+ Direct, but theoretical modeling required	+ > 10 µm	++ Noncontact	+/- Complex instrument	(21)
Confocal Raman	++ Direct, quantitative relative to keratin	+ 1 µm lateral, 5 µm depth	+ Contact probe	+ Moderate complex	This paper

Abbreviations: SEM, scanning electron microscopy; STEM, scanning transmission electron microscopy; TEWL, transepidermal water loss; NIR, near infrared; ATR-FTIR, attenuated total reflection sampling–Fourier transform infrared spectroscopy; OTTER, opto-thermal transient emission radiometry.

vibrational energy levels of the molecules. The mechanism of interaction, leading to the energy difference, involves a modulation of the electromagnetic field, because of oscillating electronic cloud, by the much smaller electromagnetic field generated by the ever-vibrating nuclei present in the molecules. By measurement of the intensity and energy of the reemitted light of different frequencies than the laser frequency, a Raman spectrum is obtained. The differences in energy correspond to transitions in vibrational energy levels. In this respect, a Raman spectrum contains the same kind of information as an infrared spectrum, but the way by which this is obtained (photon scattering) is different from IR spectroscopy (photon absorption).

In Vivo Raman Methodology

In vivo, Raman spectra are obtained by focusing a laser beam through a microscope and allowing the microscope objective to project the focused laser beam on and below the surface of the skin. Subsequently, the Raman light is measured in the backscattered direction through the same microscope objective. Numerous technical challenges have to be overcome before Raman measurements on biological tissues can be fast enough for practical use in in vivo clinical studies. General purpose Raman instruments, available in most well-equipped analytical laboratories, are not capable of practically useful measurements on skin. Recently, however, Raman instrumentation has been developed, employing advanced technologies, and made commercially available, which is capable of practical use in these demanding applications. Figure 1 shows a photo of this first commercially available Raman skin analyzer.

Most critical factors in an optimized Raman skin analyzer are selection of lasers, choice of optical materials, detector quality, opto-mechanical stability, and for practical utility, software that is easy to use and can effectively handle the large volumes of data that are generated in in vivo panel studies. Laser safety considerations also create limiting technical requirements that must be met, thereby strongly influencing the overall engineering of a Raman skin analyzer. Indeed, a capable Raman skin analyzer may be thought of as being composed of four components, each of which must meet critical requirements: (*i*) a laser light source and associated light conditioning optics, (*ii*) an NIR (the optimal wavelength applied in the measurement of skin) optimized microscopic measurement stage, (*iii*) the Raman spectrometer, and (*iv*) specialized operating and data analysis software. Each component, as well as the implications of laser safety, will now be briefly discussed below.

Laser Excitation Source and Optics

Firstly, the laser(s) used must emit light at wavelengths at which no photo(bio)chemical reactions are brought about and at which minimal fluorescence is stimulated in the skin. This places a lower limit on the usable laser wavelength at approximately 660 nm. Secondly, the Raman-scattered photons must be detected with the highest possible efficiency and the lowest possible noise. State-of-the-art technology for this purpose is a charge coupled device (CCD) detector for which the detection is limited to wavelengths shorter than about 1100 nm. Detection of a Raman spectrum in the so-called fingerprint spectral region (400–2000 cm^{-1}) therefore sets an upper limit to the laser excitation wavelength of about 900 nm.

Figure 1 The river diagnostics model 3510 skin composition analyzer is the first Raman instrument optimized for in vivo analysis of skin.

Thus, the choice of laser wavelengths is restricted to a "biological and technical window" in the NIR, approximately in the range of 660 to 850 nm. Typically, solid-state diode lasers are applied. For diffraction limited laser focusing (required for the best spatial resolution), a single mode laser is required. The laser must be stable in power output and wavelength, and its emission line must be narrow to allow for achievement of high spectral resolution. Unwanted laser diode background radiation or satellite emissions must be removed (filtered) before the laser light is injected into the microscope. The laser power out of the measurement device (the microscope) must meet the requirements derived from the laser safety limitations (see below the subsection "Laser Safety Considerations"). Finally, a strict requirement is set on all optical materials in the laser light path; only minimal fluorescence or other background contributions are allowed.

Microscope Measurement Stage
The Raman signal is collected back through the microscope objective, and the microscope must have uncompromised confocal optics. The entire optical train must very efficiently transmit the signal to the spectrometer and finally to the detector. The spatial resolution of the microscope must be better than the thickness of the SC, otherwise no information about the distribution of materials (such as water) within this skin layer is to be obtained. The best microscope objective to this end must be custom designed and optimized for the NIR wavelengths (660–950 nm). Also critical is the absence of any difference in refractive index in the optical path from the objective to the skin. In the skin analyzer (Fig. 1), this is managed by positioning the microscope objective below a measurement window of identical refractive index as the skin and the objective. The space between the objective and the measurement window is filled with a refractive index matching immersion oil. The sampled skin rests and is locally conveniently fixed on the measurement window. If, on the other hand, a large difference in refractive index is present between the microscope objective and the skin (e.g., by focusing through air), a severe degradation of depth resolution results. The microscope objective must be movable in the axial direction (z axis) under precise control. This allows spectra to be recorded at successive depths in the skin, from which composition-depth profiles are obtained. The microscope stage must allow for convenient orientation of human subjects. Usually an inverted configuration is used. The most common measurement site at present is the volar aspect of the forearm, (Fig. 1).

Raman Spectrometer
A very high laser wavelength rejection and again a high transmission at optical interfaces (low reflection and scattering losses) are required to preserve as many of the information-bearing photons as possible. Of course, the detector must also be of high performance. Any general purpose Raman spectrometer would benefit from these requirements, but in the measurement of biological samples, the information sought is often in small spectral differences. Therefore, the spectra must be of very high quality. Furthermore, these high-quality spectra must be routinely obtainable in a time scale compatible with panel studies and the patience of volunteer panelists. Hence, maximizing the signal-to-noise ratio (S/N) by employment of an optimized spectrometer design is of great importance. In a clinical research environment, data recorded today must compare meaningfully to data recorded before or after. Therefore, mechanical and optical stability and measurement repeatability are further important considerations. In clinical environments, where more than one spectrometer is in use, it is further required that results obtained on one skin analyzer will be directly and reliably comparable with results obtained on another skin analyzer. This places very high demands on the accuracy and reproducibility of instrument calibration and correction for instrument response effects.

Software
Data acquisition software for in vivo Raman measurements must have specialized features to handle the often large number of measurements in typical panel studies and to satisfy requirements that are not normally encountered in other types of Raman analysis. For example, the software must enable the operator to quickly select locations of interest on the skin surface. Also, since depth information (usually changes in composition as a function of

depth) is important, the software must incorporate a reliable and accurate means of locating the skin surface for reference. Further, the software must have minimal data acquisition "dead time" between sequential spectrum acquisitions, to maximize throughput, when thousands of spectra are typically acquired in a day. Because of the many experimental variables in a typical skin study design, the number of spectra to handle can become very large. Therefore, the data-processing software must incorporate special features. In conventional spectroscopic processing software, spectra can be manipulated and analyzed typically one by one or batch by batch. For the larger numbers of spectra, typical for in vivo studies, the time, simply to read in each single spectrum and export the result after analysis, can become prohibitive. Even in a batch-processing mode, the time to sort, select, and read in the spectra to define the batches for analysis can become a bottleneck. Therefore, the software must feature ways to enter the experimental design and use this to select and process the spectra accordingly.

Laser Safety Considerations
The International Laser Safety Standard, IEC 60825-1 (2001), prescribes maximum permissible exposure (MPE) limits for the skin, which are dependent on the wavelength of the laser light and the duration of the exposure. The MPEs for skin are formal limits based on extrapolations of exposure to sunlight and do not represent actual damage thresholds, which may be considerably higher. To provide a "flavor" for MPE magnitude, the configuration of the instrument (Fig. 1) results in an MPE limit of 30 mW for 785-nm laser excitation, and 20 mW for 671-nm excitation. These values are not to be taken in general for the wavelengths cited, but must be determined for any instrument design intended for in vivo skin analysis.

There must not be any significant risk of eye damage from exposure to the laser beam, while the measurement window is not covered by the skin to be measured. Practically speaking, laser exposure of the eye is not a difficult risk to manage in a properly designed instrument since the laser beam diverges at a high angle when emerging from the microscope objective, but the risk must nevertheless be properly managed. The instrument (Fig. 1) operates well within the limits of a class 2M laser device, which means that the instrument is eye safe. Incidental direct observation of the beam is not an eye hazard, provided that no optical instruments are used to observe the beam. Each instrument is tested for compliance with the class 2M laser device classification.

When these five elements, an appropriate laser light source, microscopic measurement stage, NIR-optimized Raman spectrometer, specialized software, and in vivo laser safety provisions, are combined in a Raman instrument, valuable information hitherto unavailable to researchers becomes accessible.

RAMAN METHODS FOR THE STUDY OF HYDRATION OF THE SKIN

Relationship Between the Raman Spectrum of Skin and the Local Water Concentration
In the Raman method for measurement of hydration of the skin, a signal is isolated from the Raman spectrum, which is mainly because of the water present in the skin. Note that the signal itself also depends on the depth from which the signal originates; signals that are recorded at greater depths will be weaker. This effect is easily understood, since the skin is not infinitely transparent for the laser and Raman light, it exhibits rather a bit turbid character. In Raman spectroscopy in general, this effect is usually compensated by dividing the measured intensity of the signal by the intensity of a reference signal that may be selected. Requirements for a good reference signal are that firstly it must be due to a substance that is present more or less homogeneously in the sample measured, and secondly, its Raman signal must be sufficiently strong. Since the reference signal is attenuated by exactly the same factor as the analyte signal (e.g., water), the division will cancel out the depth-dependent attenuation. In biological samples, often a signal due to a protein is used. The Mendelsohn group, for example, uses a signal due to phenylalanine to this end (23); others use the overall signal of keratin, which represents the major dry mass fraction in the SC (24).

In 2000, Caspers et al. published the first in vivo water concentration measurements in skin as a function of depth below the skin surface (24). In this paper, the method to calculate a water concentration in mass percentages of wet tissue is discussed in detail. It also involves internal normalization of the water signal, in this case by a signal due to keratin. In Figure 2, a
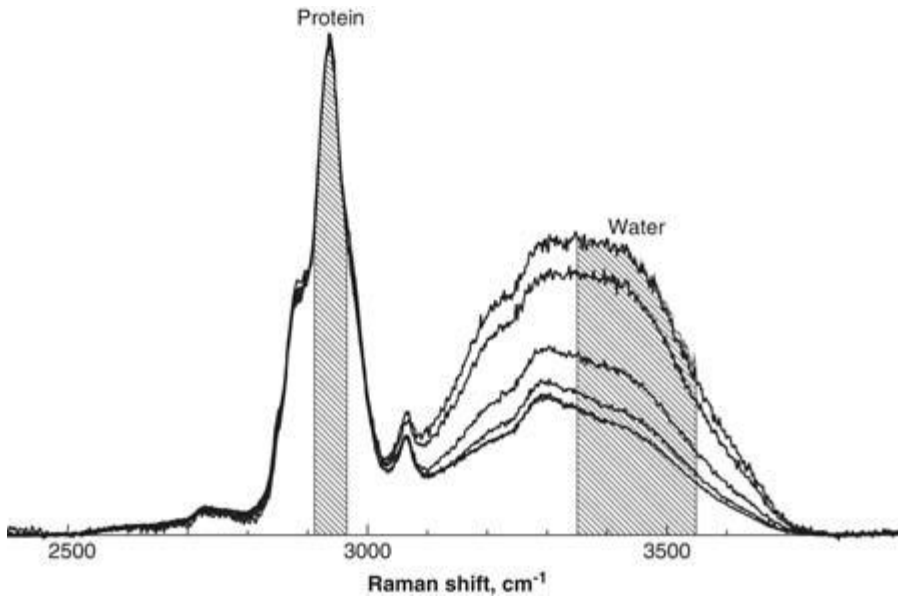
Figure 2 High wave number part of the spectral baseline corrected in vivo Raman spectrum of SC on the thenar. Indicated are integration boundaries for signals due to keratin and water.

part of a typical Raman spectrum of untreated SC skin is shown. In the Figure 2, the signals due to keratin and water are indicated. Furthermore, integration boundaries for the signals due to water and keratin are drawn after spectral baseline subtraction. From Raman spectral measurement of solutions of protein of known concentration, Caspers was able to set up a calibration, equating the ratio of Raman signal intensities due to water (W) and keratin (K) to the mass percentage of water present in the skin (for wet tissue) (24):

$$\text{water(mass\%)} = \frac{W/K}{(W/K) + R} \times 100\% \tag{1}$$

Where R is a calibration constant derived from the measurements of the protein solutions.

In Figure 3, typical water depth concentration profiles recorded within a 2×2 cm^2 area on the ventral forearm are shown.

As can be verified from Figure 3, the four repeat measurements do not coincide. This is caused by the biological inhomogeneity of the skin. This implies that for accurate water contents, repeat measurements and averaging must be carried out. In the Figure 3, the approximate SC–epidermis boundary is indicated. At this boundary, the water concentration gradient changes its slope. This feature is further discussed below.
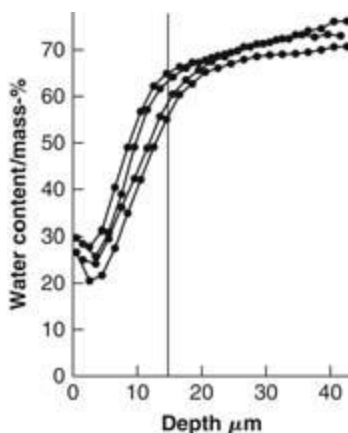

Figure 3 Measured water concentration versus depth, from confocal Raman measurements, at four locations within a small area (2×2 cm^2) on the volar aspect of the forearm. The line indicates the approximate SC–epidermis interface.

The time to record a single water depth concentration profile over about 30 μm of skin on the volar forearm (covering the SC and the upper part of the epidermis) is about 15 seconds.

In 2005, L'Oréal researchers presented in vivo results on human volar forearm skin, using an in-house built confocal Raman setup (25). The work discusses depth concentration profiles from water and other components, but for the present purpose, only the water results are highlighted. In their analysis of the Raman spectra, only a ratio between the intensities of signals due to water and a reference signal was used. The ratio chosen was taken from an older paper, on confocal Raman spectroscopy of cornea (26). No quantification of the water contents was carried out.

Validation of the Quantitative In Vivo Water Concentration Measurement

To the best of our knowledge, no independent method to quantify in vivo depth-dependent water concentration in skin exists. Therefore, validation against a "golden standard" is not possible. However, there are possibilities for comparison with in vitro methods. If we compare the water depth concentration profiles as proposed by Warner et al. in 1988 (7) to the results (Fig. 3), the agreement is striking. The two methods are completely independent. Warner et al. obtained their quantitative estimate from an area analysis of scanning transmission electron microscopy (STEM) images of thin cryosections of skin, taking into account the densities of keratin and ice. Caspers' method, on the other hand, is based on the Raman spectra of prepared solutions of proteins (24). Both methods result in a concentration in the 20% to 30% range for the outer surface (the upper layer of the SC), increasing to about 70% at the interface with the epidermis (note: the 70% concentration in Warner's method was an assumption and not a result of his method).

Very recently, Wu and Polefka presented direct validation results for extracted pigskin SC (27). Samples were equilibrated at different relative humidities and subsequently cut in half. For one half set, the absolute water concentration in the SC was determined with Karl Fischer's titration method. The other half set was analyzed according to the Raman method of Caspers (24). This approach allowed for a direct correlation of water concentrations from independent analysis methods. The correlation proved remarkably good, an R^2 of 0.989 was found. It was further noted that the precision of the Raman method for water concentrations above 30% was better than for the Karl Fischer method. In their paper, Wu and Polefka also reported correlations of conductance measurements with the Raman measurements. The same paper also covered moisturization efficacy results on pigskin SC (see the "Applications" section).

Water Concentration Gradients and Measurement of the Stratum Corneum Thickness

Knowledge of the thickness of the SC is essential in understanding the efficacies of products. Obviously, moisturization of the SC means adding water, and adding water implies adding volume. It is therefore expected that the SC will swell. The changing dimension of the SC under the action of any treatment has consequences for the calculation of efficacies; this applies to not only the degree of moisturization but also, for example, to the content of a constituent of interest before and after application.

Before confocal Raman spectroscopy became available, the shape of the water concentration gradient and its change upon treatment was already known from in vitro experiments or theoretical calculations.

STEM of biopsied and rapidly frozen human epidermis has already been applied for more then 20 years to study the water concentration gradient; see, for example, the work by Warner et al. from 1988 (7). These results showed that a water concentration gradient must reside in the SC.

In 1984, experimental dynamic water flux measurements of in vitro SC as a function of its water content enabled the calculation of water concentration gradients that must exist in the in vitro SC samples (28). These calculations were based on Fick's law of diffusion. All profiles were found to be steep and linear in the SC, and the model accurately described the swelling of the SC as a function of the water content and also as a function of the surface water content.

In 1997, Norlén et al. (6) applied light microscopy and confocal laser-scanning microscopy (CLSM) to study the swelling of extracted pieces of human SC. They found that after incubation of dried SC in distilled water for 90 minutes, the observed swellings were $26.3 \pm 16\%$ in the thickness dimension and only $4.1 \pm 1.4\%$ in the lateral dimension. Thus, swelling after addition of water to the SC mainly takes place in the thickness direction.

In Caspers' original paper (24), he pointed out that the shape of the water profiles could be linked to the SC thicknesses. At different body sites, the water concentration profiles changed slopes at different depths. Caspers confirmed the results in 2001 and pointed out that the observed steep increase in water concentration at a particular depth below the surface of the skin indicates the SC–epidermis interface (29). The general appearance of the in vivo water concentration profiles are in agreement with in vitro water concentration profiles as determined by X-ray microanalysis (7). The SC thicknesses on the thenar and forearm are approximately 110 and 15 μm, respectively. Further and conclusive confirmation of the fact that the steep increase in water concentration occurs at the boundary between SC and living epidermis was presented in 2003 by Caspers et al. (3). Boundaries, as determined by confocal video microscopy, corresponded precisely to the boundaries as derived from the water concentration profiles.

Recently, a number of groups published estimations of the SC thickness, on the basis of confocal Raman measurements and their corresponding water depth concentration profiles. Sieg et al. studied water depth concentration profiles for forearm skin (30). They propose to model the profiles with a sigmoid-like function (Weibull function). One of the fit parameters is the location of the steepest gradient; this is indicative of the SC thickness (it is not the thickness itself). Their work is further discussed in the "Applications" section.

In their study of the delivery of retinol to the viable epidermis, by confocal Raman microspectroscopy, Pudney et al. calculated the approximate location of the SC–epidermis boundary from water concentration profiles and from concentration profiles of the components of the natural moisturizing factor (NMF) recorded at the same location (31). By selecting a depth at which 30% of the maximum content of NMF is found and a second depth at which the water concentration is 55% by mass, two closely spaced locations of the interface are obtained. Subsequently, these two estimators are averaged. Although the criteria for depth selection may be arbitrary, their approach allowed for a systematic estimation of the location of the boundary for every individual measurement spot. This information was then used to verify whether the retinol was delivered to the viable cells or not.

Egawa et al. proposes yet another method to arrive at the location of the SC–epidermis boundary (32). Their estimation is taken from the depth at which the derivative of the water concentration profile is almost zero and coined this as the SC apparent thickness (SCAT; also see the "Applications" section).

The criteria discussed above to arrive at the SC thickness are not fully objective and probably do not represent the real thickness. Also, the models lack a physical rationale. Van der Pol et al. first proposed the more objective method for fitting of the water profiles, on the basis of diffusion of water through the SC–epidermis bilayer (33). The bilayer is thought of as two homogeneous media with two different (but constant) water diffusion coefficients. The water flux is considered constant. Under these conditions (Fick's law), the water concentration gradients must be linear in both media. The experimental water depth concentration profile is modeled simply with two linear functions (one in the SC and the other in the epidermis). This model function is further convoluted by an optical point spread function (a Gaussian function with a full width at half maximum of 5 μm), to account for the spatial resolution of the confocal Raman technique. The only variables for the model function are the location of the discontinuity at the interface of the SC and the epidermis and the two slopes of the water concentration gradients in both media. The method is now automated and implemented in routine moisturization efficacy studies on human panelists (34).

APPLICATIONS

Confocal Raman microspectroscopy is now a tool in the study of epidermal and dermal skin in various skin research groups. For a general review of the role of confocal Raman microspectroscopy in skin science, including the study of penetration of topically applied materials, see reference (1). In this section, the published Raman work related to the in vivo study of the moisturization of the skin is highlighted.

Moisturizing the Skin

A simple way of moisturizing is to wet it with water. In Caspers' 2000 paper, this was demonstrated using a wet towel (24). The resulting water depth concentration profiles changed

dramatically after application of the wet towel. In the SC, the water concentration increased to 50% to 60%, and swelling of the SC was noted.

Chrit et al. studied the in vivo short-term efficacy of a moisturizing cream (35). A number of 26 volunteers (Caucasian, female, dry skin) received a treatment of the volar forearm site with an emollient without hydrating agent and a treatment with a 3% glycerol-containing cream. A control measurement was included. The normalized water signal was measured at different depths after one hour of treatment. Signal intensities were then averaged over the depth range 0 to 20 μm. The glycerol-based cream induced a significant increase in average water content as compared with baseline, and at every depth between 0 and 20 μm, the water concentration was higher after the treatment. It is further noted that the shapes of the water depth concentration profiles did not exhibit a clear change of slope, at the expected depth of about 15 μm, where the SC–epidermis interface is located. This is most likely caused by a degraded optical resolution. In this study, a so-called dry microscope objective was applied; in other words, there was an air gap in between the objective and the skin. This caused a deterioration of the spatial resolution.

Sieg et al. presented an in vivo 14-volunteer study of forearm skin, but now for a cumulative treatment (3 weeks) with cosmetic moisturizers (30). The authors calculated the area under the water concentration profiles, for the entire thickness of the SC. During the treatment, the thickness of the SC changed, and this was taken into account. A formulation containing niacinamide was shown to increase the total water content of the SC much more (up to 2 or 3 times) than the other tested formulations.

Very recently, Stamatas et al. presented in vivo confocal Raman spectroscopy data of skin penetration and occlusive potential of two vegetable oils and a paraffin oil (36). Petrolatum was used as a positive control. The products were applied topically on the forearms of nine volunteers and seven infants, and Raman depth concentration profiles of both the oils and water were acquired before and at 30 and 90 minutes following application. It was shown that paraffin and vegetable oils penetrate the top layers of the SC with similar concentration profiles, a result that was confirmed both for adult and infant skin. The three oils tested demonstrated modest SC swelling (10–20%) compared to moderate swelling (40–60%) for petrolatum. The swelling was assessed using the method of van der Pol et al. (33). No statistical difference between the paraffin oil and vegetable oils in terms of skin penetration and skin occlusion was observed.

The already mentioned work by Wu and Polefka (27) included moisturization experiments using products whose effect was already known. On isolated pigskin SC samples, the following products were tested: lotion, commercial soap bar, syndet bar, non-emollient shower gel and emollient-containing shower gel. The results were consistent with what was expected. The water content on the skin treated with lotion was significantly higher than the nontreated control. Syndet bar-treated skin had significantly higher water content than soap-based bar-treated sites. Non-emollient shower gel washed sites were more moisturized than soap-based bar-treated samples. Finally, emollient shower gel-treated skin was significantly more hydrated than non-emollient shower gel–washed skin.

Water Distribution in the Skin for Different Skin Types

Understanding the hydration processes of the skin also requires knowledge of the state of the skin prior to treatment. It is likely that different types of skin will respond differently to equal treatments. Therefore, it is of interest to study differences in water distribution in the skin of human volunteers of different skin type. Such knowledge will no doubt contribute to the development of products targeted to these different skin types. The first papers using confocal Raman microspectroscopy to study different skin types are being published now.

In 2006, Matsumoto et al. presented the results of a systematic study of the water distribution in the skins of an "old" male Japanese group of volunteers ($N = 20$, average age 64.0 ± 2.5 years) and a "young" male Japanese group ($N = 20$, average age 27.8 ± 1.6 years) (37). Water concentration profiles were recorded on untreated areas on the volar aspect of the forearm, down to a depth of 200 μm; note that this is well in the dermis. Surprisingly, no differences in water concentration profile could be detected in the SC and the epidermis. However, the water content in the upper dermis was found significantly lower for the young group. Possibly, the mechanically more worn dermis of the old group contains more damages

such as voids. These voids may be filled with water. It was concluded that the water content in the dermis may be a useful parameter for evaluations of aging.

In the already referenced paper by Egawa et al. (32), the SCATs were measured at different body sites and for different panelist ages (6 male, 9 female). On the forearm, the SCAT tended to be higher for older skin, but at the cheek no age dependence was found. The volar forearm skin was hydrated with a wet cotton patch, and measurements were done after 15, 50, and 90 minutes of hydration. A swelling of the SC was observed of 4%, 40%, and 95%, respectively. This finding was in agreement with previously reported swelling of a corneocyte, using cryo-scanning electron microscopy (cryo-SEM) (38). In a later paper, Egawa and Tagami also addressed the effects of season on the distribution of water in the skin (39).

Infant skin is a subject in itself. A very large panelist study, comparing the barrier function and water-holding and water-transport properties of a group of infants ($N = 124$, age 3–12 months) and a group of adults ($N = 104$, age 14–73 years), was published by Nikolovski et al. (40). Capacitance, TEWL, and Raman measurement were employed in this study. The SC was found to be thinner for infants. The capacitance and TEWL values were higher for the infants, and the variations over the infant panelists were larger. Interestingly, as observed in the Raman water depth concentration profile, large differences were also observed in the amount of water that was absorbed after application for only 10 seconds with a wet-soaked paper towel. Whereas adult skin did not seem to absorb much water, for infant skin a rapid increase of 5% to –10% by mass of water in the outer 10 μm of the skin was observed. Desorption rates of water were also studied; the desorption rate for infants was high initially, followed by a slower rate. Adults only exhibited the slower desorption rate. It was concluded that the way the SC stores and transports water become adultlike only after the first year of life. (In this paper, the NMF contents were also compared; they were lower for infants.)

In the paper by Chrit et al. (41), an in vitro study on skin models was combined with an in vivo study on human volunteer skin, using the Raman technology. The hydration capacities of 2-methacryloyloxethylphosphorylcholine polymer (pMPC), native or microencapsulated and with or without hyaluronic acid, were investigated. The in vitro experiments on the skin models showed the best hydrating properties for the encapsulated-with hyaluronic acid formulation, which also exhibited the longest lasting efficacy. In a 26-volunteer in vivo study, using confocal Raman spectroscopy, the encapsulated-with hyaluronic acid formulation was tested and a statistically significant hydration effect was observed.

Hydration Effects in Dysfunctional Skin

The fact that too much exposure of the skin to water may have unbeneficial effects is long known, it is said to "dry out" the skin. This is already an example of dysfunctional skin. Van der Pol et al. demonstrated in 2005 the effects of hot bathing on the composition and distribution of components (among which water) in the skin (42). In this work, an interesting experiment was carried out. Following soaking the forearm in hot water for 30 minutes, Raman water depth concentration profiles were recorded at the same site (the volunteer did not move his arm) every 30 seconds, after the soaking. First of all, a relatively large swelling of the SC was observed, but more interestingly, within the first 30 minutes after soaking, the water redistributed over the SC. The water concentration decreased 5% to 10% by mass around a depth of 25 μm and increased a similar amount at a depth of about 10 μm. This phenomenon reflects *dynamically* the reduction of the barrier function (the barrier function is thought to reside at the stratum granulosum) as a result of the intense treatment with hot water.

Another way to arrive at dysfunctional skin is removal of the top part of the SC by sequential tape stripping, thereby disrupting the barrier function. In 2005, Hellemans et al. presented in vivo results on four volunteers using this approach for volar forearm and facial skin (43). The results illustrated clearly that for untreated skin, facial SC is thinner than SC of the forearm. Moreover, after tape stripping, the remaining thinner SC could be observed easily from the profiles. Remarkably, the remaining thickness of the SC for face and arm after tape stripping until TEWL = 18 g/m^2/hr is nearly identical. Right after disrupting the barrier, the water concentration over the SC was observed to be higher (as was expected). However, the recovery response after tape stripping between the external water fluxes, determined with TEWL, and the internal water content of the SC differed. A fast initial (4 hours) recovery of the TEWL was observed, whereas the internal water content stayed high, even 24 hours after

stripping. This effect may be explained by the release of the lipid content of the lamellar bodies immediately after barrier disruption. Such a "film of lipids" might keep the internal water content of the SC elevated, which in turn may facilitate the enzymatic processing required for the barrier recovery response.

Another way of compromising the integrity of the skin is to simply wipe it with acetone. The acetone will take away much of the skin lipids present at the outer surface of the SC. Initial results were obtained by River Diagnostics researchers (unpublished data). Immediately after wiping, a clear increase in water concentration over the entire SC can be observed. This indicated a reduced barrier function; the water apparently is leaking out of the epidermal layers.

It is expected that confocal Raman microspectroscopy will also find useful applications in the study of diseased skin and its treatment (e.g., atopic dermatitis and psoriasis).

DISCUSSION, CONCLUSION, AND OUTLOOK

In vivo confocal Raman microspectroscopy is a novel method that provides detailed information about the molecular composition of the skin. In this chapter, its application on the study of hydration was reviewed. Many applications so far have focused on the SC. However, the method is readily capable of measurements to a depth of greater than 150 μm into the skin–well into the dermis.

In the past decade, in vivo confocal Raman spectroscopy has made a major leap forward in sensitivity, speed of measurement, and ease of use. Raman technology has now reached a level of refinement where it can be applied in routine clinical studies. It has become fast enough to perform measurements on numbers of subjects ranging from several to several dozen per day, depending on the complexity of the study. The user interface has reached a stage of development where routine operation of the equipment by a laboratory technician is practical. Although the Raman technique has now been shown to be routinely useful in clinical settings, it is, like all measurement techniques, subject to certain limitations. It involves many measurements being made at a single location to generate composition depth profiles, whereas other techniques, such as electrical conductivity, for example, normally take only a single data point at a given location. This means that even with fast instrumentation, Raman measurements may be time consuming compared to other commonly used methods of in vivo skin analysis. That is, however, simply the price paid to obtain much greater information content. A related general issue is that in vivo tissue analysis normally requires considerable replication, by measurement of multiple locations and on multiple subjects, to achieve required statistical accuracy, given normal biological variability. This is, of course, a characteristic inherent in any human in vivo measurements, and not specific to Raman. Finally, Raman instrumentation for in vivo skin analysis is highly specialized and therefore expensive. However, as in vivo Raman microspectroscopy comes into more general use, the cost of the instruments eventually can be expected to drop as volume efficiencies are realized by manufacturers. These limitations are well compensated by the richness of information achievable and the unique ability to measure the same area of skin repeatedly and with microscopic spatial detail, allowing entirely new kinds of information to be gathered. It can be expected that this detailed and spatially resolved information and the ability to make these measurements in vivo will provide insights into the mode of action of skin hydration that have not been previously available.

The conclusion and outlook of the role of in vivo confocal Raman microspectroscopy of skin, in the study of the skin hydration process, is well captured in a citation from the work of Wu and Polefka (27): *"The unique and direct quantitative water content information provided by confocal Raman microspectroscopy offers a whole new perspective for fundamental skin moisturization studies and will play an important role in evaluating moisturizing profiles and the hydration potential of products designed."*

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