57 Skin-Whitening Agents

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INTRODUCTION

Skin hyperpigmentation is common and often causes psychosocial distress (1). Therapeutical interventions include whitening agents, chemical peels, lasers, and physical methods (1–3). Although multiple interventions are available, skin-whitening agents, due to their simplicity and convenience, continue to be the mainstay of approach to either lighten skin (individuals who wish to change or modify their skin color) in the cosmetic field or depigment skin (treatment for abnormal hyperpigmentation skin such as melasma, freckles, and actinic lentigines) in the clinical therapy. Commonly used whitening agents include hydroquinone, arbutin, kojic acid, ascorbic acid, and its derivatives. Their efficacy, mechanism, and safety have been extensively reviewed (4). This chapter revises the previous version (4) and updates current progress.

SKIN-WHITENING AGENTS

Hydroquinone

Hydroquinone (1,4-dihydroxybenzene) is used in the photographic, rubber, chemical, and cosmetic industries. In the late 1930s, it was observed that monobenzyl ether of hydroquinone, a chemical used in the manufacture of rubber, caused depigmented skin in some workers (5).

The efficacy of hydroquinone as a skin-lightening agent has been established in both human and animal studies. Clinically, hydroquinone is applied topically in the treatment of melasma, freckles, and senile lentigines as well as postinflammatory hyperpigmentation (PIH). In the United States, hydroquinone is available in concentrations up to 2% as an over-the-counter (OTC) drug and by prescription at higher concentrations (2,5).

Hydroquinone inhibits the conversion of dopa to melanin by inhibiting the tyrosinase enzyme (2,5,6). Other proposed mechanisms are inhibition of DNA and RNA synthesis, degradation of melanosomes, and destruction of melanocytes (2). Electron microscopic studies of black guinea pig skin treated with hydroquinone show the anatomic consequences of this action: (*i*) the melanosome structure is disturbed, resulting in decreased production or increased degradation of these organelles, or both; (*ii*) hydroquinone exposure can ultimately lead to melanocyte degradation; and (*iii*) keratinocytes are spared, showing no apparent injury (5).

Arndt and Fitzpatrick (7), in a non-placebo-controlled study, compared the efficacy of 2% and 5% hydroquinone cream for treatment of pigmentary disorders in 56 patients. Hydroquinone was a moderately effective depigmenting agent in 80% of cases. There was no efficacy difference between the two concentrations; however, 2% hydroquinone was less irritating than 5%.

In another non-placebo-controlled study, Fitzpatrick et al. (8) evaluated the efficacy of a 2% cream of stabilized hydroquinone in 93 patients. Of those patients, 64% showed decreasing hypermelanosis.

Kligman and Willis (9) noted enhanced efficacy with 5% hydroquinone, 0.1% tretinoin, and 0.1% dexamethasone in hydrophilic ointment for the treatment of melasma, ephelides, and PIH on adult male blacks in a non-placebo-controlled study. In contrast, they experienced poor results with each of the aforementioned as monotherapies. However, actinic lentigines were resistant.

Gano and Garcia (10) conducted a 10-week clinical trial in 20 women with melasma. Topical applications of 0.05% tretinoin, 0.1% betamethasone valerate, and 2% hydroquinone were used in a non-placebo-controlled study. There was an objective improvement rate of 65% and a subjective improvement rate of 95%. Side effects were frequent but minimal. Caution is

necessary when using potent fluorinated corticosteroids for prolonged periods on the face since it may result in epidermal atrophy, telangiectasia, rosacea-like erythemas, acne, and perioral dermatitis. Particularly, it may also exert an antimetabolic effect, resulting in decreased epidermal turnover, and, thus, may produce a mild depigmenting effect (11). However, when used in combination with tretinoin and hydroquinone in the treatment of melasma, fluocinolone acetonide 0.01% suppresses biosynthetic and secretory functions of melanocytes, and thus melanin production, leading to early response in melasma, synergy among the three agents, and no significant side effects over an eight-week period (11).

Gellin et al. (12) established a reliable in vivo method to predict the depigmenting action of chemicals on mammalian melanocytes. Black guinea pigs and black mice were used as animal models to screen the depigmenting capacity of several phenols, catechols, and organic antioxidants. Results showed that complete depigmentation on all test sites was achieved with mono-methyl ether of hydroquinone and p-tertiary butyl catechol in the black guinea pig. Less pronounced pigment loss was noted with these chemicals in black mice.

Pathak et al. (13) clinically tested the efficacy of hydroquinone in varying concentrations supplemented with corticosteroids or retinoic acid (tretinoin) in 300 Hispanic women with melasma in a non-placebo-controlled study and concluded that cream or lotion formulations of 2% hydroquinone and 0.05% to 0.1% retinoic acid provided the most favorable results. In addition, avoidance of sun exposure and constant use of broad-spectrum sunscreens are requisite for efficacy. They also suggested that patients should suspend use of oral contraceptives and other agents that promote skin pigmentation.

Sanchez and Vazquez (14) treated 46 patients with melasma, using two versions of a 3% hydroalcoholic solution of hydroquinone. In this non-placebo-controlled study, overall improvement was noted in 88% of the patients and moderate to marked improvement in 36%. Side effects were minimal. Vazquez and Sanchez (15), in a double-blind and vehicle-controlled study, compared a broad-spectrum sunscreen agent with its vehicle in the treatment of melasma in 53 patients who were concomitantly using a depigmentating solution. They reported that 96.2% of those who used the sunscreen agent showed improvement as compared with 80.7% of placebo group. These results suggested that the use of sunscreen might be necessary for efficacy in the treatment of pigmentation disorders.

Clarys and Barel (16) tested the efficacy of an ascorbate-phytohydroquinone complex in 14 patients with actinic lentigo in a non-placebo-controlled study. Objective skin color changes were evaluated with a chromameter. After one month of treatment, a clear depigmentation of the macules was measured.

Haddad et al. (17), in a double-blind, randomized, prospective study, compared the effectiveness of two products with placebo in 30 patients with melasma over three months. In group 1, a 4% hydroquinone cream was applied to one side of the face where placebo applied to opposite sides; group 2, a 5% skin-whitening complex cream was applied to one side of the face where placebo applied to opposite sides. A standard sunscreen was used daily. Group 1 (hydroquinone and placebo) presented an improvement of 76.9% with 25% side effects, and group 2 (skin-whitening complex and placebo) presented an improvement of 66.7% with 0% side effects. They concluded that both depigmentation agents were equally effective in the treatment of melasma; however, the skin-whitening complex seems to be an excellent choice since its adverse effects were nil.

Grimes (18) evaluated a microsponge formulation of 4% hydroquinone with 0.15% retinol on 28 patients of melasma and PIH in a 12-week open-label study. Patients applied the formulation on the full face twice daily (morning and evening). A broad-spectrum sunscreen was applied once in the morning, 15 minutes after application of the test product. Results showed that tested formulation produced significant improvement at all study endpoints (weeks 4, 8, and 12) when compared with baseline. The tolerance of patients to this formulation was good.

Espinal-Perez et al. (19) compared a 5% ascorbic acid cream and a 4% hydroquinone cream on a randomized split-face-designed study with 16 female patients of melasma over 16 weeks. Sunscreen was applied daily throughout the observation period. The hydroquinone side showed 93% good and excellent results, compared with 62.5% on the ascorbic acid side; however, side effects were present in 68.7% (11/16) with hydroquinone versus 6.2% (1/16) with ascorbic acid. They concluded that ascorbic acid may provide a beneficial effect on melasma, with a minimum of adverse effects when compared to hydroquinone.

Ferreira Cestari et al. (20), in a multicenter, open-label, randomized, eight-week clinical trial, compared the efficacy and safety of a triple combination (TC) cream and monotherapy with hydroquinone cream in the treatment of moderate to severe facial melasma of 120 patients. TC cream was significantly more effective than hydroquinone cream (73% vs. 49%). Adverse events (erythema, burning sensation, and desquamation) were similar in both groups.

In some cases, higher concentrations of hydroquinone may be used. The formulations contain concentrations as high as 10% combined with non-fluorinated corticoid creams with or without the additional use of tretinoin or hydroxy acids such as glycolic acid. Extemporaneously compounded preparations are often effective in patients who have failed to respond to lower concentrations of hydroquinone. With controlled use and monitoring, side effects from these preparations have proved minimal (2). However, note that hydroquinone may be quickly oxidized in such formulations.

Hydroquinone occurs in nature as the β-glucopyranoside conjugate (arbutin). Arbutin is a mild agent for treating cutaneous hyperpigmentation, including melasma and ultraviolet (UV)-induced ephelides (21). Arbutin is an active ingredient of the crude drug uvae ursi folium traditionally used in Japan and contained in the leaves of pear trees and certain herbs. Maeda and Fukuda (21) determined arbutin's inhibitory action on the melanin synthetic enzyme and its effects on melanin intermediates and melanin production in cultured human melanocytes. They indicated that the depigmentation effect of arbutin works through an inhibition of the melanosomal tyrosinase activity, rather than by suppression of the expression and synthesis of tyrosinase in human melanocytes. Arbutin was less cytotoxic than hydroquinone to cultured human melanocytes.

Adverse reactions associated with hydroquinone use include both acute and chronic complications. Among acute reactions are irritant dermatitis, nail discoloration, and PIH (5). Although generally assumed to be a common allergen, the documentation of hydroquinone allergic contact dermatitis is weak (5). Hydroquinone use can also induce hypopigmentation and, rarely, depigmentation of treated surrounding normal skin. But, these changes are temporary and resolve on cessation of hydroquinone treatment, in contrast to monobenzone use, which can cause permanent depigmentation (22). Hence, the only indication for monobenzone therapy is in the treatment of severe vitiligo.

A more recent concern regarding the use of hydroquinone is the occurrence of hydroquinone-induced ochronosis, a chronic disfiguring condition resulting, in general, from the prolonged use of high concentrations of hydroquinone (22,23).

Hydroquinone's acute and chronic toxicity toward higher terrestrial organisms appears to be minimal in humans (24,25). In an epidemiologic investigation, 478 persons employed as photographic processors showed no significant excess mortality, sickness/absence, or cancer incidence (24). The reported nephropathy and cell proliferation, as evidence of carcinogenicity, observed in Fischer 344/N rats (26,27), appears to be strain specific and sex specific (27). Hydroquinone was negative in the Ames/Salmonella and Drosophila genotoxicity assays (28). Others suggest that carcinogenic and teratogenic potentials have been inadequately studied (24,29) and that both hydroquinone and benzoquinone produce cytotoxic effects on human and mouse bone marrow cells (30). Hydroquinone in an alcoholic vehicle readily penetrates human forehead skin in vivo following a single 24-hour topical exposure; elimination was complete within five days (31). Wester et al. (32) determined the topical bioavailability, metabolism, and disposition of hydroquinone on humans in vivo and in vitro; dose recovery in urine was 45.3%, of which the majority was excreted in the first 24 hours.

Kojic Acid

Kojic acid, a fungal metabolic product, is increasingly being used as a skin-lightening agent in skin care products marketed in Japan since 1988. It was first isolated from *Aspergillus* in 1907 (33). Kojic acid suppresses free tyrosinase, mainly attributable to chelation of its copper (33–35), and it has been shown to be responsible for therapy and prevention of pigmentation, both in vitro and in vivo (34,36,37).

In Japan, it is used in nonprescription skin care products up to a concentration of 1%. To increase percutaneous absorption and thus therapeutic activity, it is usually used at the highest concentration allowed (33).

Since it is used intensively in foods (such as bean paste, soy, and sake) in some countries, particularly Japan, its oral safety has been studied.

Shibuya et al. (38) investigated the mutagenicity of kojic acid by the Ames test, by the forward mutation test in cultured Chinese hamster cells, and by the dominant lethal test in mice. They concluded that although kojic acid is a weak mutagen in bacteria, it is non-mutagenic in the eukaryotic system either in vivo or in vitro.

Abdel-Hafez and Shoreit (39) tested the mycotoxins, using the dilution plate method; kojic acid may induce some toxins. Fujimoto et al. (40) examined the tumorigenicity of kojic acid in B6C3F_1 mice. Three groups of animals were given food containing 0%, 1.5%, and 3.0% kojic acid for six weeks; mice in the groups ingesting kojic acid showed significantly higher frequency of induced thyroid tumors.

But true adverse effects after human oral ingestion have not been demonstrated. Nakagawa et al. (33) noted no signs of relapse of dermatitis or any other adverse effects on sensitized patients upon ingestion of foods containing kojic acid; however, they also noted that topical application may induce allergic contact dermatitis with sensitized patients. They postulated that kojic acid was considered to have a high sensitizing potential, because of the comparatively high frequency of contact sensitivity in patients using one or more kojic acid–containing products.

Majmudar et al. (36) used an in vitro model to evaluate the efficacy, stability, and cytotoxicity of whitening agents. They also conducted a non-placebo-controlled clinical study that indicated that kojic acid in an anhydrous base can induce more skin lightening than in an aqueous base.

Recently, Lim (37) conducted a non-placebo-controlled study to test 2% kojic acid in a gel containing 2% glycolic acid and 2% hydroquinone in 40 Chinese women who had epidermal melasma for 12 weeks. Half of the face was treated with the above formulation. The other half was treated with a formulation that was identical, except that it contained no kojic acid. Results showed similar improvement in melasma on both the sides. More than half (60%) of the melasma cleared in sides receiving kojic acid, whereas less than half (48%) cleared in the side denied kojic acid; in particular, two patients had complete clearance only in the kojic acid–treated side. However, the improvement did not show a statistical difference between the formulations.

Ascorbic Acid and its Derivatives

Ascorbic acid may inhibit melanin production by reducing o-quinones (41), so that melanin cannot be formed by the action of tyrosinase until all vitamin C is oxidized. Because vitamin C is quickly oxidized and decomposes in aqueous solution, it is not generally useful as a depigmenting agent.

Recently, stable derivatives of vitamin C have been synthesized to minimize this problem (41–44). Magnesium-L-ascorbyl-2-phosphate (VC-PMG) is a vitamin C derivative that is stable in water, especially in neutral or alkaline solution containing boric acid or its salt (41). VC-PMG is hydrolyzed by phosphatases of the liver or skin to vitamin C and thus exhibits vitamin C–reducing activity (41).

Kameyama et al. (41) investigated the effects of VC-PMG on melanogenesis in vitro and in vivo. Results from their non-placebo-controlled study suggested that the topical application of VC-PMG was significantly effective in lightening the skin in 19 of 34 patients with chloasma or senile freckles and in 3 of 25 subjects with normally pigmented healthy skin.

Other Agents

Glutathione is a ubiquitous compound found in human bodies. It has recently received attention since it possesses skin-lightening function. The proposed mechanisms of action include (*i*) direct inactivation of the enzyme tyrosinase by binding with the copper-containing active site of the enzyme, (*ii*) mediating the switch mechanism from eumelanin to phaeomelanin production, (*iii*) quenching of free radicals and peroxides that contribute to tyrosinase activation and melanin formation, and (*iv*) modulation of depigmenting abilities of melanocytotoxic agents (45). Villarama and Maibach (45) review the evidence of its involvement in the melanogenic pathway and shed light on its anti-melanogenic effects that have been documented in in vitro and in vivo studies. However, they suggested that to validate the effectiveness of glutathione, randomized, double-blind, placebo-controlled clinical studies in humans are warranted.

Since many predisposing factors—such as pregnancy or exposure to sunlight (in the UVB and UVA ranges)—may cause hyperpigmentation, various systemic drugs and natural products have been used as protective agents. These agents include chloroquine, indomethacin, vitamin C and E, fish oil, and green tea (46).

Funasaka et al. (47) demonstrated that oral vitamin E [α-tocopherol (α-T)] supplementation can improve facial hyperpigmentation: the inhibitory effect of tocopheryl ferulate (α-TF) on melanogenesis was examined biochemically using human melanoma cells in culture. α-TF, solubilized in ethanol or in 0.5% lecithin, inhibited melanization significantly, as did α-T at a concentration of 100 μg/mL, without inhibiting cell growth.

Kobayashi et al. (48) reported that neoagarobiose, a disaccharide, could be useful as a novel whitening agent because it has moisturizing and whitening effects with low cytotoxicity on B16 murine melanoma cells. However, it should be validated on human skin in vivo.

Schmaus et al. (49) recently identified a new potent lightening agent, 4-(1-phenylethyl) 1,3-benzenediol, a dihydroxylated diphenylmethane derivative. Data obtained from in vitro and in vivo studies on human skin showed good lightening effects.

Ando et al. (50) evaluated the effects of unsaturated fatty acids on UV-induced hyperpigmentation of the skin in a placebo (vehicle)-controlled study. Skin hyperpigmentation was induced on the backs of guinea pigs by UVB exposure. Oleic acid, linoleic acid (LA), and α-linolenic acid (0.5% in ethanol), or ethanol alone as a control, were then topically applied daily five times weekly for three successive weeks. Results suggest that the pigment-lightening effects of LA and α-linolenic acid are, at least in part, due to suppression of melanin production by active melanocytes and enhanced desquamation of melanin pigment from the epidermis.

A new combination product composed of 2% 4-hydroxyanisole (mequinol) and 0.01% tretinoin (all-trans-retinoic acid) in an ethanolic solution is being studied for its safety and efficacy as a topical treatment for disorders of skin hyperpigmentation (51). Fleischer et al. (51) evaluated efficacy in a controlled, double-blind trial. Subjects were randomized to treatment with the combination solution, or one of the active components (4-hydroxyanisole or tretinoin), or vehicle twice daily to all solar lentigines and related hyperpigmented lesions on the face, forearms, and backs of hands for up to 24 weeks. The combination solution (2% 4-hydroxyanisole and 0.01% tretinoin) was clinically superior to each of its active components and to the vehicle in the treatment of solar lentigines. Most skin-related adverse events were mild and were similar for both the combination solution and tretinoin treatment groups.

Bissett et al. (52) evaluated the effectiveness of a stable derivative *N*-acetyl glucosamine (NAG) to reduce facial hyperpigmentation in two separate studies. Topical 2% NAG, its vehicle control, 4% niacinamide, and a combination of 2% NAG with 4% niacinamide were compared in an eight-week, double-blind, placebo-controlled, left-right randomized, split-face clinical test. Data showed that 2% NAG was effective in improving the appearance of facial hyperpigmentation. The combination formulation provided the best results.

Hamed et al. (53) reported the efficacy and safety of deoxyarbutin, a new tyrosinase-inhibiting agent both in vitro and in vivo on human skin. They demonstrated that deoxyarbutin has the potential to be as safe and effective as a depigmenting agent and suggested that it may act an as an alternative agent to hydroquinone.

Shigeta et al. (54) evaluated the effect of liposomalization on the whitening activity of LA by using LA in ethanol, hydrogel-containing LA, and hydrogel-containing liposomal LA toward the UV-stimulated hyperpigmented dorsal skin of brownish guinea pigs. In addition, the whitening effect of LA was examined with UV-stimulated hyperpigmented human upper arm skin by using a hydrogel-containing liposomal LA (0.1% LA) and non-liposomal LA (3.0, 10.0% LA). Liposomal LA was significantly more effective than non-liposomal formulations in reducing hyperpigmentation of both pigskin and human skin.

Recently, natural substances extracted from plants have attracted attention as source of potential skin-whitening agent due to their biologically active compounds in medicine, and they may also be isolated in high quantities at low cost. Tengamnuay et al. (55) assessed the heartwood extract of *Artocarpus lakoocha* Roxb for the in vitro tyrosinase inhibitory activity and the in vivo melanin-reducing efficacy in human volunteers. They concluded that *A. lakoocha* has a promising potential for use as an effective and economical skin-whitening agent. Wang et al. (56) examined 25 traditional Chinese herbal medicines on human epidermal melanocytes

to identify the effects of skin-whitening and skin health. They found four herbal preparations to be potent tyrosinase and melanin synthesis inhibitors. However, this finding needs to be validated in vivo on human skin.

Taylor et al. (57) mentioned several combinations of whitening formulations in comparison with monotherapies such as hydroquinone and retinoids to treat the PIH. Findings suggested that 2% mequinol with 0.01% tretinoin solution is a promising alternative for the treatment of PIH.

Chemical peels are also effective and safe in treatment of pigmentation disorders. Grimes (58) investigated the clinical efficacy and safety of a new superficial salicylic acid peel in individuals of skin types V and VI. The patients were pretreated for two weeks with hydroquinone 4% prior to undergoing a series of five salicylic acid chemical peels. The concentrations of salicylic acid were 20% and 30%. The peels were performed at two-week intervals. Results suggested that superficial salicylic acid peels are both safe and efficacious for treatment of melasma and PIH.

Brief data of key skin-whitening agents are summarized in Table 1.

Table 1 Brief Data of Key Skin-Whitening Agents

Whitening agents	Study design	Models	References
Hydroquinone			
2% and 5%	Non-placebo-controlled study	In vivo in humans	(7)
2%	Non-placebo-controlled study	In vivo in humans	(8)
5%, in combination with 0.1% tretinoin and 0.1% dexamethasone	Non-placebo-controlled study	In vivo in humans	(9)
2%, in combination with 0.05% tretinoin and 0.1% betamethasone valerate	Non-placebo-controlled study	In vivo in humans	(10)
Phenols, catechols, and organic antioxidants	Screening	In vivo in black guinea pigs and black mice	(12)
2%, in combination with 0.05–0.1% retinoic acid	Non-placebo-controlled study	In vivo in humans	(13)
3%	Non-placebo-controlled study	In vivo in humans	(14)
In combination of a broad-spectrum sunscreen agent	Double-blind and vehicle-controlled study	In vivo in humans	(15)
Ascorbate-phytohydroquinone	Non-placebo-controlled study	In vivo in humans	(16)
4%, and 5% skin-whitening complex cream	Double-blind, randomized, prospective study	In vivo in humans	(17)
4%, with 0.15% retinol	Open-label study	In vivo in humans	(18)
4%, and 5% ascorbic acid cream	Randomized split-face designed study	In vivo in humans	(19)
4%, in comparsion with a TC cream	Multicenter, open-label, randomized clinical trial	In vivo in humans	(20)
Arbutin	Cell culture	In vitro in human melanocytes	(21)
Kojic acid			
1% and 2%	1) Screening 2) Non-placebo-controlled clinical study	1) In vitro in model 2) In vivo in humans	(36)
2%, in combination with 2% hydroquinone	Non-placebo-controlled study	In vivo in humans	(37)
Ascorbic acid			
10% VC-PMG	1) Cell lines and culture; 2) Non-placebo-controlled clinical study	1) In vitro mammalian tyrosinase and human melanoma cells; 2) In vivo in humans	(41)

Abbreviations: TC, triple combination; VC-PMG, magnesium-L-ascorbyl-2-phosphate.

CONCLUSIONS

The treatment of pigmentation disorders can be a long process. In general, skin-whitening agents are considered modestly effective. However, hydroquinone is still one of the most effective agents for the treatment of hyperpigmentary disorders (59). High concentrations are not recommended, except under a physician's supervision. The application of hydroquinone in combination with certain chemicals (tretinoin, salicylic acid, or corticosteroids) may enhance lightening effects. Recently, chemical peelings with kojic acid, glycolic acid, and trichloroacetic acid, either alone or in combination, have been widely introduced for treatment of hyperpigmentations (57,58,60). However, the real efficacy of whitening agents should be determined in a placebo-controlled study in humans. Non-hydroquinone agents have also been assessed (61), and results have been encouraging. Optimal whitening agents remain a future goal. Recent review of the mechanism and biological, chemical, and clinical aspects of lightening agents provides additional insights (62–64).

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58 Skin Whitening: New Hydroquinone Combination

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DISORDERS OF PIGMENTATION

Pigmentation disorders occur as a result of an increased production of melanin by melanocytes and/or an elevated transfer of melanosomes from melanocytes to basal and suprabasal keratinocytes (1–3). Melanin (eumelanin and pheomelanin) results from the hydroxylation of tyrosine to 3, 4-dihydroxyphenylalanine (DOPA) through the enzymatic action of tyrosinase and the subsequent oxidation of DOPA to dopaquinone (4). Once produced, melanin is transferred to keratinocytes or into the dermis via any of the following processes: (*i*) damage to melanocytes in the basal layer allows for phagocytization by melanophages, releasing melanin into the dermis; (*ii*) melanosomes are directly deposited, through their dendrites, into the dermis; or (3) macrophages migrate into the epidermis where melanosomes are phagocytized, returning them to the dermis.

Melasma

Difficult to treat, melasma, also known as chloasma or "mask of pregnancy," is a relatively common, chronic pigmentary condition typically seen in women of childbearing age. In fact, it is known to appear at any time during a woman's reproductive years and is often associated with pregnancy or oral contraceptive use. Melasma is more common among women of darker skin types. A small percentage of cases, approximately 10%, occur in men, most frequently in those of Middle Eastern, Caribbean, or Asian descent.

Melasma presents as often distinctly demarcated, irregularly shaped light- to dark-brown macules. The blotches usually appear on the upper lip, nose, cheeks, chin, forehead, and, occasionally, the neck. A centrofacial pattern of distribution, involving the cheeks, forehead, upper lip, nose, and chin, is the manifestation most often seen, but there are three identifiable patterns of presentation (2,5). The mandibular pattern and the malar pattern, which affects the nose and cheeks, are less common. Although it most often occurs in skin routinely exposed to the sun, there are reports in the literature of melasma appearing on the nipples and around the external genitalia (6,7).

Etiology

The cause of melasma has not yet been clearly identified, but solar exposure, genetic predisposition, and hormonal influences are considered among the most important factors (6,7). Estrogen and progesterone, nutritional deficiency, and certain antiepilepsy drugs are considered significant causal or aggravating factors in its development (4). Also, Hydantoin and Dilantin have been reported to contribute to melasma in both women and men (7,8).

A history of chronic sun exposure seems to be a necessary precondition for the development of this pigment disorder, and solar exposure is also well known to exacerbate the condition (2,4). Interestingly, in the winter months, when sun exposure is usually less frequent, melasma is typically less noticeable (4). In the absence of other compelling evidence, sun exposure is considered the primary exogenous causal factor in melasma (1,9). Data suggesting a genetic component are sparse, but a few familial cases have been reported (1).

It is not uncommon for women to develop melasma on the upper lip after hot wax application to remove unwanted hair. In fact, this phenomenon is so often reported by patients

[a]Financial conflict statement: Dr. Baumann was an investigator for Stiefel in several clinical trials.

that the author speculates that heat may play a role in melasma development as it does in erythema *ab igne* (a reticulated erythematous hyperpigmented eruption arising after chronic exposure to heat).

It has been observed that women who use oral contraceptives represent the population that most often presents with melasma (6,7). This painless but often stress-inducing condition also frequently affects pregnant women; together, women in these categories comprise the majority of melasma cases. Occasionally, there are menopausal and premenstrual presentations associated with melasma. The low incidence of melasma among postmenopausal women on estrogen replacement therapy suggests that estrogen alone is an unlikely etiological root (4). This is a highly idiopathic condition, varying from patient to patient, within individuals, and even from pregnancy to pregnancy (4). It is also characterized by a high degree of recalcitrance. Melasma may subside in the months following a patient's pregnancy or after discontinuing oral contraceptives, but may still persist, taking up to five years to resolve (7,8). An endocrine etiology has been posited by some (7), but no such mechanism has yet been proved (8). Some ovarian disorders are also correlated with an increased incidence of melasma, but no causal link has been established. The odds of experiencing initial onset of melasma are lower than the likelihood of recurrence once melasma has developed.

OTHER PIGMENTATION DISORDERS

Solar Lentigos

Long-standing data suggest that as many as 90% of elderly patients have one or more solar lentigos (10). Sun exposure, as the name suggests, is responsible for this condition, which is characterized by macular brown lesions usually 1 cm in diameter. Acute or chronic exposure to the sun can induce solar lentigos. The face and backs of hands are the areas most often affected. This condition is resistant to the Kligman formula (11).

Post-Inflammatory Hyperpigmentation

Various skin conditions, such as acne, eczema, and allergic responses, can lead to post-inflammatory hyperpigmentation, also known as post-inflammatory pigment alteration (PIPA), as can more serious cutaneous events, such as burns, surgeries, and trauma. Certain treatments for skin disease or cosmetic conditions can also engender or exacerbate discoloration (e.g., chemical peels and laser resurfacing). PIPA can occur in any skin type, but it most often affects people with darker skin types (12–14). The condition results from an elevation in melanin synthesis in response to a cutaneous attack and can be diffused or localized—its distribution depends on the location of the original insult to the skin.

PIPA presents in areas of previous inflammation as irregular, darkly pigmented splotches (15). Any area of the skin can be involved, but the disorder is especially stressful to patients when it occurs in the face. Not surprisingly then, PIPA is one of the most common conditions prompting patients to visit a dermatologist. PIPA unfortunately tends to recur in susceptible individuals (16).

Treatment

The goals of therapy are to slow the proliferation of melanocytes, inhibit the formation of melanosomes, and promote the degradation of melanosomes (17). Sun-protective behavior is a necessity. As such, patients must use a good high sun protection factor (SPF) sunscreen with UVA protection and make all reasonable efforts to avoid sun exposure. The sunscreen should be worn 24 hours a day. Other practical, behavioral elements of therapy can include UVA screens for car and home windows and protective clothing such as hats.

The Kligman Formula

The "Kligman formula" is a mixture consisting of 0.1% tretinoin, 5.0% hydroquinone, 0.1% dexamethasone, and hydrophilic ointment (11). The daily application of this combination through five to seven weeks resulted in normalized pigmentation of normal adult skin in black males treated for melasma, ephelides, and postinflammatory hyperpigmentation. The removal of one component of the therapeutic regimen resulted in less-efficacious results. The formula,

which has been very popular as a therapeutic option for melasma since its introduction in 1975, is not commercially available now, but can be formulated by a pharmacy. Unfortunately, the stability of these products formulated in a pharmacy is in question. This is because hydroquinone and tretinoin have a tendency to interact and decrease each other's effectiveness. However, the popularity of the Kligman formula led to the status of topical combination therapy as the current mainstay of melasma treatment and resulted in the development of Tri-Luma (discussed later in this chapter) in which tretinoin and hydroquinone have been successfully stabilized.

Other Topical Components

The standard products used to produce hypopigmentation include phenolic and nonphenolic derivatives. Hydroquinone and hydroquinone combination formulations are among the phenolic group; tretinoin and azelaic acid are among the nonphenolic agents (18). Typical topical preparations include hydroquinone 2% to 4%, low-potency steroids, kojic acid, deoxyarbutin, azelaic acid, hydroxy acids, and retinoids. Tretinoin 0.1% has been evaluated as a single agent in the treatment of melasma and favorably reviewed (19,20), but such monotherapy took as long as 10 months, in one study, before the condition improved. A 10-month, randomized, vehicle-controlled clinical study did show that topical 0.1% tretinoin lightened melasma in 28 black patients, with only mild side effects (20). Combination therapy has been considered the mainstay therapy for patients with any skin type, though.

Although most of the remaining discussion here will focus on recent research with a novel combination compound, it is worth noting that other tools in the dermatological armamentarium have achieved favorable results. For example, the addition of glycolic acid to hydroquinone has been shown to promote efficacy by facilitating the penetration of both agents (21). A recently evaluated cream containing 10% buffered glycolic acid, 4% hydroquinone, vitamins C and E, and sunscreen has also been shown to be safe and effective in the treatment of melasma (22). In combination with topical agents, glycolic acid peels and/or Jessner's peels can be used to accelerate the resolution of melasma. Jessner's solution and 70% glycolic acid (combined with tretinoin and hydroquinone between peels) have been shown to work equally well in the treatment of melasma (23).

Kojic acid has also been demonstrated to enhance the efficacy of topical agents. A study in Singapore followed 40 Chinese women treated with 2% kojic acid in a gel containing 10% glycolic acid and 2% hydroquinone on one half of the face and the same application without kojic acid on the other half (24). Patients were observed for 12 weeks, and they showed improvement in melasma on both sides of the face. The side treated with the combination containing kojic acid showed greater improvement, it should be noted. The melasma cleared in 24 of the 40 patients who received kojic acid as compared with 19 of 40 patients treated with the gel without kojic acid.

It is also worth noting that laser therapy has been used with some degree of effectiveness in treating several pigmentary disorders, but has not yet been established as a first-line therapy for melasma (18). However, the Fraxel laser is frequently used in light- and dark-skinned patients after topical melasma regimens have failed or in combination with topical regimens.

A PRESCRIPTION COMBINATION THERAPY: TRI-LUMA

Tri-Luma is a combination of tretinoin 0.05% (retin A), hydroquinone 4.0%, and fluocinolone acetonide 0.01% (a mild steroid). Two 8-week, multicenter, randomized, investigator-blind studies were conducted to compare the efficacy and safety of this triple-combination hydrophilic cream formulation with various dual-combination agents. The same drug concentrations and vehicles were used in all formulations. The dual combinations included tretinoin plus hydroquinone, tretinoin plus fluocinolone acetonide, and hydroquinone plus fluocinolone acetonide.

The theoretical basis for this formulation rests on the prior success of the various first-line components in dual-combination therapies, namely, hydroquinone, tretinoin, and a range of topical corticosteroids. Of particular conceptual importance in the product development are the clinical and experimental data demonstrating the effects of tretinoin and other retinoids in abrogating the epidermal atrophy that can be induced by topical corticosteroids (25,26).

A total of 641 predominantly white female adults (ranging in age from 21 to 75 years) were randomized to the various treatment groups. In both studies, all formulations were applied once daily, at night. Patients enrolled in the study represented Fitzpatrick skin types I through IV and exhibited moderate-to-severe hyperpigmentation.

Results

Significantly more of the patients treated with Tri-Luma (26.1%) demonstrated complete clearing compared with the dual-combination therapy groups (4.6%) at the end of eight weeks (27). Researchers observed complete or near complete clearing of hyperpigmentation in 77% of the aggregate Tri-Luma group compared with 42.2% for fluocinolone acetonide, 27.3% for tretinoin and fluocinolone acetonide, and 46.8% for tretinoin and hydroquinone. Side effects associated with Tri-Luma were transient and mild. The most frequently occurring adverse effects included erythema at the application site, desquamation, burning, xerosis, and pruritus.

Some authors have cautioned against the use of hydroquinone in high concentrations because of its association with inducing ochronosis. Nevertheless, hydroquinone is the most effective topically applied hypopigmenting agent approved by the Food and Drug Administration (FDA) for melasma treatment (18). No ochronosis events were observed among patients on any of the treatment regimens containing hydroquinone 4%.

The use of topical corticosteroids as therapy for melasma has also been discouraged by some authors because of the association with skin atrophy and telangiectasia (28). In fact, protracted use of potent topical corticosteroids is known to engender cutaneous atrophy. When steroids are used in combination with retinoids, however, skin atrophy does not occur. Indeed, the combination of tretinoin application with corticosteroid has been shown to ameliorate the epidermal atrophy induced by the topical corticosteroid while not reducing its activity (26) and is believed to reduce the risk of steroid-induced atrophy (25). The data from this study seem to support this fact because only one patient in the dual-therapy hydroquinone and fluocinolone acetonide group exhibited skin atrophy. Significantly, the one patient that experienced skin atrophy did not receive tretinoin. A 12-week open-label long-term safety study showed a similar safety profile as the previously described eight-week study (27).

The results of the two related studies suggest that the use of this triple-combination agent may be more effective than any of the dual-combination agents in counteracting or inhibiting the pathophysiological mechanism of melasma. Tri-Luma combines three well-established agents in a formulation that appears to be effective and safe in the treatment of melasma. This triple-combination topical therapy also shows favorable tolerability and represents a significant advance in the dermatological armamentarium for melasma.

The concept of such a triple combination is also supported by another recent study. Researchers evaluated the efficacy of a formula containing 0.1% tretinoin, 5% hydroquinone, and 1% hydrocortisone in 25 Korean female patients with melasma recalcitrant to therapy. Patients were evaluated before treatment, then instructed to apply hydrocortisone on their faces for four months and were also assessed four weeks and four months after treatment. Overall, investigators reported statistically significant depigmentation in clinical and histological studies and increased subepidermal collagen synthesis, results that were observed as early as four weeks after hydrocortisone treatment (29).

REGULATORY UPDATE: FDA AND HYDROQUINONE

On August 29, 2006, the FDA withdrew the September 3, 1982 tentative final monograph on over-the-counter (OTC) skin-bleaching products and proposed that no OTC skin-bleaching active ingredients be categorized as generally recognized as safe. Of course, prominent among such products is hydroquinone. A final ruling has not yet been handed down. Should the FDA follow through, thus changing the status of hydroquinone and other skin-bleaching agents, the effect would be to reclassify such products as "new drugs," permitting them to be used by prescription until approval of a new drug application. Until such a ruling, however, hydroquinone products remain available OTC, as this ingredient remains an important first-line therapy for pigmentary disorders. As of the date of publication, a ruling by the FDA does not appear imminent, but no timetable for a decision has been made clear.

ALTERNATIVE WHITENING AGENTS

There are several alternatives to the use of hydroquinone as a hypopigmenting agent, including kojic acid, as described earlier, deoxyarbutin, mulberry extract, Pycnogenol, and vitamin C. Recently, deoxyarbutin [4-([tetrahydro-2H-pyran-2-yl]oxy)phenol] was shown in cultured human skin cells to be less cytotoxic than hydroquinone. It was also demonstrated to exhibit significant tyrosinase-inhibiting activity, and, on xenographs, topical application of deoxyarbutin yielded observable skin lightening over an eight-week period. Further, in a clinical trial, deoxyarbutin promoted the fading of pretanned skin better than hydroquinone or no treatment, with statistically significant differences observed. Investigators concluded that these findings collectively suggest that deoxyarbutin is a potentially effective and safe tyrosinase inhibitor (30).

In a study conducted to investigate the in vitro effects of an 85% methanol extract of dried Morus alba (mulberry) leaves on melanin biosynthesis, mulberroside F (moracin M-6, 3′-di-*O*-β-*D*-glucopyranoside) isolated from the extract was found to inhibit the tyrosinase activity that converts dopa to dopachrome in the biosynthetic process of melanin. Although its activity was weaker than that of kojic acid, researchers concluded that this mulberry extract may be viable as a whitening agent (31).

A 30-day clinical trial of 30 women with melasma in which patients were given one 25-mg tablet of Pycnogenol at each meal, three times daily, was inspired by the observed efficacy of Pycnogenol in protecting against UV radiation. Investigators found that the average surface area of melasma exhibited by the subjects was significantly reduced, demonstrating that Pycnogenol could be effectively and safely used in treatment of this condition (32).

In another study in melasma patients, 16 women were instructed to apply 5% ascorbic acid (vitamin C) cream on one side of the face and 4% hydroquinone cream on the other side nightly for 16 weeks. Patients also applied sunscreen daily throughout the trial. Although the hydroquinone side was associated with better subjective improvement (93% good and excellent results vs. 62.5% for ascorbic acid), colorimetric measures indicated no statistical differences. Further, side effects were more commonly associated with hydroquinone (68.7% vs. 6.2%). Investigators concluded that while a better response was seen with hydroquinone, ascorbic acid demonstrated efficacy in treating melasma while inducing far fewer side effects, justifying its use alone or in combination therapy (33).

CONCLUSION

Many types of skin are susceptible to pigmentation disorders. Such conditions can appear especially prominent in people with dark skin. Traditionally, disorders of pigmentation have been refractory to treatment, frustrating patient and physician alike. Combination therapy, including prolonged use of topical agents, sun avoidance, and, often, in-office chemical peels, has been the mainstay. Laser treatments, with the exception of the Fraxel laser, have been of limited success. A new topical combination therapy, Tri-Luma, has shown great promise, though, in simplifying and improving treatment for these intractable disorders, particularly melasma. In addition, this new combination therapy is effective, tolerable, and easy to use.

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59 Anticellulite Products and Treatments

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INTRODUCTION

Cellulite is a localized condition of subcutaneous fat and connective tissues with the typical visual appearance of the orange peel look of the skin. Cellulite, or more correctly, gynoid lipodystrophy (GLD) affects mostly women and rarely men and is considered as a common aesthetic problem for many women. Cellulite appears generally after puberty and worsens with age. There are preferential places of cellulite: buttocks, thighs, upper part of the arms, knees, and more rarely, the lower parts of the legs and the back of the neck (Fig. 1). It is interesting to note that these preferential cellulite sites are areas in which the typical pattern of adipose deposition is observed (1). Although cellullite may be found in areas with excess adipose tissue, obesity is not necessary correlated with the presence of cellulite (1).

The aims of this chapter are to describe

1. the histological, physiological, and biochemical characteristics of subcutaneous lipodystrophy;
2. the different objective evaluation methods of lipodystrophy; and
3. the different anticellulite treatments available and their efficacy.

There have been only a few review articles on cellulite published since 2000 (1–5).

CLINICAL, VISUAL, AND TACTILE SYMPTOMS OF THE SKIN WITH CELLULITE

There are some typical symptoms, partly subjective (reported by the patients) and partly objective (observed by the investigators), which are very often reported in the case of cellulite (1).

- There is presence of the typical orange peel skin upon normal visual examination and after pinching of the skin.
- Deep palpation of the skin reveals differences in the mobility of fat tissue: presence of micro- and macronodules and fibrosclerosis. Sometimes there is presence of painful subcutaneous nodules through deep palpation.
- There are irregularities in skin surface temperature as observed by thermography. Touching the skin by hand reveals the presence of cold spots in an advanced stage.
- Clinical examination reveals venous stasis and edema.

DESCRIPTION OF THE DIFFERENT STAGES OF LIPODYSTROPHY OF FAT TISSUES

There are different stages in the progression of cellulite. It is difficult to detect cellulite by visual examination and by palpation at the first stages: orange peel skin is not permanently present, only visible after pinching the skin. The clinical symptoms are clearly more visible at later stages of cellulite: permanent orange peel, colder skin areas, diminution in mobility of fat tissue upon palpation, and increased skin sensibility.

Skin surface contact thermographic pictures using thermographic foils give an indication of the degree of cellulite, as the skin surface temperature correlates to some extent with the clinical symptoms of cellulite. On the basis of these thermographic patterns and clinical symptoms, Curri and coworkers proposed a classification of cellulite in four stages (6–11), a classification that has been confirmed by others (12–14). In normal adipose tissues, a fine mesh

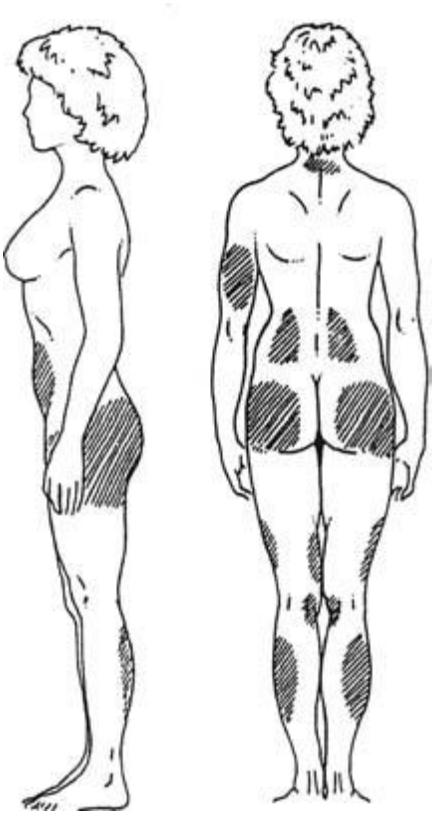

Figure 1 Preferential localizations of subcutaneous lipodystrophy in women.

of blood vessels and lymph vessels supplies this adipose tissue with the necessary nutrients and oxygen and takes care of the removal of the metabolized products. In the early stage of cellulite (stage 1), the capillary blood vessels walls become more permeable, causing leakage of blood plasma from the vessels in between the adipose tissues, which cause an edema in the adipose tissues. In addition probably, problems with the lymph circulation hampers removal of accumulating fluids. The aggregation of adipose cells and the amplification of the fibrillar network of collagen bundles interconnecting the adipose cells hamper blood circulation, leading to some hemostase (stage 2).

Adipose cells aggregates into "micronodules" surrounded by a less-mobile collagen fibers (stage 3). The size of these micronodules is of the order of millimeters. Finally, many of these micronodules aggregate into "macronodules" with larger sizes (2–20 mm) (stage 4). As nerves may be squeezed by these larger nodules, persons with severe cellulite often suffer from a sensitive to painful skin.

Stages 1, 2, and 3 of lipodystrophy are not considered clinically as pathological symptoms but more as aesthetic-cosmetic problems of the skin. Only in stage 4, some clinical symptoms such as an increased skin sensitivity, extensive fibrosclerosis of connective tissue, and very advanced edema are considered as light pathology symptoms. Furthermore, it is believed that the first stages are more or less reversible, whereas the latters stages are almost irreversible and consequently very difficult to treat.

ETIOLOGY OF CELLULITE

Cellulite is probably a multicausal condition, and many hypotheses have been proposed regarding the origin of fat lipodystrophy (1–5).

There is sexual differentiation in the histological distribution of subcutaneous fat lobules in women and in men. The differences between the sexes can be found in the structure of the

septal connective fat tissue. In women, one observes a higher percentage of septa perpendicular to the skin surface and a smaller percentage parallel to the surface as shown in men. Furthermore, in women with cellulite, deep indentations of adipose tissue into the skin were recorded. Using in vivo high frequency ultrasound imaging, Querleux et al. (15), Lucassen et al. (16), and Nuijs and Van Herk (17) confirmed an irregular dermo-hypodermal interface in women with cellulite. Mirrashed et al. (18) and Querleux (19) confirmed by magnetic resonance imaging (MRI) the existence of indentations of adipose tissue into the dermis. Also observed in women with cellulite was an increase in the thickness of the inner fat layer, a higher percentage of septa in the direction perpendicular to the skin surface. Since cellulite is widely present in women, some authors consider cellulite as a secondary sexual characteristic. Although cellulite is not always synonymous with overweight, there is clearly a relation between cellulite and hypertrophy of fat tissues.

Vascular Modifications

These consist in alterations in the microvascular network (mostly venous blood circulation) in the fat tissue leading to a venous stasis (1,2,5). The superficial microcirculation appears to be less efficient, and this results in subcutaneous edema because of the altered permeability of blood vessels and the presence of plasmatic exsudate in the subcutaneous connective tissue. This edema is probably a noninflammatory symptom. However, other authors have suggested an inflammatory basis for its pathophysiology (2). Furthermore, alterations in the reticular fibrillar network surrounding the blood vessels and adipocytes are observed. This fibrosclerosis provokes stiffening and decrease in mobility of fibers. Also, alterations in the interstitial fundamental substance (proteoglycans) are reported. GLD is probably associated with chronic venous problems. Venous insufficiency shows typical symptoms such as possible presence of telangiectasias, heaviness in the legs, cramps in the lower limbs, pain on deep palpation of the skin, and irregularities in skin surface temperature as detected by thermographic examination.

Alterations in the Matricial-Interstitial Unit Surrounding the Fat Cells (1)

The matricial-interstitial unit is formed by fibroblasts (synthesis of macromolecules of the cellular matrix), by the collagen, elastin, and reticular fiber, and by the ground substance (proteoglycans, glycoproteins). Alterations in the structure of the GAG (glycosyl amino glycan) in the perivascular tissues provoke hyperpolymerization and an increase in their hydrophilicity and the interstitial osmotic pressure: edema and hypoxia. Ryan and Curri (9,10) suggested the hypothesis of an increase in water content of subcutaneous adipose tissue in case of cellulite. An increase in the concentration of glycosyl amino glycans, presumably leading to a rise in the amount of water retained in the skin, was suggested. Querleux et al. (15) did not confirm the hypothesis of increased water content in the adipose tissue of women with cellulite except if such water would be located in the connective septa. Modifications in the structure of the proteins of the cellular matrix are observed: alterations of the fibers are followed by sclerosis.

Predisposing Factors

A genetic prediposition factor plays an important role in the development of cellulite (1). More controversial is the observation that white Caucasians tend to have more cellulite than Asians. Also, it appears that Latin women develop more GLD on the hips and thighs than Nordic women (the sale of anticellulite creams and treatments are very much in favor in the Mediterranean countries). A nonbalanced diet with excessive intake of fats and carbohydrates provokes the hypertrophy of fat tissues. A sedentary lifestyle contributes to the aggravation of cellulite, and wearing tight clothes makes venous return more difficult. Smoking provokes alterations in the microcirculation and could favor the formation of cellulite. Other coexisting disorders (hormonal, circulatory, metabolic, gynecologic, nepheotic, and gastrointestinal) may be important and contribute to the development of cellulite.

Modifications and Hypertrophy of Adipose Tissues

Although cellulite is not always synonymous with overweight (some lean persons could present symptoms of cellulite), there is a relation between cellulite and hypertrophy of fat

tissues. First, there is formation of first micronodules and later of macronodules in adipose tissues.

The combined effect of modifications and hypertrophy of adipose issues, alterations in the fibrillar connective tissue, and alterations in the microvascular venous network leads always to the presence of cellulite.

New developments in the aethiopathogenesis of cellulite have recently been described (5). It appears that three main theories on the aethiopathogenesis of cellulite have emerged. These theories indicate the following problems in the edematofibrosclerotic panniculitis (EFP):

1. A different anatomical conformation of subcutaneous tissue in women compared with men
2. A microcirculatory modification
3. An edema resulting from excessive hydropholia in the intercellular matrix

New developments have clearly emphasized the limitations of the three above-described theories. It appears that adipose tissue does not play a significant role in the onset of cellulite: it participates only as a pure inert physical function, producing mechanical tension through its hypertrophy. Today we know that the adipose organ performs complex functions by acting as a system controlling the systemic energy balance, by modulating the food intake and the metabolism of other tissues, and as a glandular system for multiple hormonal secretions. It is known that adipose tissue is able to modulate the blood flowing through it and can secrete numerous substances with the power to regulate the activity of the endothelial cells. In conclusion, these recently identified properties of the adipose tissue are also involved in the pathogenesis of cellulite (5).

OBJECTIVE EVALUATION OF THE SYMPTOMS OF GYNOID LIPODYSTROPHY

There is a variety of physical and pharmacological anticellulite treatments ranging from topical products to oral food supplements or regimens, from manual to mechanical massage, laser, infrared light, continuous or pulsed radio frequencies, etc.

As a consequence of this, there is a need for accurate, sensitive, noninvasive bioengineering methods for the quantitative evaluation of the degree of cellulite (particularly at early stages) and for the objective evaluation of the efficacy of various cosmetic treatments (2–5,14). However, the clinical evaluation of cellulite based either on direct visual examination and palpation of the orange peel skin with a diminution of the mobility of the hypodermis or photograding of photographic pictures taken under well-standardized conditions remain important. The visual evaluation is more closely related to the consumer's considerations and expectations.

The different noninvasive bioengineering measurements are as follows:

- Contact skin surface thermographic measurements using liquid crystals.
- Noncontact skin surface thermography of skin surface using infrared video camera.
- Micro blood circulation using laser Doppler image analysis.
- Ultrasonic skin analysis of skin density. Measurement of thickness of the hypodermis at 10 to 14 MHz and measurement of the surface of the interface between dermis and hypodermis at 20 MHz.
- MRI.
- Skin surface topographical imaging and fringe projection analysis.
- Macroscopic normal and digitalized photographic pictures of the skin surface.

In many studies, there is a confusion between obesity and cellulite (although adipous volume is clearly an aggravating factor for cellulite). Many patients confuse weight gain with the appearance of cellulite, and many commercial anticellulite treatments are in fact slimming treatments. As a consequence of this confusion, the use of antropometric measurements is widely applied to measure the efficacy of the various anti–weight treatments: circumference measurements of hip, both thighs, and individual.

Skin Surface Contact Thermography Using Encapsulated Liquid Crystals in the Evaluation of Cellulite

The principle of the encapsulated cholesteric liquid crystal contact thermography consists of different color plates presenting a pattern of different colors corresponding to about 3°C temperature. Application of the color sheet with uniform pressure on the skin surface and photographic recording of the thermographic pattern using a photographic camera can be made. A qualitative global analysis of the thermographic pictures in relation with the different stages of cellulite can be made (14,20–24). A cellulite-free skin surface thermography shows a uniform color pattern without hypothermic and hyperthermic areas. A cellulite skin surface thermography shows a nonuniform color pattern with the presence of hypothermic (cold spots) and hyperthermic (warm spots) areas. Quantitative analysis of the thermographic pictures can also be carried out by image analysis. Computerized color image analysis gives the mean temperature of the thermogram and respectively the number and the percentage area of the hypo- and hyperthermic areas present on a well-defined skin area. As experimentally observed, an anticellulite treatment will induce an increase in the mean temperature of the skin surface and a decrease in the percentage hypothermic zones (with a concomitant increase in the percentage hyperthermic zones).

This method is rapid, easy to use, and non-expensive for screening subjects for cellulite and for confirmation of the clinical diagnosis.

However, considering the low accuracy and reproducibility of the photographic pictures, quantitative image analysis of the thermograms is very difficult. One observes large interindividual variations in skin surface temperature (large number of subjects is necessary in a study) and long acclimatization time for temperature equilibrium of the skin (influence of external temperature). This method remains a qualitative testing of cellulite at different stages.

Skin Surface Thermography Using Infrared Thermal Imaging System in the Evaluation of Cellulite

Using an infrared video camera, an infrared thermal image of the skin surface is obtained in a noninvasive manner. The thermographic picture can be quantitatively analyzed (14,22,23).

In the validation of this infrared video imaging technique, the problems encountered with liquid crystals are same as those with the contact thermography, such as large interindividual variations in skin surface temperature, long acclimatization time for temperature equilibrium of the skin, and influence of external temperature.

Laser Doppler Imaging System in the Evaluation of Cellulite

Using a laser Doppler perfusion imager, an image of the superficial blood circulation can be obtained. The He-Ne laser light emitting at 633 nm has a penetration power in the skin of only about 300 μm (14,22,23).

This instrument measures the superficial blood flux of the skin (papillary dermis). The blood perfusion of the deeper layers of the skin such as the hypodermis cannot be measured with this technique. However, a high correlation is obtained between the skin surface thermographic pictures and the laser Doppler imaging system when studying skin with cellulite. However, the measurements are delicate (long measuring times during which the volunteer must remain immobile).

Ultrasonic Imaging of the Skin in the Evaluation of Cellulite

High-frequency ultrasound C-mode imaging (10–20 MHz) appears to be a promising method. This noninvasive method has been frequently used both clinically and in research for studying the epidermis, dermis, and hypodermis (24–35). Different authors have used the technique of the measurement of the thickness of the subcutaneous fatty layer using ultrasound imaging at 10 to 14 MHz (27–35); however, the determination of the echographic border line between subcutaneous fat and connective tissues/muscles is very delicate. As a consequence, the determination of the mean thickness of the hypodermis is not very accurate. The interface between the dermis and the subcutaneous fat can be measured using ultrasound imaging at 20 MHz (16,17). The interface between the echogenic epidermis-dermis and the hypoechoic subcutaneous fat is clearly visible, allowing measurements of skin thickness and the surface of this border.

In normal cellulite-free skin, the interface between the dermis and the fat tissue is irregular but rather smooth. In skin with cellulite, this surface is not smooth and very irregular. The surface of this interface is quantified and can be used as a measure of the degree of cellulite. Quantification of the surface of the interface between the dermis and the hypodermis (fat tissue) is possible and can be considered as a measure of the extent of cellulite (16).

Measurement of Skin Surface Topography

Cellulite skin surface presents irregularities (orange peel skin). In principle, the classical skin surface roughness measurements, which are used in cosmetic research, could be applied for studying cellulite. These involve stylus profilometry, image analysis by shadow method and optical focus laser profilometry, topographical skin imaging techniques, and fringe projections analysis (36–42). Stylus profilometry measurements are carried out on soft or hard skin replicas of general small size (2–3 cm^2 area) and have a limited vertical range of roughness capability (maximum 400–500 μm). These techniques are well suited for the determination of the microrelief of the skin surface (50–200 μm), but not for assessing the skin surface with cellulite. Optical focus laser profilometry and fringe projections analysis can be carried directly on the skin surface. The macrorelief of the skin surface can also be evaluated using an optical triangular laser profilometry. This method involves measurements on large size soft replicas with an extended vertical range of skin irregularities (up to 8–10 mm). Quantification of the skin surface macrorelief involves a computerized correction for the curvature of the skin surface with cellulite. Actually the skin surface topography of skin with cellulite could be more easily evaluated using 3-D topographical skin imaging techniques and 3-D map topography from fringe projections.

Digital Photographic Pictures of the Skin Surface

The macrorelief of the skin can be evaluated by taking digital photographic pictures under standardized experimental conditions (with tangential lighting) (1,4). These photographic pictures are then graded visually using numerical scales in double-blind manner by expert independent reviewers for the intensity of cellulite and the efficacy of various anticellulite treatments (43–45). Macroscopic digitalized video pictures (with the use of a CCD camera) of the external part of the thighs were taken after application of a gripping system around the thigh to increase the orange peel look of the skin.

In Vivo Magnetic Resonance Imaging and Spectroscopy

Recently, high-resolution MRI and localized spectroscopy data were published (15,18,19), allowing investigation of subcutaneous adipose tissue in men and in women with and without cellulite. As previously mentioned in this chapter, MRI is very efficient for measuring the thickness, surface, and volume of the adipose tissue. In women with cellulite, an increase in skin thickness and presence of deep indentations of adipose tissue into the skin were noticed. Unfortunately, because of the high cost and limited accessibility of this instrument, this promising technique will not be available for cellulite research for most laboratories and cosmetic companies.

TREATMENTS OF CELLULITE

There are numerous therapies that have been advertised and employed to treat cellulite (1,2,4). Despite the multitude of therapeutic approaches, there is little scientific evidence that any of these treatments really work. A majority of the evidence is anecdotal, subjective, or based upon patient self-evaluation. Only a few anticellulite treatments are validated using noninvasive bioengineering measurements to quantify the degree of cellulite.

The Physiotherapeutic Treatments

Physiotherapeutic treatments such as deep massage and manual and pneumatic lymph drainage stimulate the blood and lymph microcirculation and increase the removal of the extra fluid in the adipose tissues. In addition, these massage techniques will retard the further development of fibrosclerosis and the aggregation of fat cells in nodules. These physiotherapeutic treatments are generally combined with the topical use of anticellulite dermato-cosmetic products (during

massage or pre- or post-massage). Electrolipolysis and mesotherapy are invasive medical treatments of cellulite; these techniques will not be described in this chapter.

Recently, two new physiotherapeutic techniques have been developed in the treatment of cellulite (46,47). One technique combines the use of near-infrared laser light of a continuous radiofrequency wave and mechanical suction (2 rollers with suction), and another instrument combines near-infrared light with a mechanical massage apparatus. These combined physiotherapeutic instruments seem to improve the symptoms of cellulite.

The Topical Dermato-Cosmetic Products

The use of various topical products, generally applied with massage, in the treatment of cellulite and/or as slimming is known for many years (1,2,4). The most used pharmacological topic agents are xanthines, retinoids, and plant extracts. A novel approach to topical anticellulite treatments consists of combining the topical application of the pharmacological product under occlusion as enhancement (bioceramic-coated neoprene short) (48).

Xanthines are common ingredients used in anticellulite products (caffeine, aminophylline, theophylline, or plant extracts rich in xanthines) (1–4,45). Xanthines are used because of their proposed effect on adipocyte lipolysis via the inhibition of phosphoesterase, provoking an increase in AMP. In vitro metabolism studies on fat cells have shown that caffeine could slow down the lipogenesis (uptake of glucose and free fatty acids to synthesize triglycerides) and stimulate the lipolysis (degradation of triglycerides and release of the free fatty acids) in different ways. Furthermore, it appears that caffeine increases the level of a class of uncoupling proteins (UCP) in subcutaneous white adipous tissue adipocytes and may help to reduce the adipose tissue mass.

Retinoids

The use of topical retinol to improve cellulite was proposed by Kligman et al. (49,50), and they demonstrated an improvement in cellulite. Retinol will be metabolized to retinoic acid. These effects may be due to the known effects of retinoids in the dermis modifying the collagen fibers and the network of elastic fibers (1–5). It can be also noted that retinol has an "anti-adipocyte" activity by reducing the differentiation of adipocyte precursor cells in adipocytes (4).

Plant Extracts

The use of plant extracts such as *Centella asiatica*, butcher's broom, horse chesnut, ivy, Ginkgo biloba, Witch hazel, white oak, green tea, lemon, kola, fennel, alguae, barley, strawberry, marjoram, sweet clover, aloe vera, etc. should be noted. The "active" molecules of these plant extracts are probably flavonoids (rutins, rutinosides) or terpenes (ginkgolides).

These slimming/anticellulite plant extracts present properties of stimulation of the peripheral blood circulation and lymph circulation and inhibit further the fibrosclerosis of the fat surrounding collagen matrix. Various algae species such as fucus vesiculosus, laminaria flexicaulis, and ascophyllum nodosum are incorporated in anticellulite cosmetic preparations for their hypothetical beneficial effect on the skin surface. There are very few in vivo scientifically reported studies examining the effects of these plant extracts improving the condition of cellulite (4). The use of anticellulite creams containing various plant extracts seems to be acceptably safe (51); however, the risk for adverse allergic reaction must be taken into account.

ORAL TREATMENTS

Many of the above-mentioned active ingredients are also used in oral anticellulite or slimming treatments of cellulite, and similar to the topical treatments, there are very few scientifically proven clinical studies reported (2–4). These preparations contain mostly various plant extracts and xanthines (caffeine or plant extracts rich in caffeine). These anticellulite food supplements can be used alone or in combination with massage and/or topical creams. The use of plant extracts such as green tea, grape, Ginkgo biloba, and centella asiatica are particularly noticed. It is possible that both oral and topical routes may have a synergic effect and may be the best way to ameliorate the symptoms of cellulite (4).

CONCLUSIONS

Very few anticellulite studies that were performed under well-controlled experimental conditions (double-blind, vehicle-controlled, etc.) and under medical and paramedical supervision are published in the scientific literature (1–5,14).

After a critical overview of these published clinical studies, one can make the following remarks. In the case of simple trials (such as one with treated thigh and other with thigh as control), improvements are always observed. One question remains: Are the improvements the result of the combined action of massage and the active ingredients or solely the result of the massage?

In the case of more elaborated trials (double-blind and placebo-controlled), the results are variable. Some clinical studies clearly show significant improvements in the degree of cellulite found on the treated thigh compared with the placebo thigh. These improvements are significant and clearly visible (confirmed by the subjects themselves), but not very impressive. Other clinical trials indicate that similar significant improvements of cellulite were observed with the inert massage product and the massage product with the active ingredients. These trials substantiate the hypothesis that the cellulite improvements are due to physiotherapeutic treatments such as massage, lymph drainage, or thermal occlusion of the skin and not solely to the so-called active anticellulite dermato-cosmetic ingredients. The majority of cosmetic firms carry out internally clinical trials to evaluate the efficacy of their products. But the results of these clinical studies are generally not published in scientific peer-reviewed journals and are only accessible through the Internet, released press maps, and ordinary publicity in women's magazines. This information, although interesting to consult, is not considered by the author as real scientific publications. Most of the claims presented here are based on anthropometric measurements and in vitro data.

It must be noted that many anticellulite or slimming claims are based on in vitro studies. In vitro metabolism studies on adipocytes have shown that different molecules (caffeine, plant extracts, etc.) can be considered as active ingredients in order to slow down the synthesis of triglycerides and to stimulate the degradation of triglycerides in the adipocyte. However, when using these active molecules in vivo as anticellulite ingredients, one must take into account the limitations in percutaneous penetration of the active molecules through the skin to reach the hypodermis. For example, caffeine penetrates readily into the skin, but scant information is published about the penetration of these plant extracts.

Another problem is related to the concentration of the active products in commercial anticellulite products. It must be assumed that the concentrations of plant extracts are rather low considering the high cost of these extracts and the potential danger of these plant derivatives as allergens. Possibility of problems of photoallergy and photoirritation must be considered.

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60 Baby Care Products

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INTRODUCTION

A great variety of baby skin and hair care products are brought to the market. Those products claim to be specially developed for the delicate baby skin, and therefore specific requirements should be considered when developing products for the care of babies. In order to understand whether babies need different skin and hair care cosmetics than adults, it seems necessary to explain some anatomical and physiological differences of the skin and annexes between both groups. A further distinction can be made between the skin of full-term and premature babies, but in this chapter only the former group is considered. Cleansing and protective cosmetics are available for babies and their safety is in the EU based on exposure-based risk assessment for the individual ingredients as well as for the finished product. Finally, some common baby skin problems and the applicability of cosmetic products under impaired skin conditions are discussed.

ANATOMICAL DIFFERENCES BETWEEN BABY AND ADULT SKIN AND SKIN ANNEXES

A complete overview of the morphological characteristics of baby skin is present in several comprehensive handbooks (1–4). Here some key differences in comparison to adult skin and annexes are given.

In general, a full-term baby possesses all skin structures of adult skin, and anatomically these structures do not undergo dramatic changes after birth. The skin of the newborn could be considered as an "unripe" skin, which progressively adapts during the first weeks and months of life. These adaptations lay at the origin of the physiological differences observed between baby and adult skin.

- The *epidermis* of term infants is well developed and does not show much difference with that of adults (5). Only in prematures, the stratum corneum is lacking a significant barrier function (1). In the stratum germinativum, besides a majority of cylindric keratinocytes, dendritic cells including melanocytes, cells of Langerhans, and Merkel cells are present in a normal number. However, their functionality—namely photoprotection, immunological barrier, and receptor function, respectively—still has to develop progressively. The melanocytes are less pigmented, which explains the pale color of the newborn's skin and which makes sunburn an important risk factor (3).
- At the *basal membrane* of newborn skin, cohesion structures are present in a normal number in comparison with adult skin (1,5).
- In the *dermis*, numerous fibroblasts produce elastic and collagen fibers but fewer in number than in adult life. Most of the development and maturation of the elastic fibers occur after birth, and it is only at the age of three years that the elastic fibers become completely mature. It is known that the dermal matrix differs in composition depending on age. Indeed, during development, the water, glycogen, and hyaluronic acid contents of the matrix decrease, but its dermatan sulfate content increases. This difference in composition probably interferes with the particular turgescence of the newborn's skin (6).

In the embryonic dermal skin, primitive dermal vessels can be seen that differentiate into arterioles, venules, and capillaries. With respect to the vascular system, the subpapillar plexus is not yet completely developed, and the upper layer of the dermis contains a rich but disorganized capillary network, causing the erythematous aspect of the newborn. The cutaneous nerve system is also not yet finalized and may lie at the basis of masked intolerances (3,6).

- The immature *hypodermis* of baby skin consists of small lobules of roundly shaped adipoblasts that are richly vascularized. The fatty acid composition of the triglycerides is more saturated, which results in a higher fusion point of the lipids than measured for adult skin (1,5).
- The *hairs* of a newborn are well developed. Sometimes some lanugo hairs are still observed. After birth, the hairs pass from the anagenic into the telogenic phase. As a consequence, baby's hairs fall out after about eight weeks. Afterward, the hair cycle becomes similar to the one observed for adults, and hairs will be present in different phases. The hairs, however, are very thin and only faintly pigmented, but these phenomena normalize as a function of time (7).
- *Sebaceous glands*, when stimulated by the androgens originating from the mother, are well developed. Their secretions constitute the largest part of the vernix caseosa. That is why at birth, the skin is covered with a white fatty substance. The vernix caseosa is a naturally occurring fetal barrier film produced in late pregnancy (8). Besides the secretion of sebaceous and epidermal lipids, desquamation of maturing fetal corneocytes also takes part in the development of the fetal barrier. The vernix is thought to have multiple overlapping biological functions like moisturization, anti-infective, antioxidant, wound healing, and waterproofing (9,10). Because it lacks desmosomal interconnections between corneocytes, it is also referred to as the "mobile phase" stratum corneum. Removal of vernix lipids can modify the water sorption-desorption profile (11,12).

  The vernix caseosa is taken away during the first washing of the baby. After loss of this protective layer and the onset of a desquamative stratum corneum, the skin is exposed to a much dryer environment than the one present during fetal development (13). Erythema occurs that changes in appearance and gets a more marbered aspect that progressively disappears. This is an adaptation of the microvascular system (6).

  Because of the different biological effects of the vernix caseosa, the question is often raised whether it would not be better to leave this natural film on the baby instead of washing it away. Several publications investigating the effect of immediate bathing of newborns, however, are contradictory (14–17). Vernix distribution is dependent on gestational age, delivery mode, gender, race, and meconium exposure and positively affects skin hydration, skin pH, and erythema. These multiple effects would support its retention on the skin surface after birth (8). Vernix films also retain endogenous chymotrypsin, thus preventing loss of this epidermal enzyme and protecting the epidermal barrier from noxious substances (18). In this respect, the World Health Organization (WHO) developed general guidelines recommending that neonatal bathing should not be undertaken within the first six hours of birth (19).

  In certain cases, large sebaceous glands are observed together with the occurrence of the typical symptoms of so-called *acne neonatorum*. This particularly happens in male newborns and can persist for a few months. It is seen as a temporary effect of the androgens that are present in the mother's blood. Reactivation of the sebaceous glands only occurs later on, around puberty (17).
- The *hydrolipidic layer*, mainly composed of sebum from the sebaceous glands and water originating from eccrine glands and the transepidermal water loss (TEWL), is not fully developed in babies. This protective water-in-oil (w/o) mixture is sometimes even nearly absent, which also has an effect on the skin pH of the newborn (20). Consequently, the observed skin pH imbalance might be responsible for a lower capability to neutralize the alkalinization, especially seen in the diaper area due to urine and defecation.

- The *eccrine sweat glands* are normally formed but their intervention is still immature, affecting thermoregulation. *Apocrine glands* only become functional around puberty (6,21).

PHYSIOLOGICAL DIFFERENCES BETWEEN BABY AND ADULT SKIN

On the basis of TEWL and dermal absorption studies, term infants seem to possess a fully developed stratum corneum with adult barrier properties. Other parameters, such as skin thickness, skin pH, and stratum corneum hydration, show that neonatal skin is adjusting very well to the extra uterine environment. This is rather in contrast to a more steady state situation of adult skin (22).

Dermal Absorption

Dermal absorption in newborn skin is similar to that observed in adult skin. For babies, however, a number of typical risk factors exist (23–25).

1. The *surface area/body weight ratio* is 2.3-fold higher in newborns than in adults decreasing to 1.8-fold and 1.6-fold at 6 and 12 months, respectively (26). Application of the same amount of product on a similar body surface of baby versus adult could result in higher blood and tissue concentrations in the newborn. This ratio is taken up in the intraspecies factor of 10, used in exposure-based risk assessment.
2. *Pharmacokinetic parameters* differ widely between babies and adults and result in reduced clearance and/or a longer half-life of bioavailable substances, thus increasing the potential risk for adverse reactions in babies (Table 1). Premature and full-term neonates tend to show a three- to nine-times longer half-life than adults. However, once the neonatal period is over, often a greater elimination and higher clearance are observed compared with adults bringing back the normal equilibrium (26,27). As referred by Renwick et al. (28), this neonatal period would coincide with the period of lactation (26–30).
3. *In-use conditions of topical products* also play a role. Cosmetic skin care products often are applied onto large body surfaces, e.g., cleansing lotions, sunscreens, etc., increasing not only the risk for local effects but also dermal absorption and potential systemic toxicity. This factor is considered in exposure-based risk assessment.
4. The *diaper area* and nondiapered regions are indistinguishable at birth but show differential behavior over the first 14 days, with the diapered region having a higher pH and increased hydration (31). Special circumstances arise because of the close confining clothes and diapers and the uncontrolled urination and defecation. The close-fitting diaper provides a warm nutritive environment for the proliferation of bacteria (32). Because of the interaction between the urine and the feces, urease becomes activated and converts urea into ammonia, giving rise to alkaline skin pH

Table 1 Potential Differences in Pharmacokinetic Parameters Between Newborns and Adults

Parameters	Newborn	Adult
Plasma binding	+	++
Plasma protein concentration	+	++
Body water	++	+
Fat distribution	+	++
Brain development	+	++
Brain-blood barrier	+	++
Brain volume	++	+
Cyt P_{450} biotransformation	+	++
Conjugation reactions	+	++
Relative liver mass	++	+
Glomerular filtration	+	++
Tubular secretion	+	++

Source: Modified from Refs. 26–30.

levels. As a consequence, fecal enzymes such as lipases and proteases become activated and damage the fragile skin in the diaper area. Despite modern diaper technology, irritant diaper dermatitis can not completely be avoided, favoring dermal absorption of xenobiotics. A number of molecules, which historically have been used in the diaper area, are known to induce systemic toxicity and must be used very carefully and only when indicated, e.g., hexachlorophene, dichlorophene, corticosteroids, boric acid, and ethanol (24). In risk assessment of cosmetics, the margin of safety (MoS) approach is used when defining acceptable human exposure levels. When extrapolating from experimental studies to human, the magnitude of the uncertainty factor must take into account a variety of considerations, such as species differences, sensitive subpopulations, duration and route of exposure, and vehicle or matrix effects. In addition, when the diaper area is irritated, 100% dermal absorption should be used (19,33). Innovative hygiene absorbent and baby care products, however, provide an increasingly good skin compatibility profile, making the frequency and severity of diaper dermatitis declining (34,35).

Transepidermal Water Loss

The barrier function of the skin not only prevents absorption of toxic substances, but also controls TEWL. In particular when skin is damaged, excessive TEWL occurs (36–38). In a healthy, fully developed newborn, TEWL values of 6 to 8 $g/m^2 \cdot h$ water are being measured, depending on the measuring technology (39). TEWL increases proportionally with immaturity, which means that premature children have an increased evaporative heat loss and subsequently a poor temperature control (38,40). Although skin maturation occurs rapidly, fluid and electrolyte shift as well as body temperature have to be controlled frequently (41). Also increased risk of local and systemic toxicity from topically applied substances rises with increasing TEWL or barrier damage (42). In the diaper area TEWL is often defined as skin surface water loss (SSWL) and is used to measure the capability of a diaper to keep the skin dry (43,44).

Defense Against Infection: Skin Thickness, Skin pH, Stratum Corneum Hydration

The water content of the stratum corneum influences the barrier function, dermal absorption, reactivity to irritants, and the skin's mechanical properties. Although healthy infants and adults tend to have similar TEWL values, newborns (until 8–24 months) still present somewhat higher water contents in the horny layer and a greater variation than adults up to one year (38,39,45).

In newborns, skin tends to have a higher pH at birth than a few days later. Among other factors, this higher pH might reflect the influence of the vernix caseosa and the amniotic fluid (both pH values above 7) during the first days of life (38). The pH stabilizes at a slightly acidic range (pH = 5–6), although values of less than 5 also have been reported (31,38,46). Acidic skin protects against pathogenic microorganisms to which the baby is exposed after birth and serves in the defense against infections. Indeed, microbial colonization of the skin starts immediately after birth by so-called saprophytes that are not pathogenic and are credited with protective properties against some harmful microorganisms (5). They require an acidic surrounding for optimal living conditions (39). Whereas the pH value of baby skin is, after a few days, comparable to the pH value of adult skin, the buffering capacity of baby skin is much lower. Therefore, baby skin is more susceptible to pH changes induced by metabolic pathways such as the enzymatic generation of free fatty acids from phospholipids or urocanic acid from histidine, the desquamation process of the stratum corneum with formation of filaggrin and keratohyalin breakdown products, and the formation of pyrrolidone carboxylic acid and N^+/H^+ antiporter (31,47).

BABY CARE PRODUCTS FOR SKIN AND HAIR

From the anatomical and physiological differences between baby skin and adult skin, it appears that frequent contact with xenobiotics, which could damage or disrupt the barrier function of the stratum corneum and change the skin pH, may be at the basis of an increased

dermal absorption, an increased TEWL and the onset of infections (5,39,42). Therefore, exposure-based risk assessment for baby products is key to bringing safe baby cosmetics to the market.

During the development of baby products a number of criteria are taken into consideration:

-High quality of raw materials in terms of purity, stability, and microbiology via appropriate certificates of analysis.
-Skin irritation, which is dose dependent, can be controlled by avoiding well-known irritative ingredients and/or reducing concentration or frequency of application.
-Skin sensitization, triggered by an immunological response, is not restricted to the area of application. Therefore, exposure-based risk assessment is needed to exclude an induction of sensitization in particular for perfume ingredients, even when IFRA-tested or excluding the 26 allergens identified in the 7th Amendment of the EU cosmetic legislation (48).
-As is the rule for adult cosmetics, safety data of baby cosmetics are taken up in a technical information file (TIF) and the risk assessment—approved by a safety assessor —is the driving force behind the safety of baby cosmetics. Usually, special attention is given to the concentration of (*i*) reactive colorants; (*ii*) promotional additives, "natural" and "exotic" ingredients, in particular not well-identified mixtures, plant extracts, and ingredients of animal origin or a questionable, impure source; (*iii*) potential allergens, penetration enhancers, organic solvents (ethanol, isopropanol, highly reactive substances, highly detersive or foaming agents, and antiseptics in particular in daily use products); and (*iv*) concentrations of preservatives.
-It is considered to be good practice (*i*) to add antioxidants to protect unsaturated lipids from oxidative reactions; (*ii*) to adjust the pH of the final product resulting in a skin friendly pH value between 4.5 and 6 after product application; (*iii*) to add chelating or sequestering agents, when appropriate, to prevent heavy metal precipitation and protect the preservative system; and (*iv*) to use skin barrier protective ingredients.

Baby cosmetics can be subdivided in two groups, namely cleansing and protecting cosmetics.

Cleansing Cosmetics

Bath Products

Bathing a baby for five to seven minutes in lukewarm water (35–36°C) usually is sufficient (39,49). Daily bathing is general practice, but not optimal because of the risk of drying out and irritating baby's skin, in particular when aggressive anionics with high degreasing properties are involved (50). Better is to use so-called secondary tensides, including nonionics and amphoterics, or mild anionics such as sulfosuccinates, isothionates, and protein fatty acids condensates. The use of bath oil is preferred over bath foam and bath cream additives particularly when dry, sensitive skin or atopic eczema is present. For optimal effect, the baby is bathed for 5 minutes in plain water, then the bath oil is added and bathing continues for another 5 to 10 minutes (49). Also adding starch to the bath water or using starch-containing bath additives may help to restore an impaired skin barrier (51). In general, the use of bath foam is not suitable for babies because of its high content of primary tensides producing the excessive foam.

Shampoo

Baby shampoo usually has a pH of 6 to 7 and ideally should contain only mild tensides, e.g., mixtures of nonionics and amphoteric substances (7). The shampoo should not be irritating to the eyes. To avoid eye contact the viscosity of the shampoo could be increased. Parents often think that foam is important for the cleansing properties but foam has no cleansing function, and the ingredients required to produce a sufficient amount of stable foam are often quite irritating and not suitable to be used alone in baby shampoos, e.g., alkyl sulfates and alkyl ether sulfates (39,49). Furthermore, it is not necessary to wash baby's hairs every day

since they are neither dirty nor greasy. As the hydrolipidic layer is not yet formed on baby's skin, and sebum production is low, the amount of lipids distributed on the hairs is limited and is easily washed away.

Soap Bars and Syndets
Soaps (salts of fatty acids) liberate, in contact with water, alkali and increase the pH up to values of 10. Also precipitation occurs with calcium and magnesium ions from hard water. On the contrary, syndets (*syn*thetic *det*ergents) do not precipitate with hard water and have an adjustable pH to neutral or slightly acidic. As syndets cover the whole range of synthetic tensides—with exception of the legally protected soap formulations—they can be aggressive (e.g., alkyl sulfates) or mild (isothionates), depending on the choices and mixtures made. Like soaps, they can dry out the skin when not containing lubricant additives. In addition, when soap and washcloth are used in the diaper area, the buffering capacity of the skin is further damaged (35,49). Extensive washing with aggressive tensides disturbs the flora of the newborn skin and can lead to infections (15). In addition, perfumed cleansing products may cause contact allergic reactions due to enhanced skin penetration of the perfume by the presence of anionic soap ingredients (50).

Cleansing Milk
For cleansing of the baby and in particular the diaper zone, liquid cleansers based on oil-in-water (o/w) emulsions are often used, especially when water and washcloth are not well tolerated by the baby skin. Also soft tissues or towelets impregnated with these emulsions are present on the market. They are easy to use and contain anionic and/or nonionic tensides (49). When a baby is prone to contact dermatitis, it is advised to screen the ingredients list because those tissues often contain high concentrations of preservatives, necessary to prevent microbiological contamination of the tissues (52). Mineral-oil impregnated tissues can increase the presence of *Candida* in the diaper area and change the composition of the skin surface lipids.

Baby Wipes
Over the last decade disposable baby wipes have been developed as an alternative to traditional cleansing methods. They usually consist of a nonwoven carrier material soaked with an emulsion-type, watery, or oily lotion. Mineral oil wipes do not efficiently clean hydrophilic components and potentially slip over fecal contaminations. Most emulsion-type lotions are oil-in-water (o/w) and enriched with emollients and surfactants. Because of their high water content, the preservative system is very important to ensure that the product will not be contaminated during its normal lifetime. Products for sensitive skin have also been developed, which offer choice regarding the nonuse of fragrances or additives with which problems have previously occurred. Clinical studies confirmed that high-quality baby wipes are suitable for daily cleansing of the diaper area, of healthy babies as well as of babies with atopic dermatitis (35,53).

Protecting Cosmetics

Face/Body Creams and Body Lotions
Protective creams for the napkin zone are preventive or protect the skin against aggressions from urine, feces, and their interactions. Oil-in-water (o/w) creams do exist, but in case of starting skin damage mostly water-in-oil (w/o) creams or water-free ointments with talc, kaolin, and zinc oxide are advised. Allantoin, bisabolol, aloe vera extract, and silicones are often added to improve water resistance. In winter, barrier creams protect baby's face against freezing cold and wind. The lipid phase often contains petrolatum. These products are particularly effective around the nose and mouth. They usually also contain moisturizers, soothing active ingredients, and nonionic emulsifiers (39).

Powder
Talc powders are not often applied anymore in the napkin area. They absorb moisture, decrease maceration, and prevent irritation of the fragile baby skin (49). Powders,

however, pose a potential inhalation risk and can form on the skin little granules that induce friction. Furthermore, talc is susceptible to contamination with microorganisms and needs sterilization (32,54).

Sunscreens

During the past years, a steady increase of all types of skin cancer has been observed. It is, therefore, very important to inform parents and children about good sun protection. Sun exposure in childhood is seen as a risk factor for skin cancer later in life, as it is known that there is a relationship between skin burning in the prepubertal period and the occurrence of malignant melanoma 10 to 20 years later (55). Therefore, babies and infants should, in first instance, be kept out of the sun and protected by appropriate clothing and hats. Almost 90% of the clothes provide an equivalent protection to sunscreens of SPF 30 or higher, although the protection offered is dependent on weave, color, weight, stretch, and wetness (56,57). Even special protective clothes for children exist today. They have undergone special treatment to filter out UV light (58,59).

It has been reported that sunscreens often are ineffective in preventing sunburns completely, because parents tend to forget to reapply or limit the use to just the upper part of the body. The importance of using multiple sun protection methods to maximize effective sun protection clearly has to be promoted (60). Extreme care should be taken especially during the first weeks and months of life since pigmentation and thermoregulation are not yet fully developed (3). When sunscreens are used, preferably products containing UVA and UVB screens and scattering powders or a sunscreen mix with a high sun protection factor (SPF) should be applied and preferably several times a day (61).

Studies have shown that the application thickness of sunscreen products in adults usually lies between 0.5 and 1.5 mg/cm^2, although the SPF of a product is assessed in vivo at an internationally agreed application thickness of 2 mg/cm^2 (62). Application thickness has a significant effect on the expected protection of the sunscreen. A uniform layer needs to be applied, with special attention to areas like ears, neck, and feet as experience learns that these are commonly skipped (60,63).

The type of UV filter(s) used is important. In the EU, only UV filters taken up in Annex VII of the Cosmetics Directive 76/768/EEC are allowed. For babies and children often micronized and nano forms of ZnO and TiO_2 are used as an alternative to chemical sunscreens (64,65). The popularity of these products results from the fact that they are effective and thought to be safe. As nanotechnology in general is questioned with respect to human health, nanoparticles of ZnO and TiO_2 will also be reexamined for their safe use as UV filters (66).

Recently, the European Commission expressed its concern with regard to the efficacy of sunscreen products and the claims made thereto. Aiming at ensuring sufficient protection against UV light and providing proper information to the general public, Commission Recommendation 2006/647/EC unambiguously states minimum efficacy criteria for sun products: the UVB SPF must at least amount to 6 and the UVA protection factor may not be inferior to one-third of the SPF. In addition, the Commission restricts the UVB SPF values to 8 possibilities, namely 6, 10, 15, 20, 25, 30, 50, and 50+. Higher SPFs are not allowed and mentions such as "100% protection," "sunblock," or "all day prevention" are considered misleading and therefore forbidden (67).

The viscosity of sunscreens is important since the products must remain on the skin, even with bathing and sweating. Today most commercially available sunscreens have some water resistance or carry the label of being water resistant, very water resistant, or waterproof, determined in Europe according to Colipa guidelines (68).

Some of the organic UVB filters, like 4-methyl-benzylidene camphor, benzophenone-3, homosalate, octylmethoxy-cinnamate, and octyldimethyl-PABA, have been accused of being endocrine disrupters. The safety profiles of these UV screens were revised by the European Scientific Committee on Consumer Products (69) and considered to be safe for human use. In the meantime, several articles appeared, pointing to potential endocrine disrupting properties of sunscreens (70–76). The issue is not limited to sunscreens alone and appropriate in vivo tests are now being included in the risk assessment process. Efforts are also done to develop in vitro alternatives (77).

APPROPRIATE CARE OF FREQUENT SKIN PROBLEMS

Diaper Rash

Diaper rash is a common condition that refers to irritation on the groin, thighs, buttocks, and perianal area of the newborn. It is caused by the combination of incontinence and diaper use (31). Excessive wetness makes the skin more fragile, and diapers may induce an occlusive effect that makes baby skin more vulnerable. Consequently, hydrated skin is more prone to mechanical damage and chafing of the skin since an increased coefficient of friction is observed, and it may allow irritants to penetrate the stratum corneum more easily. As explained before, not only occlusion but also a higher pH can be an underlying factor, which induces several enzymes-mediated irritations. Alkalinization of the skin increases skin penetration of microorganism and activates fecal enzymes (34,78).

The most appropriate strategy for diaper rash is prophylaxis, and this includes keeping the skin dry. The selection of suitable diapers and frequent diaper changes are important because friction between skin and diaper is often an additional factor. Keeping the baby in an appropriately warmed room with naked buttocks for some limited time period is also quite effective.

In good skin care of the diaper zone, the application of emollients plays an important role, and the application of a thick layer creates an effective protective barrier. ZnO is an often-used component in diaper rash protection products. It adheres well to the injured skin, has adstringent and some mild anti-inflammatory properties, and prevents skin injury or further damage. If the diaper rash shows evidence of *Candida* infection—often seen as satellite lesions extending the rash—antifungal therapy can be indicated (5,39,78,79).

Acne Neonatorum

Mild acne may occur in newborn infants. It consists of closed comedones on the nose, forehead, and cheeks. Pustules, open comedones, and inflammatory pustules may also occur but are less frequent. The cause of neonatal acne is not clearly defined but it is believed to be secondary to the stimulation of the neonatal sebaceous glands by maternal androgens. In boys it is often more pronounced as they have some additional production of testosterone. Neonatal acne requires no treatment as the lesions spontaneously resolve within one to three months (80).

Miliaria

Miliaria is a dermatose frequently observed in neonates. It is a generic term denoting retention of eccrine sweat. Miliaria can be subdivided into three groups: *miliaria crystalline*, *miliaria rubra*, and *miliaria profunda*. The difference between these three types is the level of the skin where the obstruction of the sweat gland occurs. *Miliaria crystalline* refers to an obstruction in the stratum corneum, *rubra* an obstruction within the stratum Malpighi, and *profunda* below the dermoepidermal junction. There is no specific treatment of miliaria. Measures that can be taken consist of regulating the heat and humidity of the environment to reduce sweating. Eventually, the poral obstructions are relieved, but this can take up two or three weeks (81).

CONCLUSION

Full-term newborns have an "unripe" skin, which progressively develops toward adult skin. Anatomically spoken, the differences are limited but some minor changes occurring at birth are responsible for the physiological differences observed between baby and adult skin. The skin of newborns exhibits the same dermal absorption as adult skin, but the circumstances in newborns are very different, thus increasing the risk for dermal absorption, in particular in the napkin area. Also the thermoregulation (TEWL and sweating) of the baby is not yet fully developed, and the skin is easily invaded by infections, the latter often due to subtle pH changes and the immaturity of the defense systems of the skin. This implies that only safe cosmetics with safe ingredients should be used for newborns guaranteed by exposure-based risk assessment. During the development of new baby care products, known potentially eye or skin irritative or sensitizing ingredients are usually limited to a minimum, and profound exposure-based risk assessment has become common practice. One usually aims at simple,

pure, mild, and pathogens-free formulations. Systemic side effects are not to be expected with mild rinse-off products (shampoos, bath additives, toilet bars) but should be carefully looked for when leave-on products for babies are being developed (body milks, hydrating creams, ointments, powders, sunscreens).

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61 Cosmetics for the Elderly

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INTRODUCTION

Skin is the only organ, where signs of aging are evidently visible at the soonest in phenomena such as wrinkle formation, loss of elasticity, uneven pigmentation, loss of moisture, increased roughening, and cutaneous itching. Aging itself is understood as the result of a complex interaction of biological processes that are caused by both genetic (chronological or intrinsic aging) and environmental or behavioral processes (premature or extrinsic aging).

Current expansion of knowledge in modern bio-gerontology widely extends the theories and explanations on mechanisms of aging. They are the basis for scientific approaches in research, aiming to identify new concepts for antiaging treatment of skin. In terms of scientific research activities, the skin's accessibility to noninvasive and slightly invasive biophysical measurements and procedures is definitely advantageous for studying underlying mechanisms of cutaneous aging. In addition, experiments can also successfully be performed on cultured skin cells or on three-dimensional cultured skin models.

Today, consumers of cosmetic products are increasingly expecting a deceleration, cessation, or even a reversal of the underlying physiological processes contributing to the signs of cutaneous aging. These advancing demands require state-of-the-art technological endeavors in cosmetic research and formula development activities, with novel active ingredients, which can perfectly exert their antiaging efficacy in optimized new-formula technologies.

PHYSIOLOGICAL CHANGES IN CUTANEOUS AGING

Unlike internal organs, the skin being the outermost protective barrier is particularly exposed to external influences. As a result of environmental challenges, the aging process in skin is not only influenced by genetic, intrinsic factors, but also accelerated to a far greater extent (80%) by extrinsic factors, especially sun exposure (1). Intrinsic mechanisms of skin aging seem to be only basically involved in formation of fine lines and shallow wrinkles in advanced age. Therefore the research in cosmetic industry is focussed on the identification and qualification of new active principles mainly to fight against extrinsic factors found to be harmful for the skin. One of the most important fields of research is thereby the prevention and repair of sun-induced skin damages, which can occur through several intracellular as well as extracellular mechanisms.

Reactive Oxygen Species

It is widely accepted that UVB irradiation causes DNA damage more or less directly, whereas UVA light induces damages via the generation of reactive oxygen species (ROS) in a more indirect manner (2,3). During sun exposure, endogenous absorbers of UV light and photosensitizers in human skin (riboflavine, porphyrine, tryptophan, urocanic acid, etc.) can be involved in the generation of ROS. These UV-induced ROS are believed to be the main factors for age-related damages in skin, apparent as deep wrinkles and furrows, which are mechanistically summarized by the term "photo-aging."

Dermal Changes

With increasing age and because of UV irradiation, aging skin shows an increasing imbalance between assembly and breakdown of collagen—one of the primary compounds of the dermis—toward breakdown. This results in an overall collagen decline of approximately 1% per year per unit area of skin surface (4). The reasons for this are lower levels of new synthesized collagen, a shift in the ratio of collagen types (5), and an increased activity of collagen-degrading enzymes such as the collagenase MMP-1 (6). Aside from this, UV light also influences other important

dermal components such as elastin and glycosaminoglycans (GAGs). It causes the accumulation of elastotic material, a nonfunctional mass of elastic fibers (7) and loss of GAGs in the dermis. This lack of regeneration of the dermal connective tissue, i.e., structural reorganization of collagen and elastin, decreased GAGs, together with a reduction in the tissue fluid content and water-binding capacity, seems to play an important role in the formation of wrinkles. Even in young skin, the regeneration of collagen (over the period of months) is a relatively slow process. Thus the tensile characteristics of the skin are altered, so that it becomes generally thinner, less elastic, and less resistant to stress. Phenotypically, this extrinsically caused and accelerated premature skin aging is manifested in an advanced state as the formation of coarse, deep furrows and folds, as well as aggravated elastoses. Even though the mechanisms of both extrinsic and intrinsic skin aging cause a fundamental change in the appearance of the skin, the contribution of the extrinsic portion, however, seems to be predominant.

Epidermal Changes

A characteristic feature of aging skin is the declining ability to regenerate, being particularly evident in the longer time span needed for renewal of the epidermal layer. This so-called epidermal turnover takes about 28 days in young adult skin and may increase to 40 to 60 days with age (8). Furthermore, as skin gets older the UV-induced tanning intensity becomes more irregular, but the scientific knowledge about the physiology on development of age spots or melasma is still insufficient. The appearance of age spots can be a result of the decreasing ability of melanocytes to distribute the produced melanin packets (melanosomes) to the surrounding keratinocytes equally, or a localized overproduction of melanin. One can only speculate whether this is primarily caused by an uneven distribution of melanocytes in skin or rather a dysregulation of physiological processes in melanocytes.

Other Aspects

Furthermore the process of premature skin aging leads to an impairment of the denticulation of the epidermal/dermal junction zone and to a reduction in the number of so-called papillae, each of which harbors a blood capillary growing out of the dermis. These structural changes are considered as histological hallmarks of aging skin morphology, which is accompanied by the reduction of the capillary diameter, as well as capillary density in aged skin (9). A well-functioning blood capillary system contributes to an adequate nutrient supply to the upper skin layers, and thus to the structural integrity and complexion of skin.

The immunological defense system is also significantly reduced with increasing age. Thus intensive sun exposure can promote neoplastic cell transformation (e.g., melanoma) and the incidence of skin tumors increase with age. As a consequence protection of skin of all ages against the negative effects of sun irradiation is the most important task in keeping skin healthy and young. Often described and subjectively felt, dry aged skin cannot be only attributed to the distinctive defect of the epidermal water barrier of the horny layer. Rather, it can be attributed to regenerative processes, as well as a worsening in the water storage capacity, caused by a diminished production of cutaneous moisturization factors (e.g., amino acids, hyaluronic acid, pyrrolidon carbon acid, and glycerine) able to bind water in the horny layer. Besides the reduced water-retention capacity, the age-dependent reduction in sebum secretion of the sebaceous glands also plays a role in the formation of dry aged skin. As the sebaceous glands seem to be predominantly hormonally regulated, age-dependent decline and changes in the hormonal system worsen condition and function of aged skin.

An overt example of the endocrine influence on skin aging is the exacerbation of dry skin and increased wrinkle formation that occur with menopausal hormonal changes, and specialized hormone treatments can lead to an improvement of old skin. These new scientific insights have facilitated a scientific merging in the fields of dermatology and endocrinology.

ACTIVE COSMETIC INGREDIENTS AND THEIR POTENTIAL

Aging consumers experiencing dry skin tend to favor rich skin-care formulations that include *moisturizers* with high water-binding properties, e.g., glycerine. Increasingly, modern cosmetics attempt to satisfy these consumer demands for products with preventative or even regenerative performance. Besides preventing early skin aging, products must also smooth

or improve the appearance of wrinkles as well as retard the weakened regenerative potential of the skin (epidermal turnover). Modern skin research in cosmetic industry has already revealed several ways to specifically target the biological needs of aged skin.

In addition, to avoid intensive sun exposure, protection of the skin against UV-dependent oxidative stress can be provided by the use of products with highly efficient *UV filter* technologies. It has to be considered that older skin is even more sensitive to UV exposure. Because of the age-dependent atrophy of the skin (10) UV radiation penetrates more deeply and damages increase and can accumulate. This effect leads to a need for high photoprotection, so product formulations should employ an efficient UVA/B filter combination. Besides UVB protection, which delivers erythemal protection, UVA filter performance plays a decisive role in the prevention of photoaging, because UVA radiation is a key factor in the production of ROS and the subsequent activation of collagen degrading enzymes.

As physiological events in skin are based on physical and chemical processes including redox-cascades, the skin has developed an *antioxidative defense* system as a direct protective barrier against endogenous and exogenous environmental oxidative stress factors (e.g., UV-light). These endogenous antioxidative protectants of enzymatic and nonenzymatic antioxidant systems (11) are concentrated to a higher value in the epidermis compared with that in the dermis. Substances such as flavonoids, vitamins A, C, and E, coenzyme Q10, as well as carotinoids, are components of a healthy diet and can replenish and support the cutaneous system in its protective function. Topical application of substances such as vitamins E and C as well as, in particular, the plant-derived flavonoid derivative, α-glycosylrutin (AGR), show a large protective potential against premature UV light–induced skin aging (12). This positive activity, however, preempts that topically applied antioxidants will adequately interact with the natural endogenous redox system of the skin. Therefore, not all of the known oxidative substances achieve the desired protective effect when applied to the skin. The water-soluble and thus bio-available flavonoid AGR can build up a skin-protective depot in the living layer of the skin, in which the inherent glutathione redox system protects against oxidative damage and UV-induced inflammation is reduced (12–14). Similarly, the water-soluble antioxidant vitamin C can, among other activities, function as a co-factor in the collagen synthesis thereby supporting skin regeneration in deeper layers. Knowing the causative involvement of UV-induced oxidative stress reactions in the cutaneous aging process (15), the best prevention and radical modulation can be reached, and to some extent improved, by providing focused, customized topical treatment strategies.

For treatment of age-damaged skin, particularly for antiscaleness and antiwrinkle efficacy, countless principles are available on the market, which are based on the removal of the outer horny layers of skin (*exfoliation* or peelings). Commonly used agents are so-called α-hydroxy acids (AHAs), most often endogenous metabolites (lactate) or other naturally occurring substances such as fruit acids. Depending on the substance used, the respective depth of treatment in skin can be determined by adjusting the topical concentration and treatment time applied. The activity of these agents is generally based on induction of skin regeneration by exfoliation and subclinical inflammation, which appears to be comparative to a superficial wound-healing process.

Several *antiaging actives* are proven to have beneficial effects on skin aging. Vitamin A and its derivatives have been used as active ingredients in the cosmetic industry for many years. Their activity is essentially based on the interaction of specific nuclear receptors, whose activation regulates, e.g., collagen synthesis, improving the structure of the skin. Regrettably, besides concentration-dependent skin irritant properties, these vitamin A ingredients are also highly sensitive to light-dependent and oxidative processes, greatly reducing their activities. New cyclodextrine-based formulation technologies enable the efficient stabilization of these active ingredients without limiting their activity (16).

All cells, and thus also skin cells, need energy. It is needed to grow, for protection and repair and most important for regeneration and cell division. To maintain this capacity for cellular life the mitochondria, small intracellular organelles operating as small power plants in the cells, are imperative. Besides the mitochondrial energy supply, cells also have a system named "the creatine/phospho-creatine pathway." According to latest insights findings, this occurs in the human skin and is responsible for an extremely fast energy supply (17).

Creatine as well as another energy metabolite, co-enzyme Q10, can both be synthesized in human cells, but from the age of about 30, a reduction in the cellular concentration of these

compounds in the skin can be determined (18). As a fat-soluble oxidative substance, co-enzyme Q10 protects the cell membrane and organelles (19). It especially plays a role in the electron transport system during the energy production (ATP) in cellular respiration of the mitochondria, preventing a chronic energy deficiency in aging cells (20). The topical application of co-enzyme Q10 and creatine, respectively, can activate countless synthetic processes, ultimately resulting in a reduction of wrinkle depth by balancing energy deficits (21). Besides this, the regeneration activity of aged skin can be stimulated by the external application of these active ingredients.

There are different causes, why skin can become irritated. Independent of age, skin is more sensitive to irritation in cold, dry winter, than in summer. Especially people with so-called sensitive skin have to protect their skin against dryness, intensive sun exposure, mechanical stress, and environmental noxiousness. But also, age-related old skin is reported to be more sensitive to irritation due to restricted defense and repair mechanisms. As a consequence, a prophylactic anti-inflammatory treatment appears to be recommendable for skin of elderly.

Special skin-care regimens adapted to the specific needs of sensitive skin are developed and provided by the cosmetic industry. Actives isolated from herbal extracts (e.g., licochalcone A) proved to be effective against mechanical stress such as razor burn, but can also be effective in skin care for dry atopic skin or Rosacea.

PERSPECTIVES

The physiology of skin aging is a complex, multifaceted, and dynamic phenomenon. Even though many molecular causes of the aging process are not understood in complete detail, there is consensus that chronological age alone is not crucial to this process.

In the future, the application of modern molecular and biological methods in skin research such as the DNA chip technology (micro-arrays) and the proteomic technology will allow new insight in the aging process—genes and gene products involved with their genetic control mechanisms. These new technologies, the accessibility of the skin, and the improved possibilities to culture in vivo resembling skin models, will increasingly contribute to a better understanding of the regulation of the intrinsic and extrinsic aging process, and the positive effects of topically applied age-specific agents. In the long term, these technologies can provide new fundamental knowledge about the control mechanisms of the human aging process as a whole.

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62 Antiperspirants

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INTRODUCTION

This chapter presents an overview concerning the current knowledge of antiperspirant actives and their interactions with the human axilla. It is my intention to give the interested reader a short introduction about formulation work, drug delivery systems, and application forms developed for antiperspirant actives. The final section lists references that should be useful for anyone who wants to learn more about a specific topic of antiperspirant technology.

BIOLOGY OF SWEAT GLANDS IN THE HUMAN AXILLA

The axilla region of humans contains apocrine, eccrine, and sebaceous glands. Approximately 25,000 sweat glands/axilla can produce up to 12 g sweat/hr (1). The current understanding concerning the structure and function of sweat glands is that thermoregulation is the only aspect of the body participating in immunological, metabolic, and hormonal aspects of human life (2).

Eccrine Glands

This is the gland responsible for the majority of sweat production. It has a sensory and an excretory function and can be stimulated by emotional and thermal stimuli (3). It produces clear, colorless, and odorless liquid containing 98% to 99% water and 1% to 2% inorganic and organic compounds (4). Inorganic components include NaCl and traces of K^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, and Cu^{2+} ions. Organic components include lactic acid, citric acid, formic acid, propionic acid, butyric acid, urea, and ammonia. Underarm wetness comes mostly from the secretion of eccrine glands. Antiperspirants reduce the amount of sweat only from eccrine glands.

Apocrine Glands

Apocrine glands are apparently a relic from the phylogenetic development of man. These glands start to produce a milky, viscous fluid during puberty on special locations of the body, especially the underarm pit (5). In contrast to eccrine glands, the openings of the glands are not at the skin surface but appear at the hair follicle. Decomposition of apocrine sweat by skin bacteria is responsible for the characteristic malodor of human sweat. Apocrine sweat consists of, besides water, proteins, carbohydrates, and ammonium salts (6). Other investigators have reported that these glands secrete lipids, cholesterol, and steroids (7). Furthermore, it has been shown that androgen-converting enzymes in the apocrine glands are responsible for circulating androgens to dihydrotestosterone (5).

ANTIPERSPIRANTS

Antiperspirants are topically applied products designed to reduce underarm wetness by limiting eccrine sweat production. In the United States, these products are regulated by the Food and Drug administration (FDA) as over-the-counter (OTC) drugs, because they are intended to affect a ''function of the body'' (i.e., in this context, perspiration). Products containing antiperspirant actives have to reduce perspiration to minimum 20% in 50% of the test population under validated test conditions. Test protocols (in vivo clinical trials), to develop a safe and an effective product, have been designed to substantiate the desired claims (8–14).

Comparative quantitative determination of the activity of sweat glands on the forearm after application of aluminum chlorohydrate (ACH) solutions is now possible by combining

the classic starch iodine visualization technique with digital image analysis (15). A noninvasive optical technique that allows the analysis of the function of a number of glands, simultaneously, in vivo was recently reported (16). A new method for parallel testing of up to eight formulations on the backs of volunteers allows a very fast evaluation of product prototypes (1).

Sweat Reduction by Antiperspirants: Current Model/Theory

The reader should be aware that theories concerning the action of sweat-reducing agents depend strongly on the type of actives (aluminum salts, nonionic agents, or ionic agents). The efficacy of antiperspirants based on aluminum and/or aluminum zirconium salts can be understood by the formation of an occlusive plug of metal hydroxide in the eccrine duct (17). Tape-stripping experiments followed by analysis of transmission electron micrographs of an ACH-treated eccrine sweat gland duct show an obstructive amorphous material supporting the theory of a mechanical blockage of sweat glands from diffusion of the soluble ACH solution into the sweat gland and subsequent neutralization to a polymeric aluminum hydroxide gel (18,19). There seems to be no correlation concerning the efficacy of aluminum salts and the location of the plug in the duct, because it is known that, compared with ACH, the more effective Al-Zr compounds do not penetrate as deep as the, also highly effective, $AlCl_3$ solutions (17). The reader is referred to the literature concerning other theories of sweat reduction by aluminum salts (20).

Active Ingredients for Controlling Underarm Wetness—State of the Art

Buffered Aluminum Salts (ACH)

The first antiperspirant, Ever Dry, based on $AlCl_3$, was introduced to the market in 1903 (21). The first cream-containing aluminum sulfate was introduced during the 1930s. The acidic pH value (2.5–3.0) was a drawback of these products, leading to skin irritation in the underarm pit. History tells us that the development of antiperspirant actives with a higher pH value, so-called buffered aluminum chlorides (ACH, pH = 4.0–4.2), was an appropriate step with the additional benefit of reduced destruction of fabric clothes. The formula of this buffering salt is $[Al_2(OH)_5]^+ + (Cl^-)$, or more conveniently $Al_2(OH)_5Cl$.

The historical development from $AlCl_3$ to $Al_2(OH)_5Cl$ can be easily understood by the following consideration:

$$\mathrm{AlCl}j = {}^{x}h\ \mathrm{Al_2Cl_6}\ (\text{substitute } 5\,\mathrm{Cl^-} \text{ ions against OH ions}) => \mathrm{Al_2(OH)_5Cl}$$

$Al_2(OH)_5Cl$ is a 5/6 basic aluminum trichloride. The accepted definition of ACH is the ratio of Al to Cl = 2.1 to 1.0. Lower levels lead to ACH $[Al_2(OH)_4Cl_2]$ or to aluminum sesquichlorohydrate $[Al_2(OH)_{4.5}Cl_{1.5}]$—both actives are also generally regarded as safe (GRAS). ACH is supplied as a powder or a 50% solution in water. It can be formulated up to 25%, calculated on an anhydrous basis. The 20% aqueous solution reduces perspiration by 35% to 40% on average (22). Some dyes used in clothing may be acid sensitive and will change color when in contact with an antiperspirant.

The structure of the Lewis acid ACH is very complex, because ACH in water forms the so-called isopolyoxo cations with chloride ions as counterions (23–25). There exist several polymer equilibria of the polycationic aluminum species in water-based systems. Short-chain polycationic species are more effective in reducing sweat.

Aluminum Zirconium Chlorohydrate—Glycine Complexes (AZG or ZAG)

AZG is obtained by reaction of ACH with zirconylchloride. Reaction of the former ingredient in the presence of glycine leads to ZAG complexes. Glycine is used as a buffering agent. These antiperspirant actives form very complex polymeric structures in water. The actives are defined by the ratio of Al + Zr metal-to-chloride ratio and the Al-Zr atomic ratio. The interested reader is referred to the literature concerning available antiperspirant actives (26,27) and nomenclature of the Al-Zr complexes (21,22). These antiperspirant actives were developed especially for anhydrous formulations because they show, compared with ACH, enhanced sweat reduction (28–30). The maximal concentration of ZAG calculated on an anhydrous basis is 20%. They are not allowed to be formulated for use in aerosols.

New Concepts for Controlling Underarm Wetness

Titanium Metal Chelates

The understanding of the complex solution chemistry of aluminum-based antiperspirants gave input to the search for alternative antiperspirant salts. Titanium derivatives, like partially neutralized ammonium titanium lactate (ATL) salts, were shown to be effective in in vitro efficacy tests (31). The titanium metal chelates can be synthesized from the corresponding titanium alkoxides and organic acids allowed by neutralization with ammonia. Under acidic to neutral pH conditions, the ATL active seems to be relatively stable to hydrolysis, and therefore probably is a suitable antiperspirant active in water-based or anhydrous drug delivery systems.

Film-Forming Antiperspirant Polymers

The so-called polybarrier technology is another approach to reduce perspiration by using a polymer that forms an insoluble occlusive film barrier on the underarm skin (32). It was mentioned that the occlusive film is a barrier to the passage of moisture. The main advantages of this technology are reduced skin irritation, applicable after underarm shaving, and higher sweat reduction compared with today's classic antiperspirant salts. The preferred polymer is an olefinic acid amide/olefinic acid or ester copolymer–like octylacrylamide/acrylate copolymer (Versacryl-40). This copolymer can be used alone or in combination with PVP/eicosene copolymer in sticks, roll-ons, or alcohol-based products (33). The reduction of sweat depends on the choice of vehicle and extends in some formulations to 40%.

Lyotropic Liquid Crystals

Certain surfactant/cosurfactant combinations form in water depending on the variables of concentration/temperature instead of micelles' lamellar, hexagonal, inverted hexagonal, inverted micellar, or even cubic phases. The cubic phases can be of micellar or bicontinuous type (34). The water domains in lamellar or cubic phases can swell to a certain degree, while taking up water. The use of this swelling behavior is the basis of a patent where a surfactant/cosurfactant combination is applied to the underarm pit (35). Sweat (water) transfers the applied composition to a lyotropic liquid crystal of cubic structure, thus creating a sweat-absorbing system in the axilla. Oleic acid/glycerol monolaurate is one of the surfactant combinations in the patent. Both components are also well known as deodorizers.

DRUG DELIVERY SYSTEMS AND APPLICATION FORMS FOR ANTIPERSPIRANT ACTIVES

Antiperspirant actives can be formulated in a variety of delivery systems like anhydrous suspensions, water-or hydroalcoholic-based solutions, and emulsions. Typical application forms for antiperspirants are sticks, roll-ons, creams, pump sprays, aerosols, gels, and powders. On a global basis, the three most important product forms are sticks, roll-ons, and aerosols.

Formulation Work

After the decision for the desired application form has been made, the formulator has to decide on the vehicle system for the antiperspirant active. It is the intent of this section to summarize some of the current knowledge concerning the influence of actives with the formula, efficacy of different delivery systems, and the function of the ingredients used in antiperspirants.

Antiperspirant actives, like ACH or ZAG complexes, are soluble in water. Application of a concentrated aqueous solution of an antiperspirant active gives a rather tacky feeling (36). Reduction of tackiness can be best achieved by silicone oils (cyclomethicones) or ester oils like di-(2 ethylhexyl) adipate (27). The acidic pH value (4.0–4.2) has to be taken into account by selecting additional components for the desired drug delivery system. Loss of viscosity and problems of a final formula with color stability are often hints to change the gellant and/or perfume. Aluminum powders in anhydrous systems (aerosols and suspension sticks) often leave visible white residues on skin or clothing. Liquid emollients, like (PPG)-14 butylether or the aforementioned adipate ester, minimize these residues. Another approach is to use the solid emollient isosorbide monolaurate (ICI, Arlamol® ISML) (37). In anhydrous aerosol formulations, the ACH powder settles down and forms a hard-to-redisperse cake at the bottom

of the aerosol can. Suspending aids, like quaternium-18 hectorite or quaternium-18 bentonite, prevent settling of the antiperspirant active and additionally thicken the cyclomethicone oil phase. Usage of fine powders of ACH is another approach to overcome nature's law of gravity.

The reader should be aware that hydrophobic ingredients, like emollients, have an influence on the effectiveness of an antiperspirant active, because a cosmetic oil phase or wax can cover the pores of the eccrine duct. The efficacy of an antiperspirant active, like ACH, is higher in water-containing systems compared with anhydrous formulations. The following rules concerning efficacy might be helpful:

1. Efficacy: aqueous solution > anhydrous suspension.
2. As diffusion of an antiperspirant active in the vehicle and from the vehicle to the skin after application has to be considered, one can further differentiate the expected efficacy trends. Efficacy: aqueous solution > sprayable O/W emulsion > O/W emulsion roll-on > O/W emulsion cream.
3. It is accepted that antiperspirant actives in the outer phase of an emulsion have a higher efficacy than in the dispersed phase. Efficacy: O/W emulsion > W/O emulsion.
4. In water-free systems, the viscosity of the drug delivery system might be of relevance. Suspended ACH in anhydrous vehicles needs to be solubilized after application to the axilla by sweat (water). The effectiveness of suspension sticks depends on the rapidity of active solubilization. The usage of ultrafine powders of ACH is expected to boost efficacy compared with fine powders. Efficacy: low viscous suspension > suspension stick.

The reader is referred to the literature concerning vehicle effects on antiperspirant activity (7,38,39).

Lipophilic ingredients might have an influence on the efficacy of a product, because it is known that the water-soluble propylene glycol can form complexes or hydrogen bonds with aluminum polycationic species thereby altering the efficacy of the salt (40). Also, propylene glycol in high concentrations may result in skin irritations (41). Successful formulation work aims at finding the right viscosity for the product in the desired application form, a lower viscosity during the flow into the underarm pit, and a higher viscosity after application so that the product stays where it was applied. Conventional shear shinning flow curves are characteristic for antiperspirant products. The reader is referred to the literature concerning rheology aspects of cosmetic products (42).

Deodorant/Antiperspirant Sticks

It is at present not easy to give the reader an overview about sticks, because nowadays there exist many technologies to develop this solid delivery system. In Figure 1, an attempt was made to summarize this area. In the following section, only systems of major importance are discussed.

Sticks can be divided into different classes like suspension sticks, gel sticks, and emulsion sticks. Soft sticks have some properties of all the three categories (Fig. 1).

Suspension Sticks

Dry deodorants, or antiperspirant solids, are synonyms for an application form where the active in the form of a powder is suspended in a silicone oil phase. Stearyl alcohol is usually used as the hardening agent. The molten mass crystallizes into a matrix of stearyl alcohol saturated with the silicone oil and suspended particles (43,44). Quaternium-18 hectorite can reduce the settling of the actives. Cyclomethicones give the stick a dry, silky feel; nonvolatile oils, like PPG-14 butylether, minimize white residues on the skin (43). Low-residue sticks can be obtained by using a combination of high-and low-melting waxes and a volatile and nonvolatile silicone-oil combination (45) (Table 1).

Gel Sticks

This class can be subdivided into the following groups: white anhydrous gel sticks, clear anhydrous gel sticks, and clear water-based soap gel sticks. The last class mentioned is discussed in chapter 63.

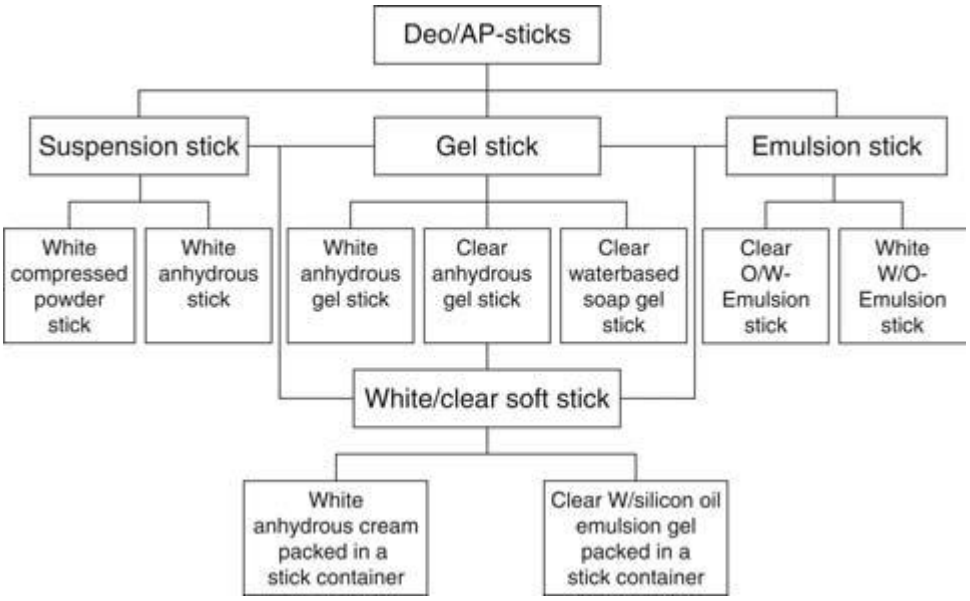

Figure 1 Overview of cosmetic deo/antiperspirant sticks.

Table 1

Suspension stick	Wt%
Stearyl alcohol	20.0
Cyclomethicone	54.0
PPG-14 butylether	2.0
Hydrogenated castor oil	1.0
Talc	2.0
Antiperspirant	20.0
Fragrance	1.0

Abbreviation: PPG, photoplethysmography.

White Anhydrous Gel Sticks

Shear solids, or ultraclear solids, are synonyms for sticks with improved washout performance compared with the classic suspension sticks. They contain *N*-acyl amino acid amides (*N*-lauroyl-L-glutamic acid dibutylamide) and 12-hydroxyacid as gelling agents for an oil phase mixture (e.g., silicone oil/mineral oil). The washout agent is an ethoxylated solubilizer, like Ceteareth-20. These white sticks turn clear after application to the skin (no-residue stick) (46).

Clear Anhydrous Gel Sticks

They are quite popular in the United States, because clarity is associated by the consumer with a lack of white residue on skin, no dangerous ingredients, and high efficacy. A typical gelling agent is dibenzylidene sorbitol [dibenzylaldehyde monosorbitol acetal, (DBMS A)]. This acetal is not stable in an acidic aqueous environment (47). The sticks usually contain a high level of alcohol and/or polyols. At high polyol concentration, the active is regarded to be solubilized instead of suspended in the gel matrix (48). An alternative gelling agent is a polyamide (49) (Table 2).

Emulsion Sticks

They can be grouped into clear O/W emulsions, white W/O emulsions, and clear W/S emulsion gels. The last mentioned is discussed below.

Table 2

White anhydrous gel sticks	Wt%	Clear anhydrous gel sticks	Wt%
N-lauroyl-L-glutamic acid dibutyl amide	5.0	Dibenzylidene sorbitol	2.0
12-Hydroxystearic acid	5.0	Dimethicone copolyol	2.0
Cyclomethicone	40.0	Di-isopropyl sebacate	2.0
Hydrogenated Polyisobutene	15.0	Glycine	1.0
Di-isopropyl myristate	15.0	Dipropyleneglycol	10.0
Antiperspirant powder	20.0	Propyleneglycol	33.0
		Antiperspirant powder	50.0

Source: From Ref. 58.

Table 3

W/O emulsion stick	Wt%
Stearyl alcohol	19.0
Volatile silicone	26.0
Mineral oil	1.0
2-Methyl-2,4 pentandiol	2.0
Polyglycerol-4 isostearate	2.0
ACH solution (50%)	50.0

Abbreviation: ACH, aluminum chlorohydrate.
Source: From Ref. 50.

Clear O/W Emulsions
They contain a high surfactant combination with the active solubilized in the external water phase. The high concentration of surfactants is a disadvantage; no products based on this technology are known to the author (47).

W/O Emulsion Sticks
The water phase containing the active is solubilized by a surfactant, like polyglycerol-4 isostearate. A typical example for an oil/wax phase combination is a mixture of silicone oil/stearyl alcohol (50) (Table 3).

Soft Sticks (Soft Solids, Smooth-Ons)

These sticks can be differentiated into two subgroups, namely, white, anhydrous creams (suspensions), and clear water-in-silicone emulsion gels. Both delivery systems are packed in a container that gives the impression of a stick. The suspension or gel is extruded onto the skin from holes in the top of the stick container to a wide smooth area around the holes.

White, Anhydrous Creams
These creams contain an antiperspirant active, a volatile and nonvolatile silicone oil, and a thickener (*N*-acyl glutamic acid amide).

Clear Water-in-Silicone Emulsion Gels
These formulations can be achieved by adjusting the refractive index of the water and silicone-oil phase. Silicone formulation aids (Dow Corning 3225 C) are mixtures of cyclomethicone and dimethicone copolyol helping to solubilize the active (7,46,48,51). Low surface tension of cyclomethicones facilitates good spreading of a product on the skin and reduces the tackiness of antiperspirant actives.

Antiperspirant Roll-Ons

Roll-on products can be differentiated into several categories (Fig. 2). O/W emulsion-based delivery systems are quite popular in Europe, whereas anhydrous suspension roll-ons or

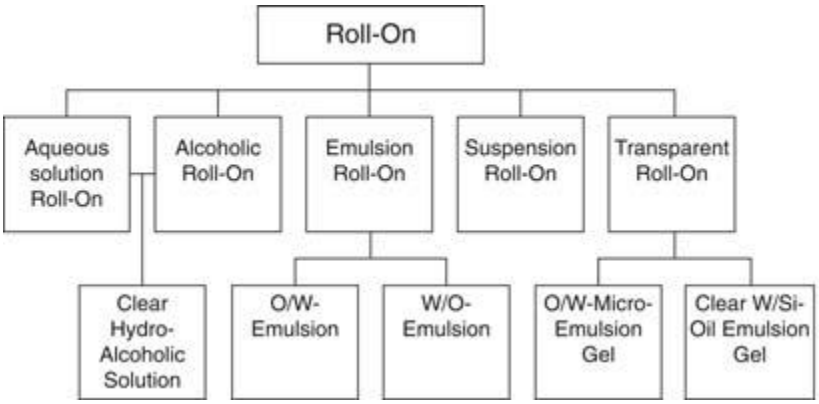

Figure 2 Overview of cosmetic deo/antiperspirant roll-on types.

transparent water-in-silicone emulsions are preferred in the United States. A new trend concerning the size of the roll-on applicator has been identified. Consumers prefer the big-ball format (3.0–3.5 cm), because of the ease of applying the product to the underarm pit (52). The popularity of roll-ons, in general, is because of the nongreasy and nonoily feel in the axilla and the good spreadability of the content on the underarm skin.

Clear Hydroalcoholic Roll-On

This delivery system contains a water/alcohol solution of the antiperspirant active thickened with a water-soluble polymer like hydroxyethylcellulose. The alcohol in the formula gives, compared with the clear aqueous solution-based roll-ons, a fresh sensation in the axilla and facilitates drying of the product. Excellent antiperspirant efficacy is another benefit of hydroalcoholic roll-ons.

O/W Emulsion Roll-On

This delivery system uses ethoxylated surfactants, like PEG-40 stearate, to solubilize an oil phase like mineral oil. The active is dissolved in the outer phase, allowing the formulation of a highly effective product. In alcohol-free formulated systems, microbiological stability has to be checked (Table 4).

W/O Emulsion Roll-On

They are weaker in efficacy because the actives are encapsulated and the external oil phase often gives a sticky feeling.

W/Si Emulsion Roll-On

Silicone oils allow products to formulate on the basis of a "W/O technology," because the skin feel is not comparable with traditional oily components, like ester oils or triglycerides. The concentration of the thickener is reduced compared with sticks based on this type. The technology is discussed under soft sticks (see p. 636).

Table 4

O/W emulsion roll-on	Wt%	Hydroalcoholic roll-on	Wt%
PEG-40 stearate	5.0	Antiperspirant active	20.0
Cetyl alcohol	3.0	PPG-5 ceteareth-20	2.0
Mineral oil	2.0	Water	35.4
Polysorbate-80	1.0	Ethanol	42.1
Glycerin	1.5	Hydroxyethylcellulose	0.5
Magnesium-aluminum silicate	0.8		
Antiperspirant active	20.0		
Water	66.7		

Abbreviations: PPG, photoplethysmography; Mg, magnesium.

O/W Micro-Emulsion Gel

An alternative approach to transparent products uses the phase inversion temperature (PIT) technology. A suitable mixture of surfactants, oils, and water is heated from 60°C to 90°C to give a W/O emulsion above the PIT. During cooling, the mixture shows phase inversion to give white or transparent O/W emulsions; O/W micro-emulsion gels are obtained in the presence of hydrophobically modified water-soluble polymers (53). The technology is explained in more detail in chapter 63.

Suspension Roll-On

The antiperspirant active in powder form is suspended in cylomethicone. The roll-on can be formulated with or without ethanol. Quaternium-18 hectorite is used as a thickener to prevent settling of the active. Consumers in the United States prefer this delivery system, as it does not give a wet feeling after application and because of the easy drying (39). Actives like ZAG complexes give high efficacy to underarm products (Table 5).

Antiperspirant Aerosols

Aerosols, in Europe and Asia, are popular delivery systems for consumers who prefer a hygienic and easy-to-use application form. Typical ingredients for aerosols include isopropyl myristate, isopropyl palmitate, volatile silicone, dimethicone, silica, clays, propylene carbonate, and ethanol. Propellants include propane, butane, and isobutane (Table 6).

As acidic aqueous ACH solutions lead to corrosion of the aerosol can, current aerosol antiperspirant products are formulated as water-free suspensions. The active is suspended as a powder in an oil phase like cyclomethicone or in a mixture of ester oils/cyclomethicone. Agglomeration of solid particles and settling of actives can be minimized by the usage of suspending agents like fumed silica (amorphous silicon dioxide) or clays (bentonite and hectorite). The clays form a weak gel in the presence of an oil phase that can be destroyed by shaking the aerosol can before usage. The gel structure is reformed on standing, thereby holding the active in suspension. Because the organoclays are agglomerated, shear is needed to deagglomerate the platelets, and a polar activator like propylene carbonate or ethanol is used to disperse them and induce the gelation of the oil phase.

The steps involved to prepare an aerosol product can be summarized in the following sequence (7):

1. Preparing bentonite or hectorite clay with the emollient in the presence of the polar activator and shearing the mixture.
2. Adding the antiperspirant active until a uniform agglomeration-free suspension is obtained.
3. Filling the concentrate into the aerosol can and adding the propellant (pressure filling).

Table 5

Suspension roll-on	Wt%
Volatile silicone	65.0
Quaternium-18 hectorite	13.5
Silica	0.5
Antiperspirant powder	20.0
Fragrance	1.0

Table 6

Antiperspirant aerosol	Wt%
Volatile silicone	13.4
Quaternium-18 hectorite	0.8
Ethanol	0.8
Antiperspirant powder	10.0
Propellant (butane/propane)	75.0

Efficacy studies of aerosols, including comparison with other drug delivery systems, have been reported in the literature (30). ZAG complexes are not allowed to be used in aerosols.

Environmental Issues

Aerosols contain volatile organic compounds (VOCs) usually in a weight ratio of propellant to concentrate of 75:25 (54). The environmental impact of VOC, like the reaction with NOX, in the presence of sunlight causes formation of unwanted ozone in the lower atmosphere. U.S. antiperspirant companies especially were forced to reduce VOC emissions by reformulating hydrocarbon propellants and/or exchanging hydrocarbon propellants with the fluorohydrocarbons, 1,1 difluorethane (Propellant 152 a) or 1,1,2,2 tetrafluorethane (Propellant 134 a). The water-soluble dimethoxyethane (DME) is another propellant that is thought to have no impact on the damage of the ozone layer (55).

The current trends in the aerosol market can be summarized as follows:

- higher ratio of concentrate/hydrocarbon propellant
- higher amount of silicone oils
- usage of 1,1 difluorethane (Propellant 152 a)
- formulations with lower vapor pressure
- usage of smaller aerosol cans

Aerosols containing 20% to 50% propellants with a concentrate-propellant ratio from 1.0 to 1.0 to 2.3 to 1.0 have been patented (56).

FUTURE TRENDS

Some new trends in the antiperspirant field concerning new actives and delivery systems have been described in this chapter. Improvements of current formulations and innovative concepts will need the ongoing investigation and better understanding of the interaction of active/vehicle and vehicle/skin. Improving efficacy and skin compatibility is another major trend in the antiperspirant field. New packaging concepts, like the extrudable gels, the big-ball applicator for roll-ons, and reduced-size aerosol cans with ozone-friendly propellants, are probably, in a few years, the state of the art. The influence of perfume components to the skin and the increasing rate of contact allergies attributable to fragrance ingredients have to be closely monitored (57).

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63 Deodorants

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INTRODUCTION

This chapter intends to give an overview on the current knowledge about the origin of underarm odor and the biology of the underarm microflora and its interaction with deodorizing agents. The contents of this chapter have been arranged in particular sequence to facilitate the understanding of rational deodorant product development.

BIOLOGY OF THE UNDERARM MICROFLORA

The resident microflora of the human underarm skin consists of up to $10^6/cm^2$ organisms, e.g., aerobic cocci, lipophilic diphtheroids, and varying species of gram-negative bacteria (1). In the axillae, two types of bacterial flora exist—coryneform bacteria and micrococcaceae such as *Staphylococcus epidermidis*. Coryneform- or *S. epidermidis-dominated* populations are characteristic for human beings. The resident microflora is a quite stable population, not varying a lot between both axillae (2). The organisms are perfectly adapted to their ecological niche with its higher pH value and higher moisture content compared with other skin areas (3). Hair in the axilla, according to the literature, is not a good substrate for bacterial growth; the bacteria prefer to reside on the underarm skin (2). Moisture is required for bacterial proliferation and is secreted especially from the eccrine sweat glands (4). The origin of strong compared with weak underarm odor is associated with a numerical dominance of coryneform bacteria (5). Components of apocrine secretion, e.g., isovaleric acid and androstenone, were proposed to contribute to axillary odor. Hydrolytic exoenzymes of skin bacteria cleave the ester bonds of odorless water-soluble precursors of androstenol to the corresponding volatile steroid (6). Other studies proposed that the key odorants are branched, straight chain, and unsaturated C6-Cn fatty acids (7). (E)-3-methyl-2-hexenoic acid (E-3M2H) is the most abundant fatty acid compared with the rest of C6-Cn fatty acids that contribute to the axillary odor bouquet. Apocrine sweat extracts have been analyzed and concentrations of 0.5 ng/^1 for androstenone and 357 ng/iL for E-3M2H were detected (8). Volatile odor molecules of E-3M2H found in sweat secretions are transported according to the authors in a nonvolatile fashion to the skin surface. Two apocrine secretion odor-binding proteins (ASOB and ASOB2) were identified, carrying 3M2H molecules to the skin surface. Coryneform bacteria liberate the odor molecules from the protein precursor/odorant complex (8).

The reader should be aware that occurrence of these chemical compounds does not mean that all of us can smell them. Individual differences in odor perception for both isomers of 3M2H (9) and for the steroid androstenone are well known (8). Approximately 50% of the adult population is not able to smell androstenones; this anosmia to androstenone—or to 3M2H—is genetically determined.

DEODORANTS

Deodorants are topically applied products designed to reduce underarm odor. They are considered in the United States as cosmetics, while antiperspirants are treated by the FDA as drugs. Deodorants tend to be less irritating than antiperspirants. In Europe, the consumers today prefer deodorants compared to antiperspirants. In the United States the trend is approximately reversed.

Concepts for Controlling Underarm Odor: State of the Art

The current knowledge of the biology of the underarm microflora and the origin of underarm odor is the basis for developing strategies against odor formation. Numerous patents and literature articles disclose the incorporation of chemical compounds for their deodorizing properties. The intention here is to describe and exemplify major strategies, but not all deodorant actives that were developed in the past.

Strategies to reduce underarm odor include the following:

- Antiperspirant active-containing deodorants
- Odor-masking deodorants
- Odor-neutralizing deodorants
- Odor-quenching deodorants
- Enterase inhibitors
- Antimicrobial active-containing deodorants

Antiperspirant Active-Containing Deodorants

Antiperspirant actives such as aluminum chlorohydrate or the Al–Zr complexes (see chap. 62) reduce the secretion of eccrine sweat. Their excellent antimicrobial properties against *S. epidermidis* and coryneform bacteria have been published (10). The acidity of the aluminum salts may be a major factor in bacterial growth inhibition.

Odor-Masking Deodorants

Fragrance compositions (such as perfumes) have been used to mask odors since ancient times. It is conventional to incorporate 0.2% to 1.5% of a perfume in body deodorants (11). They are designed to blend with the underarm odor and thus act as a masking agent. The perception of a perfume may differ significantly between individuals because of different interactions with the skin, washing habits, and specific underarm odor. The fragrance materials are blended to achieve what is known as "top note," "middle note," and "bottom note" components. The first is the refreshing note upon application while the last are the olfactoric components, which stay on after application to the underarm skin.

Perfumes with antimicrobial properties have been described in patents and in the literature (12–14). An additional benefit, especially for emulsion-based products, is that they might also act as a preservative. The increasing rate of contact allergies against fragrance ingredients should be taken into account using this approach to combat underarm odor (15).

Odor-Neutralizing Deodorants

In chapter 62, it was mentioned that odorous C6-Cn fatty acids contribute to underarm odor. Chemical neutralization with sodium bicarbonate (NaHCO3) yields the corresponding odorless soaps (16). This active, however, is not stable for a long time in aqueous compositions. Patents for deodorant applications and usage of NaHCO3, in the presence of antiperspirant actives, have been filed (17,18). Zinc carbonate–containing deodorants are also content of a patent (19).

Odor-Quenching Deodorants

Zinc Ricinoleate

Zinc salts of ricinoleic acid have no bacteriostatic or antiperspirant effect (20). They strongly bind odorous fatty acids, amines, and mercaptanes. Ligand-exchange reactions of ricinoleic acid for odor molecules are probably the reason for the quenching properties of zinc ricinoleate (21). Interactions with perfume components in a deodorant formulation may weaken the desired quenching effect of the odor molecules after topical application to the underarm.

Metal Oxides

The oxides of calcium, magnesium, and zinc form in the presence of fatty acids in the corresponding metal soaps (22). Zinc oxide particles aggregate to form a massive lump. This leads to clogging of aerosol products (23). Hybrid powders were developed in which the metal oxide covers the surface of a spherical nylon powder (23). The advantage of this technology is

the increased surface area of zinc oxide and thus enhanced odor-quenching efficacy and the reduced particle aggregation in aerosols.

Esterase Inhibitors

Zinc Glycinate

The inhibition of exoenzymes from the underarm bacteria should also result in odor reduction. Zinc glycinate has been described as a suitable active (24). Antimicrobial tests showed no inhibitory effect against *S. epidermidis* or against the lipophilic diphtheroid bacteria supporting the suggested mechanism against microbial exoenzymes.

Triethylcitrate

The optimal pH value for development of underarm odor caused by coryneform bacteria is approximately about pH 6 in axillary extracts (25). Shifting the skin surface pH to the acidic side should decrease the activity of skin esterases, which are proposed to be responsible for degradation of underarm secretions. Triethylcitrate was proposed to form citric acid by an enzymatic process on the underarm skin. In 1991, it was shown that this active has no pH-reducing effect after application to the underarm skin (26). Nevertheless, deodorants containing this active are still in the market.

Antimicrobial Active-Containing Deodorants

This approach is currently the most commonly used strategy to prevent underarm odor. Ethanol is probably one of the best-known actives for deodorization (27). Additional efficacy is normally required for a long-term deodorization, and this can be achieved by the additional usage of fragrance, an antiperspirant active, or other antimicrobial actives (famesol, phenoxyethanol, etc.).

Triclosan (2,4,40-Trichloro-20-Hydroxydiphenylether)

This active has a broad-spectrum antimicrobial activity against most gram-positive and gram-negative bacteria, molds, and yeasts. The presence of triclosan in antiperspirant sticks and roll-ons leads to a higher reduction of the bacterial microflora versus the triclosan-free antiperspirant composition (28). Triclosan is also used in skin care products, hand disinfectants, and household products (29).

Glyceryl Fatty Acid Ester

Mono- and oligoglyceryl fatty acid esters such as glyceryl monocaprylate, monocaprinate, monolaurate, and diglyceryl monocaprinate are effective deodorizers (30). Combinations of glyceryl monolaurate with farnesol and phenoxyethanol showed synergistic efficacy effects against coryneform bacteria (31). The advantage of this ingredient combination over the first generation deodorant actives such as triclosan is attributed to their higher biodegradability and their selective bacterial action. These actives are all naturally occurring in plants and animal species. In addition, it could be demonstrated that combinations of mono- and oligoglyceryl fatty acid esters with a variety of natural antimicrobials (e.g., wool wax acids) displayed a synergistic antimicrobial efficacy against underarm bacteria and serve as highly effective deodorant actives (32–35). Products containing such actives have been successfully marketed for a number of years.

Sucrose Fatty Acid Ester

The fatty acid esters of sucrose are well known as emulsifiers in food products (36). Sucrose can be substituted on eight hydroxyl groups with fatty acids. The antimicrobial potential depends strongly on the substitution degree of the sucrose. Sucrose monostearate and sucrose monolaurate have been described as deodorizers in the literature and in patents (37–39).

Glycerolether

2-Ethylhexyl glycerolether (octoxyglycerol) is a clear liquid with good solubility in cosmetic oils, polyols, and alcohol but only moderate solubility in water (0.2%). Synergistic

antimicrobial activity with other ingredients has been described (40). This active has become popular recently in European deodorant formulations.

New Concepts for Controlling Underarm Odor

Ongoing research activities focusing on a better understanding of the interaction between underarm skin/skin microflora and skin microflora/odor formation, in combination with the discovery of highly selective actives, today allow more specific designs for deodorant products. In the next sections, some of the new trends are discussed in detail. New concepts for controlling underarm odor include the following:

- Chitosan
- Bacterial enzyme inhibitors
- Odor-inhibiting precursor mimics
- Product and skin-mediated perfume transformations
- Antiadhesives

Chitosan

Chitin is a naturally occurring polysaccharide (e.g., in insects, lobster, crabs, or fungi) containing N-acetylated D-glucosamine units. Deacetylation of the amino group leads to the slightly water-soluble chitosan. The deodorizing properties of chitosan and the combination of this active with aluminum salts have been the subject of a patent (41).

Bacterial Enzyme Inhibitors

The enzyme amino acid (3-lyase) is, according to a patent filed in 1990, a catalyst for the formation of underarm odor (42). This enzyme is located in odor-releasing bacterial cells and cleaves the apocrine precursors of sweat components, such as amino acids with the structure unit COOH–CH–(NH2)–CH2–S–R, to the corresponding odorous sulfur products. Several classes of enzyme inhibitors such as derivatives of hydroxylamines, 3-substituted amino acids, cycloserine, and pyridoxal were identified.

Odor-Inhibiting Precursor Mimics

Another approach to the inhibition of the above-mentioned enzyme f-lyase is to provide an alternative substrate for the bacteria that cleave the structure unit CH(NH2) CH2–O–C(O)–R instead of the sulfur-containing amino acid sequence (43). This approach leads to the corresponding nonodorous ingredients, such as benzoic acid, or to pleasant odor-generating substances, such as phenylacetic acid.

Product- and Skin-Mediated Perfume Transformations

The physical and chemical interaction of a perfume with the underarm skin is a very complicated matter. Research activities in this area focused on the question, which components of a perfume stay on and above the skin after topical application (44). Headspace analysis is one of the techniques to gain more information concerning skin/perfume interactions. It could be demonstrated that the long lastingness of a fragrance can be achieved by using a prodrug (ester, acetale) of a perfume ingredient (45). The esters or acetales of a fragrance composition hydrolyze on human skin because of the slightly acid pH value. The hydrolysis products (acids, alcohols, and aldehydes) impart a pleasant smell to the underarm skin. These product- and skin-mediated perfume transformations are especially suitable for alkaline formulations such as soap-based deodorant sticks. The advantage of the perfume precursor approach is attributed to a prolonged fragrance impression of a deodorant after topical application to the underarm skin.

Antiadhesives

An alternative concept to reduce the amount of skin bacteria in the underarm skin is the antiadhesion approach. The understanding of the adhesion mechanisms of the resident underarm microflora to the skin surface is the basis for developing strategies against bacterial

adhesion. Numerous skin microorganisms adhere preferentially to specific sites on various body surfaces. For example, *Staphylococcus aureus* and *Pseudomonas aeruginosa* adhere to collected nasal epithelial cells (46). *Corynebacterium xerosis* binds to epidermal cells whereas yeasts species such as *Candida albicans* bind to corneocytes. Structures of the skin specifically involved in adherence to the underarm bacteria are thought to be proteins, oligosaccharide structures, lipids, and hydrophobic surfaces. Imitation of these adhesion motifs by saccharides, oligosaccharides, polysaccharides, and glycoproteins allows one to inhibit the bacterial adherence to the skin. Additionally, it was discovered recently that among others, sucrose esters such as sucrose myristate and sucrose laurate have antiadhesive properties to various microorganisms including the typical microflora of the underarm skin (47).

DRUG-DELIVERY SYSTEMS AND APPLICATION FORMS FOR DEODORANT ACTIVES

Products designed to reduce underarm odor can be formulated in a variety of delivery systems such as suspensions, water or hydroalcoholic solutions, and emulsions. Typical application forms are sticks, roll-ons, creams, pump sprays, aerosols, and gels. Sticks, roll-ons, and aerosols are discussed in detail in the chapter "Antiperspirants. Lowering the amount of an antiperspirant active, such as aluminum chlorohydrate, in an antiperspirant is one option to formulate a deodorant. In this case, the antiperspirant active has only deodorizing properties and nearly no impact on the eccrine sweat glands. Deodorants can be formulated in acidic, neutral, or alkaline environment. Designing a deodorant, the formulator should have in mind the following points:

- Long-term deodorization
- No irritation potential
- Good solubility of the active in the delivery system
- Selection of a stable fragrance
- Viscosity control of the product
- Good skin feeling of the product

Protocols for the in vitro and in vivo evaluation of deodorants have been designed. The reader is referred to the literature (48). A new method for in vivo evaluation of antimicrobial agents was recently developed, where the underarm bacteria were translocated to the forearm allowing the simultaneous evaluation of multiple deodorizers in an individual (49).

Deodorant Sticks

Deodorant sticks are solidified by 6% to 8% of sodium stearate. The deodorizing agent and a fragrance are dissolved in a hydrophilic carrier. Two stick categories can be differentiated, the ethanol-based and the propylene glycol-based sticks (50).

Transparency is usually achieved by usage of a high polyol content. Clarifying agents for sticks such as PPG-14 butylether, Cocamide DEA, Lauramide DEA, Steareth-100 have been patented (51,52). Ethanol-based sticks are preferred if it is the intent of the formulator to create a cooling sensation for the consumer. Shrinkage of the stick has to be taken into account because of evaporation of the alcohol. Propylene glycol-based sticks tend to be more resistant to shrinkage, and solubilization of a fragrance is easier in some instances (53) (Table 1).

Table 1

Deodorant stick	Wt%	Deodorant stick	Wt%
Water	16.0	Water	3.0
Ethanol	75.5	Propylene glycol	10.0
Deodorizer	1.0	Deodorizer	1.0
Sodium stearate	6.5	Sodium stearate	8.0
Fragrance	1.0	PPG-3 myristyl ether	77.0
		Fragrance	1.0

Deodorant Aerosols

Spray products containing a solution of an antimicrobial active in an ethanol and/or propylene glycol carrier, blended with a liquefied propellant, are typical for deodorant aerosols. The difference from an antiperspirant active containing aerosol is that the deodorizer is solubilized in an alcohol- or polyol-based formulation and not suspended. Deodorant sprays provide a dry skin feeling to the underarm skin because they are anhydrously formulated.

Typically, 20% to 60% of the sprayable contents of an aerosol reach the skin, because the liquefied hydrocarbon propellant vaporizes as it is sprayed (54). Propane, butane, and isobutane are the most commonly used propellants. They condense to form a clear, colorless, and odorless liquid with densities of 0.51 to 0.58 g/mL at 20°C (55). These propellants are inflammable in the presence of air or oxygen. Labeling of cosmetic aerosols concerning flammability risks of volatile organic compounds and volatile solvent abuse is discussed in detail in a recently published review (56). Aerosol containers can be fabricated from tin-coated steel, tin-free steel (chromium-coated steel), or aluminum. Numerous types of aerosol can cause corrosion, and testing for it was recently discussed in the literature (57). The environmental issues of aerosols are explained in greater detail in the chapter "Antiperspirants" (Table 2).

The formulator of an aerosol has to optimize the following parameters to get a dry deodorant product:

- Spray rate
- Spray shape
- Particle size, concentrate/propellant ratio
- Fragrance/deodorizer concentration
- Pressure of the aerosol can

Deodorant Pump Sprays

Hydroalcoholic Pump Sprays

An alternative to aerosols is pump sprays. This category is quite popular in Europe, whereas it is of lower interest for the consumers in the United States, because they tend to prefer a dry application form, like the anhydrous sticks. Pump sprays allow a good dosage of the formulation to be delivered to the underarm skin in a hygienic way. They consist of low-viscosity hydroalcoholic solutions of a deodorizer and a perfume. Usually a solubilizer, such as PEG-40 hydrogenated castor oil, is incorporated into the formulation to maintain a clear and homogeneous solution (Table 3).

Table 2

Deodorant aerosol	Wt%
Alcohol	42.0
Laureth-4	0.5
Deodorizer	1.0
Fragrance	0.5
Isobutane	47.6
Propane	8.4

Table 3

Pump spray	Wt%
Water	35.6
Alcohol	60.0
PEG-40 hyd.	2.0
Castor oil	–
Deodorizer	2.0
Fragrance	0.4

Table 4

PIT-emulsion pump spray	Wt%
Glyceryl stearate, ceteareth-20, ceteareth-10, cetearyl alcohol, cetyl palmitate (Emulgade SE)	4.5
Ceteareth-20	1.0
Dioctyl cyclohexane	5.0
Dicaprylylether	5.0
Deodorizer	2.0
Aluminum chlorohydrate	5.0
Water	77.5

Source: From Ref. 60.

PIT-Emulsion Pump Sprays

A disadvantage of hydroalcoholic pump sprays is the alcohol content in the formulation that may contribute to unwanted side reactions especially in the shaved axilla. Beiersdorf AG in Hamburg, Germany, introduced to the European market under the brand name "Nivea®" a new pump spray on the basis of an emulsion in 1995. The sprayable low-viscous deodorant is based on the phase inversion temperature (PIT) technology. Suitable mixtures of ethoxylated surfactants, oils, and water in the presence of antiperspirant and deodorizing actives are heated to 60°C to 90°C. Cooling the resulting W/O emulsion to room temperature yields, via a PIT process, a finely dispersed bluish-white O/W emulsion (58–60). The droplet size distribution of such PIT emulsions ranges from 80 to 250 nm. The above-mentioned pump spray contained a skin-friendly deodorizing combination of glyceryl monocaprinate and wool wax acids in an alcohol-free delivery system (Table 4).

Microemulsion Pump Sprays

Hydroalcoholic pump sprays are usually transparent, whereas sprayable PIT emulsions are white or bluish-white products. Sprayable alcohol-free and additionally transparent pump sprays were recently introduced into the European market (e.g., Basis pH; Beiersdorf AG, Hamburg, Germany). Transparency of an emulsion is achieved when the size of the droplets is below 100 nm. This O/W microemulsion can be obtained with and without the PIT technology but needs careful selection of ingredients and considerable fine-tuning (61). The main advantage compared with classical microemulsions is the low surfactant concentration (<10%). Furthermore, it could be demonstrated that, in the presence of hydrophobically modified water-soluble polymers, the above-mentioned technology allows the formulation of gels, sprayable gels, roll-ons, sticks, and aerosol products (62).

FUTURE TRENDS

The deodorant market has undergone some remarkable changes concerning the principles to reduce underarm odor in the last years. It is expected that the search for effective, skin-friendly actives with a highly selective action against the cutaneous underarm microflora will lead to long-lasting and safe deodorants. Improvements in understanding how microorganisms adhere to human skin should facilitate the development of new strategies to reduce underarm odor. Improvements of aerosols with no/low impact to the environment or aerosol alternatives, such as sprayable emulsions, are probably in a few years in the portfolio of every deodorant-selling company.

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64 Revulsive Products: Way of Action and Evaluation of Their Efficacy

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INTRODUCTION

In Webster's dictionary, we found the following descriptions: "revulsive"—(*i*) to pull away and (*ii*) an act or technique of turning or diverting a disease or blood from a diseased region in one part of the body to another (as by counterirritation); "rubefacient"—substance for external application that causes redness of the skin.

Revulsive products (i.e., rubefacients and urticants) are known for several clinical and nonclinical applications. Clinically, they are used in the treatment of neuropathological [diabetic neuropathy, postherpetic neuralgia (PHN)] and/or muskeloskeletal disorders (e.g., osteoarthritis, rheumatoid arthritis, muscle soreness, and back pain). Nonclinically, they are used in some sports as passive warming-up products and in the cosmetic industry as an ingredient in skin products (1).

The capital active ingredient in these topical formulations is a nicotinate derivative [methylnicotinate (MN), hexylnicotinate (HN), and benzylnicotinate (BN)] or capsaicin. Nicotinates provoke an elevation of arachidonic acid and prostaglandin levels (prostaglandin D2). The produced prostaglandins act on the neuroreticular tissue of the arteriovenous anastomoses of the dermal vascular plexuses by means of an endothelium relaxant factor. The latter provokes a relaxation of the vascular smooth muscles resulting in an augmentation of the cutaneous circulation and a flooding of the superficial veins. This nonimmunological immediate contact reaction is visible as an erythema (2).

Capsaicin is an alkaloid, derived from chili peppers, with analgesic properties. Capsaicin binds to nocisensors in the skin, excitating neurons, which results in itching, pricking, or burning with a cutaneous vasodilation (3).

Stimulation of afferent C fibers with release of substance P is hypothesized, while the desensitization after prolonged treatment is believed to occur due to a depletion of substance P (3).

The topical application of capsaicin has equally been used to study reflex mechanisms of dermal vasodilatation (4,5) and as an experimental pain inducer to study the underlying nocisensoric mechanisms (6). Threshold levels of sensitization to increasing capsaicin concentrations have been used to study sensitive facial skin (7).

Besides these clinical and practical applications, rubeficiants (especially nicotinates) are often used in more fundamental research toward percutaneous penetration processes. The quantification of the physiologically induced vascular response has been found to be a good indicator for the skin bioavailability of these topically applied substances.

The response is often quantified by measuring the perfusion of the skin microcirculation (laser Doppler velocimetry), the skin color (redness), or the skin temperature. The laser Doppler instrument measures the increased perfusion of the arterial plexuses, while the skin color is not only an indication for the increased flux in the arterial part of the skin microcirculation but also for the flooding of the venous capacitance vessels.

This chapter describes the way of action and factors influencing the pharmacodynamic response to nicotinates under different experimental conditions, followed by possible clinical applications. For capsaicin, the emphasis will be mainly on clinical applications since this revulsive product is not often used for fundamental research. Finally, the use of revulsive products in physiotherapy (sport) will be discussed.

NICOTINATES

The onset of the nicotinate contact reaction depends on the derivative used. Application of MN (hydrophilic) results in an immediate response as measured with the laser Doppler instrument, while HN (lipophylic molecule) shows a lag time up to five minutes (8).

The intensity of the reaction is concentration dependent (9,10). Concentrations between 1 and 100 mM are reported. The duration of the response varies from 20 minutes up to 60 minutes for the laser Doppler response and up to 90 minutes for the color response. An increased temperature is noticed up to 90 minutes post application (10).

The pharmacodynamic response varies in function of the anatomical skin site (11). The strongest response was recorded at the forehead and the chest, while the response was weaker at the abdomen, forearm, and thigh. A significant relation was found between the nicotinate response and skin characteristics, such as TEWL, stratum corneum hydration, skin temperature, baseline perfusion of the microcirculation, and sebum gland density.

In the experiments of Marrakchi and Maibach, reactivity was tested on different regions of the face (forehead, nose, cheek, nasolabial and perioral areas, and chin), the neck, and the volar forearm (12). Experiments were carried out on young (29.8 ± 3.9 years) and older (73.6 ± 17.4 years) population. For both the age groups, the areas on the face and neck were more sensitive to HN compared with the forearm. A different sensitivity pattern for the face was detected between the two age groups, while peak values were significantly higher in the older group for the forehead, cheek, and nasolabial area. The authors explained the differences between the age groups by the photoaging effect on the sebum glands with more and enlarged glands in the older subjects. Roskos et al. did not find an effect of age when applying MN in young and older subjects (13).

Issachar et al. found a significant correlation between the percutaneous penetration of MN and sensitive skin. The intensity of the response to the nicotinates differed significantly between normal and sensitive skin, while the duration of the inflammation was comparable (14).

Racial differences in barrier function were demonstrated by quantification of the nicotinate response with a laser Doppler instrument (15,16).

Berardesca et al. compared nicotinate responsiveness in Caucasians and black volunteers before and after a delipidation of the challenged skin area (15).

A lower cutaneous response was noticed for the blacks compared with the whites under both experimental conditions. Kompaore and Tsuruta compared Asians, blacks, and Caucasians with a nicotinate challenge. The lag time between nicotinate application and onset of the vascular response was used as an indication of barrier permeability. Permeability was strongest in Asian skin, weaker in Caucasian skin, and weakest in black skin (16).

The response to nicotinates is significantly reduced after oral treatment with anti-inflammatory drugs (17,18) and with topically applied anti-inflammatory drugs (19–23). The reduced response in the presence of topically applied anti-inflammatory drugs was used as a model to study iontophoresis (24). In these experiments, the reduction in the MN response was used as an indicator for the presence of diclofenac in the stratum corneum.

In their experiments with penetration enhancers Tanojo et al. found a reduced lag time in the HN response after pretreatment of the skin with propylene glycol. The combination of propylene glycol with oleic acid was not more effective than propylene glycol alone (25).

The microvascular sensitivity (tested with increasing nicotinate concentrations) was increased in diabetic patients compared with controls, while the maximal microvascular responses were comparable (2). Similar findings were observed by Caselli et al. in healthy control subjects and diabetic neuropathy patients (1). These authors propose the addition of MN in the moistening products used to reduce the development of diabetic foot (1,2).

A reduced response was noticed in patients with Huntington's disease (26).

CAPSAICIN

Capsaicin (trans-8-methyl-*N*-vanillyl-nonenamide) is an alkaloid derived from the common hot pepper plant of the nightshade (*solanacae*) family. Capsaicin represents the main constituent of the total pungent acid amides present in the capsicum species and is responsible for the red-hot chili taste (27).

Besides being considered as food additive, capsaicin has also gained human exposure as oral supplement or topical analgesic (28).

Capsaicin is a selective agonist for the transient receptor potential (TRP) channel. The TRPV1 receptor is a ligand-gated, nonselective cation channel expressed on a subpopulation of primary small-diameter sensory Aδ fibers and C fibers, responsive to noxious heat and mechanical and chemical stimuli (29,30). The topical application of capsaicin on the human skin excitates the TRPV1-expressing nocisensors, resulting in an itching, pricking, burning sensation with a cutaneous vasodilation, hyperalgesia, and allodynia (30). Stimulation of afferent C fibers with release of neuropeptides, predominantly substance P is hypothesized, while the desensitization after prolonged treatment is believed to occur because of a depletion of substance P (31). Capsaicin inhibits axonal transport of neurotransmitters by depressing the release of the nerve growth factor (NGF) (32).

The resulting hypoalgesia is due to degeneration of epidermal nerve fibers (31–34). The desensitization of hyperactive nociceptive sensory axons is the basis for therapeutic topical application or intra-articular injections of capsaicin (35).

Peripheral neuropathy, provoked by axonal degeneration of sensory autonomic and motor neurons of the peripheral nervous system, is a common complication of diabetes and chronic alcohol abuse. The manifestations of peripheral neuropathy classically progress from the most distal extremities. Positive symptoms are lancinating pain, paresthesia, numbness, allodynia, and burning and itching sensations (36).

The Capsaicin Study Group (1991) conducted a multicenter, double-blind, vehicle-controlled study to establish the efficacy of topical 0.075% capsaicin cream in relieving the pain associated with diabetic neuropathy. Patients ($n = 252$) were randomly assigned to the capsaicin or placebo group. Capsaicin cream or vehicle was applied on the painful areas four times a day for eight weeks. Statistical analysis showed significant difference in favor of capsaicin compared with placebo for the following parameters: pain relief, decreasing pain relief, and pain improvement on the physician's global evaluation scale. The authors asserted that topical capsaicin cream is safe and effective in treating painful diabetic neuropathy (37).

These findings corroborate the results of the placebo-controlled studies by Scheffler et al. (38) and Tandan et al. (39). They demonstrated the superiority of capsaicin cream 0.075% versus placebo in pain control and improvement of daily activity during the treatment of diabetic neuropathy.

A meta-analysis of four randomized, double-blind, placebo-controlled trials using capsaicin in the treatment of diabetic neuropathy found capsaicin to be more effective than placebo (40). This is in contrast with the findings of Low et al. (41). In their study, using a four times daily application of capsaicin cream versus a nicotinate formulation as placebo, they failed to demonstrate significant improvement in chronic distal painful polyneuropathy after 12 weeks of treatment. Besides using different pain evaluation scales, physiological functions such as sudomotor axon reflex, nerve conductance, and sensory examinations were carried out before, during, and after the experiment (41).

PHN is the most common complication of Herpes Zoster. The incidence of Herpes Zoster and its associated complications mainly occur in older patients (42). The pain associated with PHN is often referred to as neurogenic pain and generated as a result of neural dysfunction and therefore unresponsive to conventional analgesics including opiates (43).

The hypothesized mechanism for capsaicin-induced analgesia in PHN is the interference with the biosynthesis of the neuropetide substance P, which has an important role in the central transmission of nociceptive signals (44).

Two published double-blind, placebo-controlled studies evaluated the clinical effectiveness of topical capsaicin cream in the treatment of PHN. The reported results of both the studies were in favor of capsaicin (0.075%) cream versus placebo. McCleane demonstrated that the mixed application of glyceryl trinitrate and capsaicin cream was significantly more effective than placebo in reducing pain. This combination had a positive effect on the tolerability of treatment with capsaicin cream in patients with osteoarthritis (44).

Painfull cutaneous disorders such as psoriasis, nostalgia paresthetica, and atopic dermatitis are characterized by intense itching, scaling, and erythema (45,46). Nonhistaminic itching, in contrast to histaminic itching, is difficult to treat, and therapeutic applications are often uneffective (47). The release of substance P increases the vascular permeability and the number of mast cells in the skin (48). The responsiveness of several forms of urticaria to

capsaicin treatment may be related to the effects of capsaicin on the microvasculature of the skin (47). The topical application of capsaicin seems to be effective in the treatment of a variety of painful clinical conditions affecting the skin (40,47,49). However, the absence of a "burning placebo" as a control vehicle makes it difficult to conduct double-blind studies, and further research is needed to assess the clinical effectiveness of capsaicin.

Detection threshold determined by applying increasing concentrations of capsaicin on facial skin was used as an objective parameter for sensitive skin (7). Using that procedure Jourdain et al. detected subjects with low and high threshold. This threshold level corresponded with the self-declared level of sensitive skin. The authors concluded that this skin neurosensitivity test appears to be a promising tool for cosmetic diagnosis of sensitive skin (7).

However, the use of capsaicin, in some cases, is accompanied with adverse effects. Initial exacerbation of symptoms, transient burning, and redness at application site is reported by patients in various capsaicin studies. Because of the need for frequent application in the treatment procedure, these side effects may be the major reason for the poor compliance (50,51).

PHYSIOTHERAPY AND SPORT

Although revulsive products are widely used in the physiotherapy practice, few studies report on the efficacy of these products (10).

In physiotherapy, revulsive products are mainly used for the relief of joint and muscle pain. In sports, these products are often used as passive warming-up for activities in cold environments. As active ingredients, the formulations contain analgesic substances, such as salicylates, camphor, menthol, oil of turpentine, and MN or HN as vasodilatory substances (10). Their effect is mainly because of vasodilatory components provoking a thermal effect in the superficial layers of the skin. Analgesic components (e.g., salicylates) may be added in the formulations, but the target tissue of these analgesic substances is not the skin but the underlying muscle, tendon, or joint tissue. Evaluation of the efficacy of regional therapy is beyond the scope of this chapter.

Our literature search did not find reports on the efficacy of revulsive products as used in physiotherapy. We evaluated the effects of three commercially available topical products commonly used in physiotherapy practice.

The thermal effect of these products was evaluated by means of noninvasive measurements of biophysical skin properties. Product 1 was a W/O (Water/Oil) emulsion containing MN at 1.5% and methylglycolisate at 5.0% as active ingredients. Product 2 was an O/W (Oil/Water) emulsion containing MN (1.0%) and α-bisabolol as active ingredients. α-Bisabolol is the active component of camomile and has an anti-irritant effect. Product 3 was a spray containing methylsalicilate (2.0%), menthol (3.0%), and oil of turpentine (5.0%).

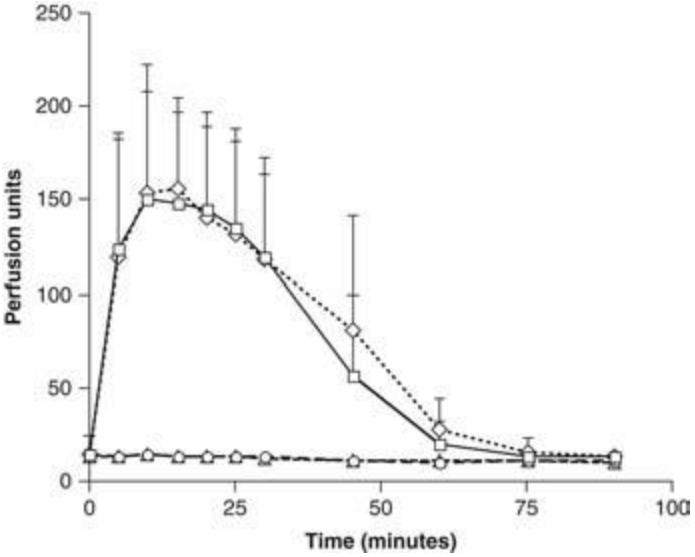

Figure 1 Perfusion of the skin microcirculation in function of time as measured after topical application of product 1 (—□—) product 2 (.....◇.....); product 3 (---o---). An untreated control is included (---△---). Mean ± s.d. ($n = 15$).

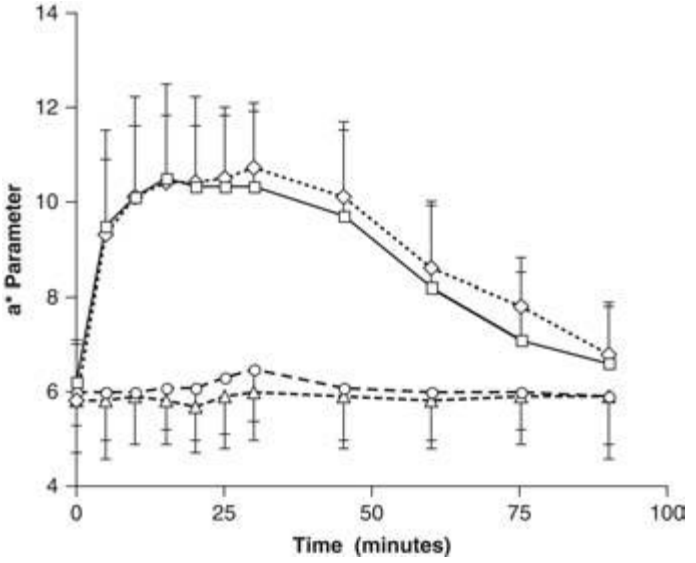

Figure 2 Skin color a* parameter in function of time as measured after topical application of product 1 (—□—) product 2 (.....◇.....); product 3 (---o---). An untreated control is included (---△---). Mean ± s.d. ($n = 15$).

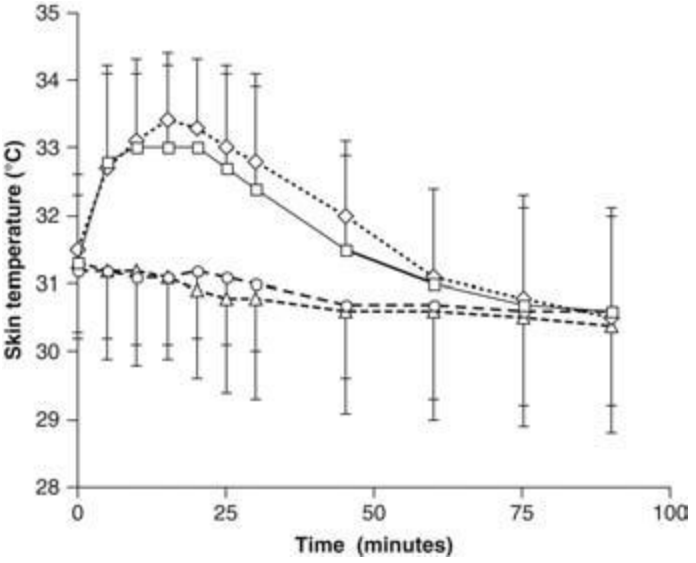

Figure 3 Skin temperature in function of time as measured after topical application of product 1 (—□—) product 2 (.....◇.....); product 3 (---o---). An untreated control is included (---△---). Mean ± s.d. ($n = 15$).

It was found that products containing nicotinates provoked a significant increase in the perfusion of skin microcirculation, a significant increase of the superficial skin temperature, and a significant reddening of the skin color (erythema) (see Fig. 1–3). The maximum increase obtained in skin temperature (±2°C) is believed to have little effect on the underlying tissues. However, the strong sensation in the skin can "pull away" the pain sensation from other tissues.

Comparison of the three measurement techniques gives information concerning the underlying mechanism of erythema production: the arterial vasodilatation causes an increased blood flow (reaching its maximum 10 minutes post application), resulting in an increased amount of blood in the superficial capacitance vessels. This results in reddening of the skin (maximum redness 15 minutes post application), and heat is lost by convection (reaching a maximum 15 minutes post application).

We assume that the effect of single application of revulsive products is mainly due to the thermal effect, which is easily quantifiable. The strong sensations in the dermal and epidermal skin layers will relieve the feeling of pain in other tissues or structures. The thermal effect is equally limited, since a maximal increase in skin temperature of ± 2°C was obtained

by nicotinate application. The heat transfer toward muscles and other structures is limited because of the hypodermal layers, containing mainly adipose tissue, which work as very good isolators (52,53). Moreover, deeper tissues have a temperature closer to the core temperature of 37°C. In our experiments, skin temperature reached about 33°C, which is lower than the temperature of deeper structures! This finding points equally to the inefficiency of these revulsive products, as passive warming-up is often used for sport activities in colder environments. On the contrary, the use of such products will provoke a greater transfer of heat toward the cold environment with a possible negative effect on the thermoregulation.

CONCLUSION

Revulsive products produce a reddening of the skin. This erythema is due to an increased perfusion of the microcirculation after a vasodilation of the arterial plexus at the different skin levels.

Nicotinates act via an endothelium relaxant factor, while capsaicin uses a neurogenic cascade with involvement of substance P.

The more clinical applications of nicotinates aim to increase the perfusion of the superficial microvasculature to obtain increased skin temperature and a kind of pain relief by "pulling away" the pain sensation located in the deeper tissues (gate control). In capsaicin treatments, desensitization is aimed by depletion of substance P at the nociceptive sensors. This can only be obtained by long-term multiple treatment regimes (up to 4 times a day for 12 weeks).

For nicotinates, no side effects are reported, while the adherence to capsaicin treatment is rather weak due to the inconvenience of the side effects at the site of application (burning, itching, etc.).

Despite nicotinates being widely used in physiotherapy (sport), there is only limited evidence for the efficiency of these treatments. Reports on capsaicin treatment indicate a moderate positive effect under different clinical situations. However, most of the designs lack an adequate placebo treatment. In the experiments of Low et al. using a nicotinate solution as "burning placebo," no difference was found between the capsaicin versus the burning placebo in the relief of pain (41). Hence, the use of nicotinates, in different clinical treatments, needs to be further elaborated. The absence of side effects may improve compliance.

Nicotinates are widely used in more fundamental research toward percutaneous penetration, since the quantification of the vascular response can be used as an indicator for the skin bioavailability. Other skin properties, such as the problem of sensitive skin can also be studied using a nicotinate challenge. In more recent reports, a capsaicin challenge has been developed to study the sensitive skin.

The instrumentation available nowadays allows a more precise evaluation and quantification of physiological responses. Using these techniques under experimental and clinical conditions may increase the knowledge and evidence in the use of revulsive products.

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65 Cooling Ingredients and Their Mechanism of Action

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INTRODUCTION

The use of "purified" cooling agents in pharmaceutical and cosmetic preparations only dates back to the late 1880s with the commercial production of menthol from Japanese peppermint (*Mentha arvensis*) oil in Japan (1). The cultivation of peppermint in Japan before the Christian era appears to predate any other country, and menthol is reputed to have been used medicinally for almost as long (2). In the Western world, it was about 1770 that the Dutch botanist, H. David Gaubius, first isolated menthol from the oil of *Mentha piperita* in Utrecht (2,3). Prior to the commercial availability of menthol, the essential oils of peppermint varieties (primarily *M.* and *M. arvensis*) were the sole source for use as cooling minty ingredients. It is significant that at the end of the 18th century only about 900 to 1400 kg of peppermint oils (both *piperita* and *arvensis*) were consumed worldwide (1). By the late 1890s, production had increased to about 175,000 kg (2). In 2007, total peppermint oil production was estimated at more than 26,000,000 kg, with about 21,500,000 kg being the oil of *M. arvensis* (commonly referred to as cornmint oil), which is used mostly for the production of natural *leavo*-menthol (4).

This chapter reviews the use of menthol and new classes of cooling agents that have been discovered since the 1970s. In addition, we briefly touch upon the efficacy of cooling agents as insect repellents. Finally, recent findings on the physiological mechanisms of cold receptors are presented.

COOLING INGREDIENTS

Menthol Background

Before World War II, production of *leavo*-menthol [hereafter referred to as (–)-menthol] was controlled exclusively by Japan and China. In 1939, Japan exported 268,920 kg of menthol, while China's exports in 1940 were 190,909 kg (1). With the advent of war, shipments to the allied countries ceased and major shortages ensued. While synthetic (–)-menthol could be produced from high citronellal feed stocks (e.g., citronella oil and citronella-type eucalyptus oils), this also was no longer an option. However, Japanese and Chinese immigrants in Brazil rapidly began planting *M. arvensis* for menthol production. In 1941, Brazil produced 5000 kg of menthol, rising to 1,200,000 kg by 1945 (1). By the 1960s, Brazil's production peaked at about 3,000,000 kg, while about the same time China began supplying menthol again.

During the 1960s, an oversupply of menthol caused the price to fall to as low as $7.70 to $8.80 per kilogram, and processors reduced production levels. This ultimately led to worldwide shortages and a price spike as high as $50 plus per kilogram in 1974 (with similar price spikes now occurring about every 10 years) (3). As menthol is a commodity, it is sometimes subject to financial speculation, which exacerbates price swings.

In 1958, India began expanding plantings of *M. arvensis*, but, until the late 1980s, the quality was highly variable and often had low menthol content. In the 1980s, new strains were introduced that gave improved oil yields and had menthol contents of 75% to 85%. By 1996, India was producing 6000 metric ton of *M. arvensis* oil and had long surpassed China as the major producer of menthol (3). In 2007, it was estimated that India would produce in excess of 20,000 metric ton of this mint oil. While the bulk of current production is used for local menthol crystallization, significant amounts of oil and crude menthol fractions are exported to Brazil, Taiwan, and Japan for further purification. The residual oil left after crystallizing

menthol still contains 35% to 45% menthol as well as menthones and other typical mint components. Much of this oil (commonly referred to as dementholized cornmint oil) is rectified by distillation and sold for use where normal peppermint oil (ex *M. piperita*) is used (toothpaste, mouthwash, etc.). In addition, some of this dementholized oil is fractionated to isolate the menthones (which can be converted by reduction into (–)-menthol) and other "natural" flavor chemicals.

During the 1970s and 1980s, a number of new routes to synthetic (–)-menthol were developed, only two of which led to long-term commercial success. These processes have been reviewed by both Leffingwell (5) and Hopp and Lawrence (6). Today, the procedure developed by Haarmann and Reimer (now Symrise) on the basis of hydrogenation of thymol to racemic *dl*-menthol followed by selective crystallization of (–)-menthol (via the benzoate ester) is the major process (7).

The Takasago process uses myrcene as the raw material, which is converted to *N*,*N*-diethylgeranylamine and then asymmetrically isomerized via the chiral rhodium (S)-BINAP (or SEGPHOS) complex to the optically active enamine of citronellal. Hydrolysis yields (+)-citronellal, which is cyclized to (–)-isopulegol by classical methods. On hydrogenation, the isopulegol gives (–)-menthol in high optical purity (8,9). An alternative starting material (instead of myrcene) is isoprene, which can be dimerized to *N*,*N*-diethylnerylamine. This material can be converted to (–)-menthol in a manner analogous to the myrcene route using rhodium (R)-BINAP as the chiral catalyst (10). Reflecting on the Takasago process, Ryoji Noyori stated in his 2001 Nobel lecture, "*This resulted from a fruitful academic/industrial collaboration*. . . ." (11).

Clark estimates that 2007 worldwide consumption of menthol from all sources (i.e., peppermint oils, natural menthol, and synthetic menthol) is 32,000 metric ton, of which 19,170 metric ton is purified menthol (4).

Table 1 provides our estimate of production in producing countries (or in the case of Symrise and Takasago, company production of synthetic (–)-menthol).

Table 2 provides the 2007 estimated worldwide usage of menthol by consumer product category—on the basis of Clark's data by region (4).

Table 1 Worldwide Sources of Menthol (2007)

Source	Metric ton
India (natural)	9,700
China (natural)	2,120
Symrise (synthetic)	3,600
Takasago (synthetic)	1,500
Other synthetic[a]	1,200
Brazil (natural)[b]	450
Taiwan (natural)[b]	300
Japan (natural)[b]	300
Total[c]	19,170

[a]Other synthetic includes menthol produced from menthone as well as racemic menthol.
[b]Primarily from *Mentha arvensis* oil or crude menthol ex India (or China).
[c]Total menthol volume based on Clark's estimate (4).

Table 2 2007 Estimated Worldwide Consumption of Menthol % by Product Category

Product category	Menthol %
Oral hygiene	28.00
Pharmaceuticals	26.60
Tobacco	25.30
Confectionaries	11.00
Shaving products	7.00
Miscellaneous	2.10

Source: From Ref. 4.

Menthol Chemistry

Menthol is a $C_{10}H_{20}O$ terpenoid alcohol (MW 156.27) with three chiral centers leading to eight possible stereoisomers (4 enantiomeric pairs). The characterization of the stereoisomeric menthols was painstakingly resolved prior to the availability of modern methods by Read (12,13). The structures of the eight enantiomers, with their optical rotations (in ethanol), are shown in Figure 1.

Only the (–)-menthol enantiomer possesses the clean desirable minty odor and intense cooling properties. For example, the (+)-menthol enantiomer is less cooling and possesses a musty off-note odor that is undesirable in most applications. This musty note is also present in racemic menthol (15). The organoleptics and cooling strengths of all of the enantiomers have been reviewed (5,6). Figure 2 provides the cooling thresholds in ppm.

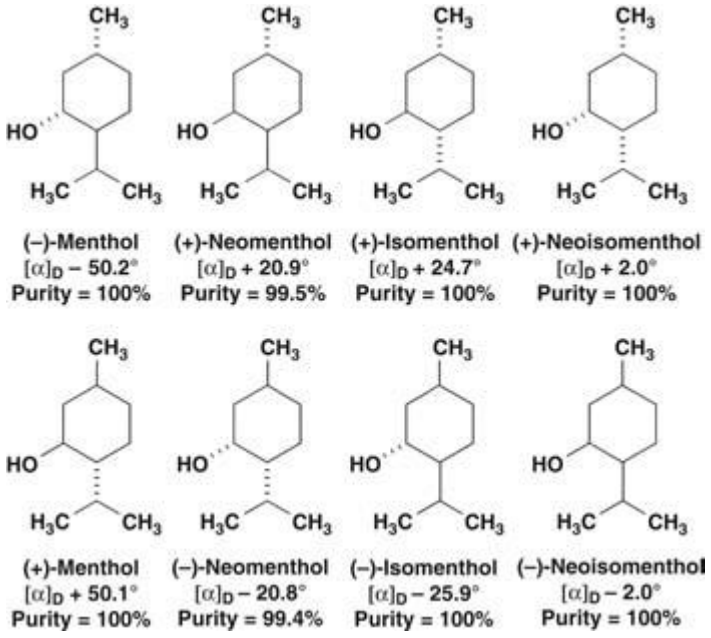

Figure 1 Stereoisomers of menthol. *Source*: From Ref. 14.

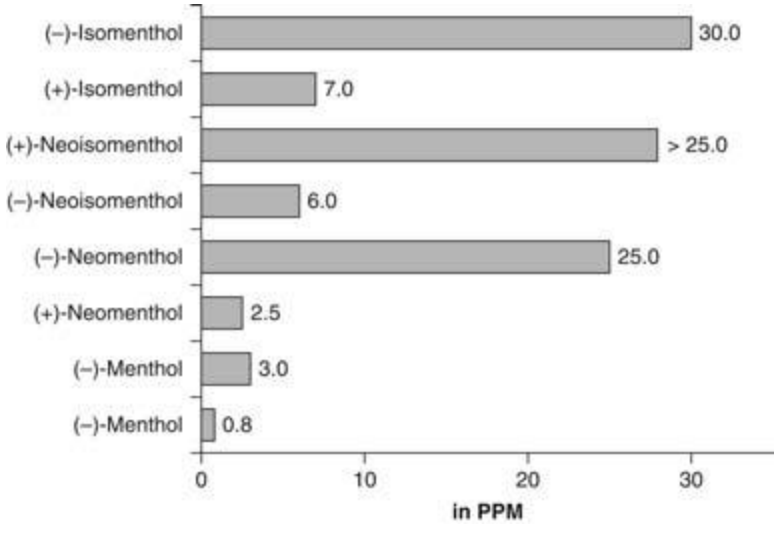

Figure 2 Cooling thresholds (in ppm) (by taste dilution). *Source*: From Ref. 14.

Table 3 Major Impurities in Synthetic and Natural Menthols

Major impurities	Synthetic %	Brazil 1 %	Brazil 2 %	China 1 %	China 2 %	India %
Menthone	0.0069	0.0258	0.0258	0.0135	0.0350	0.0295
Isomenthone	0.0000	0.0069	0.0172	0.0052	0.0123	0.0155
Menthyl acetate	0.0000	0.0100	0.0148	0.0014	0.0128	0.0048
Isopulegol	0.0022	0.1868	0.1651	0.1374	0.1914	0.1789
Neomenthol	0.0032	0.0689	0.1339	0.0951	0.0882	0.1079
Neoisomenthol	0.0000	0.0075	0.0459	0.0352	0.0177	0.0368
Isomenthol	0.0299	0.0099	0.0442	0.0296	0.0248	0.0322
Piperitone	0.0000	0.0053	0.0046	0.0018	0.0031	0.0024
Totals	0.0422	0.3211	0.4515	0.3192	0.3853	0.4080

Source: From Ref. 16.

Natural menthol ex *M. arvensis* oil is normally about 99.0% to 99.6% pure, with the remaining impurities being other constituents found in the cornmint oil. While, in most cases, the mint oil impurities contribute a pleasant peppermint aroma, certain impurities, such as mint sulfide, can also impart less desirable and harsh notes. Thus, odor discrepancies often arise when comparing samples from different companies or countries. To overcome such differences, the skilled technician can add a small percentage (e.g., 0.2–0.4%) of terpeneless peppermint oil ex *M. piperita* (or redistilled dementholized cornmint oil), which adds the desirable sweet peppermint top note. Table 3 compares the major impurities present in synthetic menthol and natural menthol samples from major producing areas (16).

Although not generally commercially available, menthol produced from *M. piperita* oil has a sweeter peppermint top note than that produced from cornmint oil (JC Leffingwell, unpublished observations).

Synthetic (–)-menthol is normally about plus 99.8% pure and has less of the minty top note present in natural menthol. Again, this can be adjusted to increase the mint character, if desired, by the addition of a small amount of terpeneless peppermint oils.

Menthol-Related Cooling Agents

Interest in menthol-related cooling agents began in the late 1950s to 1960s when several tobacco companies began to develop various esters as potential menthol release agents (17–19), some of which now appear on the flavor extract manufacturers association's GRAS list. Among those of interest today is monomenthyl succinate (MMS) (FEMA# 3810) (18), which was later patented by Mane as a cooling agent for general use (20). In addition, menthol ethylene glycol carbonate (Frescolat® MGC), with FEMA# 3805, and menthol propylene glycol carbonate (Frescolat MPC), with FEMA# 3806, were first patented as tobacco flavorants (19), again to be later patented by Haarmann and Reimer for general cooling usages (21).

A number of other menthol-related cooling agents are commercially available: menthone glycerol ketal (Frescolat MGA) (22)—both the racemic (FEMA# 3808) and *leavo* forms (FEMA# 3807); the *leavo* form appears to be the main item of commerce. This material provides a clean cooling refreshing effect and as a partial replacement of peppermint oil has been shown to provide longer-lasting sweetness and a higher cooling sensation in chewing gum (23). (–)-Menthyl lactate (Frescolat ML) is faintly minty in odor and virtually tasteless with a pleasant, long-lasting cooling effect (24). Recently, Erman has shown that the (–)-ML of commerce has the 'S' configuration for the hydroxy moiety, indicating the fact that it is produced by the esterification of (–)-menthol with (S)-(+)-lactic acid (25). 3-(l-Menthoxy) propane-1,2-diol, known as MPD, Coolact® agent 10, TK-10, and coolant agent 10, is another important commercial cooling agent, which, in contrast to menthol, is essentially odorless (26). The cooling threshold (in mouth) is 1 ppm (about 20–100% that of menthol), and the time of cold-feeling maintenance is 20 to 25 minutes for a 100-ppm solution (about twice that of menthol). While the cooling strength of Coolact agent 10 is accepted as being about 20% to 25% that of menthol, it is also noted that "in a Vaseline ointment, 3-(l-menthoxy)propane-1,2-diol shows a cool feeling 2.0 to 2.5 times stronger than that of (–)-menthol" (27). The cool-feeling intensity of the (2S) isomer is 2 to 3 times that of the (2R) isomer and 1.5 to 2 times

superior to that of the racemic modification (28). Similarly, the related menthoxyalkanols, 3-(l-menthoxy)-2-methylpropane-1,2-diol (FEMA# 3849), 3-(l-menthoxy)ethanol (Coolact 5), FEMA# 4154, 3-(l-menthoxy)propan-1-ol, and 3-(l-menthoxy)butan-1-ol have cooling properties (29). Interestingly, cooling compounds such as 3-(l-menthoxy)propane-1,2-diol and 3-(l-menthoxy)-2-methylpropane-1,2-diol when admixed with warming sensates (e.g., vanillyl butyl ether, ginger extract, or capsicum tincture) provide increased warmth and longer-lasting warmth in cosmetic and flavor systems (27,30,31). Conversely, it has also been observed that admixtures of such cooling compounds with the warming sensate vanillin-MPD (the acetal of 3-(l-menthoxy) propane-1,2-diol and vanillin), FEMA# 3904, can increase the duration of cooling sensations (32) (Fig. 3).

(–)-Isopulegol (Coolact P), FEMA# 2962, having a chemical purity of better than 99.7% and an optical purity of not less than 99.7% ee, is odorless and gives a feeling of freshness, crispness, and coolness. The cooling strength is about 20% to 30% that of (–)-menthol (33). The *p*-menthane-3,8-diols (Coolact 38D, PMD38), FEMA# 4053, consist of a mixture of (+)-*cis* and

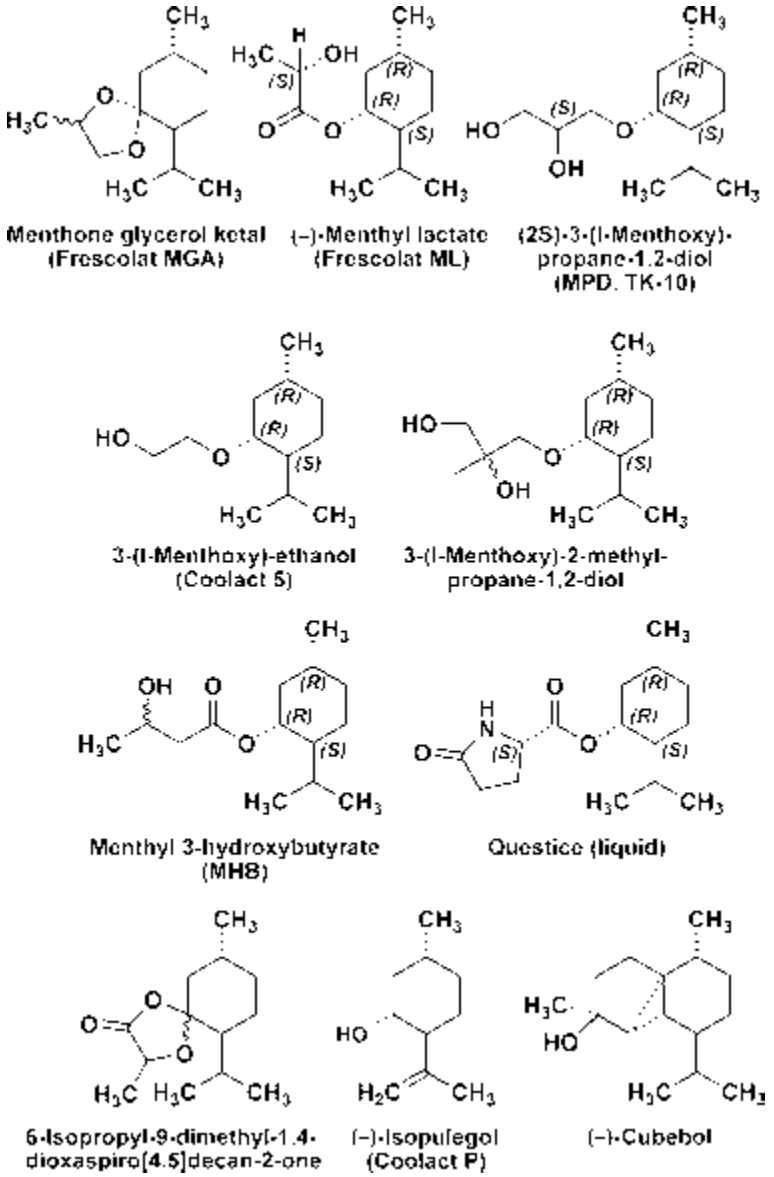

Figure 3 Menthoxy-related coolants.

(–)-*trans* PMD38 in a ratio of ~62:38 and possesses a cooling strength of about 11% that of (–)-menthol (27,34). PMD38 is a nature identical material that occurs in a number of citronellal-rich oils (e.g., *Litsea cubeba*, *Eucalyptus citriodora*) and is also effective as an insect repellant (34–36). (–)-Monomenthyl glutarate (Physcool 2, MMG), FEMA# 4006, is a nature identical cooling agent that has been found in *Litchi sinensis* accompanied by (–)-dimenthyl glutarate (37). It has been described as "probably the longest-lasting oral cooling agent that is commercially available" (38). Recently, an improved synthesis has been reported for both MMG and MMS that minimizes the amount of diester impurities (39). Similarly, (–)-MMS has been confirmed to be nature identical by its isolation from *Lycium barbarum* and *M. piperita* (37). A recent description of MMS indicates that it is virtually tasteless and has well-balanced cooling onset and length of cooling (38). Questice® (menthyl pyrrolidin-2-one 5-carboxylate) was first patented as a composition of matter that acts as a long-lasting cooling and fresh ingredient in toothpaste. The cooling properties are due to the enzymatic hydrolytic release of menthol. A liquid form was produced by reacting (–)-menthol with L-pyrrolidin-2-one carboxylic acid, while a crystalline form was produced when racemic DL-pyrrolidin-2-one carboxylic acid is employed (40). Surprisingly, it did not appear on the GRAS list until 2005 with FEMA# 2155 (41). However, it has long been employed in various cosmetics, lotions, etc. Recently, Erman has shown that the liquid form of Questice is a diastereoisomeric mixture of (–)-menthyl 5-oxopyrrolidine-2-carboxylates with a ratio of the 5S:5R configuration of ~91:8, while the solid form has a ratio of ~46:53 (25). (–)-Menthyl 3-hydroxybutyrate (MHB), FEMA# 4308, is another recent addition to the GRAS list (42). This is reported by workers at Takasago as having a long-acting excellent cooling effect and is odorless and tasteless. Potential uses include foods, drinks, cosmetics, pharmaceuticals, and cigarettes (43). Other workers indicate that the cooling effect is slightly stronger than ML (about 48% the cooling strength of menthol) (44). Firmenich workers have recently found that a diastereoisomeric mixture of the 6-isopropyl-3,9-dimethyl-1,4-dioxaspiro[4.5]decan-2-ones, prepared by reacting lactic acid with *cis* and *trans*-menthones, provides a minty, fresh, *piperita*-type flavor that is remarkable by its strength and cleanness. In combination with other cooling agents (e.g., menthyl succinate or menthol), a synergist increase in cooling strength was found. In particular, the (3S,5R,6S,9R) and (3S,5S,6S,9R) isomers are preferred (45). A patent describes the use of certain esters such as (–)-menthyl methoxyacetate and (–)-menthyl 3,6-dioxaheptanoate as cooling agents (46). In addition to the cooling properties, (–)-menthyl methoxyacetate has a head note and fruity taste resembling that of menthyl acetate, whereas (–)-menthyl 3,6-dioxaheptanoate has a bitter taste. Cubebol, a natural isolate of cubeb oil, in which it normally occurs at levels of 10% to 30% (47), is a sesquiterpenoid alcohol that has a certain stereochemical resemblance to menthol and, while not menthol derived, is included here for completeness. Cubebol has only a very weak smell and taste and provides a refreshing effect that develops in the mouth after a delay of approximately 1 to 2 minutes and lasts for approximately 30 minutes. It has applications in flavors, oral care, pharmaceutical products, etc. (48).

Carboxamide Cooling Agents

During the early 1970s, Wilkinson Sword Ltd. conducted an extensive research program in which they designed and evaluated about 1200 compounds for their cooling activity (49,50). The interest in such compounds related to cooling agents without the minty and volatile side effects of menthol, such as eye irritation, in aftershave lotions, etc. Over 25 U.S. patents were issued on these materials (51). Of these original Wilkinson Sword compounds, three were initially commercialized: WS-3 (*N*-ethyl-*p*-menthane-3-carboxamide) (52), WS-23 (2-isopropyl-*N*,2,3-trimethylbutyramide) (53), and WS-14 [*N*-([ethoxycarbonyl]methyl)-*p*-menthane-3-carboxamide] (52). WS-3 was given GRAS status (FEMA# 3455) in 1975 (54) and WS-23 (FEMA# 3804) in 1996 (55). Interestingly, WS-14 was used as a cooling agent for the Northwind cigarette introduced into test market in 1981. This test market was short lived, but it is not clear if this was because of market failure or concern that the additive testing conducted was insufficient to pass Food and Drug Administration (FDA) scrutiny (56). WS-14 is commercially available as ICE 4000 cooling sensate (57) and finds some applications as a topical cooling agent (Fig. 4).

In 2007, WS-5 [ethyl 3-(*p*-menthane-3-carboxamido)acetate], which is currently the coldest of all commercial cooling agents, was granted GRAS status as FEMA# 4309 (42). It has been found that only highly purified WS-5 is suitable for flavoring purposes (58), as less pure

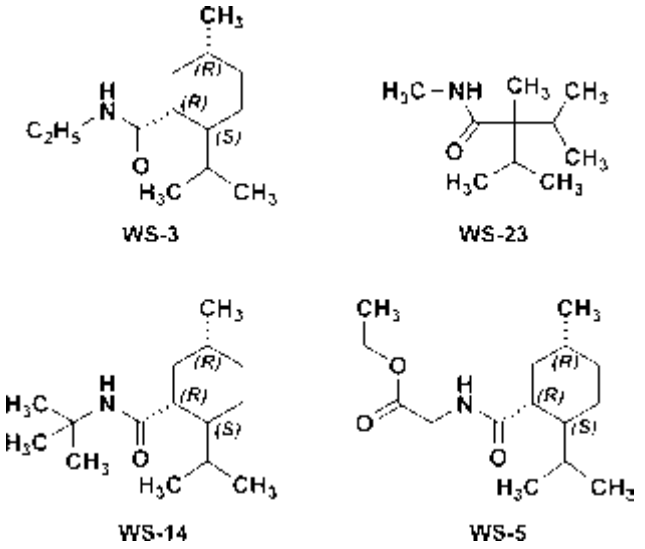

Figure 4 Successful Wilkinson sword coolants.

material exhibits a powerful bitter taste. WS-3 and WS-23 are currently the two largest volume carboxamide coolants. They are widely used in flavors, especially for chewing gum, breath fresheners, confectionaries, and oral care. They also find use in cosmetics (e.g., aftershave lotions). As both WS-3 and WS-23 are solids, there has been considerable interest in developing blends of such cooling agents that provide strong cooling but are easy to handle liquids. For example, it has been found that mixtures of ML, WS-3, and propylene glycol form stable liquid systems (59). It has also been shown that WS-3, WS-5, WS-14, and WS-23, alone or in certain combinations, when mixed with ML (or other coolants such as menthoxypropane-1,2-diol) will form stable liquid systems (60), and such mixtures often give a synergistic increase in cooling sensation. Similarly, eutectic mixtures of WS-3 and WS-23 provide liquid cooling systems (61,62), which can be used either as cooling agents or as flavor and saltiness enhancers.

Another compound that can be classified either as a carboxamide or a menthyl ester is *N*,*N*-dimethyl menthyl succinamide (FEMA# 4230 for the racemate). An International Flavors & Fragrances (IFF) patent (63) describes this as having a cooling onset time of 25 seconds with cooling duration of 11.25 minutes. The taste/sensory profile is "cooling and refreshing on tongue, palate and front gums; fruity flavor with estery top-notes and sour undertones" (at 25 ppm in water). In a chewing gum at 0.2%, it increased sweetness and exhibited a pleasant and substantive cooling effect on the tongue and roof of the mouth.

Other examples of newly discovered carboxamides coolants are a series of analogs of WS-23 [such as *N*-(2-ethoxyethyl)-2-isopropyl-2,3-dimethylbutanamide] patented by Qaroma (64) and aryl carboxamide analogs (with the reversed amide configuration) by Givaudan (65), many with cooling intensities equal to or greater than WS-23. For example, *N*-(1-isopropyl-1,2-dimethylpropyl)-1,3-benzodioxole-5-carboxamide has about 2.2 times more cooling intensity as compared with 2 ppm of menthol (Fig. 5).

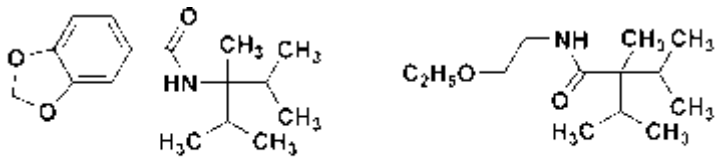

Figure 5 Recent WS-23 analogs.

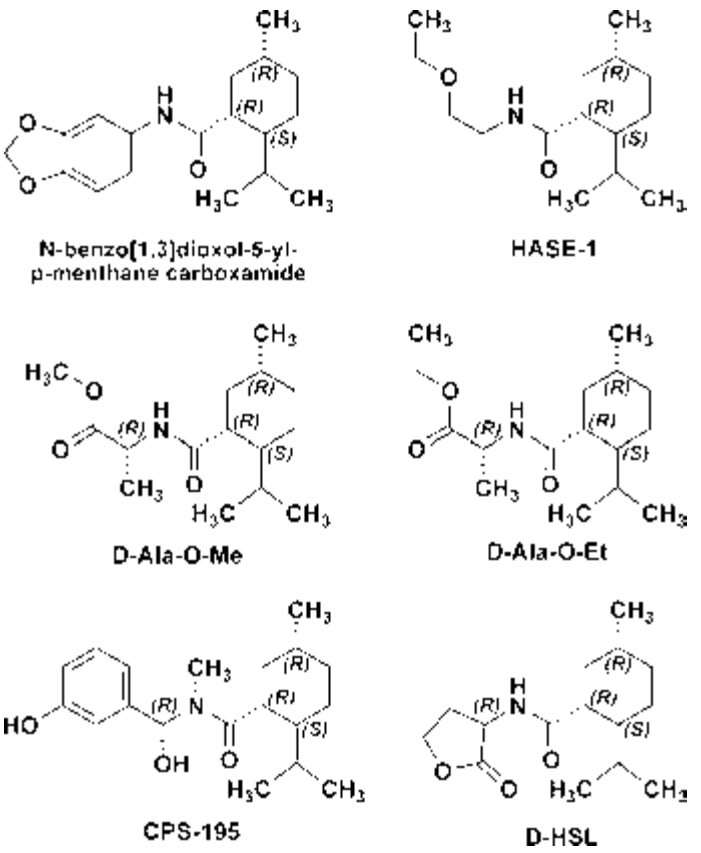

Figure 6 New *p*-menthane carboxamide coolants.

Of particular interest are various aryl *p*-menthane-3-carboxamides, such as *N*-benzo[1,3] dioxol-5-yl-3-*p*-menthanecarboxamide and *N*-benzooxazol-4-yl-3-*p*-menthanecarboxamide, which are reported to have 100 times more cooling intensity than menthol (when compared with menthol at 2 ppm) (66).

In 2004, T. Hasegawa Co. Ltd. patented a new series of strong cooling compounds on the basis of alkyloxy amides of the *p*-menthane series, which exhibit no bitterness; compound HASE-1 is an example (Fig. 6) (67).

Further, Wei (68) has shown that several materials related to WS-5 possess strong cooling with remarkable cooling longevity. For example, the methyl and ethyl ester analogs of WS-5 (referred to as D-Ala-O-Me and D-Ala-O-Et, respectively) are produced from D-alanine (rather than glycine). Similarly, when D-homoserine lactone is employed, the resultant compound is *N*-(R)-2-oxotetrahydrofuran-3-y1-(1R,2S,5R)-*p*-menthane-3-carboxamide (referred to as "D-HSL"), which also is a potent long-lasting coolant. By combining suitable sympathomimetic amine drugs that act as α-adrenergic receptor agonists to form the corresponding *p*-menthane carboxamides, Wei found certain compounds (such as L-phenylephrine *p*-menthane carboxamide, referred to as CPS-195) that were effective as long-lasting coolants and possessed additional therapeutic properties (69). The cooling duration of a number of these, applied to the skin as a 1% wt/vol in a petrolatum-based ointment, versus leading coolants is shown in Figure 7.

In the last 10 years, there has been extensive patent activity relative to physiological cooling agents. Between 1998 and 2007, more than 280 patents were issued (25,63), and from January 2005 to December 2007 more than 300 patent applications have been filed. It is beyond the scope of this article to review all of these. However, it should be noted that a recent activity trend has been the patenting of various combinations of cooling agents, both to achieve improved cooling properties and/or for liquefaction of solid coolants (59,61,70,71). For example, it has been found that blends of menthyl glutarate with low levels of

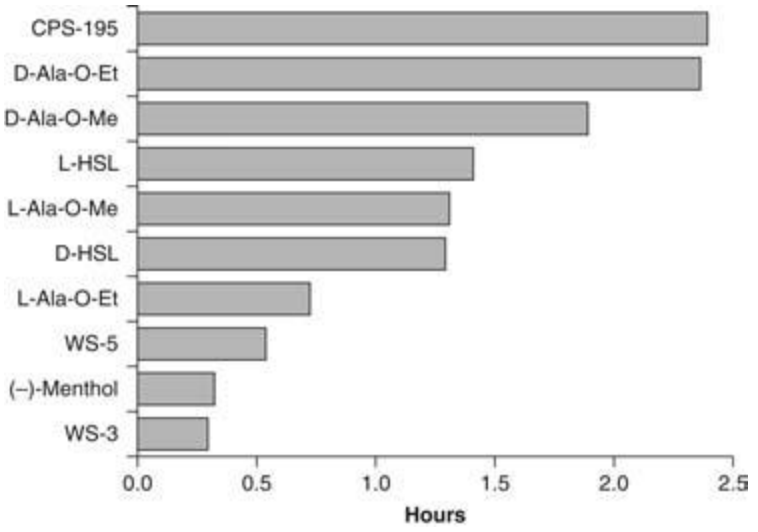

Figure 7 Topical cooling duration 1% in ointment. *Source*: From Refs. 68, 69.

(–)-isopulegol and/or PMD38 exhibit a remarkable synergistic increase in cooling in oral-care products (71).

The relative "accepted" cooling strengths of important coolants are shown in Figure 8 (72,73).

It should be noted that "accepted" cooling strengths, primarily associated with topical skin cooling, do not always agree when compared to oral sensory panel results. This is clearly shown by results obtained by Wm. Wrigley Jr. Company sensory panels comparing 5% sucrose solutions of various coolants versus 100-ppm (–)-menthol, as shown in Figure 9 (71).

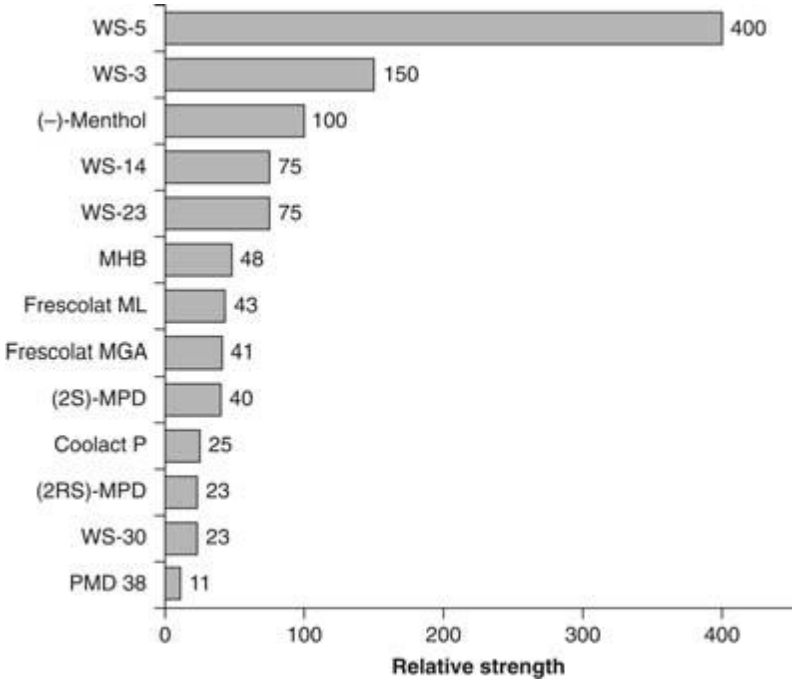

Figure 8 Approximate "accepted" cooling strengths versus menthol (as 100). *Source*: From Refs. 72, 73.

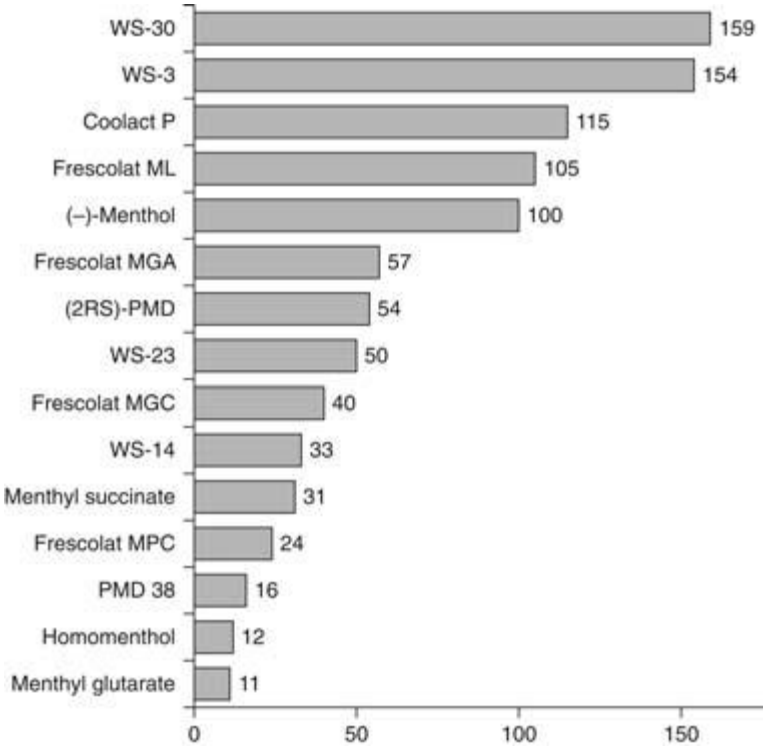

Figure 9 Relative oral cooling in 5% sucrose solutions versus 100 ppm menthol. *Source*: From Ref. 71.

COOLING COMPOUNDS AS INSECT REPELLENTS

As previously mentioned, the PMD38 have shown effectiveness as an insect repellent. Barnard has compared its efficacy against the leading insect repellants DEET, IR3535 [ethyl 3-(*N*-butyl-*N*-acetyl)-aminopropionate], and KBR3023 [sec-butyl 2-(2-hydroxyethyl)piperidine-1-carboxylate] (74,75).

Questice (menthyl pyrrolidone carboxylate) has also been patented as an insect repellent (76) and, Kalbe and Nentwig describe the use of ML or menthol glycerol acetal for repelling mites and other insects (77). Notably, Gautschi and Blondeau of Givaudan have discovered that WS-3 (*N*-ethyl-*p*-menthane-3-carboxamide) and related N-substituted *p*-menthane carboxamides have insect repelling activity against cockroaches equal to or exceeding that of DEET (diethyl-*m*-toluamide) (79). Another Givaudan patent application describes the use of a series of (–)-menthyl carbamates as insect repellents, but is silent relative to their cooling activity (80).

COLD RECEPTORS AND MECHANISM OF ACTION

The underlying process in thermoreception, whether hot or cold, is dependent on ion transport across cellular membranes. Cellular membranes consist of an oily phospholipid bilayer, which would be impermeable to ions such as K^+ or Ca^{2+}, except for receptor protein ion channels.

The flow of ions through these gated ion channels can cause rapid changes in ion concentrations, which in turn produce electrical signals that are the basis for many biological processes (80). In the case of thermoreceptors, these are activated when a thermal (or chemical) stimulus excites primary afferent sensory neurons of the dorsal or trigeminal ganglia (81).

In the last 12 years, there has been tremendous progress in determining the various receptor structural sequences. Thermoreceptors belong to the class of transient receptor potential (TRP) channels of which seven subfamilies exist (TRPC, TRPV, TRPM, TRPA, TRPP, TRPML, and TRPN). Six members of three TRP subfamilies are involved in mammalian

Table 4 Thermoreceptor Agonists

Chemical agonist (botanical source)	ThermoTRP
Capsaicin (hot chilli peppers, e.g., Tabasco®)	TRPV1
Piperine (black pepper corns)	TRPV1
Allicin (fresh garlic)	TRPV1, TRPA1
Camphor (*Cinnamomum camphora*)	TRPV3, TRPV1
Δ-9-Tetrahydrocannabinol (*Cannabis sativa*)	TRPV2, TRPA1
2-Aminoethoxydiphenyl borate (synthetic)	TRPV1, TRPV2, TRPV3
4-α-phorbol 12,13-didecanoate (synthetic)	TRPV4
(-)-Menthol (peppermint)	TRMP8, TRPV3
1,8-Cineole, eucalyptol (eucalyptus)	TRPM8
WS-3 (synthetic)	TRPM8
Icilin (synthetic)	TRPM8, TRPA1
Cinnamaldehyde (cinnamon, cassia)	TRPA1, TRPV3
Allyl isothiocyanate (mustard, horseradish)	TRPA1
Benzyl isothiocyanate (mustard, horseradish)	TRPA1
Phenethyl isothiocyanate (mustard, horseradish)	TRPA1

Abbreviation: TRP, transient receptor potential.
Source: From Refs. 80, 84.

temperature-sensitive thermoreception. The closely related TRPV analogs are activated by heat, TRPV1 (≥43°C), TRPV2 (≥52°C), TRPV3 (22–40°C), and TRPV4 (>~27°C), while TRPM8 (<~28°C) and TRPA1 (<~18°C) are activated by cold (80). Certain types of chemical agonists activate these same thermoTRP channels. TRPV1 was the first thermoreceptor characterized and is referred to as a vanilloid receptor, as it is activated by capsaicin as well as heat. The cold and menthol receptor, TRPM8, was characterized by McKemy, Neuhausser, and Julius (82) and by Peier et al. (83) in 2002. Paradoxically, the cold receptor TRPA1, which is activated by noxious cold to produce a pain-like sensation, produces a human sensorial effect often described as "hot."

Table 4 provides examples of chemical agonists that activate these thermoTRPs.

All of these thermoTRPs are gated Ca^{2+} channels consisting of six transmembrane domains (TM1–TM6) flanked by large N- and C-terminal cytoplasmic domains (80). A schematic representation is shown in Figure 10 with the putative ion channel between TM5–TM6 in TRPM8, which is activated by menthol and other cold stimuli. In the case of TRP channels, it has been shown that they can oligomerize into tetramer assemblies, which presumably modulate the calcium ion gating processes (85–87).

Much of the knowledge gained on TRP activation by chemical stimuli has been derived by genetic expression of putative receptor domains and measurement of Ca^{2+} flux intensity by fluorometric imaging assays. Behrendt et al. used this technique to screen 70 odorants and menthol-related substances for activity on the recombinant cold-menthol receptor TRPM8

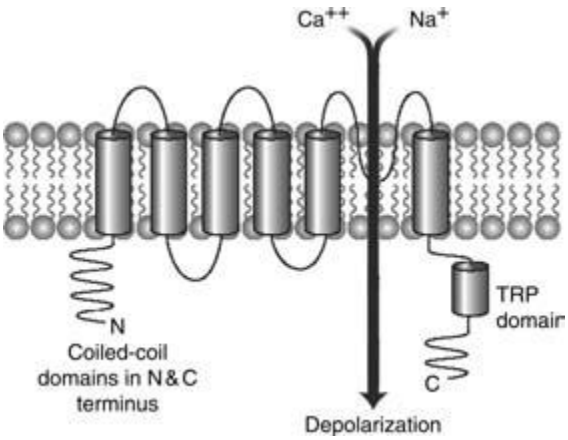

Figure 10 TRPM8 receptor channel.

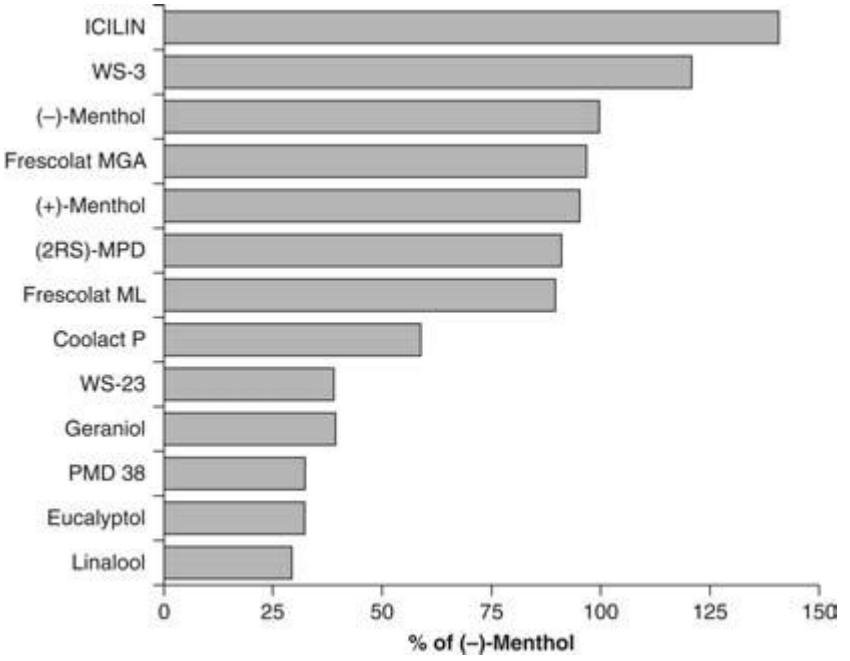

Figure 11 Efficacy of coolants on the TRPM8 receptor (by Ca^{2+} fluorometric assay). *Source*: From Refs. 72, 73, 88.

(mTRPM8), as expressed in HEK293 cells (88). The percentage efficacy of the most active candidates as compared to menthol is shown in Figure 11.

Although the fluorometric assay technique does not always translate into the human perception scale, it is already being used in industry to screen for promising new coolants (89).

In conclusion, from peppermint to menthol and to a plethora of new novel cooling compounds, we are now beginning to understand the importance of cooling substances even in the genetics of life. From early menthol-camphor-based over-the-counter (OTC) pharmaceuticals, which created famous trademarks such as Vicks® Vaporub and Mentholatum® in the early 20th century, to improved modern toothpastes, gums, breath fresheners, and cosmetic lotions, we expect this is just the beginning of even greater things for the future.

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66 Oral Cosmetics

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INTRODUCTION

As a living tissue, the skin requires nourishment to stay healthy. This nourishment cannot be provided by topical preparations only, but it largely comes from foods that are rich in vitamins, minerals, essential fatty acids, and other nutrients, which are crucial for optimal skin health and wellness. Skin functioning relies on specific nutritional needs, as evidenced by the development of skin disorders in response to various nutritional deficiencies (1,2). Supplementation with vitamins, minerals, and other dietary constituents was shown to improve skin conditions (3–5).

Cosmetics are preparations for topical use, i.e., products intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to the human body for cleansing, beautifying, promoting attractiveness, or altering the appearance. Among the products included in this definition are skin moisturizers, perfumes, lipsticks, fingernail polishes, eye and facial makeup preparations, shampoos, permanent waves, hair colors, toothpastes, and deodorants as well as any material intended for use as a component of a cosmetic product (6). Cosmeceuticals are topical preparations specifically designed to improve the appearance of aging skin (7). The term "oral cosmetics" is used in the present chapter with respect to dietary supplements, which claim to have a beneficial, physiological effect on skin, hair, or nails, i.e., these are preparations for oral use only, such as capsules, tablets, liquids, or granulates. In this chapter, we will focus on oral antiaging preparations.

AIM OF ORAL COSMETICS

Intrinsic and Extrinsic Aging

Human skin represents the body's barrier to the external environment preventing it from mechanical damage, noxious substances, invading microorganisms and radiation. Moreover, the skin plays an important role in homeostatic regulation, controlling water retention, sensory perception, and immune surveillance (8,9). Aging leads to several changes in the skin and its appendages (i.e., hair and nails). These changes can be broadly categorized as either intrinsic aging (chronobiological) or photoaging (actinic aging). Oral cosmetics can be formulated to slow down this aging process.

Intrinsic aging is, by definition, inevitable and thus not subject to manipulation through changes in human behavior. Skin that ages intrinsically is smooth and unblemished and characterized by some deepening of skin surface markings (small wrinkles). Histologically, such skin manifests epidermal and dermal atrophy, reduced numbers of melanocytes, Langerhans cells, fibroblasts, and increased cell architecture disorders (10,11). Telomere shortening combined with metabolic oxidative damage is believed to play a major role in the intrinsic aging process (11,12).

Conversely, extrinsic aging is engendered by factors that originate externally and are introduced to the human body, such as smoking, excessive alcohol consumption, poor nutrition, and chronic exposure to the sun (13). Sun exposure is considered to be the most significantly deleterious to the skin; 80% of facial aging is believed to be due to chronic sun exposure (11). Leathery surface of the skin with blotches, yellowing, and deep wrinkles comprise the clinical presentation of photoaged skin. A marked decrease in collagen,

glycosaminoglycans, and proteoglycans is observed. Losses in tone and elasticity, epidermal atrophy, and distinct alterations in collagen and elastic fibers are also associated with photoaged skin. In aged skin, collagen is characterized by thickened fibrils, organized in ropelike bundles that appear to be in disarray in comparison to the pattern observed in younger skin. Alterations in elastic fibers are so strongly associated with photoaged skin that "elastosis," an accumulation of amorphous elastin material, is considered pathognomonic of photoaged skin (9,11,14–17). Compounds, which stimulate the synthesis or inhibit the degradation of connective tissue components (e.g., collagen, keratin, and glycosaminoglycans), may slow down the aging process or even rejuvenate the dermis and its appendages. Such compounds are potential candidates to be used in oral cosmetics.

Photoaging

Extensive research in the area of photoaging over the past decade has resulted in an improved understanding of the molecular mechanism of the aging process. Ultraviolet (UV) light penetrates into the skin; depending on its wavelength, it interacts with different cells that are located at different depths. UV light of the shorter wavelengths (UVB, 280–320 nm) is mostly absorbed in the epidermis and predominantly affects epidermal cells, i.e., keratinocytes, while longer-wavelength UV light (UVA, 320–400 nm) penetrates deeper and can interact with both epidermal keratinocytes and dermal fibroblasts. Melanin pigmentation of the skin absorbs UV light and thus protects skin cells from the detrimental effects of UV exposure. Once UV light has reached the cells of the skin, the different wavelengths exert their specific effects. UVA light mostly acts indirectly through generation of reactive oxygen species (ROS). "ROS" or "pro-oxidants" is a collective term for oxygen-derived species, i.e., oxygen radicals (e.g., superoxide anion, hydroxyl radical) and certain non-radicals (e.g., peroxides) that easily convert into radicals (18). ROS exert a multitude of effects such as lipid peroxidation, activation of transcription factors, and generation of DNA damage. While UVB light can also generate ROS, its main mechanism of action is the direct interaction with DNA via induction of DNA damage (12,19–21).

The skin's enzymatic antioxidant defense includes an enzymatic and a nonenzymatic system (Table 1). Copper-zinc superoxide dismutase (SOD), manganese SOD, catalase (CAT), and the selenoenzyme glutathione peroxide (GPX) have a direct antioxidant function, i.e., SOD converts superoxide anion into hydrogen peroxide, whereas CAT and GPX degrade hydrogen peroxide into water. Nonenzymatic antioxidants are classified into two groups, namely, endogenous (e.g., glutathione, α-lipoic acid) and dietary antioxidants such as vitamins and polyphenolic compounds (e.g., flavonoids). Cases of increased ROS generation and/or a depletion of the antioxidant levels will cause oxidative stress defined as a disturbance in the balance favouring ROS generation and leading to potential tissue damage (21,22). The use of oral supplements, which contain antioxidants (e.g., polyphenols, vitamin E and A), or compounds, which stimulate the enzymatic antioxidant system (e.g., selenium compounds to stimulate GPX activity), may protect the dermis against oxidative stress and prevent tissue damage.

Immune Function and Inflammation

In the last two decades it has become clear that the skin is an essential part of the immune system (23). Reduced immune function and inflammation can alter skin condition and

Table 1 Primary Components of the Human Antioxidant Defense System

Enzymatic	Nonenzymatic
Direct antioxidant function:	*Endogenous antioxidants*:
SOD	α-lipoic acid, glutathione,
GSHPx	melatonin, coenzyme Q
CAT	
Indirect antioxidant function:	*Dietary antioxidants*:
Glutathione-S-transferase	Vitamin C and E
GSSG reductase	Polyphenolic compounds

Abbreviations: SOD, superoxide dismutase; CAT, catalase; GSHPx, glutathione peroxidase; GSSG, Diglutathione.

functioning. Sunburn is a well-known acute effect of sun exposure and is clinically visible as erythema triggered by inflammation. After a certain threshold of UV exposure is reached, delayed and prolonged vasodilatation allows the passage of lymphocytes and macrophages into the tissue, which induces inflammation. Increased dietary intake of antioxidants or oral anti-inflammatory compounds was suggested to reduce UV irradiation-induced eythrema (3), i.e., these compounds are useful in the formulation of oral cosmetics claiming specifically photoprotection.

ACTIVE COMPOUNDS IN ORAL COSMETICS

Screening of Active Compounds

Clearly, the oral route of administration requires other product characteristics compared with a classical, topical cosmetic, and key issues in the development of an oral cosmetic are toxicology, bioavailability (absorption and distribution), and metabolization of its components. In vitro studies and animal studies are useful to study the mechanism of action, but studies in humans are required to document the efficacy of oral cosmetics to validate product claims. Observational studies do not have control over product exposure (e.g., longitudinal study) and are therefore limited to identifying associations between a dietary ingredient and skin benefits; i.e., such studies cannot provide a sufficient basis to determine whether a significant correlation between a product and a benefit reflects an underlying rather than a chance relationship. Intervention studies are more reliable since the investigator can control exposure of the study population to the investigated product. Nevertheless, these clinical trials should be randomized, placebo controlled, and double blinded to minimize bias of the study results. In addition, any change in dietary habits should be avoided, and the intake of other food supplements should be controlled during the complete study. The use of validated bioengineering methods combined with both clinical observations by the investigator and observations by the participant using standardized questionnaires are highly recommended to evaluate the efficacy of the treatment. Intervention studies to study the effect on the dermis are lengthy (5–12 months), and the use of a placebo with an identical appearance (e.g., galenic form, color, taste, and odor) compared with the active product is essential to determine the seasonal influence and the subjective effect on the investigated parameters. A parallel study design (i.e., separate groups of volunteers administered different products) is preferred since seasonal influence may bias the results from crossover studies (i.e., one group of volunteers administered different products).

Vitamins

Vitamin A (retinol and β-carotene derivatives), C (ascorbic acid), and E (tocopherols) are dietary antioxidants of particular interest when formulating oral cosmetics. Combined oral supplementation with vitamins C and E as well as carotenoids provides significant antioxidant activity in human skin with demonstrated UV protection and enhancement of cutaneous immune response (8,24).

In a randomized, placebo-controlled double-blind study, a food supplement (Seresis®, Pharmaton, Switzerland) containing a combination of carotenoids (β-carotene and lycopene), vitamins C and E, selenium, and proanthocyanidins was administered for three months in healthy females, and the effect on UV-induced matrix metalloproteinases (MMP-1, MMP-9) was investigated as a marker of photoprotection (24). In fact, the expressions of MMP-1 and MMP-9 are known as markers of the activity of the endogenous defense mechanism and are mainly induced by UVB irradiation. After supplementation and following UV irradiation, a significant difference was found between active treatment and placebo for MMP-1 with an increased level in the placebo group compared with a decreased level in the active-treated group. The changes in MMP-9 showed a similar but nonsignificant trend.

La Ruche and Cesarini investigated in a double-blind, parallel, placebo-controlled trial the photoprotective effect on the skin of combined intake of α-tocopherol (14 mg) and retinol (2700 μg) for three weeks in sixteen healthy subjects. Partial protection was observed compared with placebo at a low irradiation dose (suberythemal) against the formation of sunburn cells. No difference between placebo and active treatment was observed for the minimal erythema dose (MED) of UV, which was needed to induce erythema (25).

Combined oral intake of α-tocopherol, β-carotene, lycopene, and selenium for seven weeks was found to improve the epidermal defense against UV-induced damage in an open, single-arm study in 25 healthy volunteers (26). The individual UV sensitivity was measured as the actinic erythema threshold, and skin biopsies were collected to quantify lipoperoxides and to evaluate melanogenesis. After treatment with the antioxidant complex, a significant elevation of the actinic threshold, a general reduction of UV-induced erythema, and a reduction of lipoperoxide levels were observed, respectively.

Combined intake for 50 days of high doses of α-tocopherol (2 g/day) and ascorbate (3 g/day) were found to increase the MED compared with placebo in 40 healthy volunteers (27). These observations were confirmed with lower dosages, i.e., after eight days supplementation with 671 mg vitamin E and 2 g vitamin C daily, the MED increased compared with baseline in 8 of 10 subjects, whereas in the placebo group, the MED was unchanged in 6 of 10 subjects and decreased in 4 subjects. These studies indicate that combined supplementation of moderate to high doses of vitamin E and C exerts a photoprotective effect.

Several studies indicate that combined intake of a carotene mix such as β-carotene, α-carotene, cryptoxanthin, zeaxanthin, and lutein lowers the degree of erythema after exposure to UV irradiation and that this photoprotective effect is more pronounced when carotenes are combined with vitamin E (28,29). A recent study observed a decrease in sensitivity toward UV-induced erythema after about 10 to 12 weeks of dietary intervention with carotenoids and flavonoids (30). Overall, β-carotene exhibits some photoprotection, but carotenoids like lycopene still need more investigation. Because of concern about possible negative effects from large doses, most experts agree that getting carotenoids from foods is the safest.

Vitamin C is an essential cofactor of prolyl-4-hydroxylase, an enzyme that hydroxylates prolyl residues in procollagen, elastin, and other proteins with collagenous domains prior to triple helix formation (31). Hydroxyproline in elastin has no known function, but prolyl hydroxylation is essential for efficient collagen production. However, to our knowledge no clear benefit of oral vitamin C supplementation was demonstrated in humans on collagen synthesis in the skin or in a collagen-related process such as wound healing (32,33).

Biotin is an essential cofactor for several carboxylases, which catalyze vital steps in intermediary metabolism in the skin. Deficiency of biotin is known to manifest in various skin disorders, including dermatitis, scaling, and alopecia (34). Supplementation with large doses of biotin (2.5 mg) for six to nine months in subjects with documented brittle nails resulted in an increase of nail thickness and in a reduction of splitting of the nails (35).

Minerals

Few studies have been published, which investigate the effects of oral supplementation of minerals on aged skin. Zinc is an essential element of more than 200 metalloenzymes, including the antioxidative enzyme SOD, and has anti-inflammatory actions. Zinc is a component of enzymes required for DNA replication, gene transcription, and RNA and protein synthesis (36,37). Roughened skin and impaired wound healing have been reported in association with a mild zinc deficiency, implicating changes in the skin (38).

There is a popular belief that zinc deficiency can cause hair loss, but no such correlation is found in published data for alopecia areata (39) or telogen effluvium (40).

Césarini et al. (26) demonstrated that significant photoprotection can be provided by four- to seven-week supplementation with a specific antioxidant combination of vitamins, lycopene, and selenium. Selenium is present in the form of selenocysteine in the active center of the antioxidative enzyme GPX. Selenomethionine was shown to protect skin cells from UV-induced damage, DNA oxidation, and lipid peroxidation (41). The effect on skin health and skin aging of supplements containing a combination of lycopene, lutein, β-carotene, vitamin E, and selenium was investigated in a placebo-controlled study in 39 healthy volunteers. Roughness and scaling significantly improved after 12 weeks supplementation in the active group compared with that in the placebo group (42).

Lassus published in 1993 an open study concerning the effect of silicon supplementation on the skin and hair in 50 women with biologically aged skin and fragile hair or brittle nails (43). The study showed that combined treatment of oral and topical colloidal silicic acid had a beneficial effect on biologically aged skin structure and on the condition of hair and nails. The dermal thickness increased significantly after 90 days of supplementation. In addition, the hair was significantly thicker and less fragile, and nail brittleness had improved. However, no

evidence was presented to support the fact that the colloidal silica was actually absorbed in the gastrointestinal tract; i.e., it is not clear if the observed effects on the skin are the result of the oral or the topical treatment with colloidal silicic acid. In fact, it was clearly demonstrated in other studies that polymerized forms of orthosilicic acid, such as colloidal silica, are not bioavailable (44). Furthermore, seasonal changes may have biased the observed effects on the hair, skin, and nails since no placebo control was used in this study.

Polyphenolic Compounds

Polyphenolic compounds are widely distributed in higher plants and are an integral part of the human diet. In the last decade, the antioxidant activity of flavonoids and other polyphenols such as proanthocyanidins have been studied in detail (45,46).

Silymarin is a mixture of polyphenolic flavonoids derived from the seeds of the milk thistle plant *Silybum marianu* and has been shown in several animal studies to exhibit antioxidant, anti-inflammatory, and immunomodulatory properties, which may contribute to preventing or reducing photoaging (47), especially since silybin (most active component) was demonstrated to be available in skin after oral intake.

Pycnogenol® is the registered trademark of a standardized extract obtained from the bark of French maritime pine, which contains a mixture of procyanidins, also called proanthocyanidins, and phenolic acids, which are potent radical scavengers (48,49). Proanthocyanidins can also be found in grape seed, grape skin, bilberry, cranberry, black currant, green tea, black tea, blueberry, blackberry, strawberry, black cherry, red wine, and red cabbage. Pycnogenol has been suggested to support collagen skin density as it displays physical affinity to collagen and elastin and protects it against proteolytic degradation (50,51).

The efficacy of an oral supplement containing vitamins C and E, carotenoids, selenium, zinc, amino acids and glycosaminoglycans, and blueberry extract and Pycnogenol, respectively, was tested in a double-blind, placebo-controlled study in 62 women. After a six-week supplementation, skin elasticity and skin roughness improved in the active group compared with that in the placebo group (8).

Phytoestrogens

Phytoestrogens are polyphenolic nonsteroidal plant compounds with estrogen-like biological activity, which are classified in four main groups based on their chemical structure, i.e., isoflavonoids, flavonoids, stilbenes, and lignans (52). Since phytoestrogens are structurally similar to estrogen 17-β-estradiol, they may exhibit selective estrogen-modulating activities.

For women, particularly in the postmenopausal years, acceleration of chronologic aging is enhanced by the loss of estrogen, which causes a rapid loss of collagen during the first five years after menopause. It is assumed that phytoestrogens such as soy isoflavones may mimic the effects of estrogen in skin and reduce skin changes in postmenopausal women. A double-blind study in 26 middle-aged women indicated that oral intake of 40-mg soy isoflavone aglycones per day improved the extend of fine wrinkles at the lateral angle of the eyes after a 12-week supplementation compared with baseline. However, no significant differences in fine wrinkles were found between the active treatment group and the placebo group after supplementation, which may indicate that the observed improvement in the active group was biased by seasonal changes (53).

It is well documented that systemic hormonal replacement therapy (HRT) with estrogens, or combinations of estrogen-glucocorticoid, in postmenopausal women improves the gross appearance of their skin, resulting in decreased slackness, wrinkling, and roughness. At the microscopic level, HRT seems to affect mostly dermal collagen, increasing its content and augmenting dermal thickness (8,54,55). It should be noted, however, that HRT has been correlated with increased cancer risk (56,57). Furthermore, HRT is a drug therapy and certainly cannot be categorized as an oral cosmetic.

Glycosaminoglycans

It was suggested that extracts derived from marine fish cartilage have a repairing effect on photodamaged skin. In an open study, 10 females with sun-damaged skin were treated with 0.5 g/day glycosaminoglycans derivatives (Imedeen®, Soeberg, Denmark) for 90 days (58). After 90 days of treatment, all signs of sun damage had improved, and brittleness of hair and nails was normalized in all cases. These clinical observations were confirmed by changes in skin

thickness and elasticity; however, the obtained results may have been biased by seasonal changes since a control group was missing in the study. In a second double-blind, placebo-controlled study, 30 females of the same age range and with similar signs of sun damage were treated with 0.5 g/day glycosaminoglycans derivatives or placebo for 90 days. The results in the glycosaminoglycan-treated group corresponded to those in the first study, whereas no response to treatment was observed in the placebo treatment group (58). Kieffer et al. showed that after three months of treatment with glycosaminoglycans derivatives (Imedeen) there was no significant improvement in photoaging of the skin compared with placebo or baseline. The study was continued for another nine months in an open design i.e., without the use of a control group. After one year of treatment, a significant improvement was found compared with baseline in the investigator's evaluation of fine lines and overall photoaging, and, respectively, in density measurements by ultrasound, transepidermal water loss, and skin smoothness (59).

Eskelinen and Santalahti (60) studied the effect of oral intake of natural cartilage polysaccharides (Vivida®, Helsinki, Finland) on sun-damaged skin in 15 women aged 40 to 60 years. After 90 days of treatment, significant improvements compared with baseline were found in the active group, respectively, for the clinical evaluation of skin condition (e.g., dryness, thinning, and wrinkles), epidermal and dermal thickness by ultrasound, and the erythemal index, whereas no changes were observed in the placebo group.

Choline-stabilized Orthosilicic Acid

Choline-stabilized orthosilicic acid (ch-OSA) is a specific complex with high bioavailability (61), and the effect of ch-OSA on connective tissue (e.g., bone and skin) was evaluated in animal studies and in placebo-controlled clinical studies. Ch-OSA is a specific concentrated form of orthosilicic acid with choline as stabilizing agent. Physiological concentrations of orthosilicic acid were found to stimulate collagen type I synthesis in skin fibroblasts in vitro (62). Choline is a precursor of phospholipids such as phosphatidyl choline, which is an essential component of cellular membranes.

Supplementation of young animals with low doses of ch-OSA resulted in a significant higher hydroxyproline content in the dermis (63) and increased femoral density (64,65). Oral intake of ch-OSA for 20 weeks in 50 women with photoaged skin resulted in a significant positive effect on skin microrelief and skin mechanical properties compared with that in placebo group, suggesting a regeneration or de novo synthesis of collagen fibers in the dermis. Assessment of hair and nail brittleness on a visual analogue scale indicated a significant improvement in the ch-OSA group, whereas no change was observed in the placebo group (66).

The effect of ch-OSA on hair was further investigated in a randomized, double-blind, placebo-controlled study in 48 women with fine hair. Hair morphology and tensile properties were evaluated before and after treatment with validated methods. Oral intake of ch-OSA had a positive effect on tensile strength, including elasticity and break load, and resulted in thicker hair (67). The authors suggested that the observed increase in cross-sectional area of the hair shaft after ch-OSA supplementation may be explained by a stimulation of the collagen synthesis by fibroblasts in the dermal papilla, which determine the volume of the hair follicle.

Polyunsaturated Fatty Acids

Common food sources of *n*-3 polyunsaturated fatty acids (PUFA) are cod liver oil, fish oil, and marine animals with a high amount of fat, such as mackerel, salmon, and menhaden. A few studies have assessed the photoprotective effects of dietary intakes of fish oil. Orengo et al. (68) observed a small but statistically significant increase in the MED after intake of a fish oil–enriched diet for four weeks, showing that a relatively low dose (2.8 g eicosapentaenoic acid and 1.2 g docosahexaenoic acid) of fish oil is photoprotective. In another study (69), fish oil consumption (10 g daily) was also found to reduce UV irradiation-induced erythema, but the susceptibility of the skin to lipid peroxidation increased because of the unstable nature of *n*-3 fatty acids.

Dietary fatty acids were reported to be capable of changing the fatty acid composition of membrane phospholipids of immune cells, which may modulate the function of these cells. A few studies are published, which evaluate the effect of PUFAs in delayed-type hypersensitivity (DTH) skin tests using a panel of antigens, which have generally been accepted as an important

means to monitor the cell-mediated immunity in vivo. However, variable results were obtained in these studies with respect to the effect on DTH skin reactivity, which illustrates that more research is needed to document the immunomodulating activity of PUFAs (70–72).

The most important benefit of oral *n*-3 PUFA intakes from fish oil may be ascribed to their anti-inflammatory effects. These effects of *n*-3 PUFAs have been reported to be the result of their competition with n-6 PUFAs as a substrate for cyclooxygenase and lipoxygenase, resulting in the formation of less-active prostaglandins and leukotrienes. Interference with inflammatory cascades in the skin may occur through reductions in the synthesis of pro-inflammatory lipid mediators or through reductions in the production of cytokines. Moreover, *n*-3 PUFAs are unstable and may preferably be damaged by free radicals, thereby protecting other structures from attack by free radicals. Nevertheless, to protect the skin against excessive formation of free radicals and lipid peroxidation, appropriate amounts of antioxidants should also be consumed.

Multicomponent Supplements

Murad and Tabibian conducted a randomized, single-blind study in 72 women to evaluate the effect on skin roughness of a five-week supplementation with a combination of glucosamine, amino acids, minerals, and various antioxidant compounds. Women without supplementation were used as a control group, i.e., placebo supplementation was missing in this study. A statistically significant reduction in the number of visible wrinkles and fine lines was observed in the active group but not in the control group. There was no significant change in epidermal hydration in either the control or the active study group (73).

Skovgaard et al. investigated in a placebo-controlled study the effects of a novel dietary supplement (Imedeen Prime Renewal®, Soeberg, Denmark) on skin in hundred postmenopausal women. The supplement contained soy extract, fish protein polysaccharides, white tea extracts, grape seed and tomato extract, vitamins C and E, as well as zinc and chamomile extract. The clinical grading of skin condition and the density of the skin measured by ultrasound structure improved after six months of supplementation in the active group but not in the placebo group (74).

The effect of oral intake of a combination of marine proteins with zinc, copper, vitamins C, E, B3 and B5, α lipoic acid, pine bark extract, red clover extract, tomato extract, and soya extract (DermaVite®, Florida, U.S.A.), respectively, was tested in a placebo-controlled study in 40 women with aged skin (75). There was a significant increase in skin thickness and elasticity after six months of supplementation with the active preparation but not in the placebo group. A significant improvement in global evaluation of skin condition using a visual analogue scale was also observed in the active group after six months of supplementation, whereas no change was observed in the placebo group.

Sixty-two women aged 45 to 73 years participated in a double-blind, placebo-controlled trial to evaluate the efficacy of a proprietary combination of vitamins C and E, carotenoids, selenium, zinc, amino acids, glycosaminoglycans, blueberry extract, and Pycnogenol (see description in this chapter in the sect."Polyphenolic Compounds"), respectively. Compared with that of placebo, it was found that skin elasticity and skin roughness improved significantly after 6 and 12 weeks of supplementation (8).

LEGISLATION CONCERNING ORAL COSMETICS

Oral cosmetics are dietary supplements (food supplements), i.e., the legislation on food supplements should be followed. Considerable differences in such legislation exist between different countries with respect to the maximal doses, the chemical forms of vitamins and minerals, plant species, and product claims, which are allowed to be used in and for dietary supplements. In the European Community, the legislation on both food supplements and health claims was recently harmonized for its member states when the European Commission (EC) Directive on Food supplements (i.e., Directive 2002/46/EC) and the Regulation on Nutrition and Health Claims (i.e., Regulation 1924/2006) came into force.

CONCLUSION

Sun avoidance and the use of sunscreens are well established as primary components in antiaging regimens, although these are still underappreciated by many people. Clearly, sun

avoidance is not easy to manage and is often impossible. Consumer-driven demand has led to the development of products to counteract the signs of aging skin. Functional foods positioned as beauty enhancers are a recent concept in Western countries. Bearing this in mind, the development of novel or more active cosmetics (cosmeceuticals) or dietary supplements, which specifically target the skin, hair, and nails (oral cosmetics) is one of the most exciting and promising ways in which the future of cosmetology and dietetics may address human health needs and well-being (76). Considerable research and firsthand experience of physicians have shown that using topical creams in conjunction with dietary supplements leads to superior results compared with using either skin care or supplements alone. It may come as a surprise to many consumers that only few ingredients in topical skin care products have the capacity to penetrate far enough into the dermis to ameliorate deep wrinkles. Therefore, using a combination of topical and oral cosmetics will likely be the favored recommendation in the near future to develop efficient antiaging therapies.

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67 Hair Conditioners

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INTRODUCTION

Despite myriad claimed benefits, the primary purpose of a hair conditioner is to reduce the magnitude of the forces associated with combing or brushing of the hair (1), especially when wet (2,3). This is generally accomplished by the deposition of conditioning agents that lubricate the hair fiber, diminishing surface friction and, therefore, combing forces (4).

In general, deposition of a conditioning agent also causes the hair to feel softer and more moisturized. Another secondary benefit is the reduction or prevention of flyaway hair (5), especially by cationic conditioners (6). Besides making the hair more manageable, increasing the ease of combing also improves the ability to align the hair fibers in a more parallel configuration, which can result in an increase in hair shine, even if the shine of the individual fibers is not increased (7). Some ingredients can also form a film on the hair surface that provides color retention benefits for color-treated hair (8).

A number of other benefits have sometimes been claimed or implied for conditioners, including repair of damaged hair, strengthening of hair, repair of split ends, vitamin therapy, etc. Some of these are marketing hype or are based on laboratory conditions or concentrations not found under actual usage conditions. In this chapter, we will confine ourselves to a discussion of only the observable conditioner benefits presented above. The chapter will begin with a discussion of the relationship between hair damage, conditioning, and the state of the hair surface. This will be followed by a discussion of the major classes of conditioning agents currently in use. Finally, we will end with a brief discussion of the auxiliary ingredients necessary for the production of a commercial conditioning product.

CONDITIONING AND THE HAIR FIBER SURFACE

Hair Damage

In previous chapters, it has been shown that hair fibers consist of a central cortex that comprises the major portion of the fiber, surrounded by 8 to 10 layers of overlapping cells termed "cuticle." The cortex is responsible for the tensile properties of the hair (9,10), while the state of the cuticle affects a variety of consumer-perceivable properties including hair feel, shine, combability, etc.

A major function of conditioners is to protect the hair's structural elements, especially the cuticle, from grooming damage. This type of stress, characterized by chipping, fragmenting, and wearing away of cuticle cells, is probably the single most important source of damage to the hair surface (11–13).

A rather extreme example of combing damage can be seen in Figure 1, which shows the results of an experiment in which a tress of virgin hair was washed with a cleaning shampoo and then combed 700 times while wet. Since hair is more fragile when wet (3) and combing forces are higher (2), combing under these conditions insures maximum damage. It can be seen that damage to the cuticle was extensive with many cuticle cells lifted from the surface, while others were completely torn away by the combing process.

The ability of conditioning agents to protect the hair from the above type of damage can be seen in Figure 2, which shows the results of an experiment in which a tress was washed with a high-conditioning 2-in-1 shampoo and then combed 700 times while wet. In this case, because the conditioning agents in the shampoo reduced combing forces, the hair surface is seen to be intact with evidence of only minor chipping and fragmenting of cuticle cells. This

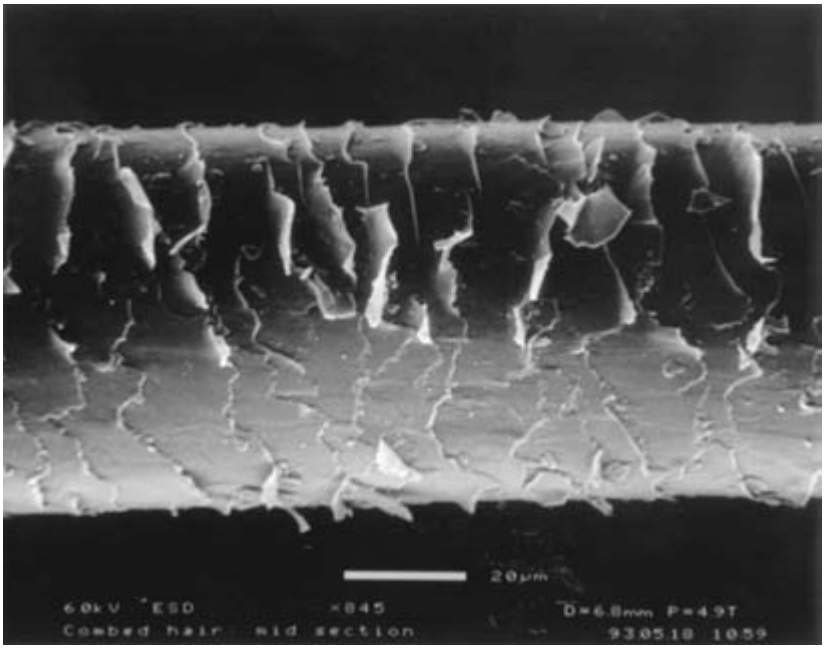


Figure 1 Typical SEM of hair taken from a tress washed with a cleaning shampoo and then combed 700 times while wet. Note raised and chipped cuticle cells and areas where cells have been completely torn away. *Abbreviation*: SEM, scanning electron micrograph.

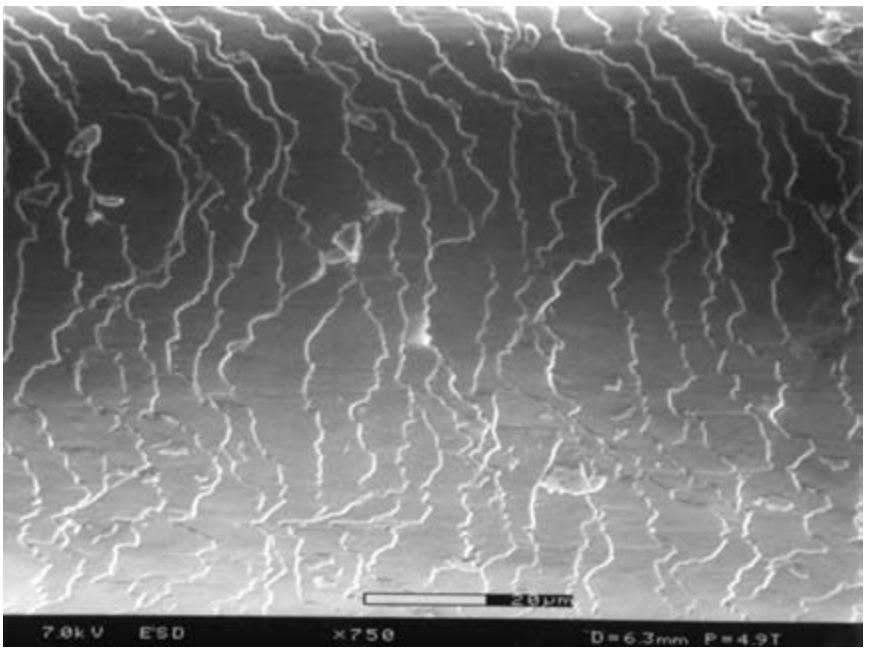


Figure 2 Typical SEM photo of hair taken from a tress washed with a high conditioning 2-in-1 shampoo and then combed 700 times while wet. Note the minimal damage compared with that in Figure 1. *Abbreviation*: SEM, scanning electron micrograph.

demonstrates the important role conditioners can play in maintaining the integrity of the hair fiber.

Heat produced by the use of appliances can also cause hair damage. Many styles require the use of blow dryers and/or curling irons, which can produce temperatures of 200°F to 400°F (14). Steam can be released from the hair fiber causing bubbling and buckling of the cuticles, especially if the hair is not completely dry while being curled. In addition to minimizing combing forces, to protect the hair from this type of damage, certain conditioning polymers can provide added protection in the presence of heat, resulting in increased characteristic life of the hair fiber (15).

Hair Damage and the Cuticle Surface

The susceptibility of a hair fiber to grooming damage and the type of conditioner most effective in preventing this damage is affected to a large degree by the nature and state of the hair surface. It is therefore helpful to precede a discussion of conditioning agents with a presentation on the hair surface and how it affects conditioner requirements and deposition.

Virgin Hair Surfaces

Hair that has not been chemically treated is termed "virgin hair." The cuticle surface of virgin hair in good condition is hydrophobic (16,17), in large part, as a result of a layer of fatty acids covalently bound to the outermost surface of the cuticle (epicuticle) (18,19). As a result of its protein structure, however, the hair surface has an isoelectric point near 3.67 (20), which insures that the surface will contain negatively charged hydrophilic sites at the ordinary pH levels of hair care products. This mix of hydrophobicity and hydrophilicity affects, of course, the types of conditioning agents that will bind to the virgin hair surface.

The situation is further complicated by the fact that the negative charge density on virgin hair increases from root to tip. This is primarily a result of oxidation of cystine in the hair to cystine S-sulfonate and cysteic acid as a result of exposure to ultraviolet (UV) radiation in sunlight (21,22). The tip portions of the hair, being older than the root portions, will have been exposed to damaging (11) UV radiation for a longer period of time and will therefore be more hydrophilic, again affecting the nature of species that can bind to these sites.

In addition to greater UV damage, the tips of hair are also subject to greater combing damage. One reason for this damage is simply that, being older, the tip portions will have been exposed to more combing. In addition, the surface friction of hair tips is higher (C. Reich, unpublished data) so that combing forces increase as one moves from root to tip. Finally, the ends of hair are subject to unusually high combing stress as a result of entangling during the combing process (2). This eventually results in destruction of the covalently bound lipid layer and a feeling of dryness at the tips. Because of this damage, the tip ends of hair require more conditioning than the rest of the fiber. Without sufficient conditioning, the cuticle layer is eventually lost, resulting in a split end. An example is seen in Figure 3, which clearly shows the exposed cortical cells.

Chemically Treated Hair Surfaces

Chemical treatments, such as perming, bleaching, and permanent dyeing, can all cause significant damage to the hair fiber (3,11,23–25). In addition to causing tensile damage, all these treatments, which include oxidative steps, modify the surface of the hair, introducing negative charges as a result of oxidation of cystine to cysteic acid (3,11,23,24,26). This can result in transformation of the entire fiber surface from a hydrophobic to hydrophilic character.

All of the above treatments also increase surface friction considerably (3,4,27,28), resulting in a significant increase in combing forces. The result is that the hair feels rough and dry and is subject to extensive grooming damage. Because of this damage, treated hair generally requires more conditioning than virgin hair. By using a conditioner, one can prolong the health of the hair fiber. It has been found that cuticle cells on damaged, chemically processed hair are in better condition when a conditioner is used as part of the grooming

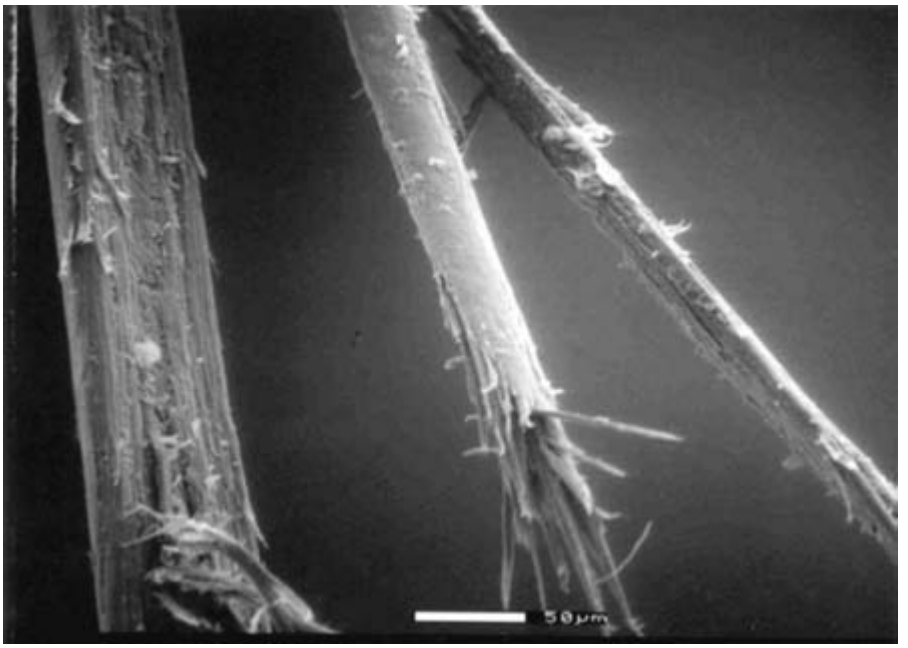


Figure 3 SEM photo of a split end. Note the exposed cortex and the complete loss of cuticle cells on the fiber surface. *Abbreviation*: SEM, scanning electron micrograph.

process (29). Therefore, using a conditioner is particularly meaningful in improving the condition and health of chemically processed hair fibers.

COMMERCIAL CONDITIONERS

The commercial hair conditioners produced to deal with the above problems have appeared in almost every conceivable form, including thick Vaseline pomades, creams, gels, mousses, lotions, and spray mists.

Categorizing by application method, conditioners have been marketed as regular rinse-off conditioners, intensive treatment conditioners, and leave-in products. The first, regular rinse-off conditioners are normally applied after shampoo, followed by a rinsing step. This is the most common form of conditioner sold.

Intensive treatment conditioners are used similarly to the above products, but are not meant for daily application. They are used, instead, for intensive treatment and a higher degree of conditioning. These products generally contain a higher level of active ingredients that are kept on the hair for a longer period of time prior to rinsing. Intensive conditioners are typically sold as thicker creams to provide the perception of higher conditioning.

Leave-in products usually are lighter and can potentially provide more significant benefits than the above rinse-off products since everything applied stays on the hair until the next shampoo. Leave-in conditioners come in various forms, such as detanglers, leave-in lotions, and sprays. They are marketed either for single application or multiple applications during the day.

Despite the wide variety of forms available, most commercial conditioners are oil-in-water emulsions in lotion form, having viscosities somewhere between 3000 and 12,000 centipoises. In addition, despite the different forms and positioning, most commercial conditioners contain the same general classes of conditioning agents with differences mainly in concentrations, numbers of different agents, and particular members of a conditioning class employed.

The major classes of conditioning agents used in commercial products are surveyed in the following sections. Example formulae taken from the patent literature are listed below for some of the various forms of conditioning products.

Hair conditioner (30)	
Ingredients	Weight (%)
Water	q.s. to 100
Stearyl alcohol	2.50
Stearamidopropyl dimethylamine	1.00
Mineral oil	0.50
Cyclomethicone	0.25
Propylene glycol	0.50
Distearyl dimonium chloride	0.75
Hydroxyethylcellulose	1.00
Citric acid	0.20
Polyvinylpyrrolidone	0.10
Formalin (preservative)	0.10
Fragrance	0.20

Deep hair conditioner (31)	
Ingredients	Weight (%)
Water	q.s. to 100
Cetyl alcohol	6.00
Stearamidopropyl dimethylamine	1.50
Mineral oil, heavy	0.50
Propylene glycol	1.00
Distearyl dimonium chloride	1.00
Citric acid	0.20
Germaben II (preservative)	0.50
Fragrance	0.40

Conditioning spray (32)	
Ingredients	Weight (%)
Trimethylolpropane triisostearate	1.00
Methyl myristate	1.00
Cetyl alcohol	1.00
Monoalkyl trimethyl ammonium chloride	0.2
Preservative	0.1
Perfume	0.1
Denatured ethyl alcohol	q.s. to 100

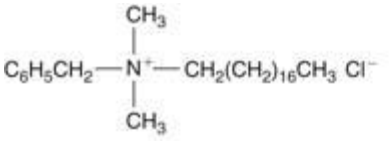
Conditioning styling gel (33)	
Ingredients	Weight (%)
Sodium PCA (50% aqueous solution)	2.00
Glycerin	1.50
Hydrolyzed collagen	0.50
Carbomer 940	0.35
SD alcohol 40	25.00
Nonionic surfactant	0.50
Fragrance	0.10
Water	q.s. to 100

KEY INGREDIENTS OF HAIR CONDITIONERS

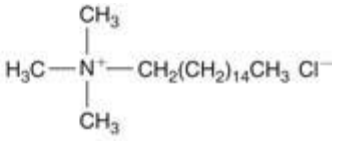
Cationic Surfactants

Cationic surfactants in the form of quaternary ammonium compounds (quats) are the most widely used conditioning agents in commercial products (34–36). Among the reasons for this popularity are their effectiveness, versatility, availability, and low cost.

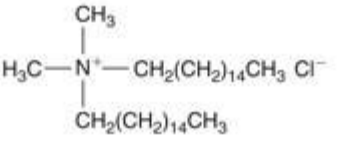
Important examples of these quats include stearalkonium chloride, cetrimonium chloride, and dicetyldimonium chloride. Different counter anions, such as chloride, bromide, and methosulfate, have been used with these materials.



Stearalkonium Chloride



Cetrimonium Chloride



Dicetydimonium Chloride

Because of the positive charge on the quats such as the ones described above, they are substantive to hair and bind to negative sites on the hair surface. Treatment with these quats results, therefore, in a hydrophobic coating on the fiber that render the hair softer and easier to comb (37). Buildup of static charge (flyaway) is also greatly reduced as a result of this surface modification (6).

Another consequence of the positive charge on quats is that deposition increases with increasing negative charge on the hair surface. This is seen in Table 1, which shows the results of an experiment in which hair tresses were treated with 1% stearalkonium chloride and then rinsed. Compared with the roots, 22% more quat was found to bind to the tips of virgin hair, while deposition of stearalkonium chloride on bleached hair was found to be more than twice that on untreated fibers.

This result is important since, as was discussed above, damaged portions of the hair, which generally carry a greater amount of negative charge from either environmental damage or chemical treatment, require a greater amount of conditioning. The fact that cationic surfactants can supply this increased conditioning makes them effective on a wide variety of hair surfaces. This is a major factor in the widespread use of these types of conditioning agents.

Research conducted at TRI/Princeton has shown that the type of deposition and degree of penetration into the hair fiber depends on the size or molecular weight of the compound. The interaction between cationic conditioners and the hair fiber mainly occurs at the surface; however, low–molecular weight materials may penetrate the interior via intercellular diffusion. Cetrimonium bromide (CETAB), e.g., can penetrate the cuticular sheath as well as cortex (39).

Conditioner Properties and Hydrophobicity

Many important properties of quaternary ammonium conditioners are related to the degree of hydrophobicity of the lipophilic portion of the surfactant. Thus, increasing the length of the

Table 1 Binding of Stearalkonium Chloride to Human Hair[a]

Type of hair	Quat deposition at roots (mg/g hair)	Quat deposition at tips (mg/g hair)
Virgin hair	0.649	0.789
Bleached hair	1.62	1.83

[a]Data taken from Ref. 38.

alkyl chain of a monoalkyl quat and, therefore, making it more hydrophobic leads to increased deposition (40–45) on hair. Cetrimonium chloride, as a result, deposits on hair to a greater extent than laurtrimonium chloride. Increasing the number of alkyl chains also increases the deposition so that tricetylmonium chloride exhibits greater deposition than dicetyldimonium chloride, which, in turn, is more substantive than the monocetyl quat.

This dependence of deposition on degree of hydrophobicity indicates that van der Waals forces play an important role in deposition of quaternary ammonium conditioners (45). This conclusion is consistent with the entropy-driven deposition demonstrated by Ohbu et al. (46) and Stapleton (47) for a monoalkyl quat and a protonated long-chain amine.

Increased hydrophobicity also correlates with increased conditioning by quats (40–43,48). Thus, cetrimonium chloride provides light to medium conditioning, while dicetyldimonium and tricetylmonium chlorides provide heavier conditioning. Detangling and wet combing, in particular, improve significantly from monocetyl to dicetyl to tricetyl quats; differences in dry combing and static charge among these compounds are not as significant.

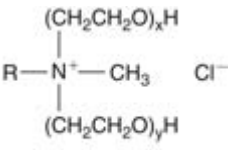
Increased conditioning with increased hydrophobicity is probably due to, in part, the increased deposition of quat on hair. Data from Garcia and Diaz (49), however, indicate greater improvements in wet combing from heavier conditioning quats even when present on the hair in much lower amounts than less hydrophobic species. The degree of hydrophobicity of a quat must, therefore, play a direct role in the conditioning efficacy of these compounds (37).

Note that on some types of hair, the greater substantivity of higher conditioning quats can lead to buildup with repeated use and result in limp, unmanageable hair. This is especially true, e.g., for untreated, fine hair. Different quats or mixtures of conditioning agents are, therefore, suitable for different uses or different types of hair. A tricetyl quat might be used, e.g., in an intensive conditioner meant only for occasional use.

The length and number of alkyl chains of quats also determines water solubility of these compounds. Monoalkyl quaternaries up to cetrimonium chloride are water soluble, e.g., distearyldimonium chloride is water dispersible, while tricetylmonium chloride is insoluble in water (43).

Compatibility with Anionics

The quaternium compounds normally used in commercial conditioners are not generally found in shampoos because of its incompatibility with common anionic detergents (44). Introducing hydrophilic groups into the quat can increase compatibility with anionics. An example is the class of ethoxylated quaternaries, termed "ethoquats." Typical members of this class are polyethylene glycol (PEG)-2 cocomonium chloride, where x + y equal 2 and R is a



Ethoxylated quaternary

C12 alkyl chain, and PEG-15 stearmonium chloride, where x + y equal 15 and R is a C18 chain.

Both of these quats are compatible with typical anionic detergents. As would be expected from the above discussion, however, introducing hydrophilic groups decreases the conditioning efficacy of these materials (40,43). They are, therefore, suitable only in light conditioning formulations. Furthermore, conditioning shampoos based on ethoquats would not be expected to be very effective as a result of low deposition of the detergent-soluble ethoquat complex.

Other detergent-soluble quats have been produced. These include alkylamidopropyl dihydroxypropyl dimonium chlorides (50), lauryl methyl gluceth-10 hydroxypropyl dimonium chloride (51), and even a hydrolyzed ginseng-saponin quaternary derived from Korean ginseng saponin (52). Although certain advantages have been claimed for these surfactants, particularly low irritation, they all suffer from much the same conditioning limitations as the ethoquats.

Other Cationic Surfactants

In addition to the above examples, numerous other cationic surfactants are in use or have been proposed for commercial products. One example of a compound that has been receiving increasing use is the behentrimonium (C22) quat. This quat exhibits significantly reduced eye and skin irritation compared with the corresponding C18 conditioner due to the longer fatty chain length. In addition, superior conditioning and thickening properties have been claimed (53).

Another interesting example is hydrogenated tallow octyl dimonium chloride (54). This material is quite substantive and provides high conditioning as a result of its two hydrophobic chains. Unlike conventional dialkyl quats, however, this particular conditioner is soluble in water as a result of branching (2-ethylhexyl) in the octyl moiety. This solubility makes the compound much easier to formulate into a commercial product.

Several patents (55–61) have disclosed immidazoline-based quats containing the immidazoline ring and fatty chains. Some patents have claimed a softening effect on fabrics or hair. Conditioner compositions using these types of quats have also been disclosed (62,63).

Concern for the environment has led to the synthesis of ester quats that exhibit increased biodegradability and environmental safety. One such example is dipalmitoylethyl hydroxyethylmonium methosulfate, an ester quat based on a partially hydrogenated palm radical (64).

Other cationic surfactants used in conditioners include quats derived from guerbet alcohols (48) (low to high conditioning depending on length of the main and side alkyl chains), distearyldimonium chloride (high conditioning), and the quaternized ammonium compounds of hydrolyzed milk protein, soy and wheat protein, and hydrolyzed keratin (varying conditioning efficacy depending on alkyl chain length).

Amines

Amines with fatty chains, such as stearamidopropyl dimethylamine, can also be found in many commercial conditioners. These types of materials become cationic after protonation at the low pH normally employed in conditioning products and therefore act as both cationic emulsifiers and conditioning agents. Neutralization is normally required to decrease the pH and convert the neutral compounds to cationic. Different acids may have different effects on the viscosity of the final product.

Lipophilic Conditioners

Quaternary ammonium surfactants in commercial products are almost never used alone. Instead, they are employed in combination with long-chain fatty conditioners, especially cetyl and stearyl alcohols (36). These fatty materials are added to boost the conditioning effects of the quaternary compounds (51). In one study, e.g., addition of cetyl alcohol to CETAB nearly doubled the observed reduction in wet combing forces on hair (65). In another study, using a novel hydrodynamic technique, Fukuchi et al. (66) found that addition of cetyl alcohol to a behentrimonium chloride formulation resulted in a significantly reduced surface friction.

Several workers have studied combinations of cationic surfactants and fatty alcohols. Under the right conditions, these mixtures have been found to form lamellar liquid crystal mesophases and gel networks (67–71) that can greatly increase viscosity and, at the same time, confer stability upon emulsions. As a result of reduced repulsion between cationic head groups when long-chain alcohols are interposed, liquid crystal formation has been observed even at low concentrations (70,71). The ready formation of these extended structures between quats and cetyl and stearyl alcohols, along with the low cost, stability, and compatibility with cosmetic ingredients of the latter are important reasons why these alcohols are so ubiquitous in conditioning formulations.

Long-chain fatty compounds are generally solids at room temperature, requiring heating to incorporate into a product. Care should be taken in manufacturing formulations so that the cooling rate is not so rapid when it interferes with liquid crystal formation. In addition, it has been claimed that improved freeze-thaw stability is conferred upon conditioners when using certain combinations of ethoxylated branched-chain fatty alcohol ethers or esters as stabilizers (72).

Other lipids found in commercial products include glycol distearate, triglycerides, fatty esters, waxes of triglycerides, liquid paraffin, etc.

Polymers

Cationic Polymers

There are numerous cationic polymers that provide conditioning benefits, especially improved wet combing and reduced static charge. Important examples of these polymers are polyquaternium-10, a quaternized hydroxyethylcellulose polymer; polyquaternium-7, a copolymer of diallyldimethylammonium chloride and acrylamide; polyquaternium-11, a copolymer of vinylpyrrolidone and dimethylaminoethyl methacrylate quaternized with dimethyl sulfate; polyquaternium-16, a copolymer of vinylpyrrolidone and quaternized vinylimidazole; and polyquaternium 6, a homopolymer of diallyldimethylammonium chloride.

By virtue of their cationic nature, the above polymers are substantive to hair. The particular conditioning effectiveness of any these materials depends on the polymer structure. In one set of studies, deposition on hair was found to be inversely proportional, roughly, to cationic charge density (73,74). This has been explained by the observation that the higher the charge density, the lower the weight of polymer needed to neutralize all of the negative charge on the hair. Once deposited, however, multiple points of electrostatic attachment make these polymers harder to remove, especially if charge density is high (38,75). Care must be taken, therefore, in formulating conditioners containing these materials to avoid over-conditioning as a result of buildup with continued use.

As with the preceding monofunctional cationics, deposition of polyquaterniums increases on treated or damaged hair (38,75). Unlike common monofunctional quats, however, the first four of the above polymers are compatible to varying degrees with anionic surfactants (75–78). As a result, such polymers are used more often in shampoos than in stand-alone conditioners, although they find some use in leave-in conditioners.

Polyquaternium-10 (PQ-10) and polyquaternium-7 (PQ-7) are two of the most frequently used polymers in commercial shampoos. Both of these polymers form negatively charged complexes (73, 75) with excess anionic surfactant, resulting in reduced deposition because of repulsion by the negatively charged hair surface. The magnitude of this effect depends on the particular anionic employed and anionic surfactant-polymer ratio. In all cases, however, conditioning from shampoos is significantly less than from stand-alone conditioners.

Despite reduced deposition, Hannah (79) has reported that polyquaternium association complexes formed with sodium lauryl sulfate resist removal from hair. Buildup and a heavy, coated feel on the hair can therefore result from conditioning shampoos containing polyquats unless they are carefully formulated.

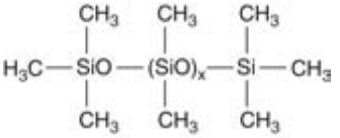
In addition to providing conditioning benefits, some polyquaternium materials have been shown to improve adhesion of the cuticle scales thereby increasing resistance to scale uplift when the hair is stressed. The same effects were observed for at least one quat—CETAB (39).

Other Polymers

In recent studies, other polymeric materials, including amphiphilc polymers (80–82), amphoteric polymers (83), block copolymers (84–86), graft polymers (87, 88), and dendrimers (89), have been investigated for use as conditioning agents, stabilizers, and deposition agents. In part probably because of cost, commercial products containing those novel polymers are rare. However, these research activities may indicate a future trend toward the use of polymers with more complicated structures in personal care applications.

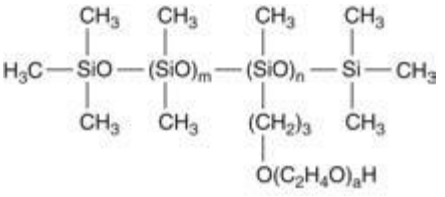
Silicones

The use of silicones in hair care products has increased considerably in the past two decades, although their first incorporation into commercial products dates back to the 1950s. Different types of silicones find use as conditioning agents in a wide variety of products including conditioners, shampoos, hair sprays, mousses, and gels (90). One of the most widely used silicones is dimethicone, which is a polydimethylsiloxane. Other important silicones are

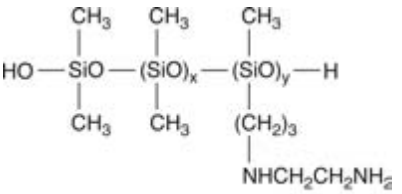


Dimethicone

dimethiconol, which is a dimethylsiloxane terminated with hydroxyl groups; dimethicone copolyol, which is a dimethylsiloxane containing polyoxyethylene and/or polyoxypropylene side chains; amodimethicone, which is an amino-substituted silicone, and silicone quats, which contain permanently quaternized ammonium groups. In general, amodimethicones and silicone quats condition better than dimethicones, which condition better then dimethicone copolyols (91). Presumably this is due to differences in substantivity from rinse-off products. Because of the increased substantivity, care should be taken with amodimethicones and silicone quats to make sure they do not build up over time. Likewise, many dimethicone copolyols are soluble in water and therefore may not be as effective in rinse-off products.



Dimethicone copolyol



Amodimethicone

Many silicones used in hair care products are insoluble and must therefore be emulsified. To increase ease of product manufacture, many suppliers offer silicones as preformed emulsions in addition to the pure material. Emulsions can vary in charge (anionic, cationic, or nonionic), size (microemulsion or macroemulsion), and how they are made (mechanical or emulsion polymerization). The factors affecting deposition of silicones from such emulsions have been reported by Jachowicz and Berthiaume (92,93) and by Hoag et al. (94).

Silicone emulsions can also vary in particle size. Typically, the smaller the size of the silicone particles, the more stable the product emulsion is. Additionally, it has been claimed that reducing the particle size also improves conditioning performance (95). If a preformed silicone emulsion is not used, particle size of the silicone droplets can be controlled by combining the correct amount of heat and shear when making the product.

Conditioning Properties of Silicones

Silicones used in hair care products possess a range of unique properties including lubricity, low intermolecular forces, water insolubility, and low surface tension. These properties permit the silicones to spread easily on the hair surface, thereby forming a hydrophobic film that provides ease of combing and imparts a smooth, soft feel to the hair without greasiness.

The relative conditioning efficacy of silicones compared with other conditioners was demonstrated by Yahagi (96), who found that dimethicone lowered frictional coefficients and surface energy of virgin hair to a greater extent than a series of cationic surfactants, including distearydimonium chloride, a very effective conditioning agent. Dimethicones with molecular weights greater than 20,000 were found to be most effective in reducing surface tension.

Nanavati and Hami (97) measured conditioning on slightly bleached European hair treated with dimethicone fluids and dimethiconol gums. Both types of silicones were found to significantly reduce combing forces on hair. Ease of wet combing was roughly the same for the two silicone treatments, while dimethiconol was found to be more effective in reducing dry combing forces.

Interestingly, under the treatment conditions employed (exposure to silicone solutions for 30 seconds followed by drying without rinsing), deposition of all silicones studied was found to nearly double if tricetylmonium chloride was present in the treatment solution.

Reduction in combing forces was also roughly doubled when silicones were deposited in the presence of quat. This latter effect was found to be synergistic; i.e., it depended on deposition of both silicone and quat, and its magnitude was greater than the sum of the individual conditioner contributions.

Wendel et al. (98) used electron spectroscopy for chemical analysis (ESCA) to demonstrate that the presence of amino groups in silicones considerably increases substantivity of these materials. This is a result of the positive charge developed by these groups at the pH commonly found in commercial products.

Comparison of conditioning effects of a series of silicone emulsions on bleached and virgin hair was carried out by Hoag et al. (94). Most of the silicones were dimethicones or amodimethicones, while emulsions were anionic, neutral, or cationic in nature. Diluted emulsions were applied directly to the hair and combing forces measured both before and after rinsing. Prior to rinsing, reduction of combing forces by most emulsions was greater than 80%. This number decreased after rinsing as a result of partial removal of deposited silicone. Unsurprisingly, the least change in ease of combing was found for cationic emulsions, especially those containing amodimethicone. Combing forces on virgin hair increased less than on bleached hair after rinsing, indicating that the silicones were more substantive to this type of hair. This is also unsurprising considering the hydrophobic nature of these conditioning agents.

Further effects of amodimethicones can be seen in work reported by Berthiaume et al. (99) who studied a series of amodimethicone emulsions in a prototype conditioner formulation. Deposition on hair from the conditioner was found to increase with increasing amine content in the silicone. This increased deposition was found, in half-head tests, to correlate with conditioning efficacy, including wet and dry combing, softness, and detangling. A microemulsion in the test series that provided high conditioning was also shown to significantly reduce the color fading caused by shampoo of temporarily dyed hair.

Other Silicones

One silicone that is widely used in conditioners to help improve wet combing is cyclomethicone, which refers to a class of cyclic dimethyl polysiloxanes ranging from trimer to hexamer. Cyclomethicone is volatile and will not remain on dry hair, especially after blow-drying. It helps other conditioning agents to disperse, however, and form films on hair. It also helps improve wet combing and provides transient shine. In addition, cyclomethicone is widely used as a solvent to reduce the viscosity of silicone gums with much higher molecular weights.

Because of its high refractive index, close to that of hair, phenyl trimethicone is commonly used in leave-in conditioners to enhance the shine of hair fibers. To improve substantivity higher molecular weight versions (Si-Tec PTM 1000, and International Specialty Products) and versions that incorporate amino groups (DC 2-2078 fluid, Dow Corning) have been produced.

Newer silicones include dimethicone copolyol phosphates, which are anionic functional silicones and fluorocarbon-modified organosilicones. The copolyol phosphates are able to complex with tertiary amines of cationic hair conditioners and form effective emulsifiers and conditioners (100). The fluorocarbon-modified silicones are very hydrophobic like dimethicone; however, they are claimed to have a lighter and more lubricious feel (100).

Interesting block copolymers with silicone blocks and organic segments have been developed for personal care applications (101). DC CE8401 from Dow Corning Co. is a commercially available example. This material has a unique structure. In contrast to traditional silicone copolyols that have a rake structure, it is a block copolymer containing silicone and polyether segments in its backbone.

Other examples of silicones include blends of these materials, having different molecular weights (102), different functional groups (103,104), and silicones with other hydrophobic oils (105). Those silicone blends have been reported to improve overall conditioning benefits.

2-in-1 Shampoos

Silicones find important application as the primary conditioning agents in 2-in-1 conditioning shampoos. On their introduction in the latter part of the 1980s, these shampoos represented a major advance in hair care technology, providing a significantly higher degree of conditioning

than was then the norm for conditioning shampoos and, at the same time, leaving a desirable soft, smooth feel on the hair.

Conditioning from 2-in-1 shampoos is expected to occur primarily at the rinsing stage, when the shampoo emulsion breaks, releasing the silicone for deposition on hair. This separation of cleaning and conditioning stages permits the shampoo to perform both functions efficiently.

The conditioning agent used most frequently in 2-in-1 shampoos is dimethicone. This silicone can provide good performance in shampoo formulations without excessive buildup on the hair (106). With advances in technology, newer formulations are now employing easier-to-process silicones, such as dimethicone emulsions, amodimethicones, dimethiconols, and copolyols as well as combinations of these different types to deliver the desired level of conditioning as well as improved product aesthetics.

The level of conditioning from 2-in-1 shampoos is lower than that from stand-alone conditioners. This is especially true for treated hair since the greater the degree of negative charge on the hair surface, the lower the substantivity of a hydrophobic material like dimethicone. Many 2-in-1 products contain polyquats, which might be expected to increase conditioning on damaged hair. In shampoos with high levels of anionic detergent, however, polyquat performance on treated hair may be no better than dimethicone as a result of formation of the negatively charged polymer complexes discussed in the section "Cationic Polymers."

Yahagi (96) studied the performance of dimethicone, amodimethicone, and dimethicone copolyols in 2-in-1 shampoos. Ease of combing was found to be similar for hair treated with shampoos containing dimethicone or amodimethicone. Unsurprisingly, soluble dimethicone copolyols did not perform well; insolubility, or at least dispersibility, was required for adequate silicone deposition. In the latter case, dimethicone copolyols were found to provide a somewhat lower level of conditioning than the other two silicones studied, especially once blow-drying was begun. Yahagi also studied silicone effects on foam volume. In these studies, dimethicone was found to significantly reduce foam volume in a model shampoo formulation, while amodimethicone and dimethicone copolyol had a minimal effect on foam.

Auxiliary Ingredients

A number of ingredients, besides conditioning actives, are added to commercial conditioners for functional, esthetic, and marketing purposes (107). These include fragrances, dyes, preservatives, thickeners, emulsifying agents, pearlizers, herbal extracts, humectants, and vitamins. Some of these are discussed in the following sections. The literature also contains many examples of such additives (36,108–112).

Preservatives

Preservatives are necessary to insure the microbiological integrity of a conditioning product. If the product contains high concentrations of ethyl alcohol (generally 20% or above), additional preservatives are not needed and the product is described as self-preserving.

For other products, a wide variety of preservatives are available; in general, combinations of different preservatives provide the broadest possible protection. Every commercial product that is not self-preserving must be carefully tested over time for adequacy of preservation. Most of the preservatives used in personal care products are described in the *Cosmetic Preservatives Encyclopedia* (110).

Thickeners

As described in the section "Lipophilic Conditioners" thickening as a result of liquid crystal formation in those products containing common quats and fatty alcohols. Cationic conditioning polymers can also act as thickeners. Many formulations may require additional thickening agents. Hydroxyethylcellulose, a nonionic cellulose ether compatible with cationic surfactants and stable over a wide pH range, is the most common thickening agent added to conditioning products (36). In addition to providing increased viscosity, this material stabilizes viscosity over time.

Polyamides may also be used to thicken formulations. A commercial product, Sepigel (which contains polyamide, laureth-7, and isoparaffin) can be used to emulsify and thicken lotion or cream conditioners. Other thickeners are described in reference (111).

Polyacrylate-based thickeners such as carbopol have been widely used in personal care products. However, in the past these thickeners have not always been compatible with cationic surfactants. Recently, new thickeners based on polyacrylate chemistry have been commercialized to address this issue. Structure Plus polymer (National Starch & Chemical Company) and Carbopol Aqua CC polymer (Noveon Inc.) are two examples that are used at low pH and show good cationic surfactant compatibility.

Humectants

Many conditioners contain humectants whose purpose is to attract moisture. Examples are propylene glycol, glycerin, honey, chitosan, and hyaluronic acid. These materials are not expected to be very effective in rinse-off products.

Emulsifiers

As discussed in the section "Lipophilic Conditioners", the fatty alcohol/quat combinations found in common conditioners confer stability on product emulsions. If necessary, other emulsifiers may be added to improve stability. Information on emulsions and emulsifiers may be found in the literature (112,113) as well as from manufacturers' technical bulletins. Most emulsifiers used in conditioners are nonionic, including ethoxylated fatty alcohols, ethoxylated fatty esters, and ethoxylated sorbitan fatty esters.

CONCLUSION

The foregoing sections have surveyed the action and properties of a diverse assortment of commercially available conditioning agents. The availability of a large selection of conditioning materials enables the formulator to tailor products to a wide variety of people having differing conditioning needs and preferences. Thus, a person having short, straight hair in good condition might desire a conditioner primarily to control flyaway. Such a need could be satisfied by one of the ethoquats, which provide light conditioning benefits together with very good static control. A person having long, heavily bleached hair, on the other hand, would require improved hair feel, ease-of-combing, and manageability. These benefits could best be provided by a trialkyl quat.

Those people sensitive to hair feel might prefer a product containing a silicone as a secondary conditioner. Other people might prefer the convenience of a 2-in-1 shampoo. In many cases, both 2-in-1 shampoos and stand-alone conditioners are used to condition the hair.

There are a number of ways in which one might satisfy the conditioning needs of a target population. It is anticipated that the information in this chapter will help the formulator to quickly choose the best conditioning system for a given purpose. It is also hoped that the material in this chapter will help the formulator to effectively evaluate new conditioning agents and even to work with synthetic chemists as well as suppliers to design new conditioning compounds to solve particular problems.

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68 Measuring Hair

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THE STRUCTURE OF HUMAN HEAD HAIR

Desmond Morris referred to humans as "Naked Apes" (1). While the body hair on most humans is vellus, we grow hair on our heads that is far longer than the more abundant coat on most other mammals. Human head hair typically ranges from 20 to more than 100 μm in diameter (2), and some people can grow their hair to lengths of more than 5 ft.

The hair shaft is composed of columnar cortical cells that are surrounded by the overlapping cuticle scales. In some hairs, there may also be a more porous area in the center called "the medulla." A transmission electron micrograph (TEM) of a horizontal cross-section through the hair follicle at the level of the reticular dermis is shown in Figure 1. The various layers of the follicle and the hair can be clearly seen. Working in from the glassy collagen layer of the dermis (D) that surrounds the follicle, the outer root sheath (ORS), Henley's (he) and Huxley's (HU) layer of the inner root sheath, the innermost cuticle of the inner root sheath (CL), and the cuticle (CU) and cortical (CO) layers of the hair shaft itself can be seen. This particular hair does not show clear evidence of a medulla.

Human head hair has 6 to 10 layers of cuticle when it emerges from the scalp (3). A cross-section of a hair stained with silver methenamine is shown in Figure 2. Each cuticle cell is connected to the cortex, and the cells overlap from the root to the tip at an angle of about 5° (4), causing the well-known directional difference in hair friction (5). Cuticle cells are flat and approximately square, being about 50 μm on a side and about 0.5-μm thick (4). Each cuticle cell is composed of a cell membrane complex (CMC) and three distinct internal layers of differing sulfur content—α-layer, exocuticle, and endocuticle. The CMC has two 3-nm thick β-layers on either side of an 18-nm thick δ-layer. The upper β-layer, which faces out from the hair, has an outer surface of 18-methyl eicosanoic acid (18-MEA) (6) that is covalently attached to proteins by thioester bonds (7,8).

Cortical cells are roughly cylindrical being 50 to 100 μm long and 3 to 6 μm in diameter. They have longitudinal flutings and may separate into smaller fingerlike structures. The cells are closely packed together in the hair shaft so that the fluted surfaces interlock putting their cell membrane complexes in contact (9).

The mechanical properties of hair are dominated by the keratin microfibrils in the cortex, while the optical and surface properties are dominated by the cuticle and particularly by the state of the 18-MEA on the surface.

DETERMINATION OF HAIR DIMENSIONS

In order to determine tensile properties such as the elastic modulus, it is necessary to accurately measure the cross-sectional dimension. Determining the cross-sectional dimensions of a hair is not always straightforward. Not only is hair a thin fiber, it is not necessarily uniform in cross section. While Caucasian hair is generally considered elliptical in shape, significant variations from ellipticity can occur. With African-American hair, the problem is compounded by the high elliptical ratio and the presence of many nonuniform shapes. There is now laser micrometer that can be used for this purpose. Dia-stron makes an instrument that can be interfaced with their automated tensile testers. Several measurements must be made along the fiber, and the fiber must be rotated to ensure measurement of the major and minor axes of the ellipse. Perhaps the most accurate method of determining the cross-sectional area of a hair is to section it and measure the diameters directly from the micrographs (10).

Figure 1 TEM of cross-section of human hair. *Abbreviation*: TEM, transmission electron micrograph. *Source*: Micrograph courtesy of Raymond Boissy.

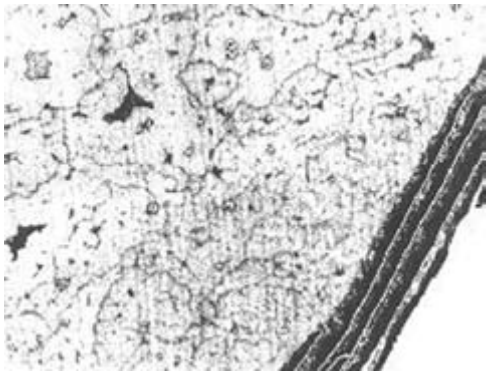

Figure 2 Cross-section of a hair stained with silver methenamine.

TENSILE PROPERTIES OF HAIR

The mechanical behavior of hair is frequently studied in extension by obtaining a stress/strain curve. Stress/strain curves for hair can be obtained by using tensile meters such as Instron or Dia-stron.

Figure 3 shows stress/strain curves for the adjacent sections from the same hair fiber in extension in water and at 50% relative humidity (RH). The curves can be characterized by three

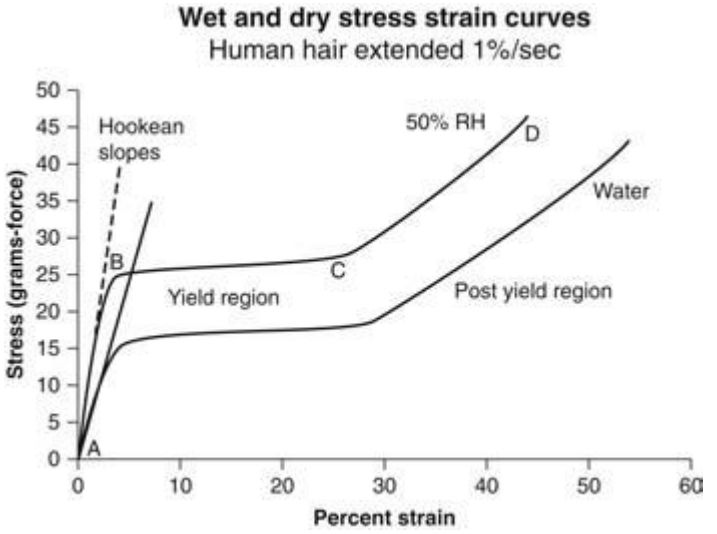

Figure 3 Stress/strain curve for hair at 50% RH. Data obtained using a Dia-stron miniature tensile tester.

different regions. The curve in the first region (A-B) is approximately linear and a slope can be determined. This part of the curve is often called "the Hookean region," and it extends to about 102% of the equilibrium length of the fiber (2% strain). The slope of the Hookean region is considerably higher in dry hair. Between 2% and 4% strain, the curve "turns over" into the yield region (B-C). In the yield region, very little increase in force is required to increase extension. In the post-yield region (C-D), which typically begins between 25% and 30% deformation, the force again increases markedly with strain. For the data presented in Figure 3, under the specified testing conditions, the slope in the post-yield region was about one-fifth of that in the Hookean region of the dry fiber. It is also observed that there is little difference in post-yield slopes between the wet and dry sections of the hair.

Published papers on the mechanical properties of keratin fibers date back to the 1920s and to the work of Speakman (11) who first reported on the effect of water on keratin mechanical properties. Since that time, extensive research on hair and wool has led to an interpretation of each region of the stress/strain curves of keratin fibers in terms of changes occurring in the molecular structure. Much of the seminal work in this area was carried out by Max Feughelman, and his book on the subject gives an excellent and detailed overview of the physical properties of keratin fibers (12).

The curve in the Hookean region (A-B) can be used to calculate Young's modulus of elasticity, E. The elastic modulus is the stress divided by the strain, so the cross-sectional dimensions of the fiber must be known accurately. The sample calculation given below was done assuming a circular hair with a diameter of 60 μm using typical numbers for a virgin hair at 50% relative humidity.

Sample calculation of the elastic modulus:

- E = stress/strain = Δ F*L/(ΔL*A).
  Δ F is change in force corresponding to the length change ΔL.
  L is length of fiber.
  A is the cross-sectional area.
- Assuming circular hair is 60 μm in diameter.
  $A = 2.83 \times 10^{-5}$ cm^2 = 2.83×10^{-9} m
- A 10-cm length is extended to 10.2 cm.
  2% extension
- Force change is 22 grams force = 0.216 N.
  1 gram force = 980 dynes = 9.8×10^{-3} N
- $E = (0.216 \times 10\ \text{cm})/(0.2\ \text{cm} \times 2.83 \times 10^{-9}\ \text{m}^2)$
- $E = 3.8 \times 10^9$ N/m^2
- 1 Pal = 1 N/m^2, 1N = 105 dynes, 1 m^2 = 10^4 cm^2
- $E = 3.8 \times 10^9$ Pa = 3.8×10^9 N/m^2 = 3.8 GPa
- In older papers, E is called Y and is reported in dynes/cm^2.
- $E = 3.8 \times 10^{10}$ dynes/cm^2

E is typically about 1.5 to 2.0 GPa for wet hair and 3.5 to 4.0 GPa for hair at 50% to 65% RH. The mechanical properties of hair or wool in the Hookean region and the effect of water on mechanical properties (Fig. 3) can be explained by the two-phase model proposed by Feughelman (13–15). Feughelman's model considers the mechanical properties of the fiber to be determined by a water-impenetrable phase, C, the microfibrils, and a water-permeable phase, M, the matrix. The microfibrils consist of α-helical proteins (keratins) aligned parallel to the fiber axis (16–19), and the matrix is composed of keratin-associated proteins (20), which are packed around the microfibrils. The composite is modeled as a fixed spring in parallel with a spring and viscous dashpot in series; the spring contributes about 1.4 GPa to the Young's modulus and is contained in the water-impenetrable microfibrils. The main resistance to extension of the microfibrils probably comes from the hydrogen bond network in the α-helical proteins (21). The matrix contributes viscous forces, which decay with time, causing stress relaxation. The viscosity of the matrix decreases greatly as the water content of the fiber increases. The two-phase model of keratin fibers accounts for the effects of water on the mechanical properties, the fact that increasing strain rate increases Young's modulus, the stress relaxation behavior in the Hookean region, and the behavior of wet, dry, and permanently set fibers in torsion (15,21).

The Yield Region

Somewhere around 2% to 3% strain, the stress/strain curve "turns over" into the yield region. Past this point, the stress does not increase markedly until about 25% to 30% extension. The mechanical properties of a fiber extended into the yield region can be recovered by relaxing the fiber in water for a few hours if the fiber is not held too long in extension and the extension is carefully confined to the yield region. This fact is of great practical importance in designing protocols to measure the effect of treatments on hair strength, as we will see. X-ray diffraction results have demonstrated that there is a progressive loss of a helical content and a concomitant increase in β-sheet as a fiber is extended through the yield region (22). By the end of the yield region, about 30% of the original α-helix has been unfolded reversibly. Mechanical behavior of keratin in the yield region can be accounted for by application of a Burte–Halsey model (23). The fiber is considered to contain a continuum of units, which can exist in a short state, A (α-helix), or an extended state, B (β-sheet), with an energy barrier between the states. The yield region corresponds to a phase transition between state A and state B at constant stress. This first-order phase transition, producing a length change at constant stress and temperature, is thermodynamically equivalent to the transformation of water to steam, producing a volume change at constant temperature and pressure.

Data from hair strained into the yield region are usually reported as either force at a given extension such as 15% or 20% or the work to extend a hair to a given extension, which is obtained by integrating the area under the stress/strain curve. Hu (24) measured the work to extend hairs to 15% and compared results for Caucasian, Asian, and African-American hair. Results for Caucasian hair, in terms of W_{15} as a function of hair diameter, are shown in Figure 4.

Table 1 shows the correlation equations for the work to extend to 20% for hair from each ethnic group. The work to extend African-American hair by 20% was found to be about two-thirds of that required for either Asian or Caucasian in agreement with other studies showing that hair of African origin is not as strong as either Asian or Caucasian hair (25).

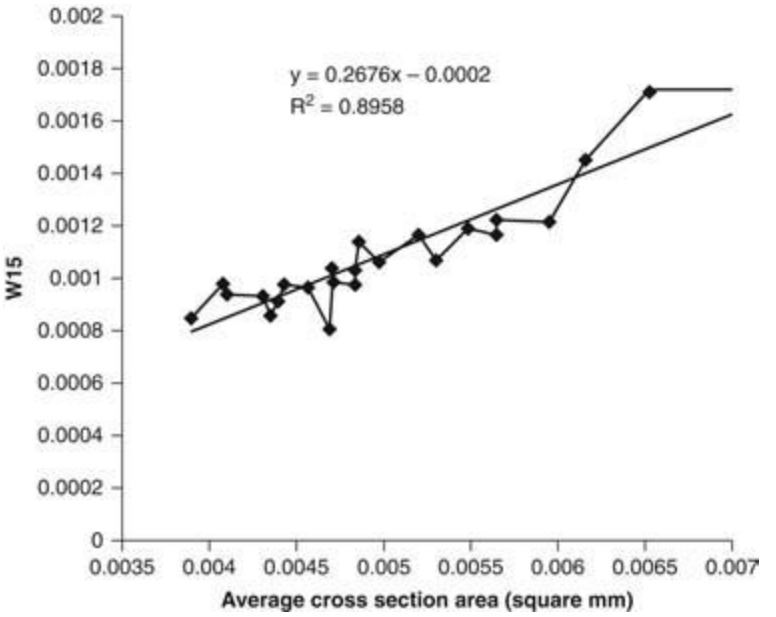

Figure 4 Work (in Joules) to extend hair by 15% versus cross-sectional area.

Table 1 Correlation of Work of Extension to Cross-section Area (A) for Different Ethnic Groups

Hair type	Correlation equation	R^2
Caucasian	W20 = 0.2676A − 0.0002	0.90
African-American	W20 = 0.1844A + 0.0002	0.83
Asian	W20 = 0.2961 − 0.0003	0.91

The Post-Yield Region

The C-D region in Figure 3 is known as the post-yield region. Speakman (11) found the post-yield slope to be independent of the water content of the fiber. This is borne out by the essential equivalence of the slopes (for dry and wet hair) of the C-D region shown in Figure 3. The increased stiffness in the post-yield region apparently results from a covalently bonded network involving cystine. The post-yield slope has been shown to be dependent on the disulfide content of the fibers (26–28).

Tensile Testers

In the past, stress/strain measurements on hair were mostly made using one of the models of the Instron® tensile tester (2,5,29,30). The Instron is a robust and versatile instrument but is more than a bit of overkill for measuring hair mechanics. In recent years, the Dia-stron miniature tensile tester (MTT) has been widely used. The instrument can be equipped with an automated sample head (31) to allow running of up to 100 hairs in one setup and can be interfaced with a laser micrometer system to automatically measure the dimensions of the hair fiber.

Software with the instrument can automatically record the stress/strain curve and report various parameters to the operator, including elastic gradient, work of extension, and breaking load. Evaluation of hair tensile properties has come a long way from the days of tediously measuring data points off a chart recorder with a ruler.

Tensile Measurements of Hair Damage

The most common use of hair tensile properties is for the evaluation of the effects of treatment on hair strength to determine the level of "damage" produced by a given treatment. The mechanical properties of wet hair are greatly affected by treatments that lead to a reduction in the number of disulfide bonds. Measurement of breaking strength may show differences between treatments if they are large and large number of hairs are run, but one must try to select hairs of approximately the same diameter for measurement, if possible. For this reason, many workers have relied on the fact that mechanical properties of hair extend into the yield region, but not beyond, and can be recovered by soaking in water. Beyak et al. (29) extended hair by 20% and measured the force. After recovery, treatments were investigated using each hair as its own control. The average change between tests for 25 untreated control hairs was only –0.33%. In contrast to this, a five-minute "cold wave" treatment led to a 12.6% decline in the force at 20% extension.

Tate et al. (32) and Robbins and Crawford (33) also used mechanical measurements in extension to study hair damage. The study by Robbins and Crawford revealed the interesting fact that significant damage to the cuticle can occur with little or no effect on the tensile properties of hair.

Gamez-Garcia described the use of short-term relaxation measurements from small deformations to assess the effect of oils, emulsions and solutions of salts, amino acids, and proteins on stress recovery in hair fiber (34). The author analyzed the relaxation curves and showed that the curves had a short-term (on the order of minutes) component and a long-term (of the order of hours) component. The medium in which the fiber is immersed was found to have a strong effect on short-term relaxation.

Hu (24) investigated the effects of heat and relaxer treatment using both breaking stress and the work to 15% extension (Fig. 4). Heat treatments were for five minutes. Relaxer treatment was with a commercial relaxer according to label directions. Data are summarized in Table 2 shown below.

Table 2 Effect of Treatment on W15 and Break Stress

Treatment	Change W15 (%)	Change break stress (%)
60°C	1.9	−5.2
115°C	−.02	−9.9
130°C	−3.5	−10.6
160°C	−5.9	−10.5
Relaxer	−25.7	−37.5

It does appear that breaking stress is more affected by heat at low temperature, and the effect of heat on breaking stress was significant at 130°C, while W15 was not significantly affected. This may be due to loss of disulfide bonds that are not extended before post-yield region is reached. To obtain this kind of result for breaking strength, the hairs must be carefully prescreened to be of approximately the same diameter.

MECHANICAL FATIGUE BEHAVIOR

Kamath et al. described an apparatus for studying the mechanical fatiguing of hair (25,32). The instrument subjects the fibers to an impact-loading mode of fatiguing at a constant load and rate of one cycle per second for up to 100,000 cycles. The strain in hair was kept within the Hookean range and the fatigue data were interpreted in terms of the following equation:

$$F(x) = A(x)^{n}$$

where $F(x)$ is the cumulative probability of failure, x is the number of cycles to failure, and A is constant. The exponent n and the number of cycles required for half of the specimens to fail (h_f) were employed to quantify the damaging effect of grooming treatments on hair. The exponent n was found to vary from approximately 0.5 ± 0.052 for untreated hair to 0.11 ± 0.098 for hair after three perming treatments. A concomitant change in half-life parameter, h_f, ranged from more than 100,000 cycles to 3000 cycles. This technique was also found to be useful in evaluating fiber damage as a result of bleaching and perming.

DYNAMIC MECHANICAL ANALYSIS

Dynamic mechanical analysis has not been employed frequently in hair studies. This was probably due to the fact that the old generation instrumentation was difficult to use, and the experiments were very time consuming.

During the last 20 years, several new instruments were introduced. They include the dynamic mechanical and thermal analyzer (DMTA) (Rheometric Scientific, Piscataway, New Jersey, U.S.) and the dynamic mechanical analyzer (Perkin Elmer DMA7), characterized by high sensitivity, broad dynamic range, and high force control.

The dynamic mechanical experiment gives information on both storage and loss modulae (or tan delta) and can provide a complete characterization of viscoelastic properties of hair fibers. The measurements can be performed as a function of time, temperature, or frequency in both stretching and bending modes of fiber deformation. For characterization of hair and hair-care products, the bending mode of operation is of particular interest because it is probably a predominant mode of deformation for "in vitro" hair on the scalp.

An example of the use of dynamic mechanical measurements in hair studies is the pH dependence of storage modulus shown in Figure 5. The data were obtained through the use of the DMTA equipped with a humidity controller and an online treatment attachment (M. Zielinski, unpublished data).

Figure 5A presents the actual DMTA trace, presented as the logarithm of bending modulus as a function of the experiment's time, obtained for a 40-fiber assembly mounted in a frame for a single cantilever bending measurement. The active length of fibers was 1 mm, with an amplitude- and frequency-held constant at 128 μm peak-to-peak and 3 Hz, respectively. The pH was adjusted with HCl and NaOH by using solutions at 22°C continuously flowing over the hair sample.

Figure 5B shows the averaged modulus data from Figure 5A plotted as a function of pH. The results demonstrate a relative constancy of bending modulus in the pH range from 3 to 10, and its decrease in both very acidic ($pH < 2$) and very basic ($pH > 12$) solutions. A similar analysis can be performed for hair exposed to chemical treatments, surfactants, or polymer solutions.

Flexabrasion Testing

Swift has pointed out that the mechanism of hair breaking on the head is probably different from simple breakage in tension (35,36). A method called "the flexabrasion test" may be

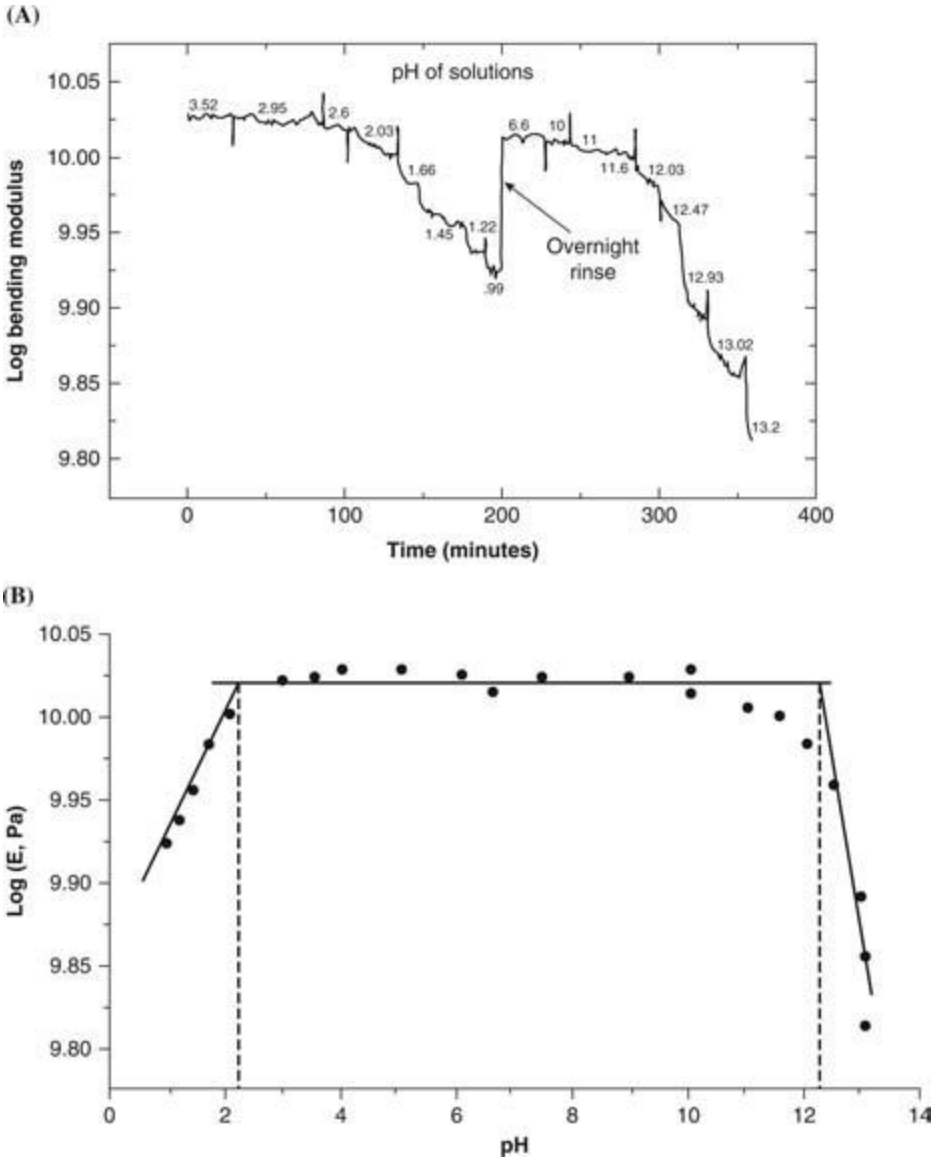

Figure 5 Dynamic mechanical analysis. (**A**) DMTA trace obtained by employing an online treatment procedure (with HCl and NaOH solutions) and shown as the logarithm of the bending modulus. (**B**) Bending modulus of wet intact hair as a function of pH. *Abbreviation*: DMTA, dynamic mechanical and thermal analyzer.

more relevant to the actual consumer experience. In this test, weighted hairs are pulled back and forth across a fine wire by a reciprocating motor as illustrated in Figure 6 on the next page.

The parameter measured is the number of cycles required to break 50% of the hairs. This number has a very high variance from hair to hair. Swift reported data were obtained using adjacent sections of the same hair for treatment and control to reduce the variance. By using three sections from each hair, a control, a damaging treatment and an intervention could be studied. Some of Swift's (36) data are presented in Table 3.

Data from this method are the basis of some rather extreme sounding claims for large increases in hair strength. It is not the tensile strength that is increased by the treatment but the resistance to fraying under repeated abrasion. This method will obviously reflect the presence of treatments that can reduce the friction between the wire and the hair.

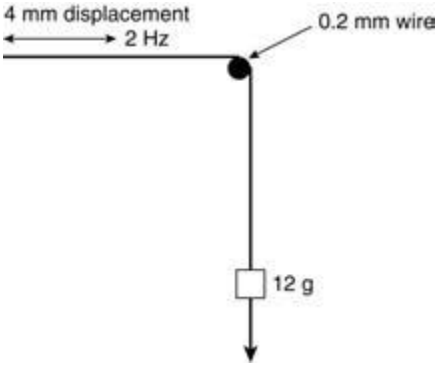

Figure 6 Illustration of the flexabrasion test.

Table 3 Effect of Conditioner on Bleached Hair by Flexabrasion

Conditioner	Bleached	Bleached + conditioner	Difference	Increase (%)	*p*
Leave on	1066.5	1621.5	555.1	52.1	0.04
Rinse off	649.7	1548.9	699.2	82.3	0.01

Torsion and Bending Measurements

Forming a curl from straight hair involves a combination of twisting and bending deformations. Response to torsion or bending stress is highly dependent on hair diameters, as both the bending and torsional moments of inertia depend on the fourth power of cross-sectional dimensions. For example, the resistance to bending of an elliptical hair is given by E^*I_b, where E is bending modulus and I_b is the bending moment of inertia, and by $(\pi/64)ab^3$, where a and b are the major and minor semidiameters of the ellipse. The torsional moment of inertia is given by $I_t = (\pi/4)(a^3b + b^3a)$, while the resistance to twisting is given by $G^*(\pi/4)(a^3b + b^3a)$, where G is the shear modulus. Because of this extreme dependence on cross-sectional dimensions, the cuticle may contribute more to torsion or bending than to extension, especially with very fine hairs.

The shear modulus of a fiber can be determined using a torsion pendulum. In this method, a small cylindrical weight is hung from the hair. Application of torque to the weight causes it to rotate back and forth. If a small white strip or a small mirror is attached to the weight as illustrated in Figure 7, the amplitude and period of the torsional deformation as the weight rotates can be easily determined. The shear modulus can be determined from the following equation:

$$G = (Ml/\omega 2)/I_t$$

where l is the fiber length, I_t is $(\pi/4)(a^3b + b^3a)$ as explained above, and M is moment of inertia of the weight. Another parameter that can be determined from a torsion pendulum is the log

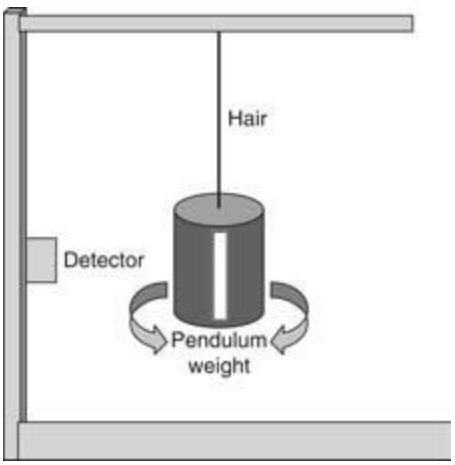

Figure 7 A schematic of a torsion pendulum for the study of hair.

decrement (δ). Log decrement is related to the log of the change in amplitude between one cycle and the next. Log decrement will be increased by treatments or conditions that either increase frictional loss or decrease the storage of elastic potential energy as the fiber twists. Pesuad and Kamath (37) have recently described such a device in detail.

Bogaty (38) first pointed out how important the behavior of hair under torsional and bending strains is to formation and maintenance of hairstyle. He reported that permanent waving decreased the torsional rigidity of hair in the wet state but actually increased it slightly at 65% RH. Harper and Kamath (39) reported similar results for bleached hair. At low RH, the shear modulus of bleached hair was higher than that for untreated hair, but above 70% RH the shear modulus of bleached hair was found to be lower than for untreated hair.

Wolfram and Albrecht (40) carried out torsional measurements on hair using a torsion pendulum. They concluded that the cuticle is very stiff in the dry state and may make a significant contribution to the torsional rigidity, especially for fine hairs. However, in the wet state, the cuticle was found to be so plasticized as to make no contribution to mechanical behavior. On the other hand, Harper and Kamath (39) and Yasuda et al. (41) reported that the cuticle makes a significant contribution to the shear modulus of dry hair.

Bending is also a key component of a hairstyle, but bending measurements are generally not simple to perform. Scott and Robbins (42) described a method for measuring the bending stiffness of hair using a balance. A long hair is draped over a small wire with small weights attached to each end. The bending stiffness can be calculated from the distance between the two ends. It is also possible to measure bending strength by a three-point beam deflection method. This method has been applied to measuring the stiffness of beard hairs (43). Another approach is cantilever beam method as applied by Yasuda et al. (41). The balanced fiber method has the disadvantage of requiring a relatively long fiber but is simpler to use. Wortman and Kure (44) used a similar fiber method to study bending relaxation during permanent waving of hair.

SPECTROSCOPY

Fluorescence Spectroscopy

Fluorescence spectroscopy is employed to measure the wavelength dependence of the intensity of emitted light as a function of the excitation wavelength. Hair and skin are characterized by strong fluorescence because of the presence of tryptophan, kynurenine, tyrosine, and phenylalanine aminoacids in the structure of keratin. Tryptophan has the strongest absorption ($\lambda_{max} = 280$ nm, $\varepsilon_{max} = 4500\ M^{-1}\ cm^{-1}$) and a high-quantum yield of fluorescence. Its fluorescence band, excited at 290 nm, has a maximum in the range from 330 to 350 nm, which is dependent on the extent of hair pigmentation.

The technique has been employed as a sensitive analytical technique to study reactions accompanying hair photo and thermal degradation (45–47). A fluorescence instrument used to study hair is typically equipped with remote fiber optics, which allows for recording the spectra directly from hair fibers. Experiments described in the literature consisted of irradiating hair with UV/visible light and recording emission spectra in a wavelength range where fluorescence emission occurs. Photodegradation studies have shown that tryptophan undergoes photodecomposition that can be quantified by the measurements of the emission intensity at 300 to 550 nm for hair before and after exposure (Fig. 8). The peaks at 350 , 420 , and 465 nm have been assigned to tryptophan, *N*-formylkynurenine, and kynurenine, respectively (45,47). The intensity of emission of all these chromophores is shown to decrease as a result of photoirradiation, with tryptophan emission undergoing the largest change. The phenomenon was shown to occur both in natural outdoor conditions and as a result of artificial light irradiations in a weatherometer. This technique can also be used to determine the extent of hair photoprotection, in terms of tryptophan damage, by incorporating photofilters in hair-care formulations such as conditioners, mousses, shampoos, and hairsprays.

IR and Raman Spectroscopy

Raman and infrared (IR) spectroscopies provide alternative ways to detect vibrational states of molecules. While transitions producing IR bands are due to vibrations of groups with a permanent dipole, those corresponding to Raman bands are due to changes in the polarizability of nonpolar groups as a result of nuclear motion.

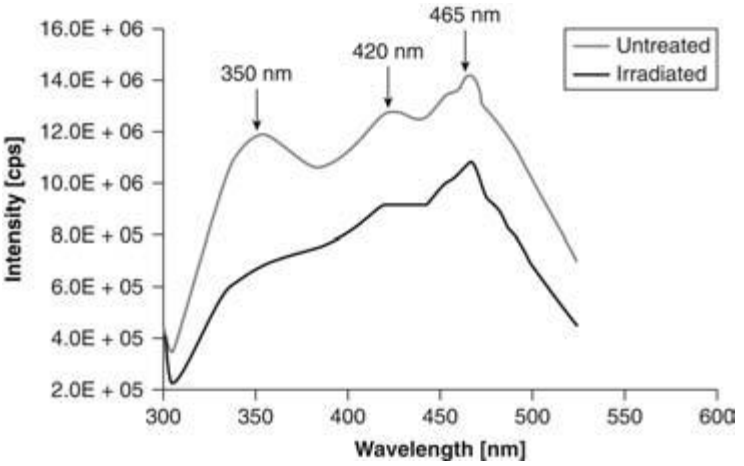

Figure 8 Comparison of the fluorescence spectra of untreated and piedmont hair irradiated for 48 hours in a weatherometer.

In recent publications on hair, fourier transform (FT)-IR spectroscopy was combined with a microscope (referred to as FT-IR microscopy), allowing researchers to examine hair shaft areas in the size range 10 to 100 nm. Bramanti et al. (48) examined micro regions of hair for anagen, catagen, and telogen hair. The data from bulb and shaft areas were analyzed in terms of relative intensities of amide II (1540 cm^{-1}) and amide III (1238 cm^{-1}) vibrations of the protein component of hair structure and O-P-O vibrations (1080 cm^{-1}) of nucleic acids. It was shown on the basis of simple spectra and their derivatives that there was a gradual change in the ratios of absorbance values for nucleic acids/proteins (A_{1080}/A_{1238}) for inferior bulb, central bulb, and suprabulbar and shaft regions for anagen, catagen, and telogen hair. It was suggested that these ratios can be used as reproducible parameters to differentiate the anagen, catagen, and telogen hair phase and to estimate the degree of hair aging.

Other reported applications of FT-IR microscopy included an estimation of the extent of hair oxidation by analyzing the intensity of a peak at 1041 cm^{-1} corresponding to cysteic acid formation in the oxidation of cystine (45).

In a typical Raman experiment, the sample is irradiated with an intense beam of light at a specified frequency u. The emitted light consists of radiation with an unchanged frequency of u (light scattering and refractive index), Raman bands with frequencies at $u + u'$ (Stokes band), and $u–u'$ (anti-Stokes band).

The main advantage of Raman spectroscopy in studies of biological molecules is the low intensity of the water spectrum. A strong IR spectrum of water overlaps the regions where biomolecules have IR absorption bands. The intensity of water spectrum in Raman spectroscopy is relatively weaker, which makes it useful for studies of proteins, etc.

For hair, Raman measurements were reported for unpigmented and bleached hair, employed to minimize the fluorescence effects predominant in more pigmented fibers (49,50). The spectra of untreated hair show a number of bands not observed in the IR analysis. They correspond to disulfide bonds (510 cm^{-1}), tyrosine (646 cm^{-1}, 853/827 cm^{-1}), phenylalanine (1003 cm^{-1}), and tryptophan (1554 cm^{-1}).

The Raman spectra could be used to assess the incurred damaged associated with hair bleaching, permanent waving, and photoirradiation:

- For bleaching, a decrease in intensity of the 510 cm^{-1} band with a concomitant increase in the intensity of a band at 1045 cm^{-1} (SO_3, cysteic acid) was observed.
- For perming, reduced hair showed a peak reduction at 510 cm^{-1} and the appearance of a peak at 2568 cm^{-1} (mercaptan).
- For photodamaged hair, analysis showed a decrease in intensity of the disulfide band with the appearance of vibrations corresponding to sulfur in various oxidation states, including a thiosulfonate bond. In addition to this, there was an increase in the intensity of a mercaptan band and a change in the amide I region corresponding to a disordered protein.

Near Infrared Spectroscopy

Near infrared spectroscopy (NIR) refers to the portion of the IR spectrum in the wavelength range from 1000 to 2200 nm (10,000 –4500 cm^{-1}). The observed bands correspond to overtones and combination of characteristic bond vibrations. The technique is typically employed to study bands corresponding to O-H from water, C-H from hydrocarbons, and N-H for proteins. It may also cause transitions of highly delocalized electronic systems, such as those present in the structure of melanin.

Several authors evaluated NIR spectroscopy for studying hair (51,52). Pande et al. (52) found the technique well suited for measuring relative moisture content of hair in situ. The NIR spectra of hair conditioned at 50% RH and dehydrated by heating to 110°C for 90 minutes are shown in Figure 9. It was concluded that peaks at 1450 and 1935nm are due to water, while the peaks at 1740 cm^{-1} are related to methylene C-H stretch, and the bands at 2051 and 1984 nm are due to protein. The 1900-nm absorption was further used to quantify the amount of water in hair at 50% RH after drying and as a result of moisture regain. The technique proved to be sensitive to detect small differences in the kinetics of moisture regain observed for hair treated with a hair-care product such as conditioner.

Another application described by Pande et al. (52) is the measurements of melanin bleaching during oxidative coloring of hair. Such measurements are possible because the synthetic hair dyes have no effect on the reflectance of hair beyond 750 nm, while natural melanin strongly absorbs in NIR range from 1000 to 1300 nm. The data revealed that the bleaching effect can be quantified in terms of melanin absorption and even small differences between bleaching products can be detected. It should be added that such measurements cannot be performed with a typical colorimeter because of interference from the synthetic dyes.

Integrating Sphere Spectrophotometry

Integrating Sphere spectrophotometry is employed to study the absorbance of light scattering samples such as dispersions of solids and liquids. In cosmetic research, one frequently deals with non-transparent materials, or chromophores incorporated in turbid formulations, or deposited on non-transparent substrates such as skin or hair. In such cases, one cannot employ routine UV-vis spectroscopy because the light scattering results in a very weak intensity of transmitted light. In order to include scattered light, a technique referred to as integrating sphere UV-vis spectroscopy is employed. In this method, the scattered light is focused by

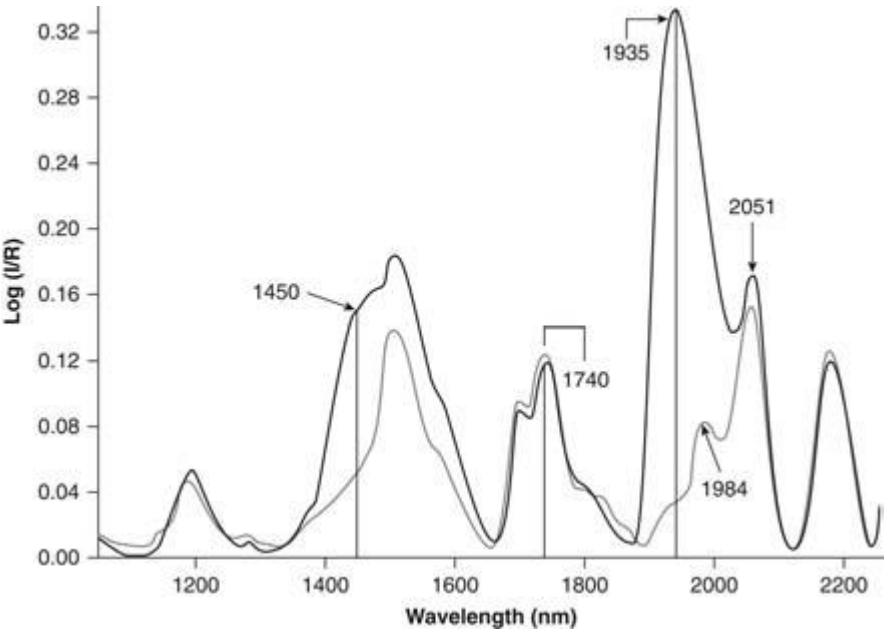

Figure 9 Near-IR absorption spectrum of unpigmented human hair (*fine line*) and the effect of water (*bold line*). *Abbreviation*: IR, infrared.

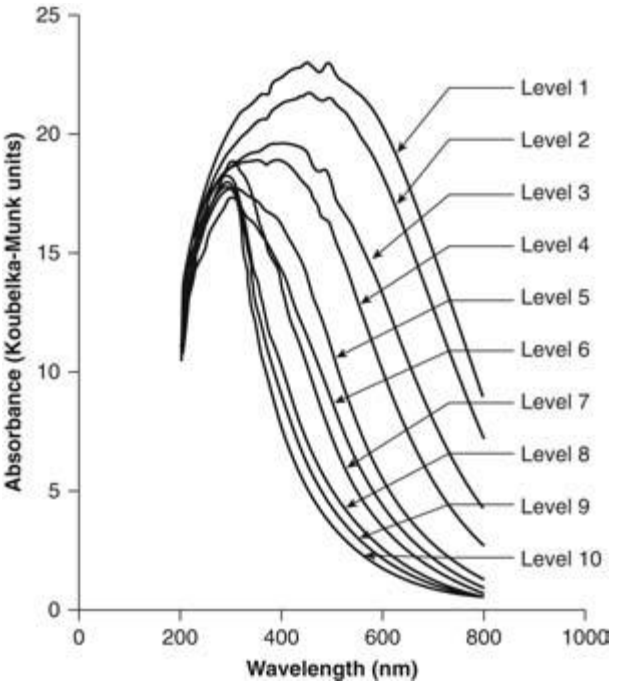

Figure 10 Absorbance (Koubelka-Munk units) as a function of wavelength for hair with various levels of pigmentation. The data were obtained by using integrating sphere UV-vis spectrophotometer in the reflectance mode. *Abbreviation*: UV, ultraviolet. *Source*: Courtesy of Johnson and Johnson Co.

reflecting from the walls of a $BaSO_4$-coated sphere. The spectra can be obtained by UV-vis spectrophotometers equipped with an integrating sphere, which operate in the transmission or reflectance mode (e.g., a Perkin Elmer Model 950). An example of the usage of the instrument in the transmission mode is testing skin-care sunscreen formulation to determine a sun protection factor (SPF) value. The tested product, which is typically a turbid formulation, is spread on the surface of a substrate (e.g., artificial skin), and the spectra are collected in the transmission mode and subsequently converted into Koubelka-Munk absorbance units. By using appropriate controls as well as the erythema action spectrum of sunlight, one can calculate the SPF value of a given product. In hair research, the technique is a quantitative tool to study hair coloration. Figure 10 presents a plot of absorbance (in Koubelka-Munk units) as a function of wavelength for hair with various levels of natural hair color classified by visual grading on the scale from 1 (black) to 10 (white). The data, such as those presented in this figure, can be further processed by, for example, mathematical spectral subtraction to derive information about the hair deposition of sunscreens, artificial hair color, color fading, etc.

X-ray Spectroscopy

The crystalline structure of keratins has been extensively studied in the past by X-ray diffraction (21,22,53,54). A new approach to this problem has been the use of synchrotron radiation, which can produce high quality X-ray diagrams in short experimental times (55).

High-resolution small angle X-ray scattering (SAXS) and wide angle X-ray scattering (WAXS) diffraction patterns were obtained by using high-intensity synchrotron radiation. SAXS diffractograms permitted the calculation of intermacrofibrillar, intermicrofibrillar, and interprotafibrillar distances (88, 67.7, and 40 Å, respectively). WAXS gave the distance between individual helices (5.15 and 9.8 Å).

The key result was that cosmetic treatments, including perming, bleaching, or a combination of both, affect not only the distances between supramolecular elements of hair structure but also the distances between individual protein chains (55). Larger distances between micro-, macro-, and protafibrils are reflected in the increased swelling of chemically treated hair, a well-known phenomenon previously described. On the other hand, an increase in interhelical separation, probably as a result of interaction with water, was unexpected because of previously accepted models that assumed that the crystalline phase of hair structure was impenetrable by water or aqueous solutions of hair treatments.

ESR

Electron spin resonance (ESR) spectroscopy measures the transitions of an unpaired electron between energy levels produced by magnetic field (56). This is due to the phenomenon that an electron spinning at a given frequency can adopt two spin orientations in a magnetic field with each characterized by a different energy. It is possible to induce transitions between electronic spin energy levels by applying electromagnetic radiation with the frequency equal to the electron's precessional frequency. A typical magnetic field range employed in an ESR experiment is from zero to a few tesla units. ESR can only be employed for the detection of unpaired electrons such as free radicals and radical ions. The key parameters employed to characterize the ESR spectrum are (*i*) electron precessional frequency given by

$$\text{Vprec} = 13.95\ [\text{GHzT}^{-1}]g\text{B}_0$$

where B_0 is the strength of the magnetic field in tesla units (T), and (*ii*) g, which is referred to as the Lande factor. The values of g vary from 2.00220 for the ethylene radical to 2.0091 for the trichloromethane radical. A typical precessional frequency for a free electron at a field strength of 0.34 T is about 9500 MHz. The population difference between electrons in different spins states is larger than in NMR experiment for protons (it can be calculated from Boltzmann distribution), and therefore, ESR spectroscopy is more sensitive than NMR Spectroscopy. An ESR spectrum can be obtained for radicals at a concentration as low as 10^{-8} M at room temperature for a volume of a few 10ths of a milliliter in both the liquid and solid state. Also, the time scale of an ESR event is about 10^{-9} seconds; thus, it is faster than that in proton NMR, so the technique can provide information about processes that are too fast for NMR analysis.

As in NMR, an ESR signal of one electron can be split by a magnetic field of neighboring hydrogens according to Pascal's triangle rule. The separation between lines in a multiplet in the ESR spectrum is termed "hyperfine coupling" and is designated by symbol a (in Gauss units). For example, for a methyl radical, the ESR signal will be a quadruplet (with the intensity ratio of 1:3:3:1) at $g = 2.00255$ and $a = 23.0$ G.

In cosmetic chemistry, the application of this technique is limited to the studies of oxidation, antioxidants, and melanin chemistry. For melanins, the ESR spectrum consists of a featureless peak with a line width of about 4 to 6 G and a g value close to 2.004 (57). There is no hyperfine coupling, and the spin concentration is very small, about 4 to 10×10^{17} spins/g. The ESR method of melanin characterization is important because this natural polymer is considered to be a photoprotective and antioxidant agent for skin and hair.

Photoirradiation of hair was also investigated by ESR using spin trapping with DMPO (5,5-dimethyl-1-pyrroline *N*-oxide), which forms DMPO-OH adduct with brown, bleached, and red hair (58). The spectral evidence confirms the formation of oxyradicals during photoirradiation. Bleached and red hair (pheomelanin) was also found to produce more oxyradicals than black hair melanin (eumelanin).

MICROSCOPY

Optical Microscopy

Optical microscopy is employed for a variety of tasks in a cosmetic laboratory. It is very useful to evaluate hair geometrical shape and dimensions, detect the presence of surface deposits, or assess a degree of fiber damage in terms of cortex integrity, state of cuticles, or the presence of split ends. Traditional light microscopy, however, has limited resolution (approximately half of the wavelength of light) and is characterized by limited depth of field. New instruments address this problem by collecting images of the object at various focal lengths and subsequently computing reconstructed in-focus image.

Scanning Electron Microscopy

Electron microscopy requires high vacuum and metal coating of a nonconductive polymer for biological samples. The resolution of this technique is limited to a few nanometers. Scanning electron microscopy (SEM) is usually not sensitive enough to detect adsorbed polymers or a surfactant layer with molecular dimensions. Newer SEM instruments offer ease of use and are capable of producing good resolution images (below a magnification of 5000×) without metal coating. They can be also equipped with energy-dispersive X-ray fluorescence detectors, which

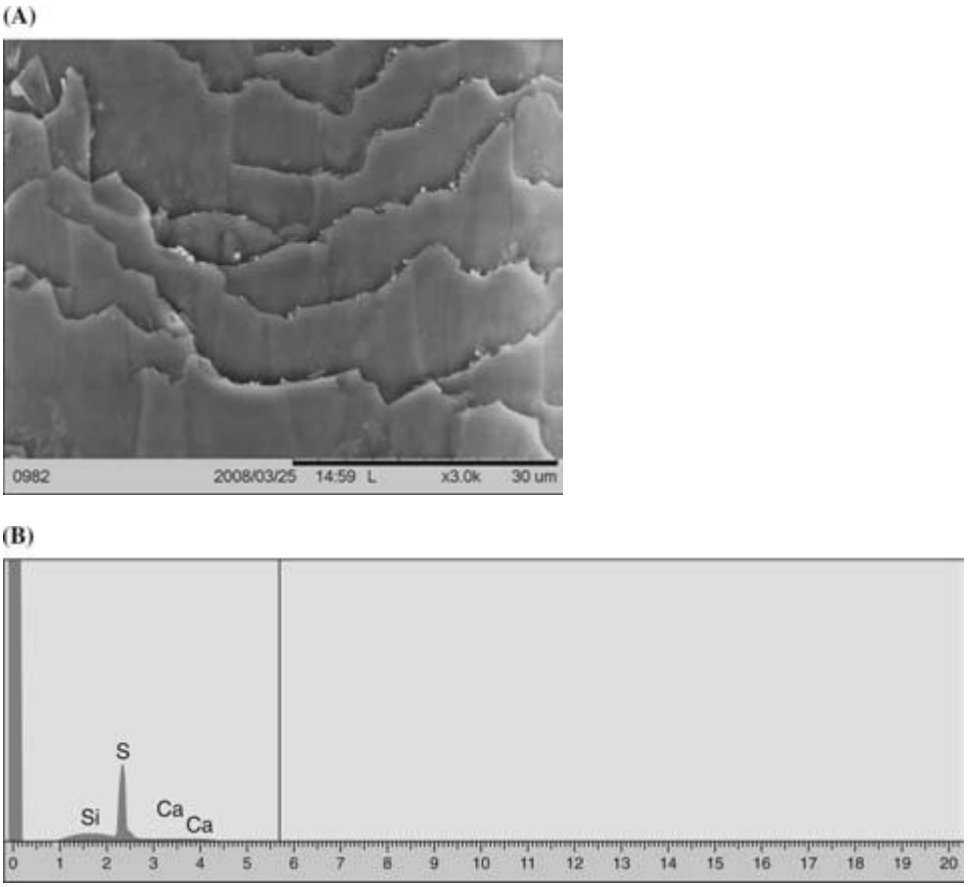

Figure 11 (**A**) SEM micrograph of hair at 3000× magnification and (**B**) results of EDS analysis of surface deposits concentrated near the cuticle edges. *Abbreviations*: SEM, scanning electron microscopy; EDS, energy dispersive spectroscopy. *Source*: Courtesy of Sunny Chen of Johnson and Johnson Co.

can display energies characteristic of the elements in the sample. The results are typically presented in the form of a histogram of signal strength as a function of energy (eV) with the detection limits ranging from 0.05% to 2%. Signal strength is related to relative concentration of a given element in the sample. Qualitative, semiquantitative, and quantitative bulk determination of elements for atomic numbers higher than 13 (all elements except H, He, Li, and Be) is possible. Energy-dispersive spectroscopy (EDS) can also provide elemental maps of the sample by identifying the elemental compositions of sample features as small as 1 μm.

Figure 11A presents an SEM image of hair (without metal coating) at a magnification of 3000× obtained by a benchtop Hitachi SEM instrument. The image illustrates the presence of granular surface deposits, which concentrate in the areas close to the cuticle edges. The use of EDS detector (Fig. 10B) indicates a high content of Si and Ca in the structure of the deposited material.

Atomic Force Microscopy

Scanning probe microscopy (SPM) can be used for imaging nonconductive surfaces of materials from the atomic to micron scale. Atomic force microscopy (AFM), and lateral force microscopy (LFM) fall under SPM designation of techniques, which also include scanning tunneling microscopy (STM), chemical force microscopy (CFM), and phase detection microscopy (PDM). These techniques can provide information about the topography and frictional and mechanical properties of a sample from the nanoscale to micron level. In both AFM and LFM, the probe, in the form of a sharp tip attached to a cantilever, scans the surface by using force on the order of 10 to 20 nN in the contact mode and 0.1 nN in the tapping mode. The latter is used to measure the surface characteristics of soft materials such as keratin fibers. In AFM, one obtains a topographical image by measuring the deflection of a soft cantilever, to

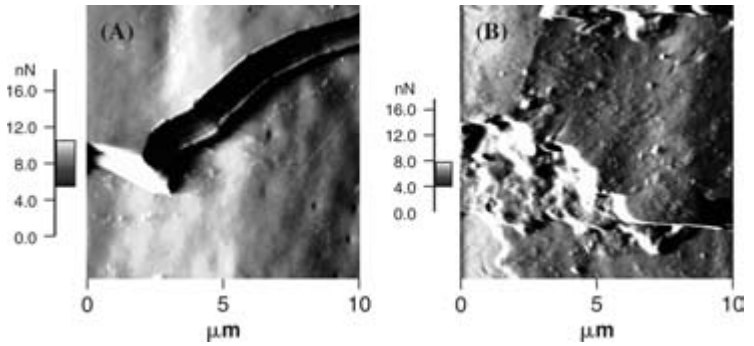

Figure 12 (**A**) Untreated hair (**B**) and hair treated with polyquaternium-28, as imaged by using error signal by LFM (64). *Abbreviation*: LFM, lateral force microscopy.

which the tip is attached, as the tip is rastered over the surface. The cantilever deflections normal to the surface are representative of topographical surface features. In LFM, one measures the torsional twisting of the cantilever as it is rastered over the surface. These lateral cantilever deflections result from drag forces between the tip and sample surface.

Both AFM and LFM have been used in cosmetic field to image hair fibers and to identify new morphological features under dry and wet conditions (59–67). O'Connor et al. (59) employed an atomic force microscope to quantitatively analyze the morphology of hair in air and water, the kinetics of hair hydration, and the effect of pH on hair morphology. Images were analyzed quantitatively by taking line cuts to illustrate height data versus position. They have shown that an average step height in the cuticle sheaths increased from 500 nm for dry hair to 1200 nm for wet fibers. By following the changes in geometrical dimensions of the cuticle height, they were also able to determine the rate constant of hydration and the effect of pH on swelling.

Goddard et al. (60,61) used an atomic force microscope in both modes (contact and tapping) to collect data on the distribution and configuration of adsorbed cationic polymers on the surfaces of hair and mica. They concluded that the hair surface is not smooth enough for quantitative analysis. Mica, on the other hand, has a model, well-characterized surface. Goddard's study of cationic polymers on mica demonstrated loop-and-trains configuration of adsorbed polymer chains, molecular weight dependence of the layer thickness, and polymer distribution as a function of charge density.

McMullen et al. (64) employed LFM to visualize deposition of cationic polymers on hair. Figure 12 presents a comparison of error signal image of untreated and co(vinylpyrrolidone—methacrylamidopropyl trimethylammonium chloride)-treated hair. The surface properties of hair treated with the polymer change significantly, with the polymer deposits taking the form of donut-shaped structures. The inside and outside diameters of the polymer deposits were determined to be 70 ± 11 nm and 202 ± 47 nm, respectively. Since the diameter of hair micropores was found to be 149 nm, it is plausible that the polymer may bind preferentially to the perimeter of the pores whose edges would presumably have a higher electric field than the more homogenous portion of the hair surface.

Confocal Microscopy

Confocal microscopy can be used to obtain specimen images that do not have out-of-focus areas. In a classical light microscope, the light illuminates a large portion of the sample, and if its geometry is not flat, a part of the image is always out of focus. Confocal light microscopy employs a focused beam of light with a reflected light passing through a pinhole in front of a detector, eliminating out-of-focus reflections. A focal plane image is generated by scanning the surface. Internal elements of a structure can also be scanned in a similar way, with the limitation being the opacity of the specimen.

Swift et al. (68) studied the penetration of fluorescently labeled proteins through intact and chemically modified hair fibers. The extent of penetration was assessed by imaging transverse sections of resin-embedded hair with a confocal laser-scanning fluorescence microscope. It was determined that the main sites for peptide deposition were endocuticle, cortex, nuclear remanants, intermicrofibrillar matrix, and cell boundaries that undergo massive swelling by water.

Corcuff et al. (69) used confocal microscopy to study the surface of dry, wet, and chemically modified hair. They claimed resolution of 0.25 versus 0.6 mm for conventional light microscopy. Their technique made possible the direct observation of sweat and sebum on hair surface and the quantitative assessment for periodic bulging of cuticles on swelling. They have also performed optical sectioning of hair samples at various depths to provide a three-dimensional reconstruction of the internal structure of hair stained with a fluorescent marker.

MICROFLUOROMETRY

The instrumental setup consists of a fluorescence illuminator, objective, interference filters, photomultiplier, and a scanning sample stage. The hair fiber was illuminated by a focused beam of light, and the fluorescence emission was monitored by a photomultiplier as the specimen was moved under the exciting light.

Weigmann et al. (70) employed microfluorometry to study deposition, substantivity, and buildup of various components of cosmetic formulations on hair. They used the sodium salt of fluorescein as a marker and assumed that the deposited film thickness, resulting from the precipitation of polymers, surfactants, and polymer-surfactant complexes, is directly proportional to fluorescence intensity. Various distributions of emitted light intensity were observed, including honeycomb patterns, which may be indicative of hair damage. Multiple treatments of hair with shampoos containing cellulose (and fluorescent marker) showed gradual increases in fluorescence intensity and an uneven distribution of surface deposits.

SURFACE ANALYSIS

Dynamic Electrokinetic and Permeability Analysis

Electrokinetic measurements have been applied in wool and hair research for some time. Recent developments, however, have made possible simultaneous measurements of electrokinetic and permeability parameters of fiber plugs in order to obtain information about the interactions of various cosmetic raw materials with hair (71–75). The technique has been termed "dynamic electrokinetic and permeability analysis" (DEPA).

The DEPA instrument consists of a streaming potential cell, conductivity meter, pressure transducer, test and treatment solution reservoirs, flow interruptor, an electronic balance, and several electric and manual valves. The most important features of the design are

- online positioning of test and treatment solution reservoirs, permitting fiber treatment within the streaming potential cell;
- the pulse mode of flow for test and treatment solutions;
- simultaneous measurement of the streaming potential, conductivity, and flow rate (permeability of the plug); and
- special software allowing flexible programming of the experimental procedures, such as the control of pressure and the timing of treatment and test cycles.

A typical experiment yields information about the electrokinetic characteristics and permeability of untreated fibers and the kinetics of sorption/desorption of cosmetic actives as a result of one or multiple treatments of hair. Figure 13 presents the results of an experiment in which hair was treated with 0.5% solutions of anionic surfactant (SLES-2), cationic surfactant cetryltrimethyl ammonium chloride, and cationic polymer cationic guar gum. The first five data points in each figure correspond to untreated hair. They are followed by a 5-minute treatment period, first measurement period of 30 minutes, a second 5-minute treatment period, and the second measurement period of 30 minutes.

Figure 13A presents the time dependence of ξ-potential and demonstrates an increase in hair-negative ξ-potential as a result of binding of SLES-2 to hair. It also shows a reversal of the surface charge for hair treated with quaternary ammonium surfactant and cationic polymer. While surfactants are rinsed off the hair after prolonged treatment with the test solution, a layer of cationic guar gum is stable and imparts a permanent positive ξ-potential to hair.

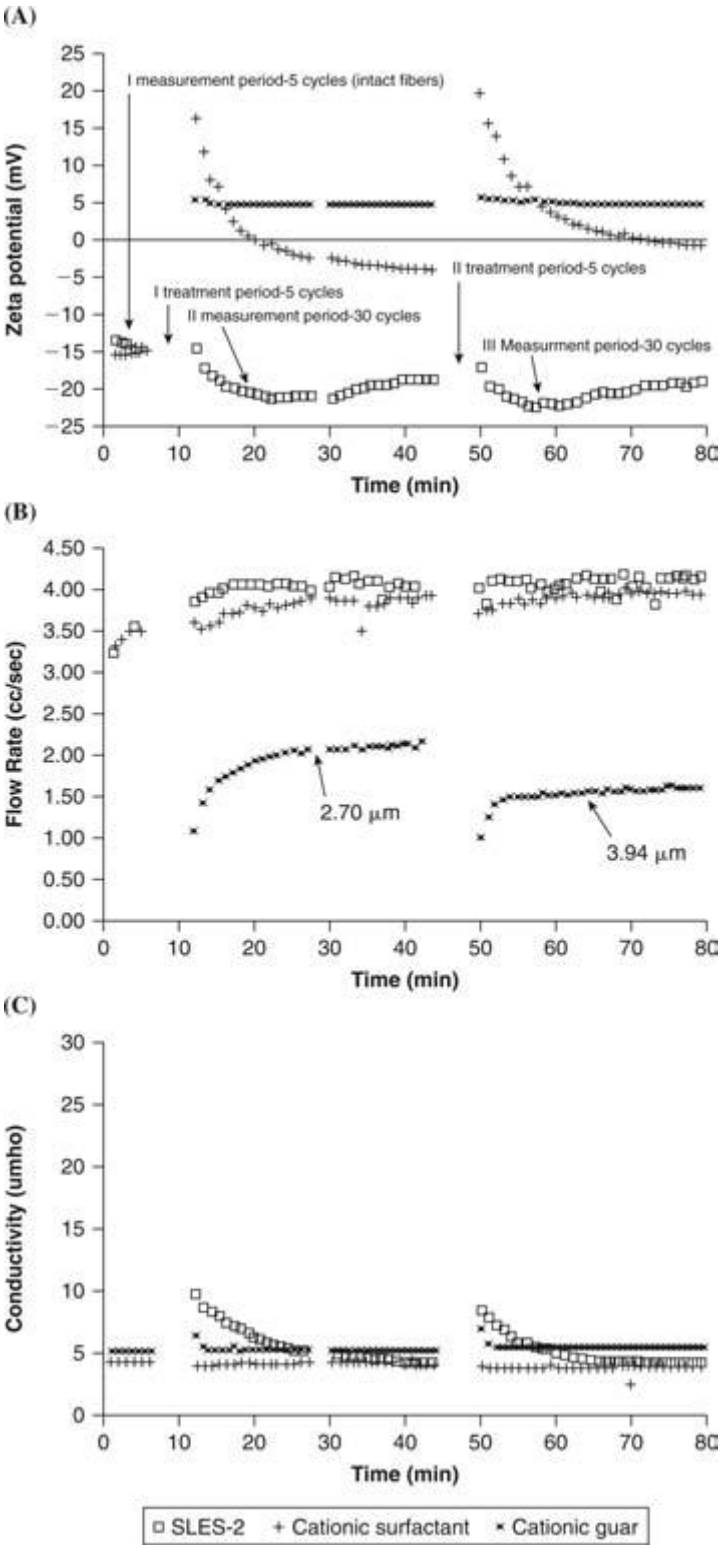

Figure 13 (**A**) ξ-potential (**B**), flow rate (**C**), and conductivity as function of time for hair treated with different classes of materials such as anionic surfactant (sodium laureth-3 sulfate), cationic surfactant (cetyl trimethylammonium chloride), and cationic polymer (cationic guar gum).

The flow rate data (Fig. 13B) show that only in the case of cationic guar gum there is a formation of a thick layer of the polymer (2.7 and 3.94 μm after the first and second treatment, respectively) on the surface of hair. This is probably due to the presence of microgels of the polysacchride in the treatment solution.

The hair conductivity is slightly reduced after quat (cetryltrimethyl ammonium chloride) treatment, a behavior typical for all cationics (Fig. 13C). A small delay in conductivity decrease for the polymer is related to the reduced flow and the resulting prolonged presence of the excess treatment solution in the plug. In contrast to this, SLES-2 slowly desorbs from hair and gives rise to increased conductivity of the plug even after extended rinsing with the test solution.

Similar data can be obtained not only for simple single component systems but also for complex, multicomponent solutions such as shampoos, conditioners, hair dyes, or any other finished cosmetic product.

The main criteria of product assessment are changes in the ς-potential, permeability, and conductivity relative to an untreated control. On the basis of these parameters, one can make conclusions about the deposition of cationic, anionic, nonionic surfactants, and polymers on hair. One can also obtain information regarding emulsions, substantivity of various treatments, their removability upon shampooing, buildup on consecutive treatments with the same formulation, and rate of desorption of residual surfactants or polymers (71–75).

The technique can also be employed to quantify the "sealing effects" produced by surfactants, polymers, and oils on dyed hair (71) or on fibers subjected to reactive treatments such as perming or bleaching (74).

A significant advantage of DEPA is that it performs the measurements on fiber assemblies rather than on single fibers, giving an average value of the assessed parameters. Also, the experimental protocols can be planned so that they simulate any sequence of operations performed on real hair, such as a combination of treatments including shampooing, conditioning, or perming.

Wettability

The use of wettability measurements to study the effect of chemical and physical treatments on the surface of hair has been described in detail by Kamath et al. (76). They basically employed a high-sensitivity balance and measured the wettability forces by immersing hair fibers into water or other liquids. An advance in this area, reported by the same authors, was the development of liquid membrane wettability scanning. In this process, a fiber is passed through a liquid membrane, and the measured wettability force is calculated from the following equation (77):

$$F = P\gamma_{LV}(\cos q_a - \cos q_r)$$

where P is the fiber perimeter, γ_{LV} is the surface tension of the membrane liquid, and q_a and q_r are the contact angles in the advancing and receding modes.

A typical experimental procedure consists of obtaining wettability scans along the length of fibers before and after treatments with solutions of conditioning actives, such as cationic cellulose or protein. Determining the value of q_r in a separate experiment, and assuming that it does not change along the fiber length, allows one to obtain a plot in which $\cos q_a$ is a function of distance. The wettability traces usually show a lot of variation along the length of a fiber so that an average value is usually employed for comparisons between intact and modified hair. It was shown that unoxidized hair, characterized by a value of $\cos q_a = -0.22 \pm 0.17$, becomes more hydrophilic after a single treatment with a solution of a cationic polymer as evidenced by an average value of $\cos q_a = -0.08 \pm 0.15$. This method was also employed to assess the effect of multiple treatments.

In current laboratory practice, automated wettability instruments are employed. They are capable of detecting a point of contact with the liquid during the fiber movement in the advancing direction, reverse the direction of movement to measure receding wettability forces, detect the point of separation from the liquid, average the forces during the fiber scan, and calculate contact angles using previously determined fiber perimeter (which is accomplished by employing wettability data for a given fiber obtained by using hexane as a solvent). Figure 14A,B shows wettability traces (wettability force as a function of distance) for virgin hair (Fig. 14A) and for bleached hair (Fig. 14B). On the basis of these measurements, the advancing contact angles were found to be 101.6° and 41.2° for intact and commercially bleached hair, respectively.

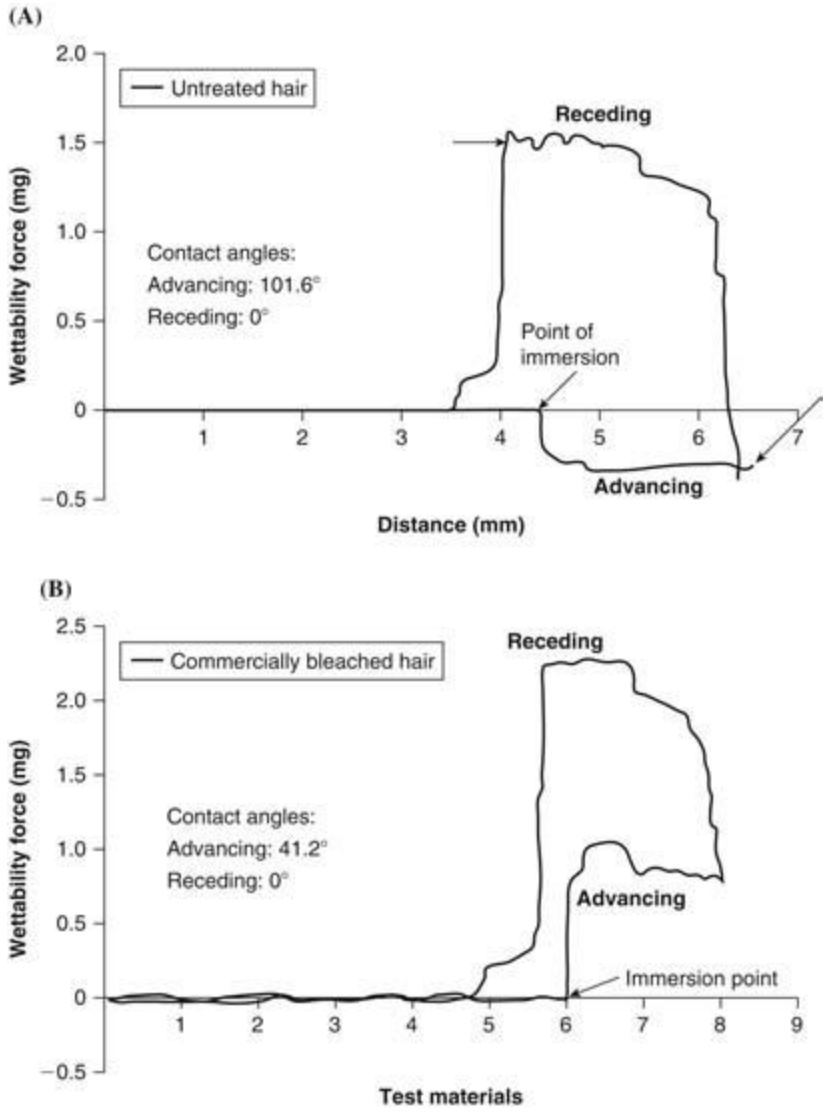

Figure 14 Wettability trace for (**A**) intact and (**B**) bleached hair.

Profilometry-FT and Fractal Analysis

Profilometry has been recently employed to study the geometrical properties of hair surface (78,79). This technique in which the surface of hair is scanned longitudinally using a wedge-shaped stylus has a resolution similar to that of optical microscopy.

A typical experimental result is a hair surface profile exhibiting a large number of random peaks and valleys ranging in size from a fraction of a micron to millimeters. The traditional way of handling these data is to calculate roughness parameters such as average roughness depth, average roughness, or geometric average roughness (78). However, these parameters are not constant and increase with an increase of the scan length, a consequence of self-similarity or fractality of hair surface profile (79).

Hair profilometric traces can be subject to Fourier transform (FT) in which the height versus length dependence is converted into intensity versus frequency spectra, where frequency is termed "spatial frequency" and is defined as number of crests per unit length. The analysis of the averaged FT spectra of hair surface showed no preferred frequencies of height variation and allowed for calculation of the fractal dimensions. They were found to be 1.31 for the high-frequency (small spatial dimension) end and 1.63 for the low-frequency (large spatial

dimension) end. This leads to the conclusion that hair is "smoother" in the probing scale from 0.5 to 5 mm than in the scale from 5 to 100 mm.

Combing Measurements

The use of quantitative combing measurements has been well established in the characterization of hair-care products. The technique has been developed over the years by Newman et al. (80), Tolgyesi et al. (81), Garcia et al. (82), and Kamath et al. (83). It is widely used in research, development, and claim substantiation.

The method consists of passing a comb through a hair tress, with a well-defined geometry, and measuring force as a function of distance. These measurements can be performed on dry or wet fibers. The parameters used for comparing product performance include the maximum combing force or combing work. The data are typically reproducible within ±20% for wet-combing measurements and ±50% for dry-combing measurements.

Jachowicz et al. recently reported a modification of the method aimed at increasing its sensitivity (84,85). The method, termed "spatially resolved combing analysis," employs special frames that allow the application of a treatment to selected areas of the fibers while shielding the remaining portions, thereby providing internal reference sections. The treatments may include thermal exposure, wet applications of cosmetic formulations or raw materials, and physical modification of hair by photoirradiation. The combing curves of hair treated in such a way, obtained by using a tensile tester such as Instron or Dia-Stron, show positive or negative peaks depending on whether the treatment results in an increase or a decrease in friction of the hair surface.

Figure 15 illustrates the application of this method to the analysis of two different conditioners on hair. Their affinity to hair is assessed by performing combing measurements

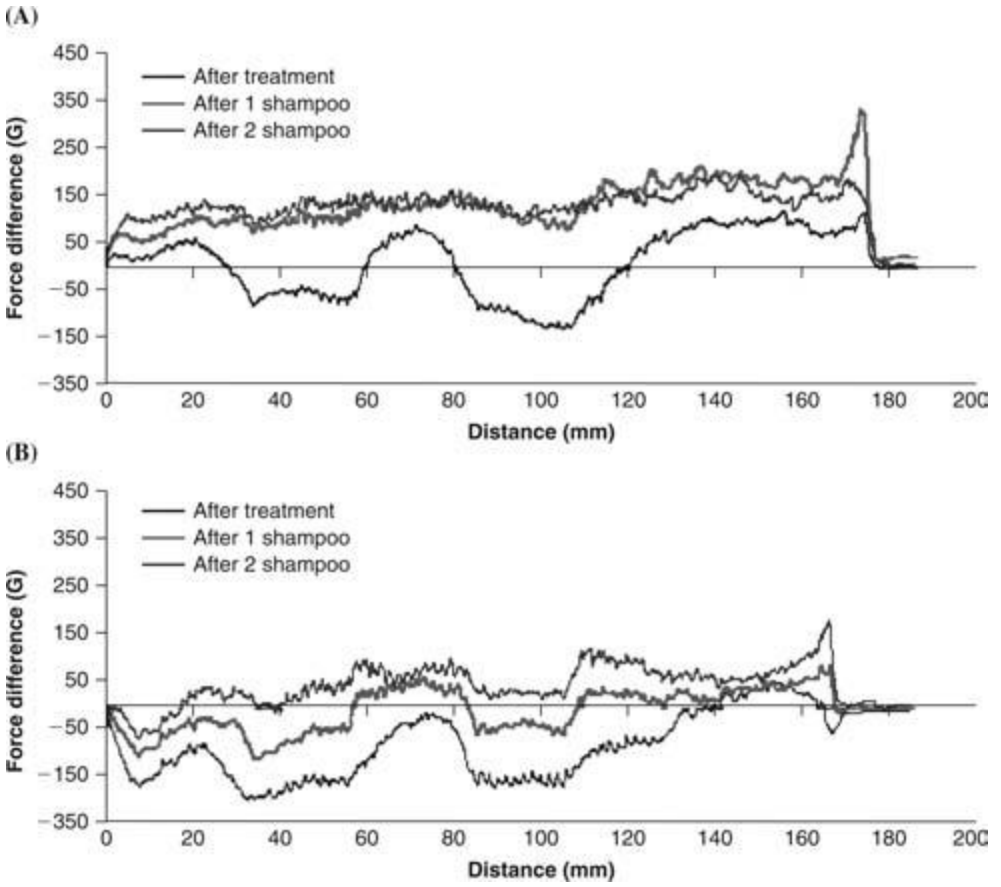

Figure 15 Differential combing curves for hair treated with (**A**) conditioning agent 1 and (**B**) conditioning agent 2 and subsequently subjected to two shampooings.

after hair treatment and after two subsequent shampooings. The presented traces are differential combing curves obtained by subtracting a curve for untreated hair from the combing trace obtained after a given treatment.

Figure 15A shows the traces of combing curves for hair treated through a two-window frame with a cationic conditioning agent, which is characterized by low affinity to hair in terms of its resistance to shampooing. Unlike a conditioning treatment, shampooing is applied to the whole tress, including untreated and conditioner-treated portions of a hair. Significant decreases in combing forces are evident in treated sections of hair, with the effect nearly completely eliminated by a single shampooing. In contrast to this, Figure 15B gives the traces obtained for a high-affinity conditioning agent, showing reductions in combing works after treatment in the window areas. The effect persists after one and two shampooings, suggesting that the conditioning agent (Fig 15B) remains adsorbed on hair surface. Notably, combing forces corresponding to untreated (shampooed only) portions of hair gradually increase probably as a result of lipid removal or adsorption of anionic surfactants from a shampoo.

Other uses of this technique include the studies of the effect of chemical treatments on hair and the analysis of hair adsorption by cationic polymers, proteins, and complexes.

TEXTURE ANALYSIS

A new tensile meter′ referred to as texture analyzer can measure the force in both the compression and tensile modes while monitoring probe displacement in relation to the sample (Texture Technologies Corporation, Scarsdale, New York, U.S.). It was developed primarily for quantitative characterization of food products in terms of texture parameters such as hardness, springiness, tackiness, and resilience.

The instrument was recently adopted for conducting quantitative analysis of hair and hair-care products by employing a procedure referred to as dynamic hairspray analysis (Fig. 16) (86–88). It involves the use of hair samples shaped into omega loops by applying a temporary wet set. Both the instrument and the sample are housed in a constant humidity chamber that can maintain relative humidity in the range from 30% to 95% at ambient temperatures. The mechanical measurements of hair loops are carried out by oscillating a plastic probe between the fiber surface and the calibration height of a few centimeters. After touching the surface of hair and sensing a trigger force (1–2 G), the probe produces an additional 1- to -4-mm deformation (6–25%) of the loop before rising to the calibration height. One-millimeter deformation is typically within the elastic limit of both untreated and resin-modified hair. On the other hand, 4-mm deformation (25%) usually results in irreversible breaking of polymer-fiber or polymer bonds in polymer-treated hair and is employed to study flexibility of styling products.

Experimental data for polymer-treated hair at high deformation of 25% are presented in a plot of force as a function of distance (Fig. 17) for the first deformation (Fig. 17A) and the first 10 consecutive deformation cycles (Fig. 17B). The data in the figures correspond to a brittle

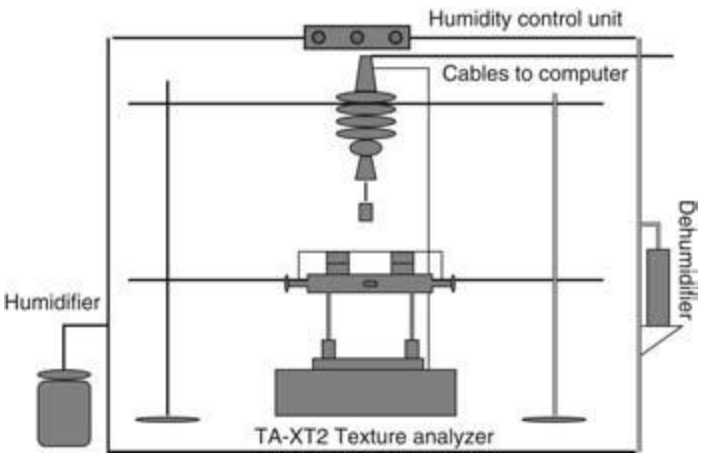

Figure 16 The experimental setup to study mechanical properties of hair-care polymers. It consists of a texture analyzer and an environmental chamber. Hair sample is in the form of omega loop placed under the probe of the texture analyzer.

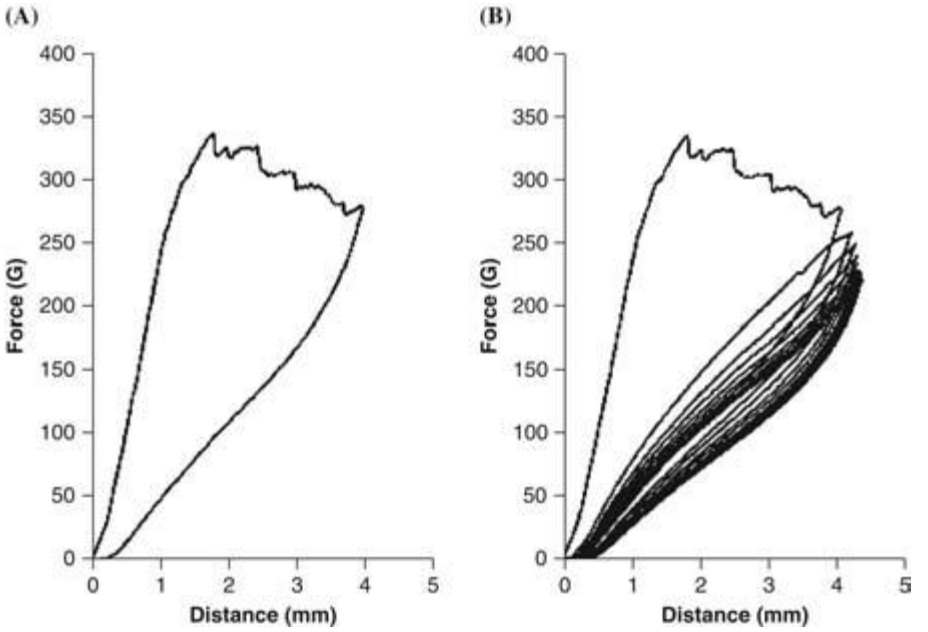

Figure 17 Force as a function of deformation for hair in the form of omega loops treated with a fixative polymer.

polymer characterized by an elastic response in the deformation range from 0 to 1 mm. It is in this deformation range that the ratio of modulae, E_{10}/E_1, is calculated (modulus is calculated as the slope of the dependence of force as a function of distance in the linear portion of the curve). E_{10} and E_1 are the modulae of the 10th and the first deformation, respectively. The ratio E_{10}/E_1 can be used as a measure of sample (hair treated with a polymer) flexibility. To further characterize the flexibility of the polymer used as a hair treatment, a parameter F_{10}/F_1 can be calculated as the ratio of the maximum force in the 10th deformation, $F_{10,}$ to the maximum force in the first deformation, F_1. As illustrated by the curves in Figure 17, at a deformation of about 2 mm in the first cycle, the polymer bonds between fibers break, resulting in a reduction of maximum force (F) in subsequent deformations. The measurements such as those presented in Figure 17 can also be used to calculate plasticity parameter of treated hair.

By using a setup shown in Figure 16, the drying of a fixative was also investigated by applying low (1%) intermittent deformations to ω-loop shaped hair tress. First, the instrument determined the properties of untreated hair; then, the fibers were treated with a fixative, and the instrument measured the changes in both tackiness of a fixative solution on the hair surface as well as mechanical stiffness of the fiber assembly as a function of drying time. The experimental procedure yields parameters such as the stiffness of untreated and resin-modified hair, duration of tack, maximum value of tack force, and time of drying. The kinetic measurements of the stiffness change can also be performed at 90% RH, resulting in information about the resistance of fixative resins to high humidity.

Other applications of this instrument include the characterization of hair (especially ethnic hair) in terms of textural parameters, analysis of skin softness, and the measurements of tactile properties of skin products. This new tensile meter can also be employed for the characterization of the textural (rheological) behavior of cosmetic formulations such as shampoos, creams, waxes, and pomades.

OPTICAL PROPERTIES AND LUSTER MEASUREMENTS

Hair luster is an important property readily assessed by a visual observation and frequently invoked in claim substantiation and advertising. It is largely dependent on the cleanliness, uniformity, and extent of damage to the hair surface. Hair luster can be affected by chemical treatments that reduce hair gloss by damaging cuticles, dissolving lipids, or changing hair

color. It can be also modified by application of shampoos, hair conditioners, or special shiner formulations.

The key papers in this area were published 30 years ago by Stamm et al. (89). Recent developments include the use of computerized goniophotometers to quantify light-scattering effects produced by single fibers (90–95) or aligned fiber tresses (96,97). The principle behind these measurements is the same as in earlier work: a light source illuminates the sample at an incidence angle, and the light intensity is recorded for different receptor angles, providing a light-scattering curve. Rotating light-scattering photometers or optical multichannel analyzers can be employed for luster measurements.

The usual criterion of gloss is the sharpness of specular reflection, which can also be quantified by defining various luster parameters given by the formula (90,93):

$$L = S/D(W_{1/2})$$

where L is luster or shine, D is the integrated diffuse reflectance, S is the integrated specular reflectance, and $W_{1/2}$ is the width of a specular peak at half height.

A different experimental approach to luster measurements was taken by Maeda et al. (96) who obtained pictures of illuminated natural hair wigs on model heads and analyzed them by using a color image processor. The data obtained by scanning across highlighted and dark areas could be presented in a format similar to a photogoniometric-scattering curve with the ability to resolve reflected light into three color signals R, G, B (red, green, and blue), or L, a, b parameters. A similar approach was taken by McMullen et al. (98,99) who employed image analysis to measure luster of hair simulated by light reflected from a curved hair tress. Hair samples were mounted side by side in a special sample holder in the form of a cylinder and illuminated by a uniform beam of polarized white light. Digital images of hair tresses were captured with a high-resolution camera and analyzed by scanning across highlighted and dark areas of the resultant image using image analysis software. Plots, similar to goniophotometric-scattering curves, were used to calculate luster values according to previously published work (89,93).

By employing all these methodologies, researchers demonstrated small variations in shine as a result of the application of shampoos with and without substantive ingredients on untreated dark brown Asian hair (90,93). Other researchers showed the effect of special shine formulations on damaged hair (91). Luster measurements were also employed to demonstrate a cuticle-abrading effect of multiple combings resulting in a shift of maximum in a light-scattering curve. Other investigations based on luster measurements documented gloss variation between root and tip sections of hair and the effect of humidity. Nagase and coworkers (100–101) and Okamoto et al. (102) have emphasized the importance of internal structure of hair to optical properties and demonstrated the presence of "glittering" patches on the hair surface caused by internal damage due to blow-drying.

Lim et al. (97) measured luster of hair tresses mounted on cylinders, similar to those reported earlier (98), with a video camera in an experimental setup called SAMBA. The authors did not provide the results of calculations of fundamental parameters, and the data interpretation is given in terms of "percentage of luster increase". It was reported that the correlation of instrumental readings and consumer evaluation ratings of luster was good.

The image analysis procedure was employed to assess the luster of natural white, light blonde, light brown, medium brown, and dark brown hair and revealed an increase in luster indices in proportion to an increase in fiber pigmentation (98,99). Figure 18 presents images of reflected light from natural white, light blonde, light brown, and dark brown hair. These images were obtained by selecting the exposure values in such a way as to visualize the details of the specular reflection band.

The light distribution curves are presented in Figure 19, and they are consistent with the visual representation of the images shown in Figure 18.

For example, one can clearly see two specular reflection bands for natural white and light blonde hair, which are evident by two peaks in the light distribution curves. The peak at 16 mm gets progressively smaller with an increase in the extent of fiber pigmentation, which indicates that it is due to reflection from the back-face of the hair fibers. The narrowest light distribution curve was obtained, as expected, for dark brown hair. Hair luster parameters, calculated according to equations published by Stamm et al. (89) and Reich and Robbins (93), are presented in Table 4.

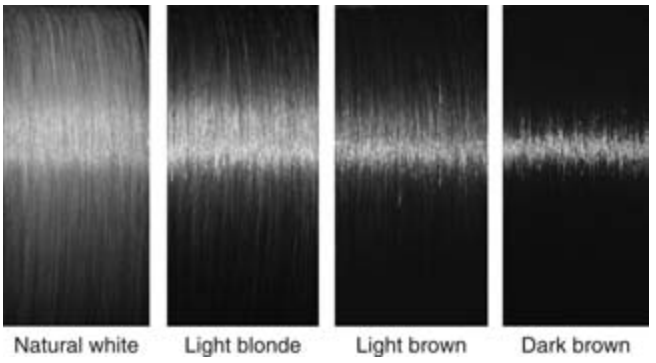

Figure 18 Images of reflected light from natural white, light blonde, light brown, and dark brown hair.

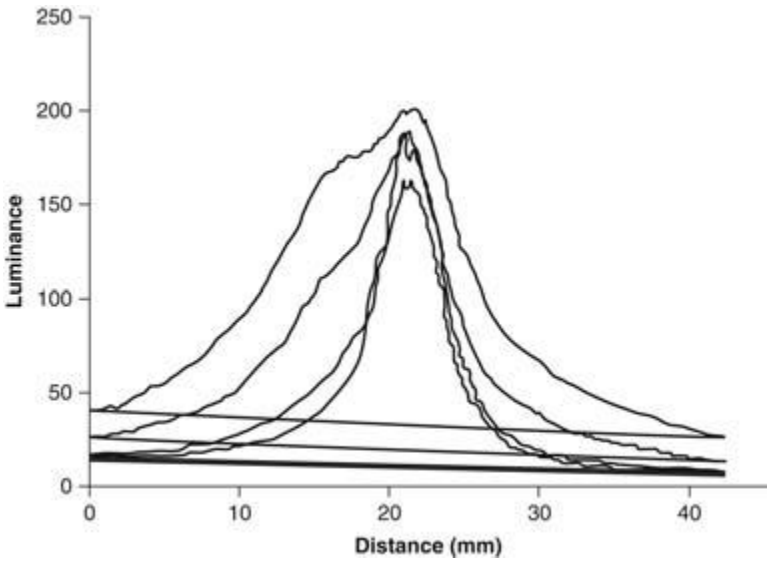

Figure 19 Light distribution curves for images of hair presented in Figure 18.

Table 4 Luster Parameters for Various Hair Types

Hair type	$W_{1/2}$ (mm)	L_{Stamm}	$L_{Reich\text{-}Robbins}$
Dark brown	5.28 ± 0.44	0.72 ± 0.020	0.67 ± 0.006
Medium brown	5.44 ± 0.16	0.72 ± 0.006	0.67 ± 0.005
Light brown	10.46 ± 0.30	0.70 ± 0.001	0.32 ± 0.008
Light blonde	14.91 ± 0.23	0.65 ± 0.005	0.19 ± 0.006
Natural white	22.78 ± 0.24	0.32 ± 0.013	0.06 ± 0.002

The calculations carried out by both formulas indicate lower luster values for fibers containing less melanin pigment, i.e., the highest luster for dark brown hair and the lowest for natural white hair. Also, $W_{1/2}$ follows the same trend, consistent with visual perception, pointing to an increase in the width of reflected light distribution for less-pigmented fibers.

Cosmetic oils such as phenyl trimethicone, amodimethicone, and castor oil were also found to increase luster of hair as a result of change in contrast between the specular and diffuse reflection (98). Styling resins such as butyl ester of PVM/MA copolymer, vinyl caprolactam/PVP/dimethylaminoethyl methacrylate copolymer, and isobutylene/ethylmaleimide/hydroxyethylmaleimide copolymer were shown to increase hair gloss by a similar

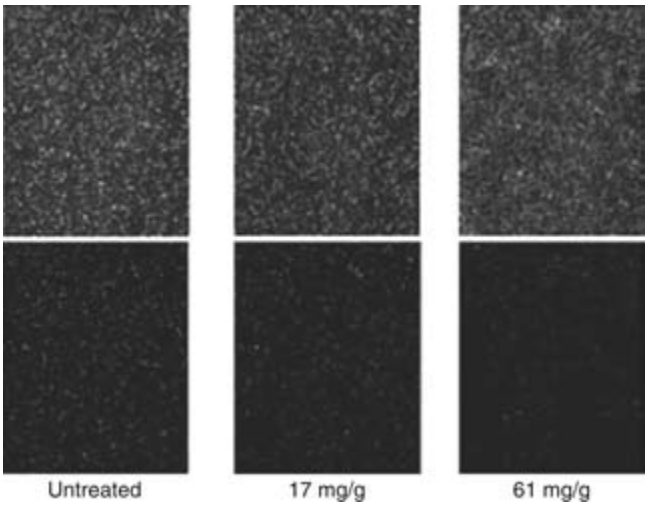

Figure 20 The effect of sebum on the luster of African hair. First row shows unprocessed images, while the second row presents corresponding images after image thresholding.

mechanism as evidenced by calculated higher values of Stamm and Reich–Robbins luster parameters (98). On the other hand, an effect of hair dulling by deposition of micronized ZnO at various concentrations as well as by synthetic sebum was also discussed (98).

African hair provided an interesting substrate for optical analysis because of the curls that are naturally present providing multiple reflection patterns (99). It is important to note that unlike straight hair, which exhibits one specular reflection band that coincides with the band on the cylinder mount, homogenous illumination of African hair with a collimated light beam results in many reflection centers of equal intensity in all regions of the sample. To quantify the multiple reflection patterns, the authors used the image analysis software, which allowed them to tally the number of reflection sites as well as to characterize the shape of the reflection. Figure 20 provides images obtained for untreated hair along with hair treated with 17 and 61 mg of artificial sebum per gram of hair. Visual inspection of the images reveals a perceived decrease in luster with increasing concentrations of sebum. Further, a decrease in luster is coupled with a decrease in the number of reflection sites. Figure 20 includes corresponding images in which all of the reflection sites have been isolated on a black background using an image threshold technique. This is accomplished by looking at a histogram corresponding to the colors present in the image and isolating the bright white light that corresponds to the reflection centers. Image file types usually have a scale from 0 to 255 to represent the colors in the image, with 0 representing the darkest colors (black) and 255 the brightest (white). By isolating values that fall in the range from 225 to 250, we can look at the brightest reflections on an entirely black background (Fig. 19), allowing us to count the total number of reflections.

As shown in Table 5, the number of reflections decreases with increasing concentrations of sebum, which was also clearly evident after visual inspection of Figure 19. It should also be added that the reflection sites could be also characterized in terms of perimeter, roundness, and compactness by using the tools of image analysis.

Table 5 Quantification of Reflection Sites on African Hair Treated with Sebum

	No. of reflections	Black (%)	White (%)
Untreated	742	98.34	1.66
17 mg/g	536	99.02	0.98
61 mg/g	273	99.67	0.33

EVALUATION OF PERMANENT WAVES

Permanent waving involves breaking disulfide bonds in hair with a reducing agent followed by reformation with a neutralizer (103,104). Evaluation of permanent waving chemistry can be carried out either by study of reduction and reoxidation rates or by measuring the permanent set achieved in the hair. Reduction rates in hair can either be determined by amino acid analysis (105–107) or by methods based on chemical stress relaxation. In chemical stress relaxation methods, a hair is stress relaxed in buffer at fixed extension until a constant level of force is reached. Addition of a reducing agent causes the stress supported by the hair to decrease as disulfide bonds are broken by reduction (108–113). Kinetics of the reaction can be followed, and some information on reaction mechanisms can be deduced. Wickett (109) introduced the term "single fiber tensile kinetics" (SFTK) to describe chemical stress relaxation.

The effect of temperature and pH on reduction with sodium thioglycolate (NaTGA) at pH 9.0 is shown in Figure 21.

The curves from pH 9.0 at 39°C and pH 10.0 at 22°C clearly show the two shapes of SFTK curves typically observed with this method. At pH 9.0, TGA follows pseudo first-order kinetics (108,109). In this model, one assumes that the reagent is in large excess, that the reaction is slow compared with diffusion, and that all stress supporting S-S are equally reactive. Then, the rate of change in S–S bonds is given by

$$\mathrm{d(S\text{–}S)/dt} = -kC_0(\mathrm{S\text{–}S})$$

where C_0 = the concentration of reducing agent and k is the reaction rate constant. If each S–S is assumed to support an equal amount of stress then the force, $F(t)$, at any time t, is given by

$$F(t) = F(0)\exp(-kC_0t)$$

And, plots of $-\ln(F(t)/F(0)$ versus t will be linear with a slope of kC_0.

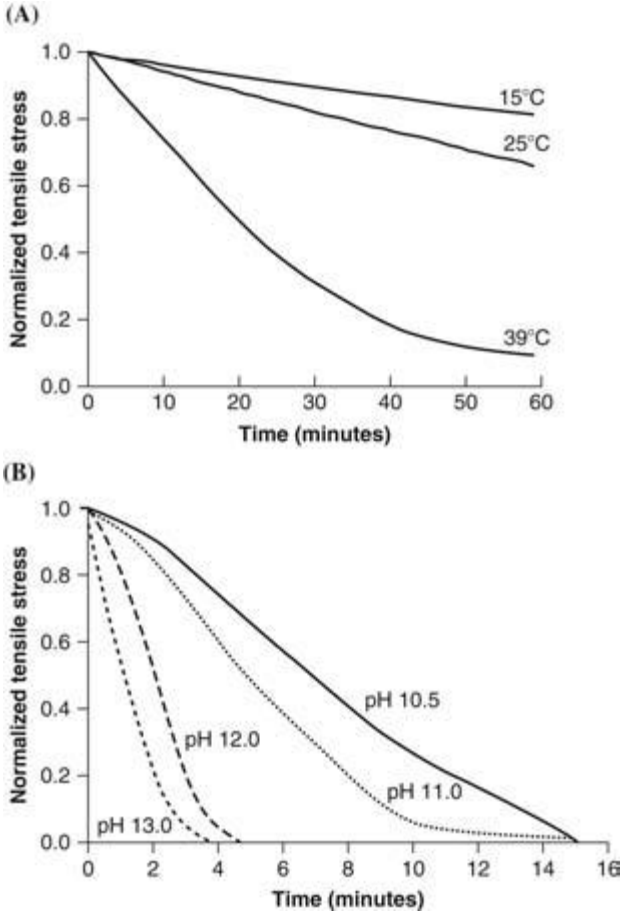

Figure 21 (**A**) Effect of temperature on SFTK reaction kinetics with NaTGA at pH 9.0. (**B**) Effect of pH on NaTGA SFTK curves at 22°C. *Abbreviations*: SFTK, single fiber tensile kinetics; NaTGA, sodium thioglycolate.

Figure 22 Pegboard for permanent wave evaluation.

The model that fits curves shaped like those for pH 10.5, as shown in Figure 21B, is more complex and assumes that diffusion is slower than reaction until some reaction has occurred, greatly speeding up diffusion. In this model, there is sharp front or moving boundary of reducing agent working its way into the hair. This model has been discussed in detail in other papers (104,109,112).

An effective way to measure the efficacy of permanent waves is the pegboard method (114), which is based on a uniform pegboard made of plastic that is 5.5-cm long and 1-cm wide and contains 14 removable pegs at a height of approximately 2 cm. The distance between each peg is about 3/10 cm. Two grams of hair are interlaced between the two peg rows without tension and secured at each end with rubber bands (Fig. 22).

The hair must be wound evenly and smoothly without any twisting. After winding, the hair is thoroughly saturated with waving lotion and the pegboard is covered and placed in a constant temperature bath at 25°C for a prescribed time. The pegboard is then removed from the bottle, rinsed thoroughly with water for 30 seconds, and then saturated fully with a neutralizer again for a prescribed time and finally rinsed with water. The rubber bands are removed from each end, and the pegs are carefully removed. The curled hair is then immersed in water for at least five minutes, and the length of the waved swatch is determined. Waving efficiency is calculated as given below.

$$100\% - [100 \times (B–A)/C–A] = \% \text{ waving efficiency}$$

A is the distance between the first and sixth peg (2.7 cm), C is the length of straight hair (14.8 cm), r, and B is the length of the curled hair swatch. Substituting these constants the equation becomes

$$100\% - [100 \times (B–2.7)/12.1] = \% \text{ waving efficiency}$$

CONCLUSION

There are a great many methods for evaluation of hair and hair-care products in the hair cosmetics laboratory. We have reviewed several of them, but there are many that we could not review in this chapter. The authors hope that the reader will find this review useful and informative and apologize for the fact that some other methods were not included.

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69 The Normal Nail

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ANATOMY

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

Two other structures deserve our attention:

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

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

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

HISTOLOGY

The Nail Plate

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

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

In electron microscopy (Fig. 4) (8), the nail plate cells appear to be made of a regular weft of keratin filaments within an interfilamentous matrix. In the upper (or dorsal) nail plate, cells are flat, their cellular membranes are discreetly indented, and they are separated from each other by ampullar dilatations. At the surface, those cells are piled up like roof tiles, which give

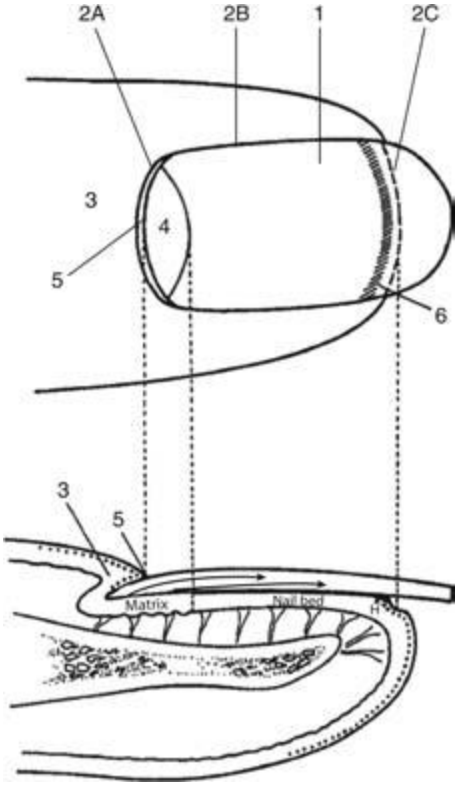

Figure 1 The normal nail: (1) nail plate, (2) nail grooves [(2**A**) proximal nail groove, (2**B**) lateral nail groove, (2**C**) distal nail groove], (3) proximal nail fold, (4) lunula, (5) cuticle, (6) onychodermal band. Small dots represent the stratum granulosum. *Abbreviation*: H, hyponychium.

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

the nail surface its smooth aspect. In the lower nail plate, cells are thicker, their cellular membranes are anfractuous, and they interpenetrate through extensions, making real anchoring knots that seem to be partly responsible for nail elasticity.

Recently, the histological structure of the nail plate has also been studied by synchrotron X-ray microdiffraction (9). Three transversal layers (characterized by different orientations of

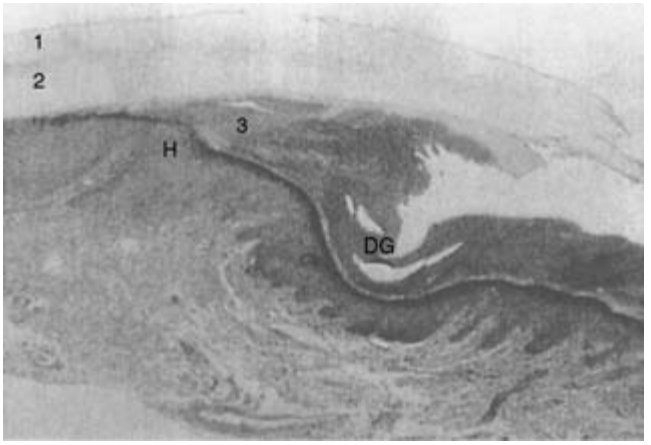

Figure 3 Longitudinal section of the distal part of the nail apparatus: (1) upper or dorsal nail plate, (2) lower nail plate, (3) subungual keratin. *Abbreviations*: H, hyponychium; DG, distal groove.

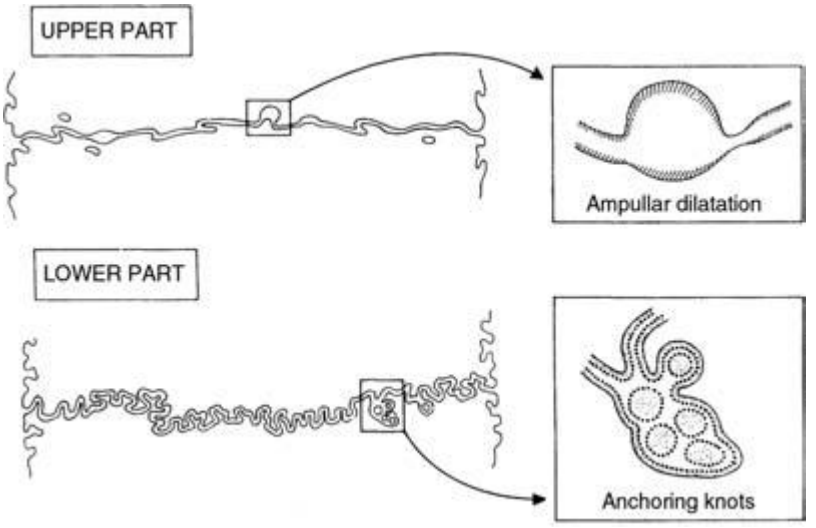

Figure 4 Schematic drawing of the cell membranes in the dorsal and ventral part of the nail plate, as observed in electron microscopic examination. *Source*: From Ref. 8.

the keratin molecules) are also identified. The outer or dorsal nail plate, which makes up the one-fourth of the nail, contains epidermal-type keratin filaments, perpendicular or parallel to the nail growth axis. The intermediate nail plate, accounting for approximately two-thirds of the nail, is the only one containing hairlike type α-keratin filaments, perfectly orientated perpendicularly to the growth axis. The very thin (one-twelfth of the nail plate only), ventral nail plate is made up of epidermal type keratin filaments, perpendicular or parallel to the nail growth axis.

In the latter study (9), and in those previously mentioned, it should be pointed out that the denominations given to the three parts of the nail are different, which could lead to confusion. In Figure 5, the correspondence between the different terms used is shown.

Other Nail Structures

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

- The PNF (Fig. 6). Its dorsal part is in continuity with the epidermis of the digit back. Its vascularization is noticeable. The capillary loops are parallel to the skin surface, which allows their in vivo examination, under a special microscope with epi-illumination.

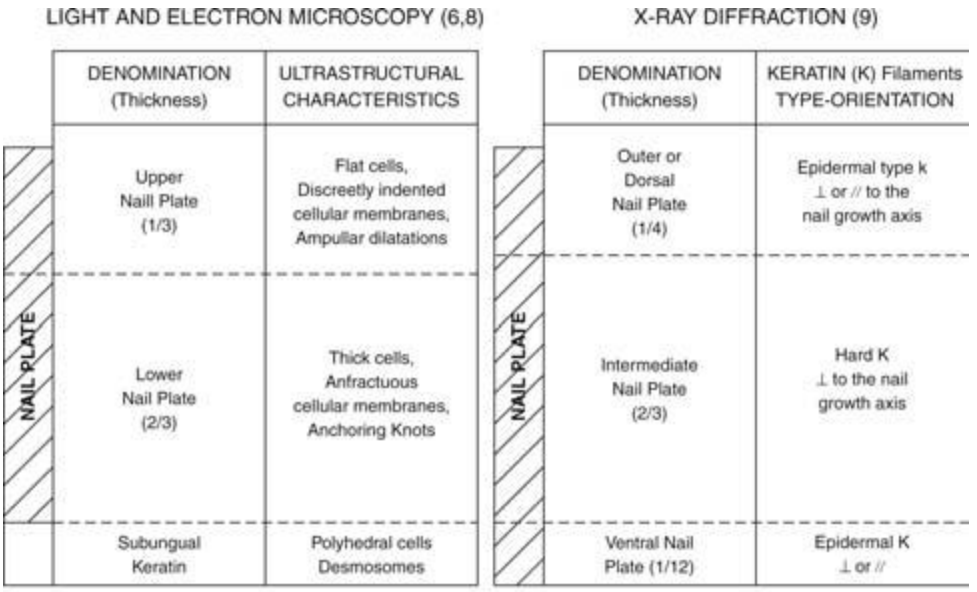

Figure 5 Nail Plate in Transversal Section.

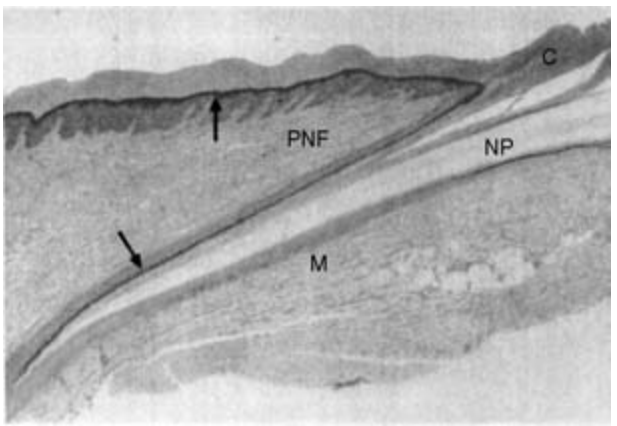

Figure 6 Longitudinal section of the proximal part of the nail apparatus. A stratum granulosum (*arrows*) is present in the dorsal and ventral part of the proximal nail fold epithelium but is absent in the matrix epithelium. *Abbreviations*: PNF, proximal nail fold; C, cuticle; NP, nail plate; M, matrix.

This technique, called "capillaroscopy," is useful in the diagnosis of Raynaud's phenomenon and connective tissue diseases. The ventral part of the PNF is a flat and rather thin epithelium that keratinizes with a stratum granulosum (SG). The latter can disappear in the most proximal part of the PNF that is the proximal matrix. The cuticle corresponds to the modified stratum corneum (SC) of the distal part of the PNF, at the angle of the dorsal and the ventral part.

- The nail matrix is a multilayered epithelium. Its keratinization process is characterized by an onychogenous zone devoid of keratohyaline granules (Fig. 6). It gives birth to the nail plate: the proximal part of the matrix gives birth to its dorsal part and the distal part of the matrix to its ventral part. Immunohistochemical studies have shown that the nail matrix epithelium is the sole site of hard keratin synthesis (10).

The epithelium of the matrix also contains melanocytes and Langerhans cells. Melanocytes are about 200/mm^2 in number (about 1150/mm^2 in the epidermis). Most of

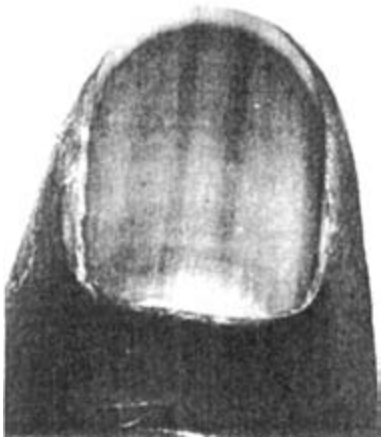

Figure 7 Multiple longitudinal melanonychia in an adult black patient.

them are dormant (11) and do not produce pigment. However, in dark-skinned individuals, longitudinal pigmented bands can be observed in nails. This racial physiological pigmentation is attributable to the activation of the matrix melanocytes and to the melanin incorporation in the nail plate (longitudinal melanonychia). It usually affects several nails and tends to become more frequent with aging; this can only be observed in 2.5% of newborn to 3-year-old black children, but in 96% of blacks older than 50 years (Fig. 7) (12).

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

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

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

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

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

PHYSICOCHEMISTRY

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

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

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

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

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

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

PHYSIOLOGY

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

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

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

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

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

ESTHETICS

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

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

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

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70 Nail Cosmetics: Handle of Skin Care

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THE MANICURE

The art of manicure is very ancient as can be testified by the Egyptian tomb of Niankhkhnum and Khnumhotep, dated approximately 2400 BC. It was discovered in the necropolis of Saqqarah in 1964. It is the tomb of two manicurist men who seem to have played an important role. Indeed, they were "Manicurist and Overseer of the Manicurists in the Palace, King's Acquaintance and Royal Confidant" (King Niouserrê, 5th dynasty, ancient empire) (1).

Nail Polish Remover

Currently, a professional manicure is made up of several steps (2):

The first one consists of removing any nail enamel remaining on the nail plate from a previous application. To do so, a cotton ball soaked with nail polish remover is used (Fig. 1). Nail enamel removers dissolve nitrocellulose and remove lipids from the nail plate. They mainly contain a mixture of organic solvents, with small amounts of oils added to counteract the drying effect of the solvents.

Typical formula (3):

- *Solvents (ca. 98%)—Example: acetone, butyl or ethyl acetate, ethoxyethanol.*
- *Lipids (ca. 2%)—Example: castor oil, lanolin oil.*

Other additives can also be found such as dyes, fragrances, preservatives, vitamins, and UV absorbers.

Nail polish removers should not be used more than once a week and should not be left too long in contact with the nails and the skin. Correctly used, they most probably do not cause nail brittleness (4).

Marketing usually insists on the fast action of the product, which is usually true. Nail polish removers specifically for brittle nails are also marketed. These are mainly nail polish removers without acetone. Actually, they are not better but are odorless.

Some varnish removers for brittle nails have a different composition, for example, on the basis of an ethoxy-diglycol solution, containing a mercapto-silanol complex (Si-nails remover®). This latter is purported to penetrate into the nail and to reinforce the disulfide linkage of the keratin. There is a lack in scientific studies, and the necessary time to remove the varnish is longer.

Cutting, Filing, Pumicing

Next, the nail is given the desired length, shape, and surface.

Therefore, the nail is cut and filed (Fig. 2). Filing is known to be less traumatic. This is probably only true when bad quality nail clippers are used.

If the nail surface is irregular, it can be trimmed, but not too much, to avoid nail plate thinning. This would be a source of nail brittleness.

Afterwards, the periungual grooves and folds are taken care of.

The nails are bathed in lukewarm, slightly soapy water. This cleans and softens the periungual tissue.

Cuticle Removers (5)

The cuticle is then covered with cuticle removers, which can be a liquid, gel, or cream. They usually contain sodium hydroxide and potassium hydroxide, in a 2% to 5% concentration.

Figure 1 Nail polish removal.

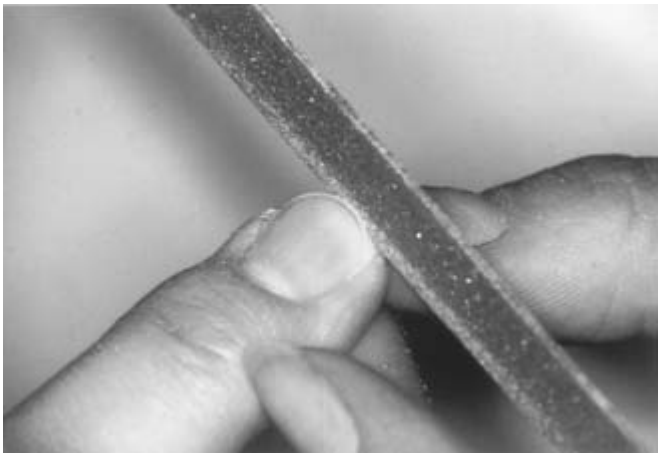

Figure 2 Nail plate filing.

α-Hydroxyacids (1–5% lactic acid, pH 3–3.7) are also used. They attack keratin by disruption of the disulfide bonds of cystine. Cuticle removers contain various additives: emollients (lanolin) or humectants (glycerin, propylene glycol) whose purpose is to decrease evaporation, increase viscosity, and reduce irritation. Preservatives, perfumes, and thickening agents can also be added.

Typical formula (3):

- *Water (ca. 90%)*
- *Softening agent (1–5 %)—Example: potassium hydroxide*
- *Thickener (0.5–1 %)—Example: sorbitol, magnesium aluminum silicate*
- *Perfume (0.1%)*

The cuticle remover increases the softening of the cuticle and of the cuticle remnants, which adhere to the nail plate surface. These are then gently pushed back with an orangewood stick covered with cotton or a rubber-ended stick (Fig. 3). Of course, the goal of this step is not to destroy the cuticle, which is a very important seal between the proximal nail fold (PNF) and the nail plate. Its destruction can lead to paronychia (periungual inflammation). Inexperienced rough handling of the cuticle may also injure the matrix below.

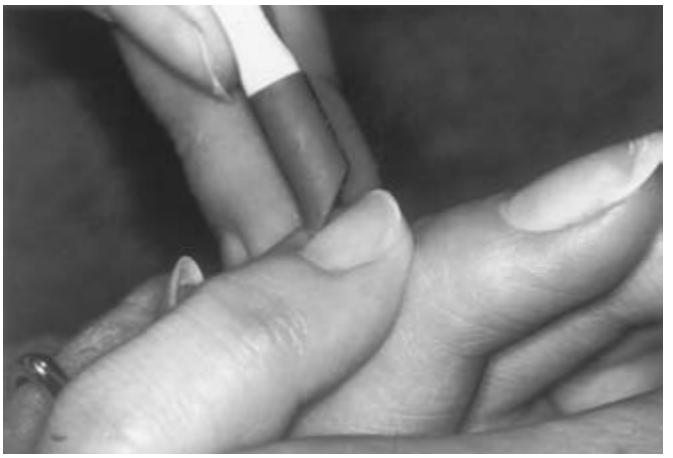

Figure 3 Rubber-ended stick, to push back the cuticle remnants.

Figure 4 Hangnails are cut with a special nipper.

The distal and lateral nail grooves are cleaned. It is most important to avoid overaggressive cleaning beneath the free edge of the nail, which can lead to onycholysis (detachment of the nail plate from the nail bed).

Finally, hang nails are cut with a special nipper (Fig. 4).

The manicure ends with a moisturising cream massage of the nails and the hands.

Buffing and Whitening

Other treatments are more rarely used: an abrasive powder, paste, or cream (stannic oxide, talc, silica, chalk, kaolin) (2) can be applied on the nail surface, which is then buffed with chamois leather. This produces a smooth and shiny nail surface, appreciated by women who do not wish to wear nail polish.

The undersurface of the nail can be painted with a whitening pencil, of which the core is kaolin, to reinforce the whiteness of the free edge.

Daily Care

Products are also marketed for daily use, which are to be used between manicures.

Cuticle softeners are not to be confused with cuticle removers. They are simply emollients to which quaternary ammonium compounds or urea are sometimes added to promote softening of the cuticles (2). These latter can then be gently reversed with the fingers.

Creams for brittle nails contain phospholipids. They are best applied after moistening the nail plate. Indeed, when a nail has been hydrated by immersion, phospholipids prevent dehydration, maintaining and increasing nail flexibility (6). Creams containing mandelic acid

are also marketed; in contrast to most α-hydroxyacid, mandelic acid would cause thickening in keratin.

Unwanted Effects of Manicure (7,8)

Unwanted effects in the nail area can mainly be attributed to technical errors. An overzealous manicure is likely an important source of microtraumatisms and can be the cause of multiple nail alterations: excessive pumicing or buffing can cause thinning of the nail plate and redness of the nail bed; repeated traumatisms on the nail matrix area can cause striate leukonychia (transverse white streaks) (Fig. 5) or Beau's lines (superficial transverse grooves).

The destruction of the cuticle leads to chronic paronychia.

The caring of the distal nail groove can cause onycholysis with proximal irregular indentation (Fig. 6), and caring of the periungual fold can cause small sores.

The damage to the cuticle, the onycholysis and the periungual sores favor bacterial and mycotic infections. The risk of transmitting infections in nail salons should not be neglected. A woman was awarded $3.1 million after contracting herpes, which spread to all 10 fingers. Precise standards of disinfection should be observed (7).

Figure 5 Leuconychia striata caused by overzealous manicure.

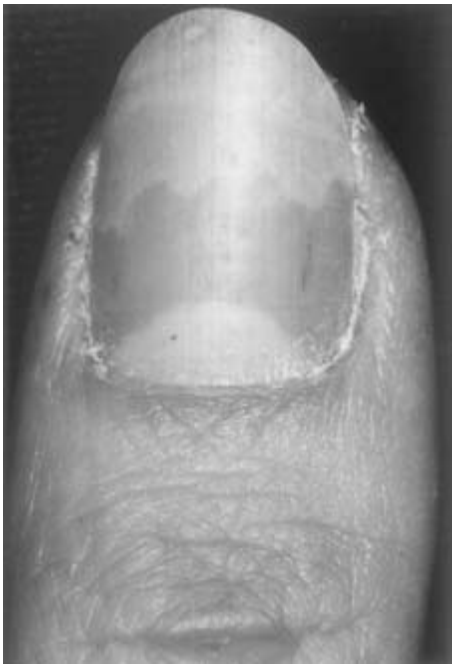

Figure 6 Onycholysis caused by excessive caring of the distal nail groove.

After contact with polish removers, the skin may have a white scaly appearance caused by stratum corneum dehydration; repeated application leads to irritant dermatitis. A single prolonged contact may even cause superficial blistering (9).

Cuticle removers should not be left too long in contact with the nail either, because they can cause irritation and should not be used on people who are susceptible to paronychia (10). In 1982, manufacturers reported receiving more complaints from cuticle removers than for nail polish removers: 2.3 and 0.33 complaints per million units sold, respectively (2).

Side effects of moisturizing creams (i.e., allergic contact dermatitis) are similar to those observed on other parts of the body.

Systemic Side Effects are Exceptional

Ingestion of nail polish remover may result in acetone intoxication, with central nervous system/respiratory depression, hyperglycemia, and ketosis (11). Inhaling nail polish removers containing toluene and aliphatic acetates (e.g., ethyl acetate) can cause central nervous system depression and a tight smothering feeling in the chest. As a consequence, excessive sniffing of polish removers could also produce toxic symptoms (9).

Classical nail polish removers should not be confused with artificial nail removers, which are much more dangerous systemically (see artificial nails/systemic side effects). In addition, nail polish removers are flammable and represent a fire hazard.

NAIL POLISH (SYNONYMS: NAIL VARNISH, NAIL ENAMEL, NAIL LACQUERS)

The concept of nail painting is not new. Numerous civilizations have used different products to enhance the beauty of nails. Henna is one of the most common product; it was probably already used in ancient Egypt and is still used today. Red balsam leaves mixed with alum were used in China, on the eve of the Mongol invasion (1250–1276) (12).

However, present polishes are quite recent; they appeared in the early 1920s. Their existence is linked to the discovery of nitrocellulose properties and to the progress made at that time in the automobile paint industry. Nitrocellulose was created by reacting natural cellulose fibers with concentrated nitric acid (HNO_3). Originally, it was used in high explosives, particularly during World War I. Later, another property of nitrocellulose was especially used: boiled in water, it decomposes enough to become soluble in organic solvents. After evaporation of the solvents, it produces a shiny and hard film. This quick-drying nitrocellulose lacquer rapidly achieved a great success in the developing car industry. Without the impetus provided by the automobile industry, it is doubtful that lacquer technology and the supply of good lacquer-grade nitrocellulose would have developed and been available for the manufacture of nail polishes (13).

For historic reasons, nail varnishes are also called “nail polishes,” “nail lacquers,” and “nail enamels” (13).

The perfect nail polish has to be easy to apply, and it should dry quickly. In addition, it should leave a shiny, smooth, even, hard, and flexible film that is able to last five days. Moreover, this polish should be removed without leaving any trace and should not have any side effects. Finally, it should be stable in the bottle and should offer a wide range of colors, enabling one to get the expected aesthetic effect.

The manufacture of nail polish is complex and potentially dangerous. It is only done in a few big factories. Nail varnishes are then contracted out by cosmetic companies, packaged, and labeled (14).

Composition (15)

The main constituent of the film that remains on the nail after drying (evaporation) is *nitrocellulose*. The film former has a lot of qualities. In particular, it is hard, tough, stable, and waterproof, but it is neither sufficiently adherent nor glossy or flexible.

To improve adherence and gloss, *film modifiers* are added. The most common one is Santolite® or toluene sulfonamide/formaldehyde resin (TSFR). TSFR is best designed under its INCI name: tosylamide/formaldehyde resin. It is the heart of the polish. As it is a potent sensitizer and contains formaldehyde, it tends to be replaced by other film modifiers that are said to be hypoallergenic: glycerophthalic polyester resin, 4-methylbenzene sulfonamide-epoxy

resin, phthalic polyester resin, polyester-saturated hydroxylated resin (16). However, in 1997, 42 nail polishes sold in Finland and belonging to 20 different brands were studied (17). They all contained TSFR, a factor of contact dermatitis, with concentrations from 0.02% to 11% in the bottle and from 0.1% to 25% in the dry polish.

With the nitrocellulose and the film modifier, the resulting film is hard, tough, adherent, and glossy. Unfortunately, it is still not flexible enough and cracks.

Plasticizers are added to increase flexibility. These are molecules with a high boiling point, which remain in the film after drying. They reportedly increase separation between the cellulose links as well as increasing the rate of solvent evaporation. Dibutyl phthalate and camphor enter this category. However, the former has been banned (65 Californian proposition in the United States). Other examples are castor oil, glyceryl tribenzoate, acetyl tributyl citrate, PPG-2 dibenzoate, glycerol, citrate esters, triacetin, and a polymer plasticizer called "NEPLAST" (a polyether-urethane) (15). While the film modifiers counterbalance the negative aspects of nitrocellulose, plasticizers modify the properties of the entire film. The glyceryl tribenzoate would even replace the film modifier in some nail polishes (15).

In the nail polish bottle, there are not only nitrocellulose, thermoplastic resin, and plasticizers but also solvents and thinners, pigments and dyes, thixotropic agents, as well as other additives.

The most commonly used *solvents* are alkyl esters (ethyl, *N*-butyl acetate) and glycol ethers (propylene glycol monomethyl ether). These molecules, which have different boiling points and evaporation rates, allow the regulation of drying time. They also allow lower viscosity, which improves brushability.

Thinners or diluents are not real nitrocellulose solvents but are miscible with them. This allows reduction of the nail varnish price. Thinners also help regulate the evaporation rates and stabilize viscosity. Indeed, a polish low in viscosity is easy to apply and leaves a homogeneous film. If viscosity is too low, the coverage of the film will be insufficient. If viscosity is too high, the film will be thick and streaky. Thinners essentially are aliphatic alcohols such as ethanol, isopropanol and butanol and aromatic hydrocarbures such as toluene. This latter, which is now considered to be cancerogenous and teratogenous, is no longer used in new nail lacquer formulations (15).

Pigments and colorants also need to draw the attention. Some are soluble in nitrocellulose and originate transparent polishes, very slightly colored. Most of them are not soluble and originate the most-used polishes: the nail enamels (13).

Example:

- *Mineral pigments: ferric ferrocyanide (Prussian blue), titanium dioxide (TiO2).*
- *Organic pigments: D&C red 6,7,34/FD&C yellow 5*

Prussian blue is used in small amounts to enhance blues and alter other shades. TiO_2 allows the attainment of pastel shades. D&C red 6 is a barium lake, D&C red 7 and 34 are calcium lakes, and D&C yellow 5 a zirconium lake, a lake being formed by precipitating a particular pigment with aluminum hydroxide to form a salt complex (15).

In pearlescent nail varnishes, there are guanine crystals, derived from scales of Atlantic herring and other fish, bismuth oxychloride, or mica coated with TiO_2.

The use of thermochrome pigments, of which the color changes according to the temperature, and photochrome pigments, of which the shade varies according to the light, offer new possibilities (18).

As a lot of pigments are insoluble, manufacturers had to cope with major problems of precipitation until new *thixotropic agents* were discovered in the 1960s (13). Thixotropic agents increase nail enamel viscosity at rest, thus preventing pigment precipitation. They, however, become fluid as soon as a mechanical constraint is exerted, either by shaking the brush or the bottle itself. This can be further improved by adding small metallic nickel (Ni) beads, often covered with plastic, in the bottle; Ni, indeed, is a common source of allergic eczema in women. Stearalkonium hectorite is the most frequently used suspending agent presenting settling out the pigments.

Theoretical composition of a nail varnish with 15% nitrocellulose, 7% resin, and 7% plasticizers is shown in Table 1.

Table 1 Nail Polish: Typical Formula. Comparison with Base Coat and Top Coat

Ingredients (approximate concentration in %)	Nail polish	Base coat	Top coat
Nitrocellulose	ca 15	↘	↗
TSFR	ca 7	↗	↘
Plasticizer	ca 7	↘	↗
Solvents–diluants	ca 70		
Butyl acetate	1.5–52		
Ethyl acetate	4–42	#	#
Isopropanol	2.7–7.7		
Toluène	20		
Suspending agent	ca 1		
Color pigments mixture	0.1		

TSFR: toluene sulfonamide formaldehyde resin; ↘: lower concentration; ↗: higher concentration; #: different concentrations
Source: From Refs. 3, 17.

Base Coats, Top Coats, Hardeners, Hypoallergenic Varnishes

Nail varnishes represent an important market estimated at $430 million a year, in the United States. (14). Besides classic nail polishes, base coats, top coats, hardeners, varnishes for brittle nails, varnishes for ridged nails, and hypoallergenic nail polishes are also marketed. What are they? All are nail varnishes with a slightly modified formulation.

The *base coats* contain more resin because they must increase the adherence of the varnish to the nail (Table 1). They contain no colorant to act as a protective antistain barrier between the nail plate and the shaded varnish (15).

On the contrary, the *top coats* contain more nitrocellulose and plasticizers because they must be tough and flexible to improve nail varnish resistance (Table 1). Often, they also contain special UV-absorbing materials, such as benzophenone 1 and 3, to help protect the underlying colored coats. Base coats and top coats also contain different proportions of thinners and solvents to ease application and to speed up drying.

Hardeners, varnishes for brittle nails, varnishes for ridged nails, etc. are in fashion. These are generally base coats to which are added nylon fibers, acrylic resins, or formaldehyde.

In hardeners, formaldehyde is in its free state, which is not to be confused with formaldehyde in the form of the TSFR in nail polishes (19). Formaldehyde is believed to crosslink protein in the nail plate, which increases surface hardness but decreases flexibility. In the long term, it may eventually be the cause of nail rigidity and cracks. Severe nail damage such as painful hemorrhages, paronychia, and nail bed involvement with subungual hyperkeratosis or onycholysis have been attributed to formaldehyde-based hardeners (Fig. 7). Lips hemorrhages are also possible in nail biters (19,20). Moreover, the presence of formaldehyde, which acts as an irritative agent, would favor TSFR sensitization (21). The presence of formaldehyde is now limited to less than 5%, and a shield for the skin should be used. Formaldehyde-containing hardeners are meant to be applied only to the free edge of the nail (19). In fact formaldehyde should be avoided.

New hardeners containing mandelic acid or silicium are marketed. In contrast to most α-hydroxyacid (AHA), acetyl mandelic acid in ISO propyl alcohol and butylenes glycol has been shown to cause thickenings in keratins. Silicium is purported to penetrate into the nail and to reinforce the disulfide linkage of the keratin. However, in both cases, biometric studies in nails are not available.

Calcium, vitamins, sulfured amino acid, and collagen can be added to the treating nail varnishes. These have probably no value. Two percent dimethyl urea is used in "Nail Intensity" by creative nail design (22). Chitosan has shown an increase in the linear nail growth in a double-blind study (23).

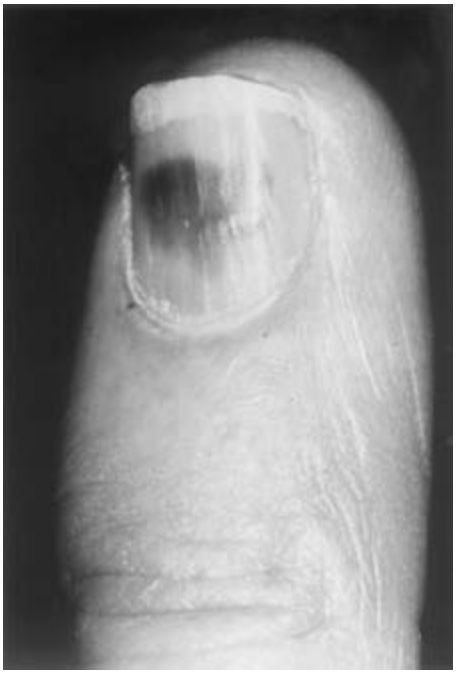

Figure 7 Subungual hemorrhage caused by a formaldehyde-based hardener.

Hypoallergenic nail polishes are polishes from which the most common substances causing allergic contact dermatitis, specially TSFR, have been removed. For example, the Pure Vernis® contains neither TSFR, nor phthalate or nickel balls. The resin is a polyester glycerophthalic resin. The plastifier is tributyl citrate acetyl. Nevertheless, these polishes do not guarantee the absence of reactions. Allergies to constituents of hypoallergenic varnishes have been described: polyester resin (24), epoxy resin (25), methyl acrylate (23), as well as rare cases of nitrocellulose allergy itself (26).

Recent Developments in Nail Polishes (14)

There is an increasing interest for quick-drying and long-lasting nail polishes, but these qualities are almost mutually exclusive. Nail polishes can be either quick drying or long lasting, not both. Quick-drying nail polishes contain low quantities of film former and are thus brittle.

Fragrances can now be added in nail polishes as well as metal or plastic glitters to give special effects.

Nail varnishes that are removed by peeling off also exist.

Water-based nail polishes have appeared. They are popular in Japan and with teenagers. As they contain water, they are prone to microbial contamination, and they have to be preserved, for example, with quaternium 15. Until now, water-based nail polishes are very brittle and crack but, according to Schoon (15), they probably constitute the future of nail polishes because a wider variety of additives could be added to them, which may eventually lead to truly preventing or help in treating common nail pathologies.

Use

After the manicure, the nail is degreased, dried, and covered with one layer of base coat, two layers of the colored nail enamel, and one layer of top coat (Fig. 8). The layers have to be thin and as uniform as possible. To do this, the quality of the nail polish and of the brush are both very important.

The varnish laying can be completed by spraying on or applying with a brush a film-drying accelerant that will protect the varnish while it is drying. (Silicone oil blends and silicone/water oil in water emulsions) (7).

The wearing of varnish is recommended for five of seven days.

Figure 8 Varnish laying.

Nail Polish Advantages

They are numerous. Firstly, nail polishes have an aesthetic advantage: they offer more refinement and elegance and allow for a wide range of personal preferences.

Secondly, the nail varnish plays a protective role: it forms a film on the nail surface, which is both tough and flexible, increasing the resistance of the nail to microtraumatisms. It also maintains a more constant degree of nail hydration.

In pathology, nail lacquers allow chromonychia and onycholysis to be hidden. Unfortunately, they can also hide a subungual melanoma and therefore delay its diagnosis (27).

Unwanted Effects of Nail Polishes (3,7,8)

They do exist but are relatively rare: in 1982, manufacturers reported receiving 0.28 complaints per million units distributed (2).

The nail polishes can cause an *orange staining of the nail plate*, prominent at the distal part. It is more frequently observed with the deeper shades of red and brown enamels and can be prevented by the former application of a base coat. But it has become rare, since soluble pigments are mainly used.

Nail lacquers can cause *keratin granulation* (Fig. 9), presenting as superficial friability. This happens when individuals apply fresh coats of enamels on top of old ones, for several weeks. Therefore, it is advisable to wear a varnish five in seven days only.

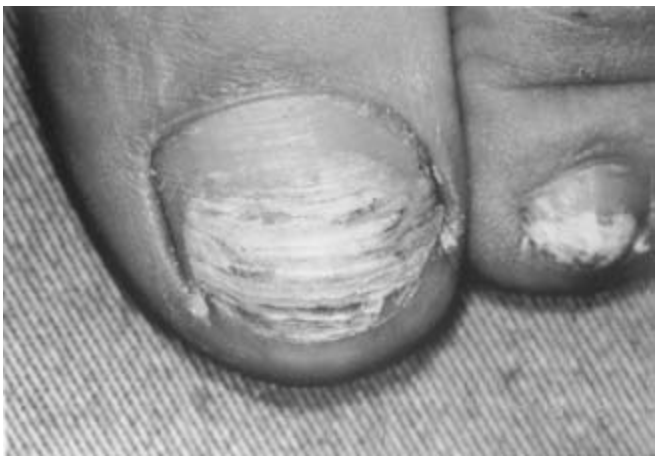

Figure 9 Keratin granulations *Source*: Courtesy of B. Richert, Belgium.

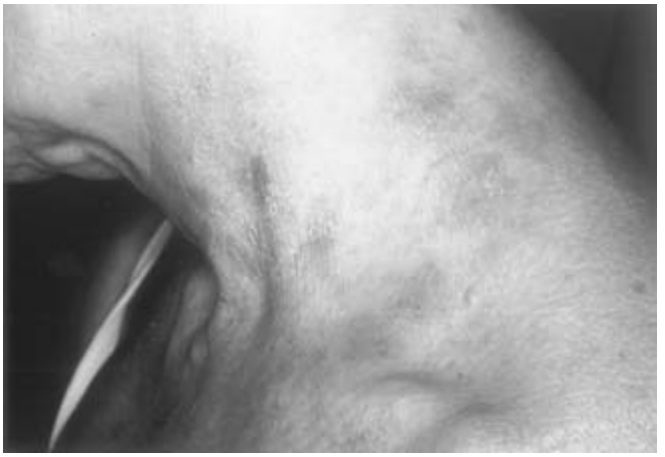

Figure 10 Eczema on the sides of the neck due to nail varnish allergy.

Nail varnishes can also cause *allergic contact dermatitis*, more rarely, contact urticaria.

Eczema breaks out especially on the eyelids, on the lower half of the face, on the sides of the neck (Fig. 10), and on the upper chest. In addition to distant (ectopic) contact dermatitis, allergic airborne contact dermatitis should be suspected when lesions on the face, neck, and ears are symmetrical. However, periungual dermatitis (28) is rare, but exists (29). The allergic contact dermatitis due to nail varnishes can have severe sociomedical consequences such as sick leave and hospitalization. Their diagnosis is easily missed (28).

The true incidence of reactions is not high: cosmetics cause 5.4% of contact dermatitis cases (30,31). Of the cosmetic reactions, nail products ranked fourth in the North American Contact Dermatitis Group study, producing 8% of the reactions (30). They ranked second, producing 13.4% of the reactions in a European study conducted by de Groot (31), in Holland. In a review of published cases, from 1925 to 1993, the most frequent allergen was by far TSFR, which was responsible for more than 347 cases (32). The guanine and the phthalates were respectively responsible for only four and three cases.

Among the most frequent allergens in cosmetics, TSFR comes in second place, after Kathon CG or paraphenylenediamine, in the studies of de Groot (31) and of Tosti (29) and in sixth place in the study of Adams and Maibach (30).

One case of both big toenail onycholysis, due to contact sensitivity to benzalkonium in a nail lacquer, has been reported (33).

Nail Polishes and Hospital

Nail polish should be removed routinely before anesthesia. Indeed, nail polishes, specially blue and green ones, significantly interfere with the measurement of oxygen saturation by pulse oximetry (34).

In health care workers, nail polish is allowed as long as it is worn on short nails and is fresh, not chipped (7). At equal length, the germ contamination rate does not seem higher for fresh painted nails than for natural ones.

THE ARTIFICIAL NAILS

Bringing very long nails into fashion may have originated from China (35). In the popular imagery, Chinese mandarins are often represented with very long nails. See, for example, the Chinese mandarin of Hergé in *Tintin and The Blue Lotus* (36).

The first artificial nails appeared in the United States, around 1935. Since then, thanks to their improving quality, they have become more and more popular. Annual business in nail salons in the United States is estimated at $6.3 billion. (14). Artificial nails were almost

exclusively applied by professionally trained manicurists in nail salons. However, professional nail applications are expensive, and artificial nail kits designed for home use are now available over the counter (37).

Composition and Techniques

To make things easier, two different kinds of artificial nails can be considered: the artificial sculptured nails and the artificial preformed nails.

Sculptured Nail Technique

In the *sculptured nail technique,* artificial nail is made of an acrylic resin obtained by blending a methyl, ethyl, or isobutyl methacrylate monomer, which comes in a liquid form, and a polymethyl or ethyl methacrylate polymer, which is a powder. The methyl methacrylate monomer, banned in certain American states because of its side effects, may still be used, especially in discount price salons. Indeed, this monomer, which smells terrible, is cheap and allows to work quickly (14).

The monomer also contains a stabilizer such as hydroquinone and *N,N*-dimethyl-*p*-toluidine as an accelerator. The polymer also contains benzoylperoxide as a polymerization initiator.

Typical formula (3):

- *Liquid: Acryl-type monomer (ca 99%). Example: methylmethacrylate monomer*
  Stabilizer (ca 1%). Example: hydroquinone
- *Powder: Acryl-type polymer (ca 97%). Example: polymethylmethacrylate*
  Polymerization initiator (ca 3%). Example: benzoylperoxide

Other components such as plasticizers, solvents, accelerators, and pigments may be included (38).

The nail plate surface is pumiced. After disinfection, a metallized paperboard template is placed to frame the new nail, and a primer is painted on the nail (Fig. 11). The latter is a highly acidic solution, most commonly methacrylic acid with a pH as low as 2. It acts as a double-sided sticky tape.

Next, several layers of the acrylic paste that has just been blended are applied. For bigger refinement, a white paste is first applied on the template and shaped (Fig. 12); then a pinker one is applied on the natural nail and the template, and again shaped (Fig. 13).

After hardening at room temperature, which occurs rapidly, the template is removed and the nail is pumiced (Fig. 14), filed (Fig. 15), and buffed, producing a long, smooth, and attractive nail.

Figure 11 A metallized paperboard template is placed. Then a primer is painted on the nail.

Figure 12 A white paste is first applied on the template.

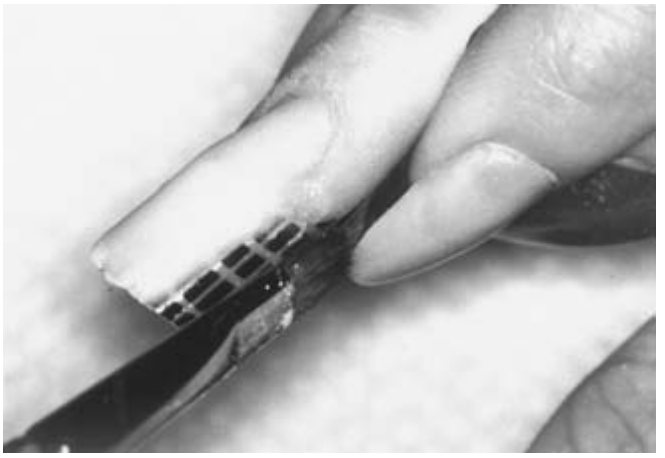

Figure 13 A pinker paste is applied on the natural nail and the template.

Figure 14 The sculptured nail is pumiced.

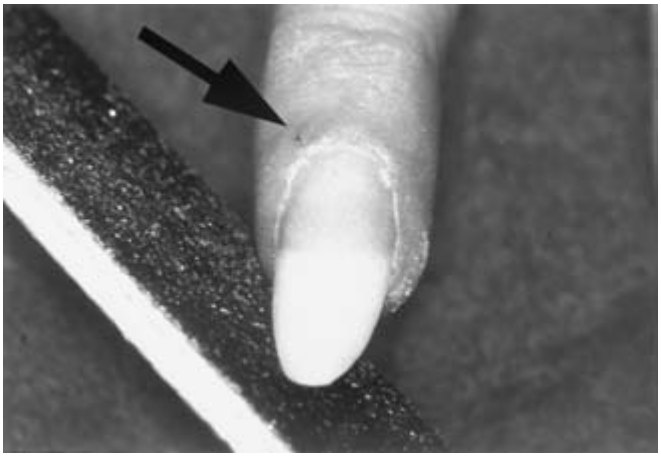

Figure 15 The sculptured nail is filed. Note the small sore in the proximal nail fold (*arrow*). The latter appeared while pumicing with the electric device shown.

This sculptured nail technique is rather difficult to perform. Its apprenticeship is long and requires good skills from the nail sculptor. However, this technique can be used on seriously damaged nail surface. Clients need maintenance filing.

Sculptured nails can be dissolved in acetone.

Artificial Preformed Nails

The second technique uses *preformed plastic tips*, which are packaged in several shapes and sizes, adapted to the different fingernails (Fig. 16).

The nail plate surface is buffed. After disinfection, the preformed plastic tip is simply fixed with cyanoacrylate glue, on the distal half of the nail (Fig. 17).

Typical formula of cyanoacrylate glue (38):

- *Ethyl cyanoacrylate* *90.6%*
- *Polymethyl methacrylate* *9%*
- *Hydroquinone* *0.4%*
- *Stabilizer (organic sulfonic acid) Trace*
- *Plasticizers and thickenings agents may be added.*

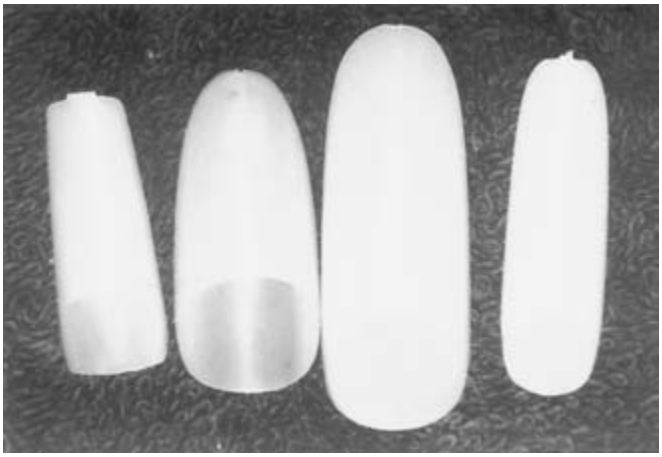

Figure 16 Preformed plastic tips.

Figure 17 Preformed plastic tips glued on the distal part of the nail.

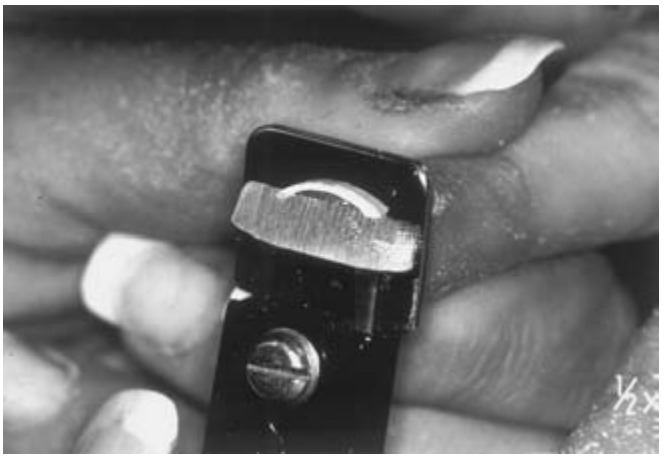

Figure 18 The artificial nail is cut with a special "guillotine clipper."

Then, the artificial nail, which is too long, is cut with a special "guillotine clipper" (Fig. 18). It is also filed and buffed to give it the desired shape and length.

At this stage, the nail surface is not smooth; it presents a bump between the proximal natural nail and the proximal border of distal artificial nail (Fig. 19). An acrylic gel is therefore painted on the nail like a nail polish (Fig. 20). It will harden, in other words polymerize, after UV exposure.

This technique is much easier to learn than the sculptured nail technique. It does not require any special skills, but there is a normal nail to be present for attachment. If not, the plastic nail will never be sufficiently glued. They should not remain for more than one or two days in time.

Light-Cured Gels

Gels are a premixed variant of sculptured nails. They are made of a mixture of acrylic monomers and polymers directly provided by the manufacturer.

They are more and more popular because they are odorless, give a more natural aspect to the nails, and do not require irritant (meth)acrylic acid as a primer.

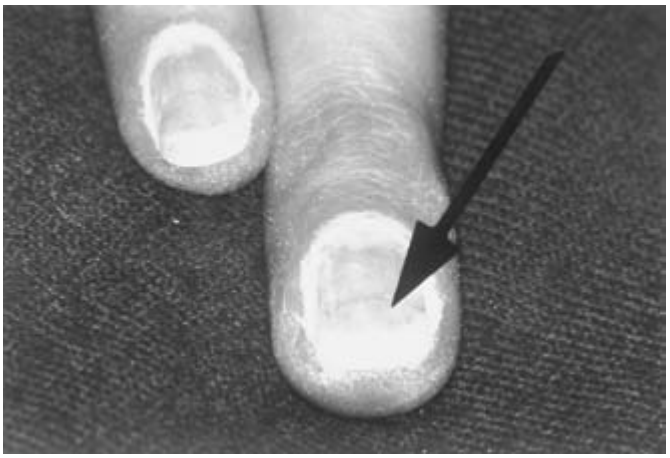

Figure 19 Bump (*arrow*) between the proximal natural nail and the distal artificial nail.

Figure 20 An acrylic gel is painted on the nail.

New gel formulations are regularly marketed, but there are two main types of gels:

1. Acrylic light-cured gels in which the polymerization or hardening is obtained by exposure to light (most often to UV). These gels may contain (meth)acrylated urethanes, triethyleneglycol dimethacrylate, methacrylated epoxy resin or hydroxyfunctional methacrylates (39), and a photoinitiator. The bonding is similar to "restorative dental bonding" commonly used by many dentists worldwide (40).

   It should be pointed out that acetone will have no effect on UV-gels in contrast to artificial sculptured nail. If a patient becomes allergic, the artificial nails must be grinded off with heavy abrasive (20).
2. Cyanoacrylate gels in which the polymerization is obtained by spraying or brushing an activator. For example: ethyl-cyanoacrylate gel; activator, *N*,*N* di-methyl paratoluidine.

There are gels with different consistencies, designed for different uses, the consistency being determined by the resin to monomer ratio. There are also colored gels.

According to their composition, the gels can be used for different purposes (20):

- For the sculptured nail technique. However, gels do not allow production of as long and resistant nails than the classical liquid-powder technique.

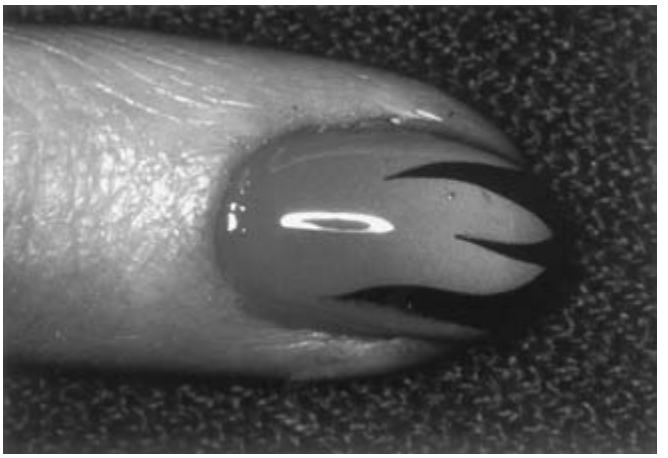

Figure 21 Air brushes made "nail art."

- Over plastic tips as we have just seen.
- To protect a natural or polished nail. This procedure is known as "nail capping."
- Capping with fabric (silk, linen, and fiberglass) adds strength and is known as "nail wrapping."
- Sprinkle resins based on cyanoacrylate resins, moisture cure, and trace quinone-type polymerization are present. Amine-based spray or brush-on polymerization activator may be used.

Advantages of Artificial Nails

Artificial nails are much more resistant than natural ones; nail polishes easily remain on them for three weeks.

They allow refinement and fantasy, which are much superior to those obtained with nail enamels. They can be decorated with jewels. Genuine work of art can be realized with special airbrushes (nail art) (37) (Fig. 21).

Artificial nails are mainly used for brittle or broken nails and for onychophagia. However, artificial nails should not be recommended in onychophagia associated with small wounds. Indeed, periungual sores that are so frequent in nail biters could favor acrylic sensitisation.

In pathology, they allow to hide more serious dystrophies than the nail polishes, such as posttraumatic permanent nail dystrophy (Figs. 22 and 23) or racket nails. After big toenail shedding in a tennis player or a skier, an artificial nail can prevent the hypertrophy of the periungual soft tissues, allowing a painless nail regrowth.

Artificial nails should not be recommended in case of nail psoriasis or lichen planus because they can worsen the condition by Koebner phenomenon (20).

Unwanted Effects of Artificial Nails (5,37)

Artificial nails are expensive and time consuming. It takes about one hour to put a set of 10 artificial nails in place, and moreover, they must be taken care of every two to three weeks. As the natural nail continues to grow, the proximal part of the artificial nail must be refilled. The adhesion between the natural and the artificial nail must also be checked; it must remain watertight.

Side effects of artificial nails can be classified in two main groups:

- Nonallergic reactions because of technical errors, bad care, or wearing of too long nails, which are most frequent
- Allergic or toxic reactions

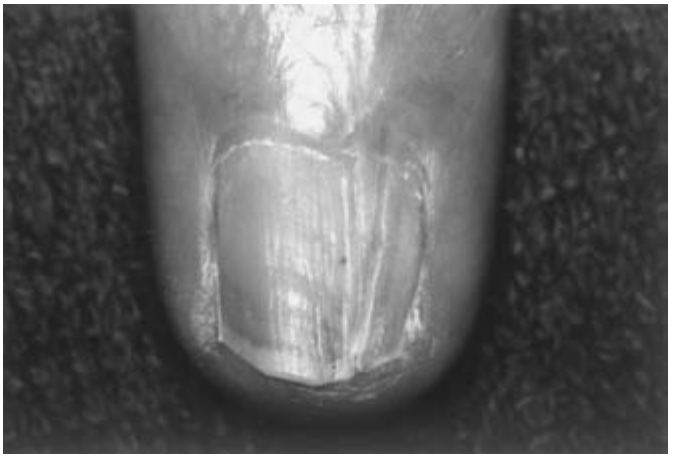

Figure 22 Posttraumatic permanent nail dystrophy.

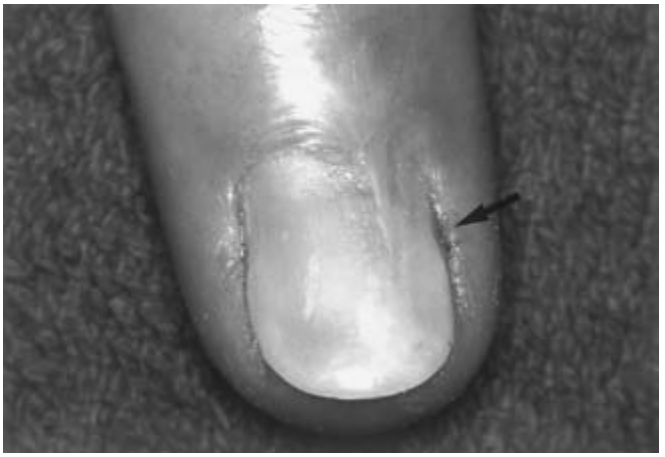

Figure 23 Posttraumatic permanent nail dystrophy as seen in Fig. 22, after application of an artificial nail. Note the small sore in the lateral nail groove (*arrow*).

NONALLERGIC REACTIONS

Technical errors or bad care cause brittleness of the natural nail by excessive filing or pumicing and inadequate use of the primer. After two or four months of wear, it is not unusual for a sculptured nail to damage the underlying natural nail. If it becomes yellow or crumbly, this means that the product was applied and maintained incorrectly. Instead of wearing prosthetic nails for no more than three consecutive months, with reapplication after one-month interval, it should be more appropriate to find a better-qualified nail technician. The problem may well not be the acrylic nail materials but rather the thinning of the nail because of over-filing with heavy abrasives. Small periungual sores (Figs. 15 and 24) are frequently observed. They could favor acrylic sensitization.

The penetration of water between the natural and the artificial nail is a frequent complication in artificial nails, which shows up as superficial nail plate discoloration (Fig. 25). Individuals in frequent contact with water, such as nurses, bartenders, and so on, have difficulty keeping on acrylic nails. This side effect is well known by nail technicians who remove the artificial nail, buff the nail surface to make the discoloration disappear, and sculpture a new, tightly adherent, artificial nail.

The wearing of too long nails favors onycholysis as well as nail fracture. It impairs finger and hand performance (41).

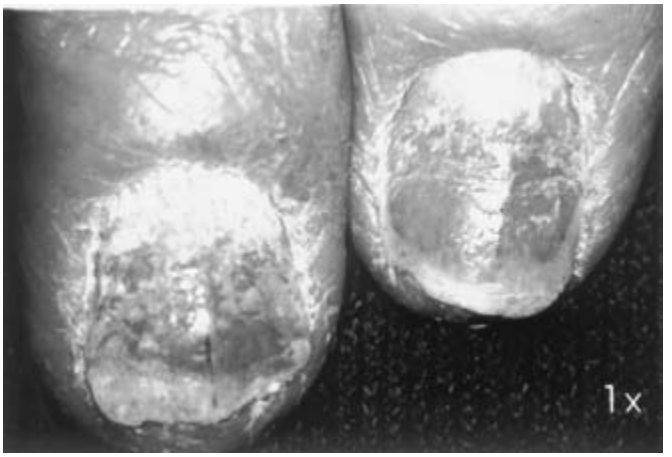

Figure 24 Severe alteration of the nail plates with distal onycholysis due to artificial nail allergy.

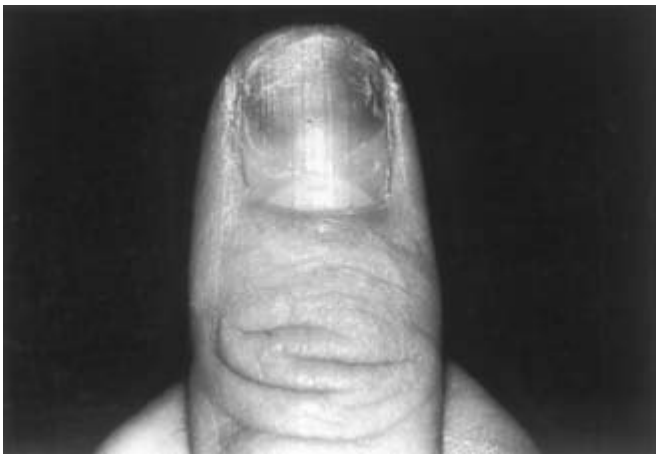

Figure 25 Superficial nail plate discoloration due to bad adherence between the normal and the artificial nail.

The wearing of too long nails, the onycholysis, the periungual sores, the penetration of water favor mycotic and microbial infections. These can be severe, especially in diabetics and immunocompromised people (42). Three cases of *Pseudomonas* corneal ulcers after artificial fingernail injuries have been described (43). There is a recent concern for microbial contamination of artificial nails in health care workers (see below).

Inappropriate Use

A case of "pseudo-onycho-palatitis" has recently been described. A mother had offered her right index finger in place of a pacifier to her baby. The mother's artificial nail became detached and was stuck on the palate. Several hours later, correct diagnosis was made when the infant was brought to hospital (44).

ALLERGIC OR TOXIC REACTIONS

Contact dermatitis to artificial nails can affect the client, less frequently the manicurist (40,45). Here, unlike nail-lacquer dermatitis, paronychia accompanied by onycholysis or subungual dermatitis are more likely to be present. Eyelid dermatitis is frequently associated. Women are usually not aware that artificial nails are a possible cause of allergy (46,47), and diagnosis is often delayed. When the use of artificial nail is discontinued, it is worth noting that it takes several months for the nails to return to normal.

Sculptured Nails

The most frequent allergen is the (meth)acrylate monomer, whereas the polymer is considered to be a weak sensitizer. Sensitization seems to be primarily caused by the monomer, which remains unpolymerized, in the final sculptured nail and in the filing dust, produced when the sculptured nail is trimmed (40). This is specially observed with self-curing resins, but even in photobonded acrylic nails monomer persists. The nail technicians should apply thin successive gel layers and expose each layer to UV, which is rarely done.

Allergic contact-type reactions were first described with methyl methacrylate monomer, but other monomers (e.g., ethyl and butyl methacrylates) can induce sensitisation, and cross-reactions also occur (39,48). They may be even stronger sensitizers than methyl methacrylate (49).

The allergic reaction usually starts two to four months, and even as long as 16 months, after the first application (37). The first symptom is an itch in the nail bed, followed by painful paronychia, which can be associated with paresthesia. Nail bed hyperkeratosis or onycholysis is frequently observed. Distant allergic contact dermatitis may affect the eyelids and the face, but more widespread lesions are also possible (50). Six cases of occupational asthma due to ethyl methacrylate have been reported in cosmetologists working with artificial nails (37).

Exceptional cases of severe paresthesia evolving for several years were described. These were accompanied by Raynaud's-like syndrome and permanent nail loss (48). They could result from a direct, toxic effect on the wounded Ranvier's cutaneous nerves in nail biters for instance. Patch tests may remain negative (51).

Prolonged paresthesia of the fingertips were also observed with photobonded acrylic-sculptured nails (39), and two natural nails had to be surgically removed because of resistant superinfections (40).

In contrast to the manufacturers' declarations, many "hypoallergenic" products continue to include acrylate functional monomers and therefore cause allergic sensitization (40).

Cyanoacrylate Nail Preparations

Initially, it was believed that the cyanoacrylates were not sensitizers. We now know that such cyanoacrylates can produce allergic reactions (39). Cyanoacrylates do not usually crossreact with the (meth)acrylate monomers used in nail preparations (38,45,48), although in a study performed by Koppula (52) ethyl α-cyanoacrylate did crossreact with several acrylates.

The cyanoacrylate glue, either used for nail wraps composed of silk, linen, fiberglass, or with plastic tips that should never cover more than distal half the nail, can be responsible for an eczema of the fingertips, with nail involvement [onycholysis, subungual hyperkeratosis (46), rough, split, deformed (53), or discolored (39) nail plates] (Figs. 25 and 26). An eyelid dermatitis as well as a nummular eczema can be present, particularly over the dorsal hand (54). More widespread eruption were also described (46); one case mimicked a small plaque parapsoriasis eruption (55).

Figure 26 Same patient as seen in Figure 25: positive patch tests with ethyl-cyanoacrylate glue and one of the patient's own product.

Persistence of the dermatitis until the nails grow out is frequently observed and is probably caused by retained adhesive. A positive patch test to nail clippings was indeed obtained in one patient, some two months after stopping the use of cyanoacrylate (46).

Patch testing is very easy, using a few drops of the cyanoacrylate adhesive, placed on the gauze portion of an adhesive plaster and allowed to dry before application (46).

Paresthesia has not yet been reported from cyanoacrylate glue.

Methacrylic Acid–Containing Primers

Nail-care products are a common cause of accidental poisoning in children. Such products accounted for 198,084 exposures (16% of exposures to cosmetics and personal-care household products) reported to the American Association of Poison Control Centers, in 1997 (56). Most nail product exposures involved either polish or nail polish removers, both of which are generally of low-order toxicity. However, products used in the application and removal of artificial nails are potentially hazardous and their packaging and labeling information are inadequate.

Methacrylic acid–containing primers are particularly involved: more than 759 exposures to methacrylic acid–containing artificial nail preparations were gathered by the American Association of Poison Control Centers. Seventy-five percent involved preschoolers younger than six years and almost 10% resulted in either moderate or severe injuries. Dermal burns were mainly reported, but burns of the airway and gastrointestinal tract with residual esophageal dysfunction were also described (57). Out of the 759 exposures, 84.9% had occurred at home, expressing the recent trend toward domestic use of artificial nail products, previously restricted to professional cosmeticians in nail salons. This home use has been done without a concomitant review of packaging safety. It is now recommended that the primers should be dispensed in child-safety containers (56).

Systemic Side Effects

Acetonitrile-containing artificial nail glue removers were removed from the market, after causing several childhood cyanide-poisoning deaths (58).

Six cases of profound methemoglobinemia were reported in children aged 13 to 27 months (59). They appeared following ingestion of small quantities of *artificial nail removers containing nitroethane*. The authors concluded that these products should be packaged in child-safety containers and properly labeled. Their availability for home use should be questioned.

N,N-dimethyl-p-toluidine can also cause methemoglobinemia when ingested (60,61).

ARTIFICIAL NAILS AND HOSPITAL

Unpolished acrylic nails do not affect pulse oximetry measurements of oxygen saturation. Theoretically, patients may not need to remove them before surgery (62); however, thick ornately painted gel false nails, which may be difficult to remove, present a real challenge to pulse oximetry (37).

There is a Real Concern for Microbial Contamination of Artificial Nails (35)

In health care workers with artificial nails, it has been shown that there is not only an increased amount of carriage of pathogen such as gram-negative bacteria (63–64) but also *Staphylococcus aureus* and yeasts, and this is observed both before and after washing the nails (65). In one study, *Serratia, Acinetobacter, and Pseudomonas* were cultured only from the fingertips of nurses wearing artificial nails (64). In a recent epidemiologic and molecular investigation of endemic *Pseudomonas aeruginosa* infection in a neonatal intensive care unit, it was shown that the use of artificial nails or nail wraps was a risk factor for colonization of the health care worker's hands (66).

Two nosocomial infectious outbreaks have been described where nurses wearing artificial nails could have played an important role in the transmission of potentially lethal infections. The first report dealt with *Serratia marcescens* infections in a cardiovascular surgery unit (67). The other report dealt with *P. aeruginosa* infection in a neonatal intensive care unit (68).

It should be added that individuals with artificial nails tend to wear their nails longer (65); they are more careful about their nails when washing their hands, and sanitary conditions for application of artificial nails, whether at the nail salon or at home, are paramount in preventing nail infections (68).

In the United States, the association of operating room nurses has guidelines concerning the nails. It is recommended that the nails should be kept short and clean. Nail polish can be used only fresh (but not chipped) and artificial nails should not be worn.

These guidelines should probably be extended to all health care workers, especially when they are dealing with immunocompromised patients. In addition, the 3-mm rule for end-of-fingernail length should be emphasized (69).

NAIL PROSTHESIS

For permanent nail degloving, following accidental or surgical origin and in congenital nail or missing digit, thimble-shaped digital fixation, for example, brings aesthetic and functional comfort to the patient (70).

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