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# Abstracts

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Review Article
Lipopeptides in cosmetics

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Lipopeptides are biosurfactants extensively used in cosmetics. The consumption of cosmetics containing lipopeptides is increasing as a result of the exceptional surface properties and diverse biological activities of lipopeptides which facilitate a vast number of applications not only in the pharmaceutics industry which includes cosmetics but also in the food industry. Cosmetics containing lipopeptides are available in various dosage forms according to their beneficial surface properties, which include anti-wrinkle and moisturizing activities and cleansing cosmetics. The microbial production of lipopeptides particularly those with biological and surface activities applicable to cosmetics are summarized based on appropriate studies and patents up to the year 2008 to manage the information and sufficiently review the data.

Two new lipoaminoacids with complementary modes of action: new prospects to fight out against skin aging

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The mode of action of two cosmetic active ingredients (AIs), palmitoyl glycine (PG) and cocoyl alanine (CA) was studied with cDNA array experiments and quantitative PCR confirmations, which were performed on experimentally aged human fibroblasts. These preliminary studies revealed complementary profiles. Thus, specific supplementary investigations were then carried out for each AI. Protocols used were based either on in vitro models: (i) biochemical assays, (ii) monolayer cell culture (primary human fibroblasts and keratinocytes) and (iii) the model of capillary-like tube formation by human endothelial cells or on ex vivo models, i.e. topically treated skin explants and both immunohistochemical and Chromameter™ investigations. New prospects are proposed to fight out against skin aging. Indeed, PG and CA showed complementary properties and thus enabled a regulation or a restoration effect on main aging-associated disorders. Thus, they can not only act on tissue architecture, cell–cell interactions and extracellular matrix protection but also on inflammation, cell longevity, skin immune system protection, skin radiance and stem cell survey. Finally, a clinical trial performed on Caucasian women confirmed AI anti-wrinkle efficacy, which was superior to that of a market reference ingredient. In the future, complementary experiments enabling a better understanding of the aging-induced decline of epidermal stem cells would be of a great interest.

A bioactive complex to protect proteins from UV-induced oxidation in human epidermis[1]

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[1] Paper was presented as a poster at the 25th Congress of the IFSCC (October 06-09, 2008, Barcelona, Spain).

UV light induces multiple damages including protein oxidation on skin. Oxidized proteins if not degraded by the proteasome would eventually accumulate causing metabolic damage, elastosis and pigment formation such as lipofuscin. During ageing, the activity of the proteasome decreases dramatically together with enzymes that protect from oxidation and as a result oxidized proteins accumulate. We have investigated a combination of Panthenyl triacetate and Ethyl linoleate (bioactive complex) to fight against protein oxidation. This complex when tested at 3% on human skin biopsies showed statistically significant protection from UV (UVA + UVB)-induced protein oxidation both in a 24-h pre-treatment before UV irradiation (72% protection, $P < 0.05$) and immediately after irradiation (78% protection, $P < 0.05$). UV light also induced a significant decrease of mRNA for protein repairing enzymes, such as Methionine Sulfoxide Reductase (MSR). The complex, given both pre- and post-irradiation, stimulated the repairing enzyme expression. We can suggest utilization of this new complex to prevent accumulation of oxidized protein as a result of skin photo-ageing and to prevent stratum corneum dehydration, skin elastosis and pigmentation formation (age spots).

## The COLIPA in vitro UVA method: a standard and reproducible measure of sunscreen UVA protection

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There is a continuing need to measure and communicate reliably the UVA protection offered by commercial sunscreens. To that end, the COLIPA (European Cosmetics Trade Association) 'In Vitro Sun Protection Methods' group has developed a new in vitro method for measuring UVA protection in a standardized, reproducible manner. The method is based on in vitro UV substrate spectrophotometry and convolution of resulting absorbance data with the action spectrum for the in vivo Persistent Pigment Darkening (PPD) endpoint to provide an in vitro UVA protection factor (UVAPF) which is correlated with an in vivo measure. This method has been published as a COLIPA guideline, used currently in European geographies for testing and labelling sunscreen products. This article summarizes two 'ring' studies, involving eight separate testing laboratories, which both defined critical parameters for the method and validated it. In Ring Study 1, eight laboratories tested the in vitro UV transmission of a total of 24 sunscreens and, from the data, a unit dose of UVA ($D_0$ of 1.2 J $cm^{-2}$) was defined to provide a single irradiation step which, by taking into account potential sunscreen photo-instability, gave the closest agreement with in vivo UVAPF values. In Ring Study 2, eight laboratories tested the in vitro UV transmission of a total of 13 sunscreens using this single irradiation step and established a very good correlation ($r^2 = 0.83$; slope = 0.84, $P < 0.0001$) between resulting in vitro UVAPF values and corresponding values derived from the in vivo PPD method. This new method, therefore, can be used to provide a reliable in vitro metric to describe and label UVA efficacy in sunscreen products, in line with the EU Commission recommendation 2006/247/EC.

## Validation of HPLC method for the simultaneous and quantitative determination of 12 UV-filters in cosmetics

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The aim of the study was the validation of a high-performance liquid chromatography (HPLC) method for the simultaneous and quantitative determination of twelve commonly used organic UV-filters (phenylbenzimidazole sulfonic acid, benzophenone-3, isoamyl p-methoxycinnamate, diethylamino hydroxybenzoyl hexyl benzoate, octocrylene, ethylhexyl methoxycinnamate, ethylhexyl salicylate, butyl methoxydibenzoylmethane, diethylhexyl butamido triazone, ethylhexyl triazone, methylene bis-benzotriazolyl tetramethylbutylphenol and bis-ethylhexyloxyphenol methoxyphenyl triazine) contained in suncare products. The separation and quantitative determination was performed in <30 min, using a Symmetry Shield® C18 (5 µm) column from Waters and a mobile phase (gradient mode) consisting of ethanol and acidified water. UV measurements were carried out at multi-wavelengths, according to the absorption of the analytes.

## Comparative evaluation of different substrates for the in vitro determination of sunscreen photostability: spectrophotometric and HPLC analyses

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Polymethylmethacrylate (PMMA) plates and Transpore™ tapes were compared as substrates for the in vitro evaluation of photostability of commercial sunscreen products. The sun care preparations were applied respectively on Transpore™ tapes and PMMA plates and their sun protection factors (SPF) and UVA protection parameters [UVA/UVB ratio, critical wavelength, UVA protection factor (UVA-PF)] were measured by transmission spectroscopy, before and after irradiation with simulated sunlight. No significant differences were observed in the UV protection parameters measured on Transpore™ tapes or PMMA plates, before exposure to the solar simulator. Conversely, after irradiation, the SPF values of the sun care products exhibited marked variations between the two substrates, the decrease in SPF being greater on PMMA plates (31.3–63.1%) than on Transpore™ tapes (10.4–23.8%). Differences between the two substrates were detected also for the UVA protection parameters, although they were significant only for the UVA-PF. The tested samples were assayed also by high-performance liquid chromatography (HPLC) to assess the extent of photodegradation of the UV filters present in the examined formulations. The results showed that for the PMMA plates, the light-induced decrease in SPF, as determined by spectrophotometry, fitted well with the percentage loss of ethyl hexyl methoxycinnamate (the only photounstable UVB filter present) measured by HPLC. Moreover, for the PMMA substrate, the UVA-PF percentage reduction was consistent with the percentage degradation of butyl methoxydibenzoylmethane (the only photounstable UVA filter present) determined by HPLC. On the other hand, poor correlation between spectrophotometric and HPLC analyses was observed on Transpore™ tapes. Therefore, PMMA plates are more reliable than Transpore™ tapes as substrates for in vitro photodegradation tests of sunscreen products by transmission spectroscopy.

## Simple and rapid analytical method for the simultaneous determination of cetrimonium chloride and alkyl alcohols in hair conditioners

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A simple method for the simultaneous determination of a cationic surfactant (cetrimonium chloride) and four non-ionic surfactants (1-tetradecanol, 1-hexadecanol, 1-octadecanol and 1-eicosanol) has been developed. Direct extraction of the analytes from the sample with methanol and a subsequent separation using reversed-phase high-performance liquid chromatography with refractive index detection are the steps followed in the procedure. The column used was a Luna C18 and the mobile phase consisted of a 0.1 M $KClO_4$ solution prepared on a 95:5 mixture of methanol and water. This solution was adjusted to pH 2.8 with phosphoric acid. Recoveries close to 100% were obtained in spiked commercial hair conditioner samples for the surfactants assayed using this method. Limits of detection were 10.4, 16.7 and 22.9 mg $kg^{-1}$ of cetrimonium chloride, 1-hexadecanol, 1-hexadecanol and 1-1-octadecanol respectively. The methodology was successfully applied to nine commercial hair conditioners of several types and different brands. All hair conditioners but one contained at least two of the surfactants included in this study.

## Antioxidant kinetics of plant-derived substances and extracts

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The antioxidant activity (AA) of substances present in several plant species has been widely studied which reflects their fundamental role in the protection of skin tissue against the harmful action of reactive oxygen species. Given the importance of effective and long-lasting protection against ultraviolet radiation, we studied the AA of several plant derivatives and extracts over time. Several chemical in vitro methods may be used to evaluate antioxidant capability, among which the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method stands out, despite its unspecificity, as the most cited and described method in the literature. In this work the AA was evaluated by measuring their capacity to reduce DPPH in 30 min, which is suggested in the literature, and additionally at different times up to 8 h from the baseline reading. The methodology used to evaluate the AA over time was validated. It is important to emphasize that this study proposes to modify the conventional DPPH method, although considered to be non-specific, to be used to test new antioxidant agents. This represents a considerable advantage because some substances show no significant activity during the first 30 min of reaction. Among other plant products, we tested a proantocyanidin-rich grapeseed extract, a hesperidin derivative, a rutin-containing ginkgo extract, a polyphenol-containing yerba maté extract and tocopheryl acetate, all of which were properly standardized. As they have different antioxidant profiles, each ingredient showed a specific behaviour over time, which may promote the selection of anti-radical compounds capable of offering protection against external agents. Combining extracts and plant derivatives that present fast, medium and slow antioxidant kinetic it is possible to create complexes capable of offering an effective protection from the moment of application up to several hours later. It is a perfectly feasible method, and such combinations prove to be more effective and have more durable effect.