

# Abstracts

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### Review Article

Importance of sunscreen products spreading protocol and substrate roughness for in vitro sun protection factor assessment

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The purpose of this study was to evaluate the impact of substrate roughness and of product spreading method on in vitro sun protection factor (SPF) measurement and to define the experimental conditions most appropriate to reach the best level of correlation to in vivo SPF. In vitro SPF assessment was carried out on 13 products (including different formulation types with SPF from 20 to 75) using various in vitro SPF protocols and comparing related predictive potential regarding in vivo SPF. In the first part, two spreading methods were compared on two types of PMMA (Polymethyl methacrylate plate with different roughness. The impact of a second spreading step after product drying was also evaluated. From the various investigated parameters, it was shown that (i) a higher roughness ( $R_a = 4, 5 \mu\text{m}$ ) was preferred for O/W formulations (ii) using a defined sequence of light linear and circular strokes was more adequate than monitoring

product spreading in terms of time and pressure (iii) both correlation to in vivo SPF and results variability were improved when a second spreading step was added. The altered protocol showed a good predictive potential regarding in vivo SPF values for O/W formulations (correlation coefficient 0.92, correlation curve slope 0.98) and coefficient of variation of in vitro results (14% of the mean SPF value) close to what is usually obtained in vivo. The repeatability of the protocol was also demonstrated. In the second part, we evaluated the impact of PMMA plate pre-treatment with paraffinum liquidum before spreading the product to get a better correlation between in vivo and in vitro SPF values for W/O formulations. This allowed us to define a protocol suitable for both O/W and W/O formulations.

### Review Article

A Hibiscus Abelmoschus seed extract as a protective active ingredient to favour FGF-2 activity in skin

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In the skin, heparin, heparan sulphate and heparan sulphate proteoglycans control the storage and release of growth

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factors and protect them from early degradation. We developed a cosmetic active ingredient containing Hibiscus Abelmoschus seed extract (trade name Linefactor™) that can maintain the FGF-2 content in the skin by mimicking the protective effect of heparan sulphate proteoglycans. By preventing the natural degradation of FGF-2, Hibiscus Abelmoschus seed extract maintains the bioavailability of this growth factor for its target cells, i.e. skin fibroblasts. Our *in vitro* evaluations showed that this ingredient exhibited heparan sulphate-like properties and dose-dependently protected FGF-2 from thermal degradation. We could also show that, in turn, the protected FGF-2 could stimulate the synthesis of sulphated GAGs, the natural protective molecules for FGF-2, thus providing a double protection. Finally, the *in vitro* results were confirmed *in vivo* thanks to a clinical study in which skin biomechanical properties and reduction in wrinkles were assessed.

An oral nutraceutical containing antioxidants, minerals and glycosaminoglycans improves skin roughness and fine wrinkles

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Various nutraceuticals (dietary supplements) are claimed to have cutaneous antiageing properties, however, there are a limited number of research studies supporting these claims. The objective of this research was to study the effectiveness of an oral nutraceutical containing antioxidants, minerals and glycosaminoglycans on cutaneous ageing. In this double-blind, placebo-controlled trial, 60 women aged 35–60 years were randomized to receive oral dietary supplement ( $n=30$ ) or placebo ( $n=30$ ), once daily for 12 weeks. The depth of skin roughness and fine wrinkles were measured using surface evaluation of skin parameters for living skin (Visioscan) at baseline, and at the 4, 8 and 12 weeks of treatment. Surface evaluation using a replica film (Visiometer) at baseline and

at the 12th week of treatment was also carried out. Statistical differences in objective skin improvement were assessed by the independent t-test. The volunteers' satisfaction was tested using the chi-squared test. The baseline depth of skin roughness and fine wrinkles in the treatment group and the placebo group were 100.5 and 100  $\mu\text{m}$ , respectively. At the end of the study, the depth of skin roughness and fine wrinkles in the treatment group showed a 21.2% improvement, whereas improvement in the control group was 1.7%. This difference was statistically significant ( $P < 0.001$ ). With regard to the volunteers' satisfaction, there was no statistically significant decrease in the homogenization of skin colour, however, a statistically significant reduction in pore size and depth of skin roughness and fine wrinkles were observed ( $P < 0.05$ ). No side effects were noted throughout the study. The oral dietary supplement containing antioxidants, minerals and glycosaminoglycans improved skin roughness and fine wrinkles but did not affect skin colour change in female volunteers.

Improvement of facial skin characteristics using copper oxide containing pillowcases: a double-blind, placebo-controlled, parallel, randomized study

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Copper plays a key role in several processes of skin formation and regeneration. Copper has been shown to be absorbed through intact skin. We hypothesized that sleeping on fabrics containing copper-impregnated fibres would have a positive cosmetic effect on the skin. The aim of this study was to confirm our hypothesis. A 4-week, double blind, parallel, randomized study was carried out in which 57 volunteers aged 40–60 years used either copper oxide containing pillowcases (0.4% weight/weight) or control pillowcases not containing copper. Photographs were taken by a professional photographer of each participant at the beginning of the study and at 2 and 4 weeks after the commencement of the study. Two expert graders (a dermatologist and a cosmetologist) evaluated the pictures for the effect on several cosmetic facial skin characteristics. The copper-containing pillowcases had a

positive effect for the following facial characteristics: reduction of wrinkles ( $P < 0.001$ ) and crow's feet/fine lines ( $P < 0.001$ ) and improvement of general appearance ( $P < 0.001$ ) at both 2 and 4 weeks. The differences were statistically significant (Wilcoxon scores and chi-squared tests). Consistent sleeping for 4 weeks on copper oxide containing pillowcases caused a significant reduction in the appearance of facial wrinkles and crow's feet/fine lines and significant improvement in the appearance of facial skin. In most trial participants, this effect was already noticeable within 2 weeks of using the copper oxide containing pillowcases.

Determination of tocopheryl acetate and ascorbyl tetraisopalmitate in cosmetic formulations by HPLC

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A rapid HPLC method was developed for the assay of tocopheryl acetate and ascorbyl tetraisopalmitate in cosmetic formulations. The validated method was applied for quantitative determination of these vitamins in simulated emulsion formulation. Samples were analysed directly on a RP-18 reverse phase column with UV detection at 222 nm. A mixture of methanol and isopropanol (25 : 75 v/v) was used as mobile phase. The retention time of tocopheryl acetate and ascorbyl tetraisopalmitate were 3.0 min and 5.9 min, respectively. Recovery was between 95% and 104%. In addition, the excipients did not interfere in the analysis. The method is simple, reproducible, selective and is suitable for routine analyses of commercial products.

Optimization of preservatives in a topical formulation using experimental design

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Optimizing the preservative regime for a preparation requires the antimicrobial effectiveness of several preservative combinations to be determined. In this study, three preservatives were tested: benzoic acid, sorbic acid and benzylic alcohol. Their preservative effects were evaluated using the antimicrobial preservative efficacy test (challenge-test) of the European Pharmacopeia (EP). A D-optimal mixture design was used to provide a maximum of information from a limited number of experiments. The results of this study were analysed with the help of the Design Expert® software and enabled us to formulate emulsions satisfying both requirements A and B of the EP.

Ultrastructural assessments of the melanosome distribution patterns and pigmentation features in human epidermal cells after UV irradiation and kojic acid treatment

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It is well-known that skin pigmentation depends, among others, on number, aggregation and distribution of melanosomes in the epidermis. Here we describe a

correlative microscopy-based ultrastructural approach that investigates the spatial distribution and pigmentation features of the melanosomes within melanocytes and keratinocytes. Data obtained from control skin, ultraviolet (UV)-stimulated tissue and kojic acid-treated UV-irradiated explants are compared. We introduce original parameters for the evaluation of the aggregation and pigmentation features of the melanosomes: the aggregation and pigmentation indexes. The aggregation index evaluates the presence of clustered melanosomes when the pigmentation index expresses the electron-density level of the pigment granules. The present study demonstrates that the last

parameters clearly express histological effects induced by UVB irradiation. Results indicate that UV light did not change the number of melanosomes within either melanocytes or keratinocytes, but it definitely modified the distribution patterns of the pigment granules in both cell types. It also enhanced the pigmentation state of the epidermal cells. Moreover, statistical analysis concerning keratinocytes discloses a significant decrease in the mean pigmentation index when explants exposed to UV light were treated with kojic acid. Obviously, the present numerical findings point out the relevance of the introduced parameters to characterize the pigmentation state of skin.