

Abstracts

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Review Article

A review of ageing and an examination of clinical methods in the assessment of ageing skin. Part I: Cellular and molecular perspectives of skin ageing

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The ageing process is noticeable within all organs of the body and manifests itself visibly in the skin. Skin ageing is influenced by several factors including genetics, environmental exposure, hormonal changes and metabolic processes. Together these factors lead to cumulative alterations of skin structure, function and appearance. The functioning of the central nervous, immune, endocrine and cardiovascular systems, as well as the skin is also impaired with age. Chronologically, aged skin is thin, relatively flattened, dry and unblemished, with some loss of elasticity and age-related loss of architectural regularity. General atrophy of the extracellular matrix is reflected by a decrease in the number of fibroblasts. Reduced levels of collagen and elastin, with impaired organization are primarily because of

decreased protein synthesis affecting types I and III collagen in the dermis, with an increased breakdown of extracellular matrix proteins. Oxidative stress is considered of primary importance in driving the ageing process. The original free radical theory of ageing purported that the molecular basis of ageing was derived from a lifetime accumulation of oxidative damage to cells resulting from excess reactive oxygen species (ROS) produced as a consequence of aerobic metabolism. Although the skin possesses extremely efficient antioxidant activities, during ageing, ROS levels rise and antioxidant activities decline. The ROS are necessary in multiple MAP kinase pathways and the induction of AP-1, in turn, up-regulates expression of matrix- metalloproteinases providing a plausible mechanism for the increased collagen degradation in aged human skin.

Review Article

A review of ageing and an examination of clinical methods in the assessment of ageing skin. Part 2: Clinical perspectives and clinical methods in the evaluation of ageing skin

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With the advancement of skin research, today's consumer has increased access to technological information about ageing skin and hair care products. As a result, there is a rapidly increasing demand for proof of efficacy of these products. Recognizing these demands has led to the development and validation of many clinical methods to measure and quantify ageing skin and the effects of anti-ageing treatments. Many of the current testing methods used to research and evaluate anti-ageing product claim to employ sophisticated instruments alongside more traditional clinical methods. Intelligent use of combined clinical methods has enabled the development of technologically advanced consumer products providing enhanced efficacy and performance. Of non-invasive methods for the assessment and quantification of ageing skin, there is a plethora of tools available to the clinical researcher as defined by key clinically observed ageing parameters: skin roughness and surface texture; fine lines and wrinkles; skin pigmentation; skin colour, firmness and elasticity; hair loss; and proliferative lesions. Furthermore, many clinical procedures for the evaluation of ageing skin treatments are combined with invasive procedures, which enable added-value to claims (such as identification and alteration of biochemical markers), particularly in those cases where perception of product effect needs additional support. As discussed herein, clinical methods used in the assessment of skin ageing are many and require a disciplined approach to their use in such investigations.

The perception threshold measurement can be a useful tool for evaluation of sensitive skin

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Sensitive skin is characterized by subjective symptoms that are hard to quantify. However, a neurobiological

approach could improve our understanding of the nature of skin sensitivity. In this study, we measured the sensory perception of well-controlled electric currents on the skin that stimulated sensory nerve fibres such as the myelinated A fibre, A delta fibre and unmyelinated c-fibre. The sensory perception thresholds were obtained quantitatively from subjects with sensitive-prone skin and controls. Application of 0.075% capsaicin, known to stimulate the nociceptor c-fibre, was topically applied; then the sensory perception thresholds were measured to determine whether the exposure to nociceptive stimulation could affect the subsequent sensory perception. The results showed that the perception thresholds of skin sensitive-prone subjects were low for the c-fibre measurements at 5 Hz electric current stimulation. Furthermore, a wide variation in sensory perception was noted in the skin sensitive-prone subjects after topical application of capsaicin. In conclusion, the abnormal sensory perception in individuals with sensitive skin appears to be related to neurological instability, where c-fibre nociception plays a role. Thus, quantitative sensory perception threshold measurement was found to be a useful method for the identification of skin sensitive-prone subjects.

Cocoa polyphenols and their influence on parameters involved in ex vivo skin restructuring

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Polyphenols in general are compounds that are known to promote health and have a preventive effect against various chronic diseases. The influence of cocoa polyphenols on skin, however, has scarcely been studied from a histological point of view. The aim of this study is to assess the influence of cocoa polyphenols on several indicators of skin elasticity and skin tonus, namely, glycosaminoglycans and collagen I, III and IV. This was carried out by using a model of ex vivo human skin explants maintained in survival, on which a cocoa polyphenols extract was applied. After processing by standard histological techniques (fixation, paraffin embedding, sectioning, staining, immunostaining and microscopical observation), the influence of cocoa polyphenols on the evaluated parameters was quantified by image

analysis. The results obtained show that cocoa polyphenols exhibit a positive action on the parameters assessed, and the dose at which they improve the most parameters associated with skin tonus and elasticity was determined. Their activity was compared with a commercially available product, and the results obtained show that their efficacy is equivalent. Moreover, an enhancing effect of cocoa butter on activity of cocoa polyphenol was highlighted. Now that the properties of cocoa polyphenols on ex vivo skin restructuring parameters have been assessed, the next step could include their evaluation in vivo.

Properties of a new hydrotrope hydrophobic molecule and its potential applications

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In the present contribution, the properties of dipropylene glycol isobornyl ether (Pribalance_) are discussed, especially in the context of microemulsion and emulsion formulations. Pribalance_ is a new low-toxic anti-foaming hydrotrope with excellent co-surfactant properties that has some similarities with long-chain alcohols, but in contrast to them, it is liquid at room temperature. In combination with another, more hydrophilic cosurfactant, it allows significant amounts of oil to be solubilized in water. Possible applications such as in cosmetics, as an anti-foaming agent or as additive to cooling lubricants are discussed. Further potential applications are plasticizers, fermentation systems, agrochemicals and waste-water treatments.

Screening of Nepalese crude drugs traditionally used to treat hyperpigmentation: in vitro tyrosinase inhibition

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South-East Asian population is daily exposed to strong sunlight. As a result, the majority of population will have darker, ethnic skin. Moreover, many people suffer from dark spots, hyperpigmentation, which is considered to be a skin disorder and causes psychological disturbance. To treat dark spots, most of the population will still rely on traditionally used crude drugs, knowledge about which is transferred from generation to generation. Fifty-two crude drugs were selected based on the survey performed among local healers and beauticians of different ethnic origin. These crude drugs were screened for mushroom tyrosinase inhibitory activity, as tyrosinase inhibitors are becoming increasingly important as cosmetic and medicinal products, primarily to control hyperpigmentation. Among the tested crude drugs, methanolic extracts of *Glycyrrhiza glabra*, *Morus alba*, *Syzygium aromaticum*, *Citrus aurantifolia*, *Cypraea moneta*, *Punica granatum* and *Citrus aurantium*, at the final concentration of 50 lg mL⁻¹, showed mushroom tyrosinase inhibitory activity of 78.9%, 71.0%, 69.4%, 59.0%, 56.0%, 53.4% and 51.9%, respectively, with 91.4% inhibitory activity of kojic acid taken as positive control. To our knowledge, this is the first report that extracts of *Cypraea moneta* shell and *Syzygium aromaticum* flowering bud have tyrosinase inhibitory activity. These potent extracts were further evaluated at different concentration. The final concentration of the extracts in reaction mixtures was 50, 25 and 5 lg mL⁻¹ for the initial concentration of 1000, 500 and 100 lg mL⁻¹, respectively. They showed concentration-dependant inhibition of mushroom tyrosinase. Those extracts expressing relatively weak tyrosinase inhibitory activity may act through different inhibition pathway which is not based on tyrosinase activity. Further evaluation of the most potent tyrosinase inhibitors in in vivo conditions would be recommended.

Adaptation of the protocol for determining in vitro the sun protection factor of anti-solar sticks

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Apart from the protection offered by clothing, the application of sunscreen products suited to each type of skin constitutes one way for decreasing the frequency of skin cancers nowadays. After having adapted an *in vitro* method for determining the efficacy of sunscreens in emulsion form, we wished to transpose this technique by adapting it for the anti-solar sticks for the evaluation of sun protection factor (SPF) using a spectrophotometer equipped with an integrating sphere. To do this, we tested 14 products in the market as well as sticks that we ourselves fabricated in the laboratory. In a base common to all of these sticks, we added organic (13 filters tested) and inorganic (two filters tested, titanium dioxide and zinc oxide) to their maximum permitted concentration in the European Union. In parallel, emulsions containing the same filters at the same percentage of use were studied; to be in keeping with the results on the products packaging on the one hand, and with the results obtained for the emulsion form on the other hand, we were able to determine the optimal mass which needed to be placed on the support used the *in vitro* test to determine the SPF.

Simultaneous determination of 21 preservatives in cosmetics by ultra performance liquid chromatography

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An ultra performance liquid chromatography (UPLC) method has been developed for the simultaneous determination of 21 preservatives: 2-methyl-4-isothiazoline-3-ketone, bronopol, 5-chloro-2-methyl-4-isothiazoline-3-ketone, benzylalcohol, 2-phenoxyethanol, methyl-p-hydroxy benzoate, ethyl-p-hydroxy benzoate, methyl benzoate, 4-hydroxybenzoic acid iso-propyl ester, propyl-p-hydroxy benzoate, 4-chloro-3-methylphenol, ethyl benzoate, 2-phenylphenol, 4-hydroxybenzoic acid iso-butyl ester, butyl-p-hydroxy benzoate, 4-chloro-3,5-dimethylphenol, phenyl benzoate, 2,4-dichloro-3,5-dimethylphenol, 2-benzyl-4-chlorophenol, triclocarban

and triclosan in cosmetics. A Waters ACQUITY UPLC BEH C18 column was used with 0.1% formic acid solution as the mobile phase under the condition of gradient elution. Preservatives were extracted with methanol by ultrasonicator, and then they were analysed by UPLC-PDA detector. All these preservatives were baseline separated in 8.5 min. The pre-treatment method of samples and the chromatographic condition of analysis were critically examined in this study. The recoveries ranged from 90.5 to 97.8%, with RSD values below 3.2%, and all correlation coefficients (*r*) were no less than 0.9997. Thus, this method could be used for analysing the preservatives in cosmetic products.

Validation of HPLC method for quantitative determination of Tinosorb_S and three other sunscreens in a high protection cosmetic product

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A chromatographic method (high performance liquid chromatography) with a diode array detector was developed for simultaneous assay of Tinosorb_S (bisethylhexyloxyphenol methoxyphenyl triazine) with three other sunscreen agents [benzophenone-3, butyl methoxydibenzoylmethane (avobenzone) and ethylhexyl methoxycinnamate] in high protection sunscreen. Separations were performed on a RP-18 Nucleodur_Gravity_column (150 × 4.6 mm, 5 μm) eluted with a ternary gradient mixture constituted of tetrahydrofuran, acetonitrile and an aqueous solution of acetic acid. The quantitative analysis was achieved with internal calibration performed with octyl dimethyl paraaminobenzoate (PABA) at 330 nm. In accordance with the analytical references (SFSTP, ICH, ISO...), the accuracy of the method was evaluated using a statistical approach of the validation parameters (specificity, response function, linearity, precision and trueness). For each studied ultraviolet filter, an accuracy profile was determined on a predicted range. These profiles show a graphical representation of the recovery percentage and confidence limits centred on 100%. The method is validated and can be used for analysis in cosmetic sunscreen products.