



## On the assessment of photostability of sunscreens exposed to UVA irradiation: From glass plates to pig/human skin, which is best?

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### ABSTRACT

Photostability of sunscreens is a great area of interest since several sunscreens on the market are photounstable, and this is primarily a problem concerning the UVA region (320–400 nm). Here we report a comparative study on the photostability assessment of two commercial sunscreens with same SPF, spread onto glass plates or onto full thickness pig ear skin or human/pig SCE membranes, and exposed to 183 kJ/m<sup>2</sup> UVA. Absorbance spectra and lipid peroxidation (measured by TBARS production) were determined. The results indicate: (a) sunscreen performance consequent to UVA exposure is independent of whether it is spread onto a non-biological and chemically inert substrate such as glass, or on biological substrates such as skin/SCE membranes; (b) despite the same SPF, sunscreen performance and photostability can be very different; (c) the data on human SCE membranes are similar to those on pig SCE membranes, indicating the suitability of the latter as a model for human skin. However, since the results obtained using skin membranes, akin to the more realistic conditions of use in vivo, do not substantially differ from those obtained on glass plates, the method proposed here using the latter may be applied for rapid, inexpensive, efficacy screening of photostability of sunscreens. Photostability testing should be a mandatory requirement for safer sunscreen protection products, since the results clearly show that some are still far from perfect.

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### 1. Introduction

The use of sunscreens is the most popular, universal method for preventing skin damage caused by sun over-exposure, which manifests itself as sunburn/erythema in the short-term and photocarcinogenesis/photoageing in the long-term (Clydesdale et al., 2001; Trautinger, 2001; Ullrich, 2007). The active ingredients in sunscreens are a mixture of UV filters designed to absorb/reflect/scatter the UVB rays (290–320 nm), UVA rays (320–400 nm) or both, thus reducing the amount of UV light reaching the viable skin layers (Palm and O'Donoghue, 2007). Most UV filters are sufficiently, photochemically stable, i.e. their absorbance spectra remain relatively unchanged during UV exposure. However, it is well known that some common ones are not. Their

absorbance spectra change following UV exposure and this leads to a loss in absorbance which ultimately translates into reduced photoprotection of the sunscreens containing them (Bonda, 2005; Dondi et al., 2006). In addition, in some cases chemical photoinstability is accompanied by the formation of photoproducts, free radicals, reactive oxygen species (ROS) which may not only interact with other co-formulated ingredients of sunscreen products, but also with skin constituents such as lipids, proteins, and nucleic acids (Allen et al., 1996; Butt and Christensen, 2000; Karlsson et al., 2009; Schwack and Rudolph, 1995). Hence, obtaining knowledge on the photostability of individual UV filters and, more importantly, of their photochemical behaviour when combined in a sunscreen, should be worthwhile pursuing for product safety and skin photoprotection. In fact, there is plentiful literature on the behaviour of individual UV filters but their performance may change when co-formulated with others in a sunscreen product (Damiani et al., 2007; Dondi et al., 2006; Maier et al., 2001; Roscher et al., 1994; Tarras-Wahlberg et al., 1999). Unfortunately, the majority of sunscreens on the market do not have a photostability label, since this is not a regulatory requirement for marketing, but only a SPF

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(sun protection factor) label which is an indicator only for protection against erythema, largely caused by UVB wavelengths (EC, 2007; Stanfield et al., 2010). No information is given on protection against UVA wavelengths which penetrate much deeper into the dermal skin layers than UVB ones. Furthermore, some studies have demonstrated that sunscreens' photostability is primarily a problem concerning the UVA region (Hojerova et al., 2011; Maier et al., 2001). High SPF value sunscreens imply that the UV filters present in them should remain photostable for the entire period of sunlight exposure providing long-lasting photoprotection. However, this may not be the case if the wrong combination of UV filters is present in the sunscreen and if they are prone to photoinstability.

At this regard, we recently developed a simple and effective method for assessing photostability and photoinduced ROS generation in sunscreens containing individual UV filters and their combination (Damiani et al., 2010). However, sunscreens were not applied to skin itself but on glass plates, hence the information obtained may not totally reflect the true behaviour of sunscreens when applied to skin. It is known that sunscreen performance is dependent on whether it is as a thin film or disrupted such as in real application to the irregular surface of the skin (Farr and Diffey, 1985; Haywood, 2006). Furthermore, the different pigmentation of skin (the UV and visible light reflected, scattered, absorbed and dissipated by chromophores in various layers of skin depending on the different skin types/tones) may affect the true behaviour of sunscreens. Based on our previous method, the present study takes one step further to gain more realistic information on the photostability of UV filters present in sunscreens. For this purpose, the behaviour of two commercial sunscreens with same SPF was investigated on human stratum corneum/epidermis (SCE) membranes, and for the first time, on pig SCE membranes, in addition to full-thickness pig ear skin, and exposed to UVA. Pig skin was used in this study since it shares many similarities to that of human skin including follicular structure, and has been used for in vitro skin penetration of UV filters as well as many other compounds (Jacobi et al., 2007; Weigmann et al., 2009). In addition, the behaviour of the sunscreens was compared with that obtained from their application as a thin film on glass plates, according to our previous method (Damiani et al., 2010).

## 2. Materials and methods

Two commercial sunscreens currently available on the European market were purchased from local stores and selected on the basis of their equal high SPF (SPF 30), but with a different combination of UV filters, as indicated on the product label, in the following order of appearance and therefore of concentration, as follows: cream A: OMC (ethyhexyl methoxycinnamate, UVB filter), TiO<sub>2</sub> (titanium dioxide, UVA/B filter), BMDBM (butyl methoxydibenzoylmethane, UVA filter); cream B: OCT (octocrylene, UVB filter), BP-3 (benzophenone-3, UVA filter), BMDBM, EHS (ethyhexyl salicylate, UVB filter). All other reagents and solvents were purchased from Sigma–Aldrich Chemical Co. (Milan, Italy).

### 2.1. Preparation of substrates and products application

Samples of adult human skin (mean age 36 ± 8 years) were obtained from breast reduction operations and treated as previously reported (Puglia et al., 2012). Briefly, subcutaneous fat was carefully trimmed and the skin was immersed in distilled water at 60 ± 1 °C for 2 min, after which SCE were removed from the dermis using a dull scalpel blade (Kligman and Christophers, 1963). Epidermal membranes were dried in a desiccator at ~25% relative humidity. The dried samples were wrapped in aluminium foil

and stored at 4 ± 1 °C until use. Previous research work demonstrated the maintenance of SC barrier characteristics after storage under the reported conditions (Swarbrick et al., 1982). Besides, preliminary experiments were carried out in order to assess the barrier integrity of SCE samples by measuring the in vitro permeability of [<sup>3</sup>H]water through the membranes using the Franz cell method. The value of calculated permeability coefficient (*P*<sub>m</sub>) for [<sup>3</sup>H]water agreed well with those previously reported (Bronaugh et al., 1986).

Pig SCE membranes were obtained in a similar way as described above. Briefly, pig ears that had not been scalded, were obtained from freshly killed animals (Large White breed, 9–10 months old) from a local abattoir, and treated in the following way in a cold room: the ears were washed with cold, distilled water and hairs carefully removed using an electric hair clipper for better distribution during sunscreen application. The underlying fatty tissue and cartilage was removed with a scalpel and the full-thickness skin was either treated as described above for human skin to obtain pig SCE membranes or laid out on a polystyrene tray, covered with a plastic bag and stored at –20 °C until ready for use for a period that did not exceed 2 months.

Prior to use, the SCE membranes and full-thickness pig skin were cut into samples 4 cm<sup>2</sup> in size and placed on a petri dish containing filter paper imbibed with a sufficient amount of PBS (phosphate buffered saline) such that only the underside of the skin was in contact with PBS. Sunscreens (15 µl) were then applied to the skin samples using a Microman positive displacement pipette which corresponded to 8 mg (2 mg/cm<sup>2</sup> as recommended by the COLIPA sun protection factor test method (COLIPA, 2006)). The same amount was applied to glass plates of the same dimensions as the skin samples. The sunscreens were spread over the different supports with a gloved finger using a light, circular, rubbing motion for uniform distribution, and left at room temperature in the dark for 20 min. For each cream two samples were always prepared, for and without UVA exposure. In parallel, skin samples without cream were also tested, for and without UVA exposure. Furthermore, human SCE membranes were from two different individuals, therefore one was used for all experiments concerning cream A and the other sample for all those concerning cream B. The same criteria were also used for pig SCE membranes which were from two different animals.

### 2.2. Irradiation source and protocol

A commercial UVA sun lamp, Philips Original Home Solarium (model HB 405/A: Groningen, Holland) equipped with a 400 W ozone-free Philips HPA lamp, UV type 3 was used for UVA irradiation. The output was measured with a UV Power Pack Radiometer (EIT Inc., Sterling, MA) while the emission spectrum was checked using a StellarNet portable spectroradiometer (Tampa, FL). The lamps emission spectrum has been reported elsewhere (Venditti et al., 2008), and shows that of the total light emitted between 300 and 400 nm, <1.5% is below 320 nm, hence the UV source is essentially a UVA one. The lamp was always pre-run 10 min to allow the output to stabilize. The petri dish containing the samples were then placed on ice at a distance of 20 cm from the light source and irradiated for 10 min which corresponded to a UVA dose of 183 kJ/m<sup>2</sup>. This dose is approximately equivalent to 60 min of sunshine at the French Riviera (Nice) in summer at noon (Seite et al., 1998). For cream A, a kinetic analysis was also carried out between time 0 and 10 min, for monitoring product stability every 2.5 min, within the selected time course. For each irradiated sample, a non-irradiated one serving as control was kept in the dark for 10 min at room temperature.

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