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Reliable and fast sensor for *in vitro* evaluation of solar protection factor based on the photobleaching kinetics of a nanocrystalline TiO_2/dye UV-dosimeter

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ABSTRACT

A reliable and fast sensor for *in vitro* evaluation of solar protection factors (SPFs) of cosmetic products, based on the photobleaching kinetics of a nanocrystalline TiO_2/dye UV-dosimeter, has been devised. The accuracy, robustness and suitability of the new device was demonstrated by the excellent matching of the predicted and the *in vivo* results up to SPF 70, for four standard samples analyzed in blind. These results strongly suggest that our device can be useful for routine SPF evaluation in laboratories devoted to the development or production of cosmetic formulations, since the conventional *in vitro* methods tend to exhibit unacceptably high errors above SPF ~30 and the conventional *in vivo* methods tend to be expensive and exceedingly time consuming.

1. Introduction

Sun protection products are cosmetic formulations for topic use having the capacity to absorb/reflect ultraviolet radiation (UVB (280–315 nm) and UVA (315–400 nm)). Non-toxic and photostable efficient inorganic (particularly TiO_2 and ZnO powders) and organic (*para*-aminobenzoic acid (PABA), 3-(4-methylbenzilidene)-camphor (4-MBC), octyl-methoxicynamate (OMC), octyl-dimethyl-PABA (OD-PABA), benzophenone-3 (Bp-3), homosalate (HMS), among others) UV-light absorbers are generally used as sunscreens [1–3], in order to reduce the deleterious effects of excessive exposure to sunlight on the skin, such as buildup of sunburned cells, immunosuppression, induction of carcinogenesis, photoaging, solar keratosis, alteration in fibroblasts, mutation of p53 gene, etc. [4–7]. Thus, the correct evaluation of the effectiveness of cosmetic sun protection products is of great importance, being a significant public health issue particularly as a consequence of the continuous degradation of the upper atmosphere ozone layer.

The SPF of cosmetic preparations is currently being determined based on internationally recognized and validated methodologies. There are two well accepted methods for the determination of SPF: the methodology proposed by the Food and Drug Administration of USA [8], and the International sun protection factor test method [9], recognized by the European Community, Japan, CTFA-South Africa and CTFA-USA. These clinical evaluation methodologies are based on the irradiation of human volunteers with UV-light, in the absence and presence of sun protection products. However, such *in vivo* methods are expensive, exceedingly time consuming and in some sense affront ethical principles because of the exposure of human volunteers to invasive tests. For this reason, reliable *in vitro* methods have been pursued for the determination of SPF [10–12].

The erythema formation is however another relevant and complex photochemical process to be considered. As a matter of fact, it depends on many factors and some drawbacks of the *in vitro* methods still remain unsolved [13–16], precluding their approval in substitution to the currently used *in vivo* assays. Generally, they are based on the correlation of the *in vivo* assays with parameters determined from the UV absorption spectra of sun protection products, with or without corrections for the skin erythema action spectrum and the photodecomposition of sunscreens [17]. Thus, there is great interest from the pharmaceutical and cosmetic industries, as well as of the government bodies all over the world, for the development of reliable noninvasive sensors and methodologies for evaluation of SPF.

On the other hand, nanocrystalline TiO_2 is being extensively exploited as white pigment, as photocatalyst for decomposition of organic contaminants and environmental remediation [18], as superhydrophillic self-cleaning coatings [19], as sunscreen in cosmetics [1] and as high surface area interfaces responsible for the photoinduced charge separation process in dye sensitized solar cells [20–22]. We brought those works to a new ground by chemically mapping [23] the TiO_2/molecule interface using confocal Raman microscopy, and developing new type of devices [24] such as photoelectrochemical XOR logic gates [25] and personal UV-

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dosimeters [26]. Now we describe a SPF sensor based on the photobleaching kinetics of that effective visual UV-dosimeter, a new concept for the determination of the sun protection factor. The interesting feature of this nanocrystalline TiO_2/dye interface device is that it possesses a UV-induced photobleaching efficiency spectrum matching the erythema response spectrum of human skin, thus providing a reliable evaluation of the sun protection factor of cosmetic formulations.

2. Experimental

2.1. Preparation of the dye

The bis-[4′-(4-bromophenyl)-2,2′:6′,2″-terpyridine]iron(II) hexafluorofosfate complex, $[Fe(Br\text{-}ph\text{-}terpy)_2](PF_6)_2$, was prepared according to a previously described method [26], by reacting 0.25 g (0.90 mmol) of $FeSO_4{\cdot}7H_2O$ dissolved in 25 mL of Milli-Q water with 0.70 g (1.80 mmol) of 4′-(4-bromophenyl)-2,2′:6′,2″-terpyridine (Br-ph-terpy), previously dissolved in 20 mL of a CH_2Cl_2/ethanol mixed solvent. The reaction mixture was stirred for 1 h and an excess of aqueous NH_4PF_6 solution was added and kept in the refrigerator overnight. The solid was filtered out, washed with water and dried under vacuum. Yield = 90%. Analysis (exp. (calc.)): C = 43.4 (44.8); H = 2.5 (2.5); N = 7.5 (7.5). Mass spectrum: m/z (z = 2) (calc./found): 416.2/416.0.

The elemental analyses were carried out in a Perkin Elmer 2400 instrument, at 925 °C, in air. The mass spectra were obtained using a Bruker Daltonics Esquire 3000 Plus Qq-TOF instrument. The samples were prepared in methanol and inserted in the spectrometer using a syringe-pump (180 μL h^{-1}), and the ESI-MS registered (capillary voltage = 4 kV and cone voltage = 20 V). The UV–Vis spectra were obtained in a FieldSpec FS3 fiber optic spectrophotometer from Analytical Spectral Devices (350 to 2500 nm).

The UV-dosimeters were prepared by following a method [26] previously described by our group. Optical microscopy glass plates were thoroughly cleaned by washing with acetone, isopropanol and water. Two columns of three 1.2 cm × 1.2 cm squares was delimited using scotch tape. Then, a TiO_2 (Degussa P25) slurry [23] was dispersed using the doctor blade method, dried in air and fired at 450 °C for 30 min. Then, each piece was cut and separated using a diamond cutter, dipped into a 1×10^{-4} mol dm^{-3} solution of β-carboxymethylcyclodextrin (CMCD) in methanol, thoroughly washed with the same solvent to remove the excess and dried in air. Finally, they were kept overnight in a 1×10^{-4} mol dm^{-3} ethanol solution of $[Fe(Br\text{-}ph\text{-}terpy)_2](PF_6)_2$ complex, carefully washed with the same solvent and allowed to dry in air. The white films became purple after the host–guest interaction of the iron complex with the CMCD molecules adsorbed onto the nanocrystalline TiO_2 surface (Fig. 1).

2.2. SPF by the in vivo method

The SPFs were determined using a properly collimated and calibrated xenon arc lamp, and dispersing the formulations on the skin according to the standard procedure, ensuring a homogeneous distribution and coverage of 2.0 mg cm^{-2} (±2.5%). Drops of formulation were carefully distributed along each area with a syringe or pipette and uniformly spreading with a finger for 20–50 s. Then, the film of the cosmetic product was dried open to air for 15–30 min before exposure to the UV-light.

The irradiation was carried out to evaluate the minimal erythema dose (MED) of (a) non-protected, (b) protected with a reference formulation (with known SPF) and (c) protected with a cosmetic formulation sample, using three suitable areas on the back of each volunteer. Each of those areas received six incremental dosages of UV-radiation (circular subareas of A = 1.0 cm^2) according to a pre-determined power progression (1.25 for SPF < 25 and 1.12 for SPF > 25). The irradiance of each waveguide of the solar simulator was calibrated using the erythema-effective UV-detector PMA 2103LLG from Solar Light Company. The MED were determined by visual evaluation of the dose corresponding to the area with the slightest but clearly defined redness of the skin, similar to that obtained at the non-protected area (16–24 h after irradiation) by a trained professional, in a room with appropriate illumination (at least 450 lux). The analyses were carried out on the back, in the area in between the line of scapula (or shoulder blade) and the waist, of a minimum of 10 and a maximum of 25 no-tanned volunteers belonging to Fitzpatrick's skin phototype I–III or with individual typological angle (ITA°) superior to 28.

The minimal erythema dose of the non-protected area, MED_{NP}, was first estimated in order to centralize the range of UV dose that will be employed in the analysis, according to the colorimetric method proposed by Mérot and Masson, and adopted by COLIPA since 1994 (sun protection factor test method, COLIPA Publication, Ref 94/289, 1994). The method is based on the interpolation of DEM_{NP} *versus* ITA° for a large data set. The exposition time was calculated multiplying the time necessary to reach the MED in the non-protected area by the SPF expected for the formulations. After irradiation, the exposed areas were cleaned with a cotton pad humidified with a remover lotion. All *in vivo* assays were carried out according to the International sun protection factor test method (ISPF) of 2003, revised in 2006.

2.3. In vitro evaluation using the SPF sensor

All measurements were carried out using a Singleport 16S-150 solar simulator from Solar Light Company, Philadelphia, PA, equipped with a 150 W xenon lamp, a dichroic mirror and a 1.0 mm thick WG320 Schott filter. The cosmetic formulations were homogeneously spread on 2.0 cm^2 round shaped pieces of artificial skin (VitroSkin®), mounted on a circular holder, using teflon O-rings, as shown in Fig. 1. The measurements were carried out by positioning the VitroSkin® substrate perpendicularly to the UV-beam and 5 cm away from the solar simulator. The titanium dioxide UV-dosimeter was positioned just behind, ensuring that the photobleaching is solely promoted by the dose of the UV-light impinging on it after passing through the cosmetic formulation layer and the VitroSkin® substrate. The visible spectra were registered as a function of the irradiation time using the FieldSpec fiber optic spectrophotometer, positioned behind the UV-dosimeter and parallel to the light beam. The photobleaching kinetic curves were obtained by plotting the absorbance at 572 nm (assigned to the MLCT band of the iron complex) as a function of the irradiation time. For this purpose, the Schott UG11 filter was removed to add visible light to the beam. It is important to note that the spectral profile in the UVA/UVB region was not significantly affected by this procedure, as confirmed using the FieldSpec spectroradiometer. In addition, the adequacy of the solar simulator to the limits of irradiance was ensured measuring the percentage variation of its Relative Cumulative Erythema Efficiency (%RCEE) according to the International SPF Test Method (2006), in the COLIPA Guidelines for monitoring UV radiation sources. The presence of visible light was previously shown to have no significant influence on the UV-dosimeter response, thus ensuring the quality of the measurements. The light power was adjusted before each measurement using the erythema-effective UV-detector PMA 2103LLG.

The measurements were carried out according to the scheme shown in Fig. 1. Note that the light output from the solar simulator and the fiber optic probe of the spectrophotometer were placed in line, and the substrate/cosmetic formulation oriented perpendicu-