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Assessment of the photo-degradation of UV-filters and radical-induced peroxidation in cosmetic sunscreen formulations

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Abstract

Photo-instability of common UV-filters is a well documented phenomenon. This study develops a method for concomitant measurement of photostability and photo-induced ROS generation in cosmetic formulations. Oil-in-water formulations containing three common UV filters (OMC, BMDBM, EHT), individually or combined, were further supplemented with phosphatidylcholine and exposed to UVA. All filters show spectral decrease after UVA exposure. OMC and EHT do not induce significant lipid-peroxidation (as measured by TBARS production) while BMDBM does. In the latter case, this is reduced when BMDBM is combined with OMC but not with EHT. Neither OMC nor EHT stabilize BMDBM with respect to loss of absorbance. ROS-generation assessed via TBARS formation was supported by EPR experiments. The UV-induced changes in UV-filter performance, as monitored in this model formulations and in commercial sunscreens, demonstrate that this is a simple and effective method for stability assessment of sunscreen filters under conditions of use.

Keywords: *UV-filters, photostability/instability, lipid peroxidation, sunscreen formulations, UV protection.*

Introduction

UV-filters are the key ingredients of cosmetic sunscreen formulations that absorb specific wavelengths of ultraviolet radiation, UVA (320–400 nm), UVB (290–320 nm) or both, making the sunscreen protective against UV-induced skin damage [1]. Sunscreens should ideally protect from sunburn/erythema induced by UVB [2,3] and from the genotoxic/oxidant effects of both UVA and UVB [4,5] which contribute to skin photodamage (photoageing, photocarcincogenesis, immunosuppression) [6,7]. For a sunscreen's optimum performance and efficacy, the first and foremost requirement is that the UV-filters should remain stable during the entire period of exposure. The light absorbed should ideally be dissipated into heat in the case of soluble filters or absorbed, reflected and scattered in the case of particulate filters [8]. However this is not always the case: some common UV-filters are photo-unstable, a well documented phenomenon that can be induced by UV doses similar to those acquired during sunbathing [9–13]. If UV-filters are photochemically unstable, this leads to loss in absorbance following their UV exposure, which ultimately translates into reduced photoprotection. The absorbance spectrum of photo-unstable organic UV-filters changes during UV exposure due to structural transformations or degradation, leading in the latter case to photoproduct(s) [14]. These products, which can also include free radicals [15–17], may interact with other co-formulated UV-filters, other ingredients of sunscreen products, oxygen and even skin constituents (e.g. lipids, proteins, nucleic acids), thus altering the performance of sunscreens and possibly inducing oxidative damage on

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skin structure and/or function [18,19]. Evaluating the fate of UV-filters upon dissipation of absorbed UV energy is therefore critical.

In this regard, we previously developed a method for examining the spectral stability and photo-induced radical generation of several UV-filters incorporated into phosphatidylcholine (PC)-based liposomes exposed to UVA [20]. This system revealed that (i) in many cases, a photo-unstable absorbance spectrum of UV-filters goes along with enhanced levels of lipid peroxidation, a well known radical-based process, and that (ii) spectral stability does not necessarily exclude radical generation. Such information obtained from this and other spectroscopic and photochemical investigations is important for understanding the photochemical behaviour of UV-absorbing molecules [21–24]. However, such studies have certain methodological drawbacks: the experiments are usually carried out on single filters in more or less dilute solutions, i.e. much lower than use concentrations and in the absence of further formulation constituents. Instead, under actual conditions of use, several UV-filters are normally present at relatively high concentrations in an emulsion composed of a polar phase and an apolar one and containing, besides the typically apolar UV-filters, water, surfactants, thickeners, cosmetic oils and other cosmetic actives [25]. Hence, all these ingredients may interfere with the UV behaviour of the filter molecules. Those studies that describe the UV behaviour of finished sunscreen products concentrate on stability of the transmission spectra, parallelled at times with HPLC, GC/MS analysis for the chemical stability of the filter molecules [10,13,26–30]. They do not address the existence and consequences of dissipation-related radical processes. In order to obtain more realistic information, we here aimed at transferring the simple method for the assessment of both photo-stability and photo-induced radical generation of UV-filter molecules, already measured under dilute conditions in PC liposomes [20], to UV-filter molecules in cosmetic formulations. To this end, PC was incorporated during the sunscreen preparation to be used as a marker for lipid peroxidation. Since PC is very susceptible to oxidation, any reactive oxygen species (ROS) generated within the sunscreen formulation should oxidize the lipid whose oxidation products can then be easily detected and quantified via TBARS as described. The method proved feasible also for commercial sunscreens which often contain PC or other unsaturated lipids.

Materials and methods

The following UV-filters were supplied by CIBA Inc. (Basel, Switzerland); ethylhexyl *p*-methoxycinnamate (OMC), butylmethoxydibenzoylmethane (BMDBM) and trianilino *p*-carboxyethylhexyl triazine (EHT). L-α-phosphatidylcholine (P2772: Type XI-E) as well as all other reagents and solvents were purchased from Sigma-Aldrich Chemical Co. (Milan, Italy). Three commercial sunscreens were purchased from local stores and tested within 3 months from purchase. The labelled SPF's (Sun Protection Factor) and the UV active ingredients (INCI) of the three products are as follows: Cream A (SPF 25) ethylhexyl methoxycinnamate, benzophenone-3, butylmethoxydibenzoylmethane, octocrylene, titanium dioxide; Cream B (SPF 25) ethylhexyl methoxycinnamate, butylmethoxydibenzoylmethane, octocrylene; Cream C (SPF 30) ethylhexyl methoxycinnamate, titanium dioxide, butylmethoxydibenzoylmethane.

Preparation of formulations

The various UV-filters or their combinations were integrated in the same oil-in-water based formulations, which were obtained as here briefly described: Phase A [(5% tribehenin PEG-22 esters, 0.5% stearyl alcohol, 5% dibutyl adipate, 3% triheptanoin, 5% PPG-2-myristyl ether propionate, 0.4% phosphatidylcholine) with 0.5%, 2% or 4% individual UV-filter plus 5.5%, 4% or 2% caprylic/capric triglycerides or 2% UV-filter 1, 2% UV-filter 2 plus 2% caprylic/capric triglycerides (for combined filters)] and phase B (3% glycerol, 0.2% disodium EDTA and deionized water making up to 100%) were heated separately to 70–75°C until homogeneous. Afterwards, 0.6% sodium polyacrylate was added to phase B and homogenized with an Ultra-Turrax T50 at 5000 rpm (Janke & Kunkel GmbH & Co., Staufen, Germany). Then A was added into B and mixed until homogenous at 7500 rpm. The emulsion was moderately stirred until the temperature dropped to 60°C, (Eurostar digital and paddle mixer by Janke & Kunkel GmbH & Co., Staufen, Germany). Then phase C (5% cyclohexasiloxane+cyclopentasiloxane) was added to the emulsion and continuously stirred until ambient temperature was reached. At this point, phase D (1% phenoxyethanol (and) methylparaben (and) ethylparaben (and) butylparaben (and) propylparaben (and) isobutylparaben) was added to the emulsion and homogenized again under stirring.

UV exposure

Of each formulation, 50 ± 2 mg were spread (2 mg/cm^2) onto 5 × 5 cm glass plates with a gloved finger and left to dry at room temperature in the dark for 30 min before exposure. The amount loaded was that recommended by the COLIPA sun protection factor test method [31]. For UVA exposure, the plates were placed on a brass block embedded on ice at a distance of 20 cm from the light source. A commercial UVA sun lamp, Philips Original Home Solarium (model

HB 406/A; Philips, Groningen, Holland) equipped with a 400 W ozone-free Philips HPA lamp, UV type 3 was used for irradiation. The output of UVA was measured with a UV Power Pack Radiometer (EIT Inc, Sterling, MA). The emission spectrum of the UVA lamp was further checked using a StellarNet portable spectroradiometer (Tampa, FL) which confirmed the manufacturer's declaration. The emission of UV light below 320 nm was <1.5% of the total emitted between 300–400 nm, hence the UV source is essentially a UVA one. The lamp was always pre-run for 10 min to allow the output to stabilize. Samples were irradiated for 15 min corresponding to an incident dose of UVA of 275 kJ/m^2, i.e the dose approximately equivalent to ~90 min of sunshine at the French Riviera (Nice) in summer at noon [32]. The temperature of the plates during irradiation, as measured with a thermocouple was 35 ± 2°C.

For each irradiated sample, a non-irradiated sample serving as (non-irradiated) control was kept in the dark at room temperature for 30 min.

Optical absorption spectra

Irradiated and control formulations were collected by immersing the plates in 10 ml ethyl acetate for 30 min. From this organic solution, 50 µl were added to 2450 µl ethyl acetate in a quartz cuvette and its absorption spectra was measured on a UV Kontron 941 spectrophotometer against a blank containing ethyl acetate.

Evaluation of lipid peroxidation

The remaining dissolved formulation was evaporated under vacuum by Rotavapor. To the residue, 3 ml of TBA-TCA-HCl (0.375% w/v TBA (thiobarbituric acid), 15% w/v TCA (trichloroacetic acid), 0.2 M HCl) was added followed by BHT (butylated hydroxytoluene) 0.1 mM to prevent possible peroxidation of phosphatidylcholine during the TBA assay. The samples were heated for 30 min at 95°C followed by cooling and centrifugation. The absorbance of the pink chromophore formed in the supernatant by TBA and aldehydic breakdown products upon heating was measured at 532 nm and compared to that of a calibration curve of 1,1,3,3-tetraethoxypropane reacted with TBA. The absorbance of aldehydic breakdown products of lipid peroxidation (TBA reactive substances = TBARS) [33] was related to that of the respective amount of malondialdehyde in the calibration curve.

EPR spectroscopic measurements

EPR measurements were performed with an X-band Bruker EMX EPR spectrometer (Bruker, Karlsruhe, Germany) equipped with a TE102 resonator (Bruker ER 4102ST, 50% optical transmittance). The samples were irradiated at 293 K directly inside the EPR spectrometer microwave cavity and the EPR spectra were recorded *in situ* with the following instrumental settings: 9.78 GHz microwave frequency, 5 mW microwave power, 100 G sweep width, 5×10^3 receiver gain, 2 G modulation amplitude, 41 s scan time, 1.28 ms time constant. As irradiating source, a 500 W Xe/Hg lamp (Oriel 66905, Oriel Corporation, USA) coupled with an IR-block filter (Oriel 61945, Oriel Corporation, USA) to avoid thermal effects was used. Light was passed through a Pyrex glass filter (1 mm thick) to eliminate the radiation wavelengths below 300 nm. Sunscreen formulations (80 mg) were diluted with 900 µL of water and to this 20 µL of 1 mM TEMPOL (4-hydroxy 2,2,6,6-tetramethylpiperidine-N-oxyl) aqueous stock solution was added. The mixture was degassed with argon, transferred into a quartz flat cell and irradiated in the EPR cavity. The low field line of the TEMPOL triplet was double integrated and the relative amplitude decrease was measured during 20 min of irradiation by means of WinEPR program.

Appropriate controls were carried out throughout all the experiments described above. The data reported represent average values from at least three independent experiments. Statistical analysis was performed using the student's *T*-test.

Results and discussion

In the first part of this study, we examined (i) the spectral behaviour of three common UV-filters incorporated into the same PC-supplemented sunscreen formulations and (ii) the lipid peroxidation levels of these same formulations, when exposed to UVA radiation. Three concentrations of UV-filters were employed: 0.5%, 2% and 4%, which all fall within the levels permitted in the European Union, i.e. for OMC up to 10% and for BMDBM and EHT up to 5%. When combinations of two filters were used, these were each present at a concentration of 2%.

Figure 1 shows the spectra of OMC before and after UVA exposure, extracted with ethyl acetate from the formulations containing decreasing concentrations of this UV-filter. The spectral absorbance relates directly to the concentrations of OMC in the formulations, i.e. levels of absorbance for non-exposed formulation at 4% is twice that at 2% which in turn is four times that at 0.5%, thus demonstrating that the extraction method employed is efficient. The same behaviour was also observed for the other two filters (not shown). In addition, a decrease in absorbance without changes in the spectral profile was observed for each concentration of OMC after UVA exposure. This is attributable to the *cis-trans* photo-isomerization and possibly [2+2] cyclo-addition of OMC reported earlier [20,34–36].

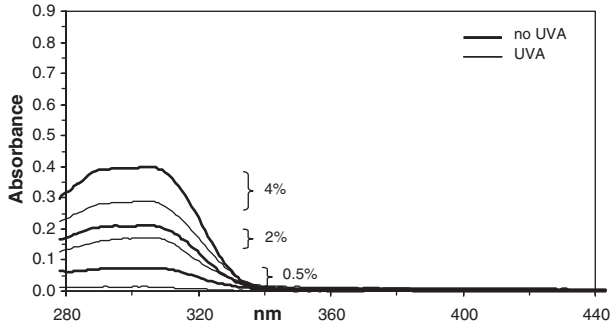

Figure 1. UV-absorption spectra of PC-supplemented sunscreen formulation containing different concentrations of OMC before and after UVA exposure followed by extraction with ethyl acetate. See Materials and methods for experimental details.

Figure 2 documents the UV absorbance spectra of the three UV-filters (OMC, BMDBM, EHT each formulated at 4%) before and after UVA exposure. The decrease in spectral absorbance of OMC, which is the most popular UVB filter used in sunscreens, has already been commented on above. For BMDBM, which is the most widely used UVA filter due to its broad UVA spectrum coverage, the exposure to UVA leads to a substantial decrease in spectral absorbance. This loss in photo-stability is in accordance with the earlier reports on BMDBM when in certain sunscreen formulations [10,37] and is attributed to the well-established keto-enol isomerization upon UV exposure (the diketo tautomer of BMDBM absorbs at 260 nm) followed by photo-cleavage leading to radical generation [16,22,38]. In the case of EHT, its characteristically very high molecular absorbance slightly decreases after exposure to UVA without changes in spectral profile.

For achieving a protection spectrum spanning both the UVA and UVB region of the solar spectrum and to reach higher overall absorbance, sunscreens are commonly formulated as a combination of two or more UV-filters [39]. Hence the two UVB filters (OMC, EHT) were combined with the UVA one used in this study (BMDBM) and irradiated accordingly. Their absorbance spectra are reported in Figure 3. Here we can observe that in the combination BMDBM+OMC there is broad spectrum coverage but its absorbance is reduced upon exposure. This is expected since this combination is known to be spectroscopically unstable if specific stabilizing molecules are absent and in accordance with earlier reports [9,40]. These two filters may undergo an irreversible [2+2] cycloaddition reaction, yielding a mixture of diketones whether in apolar solvents, neat form or commercial sun creams [11]. With the other broad spectrum combination BMDBM+EHT, which displayed a much higher absorbance in the UVB range due to the presence of EHT, a similar decrease in absorbance after UVA exposure was observed. This decrease can be attributed to the inherent photo-instability of the two filters, especially of BMDBM, present in the combination and already commented on above.

Besides monitoring spectral stability of the UV filters in the formulations, the oxidative status of these same formulations were also tested before and after UV exposure. Since the formulations were supplemented with PC, any UV- and/or UV-filter-induced ROS generated within the sunscreen formulation will increase lipid peroxidation and eventually contribute to increasing the TBARS levels. Moreover, the butylated hydroxytoluene (BHT) present during the test itself will ensure that the levels of TBARS measured derive exclusively from peroxide decomposition of oxidized PC following UVA exposure. The measurement of TBARS has its limitations such as its unsuitability for measuring and comparing levels of lipid peroxidation *in vivo* and between biological tissues or body fluids with different fatty acid compositions, as well as the uncertainty of the identity of the chromogen measured [41]. However, it is valuable as a simple means of determining lipid peroxidation in isolated lipid systems, as in our case. Figure 4A shows the contribution of the individual UV-filters at the three concentrations tested to UVA-induced lipid peroxidation. UVA was chosen since it is the principal UV component in sunlight (>95%) and promotes and propagates lipid peroxidation much more efficiently than UVB [42,43]. Accordingly, the graph shows already a significant increase in TBARS in the irradiated formulation supplemented with PC in the absence of UV-filters, yet not without PC, indicating that the increase in TBARS levels observed derives exclusively from the breakdown products of PC (among which is malondialdehyde, the most abundant aldehyde resulting from lipid peroxidation [41]). TBARS levels in the presence of OMC at all concentrations tested remained in the range of the irradiated control, indicating that no ROS are generated in the presence of this UV-filter, i.e. no PC/OMC interaction. It is noteworthy that this molecular integrity of OMC in formulation, assessed indirectly via quantification of UVA-induced PC peroxidation, is not correlated with its decrease in spectral absorbance (Figures 1 and 2), which confirms our previous results, i.e. spectral lability does not necessarily have to lead to radical generation and molecular decay [20]. In the case of formulated EHT, the level of TBARS compares well to those measured in the irradiated control without UV-filter (CNTRL). This implies that EHT *per se* does not lead to the generation of ROS when formulated at these concentrations, a result which is strikingly different from what had been observed in our previous investigation [20]. In fact, at 100 μM concentration in liposomes suspended in PBS and exposed to UVA, EHT remarkably increased lipid peroxidation to the same extent as BMDBM and this suggested that ROS may be generated from EHT as a consequence of inefficient energy dissipation

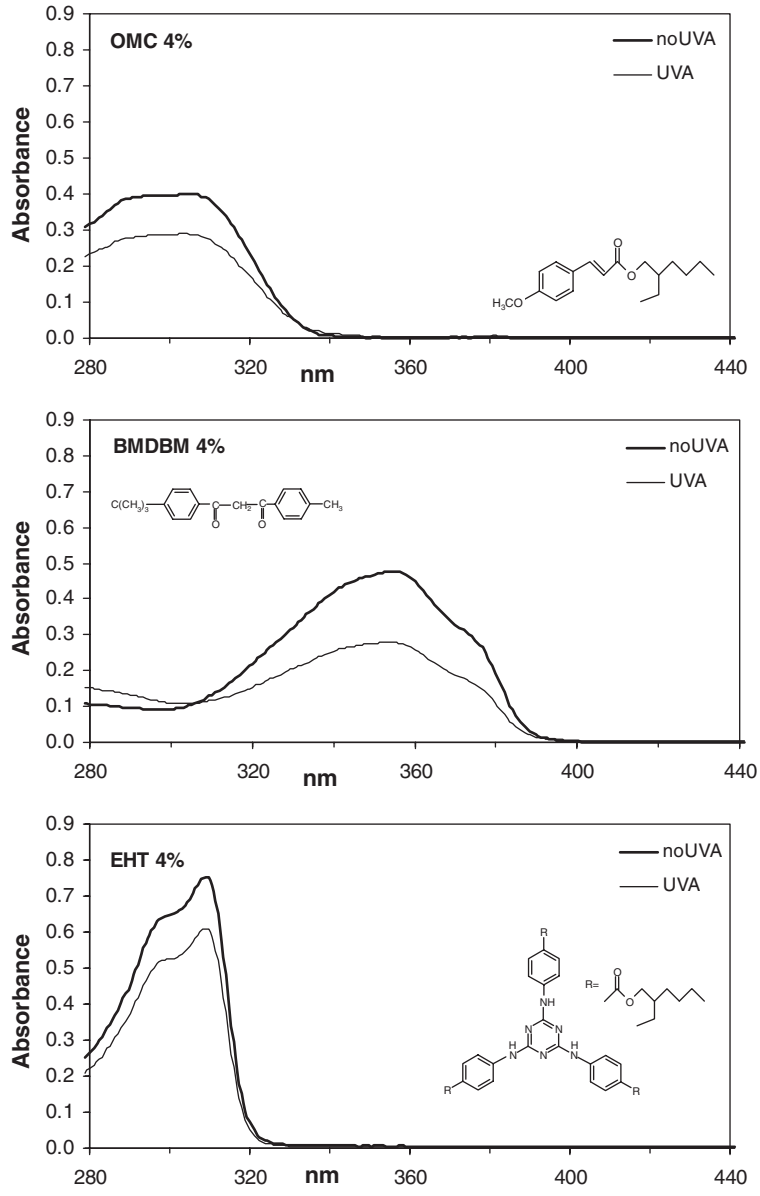

Figure 2. UV-absorption spectra of sunscreens containing 4% concentrations of UV-filters before and after UVA exposure followed by extraction with ethyl acetate. See Materials and methods for experimental details.

mechanisms when present at low concentrations. In the current experiment, EHT is present at a much higher concentration (~400-fold) and as a thin film, hence it is very likely that the dissipation mechanisms of the absorbed energy is more efficient. It is in fact known that at higher concentrations there is a self-protecting effect of EHT due to the increased optical density and this in turn increases the half-life of any photoreaction [44]. Another reason for the conflicting results with our previous measurements in UV-filter liposome preparations [20] could be due to greater oxygen availability (the exposed lipid surface area and oxygen availability is possibly greater in liposomes than in the thin film), since lipid peroxidation requires oxygen. The slight decrease in spectral absorbance after UVA exposure for EHT (Figure 2) correlates well with the observations reported above. With formulated BMDBM, however, there is a significant increase in lipid peroxidation due to ROS generated from UVA exposure of this filter. In this case, increased TBARS also correlates with the observed loss in spectral stability (Figure 2). This again is in accordance with what had been observed in our previous investigation where UV-filters had been incorporated into liposomes and exposed to UVA [20]. Figure 4B compares the levels of TBARS achieved after exposure to UVA in formulations containing a combination of UVA and UVB filters with formulations of each individual UV-filter at 2% concentration. The combination of BMDBM+OMC shows lower peroxidation levels than BMDBM+EHT. Furthermore, the TBARS levels are lower in the BMDBM+OMC combination than with BMDBM alone. Although this combination is not photo-stable from the absorbance

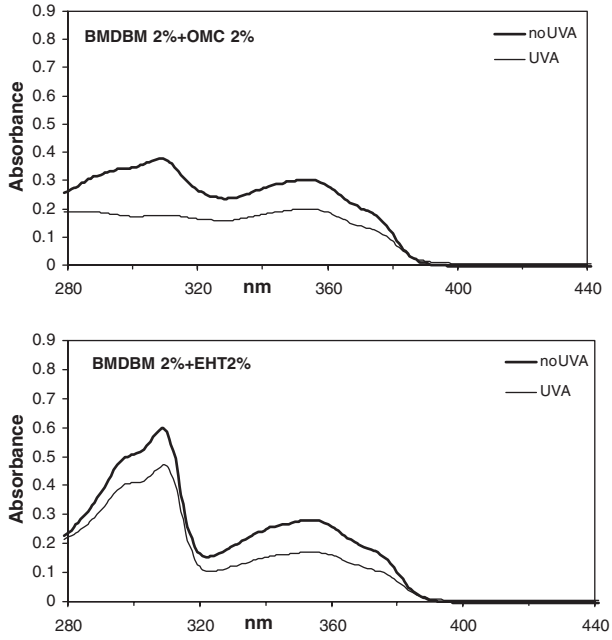

Figure 3. UV-absorption spectra of sunscreens containing combinations of different UV-filters each individually present at 2% concentration, before and after UVA exposure followed by extraction with ethyl acetate. See Materials and methods for experimental details.

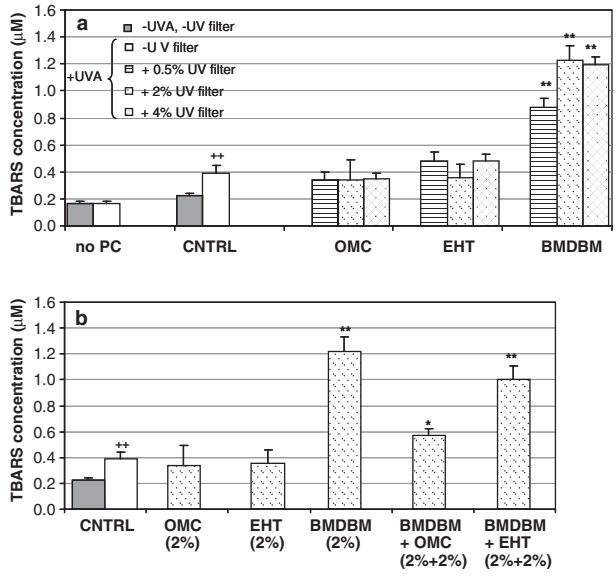

Figure 4. Concentration of TBARS determined in sunscreens containing different UV-filters or their combinations after extraction with ethyl acetate followed by subsequent treatment for detection of TBARS. (A) TBARS levels after UVA exposure of sunscreens containing three different UV-filter concentrations; (B) TBARS levels after UVA exposure of sunscreens containing 2% UV-filter and their combinations each individually present at 2% concentration. $*p < 0.05$, $**p < 0.01$ vs irradiated control without UV-filter (white bar). $^{++}p < 0.01$ vs non-irradiated control without UV-filter (grey bar). See Materials and methods for experimental details.

point of view (Figure 3), the low TBARS levels imply that in this combination lower levels of ROS are being generated, leading to less oxidation of lipid-based substrates than when BMDBM is present alone or in combination with EHT. In this latter mixture, the high TBARS levels are most likely due to the contribution of BMDBM breakdown alone. The BMDBM breakdown proceeds via cleavage of the BMDBM diketone, thus giving rise to radical generation. In the BMDBM+OMC mixture, less ROS are generated because the [2+2] cyclo-addition of OMC and BMDBM does not involve radical formation and competes with the cleavage of the BMDBM diketone.

The TBARS data which are indirectly a measure of free-radical induced lipid peroxidation were further supported by the EPR experiments. Any carbon-centred radicals generated from the sunscreen formulations during UV-irradiation should be trapped by the nitroxide function of TEMPOL to give an EPR-silent species, leading to a decrease in intensity of the TEMPOL EPR signal [45]. Although this method is not specific with regard to the identity of the radical trapped, it is nevertheless useful for determining the extent of ROS production from a complex system such as a sunscreen, by monitoring signal intensity. This signal was followed for 20 min in each case and, as shown in Figure 5, there was an abrupt decline after only 5 min of UV exposure in the case of BMDBM and BMDBM+EHT indicating a high yield of radicals which then levelled off. This is in line with the high levels of TBARS detected in these two formulations (Figure 4). The decline in EPR signal was similar for the remaining formulations, which again is in good agreement with the levels of TBARS detected in these cases (Figure 4).

Since many commercial sunscreen formulations do contain lipid-based substrates which can be susceptible to oxidation, we also tested three commercial sunscreens which all contained BMDBM and OMC for UVA-induced spectral changes and TBARS production as described above. Figure 6 documents for all three a loss in absorption after UVA exposure accompanied by lipid peroxidation. The drop in spectral

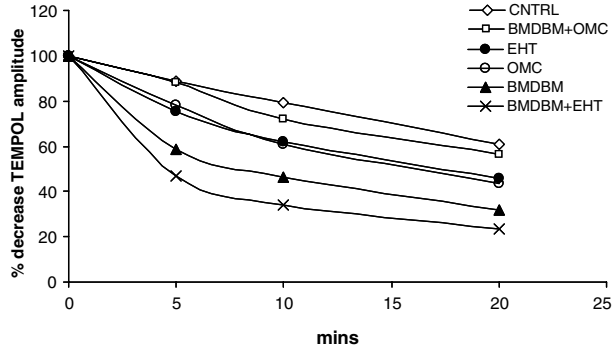

Figure 5. Percentage of the relative amplitude decrease of the low field line of the TEMPOL (20 μM) triplet EPR signal, doubly integrated, during 20 min of UVA irradiation in the presence of different UV-filters at 4% concentration in sunscreens or their relative 2% combinations. See Materials and methods for experimental details.

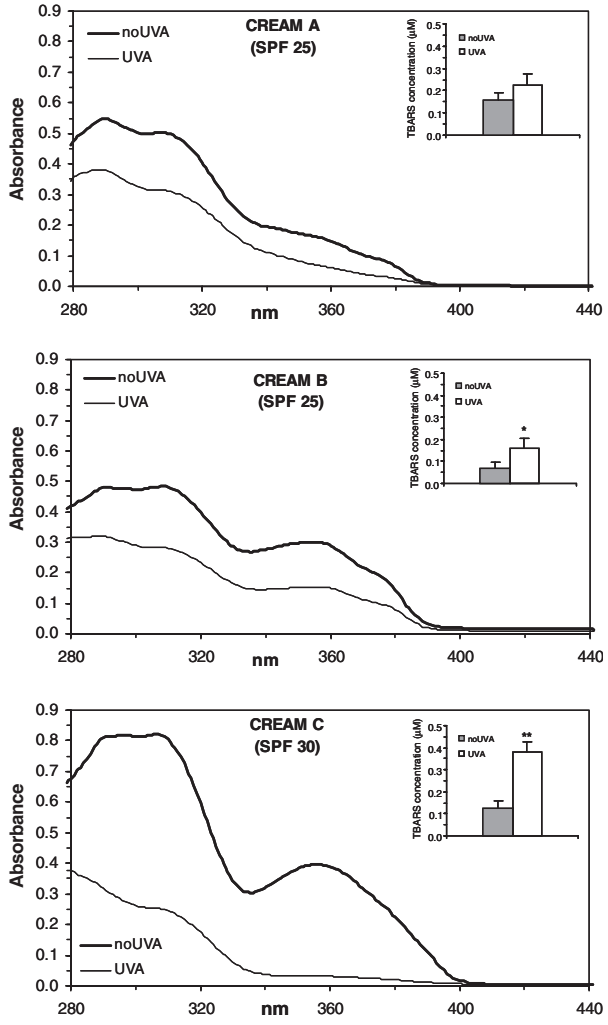

Figure 6. UV-absorption spectra of three commercial sunscreens before and after UVA exposure followed by extraction with ethyl acetate. Inserts: Concentration of TBARS determined in each commercial sunscreen before and after UVA exposure and after extraction with ethyl acetate followed by subsequent treatment for detection of TBARS. $*p < 0.05$; $**p < 0.01$ vs the respective non-irradiated control. See Materials and methods for experimental details.

absorbance was parallelled by an increasing difference in TBARS production from A to C, with increase in TBARS proving statistically significant for B and C. This is in line with the fact that sunscreen A contains benzophenone-3 and octocrylene which both stabilize BMDBM, B contains octocrylene, while C does not contain any stabilizer [14,46]. It is noteworthy that sunscreens A and B (having the same labelled SPF) exhibit similar absorbance levels (~0.5) in the UVB range but different ones in the UVA range. This is not surprising since SPF is based primarily on measuring protection from UVB-induced erythema and follows an internationally accepted procedure [31], whereas measurement of UVA protection is only recently becoming progressively standardized by the cosmetic industries following the increased knowledge on the role of UVA in photoageing and photocarcinogenesis. Sunscreen C, having a higher SPF, also shows a higher absorption since it contains higher concentrations of active UV-filters. The loss in absorbance for this product after UVA exposure is, however, remarkable, especially in the UVA range, and likely due to the absence of stabilizers. Secondly, sunscreen C contains titanium dioxide which may also affect the performance of this sunscreen. If this still retains some photocatalytic activity according to the type of coating it has, it may interact with the other active ingredients of the sunscreen leading to rapid photodegradation [47]. The lipid peroxidation levels in the sunscreens should also be affected by the antioxidants present in the formulation [48]. Although both products A and C contained tocopheryl acetate and retinyl palmitate, these vitamins are not active in their ester form. In contrast, sunscreen A also contained vitis vinifera, a grape seed extract rich in polyphenols and other antioxidants [49,50], that may have contributed to protecting this sunscreen against UVA/filter-induced lipid peroxidation. In addition, sunscreen A, having less BMDBM than sunscreen C in its formulation, will generate less radicals upon UV exposure, hence indirectly lower TBARS levels.

Conclusions

Summarizing, the simple method of testing UV-filters incorporated into PC liposomes following UVA exposure [20] has now been further developed to determine their photo-stability under conditions that parallel those encountered upon typical conditions of usage. Three representative UV filters incorporated into PC-supplemented sunscreen formulations and exposed to UVA as a thin film were investigated by absorbance spectroscopy and by indirect measurement of radical processes. It is shown that all the filters (individually) are not stable to UVA as determined by UV absorbance alone. OMC and EHT (individually) do not induce significant lipid-peroxidation (as measured by increased TBARS) but decrease TEMPOL radical signal. BMDBM induces significant TBARS production (and decrease in the TEMPOL signal), which is reduced when it is combined with OMC but not with EHT (substantiated by EPR measurements), suggesting stabilization of BMDBM by OMC with respect to induction of radical-induced peroxidation. Neither OMC nor EHT stabilize BMDBM with respect to loss of absorbance. These findings obtained from formulated UV filters at use concentrations, i.e. with filter molecules at least 200× more concentrated than in solution and exposed as a thin film, are more representative than those that were previously obtained in liposomes. In addition they support our earlier findings that the behaviour of UV-irradiated molecules cannot be predicted on the basis of their UV absorbance alone [20]. The concomitant measurement of other parameters, such as the generation of ROS, may be useful.

Three commercial sunscreens, rated SPF 25+, were also tested and show loss of UV absorbance upon UVA irradiation, with sunscreen C (lacking photostabilizers) being least photostable both with respect to loss of absorbance and lipid peroxidation. A significant loss in spectral absorbance of sunscreens in use may have an impact on their UVA screening efficacy (and claims for UVA protection), while photo-induced lipid peroxidation in the base cream may compromise other ingredients of the sunscreen formulation. In the latter case, this may not be relevant if the sunscreen remains in the superficial dead layer of skin (stratum corneum), but it may be potentially relevant to skin damage if the cream is rubbed into the skin (as currently recommended) and contacts living cells of the epidermis. Within this respect, the use of antioxidants in commercial sunscreen formulations does not necessarily guarantee that the finished product is spectroscopically photostable and less oxidizable, whereas the presence of molecules acting as photostabilizers substantially improved the stability.

It is worth bearing in mind that since sunscreens were not applied to skin itself, the information obtained from our previous study on UV filters in liposomes [20] and from this present study of formulated UV filters exposed as a thin film on glass plates may not totally reflect the true behaviour of sunscreens when applied to skin. Sunscreen performance is known to be dependent on whether it is as a thin film or disrupted such as in real application to the irregular surface of the skin [51,52]. Therefore it is extremely important that the photostability of UV-filters is studied in sunscreens instead of isolated filter preparations, and ideally (where possible) as applied to skin. Furthermore, the chromatographic effect 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) and the skin's in-built antioxidant defensive system, may all affect the true behaviour of sunscreens. Hence, without appropriate *in vivo* experiments it is difficult to predict whether photo-induced ROS-formation from UV-filters will have a direct impact on skin cells and function.

Overall, the method proposed here may, however, prove useful for formulators during the screening stage to help optimize which are the best UV-filter combinations, antioxidants and photostabilizers that can be mixed together for achieving more efficient and safer sunscreen products. This simple technique may also be valuable to compare the photostabilities of different sunscreen products already present on the market.

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