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# Changes in ultraviolet absorbance and hence in protective efficacy against lipid peroxidation of organic sunscreens after UVA irradiation

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**Abstract**

Owing to the spectral distribution of solar UV, the UVA component of sunlight is now believed to be the main cause of photoaging and photocarcinogenesis and is much more effective than UVB in inducing peroxidative damage. Consequently, most skin care cosmetic products now include UVA filters in their formulations along with UVB filters. These modern sunscreens should provide and maintain their initial absorbance, hence protection, throughout the entire period of exposure to sunlight. However, not all UVA and UVB filters are sufficiently photostable. In this study, we examine the correlation between the photochemical degradation of sunscreen agents under UVA irradiation, with particular reference to the UVA-absorber 4-*tert*-butyl-4′-methoxydibenzoylmethane, alone and in combination with other organic UV filters (2-ethylhexyl 4 methoxycinnamate and 2-ethylhexyl 2-cyano-3,3-diphenylacrylate) and their ability to prevent UVA-induced lipid peroxidation. Since antioxidants are also added to formulations to deactivate free radicals generated during UVA exposure, vitamin E and the synthetic antioxidant, bis(2,2,6,6-tetramethyl-1-oxyl-piperidine-4-yl)sebacate, a nitroxide derivative, were also included in this study. By using simple in vitro tests, the results show that a decrease in spectral absorbance of the UV filters correlates in most cases with increased UVA-induced lipid peroxidation; this depends on the specific UV absorber analysed and also on whether they are alone or in combination. Furthermore, the combined presence or absence of antioxidants has a profound effect on this oxidative event. In particular, the nitroxide appears to be a more efficient photo-antioxidant than vitamin E. Similar experiments were also performed under natural sunlight and the results obtained did not differ substantially from those performed under UVA. The results presented and discussed in this work may help in understanding the effects of UVA/UVB absorbers and antioxidants upon the level of UV-induced ROS generated under UVA exposure and in natural sunlight which could be relevant for improving the photoprotection and efficacy of skin care cosmetic formulations.
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*Keywords:* 4-*tert*-Butyl-4′-methoxydibenzoylmethane; 2-Ethylhexyl 4 methoxycinnamate; 2-Ethylhexyl 2-cyano-3,3-diphenylacrylate; Nitroxide; Antioxidants; Sunscreens; UVA

## 1. Introduction

The use of sunscreens is the most efficacious means of reducing acute and chronic adverse effects of solar UV radiation to skin when exposed to sunlight outdoors. However, protection against indoor exposure is also relevant since UVA radiation (320–400 nm), which makes up 95% of natural sunlight, is not filtered by standard glass (i.e., automobile windows, conservatories, verandas and windows in general). UVA radiation is the main cause of photoaging and tumour promotion originating strand breaks and oxidation of nucleic acids because of its capacity to reach dermal layers, unlike UVB (290–320 nm) which is absorbed in the epidermis [1–3]. To achieve greater protection against the damaging effects induced in skin by

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UVA, the majority of daily skin care cosmetic products now contain UV absorbers [4,5]. However, although sunscreens are useful because they efficiently protect skin against erythema and edema and probably prevent squamous cell carcinoma [6–9], they also seem to provide much less protection than expected [10–13]. In fact, the Sun Protection Factor (SPF) reported on sunscreens, which is a measure of their biological activity (prevention of erythema), may significantly change due to several reasons such as de-emulsification and absorption of the sunscreen when applied to a substrate [14]. Thermally induced changes in sunscreen chemicals and evaporation of the sunscreen, both of which could be affected by substrate and drying out of the epidermis, may also influence the SPF [14]. But probably, the most important factor which may modify the sunscreen's efficiency is the photochemical instability of sun-care products containing various sun-blocking ingredients. A recent in vitro systematical investigation of 16 original commercial sunscreens exposed to increasing standard erythema doses of solar-simulated radiation, revealed that neither complex combination of organic filters nor addition of inorganic filters could prevent the undesired photoinactivation [15]. Furthermore, the authors found that there was a high photostability of sunscreens in the UVB, but a considerable loss in the UVA region. This photo-decomposition of the UV filters results in the formation of free radicals and other reactive/toxic intermediates which may directly or indirectly initiate skin damage [16–22]. Moreover, the breakdown of the UV filter decreases the UVA absorptive capacity resulting in an increase of the direct UVA-induced skin damage. A classical example is represented by 4-*tert*-butyl-4′-methoxydibenzoylmethane (commercially known as Parsol 1789 or Avobenzone) which is one of the most common UVA filters present on the market because of its high absorptive capacity over almost the entire UVA range. This compound produces free radicals when activated by UVA [17,22,23] that lead to a reduction in photo-protective power and to an increased potential to damage biologically relevant molecules, such as proteins [24], plasmid DNA [25] and more recently, cultured keratinocytes [26]. The photo-decomposition of sunscreens may hence affect their protection against UV-induced skin damage. In this study, we first investigated the UV spectrum changes of 4-*tert*-butyl-4′-methoxydibenzoylmethane alone and in combination with other organic filters commonly present in sunscreens, before and after UVA irradiation (Fig. 1). Since the UV-protection spectrum of a sunscreen formulation is usually attained by a mixture of UV-filters, the compatibility between the different UV-filters is important. Secondly, we examined if there was a correlation between the UV spectra changes of the sunscreens after UVA irradiation and the degree of protection offered by these compounds against UVA-induced lipid peroxidation. Since antioxidants have recently been added to formulations to deactivate the levels of reactive oxygen species (ROS) produced during UV irradiation [27,28], the natural antioxidant, vitamin E and the synthetic antioxidant, bis(2,2,6,6-tetramethyl-1-oxyl-piperidine-4-yl)sebacate were also included in the study.

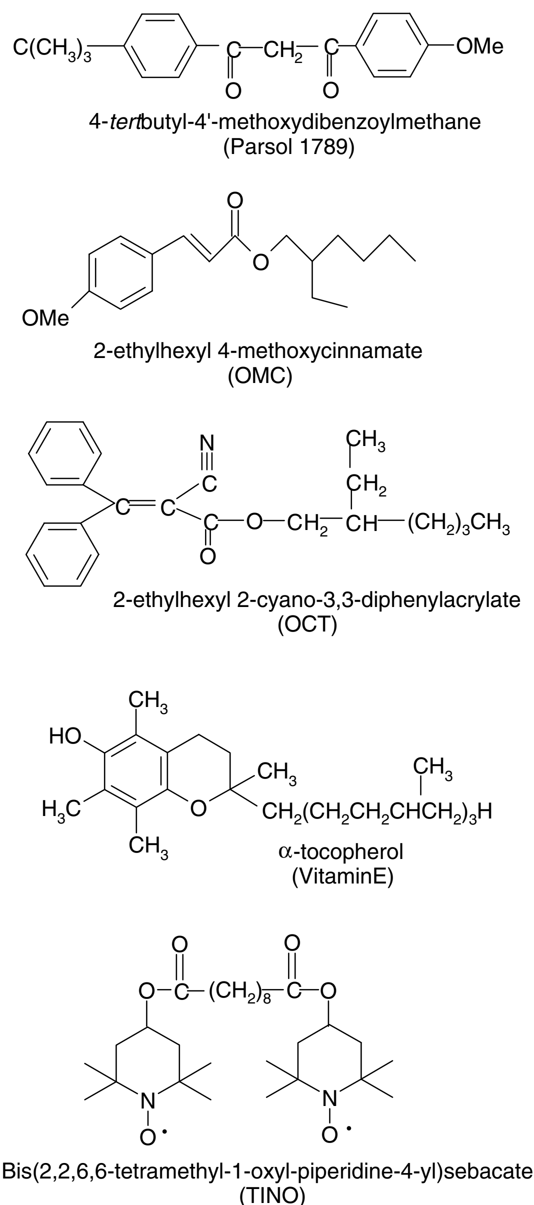


Fig. 1. Chemical structures of the compounds studied.

## 2. Materials and methods

4-*tert*-Butyl-4′-methoxydibenzoylmethane (here abbreviated as Parsol) was obtained in the form of Eusolex 9020 from Merck (Darmstadt, Germany) and its identity was confirmed by NMR while bis(2,2,6,6-tetramethyl-1-oxyl-piperidine-4-yl)sebacate (here abbreviated as TINO) was a gift from Ciba Specialty Chemicals (Basel, Switzerland). L-α-Phosphatidylcholine (P2772: Type XI-E), vitamin E, vitamin E acetate, 2-ethylhexyl 4-methoxycinnamate (octylmethoxycinnamate; here abbreviated as OMC), 2-ethylhexyl 2-cyano-3,3-diphenylacrylate (octocrylene; here abbreviated as OCT) as well as all other reagents and

solvents were purchased from Sigma–Aldrich Chemical Co. (Milan, Italy) (see Fig. 1).

As UVA irradiating source, a commercial 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, delivering a flux of 23 mW/cm$^2$ between 300 and 400 nm, at a distance of 20 cm was used. It was always pre-run for 15 min to allow the output to stabilize. The dose of UVA was measured with a UV Power Pack Radiometer (EIT Inc.).

### 2.1. Optical absorption spectra

Ten mM stock solutions of the photoactive ingredients were prepared in acetonitrile. Appropriate amounts were then added to 5 mM phosphate buffer, 0.9% NaCl, 0.1 mM EDTA, pH 7.4 (acetonitrile < 2% v/v) and mixed thoroughly to reach final concentrations of 100 μM for Parsol, vitamin E, and vitamin E acetate and 200 μM for OMC and OCT in a final volume of 3 ml. The solutions were then transferred to a 24 multi-well plate for cell cultures (Orange Scientific, Cambrex BioScience, Walkerville, Inc.) which was placed on a brass block embedded on ice at a distance of 20 cm from the light source. The multi-well plate was covered with a 2-mm thick quartz slab to prevent any evaporation. The incident dose of UVA received from above by the samples was 410 kJ/m$^2$. After illumination, 2.4 ml of sample were collected from each well and extracted with the same volume of ethyl acetate. The organic phase was separated and its absorption spectrum was then run on a UV Kontron 941 spectrophotometer. For the non-illuminated samples, the same procedure was followed for the same length of time except that the samples were exposed to direct artificial laboratory working light.

### 2.2. Peroxidation of multilamellar phosphatidylcholine (PC) liposomes induced by UVA

PC multilamellar liposomes were prepared as follows. The desired amount of egg PC in chloroform was added to a glass test-tube kept in an ice bath and the solvent was thoroughly removed under a stream of nitrogen. When compounds were to be tested, either alone or in combination, the desired amount of an acetonitrile solution of the compound was introduced into another glass test-tube and after solvent evaporation, egg PC was added and subjected to the same procedure as described above. The lipid films prepared were each dispersed in 1.5 ml of 5 mM phosphate buffer, 0.9% NaCl, 0.1 mM EDTA, pH 7.4 and vortexed for 10 min until a white, homogeneous, opalescent suspension was obtained. The final concentration of PC in the resulting multilamellar liposomal dispersion was 3.5 mM. Each sample was then aliquoted into two parts (700 μl each) and transferred into a multi-well plate, covered with a 2-mm thick quartz slab to prevent any evaporation and exposed to UVA as described above. The incident dose of UVA received from above by the samples was 275 kJ/m$^2$. At the end of UVA exposure, the extent of lipid peroxidation was assessed using a modified method of the thiobarbituric acid (TBA) assay [29]. In this procedure, 2 ml of TBA–TCA–HCl (0.375% w/v TBA, 15% w/v TCA, 0.2 M HCl) was added to 600 μl of sample containing BHT 0.3 mM to prevent possible peroxidation of liposomes during the TBA assay. The samples were heated for 20 min at 95 °C followed by cooling and centrifugation. The absorbance of the pink chromophore of the supernatant developed upon heating, was measured at 535 nm.

When natural sunlight was used as illumination source, the same procedure as above was followed except that the samples were exposed to direct natural sunlight, for 30 min between 13.45 and 14.15, at sea level (Ancona, Italy), during the hot summer week of 19th–23rd July, 2004. The dose corresponding to 30 min of sunlight in Ancona situated at 43° latitude has been estimated to be around 90–100 kJ/m$^2$ based on the fact that 180 kJ/m$^2$ of UVA are equal to about 1 h of sunshine at the French Riviera (Nice) at noon which is also situated at 43° latitude [30].

Appropriate controls were carried out throughout all the experiments described above and the results reported are an average of at least three independent experiments each performed in duplicate. Statistical comparisons were performed by calculating the percentage difference between the data to be compared, and the level of significance for all the comparisons was set at >5%.

## 3. Results

The main aim of this study is to determine if a correlation exists between the photochemical degradation of UV-filters, either alone or in combination, and their efficacy to prevent photo-oxidative damage to lipids induced by UVA.

Fig. 2A–F shows the spectra of the compounds tested when exposed to 410 kJ/m$^2$ UVA in buffer and after solvent extraction. The reason for using buffer was to maintain the same medium as the peroxidation experiments so that comparisons between the optical absorption experiments and lipid peroxidation could be meaningful. In addition, twice the amount of OCT and OMC compared to Parsol were used to respect the ratios of these compounds found in suncare products (Europe: max. conc. Parsol = 5%, OMC = 10%, OCT = 10% while in the USA: max. conc. Parsol = 3%, OMC = 7.5% and OCT = 10%).

As expected, the strong absorption in the UVA region of Parsol (Fig. 2A) is greatly reduced after exposure to UVA and a new absorption band appears in the UVB region similarly to the findings of Tarras-Wahlberg et al. performed in petroleum jelly [31]. This is most likely due to its photodecomposition following keto-enol isomerization as has been amply documented [17,32,33]. With OCT, which is a stable UVB filter, the reduction in the degree of absorption after UVA exposure is not as remarkable as with Parsol and no spectrum changes are observed (Fig. 2B). Instead, a strong decrease in spectral absorption is observed for OMC, a common UVB filter incorporated into over 75%

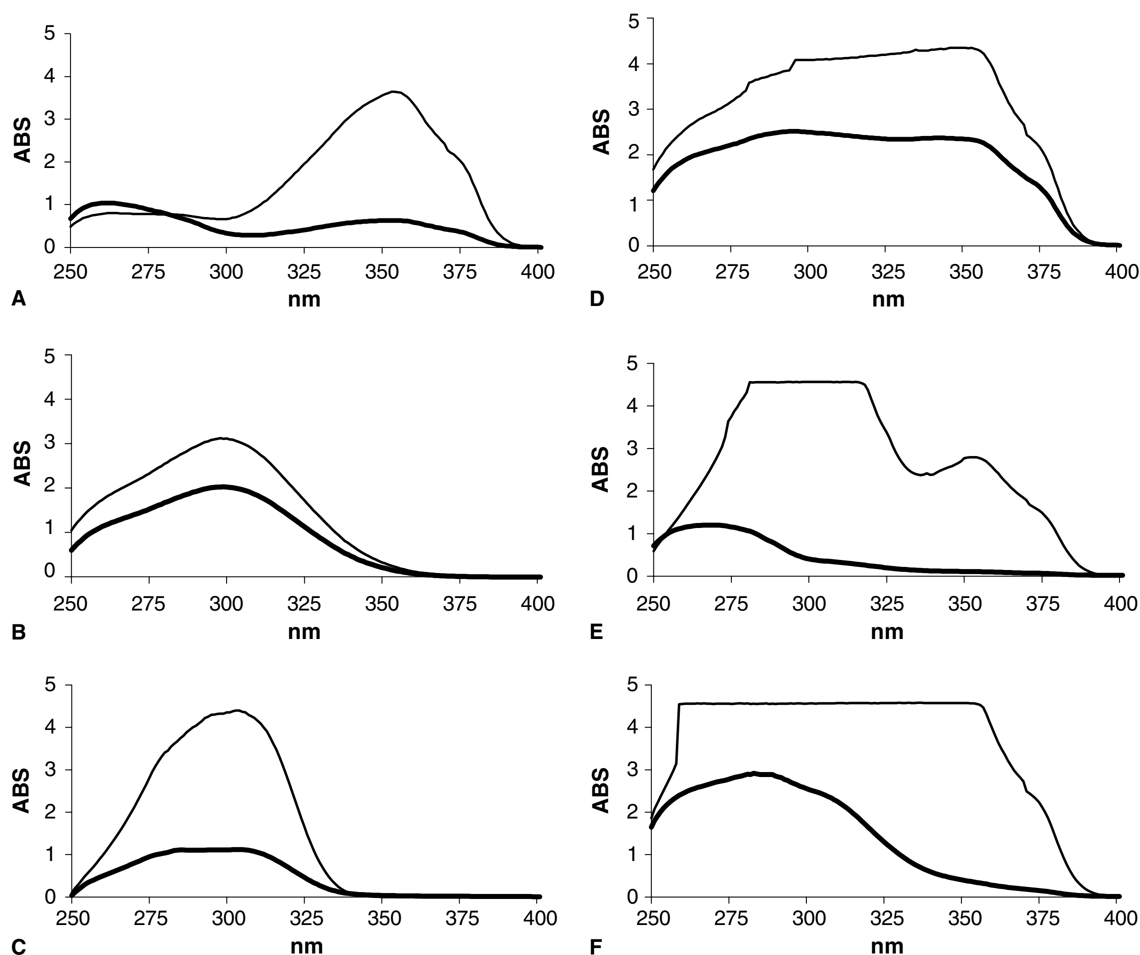


Fig. 2. Absorption spectra (ABS) of compounds, before (thin line) and after (thick line) irradiation with 410 kJ/m$^2$ UVA. (A) = 100 μM Parsol, (B) = 200 μM OCT, (C) = 200 μM OMC, (D) = 100 μM Parsol + 200 μM OCT, (E) 100 μM Parsol + 200 μM OMC, (F) = 100 μM Parsol + 200 μM OCT + 200 μM OMC. See Section 2 for experimental details.

of sunscreen-containing formulations (Fig. 2C). In fact, this UVB filter has been shown to be light sensitive and a decrease in UV absorption efficiency upon light exposure which results from *cis/trans* photo-isomerization and possibly [2 + 2] cyloaddition at wavelengths above 300 nm, has been reported [31,34,35]. The resulting *cis*-isomer absorbs at the same wavelength, but it has a reduced extinction, thus giving lower spectrophotometric values which could account for the result observed here. Figs. 2D–F show the spectral changes upon combination of Parsol with the two UVB filters. These experiments were performed because some filters may affect the stability of others, hence the resulting stability may change in different filter combinations. In the controls (non-illuminated samples = thin line) the overall absorbance reaches a plateau because the absorbance of a combination of compounds is additive. The presence of OCT remarkably improves the UV-absorbance capacity of Parsol where only ~30% reduction in the UVA range is seen after UVA exposure (Fig. 2D) compared to when Parsol is illuminated alone (Fig. 2A). OCT is often used in combination with this UVA filter in some formulations because it is able to stabilize it and this simple experiment proves this [36]. It is believed that Parsol is able to transfer absorbed UV energy to other UV filters such as OCT and 4-methylbenzylidene camphor instead of undergoing alternative reactions which result in a loss of sunscreening efficiency. The combination of Parsol with OMC leads to strong spectral changes both in absorbance and in shape (Fig. 2E). The resulting spectra is the same as that obtained with Parsol alone after illumination (Fig. 2A) except that there is a further decrease in absorbance in the UVA range. Fig. 2F shows that the addition of OCT improves the latter result in the UVB range, but absorbance in the UVA is lost (compare Fig. 2D with F). Addition of 100 μM vitamin E to the above combinations did not affect any of the spectral changes observed (results not shown), primarily because the absorbance of vitamin E is negligible in the UVA. This implies that vitamin E does not influence the decomposition products and/or photoadducts deriving from the filters after UVA exposure.

It is known that compared with UVB, UVA generates more oxidative stress, and at levels found in sunlight, it is ten times more efficient than UVB at causing lipid peroxidation leading to plasma membrane damage [37–39]. Therefore, the effects of the same filters used above, alone and in combination, in addition to vitamin E and vitamin

E acetate, on this oxidative event was studied in vitro using liposomes as membrane models. The extent of the oxidative process was determined through the popular method of evaluating the aldehydic breakdown products (TBARS) produced during lipid peroxidation using the TBA assay [40]. Fig. 3 shows the level of TBARS measured in liposomal suspensions before and after exposure to 275 kJ/m$^2$ UVA in the absence and presence of tested compounds. UVA induces a 3-fold increase in lipid peroxidation in this experimental system. The presence of Parsol significantly enhances this process, OCT has no effect, whereas OMC significantly inhibits it. The result observed with Parsol once again confirms that free radicals are produced during UVA illumination of this filter [17,25] which contribute to exacerbating the free radical chain reaction of lipid peroxidation. The UVB filter, OMC seems to slightly protect UVA-induced lipid peroxidation. Although it does not absorb in the UVA, except for a very small fraction between 320 and 330 nm, its spectral behaviour (Fig. 2B) shows that there is a decrease in its absorbance which does not necessarily imply photoinstability. OMC undergoes *cis*/*trans* isomerization which can be considered a very efficient way of dispersing the absorbed energy and this may explain the slight protection observed here: part of the UVA energy is attenuated by OMC so less lipids are oxidized. The presence of vitamin E alone strongly inhibits UVA-induced lipid peroxidation bringing the level of TBARS to almost control levels. This is not due to its filtering capacity since it does not absorb in the UVA range but rather to its ability to react with ROS induced by UVA exposure [41,42]. Its acetylated derivative, vitamin E acetate does not inhibit lipid peroxidation as expected because the antioxidant activity of vitamin E is due to hydrogen donation from the hydroxyl group on the benzene ring. If this is no longer available through esterification then radical-scavenging cannot occur [41]. With the combination Parsol/OCT, there was a significant reduction in TBARS vs. Parsol alone in accordance with what was observed and discussed on Fig. 2D, though the level of TBARS is still relatively high compared to the illuminated control. In the liposomes containing Parsol/OMC there was no significant difference in lipid peroxidation levels with respect to Parsol alone, and this again is in line with the results reported in Fig. 2E. The combination Parsol/vitamin E lead to a significant decrease in TBARS compared to the illuminated control. However, the level of oxidation is two-fold higher with respect to the samples containing vitamin E only. Increasing the amount of vitamin E to 200 μM did not lead to any further decrease in lipid peroxidation (result not shown). Since all antioxidants have a pro-oxidant action depending on the concentrations used, a higher concentration of vitamin E in our system probably leads to more tocopheroxyl radical being generated which might exacerbate lipid peroxidation with no beneficial effects [43]. In the system Parsol/OCT/OMC/Vitamin E there was a further significant reduction in TBARS compared to Parsol/Vitamin E and this is most likely due to the fact that OCT in part stabilizes Parsol leading to a reduction in radicals produced, while the presence of OMC should have no influence on this system.

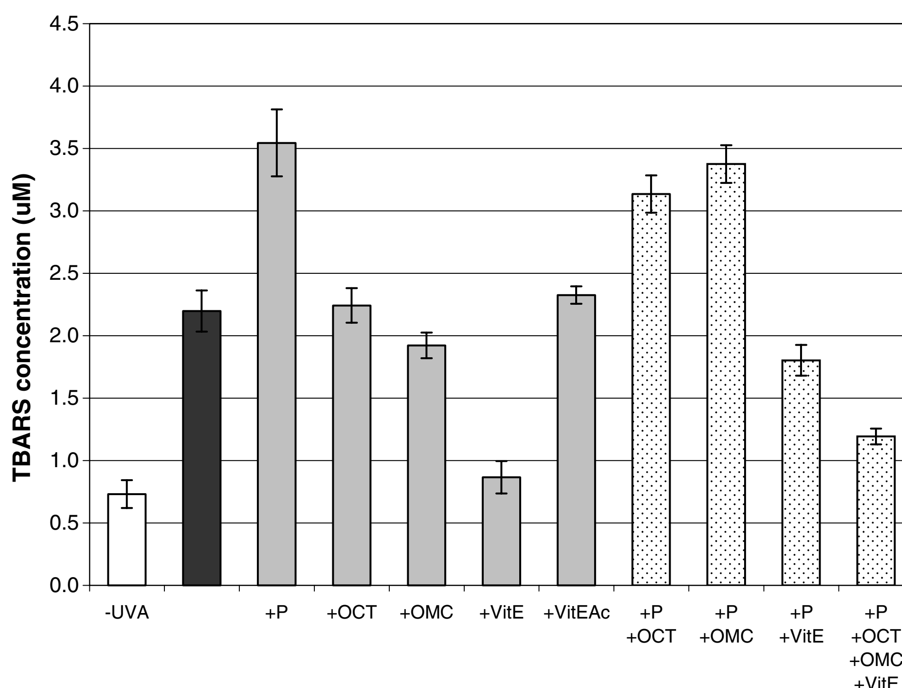


Fig. 3. Concentration of TBARS determined in PC multilamellar liposomes (3.5 mM) in PBS, 0.1 mM EDTA, after irradiation with 275 kJ/m$^2$ UVA. White bar = no UVA exposure, black bar = UVA exposure, remaining bars = UVA exposure in the presence of 100 μM Parsol (P), 200 μM OMC, 200 μM OCT, 100 μM vitamin E (VitE), 100 μM vitamin E Acetate (VitEAcet.) and the various combinations at the aforementioned concentrations.

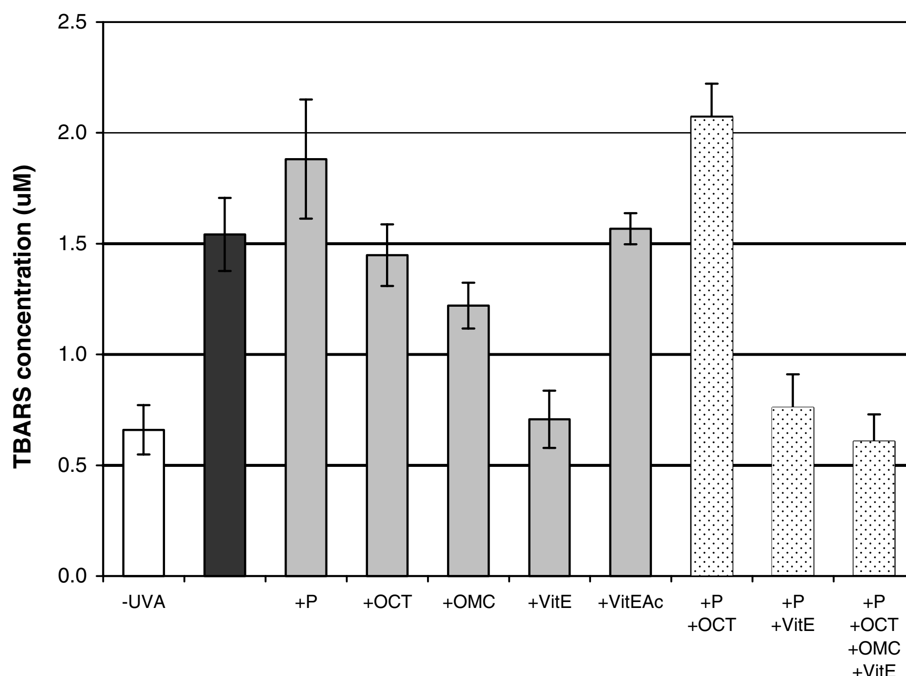


Fig. 4. Concentration of TBARS determined in PC multilamellar liposomes (3.5 mM) in PBS, 0.1 mM EDTA, after exposure to 30 min of natural sunlight. White bar = no sunlight exposure, black bar = sunlight exposure, remaining bars = sunlight exposure in the presence of 100 μM Parsol (P), 200 μM OMC, 200 μM OCT, 100 μM vitamin E (VitE), 100 μM vitamin E Acetate (VitEAcet.) the various combinations at the aforementioned concentrations.

For transference and comparison of the in vitro results obtained with UVA exposure to a more realistic situation, we performed similar in vitro lipid peroxidation experiments under natural sunlight (Fig. 4). Thirty minutes exposure to natural sunlight lead to a significant increase in the level of TBARS though the overall levels were approx. 30% less compared to 20 min UVA exposure. Longer exposure times to natural sunlight would have probably lead to similar levels of TBARS as those under UVA, but this was not the aim of the investigation. The results obtained on the samples containing the UV filters, vitamin E and vitamin E acetate alone do not differ substantially from those obtained under UVA exposure. In the samples with the combination Parsol/OCT, there are no significant differences with respect to Parsol alone contrarily to what was observed under UVA illumination. It is noteworthy that under natural sunlight, the extent of TBARS formation in liposomes in the presence of Parsol is slightly lower compared to the same samples exposed to UVA, which probably means that less Parsol is degraded under natural sunlight, hence the stabilizing effect of OCT becomes less noticeable. Samples with the combination Parsol/vitamin E under natural sunlight show a significant reduction in lipid peroxidation and the TBARS levels are reduced to almost control values. This result is more remarkable compared to what was observed under UVA illumination. It appears that for the length of sunlight exposure employed in this study, less Parsol seems to photodecompose, which translates into less radicals being produced; the amount of vitamin E in this system is therefore sufficient to totally inhibit sunlight + Parsol-induced lipid peroxidation. The addition of OMC and OCT to this latter system lead to a further decrease in lipid peroxidation compared to when they were not included, for the same reasons discussed earlier.

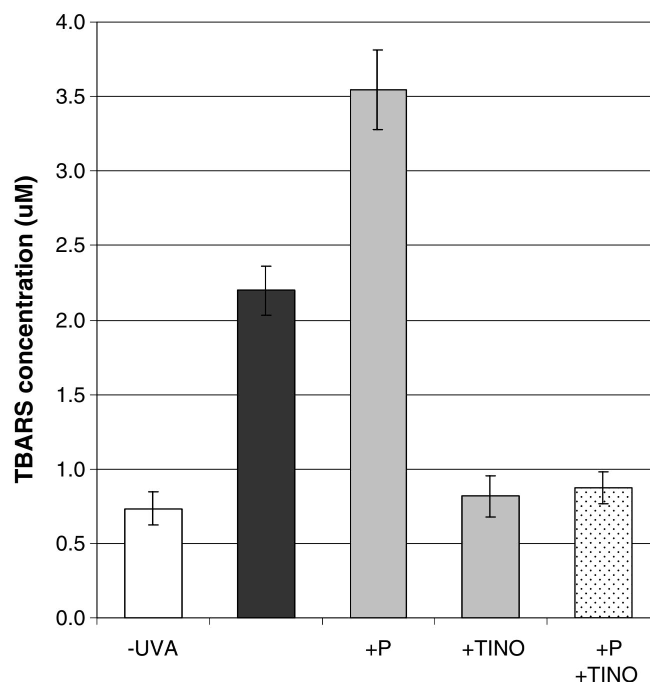


Fig. 5. Concentration of TBARS determined in PC multilamellar liposomes (3.5 mM) in PBS, 0.1 mM EDTA, after irradiation with 275 kJ/m$^2$ UVA. White bar = no UVA exposure, black bar = UVA exposure, remaining bars = UVA exposure in the presence of 100 μM Parsol (P), 100 μM TINO and the combination P + TINO at the aforementioned concentrations.

In Fig. 5, the results of the effects of the nitroxide TINO on liposomes exposed to UVA are reported. The presence of TINO drastically reduces the formation of TBARS down to control levels. This has previously been observed and reported by us [44]. However, here we wanted to investigate the effects of this antioxidant in the presence of Parsol. The results show that even when Parsol is present, 100 μM TINO is capable of totally inhibiting lipid peroxidation, thereby reducing the oxidative effect induced in lipid systems by the UVA-absorber under illumination. These findings seem to indicate that this antioxidant is more effective than vitamin E at the same concentrations, in reducing the photo-oxidative effect of Parsol.

## 4. Discussion

The main and immediate damage inflicted by UVA light to cells is produced from free radicals and other oxidative species which may directly or indirectly be responsible for the erythemal reaction too [45,46]. Understanding the effects of UVA/UVB absorbers and antioxidants upon the level of UV-induced ROS generated under UVA exposure and in natural sunlight, is useful for comprehending the efficacy of skin care cosmetic formulations and may lead to improved photoprotection. This is especially important considering the several reports in the literature which show that UV absorption spectra of sunscreen agents following UVA irradiation are changed in many instances due to photoinstability [15,22,31–33,47]. Neither, the combination of various organic filters nor the addition of inorganic filters seem to guarantee photostability. Recent research by Haywood et al. [48] also indicates that sunscreen users are little protected against UVA free-radical production and the damaging effects of UVA. To gain more insight into the behaviour of UV absorbers, we investigated whether the spectral properties of common sun filters, alone and in combination after UV exposure, may correlate with their ability to prevent UVA-induced ROS generation measured as a reduced photo-oxidative damage to lipids.

For this purpose, simple in vitro experiments were performed. Despite the simplicity of these tests, the spectral profiles obtained on the UV-absorbers alone are in total accordance with literature data using other, sometimes more sophisticated means of investigation [15,31,47]. However, here we show for the first time the spectral profiles of the UV absorbers when they are combined together and the results correlate well with those obtained by others on the combination of UV filters in formulated model sunscreens and in sunscreens themselves [15,47]. More specifically, the combination of Parsol, the most widely used UVA absorber, with OMC, the most popular UVB absorber, leads to a decrease in absorbance over the entire UV range. It has been hypothesized that the photosensitivity reactions induced by Parsol may be responsible for the photodecomposition of certain UVB filters and the results reported in this study confirm this. Once Parsol has undergone photoreactions it can react with OMC to form cycloaddition products and perhaps other photoadducts, which may contribute to the overall decrease in absorbance observed [47,49]. The results also confirm that OCT, which is itself a UVB absorber stabilizes Parsol, but when in combination with OMC, this effect is reduced. This agrees with the statements of others where the combination of Parsol and OCT becomes less stable when OMC is present [47].

The results on UVA-induced lipid peroxidation were obtained by evaluating the extent of TBARS formation which mainly arises from the reaction of breakdown products of lipid peroxides, principally malondialdehyde (MDA) with TBA. MDA is in many instances the most abundant individual aldehyde whose production has been suggested to involve the formation of cyclic peroxides and endoperoxides that undergo fragmentation during lipid peroxidation. Several other compounds (e.g., deoxyribose, methionine, proline, adenosine, DNA) under appropriate conditions also form pink TBA complexes [50], however in our isolated liposomal system, TBARS can derive only from the breakdown of peroxides formed during PC peroxidation. The results reported in Figs. 2–4 reveal that a decrease in spectral absorbance of UV absorbers correlates in most cases with increased lipid peroxidation with the exception of OMC which undergoes a photo-induced isomerization instead of photodegradation. Therefore, the extent of UVA-induced lipid peroxidation appears to depend on the specific UV absorber present and also on whether they are alone or in combination. In addition, the combined presence or absence of antioxidants has a profound effect on this oxidative event.

It has been reported that UVA generates a peroxidative process in cultured human skin fibroblasts and in keratinocytes and that this radical process alters the plasma membrane [37–39]. The same researchers also proved that the chain-breaking antioxidant vitamin E inhibited this radical process [37]. Here, using liposomes as model membrane systems, we show that vitamin E leads to a significant reduction in lipid peroxidation and this is evidence that the UVA-induced changes in the liposomes is indeed due to a radical process. It's acetylated derivative is inactive as such which demonstrates that the free phenolic OH group of vitamin E is essential for antioxidant activity. Therefore, the ample use in cosmetic formulations of vitamin E acetate, used because it is more stable than vitamin E, may be beneficial only if it is bioconverted into the active form and this has only recently been demonstrated in viable human skin [51]. The use of vitamin E in combination with Parsol appears to greatly reduce the latter's photo-oxidative effect, however, the level of TBARS reached are higher compared to when vitamin E is present alone. This possibly signifies that more radicals are produced when Parsol is present leading to more lipids being oxidized, hence vitamin E has to scavenge not only the radicals generated by UVA exposure but also those induced by the photo-decomposition of Parsol. A similar

behaviour has previously been observed by us when Parsol was co-incubated with the antioxidant glutathione in cultured keratinocytes exposed to UVA. Glutathione alone was able to reduce intracellular oxidative stress induced by UVA but its positive effect was nullified when Parsol was present [26].

When the nitroxide TINO was tested in combination with Parsol, total inhibition of lipid peroxidation was achieved. This is primarily due to the excellent radical scavenging properties of nitroxide radicals. These are a group of compounds whose protective effects in a multiplicity of biological systems at the molecular, cell, organ, and whole-body levels against oxidative stress, have been widely established [52–54]. The reasons underlying their success is that nitroxides are extremely effective modulators of processes mediated by paramagnetic species (radicals and transition metals) which makes them useful for probing reactions and processes associated with free radicals. Here we show that these compounds are also extremely efficient in reducing photo-oxidative damage even in combination with photolabile UV-absorbers. It is worth recalling that the nitroxide TINO is a derivative of a hindered amine light stabilizer (HALS-1). These HALS are extensively employed in polymers to prevent photooxidation [55,56] and their stabilizing effect is attributed to the activity of the nitroxide radical derived from the parent amine as described previously by us [44]. Bernstein et al. [57] have also demonstrated that the nitroxide Tempol affords protection against UV radiation in a transgenic murine fibroblast culture model of cutaneous photoaging. Therefore the results reported in this study further support the notion that antioxidants, such as nitroxides, may provide useful supplementation to sunscreen protection against photocarcinogenesis and photoaging in skin.

Since the study of the photodegradation of sun filters under realistic conditions such as sunlight is important for understanding the behaviour and photostability of common sunscreens, an aspect in this field of research which should be investigated deeper, experiments were also performed under natural sunlight. Although TBARS formation was reduced in all cases after sunlight exposure with respect to UVA, the results practically do not differ from the experiments performed under UVA.

In conclusion, the results indicate that the photostability of sunscreens is not predictable on the basis of the constituent UV absorbers alone. The mutual presence of individual UV absorbers, as well as further additives, such as antioxidants and solvents, in suncare compositions could significantly influence the photochemical response of sunscreens. Therefore the characterisation of the photostability of suncare products should be recommended before marketing especially because their efficacy is based mainly only on protection against UVB-induced erythema. Protection against ROS should also be taken into account since the UVA/free-radical protection currently provided by sunfilters appears to be inadequate. This is evident in the work presented here and lends support to the results of Haywood et al. [48] and to those recently published by Zastrow et al. [58] who have proposed a new sun protection factor based on free radicals generated by UV irradiation. The use of simple in vitro assays (UV spectral absorbance properties combined with liposomal lipid peroxidation) as employed in this study, could provide an initial screening step for determining efficiently and rapidly the efficacy of sun-filter combinations in conjunction with appropriate concentrations of suitable antioxidants, before passing on to more complex means of investigation. The results reported here may also be relevant, considering that certain sunscreen agents including Parsol [59–61], and antioxidants are absorbed through human skin following topical application, especially with the aid of liposomes and nanotopes [62], and that skin surface lipid fractions represent the first target of UV irradiation of the skin. Since human exposure to UVA is increasing due to the use of modern tanning equipment (sunbeds and sunlamps) where the application of sunscreens is encouraged before exposure, the search for appropriate and more efficient UVA-blockers combined with efficient antioxidants and stabilizers should be a forthcoming priority in sunscreen research. The addition of nitroxide radicals as novel antioxidants in formulations aimed at reducing photo-induced skin damage may be envisaged.

## 5. Abbreviations

BHT butylated hydroxytoluene
HALS hindered amine light stabilizers
MDA malondialdehyde
OMC 2-ethylhexyl 4-methoxycinnamate
OCT 2-ethylhexyl 2-cyano-3,3-diphenylacrylate
ROS reactive oxygen species
TBA thiobarbituric acid
TBARS thiobarbituric acid reactive substances
TCA trichloroacetic acid
TINO bis(2,2,6,6-tetramethyl-1-oxyl-piperidine-4-yl) sebacate

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