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abstract

The impact of UV-filter combination on the number of free radicals generated in sunscreen formulations and the skin following UV-VIS irradiation was assessed via electron spin resonance spectroscopy using a spin-probing approach. Four UV-filter combinations that differed in their photostability and range of UVA absorbance coverage were investigated. Fewer free radicals were generated in the sunscreen formulation when a photostable UVA filter system was used, compared to a stabilized UVA filter system. Additionally, fewer free radicals were generated in the skin when a sunscreen with long UVA protection extending to the short visible range was used, compared to a sunscreen with minimal UVA protection. This study showed that the photostability of a UV-filter system is central to the generation of free radicals in a formulation, and that long UVA-blue light protection is key for avoiding the generation of free radicals in the skin. A sunscreen product exhibiting both photostable UVA protection and long UVA-blue light protection is therefore the most appropriate choice for protecting against ROS-induced skin damage.

Introduction

Knowledge of skin and health-related photodamage grows with each passing decade. Beyond the first visible damage, i.e. sunburn, DNA damage occurring immediately after sun exposure only becomes visible years later. DNA damage can include nucleus DNA damage related to UVB-induced breakage of DNA strands [1, 2], as well as mitochondrial DNA damage, primarily via UVA-induced free radicals [3]. Zastrow *et al.* published a spectrum exhibiting the effectiveness of free radical formation from 290 to 700 nm [4] and showed that both UV and visible (VIS) radiation account for the generation of free radicals, with UVA radiation being the biggest contributor.

The well-known photo-instability of some UV filters used around the world is problematic due to the formation of damaging free radicals. This is the case with the UVA filter Butyl Methoxydibenzoylmethane (BMDBM), which undergoes an irreversible photodegradation of its keto isomer in the triplet state via a Norrish type I cleavage, resulting in the formation of benzoyl and phenacyl radicals [5, 6]. In addition to BMDBM, the water-soluble UV filter Phenylbenzimidazole Sulfonic Acid (PBSA) has also been shown to convert into an organic PBSA* radical and to produce superoxide and hydroxyl radicals that could trigger further chain reactions as well as highly reactive singlet oxygen [7]. The generated free radicals can result in additional free radical chain reactions through interaction with either photocatalytic formulation ingredients or, even worse, skin-photosensitizing molecules. Photostabilization of BMDBM was shown to be effective in triplet-triplet energy transfer from the excited keto form to a quenching molecule such as the UV filters Octocrylene and Bis-Ethylhexyloxyphenol Methoxyphenyl Triazine (BEMT) [8, 9]. However, the current trend is to avoid octocrylene due to environmental or health-related issues [10-12].

The overall objective of the present study is to evaluate the impact of UV-filter combination on the generation of free radicals. In an initial study, we first assessed the influence of the UV-filter system on the number of free radicals generated in the sunscreen formulation following UV irradiation; in a second study, we assessed the number of free radicals generated in the skin after UV-VIS irradiation. In both studies, the assessment of the free radicals was performed using electron spin resonance (ESR) spectroscopy measurements with a spin-probing approach.

Materials and Methods

Sunscreen Formulations

The investigated sunscreens differed exclusively in their UV-filter combinations. **Tab. 1** describes the formulation chassis used for both studies, consisting of a standard oil-in-water emulsion; **Tab. 2** describes the investigated UV-filter combinations.

The formulation chassis is based on emulsifiers and emollients commonly used in the sun care industry; they have been chosen to ensure a stable formulation and full solubilization of the UV-filters, regardless of the combination tested.
The UV-filter combinations were all designed to provide an SPF of 30, but to differ in the UVA protection, from low to very high UVA protection. Sunscreen formulation 214-1-5 would not fulfill the European UVA requirement; sunscreens 214-1-1 and 214-1-2 would fulfill it; whereas sunscreen 214-1-3 would provide a much higher UVA protection than the European requirement, extending to the blue light spectral range (high energy visible, or HEV). Furthermore, the sunscreens differed in the UVA filter employed, which was BMDBM for sunscreens 214-1-5 and 214-1-1 and DHHB for sunscreens 214-1-2 and 214-1-3. In addition to the sunscreen formulations, the study involved a placebo formulation that did not contain any UV filters.

Phase	Trade Name	INCI	wt %
A	Emulgade® Sucro [1]	Sucrose Polystearate (and) Hydrogenated Polyisobutene	3.00
	Eumulgin® Prisma [1]	Disodium Cetearyl Sulfosuccinate	1.00
	Lanette® O [1]	Cetearyl Alcohol	1.50
	Cetiol® CC [1]	Dicaprylyl Carbonate	5.00
	Cetiol® Sensoft [1]	Propylheptyl Caprylate	5.00
	Cetiol® B [1]	Dibutyl Adipate	12.00
		Preservative	qs
	UV-filter system		qs
B	Water		Qsp 100 %
	Glycerin	Glycerin	2.00
	Rheocare® XGN [1]	Xanthan Gum	0.20
	Edeta BD [1]	Disodium EDTA	0.20
C	UV-filter system		qs
D	Cetiol® Ultimate [1]	Undecane, Tridecane	2.00

[1] from BASF SE (Ludwigshafen, Germany)

Tab. 1 Chassis of the sunscreen formulations used in both studies.

Measurement of Free Radicals

The free radicals generated under light exposure were measured via electron spin resonance (ESR) spectroscopy (MiniScope MS300, Magnettech GmbH Berlin, Germany). For both studies, a spin-probing approach was used to measure the free radicals generated either in the formulation (study 1) or in the skin (study 2). The organic nitroxide free radical (PCA, (2,2,5,5-tetramethyl pyrrolidine N-oxyl, Sigma-Aldrich, Munich, Germany) was used as spin probe;

	Placebo	Unstable UVA	Minimally stabilized UVA	Photo-stable UVA 1/3	Photo-stable long UVA–HVE
Formulation ID / UV filters	214-1-4	214-1-5	214-1-1	214-1-2	214-1-3
Diethylamino Hydroxybenzoyl Hexyl Benzoate (DHHB) [1]	–	–	–	4.00	4.00
Butyl Methoxydibenzoyl Methane (BMDBM)	–	4.00	4.00	–	–
Ethylhexyl Triazone (EHT) [2]	–	2.00	2.00	4.00	0.50
Bis-Ethylhexyloxyphenol Methoxyphenyl Triazine (BEMT) [3]	–	1.00	2.50	2.20	1.00
Methylene Bis-Benzotriazolyl Tetramethylbutylphenol (MBBT) [4]	–	–	–	–	8.00
Tris-Biphenyl Triazine (TBPT) [5]	–	–	–	–	3.00
Ethylhexyl Salicylate (EHS)	–	5.00	5.00	5.00	3.50
Phenylbenzimidazole Sulfonic Acid (PBSA)	–	3.00	2.00	–	–
SPF calculated [6]	–	27	30	30	30
UVA-PF calculated [6]	–	7.4	9.9	10.5	26.8

[1] Uvinul® A Plus, [2] Uvinul® T150, [3] Tinosorb® S, [4] Tinosorb® M, [5] Tinosorb® A2B, [6] with the BASF sunscreen simulator [13]

Tab. 2 Composition of UV-filter combinations in % by weight.

it remains stable over time but readily reacts when free radicals are generated and is reduced to the ESR-silent hydroxylamine.

Study 1

For the measurement of free radicals in the formulation, the formulas were diluted 1:10 in water and PCA was added to a final concentration of 0.01 mM. Quartz ESR capillary tubes were filled with 40 µL of the resulting solutions, and the PCA signal intensities were determined before and after various doses of UV radiation (280-400 nm). UV irradiation of the samples was performed using a UV solar simulator 300 W Oriel (Newport). The irradiances as integrated values over the spectral ranges were E (UVB = 280-320) = 23.5 W/m² and E (UVA = 320-400nm) = 180 W/m². To test the effect of different UV doses, the irradiation time was varied and the samples exposed to up to 10 minutes of UV irradiation (= 13.9 J/cm²). The emitting intensity was controlled before each measurement. The free radicals induced in the formulation after UV exposure react with the PCA probe, reducing it to an ESR-silent hydroxylamine. The signal intensity decay was measured as a function of UV exposure doses. The quantity of free radicals was calculated from the area under the curve (AUC).

Moreover, analysis of the kinetic parameters of the radical generation, as a function of UV irradiation time, allowed better understanding of the direct or indirect photocatalytic reactions within the products.

Study 2

For the measurement of free radicals in the skin, a pig skin model was used to simulate human skin. Numerous reports suggest anatomical, physiological and biochemical similarities in man and pig [14], and pig skin provides reproducible results when studying photochemical and phototoxicological processes [15]. Pig skin is a waste product of the meat industry and samples were obtained from a local butcher. For the study, pieces of pig skin measuring 1×1 cm were placed in petri dishes (epidermal side up, in immediate contact with air) and on filter paper, and soaked in the PBS solution containing the PCA nitroxyl probe as a free radical trap. Following this, the relevant sunscreen formulations were applied to the epidermis (2 mg/cm²). Skin samples were kept in the dark for 20 minutes, allowing equilibrium of the applied sunscreen film. After the treatment time, a punch biopsy (Ø 4 mm) was extracted and inserted into an ESR tissue cell (Magnettech GmbH Berlin Germany). The PCA signal intensity was monitored before UV exposure and after 10 minutes of UVA-VIS irradiation. The UV irradiation of the pig skin substrate was performed using a solar simulator (SOL 2, Hönle). The irradiances as integrated values over the spectral ranges with the 360 nm cut-off filter (ITOS N-WG360 Farbglas Schott) were E (UVB=280-320) = 0 mW/cm² and E (UVA = 320-400nm) = 9.4 mW/cm². No irradiation in the UV wavelength was measured using the 830 nm cut-off filter (LOT-Quantum design 830FG07). To test the effect of different UV doses, the irradiation time was varied. The emitting intensity was controlled before each measurement. The free radicals induced in the skin after UV-VIS exposure reacted with the PCA probe, reducing it to an ESR-silent hydroxylamine. The signal intensity decay was measured as a function of UV exposure doses directly inside the skin biopsies. The results are expressed as the percentage of free radicals induced after each UV dose. All values were normalized to placebo-treated skin. Each value is the result of a minimum 4 independent measurements on 3–5 different skin biopsy samples.

UV and Blue Light Transmittance Measurements with Photostability Assessment

The transmittance profile of the investigated sunscreens applied on a roughened PMMA plate in an amount of 1.3 mg/cm² (SB6 from HelioScreen Labs, FR) was measured from 290 to 450 nm using Labsphere UV-2000S equipment (Labsphere Inc, USA). The photostability was assessed by comparing the transmittance spectrum before and after irradiation, with the dose calculated according to ISO 24443 [16].

Furthermore, using the spectral transmittance data, the blue light protection can be calculated according to Eq. 1:

$$\text{Blue light protection } (\%) = \left[1 - \frac{\sum_{400}^{450} T(\lambda)}{n}\right] \cdot 100$$

where, T(λ) is the transmittance at wavelength λ and n is the total number of wavelengths between 400 and 450nm.

Results and Discussion

Photostability Profile of Investigated Sunscreen Formulations

Fig. 1 displays the UV absorbance spectra of the investigated sunscreen formulations before and after UV irradiation.

Fig. 1 clearly shows that sunscreens 214-1-2 and 214-1-3 based on DHHB are fully photostable and that sunscreens 214-1-1 and 214-1-5 based on BMDBM undergo a more or less significant decrease in performance following irradiation. A common and efficient way to photostabilize BMDBM by quenching its excited triplet state involves using Octocrylene [8] and Tinosorb® S [9]. The current trend, however, is to avoid octocrylene due to environmental and health-related issues [10–12]. Therefore, in the present study, the formulations were specifically developed without octocrylene to reflect current market trends. These results show that, used in isolation, Tinosorb® S was not able to efficiently photostabilize BMDBM.

Study 1: Free Radicals Generated in the Formulation

The number of free radicals induced in the formulation after UV irradiation is summarized in **Tab. 3** for each investigated UV-filter combination.

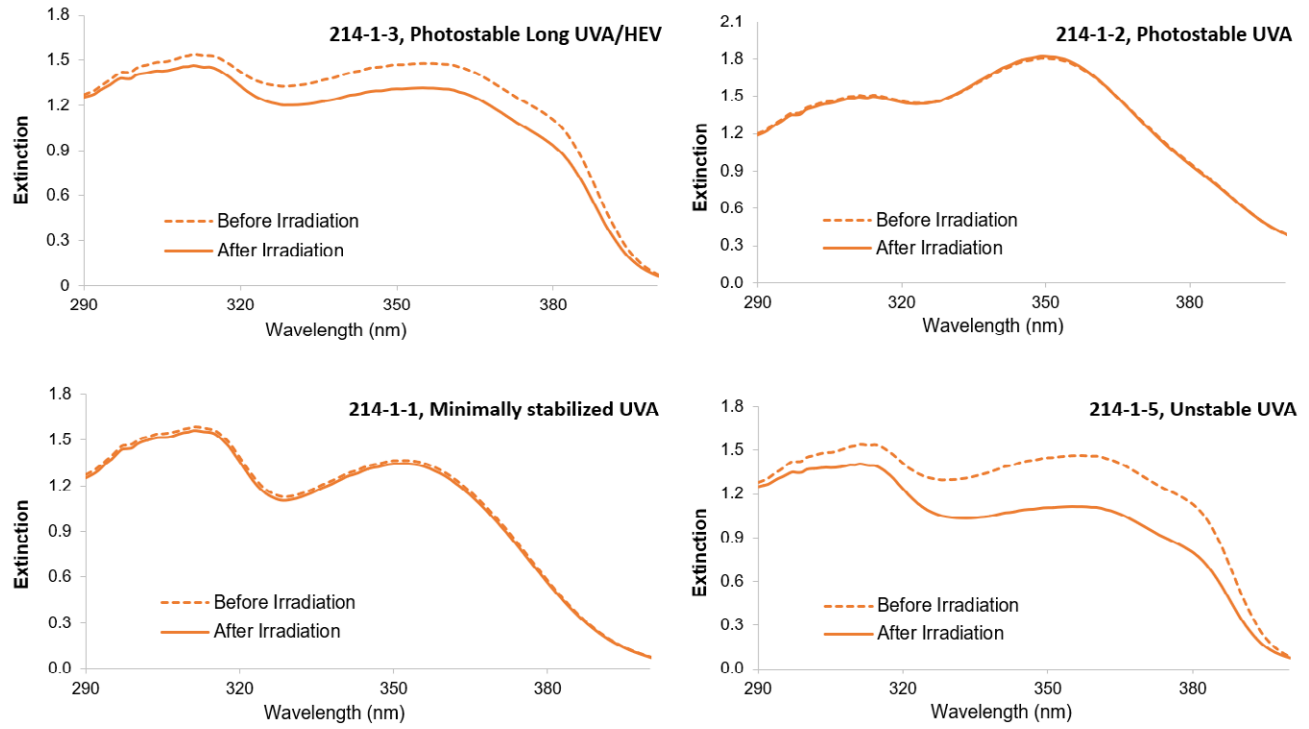

Fig. 1 Photostability profile of the investigated sunscreens.

The number of free radicals generated in the placebo formulation (214-1-4) was minimal; few to zero free radicals were formed (**Tab. 3**). The formulation chassis used for the present investigation is, therefore, not a source of free radical formation. The same result was obtained with the two sunscreens based on the photostable UVA filters Uvinul® A Plus (DHHB) and Tinosorb® S (BEMT) (214-1-2 and 214-1-3), with the number of free radicals equaling that of the placebo. No free radicals were formed in UV-irradiated sunscreens containing photostable UVA filters. Conversely, the same formulation chassis based on a combination of BMDBM and PBSA (sunscreens 214-1-1 and 214-1-5) led to a signification formation of free radicals after UV irradiation. Both UV filters were previously shown to produce free radicals after UV exposure [5-7]. The differences between the unstable UVA and minimally stabilized UVA formulation can also be highlighted by analyzing the kinetic parameters of the radical generation with increasing UV irradiation times (**Fig. 2**). The highly unstable formulation generates more radicals after a given UV dose (50%) compared to the minimally stabilized formulation (x_0 = 22.3 and 19.1, respectively).

This corroborates the results of an earlier investigation in which two sunscreens with different UV-filter combinations were compared with respect to the quantity of free radicals generated in the formulation and the elicitation of *Acne aestivalis* in sensitive subjects. The sunscreen including BMDBM and PBSA induced a higher number of free radicals in the UV-exposed formulation that was linked to its photo-unstable profile. Furthermore, the generation of free radicals was positively correlated with the elicitation of *Acne aestivalis* in

Formulation ID	Sunscreen label	% of induced free radicals
214-1-4	Placebo	2.7 ± 0.3
214-1-5	Unstable UVA	39.3 ± 0.5
214-1-1	Minimally stabilized UVA	33.5 ± 2.1
214-1-2	Photostable UVA	1.0 ± 0.1
214-1-3	Photostable long UVA/HEV	1.2 ± 0.7

Tab. 3 Relative quantity of free radicals, N = 2.

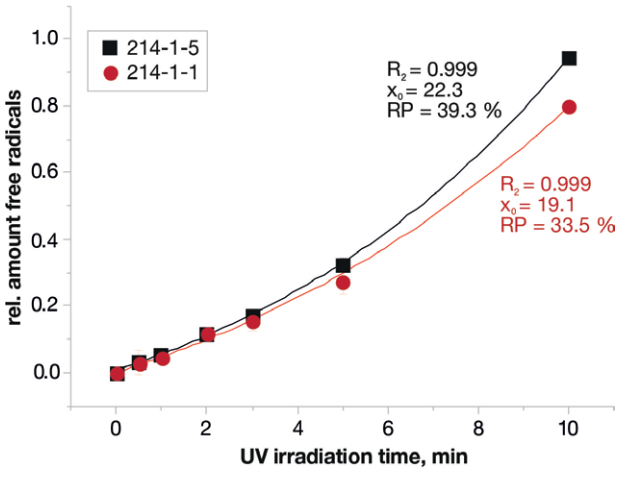

Fig. 2 Free radical formation in the unstable sunscreens as a function of UV irradiation time. Data fitted with a Boltzmann sigmoidal algorithm.

Formulation ID	Sunscreen label	% of induced free radicals ± sd after 10 min
214-1-4	Placebo	41 ± 6
214-1-5	Unstable UVA	32 ± 0.8
214-1-1	Minimally stabilized UVA	30 ± 8
214-1-2	Photostable UVA	24 ± 4
214-1-3	Photostable long UVA/HEV	10 ± 5

Tab. 4 Means and standard deviations for N = 3-5.

sensitive subjects in this specific sunscreen. Conversely, no adverse skin reaction was observed for the sunscreen containing the photostable UVA filter DHHB [17]. In an unpublished work, the concentration of squalene monohydroperoxyde was measured *in vivo* after a single UVA irradiation of 20 joule, applying either a sunscreen containing DHHB or BMDBM as UVA filter (like the sunscreens in the present study). Squalene monohydroperoxyde resulting from squalene oxidization is a suitable indicator for measuring oxidative damage and anti-oxidative effect. In this unpublished study, the level of squalene monohydroperoxyde reached 367 ng/mg for the sunscreen containing BMDBM/PBSA and 348 ng/mg for the sunscreen containing DHHB and Tinosorb® M.

Viewed together, these results confirm that the UV filter can be a major cause of free radical generation and, in particular, highlight the importance of the photostability of the UV-filter system. Additionally, the free radicals produced using an inappropriate photo-unstable UV-filter system may react with photocatalytic ingredients present in the formulation, which in turn may affect the integrity or efficacy of the formulation or, even worse, react with sensitive skin molecules and produce undesirable reactions.

Study 2: Free Radicals Generated in the Skin

The number of free radicals induced inside the skin after UV irradiation is summarized in **Tab. 4** for each applied sunscreen. The number of free radicals generated in the skin was highest for the placebo (214-1-4). Since the placebo does not contain any UV protection, the UV radiation penetrates the skin where it is able to produce free radicals. With the application of sunscreens with a UVA protection of 1/3 of the SPF, the number of free radicals is reduced. There is approximately a 25% reduction with a sunscreen based on BMDBM (214-1-1 & 214-1-5) and a 40% reduction with a sunscreen based on DHHB (214-1-2). The greatest protection against free radical generation in the skin was obtained with a sunscreen with a long UVA and blue light protection (214-1-3), with a 75% reduction in induced free radicals generated in the skin compared with the placebo. Both sunscreens 214-1-2 and 214-1-3 are based on the same photostable UV filter combination; the difference is the addition of organic particulate UV filters Tinosorb® M and Tinosorb® A2B in the photostable long UVA/HEV sunscreen. These results clearly show that increased protection against long UVA and blue light results in significantly better radical protection.

Viewed together, both studies demonstrate that using an inert formulation chassis, avoiding any photocatalytic UV filter and adding organic particulate UV filters enables a product to be optimized with respect to preventing free radical formation in the formulation and the skin.

Absorbance Profile of Organic Particulate UV Filters

Organic particulate UV filters are a special category: they feature UV-absorbing chromophores but are also able to reflect and scatter light because they are in a particulate form [18].

Fig. 3 displays the long UVA-blue light transmittance (in %) of Tinosorb® M and Tinosorb® A2B organic particulate filters at different concentrations. **Fig. 3** clearly shows that the long UVA-blue light transmittance can be lowered using either Tinosorb® M or Tinosorb® A2B. This effect is linked to their ability to reflect and scatter light and is concentration-dependent; the higher the concentration, the lower the blue light transmittance. The lower transmittance of between 400 and 405 nm for Tinosorb® M compared to Tinosorb® A2B is due to the absorbance tail of Tinosorb® M in the long UVA. To verify the significance of this effect when the particulate UV filter is used in isolation, the blue light

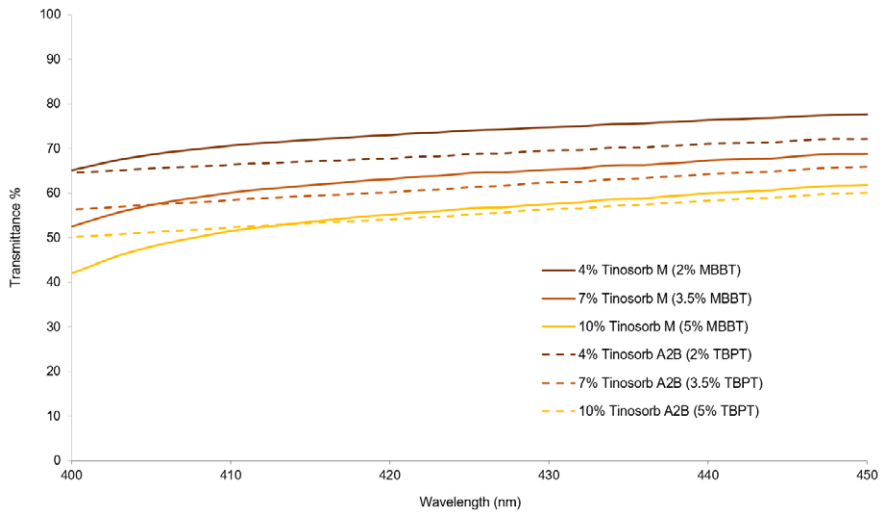

Fig. 3 Long UVA-blue light transmittance of Tinosorb® M and Tinosorb® A2B at 4%, 7% and 10%.

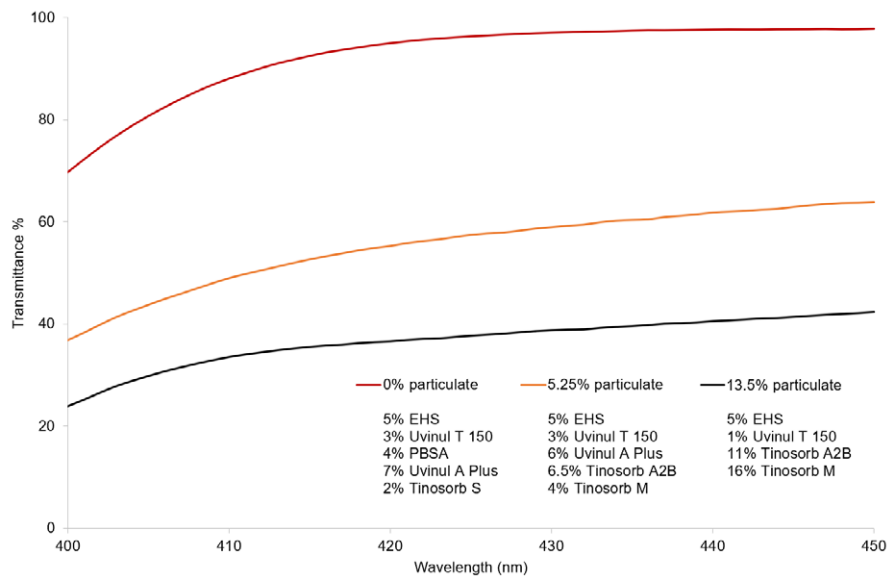

Fig. 4 UVA-blue light transmittance of UV-filter combinations with varying quantities of particulate organic UV filters: from 0% (top curve), to 5.25% (middle curve) and 13.5% (bottom curve) of organic particulate UV filters.

transmittance of sunscreens was measured and compared using market-relevant UV-filter combinations that differed in their quantity of organic particulate UV filters (**Fig. 4**).
The results displayed in **Fig. 4** confirm the observations above. The addition of organic particulate UV filters in a sunscreen offers a blue light protection. As above, the effect depends on the concentration. The blue light protection factor increases from 7% to 41% and 63% using 0%, 5.25% and 13.5% organic particulate filters, respectively.

Conclusions

The first study highlighted that the quality of the filter system with respect to photostability is central to free radical generation in sunscreen formulations. Only a photostable UV-filter system can avoid the formation of undesirable free radicals and the subsequent chain reactions and adverse skin effects.
The second study highlighted that the broadness of a filter system's protection against long UVA and visible light ranges is key to preventing free radical generation in the skin. The photostable product with long UVA and blue light protection led to a significant reduction in the formation of damaging free radicals. This is particularly significant for anti-ageing daily use products, seeing as free radicals are linked to UVA and visible exposure.

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