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Antioxidants for the Stabilization of Sunscreen

Introduction

To preclude sunburns and protect people from serious skin damage, sunscreens must possess several attributes. They have to be photostable (ideally 100%) and they have to dissipate the absorbed energy efficiently through photophysical and photochemical pathways that rule out the formation of singlet oxygen, other reactive oxygen species, and other harmful reactive intermediates **(Fig. 1)**.

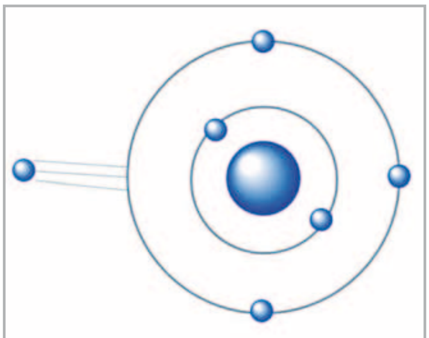

Fig. 1 The generation of free radicals by adsorption of an electron

They should not penetrate the skin, and should not be transported into the human cells where they can cause deleterious damage to DNA. Sunscreens should also minimize the extent of UVB and UVA radiation that might reach DNA in cell nuclei (1-4). With few exceptions, sunscreens contain chemical filters (organic; absorb mostly UVB radiation) and physical filters (e.g., TiO_2 and ZnO). The latter have been said to blockUVB/UVA sunlight through reflection and scattering (5, 6). If this were so, since reflection and scattering are physical phenomena, the term physical UV filters was coined. However, these inorganic UV filters also absorb considerable UV-radiation.

Abstract

Different sunscreen formulations have been investigated regarding their radical generation under UV radiation. Organic and inoraganic UV-filter molecules can generate free radicals during UV-radiation. Whereas organic UV-filters are marked by their degradation under UV-light, inorganic filters are marked by their photocatalytic reactions. The generation of free radicals inside a cosmetic formulation leads to the de-stabilization of the entire cosmetic preparation. Antioxidants can help to overcome this problem, neutralizing the UV-induced free radicals. Polyphenolic compounds, as Green tea extracts, are efficient radical quenchers. The amount of radicals generated with and without the addition of antioxidants can be quantified by Electron Spin Resonance (ESR) spectroscopy and is expressed by the Radical Generationg Factor (RGF).

Minerals such as titanium dioxide, TiO_2, and zinc oxide, ZnO, are well known active semiconductor photocatalysts used extensively in heterogeneous photocatalysis to destroy environmental pollutants that are organic in nature. They are also extensively used in sunscreen lotions as active broadband sunscreens that screen both UVB (290-320 nm) and UVA (320-400 nm) sunlight radiation and as high SPF makers. When so photoactivated by UV light, however, these two particular metal oxides are known to generate highly oxidizing radicals (·OH and $O_2^{-\bullet}$) and other reactive oxygen species (ROS) such as H_2O_2 and singlet oxygen, $^1O_2$, which are known to be cytotoxic and/or genotoxic. Hydroxyl(·OH) radicals photogenerated from photoactive TiO_2 specimens extracted from commercial sunscreen) induce damage to DNA plasmids *in vitro* and to whole human skin cells in cultures.
In contrast to the physical UV-blockers, chemical UV absorbers have to convert the UV light into chemical energy. During this conversion and the following molecular re-arrangements, the excited molecules formed by absorption of UV radiation return to the basal state by different radiative and non-radiative decay mechanisms. Some of these mecanisms can affect their activity, leading to the formation of new compounds by photoaddition, substitution, cycloaddition, isomerization, photofragmentation reactions, etc. The molecular re-arrangements are strongly depending on the chemical environment of the UV-blockers, on their solvent compounds, purity, and concentration.
These new compounds can be inactive (they do not absorb the UV radiation) or

favor the degradation of other compounds present inside the formulation. In both cases, the photoprotective effect of the sunscreen is compromised.
Eleven commercial sunscreen were tested (7, 8) for their free radical generation during UV-radiation. The results are expressed in percentage as Radical Generating Factor (RGF). The aim of the present study was the characterization of the radical generation inside the formulations and the radical reduction due to antioxidants. Therefore, five antioxidative raw materials (tocopherol, green tea extract, honeybush extract, pomegranate extract, and rooibos extract) typically used in cosmetic formulations (9), were added to the tested sunscreens and the radical formation during UV-exposure was determined. The antioxidative power (AP) of the cosmetics was determined by an ESR spectroscopy method (10). The comparison between the amounts of free radicals generated with and without antioxidants led to a rating of antioxidative actives that might be useful for the stabilization of sunscreen products.

Materials and Methods

Chemicals

The nitroxide 2,2,5,5 tetramethylpyrrolidine-*N*-oxyl (PCA) was obtained from Sigma-Aldrich (Germany). A final concentration of 1 mM PCA solution was used.
Eleven different sunscreens (market products) were used. The content of the UV-filter is indirect proportional to the ordinal number. All containing UV-filters in an unknown concentration and are marked in the INCI declaration **(Table 1)**. Antioxidants green tea, pomegranate, honeybush, and rooibos extract idoneous for cosmetic preparations were purchased from different suppliers. D-α-tocopherol was purchased from Sigma-Aldrich (Munich, Germany) at the highest purity grade available.

Free radical indicator

Oxygen and carbon centered free radicals generated in skin during UV irradiation were detected by using a radical trap on the basis of nitroxyl compounds (2,2,5,5 tetrametylpyrrolidine-*N*-oxyl - PCA). The RGF (Radical Generating Factor) shows the increase of generated free radicals indicated as a percentage (%).

UV irradiation

The UV-irradiation was performed with xenon arc lamp Solar Simulator from Newport-ORIEL Product Line 81260 (US, Newport Solar Simulators – product specifications) equipped with a 300W Xenon lamp supplying an irradiance in the plane of the sample of 16,5 mW/ cm² for UVA (330-400 nm) and 5,0 mW/cm² for UVB (290-330 nm). The 81260 has a UVB/UVA dichroic mirror as a standard device. It passes 280 to 400 nm and greatly reduces the VIS and IR output of the lamp. The measurements were performed with an UV-Meter-BASIC (hönle UV technology, Germany). The UV solar simulator emits a continuous spectrum with no gaps or extreme peaks of emission in the UV region. The output from the solar simulator is stable, uniform across the whole output beam and suitable filtered to create a spectral quality that complies

Code	SPF	TiO_2	BMDBM	OC	EHS	BEMT	EHT	TDSA	HS	PBSA	DHHB	EHMC
A	25	3	4	1	2							
B	25	3	2	1		4	5					
C	30	3	4	1	2							
D	30	3	4	1	2							
E	30	2		1							3	
F	50+	2	3	1								
G	20	3	2	1								
H	20	2	4	3	1	5	6	7				
I	15	3	4	1	2							
J	30	3	2	1				4				
K	50+	5	2	1		3			4	6	7	8

List of abbreviation

BEMT	Bis-Ethylhexyloxyphenolmethoxyphenyl triazine
BMDM	Butyl methoxydibenzoylmethane
DHHB	Diethylamino Hydroxybenzoyl Hexyl Benzoate
EHMC	Ethylhexyl methoxycinnamate
EHS	Ethylhexyl salicylate
EHT	Ethylhexyl triazone
HS	Homosalate
OC	Octocrylene
PBSA	Phenylbenzimidazole sulfonic acid
TDSA	Terephtalylidene dicamphorsulfonic acid

Table 1 UV-ilter content for eleven assorted sunscreen

with the required acceptance limits. The RCEE% values are in the acceptance limits.

ESR Spectrometer
A X-band ESR spectrometer Miniscope 300 Magnettech GmbH, Berlin, Germany was used for *in vitro* detection of free radicals. A conventionel quartz tube with a inner diameter of 1 mm from Magnettech for aques measurements was applied.

Experimental process
500 mg of the sunscreen were solved in 1000 mg of distilled water. 15 µl of 10 mM PCA were mixed under continuous stirring during UV-irradiation with the solar simulator. The PCA signal is measured before and after the UV irradiation of 5 minutes.
The signal is reduced if free radicals are generated. If the signal is not reduced (RGF = 0), there is no generation of free radicals.
The addition of antioxidants (9) can give the atom the missing electron back to fill in the electrons **(Fig. 2)**. Adding antioxidants to the sunscreen formulation can reduce the amount of UV-induced free radicals.
50 mg of the antioxidant stock solutions (10% concentrated in water) were added to 450 mg of the sunscreen. This preparation was added to 1000 mg of water. The final antioxidant concentration in the samples was 0.3 %.

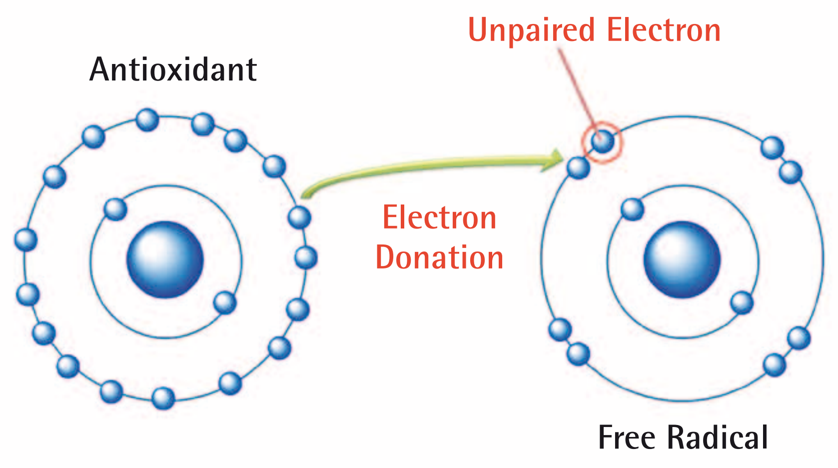

Fig. 2 The effect of antioxidants for neutralizing of free radicals

Different antioxidants were used which have a dfferent AP (10).

Results

Table 2 shows the RGF values (%) of 11 products, which can be divided into two main groups concerning their radical

			RGF (%) Antioxidants added to the final formulations				
Code	SPF	Pure product	Honey Bush Extract 1%	Vit. E (Toco.) 1%	Green Tea Extract 1%	Pomegranate Extract 1%	Rooibos Extract 1%
	AP(AU) t_r(min)	0 -	10.290 0,97	40.400 0,33	112.400 0,33	70.955 0,40	10.788 0,72
A	25	22	11	20	0	13	10
B	25	28	18	21	0	16	12
C	30	25	12	25	0	14	9
D	30	25	10	25	0	10	5
E	30	26	20	22	0	15	4
F	50+	25	8	20	0	17	13
G	20	10	2	10	0	10	10
H	20	0	0	0	0	0	0
I	15	10	6	10	0	15	10
J	30	3	0	5	0	3	3
K	50+	5	1	3	0	3	0

Table 2 RGF(%) after 5' UV and application of antioxidants

generation during UV-irradiation. The products in the first group marked by the capital letters A-F show a radical generation of approx. 25%. This important radical generation will lead to radical chain reactions inside the formulation causing the decay of the concentration of UV-filters on the skin with consequent reduction of the SPF.

The products in the second group G-K show low or no radical generation. In these formulations the UV-blockers are assumed to be stabilized. The photo-unstable BMDBM has been stabilized by octocrylene and/or the TiO_2 is perfectly coated inside the formulation.

The effect of different antioxidants for radical removing is also shown in **Table 2**. The lipophilic Vit.E with an AP= 404.000 and a reaction time t_r= 0,33 min has no or minimal effect on the radical status. Lipophilic antioxidants show no or only minimal protection against oxygen radicals. This is also valid for all other lipohilic antioxidants.

The three hydrophilic organic antioxidants honey bush with AP=102.900; t_r= 0,97min; pome granate with AP=709.550; t_r=0,40min and roibos with AP=107.880; t_r=0,72 min show a moderate behavior in fighting against free radicals. Only green tea extract with an AP= 1.124.000; t_r= 0,33min shows a absolute radical elimination capacity in all sunscreens. This elimination of UV-induced free radicals enhances the stability of the whole formulation.

Discussion

Eleven products were tested regarding their radical generating properties.

Ten products contain TiO_2, but nothing is known about their concentration and the character of coating.

6 products show a high radical generation superior to 20%. The Radical Generation Factor RGF (%) depends mainly on the coating character of the inorganic UV blocker, on the combination of organic UV-filters, and on the formulation properties. All products contain the organic filter BMDBM which is unstable under UV exposition. Avobenzone can degrade faster in light in combination with mineral UV absorbers like zinc oxide and titanium dioxide, though with the right coating of the mineral particles this reaction can be reduced (11, 12).

The method herein described aims to detect the UV-induced free radicals inside a cosmetic formulation. The higher is the Radical Generation Factor (RGF), the higher is the amount of UV-induced free radicals and the higher is the risk of photo-oxidative reactions that lead to instability of the formulation. It is important to mention that the free radicals measured are generated inside the cosmetic formulation and not inside the skin. Free radicals due to components of a sunscreen can be generated only if these components penetrate into the skin. There is no reason to assume that

this happens in intact skin. Nevertheless, the efficacy of sunscreens is one of the most important skin protection tools and the stability of those products should be guaranteed and optimized.
The newest trend in modern sunscreen is the addition of powerful antioxidants. The aim is to add the beneficial effects of radical scavengers to the skin. However, to be effective against free radicals in the skin, these antioxidants should penetrate into the skin and remain there active for a prolonged time, at least the permeation time on the sun. The antioxidants should therefore a) penetrate, b) remain active for several hours, c) be photostable, d) be active against different radical species. We believe that antioxidants in sunscreens may be able to protect the formulation against photo-induced radical reactions, that take place in any sunscreen due to the activity of the inorganic and organic UV-filters. The effect of the antioxidative activity in neutralizing the UV-induced free radicals depends on several parameters, as the hydrophilic or lipophilic solubility, the reactivity towards determined radical species, or the radical-inhibiting mechanisms. In the present work some antioxidative active natural extracts were particularly able to quench the radical reactions inside the formulation. The efficacy of each antioxidant should be evaluated inside the specific sunscreen, since the influence of the formulation is one of the main parameters of radical generation and propagation.

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