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Distribution of sunscreens on skin

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Abstract

The effectiveness of sunscreens was originally achieved by incorporation of soluble organic UV absorbers such as cinnamates and others into cosmetic formulations. Determinations of the sun protection factor (SPF) of emulsions containing different organic UV absorbers clearly indicate that the efficacy depends on the absorption characteristics of each single UV filter substance. Nowadays, micronised pigments such as titanium dioxide or zinc oxide have also been found to be protective against harmful UV rays. Our investigations using optical and electron microscopy proved that neither surface characteristics, particle size nor shape of the micronised pigments result in any dermal absorption of this substance. Micronised titanium dioxide is solely deposited on the outermost surface of the stratum corneum and cannot be detected in deeper stratum corneum layers, the human epidermis and dermis.

Keywords: Sunscreens; Sun protection factor; Electron microscopy; Nanoparticles; Titanium dioxide

Contents

1. Introduction

Modern sunscreen formulations deliver protection against both parts of the terrestrial UV radiation, the short wavelength UV range (UVB: 290–320 nm) and the longer wavelength range (UVA: 320–400 nm). The use of sunscreens should also be recommended in order to counteract against all kind of UV-induced skin damage such as photoallergies, skin wrinkles, sunburn or even skin cancer [2–5]. Obviously,

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visible changes of the skin such as sunburn and the generation of fine lines and wrinkles are directly linked to the quantity of light to which the skin was previously exposed to.

The following studies were performed in order to investigate effects of UVB and UVA filters on the sun protection factor. The sun protection factor and the efficacy of sunscreens depend on the distribution of the sunscreen on the skin. In order to determine the distribution behaviour we chose inorganic nanoparticles such as titanium dioxide and zinc oxide as ideal UV-filter substances which can easily be detected by modern analytical techniques. Micronised titanium dioxide is widely used in cosmetic sunscreens because of its broadspectrum UV-absorption and its high esthetical acceptance. We used electron microscopy (TEM) visualisation and light microscopic investigations of three different types of titanium dioxide in order to determine the influence of different parameters such as surface coating, particle size or shape of the micronised pigments on the dermal absorption behaviour of these substances.

2. Methods and material

The sun (burn) protection factor (SPF) is the main indication of the efficacy of suncare products. It is measured by determination of the dose which is required to induce a just perceptible redness (MED = minimal erythema dose) on untreated and on sunscreen-treated skin. The SPF is defined as the ratio between the MED protected and the MED unprotected. All described SPF data were determined according to the Colipa sun protection factor test method established in 1994 [6].

In order to assess the distribution behaviour of sunscreens on the skin, we chose three different types of micronised titanium dioxides for our investigations using optical and electron microscopy. Due to their surface modification the titanium dioxides have to be formulated in different ways, either in the water or in the oil phase. Therefore, one might expect a different distribution behaviour on the skin.

One variant of the micronised titanium dioxide (T805, Degussa, Hanau, Germany) is hydrophobically coated with trimethyloctylsilane and is cubic in shape with a mean particle size of 20 nm. The second preparation of the ultra-fine titanium dioxide (Eusolex T-2000, Merck, Darmstadt, Germany) is coated with non-covalently bound Al_2O_3 (8–11%) and SiO_2 (1–3%) and exhibits amphiphilic characteristics. Primary particles of Eusolex T-2000 have a mean size of 10–15 nm; these, however, aggregate secondarily to needle-shaped crystals sized roughly 100 nm. Finally, Tioveil AQ-10P (Solaveil, London, UK), a hydrophilic dispersion of titanium dioxide in water and propylene glycol, with alumina (4.25%) and silica (1.75%) as coating elements, was used in this study. The micronised titanium dioxide crystals in Tioveil AQ-10P have a mean particle size of 100 nm and are needle-shaped. Consequently, the particles differ significantly with respect to their behaviour in emulsions, which might be relevant after topical application onto human skin as well.

The test emulsions contained (INCI nomenclature) water, caprylic triglyceride, glycerin, butylene glycol, glyceryl stearate, octyldodecanol, dicaprylyl ether, cyclomethicone, stearic acid, trisodium EDTA, cetearyl alcohol, carbomer, lanolin alcohol and 4% ultra-fine titanium dioxide in either of the above-mentioned variants. In the placebo-emulsion, titanium dioxide was substituted by an increase of 4% in water content.

All test emulsions were applied on distinct areas on the forearm of a human volunteer. The areas were circular in shape with a diameter of 1.9 cm, rendering a total size of 11.3 cm^2; 45 mg of the respective test emulsion was applied on each test area corresponding to a concentration of 4 mg emulsion per cm^2 or 160 μg titanium dioxide per cm^2. The test emulsions were applied non-occlusively for a period of 6 h. Application areas were protected by rings covered with gauze in order to prevent abrasion of the test substance by accidental friction. Punch biopsies (2 mm in diameter) were subsequently taken from the centre of the respective test area under local anaesthesia.

The skin biopsies were incubated for a period of 36 h at 4 °C in Karnovsky's fixative containing 1% paraformaldehyde, 1.25% glutaraldehyde and 5 mM $CaCl_2$ in 0.16 M sodium cacodylate buffer (pH 7.3). After rinsing the skin samples twice with 0.16 M sodium cacodylate buffer (10 min each), post-fixation was performed for 1 h at RT in a 1% osmium tetroxide solution in 0.1 M cacodylate buffer (pH

7.3) containing 0.14 M potassium ferrocyanide(VI). After two more rinses with buffer (5 min each), dehydration was carried out in an ethanol series at room temperature (50, 70, 95, 99% 10 min each, and four times for 20 min at 100%). Then the specimens were immersed twice in propylene oxide for 5 min each. This was followed by incubation in mixtures of propylene oxide and epon (2:1 and 1:2), and twice in pure epon for 1 h each. Polymerisation took place at 60 °C overnight.

Either ultra-thin (50–100 nm) or semi-thin (500 nm) sections were cut from the embedded chemically fixed skin samples using a FC 4E ultramicrotome (Reichert, Germany) equipped with a diamond knife. Ultra-thin sections were collected on formvar/carbon-coated copper grids, counterstained with uranyl acetate as well as lead citrate, and investigated using a transmission electron microscope (912 WLeo, Oberkochen, Germany) at an acceleration voltage of 80 kV. Semi-thin sections were collected on microscopic slides using a copper loop and dried at 60 °C. Beforehand, a drop of 15% ethanol was placed on the slide in order to reduce surface tension. Afterwards, sections were stained with toluidine blue and pyronin G (Sigma, Deisenhofen, Germany) for examination under a light microscope (Axioskop, Zeiss, Oberkochen, Germany).

3. Results and discussion

3.1. Efficacy studies

Considering the action spectrum of the erythema reaction which is determined on human skin with the maximum at 307 nm [1], UVB filters are expected to be protective. In the first study we investigated the sunburn protection of single UVB filters. The chosen UVB filter ethylhexyl triazine (EHT), methylbenzylidene camphor (MBC) and octocrylene (OC) differ significantly in their absorption potential. The SPF measurements were performed with o/w emulsions containing 4% of each filter. Fig. 1 shows the results and the specific absorbance values.

The SPF data excellently correlate with the absorbance characteristics of the single filters. Ethylhexyltriazine with its very high specific absorbance value ($E^{\%}$) of 1580 yields the highest SPF followed by methylbenzylidene camphor and octocrylene. The relationship between the absorbance coefficient, which is measured in non-physiological, organic solvents, and the SPF measured on human skin contradicts the often suggested interaction between UV filter and skin.

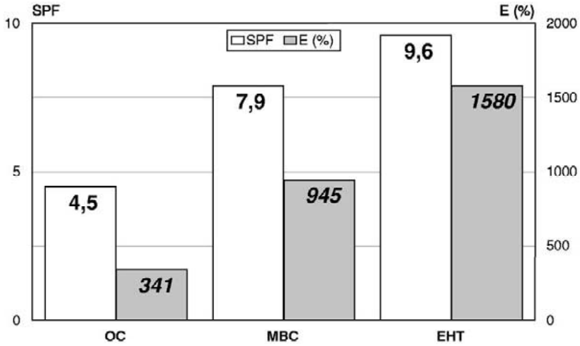

Fig. 1. SPF measurement of o/w emulsions with 4% UVB filter.

In a second experiment we analysed the effect of combinations of UV filters on the SPF. Two effective UVB filters, methylbenzylidene camphor (MBC) and ethylhexyltriazine (EHT), were formulated into o/w emulsions solely or in combination with the UVA filter butylmethoxydibenzoylmethane (BMDBM) which has its absorbance maximum at 360 nm far away from the maximum of the erythema action spectrum (307 nm). Fig. 2 shows the results of the efficacy measurements.

The data clearly indicate that the erythema protec-

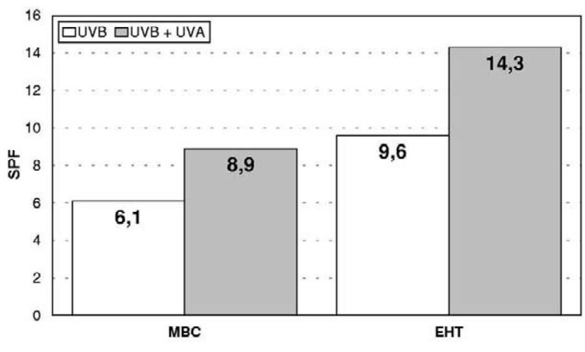

Fig. 2. SPF measurement of o/w emulsions with UVB and UVA filters.

tion is significantly influenced by the UVA filter BMDBM. The SPF boosting effect is observed in both combinations at a similar level. Consequently, a substance specific chemical interaction between the UVA and UVB filter is unlikely. But nevertheless the results underline that the use of UVA filters is recommended to reach higher SPF values.

Finally, we investigated whether there is an influence of the cosmetic formulation on the SPF. For this purpose, we chose a defined combination of UVB and UVA filters and formulated it into different vehicles. The SPF results are shown in Fig. 3.

Obviously we found a remarkable effect resulting from the different vehicles. Whereas the w/o formulation led to an increase of the SPF of approximately 40% compared to the o/w emulsion. In case of the emulsifier free hydrodispersion, we saw a loss of efficiency. We assume that differences in the remaining film thickness on the skin are responsible for the deviations in the SPF.

In order to determine the efficacy and distribution of different types of UV-active material, we performed studies using oil-in-water emulsions containing three different micronised titanium dioxides.

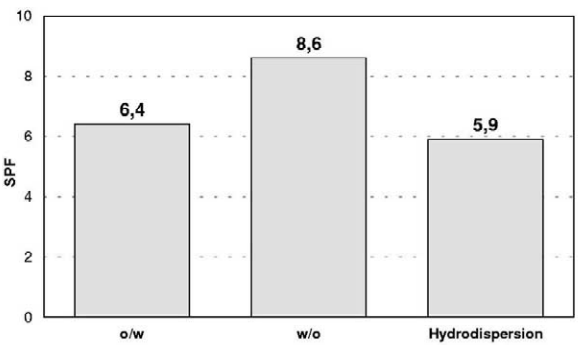

Fig. 3. Efficiency of UV filters in different vehicles.

These particles can easily be detected by optical and electron microscopy.

3.2. Electron and optical microscopy

Our investigation was set up to further evaluate the dermal absorption and penetration properties of different types of micro-fine titanium dioxide. Three sunscreen emulsions containing different types of micronised titanium dioxide (Table 1) were investigated.

Evaluation by light microscopy showed that micronised titanium could already be visualised with 1000-fold magnification. The reason for this is that the primary particles tend to form secondary agglomerates that are partially longer than the wavelength used for visualisation. A comparison of the micrographs of human skin samples 6 h after application of emulsions containing the three different types of micronised titanium dioxide used in this study can be seen in Figs. 4 and 5.

In all cases, the micronised titanium dioxide containing test emulsion forms an almost continuous film on the outermost layer of the human stratum corneum. Occasionally, however, the emulsion did not directly adjoin to the stratum corneum surface because desquamation due to preparation artefacts had taken place. Stratum corneum thickness in all sections examined was around 15 μm whereas the emulsion film varied in thickness between 5 and 10 μm depending on the type of micronised titanium dioxide used.

Contrary to the light microscopic evaluation, examination by electron microscopy (TEM) allowed an unequivocal discrimination of the micronised titanium dioxide particles from other apparently similar structures of equal density and morphology. TEM confirmed the impression gained by light

Table 1
Investigated types of micronised titanium dioxides

	Surface	Size (nm), shape	Coating elements
T 805	Hydrophobic	20, cubic	Ti/Si
Eusolex T-2000	Amphiphilic	100, needles	Ti/Al//SiO_2
Tioveil AQ	Hydrophilic (Disp.)	100, needles	Ti/Al//Si

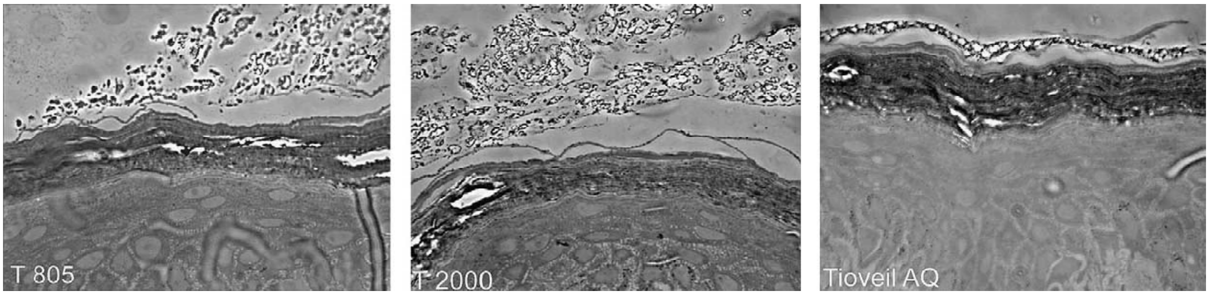

Fig. 4. Light micrograph of a semi-thin section of human skin vertical to the surface. An oil-in-water emulsion containing 4% titanium dioxide {(a) pigment T 805; (b) pigment T 2000; (c) pigment Tioveil AQ} was topically applied for 6 h.

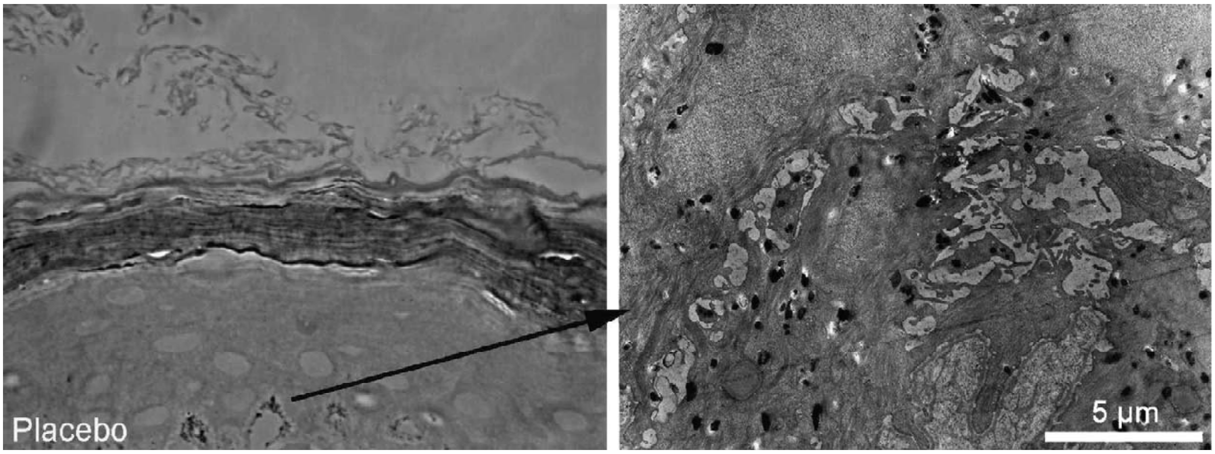

Fig. 5. Light micrograph of a semi-thin section of human skin vertical to the surface. An oil-in-water emulsion without titanium dioxide was topically applied for 6 h.

microscopic evaluation that titanium dioxide particles were solely located on the outermost surface of the stratum corneum and were not absorbed by deeper s.c. layers, epidermis and dermis. The histologically similar dense structures in the stratum granulosum and stratum basale could be clearly identified as melanosomes, as can be seen in Fig. 6 showing the skin section pre-treated with placebo-emulsion only.

TEM also documents the crystal shape and size of the three different types of micronised titanium dioxide used in this study (Fig. 7). Titanium dioxide pigments could be found in association with the fixed lipids of the emulsion on the surface of the stratum corneum. The pigments themselves were only rarely seen in singular structures but tended to form secondary agglomerates that were cubic or needle-like in shape varying in size between 20 and 100 nm.

4. Conclusion

Overexposure to UVB radiation (290–320 nm) as well as to UVA radiation (320–400 nm) causes several harmful changes in the structure and functionality of human skin. Sunscreens do protect effectively the skin against most of these reactions. In studies where we used a single UVB filter we

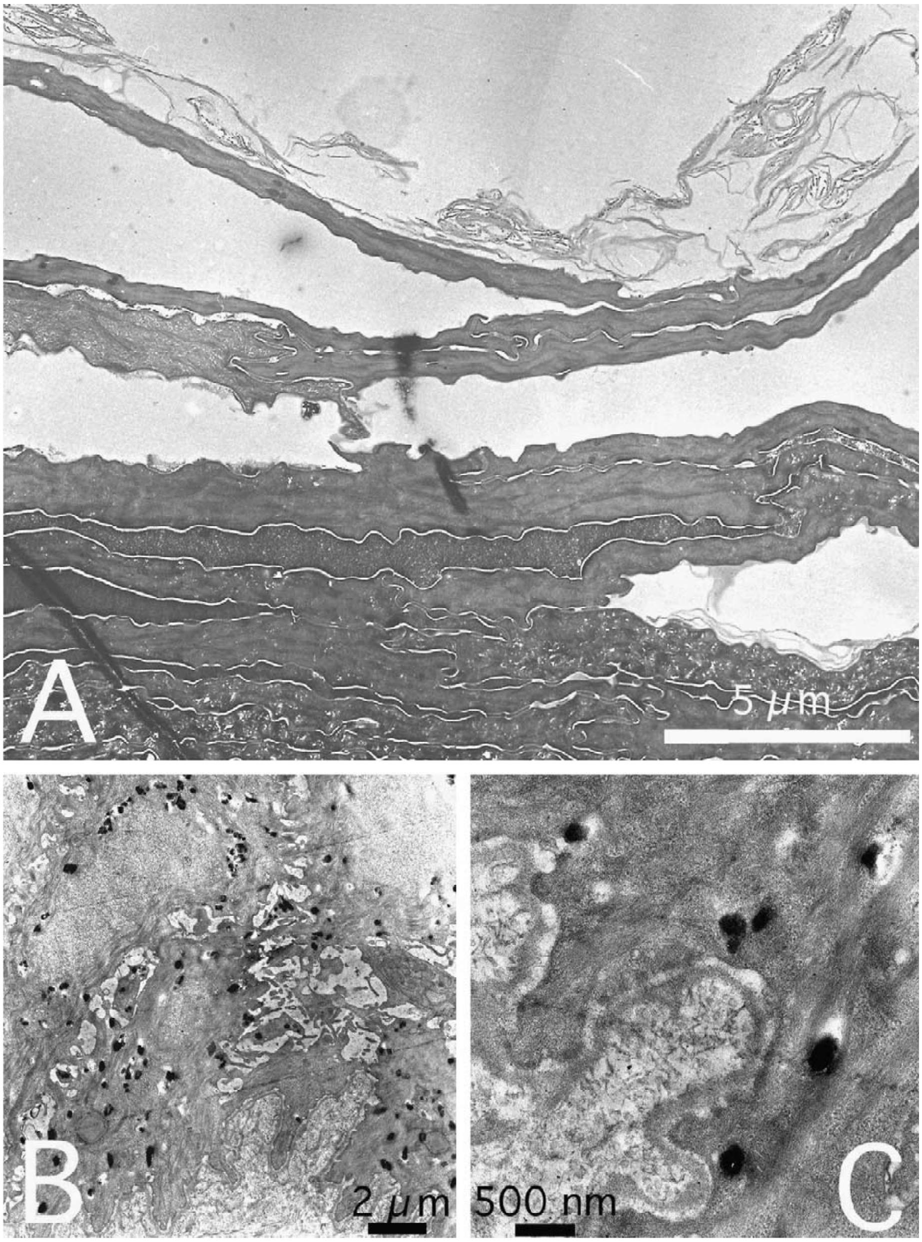

Fig. 6. Transmission electron micrograph of human skin vertical to the skin's surface (A). An oil-in-water emulsion without titanium dioxide was topically applied. The histologically similar dense structures in the stratum granulosum and stratum basale could clearly be identified as melanosomes (B and C).

demonstrated that the efficacy was well correlating with the specific absorbance coefficient of the evaluated UV filters. When combinations of UV filters were investigated, we also found an excellent correlation between the sun protection factor (SPF) and the protection against other UV-induced alterations of the human skin such as photoallergies, premature skin ageing and skin wrinkles. The data indicated

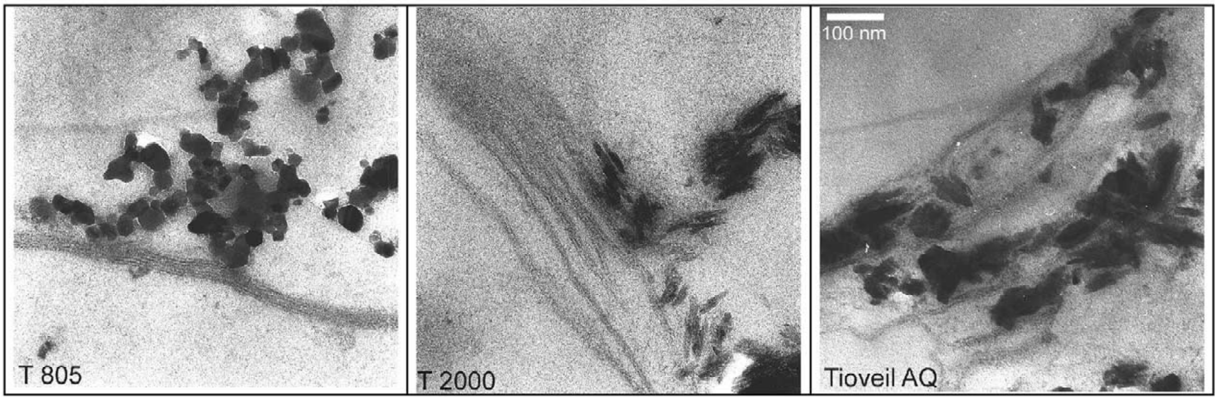

Fig. 7. Transmission electron micrograph of human skin vertical to the skin's surface with typical crystal structures of micropigments. An oil-in-water emulsion containing 4% titanium dioxide was topically applied.

that absorption of light is the most important function of UV filters covering the top of the skin. If one considers this reduction of transmitting light being the general mechanism, the protection of sunscreens can be predicted for all reactions which action spectrum is covered by the absorption spectrum of the complete sunscreen.

In order to demonstrate the effect of the cosmetic vehicle and the distribution of sunscreens on the skin on the efficacy of sunscreens we performed studies using oil-in-water emulsions containing different types of micronised titanium dioxides. The analysis of these test emulsions by light and electron microscopy (TEM) revealed that the titanium dioxide pigments were located exclusively on the outermost layer of the human stratum corneum only. TEM visualisation of the different application forms of titanium dioxide proved that neither surface characteristics, particle size nor shape of the micronised titanium dioxide result in any dermal absorption of this substance. These findings underscore the safe use of micro-fine titanium dioxide for topical application to humans.

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