Synergy effects between organic and inorganic UV filters in sunscreens

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Abstract. The influence of the synergy effects between organic and inorganic UV filter substances on the sun protection factor (SPF) of topically applied sunscreen formulations is investigated. The medium is considered to have reflection, absorption, and scattering properties. The distribution of photons in this medium is investigated by Monte Carlo calculation. Typical optical parameters of the skin and substances are used to characterize the synergy effect. The results of the model calculation are checked by *in vitro* and *in vivo* measurements investigating the influence of different types of scattering microparticles on the absorption efficacy of topically applied formulations. It is found that the inorganic filter substances act as scattering microparticles in the upper skin layers. They increase the optical pathway of the photons in the topically applied absorbing formulation also localized there. In this way, more photons are absorbed, increasing the SPF. The results obtained are important for the optimization of the SPF of sunscreen formulation containing organic and inorganic UV-filter components.
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Keywords: sunscreen; sun protection factor; UV filter; microparticles; TiO_2; reflection; ultraviolet; filtering; scattering; absorption.

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1 Introduction

Sunscreens are becoming increasingly important as protection measures against UV radiation, on account of the increasing spare time of the population and the growing popularity of holidays on the beach. Modern sunscreens usually contain organic UV-filter substances such as butyl methoxydibenzoylmethane (BMDBM), 4-methylbenzylidene camphor (MBC), and the inorganic UV-filter substances TiO_2 and ZnO. After topical application and equilibration, these UV filters are located in the upper part of the stratum corneum where they form a protective layer[1,2] or, especially in the case of titanium dioxide, they are said not even to enter the skin.[3–6] The efficacy of sunscreen products is characterized by the sun protection factor (SPF). Usually, the SPF of a formulation containing organic and inorganic filter compounds is higher than the sum of the sun protection factors of the separate UV-filter substances.[7,8] Therefore, there is a synergy effect between organic and inorganic UV-filter substances.

The aim of our investigations was to analyze the reason for this synergy effect: therefore, theoretical and experimental studies were carried out to solve this task.

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2 Methods

2.1 *Model Calculation Using the Monte Carlo Method*

The distribution of photons in a medium similar to human skin was investigated by Monte-Carlo calculation.[9] The medium was considered to possess reflection, absorption, and scattering properties. Typical optical skin parameters were used to characterize this medium.[10]

It was investigated how the absorption of photons changes the medium if an absorber and scattering particles were added. The investigations were carried out in the visible part of the spectrum using the absorption parameter of the dye Evans blue and scattering and reflection parameters of TiO_2 microparticles, which were tested in the *in vitro* experiments. In this spectral region, the TiO_2 microparticles have only scattering but not absorption properties. In this case, the effect of absorption and scattering can be separately investigated by changing the concentration of Evans blue or microparticles.

In the calculation, the absorption of the medium was increased by increasing the concentration of Evans blue, which corresponds in practice to the topical application of an organic UV filter onto the skin.[11] Additionally, the reflection and scat-

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Table 1 Overview of the microparticles used in the experiments.

Type of microparticles	Composition/parameters
Type 1	Light diffusing pigment (INCI: silica, titanium dioxide, alumina).
Type 2	Eusolex® T-S (INCI: titanium dioxide, alumina, stearic acid).
Type 3	TiO_2 with high bulk density (INCI: titanium dioxide, alumina, simethicone).
Type 4	Hydrophobic ZnO for cosmetic use (INCI: zinc oxide, trimethoxycaprylylsilane).
Type 5	Spherical silica particles (particle size 500 nm, INCI: silica).
Type 6	Eusolex® T-2000 (INCI: titanium dioxide, alumina, simethicone).

tering properties of the medium were increased by increasing the concentration of microparticles, which correspond to the application of microparticles (inorganic filter substances) onto the skin. The changes in the absorption properties of the organic filter substances were analyzed depending on the concentration of the microparticles.

2.2 *In vitro Measurements*

2.2.1 *Tissue samples*

In the first step, the synergy effect between organic absorbers and microparticles was investigated *in vitro* using human split skin. In the second phase, split skin freshly obtained from pig ears was used. Approval was obtained for these experiments from the Veterinary Board of Control, Berlin Treptow-Köpenick.

All tissue samples had a thickness of 0.3 mm and were cut using a dermatome (GA 140, Aesculap AG, Tuttlingen, Germany). Samples of identical size were used in all experiments. Therefore, tissue samples with a diameter of 14 mm were punched from the split skin.

2.2.2 *Topically applied organic and inorganic substances*

The inorganic particles (received by Merck KGaA, Darmstadt, Germany) used in the experiments are listed in Table 1.

All microparticles, excluding type 5, have absorption bands in the UV range. Therefore, the effect of the increase in absorption efficacy caused by scattering microparticles was not investigated in the UV but in the visible range. Here no superposition of the absorption bands of the organic and inorganic components could be observed. Evans blue was selected as an absorbing organic component. The substance has a characteristic absorption band at 600 nm.

Six different types of formulation were prepared. The first formulation consisted of an oil and water (o/w) emulsion. The second type of the formulation was identical to formulation 1. Additionally, 10% of different types of microparticles listed in Table 1 were added to this formulation. The third and fourth formulations were identical to formulations 1 and 2. Additionally, 1% of Evans blue was added. The fifth formulation was based on formulation 1. Additionally, it contained 2% of Eusolex® 9020 (BMDBM, Avobenzone), and 4% Eusolex® 6300 (MBC). The sixth formulation was based on formulation 5. Additionally, it contained 10% of microparticles (spherical silica particles, type 5).

The compositions of the six formulations are summarized in Table 2.

2.2.3 *Application of the formulation*

The formulation was applied onto the split skin at a concentration of 2 mg/cm^2 following the COLIPA standard.[12] The homogeneous application of such a small amount on a small skin sample (∅ 14 mm) became difficult. Therefore, a special application system was developed (Fig. 1), which consists of an optical quartz cell formed like a ''pot'' with a flat bottom (optical window). The bottom has a diameter of 14 mm, identical to the diameter of the skin samples. The optical cell was put on a balance (BP211 D-OCE, Satorius GmbH, Göttingen, Germany). 3.5 mg of the formulation (corresponding to 2 mg/cm^2) were applied to this optical cell. Subsequently, the skin sample was placed onto the optical cell so that the skin surface was faced to the formulation. Using a small ''stamp,'' which fit exactly into the opening of the optical cell, the skin

Table 2 Composition of different formulations used in the experiments.

Number of formulation	o/w emulsion	2% of Eusolex® 9020 (BMDBM, avobenzone), 4% Eusolex® 6300 (MBC)	10% microparticles type 1 through 5	1% Evans blue
Formulation 1	X			
Formulation 2	X		X	
Formulation 3	X			X
Formulation 4	X		X	X
Formulation 5	X	X		
Formulation 6	X	X	type 5	

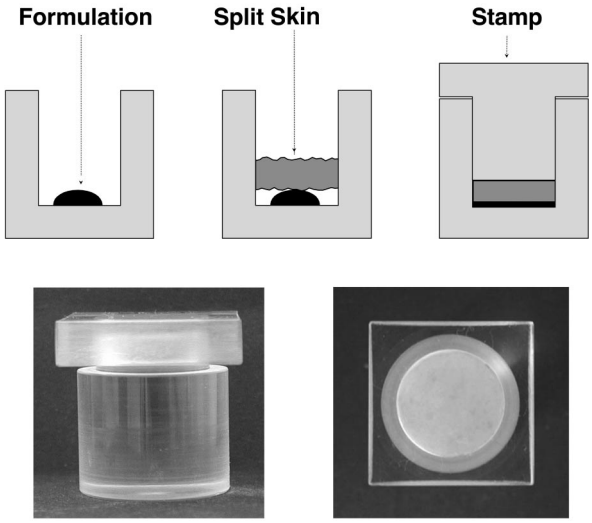

Fig. 1 System for the homogeneous application of formulation on the skin samples.

was gently pressed to the bottom of the pot. In this way the formulation was distributed homogeneously between the optical window and the skin surface. The stamp was removed and the opening was closed with a thin optical quartz window. After a penetration time of 6 h, the optical cell was put into the spectrometer and the spectrum was measured.

2.2.4 *Absorption measurements*

The absorption measurements were carried out in the spectral range of 280 nm up to 800 nm using a spectrometer M 80 (Perkin Elmer, Göttingen, Germany). The measuring spot was 6×8 mm^2. Because of the scattering properties of the tissue and the microparticles, the light transmitted through the samples was collected by an Ulbricht sphere containing the detector. The absorption of Evans blue was detected by determining the difference spectra of formulations 1 and 3 (Evans blue absorption without microparticles) and of the formulations 2 and 4 (Evans blue absorption with microparticles). The principle of the determination of the absorption of Evans blue in the skin samples is demonstrated in Fig. 2, where the spectra of the formulations 1 and 3 are presented. The absorption band of Evans blue at 600 nm is seen in the spectrum obtained from formulation 3. The spectrum of formulation 1 was used for base line correction. In analogy, the Evans blue absorption applied in formulation 4 containing microparticles was determined using formulation 2 for base line correction.

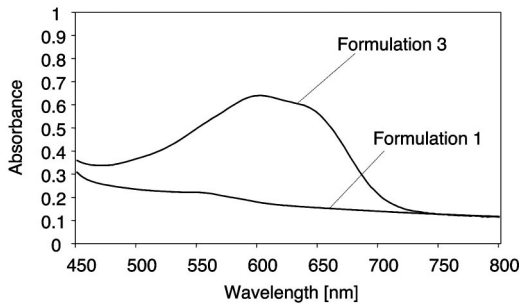

Fig. 2 Determination of absorption of Evans blue by calculation of the difference spectrum of the spectra obtained from formulation 1 (emulsion) and formulation 3 (emulsion+Evans blue).

The increase in the absorption caused by the microparticles was determined by comparison of the absorption of Evans blue in formulation 3 (without particles) and formulation 4 (with particles).

The formulation 3 and all types of formulation 4, containing different types of microparticles, were applied and analyzed on ten skin samples. The main value and the standard deviation were determined.

2.3 *In vivo Measurements*

2.3.1 *Topically applied organic and inorganic substances*

In the second part of the experiments, we checked whether the changes of the visible absorption of the samples containing Evans blue on split skin could be reproduced with the UV filters (2% BMDBM and 4% MBC) in the UV range. Microparticles of type 5 (silica microparticles) were used in these experiments, which have no absorption in the spectral range of the UV filters. Formulations 5 and 6 were applied on the back of six volunteers at a concentration of 2 mg/cm^2 following the COLIPA protocol.[12] The SPF was determined for formulations 5 (without microparticles) and 6 (with SiO_2 microparticles, type 5) one hour after application.

2.3.2 *SPF determination*

The minimal erythema dose (MED) was determined on the back of volunteers using the sun simulator ETG 1 (Fa. A.L.T. Lichttherapietechnik GmbH, Zörbig, Germany).[11] This device was equipped with a lamp TL 4 W/12 (Fa. Philips, Hamburg, Germany). The time of irradiation was calculated on the basis of the skin phototype, taking into consideration the Fitzpatrick scale.[13] All MED measurements started 1 h after application of the emulsions to the skin. The erythema was determined 24 h later using the colorimeter ''spectropen'' (Fa. Dr. Lange, Berlin, Germany). The SPF of formulations 5 and 6 was determined using the results of the MED measurements.

The SPF determination was repeated for every formulation on six volunteers aged between 26 and 35 years. Ethical approval had been obtained for these experiments from the Ethics Commission of the Medical Faculty Charité.

2.3.3 *Sunscreen distribution on the skin*

Tape stripping can be used to determine the homogeneity of the distribution of topically applied substances on the skin.[14–16] The homogeneity of the distribution of formulations 5 and 6 used for SPF determination on the skin was investigated by laser scanning microscopy (LSM 2000, Carl Zeiss, Germany). 0.1% of the fluorescent dye sodium fluorescein (MERCK-Schuchardt, Hohenbrunn, Germany) was added to both formulations.[17,18]

The formulations were applied onto the skin following the COLIPA protocol.[12] After 1 h, the tape strips were removed from the treated skin areas following the previously described protocol[2] using *tesa* tapes (number 5529, Beiersdorf AG, Hamburg, Germany). The homogeneity of distribution of the

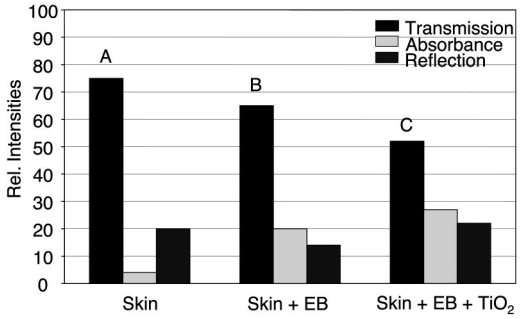

Fig. 3 Results of Monte-Carlo calculations describing the photon distribution in a skin layer after application of absorbing substances and scattering and reflecting microparticles.

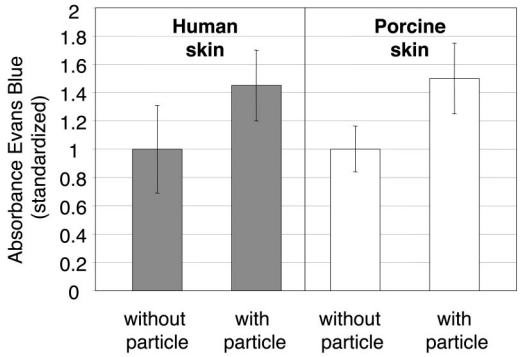

Fig. 4 Comparison of the Evans blue absorption with and without addition of microparticles measured on human and porcine skin.

dye on the removed corneocytes was analyzed by fluorescence measurements using the LSM excitation wavelength at 488 nm.

3 Results

3.1 *Model Calculation*

The results of the Monte Carlo calculation are presented in Fig. 3. The bar groups describe the transmission, absorption, and reflection properties of the skin at 600 nm. This wavelength corresponds to the absorption band of Evans blue. The relative transmission, absorption, and reflection values for the nontreated skin are presented by the bar group A. If Evans blue (EB) was added to the system, the absorption of the skin surface consequently increased [see graph (b) in Fig. 3].

The addition of TiO_2 microparticles to the skin slightly increased the reflection of the skin. Additionally, the skin absorption determined via the absorption of Evans blue increased if microparticles were added [see graph (c) in Fig. 3]. In this case, the skin absorption increased by 40% because of the scattering and reflection properties of the microparticles [compare (b) and (c) in Fig. 3].

3.2 *In vitro Measurements*

3.2.1 *Comparisons between human and porcine skin*

The influence of coated TiO_2 microparticles (type 6) on the absorption of Evans blue was analyzed using six samples of human and porcine split skin obtained from pig ears. The results are presented in Fig. 4. For better comparison, the absorption of Evans blue in the nonparticle-containing formulation 3 was standardized to a value of 1. The absorption of the same amount of Evans blue in the particle-containing formulation 4 was related to this standard. The absorption of Evans blue increased if the microparticles were added to the formulation. Identical results were obtained for human and porcine skin.

3.2.2 *Influence of different types of microparticles*

The influence of different types of microparticles on the absorption of Evans blue is demonstrated in Fig. 5. The absorption of Evans blue without microparticles (formulation 3) was standardized to 1 (bar A in Fig. 5). The Evans blue absorption increased up to 150% if silica microparticles covered with TiO_2 (type 1) were added (bar B in Fig. 5). TiO_2 microparticles with a high reflection coefficient (type 2) increased the Evans blue absorption up to 174% (bar C in Fig. 5). On the other hand, the Evans blue absorption did not change if coated TiO_2 microparticles with a different structure and a low reflection coefficient (type 3) were added (bar D in Fig. 5). ZnO microparticles in the configuration used (type 4) increased the Evans blue absorption up to 125% (bar E). Pure silica microparticles with a special high reflection coefficient (type 5) increased the Evans blue absorption up to 180% (bar F).

3.3 *In vivo Measurements*

3.3.1 *SPF determination*

SPF measurements were carried out to confirm the *in vitro* results concerning the influence of microparticles on the absorption of a topically applied substance *in vivo*. The measurements were carried out using formulation 5 (emulsion+UV filters) and formulation 6 (emulsion+UV filters+microparticles). These silica microparticles (type 5) showed a high influence on the Evans blue absorption in the previous experiments, and have no absorption in the spectral region of the UV filter substances. In Fig. 6, the sun protection factors are presented, which were obtained for the UV filter containing formulations with and without silica microparticles. The SPF values are compared with the results of the

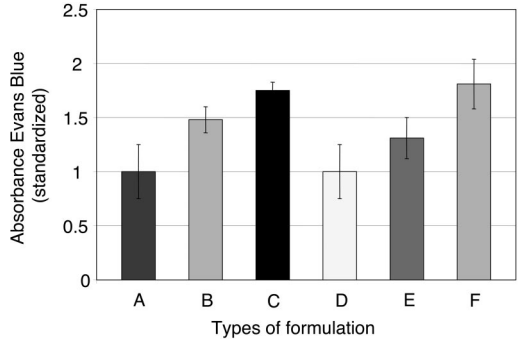

Fig. 5 Influence of different types of microparticles on the absorption of Evans blue: A is formulation 3, B is formulation 4+microparticles type 1, C is formulation 4+microparticles type 2, D is formulation 4+microparticles type 3, E is formulation 4+microparticles type 4, and F is formulation 4+microparticles type 5.

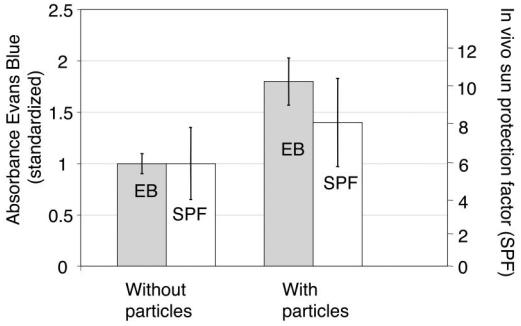

Fig. 6 *In vivo* SPF and Evans blue absorption obtained with and without silica microparticles (type 5).

in vitro Evans blue absorption measurements. Similar to the Evans blue absorption, the SPF increased if microparticles were added.

3.3.2 *Sunscreen distribution on the skin*

It was checked whether the microparticles have an influence on the distribution of the formulations on the skin. The distribution of formulations 5 and 6 on the skin could be considered to be identical. The distribution of the fluorescent dye sodium fluorescein on the removed tape strips is presented in Figs. 7(a) and 7(b). No differences in the distribution of formulations 5 and 6 were observed.

4 Discussion

4.1 *Monte Carlo Calculation*

The Monte Carlo calculation demonstrates that microparticles with scattering properties can increase the absorption of photons in an absorbing medium. This effect is caused by the increase of the optical pathway of photons in the absorbing medium because of scattering, as is schematically presented in Fig. 8.

From Fig. 8 it becomes clear that the effect had to be measured using an Ulbricht sphere.[10] In this case, all the scattered light would be detected.

The Monte Carlo calculation presents an estimation of the synergy effect between organic and inorganic filter substances topically applied. Regardless of the application of estimated spectroscopic parameters (absorption, reflection, and scattering), the calculations of the experimentally observed effect of the increase in the absorption of Evans blue (see Fig. 5) and of the SPF (see Fig. 6) were well described.

4.2 *Measurements on Human and Porcine Tissue*

From the results presented in Fig. 4, it can be seen that there are no differences in the absorption properties of the topically applied formulation when human or porcine tissue is used. Therefore, the experiments can be performed *in vitro* on porcine skin. This tissue is readily available and easy to handle.

4.3 *Influence of Different Types of Microparticles on the Absorption of Evans Blue*

In agreement with the Monte Carlo calculations, the absorption of Evans blue increased if microparticles with scattering properties were added to the formulation. All the Evans blue

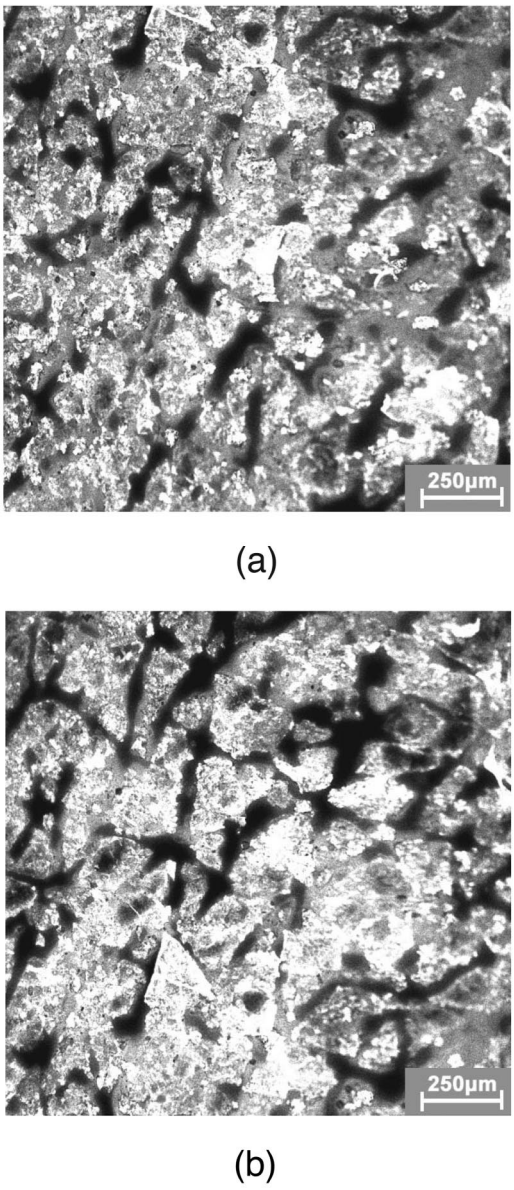

Fig. 7 Distribution of the fluorescent dye sodium fluorescein added to formulations 5 and 6 on the removed tape strips: (a) formulation 5 and (b) formulation 6.

absorption measurements were carried out in the visible part of the spectrum, where the microparticles have no absorption but only scattering properties. Microparticles with different scattering properties influence the Evans blue absorption to a different extent. The best results were obtained with silica microparticles (type 5). These microparticles have a diameter of 500 nm and high scattering properties. They increased the Evans blue absorption up to 180%. Similar results were obtained using TiO_2 microparticles (type 2) used in commercial sunscreens. With microparticles based on zinc oxide only, a medium absorption can be achieved.

The medium absorption of the microparticles based on TiO_2 with a high bulk density (type 3) might be caused by a higher secondary particle size (agglomerates) compared to other high-grade titanium dioxide types (e.g., type 2). The

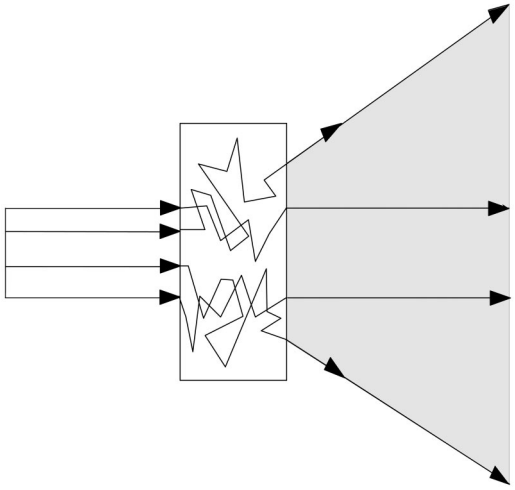

Fig. 8 Increase in the optical pathway of photons in an absorbing medium on account of scattering.

distribution of the secondary particle size depends on the energy input during processing of the emulsions. But all types of microparticles used in the present work were introduced into the formulations with more or less the same energy. Higher bulk density material would at least need a higher energy input to lead to a similar degree of distribution.

In this way, the synergy effect could be studied under definite conditions, without taking into consideration a superposition of the absorption of Evans blue and the microparticles. It can clearly be seen from Fig. 5 that the light scattering caused by the microparticles increased the number of photons absorbed by Evans blue.

4.4 *SPF Determination*

The aim of the SPF determination was to reproduce the results of the *in vitro* measurements obtained in the visible part of the spectrum under *in vivo* conditions in the UVB part of the spectrum. In the case of the silica microparticles (type 5), only the scattering properties of the microparticles but not their absorption had to be considered. From Fig. 6, it can be seen that the SPF increased by a factor of 1.4 after the addition of these microparticles (type 5, silica microparticles) to the formulation containing chemical UV filters (formulation 5). Both *in vitro* experiments based on the Evans blue absorption measurements, and the *in vivo* measurements based on the SPF determination, show an increased absorption of photons by the organic absorbers caused by scattering processes. The increase in SPF by a factor of 1.4 is lower than the increase of the Evans blue absorption (factor of 1.8) but still significant.

An influence on the distribution of the formulations on the skin, based on the difference in the SPF values, could be ignored because of the identical distribution demonstrated by the results of the fluorescence measurements shown in Fig. 7. Thus, the increase in SPF in the case of silica microparticles being added to the formulation is only determined by scattering properties of the microparticles.

Taking into consideration the obtained results, the proposed *in vitro* method can be used to screen the protection properties of sunscreens containing different types of microparticles and organic UV filter substances. In this way, *in vivo* SPF measurements based on erythema formation can be avoided.

In contrast to the SPF determination, the results of the *in vitro* measurements can be immediately obtained, whereas the results of the SPF measurements are available only after 24 h. Additionally, in the case of SPF measurements, the minimal erythema dose (MED) has to be determined by means of pretests for every volunteer.

5 Conclusion

Microparticles characterized by high scattering efficiencies increase the optical pathway of photons in absorbing media. In the case of topically applied sunscreens containing organic UV filter substances, the addition of microparticles increases the UV protection of these sunscreens, even if the microparticles have no absorption bands in the UVB and UVA. This means that micronized titanium dioxides, such as Eusolex® T-S, currently used in cosmetic formulations, do not only act through their own UV absorption capacity. Their advantage is the synergistic effect caused by light scattering in combination with traditional organic UV filters to reach high SPF values. As found in our study, spherical silica powders or other microparticles with special light scattering properties also can be used in cosmetic formulations to support their performance.

The efficacy of the microparticles in connection with the SPF increase could easily be tested by using the *in vitro* method presented. The results correlate to the values obtained *in vivo* by SPF determination.

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