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Influence of nonhomogeneous distribution of topically applied UV filters on sun protection factors

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Abstract. The aim of the present study is the development of a method to determine quantitatively *in vivo* the influence of homogeneity of the distribution of sunscreen containing UV filters on the sun protection factor (SPF). The SPF of a sunscreen applied either topically or inside an optical cell (pure or in a solvent) fixed above the skin is determined *in vivo*. In both cases, *in vivo* measurements using the erythema formation are carried out. Identical optical parameters of the skin are realized in both experiments. In addition, both *in vitro* (using tape stripping) and *in vivo* microscopic measurements are performed to analyze the homogeneity of distribution of the topically applied substances. An SPF of 8 is measured in the experiment applying the UV filters topically, whereas this value increases by a factor of 10 if the same amount of filter substances is distributed homogeneously in solution inside the optical cell. Tape strips removed from skin treated with the sunscreen reflect the inhomogeneous distribution of the topically applied substances on the skin. The direct correlation of homogeneity of distribution with the SPF opens up the possibility to increase the SPF by optimizing the formulation. [DOI: 10.1117/1.1805557]

Keywords: sunscreen; stratum corneum; laser scanning microscopy; minimal erythema dose.

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1 Introduction

The protective ability of sunscreens is commonly characterized by the sun protection factor (SPF), measuring the UVB-induced erythema solare.[1–3] Several attempts have been undertaken to develop an *in vitro* method for the SPF determination, because the produced erythema represent a skin damage. Usually, the *in vitro* measurements were carried out spectroscopically. The sunscreen was applied in an optical cell and the transmission was measured using a spectrometer. In general, the specific absorption intensity of the UV filter substances and the amount applied were considered to be the decisive parameters for the determination of the protection efficacy.[4,5]

The SPF values determined *in vivo* are considerably less than the values determined *in vitro*.[6–9] These results are in agreement with the observation that applied sunscreens can be accumulated in the furrows and wrinkles of the human skin.[9] The differences between the *in vitro* and *in vivo* results are not only caused by the differences in the homogeneity of the distribution but also by the changes of the optical properties of the skin treated with a sunscreen. The scattering properties of the stratum corneum can be significantly reduced if a cosmetic formulation is applied.[10,11] This results in an increased transmission of the skin.[12,13] In the present study, a method to determine the influence of the homogeneity of the distribution of UV filter substances on the SPF is described, taking into consideration the mentioned parameters that can affect the measurement. Therefore, two different modes for application of the UV filter substances were compared. In the first case, the substances were directly applied onto the skin. In the second case, the filter substances were applied inside an optical cell above the skin, while the emulsion not containing UV filters was applied onto the skin. In this way, a homogeneous distribution of the UV filters was realized. In both cases, the SPF values were determined via the minimal erythema dose (MED), avoiding influences of the formulation on the optical properties of the skin. In addition, the distribution of the topically applied formulation was studied by laser scanning microscopy *in vivo* and *in vitro* using the tape stripping method.

2 Material and Methods

2.1 *Volunteers*

The investigations were performed using healthy male and female volunteers of skin phototypes 1 through 3,[14] aged 26±9 years (protocol 1) and 23±1 year (protocol 2). Approval was obtained for these experiments from the Ethics Committee of the Charité Hospital, Berlin.

2.2 *Materials*

A sunscreen consisting of an o/w emulsion containing the UV filters octylmethoxycinnamate (7%) and butyl methoxydiben-

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zoylmethane (1.5%) with a declared SPF of 8 was used in the experiments. The same o/w emulsion without UV filters was used as a control in the experiments.

The sunscreen was dissolved in a mixture of chloroform/methanol=2:1 (both UVASOL, Merck, Darmstadt, Germany) for the application in optical cells. In addition, the sunscreen was mixed with the emulsion at a ratio of 1:1 (sunscreen/emulsion mixture) for the application in an optical cell.

In the case of laser scanning microscopy (LSM), the fluorescent dye curcumin (Merck-Schuchardt, Hohenbrunn, Germany) was added at a concentration of 0.2% to the sunscreen to visualize the substance distribution on the skin.

2.3 *Tape Stripping*

Tape strips were removed from the skin treated with the sunscreen using tesa film (number 5529, Beiersdorf, Hamburg, Germany). The tapes were pressed onto the skin using a roller and removed with one quick movement, as described previously.[15,16]

2.4 *Laser Scanning Microscopy*

The distribution of the sunscreen containing curcumin on the removed tape strips was investigated by fluorescence measurements (excitation wavelength 488 nm, detection of the fluorescence signal at 600 nm) using the LSM 2000 (Carl Zeiss, Jena, Germany).

The *in vivo* measurements were performed using a dermatological LSM system (Optiscan Limited, Melbourne, Australia), which can be applied directly to the skin.

2.5 *Application Protocol and Determination of the SPF*

The SPF determination was carried out in accordance with the COLIPA protocol[1] based on the ratio of the minimal erythema dose (MED) with and without sunscreen application. The MED was determined on the back of the volunteers using the sun simulator ETG 1 (Fa. A.L.T. Lichttherapietechnik GmbH, Zörbig, Germany). The device was equipped with a lamp TL 4 W/12 to 8.09 W/m^2 (Fa. Philips, Hamburg, Germany). The correlation of the UV spectrum to the sun spectrum of this lamp was evaluated by Meffert[17] and Piazena.[18] The spectral characteristics of the used UV lamp do not influence the SPF ratio if the same sunscreen is applied in different forms.[19] The sun simulator emits a parallel light beam. The time of irradiation was calculated on the basis of the skin phototype in accordance with the scale by Fitzpatrick.[14]

Erythema were determined 24 h after irradiation using the colorimeter Spectropen (Fa. Dr. Lange, Berlin, Germany). Then, the length of the erythema was measured. The MED was determined on the basis of this erythema length in dependence on the UV dose applied. The MED was determined for untreated skin on the back, and for skin after the direct application of the sunscreen, as well as for skin covered by optical cells containing the sunscreen incorporated in the emulsion or solved form.

Irradiation started 1 h after application of the formulations, to make sure that the sunscreen had achieved the maximal homogeneity of distribution on the skin. The measurements were realized with an experimental arrangement as illustrated in Fig. 1. Two different protocols were used.

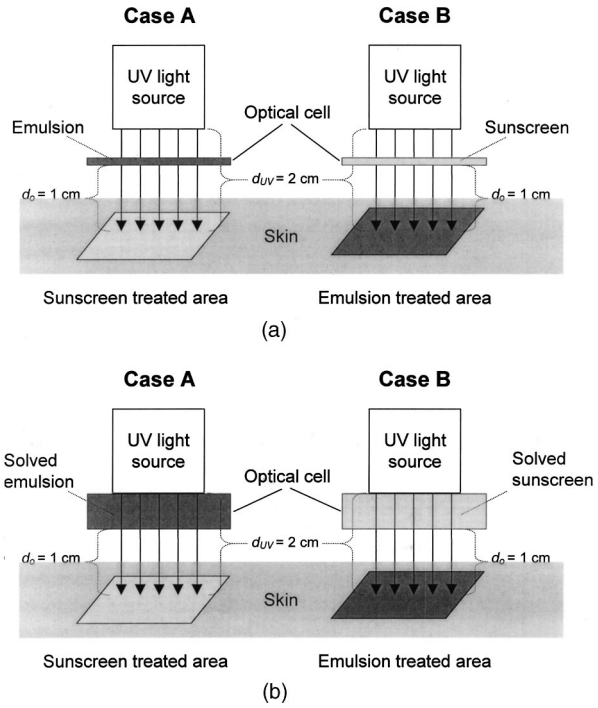

Fig. 1 Arrangement for the determination of the SPF. (a) Protocol 1: direct application, cell length $d=1$ mm. (b) Protocol 2: dissolved form, cell length $d=1$ cm.

The first protocol (protocol 1, 6 volunteers) was established to compare the sunscreen applied topically onto the skin, with the emulsioned form applied into the optical cell [Fig. 1(a)]. The optical cell consisted of two quartz plates with a distance holder in-between. Because it was difficult to distribute the small amount of 2 mg/cm^2 sunscreen homogeneously in the optical cell, the sunscreen was diluted with the corresponding emulsion in the ratio 1:1. Before the irradiation experiments started, ten samples of this mixture were analyzed using a spectrometer to ensure that the UV filter substances were distributed homogeneously in the mixture.

In case **A**, the sunscreen was applied directly onto the skin (2 mg/cm^2) and the optical cell was filled with the emulsion (4 mg/cm^2). Due to this amount, the thickness of the optical cell was 1 mm. The optical cell was positioned 1 cm above the skin surface. Case **B** was realized by applying the emulsion onto the skin (2 mg/cm^2) and filling the mixture of sunscreen and emulsion into the cell (4 mg/cm^2). In this way, identical optical parameters of the skin were obtained. The distance of the radiation source to the skin was 2 cm.

A second protocol (protocol 2, 6 volunteers) was established to compare the sunscreen applied topically onto the skin, with the solved one applied into the optical cell [Fig. 1(b)]. Therefore, eight quartz cells with a thickness of 10 mm (Type 100-QS, Fa. Hellma, Jena, Germany) were fitted together to form one large cell (total area: 4×8 cm^2) using a frame. In case **A**, this setup was used to determine the SPF obtained after the application of the sunscreen directly onto the skin (2 mg/cm^2) and filling the solved emulsion into the optical cell. The concentration of the solved emulsion was 2 mg/ml, which corresponds to 2 mg/cm^2. Due to this amount, the thickness of the optical cell was 1 cm. In case **B**, vice

Table 1 Overview on the application protocols for the SPF determination.

		Substance applied	
Protocol	Case	onto the skin	into the optical cell
1	**A**	Sunscreen (2 mg/cm^2)	Emulsion (4 mg/cm^2)
	B	Emulsion (2 mg/cm^2)	Sunscreen/emulsion mixture (1:1) (4 mg/cm^2)
2	**A**	Sunscreen (2 mg/cm^2)	Dissolved emulsion (2 mg/cm^2)
	B	Emulsion (2 mg/cm^2)	Dissolved sunscreen (2 mg/cm^2)

versa, the sunscreen was filled as a homogeneous solution into the optical cell (2 mg/cm^2), while the emulsion was applied onto the skin (2 mg/cm^2). The distance of the radiation source to the skin was 2 cm.

Thus, in both protocols, identical optical conditions were realized in cases **A** and **B**. Only the positions of the UV filter substances were different. The application protocols are summarized in Table 1. The substances were applied on the back of volunteers on an area of 8×10 cm^2, which was marked with a permanent marker. The formulations were applied with a syringe and distributed homogeneously with a saturated gloved finger. Formulations were rubbed in for approx. 30 s. Thereafter, the volunteers rested for 1 h without sweating and without covering the test area with textiles until the irradiation started. Cases **A** and **B** were always compared on the same volunteer.

2.6 *Statistics*

Statistical analysis was performed with the software program SPSS®. The Wilcoxon test was utilized to analyze the SPF obtained with protocols 1 or 2 depending on the case (**A** or **B**). The SPF of both groups of volunteers, differing in the protocol (case **A**, protocols 1 and 2), were compared using the Mann-Whitney test.

3 Results

3.1 *In Vivo Measurements Using Laser Scanning Microscopy*

The penetration kinetics of the o/w sunscreen emulsion containing a fluorescent dye (curcumin) were investigated on the same skin area of the forearm at different times after application. The fluorescence images of the stratum corneum of a volunteer, obtained 5 and 20 min after application, are presented in Fig. 2. The fluorescent dye was mainly located in the furrows and in the lipid layer around the first layer of the stratum corneum, 5 min after application. Later, it could also be detected around deeper corneocyte layers. After 20 min, four layers of the stratum corneum became visible. The shifted structure of the stratum corneum could be clearly distinguished.

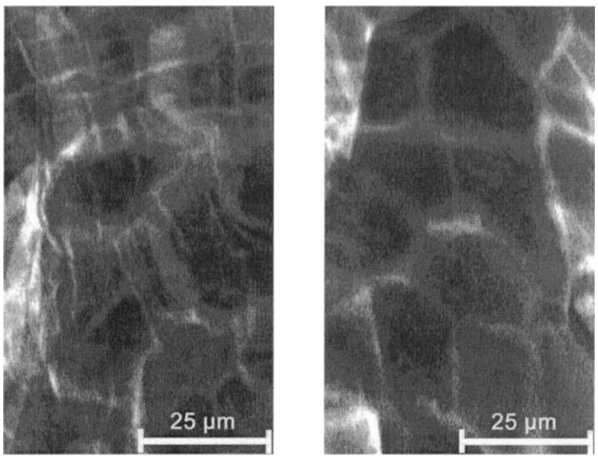

Fig. 2 Deeper cell layers of the stratum corneum become visible during penetration of the fluorescent dye into the horny layer (a) 5 min after application and (b) 20 min after application.

20 min after application, the distribution of the formulation within the skin was very nearly constant. Only slight changes were observed up to 45 min after dye application (data not shown). Therefore, the following measurements were carried out 1 h after the topical application of the sunscreen when the maximal homogeneity of the distribution of the sunscreen on the skin was certain.

3.2 *Comparison of In Vivo and In Vitro Measurements Using Laser Scanning Microscopy*

Tape strips were removed from the skin 1 h after treatment with the o/w sunscreen emulsion containing curcumin. The distribution of the dye on the tape strips was immediately analyzed by LSM measurements after removal. In addition, the distribution of the dye was determined *in vivo* to make sure that the *in vitro* measurements reflect the real distribution of substances on the skin. In Fig. 3, typical results of these measurements are shown. A similar nonhomogeneous distribution was observed on the tape strips and on the living skin. In both cases, the dye was mainly located in the lipid layers around the corneocytes. The highest amount was detected inside the furrows of the skin *in vivo* [Fig. 3(b)].

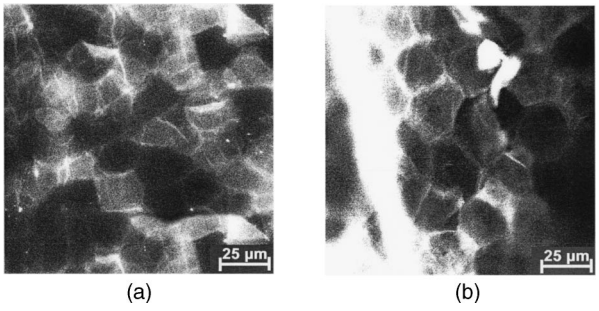

Fig. 3 Distribution of topically applied curcumin (white fluorescence signal) determined by laser scanning microscopy: (a) *in vitro,* measured on a removed tape strip and (b) *in vivo,* measured on the forearm.

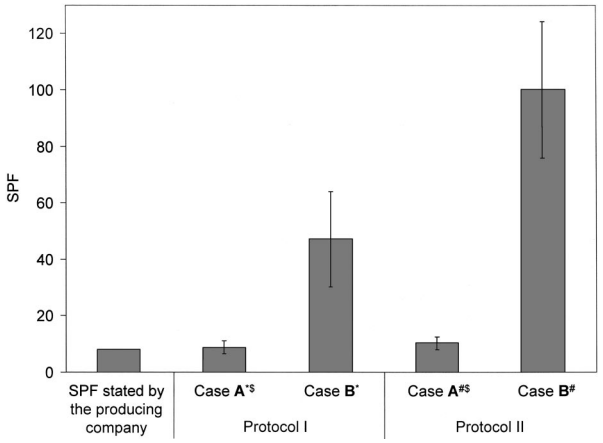

Fig. 4 Comparison of the SPF obtained with the different protocols [Protocol 1: sunscreen applied onto the skin (case **A**) and into the optical cell (case **B**); Protocol 2: sunscreen applied onto the skin (case **A**) and dissolved in a solvent into the optical cell (case **B**)]. $^{*}p=0.035$ (Wilcoxon test), $^{\#}p=0.035$ (Wilcoxon test), $^{\S}p=0.337$ (Whitney-Mann test).

3.3 *Measurements of the Sun Protection Factor*

In primary experiments, it was established that the solvents used in the experiments did not change the position and intensity of the characteristic absorption bands of the filter substances, in comparison to the original sunscreen. The results of the SPF determination are presented in Fig. 4 for cases **A** and **B** of both protocols, after direct application of the UV filters onto the skin and into the optical cell above the skin. The results were compared to the SPF, as declared by the company producing the sunscreen. The SPF of 8 was reproduced with both protocols (10.3 ± 2.2 and 8.8 ± 2.3, respectively) taking into consideration case **A** ($p>0.05$). Significantly higher SPF values were obtained in both protocols for case **B** ($p<0.05$ for both protocols).

In Table 2, the ratio of the SPF values measured for cases **A** and **B** following identical protocols is summarized. A drastic increase in the SPF, by a factor of approximately 5, was observed when the emulsion was located inside the optical cell above the skin surface. This difference was increased to 10 when the optical cell was filled with the solved sunscreen emulsion.

4 Discussion

4.1 *Nonhomogeneity of Distribution*

Both *in vivo* as well as *in vitro*, the dye applied in a sunscreen was mainly located around the corneocytes and inside the furrows (see Fig. 3). These results are in agreement with the reported observations, which illustrate that topically applied substances were inhomogeneously distributed on the skin *in vivo*[9,18] and *in vitro*.[20] *In vivo* measurements using laser scanning microscopy demonstrated that the sunscreen penetrated into the upper part of the stratum corneum. After 20 min it was located around the upper layers of the corneocytes without further changes of the distribution in the skin.

These results demonstrate that the sunscreen should be applied approximately 20 min earlier onto the skin before going into the sun, as recommended by most of the producers. Only in this case can maximum sun protection of the formulation be obtained. Therefore, the investigations in the present study were started 1 h after application of the sunscreen, thus the maximum protection efficacy of the sunscreen was achieved.

In addition, it has been shown that the distribution of the sunscreen on the removed tape strips reflects the real (actual) situation on living skin (see Fig. 3). This effect can be used for the *in vitro* determination of absorption properties of sunscreens applied topically when taking the actual distribution on the skin into consideration. An application of this effect for SPF determination will be an object of further investigation. This method may be more precise than model calculations, if an artificial nonhomogeneous distribution of sunscreens is given to simulate the skin surface structure.[6,21]

It is well-known in spectroscopy that a disturbance of the homogeneous distribution reduces the intensity of the absorption. Indeed, an influence of the *in vivo* distribution of the UV filter substances on their protection properties has been reported.[8,9] These results were quantitatively tested *in vivo* by SPF measurements in the present study.

4.2 *Measurements of the Sun Protection Factor*

The results given in Fig. 4 and Table 2 describe the difference of the SPF up to 1 order of magnitude, depending on the distribution of the UV filters. In these experiments, only the homogeneity of the distribution of the identical concentrations of UV filter substances per cm^2 varied. All other experimental conditions used for the SPF determination were constant, even the changes in the optical properties of the skin after application of an emulsion as reported by Tuchin et al.[10] and Lademann et al.[11] This can be realized only by *in vivo* measurements using the erythema in both experiments as an indicator.

The SPF increased by 1 order of magnitude if the sunscreen was solved in chloroform/methanol and put into the optical cell above the skin (protocol 2). These significant changes were caused by the nonhomogeneous distribution of the filter substances on the human skin after topical application, compared to the homogeneous distribution in the optical cells.

The results obtained using protocol 1 demonstrate that the UV filter substances were not only distributed on the skin nonhomogeneously, but also to a much lower extent in the sunscreen sample used. The ratio of the SPF (case **B**) to the SPF (case **A**) given in Table 2 is decreased to 5.3, reflecting a lower degree of homogeneity of the sunscreen in the emulsion in comparison to the solution.

This means that in normal use, the nonhomogeneous distribution of the UV filter substances within the stratum corneum, after application of a sunscreen, reduced the protection potential by a factor of 10 in comparison to the optimum.

Table 2 Relation of the SPF determined in cases **A** and **B** with the different protocols.

Protocol	Relation SPF (case **B**)/SPF (case **A**)
1	5.3 ± 1.2
2	10.9 ± 1.3

These results quantitatively confirm the findings of Brown,[9] O'Neill,[6] and Sayre et al.,[8] who found less SPF values *in vivo* than in *in vitro* experiments. The slight difference between the measured SPF (case **A**, both protocols) and the declared SPF might be the result of a higher degree of homogeneity in distribution achieved during the 1 h after application compared to the 15 min as recommended by the COLIPA.[1] The COLIPA protocol[1] is usually used by companies to determine the SPF of commercial products.

The *in vivo* method, described herein, allows the qualitative and quantitative determination of the influence of the nonhomogeneity of the distribution of a sunscreen onto the SPF for defined formulations. Therefore, it is a future challenge for pharmaceutical and cosmetic research to increase the SPF of a sunscreen by optimizing the formulation in terms of homogeneity to significantly increase the efficacy of sunscreens after topical application.

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