Comparison of human and porcine skin for characterization of sunscreens

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Abstract. The universal sun protection factor (USPF) characterizing sunscreen efficacy based on spectroscopically determined data, which were obtained using the tape stripping procedure. The USPF takes into account the complete ultraviolet (UV) spectral range in contrast to the classical sun protection factor (SPF). Until now, the USPF determination has been evaluated only in human skin. However, investigating new filters not yet licensed excludes *in vivo* investigation on human skin but requires the utilization of a suitable skin model. The penetration behavior and the protection efficacy of 10 commercial sunscreens characterized by USPF were investigated, comparing human and porcine skin. The penetration behavior found for typical UV filter substances is nearly identical for both skin types. The comparison of the USPF obtained for human and porcine skin results in a linear relation between both USPF values with a correlation factor $R^2=0.98$. The results demonstrate the possibility for the use of porcine skin to determine the protection efficacy of sunscreens.
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1 Introduction

Recently, a new spectroscopic method was described investigating removed tape strips taken from human skin after topical application of sunscreens with ultraviolet (UV)/visible (VIS) spectroscopy. As a result of these investigations, the universal sun protection factor (USPF) was proposed.[1] This factor quantifies the protective ability of sunscreens in the entire UV spectral range noninvasively. The applicability of the described protocol was investigated on human skin under *in vivo* conditions. Therefore, it is limited to sunscreen formulations approved for application on human skin. During the search for and investigation of new types of filters, at an early stage of development and not yet licensed, an analysis of the protection efficacy of these sunscreens on model tissue would be helpful. In the present study, porcine skin was tested as a model system for the assessment of the sunscreen efficacy.

Different studies, including general considerations as well as the discussion of special methods, demonstrate that the porcine skin is well suited as a model for human skin. A current overview on dermal absorption is given in a guidance document of the European Commission (EC).[2] In addition to the experimental conditions and properties of the active substance, the interspecies differences are well documented. In this context, porcine ear is described as a well-suited subject for skin investigations.

A detailed review,[3] summarizing the results found over decades, describes a lot of similarities between porcine and human skin in different fields but also some dissimilarity. Anatomical, physiological, and biochemical similarities are cited, and various uses of the pig as a model for humans in the investigation of skin permeation are reviewed. Nevertheless, the necessity to verify each new system is emphasized.

Special investigations concern, e.g., tape stripping in combination with measurements of the trans epidermal water loss (TEWL) and impedance spectroscopy,[4] light microscopy, transmission electron microscopy, and cryoscanning electron microscopy:[5] studies with diffusion cells,[6–8] penetration studies applying laser scanning microscopy and biopsies[9] as well as the comparison of stratum corneum (SC) thickness, diameters of hair, and infundibula.[9]

In the present study, we investigated to what extent porcine skin can be used to assess the efficacy of sunscreens by the newly proposed universal sun protection factor (USPF).[1,10–12] Preparing these measurements, the penetration profiles of UV filter substances into the *stratum corneum* was determined comparing both skin types, because the USPF determination is based on the analysis of the penetration and distribution of sunscreens in the skin.

The USPF value characterizes the sunscreen efficacy objectively in the entire UV range. This is highly important, because on the one hand, sun irradiation is essential for hu-

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man life,[13] but on the other hand, at high dosages, it can be dangerous to human health.[14] It is well known that UVB (320 to 400 nm) and UVA (280 to 320 nm) irradiation can produce sunburn,[15] immunosuppression,[15] an enhanced free radical formation,[16,17] and the appearance of skin cancer,[14] while skin aging is caused mainly by UVA light.[18]

A high number of different commercial sunscreens are available to protect the human skin from these damages. Up to now, they have been characterized by the traditional sun protection factor (SPF). The SPF is the relation between the minimal erythema dose of the untreated and sunscreen-protected skin.[19,20] In principle, the values describe the length of time individuals can stay in the sun after sunscreen application, in relation to the unprotected skin. The correlation of the SPF to one special biological effect, erythema formation, has however the disadvantage that it is related only to the UVB spectral range. The SPF contains no information about UVA, which also causes damage to the skin including skin cancer. Therefore, the European Commission (EC) and the American Food and Drug Administration (FDA) require the development of noninvasive objective methods for the determination of the sun protection efficacy in the whole UV spectral range.[20]

The most efficient and simplest method to quantify sunscreen efficacy seems to be the direct analysis of the samples in cells by spectroscopic measurements. Unfortunately, this would not reflect the nonhomogeneous distribution of the UV filter on the human skin, due to the fact that the distribution of sunscreens in solution is homogeneous. The use of PMMA plates with a specially structured surface is one possibility to overcome this problem. This technique has already been applied in different investigations.[21,22]

The USPF represents a newly developed *ex vivo* method, which combines *in vivo* tape stripping with *in vitro* spectroscopic measurements. In this case, the real UV filter distribution determined by the skin structure is transferred undisturbed to the tape strips, which are analyzed spectroscopically.[23] The obtained data contain information about the real absorption properties of the sunscreens under *in vivo* conditions in the UVA and the UVB ranges. These are the basis to calculate a sum transmission, used for the definition of a universal sun protection factor (USPF).[1,10] This value describes the protection properties of sunscreens, not only in the entire UV but also in the visible and infrared (IR) spectral ranges. This is of particular importance, as recently it could be demonstrated that visible and IR irradiation of the sun can also cause the formation of free radicals and skin damage.[24] Furthermore, the USPF is a measuring value, taking into account the intensity and energy of photons reaching the living cells of the skin, independent of any biological response of the human body.

2 Materials and Methods

Different sunscreens were applied both on human skin *in vivo* and on porcine skin *in vitro*. After a penetration time of 1 h, tape stripping was performed. The removed tape strips were analyzed immediately by spectroscopic measurements, in order to determine the amount of removed corneocytes and the absorption of the UV filter substances in the different depths of the *stratum corneum*. These data were used to generate penetration profiles and to calculate the USPF, comparing human and porcine skin.

2.1 *Volunteers*

The study was carried out testing each sunscreen on the flexor forearm of six healthy volunteers, aged between 23 and 45 years (skin photo-types[25] I to III), at the Charité—Universitaetsmedizin Berlin, Department of Dermatology, Berlin, Germany. Ethical approval for these experiments had been obtained from the Ethics Committees of the Charité Hospital.

2.2 *Porcine Skin*

The ears of freshly slaughtered pigs (German domestic pigs, 6 months old) were obtained from the local butcher. Permission had been obtained from the Supervisory Office for Veterinary Medicine, Berlin, Germany. Each sunscreen was investigated using six different porcine ears.

2.3 *Sunscreen Application*

The investigations were performed, using the following sunscreens:

- COLIPA Low SPF Standard DIN Standard K17N SPF 4.4 (BASF, Ludwigshafen, Germany)
- Nivea Sun Feuchtigkeits-Sonnenmilch SPF 8, SPF 12, SPF 30, and SPF 50 (Beiersdorf AG, Hamburg, Germany)
- COLIPA High SPF Standard SPF 15.5 (BASF, Ludwigshafen, Germany)
- NIVEA Sonnen-Milch SPF 8, and SPF 20 (Beiersdorf AG, Hamburg, Germany)
- Protection Body Cream Lancaster SPF 30 (Lancaster, Monaco)
- Anthélios S SPF 40 (La Roche-Posay, France)

After cleaning the skin of the human flexor forearm with cold water and drying with a paper towel, an area of 8 $\times 10$ cm^2 was marked and 2 mg/cm^2 of the formulation was applied. The porcine ears were fixed on a polystyrene support covered with aluminum foil and washed with water and dried with paper towels. The hairs of the ears were carefully removed by scissors to avoid later disturbances in the nonhomogeneous distribution of UV filter on the removed tape strips. 2 mg/cm^2 of the sunscreens were applied to the ear on marked areas of 40 cm^2.

2.4 *Tape Stripping*

The tape stripping commenced one hour after sunscreen application and was carried out as described previously.[26] The adhesive tapes (*tesa* film, no. 5529, Beiersdorf, Hamburg, Germany; width: 19 mm) were pressed onto the human skin by a stamp (pressure: 15 kp/cm^2) and quickly removed with one movement.

In both protocols, 10 tapes were taken from each skin area investigated, and the absorption spectra were recorded immediately. Ten tapes were also removed from an untreated skin area for the correction of the absorption spectra obtained from the treated skin, as will be described in the following paragraph.

2.5 *Determination of the Sum Transmission*

After removal from the skin, each tape strip was fixed onto a metal support and analyzed in a spectrometer, together with an empty tape as reference. The measurements to calculate the sum transmission were performed immediately after removal of the tape strips (within 15 seconds), in order to neglect the diffusion of the UV filter into the tape.[23] The absorption spectra of the sunscreens were recorded between 240 and 500 nm using the UV/VIS spectrometer Lambda 5 (PerkinElmer, Frankfurt/Main, Germany) with an integrating sphere and a quadratic beam diameter of 8×10 mm^2.

The software UV Winlab Version 2.70.01 (PerkinElmer, Frankfurt/Main, Germany) was used to calculate the essential spectroscopic data. The spectra of the treated skin were corrected by subtracting the spectra obtained for the untreated skin, in order to suppress the influence of the absorption of the corneocyte aggregates and the proteins.

2.6 *Determination of the Penetration Profile*

The determination of the penetration profiles has been described previously.[23] For this procedure, two points of information are necessary: the amount of corneocytes removed with each tape strip and the corresponding amount of sunscreen on the single removed tape strip.

2.6.1 *Determination of the stratum corneum amount*

The pseudo-absorption of the corneocytes[26] based on the absorption, scattering, and reflection of the corneocytes is the basis for the quantification of the amount of *stratum corneum* fixed on the individual tape strips. Former investigations ensured that these spectroscopic data correlate to the mass of the horny layer particles, both of human skin[23,26] as well as of porcine skin,[27] with a correlation coefficient $\geqslant$0.95 up to 0.99. In addition, a clear relation was found between the pseudo-absorption and two protein-specific absorptions (the original absorption at 278 nm and the absorption after staining at 652 nm),[28] confirming that the values used are well suited to quantify the *stratum corneum* removed by tape stripping.

The pseudo-absorption of the corneocytes must be measured at a wavelength not influenced by absorbing substances in the investigated systems. For the sunscreens, this was guaranteed at 430 nm. The measurements were carried out using the linear UV/VIS spectrometer Lambda 20 (PerkinElmer, Frankfurt/Main, Germany) with a quadratic beam diameter of 8×10 mm^2. The obtained data were used to calculate the relative position inside the horny layer (left axes in Figs. 1 and 2) from where the individual tape strips had been removed.

2.6.2 *Determination of the substance concentration*

The tape strips taken from the sunscreen-treated areas were cut to a constant size of 1.45×2.8 cm^2, using a template. Each tape was placed in a test tube filled with 4.06 ml ethanol (1 ml/cm^2). The tubes were then treated for 10 min in an ultrasonic bath (Sonorex, Super RK102H, Bandelin Electronic, Berlin, Germany). Solutions were purified by centrifugation for 10 min (Centrifuge MR 1812, Jaouan GmbH, Unterfaching, Germany). Spectra of solutions were recorded between 240 and 600 nm. The concentration of the substance

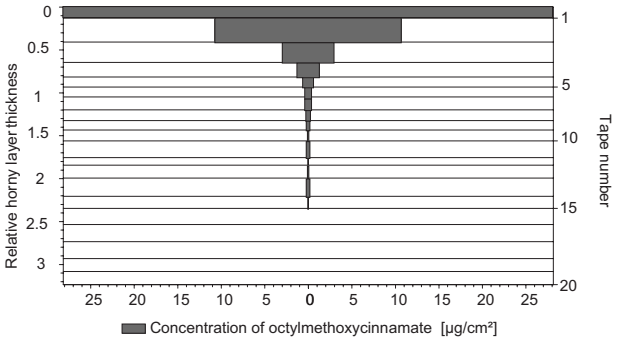

Fig. 1 Penetration profile of the UV filter octylmethoxycinnamate (Nivea Sun Feuchtigkeits-Sonnenmilch SPF 8) applied to human skin.

on each tape strip was determined by its maximum absorbance using a calibration curve according to the Beer-Lambert rule. The penetration profile was calculated by correlating the amount of the UV filter substances to the depth in the *stratum corneum*, from where the corresponding tape strips were removed.

2.7 *Sum Transmission Spectra and Average Sum Transmission Values*

In Figs. 3 and 4, the determination of these spectra is demonstrated using individual measurements of two different sunscreens for volunteers and for porcine skin.

The uppermost spectrum in each diagram represents the situation of the first tape. The following spectra were calculated cumulatively by adding the values of all foregoing tape strips up to each individual tape strip. The lowest spectrum corresponds to the sum of the spectra of the tape strips 1 to 8 or 10 depending on the individual penetration profile.

The area under the last curve corresponds to the relative amount of UV photons reaching the deeper parts of the skin after sunscreen application. Dividing this area by 120—the length of the UV range (280 nm to 400 nm)—results in an average sum transmission value. This mean value characterizes the relative remaining average intensity of photons in the complete UV range after sunscreen application.

The discussion of the limitations of the described spectroscopic method comparing human and porcine skin needs to

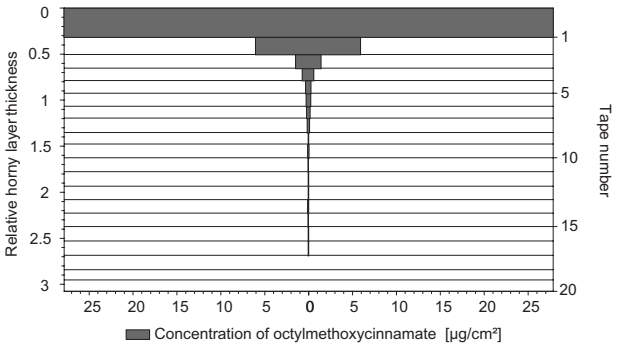

Fig. 2 Penetration profile of the UV filter octylmethoxycinnamate (Nivea Sun Feuchtigkeits-Sonnenmilch SPF 8) applied to porcine ear skin.

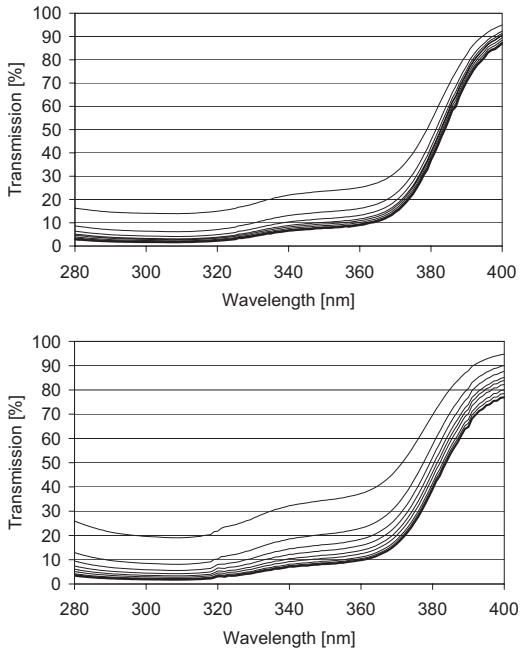

Fig. 3 Comparison of the sum transmission curves of the sunscreen Nivea Sun Feuchtigkeits-Sonnenmilch SPF 8 obtained for *ex vivo* measurements on human skin (upper diagram) and for *ex vitro* measurements on porcine ear skin (lower diagram).

consider the different parameters influencing the measured values. There are two method-correlated parameters:

• The total amount of applied sunscreens located inside the investigated skin area is not absolutely constant. Therefore, small differences of the sum transmission may emerge. The sum absorbance measured after extraction of all removed tape strips reflects the amount of sunscreen removed from the investigated skin area allowing the correction of existing differences if necessary.

• The influences of the tape stripping process and the spectroscopic measurement were controlled by measuring samples taken from adjacent skin areas. The standard deviation was found at 5.7% (Ref. 1).

Another parameter influencing the individually measured values is a sample-correlated one. As described previously, the experimental results[10] clearly demonstrate that the skin profile has a significant influence, resulting in a variation of the measured data.

An adjustment of these statistical parameters is reached by calculating the average values of the discussed data. Usually, the measurement of six individual samples is sufficient to adjust the existing differences. These parameters influence the results obtained with both skin types in the same extent.

2.8 *Universal Sun Protection Factor*

In order to obtain a measured value with the same information as the classical SPF, a universal sun protection factor was proposed earlier.[1] A lowering of the incident irradiation intensity, for example, to 10%—described by the average sum transmission—lengthens the time to be exposed to sunlight without danger by a factor of 10. Dividing 100 by the average sum transmission value, the universal sun protection factor is determined (USPF=100/average sum transmission). This value is independent of any biological response after UV irradiation and considers the UVB as well as the UVA range.

3 Results and Discussion

3.1 *Penetration Profiles*

Penetration profiles were determined for all investigated sunscreens after application on human and porcine skin. In Figs. 1 and 2, two typical examples of penetration profiles of one typical sunscreen (Nivea Sun Feuchtigkeits-Sonnenmilch SPF 8 with the UV filter octylmethoxycinnamate) obtained on human and porcine skin are shown. Similar penetration profiles were obtained for the other investigated sunscreens. In all cases, the sunscreens were located in the upper layers of the stratum corneum, which is in agreement with findings reported in the literature for human skin.[1,26] Up to 10 tape strips were sufficient to remove the UV filter completely. The differences between human skin under *in vivo* conditions and porcine skin *in vitro* are not significant. There is a tendency to a slightly deeper penetration in some porcine skin samples, which can be explained by the larger orifices of the hair follicles.

The determination of the penetration profiles was a necessary step to obtain information about the extent to which porcine tissue is comparable with human skin when assessing the efficacy of sunscreens. No significant differences for the profiles obtained for both skin types were observed. This was the first requisite to take porcine skin as a model for human skin, quantifying the protective ability of not yet licensed sunscreens by spectroscopic data.

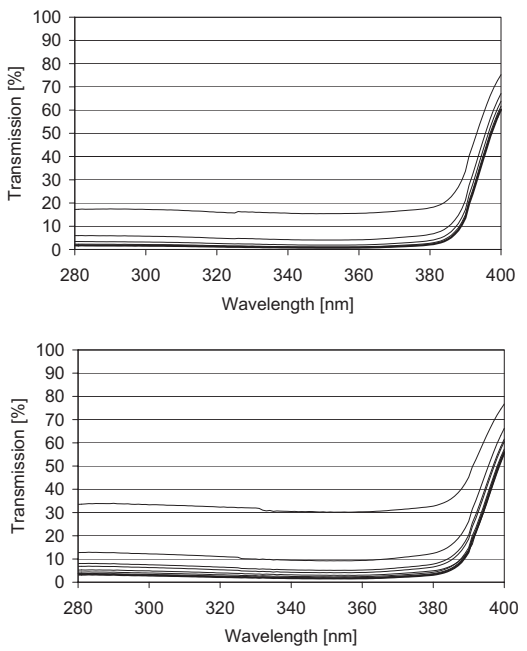

Fig. 4 Comparison of the sum transmission curves of the sunscreen Nivea Feuchtigkeits Sonnen-milch SPF 20 obtained for *ex vivo* measurements on human skin (upper diagram) and for *ex vitro* measurements on porcine ear skin (lower diagram).

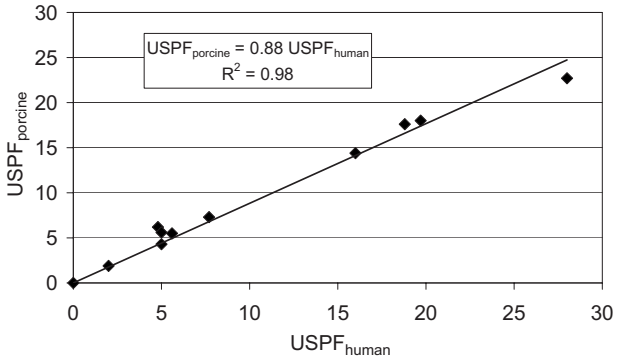

Fig. 5 Comparison of the $USPF_{porcine}$ determined *in vitro* from porcine ear skin and $USPF_{human}$ determined *ex vivo* from human skin.

3.2 *Sum Transmission Spectra and Average Sum Transmission*

The average sum transmission calculated on the basis of the sum transmission spectra quantifies objectively the protection efficacy of the applied sunscreens in the investigated systems. In Figs. 3 and 4, the sum transmission spectra are given for the sunscreens Nivea Sun Feuchtigkeits-Sonnenmilch SPF 8 and Nivea Sun Feuchtigkeits-Sonnenmilch SPF 20. The removed tape strips contain the complete amount of UV filter applied with each sunscreen. This allows quantifying the remaining radiation intensity after sunscreen application by the area under the last curve.

The typical examples of the sum transmission spectra obtained on human and porcine skin for two sunscreens show in an exemplary manner that no differences between the spectra of both skin types exist. This was the second requisite to demonstrate that porcine skin is a good tissue model to characterize sunscreen products by spectroscopic measurements.

3.3 *Universal Sun Protection Factor*

The obtained spectroscopic data are well suited to calculate universal sun protection factors also on the basis of porcine skin. The USPF values were determined for each sunscreen on several porcine and human skin samples. The average values were determined for each sunscreen and both types of tissue. In Fig. 5, these USPF values obtained *ex vivo* on human skin and *in vitro* on porcine ear skin are compared for each sunscreen investigated. A total of 60 pig ear samples and 60 volunteers were analyzed. A good correlation with $R^2 = 0.98$ was obtained between the USPF values determined on both tissue systems. The result clearly demonstrates that the described way to adjust the effects of the discussed parameters influencing the individual measurements is well suited to obtain data of sufficient accuracy. The relation of the $USPF_{porcine}$ and $USPF_{human}$ is described by the linear function:

$$USPF_{porcine} = 0.88 USPF_{human}. \quad (1)$$

The gradient of the line describing the correlation is 0.88, which means that the USPF values on human skin are only slightly higher than the USPF values determined on porcine skin.

These relatively small differences obtained with the applied protocol are understandable, considering the parameters influencing the measured spectroscopic data. On the one hand, the porcine skin has broader furrows than human skin, resulting in a higher homogeneous distribution of the UV filter on the skin and with a higher protective ability ($USPF_{porcine} > USPF_{human}$).

On the other hand, the covering of corneocytes on the tape strips taken from porcine skin is lower, in comparison to human skin. This effect enhances the measured transmission, resulting in lower protection ability ($USPF_{porcine} < USPF_{human}$).

Both effects have an opposite influence on the USPF values, so that they partly compensate each other.

The results of the study confirm that in the considered field, in agreement with the general situation described previously,[2,3] porcine ears are best suited as model skin for the determination of the protection behavior of sunscreens applying the given protocol. This is of high importance for the investigation of newly developed UV filter substances, which are not licensed and, therefore, could not be evaluated because of customer safety aspects. The determination of the USPF allows determining their efficacy, as well as their behavior in different formulations during the process of the development and optimization of sunscreens. This possibility would increase the cost-effectiveness of the development and optimization process.

Assessing the obtained results, it must be taken into consideration that the USPF can be determined without irradiation of the skin by UV light, i.e., noninvasively, without damaging the human skin. In contrast to the traditional SPF, this allows the characterization of the protection efficacy of the sunscreen in the complete spectral ranges, UVB and UVA. It can even be applied for the determination of the protection properties of formulations in the visible and infrared parts of the spectrum. This is of special interest, because recently it was demonstrated that in these spectral ranges also, significant amounts of radicals were formed by sun irradiation.[15] This means that the USPF method fulfils all the requirements of the European Commission (EC) and the FDA concerning the noninvasive protection assessment of sunscreens in the whole spectral range of sun irradiation, enlarged by the possibility to apply porcine skin for these investigations.

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