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Abstract. Sun protection factor (SPF) frequently differs between sunscreens containing the same composition of ultraviolet (UV) filters that primarily define sunscreen efficacy. We tested the hypothesis that the thickness frequency distribution of the sunscreen film is also responsible for and can explain the divergence in the measured SPF. For this, we developed a method to measure film thickness from the difference of topography before and after application of 2 mg/cm² of sunscreen on pig ear epidermal membrane. The influence of five vehicle formulations and of application pressure and spreading time on mean thickness (S_{mean}), S_{mean} to median ratio, and SPF *in vitro* was investigated. The vehicle had a significant impact, low vehicle viscosity resulting in a smaller S_{mean}, larger S_{mean} to median ratio, and lower SPF *in vitro* than high viscosity; continuous oil phase produced the largest S_{mean} and SPF values. A long spreading time reduced S_{mean} and SPF and increased application pressure reduced SPF. There was a positive correlation between S_{mean} and SPF *in vitro*, underlining the relevance of film thickness for interpreting UV protection differences of formulations with the same filter composition. This work demonstrated a strong influence of vehicle and application conditions on sunscreen efficacy arising from differences in film thickness distribution. © *2014 Society of Photo-Optical Instrumentation Engineers (SPIE)* [DOI: 10.1117/1.JBO.19.11.115005]

Keywords: sun protection factor *in vitro*; film thickness; film homogeneity; pig ear skin; vehicle; sunscreen application.

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1 Introduction

Topically applied sunscreens constitute a suitable and commonly employed measure to protect skin from sun damages.[1,2] Efficacy of sunscreens in terms of sun protection factor (SPF), ultraviolet A (UVA) protection, photostability, and balanced absorbance depends primarily on the intrinsic absorbance and photostability properties of ultraviolet (UV) filters contained in the product in conjunction with the used concentration.[3,4] The ideal sunscreen achieves balanced protection by equally attenuating ultraviolet B (UVB) and UVA radiations, similar to the protection afforded by clothing and shade.[5,6] Therefore, an appropriate UV filter system should combine UVB and UVA filters to achieve an optimized UV shield.[7] Reasonably, the amount of product applied also affects protection.[8–12] However, SPF frequently differs between sunscreens with different vehicle formulations containing the same filter composition,[3,13] yet the cause of this difference has not been investigated. Also, *in vitro* interlaboratory trials with the same sunscreen have produced variable results[14] and the application procedure was further found to influence the measured SPF.[15,16] In addition to the absorbing property of the UV filters and the amount of applied product, the homogeneity of distribution of the sunscreen was found to play an important role with respect to SPF *in vivo*.[17] The ideal situation for optimal performance is to achieve a film with uniform thickness, resembling the perfectly homogeneous distribution of a solution of UV filters in an optical cell. Understandably, this condition can never be reached under *in vivo* condition of application due to the skin surface topography. Skin relief shows ridges and furrows that preclude the formation of an even sunscreen film.[18] In addition, manual application makes it practically impossible to achieve a uniform film. This irregularity of the film thickness is probably a cause of the reported experimental variability of SPF and was suggested to be responsible for the divergence of orders of magnitude between predictions based on UV transmission of dilute transparent filter solutions and clinical study results.[19]

The aim of the present work was to understand the relationship between film thickness frequency distribution and efficacy of sunscreens. To this end, we developed a method for determining the precise thickness distribution of the applied sunscreen film based on topographical measurements with high spatial resolution. We used the epidermal membrane of pig ear skin as a biological substrate for *in vitro* sunscreen application as we recently showed that using this substrate for SPF *in vitro* testing provided better prediction of SPF *in vivo* than conventionally used synthetic substrates. Substrate-to-product affinity rather than topography was discussed to be responsible for this better prediction of SPF *in vivo*.[20] The skin of pig ear has also been used for *in vitro* assessment of UV-induced damages on DNA,[21] UV filter penetration,[22,23] and sunscreen photostability tests.[24] Using the developed film assessment method we investigated the sunscreen film residue in terms of thickness and homogeneity of distribution for five sunscreen vehicles and different application conditions. In parallel, we measured SPF *in vitro* on the same preparations to determine UV protection efficacy. The

impact of vehicles with the same UV filter combination and of the application conditions on film parameters and SPF *in vitro* as well as the correlation between film parameters and SPF *in vitro* was then assessed. Identification of formulation and application related factors that may impact film characteristics and UV protection was a further goal of the present work. This is put forth as a fundamental aspect for understanding the mechanism of sunscreen efficacy.

2 Materials and Methods

2.1 Chemicals and Equipment

The following reagents were used: potassium carbonate from Sigma-Aldrich, St. Gallen, Switzerland; Tinosorb S abbreviated BEMT (INCI, bis-ethylhexyloxyphenol methoxyphenyl triazine), Uvinul N539T abbreviated OCR (INCI, octocrylene), Salcare SC 91, Cetiol AB, Lanette O, Dehymuls LE, Edeta BD, all from BASF SE, Ludwigshafen, Germany; Eusolex 232 abbreviated PBSA (INCI, phenylbenzimidazol sulfonic acid) from Merck, Darmstadt, Germany; Parsol 1789 abbreviated BMDBM (INCI, butyl methoxydibenzoylmethane), Amphisol K from DSM, Kaiseraugst, Switzerland; Neo Heliopan OS abbreviated EHS (INCI, ethylhexyl salicylate) from Symrise, Holzminden, Germany; Arlacel 165 from Croda, East Yorkshire, England; Keltrol RD from CP Kelco, Atlanta, Georgia; Carbopol Ultrez 10, Carbopol Ultrez 21 from Lubrizol, Brussels, Belgium; Tegin OV from Evonik Industries, Essen, Germany; Paracera M from Paramelt, Heerhugowaard, The Netherlands; beeswax white from Koster Keunen, Bladel, The Netherlands; glycerin from Sigma-Aldrich; tris amino ultrapure from Angus, Buffalo Grove, Illinois; Phenonip from Clariant, Muttenz, Switzerland.

Quartz plates with a size of 4.2×4.2 cm^2 were obtained from Hellma Analytics, Zumikon, Switzerland.

The following equipment was used: electric shaver (Favorita II GT104, Aesculap, Germany), epilator (Silk-épil7 Xpressive Pro, Braun, Germany); water purification device (Arium 61215, Sartorius, Goettingen, Germany); precision balances (XS105 Dual range and XA3001S, Mettler-Toledo, Columbus, Ohio); surface metrology instrument (Altisurf 500, Altimet, Thonon-les-Bains, France); UV transmittance analyzer (Labsphere UV-2000S, Labsphere Inc., North Sutton, New Hampshire).

The following software packages were used: BalanceLink (Mettler Toledo, Columbus, Ohio) with balance XA3001S for the recording of pressure during spreading of sunscreen; Phenix and Altimap (Altimet, France) for topographical measurement and evaluation, respectively; UV-2000 (Labsphere Inc.) for UV transmittance measurement; Statgraphics centurion XVI software (Statpoint Technologies, Inc., Warrenton, Virginia) for statistical evaluation.

2.2 Preparation of Skin Substrate

We used the epidermal membrane of pig ears as a biological substrate for sunscreen application as described before.[20] Ears of freshly slaughtered pigs were obtained from the local slaughterhouse (Basel, Switzerland) not more than a few hours postmortem. The study did not require the approval of the ethics committee of animal research as the ears were taken from pigs not specifically slaughtered for the purpose of this study. The epidermal membrane was isolated using a heat separation procedure. The full skin was immersed in a water-bath at 60°C for

90 s. The epidermal membrane was separated from the dermis by gentle peeling off, cut to a dimension of 2×2 cm^2, laid flat on quartz carrier plates, and stored at 4°C in a desiccator over saturated potassium carbonate solution until use.

2.3 Characterization of Sunscreen Formulations

We assessed SPF *in vitro* and the film thickness distribution of five different sunscreens. The formulations included an oil-in-water cream (OW-C), an oil-in-water spray (OW-S), a water-in-oil emulsion (WO), a gel (GEL), and a clear lipo-alcoholic spray (CAS). They contained the same UV filter combination and emollient. The filter system was composed of 8 wt. % OCR, 5 wt. % EHS, 2 wt. % BMDBM, 1 wt. % BEMT, and 1 wt. % PBSA. Based on this UV filter composition, an SPF *in silico* of 25 was calculated with the BASF sunscreen simulator.[25] The detailed composition of the sunscreens and their respective SPF *in vivo* values are given in Table 1. SPF *in vivo* values were measured in accordance with ISO24444:2010 guidelines.[26]

The sunscreens showed different rheological characteristics (Fig. 1). GEL had the highest shear viscosity followed by OW-C and WO, whereas OW-S and CAS were much less viscous. Viscosity of all sunscreens decreased with increasing shear rate whereas hysteresis depended on the formulation.

2.4 Application of Sunscreen

We applied 2.0 mg/cm^2 of sunscreen nominally corresponding to a film thickness of 20 μm. The sunscreen was applied in form of 20 to 30 small drops evenly distributed over the skin surface and manually spread with the fingertip using a presaturated finger coat. Two spreading procedures were employed. In the first, the sunscreen was spread on the specimen with light circular movements followed by left-to-right linear strokes from top to bottom starting at each side of the specimen (designated spreading 1); in the second, the complete linear stroke step was repeated four times (designated spreading 2). Spreading procedure 2 resulted in a longer application time. Furthermore, the pressure used to distribute the product was varied for spreading 1 between low and high, corresponding to a force of 100 ± 14 and 281 ± 35 g, respectively. These values represent extremes used in the authors' laboratory with this substrate preparation. The two pressure and spreading conditions were used solely with the gel formulation (GEL). All other sunscreen formulations were applied with high pressure and spreading procedure 1.

2.5 Measurement of the SPF In Vitro Using Spectral Transmission of Ultraviolet Radiation

Measurement of SPF *in vitro* is based on diffuse UV transmission spectroscopy according to the approach proposed by Sayre et al.,[27]

$$\text{SPF}_{in\ vitro} = \frac{\sum_{290\ nm}^{400\ nm} S_{er}(\lambda).S_s(\lambda)}{\sum_{290\ nm}^{400\ nm} S_{er}(\lambda)S_s(\lambda).T(\lambda)}, \quad (1)$$

where $S_{er}(\lambda)$ is the erythema action spectrum[26] as a function of wavelength λ, $S_s(\lambda)$ is the spectral irradiance of the UV source at wavelength λ,[28] and $T(\lambda)$ is the measured transmittance of the light through a sunscreen film applied on a suitable UV transparent substrate at wavelength λ.

For SPF determination, the spectral UV transmittance was registered from 290 to 400 nm in 1-nm increment steps through

Table 1 Composition (wt. %) and sun protection factor (SPF) *in vivo* of investigated sunscreens.

Sunscreen designation		Oil-in-water cream (OW-C)	Oil-in-water spray (OW-S)	GEL	Water-in-oil emulsion (WO)	Clear lipo-alcoholic spray (CAS)
[a]SPF *in vivo* ± SD		38.8 ± 8	24 ± 5	19.4 ± 5	19.5 ± 3.1	17.8 ± 2.2
Ingredient type	Trade name	Composition (wt. %)				
Emulsifier system	Arlacel 165	1.5	—	—	—	—
	Amphisol K	1.5	2.5	—	—	—
	Dehymuls LE	—	—	—	1.0	—
	Tegin OV	—	—	—	2.0	—
Thickener system	Lanette O	0.5	—	—	0.5	—
	Keltrol RD	0.15	—	0.15	—	—
	Salcare SC 91	1.8	—	1.8	—	—
	Carbopol Ultrez 10	—	—	0.2	—	—
	Carbopol Ultrez 21	—	—	0.15	—	—
	Paracera M	—	—	—	0.5	—
	Beeswax	—	—	—	1.0	—
Emollient	Cetiol AB	5.0	5.0	5.0	5.0	5.0
Filter system	Mixture of UV filters	17.0	17.0	17.0	17.0	17.0
Neutralizing agent	Tris Amino Ultra Pure	qs	qs	qs	qs	—
	Neutrol TE	—	—	—	—	qs
Additional ingredients	Glycerin	3.0	3.0	3.0	3.0	—
	Edeta BD	0.2	0.2	0.2	0.2	—
	Phenonip	1.0	1.0	1.0	1.0	—
	Water	qs 100%	qs 100%	qs 100%	qs 100%	—
	Ethanol	—	—	—	—	qs 100%

[a]SPF *in vivo* and standard deviation evaluated in accordance with ISO24444:2010 guidelines with $n = 5$.

skin substrate preparations before and after application of a sunscreen using Labsphere UV-2000S. The UV transmittance of four positions per 2×2 cm^2 skin substrate was measured to virtually cover the complete surface area of the preparation.

The blank transmittance spectrum was recorded at first for each single position before sunscreen application followed by topographical measurement of the bare skin (see Sec. 2.6). Subsequently, sunscreen was applied and topographical measurement was performed again. After completion of topographical measurement which lasted ~4 h, UV transmission through the sunscreen-covered skin substrate was measured. Stability of SPF *in vitro* values over 4 h was checked and confirmed for all sunscreens (data not shown).

2.6 Assessment of the Sunscreen Film

The layer of sunscreen applied on the pig skin substrate was investigated using topographical measurements with an optical probe based on the white light chromatic aberration principle (Altisurf 500 instrumentation). The instrumentation allowed noncontact surface topography measurement and analysis. The employed optical sensor yielded an axial resolution (z) of 5 nm and a lateral resolution (x–y) of 1.1 μm. The motorized x–y stage permitted scanning of samples in the millimeter range. Skin preparations on quartz plates were fixed on the stage using a custom made holder.
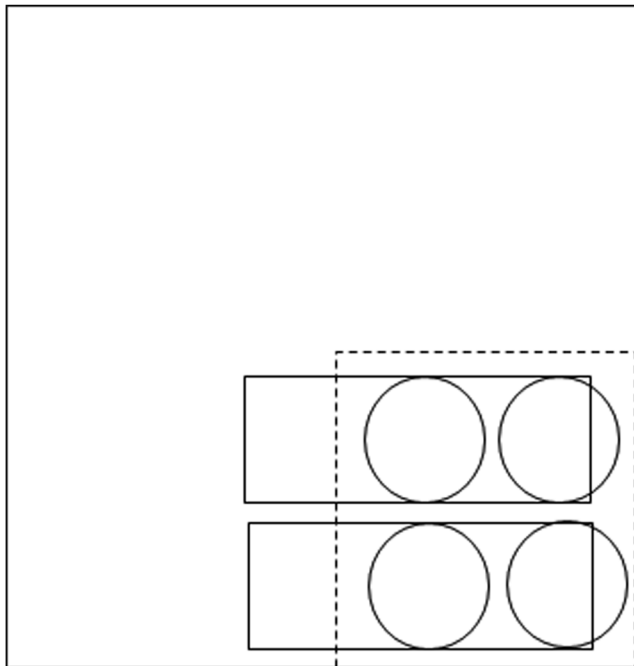
Surface topography of bare skin and skin covered with sunscreen was measured in order to assess the sunscreen film. Skin preparations were removed from the desiccator and allowed to equilibrate for 12 h next to the device at room conditions before starting topographical measurements. Repeated measurements on bare skin using the "loop" option of the operating software revealed that this equilibration was necessary to allow stabilization of the surface position along the z axis (data not shown). After measuring the surface topography of bare skin, sunscreen was applied, equilibrated for 15 min, and the same area was scanned again.
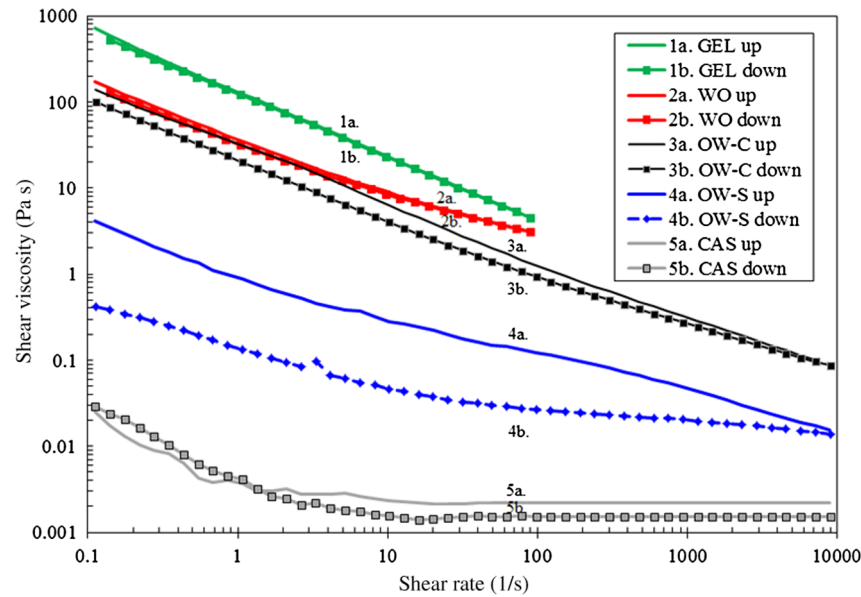
Fig. 1 Rheological behavior of sunscreens measured with AR-G2 rheometer (TA instrument, New Castle, Delaware), CP 4 deg /40 mm, gap 100 μm, $T = 23°C$.

Figure 2 illustrates the area of topographical and UV transmittance measurements.

Topographical measurements were performed on two rectangular areas ($\sim 23 \times 8$ mm^2) per specimen (Fig. 2). A part of the rectangular area (about 5×8 mm^2 on left hand side) corresponded to quartz without skin and served as a reference. The skin area (right hand side of the rectangle) measured about 18×8 mm^2. Topography was recorded in lines each extending over the quartz and the skin part of the rectangle with an increment step of 10 μm. The rectangular areas were scanned with lines in 10-μm intervals resulting in 1,840,000 single-measurement points per rectangle.

The raw data of the topographical measurements were redressed by a line-by-line leveling correction of each rectangular surface to the same x-y plane using the quartz part of each measured line (left side of rectangle, Fig. 2). This redressing procedure was carried out with the data of bare skin and skin covered with sunscreen and was essential in order to correct for variation due to positioning and due to environmental factors changing in the course of the experiment. Each rectangular surface area was divided into two zones of 8×8 mm^2 coinciding with the four positions (circles) of UV transmittance measurements (Fig. 2). The film thickness of the applied sunscreen was obtained as the difference of the redressed skin topography data with and without sunscreen computed for each single-measurement point. The result was expressed as a distribution of frequencies of film thickness over the measured surface area normalized to 100% and is referred to as thickness distribution curve. A threshold of 0.5% of area under the curve was applied to remove extreme values at both ends of the film thickness distribution. To validate this measurement and calculation method, a surface area of bare skin was measured twice and the film thickness was computed. The result was found to be centered around 0 μm ($n = 8$), confirming the validity of the method for measurement of the sunscreen film thickness distribution on skin.

Data extracted from the distribution curve and serving to characterize the applied sunscreen film are given in Table 2.

S_{mean} is the frequency-weighted average thickness. The S_{mean} to median ratio of the thickness distribution is a measure of skewness of distribution and is used as an expression of film homogeneity; the smaller this ratio the greater the homogeneity of the film. The Abbott–Firestone curve is commonly used in surface metrology[29] and is employed here to depict the experimentally determined thickness distribution, indicating thickness and uniformity of the applied product layer.

Fig. 2 Illustration of areas for topographical and UV transmittance measurements; the big square corresponds to the carrier quartz plate, the dotted small square to the skin surface area with a dimension of 2×2 cm^2, the four circles correspond to the areas of UV transmittance measurements [sun protection factor (SPF)] with a diameter of 1 cm and the two rectangles to the two areas of topographical measurements.

Table 2 Data extracted from the thickness distribution curve of applied product.

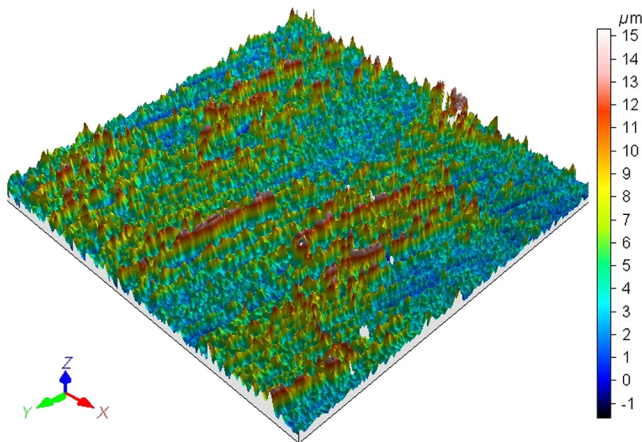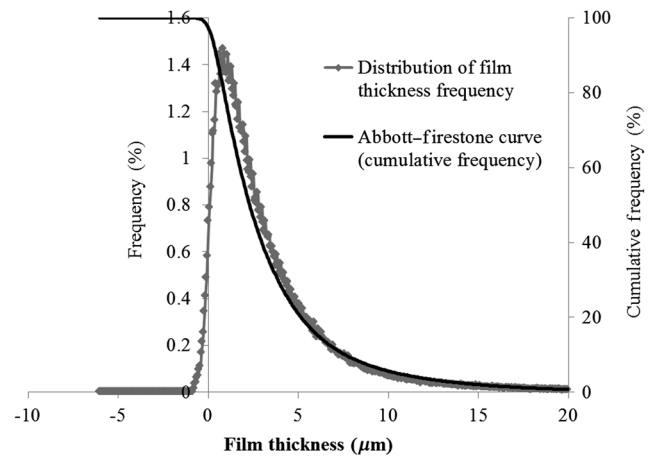| Parameter	Meaning
S_{mean} (μm)	Average of film thickness over the measured area
S_{mean} to median ratio	Indicator of film homogeneity
Abbott–Firestone curve	Cumulative frequency of occurrence of film thickness

2.7 Statistical Analysis

Statistical analysis was performed using Statgraphics centurion XVI software (Statpoint Technologies, Inc., Warrenton, Virginia). The impact of formulation vehicle on SPF *in vitro* and on film parameters was assessed with a Kruskal–Wallis non-parametric test, and the impact of application conditions was assessed with a Mann–Whitney U test, both with a statistical significance at 5% confidence level. In case Kruskal–Wallis test revealed a statistically significant difference among sunscreens for an investigated parameter, a multiple pairwise comparison test using Bonferroni approach was performed to identify which sunscreens significantly differed from which other. Correlations between film parameters and SPF *in vitro* values within each formulation were assessed using Spearman's rank correlation coefficient test.

3 Results

3.1 Film Assessment

The film thickness distribution of sunscreen, extracted from the topographical measurements, is visualized three-dimensionally for qualitative assessment in Fig. 3 and is quantitatively displayed as a distribution curve of thickness frequency. From the distribution curve, the Abbott–Firestone curve (cumulative frequency) was deduced (Fig. 4).

Thickness distribution was always positively skewed, the degree of skewness varying between the different sunscreens. In the example of Fig. 4, the most frequently occurring film thickness was in the range of 2 to 4 μm while a thickness as
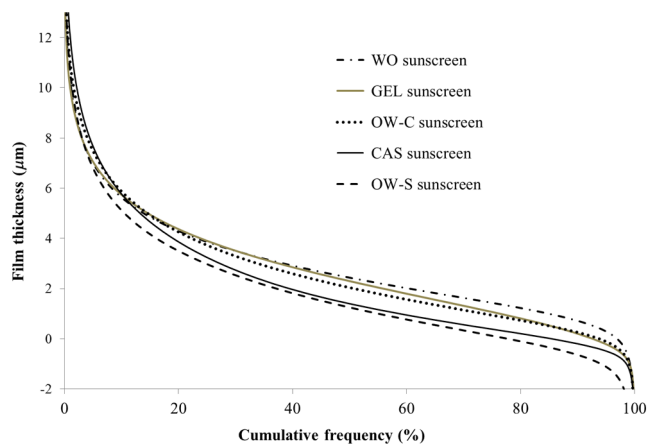

Fig. 4 Example of distribution of film thickness frequency and Abbott–Firestone curve of OW-C sunscreen.

large as 10 to 13 μm was recorded. A small percentage of the area under the thickness distribution curve lay below a film thickness of 0 μm, which was likely due to experimental error. This was included in the calculation of the S_{mean} value.

3.2 Impact of Vehicle on Film Parameter Values and SPF In Vitro

Figure 5 gives the average of the Abbott–Firestone curves of all measurements with each investigated sunscreen using high pressure and spreading 1 conditions of application.

The Abbott–Firestone profiles differed considerably between the sunscreens (Fig. 5). Film thickness was different for the different vehicles and decreased roughly in the order WO > GEL > OW-C > CAS > OW-S. For WO for example, a film thickness of 2.41 μm corresponds to 50% of the cumulative thickness frequency meaning that 50% of the measured surface area of the sample exhibited a film thickness greater than 2.41 μm. As a comparison, 50% of the measured area of OW-S exhibited a thickness greater than merely 1.20 μm. Moreover, the shape of the curve differed between the used vehicles, the WO, for example, showed a more flat-shaped profile compared to CAS (Fig. 5).

Fig. 3 Example of three-dimensional visualization of film thickness distribution of oil-in-water cream (OW-C) sunscreen.

Fig. 5 Abbott–Firestone profiles of investigated sunscreens applied with high pressure and spreading 1.

Table 3 Medians of SPF *in vitro*, S_{mean}, and S_{mean} to median ratio of thickness distribution with interquartile range Q1 to Q3 (in brackets) for investigated sunscreens with high pressure and spreading 1.

Sunscreen	SPF *in vitro*	S_{mean} (μm)	S_{mean} to median ratio
OW-C ($n = 27$)	33 (30 to 48)	2.3 (2.0 to 2.7)	1.30 (1.25 to 1.44)
OW-S ($n = 20$)	16 (13 to 26)	1.6 (1.2 to 2.0)	1.41 (1.30 to 1.96)
GEL ($n = 28$)	28 (20 to 34)	2.6 (2.4 to 3.1)	1.19 (1.16 to 1.23)
WO ($n = 24$)	72 (55 to 85)	2.9 (2.6 to 3.2)	1.19 (1.17 to 1.21)
CAS ($n = 20$)	14 (7 to 20)	2.2 (1.7 to 2.6)	1.71 (1.44 to 1.99)

Table 4 Impact of vehicle on SPF *in vitro*, S_{mean}, and S_{mean} to median ratio of thickness distribution.

Parameter	Statistically significant difference[a]
SPF *in vitro*	Yes ($p < 0.05$)
S_{mean}	Yes ($p < 0.05$)
S_{mean} to median ratio	Yes ($p < 0.05$)

[a]Between the different formulations on SPF *in vitro*, S_{mean}, and S_{mean} to median ratio of thickness distribution at 5% confidence level (Kruskal—Wallis).

These differences between the vehicles are reflected by the calculated film thickness distribution parameters S_{mean} and S_{mean} to median ratio. Table 3 gives the values of the median and interquartile range for the film parameters of all individual measurements of each investigated sunscreen. Also, SPF *in vitro* values of these sunscreens are given in Table 3.

SPF *in vitro* varied markedly between the investigated sunscreen formulations attaining values from 14 for CAS to 72 for WO. SPF *in vitro* values are compared to the SPF *in vivo* in Fig. 6. SPF *in vitro* values generally approached SPF *in vivo* and, considering the declared variation range, a satisfactory agreement between SPF *in vitro* and *in vivo* for spreading 1 and a high pressure condition is found. WO sunscreen was an exception, with surprisingly low and high SPF *in vivo* and SPF *in vitro,* respectively. *In silico* estimation of SPF gave a value of 25 (Fig. 6). This computational approach takes into account the absorbance spectrum of each UV filter, their photostability and mutual stabilization or de-stabilization, and their distribution in the phases of the vehicle and uses the Gamma

distribution function to describe film irregularity.[30] The estimated value lies within the range of the experimental values of all vehicles, yet the *in silico* calculation cannot predict the effect of formulation on SPF. In Fig. 6, the S_{mean} of the formulations is also visualized.

The impact of the vehicle on SPF *in vitro* and film parameters was evaluated with a Kruskal–Wallis test (Table 4).

This statistical test revealed a significant effect of the vehicle on all tested parameters. To identify which sunscreens significantly differed from each other with respect to the studied parameters, a multiple pairwise comparison test based on the Bonferroni approach was employed. The results are given in Tables 5–7.

This multiple comparison test resulted in a group classification of the investigated sunscreens. Formulations of one group differ statistically from those of another group while formulations that belong to the same group do not differ significantly from each other with respect to the considered parameter. When the same formulation is contained in two different groups it does not differ significantly from the formulations of either group. The number of groups was different for the tested parameters;
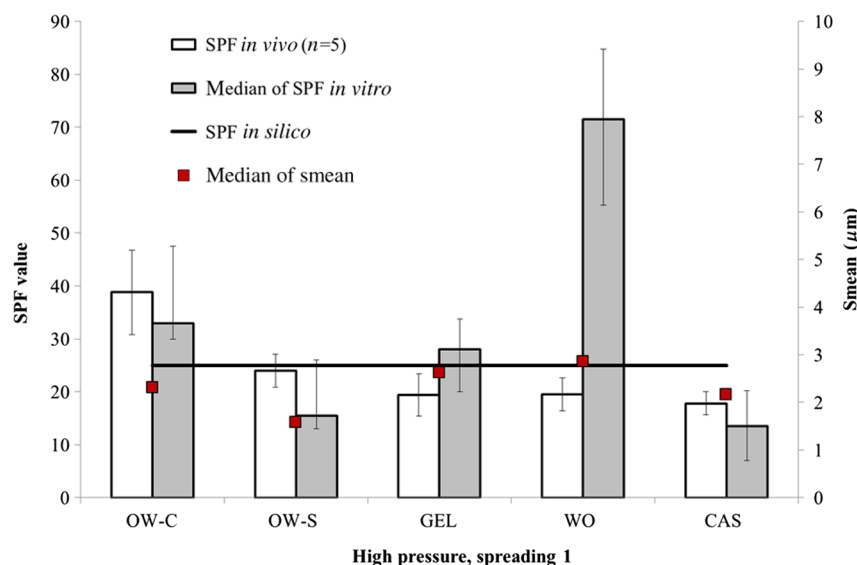
Fig. 6 SPF *in vivo* (white columns) with standard deviation (bars), medians of SPF *in vitro* (gray columns) with interquartile values (bars) for OW-C ($n = 27$), OW-S ($n = 20$), GEL ($n = 28$), WO ($n = 24$), CAS ($n = 20$), SPF *in silico* (black horizontal line), and medians of S_{mean} values (squares) of the same sunscreens applied with high pressure and spreading 1.

Table 5 Multiple pairwise comparison test using Bonferroni approach for SPF *in vitro*.

Group classification[a]	WO	OW-C	GEL	CAS	OW-S
Group 1	X				
Group 2		X	X		
Group 3			X	X	X

[a]Sunscreens that were nonsignificantly different from each other with respect to SPF *in vitro* were assigned to the same group.

Table 6 Multiple pairwise comparison test using Bonferroni approach for S_{mean}.

Group classification[a]	WO	GEL	OW-C	CAS	OW-S
Group 1	X	X			
Group 2		X	X		
Group 3			X	X	
Group 4				X	X

[a]Sunscreens that were nonsignificantly different from each other with respect to S_{mean} were assigned to the same group.

**Table 7** Multiple pairwise comparison test using Bonferroni approach for S_{mean} to median ratio.

Group classification[a]	WO	GEL	OW-C	CAS	OW-S
Group 1	X	X	X		
Group 2				X	X

[a]Sunscreens that were nonsignificantly different from each other with respect to S_{mean} to median ratio were assigned to the same group.

two, three, and four groups were found for S_{mean} to median ratio, SPF *in vitro*, and S_{mean}, respectively. A small number of groups mean less difference between the sunscreens.

WO yielded a significantly higher SPF *in vitro* than all other sunscreens, a greater S_{mean} than OW-C, CAS, and OW-S and a smaller S_{mean} to median ratio than CAS and OW-S. OW-C gave a higher SPF *in vitro* and a smaller S_{mean} to median ratio than CAS and OW-S. The GEL and OW-C formulations did not significantly differ from each other with respect to any of the criteria. Also, CAS and OW-S did not differ from each other. OW-C yielded a greater SPF *in vitro*, a greater S_{mean} and a smaller S_{mean} to median ratio than OW-S, which was interesting since these two sunscreens varied only in their content of thickeners, hence their viscosity characteristic.

Finally, the correlation of the SPF *in vitro* with both film parameters for the individual measurements within each sunscreen was evaluated using a Spearman rank correlation test. A significant positive correlation between SPF *in vitro* and S_{mean} was found for every sunscreen formulation ($p < 0.05$). A negative correlation was found between SPF *in vitro* and S_{mean} to median ratio for WO; OW-S, and CAS ($p < 0.05$), whereas no correlation was found for OW-C and GEL.
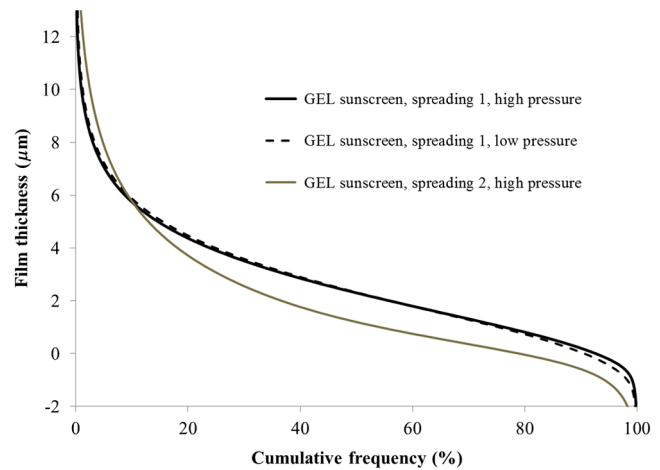
Fig. 7 Abbott–Firestone profiles of GEL sunscreen applied with two pressure (high and low) and spreading (1 and 2) conditions.

3.3 Impact of Pressure and Spreading Procedure on Film Parameter Values and SPF In Vitro

In addition to the vehicle, the impact of the application conditions, i.e., spreading procedure and pressure on film parameters and SPF *in vitro*, was studied using the GEL sunscreen. In total, three conditions of application were investigated, spreading 1 with high pressure, spreading 1 with low pressure, and spreading 2 with high pressure.

Figure 7 shows the average of the Abbott–Firestone curves of the GEL sunscreen for each application condition, and Table 8 gives the median and interquartile range values of SPF *in vitro* and the film parameters for the investigated conditions.

It is evident from Fig. 7 that the shape of the Abbott–Firestone curve of GEL sunscreen is different between spreading 2 and spreading 1, while no difference was found between low and high pressure using spreading 1. The differences of the Abbott–Firestone curves are reflected in the S_{mean} and S_{mean} to median ratio.

SPF *in vitro* data measured for each condition of application were compared to the SPF *in vivo* for GEL sunscreen (Fig. 8). From this evaluation, spreading 2 with high pressure seems to give a better approximation of the SPF *in vivo*. However, as this condition could not be practically applied to all types of formulation, spreading 1 with high pressure was used as an alternative in the investigation of the different vehicles (Sec. 3.2).

The impact of spreading (procedure 1 versus 2) and pressure (low versus high) on SPF *in vitro* and film parameters were evaluated using the Mann–Whitney U test (Table 9).

Spreading 2 showed a significantly smaller film thickness (S_{mean}) and a larger S_{mean} to median ratio compared to spreading 1 (Tables 8 and 9). Both spreading and pressure had a significant effect on SPF *in vitro*. For spreading 2 compared to spreading 1 and high compared to low pressure a reduction of SPF *in vitro* was found. Film parameters were not significantly influenced by pressure.

4 Discussion

This work tests the hypothesis that the film thickness distribution can be used to explain the variation of SPF between sunscreen vehicles and application conditions. For this purpose, the accurate measurement of film thickness was necessary.

Table 8 Medians of SPF *in vitro*, S_{mean}, and S_{mean} to median ratio with interquartile range Q1 to Q3 (in brackets) for investigated conditions of application of the GEL sunscreen.

Application of GEL sunscreen	SPF *in vitro*	S_{mean} (μm)	S_{mean} to median ratio
Spreading 1, high pressure, $n = 28$	28 (20 to 34)	2.6 (2.4 to 3.1)	1.19 (1.16 to 1.23)
Spreading 1, low pressure, $n = 24$	39 (30 to 54)	2.7 (2.4 to 3.1)	1.19 (1.17 to 1.21)
Spreading 2, high pressure, $n = 24$	20 (15 to 25)	1.9 (1.5 to 2.3)	1.57 (1.50 to 1.91)
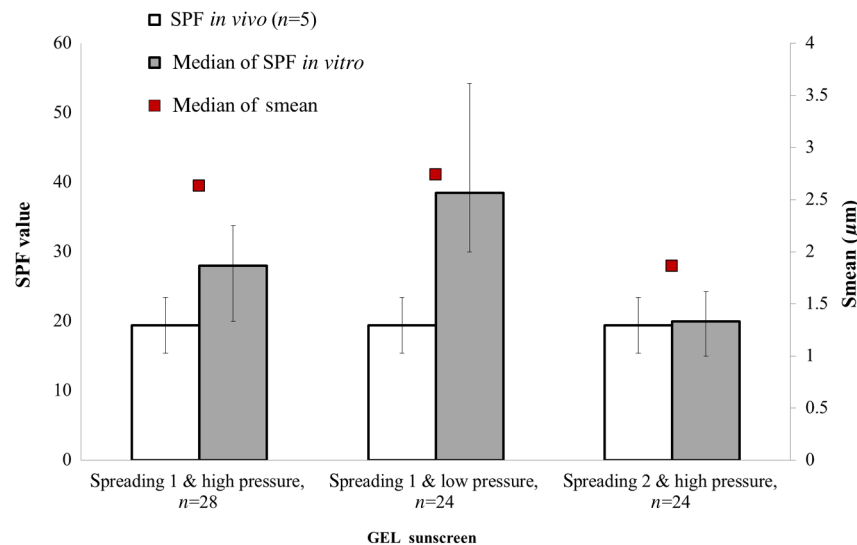
Fig. 8 SPF *in vivo* (white columns) with standard deviation (bars), medians of SPF *in vitro* (gray columns) with interquartile values (bars), and medians of S_{mean} values (squares) of the GEL sunscreen for different application conditions.

Many techniques for assessing the film distribution of an applied sunscreen have been used providing merely qualitative or some quantitative information about its distribution. For qualitative assessment, fluorescence resulting either from a UV filter or from an added fluorescent marker was used to visualize the homogeneity of distribution of the applied product.

Table 9 Impact of application conditions on SPF *in vitro*, S_{mean}, and S_{mean} to median ratio of thickness distribution of GEL sunscreen.

Application condition	Parameter	Statistically significant difference[a]
Spreading (1 versus 2)	SPF *in vitro*	Yes ($p < 0.05$)
	S_{mean}	Yes ($p < 0.05$)
	S_{mean} to median ratio	Yes ($p < 0.05$)
Pressure (low versus high)	SPF *in vitro*	Yes ($p < 0.05$)
	S_{mean}	No ($p > 0.05$)
	Smean to median ratio	No ($p > 0.05$)

[a]Between tested application condition (either spreading or pressure) and SPF *in vitro*, Smean, Smean to median ratio at 5% confidence level (Mann–Whitney U test).

Sunscreen distribution was evaluated *in vivo* using an appropriate illumination source optionally combined with photography[31–33] or multiphoton tomography;[34] for *in vivo* and on tape strips evaluation the use of laser scanning microscopy[17] was also reported. Alternatively, for sunscreens containing titanium dioxide as a UV filter, light microscopy on cross sections of skin biopsies[3] was used that gave a rough estimation of the thickness layer based on the visualization of titanium dioxide particles; optical coherent tomography[35] was also used on intact skin that detected the distribution of titanium dioxide particles within the sunscreen layer. For quantitative assessment, the use of *in vivo* fluorescence spectroscopy gave indirect information about the film thickness by converting the fluorescence intensity into an equivalent thickness of an applied product.[16,36] When sunscreens are not intrinsically fluorescent, this technique requires the addition of a fluorescent agent which, however, often produced inconclusive results because of immiscibility or interference issues.[37] An alternative approach reported the use of an *in vivo* skin swabbing technique in conjunction with sunscreen quantification by UV spectroscopy to evaluate the thickness of the film.[38] None of above mentioned methods, however, provided a full characterization of the sunscreen film in terms of thickness and homogeneity of distribution.

In our work, we started from an approach based on topographical measurements. This method was used before on skin replicates and provided a semiquantitative assessment of

film thickness.[39] In contrast to that work, we used a biological substrate for the application of sunscreen to reproduce as closely as possible the product-to-substrate adherence relevant for the *in vivo* situation. In addition, by developing a reference-corrected measurement protocol and quantitative data evaluation the complete thickness distribution could be determined. Topographical evaluation was combined with measurement of SPF *in vitro* both of which were performed in the same position and nearly the same surface area making it possible to reveal existing correlations.

The composition of the five studied vehicles principally differed in the thickener and emulsifier system, the UV filter combination remaining the same. The formulation of the vehicles had a significant effect on S_{mean} and S_{mean} to median ratio (Tables 6 and 7). Of the two sunscreens, OW-C and OW-S, which mainly differed in their thickener system, OW-S showed a significantly smaller S_{mean} and greater S_{mean} to median ratio than OW-C. The thickeners Lanette O, Keltrol RD, and Salcare SC 91 contained in OW-C but not in OW-S corresponded to a relative weight difference of only 10% in the remaining film on the skin surface of OW-S versus OW-C, but they appear to be responsible for the significant difference of film thickness and homogeneity between the two sunscreens. This indicates that thickeners which enable the formation of a firm film upon spreading also lead to a thicker and more homogeneous film. OW-S and CAS did not differ in their S_{mean} and S_{mean} to median ratio both yielding a smaller S_{mean} and larger S_{mean} to median ratio than the other vehicles. This also seems to be related to the absence of thickeners in both formulations. The emulsifier, that was present in the OW-S emulsion, but not in CAS which was a mono-phase, seems to play a minor role for the S_{mean} and the S_{mean} to median ratio. The same observation is true for OW-C and GEL sunscreens that did not statistically differ in S_{mean} and S_{mean} to median ratio, with both containing thickeners but only OW-C containing emulsifiers. WO had a statistically larger S_{mean} than OW-C, OW-S, and CAS which might be related to its continuous oil phase; yet it did not show a significant difference to GEL. With respect to S_{mean} to median ratio, the low viscosity vehicles CAS and OW-S showed a higher positively skewed thickness distribution, hence a greater nonhomogeneity of film than the high viscosity vehicles WO, OW-C, and GEL. It should be pointed out that S_{mean} differences between the vehicles were not due to differences in mass loss during application.

The formulation of the vehicles had a significant effect on SPF *in vitro* (Table 5). It appears that large and small S_{mean} values among vehicles corresponded, respectively, to high and low SPF *in vitro*. Therefore, the differences in SPF between vehicles may be discussed in relation to the film parameter S_{mean}. For this, we consider that a smaller S_{mean} is connected to a greater occurrence of small film thicknesses and that light transmittance, which is inversely proportional to SPF, increases exponentially with decreasing film thickness. OW-S and CAS for instance, exhibited the smallest S_{mean} values and yielded also the lowest SPF. These two sunscreens which lacked thickeners and had the lowest viscosity compared to the rest may leave larger areas of ridges virtually uncovered while accumulating in the furrows thus leading to a low SPF. Therefore, the presence of thickeners in the formulation seems to be a prevailing prerequisite for UV efficacy. Further, WO exhibited both the largest S_{mean} value and the highest SPF. This is consistent with minimal surface area with very small film thickness that would be virtually unprotected. Furthermore and in contrast to the other sunscreens, the UV filters of WO are distributed in the continuous phase which does not evaporate, forming a uniform protecting film with the help of the thickeners. An increase of about 45% of SPF *in vitro* was found for the WO sunscreen compared to OW-C, which is in line with data previously reported on sunscreens with smaller SPF values.[3] CAS and OW-S as well as OW-C and GEL did not differ with respect to any of the tested criteria and can be considered as very similar in terms of film forming ability and SPF efficacy. Taken together, the SPF variation observed between sunscreens containing the same filter composition is proposed to arise from the difference in their film thickness distribution.

Within every sunscreen, S_{mean} positively correlated with SPF *in vitro*. Further, S_{mean} to median ratio showed a negative correlation with SPF *in vitro* for three of the five sunscreens. This demonstrates the significant connection between the film formation and sun protection efficacy and supports the observation discussed above about the differences between sunscreens. The present data addressing film formation and thickness distribution go beyond the previous studies, which showed that film thickness resulting from a different application amount of sunscreen strongly impacts SPF efficacy.[8,10]

In addition to the vehicle formulation, this work demonstrated using the GEL that application conditions can significantly impact sunscreen performance. We found that a longer
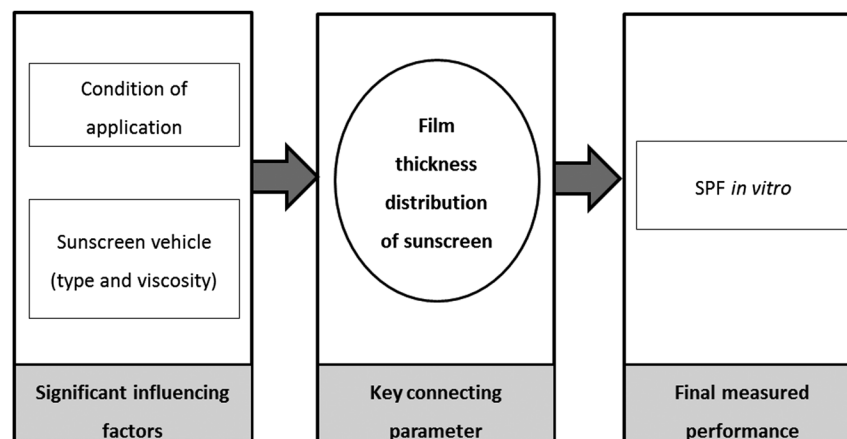

Fig. 9 Connections between influencing factors, film distribution, and SPF efficacy.

spreading time resulted in a larger S_{mean} to median ratio, a smaller S_{mean} and smaller SPF *in vitro* values (Table 8) further corroborating the correlation between film characteristics and sunscreen efficacy; also, an increase of pressure by 180 g resulted in a significant decrease in SPF values. Interestingly, this effect of prolonged and high pressure application was analogous to that elicited by low viscosity formulations, which might be related to a thinning of the GEL under these application conditions. The effect of application conditions on the performance of the other vehicles still needs to be investigated. Some authors reported that even a change in pressure of 50 g led to a different SPF *in vitro* when using synthetic plates as a substrate.[40] Former studies reported that a more rubbed application led to a smaller SPF *in vivo*[15] and a crude compared to a careful application to a smaller cream thickness.[36] Finally, more recently, the effect of careful versus crude spreading of sunscreen on the magnitude of erythema occurrence was simulated, and underlined the "importance of homogeneity of spreading on the level of delivered protection.[41]"

Figure 9 summarizes the connection between the influencing factors, i.e., application condition and vehicle, the film distribution and the measured SPF *in vitro* of sunscreens.

5 Conclusion

The type and the viscosity of sunscreen vehicles and application conditions play a role for the film thickness parameters that finally influenced the SPF efficacy. High application pressure, long spreading time, low viscosity of formulation, and/or absence of thickeners were shown to unfavorably impact UV protection. As the application condition can, in principle, be fixed, the impact of a vehicle on the formed film can now be investigated during the product development step. Sunscreen composition might be optimized accordingly to achieve a large film thickness with a uniform distribution with minimization of the small thickness fraction of the film being crucial for ultimate sunscreen performance. Development of a method to quantify the film thickness distribution of sunscreen on skin was shown to be essential for understanding the mechanism influencing UV efficacy.

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