Development of a Synthetic Substrate for the in vitro Performance Testing of Sunscreens

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Keywords

Sun protection factor · In vitro testing · Surface free energy · Surface roughness · Ethylene polymer derivative

Abstract

Purpose: Polymethylmethacrylate (PMMA) plates generally used for the in vitro testing of sunscreens have failed to yield a satisfactory correlation between the sun protection factor (SPF) in vitro and that in vivo. In the present study, various polymers were investigated as alternative substrates to PMMA plates. **Procedures:** In total, 14 polymers were tested in terms of ultraviolet (UV) transparency by transmission spectroscopy, and surface properties by contact angle measurement. The polymers that were UV transparent and that showed surface properties similar to human skin were mold-injected in casts with various degrees of roughness to obtain corresponding substrate plates. To assess them, the in vitro SPF of 1 sunscreen was measured on a mold-injected PMMA plate as well as on the proposed polymer plates and compared to the in vivo SPF. **Results:** Four polymers showed a UV transmittance at 310 nm of more than 50%, as well as a total surface free energy close to the reported value for human skin. Of these, 2 provided an in vitro SPF matching the

in vivo SPF contrary to the mold-injected PMMA plate for the tested oil-in-water sunscreen. **Conclusion:** This work demonstrates the possibilities of using alternative polymers for synthetic substrates for in vitro sunscreen testing.

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Introduction

Globally, there is growing interest in developing sunscreens with increased photoprotection, claiming sun protection factor (SPF) values as high as 50+. In Europe, sunscreens with SPF 50 or higher represent approximately 36% of the suncare products launched in 2015 compared to only 4% in 2007 and 16% in 2009 [1; search criteria: EP, sun protection, SPF 50 and higher, 2007–2015]. The SPF value remains the principal criterion for indicating the performance of a sunscreen. The only approved method for SPF testing is based on the in vivo assessment of the erythral response on human volunteers following an ultraviolet (UV) irradiation [2]. Besides the drawbacks of being costly and time consuming, in vivo methods are also ethically questionable, which explains the considerable interest in developing an in vitro approach for SPF testing. Conse-

Table 1. Chemical characteristics of the investigated polymers

Trade name	Supplier	Chemical characteristics	Product description
Elvax 150W	Dupont	Ethylene-vinyl acetate copolymer	Vinyl acetate 32%
Elvax 250A	Dupont	Ethylene-vinyl acetate copolymer	Vinyl acetate 28%
Elvax 420A	Dupont	Ethylene-vinyl acetate copolymer	Vinyl acetate 18%
Luwx EVA3	Dupont	Ethylene-vinyl acetate copolymer	Vinyl acetate 13–15%
Luwx EVA1	Dupont	Ethylene-vinyl acetate copolymer	Vinyl acetate 10.5%
Elvaloy 742	Dupont	Ethylene vinyl acetate/carbon monoxide copolymer	
Nucrel 0910	Dupont	Ethylene methacrylate acid copolymer	Methacrylic acid 15%
Nucrel 925	Dupont	Ethylene methacrylate acid copolymer	Methacrylic acid 8.7%
Polystyrol 495F	BASF	Polystyrene	Styrene-butadiene polymer 92–100%
Ultramid Seal-Fit	BASF	Amorphous copolyamide	Polyamide 66/6 copolymer
Elastollan 695A10	BASF	Polyurethane	Thermoplastic polyester
Elastollan 1185A10	BASF	Polyurethane	Thermoplastic polyether
Elastollan L1160D10N	BASF	Polyurethane	Aliphatic thermoplastic polyurethane

quently, much work is ongoing to find an in vitro procedure for SPF determination involving sunscreen manufacturers as well as raw material suppliers, test institutes, cosmetic national associations, or international organizations (such as ISO) [3–6]. The influence of various experimental factors on in vitro SPF measurement has been investigated. The amount of product applied [7, 8], the application procedure [9], the force applied during spreading [10, 11], and the type of in vitro substrate have all been shown to impact the in vitro SPF value. Especially careful consideration has been given to the effect of the substrate for SPF measurement, especially its origin, either biological or synthetic [4, 12–14], its roughness [15], and its surface properties, indicating the affinity of the sunscreen for the in vitro substrate [16]. A substrate made from polymethylmethacrylate (PMMA) is currently favored as it is easy to handle, does not require any preparation, and can be manufactured reproducibly [4]. However, several studies have reported a lack of affinity with the spreading of sunscreen formulations using mold-injected PMMA plates, causing an irregular film structure and subsequently a lower in vitro performance compared to the in vivo performance. Differences in the affinity of the sunscreen formulation for PMMA mold-injected plates compared to human skin is assumed to be the cause of the discrepancies between in vitro and in vivo SPF results. In other words, the currently employed PMMA plates do not simulate human skin properly [13]. Some means of increasing the adherence of the sunscreen product on these plates have been proposed [16, 17]. However, they are formulation dependent, and therefore not a practicable solution. As stated by Pissavini et al. [18] “no ideal in vitro substrate exists,” yet.

Table 2. Characteristics of the mold-injected PMMA plate currently used in the industry

Name	HD6
Manufacturer	HelioScreen Labs
Manufacturing process	Mold injection
Surface size as supplied	4.7 × 4.7 cm
Roughness given by the supplier (Ra or Sa)	Ra = 4.5 μm
Amount for SPF in vitro testing	1.3 mg/cm ²

In the present work, we examined polymers of various chemical structure to find a new substrate material for the in vitro testing of sunscreens. A prerequisite for a substrate plate for this testing is UV transparency between 290 and 400 nm. The objective consisted in finding a polymer that is UV transparent and that shows surface properties close to human skin compared to the conventionally used PMMA polymer. A substrate with a closer resemblance to human skin is expected to more suitably simulate the in vivo product-to-substrate affinity and result in a more accurate prediction of SPF. Surface properties were assessed by measuring the contact angle and calculating the surface free energy (SFE). Screening tests including the assessment of UV transmittance and surface properties were performed to select appropriate polymers as substrates for the in vitro testing of sunscreens. To validate the suitability of the polymers, the in vitro SPF of a sunscreen formulation was measured using the new developed polymer substrate.

Table 3. Photographs, 3-dimensional view, and roughness Sa of the HD6 PMMA plate and each investigated cast (example for Nucrel 925 polymer)

	Photo taken from above	Three-dimensional view ¹	Roughness parameters ² , μm
Cast 1			Sa = 29
Cast 2			Sa = 28
Cast 3			Sa = 14
HD6			Sa = 6

¹ Surface texture analysis instrument (Altisurf 500), 12.5 × 12.5 mm scanned in 10- μm incremental steps using a 2.5-mm filter.
² Sa, arithmetical mean height over an area ($n = 5$).

Table 4. UV filter composition and SPF in vivo and in silico of the investigated sunscreen

Sunscreen designation		O/W
Filter composition, wt%	DHHB	6.0
	BEMT	1.8
	EHMC	10.0
Performance	SPF in vivo $\pm$ SD ¹	30.5 $\pm$ 4.9
	SPF in silico ²	32

DHHB, diethylamino hydroxy hexyl benzoate; BEMT, bis-ethylhexyloxyphenol methoxyphenyl triazine; EHMC, ethylhexyl methoxycinnamate.

¹ SPF in vivo was evaluated in accordance with ISO24444:2010 guidelines with $n = 5$.

² SPF in silico was calculated with the BASF sunscreen simulator.

Materials and Methods

Chemicals and Equipment

The following equipment was used: a UV transmittance analyzer (Labsphere UV-2000S, Labsphere Inc., North Sutton, NH, USA), a contact angle measuring instrument (Easy Drop, Krüss GmbH, Hamburg, Germany), a surface texture analyzer (Altisurf 500, Altimet, Thonon-les-Bains, France), and hydraulic press system equipment with 2 electric heating plates (from 1979, Wickert, Landau, Germany).

The following software packages were used: UV-2000 (Labsphere Inc.) for the transmittance measurement, Drop Shape Analysis DSA1 (Krüss GmbH) for the determination of the SFE, and Altimap (Altimet) for topographical measurement and evaluation.

Synthetic Substrates

The tested polymers included ethylene copolymers, polyurethane, polystyrene, and polyamide polymers; their chemical characteristics are detailed in Table 1. For the screening of the UV transparency and surface properties of the proposed polymers, a plate was produced by a high-pressure process resulting in a 2-mm-thick flat sheet using a hydraulic press system. The polymer raw material supplied in the form of granulates was heated to 150°C, pressed for 6 min at 150 bar using the hydraulic press system, and cooled down for 6 min while maintaining a pressure of 150 bar.

For the in vitro SPF measurement, selected polymers were mold injected in a homemade cast in hot work tool steel (DIN X38CrMoV5-1) producing plates with a flat side and a roughened side. Three casts with varying patterns and roughness were considered. Furthermore, a mold-injected PMMA plate that is currently used in the industry as a standard synthetic substrate was also included in the study (Table 2). Pictures of the mold-injected roughened plates, their 3-dimensional view, and their roughness parameter S_a (calculated as the arithmetical mean height over an area) [19], are given in Table 3.

Biological Pig Skin Substrate

The stratum corneum of the ears of freshly slaughtered pigs was prepared as described previously [13]. Due to its similarity to human skin, skin from pig ears served as the control substrate to set the target range of SFE values for the new proposed synthetic substrate.

Sunscreen Formulation

Table 4 details the composition of the UV filtering system along with the in vivo SPF and in silico SPF of the investigated oil-in-water (O/W) sunscreen formulation. SPF in silico was calculated using the BASF sunscreen simulator [20]. The full formulation composition is given in Appendix 1.

Spectral Transmission of UV Radiation

Measurement of Substrate UV Transparency

The UV transparency of the proposed synthetic substrates was determined by transmission measurement through the bare flat polymer plate in the spectral range 290–400 nm in incremental steps of 1 nm. A spectral transmission measurement with a bare quartz plate served as the blank transmittance spectrum.

Measurement of the in vitro SPF

Determination of the in vitro SPF is based on UV transmission measurements through a layer of sunscreen applied on a suitable UV transparent substrate, and is described by the following relationship [21]:

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

where, $s_{\text{er}}(\lambda)$ is the erythema action spectrum [2], $S_s(\lambda)$ is the spectral irradiance received from the UV source [2], and $T(\lambda)$ is the light transmittance measured from 290 to 400 nm before and after sunscreen application. We applied 1.3 mg/cm² of sunscreen on the PMMA HD6 plate versus 2 mg/cm² on all of the studied polymer plates. The difference in the amount of product applied is related to the difference in the roughness between the substrates. The casts used for producing the plates of the investigated polymers show a roughness close to that of human skin, which explains the use of the same application amount as for the in vivo SPF determination, equaling 2 mg/cm².

Surface Properties Assessment

Wettability and adhesion properties are dependent on the SFE of the surface. The SFE of a solid can be estimated from the interactions between the surface and probe liquids of different interfacial properties by measuring the contact angle at the solid/liquid interface, and is given in mN/m. Young described the relationship between the contact angle, θ , surface tension of the liquid, γ_L , interfacial tension between the liquid and solid, γ_{SL} , and SFE of the solid, γ_S :

$$\gamma_S = \gamma_{SL} + \gamma_L \cos \theta, \quad (2)$$

where, γ_S and γ_{SL} are unknown. To calculate the SFE of a solid, several approaches have been proposed taking the Young equation as a basis. In our study, the SFE was calculated according to the Owens, Wendt, Rabel, and Kaelble (OWRK) method, which is derived from the Fowkes approach. In this model, the surface tension of the liquid, γ_L , and the SFE of the solid, γ_S , is considered as the

sum of 2 independent components: surface energy due to dispersive interactions and surface energy due to polar interactions, each representing a particular intermolecular force [22, 23]. In this 2-component model, the estimation of the SFE of the solid requires the measurement of the contact angle of at least 2 probe liquids with known dispersive and polar parts of their surface tension in order to calculate the interfacial tension between the solid and a liquid. Subsequently, the contact angle was measured optically using the sessile drop method on the flat surface of the synthetic polymer plates using Easy Drop from the Krüss instrument. Water and diiodomethane were used as polar and apolar probe liquids, respectively. A drop of probe liquid with a volume of 2 μ L was applied to the substrate surface using a microsyringe. Then, after waiting for 5 s, the contact angle was measured using the Tangent Method-2 of the Drop Shape Analysis software. The value used for the calculation of the SFE was the average of 5 individual measurements. We selected a range of SFE values to be targeted for the proposed synthetic substrates (Table 5) based on the SFE values of human skin [24, 25]. The approach for calculating the SFE using the measured contact angle values is given in Appendix 2.

Statistical Evaluation

Statistical analysis was performed using the SAS software package, version 9.4 (TS1M2). The statistical significance of the differences between the SPF values of tested substrates, comprising both experiments done in vitro on polymer plates and in vivo on human skin, was assessed using a Kruskal-Wallis nonparametric test with a 5% confidence level. The Dwass, Steel, Critchlow-Fligner (DSCW) nonparametric 1-factorial comparison test with a 5% confidence level was used to identify substrates that significantly differed from each other.

Results and Discussion

Spectral UV Transparency of New Proposed Synthetic Substrates

To calculate the SPF in vitro (Equation 1) the inverse of the measured UV transmittance ($1/T$) is weighted with the erythral effectiveness spectrum (EES). The EES shows that UVB rays (290–320 nm) are the most erythemogenic rays, with the apex of the EES occurring at around 310 nm [26, 27]. Accordingly, UV transparency especially in the UVB range is a prerequisite for a new polymer substrate for the in vitro testing of sunscreens. A minimal UV transmittance of 50% at 310 nm through the bare polymer plate seems reasonable for sufficient light to be transmitted through the sunscreen-covered polymer plate and detected, ensuring the best determination of SPF in vitro. The measured UV transmittance spectra through the flat surface plates of proposed polymers are displayed in Figure 1. It is evident that UV transmittance profiles strongly differ between the studied polymers. Polystyrene (Polystyrol 495F), polyamide (Ultramid Seal-Fit), polyurethanes (Elastollan 695A10 and Elastollan

Table 5. Surface properties of human skin and targeted range for proposed synthetic substrates

Surface properties of human skin	Selected target range for proposed synthetic substrates, mN/m
Total SFE	31–41
Dispersion fraction	28–37
Polar fraction	2–6

Table 6. Contact angle measurements for the tested substrates

Tested substrate	Contact angle, °		
	water	diiodo-methane	1-bromo-naphthalene
Elvax 150W	94.2±2.20	62.4±0.49	–
Elvax 250A	84.7±0.73	47.4±0.41	–
Elvax 420A	86.4±0.58	45.2±0.75	–
Luwax EVA1	75.0±1.31	39.4±1.12	–
Luwax EVA3	76.3±1.77	38.3±1.57	–
Elvaloy 742	103.0±1.33	71.8±1.66	–
Nucrel 0910	88.4±0.83	50.9±1.39	–
Nucrel 925	88.3±0.62	51.4±1.15	–
Polystyrol 495F ¹	84.2	34.7	–
Ultramid Seal-Fit ¹	91.1	48.5	–
Elastollan 695A10	80.3±1.53	–	13.7±1.02
Elastollan 1185A10	99.7±2.04	–	61.4±1.83
Elastollan L1160D10N	72.3±1.66	–	20.6±2.35
HD6 PMMA plate ²	69.1±0.82	24.4±0.76	–
Pig skin stratum corneum	81.8±2.98	46.0±0.90	–

$n = 5$, unless indicated. ¹ $n = 1$. ² $n = 3$.

1185A10), and the ethylene-vinyl acetate copolymers Luwax EVA1, Luwax EVA3, and Elvaloy 742 show low to zero UVB transmission (290–320 nm). Consequently, this deficit in UVB transparency makes them inappropriate as a new synthetic substrate for in vitro testing of sunscreens. The 2 ethylene-vinyl acetate copolymers Nucrel 0910 and Elvax 420A show a transmittance of approximately 60–65% at 310 nm, whereas the most promising polymers were Nucrel 925, Elvax 150W, and Elvax 250A, with transmittance attaining 80–90% at 310 nm.

The UV transmittance of the most promising candidates (Elvax 150W, Elvax 250A, Elvax 420A, Nucrel 0910, and Nucrel 925) was measured on plates with roughened surfaces obtained with cast 3 to check the impact of the roughening on UV transmittance (Fig. 2). For comparison purposes, the transmittance of a glycerin-covered

Fig. 1. UV transmission through a bare flat plate for the proposed polymers (thickness 1 mm) and through a glycerin-covered plate for the PMMA HelioScreen HD6.

Fig. 2. UV transmission through a bare flat (thickness 1 mm) and roughened plate (cast 3, thickness 2 mm) for the selected polymers and through a glycerin-covered plate for the PMMA HelioScreen HD6.

HelioScreen HD6 PMMA plate is also shown and achieved roughly 80% at 310 nm. Compared to the plate with a flat surface, the roughened plates resulted in a smaller UV transmittance for all polymers. The reason for this is 2-fold. Firstly, the roughened plates were thicker (2 mm) than the pressed flat plate (1 mm), and light transmit-

tance is dependent on the thickness of the layer to be penetrated. Secondly, a roughened surface scatters incident light, resulting in a smaller intensity of light transmitted through the substrate.

Although the UV transmittance of the studied polymers was lowered using roughened plates compared to

Fig. 3. SFE (columns) with SD (bars; $n = 5$) of the investigated substrates. The black rectangle corresponds to the target range of the SFE for the studied polymers. For the PMMA HelioScreen HD6 plate, measurement was performed on the flat reverse side of the plate; for the other studied polymers the measurements were performed on homemade flat surface plates. Values for human skin were obtained from the literature [24, 25].

flat plates, the transmittance was greater than 50% at 310 nm for the Elvax grades and Nucrel 925, and borderline for Nucrel 0910. Thus, in respect of the UV transparency requirement, the 5 retained candidates comprised the 3 ethylene-vinyl acetate copolymers Elvax 150W, Elvax 250A, and Elvax 420A, together with the 2 ethylene methacrylate acid copolymers Nucrel 0910 and Nucrel 925.

Surface Property Assessment of the New Proposed Synthetic Substrates

To estimate the SFE of the proposed polymers, contact angle measurements of the 2 probe liquids, water and diiodomethane, were made. The measurements were performed on the flat surface plates of the tested polymers since surface roughness may affect contact angle measurements [28] and generate misleading results. The measured contact angle values are given in Table 6.

The values of the total SFE, and the dispersive and polar parts of the surface energy are shown in Figures 3–5, respectively, for the pig ear skin, HD6 PMMA plate, and new proposed synthetic polymers. The black rectangles in

Figures 3–5 correspond to the target range selected in accordance with the data reported for human skin [24, 25]. The results show that pig ear epidermis yielded SFE values matching the data available for human skin. This suggests that pig ear skin imitates human skin appropriately in terms of adherence characteristics and confirms its suitability as a substrate for the in vitro testing of sunscreens [13]. However, using this substrate as a model requires time-consuming preparation and is not appropriate for routine tests. Moreover, the standardization of a model from biological origins is rather problematic; therefore, a synthetic substrate is favored. Regarding the HD6 PMMA plate currently used as an in vitro substrate, it exhibited an SFE that was substantially greater than the SFE reported for human skin. Previous work has highlighted the issue of a lack adherence of the sunscreen product on the mold-injected PMMA plates during application, resulting in a nonuniform film and subsequently a poor in vitro SPF for some formulations [16, 17]. Accordingly, the discrepancy observed between SPF in vitro using PMMA plates and SPF in vivo is hypothesized to be

Fig. 4. Dispersive fraction of the surface energy (columns) with SD (bars) for the investigated substrates ($n = 5$). The black rectangle corresponds to the target range of the dispersive part of the SFE for the studied polymers. Values for human skin were obtained from the literature [24].

Fig. 5. Polar fraction of the surface energy (columns) with SD (bars) for the investigated substrates ($n = 5$). The black rectangle corresponds to the target range of the polar part of the SFE for the studied polymers. Values for human skin were obtained from the literature [24].

Fig. 6. Average in vitro SPF (columns) and SD (bars) values for Nucrel and Elvax grades and for the HD6 PMMA plate with 1 operator ($n = 27$ for each). The y axis was purposely cut at the value of 200. The in vivo SPF value was equal to 30.5, the black rectangle box corresponds to the value of the in vivo SPF $\pm 20\%$.

related to the difference in surface properties between PMMA plates and human skin. Conversely, of the 13 proposed polymers, the 2 ethylene-vinyl acetate copolymers Elvax 250A and Elvax 420A, as well as the 2 ethylene methacrylate acid copolymers Nucrel 0910 and Nucrel 925, attained the targeted range set for the total, dispersive, and polar part of SFE. Thus, from the proposed polymers, these were best able to mimic the surface properties of human skin and, therefore, appear to be the most appropriate for the in vitro testing of sunscreens. Despite the high UV transparency of Elvax 150W, this polymer was rejected since the measured SFE values were below the targeted SFE values.

SPF in vitro Determination

From all the investigated polymers, ethylene-vinyl acetate copolymers Elvax 250A and Elvax 420A, in addition to the ethylene methacrylate acid copolymers Nucrel 0910 and Nucrel 925, met the requirements with respect to UV transparency and surface property parameters. Therefore, they served as substrates for the in vitro testing of the performance of a sunscreen formulation. The 4 polymers were mold injected in 3 different casts, producing plates with a flat and a rough side with differing roughness patterns (Table 3), which resulted in a total of 12 variant plates. The SPF in vitro obtained using the 12 plates as well as the HD6 PMMA plate were compared to the SPF in vivo for the tested sunscreen (Fig. 6).

The Kruskal-Wallis test revealed a statistically significant effect of the substrate (comprising in vitro polymer

Table 7. Nonparametric 1-factorial comparison using the DSCF test at a 5% confidence level

Substrate	Cast	Group letter			
In vivo			d	e	g
HD6					h
Nucrel 0910	1			e	
	2			e	
	3			e	g
Nucrel 925	1				g
	2			e	f
	3			f	g
Elvax 250A	1	b	c	d	
	3	a			
Elvax 420A	1		c		
	2	b			
	3	b			

Substrates that were nonsignificantly different from each other with respect to the SPF value share the same letter. $n = 27$ for polymer substrates, $n = 5$ for in vivo testing.

plates and in vivo human skin) on the SPF value ($\log_{10}$ -transformed SPF values). The DSCF method was employed to identify which substrates significantly differed from each other. This test resulted in a group classification in which substrates sharing the same letter are not significantly different (Table 7).

The relative standard deviation (SD) was comprised between 29 and 36%, 16 and 40%, 10 and 56%, and 16 and

32% for Nucrel 0910, Nucrel 925, Elvax 250A, and Elvax 420A, respectively, independently from the cast. However, cast 3 tended to produce a lower SD, with values of 31, 16, 10, and 17% for Nucrel 0910, Nucrel 925, Elvax 250A, and Elvax 420A, respectively. This is similar to the HD6 PMMA plate, which showed a relative SD of 14% for the tested sunscreen.

Of the proposed polymer substrates, the Elvax 250A and 420A plates produced in vitro SPF values that were beyond the required range, at about 5–6 times greater (Fig. 6) than and significantly different (Table 7) from the in vivo SPF, making these polymers inadequate for SPF in vitro testing. In contrast, the in vitro SPF achieved with the Nucrel 0910 and Nucrel 925 polymers did not statistically differ from in vivo SPF independently from the cast (Table 7). In comparison, the available mold-injected HD6 PMMA plate yielded an SPF in vitro value that was significantly lower than the SPF in vivo value (Fig. 6; Table 7).

Taken together, the polymer Nucrel 925 mold injected in cast 3 was the most promising substrate. It allowed a UV transmission value through the roughened plate higher than 50% at 310 nm, reached the target value for the SFE, and produced an in vitro SPF value for the tested sunscreen that was not statistically significantly different from the in vivo SPF value and had a low relative SD. Despite this encouraging outcome, it should be remembered that the present SPF evaluation was carried out us-

ing only 1 O/W sunscreen with 1 operator. Therefore, to validate these findings further investigations are required, such as testing the reproducibility between several operators. It is also mandatory to extend the study to an increased number of formulations differing in their type (W/O, O/W, or monophasic, etc.) or in their viscosity (spray, cream, etc.).

Conclusion

Four out of the screened 14 polymers showed appropriate UV transparency and SFE, including the 2 ethylene-vinyl acetate copolymers, Elvax 250A and Elvax 420A, as well as the 2 ethylene methacrylate acid copolymers, Nucrel 0910 and Nucrel 925. The 2 latter appeared to deliver an in vitro SPF value that did not differ from the in vivo SPF value compared to the conventionally used PMMA plate for the tested O/W formulation. This experiment revealed that using a mold-injection process with a polymer exhibiting surface properties close to human skin can result in plates providing suitable SPF predictions. This work provides new insight into the in vitro testing of sunscreens by showing the possibility of using alternative polymers for producing an appropriate synthetic substrate.

Appendix 1

Composition of the Tested O/W Sunscreen

Phase	Trade name	INCI	% by weight
A	Eumulgin SG	Sodium stearyl glutamate	1.00
	Cetiol CC	Dicaprylyl carbonate	5.00
	Cetiol B	Dibutyl adipate	4.00
	Cutina PES	Pentaerythrityl distearate	3.00
	Uvinul A Plus	Diethylamino hydroxybenzoyl hexyl benzoate	6.00
	Uvinul MC80	Ethylhexyl methoxycinnamate	10.0
	Tinosorb S	Bis-ethylhexyloxyphenol methoxyphenyl triazine	1.80
B	Water	Water	QSP 100%
	Glycerin	Glycerin	3.00
	Keltrol CG-RD	Xanthan gum	0.20
	Edeta BD	Sodium EDTA	0.20
C	Cosmedia ACE	Sodium polyacrylate (and) dicaprylyl carbonate (and) polyglyceryl-3 caprate	1.00
	Orgasol Caresse	Polyamide-5	3.00

Appendix 2

Calculation of the SFE Using the Measured Contact Angle Values according to the OWRK Approach

The SFE of a solid can be assessed indirectly by determining its wettability by measuring the contact angle (θ) of liquids with a known surface tension.

Fowkes divided the SFE of a solid and liquid into 2, dispersive and polar, parts. However, Fowkes expressed the contact angle of a liquid on a solid only in terms of dispersive force contributions, considering the dispersion forces being predominant in the solid-liquid interface, resulting in the following approximation:

$$\gamma_L (1 + \cos\theta) = 2\sqrt{\gamma_S^d \times \gamma_L^d}, \quad (a)$$

where, γ_L is the surface tension of the liquid and γ_L^d the dispersive fraction of the surface tension (or SFE) of the liquid, which are known values, θ is the measured contact angle of the liquid on the solid, and γ_S^d the unknown value for the dispersive fraction of the SFE of the solid. It is possible to determine γ_S^d measuring the contact angle of an apolar liquid.

Using the Fowkes approach, the OWRK determines the dispersive and polar parts of the SFE of liquids and solids, resulting in following equation:

$$\gamma_L (1 + \cos\theta) = 2\sqrt{\gamma_S^d \times \gamma_L^d} + 2\sqrt{\gamma_L^p \times \gamma_S^p}, \quad (b)$$

where, γ_L^p is the polar fraction of the surface tension (or SFE) of the liquid, and γ_S^p the polar fraction of the SFE of the solid.

Example of Calculating the SFE of a Solid Using the OWRK Approach

The values for γ_L^d , γ_L^p , and γ_L of the standard liquids used in our evaluation, water, diiodomethane, and 1-bromonaphthalene, are known and given in the table below (mN/m):

	γ_L^d	γ_L^p	γ_L
Water	21.8	51	72.8
Diiodomethane	48.5	2.3	50.8
1-Bromonaphthalene	44.6	0	44.6

Firstly, using an apolar liquid, the polar fraction ($2\sqrt{\gamma_L^p \times \gamma_S^p}$) in Equation b equals zero and the dispersive fraction γ_S^d of the SFE of the solid can be determined:

$$\begin{aligned} \gamma_L (1 + \cos\theta) &= 2\sqrt{\gamma_S^d \times \gamma_L^d} \\ \gamma_S^d &= [\gamma_L (1 + \cos\theta) / 2\sqrt{\gamma_L^d}]^2. \end{aligned}$$

Secondly, using a polar liquid, the polar fraction γ_S^p of the SFE of the solid can be calculated:

$$\begin{aligned} \gamma_L (1 + \cos\theta) &= 2\sqrt{\gamma_S^d \times \gamma_L^d} + 2\sqrt{\gamma_L^p \times \gamma_S^p} \\ \gamma_S^p &= [(\gamma_L (1 + \cos\theta) - 2\sqrt{\gamma_S^d \times \gamma_L^d}) / 2\sqrt{\gamma_L^p}]^2. \end{aligned}$$

Finally, γ_S , the total SFE of the solid, can be calculated:

$$\gamma_S = \gamma_S^d + \gamma_S^p.$$

Acknowledgements

I would like to thank Oliver Reich for the preparation of the flat substrates, and express a big thank you to Susanne Zeiher for her great support in the preparation of the molded plates.

Also, thanks to Bernd Herzog and Marcel Schnyder for their valuable input, and to Andreas Buechse for his support in the statistical evaluation. Finally, a thank you to Sophie Bistac-Brogly and Jean-François Stumbe from the LPIM Université de Haute-Alsace, Mulhouse-Colmar, France, for the discussions related to this work, as well to the students Cécile Patenôtre and Justine Toubeau de Maisonneuve.

Statement of Ethics

No approval from the ethics committee of animal research was needed for this study as the ears were taken from pigs not slaughtered for the purpose of this study.

Disclosure Statement

The authors declare no conflicts of interest.

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