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A new chemical approach to optimize the *in vitro* SPF method on the HD6 PMMA plate

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Synopsis

In a previous study, we demonstrated that control of the roughness of molded PMMA plates improves *in vitro* SPF reproducibility. However, *in vitro*/*vivo* deviations are still observed.

Sunscreens show different behavior during spreading on the HD6 surface according to the formulation, resulting in a more or less homogenous distribution. The hydrophilic nature of HD6 appears to contribute significantly during spreading. Two different sunscreens offering a homogenous and non-homogenous distribution were investigated to check if the interfacial tension between product and substrate has a real influence on the spreading quality. Using microscopic observations, we attempted to correlate the *in vitro* SPF results with the product's spreading property.

In order to reduce this interfacial tension, an HD6 pretreatment with an amphoteric surfactant, cocamidopropyl betain, was performed. *In vitro* SPF on "pretreated HD6" was examined using a cohort of 30 products. This pretreatment led to reliable results, demonstrating good association with the *in vivo* SPF.

INTRODUCTION

The challenge facing the cosmetics industry is to develop innovative, efficient, sunscreen products that conform with European recommendations with a minimum of time and cost. In this regard, a reliable spectroscopic *in vitro* method is an essential tool. The advantages are well known. It is fast and relatively inexpensive, and moreover it offers complete spectral information on products. And importantly, there are no ethics problems (1–3).

The EU recommendation on sun protection products, published in September 2006, recommends the use of *in vitro* methods. Although Colipa succeeded in establishing a UVA *in vitro* method (1), the *in vitro* SPF determination remains a challenge. Indeed, different working groups such as ISO, DGK, and even Colipa concentrated their efforts on developing an *in vitro* reliable method with little success. All the ring tests performed led to the same conclusions: whatever the method used or the parameters chosen, inter-laboratory variability remains a major problem.

The actual situation is the following: The *in vitro* SPF determination is accessible to every laborator, but the lack of control of the variables influencing the results can lead to poor results. The technique is very sensitive to different parameters (4). This may explain the

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deviation sometimes noted between the *in vitro* and *in vivo* results. Among the factors impacting on the *in vitro* SPF results, the most common are the device used to measure the transmission spectra, the amount of product applied, the application, the experience of the operator in the spreading process, and the substrate used to apply the product, or more precisely its roughness characteristics.

One objective of Coty is to control more carefully these factors. Particular attention is paid to the substrate used for *in vitro* tests. In a previous study, we demonstrated how *in vitro* SPF reproducibility (5) can be improved by controlling the substrate's surface microtopography of the injected PMMA plates. As the roughness affects the SPF value, this factor must be fully controlled (6), but in spite of a constant PMMA roughness control, *in vitro*/*vivo* deviations are still observed. These experiments showed different product behavior during the product's application on the surface of the HD6 PMMA, although the roughness remained constant.

These phenomena clearly show that the physical properties of the PMMA molded plate are essential but are not the only substrate characteristics that play a key role in SPF results. In the course of our research on improving the *in vivo*/*vitro* correlation, the "physicochemical aspect" of the spreading process on the HD6 substrate was investigated. The cases of two different sunscreens offering a good and a bad adherence on the chosen substrate were compared. The objective was to study how the interfacial tension (I.T.) between product and HD6 molded plates can affect SPF results.

The following study raises the possibility of modifying this I.T. by the use of a specific HD6 pretreatment with an amphoteric surfactant, cocamidopropyl betain. This paper describes how the amphoteric pretreatment led to a more universal *in vitro* SPF method suitable for every type of product tested, resulting in more reliable results.

MATERIALS AND METHODS

SUBSTRATE USED FOR *IN VITRO* SPF MEASUREMENT

At the present time, PMMA is internationally recognized as a reliable substrate for *in vitro* sunscreen assay (1). The first generation of PMMA plates was sandblasted, but in 2008 a new type of PMMA plate was introduced using injection molded manufacturing. Such PMMA plates offer a better batch-to-batch reproducibility. For this reason, the present study used the high-roughness molded PMMA plates supplied as Helioplate® HD6 (Helioscreen®, Creil, France). Each batch is validated by a control chart including ten roughness parameters (5).

SUNSCREEN PRODUCTS SELECTED FOR *IN VITRO* SPF EVALUATION ON HD6

The *in vitro* SPF values of two different sunscreens, A and B, were investigated on the HD6 with exactly the same spreading procedure. Product A is an O/W emulsion, whereas product B is a polymer gel. Both products were selected for this study because of their different *in vitro* behavior during spreading on HD6 as described in Table I. The second criterion of selection was the comparison of the *in vivo* to the *in vitro* SPF values.

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Table I
Sample A and B Description

Products	*In vivo* SPF	Base	Adherence product/substrate	*In vitro* SPF on HD6 vs *in vivo*
Sample A	16	Steareth-21, Steareth-2	Bad	Poor correlation
Sample B	30	Acrylate polymer	Good	Good correlation

STUDY OF AN AMPHOTERIC HD6 PRETREATMENT

Surfactants properties. The aim of the present study was to control more reliably the interaction between products and the HD6 substrate during spreading. In this regard, the properties of the surfactant were used in order to modify the molded PMMA plates on a more strongly hydrophilic surface. Indeed, surfactants are usually amphiphilic compounds (containing both hydrophobic and hydrophilic groups). Very few amounts of surfactant can modify the interfacial tension, particularly those of water, by adsorbing at the interfaces thanks to their amphoteric nature. Interfaces are characterized by an interfacial level of energy that depends on the properties of the two separated phases (chemical composition and the nature of both phases) (7–10). As a result of their properties, surfactants are able to increase surface wetability, which corresponds to the ability of a drop to spread on a solid surface (Figure 1).

Wettability properties have great application in the areas of painting and surfaces (11,12), and the interfacial tensions between the different phases are related by the following equation:

$$\gamma_{SL} = \gamma_{SV} - \left(\gamma_{LV} * \cos\theta\right)$$

where

γ_{SV} = interfacial tension of the interface solid/gas
γ_{SL} = interfacial tension of the interface solid/liquid
γ_{LV} = interfacial tension of the interface liquid/gas

The deposition of a drop of sunscreen on the HD6 offers different degrees of wettability according to the product's nature: the more the contact angle formed on the PMMA

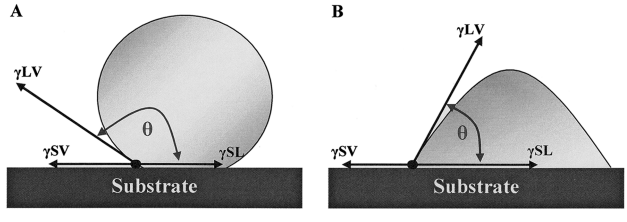

Figure 1. A. High interfacial tension. B. Low interfacial tension.

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increases, the more the cosine of this angle becomes small or even negative, and therefore the more the interfacial tension of the interface solid/liquid is important. Conversely, the more the contact angle decreases, the more the interfacial tension of the interface solid/liquid also decreases.

Wettability measurements. Wettability of untreated and pre-treated HD6 was tested by deionized water to evaluate the properties of the surface and the variation brought by the amphoteric treatment. Then the contact angles (θ) formed after the deposition of A and B on the HD6 with and without pretreatment were measured to compare the interfacial tension of both products according to the substrate properties.

Measurements were performed by the Laboratory of Chemistry of Organic Materials and Metal, University of Nice-Sophia Antipolis. The contact angles (θ) were measured by a goniometer (Krüss DSA-10 contact angle goniometer). Drops of water and cream were deposited using a syringe controlled by a computer (fixed volume) on the surface of the HD6 plate. The contact angle was determined from images captured by the computer via a camera (software drop shape analysis).

SURFACTANT SELECTED FOR HD6 PRETREATMENT, COCAMIDOPROPYL BETAIN

The amphoteric surfactant, cocamidopropyl betain (C.B.) (Figure 2) was selected as a pretreatment to decrease the hydrophobicity of the HD6 PMMA plates. (commercial name: TEGO® Betain F50). Supplied by Evonik Goldschmidt Industries, cocamidopropyl betain is more highly concentrated than common products incorporating 30% of raw material (13).

IN VITRO SPECTROSCOPIC MEASUREMENTS ON HD6 AND PRETREATED HD6

All the spectroscopic data used in this study were based on transmission measurements of suncare products applied on HD6 with or without amphoteric pretreatment. The transmission spectrum for each area measured was determined, and then absolute protection factors like SPF were calculated by combining the UV transmission spectrum of the sunscreen preparation with a specific biological action spectrum and a relevant sun emission spectrum.

Operating conditions. A Labsphere® UV-2000 S Transmittance analyzer was used in the determination of the diffuse transmission spectrum of UV radiation through the substrate

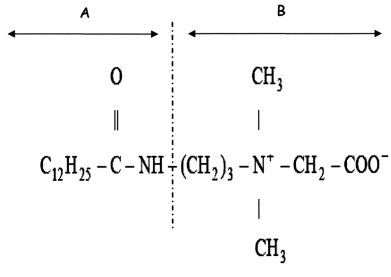

Figure 2. Chemical structure of cocamidopropyl betain. A. aliphatic tail. B. Polar head.

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before and after application of the sunscreen. A PMMA plate covered with 10 mg of C.B. was used to obtain the blank transmittance spectrum from 290 to 400 nm in steps of 1 nm. Preliminary studies showed that the optimal amount of sunscreen per surface unit on these high-roughness plates is 1.3 mg/cm^2 (5).

Twelve milligrams of C.B. were spread with a saturated fingercot until a homogenous distribution was achieved over the whole surface. A period of ten minutes is necessary for C.B. stabilization. Samples A and B were applied in parallel on the HD6 PMMA with and without C.B. pretreatment.

The sunscreen product was spread over the whole surface by means of light strokes with a fingertip "presaturated" with the product. The different formulae studied were spread with the same protocol. The sample thus obtained was allowed to settle for 15 minutes in the dark at room temperature to ensure a leveling of the formula. A total of 9 UV transmission spectra (from 290 to 400 nm, 1-nm increment steps) were recorded on each plate at different locations.

ROUGHNESS MEASUREMENT OF FILM PRODUCT SPREAD ONTO HD6 PLATES

Roughness of the substrate. The roughness of the HD6 substrate was measured by noncontact surface topographic analysis using an "Altisurf 500" Lab-workstation from Altimet™, France. The system is composed of an optical sensor, a motion controller, an x-y translation stage, and a microtopography software Mountain Altimap module. The confocal optical sensor "Altiprobe" is based on a white light chromatic aberration principle that allows a high resolution: 10 nm vertical and 1 μm horizontal. Five different areas of exactly 10×5 mm were analyzed according to the scheme illustrated in Figure 3.

Surface topography parameters. As described in our previous publications (5,14), the control chart enables the roughness of an HD6 surface to be characterized. In the present publication, the control chart of the HD6 substrate with and without C.B. pretreatment was determined. For each covered PMMA plate, five areas were analyzed. We obtained the respective values for the ten parameters for every location, following which we determined the mean of five values obtained for the five different sites studied.

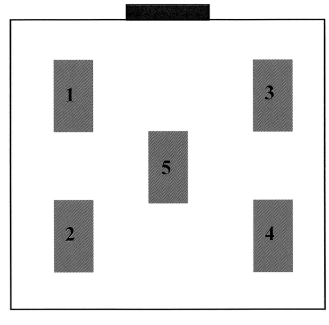

Figure 3. Localisation of the five areas measured with an "Altisurf 500" Lab-workstation.

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MICROSCOPIC ANALYSIS

The behavior of samples A and B during spreading were followed by microscopy to understand more fully the role of pretreatment on product distribution. The microscopic observations were performed with a microscope (Axio Imager, Carl Zeiss S.A.S.) in conjunction with a camera (Axio Cam MRc.) The images were then analyzed with Axio Vision software.

RESULTS AND DISCUSSION

Different *in vitro* methodologies have already been proposed by the industry to assess the *in vitro* SPF of suncare products, but all the methods proposed showed poor correspondence between *in vitro* and *in vivo* values. The limitations of the existing *in vitro* methods were explored and showed the importance of the substrate used.

A previous study, using the injected molded plates, revealed that results can be improved by controlling the physical characteristic (the roughness) of the plates. Nevertheless, this step seems to be necessary, but not sufficient, to have complete control of the technique, as HD6 fails to reproduce the *in vivo* results in some cases.

The question is why the HD6 sometimes fails to give reliable results. The present study investigates the chemical aspect of the spreading of different products on HD6 plates, offering either poor or good correlation with the *in vivo* results. The aim is to compare the interactions between product and substrate in both cases.

First, the criteria of transparency and roughness were controlled on both types of plates (treated and untreated) in order to reproduce exactly the same experimental conditions and adhere to the Colipa guidelines concerning the transparency and the roughness control chart (5).

OPTICAL TRANSMISSION GUIDELINES

According to Colipa guidelines, the minimum average optical transmission requirement through a substrate treated with glycerin is 60% at 290 nm, 69% at 300 nm, and 81% at 320 nm. The present study complied with these recommendations. The optical characteristics of cocamidopropyl betain are compared with glycerin in Table II.

The results of transmission obtained with the cocamidopropyl betain-treated HD6 plates showed that the transparency required is appropriate for UV analysis. This amphoteric pretreatement can be introduced in an *in vitro* UV spectroscopic method.

Table II
Percentage of Transmission at 290 nm, 300 nm, and 320 nm for the PMMA Plate HD6 Treated with C.B.

Treatment	PMMA HD6 + glycerin			PMMA HD6 + TegoBetain F50		
Wavelength (nm)	290	300	320	290	300	320
% Transmittance	66.7	71.8	83.1	68.5	73.6	84.9

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INFLUENCE OF C.B. PRETREATMENT ON ROUGHNESS OF THE HD6 SUBSTRATE

The second criterion checked was the surface roughness in the presence of the cocamidopropyl betain film. The values obtained (Table III) were compared with those obtained with a petrolatum film applied on the HD6. The amphoteric pretreatment has a lower influence on the roughness substrate compared with petrolatum pretreatment. The C.B. treatment makes a very thin film whose roughness will not affect the SPF results.

INFLUENCE OF C.B. PRETREATMENT ON THE WETTABILITY OF THE HD6 SUBSTRATE

The contact angles between a 10-µl drop of ionized water and HD6 PMMA plates with and without pretreatment were measured. The values (Table IV) show the hydrophilic nature of PMMA (contact angle of 63°). In the presence of C.B. pretreatment the substrate becomes highly hydrophilic (θ=14.9).

INFLUENCE OF C.B. PRETREATMENT ON THE SPREADING OF TWO SELECTED PRODUCTS

SPF investigations were performed with two different bases, an O/W emulsion and a gel. Both these formulae were chosen according to their respective poor and good correlations with the *in vivo* values.

Sample A with poor in vitro/vivo correlation. Product A is selected in this study because HD6 plates failed to reproduce the *in vivo* SPF value. It is interesting, however, to measure the SPF of such a product on the amphoteric pretreated HD6 in order to compare the results. The *in vitro* SPF results (Table V) obtained with the same operator on untreated plates (5.59) were much lower than those obtained on the pretreated HD6 (18.46), which are in line with the *in vivo* values. It is therefore necessary to understand why untreated HD6 failed to give a result close to the *in vivo* value, whereas it works on the pretreated plates in the case of sample A.

Table III
Mean Values of the Different Roughness Parameters for the Untreated PMMA and Pretreated PMMA Plates

Roughness parameters	Ra	Rp	Rv	Rdq	Rsk	Rku	A1	A2	Ssc	Vvv
Untreated PMMA plate	4.70	10.80	12.83	10.60	-0.22	3.64	213.83	550.83	0.032	9.50E-07
PMMA plate + TegoBetain F50	3.54	9.38	8.43	6.91	0.30	3.87	309.40	296.00	0.017	6.54E-07
PMMA plate + petrolatum	2.42	6.85	4.57	3.59	0.78	4.45	279.00	99.46	0.008	4.69E-07

Table IV
Values of the Contact Angle (θ) between Deionized Water and the Substrate before and after Pretreatment

10 µl deionized water	Untreated HD6	Pretreated HD6
Contact angle θ	63	14.9

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Figure 4a shows a clear separation of both phases of emulsion A, in contact with the molded plate. After stabilization, it results in multiple non-covered areas; the poor coverage of the surface may explain why the SPF value is lower than the results expected with the filters used (Table V). A poor distribution reduces the intensity of the absorption (15). Figure 4b shows that in the presence of C.B. pretreatment, a slight separation of the product phases during spreading on the PMMA is observed by microscopic visualization. After stabilization, it results in a uniform covered area and a good filter distribution, which could explain the higher SPF corresponding to the *in vivo* value.

WETTABILITY MEASUREMENTS BETWEEN SAMPLE A AND THE UNTREATED AND PRETREATED HD6

The contact angles between sample A and the HD6 plates were measured with and without C.B. The values reported in Table VI significantly decrease in the presence of C.B., reflecting a better contact between product A and the HD6 PMMA substrate in the presence of the 12-mg C.B. treatment. The C.B. pretreatment improves the attraction of sample A on the PMMA surface, resulting in a better distribution and a higher *in vitro* SPF.

Sample B with good in vitro/vivo correlation. In contrast to sample A, sample B yields results in line with the *in vivo* value (Table VII) whatever the level of the hydrophilicity of the HD6.

MICROSCOPIC OBSERVATIONS

In the case of untreated as well as treated PMMA plates, both phases of product B remain homogenous (Figure 5) during the spreading on PMMA. After stabilization, it results in a homogenous film distribution. The distribution of sample B is homogenous in both cases, which explains the similar SPF values.

In the case of sample B, the wettability measurements are also in accordance with the *in vitro* SPF results. In fact, the lowest value of the contact angle measured on an untreated

Table V
Mean Values of *In Vitro* SPF for Sunscreen Product A

Sample A (*in vivo* SPF = 16)	Untreated PMMA plate	PMMA plate + TegoBetain F50
Mean SPF	5.59	18.46
Std. SPF	0.36	1.2
Cov. SPF	6.50%	6.52%

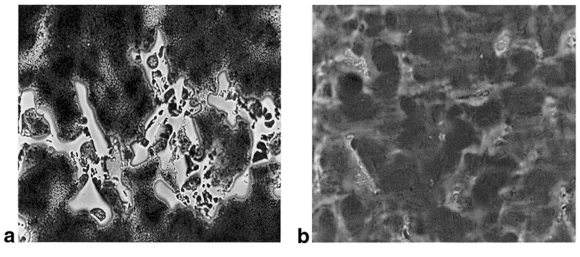

Figure 4. (a) Microphotography on untreated HD6. (b) Microphotography on C.B.- treated HD6.

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PMMA plate is significantly different from the value obtained with sample A. Therefore, the lowest interfacial tension of the interface solid/liquid explains the good wettability of sample B when it's applied on an untreated PMMA plate (Table VIII). The examination of this value between the sunscreen product and the PMMA surface after C.B. pretreatment explains the increase in the homogeneity of the sunscreen and the lower covariance obtained (large decrease in the contact angle). In addition, the finding of an SPF value almost equivalent with or without pretreatment for sample B demonstrates that the C.B. film does not affect the microtopography of the substrate and, as a consequence, conforms with the criteria of the control chart.

WETABILITY MEASUREMENTS BETWEEN SAMPLE A AND THE UNTREATED AND PRETREATED HD6: EXTENSION OF THE COHORT OF FORMULATIONS

The study was extended to 30 sunscreen formulations coming from internal development, as well as to competitive products, in order to have a wide range of galenic properties. The *in vivo* SPF value or claimed SPF is known for each formulation. When one considers the protocol without C.B. pretreatment, even if the R^2 is low (0.17), the overall

Table VI
Values of the Contact Angle (θ) between Sunscreen Product A and the Substrate before and after Pretreatment

Sample A	Untreated HD6	HD6 + C.B.
PMMA plate n° 1	63°	45°
PMMA plate n° 2	64.4°	47°
PMMA plate n° 3	62.8°	51°
Mean of the contact angle (θ)	63.4°	47.6°

Table VII
Mean Values of *In Vitro* SPF for Sunscreen Product B

Sample B (*in vivo* SPF = 30)	Untreated PMMA plate	PMMA plate + TegoBetain F50
Mean SPF	35.22	36.91
Std. SPF	3.69	1.17
Cov. SPF	10.49%	3.16%

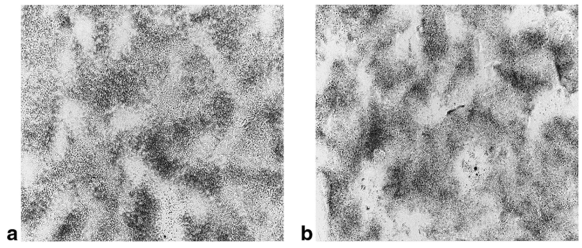

Figure 5. (a) Microphotography on untreated HD6. (b) Microphotography on pre-treated HD6.

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Table VIII
Values of the Contact Angle (θ) between Sunscreen B and the Substrate before and after Pretreatment

Sample B	Untreated PMMA plate	PMMA plate + TegoBetain F50
PMMA plate n° 1	56.7°	44.8°
PMMA plate n° 2	53°	40.5°
PMMA plate n° 3	58.2°	42.6°
Mean of the contact angle (θ)	55.9°	42.6°

correlation for 21 of the 30 products tested is acceptable. Only nine products appeared to be outliers.

The significant improvement in the *in vitro*/*vivo* correlation by the use of the pretreatment is shown in Figure 6, which demonstrates that the C.B. limits the aberrant values sometimes obtained with the classic application procedure of *in vitro* SPF evaluation. The pretreatment investigation shows the benefits in the quality of spreading in terms of interfacial tension between product and substrate, and in wettability during spreading on the PMMA plate. Hence there is a better *in vitro*/*in vivo* correlation.

CONCLUSIONS

In vitro spectroscopic methods are very useful tools in the development of sunscreen products. The goal of industry is to find a universal SPF *in vitro* method. Nowadays it remains a challenge because of the multiple parameters that impact upon the results. Among these factors the substrate used is one of the most important, which is why a big effort was made to understand more clearly its role.

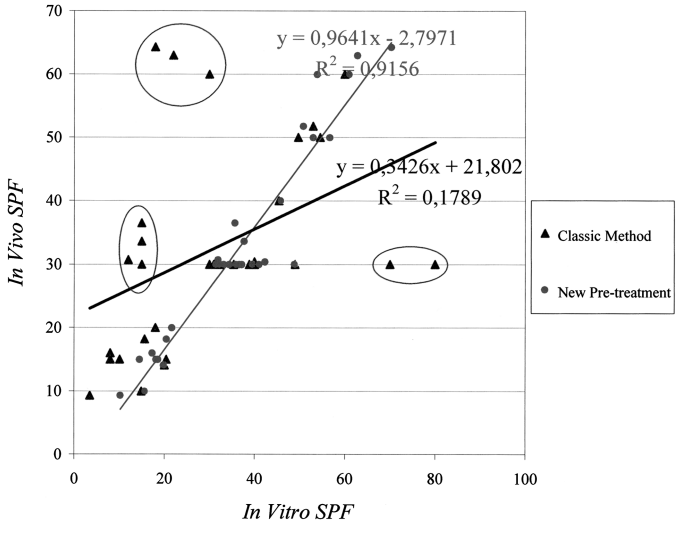

Figure 6. Correlation of *in vivo*/*in vitro* SPF: untreated HD6 and pretreated HD6.

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A previous study showed the importance of the physical characteristics of the surface substrate used. The reproducibility of the *in vitro* SPF results can readily be improved by controlling roughness by using the HD6 molded plates. Although the method proposed on the HD6 plates showed good correlation with the *in vivo* results for the majority of products, we still observed deviations for certain types of products.

In order to go further into the reliability of the method, we investigated the behavior of the formulae on the HD6 during spreading. Upon consideration of the hydrophilicity level of the molded plate, the present study was inspired by the interfacial tension phenomena that are present between product and substrate. With the aim of modifying this interfacial tension, an amphoteric pretreatment with C.B. was investigated.

The results obtained showed the benefits in the spreading quality and, as a consequence, in the *in vitro*/*in vivo* correlation. In the case of high interfacial tension between product and HD6 PMMA plates, it will decrease to enable better adherence so as to obtain suitable spreading. In the case of low interfacial tension, it isn't affected, but it slightly improves the repartition of the vehicle, resulting in better homogeneity of the results on the whole surface without any influence on the SPF result. With this pretreatment, the chemical characteristics of the spreading were improved in terms of wettability and in adherence of the product onto the HD6 PMMA. Of the 30 products tested, the C.B. pretreatment improved the results for the nine non-correlated SPF values obtained without pretreatment. Interestingly, the C.B. application had no effect on the SPF values of the 21 well-correlated products. The C.B. pretreatment is, however, suitable for both correlated and non-correlated products.

The control of the chemical aspect of spreading enabled us to access a more universal method, adapted for each type of vehicle (O/W, W/O, gel, oil). The future goal is to evaluate the pretreatment technique at different laboratories in order to extend the results and validate inter-laboratory variability.

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