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Comparison of two *in vitro* methods to evaluate the water resistance of sunscreens

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For a long time, the water resistance of sunscreens has been determined *in vivo*, according to Colipa's (Comité de Liaison des Industries de la Parfumerie) procedure. This method is not so ethical as healthy volunteers are irradiated, and can be replaced by an *in vitro* method which is easy and quick to perform. The objective of this work was to correlate the experimental device proposed by Choquenet *et al.* and the dissolutest (Sotax® AT6). This equipment is used in the pharmaceutical industry to control the tablets. The experimental conditions have been fixed to correlate the results obtained with both methods. The stirring speed for the dissolutest was fixed at 75 rpm, which is the speed value recommended by the European Pharmacopeia to study the dissolution over time of tablets.

1. Introduction

To stay as long as possible on the surface of the skin, a sun-care product should be water resistant, in order to resist bathing as well as sweat (Leroy and Deschamps 1986; Moloney et al. 2002; Poh Agin 2006). It is good to remember that sweat is 99% water in composition (Saga 2002). Currently, the method used predominantly to determine the water resistance is the one proposed by the Colipa. It is an *in vivo* method which can raise ethical issues as the subjects are exposed to irradiation. A certain number of *in vitro* procedures have been put in place to avoid volunteers' irradiation; additionally costs are decreasing with reducing the duration of the test. Another important point to take into account is that this *in vitro* method can be used during the development of a sunscreen (Stokes et al. 1998; Pissavini et al. 2007; Ahn et al. 2008).
We compared the previous established procedure (Choquenet et al. 2008) with an experimental method using a common device of the pharmaceutical industry, the dissolutest.

2. Investigations and results

2.1. Results concerning the efficacy

The sun protection factor data and critical wavelengths (λ_c) obtained before immersion are presented in Table 1. The data obtained for the benchmarks tested matched the values claimed on the packaging. For the water resistance standard formulation Colipa, the results were identical to the Colipa requirement, namely a SPF between 12 and 15.
Concerning the sunscreens formulated in the laboratory, we observed that the excipient influenced the SPF obtained. With the same combination of sun filters, the SPF measured could vary from 53 to 65. The consequences will be important: it will determine the category of the sun products tested. If the SPF measured is 53, the value claimed would be 50 (high protection); in case of 65, it would give a SPF 50 + (very high protection).

Table 1: Efficacy measured of the formulations tested

	Bioblock IK mixer		Sotax	
Products	$SPF_0 \pm SD$	λ_c (nm)	$SPF_0 \pm SD$	λ_c (nm)
Colipa standard	12.95 ± 2.85	353	14.00 ± 1.51	353
Nivea SPF 50 +	65.58 ± 7.39	381	61.88 ± 7.85	380
Uriage SPF 50 +	74.26 ± 9.67	381	74.16 ± 7.95	381
Vichy SPF 50 +	72.69 ± 9.32	377	75.16 ± 12.62	377
Noreva SPF 50 +	62.76 ± 4.88	381	65.85 ± 6.97	381
Bioderma SPF 20	28.46 ± 4.18	380	27.14 ± 3.52	380
Emulsion 1	64.91 ± 6.52	375	63.44 ± 8.15	375
Emulsion 2	56.54 ± 5.7	375	58.71 ± 7.22	374
Emulsion 3	56.97 ± 4.83	374	52.79 ± 4.57	375

2.2. Results concerning water resistance

In each case, the prerequisites have been verified. In fact, the measurement uncertainty was systematically below 17% of the SPF, and the standards deviations measured were small.
The water resistance data after 40 min are presented in Table 2, in percentage. We can conclude that the two methods correlate, as the percentages of the water resistance obtained are very similar. Not all the raw materials claiming a water resistance property were found to have it (Table 2). The Antaron® V-220 does not present any interest in the formulations of sunscreens as its efficacy in terms of water resistance has not been demonstrated; moreover it is potentially allergenic (Gallo et al. 2004). Only Cosmedia® DC can increase the water resistance of the formulations by 25%.

Table 2: Results of water resistance

	Bioblock IKA mixer	Sotax
Products	WRR_{40} (%)	WRR_{40} (%)
Colipa standard	65	69
Nivea SPF 50 +	88	86
Uriage SPF 50 +	91	92
Vichy SPF 50 +	138	129
Noreva SPF 50 +	96	98
Bioderma SPF 20	109	102
Emulsion 1	63	63
Emulsion 2	79	72
Emulsion 3	54	58

3. Discussion

In the cosmetic industry people are looking more and more at alternative methods to measure the efficacy and the water resistance of suncare products. Concerning the water resistance, different procedures have been proposed to correlate with the *in vivo* method. Certain parameters are essential: the immersion time and the stirring inside the bath vessel (Gupta and Zatz 1999), and the temperature should be constant and identical as the one requested in the *in vivo* procedure (Stokes and Diffey 1999).

This work allowed us to correlate the use of the dissolutest with an established method.

4. Experimental

4.1. Chemicals

The composition on sun filters of the benchmarks is presented in Table 3. The formulation proposed by the Colipa as a standard for the water resistance test and the emulsions prepared in the laboratory are described in Table 4 and Table 5 respectively. Two film former hydrophobic polymers, Cosmedia® DC and Antaron® V-220F (Table 6), claiming to increase the water resistance of sunscreens, have been incorporated in the formulation developed in the laboratory, at 3 and 5% (m/m) respectively as recommended by the suppliers, and tested.

Table 4: Colipa's water resistant reference sun product

Ingredients (INCI name)	% (m/m)	Suppliers
Lanolin	4.50	Cooper
Theobroma Cacao	2.00	Sederma
Glyceryl Stearate	3.00	Gattefossé
Stearic Acid	2.00	Cooper
Octyldimethyl PABA	7.00	Symrise
Benzophenone-3	3.00	Symrise
Sorbitol (70%)	5.00	Roquette
Sodium Propylparaben	0.10	Merck
Sodium Methylparaben	0.30	Cooper
Triethanolamine	1.00	Cooper
Benzyl alcohol	0.50	Janssen Chemica
Aqua	qsp 100.00	

4.2. Water resistance determination

The emulsions developed in the laboratory were prepared as described: the lipophilic and hydrophilic phases were heated separately in a waterbath (Memmert, Grosseron, St Herblain, France). When both phases are at the same temperature, they are mixed together under a stirrer (Bioblock IKA Yellow Line OST Basic Overhead stirrer), under 800 rpm speed. The filters and polymers are incorporated inside the oil phase.

To measure the water resistance, all the products tested are applied on a 25 cm^2 polymethylmethacrylate plate (Europlast, Aubervilliers, France), with a roughness of 6 microns, at the rate of 0.6 $mg.cm^{-2}$, as described previously (Couteau et al. 2007). Whatever stirring devices used, the plates are immerged inside a waterbath with a controlled temperature of $29 \pm 2\,^{\circ}C$ 2 times, during 20 min, with a drying phase in between.

Two different stirring devices have been tested (Fig.). The first one is a polycarbonate bath (Cuve IKA® Werke EH4.1, Grosseron, St Herblain, France) equipped with an immersion thermostat (IKA® Yellow Line Basic EH4 basic immersion thermostat, Grosseron, St Herblain, France [pump type: pressure pump/pump pressure max. (0 litres discharge flow): 0.08 bar/pump pressure max. (0 bar back pressure): 5 L/min]) (Choquenet et al. 2008). The second equipment is commonly in the pharmaceutical industry to test the dissolution of tablets (Sotax AT 6, Sotax AG, Switzerland).

Table 3: Composition of the sunscreens tested

Trade name	Sun filters used
Nivea SPF 50 +	Octocrylene, butylene glycol dicaprylate/dicaprate (and) titanium dioxide (and) silica (and) polyglyceryl-2 dipolyhydroxystearate, butyl methoxydibenzoylmethane, bis-ethylhexyloxyphenol methoxyphenyl triazine, homosalate, sodium phenylbenzimidazole sulfonate, dDiethylhexylbutamido triazone, ethylhexyl methoxycinnamate
Uriage SPF 50 +	Methylene bis-benzotriazolyl tetramethylbutylphenol, octocrylene, ethylhexyl triazone, butyl methoxydibenzoylmethane
Vichy SPF 50 + Kids Lait (very water resistant)	Ethylhexyl salicylate, titanium dioxide, butyl methoxydibenzoylmethane, octocrylene, bis-ethylhexyloxyphenol methoxyphenyl triazine, terephthalylidene dicamphor sulfonic acid, ethylhexyl triazone, drometrizole trisiloxane
Noreva 50 + Spray (water resistant)	Ethylhexyl salicylate, titanium dioxide, butyl methoxydibenzoylmethane, octocrylene, bis-ethylhexyloxyphenol methoxyphenyl triazine, terephthalylidene dicamphor sulfonic acid, ethylhexyl triazone, drometrizole trisiloxane
Bioderma Photoderm SPF 20 (water resistant)	Homosalate, octocrylene, butyl methoxydibenzoylmethane, ethylhexyl salicylate, methylene bis-benzotriazolyl tetramethylbutylphenol, phenylbenzimidazole sulfonic acid, bis-ethylhexyloxyphenol methoxyphenyl triazine

Table 6: Raw materials for water resistance

Trade name	Ingredients (INCI name)	Use level (%)	Suppliers
Cosmedia DC	Hydrogenated Dimer Dilinoyleyl/Dimethylcarbonate Copolymer	3.00	Cognis
Antaron V-220F	VP/Eicosene Copolymer	5.00	ISP

(A)

(B)

Fig.: Experimental devices used (a) Bath vessel IKA, (b) Sotax AT6

Before the immersion, and then every 20 min, *in vitro* SPF (Sun Protection Factor) measurements have been done. Three plates were prepared for each product to be tested and 9 measures were performed on each plate. Transmission measurements between 290 and 400 nm were carried out using a spectrophotometer equipped with an integrating sphere (UV Transmittance Analyzer UV1000S, Labsphere, North Sutton, US). The calculations for either term use the same relationship:

$$SPF = \sum_{290}^{400} E_\lambda S_\lambda \Delta_\lambda / \sum_{290}^{400} E_\lambda S_\lambda T_\lambda \Delta_\lambda \quad (1)$$

where E_λ is CIE erythemal spectral effectiveness, S_λ is solar spectral irradiance and T_λ is spectral transmittance of the sample (Diffey and Robson 1989).

Table 5: Sunscreen formulated in the laboratory

Ingredients (INCI name)	% (m/m)	Suppliers
Lanolin	4.50	Cooper
Theobroma Cacao	2.00	Sederma
Glyceryl Stearate	3.00	Gattefossé
Stearic Acid	2.00	Cooper
Octocrylene	8.00	Merck
Diethylaminohydroxybenzoyl Hexylbenzoate	8.00	BASF
Homosalate	4.00	Merck
Octylmethoxycinnamate	8.00	BASF
Sorbitol (70%)	5.00	Roquette
Sodium Propylparaben	0.10	Merck
Sodium Methylparaben	0.30	Cooper
Triethanolamine	1.00	Cooper
Benzyl alcohol	0.50	Janssen Chemica
Aqua	qsp 100.00	

According to Colipa method, the test will be considered acceptable if the 95% confidence interval on the mean SPF_0 is within ± 17% of the mean SPF_0 that is so say with d equal to:

$$\frac{t.s}{\sqrt{n}} \leq 0.17 \times SPF_0 \quad (2)$$

where t is the value from the Student-t distribution table with n-1 degrees of freedom at a probability level $P = 0.95$.
The percentage Water Resistance Retention (%WRR) value for each plate should be calculated according to the formula below:

$$\%WRR = \frac{(SPF_{40} - 1)}{(SPF_0 - 1)}.100 \quad (3)$$

where SPF_{40} is SPF after 40 min water immersion and SPF_0 is SPF initially measured. According to Colipa, a product will be considered water resistant if the value is greater than or equal to 50% after 40 min of immersion.

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