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Development of an *in vitro* test to determine the water-resistance of sunscreens

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The aim of this work was to develop an *in vitro* method for testing the water resistance of sun products. For comparison, we used the *in vivo* method put forward by the Colipa. Standards and commercial products were successive baths. The Sun protection factor of these different creams was determined *in vitro* both before and after the baths. The result was that two successive baths of 20 min in moderately shaken distilled water, at 29 ± 2 °C and may substitute the *in vivo* test.

1. Introduction

External photoprotection, designed to avoid actinic erythema is given by two classes of substances: screens and filters which are incorporated into excipients allowing us to obtain liquid forms (waters and oils), thick forms (gels, milks and creams) and solid forms (sticks). The active ingredients used (filters or screens) must have two particular qualities: they must be residual, that is to say that they must keep their filtration capacity as long as possible after being applied to the skin – this notion of photostability was the subject of a previous piece of work (Couteau et al. 2007a) – and they must be substantive, that is to say that they must be capable of fixing themselves at the level of the superficial layers of the epidermis without penetrating deeply into the skin. This substantivity is closely linked to the water resistance (Diffey 2001; Poh Agin 2006). It is indeed important that the excipient should stay on the skin as long as possible without being eliminated by the bathing water or by sweat (Leroy and Deschamps 1986; Moloney et al. 2002). The current reference method is the Colipa method, which is an *in vivo* method carried out on healthy volunteers. This method is very restrictive and poses an ethical problem due to the fact that the subjects are exposed to radiation. In order to compensate for these two drawbacks, an *in vitro* method of evaluating the water resistance of sun products has been developed by using the reference product recommended by the Colipa and by testing products which we prepared as well as products available on the market.

2. Investigations, results and discussion

The choice of the method of immersion, with or without agitation, using distilled water or salt water, was made by analysing the results obtained in each case (Table 1). The Colipa standard must give a Sun protection factor (SPF) of between 12 and 15 included, which is the case here. The SPF of the standards formulated fulfils the condition of acceptability of the SPF_0 as d is strictly inferior to $0.17 \times SPF_0$. The three protocols used allow us to obtain in each of the 3 cases a value of [%WRR–d] strictly greater than 50%. We chose the most drastic protocol, which was the use of distilled water and shaking.

Table 1: Results obtained according to experimental conditions

	Stagnant distilled water	Distilled water (stirred)	Salt water (stirred)
SPF_0 Mean ± SD	14.78 ± 1.16	15.64 ± 1.14	12.60 ± 1.66
SPF_{40} Mean ± SD	12.81 ± 0.94	10.96 ± 0.90	11.11 ± 1.47
d	0.47	0.46	0.67
$0.17 \times SPF_0$	2.51	2.66	2.14
[%WRR–d]	85.46	67.8	86.78

Table 2: Variation of the PF-UVA of the Colipa standard after immersion

	Distilled water (stirred)
$PF\text{-}UVA_0$ Mean ± SD	4.15 ± 0.18
$PF\text{-}UVA_{40}$ Mean ± SD	2.73 ± 0.13
d	0.07
$0{,}17 \times PF\text{-}UVA_0$	0.67
[%WRR–d]	76.73

Table 3: Results of water resistance concerning the O/W emulsion

	SPF_0 Mean ± SD	SPF_{40} Mean ± SD	d	$0.17 \times SPF_0$	[% WRR–d]
O/W Emulsion (HMS 8%)	3.45 ± 0.30	1.61 ± 0.03	0.12	0.59	24.89

Table 4: Results of water resistance concerning different filters incorporated in the reference formula (UVB range)

Filter (concentration)	SPF_0 Mean ± SD	SPF_{40} Mean ± SD	d	$0.17 \times SPF_0$	[% WRR–d]
Isoamyl p-methoxycinnamate (10%)	11.32 ± 1.24	9.21 ± 0.63	0.49	1.92	79.39
Octocrylene (10%)	14.78 ± 2.33	12.57 ± 1.55	0.92	2.51	83.57
Octyl dimethyl PABA (8%)	11.15 ± 0.53	10.18 ± 0.41	0.21	1.90	90.34
Benzophenone-4 (5%)	4.49 ± 0.31	1.13 ± 0.02	0.12	0.76	3.72
PEG-25 PABA (10%)	4.10 ± 0.16	1.12 ± 0.03	0.06	0.70	3.86

Table 5: Results of water resistance concerning different filters incorporated into the reference formula (UVA range)

Filter (concentration)	P-UVA_0 Mean ± SD	P-UVA_{40} Mean ± SD	d	$0.17 \times$ P-UVA_0	[% WRR–d]
Isoamyl p-methoxycinnamate (10%)	3.05 ± 0.15	2.65 ± 0.08	0.06	0.52	80.47
Octocrylene (10%)	4.08 ± 0.21	3.46 ± 0.21	0.08	0.69	79.82
Octyl dimethyl PABA (8%)	2.22 ± 0.04	2.05 ± 0.04	0.02	0.38	86.06
Benzophenone-4 (5%)	3.09 ± 0.25	1.06 ± 0.11	0.10	0.53	2.84
PEG-25 PABA (10%)	1.60 ± 0.30	1.06 ± 0.01	0.12	0.27	10.00

The notion of water-resistance is defined by the Colipa in the UVB range. It seemed important to us to carry out measures in the UVA range as well, so that we could know the behaviour of a sun product classed as water resistant on the whole of the UV spectrum. The results shown in Table 2 tell us that the Colipa standard is also water resistant in the UVA range.

For the O/W emulsion containing 8% of homosalate, the condition required concerning the confidence interval of the SPF_0 is fulfilled. This O/W emulsion, which washes off with water, turned out indeed to be non-water resistant (Table 3).

Tables 4 and 5 show that the formulae with liposoluble filters proved to be water resistant. This is not the case with the water-soluble filters tested.

The twelve products available on the market which were labelled water-resistant proved to be so, both in the UVB range (Table 6) and in the UVA range (Table 7). In addition, the products comply concerning the SPF/PF-UVA ratio and the critical wavelength (Table 8).

This method, which takes into account the water resistance in the UVB and UVA ranges, could constitute an interesting alternative to the *in vivo* test.

Table 6: Determination of the water resistance of products on the market (UVB range)

Product	SPF_0 Mean ± SD	SPF_{40} Mean ± SD	d	$0.17 \times SPF_0$	[% WRR–d]
1	22.10 ± 3.03	12.59 ± 1.36	1.36	3.76	54.58
2	31.14 ± 4.15	20.70 ± 2.59	1.64	5.29	64.71
3	44.58 ± 4.34	32.41 ± 3.05	2.11	7.58	71.05
4	20.81 ± 2.51	11.71 ± 1.34	1.39	3.54	53.60
5	29.31 ± 3.02	15.78 ± 1.63	1.19	4.98	51.80
6	25.78 ± 3.02	14.92 ± 1.17	1.19	4.38	55.88
7	56.37 ± 6.08	45.64 ± 4.54	3.20	9.58	78.97
8	40.61 ± 7.05	33.00 ± 5.16	2.79	6.90	79.48
9	28.91 ± 3.65	21.21 ± 3.02	1.44	4.91	71.65
10	33.19 ± 3.19	25.00 ± 1.87	1.26	5.64	74.08
11	28.00 ± 1.66	20.40 ± 1.44	0.66	4.76	71.49
12	35.44 ± 2.53	33.74 ± 2.01	1.00	6.02	94.56

Table 7: Determination of the water resistance of products on the market (UVA range)

Product	PF-UVA_0 Mean ± SD	PF-UVA_{40} Mean ± SD	Incertitude	$0.17 \times$ PF-UVA_0	[% WRR–d]
1	13.50 ± 1.43	8.12 ± 0.60	0.68	2.30	56.78
2	15.59 ± 1.52	11.84 ± 1.13	0.60	2.65	74.01
3	20.49 ± 1.76	16.37 ± 1.60	0.70	3.48	78.46
4	12.86 ± 1.66	7.58 ± 0.94	0.66	2.19	55.24
5	15.54 ± 1.38	9.01 ± 0.78	0.55	2.64	54.89
6	16.21 ± 1.47	11.82 ± 0.88	0.58	2.76	70.91
7	42.52 ± 3.98	34.12 ± 2.92	1.97	7.23	78.90
8	20.63 ± 2.57	15.15 ± 1.66	1.29	3.51	71.71
9	9.98 ± 0.94	7.63 ± 0.70	0.37	1.70	73.65
10	11.64 ± 0.77	8.87 ± 0.45	0.30	1.98	73.85
11	9.68 ± 0.46	7.44 ± 0.31	0.18	1.65	74.12
12	13.18 ± 0.83	11.79 ± 0.66	0.33	2.24	88.42

Table 8: Determination of indicators of conformity of products on the market

Product	SPF_0/PF-UVA_0	SPF_{40}/PF-UVA_{40}	Critical wavelength t_0	Critical wavelength t_{40}
1	1.64	1.55	380	380
2	2	1.75	378	379
3	2.18	1.98	379	379
4	1.62	1.54	380	380
5	1.89	1.75	382	383
6	1.59	1.26	378	381
7	1.33	1.34	382	381
8	1.97	2.18	378	376
9	2.9	2.78	373	372
10	2.85	2.82	376	374
11	2.89	2.74	373	372
12	2.69	2.86	377	375

3. Experimental

3.1. Chemicals

Dimethicone (Abil® WE 09) was obtained from Goldschmidt (Montigny-le-Bretonneux, France). Cetiol® HE, stearic acid, lanolin, cocoa butter, glyceryl stearate, glycerin, sorbitol, benzyl alcohol, parabens and triethanolamin (TEA) were purchased from Cooper (Melun, France). Xanthan gum (Keltrol® BT) was obtained from Kelco (Lille Skensved, Denmark). The filters which were tested are shown in Table 9. Polymethylmethacrylate (PMMA) plates were purchased from Helioscience (Creil, France). Powder-free latex finger cots were obtained from Cooper (Melun, France).

Table 9: Origin of the UV filters tested

INCI name	Trade name	Supplier
Homosalate	Eusolex HMS	Merck
Oxybenzone	Néohéliopan BB	Symrise
Octocrylene	Uvinul N539T	BASF
Isoamyl p-methoxycinnamate	Néohéliopan E1000	Symrise
PEG-25 PABA	Uvinul P25	BASF
Octyl dimethyl PABA	Eusolex 6007	Merck
Benzophenone-4	Uvinul MS 40	BASF

Table 10: Water resistant reference sun product

Ingredients	INCI Name	% w/w
Phase 1	Lanolin	4.5
	Theobroma cacao	2.0
	Glyceryl stearate	3.0
	Stearic acid	2.0
	Octyl dimethyl PABA	7.0
	Benzophenone-3	3.0
Phase 2	Water	71.6
	Sorbitol	5.0
	Triethanolamine	1.0
	Methylparaben	0.3
	Propylparaben	0.1
Phase 3	Benzyl alcohol	0.5

3.2. Preparation of water resistant reference sun product

The reference cream was prepared in the laboratory using the formula proposed by the Colipa (Table 10).

3.3. Preparation of sun products

The O/W emulsion usually used in the laboratory (Couteau et al. 2007b) for trials concerning efficacy and in which 8% of homosalate (HMS) was incorporated, was used as a negative control for water resistance. In addition, different filters were tested by incorporating them in the reference cream.

3.4. Study of effectiveness

To develop the *in vitro* test of water resistance, we took inspiration from the protocol proposed *in vivo* by the Colipa. The method is based on the comparison of the SPF determined before and after the immersion of the subjects in a spa, jacuzzi or a bath, at the rate of 2 successive baths of 20 min each (the water temperature being fixed at 29 ± 2 °C) followed by a drying period. For our part, we worked with PMMA plates, a support on which we applied the cream to be tested. Product (30 mg exactly weighed) was spread on PMMA plates over the whole surface (25 cm) using a cot-coated finger. Polymethylmethacrylate (PMMA) plates were purchased from Helioscience (Creil, France). An amount of 15 ± 0.2 mg remained on the finger cot. SPF_0 and $PF\text{-}UVA_0$ of the creams was measured *in vitro* before immersion. 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 or 320 and 400 nm were carried out using a spectrophotometer equipped with an integrating sphere (UV Transmittance Analyzer UV1000S, Labsphere, North Sutton, US). The standard used was the 8% homosalate standard mandated by the US Food and Drug Administration Sunscreen Monograph. The calculations for either term use the same relationship:

$$SPF = \sum_{290}^{400} E\lambda S\lambda d\lambda / \sum_{290}^{400} E\lambda S\lambda T\lambda d\lambda \quad (1)$$

$$PF - UVA = \sum_{320}^{400} E\lambda S\lambda d\lambda / \sum_{320}^{400} E\lambda S\lambda T\lambda d\lambda \quad (2)$$

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).

The PMMA plates, placed on a stainless steel rack, (haemolysis tube rack 12T 3 × 4, Grosseron, St Herblain, France) are immersed in a polycarbonate bath (Cuve IKA® Werke EH4.1, Grosseron, St Herblain, France) equipped with an immersion heater (thermoplongeur Yellow line Basic ET, Grosseron, St Herblain, France) filled with distilled water or salt water simulating sea water, at a rate of 2 successive baths of 20 min each followed by a drying period. This system allowed us to maintain the water temperature at 29 ± 2 °C with the possibility of creating a moderate agitation (pumping head of 5 $L.min^{-1}$). SPF and PF-UVA were then measured again using the same protocol as before. The test was considered acceptable if the 95% confidence interval on the mean SPF_0 was within ±17% of the mean SPF_0 that is so say with d equal to:

$$\frac{t.s}{\sqrt{n}} \leq 0.17 \times SPF_0 \quad (3)$$

where t is the value read in the table of student for n^{-1} degrees of freedom and P = 0.95.

The individual percentage water resistance retention ($\%WRR_i$) value for each plate was calculated according to the formula:

$$\%WRR_i = \frac{(SPF_{40'} - 1)}{(SPF_0 - 1)} \times 100 \quad (4)$$

where SPF_{40} is SPF after 40 min water immersion and SPF_0 is the SPF initially measured.

The mean percentage water resistance retention (%WRR) is expressed as the arithmetic mean of the three %WRR values ($\%WRR_i$).

The 90% unilateral confidence interval for the mean %WRR was calculated as: [%WRR−d] with d calculated as:

$$d_1 = \frac{t_u \times s}{\sqrt{n}} \quad (5)$$

where s is the standard deviation, n the number of plates tested (n = 3) and t_u is t value from the "one-sided" Student-t distribution table at a probability level P = 0.10 and with n−1 degrees of freedom.

According to the Colipa, a product will be considered water resistant if the value for the 90% lower unilateral confidence limit [%WRR−d] is greater than or equal to 50%.

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