A Laboratory Method for Measuring the Water Resistance of Sunscreens

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Normal summer activities put a great deal of stress upon sunscreen products, particularly water exposure through swimming and sweating. Most sunscreen products are therefore designed to be water resistant. Regulatory agencies around the world, including the US Food and Drug Administration (FDA) and the European Cosmetic, Toiletry and Perfumery Association (COLIPA), have defined protocols to assure consumers that a claim of water resistance has been substantiated. Although protocols differ around the world, they all require testing on a significant number of people, which makes the testing expensive and time-consuming. The sunscreen formulator and chemist need a rapid and cost-effective method to determine at least a comparative level of water resistance in the laboratory in order to determine project progress.

A number of papers[1-3] have been published on in vitro methods for the measurement of water resistance. The first challenge for any in vitro method is to select a substrate that properly models at least the physical properties of skin. This requirement eliminates some convenient substrates such as polyethylene film and Transpore tape.[a] Although the latter is used for laboratory SPF determination,[4] its porous nature creates an artificial opportunity for sunscreen retention when immersed in water. Therefore, a number of methods have focused on the use of real skin from both human and animal sources.

For our purposes, actual skin does not meet the criteria for cost-effectiveness due to limited shelf life (decomposition), cost, and/or availability.

Model skin substrates must simulate the heterogeneous mixture of lipid and protein, the overall hydrophobic behavior of the skin, and its topology, which can vary by ±50 microns or more. One of the first to do so was from Charkoudian,[5] who described a composition and process to make a model skin surface. Although this material meets the requirements described above, it lacks the mechanical strength to be manipulated for water-resistance testing.

A material of similar composition is Vitro-Skin[b] from IMS, Inc. This product, made of cross-linked collagen with added lipids, does have mechanical integrity. Vitro-Skin simulates the properties of composition, wettability, pH, ionic strength and surface topography of human skin. Indeed, IMS has developed a water-resistance test based on their Vitro-Skin material using the Optometrics[b] SPF 290 to measure the SPF of the product on the skin before and after water immersion.[6]

We have found that the normally opaque Vitro-Skin becomes optically transparent upon hydration. This lets us use a normal UV-VIS spectrophotometer to measure the sunscreen present before and after water immersion, and allows the use of smaller samples and measurements with greater precision than we experienced with the Optometrics SPF 290. Here we describe this new method and its validation with in vivo data as well as a comparison of some common water-resistant technologies for sunscreen formulations.

Key words

in vitro testing, sunscreen, water resistance, model skin substitute

Abstract

A new laboratory in vitro method for the measurement of sunscreen water resistance uses spectrophotometric analysis of a model skin substitute before and after 80-minute immersion. It gives good correlation with the FDA's 80-minute immersion SPF results (very water resistant). The method is especially good for screening new formulations or water-resistant technologies.

Materials and Methods

Sunscreen compositions: Medium sun protection factor (SPF) sunscreen formulations based on a lamellar gel structuring system (Table 1) were used for the validation of the in vitro water-resistance method described here. The method was then used to examine the effect of some commercial water-resistant technologies in a medium SPF non-ionic formulation (Table 2). This formulation

[a] *Transpore is a registered trademark of 3M Co., St. Paul, MN USA.*

[b] *Vitro-Skin is a trademark of IMS, Inc., Milford, CT USA.*

[c] *Optometrics is a trademark of Optometrics USA, Inc., Ayer, MA USA.*

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was chosen because non-ionic-based emulsions are well known to be difficult to make water-resistant.

Sunscreen preparation: Sunscreen formulations were made using the following procedure. Water and hexylene glycol of Phase A were combined at room temperature. Carbomer was slowly sprinkled onto the surface while stirring. After incorporating all the carbomer, Phase A was heated to 70-75°C with stirring. Phase B was prepared separately, heated to 75-80°C and stirred until uniform. Phase B was slowly added to Phase A with homogenization at 70°C. When the mixture appeared uniform, Phase C was added with homogenization. After achieving uniformity again, the heat was turned off and mixing was switched to sweep at 60°C. Sweep mixing was continued throughout cool-down. Phase D was added with stirring at 45°C. Finally, water was added to make up for loss during heating and stirred to room temperature.

In Vitro Testing Methodology

Before the application of sunscreens, large strips (20x10 cm) of Vitro-Skin N-19 were hydrated following the manufacturer's recommendations (16-18 hours at 90-95% relative humidity at room temperature).

A measured amount (6-7 mg) of formulation was applied on pre-hydrated pieces of Vitro-Skin (28x38 mm) that were mounted in 35 mm slide mounts. The emulsion was carefully spread using a rubber-gloved finger with initial circular and then linear motion for approximately one minute. Samples were then placed in a humidity chamber (90-95% relative humidity at room temperature) for 20 minutes to allow for emulsion coalescence.

Four UV spectra were collected per sample in the wavelength range of 250-350 nm using a spectrophotometer.[d] Each spectrum was collected after a 90° rotation of the sample giving four separate area scans for each sample. Samples were scanned against the untreated reference sample in the reference beam of the two-beam spectrophotometer, as demonstrated in Figure 1. Absorbance readings were taken at 310 and 291 nm and were labeled as the initial absorbance, A_i.

Samples were then immersed in a temperature-controlled water bath (25 ± 0.2°C) for 80 minutes with constant mixing using a paddle type impeller at 50 rpm, as shown in Figure 2. The volume of the water bath was large enough (2000 ml) to prevent a high concentration of dispersed sunscreen and possibility of re-adsorption.

After immersion, the samples were taken out of the water, lightly shaken to remove the largest water droplets and hung in the air in a climate-controlled room at 50% relative humidity for 30 minutes. Afterward, the samples were placed back into

[d] *Cary 1-E Spectrophotometer, Varian Corp., San Fernando, CA USA*

Table 1. Medium SPF sunscreens for in vivo and in vitro water-resistance measurements (percentages are wt %)

		A	B	C	D	E
A	Water (aqua), deionized	69.1%	67.1%	68.1%	68.1%	68.1%
	Hexylene glycol	2.0	2.0	2.0	2.0	2.0
	Carbomer	0.2	0.2	0.2	0.2	0.2
B	Octyl methoxycinnamate	7.5	7.5	7.5	7.5	7.5
	Benzophenone-3	3.0	3.0	3.0	3.0	3.0
	Octyl salicylate	3.0	3.0	3.0	3.0	3.0
	Octyl palmitate	6.0	6.0	6.0	6.0	6.0
	Cetearyl alcohol	0.2	0.2	0.2	0.2	0.2
	Lamellar gel structurant*	3.0	5.0	3.0	3.0	3.0
	PVP/hexadecene copolymer	-	-	1.0	-	-
	PVP/eicosene copolymer	-	-	-	1.0	-
	Tricontanyl PVP	-	-	-	-	1.0
C	Triethanolamine	0.2	0.2	0.2	0.2	0.2
	Water (aqua), deionized	5.0	5.0	5.0	5.0	5.0
D	Diazolidinyl urea (and) iodopropynyl butylcarbamate	0.3	0.3	0.3	0.3	0.3
	Phenoxyethanol (and) isopropylparaben (and) isobutylparaben (and) butylparaben	0.5	0.5	0.5	0.5	0.5
		100.0	100.0	100.0	100.0	100.0

* Stearic acid (and) behenyl alcohol (and) glyceryl stearate (and) lecithin (and) cetyl alcohol (and) myristyl alcohol (and) lauryl alcohol (and) palmitic acid

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the humidity chamber for 120 minutes. Final absorbance readings, A_f, were taken in the same manner as the initial ones. The percentage of water resistance was calculated as $(A_f / A_i) \times 100$.

Samples were run in quadruplicates giving 32 readings (4 samples x 4 orientations x 2 wavelengths) for each formulation tested. It is important that the samples remain hydrated; if they lose too much moisture, the Vitro-Skin becomes opaque and unsuitable for spectrophotometric analysis. Each blank control sample (without sunscreen) was treated and measured in exactly the same way. The control sample was immersed in a separate water bath, as shown in Figure 2, to ensure no sunscreen transfer from formulation-treated samples.

Results and Discussion

Comparing in vivo and in vitro test methods: The in vivo water resistance of the series of sunscreen formulations in Table 1 was determined according to the FDA method using five human panelists of different skin types for the very-water-resistant protocol (80-minute immersion). This series shows the water-resistance effect of a lamellar gel structuring system at 3% (A) and 5% (B), and when the 3% lamellar gel formulation is augmented with 1% of three alkylated PVP polymers: PVP/hexadecene copolymer (C), PVP/eicosene copolymer (D), and tricontanyl PVP (E). The in vivo performance is reported in Figure 3 as a percentage of SPF before and after the 80-minute immersion. For example, formulation C gave an average pre-immersion SPF of 16.5 and average post-immersion SPF of 9.2 giving an average retention of 55.8 %. The initial SPF of all five formulations was between 16.5 and 18.0.

When the same formulations were tested in the new method described here, the agreement, as a percentage of retained ma-

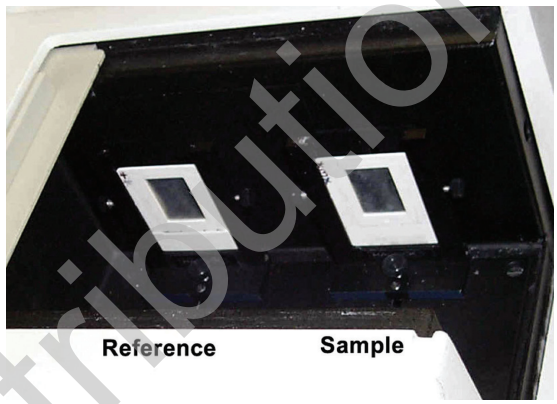

Figure 1. Placement of Vitro-Skin sample and reference in the spectrophotometer sample compartment

Table 2. Medium SPF nonionic sunscreen for in vitro water-resistance measurements (percentages are wt %)

		F	G	H	I	J	K	L
A	Water (aqua), deionized	65.0%	64.0%	64.0%	64.0%	64.0%	64.0%	64.0%
	Hexylene glycol	2.0	2.0	2.0	2.0	2.0	2.0	2.0
	Carbomer	0.2	0.2	0.2	0.2	0.2	0.2	0.2
B	Octyl methoxycinnamate	7.5	7.5	7.5	7.5	7.5	7.5	7.5
	Benzophenone-3	3.0	3.0	3.0	3.0	3.0	3.0	3.0
	Octyl salicylate	3.0	3.0	3.0	3.0	3.0	3.0	3.0
	Octyl palmitate	6.0	6.0	6.0	6.0	6.0	6.0	6.0
	PEG-20 stearate	2.0	2.0	2.0	2.0	2.0	2.0	2.0
	Glyceryl stearate (and) laureth-23	5.0	5.0	5.0	5.0	5.0	5.0	5.0
	PVP/eicosene copolymer	-	1.0	-	-	-	-	-
	Polybutene, hydrogenated	-	-	1.0	-	-	-	-
	Adipic acid/diethylene glycol/ glycerin crosspolymer	-	-	-	1.0	-	-	-
	Synthetic wax	-	-	-	-	1.0	-	-
	C_{30-38} olefin/isopropyl maleate/ MA copolymer	-	-	-	-	-	1.0	-
	Diglycol/CHDM/isophthalates/ SIP copolymer	-	-	-	-	-	-	1.0
C	Triethanolamine	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Water (aqua), deionized	5.0	5.0	5.0	5.0	5.0	5.0	5.0
D	Diazolidinyl urea (and) iodopropynyl butylcarbamate	0.6	0.6	0.6	0.6	0.6	0.6	0.6
	Phenoxyethanol (and) isopropylparaben (and) isobutylparaben (and) butylparaben	0.5	0.5	0.5	0.5	0.5	0.5	0.5
		100.0	100.0	100.0	100.0	100.0	100.0	100.0

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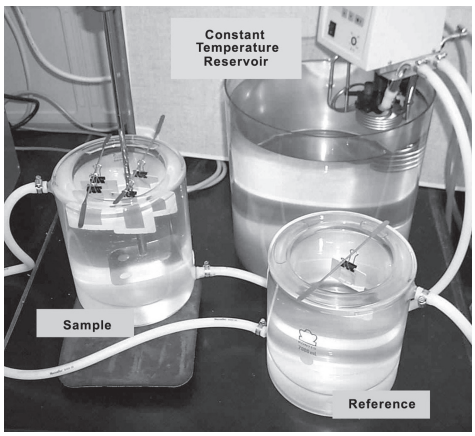

Figure 2. Immersion apparatus for the in vitro measurement of water resistance

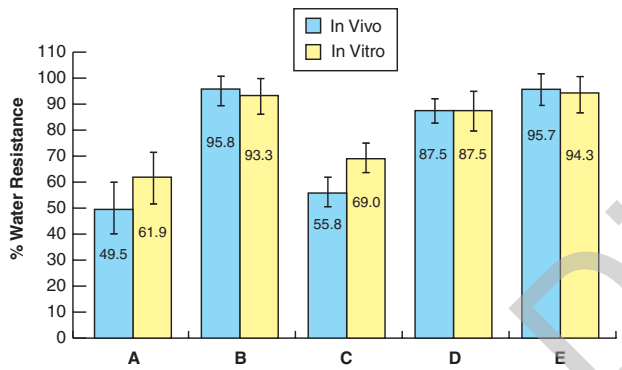

Figure 3. Comparison of FDA in vivo 80-minute immersion results with in vitro 80-minute immersion results for formulations shown in Table 1

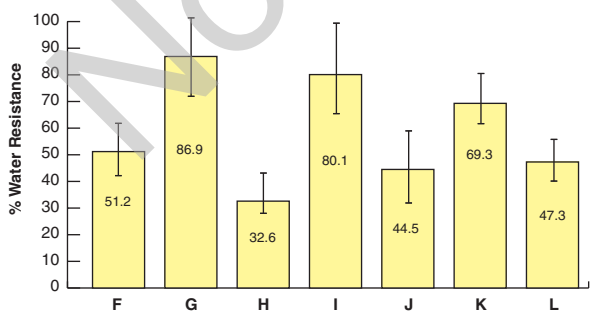

Figure 4. Comparison of water-resist technologies in the medium SPF nonionic formulations shown in Table 2

terial, was very good. Not only does the new method provide similar accuracy, it also gave standard deviations on the same order as the FDA in vivo method. It was also important that there was agreement between formulations that retained most of their activity (B, D and E) and those that lost about half of their activity (A and C). Best agreement was seen for the formulations that were very-water-resistant (B, D and E). There was more uncertainty with the formulations that lost significant amounts of activity (A, C), which can probably be attributed to the mechanisms expected for the loss of materials that are inhomogeneous in character.

Since this method is designed to rapidly screen formulations for water resistance, best agreement with the most water-resistant samples is most desirable. Those that are not water-resistant are selected away from further examination, as desired. The original study showed that for a medium SPF (~15) formulation, 3% of the lamellar gel structurant and 3% lamellar gel structurant with 1% PVP/hexadecene copolymer did not meet the requirements for very-water-resistant claims at an SPF of 15. Formulations with 3% of the lamellar gel structurant and 1% PVP/eicosene copolymer and 1% tricontanyl PVP, as well as a 5% level of the lamellar gel structurant, easily met the criteria for a very-water-resistant SPF 15 claim. The same conclusion could be reached much more quickly and inexpensively in the laboratory using the new method described here. Then only the final selected formulation need be tested in vivo for the final label claim.

In vitro testing of four commercial sprays: Sunscreen spray formulations have become very popular recently. We tested four commercial spray formulations that claimed to be waterproof or very water resistant. Table 3 summarizes the results of the testing of the four sprays in the new method described here.

Product 1 claimed a waterproof SPF of 8 and we found retention of 85.4% of the initial absorbance. Product 2 claimed a very-water-resistant SPF of 25 and we found 99.5% retention of the initial absorbance. Product 3 claimed a very-water-resistant SPF of 30 and we found 98.0% retention of the initial absorbance. Product 4 claimed a waterproof SPF of 30 and we found 96.2% retention of the initial absorbance. Since these products come under FDA regulation, they should deliver on their claims; therefore we believe this gives an independent confirmation of the correlation of this method with in vivo testing.

In vitro comparison of seven water-resistance technologies: Finally, we looked at a simple medium SPF nonionic formulation (Table 2) to screen the performance of a number of technologies that are marketed for sunscreen water resistance at a level of 1%. The level of 1% was selected to show differ-

Table 3. In vitro test results for four commercial spray products

Product	SPF	Claim	% retained
1	8	waterproof	85.4 ± 8.1
2	25	very water resistant	99.5 ± 0.9
3	30	very water resistant	98.0 ± 3.9
4	30	waterproof	96.2 ± 2.4

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ences, not to generate very-water-resistant formulations. Nonionic formulations are known to be difficult to make water-resistant, probably due to the excellent detergency of the nonionic emulsifiers. This was confirmed in our study.

Formulation F, the control nonionic formulation, retained only 51.2% of the initial absorbance. Hydrogenated polybutene (H) retained less than the control at 32.6% of the initial absorbance. Synthetic wax (J) and diglycol/CHDM/isophthalates/SIP copolymer (L) were similar to the control at 44.5% and 47.3% retention of initial absorbance, respectively. Three technologies improved retention: C_{30-38} olefin/isopropyl maleate/MA copolymer (K), adipic acid/diethylene glycol/glycerin crosspolymer (I), and PVP/eicosene copolymer (G). The PVP/eicosene copolymer gave the best retention at the 1 wt % level. Several other waterproofing technologies could not be evaluated in this model because they were incompatible with the system. Results are shown in Figure 4.

Conclusion

In this paper we have described a new laboratory in vitro test method for determining the water resistance potential of sunscreen formulations. This method is rapid, accurate and inexpensive, based on its results compared to in vivo results for the same formulations. The method is easily suited to most laboratory environments requiring only a thermostated water bath, glass-jacketed vessels, constant humidity chambers, and a UV-visible spectrophotometer. The substrate is readily available. We have found the method fast and reliable for screen both sunscreen formulations and water-resist technologies.

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