

Resistance of Liposomal Sunscreen Formulations against Plain Water as well as Salt Water Exposure and Perspiration

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Key Words

Liposomal sunscreen · Sun protection factor · Water resistance · Sweat resistance

Abstract

The present in vivo investigation using a total of 30 healthy adult volunteers with Fitzpatrick skin type II examines the persistent efficacy of sunscreens using liposomal suspensions as the vehicle. Based on the COLIPA guidelines, the protective effect of a single application of 4 different liposomal sunscreen formulations (sun protection factors, SPFs: 50+, 30, 25 and 15) against sunburn at the recommended amount of 2 mg/cm² was determined after exposure of the skin to plain water and salt water and after profuse perspiration. Under the influence of plain water, salt water and sweating, the SPF values of sunscreen 1 (labeled SPF of 50+) were reduced only marginally to 97, 96 and 99%, respectively, those of sunscreen 2 (labeled SPF of 30) to 97, 96 and 99%, respectively, those of sunscreen 3 (labeled SPF of 25) to 90, 83 and 91%, respectively, and those of sunscreen 4 (labeled SPF of 15) to 96, 96 and 95%, respectively. This set of data shows that despite plain water and salt water immersion or profuse sweating, the liposomal sunscreen formulation may deliver a long-lasting protective effect in everyday situations encountered by outdoor workers or during leisure activities.

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Introduction

The main rationale for using sunscreens to protect the skin from excessive sunlight is to prevent sunburn. Furthermore, chronic skin damage – especially non-melanoma skin cancers induced by sunlight and their precursors, as well as cutaneous changes associated with premature skin aging due to light exposure (photoaging) – should be prevented [1].

Sun protection factor (SPF) is a universal indicator of the efficacy of sunscreen preparations against sunburn. It is determined by assessing individual sensitivity to sunburn by ultraviolet (UV)-stimulated radiation with or without concomitant use of sunscreens. It is defined as the minimal erythema dose (MED) in sunscreen-protected skin divided by the minimal erythema dose in non-sunscreen-protected skin [2] or, in other words, as the ratio from the energies necessary to induce a minimum erythema response with and without sunscreen applied to the skin. It has to be stressed that the SPF is a factor measuring mainly the UVB protection of a sunscreen formulation since UVB is responsible for 80–90%

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of sunburns. In contrast, the protective effect of a sunscreen against the UVA component of sunlight – which significantly influences chronic skin damage corresponding to photoaging characterized by wrinkles, melanic freckles and a dry appearance as well as photocarcinogenesis [2] – has not been determined. The effects of infrared radiation – which might be involved in photoaging and possibly in photocarcinogenesis [3, 4] – are also inadequately reflected. However, no generally accepted and validated test systems are currently available for determining the protective effect of a sunscreen against UVA and infrared radiation that are comparable with test systems determining the erythral response of skin exposed to UVB [2].

Traditionally, the level of sun protection against UVB has been estimated using the *in vivo* SPF test which determines the erythral response of the skin of healthy volunteers to UV radiation from an artificial source under or without the influence of a sunscreen. One well-established tool that is in accordance with current statutory provisions is the International SPF Test Method by the European Cosmetics Association (COLIPA), the Cosmetic, Toiletry and Fragrance Association of South Africa, the Japan Chemical Industry Association and the Cosmetic, Toiletry and Fragrance Association [5]. However, this *in vivo* method does not take into consideration that the protective effect of a sunscreen is influenced by several exogenous factors. Water resistance plays a special role in this regard [6], which in turn is strongly dependent on the formulation of the sunscreen preparation [7–9]. For example, liposomal suspensions as vehicles for UV filters are particularly capable of transferring light protection substances into the horny layer (stratum corneum) [10]. This provides the opportunity to formulate sunscreens with improved water resistance [11]. Fortunately, processes have been developed that allow the validated measurement of water resistance of a sunscreen *in vivo*, whereas the *in vitro* procedures currently available are only able to deliver rather rough estimates of water resistance [12–15].

The COLIPA guidelines for evaluating sun product water resistance [16] are commonly used in Europe, in particular in the EU where a sunscreen is defined as water resistant or very water resistant if the SPF value after water exposure of 40 min (2 × 20 min) or 80 min (4 × 20 min) is similar compared to the SPF value determined before water exposure, respectively [11]. However, the established *in vivo* testing procedure by COLIPA does not take into consideration the influence of salt water or sweating upon the protective effect of sunscreens – fac-

tors most relevant in real-life situations such as a day on the beach or in the mountains. The present study examines (on the basis of the COLIPA guidelines) how the protective effect of liposomal sunscreens, which per se have pronounced substantivity, changes under the influence of plain water, salt water and sweating.

Materials and Methods

To determine the SPF of the liposomal sunscreen formulations to be tested under the influence of plain water, salt water or defined sweating, incremental series of delayed erythral responses were induced on a number of small sub-sites on the skin of healthy human subjects based on the International SPF Test Method [5] and a modification of the COLIPA Guidelines for Evaluating Sun Product Water Resistance [16].

The test was performed at AMA Laboratories (New City, N.Y., USA). Spirig Pharma Ltd., Egerkingen, Switzerland, acted as the sponsor. The test was restricted to the area of the back between the waist and shoulder line. In general, an area of each subject's skin was exposed to ultraviolet light without any protection and another area was exposed after application of a sunscreen to be tested. At least one further area was exposed after application of an SPF reference sunscreen formulation (see below under the heading 'test preparations'). By incrementally increasing the UV dose, varying degrees of skin erythema were generated. Sixteen to 24 h after UV radiation, the delayed erythral responses were visually assessed for redness intensity, by the judgment of a trained evaluator based on a visual grading scale from 0 (no erythema) to 5 (erythema and edema/vesicles). An individual SPF value for a test preparation was defined as the ratio of the MED (i.e. the lowest UV dose that produces the first distinct erythema) on preparation-protected skin (MED_p) to the MED on unprotected skin (MED_u) of the same subject.

Test Subjects

Thirty healthy adult volunteers with Fitzpatrick skin type II and free of any cutaneous or internal disorders were enrolled in the study. The volunteers were divided into 3 groups of 10 persons, each of which had to pass 4 test runs with the 4 formulations to be tested – one group in respect of plain water resistance (age 25–56 years; 2 males, 8 females), one in respect of salt water resistance (age 19–57 years; 3 males, 7 females) and one in respect of sweat resistance (age 18–52 years; 5 males, 5 females). Individuals who were under a doctor's care or were currently taking any medications that may mask or interfere with the test results as well as individuals with blemishes, nevi, sunburn, suntan, scars, moles, active dermal lesions and/or uneven pigmentation in sites to be tested were excluded. The study was performed following the Declaration of Helsinki and the Guidelines for Good Clinical Practice. Test protocol and informed consent procedures were approved by the institutional review board (American equivalent of an ethics committee) of AMA Laboratories.

Light Source

The artificial light source employed was a 150-Watt Xenon Arc Solar Simulator (Solar Light, Philadelphia, Penn., USA; model

Table 1. Sunscreens and ingredients of the test preparations and controls

Sunscreen 1	bis-ethylhexyloxyphenol methoxyphenyl triazine; butyl methoxydibenzoylmethane diethylhexyl butamido triazone; ethylhexyl salicylate; methylene bis-benzotriazolyl tetramethylbutylphenol; acrylic acid/VP crosspolymer; alcohol; aloe barbadensis gel; aqua; C12-C15 alkyl benzoate; cetyl alcohol; cetyl phosphate; decyl glucoside; dibutyl adipate; dimethicone; lecithin; methylparaben; pentylene glycol; propylene glycol; propylparaben; sclerotium gum; sorbitol; tocopherol; triethanolamine; xanthan gum
Sunscreen 2	bis-ethylhexyloxyphenol methoxyphenyl triazine; diethylamino hydroxybenzoyl hexyl benzoate; ethylhexyl methoxycinnamate; methylene bis-benzotriazolyl tetramethylbutylphenol; alcohol; aloe barbadensis gel; aqua; BHT; C12-15 alkyl benzoate; carbomer; cetyl alcohol; cetyl phosphate; decyl glucoside; dimethicone; ethylhexyl salicylate; glycerin; lecithin; methylparaben; panthenol; propylene glycol; propylparaben; sorbitol; tocopherol; triethanolamine; xanthan gum
Sunscreen 3	bis-ethylhexyloxyphenol methoxyphenyl triazine; diethylamino hydroxybenzoyl hexyl benzoate; ethylhexyl methoxycinnamate; ethylhexyl salicylate; methylene bis-benzotriazolyl tetramethylbutylphenol; alcohol; aloe barbadensis gel; aqua; BHT; carbomer; cetyl alcohol; cetyl phosphate; decyl glucoside; dicaprylyl maleate; dimethicone; lecithin; methylparaben; propylene glycol; propylparaben; sorbitol; tocopherol; triethanolamine; xanthan gum
Sunscreen 4	bis-ethylhexyloxyphenol methoxyphenyl triazine; butyl methoxydibenzoylmethane; dicaprylyl maleate; ethylhexyl salicylate ethylhexyl triazone; alcohol; aloe barbadensis gel; aqua; C12-15 alkyl benzoate; carbomer; cetyl alcohol; cetyl phosphate; dimethicone; lecithin; methylparaben; propylparaben; sorbitol; tocopherol; triethanolamine
SPF 15 standard (P3)	see International SPF Test Method [5]
SPF12/15 very water-resistant standard (P2)	see Guidelines for Evaluating Sun Product Water Resistance [16]
Sweat-resistant SPF 15 in-house control	aloe baradensis leaf extract; avobenzene; barium sulfate; benzyl alcohol; carbomer; nocos nucifera (coconut) oil; dimethicone; disodium EDTA; fragrance; homosalate; methylparaben; mineral oil; octadecene/MA copolymer; octisalate; octocrylene; oxybenzone; polyglycerceryl-3 distearate; propylparaben; simmondsia chinensis (jojoba) seed oil; sorbitan isostearate; sorbitol; stearic acid; tocopherol (vitamin E); triethanolamine; VP/eicosene copolymer; water

14 S, 15 S or 16 S) possessing a continuous emission spectrum in the UVB range of 290–320 nm. This light source was selected on the basis of its black body radiation temperature of 5,727 °C, which is known to produce continuous UV spectra of all wavelengths, being substantially equivalent to that of natural sunlight [17]. The device was additionally equipped with a diachronic mirror reflecting all radiation below 400 nm and a 1-mm Schott WG-320 filter absorbing all radiation below 290 nm. The result was a simulation of the solar UVA-UVB spectrum. In addition, a 1-mm thick UG filter (black lens) was added to remove reflected heat (i.e. infrared light above 700 nm) and remaining visible radiation. The employed Solar Simulator meets the requirements of the generally used standard. UVB radiation was monitored continuously during exposure.

Test Preparations

Test preparations encompassed several liposomal sunscreen formulations provided by Spirig Pharma AG: Daylong® extreme with an SPF of 50+ (in the following referred to as sunscreen 1), Daylong® Kids with a SPF of 30 (in the following referred to as sunscreen 2), Daylong® ultra with a SPF of 25 (in the following referred to as sunscreen 3), Daylong® SPF 15 with a SPF of 15 (in the following referred to as sunscreen 4) and as controls the SPF 15 standard (P3) as described by the International SPF Test Method [5] and the SPF12/15 very water resistant standard (P2) as described by the COLIPA guidelines [16]. For the test for sweat resistance, a SPF 15 in-house control was used that is known to be sweat resistant (table 1) as there is no sweat-proof control described by COLIPA.

Static SPF Determination

Rectangular test sites were delineated within an area of 30–60 cm² on the back of the volunteers. Test preparations and SPF 15 standard (P3) as a control for the static SPF determination were applied to test areas at a dose of 2 mg/cm² (±2.5%). Evenness of application was verified using a Wood's lamp. Fifteen minutes after application, the test sites received a series of 5 progressive UV light exposures based upon previously determined MEDs bracketing the intended SPF. The exposures for the test material and the SPF 15 standard (P3) were calculated from previously determined MEDs and the expected SPF with a maximum geometric progression of 1.12 for expected SPF >25 and a maximum geometric progression of 1.25 for expected SPF ≤25, as described by the International SPF Test Method [5].

Test for Plain Water Resistance

The test was employed to determine the substantivity of the 4 liposomal sunscreen products listed above and their corresponding ability to resist plain water immersion as described by the COLIPA guidelines [16]. On the day of the test, 2 sites were selected, one serving as an untreated unprotected area of the skin (to determine MED_u) and the other to expose the test preparation treated site after plain water exposure to determine MED_p. An adjacent test site was designated to determine the SPF of the SPF12/15 very water resistant standard (P2) after plain water immersion.

Test preparations and SPF12/15 very water resistant standard (P2) were applied to test areas at a dose of 2 mg/cm² (±2.5%). After application and a drying time of 15 min, the individuals had to undergo a defined plain water immersion procedure of altogether 80 min (20 min in a circulating whirlpool maintained at 29 ± 2 °C, 15

min drying time without toweling, then again 20 min in the whirlpool and 15 min drying time without toweling). Subsequently, the test sites received a series of 5 progressive UV light exposures as described above. Volunteers were instructed to return to the testing facility 20–24 h after exposure, and the delayed erythematous responses were evaluated using a visual grade scale (0: no erythema, 1: minimal erythema, 2: slight erythema, 3: well-defined erythema, 4: erythema and edema; 5: erythema and edema/vesicles).

Test for Salt Water Resistance

This test was performed as described above for the test for plain water resistance with the exception that (in deviation from the COLIPA guidelines [16]) the water in the whirlpool was adjusted to a salinity of 3.5% representing the average salinity of the world's oceans.

Test for Sweat Resistance

This test was employed to determine the substantivity of the 4 liposomal sunscreen products listed above and their corresponding ability to resist perspiration. On the day of the test, two sites were selected, one serving as an untreated unprotected area of the skin to determine MED_u and the other to expose the test-preparation-treated site after sweating to determine MED_p . An adjacent test site was designated to determine the SPF of the sweat-resistant in-house control after perspiration.

Test preparations and SPF 15 in-house control were applied to test areas at a dose of 2 mg/cm^2 ($\pm 2.5\%$). After a drying time of 20 min, the individuals had to run a defined perspiration procedure. They were placed into a controlled environment chamber ($35\text{--}38^\circ\text{C}$, relative humidity $80 \pm 2\%$) and were allowed to sweat. When the volunteers started to sweat profusely – defined by drops or rivulets of sweat running down the test sites – they had to stay in the chamber for a further 60 min. The pulse and oral temperature of the volunteers were taken every 15 min to monitor each person's heat stress. Afterwards, the test sites were air-dried for 20 min and then received a series of 5 progressive UV light exposures as described above. Evaluation of delayed erythema was also carried out as described earlier.

Calculation of SPF and Statistics

The SPF result for the given test preparation – under static conditions and under the influence of plain water, salt water and perspiration – was calculated as the arithmetical mean of all valid individual SPF values. The number of valid individual SPF values in each test run was 10 according to the International SPF Test Method [5]. The test result was only considered as acceptable if the 95% confidence limits for the mean SPF fell within the range of $\pm 17\%$ of the mean SPF. Calculations and statistics were carried out according to the International SPF Test Method [5].

Results

Subjects

In total, 30 healthy subjects were enrolled in the study as intended. All of them achieved accepted erythema responses on all test area sites and data from all subjects could be evaluated.

Determination of Static SPF Values

In the 4 sunscreens to be tested (applied in the recommended amount), the calculated SPF values turned out to be as high as or in most cases even higher than the labeled SPFs. For sunscreen 1 with a labeled SPF of 50+, mean static SPFs of 60.0 ± 3.5 (in the test for plain water resistance), 60.0 ± 3.5 (in the test for salt water resistance), and 58.7 ± 3.5 (in the test for sweat resistance) were determined, respectively. For sunscreen 2 with a labeled SPF of 30, a mean static SPF of 35.7 ± 2.1 was determined in the tests for plain water, salt water and sweat resistance, respectively. For sunscreen 3 with a labeled SPF of 25, a mean static SPF of 32.9 ± 3.3 was determined in the tests for plain water, salt water and sweat resistance, respectively. For sunscreen 4 with a labeled SPF of 15, a mean static SPF of 21.2 ± 2.5 was determined in the tests for plain water, salt water and sweat resistance, respectively (tables 2–4).

Test for Plain Water Resistance

The SPF determination for sunscreen 1 when tested on 10 subjects as described above under the provisions of a very water resistant claim yielded a mean value of 58.0 ± 3.5 compared to the mean static SPF of 60.0 ± 3.5 . The 95% CI of very water resistant SPF was $\pm 4.1\%$ compared to $\pm 4.2\%$ for mean static SPF corresponding to a reduction in SPF of 3.3% as a consequence of the previous plain water exposure (table 2). The SPF determination for sunscreen 2 under the provisions of a very water resistant claim yielded a mean value of 33.7 ± 2.6 compared to the mean static SPF of 35.7 ± 2.1 being related to a reduction in SPF of 5.6% as a consequence of previous plain water exposure. The 95% CI of very water resistant SPF was $\pm 5.5\%$ compared to $\pm 4.2\%$ for mean static SPF (table 2). The SPF determination for sunscreen 3 under the provisions of a very water resistant claim yielded a mean value of 29.4 ± 3.0 compared to the mean static SPF of 32.9 ± 3.3 corresponding to a reduction in SPF of 10.3% as a consequence of previous plain water exposure. The 95% CI of very water resistant SPF was $\pm 7.3\%$ compared to $\pm 7.2\%$ for mean static SPF (table 2). The SPF determination for sunscreen 4 under the provisions of a very water resistant claim yielded a mean value of SPF of 20.2 ± 2.3 compared to the mean static SPF of 21.2 ± 2.5 being related to a reduction in SPF of 4.2% as a consequence of previous plain water exposure. The 95% CI of very water resistant SPF was $\pm 8.2\%$ compared to $\pm 8.4\%$ for mean static SPF (table 2). In the tests for plain water resistance, the mean value of the P3 SPF standard on the same panel was 17.7 ± 1.8 under static conditions, and the mean

Table 2. SFF determination (means \pm SD): test for plain water resistance

	Static	After immersion
Sunscreen 1 (SPF 50+)	60.0 \pm 3.5	58.0 \pm 3.3
Sunscreen 2 (SPF 30)	35.7 \pm 2.1	33.7 \pm 2.6
Sunscreen 3 (SPF 25)	32.9 \pm 3.3	29.4 \pm 3.0
Sunscreen 4 (SPF 15)	21.2 \pm 2.5	20.2 \pm 2.3
Standard P3 (SPF 15)	17.7 \pm 1.8	
Standard P2 (SPF 12/15)		14.1 \pm 1.4

Table 3. SFF determination (means \pm SD): test for salt water resistance

	Static	After immersion
Sunscreen 1 (SPF 50+)	60.0 \pm 3.5	57.4 \pm 2.8
Sunscreen 2 (SPF 30)	35.7 \pm 2.1	33.0 \pm 2.4
Sunscreen 3 (SPF 25)	32.9 \pm 3.3	27.5 \pm 3.2
Sunscreen 4 (SPF 15)	21.2 \pm 2.5	20.2 \pm 2.3
Standard P3 (SPF 15)	16.1 \pm 1.8	
Standard P2 (SPF 12/15)		14.1 \pm 1.4

Table 4. SFF determination (means \pm SD): test for sweat resistance

	Static	After perspiration
Sunscreen 1 (SPF 50+)	58.7 \pm 3.5	58.0 \pm 3.3
Sunscreen 2 (SPF 30)	35.7 \pm 2.1	33.7 \pm 2.6
Sunscreen 3 (SPF 25)	32.9 \pm 3.3	30.0 \pm 2.7
Sunscreen 4 (SPF 15)	21.2 \pm 2.5	20.2 \pm 2.3
Standard P3 (SPF 15)	16.9 \pm 2.0	
In-house control (SPF 15)		16.1 \pm 1.8

SPF of the very water resistant standard on the same panel was 14.1 \pm 1.4 after immersion (table 2).

Test for Salt Water Resistance

The SPF determination for sunscreen 1 when tested on 10 subjects as described above under the provisions of a very water resistant claim and salt water conditions yielded a mean value of 57.4 \pm 2.8 compared to the mean static SPF of 60.0 \pm 3.5 corresponding to a reduction in SPF of 4.4% as a consequence of previous salt water exposure. The 95% CI of very water resistant SPF was \pm 3.5%

compared to \pm 4.2% for mean static SPF (table 3). The SPF determination for sunscreen 2 under the provisions of a very water resistant claim and salt water conditions yielded a mean value of 33.0 \pm 2.4 compared to the mean static SPF of 35.7 \pm 2.1 being related to a reduction in SPF of 7.7% as a consequence of previous salt water exposure. The 95% CI of very water resistant SPF was \pm 5.2% compared to \pm 4.2% for mean static SPF (table 3). The SPF determination for sunscreen 3 under the provisions of a very water resistant claim and salt water conditions yielded a mean value of 27.5 \pm 3.2 compared to the mean static SPF of 32.9 \pm 3.3 corresponding to a reduction in SPF of 16.6% as a consequence of previous salt water exposure. The 95% CI of very water resistant SPF was \pm 8.3% compared to \pm 7.2% for mean static SPF (table 3). The SPF determination for sunscreen 4 under the provisions of a very water resistant claim and salt water conditions yielded a mean value of 20.2 \pm 2.3 compared to the mean static SPF of 21.2 \pm 2.5 being related to a reduction in SPF of 4.2% as a consequence of previous salt water exposure. The 95% CI of very water resistant SPF was \pm 8.2% compared to 8.4% for mean static SPF (table 3). In the tests for salt water resistance, the mean value of the P3 SPF standard on the same panel was 16.1 \pm 1.8 under static conditions and the mean SPF of the very water resistant standard on the same panel was 14.1 \pm 1.4 after immersion (table 3).

Test for Sweat Resistance

The SPF determination for sunscreen 1 when tested on 10 subjects as described above under the provisions of a defined perspiration procedure yielded a mean value of 58.0 \pm 3.3 compared to the mean static SPF of 58.7 \pm 3.5 corresponding to a reduction in SPF of 1.2% as a consequence of previous sweating. The 95% CI of very water resistant SPF was \pm 4.3% compared to \pm 2.4% for mean static SPF (table 4). The SPF determination for sunscreen 2 under a defined perspiration procedure yielded a mean value of 33.7 \pm 2.6 compared to the mean static SPF of 35.7 \pm 2.1 corresponding to a reduction in SPF of 5.6% as a consequence of previous sweating. The 95% CI of very water resistant SPF was \pm 4.2% compared to \pm 3.9% for mean static SPF (table 4). The SPF determination for sunscreen 3 under the provisions of a defined perspiration procedure yielded a mean value of 30.0 \pm 2.7 compared to the mean static SPF of 32.9 \pm 3.3 corresponding to a reduction in SPF of 8.8% as a consequence of previous sweating. The 95% CI of very water resistant SPF was \pm 7.7% compared to \pm 4.3% for mean static SPF (table 4). The SPF determination for sunscreen 4 under the provi-

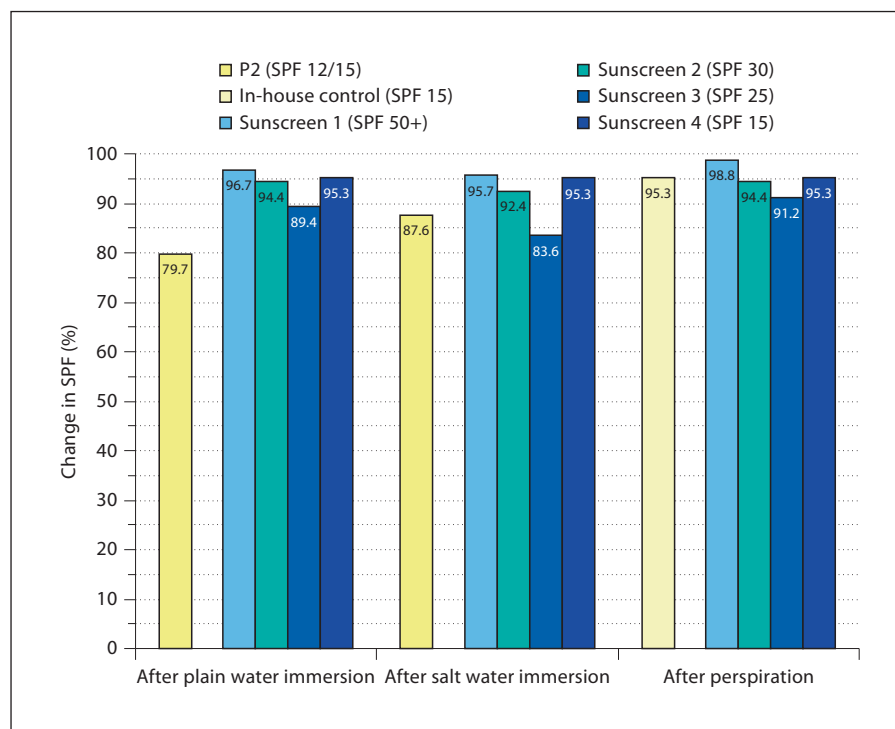


Fig. 1. Change in mean SPF in relation to the initial static SPF (set at 100%) under the influence of plain water and salt water immersion and perspiration.

sions of a defined perspiration procedure yielded a mean value of 20.2 ± 2.3 compared to the mean static SPF of 21.2 ± 2.5 corresponding to a reduction in SPF of 4.7% as a consequence of previous sweating. The 95% CI of very water resistant SPF was $\pm 8.9\%$ compared to $\pm 6.6\%$ for mean static SPF (table 4).

In the tests for sweat resistance, the mean value of the P3 SPF standard on the same panel was 16.9 ± 2.0 under static conditions, the mean SPF of the SPF (15) in-house control on the same panel was 16.1 ± 1.8 after perspiration (table 4).

Figure 1 summarizes to what degree the SPF of the 4 tested liposomal sunscreens changed under the influence of a defined exposure to plain water, salt water or sweat, whereby the static SPF of each of the sunscreens – that is, the SPF without intervention – is set at 100% (fig. 1).

Discussion

The value of an SPF on the label of a sunscreen usually is challenged by environmental factors that are normally not taken into account during SPF measurements in the laboratory [18]. The concept of ‘substantivity’ reflects the property of a sunscreen to maintain its degree

of protection under the influence of such retrenching exogenous conditions, such as repeated water immersion or sweating. Due to outdoor use of sunscreens in conditions where (salt) water immersion and abundant sweating are common, knowledge about water- and sweat-resistance of a sun protection formulation is very important [19].

The purpose of this study was to investigate the substantivity of 4 different liposomal sunscreen formulations after a defined immersion procedure in plain water or salt water over a total of 80 min or a defined perspiration procedure over ≥ 60 min using SPF as the most valid current measure of the efficacy of a sunscreen preparation against sunburn. In the context of pertinent testing, the conventional standard preparations with the particular specificity were included as controls. As the capacity for the testing was limited, a conventional sunscreen preparation without any such specification was not included. However, it would be clearly of interest in the future to check for superiority with the liposomal sunscreen preparations for general use as compared to a conventional ‘general use’ sunscreen preparation.

It could be shown that the SPF values of all 4 liposomal sunscreen formulations decreased only marginally under the influence of plain water, salt water or sweat. For sunscreens 1, 2 and 4, $>92\%$ of the protective effect against

sunburn was maintained, and $\geq 83\%$ of the protective effect was maintained for sunscreen 3 (fig. 1).

With regard to their *in vivo* protective effect, liposomal formulations have repeatedly been shown to be superior to other formulations such as oil-in-water or water-in-oil emulsions [7, 20]. Although sunscreens with a water-in-oil basis were shown to be resistant vis-à-vis a defined water treatment very similar to those being used in this study [7], their protective effect already declined by 33% after 30 min of intensive sweating [20]. The protective effect of oil-in-water emulsions was even cut in half (reduction by 49%) under the influence of 30 min of sweating [20]. Other authors found reductions in the SPF with the sunscreen preparations (2 creams and 1 lotion) that they tested of approximately 20–30% after the test persons had finished a swimming pool exercise [8]. Even if in the view of the authors these values prove the high substantivity of the sunscreens investigated, these showed a clear inferiority compared to the liposomal sunscreens tested in this study.

Up to the present, there are not many studies addressing the substantivity of sunscreens under conditions that are close to real life conditions. The COLIPA guidelines, which served as the basis for the tests presented here, are not intended to reflect the reality of a day at the beach or in the mountains. In fact, in reality even further factors have to be considered, such as not applying enough of the sunscreen [21–23], which undoubtedly further impair sun protection [24].

Bodekær et al. [25] made the first attempt to determine the persistence of sunscreens during a day with physical activity, wearing a T-shirt, bathing and toweling. They wanted to clarify the controversial issue of whether sun lotion reapplied throughout a day in the sun and after vigorous activity guarantees optimal sun protection [26–29]. They discovered that the SPF values linked to a single application of an inorganic water-resistant sun lotion in the recommended amount of 2 mg/cm^2 after 4 h of physical activity and heat were reduced by 38%. Another 4 h of physical activity, heat and an additional bathing procedure including toweling in fact led to further reduction in SPF of 55%, whereas the bathing and toweling procedure did not significantly contribute to the reduction in SPF [25].

Because of the different test conditions, and especially because of the different periods of time, the results of Bodekær et al. [25] are certainly not comparable to the data obtained by the investigation presented here. However, it is noteworthy that the SPF in the study of Bodekær et al. [25] already diminished by 63% as a result of 4 h of

physical activity and heat. In contrast, in our study after altogether 80 min of exposure vis-à-vis plain water or salt water (20 min in a circulating whirlpool maintained at $29 \pm 2^\circ\text{C}$, 15 min drying time without toweling, then again 20 min in the whirlpool and 15 min drying time without toweling), or after at least 1 h of strong sweating, only a marginal reduction in SPF (up to a maximum of 8%) was determined, with the exception of sunscreen 3 with an SPF reduction of a maximum of 17%. Based on the fact that the reduction in the SPF over time takes an exponential course [25], the relatively large reduction in SPF seems to occur within the first 1–2 h after application.

On the basis of these considerations, the liposomal sunscreens tested in this investigation appear to be potentially superior to the sun lotion used by Bodekær et al. [25] with regard to substantivity. Inorganic filters in sunscreens are not able to penetrate into the skin [29] and hence tend to be removed by exogenous factors whereas liposomal sunscreen formulations deliver high quantities of organic filters to their effective target layers in the upper stratum corneum [11] without any notable penetration into deeper skin layers [9, 10, 30–32]. Based on the available data and due to fundamental considerations, it is tempting to speculate that in the case of the liposomal sunscreens tested in this study a one-time application 15 to 30 min prior to sun exposure might well be sufficient to protect against sunburn for a prolonged period of time – even given plain water and salt water immersion or profuse sweating.

Conclusion

With this investigation based on the COLIPA guidelines, we have demonstrated that the 4 different liposomal sunscreens are able to maintain their degree of protection against sunburn for a prolonged period of time – even under the influence of retrenching exogenous conditions such as plain water and salt water immersion as well as perspiration. Thus a strong substantivity of liposomal sunscreens could be demonstrated.

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