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Assessment of *in vitro* Sun Protection Factor (SPF) and rheological profile of commercial infant sunscreens

Fernanda Daud Sarruf[1]; Daniela D'Almeida Peres[1,*]; Deborah de Oliveira Nishikawa[1]; Vladi Olga Consiglieri[1]; Telma Mary Kaneko[1]; Maria Valéria Robles Velasco[1]; André Rolim Baby[1]

[1] Departamento de Farmácia, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo - SP, Brasil.

ABSTRACT

This research focused on the measurement of spectral transmittance using an integrating sphere to estimate the Sun Protection Factor (SPF) of commercial sunscreens oriented for child use. Commercially available SPF 30 sunscreens (A, B and C) were selected and assessed by the following methodology: pH measurement; rheological profile analysis; and *in vitro* SPF estimation by spectroscopy. Samples A, B and C exhibited, respectively, pH values of 7.8, 7.4 and 7.0. The rheological profile identified the systems as pseudoplastic non-Newtonian semisolids with hysteresis loops. The t-test ($p < 0.05$) was used to confront the claimed SPF (SPF = 30) with that estimated by spectroscopy. *In vitro* tests are considered of utmost importance in the cosmetic field, since their results may be used to validate submission to subsequent *in vivo* efficacy experiments. According to our results, sunscreens A, B and C generated SPF values without any significant difference from those claimed on the commercial sunscreen labels. The measurement of spectral transmittance with an integrating sphere proved to be a convenient, fast and reproducible method for SPF estimation.

Keywords: Sunscreen. *In vitro* SPF. Rheology. Spectral transmittance. Spectroscopy. UV radiation

INTRODUCTION

The sunlight that reaches the Earth's surface is composed of ultraviolet (200-400 nm), visible (400-800 nm) and infrared (> 800 nm) radiation. The UV radiation is a restricted portions of the electromagnetic spectrum that is traditionally divided into regions labeled UVC (200-290 nm), UVB (290-320 nm) and UVA (340-400 nm). The UVA region is also subdivided into UVA1 (340-400 nm) and UVA2 (320-340 nm) (Velasco *et al.*, 2006; DHHS, 2011).

Corresponding Author: Daniela D'Almeida Peres - Laboratório de Pesquisa e Desenvolvimento de Produtos Cosméticos - Departamento de Farmácia, Faculdade de Ciências Farmacêuticas - Universidade de São Paulo - Av. Prof. Lineu Prestes, 580, Bl. 15 - Conjunto das Químicas - Cidade Universitária CEP.05508-900 - São Paulo - SP - Brasil - telefone: +55 (11) 3091-2358/ +55(11)8515-6263 - fax: +55(11)3815-4418 - e-mail: daniela.peres@usp.br

Skin cancer is a significant public health problem and its incidence, morbidity and mortality have increased in recent years. UV radiation is the main etiologic agent in many different types of skin cancer. Even though skin cancer has an excellent prognosis when detected in the early stages, it can and should be prevented with adequate sun protection. The use of sunscreens is thus very important to prevent the growth in the incidence of skin cancer (Kolm *et al.*, 2010; Narayanan *et al.*, 2010).

As well as aiding in the reduction of skin cancer incidence, sunscreens slow the appearance of skin aging signs, as they reduce the amount of UV radiation that reaches the skin. They are mainly employed to prevent the formation of erythema due to sun exposure. In particular, the effects of UVB radiation are cumulative, which suggests a need for sun protection from early childhood (Velasco *et al.*, 2006; Chang *et al.*, 2010).

In vitro spectrophotometric tests are based on the analysis of the reflection spectrum, collected in a diffuse reflectance spectrophotometer. Sunscreen samples are spread thinly over a suitable surface, avoiding the need to make samples solutions and enabling the evaluation of sunscreens containing physical UV filters (Mansur *et al.*, 1986; Diffey, 1997).

The present research was aimed at determining the *in vitro* sun protection factor (SPF) of commercial sunscreen products for child's use (infants and toddlers), by measuring their spectral transmittance with an integrating sphere technique.

MATERIAL AND METHODS

Three commercially available SPF 30 sunscreens (A, B and C) for babies and children were selected for the assessment of pH, rheological profile and *in vitro* SPF. The UV filters in each sunscreen are listed in Table 1.

The pH was measured with a Quimis® pHmeter. The electrode was introduced into each sample and the reading performed after stabilization.

The rheological profile was assessed with a rheometer (Physica® MCR 300 - Anton Paar) operated in cone-plate geometry (cone diameter of 25.0 mm and angle of 1° between cone and plate) (Figueiredo *et al.*, 2008). Samples of approximately 0.5 g were tested at 22.00 ± 0.01°C and the flow curve recorded at rotational speeds varying from 0.1 to 200.0 s^{-1}. The apparent viscosity was determined at maximum shear

rate (200.0 s^{-1}). Measurements were made under controlled laboratorial conditions: 22,0 ± 0,5°C and 40 - 50% relative humidity.

The viscosity and hysteresis area results were tested statistically with the software package Statistica® 7.0; viscosity was analyzed by the Kruskal-Wallis test and hysteresis area by analysis of variance (ANOVA) followed by the Fischer test. Significance was accepted when $\alpha = 0.05$.

The photoprotective efficacy was assessed *in vitro* by determining the SPF value of the formulations, which was calculated from the spectral transmittance measured with an integrating sphere (Labsphere® UV-2000S Ultraviolet Transmittance Analyzer). The SPF on the labels (SPF = 30 for all products tested) was then compared with the *in vitro* SPF value by the Student *t*-test for two samples ($p < 0.05$).

The samples were accurately weighed and uniformly spread with a gloved finger on the rough side of PMMA (poly methyl methacrylate) plates at a rate of 0.75 mg/cm^2. The samples were then allowed to dry for 25 minutes protected from the light (Springsteen *et al.*, 1999; Velasco *et al.*, 2006). Measurements were made under the following laboratory conditions: 22,0 ± 0,5°C and 40 - 50% relative humidity.Duplicate plates were prepared and nine different points were measured on each sample (n = 18).

Table 1. Qualitative composition of commercial infant sunscreens A, B and C

Sunscreen	UV filters (INCI)a
A	homosalate, ethylhexyl salicylate, benzophenone-3 and ethylhexyl methoxycinnamate
B	zinc oxide and titanium dioxide
C	bis-ethylhexyloxyphenol methoxyphenyl triazine, ethylhexyl salicylate, octocrylene, ethylhexyl methoxycinnamate and titanium dioxide (and) alumina (and) simethicone

[a] Qualitative composition in accordance with INCI (*International Nomenclature of Cosmetic Ingredients*)

RESULTS

The pH results are shown in Table 2.

The rheograms of formulations A, B and C are plotted in Figure 1 and Table 3 contains the average apparent viscosity and hysteresis area of these sunscreens.

Table 2. pHmeter measurements of commercial infant sunscreens A, B and C

Formulation	pH
A	7.8
B	7.4
C	7.0

Table 3. Average and standard deviation of the apparent viscosity (mPa/s; n=10) and hysteresis area (Pa/s; n=2) of commercial infant sunscreens A, B and C calculated from their rheograms

Formulation	Apparent Viscosity* (mPa/s; n=10)	Hysteresis Area* (Pa/s; n=2)
A	426,20a,b ± 11,42	756,70a ± 118,65
B	1003,50a ± 14,60	4757,80b ± 83,11
C	254,10b ± 2,47	519,16a ± 75,99

* Different letters beside the numbers indicate statistically different values.

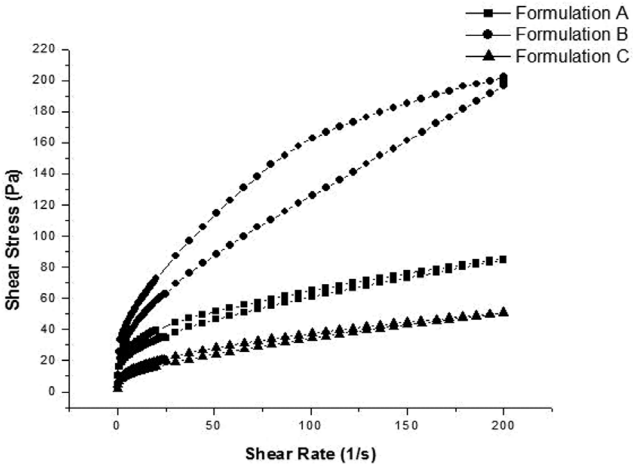

Figure 1. Rheograms of commercial infant sunscreens A, B and C

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Statistical analysis of the rheological profiles showed that formulation B had the highest apparent viscosity (1003.50 ± 14.60 mPa/s) while formulations C and A had lower values that were not significantly different (respectively 254.10 ± 2.47 and 426.20 ± 11.42 mPa/s).

Formulation B also showed the highest hysteresis area (4757.80 ± 83.11 Pa/s), while C and A had lower values, again without significant difference (respectively 519.16 ± 75.99 and 756.70 ± 118.65 Pa/s). The results obtained for formulation B were statistically different from those for C and A.

The spectroscopic technique involves the measurement of the diffuse transmittance of a thin layer of sample, spread on a specific substrate, in the ultraviolet spectrum, encompassing the UVA and UVB regions (Springsteen *et al.*, 1999; Velasco *et al.*, 2006; Meireles *et al.*, 2007).

According to the transmittances measured for the commercially available sunscreens, samples A, B and C all generated SPF values without significant difference from those on the reported on the labels (Table 4**)**.

Table 4. *In vitro* SPF values of commercial infant sunscreens A, B and C, determined by spectral transmittance with an integrating sphere, and calculated *p-value*

Formulation	In vitro SPF	p-value
A	45,0 ± 4,2	0.1256
B	35,0 ± 1,4	0.1256
C	39,5 ± 2,1	0.0996

DISCUSSION

By analyzing the pH results (Table 2), it could be concluded that all the test samples were compatible with children's skin, having a neutral pH, around 7.0 (Meireles *et al.*, 2007).

The rheological profiles (Figure 1 and Table 3) allowed the products to be classified as pseudoplastic non-Newtonian semisolid systems with hysteresis. Sunscreen formulations with a pseudoplastic flow produce a coherent protective film covering the skin surface with evenly distributed UV filters and this characteristic helps to promote a higher SPF. Newtonian materials do not behave in this way, so that the protective film over the skin can be uneven, in accordance with its low viscosity. Pseudoplastic material, however, can break down under shear, for easy spreading, and after being spread the film may gain viscosity instantaneously to resist running (Gaspar & Maia Campos, 2003).

There is an optimal value for thixotropy (hysteresis area), in order to achieve the highest possible SPF. At lower thixotropy values, the spreadability is insufficient to permit good distribution of the sunscreen. Above the optimum thixotropy value, there is insufficient recovery of the structure to obtain an evenly distributed film, as it continues to flow into the wrinkles of the skin (Gaspar & Maia Campos, 2003). Dahms (1994) showed that emulsions that have high thixotropy show low SPF values. Thus, ideal formulations would be those that exhibit a low degree of thixotropy.

Therefore, although all formulations had the same label SPF, the best formulations, according to Dahms (1994) were A and C, which had lower thixotropy, as shown in Tables 3 and 4.

The *in vitro* photoprotection test method allows the determination of the protection against UVA and UVB radiation supplied by sunscreen active compounds or final products (Velasco *et al.*, 2006). *In vitro* SPF studies are of the utmost importance in the cosmetic field, since their results may be used to give prior validation for submission to *in vivo* efficacy experiments, thus ensuring that only the formulations with favorable responses go to the *in vivo* test, in compliance with safety standards (Santos *et al.*, 1999; Velasco *et al.*, 2006).

The SPF results obtained **(**Table 4**)** were not statistically different to the ones claimed on the labels, proving that the measurement of spectral transmittance with an integrating sphere is a convenient, fast and reproducible methodology for SPF estimation of cosmetic formulations.

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RESUMO

Avaliação do fator de proteção solar (FPS) in vitro e perfil reológico de fotoprotetores comerciais infantis

A pesquisa tem como objetivo a aplicação da transmitância espectral utilizando uma esfera de integração para estimar o Fator de Proteção Solar (FPS) de fotoprotetores comerciais para o uso infantil. Fotoprotetores com FPS 30 (A, B e C), disponíveis comercialmente, foram selecionados para avaliação conforme as seguintes metodologias: valor de pH, perfil reológico, e valor de FPS *in vitro* por espectroscopia. As amostras A, B e C apresentaram, respectivamente, os seguintes valores de pH: 7.8, 7.4 e 7.0. O perfil reológico identificou os sistemas como semissólidos pseudoplásticos não-Newtonianos, com área de histerese. O teste-T ($p < 0.05$) foi utilizado para confrontar o FPS alegado (FPS = 30) com o estimado por espectroscopia. Estudos *in vitro* foram considerados de grande importância para a área cosmética, podendo corroborar, previamente, a realização dos estudos de eficácia *in vivo*. De acordo com os resultados, as amostras A, B e C apresentaram valores de FPS sem diferença estatisticamente significativa, quando comparados àqueles citados nos rótulos. A transmitância espectral utilizando esfera de integração provou ser uma metodologia conveniente, rápida e reprodutível para a avaliação estimada do FPS.

Palavras-chave: Fotoprotetor. Espectroscopia. FPS *in vitro*. Radiação UV. Reologia. Transmitância espectral.

REFERENCES

Chang NB, Feng R, Gao Z, Gao W. Skin cancer incidence is highly associated with ultraviolet-B radiation history. Int J Hyg Environ Health. 2010;213(5):359-68. DOI: 10.1016/j.ijheh.2010.06.006.

Dahms GH. Einfluss der Thixotropie auf die Lichtschutzwirkung von Sonnenschutzemulsionen. *Parfuem Kosmet.* 1994; 75(10):675-679.

Department of Health and Human Services – DHHS. Food and Drug Administration - FDA. 21 CRF parts 201 and 310. Labeling and effectiveness testing; sunscreen drug products for over-the-conter human use; Final Rule. Silver Spring, Federal Register. 2011;76(117):46 p.

Diffey BL. Indices of protection from *in vitro* assay of sunscreens. In: Lowe NJ, Shaath MA, Pathak MA. Sunscreens development, evaluation, and regulatory aspects. New York: Marcel Dekker; 1997. p. 589-600.

Figueiredo EM, Areas JAG, Areas EPG. Rheology of decane/water and triglyceride/water emulsions stabilized by β-casein and sodium caseinate. J Brazil Chem Soc. 2008;19(7):1336-1346.

Gaspar LR, Maia Campos PMBG. Rheological behavior and the SPF of sunscreens. Int J Pharm. 2003;250(1):35-44.

Kolm I, Hofbauer G, Braun RP. Early diagnosis of skin cancer. Ther Umsch. 2010;67(9):439-446.

Mansur JS, Breder MNR, Mansur MCA, Azulay RD. Determinação do fator de proteção solar por espectrofotometria. An Bras Dermatol. 1986;61(4):121-124.

Meireles C, Hergy F, Mousinho M, Afonso S, Rosado C. Caracterização da pele infantil e dos produtos cosméticos destinados a esta faixa etária. Rev Lusófona C&T Saúde. 2007;1(4):73-80.

Narayanan DL, Saladi RN, Fox JL. Ultraviolet radiation and skin cancer. Int J Dermatol. 2010;49(9):978-986.

Santos EP, Freitas ZM, Souza KR, Garcia S, Vergnanini A. *In vitro* and *in vivo* determinations of sun protection factors of sunscreen lotions with octylmethoxycinnamate. Int J Cosmet Sci. 1999;21(1):1-5. DOI: 10.1046/j.1467-2494.1999.181658.x.

Springsteen A, Yurek R, Frazier M, Carr KF. In vitro measurement of sun protection factor of sunscreens by diffuse transmittance. Anal Chim Acta. 1999;380(1-2):155-164.

Velasco MVR, Sarruf FD, Salgado-Santos IMN, Haroutiounian-Filho CA, Kaneko TM, Baby AR. Broad Spectrum Bioactive Sunscreens. Int J Pharm. 2008;363(1-2):50-7. DOI: 10.1016/j.ijpharm.2008.06.031.

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