Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 297 (2005) 197–203
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

The effect of packaging materials on the stability of sunscreen emulsions

Maria Inês R.M. Santoro*, Daniella Almança Gonçalves Da Costa E. Oliveira, Erika R.M. Kedor-Hackmann, Anil K. Singh

Department of Pharmacy, Faculty of Pharmaceutical Sciences, University of São Paulo, Caixa Postal 66083, CEP 05315-970 São Paulo, SP, Brazil

Received 3 November 2004; received in revised form 30 March 2005; accepted 30 March 2005

Abstract

The purpose of this research was to study the stability of a emulsion containing UVA, UVB and infrared sunscreens after storage in different types of packaging materials (glass and plastic flasks; plastic and metallic tubes). The samples, emulsions containing benzophenone-3 (B-3), octyl methoxycinnamate (OM) and Phycocorail®, were stored at 10, 25, 35 and 45 °C and representative samples were analyzed after 2, 7, 30, 60 and 90 days period. The stability studies were conducted by analyzing samples at pre-determined intervals by high performance liquid chromatography (HPLC) along with periodic rheological measurements.

Keywords: Cosmetic products; Emulsions; Sunscreens; Packaging materials; HPLC; Rheology

1. Introduction

Sun radiations are necessary for all living organisms, although it presents a threat to the overall health of human skin, especially through ultraviolet radiation. The mass destruction of atmospheric ozone layer presents threat to natural protection system from UV radiation and as a result a potential harmful effect on human skin (Epstein, 1990; Krutmann, 2000; Osterwalder et al., 2000; Parisi and Wong, 2000; Romanowski and Schueller, 2000; Molen et al., 2001).

In order to minimize the effects of UV radiation, the use of sunscreens in cosmetic preparations has been increasing. Many new cosmetic products containing sunscreens are being developed and are commercially available. Consequently, there is a need for development and validation of analytical methods for quantitative determination of sunscreen agents in cosmetic products. High performance liquid chromatography (HPLC) is the most used chromatographic method for qualitative and quantitative determination of sunscreen agents in cosmetic products (Rastogi and Jensen, 1998;

* Corresponding author. Tel.: +55 11 309 13648; fax: +55 11 309 13648.
E-mail address: ines@usp.br (M.I.R.M. Santoro).

0378-5173/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2005.03.021

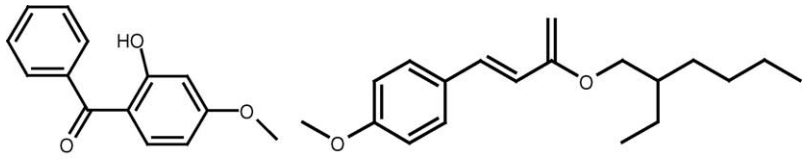

Fig. 1. Chemical structures of benzophenone-3 and octyl methoxycinnamate.

Vanquerp et al., 1999; Portad et al., 1999; Santoro et al., 2000; Scalia, 2000; Dutra et al., 2002).

The purpose of this research was to prepare emulsions containing UVA, UVB and infrared sunscreens and to study their stability after storage in different types of packaging material (glass and plastic flasks; plastic and metallic tubes). The emulsion samples containing benzophenone-3 (B-3), octyl methoxycinnamate (OM) (Fig. 1) and Phycocorail®, were stored at 10, 25, 35 and 45 °C and analyzed after 2, 7, 30, 60 and 90 days period by rheological measurements and after 2, 7, 30 and 60 days period also by HPLC in order to proceed the stability studies.

2. Material and methods

2.1. Standards, reagents and solvents

All reagents and solvents were of analytical grade or of HPLC grade. Standards: B-3 and OM (Neo Heliopan AU®) were acquired from Galena, São Paulo, Brazil. Solvents: methanol (Omnisolv®, Merck®); water from a Milli-Q® Plus Water Purification System (São Paulo, SP, Brazil); packaging materials with 60 g capacity each: glass (type II soda-lime type, treated with SO_2) and plastic (polypropylene) flasks, plastic (polypropylene) and metallic (aluminum) tubes were acquired from local industries.

2.2. Sample

The O/W emulsion used in this research was constituted of: phase A – silicone oil, 3.00% (w/w); gliceryl monostearate, 2.00% (w/w); cetostearyl alcohol, 2.00% (w/w); mineral oil, 3.00% (w/w); Polawax®, 2.50% (w/w); Chemynol®, 0.80% (w/w); BHT, 0.05% (w/w); B-3, 2.00% (w/w); OM, 4.00% (w/w); Tiosorb TG®, 2.00% (w/w); Phycocorail®, 0.50% (w/w); phase B – propyleneglycol, 5.00% (w/w); Veegum ultra®, 0.50% (w/w); xanthan gum, 0.30% (w/w); imidazolylurea, 0.50% (w/w); distilled water q.s. 100% (w/w). Placebo (emulsion base) was also prepared containing all the ingredients without the sunscreens.

The components of phase A (oil phase) were weighed and triturated in a beaker, with exception of Chemynol®. In another beaker, xanthan gum and distilled water of phase B (aqueous phase) were mixed to obtain a gel like consistency. Immediately after, other components of phase B were added to it. Phase A and phase B were heated separately until 75–80 °C. Emulsions were prepared by adding phase B into A with constant stirring. When the mixture temperature reached around 40 °C, Chemynol® was added and stirring maintained until the emulsion reached room temperature. Samples were taken and stored at room temperature (25 ± 1 °C), 10, 35 and 45 °C separately during 2, 7, 30, 60 and 90 days.

2.3. Apparatus

Liquid chromatographic system CG Model 480C, equipped with a variable UV detector, connected to an electronic integrator Model CG-200 and manual injection valve fitted with a 20 μL sample loop (Instrumentos Científicos CG Ltd., São Paulo, SP, Brazil). Rotational viscosimeter Brookfield-RVT with SC4-29 spindle and sonicator, Thornton T-14.

2.4. Methods

2.4.1. HPLC operating conditions

LiChrospher® 100 RP-18 column Merck®, particle size 5 μm, 125 mm × 4 mm i.d.; mobile phase methanol–water (87:13 v/v); flow rate of 1.0 mL/min;

UV detection at 290 nm; room temperature (25 ± 1 °C). All solutions and solvents were filtered through a Millipore® filter membrane, pore size 0.45 μm and vacuum degassed by sonication before use.

2.4.2. Standard solution

The standard solution containing B-3 and OM were prepared in methanol. After adequate dilutions the final concentration of B-3 and OM were, 10.0 and 20.0 μg/mL, respectively.

2.4.3. Sample solution

Sample solutions were prepared in methanol. An amount of sample containing 5.0 mg of B-3 and 10.0 mg of OM was weighed and transferred to a 100 mL volumetric flask; 50 mL of methanol was added and the mixture was sonicated during 20 min. The volume was completed with methanol, the solution was filtered, and after adequate dilutions, a final solution containing 10.0 μg of B-3/mL and 20.0 μg of OM/mL was obtained.

2.4.4. Placebo solution

A similar process was adopted, as described for sample solution preparation to obtain placebo solution. Placebo matrix equivalent to 250.0 mg of emulsion was used to prepare placebo solution.

2.4.5. Method validation

2.4.5.1. Linearity. Standard curves were obtained by analyzing, in triplicate, eight different concentrations, ranging from 4.0 to 18.0 μg/mL of B-3 and from 8.0 to 36.0 μg/mL of OM. All the solutions were prepared by diluting a standard solution mixture (50:100 μg/mL of B-3 and OM, respectively) in methanol with triplicate injection of each solution into the chromatographic system. The calibration curves were constructed by plotting the peak areas against concentration.

2.4.5.2. Detection limit (DL) and quantitation limit (QL). The DL and QL were determined based on standard deviation amongst responses and slope of the regression equation of a curve constructed at lower concentration levels (2.0, 4.0, 6.0, 8.0 and 10.0 μg/mL of B-3 and 4.0, 8.0, 12.0, 16.0 and 20.0 μg/mL of OM. DL and QL were expressed as 3 times and 10 times of the fraction, standard deviation/slope, respectively (ICH Q2A, 1995; US Pharmacopeia, 2002).

2.4.5.3. Precision. The precision of the method was determined by replicate ($n = 10$) injection of the sample solutions into the chromatographic system. A standard solution of each compound was also injected, in triplicate, into the system. The precision of the method was expressed as degree of repeatability of responses (ICH Q2A, 1995; US Pharmacopeia, 2002).

2.4.5.4. Accuracy. The accuracy was determined by recovery tests, performed by adding known amounts of standard solutions to the sample solution. The test was performed based on guidelines published by AOAC International (AOAC International, 1990). Concisely, known amounts of B-3 (6.0, 8.0 and 10.0 μg/mL) and OM (12.0, 16.0 and 20.0 μg/mL) standard solutions were added to sample solutions. The resulting spiked samples were submitted to analysis with triplicate injection of each solution. The amount of standard analyte recovered is determined by comparing responses of spiked sample with those of sample without spiking.

2.4.5.5. Specificity. The specificity was determined by comparing the results obtained by analysis of placebo formulation, a simulated sample and a standard solution containing referred sunscreens (ICH Q2A, 1995; US Pharmacopeia, 2002).

2.4.6. Rheological measurements

Rheological studies were conducted by transferring accurately weighed 13 g of emulsion sample to stainless steel cup with SC4-29 spindle. The samples were subjected to velocity gradient from 0.125 to 25.0 s^{-1} in a gradual manner. The torque time was fixed to 2 min in all cases and the readings were taken at room temperature (25 ± 1 °C).

3. Results and discussion

3.1. Rheology

Results of rheological studies in glass pot packaging material can be observed in Fig. 2. Same studies were conducted with plastic pots, plastic tubes and metallic tubes (graphs not presented). The solar emulsion studied presented pseudoplastic behavior, exhibiting a reduction in viscosity when the spread-

ing/shearing velocity increases. This emulsion also presented thixotropic behavior, which is defined as the capacity of a system to present lowest viscosity when subjected to shear and its capacity to recover its original structure during a defined period after the shear is removed. Samples stored at 35 °C presented similar behavior as those presented by stored at room temperature. A decrease in viscosity was observed with an increase in the storage temperature. A decrease in thixotropy was observed in samples stored at 10 °C when compared with those maintained at room temperature (25 ± 1 °C).

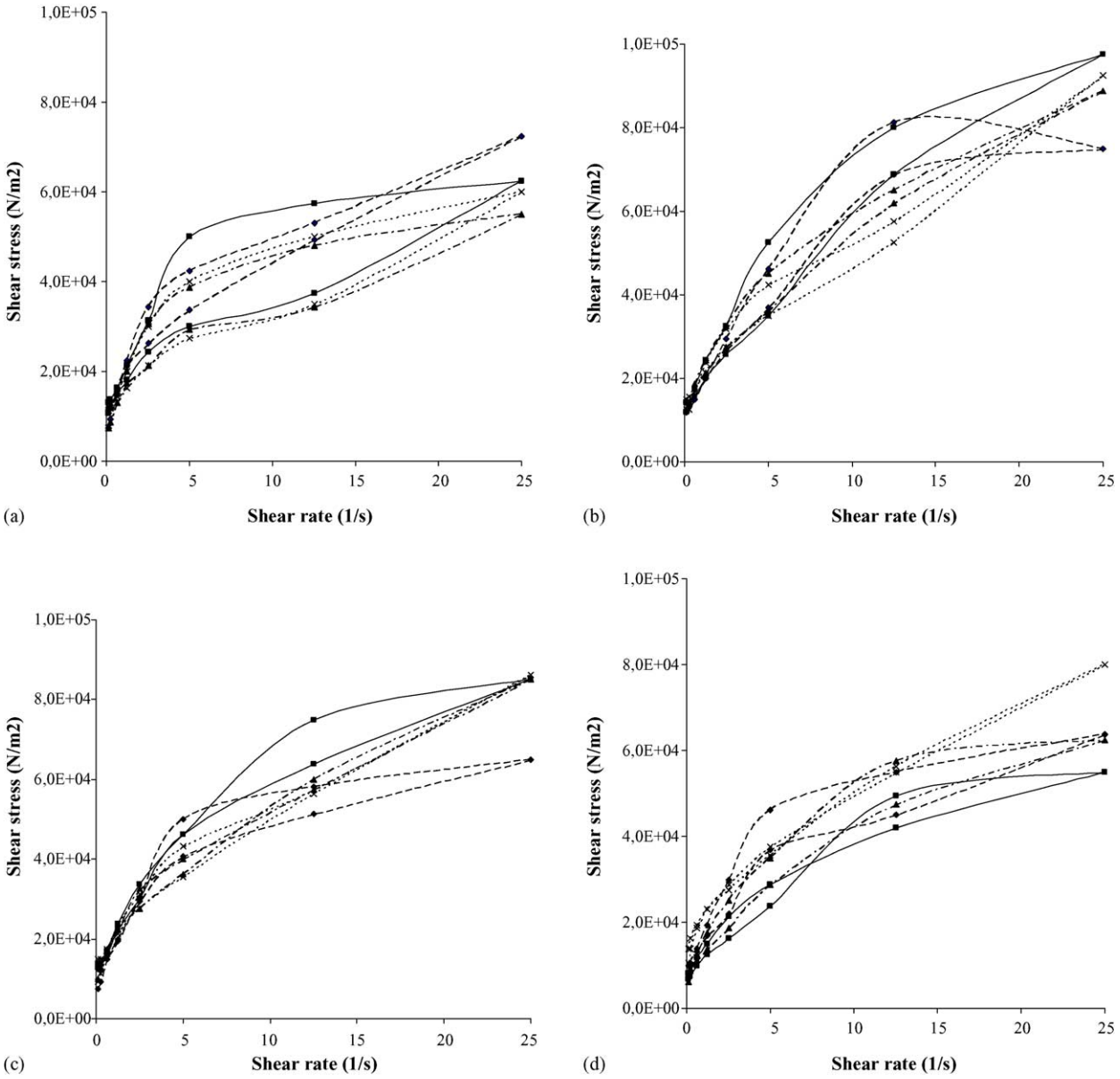

Fig. 2. Rheology of sunscreen emulsion samples stored at selected temperature conditions: 10 °C (a); 25 °C (b); 35 °C (c); 45 °C (d) during 90 days study period: (♦) 7 days; (■) 30 days; (▲) 60 days; (×) 90 days in glass pot packaging materials.

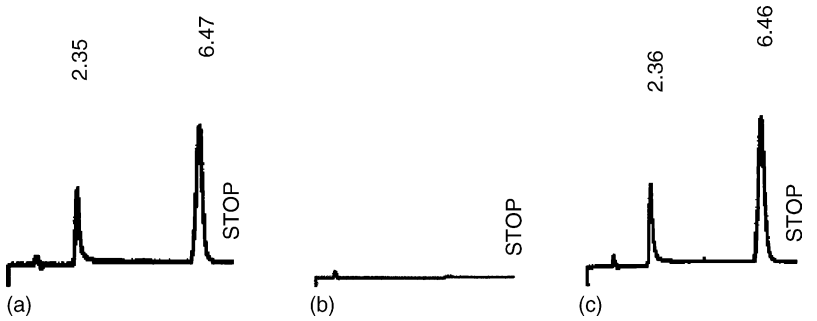

Fig. 3. Chromatograms of sample (a), placebo formulation (b) and standards (c), using a LiChrospher® 100 RP-18 Merck® column, particle size 5 μm, 125 mm × 4 mm i.d., methanol–water (87:13 v/v) as mobile phase, flow rate 1.0 mL/min, UV detection at 290 nm and room temperature (25 ± 1 °C).

3.2. High performance liquid chromatography (HPLC)

The system suitability is integral part of method development and its validation. Chromatographic conditions were adjusted in order to optimize selectivity, resolution and elution time. The peaks were well resolved within 7 min as shown in Fig. 3. An analytical method should be validated in order to obtain reliable results. The linearity of an analytical method is its ability to produce responses proportional to the analyte concentration within a given range (ICH Q2B, 1996; Jenke, 1996; Krull and Swartz, 1999; US Pharmacopeia, 2002). The calibration curves were constructed by plotting the peak areas against concentration (μg/mL). The linear regression data for each sunscreen is presented in Table 1. The correlation coefficient data substantiate official literature recommendations (Garfield, 1991). The DL and QL were determined and the results are presented in Table 1.

The precision of the proposed HPLC method was established through repeatability of the responses, expressed as relative standard deviation (R.S.D.). In pharmaceutical analysis, a R.S.D. less than 2% substantiates the precision of an analytical method (Jenke, 1996; Krull and Swartz, 1999; Santoro et al., 2000). The mean inter-day repeatability results ($n = 10$) are presented in Table 1.

For pharmaceutical applications, the percentage of recovery must be within an interval of 98 and 102% to prove the accuracy of a method (Jenke, 1996). The experimental results were considered satisfactory, as the sample used in this work was a cosmetic preparation (Azevedo et al., 1999). The average recovery data is presented in Table 1.

Table 1
Statistical results obtained in the validation of the HPLC method

Statistical data	Benzophenone-3	Octyl methoxycinnamate
Concentration range (μg/mL)	4.0–18.0	8.0–36.0
Number of injections	8	8
Slope	2120.6	3202.66
Intercept	−612.35	821.5
Coefficient of correlation	0.9999	0.9999
Detection limit (μg/mL)	0.11	0.59
Quantitation limit (μg/mL)	0.38	1.98
Confidence limit[a] (%)	99.88 ± 0.42	100.68 ± 0.32
Precision (R.S.D.)[a]	0.58	0.45
Accuracy (average recovery) (%)[b]	101.88	98.28

[a] Average of 10 determinations.
[b] Mean of three determinations, at three concentration levels.

The specificity of a method is its ability to produce reliable results in the presence of impurities and excipients of the formulation (ICH Q2B, 1996). The results obtained in the analysis of placebo showed that the emulsion excipients do not interfere in the analysis of analytes (Fig. 3).

The results obtained with the emulsion stored in glass flasks showed better profile, at all temperatures and for a longer period (Table 2). The emulsions stored in metallic tube, at room temperature showed good stability (Table 3), nevertheless at all other temper-

Table 2
Results of quantitative HPLC determination of B-3 and OM in samples stored in glass and plastic flasks at 10 °C, room temperature (25 ± 1 °C), 35 and 45 °C, during 2, 7, 30 and 60 days

Sample	Temperature (°C)	Storage period (glass flask)				Storage period (plastic flask)			
		2	7	30	60	2	7	30	60
B-3	10	nd[a]	95.95	98.90	97.00	nd[a]	97.79	98.00	93.90
	25	100.98	100.35	98.95	100.29	102.28	101.79	98.19	96.07
	35	nd[a]	99.38	97.97	101.70	nd[a]	103.86	102.60	100.70
	45	nd[a]	101.05	101.33	99.59	nd[a]	104.40	103.60	104.15
OM	10	nd[a]	92.52	99.10	96.90	nd[a]	96.77	98.90	94.41
	25	100.01	100.89	97.52	100.67	100.02	103.32	98.25	98.64
	35	nd[a]	99.15	98.80	102.71	nd[a]	101.73	102.10	101.81
	45	nd[a]	100.08	100.85	101.39	nd[a]	104.05	103.45	107.78

[a] Not determined.

Table 3
Results of quantitative HPLC determination of B-3 and OM in samples stored in metallic and plastic tubes at 10 °C, room temperature (25 ± 1 °C), 35 and 45 °C, during 2, 7, 30 and 60 days

Sample	Temperature (°C)	Storage period (metallic tubes)				Storage period (plastic tubes)			
		2	7	30	60	2	7	30	60
B-3	10	nd[a]	94.19	93.68	92.16	nd[a]	99.97	98.42	96.0
	25	99.50	100.60	99.46	101.65	103.99	102.89	100.70	99.00
	35	nd[a]	99.23	98.24	94.89	nd[a]	105.88	99.70	99.04
	45	nd[a]	102.34	102.10	95.59	nd[a]	104.50	102.28	102.22
OM	10	nd[a]	96.33	96.05	93.66	nd[a]	99.85	98.65	95.99
	25	100.32	100.88	100.79	102.73	102.34	103.72	102.20	101.34
	35	nd[a]	98.79	99.20	94.43	nd[a]	103.16	103.05	105.56
	45	nd[a]	101.35	100.95	96.58	nd[a]	105.89	102.55	103.48

[a] Not determined.

ature conditions, the quantity of both sunscreens decreased with time, reaching to 92.16% of B-3 within 60 days.

The emulsions stored in plastic containers maintained their stability at 35 °C and at room temperature, nevertheless alteration in the sunscreen concentrations were observed at 10 and 45 °C.

Tables 2 and 3 show the results obtained in the quantitative determination of B-3 and OM in samples stored in glass and plastic flasks and in metallic and plastic tubes, stored at 10 °C, room temperature (25 ± 1 °C), 35 and 45 °C, during 2, 7, 30 and 60 days. Even though there were small variations in the B-3 and OM concentration at temperature studied, all analytical results obtained were in accordance with pharmacopeial specifications during 60 days study period.

4. Conclusions

The proposed HPLC method enabled the separation and quantitative determination of B-3 and OM present in sunscreens. The method was successfully applied in the stability studies of the emulsions. The method is simple, precise and accurate; there was no interference from formulation components. The sample emulsions stored at different temperatures presented similar rheological behavior, at least during the period of the study (3 months). Most of the samples showed a pseudoplastic non-newtonian thixotropic profile. There were no significant changes in the physical and chemical stability of emulsions stored in different packaging material. The studied glass and plastic packaging materials were found adequate for storing referred solar protector emulsions.

Acknowledgment

The authors wish to acknowledge the "Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), São Paulo, SP, Brazil, for the financial support.

References

Association of Official Analytical Chemists (AOAC) International., 1990. Official Methods of Analysis of the Association of Official Analytical Chemists. 15th ed. Arlington, pp. 15–17.

Azevedo, J.S., Viana Jr., N.S., Soares, C.D.V., 1999. UVA/UVB sunscreen determination by second-order derivative ultraviolet spectrophotometry. Il Farmaco 54, 573–578.

Dutra, E.A., Kedor-Hackmann, E.R.M., Santoro, M.I.R.M., 2002. Validation of a high performance liquid chromatography method for sunscreen determination in cosmetics. Int. J. Cos. Sci. 24, 97–102.

Epstein, J.H., 1990. Biological effect of sun light. In: Lowe, N.J., Shaath, N.A. (Eds.), Sunscreens: Development, Evaluation and Regulatory Aspects. Marcel Dekker Inc., New York, pp. 43–54.

Garfield, F.M., 1991. Quality Assurance Principles for Analytical Laboratories. Association of Official Analytical Chemists International, Arlington.

ICH Q2A., 1995. Text on Validation of Analytical Procedures. Geneva.

ICH Q2B., 1996. Validation of Analytical Procedures: Methodology. Geneva.

Jenke, D.R., 1996. Chromatographic method validation: a review of current practices and procedures. II. Guidelines for primary validation parameters. J. Liq. Chromatogr. Relat. Technol. 19, 737–757.

Krull, I.R., Swartz, M., 1999. Analytical method development and validation for the academic research. Anal. Lett. 32, 1067–1080.

Krutmann, J., 2000. Photoaging, photodermatosis and photocarcinogenesis: recent advances in understanding the detrimental effects of ultraviolet-A radiation on human skin. IFSCC Mag. 3, 51–54.

Molen, R.G., Out-Luiting, C., Claas, F.H.J., Norval, M., Koerten, H.K., Mommaas, A.M., 2001. Ultraviolet-B radiation induces modulation of antigen presentation of Herpes simplex virus by human epidermal cells. H. Immunol. 62, 589–597.

Osterwalder, U., Luther, H., Herzog, B., 2000. Novo protetor UVA. Cosmet. Toilet. Ed. Port. 12, 52–59.

Parisi, A.V., Wong, J.C.F., 2000. An estimation of biological hazards due to solar radiation. J. Photochem. Photobiol. B: Biol. 54, 126–130.

Portad, G., Laugel, C., Baillet, A., Schaefer, H., Marty, J.P., 1999. Quantitative HPLC analysis of sunscreens and caffeine during in vitro percutaneous penetration studies. Int. J. Pharm. 189, 249–260.

Rastogi, S.C., Jensen, G.H., 1998. Identification of UV filters in sunscreen products by high-performance liquid chromatography-diode array detection. J. Chromatogr. A 828, 311–316.

Romanowski, P., Schueller, R., 2000. Introdução aos produtos fotoprotetores. Cosmet. Toilet. Ed. Port. 12, 60–67.

Santoro, M.I.R.M., Silva, F.C.F., Kedor-Hackmann, E.R.M., 2000. Stability analysis of emulsions containing UV and IR filters. Cosmet. Toilet. 115, 55–62.

Scalia, S., 2000. Determination of sunscreen agents in cosmetic products by supercritical fluid extraction and high-performance liquid chromatography. J. Chromatogr. A 870, 199–205.

US Pharmacopeia XXIV., 2002. US Pharmacopeial Convention, Rockville, MD.

Vanquerp, V., Rodriguez, C., Coiffard, C., Coiffard, L.J.M., Roeck-Holtzhauer, Y., 1999. High-performance liquid chromatographic method for the comparison of the photo stability of five sunscreen agents. J. Chromatogr. A 832, 273–277.