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Broad spectrum bioactive sunscreens

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ABSTRACT

The development of sunscreens containing reduced concentration of chemical UV filters, even though, possessing broad spectrum effectiveness with the use of natural raw materials that improve and infer UV absorption is of great interest. Due to the structural similarities between polyphenolic compounds and organic UV filters, they might exert photoprotection activity. The objective of the present research work was to develop bioactive sunscreen delivery systems containing rutin, *Passiflora incarnata* L. and *Plantago lanceolata* extracts associated or not with organic and inorganic UV filters. UV transmission of the sunscreen delivery system films was performed by using diffuse transmittance measurements coupling to an integrating sphere. *In vitro* photoprotection efficacy was evaluated according to the following parameters: estimated sun protection factor (SPF); Boot's Star Rating category; UVA/UVB ratio; and critical wavelength (λ_c). Sunscreen delivery systems obtained SPF values ranging from 0.972 ± 0.004 to 28.064 ± 2.429 and bioactive compounds interacted with the UV filters positive and negatively. This behavior may be attributed to: the composition of the delivery system; the presence of inorganic UV filter and quantitative composition of the organic UV filters; and the phytochemical composition of the *P. incarnata* L. and *P. lanceolata* extracts. Among all associations of bioactive compounds and UV filters, we found that the broad spectrum sunscreen was accomplished when 1.68% (w/w) *P. incarnata* L. dry extract was in the presence of 7.0% (w/w) ethylhexyl methoxycinnamate, 2.0% (w/w) benzophenone-3 and 2.0% (w/w) TiO_2. It was demonstrated that this association generated estimated SPF of 20.072 ± 0.906 and it has improved the protective defense against UVA radiation accompanying augmentation of the UVA/UVB ratio from 0.49 to 0.52 and λ_c from 364 to 368.6 nm.

1. Introduction

Sunscreens are mainly used to prevent erythema formation from sun exposure. The decrease in amount of UV radiation reaching the skin through sunscreens may further reduce risk of sun-induced skin cancer. The UV spectrum is conveniently divided into three groups based on wavelength: UVC (100–290 nm), UVB (290–320 nm) and UVA (320–400 nm). UVA is further subdivided into UVA2 (320–340 nm) and UVA1 (340–400 nm). Solar UV radiation at the Earth's surface is approximately 90–99% UVA and 1–10% UVB (COLIPA, 2006; Verschooten et al., 2006; Faurschou and Wulf, 2007).

UVB radiation primarily causes photocarcinogenesis due its direct interaction with cellular DNA and subsequent formation of cyclobutane pyrimidine dimmers and thymine glycols, but there are several reasons why investigation of the role of UVA is also relevant. Major consequence of cumulative UVA radiation is the generation of reactive oxygen species which, however, can also induce cancer, for instance, generating oxidized DNA base derivatives, like 8-hydroxydeoxyguanosine, and altered tumor suppressor genes, like p53 (Seité et al., 2000; Heinrich et al., 2004; Vielhaber et al., 2006).

It has been demonstrated, in human fibroblast model, the induction of 8-hydroxydeoxyguanosine after radiation from UVA1 (>334 nm) up to near visible light (434 nm). Whereas UVB effects are mainly restricted to the epidermis, UVA radiation directly affect the dermal compartment and is therefore thought to be the major factor responsible for photoaging of human skin. It had been shown that the UVA1 accounts for damaging effects in human dermal fibroblasts, as induction of cytokines, matrix metalloproteinases, and mtDNA mutations. Of these, the induction of collagenase (matrix metalloproteinase-1; MPP-1) which degrades collagen-type I, the major constituent of the connective tissue, is of particular significance since the extent of collagen I reduction

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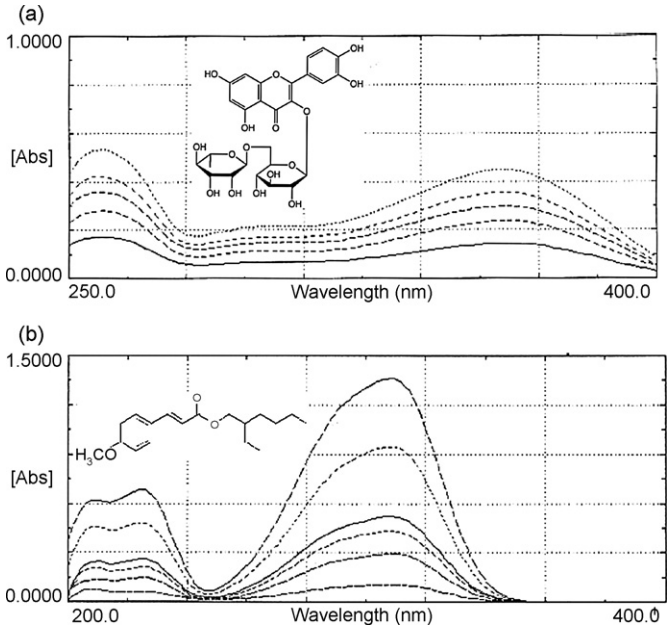

Fig. 1. Absorbance profiles of (a) rutin (5.0–15.0 μg mL^{-1}) and (b) ethylhexyl methoxycinnamate (3.5–15.0 μg mL^{-1}) in 99.5% ethanol (Velasco et al., 2008). [Abs]: absorbance.

correlates with photodamage in human skin (Persson et al., 2002; Vielhaber et al., 2006).

Recently, there is a world trend to the development of sunscreens containing reduced concentration of chemical UV filters, even though, possessing broad spectrum anti-UV radiation effectiveness (Velasco et al., 2008), and natural or bioactive products have been the focus of several researches due to the presumable safe utilization, ecological issues (sustainability), and minimum ambient impact (Banov et al., 2006; Rolim et al., 2006). Oriented to the sunscreen development, the use of natural raw materials that infers UV absorption and skin protection against UVB and UVA radiation is of great interest, associated with the benefits of these products and compliance of the consumers (Tabrizi et al., 2003).

Polyphenolic compounds exhibit a wide number of biological properties as an outcome of their different *in vivo* action mechanism. Their several pharmacological activities include: antiallergic, antiinflamatory, hepatoprotective, vasoactive, antithrombotic, antioxidant, free radical scavenging, antitumor, antibacterial and antiprotozoal, among others (Movileanu et al., 2000; Baby et al., 2006). Some studies have also been performed on various plants such as *Aloe*, *Helichrysum*, *Chamomile*, *Hamamelis*, *Cinnmomum*, *Camellia*, *Rosa*, *Ginkgo* and *Polypodium*, among others (Tabrizi et al., 2003; Kullavanijaya and Lim, 2005; Maia Campos et al., 2006; Yusuf et al., 2007). Due to the structural similarities between polyphenolic compounds, such as flavonoids, and organic UV filters, in addition to the antioxidant and absorbance spectrum profiles of these bioactive compounds, illustrated in Fig. 1, they might exert photoprotection activity (Velasco et al., 2008).

The objective of the present research work was to develop sunscreen delivery systems containing, as bioactive compounds, rutin, *Passiflora incarnata* L. and *Plantago lanceolata* extracts associated or not with UVB and UVA organic and inorganic UV filters. Bioactive sunscreens had their photoprotection effectiveness obtained *in vitro* by diffuse transmittance measurements. *P. incarnata* L. and *P. lanceolata* extracts were selected as potential bioactive agents due to their phytochemical compositions possessing considerable content of polyphenolic compounds. Phytochemical trials also identified the following constituents for these extracts: alkaloids, flavonoids, tannins, carbohydrates, glycosides, benzoflavone, volatile substances and amino acids, among others (Dhawan et al., 2004; Brady et al., 2003).

2. Experimental

2.1. Development of prototype sunscreens: dimethicone-polymer delivery systems

Sun-care delivery systems were developed as innovative dimethicone-polymer emulsions obtained by cold process with composition described in Table 1. The design of the delivery systems was based on sun-care emulsions reported by Velasco et al. (2008). *Mangifera indica* seed (mango) butter (and) dimethicone (and) ceteth-20 (and) steareth-21 (DC® 7–3123 Mango Blend Emulsion), *Butyrospermum parkii* (shea butter) (and) dimethicone (and) ceteth-20 (and) steareth-21 (DC® 7–3121 Shea Blend Emulsion) and sodium polyacrylate (and) dimethicone (and) cyclopentasiloxane (and) trideceth-6 (and) PEG/PPG-18/18 dimethicone (DC® RM 2051 Thickening Agent) were gifts from Dow Corning (Brazil). Alcohol denat., BHT (butylated hydroxytoluene), propylene glycol, sodium hydroxide and citric acid were purchased from Lab-Synth (Brazil). Sodium lauryl sulfate (Genapol® LSS), ammonium acryloyldimethyltaurate/VP copolymer (Aristoflex® AVC), PEG-8 (Carbowax® PEG 400) and disodium EDTA (Uniquelan® Na2S) were acquired from Pharmaspecial (Brazil). 2-Bromo-2-nitropropane-1,3-diol (Protectol® BN) was kindly provided by Croda (Brazil). Raw materials were of analytical, cosmetic or pharmaceutical grade and used as received, without any further purification.

Table 1
Qualitative and quantitative composition (%, w/w) of the delivery systems to be incorporated with the bioactive compounds and organic and inorganic UV filters

Components (INCI)[a]	Composition (%, w/w)
Mangifera indica seed (mango) butter (and) dimethicone (and) ceteth-20 (and) steareth-21	1.5
Butyrospermum parkii (shea butter) (and) dimethicone (and) ceteth-20 (and) steareth-21	1.5
Sodium polyacrylate (and) dimethicone (and) cyclopentasiloxane (and) trideceth-6 (and) PEG/PPG-18/18 dimethicone	1.5
Alcohol Denat.	15.0
Sodium lauryl sulfate	0.5
BHT	0.05
Ammonium acryloyldimethyltaurate/VP copolymer	3.0
Propylene glycol	3.0
PEG-8	3.0
Disodium EDTA	0.05
2-Bromo-2-nitropropane-1,3-diol	0.03
Sodium hydroxide ou citric acid	pH 6.0 ± 0.5
Aqua	q.s. 100.0

[a] Qualitative composition was reported in accordance with INCI (*International Nomenclature of Cosmetic Ingredient* – http://ec.europa.eu/enterprise/cosmetics/inci/inci_2006.pdf).

Table 2
Total flavonoids (%, w/w), expressed in rutin, of *Passiflora incarnata* L. dry extract and *Plantago lanceolata* hydroglycolic extract

Bioactive extracts	*Passiflora incarnata* L.	*Plantago lanceolata*
Total flavonoids (%, w/w)	5.951 ± 0.146	3.591 ± 0.109

As bioactive compounds, rutin (Henrifarma, Brazil), *P. incarnata* L. dry extract (Grupo CentroFlora, Brazil) or *P. lanceolata* hydroglycolic extract (Phytessence® plantago, Croda Brazil) were added to the delivery systems and they were associated or not with organic and inorganic UV filters. Ethylhexyl methoxycinnamate (UVB organic filter, Uvinul® MC 80, Basf, Brazil), benzophenone-3 (UVA organic filter, Uvinul® M 40, Basf, Brazil) and titanium dioxide (inorganic filter, Henrifarma, Brazil) were selected due to their wide utilization, as anti-UV radiation actives, in commercially available sunscreen products (Gaspar and Maia Campos, 2003; Serpone et al., 2006; More, 2007; Velasco et al., 2008).

Bioactive compounds, specifically dry and hydroglycolic extracts, prior to inclusion into the dimethicone-polymer delivery systems, had their content of total flavonoids (expressed in rutin) quantified (%) according to UV spectrophotometric method at 361.0 nm with solvent composed of 95.0% ethanol–0.02 M acetic acid (99:1, v/v) and, as reference, standard rutin 96.1%, NF, XI. The analytical method was previously validated and described elsewhere (Rolim et al., 2005; Baby et al., 2006). The content of total flavonoids (%, w/w), expressed in rutin, of *P. incarnata* L. dry extract and *P. lanceolata* hydroglycolic extract was presented in Table 2.

The content of total flavonoids from the extracts was achieved to allow the incorporation of analogous bioactive concentration, comparable to isolated rutin equal to 0.1% (w/w). Table 3 reports associations and proportions of the bioactive compounds and UV filters.

2.2. Substrate treatment

Rough exterior synthetic collagen-type sheet (Vitro-Skin®, IMS Inc., USA), mimicking human's skin surface geometry, was treated as described by Diffey et al. (2000), and Velasco et al. (2008), prior to the assessment of *in vitro* photoprotection efficacy. Collagen-type sheets were hydrated by complete immersion in distilled water for 24 h. Once hydrated, it acquired texture similar to human epidermis and seemed to assist break down of sunscreen emulsions likewise as in human skin (Springsteen et al., 1999). After hydration, substrate was placed in temperature- (20.0 ± 2.0 °C) and humidity-controlled environment until the spectrophotometric analysis of the sunscreen delivery systems.

2.3. In vitro photoprotection efficacy

Delivery system samples of 70.0 mg were accurately weighed and uniformly applied on the treated collagen-type substrate as a thin homogeneous film of 2.0 μg cm^{-2} with 5.0 mm thickness. Samples were gently rubbed on the substrate rough side employing circular motions, from edges to center, by a saturated glove-coated finger. Then, sample films over substrate were allowed to dry under room temperature (20.0 ± 2.0 °C), protected from light exposure, for 20 min. This rest condition is also relevant to allow fully breaking of dimethicone-polymer delivery systems (Springsteen et al., 1999; Diffey et al., 2000; Couteau et al., 2007; Velasco et al., 2008).

The principle of the analysis is based on diffuse transmittance measurements in which light scattered at different angles is collected using an integrating sphere (Springsteen et al., 1999; Gers-Barlag et al., 2001). UV transmission of the sunscreen delivery system films was performed by using an UV-1000S Ultraviolet Transmittance Analyzer equipped with integrating sphere and xenon flashlamp (Labsphere®, USA). Spectra are obtained with two photodiode array spectrographs. Transmittance values were registered on the interval of wavelength ranging from 290 to 400 nm. After the analysis, *in vitro* photoprotection efficacy was evaluated according to the following parameters: UVB efficacy by estimated sun protection factor (SPF); UVA efficacy by Boot's Star Rating and UVA/UVB ratio; and critical wavelength (λ_c). Replicates of five were performed (Springsteen et al., 1999; Diffey et al., 2000; FDA, 2007; Velasco et al., 2008).

2.4. Theoretical background

The *in vitro* photoprotection test method allows us to determine the performance of sunscreen actives or final products against UV radiation, ranging from UVB to UVA (Heinrich et al., 2004).

The technique involves measuring the diffuse transmittance of a defined layer of the tested sample in the UV spectrum. Estimated SPF (Eq. (1)) is calculated as follows (Springsteen et al., 1999; Gers-Barlag et al., 2001):

$$\mathrm{SPF} = \frac{\int_{280\,\mathrm{nm}}^{400\,\mathrm{nm}} E_\lambda S_\lambda \, \mathrm{d}\lambda}{\int_{280\,\mathrm{nm}}^{400\,\mathrm{nm}} E_\lambda S_\lambda T_\lambda \, \mathrm{d}\lambda} \quad (1)$$

where E_λ: spectral irradiance of used light spectrum at wavelength λ; S_λ: erythemal action spectrum at wavelength λ; T_λ: measured sample transmittance at wavelength λ.

Table 3
Concentration (%, w/w) and associations of bioactive compounds and organic/inorganic UV filters

SDS	Concentration (%, w/w)					
	R	Pi	Pl	EHMC	BZP	TiO_2
263 (control)	Zero					
264		Zero		3.5	1.0	1.0
265		Zero		7.0	2.0	2.0
266	0.1			Zero		
269	0.1		Zero	3.5	1.0	1.0
270	0.1		Zero	7.0	2.0	2.0
267	Zero	1.68[a]			Zero	
271	Zero	1.68[a]	Zero	3.5	1.0	1.0
268	Zero	1.68[a]	Zero	7.0	2.0	2.0
272	Zero		2.78[b]		Zero	
273	Zero		2.78[b]	3.5	1.0	1.0
274	Zero		2.78[b]	7.0	2.0	2.0

SDS: sunscreen delivery systems; R: rutin; Pi: *Passiflora incarnata* L. dry extract; Pl: *Plantago lanceolata* hydroglycolic extract; EHMC: ethylhexyl methoxycinnamate; BZP: benzophenone-3; TiO_2: titanium dioxide.

[a] Concentration of the *Passiflora incarnata* L. dry extract equivalent in 0.1% (w/w) isolated rutin

[b] Concentration of the *Plantago lanceolata* hydroglycolic extract equivalent in 0.1% (w/w) isolated rutin.

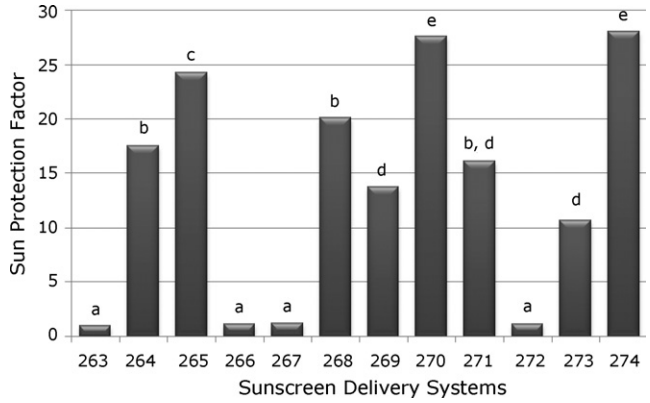

Fig. 2. *In vitro* SPF values of the sunscreen delivery systems (263–274: delivery systems). Equal letters over the bars indicate statistical similarities ($p < 0.05$).

To calculate critical wavelength (λ_c), the area under the absorbance curve (AUC) is set at 100%. λ_c is the wavelength at which 90% of the AUC is reached in the range 290–400 nm. According to FDA (2007), a five-point scale ranging from 0 to 4 is used to classify products: 0 ($\lambda_c < 325$ nm); 1 ($325 \leq \lambda_c < 335$), 2 ($335 \leq \lambda_c < 350$), 3 ($350 \leq \lambda_c < 370$), 4 ($370 < \lambda_c$). Springsteen et al. (1999), classified as a broad spectrum sunscreen the product that achieved λ_c higher than 370 nm. The following Eq. (2) (critical wavelength) is applied (Diffey et al., 2000; Gers-Barlag et al., 2001; Heinrich et al., 2004):

$$\int_{290\text{nm}}^{\lambda_c} A(\lambda) * \delta\lambda = 0.9 * \int_{290\text{nm}}^{400\text{nm}} A(\lambda) * \delta\lambda \quad (2)$$

where A: absorbance.

The UVA/UVB ratio is a criterion that reflects the ratio of the integral UVA to UVB attenuation. The closer the ratio is to 1, the more similar are the integral UVA- and UVB-attenuation values. For its determination, the Eq. (3) (UVA/UVB ratio (R)) is employed (Gers-Barlag et al., 2001; Heinrich et al., 2004):

$$R = \frac{\int_{320\,\text{nm}}^{400\,\text{nm}} A(\lambda)\,\mathrm{d}\lambda / \int_{320\,\text{nm}}^{400\,\text{nm}} \mathrm{d}\lambda}{\int_{290\,\text{nm}}^{320\,\text{nm}} A(\lambda)\,\mathrm{d}\lambda / \int_{290\,\text{nm}}^{32\,\text{nm}} \mathrm{d}\lambda}. \quad (3)$$

2.5. Statistical treatment

One-way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison Test ($p < 0.05$) was performed by using GraphPad® Prism version 5.00 for Windows®, GraphPad® Software, San Diego, California, USA.

3. Results and discussion

During recent years, knowledge of the effects of UVB and UVA radiation from the sun on human skin has increased significantly. UV exposure of human skin induces multiple deleterious in the epidermis and dermis, and this type of radiation appears to have a dual role in the induction of skin cancers as it may cause several varieties of direct DNA damage plus suppress the immune response to developing skin cancers (FDA, 1999; Seité et al., 2000). The authentic necessity of human skin protection against solar radiation has led to the concerning and upgrading of the development of broad spectrum sunscreens highly effective over the UVB-UVA absorbing range. Modern sunscreen products are also intended to counteract several kinds of UV-induced skin damage such as photoallergies, skin wrinkles and dryness, sunburn and even skin cancer. From the Cosmetic Science viewpoint, the cumulative radiation exposure of skin is directly linked to the generation of fine lines and wrinkles

Table 4
In vitro estimated SPF values of sunscreen delivery systems

Sunscreen delivery systems (SDS)											
SDS-263	SDS-264	SDS-265	SDS-266	SDS-267	SDS-268	SDS-269	SDS-270	SDS-271	SDS-272	SDS-273	SDS-274
SPF											
0.972 ± 0.004	17.48 ± 0.438	24.256 ± 3.276	1.122 ± 0.008	1.192 ± 0.020	20.072 ± 0.906	13.748 ± 1.097	27.574 ± 2.056	16.106 ± 0.870	1.128 ± 0.011	10.730 ± 0.369	28.064 ± 2.429

SPF: estimated sun protection factor.

(Schulz et al., 2002; Velasco et al., 2008). Therefore, sun-care formulations are supposed to deliver to the skin multi-aggregate benefits, such as: emolliency, moisture, free radical scavenging protection, UV filter non-migration, anti-aging prevention, UV substantivity (adhesion) to the skin, well-being and environment protection, among other.

In vitro studies have been considered of utmost importance to elucidate the potential of some active substance additions to cosmetic formulations. Results from *in vitro* assessments may previously validate submission to *in vivo* efficacy experiments through non-clinical and clinical studies (Santos et al., 1999; Maia Campos et al., 2006). Regarding sun-care experiments, it is also a safety issue, since only *in vitro* positive responses will direct the future of the *in vivo* tests.

Sunscreen delivery systems obtained SPF values ranging from 0.972 ± 0.004 (SDS-263) to 28.064 ± 2.429 (SDS-274), determined by diffuse transmittance analysis coupling with integrating sphere. Bioactive compounds interacted with organic and inorganic UV filters positive and negatively, and photoprotection efficacy was dependent of the nature of the bioactive compound and UV filter concentrations. Fig. 2 and Table 4 summarize SPF values of sunscreen delivery systems containing bioactive compounds associated or not with UV filters.

Highest SPF values (statistically significant and equivalent) were synergistically reported when rutin (SDS-270; SPF = 27.574 ± 2.055) or *P. lanceolata* hydroglycolic extract (SDS-274; SPF = 28.064 ± 2.429) were associated with 7.0% (w/w) ethylhexyl methoxycinnamate, 2.0% (w/w) benzophenone-3 and 2.0% (w/w) TiO_2. Delivery systems containing UV filters at high concentrations (SDS-265) have accomplished SPF of 24.256 ± 3.276 and, at low concentrations (SDS-264), 17.480 ± 0.438. Fig. 3 shows the absorbance spectrum profiles of the sunscreens delivery systems.

Unpredictably, *P. incarnata* L. dry extract interacted negatively with UV filters at high concentrations (SDS-268), provoking significant reduction of the SPF value to 20.072 ± 0.906. Similar profile was observed for *P. lanceolata* hydroglycolic extract (SDS-273; SPF = 10.730 ± 0.369), in which UVB protection was decreased in comparison with SDS-264. This behavior may be attributed to four main factors: composition of the delivery system, as a vehicle; presence of inorganic UV filter (TiO_2); quantitative composition of the organic UV filters; and phytochemical composition of the *P. incarnata* L. and *P. lanceolata* extracts.

Formerly reported, rutin has statistically improved the SPF value of a sunscreen containing 3.5% (w/w) ethylhexyl methoxycinnamate and 1.0% (w/w) benzophenone-3, in comparison to a similar preparation bioactive-free, from 7.34 ± 0.24 to 9.97 ± 0.18. It is made noteworthy that the stated formulations were not incorporated with TiO_2. Divergently with this present research, when rutin was associated with organic UV filters at high concentrations, SPF augmentation was not observed (SPF rutin-free = 14.63 ± 0.05; SPF rutin = 14.94 ± 1.41; data reported previously by Velasco et al., 2008).

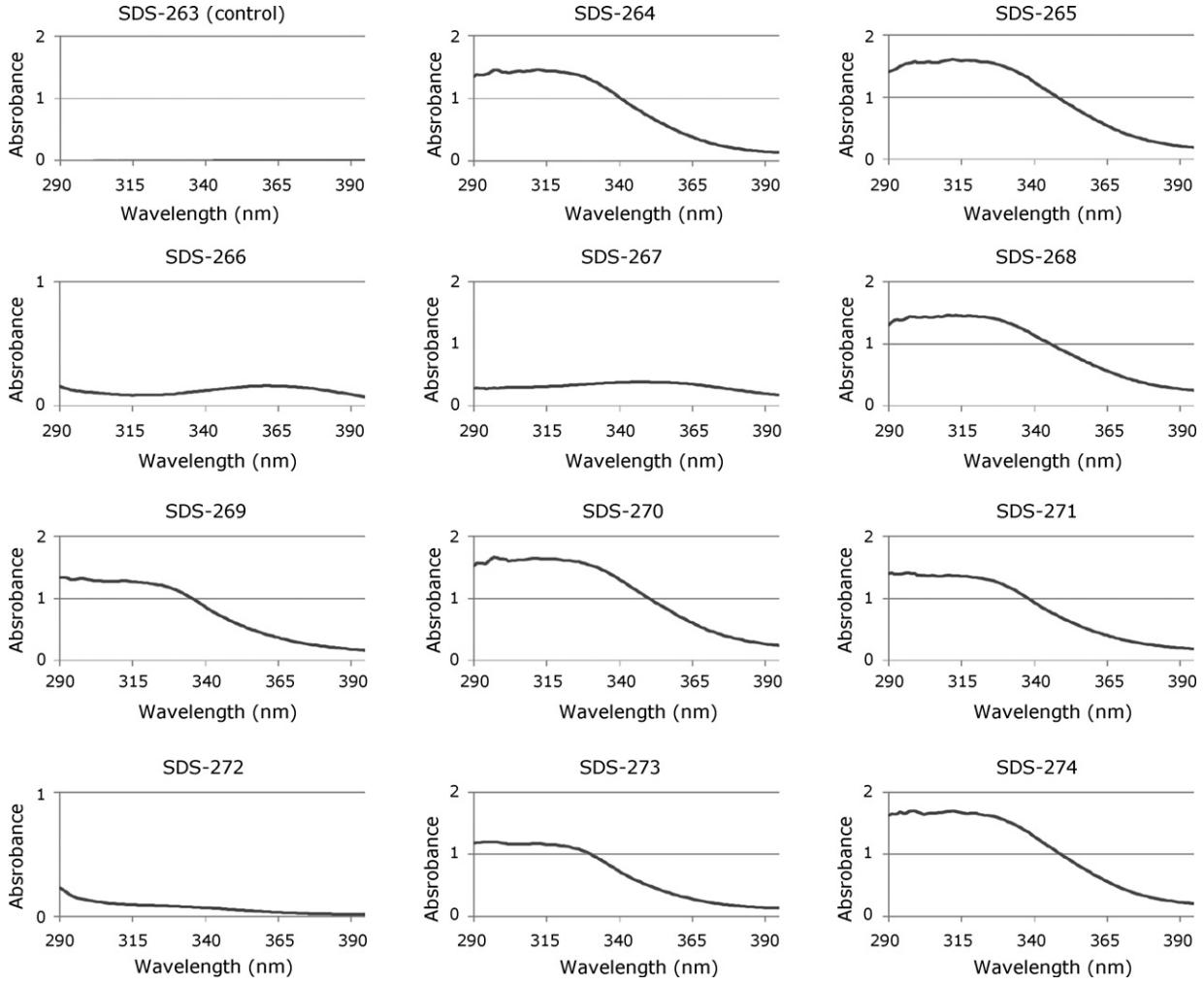

Fig. 3. Absorbance spectrum profiles of the sunscreens delivery systems (263–274: delivery systems).

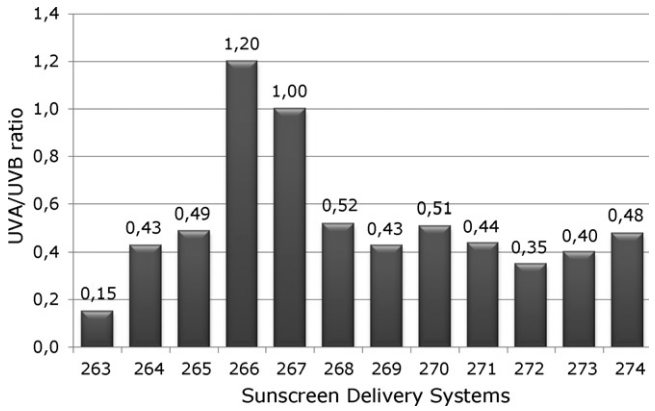

Fig. 4. UVA/UVB ratio of the sunscreen delivery systems (263–274: delivery systems).

Organic UV filters are sun-care active substances existing, generally, as aromatic compounds conjugated with a *para* or *ortho* electron-releasing group and an electron-acceptor one. This chemical structure favors electron delocalization and transfer from the electron-releasing to the electron-acceptor group. Quantum mechanical calculations have shown that this electron delocalization energy corresponds to the energy of the radiation quanta present in the UVB and UVA region. Complementing, organic UV filters decrease the dose of UV light reaching human skin by absorbing the radiation via electron promotion from a lower energy molecular orbital (LUMO) to a higher energy one (HOMO) (Wolf et al., 2001; Scalia et al., 2002; Flor et al., 2007). Thus, it may be suggested that the phytochemical composition of the extracts, considering the presence of flavonoids as electronegative polyphenolic compounds (Martins et al., 2004; Mielczarek, 2005; Mukherjee et al., 2005; Teixeira et al., 2005), could have stabilized the organic UV filter molecules, elevating the amount of energy between LUMO and HOMO. Since the energy is inversely proportional to wavelength, the latter was diminished which might have caused a dislocation of the maximum wavelength to values inferior than 290 nm, consequently, provoking the decline of the SPF. Also, the opposite behavior might have occurred and favored UVA protection effectiveness and, accordingly, maximum wavelength could have reached values superior than 320 nm which might justify some improvement of the sunscreen delivery systems against UVA radiation. From a practical application perspective, it was noticed that associating UV filters with bioactive compounds in a complex medium, as an emulsion, led to a distinctive photochemistry profile, different from the photochemistry of isolated compounds. The reactions occurred on the associations, as also observed by Dondi et al. (2006), involving ethylhexyl methoxycinnamate and avobenzone, as UV filters, were concentration- and medium-dependent, which were consistent with our experimental results.

SDS-263 (control), SDS-266, SDS-267 and SDS-272 generated SPF statistically alike of, approximately, 1. Even so, SDS-266 and SDS-267 obtained UVA/UVB ratios of 1.20 and 1.00, respectively, and, unexpectedly, SDS-272 offered UVA/UVB ratio of 0.35. Control provided 0.15 for this parameter. Fig. 4 presents the UVA/UVB ratio results. The UVA/UVB ratio derived from extinction spectra of sunscreen formulations. It is a result of the reduction of the complete spectral information to one number, characterizing, in some way, the shape of a spectrum in terms of UVA coverage in relation to the amount of UVB coverage (Herzog et al., 2002). Rutin and *P. incarnata* L. dry extract have increased UVA protection, in comparison with the control, when incorporated alone on the sunscreen delivery systems, whereas *P. lanceolata* hydroglycolic extract has not improved this relevant defense against UVA radiation. Although the bioactive

Table 5
Boot's Star Rating system and its relation with sunscreen claim for UVA protection (Springsteen et al., 1999)

Star category	Sunscreen claim related to UVA protection
No stars	No claim (too low for UVA claim)
★	Minimum
★★	Moderate
★★★	Good
★★★★	Superior
★★★★★	Ultra

sunscreens were developed with identical concentration of isolated natural or botanical raw materials (total flavonoids, expressed in rutin), it was observed, through our results, that the composition of the extracts played a major role on sunscreen effectiveness.

In our previous study (Velasco et al., 2008), 0.1% (w/w) rutin incorporated to an O/W emulsion developed with cetearyl alcohol (and) dicetyl phosphate (and) ceteth-10 phosphate has achieved UVA/UVB ratio of 0.95, inferior but compatible with the present research work. However, the emulsified phosphate-base system (control) was superiorly defensive against UVA radiation, with UVA/UVB ratio of 0.77, when compared to this authentic dimethicone-polymer delivery system.

According to the Boot's Star Rating system related to UVA effectiveness, sunscreen delivery systems were classified from non-protective against UVA to ultra-protective. Table 5 describes Star Rating categories and their association with sunscreen claim for UVA protection (Springsteen et al., 1999). Delivery systems developed with organic and inorganic UV filters (SDS-264 and SDS-265) have reached moderate-protection against UVA radiation. Independently of the UV filter concentration, the maximum UVA protection attained was moderate. Rutin (SDS-266) and *P. incarnata* L. dry extract (SDS-267) have provided UVA ultra-protection when alone at the delivery systems. *P. lanceolata* hydroglycolic extract (SDS-272) alone just reached UVA minimum-protection, what allowed us to suggest that this extract, despite its content of total flavonoids, expressed in rutin, was ineffective as an UVA filter. It was noticed that UV filters interacted negatively with rutin (SDS-269 and SDS-270) and *P. incarnata* L. dry extract (SDS-271 and SDS-268) concerning the defense against UVA radiation. This negative interaction seemed to be qualitative and independent of the UV filter concentrations, since reduction of UVA protection occurred

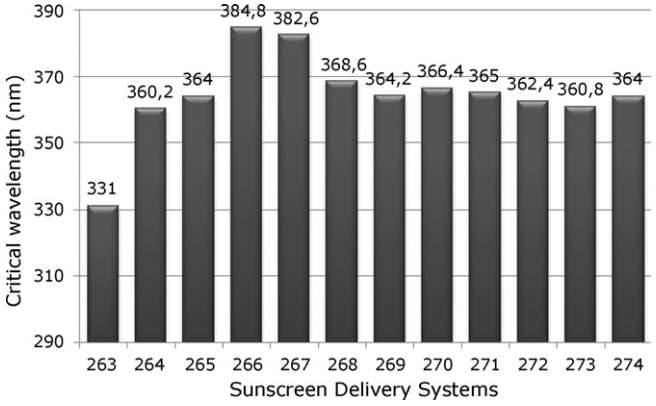

Fig. 5. Critical wavelength (λ_c, nm) of the sunscreen delivery systems (263–274: delivery systems).

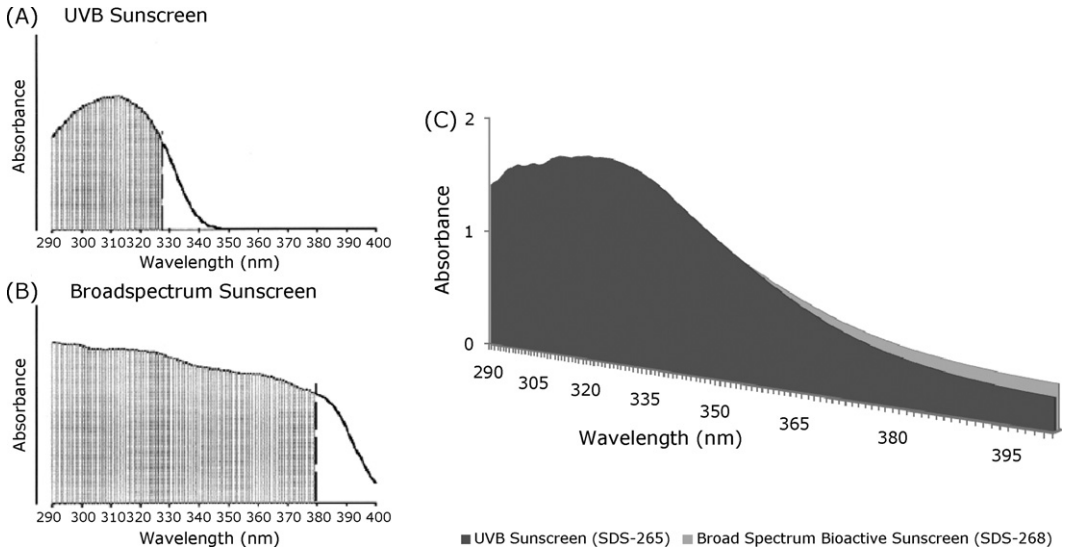

Fig. 6. Literature data (A and B) compared to the results obtained wherein (C) in relation to a UVB (SDS-265) and a broad spectrum bioactive sunscreen containing *P. incarnata* L. dry extract (SDS-268). A: UVB sunscreen; B: broad spectrum sunscreen (Diffey et al., 2000); and C: experimental results where dark gray represents the UVB sunscreen and the light gray, with the higher absorbance at UVA region (>320 nm), represents the broad spectrum bioactive sunscreen (note the absorbance extension in light gray).

simply by the presence of the UV filters with rutin and *P. incarnata* L. bioactive compounds. UV filters in the presence of *P. lanceolata* hydroglycolic extract have not shown interaction, since SDS-273 and SDS-274 have neither improved nor decreased the Star Rating category: it was kept identical to their similar sunscreen delivery systems, SDS-264 and SDS-265 (UVA moderate-protection).

We have also observed the effect of the delivery system on the UVA protection effectiveness. This new dimethicone-polymer delivery system (SDS-263) achieved UVA non-protection category, as could be estimated. On the other hand, a phosphate-base system mentioned earlier (Velasco et al., 2008), as an emulsion vehicle without addition of bioactives or UV filters, obtained UVA good-to superior-protection (data not shown). It has demonstrated the choice relevance prior to select and to develop a sunscreen vehicle. Presently, UV filters are contained in several types of vehicles, each one presenting peculiar characteristics. Among these vehicles: aqueous and hydroalcoholic gels; emulsions (lotions and creams); oils; sprays and aerosols; and wax sticks. Gupta (1998), and Palm and O'Donoghue (2007), described advantages and disadvantages for each vehicle intended to contain UV filters. Emulsified systems are largely used to deliver UV filters, being considered popular and versatile vehicles with easy application. Viscosity and sensorial properties are conveniently adjusted allowing the improvement of the consumer adherence. But, as wherein presented, emulsified systems may favor or inhibit anti-UV protection, which is dependent of their excipients. Emulsions developed with phosphate-base emulsifiers seemed to achieve better profile for UV filter vehicles.

Critical wavelength (λ_c) is an alternative test to Boot's Star Rating system that straightforwardly identifies if a sunscreen presents or not UVA protection. By this assessment, a given sun-care product may be qualified as broad spectrum sunscreen, according to a minimum value of λ_c. Basically, the idea of this parameter is the uniformity evaluation of the sunscreen absorbance spectrum (total reliance on the curve shape) based on the integral of the spectral absorbance curve that reached 90% of the integral from 290 to 400 nm (Springsteen et al., 1999; Diffey et al., 2000). Fig. 5 concisely presents the λ_c results of the sunscreen delivery systems. Diffey et al. (2000), and Nash et al. (2006), exemplified the difference between an UVB sunscreen and a broad spectrum one. Illustratively, Fig. 6 compares literature data (Diffey et al., 2000) and the results obtained wherein in relation to an UVB (SDS-265) and a broad spectrum bioactive sunscreen containing *P. incarnata* L. dry extract (SDS-268).

The sunscreen delivery systems that attained λ_c of 370 nm or more were those developed with rutin (SDS-266) and *P. incarnata* L. dry extract (SDS-267) alone and the one containing high concentration of organic and inorganic UV filters associated with *P. incarnata* dry extract (SDS-268), that achieved λ_c of 368.6 nm, which was statistically equal to 370 nm. All other sunscreens have not reached critical wavelength superior than 366.4 nm and SDS-263 (control) obtained 331 nm. These results are corroborated by Diffey et al. (2000), who noticed that UV filter concentration and appropriate formulation design are qualitative and quantitatively essential to successfully produce a true broad spectrum sunscreen and the empirical addition of UVA organic and inorganic filters would not ensure a sun-care product presenting such characteristic.

4. Conclusion

The development of optimal sunscreen delivery systems is an incessant challenge to the Cosmetic Sun-care Research and Development, in addition to the search of more sophisticated and effective formulations that provide broad spectrum UV protection (Rhodes and Callaghan, 2004). By the exposed, among all associations of bioactive compounds and UV filters, we found that the broad spectrum sunscreen was accomplished when *P. incarnata* L. dry extract was in the presence of 7.0% (w/w) ethylhexyl methoxycinnamate, 2.0% (w/w) benzophenone-3 and 2.0% (w/w) TiO_2. It was demonstrated that this association generated estimated SPF of 20.072 ± 0.906 and it has improved the protective defense against UVA radiation, accompanying augmentation of the UVA/UVB ratio from 0.49 (analogous delivery system not added with the dry extract) to 0.52 and λ_c from 364 to 368.6 nm. The photochemistry profile exhibited by all associations of bioactive compounds with UV filters was generated by a complex mechanism, involving chemical interactions not yet elucidated.

Effectiveness analyses of sunscreen products involved the interpretation of several critical parameters to broadly determine the anti-UV protection, like: estimated SPF, Boot's Star Rating category, UVA/UVB ratio and critical wavelength. According to our results, it was also noticed that the photoprotection profile of a sunscreen

product would only be fully obtained if those parameters, revealed earlier, were treated concomitantly.

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References

Baby, A.R., Maciel, C.P.M., Kaneko, T.M., Velasco, M.V.R., 2006. UV-spectrophotometric determination of bioflavonoids from a semisolid pharmaceutical dosage form containing *Trichilia catigua* Adr. Juss (and) Ptychopetalum olacoides Bentham standardized extract: analytical method validation and statistical procedure. J. AOAC Int. 89, 1532–1537.

Banov, D., Baby, A.R., Bosco, L.M., Kaneko, T.M., Velasco, M.V.R., 2006. Caracterização do extrato seco de *Ginkgo biloba* L. em formulações de uso tópico. Acta Farm. Bonaerense 25, 219–224.

Brady, C.A.L., Gill, R.A., Lynch, P.T., 2003. Preliminary evidence for the metabolism of benzo(a)pyrene by *Plantago lanceolata*. Environ. Geochem. Health 25, 131–137.

COLIPA, 2006. COLIPA Guidelines: International Sun Protection Factor (SPF) Test Method.

Couteau, C., Pommier, M., Paparis, E., Coiffard, L.J.M., 2007. Study of the efficacy of 18 sun filters authorized in European Union tested *in vitro*. Pharmazie 62, 449–452.

Dhawan, K., Kumar, S., Sharma, A., 2004. *Passiflora*: a review update. J. Ethnopharmacol. 41, 87–91.

Diffey, B.L., Tanner, P.R., Matts, P.J., Nash, J.F., 2000. *In vitro* assessment of the broad-spectrum ultraviolet protection of sunscreen products. J. Am. Acad. Dermatol. 43, 1024–1035.

Dondi, D., Albini, A., Serpone, N., 2006. Interactions between different solar UVB/UVA filters contained in commercial suncreams and consequent loss of UV protection. Photochem. Photobiol. Sci. 5, 835–843.

Faurschou, A., Wulf, H.C., 2007. The relation between sun protection factor and amount of sunscreen applied *in vivo*. Br. J. Dermatol. 156, 716–719.

FDA, 1999. Department of Health and Human Service. Food and Drug Administration. 21 CFR Parts 310, 352, 700 and 740. Sunscreen Drug Products for Over-the-Counter Human Use; Final Monograph.

FDA, 2007. Department of Health and Human Service. Food and Drug Administration. 21 CFR Parts 347 and 352. Sunscreen Drug Products for Over-the-Counter Human Use; Proposed Amendment of Final Monograph; Proposed Rule.

Flor, J., Davolos, M.R., Corrêa, M.A., 2007. Protetores solares. Quim. Nova 30, 153–158.

Gaspar, L.R., Maia Campos, P.M.B.G., 2003. Rheological behavior and SPF of sunscreens. Int. J. Pharm. 250, 35–44.

Gers-Barlag, H., Klette, E., Bimczok, R., Springob, C., Finkel, P., Rudolph, T., Gonzenbach, H.U., Schneider, P.H., Kockott, D., Heinrich, U., Tronnier, H., Bernklau, R., Johncock, W., Langner, R., Driller, H.J., Westenfelder, H., 2001. *In vitro* testing to assess the UVA protection performance of sun care products. Int. J. Cosmet. Sci. 23, 3–14.

Gupta, V.K., 1998. Effect of formulations on skin permeation and substantivity of chemical sunscreens. Doctoral Thesis. Graduate School New Brunswick Rutgers (Graduate Program in Pharmaceutical Sciences, The State University of New Jersey), New Jersey.

Heinrich, U., Tronnier, H., Kockott, D., Kuckuk, R., Heise, H.M., 2004. Comparison of sun protection factors determined by an *in vivo* and different *in vitro* methodologies: a study with 58 different commercially available sunscreen products. Int. J. Cosmet. Sci. 26, 79–89.

Herzog, B., Mongiat, S., Deshayes, C., Neuhaus, M., Sommer, K., Mantler, A., 2002. *In vivo* and *in vitro* assessment of UVA protection by sunscreen formulations containing either butyl methoxy dibenzoyl methane, methylene bis-benzotriazolyl tetramethylbutylphenol, or microfine ZnO. Int. J. Cosmet. Sci. 24, 170–185.

Kullavanijaya, P., Lim, H.W., 2005. Photoprotection. J. Am. Acad. Dermatol. 52, 937–958.

Maia Campos, P.M.B.G., Gianeti, M.D., Kanashiro, A., Lucisano-Valim, Y.M., Gaspar, L.R., 2006. *In vitro* antioxidant and *in vivo* photoprotective effects of an association of bioflavonoids with liposoluble vitamins. Photochem. Photobiol. 82, 683–688.

Martins, H.F.P., Leal, J.P., Fernandez, M.T., Lopes, V.H.C., Cordeiro, M.N.D.S., 2004. Toward the prediction of the activity of antioxidants: experimental and theoretical study of the gas-phase acidities of flavonoids. J. Am. Soc. Spectrom. 15, 848–861.

Mielczarek, C., 2005. Acid-base properties of selected flavonoid glycosides. Eur. J. Pharm. Sci. 25, 273–279.

More, B.D., 2007. Physical sunscreens: on the comeback trail. Indian J. Dermatol. Venereol. Leprol. 73, 80–85.

Movileanu, L., Neagoe, I., Flonta, M.L., 2000. Interaction of the antioxidant flavonoid quercetin with planar lipid bilayers. Int. J. Pharm. 205, 135–146.

Mukherjee, S., Mukherjee, A., Saha, A., 2005. QSAR modeling on binding affinity of diverse estrogenic flavonoids: electronic, topological and spatial functions in quantitative approximation. Theochem. 715, 85–90.

Nash, J.F., Tanner, P.R., Matts, P.J., 2006. Ultraviolet A radiation: testing and labeling for sunscreen products. Dermatol. Clin. 24, 63–74.

Palm, M.D., O'Donoghue, M.N., 2007. Update on photoprotection. Dermatol. Ther. 20, 360–370.

Persson, A.E., Edström, D.W., Bäckvall, H., Lundeberg, J., Pontén, F., Ros, A.-M., Williams, C., 2002. The mutagenic effect of ultraviolet-A1 on human skin demonstrated by sequencing the p53 gene in single keratinocytes. Photodermatol. Photoimmunol. Photomed. 18, 287–293.

Rhodes, L.E., Callaghan, T.M., 2004. Beyond sun protection factor. Int. J. Cosmet. Sci. 26, 207–214.

Rolim, A., Maciel, C.P.M., Kaneko, T.M., Consiglieri, V.O., Salgado-Santos, I.M.N., Velasco, M.V.R., 2005. Validation assay for total flavonoids (as rutin equivalents) from *Trichilia catigua* Adr. Juss (Meliaceae) (and) Ptychopetalum olacoides Bentham (Olacaceae) commercial extract. J. AOAC Int. 88, 1015–1019.

Rolim, A., Oishi, T., Maciel, C.P.M., Zague, V., Pinto, C.A.S.O., Kaneko, T.M., Consiglieri, V.O., Velasco, M.V.R., 2006. Total flavonoids quantification from O/W emulsion with extract of Brazilian plants. Int. J. Pharm. 308, 107–114.

Santos, E.P., Freitas, Z.M., Souza, K.R., Garcia, S., Vergnanini, A., 1999. *In vitro* and *in vivo* determinations of sun protection factors of sunscreen lotions with octylmethoxycinnamate. Int. J. Cosmet. Sci. 21, 1–5.

Scalia, S., Casolari, A., Iaconinoto, A., Simeoni, S., 2002. Comparative studies of the influence of cyclodextrins on the stability of the sunscreen agent. 2-Ethylhexyl-*p*-methoxycinnamate. J. Pharm. Biomed. Anal. 30, 1181–1189.

Schulz, J., Hohenberg, H., Pflücker, F., Gärtner, E., Will, T., Pfeiffer, S., Wepf, R., Wendel, V., Gers-Barlag, H., Wittern, K.-P., 2002. Distribution of sunscreens on skin. Adv. Drug Deliv. Rev. 54, S157–S163.

Seité, S., Moyal, D., Verdier, M.-P., Hourseau, C., Fourtanier, A., 2000. Accumulated p53 protein and UVA protection level of sunscreens. Photodermatol. Photoimmunol. Photomed. 16, 3–9.

Serpone, N., Dondi, D., Albini, A., 2006. Inorganic and organic UV filters: their role and efficacy in sunscreens and suncare products. Inorg. Chim. Acta 360, 794–802.

Springsteen, A., Yurek, R., Frazier, M., Carr, K.F., 1999. *In vitro* measurement of sun protection factor of sunscreens by diffuse transmittance. Anal. Chim. Acta 380, 155–164.

Tabrizi, H., Mortazavi, S.A., Kamalinejad, M., 2003. An *in vitro* evaluation of various Rosa damascena flower extracts as natural antisolar agent. Int. J. Cosmet. Sci. 25, 259–265.

Teixeira, S., Siquet, C., Alves, C., Boal, I., Marques, M.P., Borges, F., Lima, J.L.F.C., Reis, S., 2005. Structure-property studies on the antioxidant activity of flavonoids present in diet. Free Radic. Biol. Med. 39, 1099–1108.

Velasco, M.V.R., Balogh, T.S., Pedriali, C.A., Sarruf, F.D., Pinto, C.A.S.O., Kaneko, T.M., Baby, A.R., 2008. Associação da rutina com *p*-metoxicinamato de octila e benzofenona-3: avaliação in vitro da eficácia fotoprotetora por espectrofotometria de refletância. Lat. Am. J. Pharm. 27, 23–27.

Verschooten, L., Declercq, L., Garmyn, M., 2006. Adaptive response of the skin to UVB damage: role of the p53 protein. Int. J. Cosmet. Sci. 28, 1–7.

Vielhaber, G., Grether-Beck, S., Koch, O., Johncock, W., Krutmann, J., 2006. Sunscreens with an absorption maximum of ≥360 nm provide optimal protection against UVA1-induced expression of matrix metalloproteinase-1, interleukin-1, and interleukin-6 in human dermal fibroblast. Photochem. Photobiol. Sci. 5, 275–282.

Wolf, R., Wolf, D., Morganti, P., Ruocco, V., 2001. Sunscreens. Clin. Dermatol. 19, 452–459.

Yusuf, N., Irby, C., Katiyar, S.K., Elmets, C.A., 2007. Photoprotective effects of green tea polyphenols. Photodermatol. Photoimmunol. Photomed. 23, 48–56.