Multifunctional Cosmetic Containing Blueberry and Tinosorb M®-Loaded Microparticles Improves Sunscreen Performance

Daiana Schiavon1, Daniela Novello Martin1, Gabriela Brocco1, Júlia Scherer Santos2, Ana Paula Anzolin3, Luciana Grassiotin Rossato-Grando1,4, Hamid Omidian5, Charise Dallazem Bertol1,3,5*

1Curso de Farmácia, Universidade de Passo Fundo, Passo Fundo, Brasil.
2Curso de Farmácia, Centro Universitário União Dinâmicas das Cataratas, Foz do Iguaçu, Paraná, Brasil.
3Programa de Pós-Graduação em Envelhecimento Humano, Universidade de Passo Fundo, Passo Fundo, Brasil.
4Programa de Pós-Graduação em Bioexperiimentação, Universidade de Passo Fundo, Passo Fundo, Brasil.
5Pharmaceutical Sciences, Nova Southeastern University, Fort Lauderdale, Florida, USA.

Article info
Article History:
Received: 22 Jan. 2019
Revised: 22 Apr. 2019
Accepted: 4 May 2019
epublished: 1 June 2019

Keywords:
• Sunscreen
• Blueberry
• Microparticles
• Stability
• Tape stripping

Abstract
Purpose: We aimed to evaluate the effect of blueberry extract and microparticles (MPs) on sunscreen performance of multifunctional cosmetics. Octocrylene (OCT), benzfophenone-3 (BENZ-3) and Tinosorb® M (MBBT) were employed as UV filters.
Methods: An in-silico modeling was used to determine the UV filters concentrations to obtain high values of sunscreen protection factor (SPF) and UVA protection factor (UVA-PF). MBBT and blueberry-loaded microparticles (MPs) were prepared by spray-drying. OCT and BENZ-3 were added in the oil phase of cosmetics. Cosmetics A and B contained MPs and MBBT, respectively, and cosmetic C was prepared without MP. Characterization, physicochemical stability and in vitro SPF were performed. UV filters distribution in human stratum corneum (SC) for each cosmetic was performed. Anti-oxidant activity of blueberry extract was evaluated.

Results: Sunscreen combination with the highest SPF was selected for formulations. Formulations A and B maintained their rheological behavior over time, unlike formulation C. In vitro SPF for formulations A, B and C were 51.0, 33.7 and 49.6, respectively. We also developed and validated a method for analysis of the UV filters by HPLC/PDA suitable for the in-vivo assay. In Tape stripping test, MBBT showed SC distribution similar for all cosmetic formulations. OCT and BENZ-3 distribution to formulation A and C was also similar. Blueberry extract showed antioxidant capacity of 16.71 μg/mL equivalent to vitamin C.

Conclusion: Cosmetics containing MPs presented better physical stability. Blueberry increased the photoprotective capacity of the formulations and added extra benefits due to its anti-oxidant and anti-aging properties.

Introduction
A good sunscreen is expected to block the UV penetration and to prevent its acute and chronic damages and ultimately skin cancer.¹ Sunscreens are characterized by their sunscreen protection factor (SPF) and physiochemical properties. Apart from the active ingredient, a sunscreen may contain other additives such as moisturizing agents, antioxidants, and repellents which enhance its photoprotective properties and encourage its frequent use.¹ Sunscreens containing natural anti-oxidants are in line with the current trend of developing multifunctional cosmetics or “smart” products that can offer extra benefits such as antioxidant and anti-inflammatory properties.²–³

Blueberry (Vaccinium sp.) from Ericaceae, is known as the fruit of longevity due to its high polyphenols and anthocyanins (mainly delphinidin and malvidin) content with antioxidant function⁴ as well as vitamins, minerals, and resveratrol among others.⁷ Besides its anti-aging function, polyphenol can increase the photoprotective potential of the formulations.⁸ Blueberry is a potential anti-cancer⁹–¹¹ and can reduce tumor proliferation in murine melanoma cells¹² and reduce phototoxing effect and free radical generation caused by UVB radiation on human dermis cells¹³ and on keratinocytes.¹⁴

In sunscreen formulations, MPs can delay the penetration of the active, allowing photoprotection or a longer period of action.¹⁵,¹⁶ Moreover, they increase photostability, protect against oxidation, reduce odors of compounds, avoid incompatibilities, and reduce allergies and dermatitis caused by sunscreens.¹⁶,¹⁷

This research intended to develop and evaluate the effect of blueberry and the role of MPs in three multifunctional...
cosmetics sunscreen formulations performance. Sunscreen formulations was composed of Octocrylene (OCT), Benzophenone-3 (BENZ-3), and Tinosorb® M (MBBT - Bisocitrizole or 2,2'-methylene-bis-(2H-benzotriazol-2-yl)-4-(tetramethyl-butyl)-1,1,3,3-phenol).

**Material and Methods**

**Determination of the concentration of the UV filters and SPF / UVA-PF in-silico**

The amounts of OCT, BENZ-3 and MBBT used in the formulations were at the permitted levels. Different concentrations of three UV filters were evaluated by the in-silico Online BASF Sunscreen simulator, obtaining solar protection factor for UVB (SPF) and protection factor for UVA (UVA-PF). Combination 1 was made of 3% OCT, 8% BENZ-3 and 6.4% MBBT (total of 15.4% UV filters), and Combination 2 was made of 6% OCT, 8% BENZ-3 and 10% MBBT (total of 24% UV filters).

**Materials**

The compounds used in the formulations were purchased in different suppliers. OCT (98.4% v/v, supplied by CosmeTrade Commercial, Porto Alegre/RS/Brazil), MBBT (59.42% w/v, D’Altomare Química, Santo Amaro/SP/Brazil, Manufacturer BASF), BENZ-3 (99.80%, Audaz São Paulo /SP/Brazil), blueberry extract (9.68% of anthocyanins, Viafarma Supplier, Manufactured by Quimer, São Paulo/SP/Brazil), hydroxypropyl methylcellulose (HPMC) (Methocel K15M®, Colorcon, Cotia/ SP), butylhydroxytoluene (BHT) (Alpha Química, Porto Alegre/RS), ethylenediamine tetra acetic acid (EDTA) (Synth, Diadema/SP), imidazodinylurea (Audaz Brasil, São Paulo/SP), octyl stearatate (Alpha Química, Porto Alegre/RS), polysorbate 80 (Neon) and Lanette N (Alpha Química, Porto Alegre/RS) were used. Solvents and reagents used include N, N-dimethylformamide (Dynamic), methyl alcohol (Dynamic), acetonitrile high-performance liquid chromatography (HPLC) grade (Sigma-Aldrich), glacial acetic acid (Audaz, Brasil, São Paulo/SP), butylhydroxytoluene (BHT) (Alpha Química, Porto Alegre/RS/Brazil), octyl stearate (Alpha Química, Porto Alegre/RS/Brazil), hydroxypropyl methylcellulose (HPMC) (Methocel K15M®, Colorcon, Cotia/ SP), butylhydroxytoluene (BHT) (Alpha Química, Porto Alegre/RS), ethylenediamine tetra acetic acid (EDTA) (Synth, Diadema/SP), imidazodinylurea (Audaz Brasil, São Paulo/SP/Brazil), octyl stearatate (Alpha Química, Porto Alegre/RS), polysorbate 80 (Neon) and Lanette N (Alpha Química, Porto Alegre/RS) were used. Solvents and reagents used include N, N-dimethylformamide (Dynamic), methyl alcohol (Dynamic), acetonitrile high-performance liquid chromatography (HPLC) grade (Sigma-Aldrich), glacial acetic acid (Audaz, Brasil, São Paulo/SP), and ultra-pure water in a Direct-Q® system (Millipore, USA).

**Preparation of microparticles (MPs)**

The concentrations of the components were defined considering the maintenance of the same concentration of UV filters in all formulations providing an adequate SPF/ UVA-PF (see in the silico mathematical modeling). The other compounds followed the permissible concentrations in the legislation, and are commonly used. Pilot formulations were developed to obtain a homogeneous formulation capable to incorporate the compounds.

Two MPs formulations named MP$_{MBBT-B}$ and MP$_{MBBT}$ (without blueberry) were prepared and composed of HPMC, polysorbate 80, MBBT and blueberry (B) and water to make a 100% composition (according to Table 1). Initially, three distinct phases were prepared in water-bath under heating at 70 °C and stirring for 90 min and then pooled together.

A spray dryer (LabMaq, MSD 1.0) was used to dry the samples and to obtain MPs (inlet temperature of 115°C, flow rate of 0.6 L/h). The vials containing samples were sealed and stored in a desiccator followed by drying. A scanning electron microscopy (SEM) (Vega LM3/Tescan Oxford EDS Instrument) was used to study morphology. Samples were mounted with carbon adhesive on an aluminum holder, covered with gold in a metallizer Quorum (Q150R ES), and photographed at 20 kV.

**Preparation of multifunctional cosmetic sunscreens containing UV filters and blueberry extract**

Three emulsions formulations were prepared and named as formulation A, B and C. Formulations A and B presented MPs in their composition. Formulation C did not contain any MPs. UV filters and the additives content were kept constant in all formulations. Blueberry extract was present in the formulations A and C (Table 2).

The components were weighed, separated according to the phase, and heated to 70-75°C. The aqueous phase 1 was composed of EDTA, imidazodinylurea, MBBT, polysorbate 80 and water. EDTA was used as chelating agent, imidazodinylurea was employed as the preservative, MBBT as UVA/UVB filter, polysorbate 80 was employed as surfactant in order to increase MBBT solubilization. The phase 2 contained octyl stearatate, OCT, BENZ-3, BHT and water. Octyl stearatate was used as solubilizer of OCT and BENZ-3. OCT and BENZ-3 were used as UVB filters. BHT was applied as antioxidant to prevent blueberry oxidation. Phase 1 was poured into the phase 2 and vigorously stirred. Phase 3 was added to formulations A, B and C previously prepared. MP powder MP$_{MBBT-B}$ and MP$_{MBBT}$ were added to formulation A and B respectively. Regarding to emulsion C, phase 3 was prepared under heating and stirring and then added to formulation C.

**Characterization and physicochemical preliminary stability assessment of multifunctional formulations A, B and C**

The formulations A, B and C were characterized for their organoleptic characteristics, pH at 5% (w/v, in water)

---

**Table 1.** Composition of blueberry-loaded microparticles (MP$_{MBBT}$) and MBBT-loaded microparticles (MP$_{MBBT}$).  

| Components         | MP$_{MBBT}$ | MP$_{MBBT}$ |
|--------------------|-------------|-------------|
| Phase 1            |             |             |
| HPMC               | 0.25%       | 0.25%       |
| Water              | up to 30%   | up to 30%   |
| Phase 2            |             |             |
| MBBT               | 6%          | 6%          |
| Polysorbate 80     | 3%          | 3%          |
| Water              | up to 30%   | up to 30%   |
| Phase 3            |             |             |
| Blueberry Extract  | 2.5%        | -           |
| Polysorbate 80     | 3%          | 3%          |
| Water              | up to 40%   | up to 40%   |
In-vivo UV filters quantification from multifunctional formulations in stratum corneum (SC) by tape stripping

*In-vivo* assays were performed to determine cutaneous penetration of the sunscreen formulations A, B and C. Ten male/female volunteers 18-50 years old with skin phototype II, III, IV were included in this study. The exclusion criteria were volunteers allergic to sunscreens/ the components of the formulations/ adhesive tape, those using any skin sensitizing medication, and those with dermatoses, skin cuts in the area of application, and previous history of skin cancer. Based on previous studies, the contact time of the formulations with the skin was set at 30 minutes. Volunteers washed their forearms with water and neutral soap, and an area of 4 cm² was prepared for the application. All volunteers received a dose of 2 mg/cm² of each formulation at different sites of their forearm.

After 30 minutes, the Tape Stripping technique was performed to remove the SC. With a little pressure, a piece of tape was placed on the area containing formulation and it was later removed. This cycle was repeated using 5 pieces of adhesive tape. The tapes were placed in a beaker containing an approximately 10 mL of N, N-dimethylformamide diluent, and sonicated for 10 minutes. The volume was adjusted using a 10 mL HPLC volumetric flask, filtered and transferred to HPLC. After the process, the volunteers washed the forearm to remove

---

**Table 2. Composition of multifunctional cosmetics A, B, and C**

| Components                          | A (%) | B (%) | C (%) |
|-------------------------------------|-------|-------|-------|
| Ethylenediamine tetra acet acid (EDTA) | 0.11  | 0.11  | 0.11  |
| Imidazolidinylurea 50% (w/w)         | 0.6   | 0.6   | 20    |
| MBBT, %                             | -     | -     | 20    |
| Polysorbate 80, %                   | -     | -     | 3     |
| Water, %                            | 45.50 | 48.00 | 23.17 |
| Octyl stearate, %                   | 3     | 3     | 3     |
| Lanette N, %                        | 8     | 8     | 8     |
| Phase 2                             |       |       |       |
| OCT, %                              | 6     | 6     | 6     |
| BENZ- 3, %                          | 8     | 8     | 8     |
| Butylhydroxytoluene (BHT), %        | 0.05  | 0.05  | 0.05  |

2.5 g blueberry extract, 20 g MBBT, 6 g polysorbate 80, and 0.25 g HPMC.
3.20 g MBBT, 6 g polysorbate 80, and 0.25 g HPMC.

---

**Advanced Pharmaceutical Bulletin, 2019, Volume 9, Issue 2**

---
Normality was assessed by D’agostino Pearson’s test. The results were analyzed by one-way ANOVA followed by the Friedman test ($P \leq 0.05$).

**Antioxidant activity of the blueberry extract**

The sequestering activity of DPPH (1,1-diphenyl-2-picrylhydrazyl) radical was determined.\textsuperscript{26,27} DPPH (0.1 mM) was prepared in 80% methanol (v/v in water). A 0.1 mL aliquot of the blueberry extract (1 mg/mL in 80% methanol) was added to 2.9 mL of the DPPH, homogenized and kept at room temperature in the dark for 30 min. Using a UV spectrophotometer (Lambda 20/Perkin Elmer spectrophotometer), the absorbance was measured at 517 nm. A solution containing methanol and DPPH was used as control. The calibration curve was obtained with vitamin C solutions (40.0-120.0 μg/mL). The sequestering activity of the DPPH radical by the extract was expressed as μg/mL antioxidant capacity equivalent to vitamin C.

**Results and Discussion**

**In-silico determination of the concentration of the UV filters and SPF/UVA-PF**

In-silico and in-vitro tests are important tools in the development of sunscreens to determine preliminary qualitative and quantitative concentrations of the UV filters.\textsuperscript{28} The use of BASF Sunscreen Simulator showed good correlations with in-vivo results, representing a valuable tool in the development of sunscreens.\textsuperscript{29}

Radiation blocking capability for the combinations 1 and 2 were 96% and 98%, respectively. The in-vitro UVA-PF and in-vitro UVA-PF for the combinations 1 and 2 were 13/16 and 23/21, respectively. Critical wavelength for both combinations was 380 nm, a wavelength showing suitable UVA protection. Combination 1 and 2 presented SPF of 30 and 50, respectively. The combinations 2 presented a higher SPF/UVA-PF due to the higher UV filters concentrations. For this reason, this combination was selected for further studies. The legislation requires at least an SPF of 6, an UVA-PF corresponding to 1/3 of the SPF and a critical wavelength of at least 370 nm which were found in both combinations. The UV filters selected for this study are safe; OCT is a liposoluble and photostable; BENZ-3 is a commonly used UVB filter; and MBBT is a photostable sunscreen with low risk of skin penetration.\textsuperscript{28,30}

**Characterization of MBBT and blueberry-loaded microparticles (MP\textsubscript{MBBT+B}) and MBBT-loaded microparticles (MP\textsubscript{MBBT})**

Initially, in an attempt to prepare MP containing the three UV filters used in this study, we employed the solvent evaporation methodology. We prepared an emulsion. In the oily phase, we added OCT and BENZ-3. In the aqueous phase we added MBBT and blueberry extract. However, during the use of rotary evaporator to remove acetone used as solvent, a sticky composition was obtained.

In the technique used in this work, only the MBBT and the blueberry extract were microencapsulated. Before drying, the MP were milky in appearance and runny. MP\textsubscript{MBBT+B} and MP\textsubscript{MBBT} were slightly pink and white, respectively. The MP turned into a cohesive mass due to the presence of MBBT.

Figure 1 shows the SEM micrograms of both compositions. Formulation MP\textsubscript{MBBT+B} showed microspheres characteristics with the roughest and most clumped wall, while MP\textsubscript{MBBT} (without blueberry extract) had smoother surface with characteristics similar to microcapsules. The size of MP\textsubscript{MBBT+B} was in the range of

![Figure 1. SEM of the microparticles containing 2.5% of blueberry extracts and 20% MBBT (A, B, C) (MP\textsubscript{MBBT+B}), and white microparticles containing 20% MBBT (D, E, F) (MP\textsubscript{MBBT}) at 100× (A, D), 500× (B, E) and 1000× (C, F) magnifications.](image-url)
100 to 200 µm, because these MPs formed cluster. The size of MP_{MBBT} was lower than 100 µm (Figure 1).

**Characterization, and physiochemical preliminary stability assessment of multifunctional sunscreen formulations**

Formulations were homogenous and those containing blueberry were slightly pink. All formulations were easy to spread on the skin and had a characteristic odor of sunscreens. All pH values were in the range from 7.00 to 7.60, compatible with the area of application. Statistically, there was no pH difference between formulations at time 0 and after 90 days. The centrifugation behavior of the samples was also the same after 90 days as evidenced by no phase separation.

Figure 2 shows general rheological behavior of the multifunctional sunscreens formulations at the shear rates of 0-100 rpm, at the time of preparation and ninety days post-preparation. Formulations A and B remained stable after ninety days, and the formulation B showed superior stability compared to the formulations A and C. The rheogram also shows that the yield value of the formulations C was three times higher than those of the two other formulations, suggesting that the formulations C had a greater spreadability. However, the formulation C statistically experienced decrease in viscosity after 90 days, demonstrating the decrease of physical stability during the study. Formulations A and B displayed a very similar flow behavior. All three formulations showed a yield value (plastic flow) followed by a pseudoplastic flow which was more notable in the case of formulations C. All three formulations displayed hysteresis over the range of the shear rates studied, and the hysteresis was greater for the formulations C compared with the other two. Formulation B didn’t present hysteresis immediately after preparation. All three formulations showed thixotropic behavior, a desirable feature promoting greater photoprotection.

As showed in Table 3, rheological behavior of the Formulations A and B best fitted a Casson model while formulation C best fitted as Ostwald model. Formulations containing MPs presented plastic behavior and the formulation without MPs presented pseudoplastic behavior. The presence of MPs therefore, altered the rheological behavior. Nevertheless, both models have been previously used for sunscreen formulations. Pseudoplastic behavior promotes the formation of a homogeneous film on the skin, ensuring adequate sun protection.

**Determination of the in-vitro SPF of formulations**

The in-vitro SPFs for the formulations A, B and C were 41.51±3.48, 33.65±2.67, and 49.58±2.83, respectively. All formulations were statistically different. The blueberry extract present in the formulations A and C improved the SPF. MPs did not improve the SPF. The in-vitro SPF technique does not allow detecting the interaction of the particles with the skin, including the formation of a film that acts as a physical filter. In this way, this assay should be used with other tests to demonstrate the effectiveness of the MP sunscreens.

**In-vivo UV filters quantification from multifunctional formulations in stratum corneum (SC) by tape stripping**

The effectiveness of the sunscreens is determined by the SPF and UVA-PF. The official test to determine the SPF is to expose the volunteers to UV radiation. By exposing an unprotected area of the skin to a UV light, and covering another area with sunscreen, the erythematous dose (for UVB radiation) or the minimum pigment dose (for UV A radiation) can be determined. The tape stripping technique can also be used for in-vivo evaluation. This technique can quantify the amounts of the active substance retained in the SC and is minimally invasive because the removed SC can rapidly be reconstituted without damaging the epidermis and dermis. The SC corresponds to the target site of sunscreens. Therefore, tape stripping can be used to predict sunscreen efficacy.
Statistically the MBBT results showed a normal distribution while OCT and BENZ-3 were not normal. The amounts of the MBBT found in the SC was similar among the formulations A, B and C. Therefore, the presence of MPs did not influence MBBT SC distribution. Regarding to BENZ-3 and OCT SC distribution from formulation A and C a similar profile was observed, which is expected since BENZ-3 and OCT are not microencapsulated. Unlike, BENZ-3 and OCT SC distribution from formulation B was statistically lower. The presence of blueberry may have affected OCT and BENZ-3 content in SC since those UV filters showed a similar distribution in formulations A and C (Figure 3).

Cosmetic formulations containing MBBT+B showed a similar skin profile distribution to cosmetic containing non-microencapsulated ingredients. Despite this, topical application of MP formulations may increase protection against erythema fulfilling the role of MP systems as suitable for sunscreens.

**Conclusion**

Multifunctional cosmetics sunscreen were successfully prepared. All formulations displayed pH skin compatible, a combination of plastic flow, pseudoplasticity and thixotropy, a very desirable flow property in the preparation, application, and performance of sunscreen formulations. Cosmetics containing blueberry and MBBT-loaded MPs showed anti-oxidant activity and improved physicochemical stability. All formulations presented high values of SPF, mainly, the cosmetic containing blueberry and MBBT-loaded MPs. MPs increased the stability and the blueberry increased the photoprotective and anti-oxidant capacity. Therefore, cosmetic containing MBBT and blueberry-loaded MPs presented the best performance as sunscreen.

**Ethical issues**

The work was approved by the Research Ethics Committee according to approval number: 58224116.0.0000.5342, and the volunteers signed the consent form. Procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration.

**Conflict of Interest**

The authors report no conflict of interest.

**Acknowledgements**

This work was supported by Project "Implantação do Laboratório do Laboratório de Protetores Solares".

| Models                | Equations                                      | Freshly prepared | After 90 days |
|-----------------------|------------------------------------------------|------------------|---------------|
|                       |                                                 | A                | B          | C     | A       | B     | C     |
| Bingham               | \( \tau = \tau_0 + \eta \gamma \)             | 0.9740± 0.0065   | 0.9764± 0.0063 | 0.9335± 0.0138 | 0.9704± 0.0055 | 0.9764± 0.0057 | 0.9317± 0.0232 |
| Casson                | \( \tau^0 = \tau_0^{1.4} + \eta^{0.5} \gamma^{1.4} \) | 0.9969± 0.0009   | 0.9978± 0.0006 | 0.9804± 0.0074 | 0.9956± 0.0018 | 0.9966± 0.0020 | 0.9792± 0.0107 |
| Ostwald               | \( \tau = K \gamma^0 \)                        | 0.9419± 0.0260   | 0.9706± 0.0198 | 0.9948± 0.0044 | 0.9925± 0.0022 | 0.9965± 0.0020 | 0.9984± 0.0008 |
| Herschel–Bulkley      | \( \tau = \tau_0 + K \gamma^0 \)             | 0.7665± 0.0683   | 0.8487± 0.0113 | 0.7741± 0.0131 | 0.8992± 0.0361 | 0.9907± 0.0088 | 0.8446± 0.0787 |

Where \( \tau \) is the shear stress; \( \tau_0 \) is the critical shear stress; \( \eta \) is the viscosity; \( \gamma \) is the shear rate, \( K \) is the consistency and \( n \) is the power law index. Results are expressed as regression coefficient (n = 3, mean ± SD).

**Antioxidant activity of blueberry extract**

Using a DPPH assay, it was found that blueberry extract has an antioxidant capacity with a value of 16.71 μg/mL equivalent to vitamin C, indicating a potential anti-aging action. In the in-vitro SPF assay, it was observed that the blueberry extract also improved the photoprotective capacity of the formulations.
Sunscreens containing blueberry

References
1. Schalka S, Steiner D, Raveli FN, Steiner T, Terena AC, Marcon CR, et al. Brazilian consensus on photoprotection. An Bras Dermatol. 2014;89(6):1–74. doi: 10.1590/abd1806-4841.20143971
2. Ferrari M, Oliveira MSC, Nakano AK, Rocha-filho PA. Determinação do fator de proteção solar (FPS) in vitro e in vivo de emulsões com óleo de andiroba (Carapa guianensis). Rev Bras Farmacogn. 2007;17(4):626–30. doi: 10.1590/S0102-695X2007000400023
3. de Souza FP, Campos GR, Packer JE. Determinação da atividade fotoprotetora e antioxidante em emulsão contendo extrato de Malpighia glabra L. - Acerola. Rev Ciências Farm Basica Apl. 2013;34(1):69–77.
4. Velasco MVR, Balogh TS, Pedriali CA, Sarruf FD, Pinto CASO, Kaneko TM, et al. 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. 2008;27(1):23–7.
5. Brasil, Ministério da Saúde, Agência Nacional de Vigilância Sanitária. Resolução da Diretoria Colegiada. RDC 30 de 1 de Junho de 2012. Aprova o Regulamento Técnico Mercosul sobre Protetores Solares em Cosméticos e dá outras providências. Available from: http://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2012/rdc0030_01_06_2012.html. Accessed 10 Jan 2015.
6. Wang L, Gao S, Jiang W, Luo C, Xu M, Bohlin L, et al. Antioxidative dietary compounds modulate gene expression associated with apoptosis, DNA repair, inhibition of cell proliferation and migration. Int J Mol Sci. 2014;15(9):16226–45. doi: 10.3390/ijms150916226
7. Spagolla LC, Santos MM, Passos LML, Aguiar C. Extração alcoólica de fenólicos e flavonóides totais de mirtilo "Rabbiteye" (Vaccinium ashei) e sua atividade antioxidante. Rev Ciências Farm Basica Apl. 2008;30(2):187–91.
8. Rosa MB da, Oliveira TG de, Carvalho CA de, Silva FD, Carvalho LM de, Nascimento PC, et al. Estudo espectrofotométrico da atividade foto-protetora de extratos aquosos de Achillea millefolium, Brassica oleracea var. capitata, Cyperus rotundus, Plectranthus barbatus, Porophyllum ruderale (jacq.) e Sonchus oleraceus. Rev Eletrônica Farmácia. 2008;5(1):101–10. doi: 10.5216/rev.v5i1.4620
9. Katsube T, Tabata H, Ohta Y, Yamasaki Y, Anuurad E, Shiwaku K, et al. Screening for antioxidiant activity in edible plant products: comparison of low-density lipoprotein oxidation assay; DPPH Radical scavenging assay, and Folin-Ciocalteu assay. J Agric Food Chem. 2004;52(8):2391–6. doi: 10.1021/jf035372g
10. Aiyer HS, Vadhanam MV, Stoyanova R, Caprio GD, Clapper ML, Gupta RC. Dietary berries and ellagic acid prevent oxidative DNA damage and modulate expression of DNA repair genes. Int J Mol Sci. 2008;9(3):327–41.
11. Adams LS, Phung S, Yee N, Seeram NP, Li L, Chen S. Blueberry phytochemicals inhibit growth and metastatic potential of MDA-MB-231 breast cancer cells through modulation of the phosphatidylinositol 3-kinase pathway. Cancer Res. 2010;70(9):3594–605. doi: 10.1158/0008-5472.CAN-09-3565
12. Bunea A, Rugină D, Sconța Z, Pop RM, Pintea A, Socaciu C, et al. Anthocyanin determination in blueberry extracts from various cultivars and their antiproliferative and apoptotic properties in B16-F10 metastatic murine melanoma cells. Phytochemistry. 2013;95:436–44. doi: 10.1016/j.phytochem.2013.06.018
13. Bae JY, Lim SS, Kim SJ, Choi JS, Park J, Ju SM, et al. Bog blueberry anthocyanins alleviate photoaging in ultraviolet-B irradiation-induced human dermal fibroblasts. Mol Nutr Food Res. 2009;53(6):726–38. doi: 10.1002/mnfr.200800245
14. Svobodová A, Zdařilová A, Vostálová J. Loniceracaruella and Vaccinium myrtillus fruit polyphenols protect HaCaT keratinocytes against UVB-induced phototoxic stress and DNA damage. J Dermatol Sci. 2009;56(3):196–204. doi: 10.1016/j.jdermsci.2009.08.004
15. Wu X, Guy RH. Applications of nanoparticles in topical drug delivery and in cosmetics. J Drug Deliv Sci Technol. 2009;19(6):371–84. doi: 10.1016/j.jtss.2009.05.008
16. Patravale VB, Mandawade GD. Novel cosmetic delivery systems: An application update. Int J Cosmet Sci. 2008;30(1):19–33. doi: 10.1111/j.1468-2494.2008.00416.x
17. Jain SK, Jain NK. Multiparticle carriers for sun-screening agents. Int J Cosmet Sci. 2010;32(2):89–98. doi: 10.1111/j.1468-2494.2010.00547.x
18. Brasil, Ministério da Saúde, Agência Nacional de Vigilância Sanitária, Resolução da Diretoria Colegiada. RDC 69 de 23 de Março de 2016. Regulamento Técnico Mercosul sobre lista de filtros ultravioletas permitidos para produtos de higiene pessoal, cosméticos e perfumes. Available from: http://portal.anvisa.gov.br/documents/10181/2721567-procedures-d5fb92b3-6c6b-4130-8670-10181/2863150.http://bvsms.saude.gov.br/ Vitronelectrónica Eletrônica Farmácia.
19. Mansur J de S, Breder MNR, Mansur MC d’Ascenção, Azulay RD. Determinação do fator de proteção solar por espectrofotometria. An Bras Dermatol. 1986;61(3):121-4.
20. Brasil, Ministério Da Saúde. Agência Nacional De Vigilância Sanitária. Resolução da Diretoria Colegiada. RDC 166 de 24 de Julho de 2017. Dispõe sobre a validação de métodos analíticos e dá outras providências. Available from: http://portal.anvisa.gov.br/documents/10181/2721567/RDC_69_2016_COMP.pdf/d5f9b2b3-6c6b-4130-8670-4e3263763401. Accessed 05 May 2016.
21. ICH - International Conference on Harmonization. Validation of Analytical Procedures: Text and Methodology Q2 (R1). ICH Steer Committee, Switz; 2005. Available from: https://www.ema.europa.eu/documents/scientific-guideline/ich-q-2-r1-validation-analytical-procedures-text-methodology-step-5_en.pdf. Accessed 12 Feb 2015.
22. Lazar GM, Fructus AE, Baliet A, Bocquet JL, Thomas P, Marty JP. Sunscreens' photochemical behaviour: in vivo evaluation by the stripping method. Int J Cosmet Sci. 1997;19:87–101. doi: 10.1046/j.1167-2494.1997.171703.x
23. Wissing SA, Müller RH. Solid lipid nanoparticles as carrier for sunscreens. In vitro release and in vivo skin penetration. J Control Release. 2002;81(3):225–33. doi: 10.1016/S0168-
25. Villa D de. Avaliação da quantidade e uniformidade do filtro solar quando aplicado na pele de adolescentes e adultos jovens após aplicação simples e reaplicação, através da técnica de Tape- Stripping. Universidade Federal do Rio Grande do Sul, Porto Alegre, Brasil; 2010. http://www.lume.ufrgs.br/handle/10183/23001. Accessed 10 Jun 2017.

26. Kim D-O, Lee KW, Lee HJ, Lee CY. Vitamin C Equivalent Antioxidant Capacity ( VCEAC ) of Phenolic Phytochemicals. *J Agric Food Chem.* 2002;50:3713–7. doi: 10.1021/jf020071c

27. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. *LWT - Food Sci Technol.* 1995;28(1):25-30. doi: 10.1016/S0023-6438(95)80008-5

28. Pupo M. Tratado de Fotoproteção. iPupo; 2012.

29. Santos Caetano JP, Abarca AP, Guerato M, Guerra L, Schalka S, Perez Simão DC, et al. SPF and UVA-PF sunscreen evaluation: are there good correlations among results obtained in vivo, in vitro and in a theoretical Sunscreen Simulator? A real-life exercise. *Int J Cosmet Sci.* 2016;576–80. doi: 10.1111/ics.12322

30. Pereira N, Coutinho I, Andrade P, Gonçalo M. The UV filter Tinosorb M, containing decyl glucoside, is a frequent cause of allergic contact dermatitis. *Dermatitis.* 2013;24(1):41–3. doi: 10.1097/DER.0b013e31827cd36f

31. Gaspar LR, Maia Campos PMBG. Rheological behavior and the SPF of sunscreens. *Int J Pharm.* 2003;250(1):35–44. doi: 10.1016/S0378-5173(02)00462-3

32. Nascimento PD, Isaac VLB. Uso da reologia e do FPS para avaliação de estabilidade de cosmético fotoprotetor. *Rev Ciências Farm Básica Apl.* 2016;37(1). Available from: http://seer.ufscar.br/rcfba/index.php/rcfba/article/view/522/326. Accessed 30 May 2017.

33. Monteiro MS, Ozzetti RA, Vergnanini AL, de Brito-Gitirana L, Volpato NM, de Freitas ZM, et al. Evaluation of octyl p-methoxycinnamate included in liposomes and cyclodextrins in anti-solar preparations: preparations, characterizations and in vitro penetration studies. *Int J Nanomedicine.* 2012;7:3045-58. doi: 10.2147/IJN.S28550

34. Patel M, Jain S, Yadav A, Gogna D, Agrawal G. Preparation and characterization of oxybenzone-loaded gelatin microspheres for enhancement of sunscreening efficacy. *Drug Deliv.* 2006;13(5):323–30. doi: 10.1080/10717540500398175

35. Escobar-Chávez JJ, Merino-Sanjuán V, López-Cervantes M, Urban-Morlan Z, Piñón-Segundo E, Quintanar-Guerrero D, et al. The tape-stripping technique as a method for drug quantification in skin. *J Pharm Pharm Sci.* 2008;11(1):104–30.