ZnO and quercetin encapsulated nanoparticles for sun protection obtained by miniemulsion polymerization using alternative co-stabilizers

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Abstract
The present work evaluated the encapsulation of ZnO and quercetin in PBMA-PMMA-PS using the miniemulsion polymerization technique, aiming to develop nanoparticles with sun protection factor (SPF) and antioxidant activity (AA) for application in photoprotective lotions. In both formulations, octocrylene and green coffee oil were tested as co-stabilizing agents of the miniemulsion, being also encapsulated in the NPs, contributing to the SPF and AA of the obtained latexes. Spherical nanoparticles of homogeneous size, from 169 to 346 nm, and regular surfaces were obtained, remaining stable for at least 30 days. The encapsulation efficiency on the formulations tested was from 59 to 87% for ZnO, 47 to 51% for quercetin, 80 to 92% for octocrylene and 90 to 92% for green coffee oil. The quercetin and green coffee oil presented high antioxidant activity when encapsulated in polymeric NPs. The values of in vitro SPF was very good for formulations containing NPs-ZnO, with the best result for the simultaneous nanoencapsulation of ZnO and octocrylene (SPF 29 ± 5). The application of the NPs of quercetin and green coffee oil may promote an increment on SPF in vivo, reducing the damage caused to the skin by UV radiation, beyond the ability to scavenge the free radicals generated by ZnO.

1. Introduction

The consumption of sunscreen to protect against acute and chronic adverse effects of solar radiation has been increasing in recent years. Initially filters were used only to protect the skin against erythematous response, but today sunscreens have also been used to prevent early photoaging, photosensitivity, skin cancer and free radical damage. The aim is to protect the skin from both UVA and UVB radiation, reducing their harmful effects on the skin. Thus, an efficient sunscreen should not only prevent a possible sunburn, but also reduce the accumulation of injuries caused by the whole range of UV radiation, thus preventing the risk of fatal changes and the emergence of chronic diseases [1]. The sunscreen must be photochemically stable when exposed to sunlight, easily and permanently disperse in the vehicle, remain on the skin after perspiration or contact with water, be nontoxic and do not cause irritation or contact allergy.

The mechanism of protection against ultraviolet (UV) radiation used in sunscreens can be obtained through inorganic (physical protection) and organic compounds (chemical protection) [1]. Zinc oxide (ZnO) and titanium dioxide (TiO₂) are the inorganic compounds most used in sunscreen formulations [2], associated with some synthetic organic filters, such as octocrylene [3], to obtain efficient solar protection factor (SPF) values. Inorganic compounds have the advantage to have low allergenic potential, when compared to the organic compounds. However, they are usually opaque when applied to the skin [2]. Another drawback of inorganic compounds as ZnO and TiO₂ is the generation of free radicals when exposed to UV radiation [4]. In this sense,
encapsulation of inorganic nanoparticles becomes a promising technique to reduce the formation of free radicals when exposed to UV radiation, beyond the contribution to the translucent appearance of the Sunscreens [4, 5]. Also, the encapsulation of organic compounds may improve photostability, photo protection and safety of the compounds [6]. There are several techniques used to encapsulate compounds of interest, depending on the size, shape, physical and chemical properties of the particles to be obtained, application and final process costs. Miniemulsion polymerization is an \textit{in situ} encapsulation technique, where polymer synthesis and encapsulation of the component of interest are performed in a single step [7]. Some authors have evaluated the use of alternative co-stabilizers, such as andiroba oil [8, 9]; jojoba oil [8]; clove oil [10]; sunflower seed oil and linoleic acid [11]; linseed oil [9]; argan oil and coconut oil [12], being encapsulated as compounds of interest besides stabilizing the miniemulsion.

Recent studies have reported the synergic effects of natural plant-derived compounds when applied in sunscreens and cosmetics improving the SPF and acting as antioxidant components, scavenging free radicals and preventing from skin photoaging [13, 14]. Herbal cosmetics can protect the skin from solar radiation because they contain polyphenols such as flavonoids and carotenoids [13]. Curcumin, quercetin and resveratrol are examples of polyphenols with verified antioxidant and photoprotective potential [15]. Quercetin (QUE) is a flavonoid present in some natural products such as onion, cabbage, broccoli, apple [13], Ginkgobiloba [16], grape and red wine [17]. This flavonoid has antioxidant, antiallergic, anti-inflammatory, antiplatelet, antimicrobial, antineurodegenerative, antitumor and antiviral activity [15]. Quercetin is widely used in cosmetic formulations and its antioxidant and SPF potential have been investigated [15, 18–20]. However, temperature and light are factors that may cause a decrease in its desired antioxidant activity. Therefore, certain precautions are required during storage [20]. In this sense, encapsulation of quercetin is an alternative to protect it, preventing its photo degradation under UV radiation [21].

Plant oils are also commonly used to prevent skin aging as they contain antioxidant agents that minimize free radical activity. Pomegranate seed oil [22, 23] and green coffee oil [24, 25] are some examples that showed potential antioxidant and photoprotective activity in previous studies. Green coffee oil (GCO) is a natural source of antioxidants by the presence of chlorogenic acids, and also tocopherol, capable of reducing the production of free radicals. It is a very lipophilic and hardly washable substance [26].

Thus, the present work aims the encapsulation of ZnO and quercetin in polymeric nanoparticles, by miniemulsion polymerization technique, using octocrylene and green coffee oil as co-stabilizers to obtain high antioxidant activity and SPF, for application in sunscreen formulations. In addition, this work proposed the use of a copolymer with lower glass transition temperature [27] as encapsulating agent, aiming a better spreadability and retention of latex for application on the skin.

2. Experimental

2.1. Materials

The polymerization reactions were performed with the monomers butyl methacrylate (BMA, $\text{C}_8\text{H}_{14}\text{O}_2$) (Sigma-Aldrich, PA), methyl methacrylate (MMA, $\text{C}_5\text{H}_8\text{O}_2$) (Vetec, PA) and styrene (STY, $\text{C}_8\text{H}_8$) (Innova SA). Crodamol GTCC (Alpha Chemistry), Univil N539T octocrylene (OC) (BASF) and green coffee oil (GCO) (Mundo dos Óleos, Brazil) were used as co-stabilizers. Zinc oxide (ZnO) nanoparticles (Sigma-Aldrich) were modified using 3-trimethoxysilyl propyl methacrylate (MPS) (Sigma-Aldrich). Lutensol AT50 (Basf) and potassium persulfate (KPS, $\text{K}_2\text{S}_2\text{O}_8$) (Vetec) were used as surfactant and initiator, respectively, to encapsulate NPs-ZnO. For quercetin dihydrate (QUE) (Sigma Aldrich) encapsulation, initiator 2,2′-azobis(2-methylpropionitrile) (AIBN) (Vetec PA) and surfactants Tween 80 and Span 80 (Vetec) were used. Methanol ($\text{CH}_3\text{OH}$) and ethanol ($\text{C}_2\text{H}_5\text{OH}$) were purchased from Neon. Acetic acid ($\text{CH}_3\text{COOH}$) and sodium bicarbonate (NaHCO$_3$) were purchased from Vetec. All reagents were used as received.

2.2. Experimental procedure

2.2.1. Encapsulation of quercetin and ZnO by miniemulsion polymerization

The formulations (1 to 5) for encapsulation of quercetin are described in table 1. Experimental procedure was adapted from Bernardy et al [28]. First, quercetin was solubilized in the co-stabilizer (crodamol, green coffee oil or octocrylene) using ultrasonic disperser (Fisher Scientific, Sonic Dismembrator Model 500) with 40% of amplitude for 4 min (10 s on/10 s off). After that, the organic phase and aqueous phase were prepared in two different flasks by magnetic stirring for 30 min. The miniemulsion was prepared mixing both phases together for 30 min in magnetic stirring and, after that, using the ultrasonic disperser for 6 min (10 s on/10 s off) with amplitude of 70%. The polymerization reactions were carried out in a thermostatic bath at 70 °C for 5 h.

Before encapsulation of the ZnO nanoparticles, their surface was modified using MPS [29]. 5 g of the ZnO nanoparticles were dispersed in 250 ml of an aqueous solution of ethanol (90% v/v), using the ultrasonic bath...
Unique, Maxiclean Model 750 for 60 min and then the ultrasonic disperser for 60 s (10 s on/10 s off) with 70% of amplitude. 2.5 g of MPS were also mixed with 250 ml of ethanol/water solution 90:10 under magnetic stirring for 60 min; the pH of the solution was adjusted to 4.2 using acetic acid. Then, both solutions were mixed and kept under magnetic stirring at 50 °C for 21 h. The modified NPs-ZnO were centrifuged at 4000 rpm for 30 min (Centribio 80-2B centrifuge), washed with ethanol, dried and macerated. Next, the modified NPs-ZnO were encapsulated by miniemulsion polymerization, according to the formulations 6 to 9 (table 2).

Experimental procedure was adapted from Frizzo et al [29]. Aqueous phase was prepared with 30 min of magnetic stirring, while the organic phase was prepared in ultrasound bath for 30 min. Both phases were mixed and miniemulsion was obtained after 4 min in ultrasonic disperser (70% of amplitude, 10 s on/10 s off). Then, the initiator KPS was added and polymerization reaction was performed in a round bottom flask, under magnetic stirring at 70 °C for 4 h.

### Table 1. Formulations used in the miniemulsion polymerization reactions for encapsulation of quercetin.

| Samples | 1 (blank) | 2 (QUE) | 3 (QUE/GCO) | 4 (QUE/OCT) | 5 (QUE/GCO/OCT) |
|---------|-----------|---------|--------------|-------------|-----------------|
| **Aqueous phase (g)** | | | | | |
| Water | 12.0 | 12.0 | 12.0 | 12.0 | 12.0 |
| Tween 80 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| **Organic phase (g)** | | | | | |
| QUE | — | 0.015 | 0.015 | 0.015 | 0.015 |
| Crodamol | 1.5 | 1.5 | 0.75 | — | — |
| GCO | — | — | 0.75 | — | 0.75 |
| OCT | — | — | — | 1.5 | 0.75 |
| BMA | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 |
| MMA | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| STY | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| AIBN | 0.035 | 0.035 | 0.035 | 0.035 | 0.035 |
| Span 80 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |

QUE: quercetin; GCO: green coffee oil; OCT: octocrylene; BMA: butyl methacrylate; MMA: methyl methacrylate; STY: styrene; AIBN: 2,2’-azobis(2-methylpropionitrile).

### Table 2. Formulations used in the miniemulsion polymerization reactions for encapsulation of NPs-ZnO.

| Samples | 6 (blank) | 7 (NPs-ZnO) | 8 (NPs-ZnO/OCT) | 9 (NPs-ZnO/GCO) |
|---------|-----------|-------------|-----------------|-----------------|
| **Aqueous phase (g)** | | | | |
| Water | 7.8 | 7.8 | 7.8 | 7.8 |
| Lutensol AT50 | 0.09 | 0.09 | 0.09 | 0.09 |
| KPS | 0.06 | 0.06 | 0.06 | 0.06 |
| **Organic phase (g)** | | | | |
| NPs-ZnO | — | 0.63 | 0.52 | 0.63 |
| Crodamol | 0.35 | 0.35 | — | — |
| GCO | — | — | — | 0.35 |
| OCT | — | — | 0.52 | — |
| BMA | 1.25 | 1.25 | 1.25 | 1.25 |
| MMA | 0.42 | 0.42 | 0.42 | 0.42 |
| STY | 0.42 | 0.42 | 0.42 | 0.42 |

KPS: potassium persulfate; NPs-ZnO: zinc oxide nanoparticles modified with 3-trimethoxysilyl propyl methacrylate; GCO: green coffee oil; OCT: octocrylene; BMA: butyl methacrylate; MMA: methyl methacrylate; STY: styrene.
2.2.2. Characterization of the nanoparticles
Particle size and polydispersity index of the nanoparticles were measured by dynamic light scattering (DLS—Zeta Sizer Nano S, Malvern). Analyses were carried out in triplicate at room temperature (25 °C). Transmission Electron Microscopy (TEM, JEOL, JEM 2100 F, 100 kV) was employed to verify the nanoparticles morphology. The latex was diluted in distilled water 1:100 and one single drop of latex was placed on a carbon-coated copper grid (300 mesh) and dried at room temperature.

2.2.3. Recovery and encapsulation efficiency of quercetin
Since quercetin can destabilize and precipitate from the latex, the recovery was determined as the amount of quercetin incorporated in the stable part of the latex, which can be either encapsulated or not. The recovery of quercetin on the latex was estimated diluting 500 μl of the sample in 9.5 ml of methanol. After 24 h, the mixture was centrifuged at 4,000 rpm during 30 min. An aliquot of supernatant was measured at 370 nm by spectrophotometer UV–vis (HITACHI, U-1900). The recovery of quercetin was calculated by the difference between the concentration added to the formulation and the concentration measured in the supernatant. Within the amount of quercetin recovered in the latex, the non-encapsulated quercetin was quantified to calculate the encapsulation efficiency. The non-encapsulated quercetin was determined by ultrafiltration of 500 μl of NPs solution in an eppendorf with an Amicon Ultra 0.5 filter (Millipore®, 100 kDa) during 30 min at 13,400 rpm. An aliquot of permeate was diluted in methanol and analyzed at 370 nm using spectrophotometer UV–vis. Encapsulation efficiency was calculated by the difference between the recovered quercetin and the non-encapsulated quercetin.

2.2.4. Encapsulation efficiency of Nps-ZnO
The encapsulation efficiency of NPs-ZnO was calculated indirectly, considering that non-encapsulated ZnO precipitates (the density of ZnO is about 5 times higher than the density of polymer). The latex containing ZnO encapsulated in BMA-PMMA-PS was left during 48 h under refrigeration to precipitate the non-encapsulated ZnO. The precipitate was dried at 60 °C for 24 h and then kept in a muffle furnace (Marqlabor) at 600 °C for 1 h. The mass of the samples was determined before and after the muffle, and the % of encapsulated ZnO-NPs was obtained.

2.2.5. Encapsulation efficiency of green coffee oil and octocrylene
The non-encapsulated octocrylene (OCT) and green coffee oil (GCO) was determined by ultrafiltration of 500 μl of NPs solution in an eppendorf with an Amicon Ultra 0.5 filter (Millipore®, 100 kDa) during 30 min at 13,400 rpm. An aliquot of the permeate was diluted in ethanol and analyzed at 302 nm (HITACHI, U-1900). The encapsulation efficiency was calculated by the difference between the concentration added to the miniemulsion and the concentration measured in the supernatant.

2.2.6. In vitro SPF
In vitro SPF was analyzed by an UV transmittance analyzer (Labsphere R UV–2000 S, Sutton, Vermont, United States) and PMMA plates (50 × 50 mm) (Helioplate HD 6, Sutton, Vermont, United States) with an area of 25 cm². A first measurement was performed on a plate covered with glycerin but without product to make the first measurement. The amount of 50 mg (2.0 mg cm⁻²) of each latex was applied in a large number of small drops evenly distributed over the whole surface of the plate [30]. After application, the latex drops were immediately spread over the whole plate surface using a finger with circular movements in order to obtain a uniform film, during 20 s. After drying for 15 min in a dark chamber, the samples were analyzed. SPF values for each formulation were determined in triplicate of nine determinations [29, 30].

SPF in silico was calculated using the BASF sunscreen simulator [31, 32]. The virtual lab provides estimation of the Sun Protection Factor (SPF). It easily helps the formulator to compose an optimal combination of the UV filters in the development of the Sunscreen formulation. Thus, BASF sunscreen simulator shortens the work by providing realistic estimations of the final product performance [31].

As the NPs were very diluted in the latex, SPF of concentrated samples was also analyzed. For this, the samples were concentrated 1.5 × (by mass) using centrifugation process. Part of the supernatant was removed, and the precipitated nanoparticles were dispersed again using ultrasonic disperser (Fisher Scientific, Sonic Dismembrator Model 500) with 40% of amplitude for 1 min (10 s on/5 s off).

2.2.7. Antioxidant assay by DPPH
Antioxidant activity of free and encapsulated quercetin and green coffee oil was measured based on its ability to transfer electrons or hydrogen atom to 2,2-diphenyl-1-picryl hydrazyl (DPPH·), forming a non-radical form 2,2-diphenyl-1-picryl hydrazine (DPPHH) [10]. Briefly, 1 ml of sample at different concentrations was mixed with 400 μl of a solution of DPPH and ethanol (0.3 mM) and placed in a dark room, at 25 °C for 60 min. The
polymerization. The diameter of droplets after sonication was observed.

### 3.1. Characterization of the nanoparticles

Encapsulation of quercetin and ZnO nanoparticles was successfully performed through miniemulsion polymerization. The diameter of droplets after sonication (Dg, table 3) was in the range 169–346 nm; diameters higher than 300 nm were obtained only for droplets with NPs-ZnO (samples 6 to 9), due to the presence of inorganic particles. NPs-ZnO used in this work presented rod shape with several sizes higher than 300 nm were obtained only for droplets with NPs-ZnO. NPs-ZnO used in this work presented rod shape with several sizes.

Table 3. Intensity mean diameter and polydispersity of droplets (Dg, PdIg) measured after sonication; intensity mean diameter and polydispersity of particles (Dp, PdIp) measured at the end of the reaction and after 30 days of storage.

| Samples       | Droplets Dg (nm) | PdIg | Particles Dp (nm) | PdIp | Particles after 30 days Dp (nm) | PdIp |
|---------------|------------------|------|------------------|------|-------------------------------|------|
| 1 (blank)     | 294.0 ± 1.4      | 0.177| 199.4 ± 1.3      | 0.133| 178.3 ± 0.9                   | 0.075|
| 2 (QUE)       | 169.9 ± 1.4      | 0.141| 166.0 ± 0.7      | 0.053| 162.3 ± 0.5                   | 0.052|
| 3 (QUE/GCO)   | 228.7 ± 4.3      | 0.139| 199.3 ± 1.0      | 0.188| 161.0 ± 1.2                   | 0.139|
| 4 (QUE/OCT)   | 197.7 ± 0.8      | 0.150| 184.1 ± 0.9      | 0.031| 182.9 ± 1.1                   | 0.048|
| 5 (QUE/OCT/GCO)| 187.7 ± 0.9     | 0.140| 168.1 ± 0.4      | 0.150| 163.7 ± 1.6                   | 0.123|
| 6 (blank)     | 175.4 ± 0.5      | 0.104| 193.3 ± 1.3      | 0.014| 193.3 ± 1.2                   | 0.013|
| 7 (NPs-ZnO)   | 346.2 ± 1.4      | 0.405| 194.6 ± 2.4      | 0.138| 173.4 ± 0.1                   | 0.028|
| 8 (NPs-ZnO/OCT)| 326.1 ± 4.6     | 0.235| 217.0 ± 0.8      | 0.046| 208.0 ± 0.2                   | 0.041|
| 9 (NPs-ZnO/GCO)| 282.2 ± 2.8     | 0.260| 194.4 ± 1.2      | 0.101| 184.1 ± 0.5                   | 0.051|

Absorbance of the samples was measured at 517 nm using a spectrophotometer against the blank samples, in which ethanol was mixed with the DPPH solution at the same proportions. The ability of the sample to scavenge the DPPH radical was calculated using equation (1) where, Absc is the absorbance of control of DPPH solution at 0.3 mM, Abs is the absorbance of the sample after reacting with DPPH and Absb is the absorbance of the blank. Determination of EC50 concentration required to reduce the DPPH radicals by 50% was carried out by varying the sample concentration.

\[
\text{DPPH SA\%} = 100 - \left( \frac{(\text{Abs}_{c} - \text{Abs}_{b}) \times 100}{\text{Abs}_{c}} \right)
\]  

### 2.2.8. Spreadability and retention test

Spreadability and retention of the latex was assessed by comparing sample 1 (blank nanoparticles, produced with monomers BMA/MMA/STY) with sample 1* (blank nanoparticles, produced with monomers MMA/STY). Sample 1* was prepared exactly as sample 1, except for monomer composition, that was changed to 50% of MMA and 50% of STY (no BMA used). For retention tests, Transpore® 3 M acrylic adhesive microperforated polyethylene tape (100 × 4.5 mm) was attached to glass plates. In the first test, 500 μl of both latexes were placed on the tape and allowed to spread naturally. In the second test, 100 μl of the latexes were deposited on the tape and spread with circular movements (simulating the spreadability of a sun protection lotion on the skin). All the samples were submitted to air convection for drying. After 1 h, the tapes were removed from the plates to assess latex retention capacity. For spreadability tests (adapted from Parente et al [33]), two drops of both latexes were placed on the Transpore® tape attached externally to glass plates. Then, a second glass plate was placed over the plate containing the sample, without applying force, leading to sample spread. The results of these procedures were analyzed visually. For retention test, it was observed the rupture of the dried film during tape movement and its retention on the tape. For spreadability test, the spreading area of the latex on the tape was observed.

### 3. Results and discussion

#### 3.1. Characterization of the nanoparticles

Encapsulation of quercetin and ZnO nanoparticles was successfully performed through miniemulsion polymerization. The diameter of droplets after sonication (Dg, table 3) was in the range 169–346 nm; diameters higher than 300 nm were obtained only for droplets with NPs-ZnO (samples 6 to 9), due to the presence of inorganic particles. NPs-ZnO used in this work presented rod shape with several sizes from 10 nm to up to 200 nm [29]. After reaction, the obtained sizes for nanoparticles (Dp, table 3) were between 166 and 200 nm for formulations encapsulating quercetin (samples 1 to 5) and between 193 and 217 nm for formulations encapsulating ZnO (samples 6 to 9). These diameter values are in agreement with those observed in TEM micrographs (figure 1), obtained for samples 1, 2 and 3. In general, all samples indicated the presence of spherical NPs, with regular surfaces and homogeneous size. The diameter of the particles after 30 days of storage at approximately 8 °C was similar to that measured at the end of the reaction (table 3), showing the stability of the obtained nanoparticles. Also, it was not observed significant variation in particle diameter and stability as a function of the type of co-stabilizer: crodamol, green coffee oil and octocrylene. Thus, green coffee oil and...
octocrylene presented good results as alternative co-stabilizers, and it was possible to substitute up to 100% of the crodamol by these compounds, as observed for samples 4, 5, 8 and 9.

3.2. Recovery and encapsulation efficiency of quercetin
After encapsulation by miniemulsion polymerization, from 78.7 to 91.1% of quercetin was recovered into the latex. The stability of the latex was then analyzed during 30 days, showing that part of the quercetin precipitated in the flasks with time, resulting in quercetin recovery between 46.4 and 51.4% for samples 2 to 5 after 15 days (this value did not change for 15 to 30 days). The recovery corresponds to the amount of quercetin that did not precipitate and, so, remains in the stable part of the latex. 99%–100% of the quercetin that was recovered after 15 days, was encapsulated inside polymeric nanoparticles. So, the calculated encapsulation efficiency of quercetin (QUE) varied from 46.9 to 51.4%, for samples 2 to 5 (table 4). The higher encapsulation efficiency was obtained when both octocrylene and green coffee oil were used as co-stabilizers (sample 5). Bernardy et al [28] encapsulated quercetin in PMMA nanoparticles obtaining up to 28% of encapsulation efficiency using lecithin as surfactant and crodamol as co-stabilizer, for the same initial amount of quercetin as used in this work.

3.3. Encapsulation efficiency of NPs-ZnO
NPs-ZnO have a high specific mass and when non encapsulated, they tend to precipitate from the latex. Thus, this analysis must be interpreted in a qualitative way. Samples 7, 8 and 9 presented 75.3%, 87.2% and 59.1% of ZnO encapsulation efficiency, respectively (table 4). Thus, octocrylene used as co-stabilizer provided the best result to NPs-ZnO encapsulation (sample 8). In previous work [29], using PMMA-PS matrix and adding up to 20% NPs-ZnO, it was obtained 71% of encapsulation using crodamol and 96% using octocrylene as co-stabilizers by miniemulsion polymerization. When green coffee oil was used as co-stabilizer (sample 9), the NPs-ZnO encapsulation efficiency was lower; however, higher antioxidant activity should be obtained for this sample due to GCO activity (see results for antioxidant activity—table 6).

3.4. Encapsulation efficiency of green coffee oil and octocrylene
The encapsulation efficiency of green coffee oil was very high, with 92.2% and 91.9% for reactions encapsulating quercetin (samples 3 and 5) and 89.5% for reaction encapsulating Nps-ZnO (sample 9) (table 4).
Table 5. Solar protection factor (SPF) of the latexes obtained after miniemulsion polymerization and after concentration of the latex (1.5 ×).

| Samples          | Latex BASF Sunscreen Simulator | SPF Labsphere b | Concentrated Latex SPF Labsphere b |
|------------------|--------------------------------|-----------------|-----------------------------------|
| 1 (blank)        | —                              | 2 ± 1           | 3 ± 1                             |
| 2 (QUE)          | —                              | 2 ± 1           | —                                 |
| 3 (QUE/GCO)      | —                              | 2 ± 1           | —                                 |
| 4 (QUE/OCT)      | 10 ± 2                         | 10 ± 2          | 16 ± 4                            |
| 5 (QUE/OCT/GCO)  | 6 ± 1                          | 6 ± 1           | 7 ± 1                             |
| 7 (NPs-ZnO)      | 5 ± 1                          | 5 ± 1           | —                                 |
| 8 (NPs-ZnO/OCT)  | 11 ± 3                         | 11 ± 3          | 29 ± 5                            |
| 9 (NPs-ZnO/GCO)  | 5 ± 1                          | 5 ± 1           | —                                 |

* SPF in silico was calculated with the BASF Sunscreen Simulator.

b SPF in vitro was measured in triplicate of nine determinations using a Labsphere (UV transmittance analyzer).

Encapsulation efficiencies were obtained also for octocrylene, with 92.4% and 89.8% for samples 4 and 5 (encapsulating quercetin) and 80.4% for sample 8 (encapsulating NPs-ZnO). These compounds were found to be good co-stabilizers and remained encapsulated as compounds of interest, probably increasing SPF and antioxidant activities of the nanocapsules, besides other benefits of green coffee oil. Wagemaker et al. [24, 34, 35] developed cosmetic formulations containing coffee oil and observed an increase of SPF among other beneficial effects on skin due to the addition of coffee oil.

3.5. In vitro SPF

SPF analyses showed that the latexes obtained in this work are very promising for sunscreen application (table 5). SPF values were estimated in BASF sunscreen simulator [31] (in silico) and experimentally measured using Labsphere (UV transmittance analyzer) [30].

A SPF value of 10 was obtained for nanoparticles containing octocrylene and quercetin (sample 4), the same as calculated from BASF simulator for free octocrylene. As the NPs were very diluted in the latex, the samples were concentrated by centrifugation, to perform a second SPF test. When this latex was concentrated, resulted in SPF value of 16, very good for sun protection. For formulation replacing a part of octocrylene by green coffee oil (sample 5), the SPF reduced to 4, and after concentration the latex presented SPF = 7. NPs containing GCO and quercetin (samples 2 and 3) also did not show an increase in SPF; SPF = 2 was obtained for these formulations, which is ascribed for blank polymer nanoparticles (sample 1). Meanwhile, these nanoparticles are expected to exhibit good antioxidant activity that may contribute to increase SPF in vivo, being also interesting for application in photoprotective formulations. SPF studies in humans should be performed to confirm the hypothesis that GCO added in sunscreens formulations is capable of increasing SPF in vivo.

Pereda et al. [36] evaluated in vivo the photoprotective efficacy of a formulation containing 5% green coffee oil by topically applying the product to volunteers and achieved a 28% increase in SPF compared to the placebo group. The study showed the anti-inflammatory effect presented by GCO by reducing the erythema formed in the skin exposed to UV rays.

Wagemaker et al. [24] evaluated the use of coffee oil for cosmetic and photoprotective formulations and also obtained low SPF values measured by in vitro methods (SPF < 2). However, in vivo experiments performed on mice showed that the skins of animals treated with formulations containing 5 and 10% of coffee oil had fewer cells in apoptosis and thinner epidermis, indicating that coffee oil could protect the skin from damage caused by ultraviolet radiation. The oil promoted protection, hydration, maintenance, improvement of the skin barrier and significant increase of the antioxidant and photoprotective activities of the formulations [24, 35].

Nanoparticles encapsulating NPs-ZnO (sample 7) presented the same SPF as calculated by BASF simulator for free ZnO (SPF = 5), showing that photoprotective property is maintained after polymerization reaction. It is important to mention that the total amount of ZnO in these latexes was only 4 wt%. These nanocapsules are very promising since ZnO nanoparticles can generate free radicals under UV radiation, so that encapsulating this compound prevent radicals’ formation [4]. Sample 8, containing NPs-ZnO and octocrylene, presented SPF equal to 11, indicating that the simultaneous encapsulation of octocrylene and NPs-ZnO in the polymer nanoparticles increased in 6 the SPF of the formulation. After concentration of the latex, the SPF value was 29, considered excellent for use as photoprotective lotion. The concentration of both NPs-ZnO and octocrylene was about 6 wt% in this latex after concentration. Frizzo et al. [29] encapsulated simultaneously NPs-ZnO and octocrylene in PMMA/PS nanoparticles and incorporated the obtained nanoparticles into Artistilox AVL® gel. The formulation containing 10% of ZnO-Oct-PMMA/PS nanoparticles exhibited SPF of 18. The formulation containing 20% of ZnO-Oct-PMMA/PS nanoparticles presented a SPF of 32.
Table 6. EC50 values for free quercetin, free green coffee oil and latexes obtained by miniemulsion polymerization encapsulating quercetin (QUE) and green coffee oil (GCO).

| Compounds          | EC50 (μg.ml\(^{-1}\)) |
|--------------------|------------------------|
| Quercetin          | 2.9                    |
| Green coffee oil   | 433.5                  |
| Samples            | EC50 (mg.ml\(^{-1}\))  |
| 1 (blank)          | —                      |
| 2 (QUE)            | 5.6                    |
| 3 (QUE/GCO)        | 3.5                    |
| 4 (QUE/OCT)        | 5.6                    |
| 5 (QUE/OCT/GCO)    | 3.5                    |

* no antioxidant activity.

Sample 9, encapsulating both NPs-ZnO and green coffee oil, presented an SPF of 5, the same result obtained for sample 7, containing only ZnO, showing that GCO did not contribute to the increase of SPF. As reported in the literature [36], an increase of 28% in SPF was observed on a formulation containing 5% green coffee oil by topical application in volunteers. Other study from literature [24] evidenced an increase of up to 3.5 in the SPF value when coffee oil was added to the formulations, evaluated by the anti-inflammatory effect through the reduction of erythema formed on skin exposed to UV rays. Thus, although in the present work green coffee oil did not contribute to the SPF value measured in vitro, it can be used in photoprotective formulations of nanoparticles containing ZnO and octocrylene. In vivo SPF studies should be performed with formulations containing or not green coffee oil to confirm the contribution of this natural active to in vivo SPF increase.

3.6. Antioxidant assay by DPPH
Antioxidant activity of free quercetin was analyzed by DPPH in the range 0.1 to 5 μg.ml\(^{-1}\) in methanol, and EC50 of 2.9 μg.ml\(^{-1}\) was obtained (table 6), which was similar to values found in the literature. Some EC50 values for quercetin reported in the literature are 1.6 μg.ml\(^{-1}\) [37, 38] and 3.5 μg.ml\(^{-1}\) [39]. Due to its high antioxidant activity, quercetin is widely used as a standard on DPPH analysis. As the latexes obtained in this work (samples 2, 3, 4 and 5) contained about 0.05 wt% of quercetin, the EC50 for these latexes was quantified in mg.ml\(^{-1}\). Latex without quercetin (sample 1) presented no antioxidant activity at the concentrations studied. EC50 for the latexes with encapsulated quercetin (samples 2 and 4) was found to be 5.6 mg.ml\(^{-1}\), showing that quercetin maintained its antioxidant activity after encapsulation. Bernardy et al [28] reported that quercetin may be partially consumed during polymerization reactions, as it is an antioxidant and consequently may be reacting with the radicals present in the reaction, losing its activity. This effect was not observed in the present work.

The antioxidant activity of the green coffee oil was also evaluated, resulting in EC50 value equal to 433.5 μg.ml\(^{-1}\). For the latexes containing quercetin (0.05 wt%) and GCO as co-stabilizer (4.5 wt%) (samples 3 and 5), the EC50 obtained was 3.5 mg.ml\(^{-1}\), indicating that GCO provided a significantly increase on the antioxidant activity, when compared to sample 2 with only quercetin. It means that, comparing the same concentration of both latexes, the simultaneous encapsulation of quercetin and green coffee oil provided an antioxidant activity 25.5% higher than for nanoparticles encapsulating only quercetin.

3.7. Spreadability and retention test
The retention and spreadability of the obtained latexes were visually assessed using Transpore® tape as a skin-simulating application surface. Latexes prepared with monomers BMA, MMA and STY (sample 1) were compared to the latex obtained with only MMA and STY (sample 1’), which are monomers usually applied.

Latexes prepared with BMA/MMA/STY presented better retention and spreadability than latex prepared without BMA (figure 2). It was observed that the film formed after drying sample 1” ruptured during tape movement and did not obtain good retention on the tape (figure 2, left side), while sample 1 showed adherence and much higher retention on the tape, for both retention tests with 100 or 500 μL of latex. The sample 1, containing BMA, resulted in a film with greater flexibility. In addition, film formed with 500 μL of sample 1” was opaque, while the film formed with 500 μL of sample 1 (with BMA) was translucent. Testing the spreadability of the latexes, it was observed that sample 1 (with BMA) presented higher spreadability than sample 1” (without BMA), since the spreading area on the tape obtained for sample 1 was three times higher (figure 2, right side). Thus, adding BMA to the latex formulation promoted better retention, flexibility and spreadability of the final latex, which are important characteristics for sunscreen and general cosmetic applications.
4. Conclusion

ZnO nanoparticles and quercetin were encapsulated in polymer nanoparticles using the miniemulsion polymerization technique. The obtained nanoparticles presented between 160–230 nm, with spherical, homogeneous and regular surface, being stable for at least 30 days. Octocrylene and green coffee oil were successfully used as co-stabilizers, being also encapsulated as compounds of interest in the formulation. When using BMA as monomer in the formulation (60 wt% BMA, 20 wt% MMA and 20 wt% STY), the obtained latexes presented good spreadability and retention for skin application. Quercetin maintained high antioxidant activity when encapsulated in polymeric NPs, and obtained even better results when encapsulated simultaneously with green coffee oil. The value of SPF measured in vitro was very good for formulations containing NPs-ZnO or octocrylene, obtaining SPF = 29 for latex with simultaneous encapsulation of 6 wt% of NPs-ZnO and 6 wt% of octocrylene. The encapsulation of quercetin and green coffee oil with ZnO and octocrylene in sunscreen formulations may contribute to the increase of in vivo SPF, combating free radicals and reducing the damage caused by UV radiation.

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Conflict of interest

The authors declare that they have no conflict of interest.

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