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Research Article

Nanoencapsulation of Rose-Hip Oil Prevents Oil Oxidation and Allows Obtainment of Gel and Film Topical Formulations

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Abstract. The rose-hip oil holds skin regenerating properties with applications in the dermatological and cosmetic area. Its nanoencapsulation might favor the oil stability and its incorporation into hydrophilic formulations, besides increasing the contact with the skin and prolonging its effect. The aim of the present investigation was to develop suitable rose-hip-oil-loaded nanocapsules, to verify the nanocapsule effect on the UV-induced oxidation of the oil and to obtain topical formulations by the incorporation of the nanocapsules into chitosan gel and film. The rose-hip oil (500 or 600 μL), polymer (Eudragit RS100®, 100 or 200 mg), and acetone (50 or 100 mL) contents were separately varied aiming to obtain an adequate size distribution. The results led to a combination of the factors acetone and oil. The developed formulation showed average diameter of 158±6 nm with low polydispersity, pH of 5.8±0.9, zeta potential of +9.8 ±1.5 mV, rose-hip oil content of 54±1 μL/mL and tendency to reversible creaming. No differences were observed in the nanocapsules properties after storage. The nanoencapsulation of rose-hip oil decreased the UVA and UVC oxidation of the oil. The chitosan gel and film containing rose-hip-oil-loaded nanocapsules showed suitable properties for cutaneous use. In conclusion, it was possible to successfully obtain rose-hip-oil-loaded nanocapsules and to confirm the nanocapsules effect in protecting the oil from the UV rays. The chitosan gel and film were considered interesting alternatives for incorporating the nanoencapsulated rose-hip oil, combining the advantages of the nanoparticles to the advantages of chitosan.

KEY WORDS: chitosan film; chitosan gel; polymeric nanocapsules; rose-hip oil; UV-induced oxidation.

INTRODUCTION

The rose-hip or rosa mosqueta oil is mainly obtained from the seeds of the plant *Rosa rubiginosa* found in Central Europe and the Andean region (1). This oil, presenting a greasy non-volatile aspect, is widely composed of unsaturated fat acids like the linoleic, oleic, and linolenic acids, and also of poliphenols, carotenoids, and transretinoic acid, representing therefore an alternative for accelerating the skin regenerating process (1–3). Pigmentation, ulceration, and scaring problems can be treated with rose-hip oil (4). Its use includes dermatological and cosmetic applications, used in its plain form or in formulations at concentrations ranging from 2 to 30%.

Nanoparticles for dermatological and cosmetic use have been extensively studied, in the past years (5–7). The nanoencapsulation advantages include a more strict contact with the *stratum corneum*, due to a great skin adhesion of the nanostructures (8) and a controlled release of the active substances (9). Those advantages lead to a more prolonged action (10,11) and increase in efficacy (12). Due to the size of around 300 nm, those nanoparticles used on the skin are not able to cross the intact *stratum corneum* (7). Benefits regarding the formulating process and stability achievement should also be taken into consideration. Oil-loaded nanocapsules in aqueous suspension represent an easy way to incorporate oils of interest in cosmetic formulations, especially formulations containing low lipid content, such as gels. Although it was previously proven that the nanoencapsulation protects active substances from physical or chemical degradation (13–15), the protection of the nanocapsules to the degradation of the oily core has not been evaluated. This is of even more interest when the oil core is used by its pharmacological or cosmetic properties, which is the case of rose-hip oil.

The nanoencapsulation of rose-hip oil, among other vegetable oils, has been previously proposed by our research group using Eudragit RS100® as polymer (16). The polymer was chosen due to its biocompatibility and cationic properties, which increase skin adhesion (8). However, such nanocapsule suspension presented some aspects to be improved: particles in the micrometer range, a large size distribution, and high

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creaming of the nanocapsules. Nevertheless, the viscosity of formulations were observed to be very low, similar to the viscosity of pure water (16), not favoring the topical application. In this context, the obtainment of final formulations is considered essential; therefore, the selection of an adequate semisolid vehicle is a crucial step.

Chitosan, a hydrogel-forming and film-forming polymer (17), has been proposed as gel vehicle for polymeric nanocapsules (8), liposomes (18), niossomes (19), and solid lipid nanoparticles (20), as well as film vehicle for polymeric nanoparticles (21) and silver nanoparticles (22). Some interesting properties of this polymer such as bioadhesion, biocompatibility, and wound healing properties (23–25) turn chitosan formulations into great candidates for the topical application of rose-hip-oil-loaded nanocapsules.

Thus, the objective of the present article was to improve the nanoencapsulation of rose-hip oil in terms of homogeneity of nanocapsule sizes avoiding the presence of particles with more than 1000 nm, by varying the component contents. Also, the effect of the nanocapsules on the UVA- and UVC-induced oxidation of the rose-hip oil and the development of final topical gel and film formulations, using the biopolymer chitosan, was investigated.

MATERIALS AND METHODS

Materials

The rose-hip oil was purchased from Embacaps (Porto Alegre, Brazil). Eudragit RS 100® (approximate molecular weight of 32,000 g/mol) and polysorbate 80 were purchased from Degussa (Darmstadt, Germany) and Labsynth (São Paulo, Brazil), respectively. Chitosan (medium molecular weight and deacetylation degree of 77%) and tripolyphosphate were obtained from Sigma-Aldrich (São Paulo, Brazil). Lactic acid at a concentration of 85%, glycerin, decamethylcyclopentasiloxane or volatile silicone fluid, and sodium pyrrolidone carboxylic acid (Na-PCA) were obtained from Via Farma (São Paulo, Brazil). Acetone and ethyl acetate of analytical grade were obtained from Vetec (Rio de Janeiro, Brazil), while acetonitrile of HPLC grade was obtained from Tedia (Sao Paulo, Brazil).

Nanocapsule Production and Formulation Improvement

The nanocapsule aqueous suspensions were obtained by means of the interfacial deposition of pre-formed polymer method in which an organic phase containing the organic solvent (acetone), the rose-hip oil and the nanocapsule shell-forming-polymer (Eudragit RS100®), was injected into an aqueous phase containing polysorbate 80 as hydrophilic surfactant. The acetone and part of the water (until volume of 10 mL) were then eliminated at a rotary evaporator (R-114, Büchi).

Based on the previous formulation of nanoencapsulated rose-hip oil, obtained with slight modifications from CONTRI and co-workers (16) ($NC_{Original}$), three components, the rose-hip oil (NC-A), the polymer (NC-B), and the acetone (NC-C), were varied in order to attempt an improvement of the formulation regarding the percentage of microparticles in the formulation and the homogeneity of the system, observing the average nanoparticle diameter, as well (Table I). Such data [percentage of particles above 1000 nm, D (4,3), and SPAN value] were obtained by means of laser diffraction analyses after dilution in ultrapure water (Mastersizer, Malvern), using the refraction index of Eudragit RS100® (1.38). Based on the results, we developed a more suitable formulation ($NC_{Improved}$) which was further analyzed as described below.

The polydispersity values (SPAN values) were determined as follows (Eq. 1):

$$SPAN = \frac{(D_{0.9} - D_{0.1})}{D_{0.5}} \quad (1)$$

where $D_{0.1}$, $D_{0.5}$, and $D_{0.9}$ are the diameters that correspond to 10, 50, and 90%, respectively, in the size distribution.

Nanocapsule Characterization

The improved nanocapsule ($NC_{Improved}$) formulation was further characterized in terms of average diameter, polydispersity, zeta potential, nanocapsule concentration, oil content, and pre-stability. The diameter and polidispersity were evaluated by light scattering (Zetasizer, Malvern) after dilution in ultrapure water (1:500 *v*/*v*), using the refraction index of 1.38. To guarantee that the oil was encapsulated within the nanocapsules, three consecutive measurements of the size distribution profiles were analyzed. Dynamic systems present different profiles among the measurements, which would indicate formation of nanocapsules and nanoemulsion simultaneously, while stable formulations present similar profiles among the consecutive measurements (26). The zeta potential was evaluated by eletroforetic mobility (Zetasizer, Malvern) after dilution in 10 mM NaCl solution (1:500 *v*/*v*). The pH was analyzed by potentiometry (Micronal) by direct measurement in the suspensions. The number of particles per milliliter (concentration) was determined by turbidimetry at 395 nm (spectrophotometer Femto 600 Plus) by several dilutions in ultrapure water. The determinantion of the oil content was performed by a matrix superposition method at 269 nm (spectrophotometer Femto 600 Plus) by dilutions in ethyl acetate/acetonitrile. The morphology of the nanocapsules was evaluated by transmission electron microscopy (TEM; JEM 1200 Exll operating at 80 K). The aqueous suspension sample was deposited on a Formvar/Carbon grid and negatively stained with uranyl acetate solution (2%*w*/*v*).

The pre-stability of the aqueous nanocapsule suspension was analyzed by its resistance to centrifugation (MTDIIIPLUS, 30 min under 1210×*g*), by its tendency to physical instability phenomena using the multiple light scattering technique (Turbiscan LAB, Formulaction, at 25°C for 12 h), and by the evaluation of the formulation properties under storage at room temperature and at 40°C. After 30 days, in the mentioned conditions, the formulation was again characterized in terms of average diameter, polydispersity, zeta potential, and pH, as described above.

In Vitro Oxidation Study

The effect of the nanoencapsulation on the rose-hip oil oxidation process was evaluated (n=3), considering the possibility of increasing the formulation stability under UV radiation. The rose-hip oil oxidation under UVA and UVC lights

Table I. Composition of Nanocapsule Colloidal Dispersion (10 mL)

Formulation	Acetone (mL)	Polymer (Eudragit RS 100®) (mg)	Rose-hip oil (μL)	Ultrapure water (mL)	Polysorbate 80 (mg)
$NC_{Original}$	54	200	660	106	152
NC-A	54	200	500	106	152
NC-B	54	300	660	106	152
NC-C	100	200	660	106	152
$NC_{Improved}$	100	200	500	106	152

Underlined numbers indicate the changes performed in relation to the prior formulation ($NC_{Original}$)

was studied by means of the lipoperoxidation process, quantifying the fluorescent product obtained by the reaction of malondialdehyde and thiobarbituric acid at 532 nm by UV spectroscopy (27). The malondialdehyde is one of the most frequently used indicators of lipid peroxidation.

The nanocapsule aqueous suspensions ($NC_{Improved}$) were placed under UVA and UVC light sources for 24 h. At predetermined time points, aliquots from the formulations were withdrawn and mixed with trichloreacetic acid (12% *w/v* in water) and thiobarbituric acid (0.73% *w/v* in water). The fluorescent color appeared when the formulations were placed in a water bath at 100°C for 30 min. The mixture obtained was centrifuged for 12 min at 10,000 rpm and 25°C. The supernatant was obtained and analyzed regarding its absorbance at 532 nm. The nanocapsule aqueous suspension was compared to a dispersion of the oil in water and polysorbate 80, at the same concentrations of rose-hip oil and surfactant. The values obtained were discounted from the values obtained at time zero (treating the formulations in similar conditions in except for the period under UV radiation). It is important to notice that, previously to the quantification, growing fluorescence curves were obtained by adding different concentrations of malondialdehyde aqueous solution (obtained by a standard solution) at 100°C and centrifugating afterward. The linearity was obtained in the range from 0.0036 to 0.022 mM of malondialdehyde.

Development and Characterization of Final Dermal Formulations Based on Chitosan

Based on the fact that the aqueous nanocapsule suspensions developed are difficult to apply on the skin, besides the possibility of instability and microbial contamination, two final formulations based on chitosan (gel and film) were proposed for the cosmetic and dermatological use of rose-hip oil (Table II). The preparation procedure is described below.

Gel

The chitosan gel containing rose-hip oil nanocapsules was obtained by simple mixing of chitosan, nanocapsule aqueous suspension, lactic acid, and sodium pyrrolidone carboxylic acid. Then, volatile silicone and diazolidinyl urea were added to the formulation (28). The innovative hydrogel was obtained in triplicate of batches. The characterization of the gel formulation was performed regarding size distribution, viscosity, pH, and pre-stability. The size distribution analysis was evaluated by laser diffraction (Masterizer, Malvern) performing a dilution in ultrapure water. The viscosity was assessed by rotary viscosimetry (Brookfield® LV-DV-II+Pro, spindle SC4-25 at 25°C). The pH value was evaluated by potentiometry (Micronal) after dilution in ultrapure water (1:10 *w/v*). The tendency to instability phenomena was analyzed by multiple light scattering (Turbiscan LAB, Formulaction) by direct measurement at 25°C for 12 h.

Film

The chitosan film containing rose-hip oil nanocapsules was obtained by mixing of chitosan, nanocapsule aqueous suspension, lactic acid, and glycerin, followed by solvent casting (17). The water was eliminated by keeping 1 g of the formulation in recipients of 3 cm of diameter under desiccator for 15 days. After drying, tripolyphosphate aqueous solution was added to each recipient, increasing the rigidity of the films and allowing the removal from the recipients. The innovative film containing nanocapsules was obtained in triplicate of batches. The characterization of the film was performed regarding weight, thickness, pH, presence of nanocapsules, and water absorption capacity. The thickness was assessed by direct measurements of the films formed using a dial thickness gauge (No. 7301®, Mitutoyo, Japan). The pH and presence of nanocapsules were determined, after disruption of the films with ultrapure water and vortex mixing, by potentiometry (Micronal) and by laser diffraction (Masterizer, Malvern), respectively. The water absorption was determined by keeping films immersed in ultrapure water (37°C). Then, at predetermined times, the film weights were determined.

Statistical Analyses

The statistical analysis of the characterization properties were performed by ANOVA, followed by Dunnet's test, for multiple groups and Student's *t* test for two groups. In both cases, differences were considered significant for α=0.05.

Table II. Composition of Dermal Chitosan Formulations Containing Rose-Hip-Oil-Loaded Nanocapsules

Components	Gel	Film
Chitosan	2.5%	1%
Lactic acid	1%	1%
Carboxylic pirrolidon acid sodium salt	2%	–
Decamethylcyclopentasiloxane	3%	–
Diazolidinyl urea	0.5%	–
Glycerin	–	10%
Tripolyphosphate	–	2%
Rose-hip oil (nanoencapsulated)	5%	5%

RESULTS AND DISCUSSION

Nanocapsule Improvement

The changes in the previous developed formulation ($NC_{Original}$) (16) were performed in order to obtain higher homogeneity in terms of nanoparticle sizes, also aiming to exclude the simultaneous presence of particles of more than 1000 nm. Therefore, the contents of three components of the organic phase were varied, compared to the prior formulation. Changing the amount of rose-hip oil (NC-A), polymer (NC-B), and acetone (NC-C), three results were evaluated: the SPAN value, the percentage of microparticles, and the average diameter. The values observed for the triplicate of batches are shown in Fig. 1.

When the content of acetone was increased (NC-C), the SPAN value is significantly decreased (Fig. 1a). On the other side, decreasing the amount of oil (NC-A), the SPAN value is significantly decreased. Similar results are obtained for the percentage of microparticles (Fig. 1b) and average diameter (Fig. 1c). The SPAN value reduced 1.5 and 1.9 times by decreasing the oil and increasing the acetone, respectively. The microparticle percentage reduced 2 times and 11.8 times by decreasing the oil and increasing the acetone, respectively. The average diameter reduced 1.4 and 1.8 times by decreasing the oil and increasing the acetone, respectively. Those findings indicate that smaller oil droplets are obtained in the organic phase by reducing the oil and increasing the acetone. Such smaller droplets in the organic phase lead to smaller average diameters, improving the homogeneity of the system. The encapsulation efficiency is probably affected by increasing the acetone in the organic phase, since, as previously mentioned, smaller oil droplets are formed during particle production. The increase in the amount of acetone is not considered a production problem because the acetone is completely evaporated during the process and could be reused. Also, it is important to notice that the increase in particle sizes due to increase of oil content suggests that the rose-hip oil is encapsulated within the nanocapsules. The polymer content (NC-B) did not show influence on the three results analyzed. The excess of the polymer in relation to the original formulation was probably not able to improve the formulation since residual polymer was observed after evaporating the solvents under reduced pressure, for the formulations with 300 mg of Eudragit RS 100®. Therefore, the original amount of polymer was considered ideal, since it was able to be successfully placed around the oil droplets.

From the results obtained after the component changes, it was possible to conclude that another formulation, called $NC_{Improved}$, combining the changes performed in the oil and in the acetone content should be prepared. Increasing the amount of acetone (from 54 to 100 mL) and simultaneously decreasing the amount of oil (from 660 to 500 μL) led to smaller particles sizes (from 333±37 to 162±10 nm), lower SPAN values (from 3.2±0.7 to 1.5±0.1), and lower percentage of microparticle (from 4.7±0.8 to 0.2±0.3%), improving the formulation. The average diameter and the SPAN value were reduced 2.1 times while the percentage of microparticles was reduced in 23.5 times, respectively after formulation improvement.

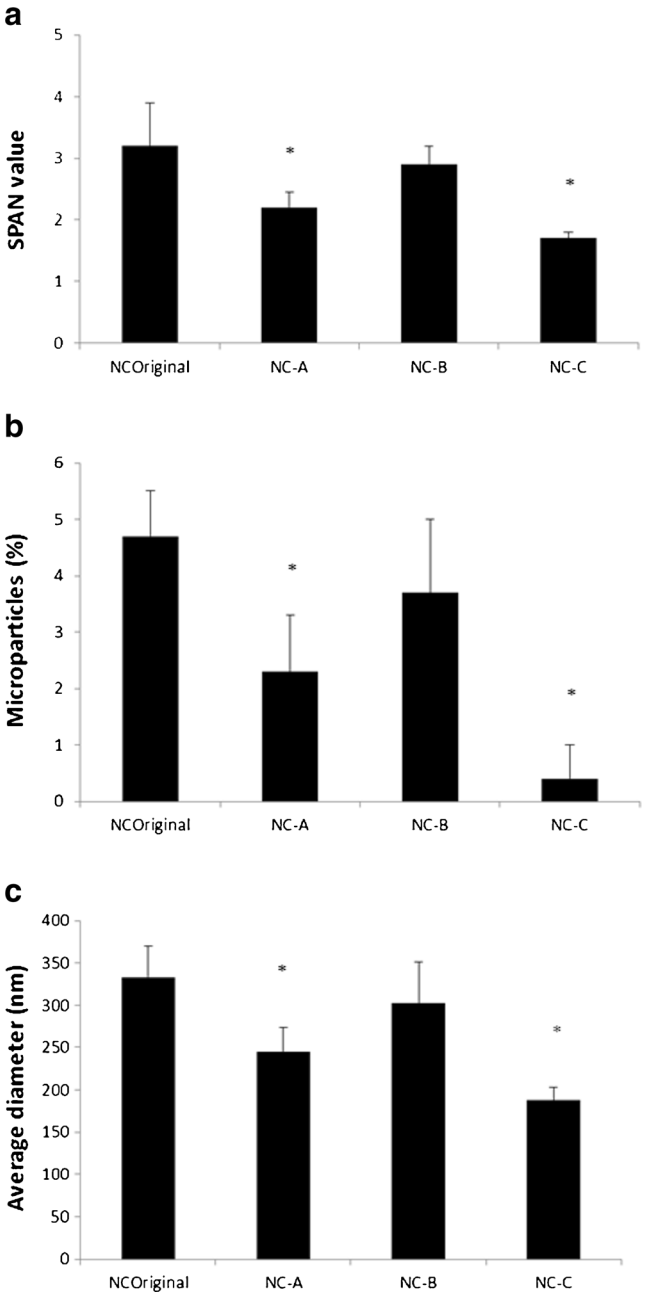

Fig. 1. SPAN values (**a**), percentage of microparticles (**b**), and average diameter (**c**) of nanocapsule colloidal dispersion. *Statistical difference compared to $NC_{Original}$ ($p<0.05$)

Nanoparticle Properties

The improved nanocapsule aqueous suspension was further characterized. The nanocapsule presented average diameter of 158±6 nm by means of light scattering analyses, similar to the value found by laser diffraction (Nanocapsules improvement). When comparing the three consecutive measurements of the size distribution profiles of the improved formulation, very similar profiles were observed indicating stable systems regarding the particle size. Therefore, it is concluded that the oil is encapsulated within the nanocapsules. The polydispersity index obtained by the mentioned

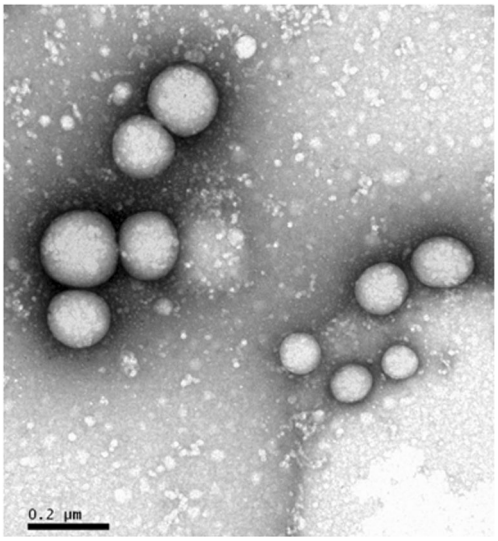

Fig. 2. Transmission electron microscopy image of rose-hip-oil-loaded nanocapsules

technique was 0.12±0.02, proving a great homogeneity in the system regarding the nanocapsule sizes. By means of electron microscopy, the nanocapsules were found to be spherical, with homogeneous size, and values close to that obtained by laser diffraction and light scattering (Fig. 2). The zeta potential was found to be +9.8±1.5 due to the cationic properties of Eudragit RS 100®, used as nanocapsule shell. The positive zeta potential can increase the contact of the nanocapsules with the skin, due to negative charge of the tissue, as previously observed for the vaginal mucosae (29). The value obtained for the pH was 5.8±0.9, which is in agreement with previous similar nanocapsule aqueous suspensions (30). The nanocapsule aqueous suspension presented $5.37\pm0.46\times10^{13}$ particles per milliliter, a higher value than the ones previously obtained by encapsulating vegetable oils in Eudragit RS100® nanocapsules (16). This result was expected since the volumetric fraction was two times higher and the acetone and oil amounts were changed, leading to particles smaller in size and higher in number.

Regarding the rose-hip oil content in the formulation, a UV methodology was previously validated. The linearity was obtained by adding a constant amount of polymer and polysorbate 80 (the same amount as present when the oil amount in the formulation was determined) and adding different amounts of rose-hip oil from a standard solution (100 µL/mL) to the five different curve points (1 to 7 µL/mL). The linearity of the method was confirmed (correlation coefficient of more than 0.99), and the method was considered precise and accurate. Using the referred validated methodology, the amount of rose-hip oil in the formulation was found to be 54±1 µL/mL (108% of the theoretical value). The percentage of rose-hip oil in the improved nanocapsule formulation was 5%, which is in the range of usual topically applied formulations containing the oil. Therefore, by means of nanocapsule improvement, it was possible to successfully encapsulate a high amount of vegetable oil indicating the obtainment of an efficient skin regenerating formulation. Eudragit RS 100® nananocapsules present great potential for controlled delivery of lipophilic substances as previously proven with capsaicinoid-loaded nanocapsules (31), which suggests that the actives present in the rose-hip oil, such as transretinoic acid, will be delivered in a controlled way when applied on the skin.

The nanocapsule formulation was additionally characterized in terms of pre-stability. The formulation was analyzed by centrifugation and by multiple light scattering (predetermined time points for a total period of 12 h), to investigate its tendency to physical instability phenomena, such as sedimentation, creaming, or particle agglomeration. No instability phenomena were observed after the centrifugation process, suggesting adequate oil nanoencapsulation. Regarding multiple light scattering analysis, Fig. 3 shows the variation of the backscattering signal relative to the first measurement (ΔBS). The light backscattering is shown since the transmittance was null (data not shown). It is possible to observe an increase in the ΔBS with the time, at the top of the cell. The opposite occurred at the bottom of the cell, where a decrease in ΔBS is observed as a function of the time. This is a typical creaming profile, indicating the migration of particles to the top of the cell, as previously described (16).

In order to verify if the creaming was reversible for the nanocapsule suspension here described, the nanocapsule basic properties (average diameter, polydispersity, and zeta potential) were again measured after 30 days under storage at room temperature and at 40°C. The formulations were slightly homogenized by hand and analyzed (Table III). No significant differences were observed in the majority of nanocapsule properties after storage, confirming that the creaming did not influence the average size, the homogeneity regarding

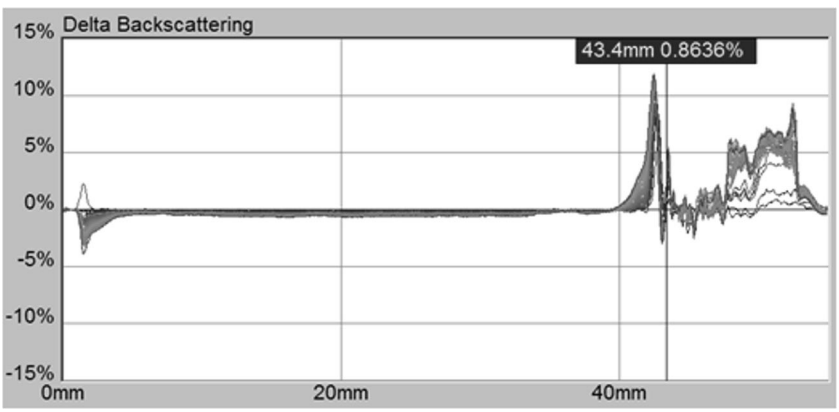

Fig. 3. Delta backscattering analysis of rose-hip-oil-loaded nanocapsule colloidal dispersion

Table III. Properties of Rose-Hip-Oil-Loaded Nanocapsule Colloidal Dispersion After Storage at Room Temperature and at 40°C

Storage period/condition	Size (laser diffraction)	SPAN	Size (light scattering)	PDI	Zeta potential	pH
30 days/room temperature	161±9	1.4±0.1	158±3	0.13±0.01	9.2±0.7	5.4±0.3
30 days/40°C	146±7	1.2±0.1	155±2	0.14±0.07	10.2±0.8	4.5±0.1*

*Statistical difference compared to the value obtained right after production

particles sizes, or the surface charge, being considered a reversible physical instability phenomenon. The pH values, also measured after storage period, presented significant decay only when stored at 40°C. Such decay was previously described for similar nanocapsules, and it is probably due to the partial hydrolysis of the polymer side groups not causing short-time destabilization system (16).

Effect of the Nanocapsules in Protecting the Rose-Hip Oil from Oxidation due to UV Light

The nanocapsule aqueous suspension and a dispersion of oil in the same amount of polysorbate 80 and water were put under UVA and UVC light for 24 h. Aliquots were withdrawn and analyzed regarding the presence of malondialdehyde, after its reaction with tiobarbituric acid, forming a colored compound. The unsaturated fatty acids are probably the oxidized compounds. The malondialdehyde content (mM) as a function of the time (Fig. 4) was determined by means of a pre-validated methodology. Considering the UVA light, there is a significant difference between formulations when aliquots were withdrawn after 12 and 24 h under light. For the UVC light, there is a significant difference between formulations when aliquots were withdrawn after 6, 8, 12, and 24 h under light. In both conditions, the nanoencapsulated formulation presented less amount of malondialdehyde in the time intervals mentioned. So, the nanoencapsulation was able to protect the oil from oxidation, leading to a formulation of high stability. The property of the nanocapsules in reducing the degradation of encapsulated drug under UV light was previously shown (13,15). However, no data about the oxidation of vegetable oils and the influence of the nanocapsules on this parameter were described so far. Such increase in the stability of vegetable oils is of extreme importance since their oxidation might lead to loss in activity besides formation of unpleasant odor.

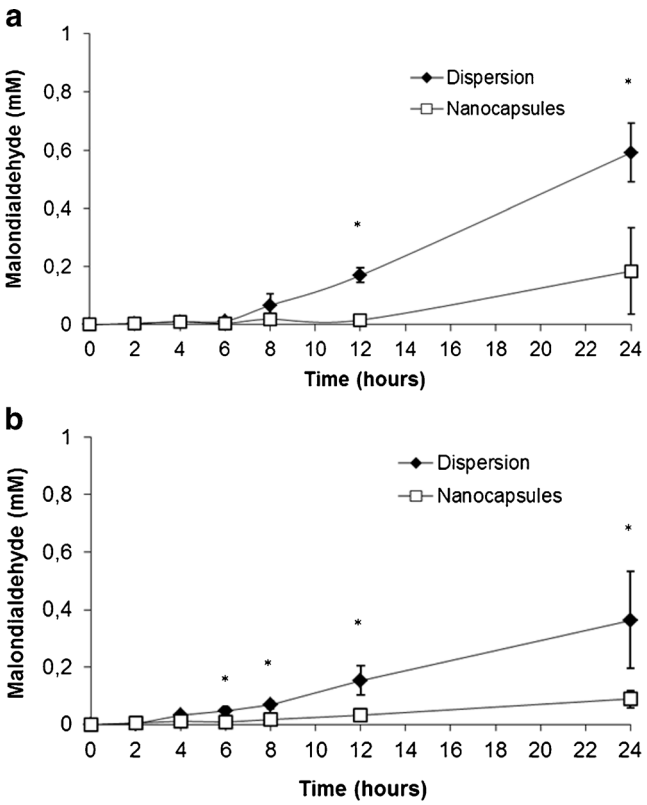

Fig. 4. Malondialdehyde formed as a function of time under UVA (**a**) and UVC (**b**) light. *Statistical difference between free oil dispersion and nanocapsules ($p<0.05$)

Development of Final Formulation for Cutaneous Use of Rose-Hip-Oil-Loaded Nanocapsules

Final dermal formulations based on chitosan and containing rose-hip-oil-loaded nanocapsules were successfully obtained as shown in Fig. 5. Chitosan was chosen as hydrogel-forming and film-forming polymer due to its wound healing and adhesion properties (23,24,32). Chitosan gel containing polymeric nanocapsules was already described by our research group, presenting great skin adhesion (8) and non-irritant properties (33). Moreover, the adjuvants sodium pyrrolidone carboxylic acid (Na-PCA) and volatile silicone were added to the gel aiming to improve the sensorial properties of the formulation, as previously described (28). However, as far as we know, this is the first time that chitosan films with polymeric nanocapsules are produced.

The chitosan hydrogel (Fig. 5a) presented opaque aspect, due to the presence of nanocapsules, adequate homogeneity, and apparent good spreadability on the skin. The chitosan film

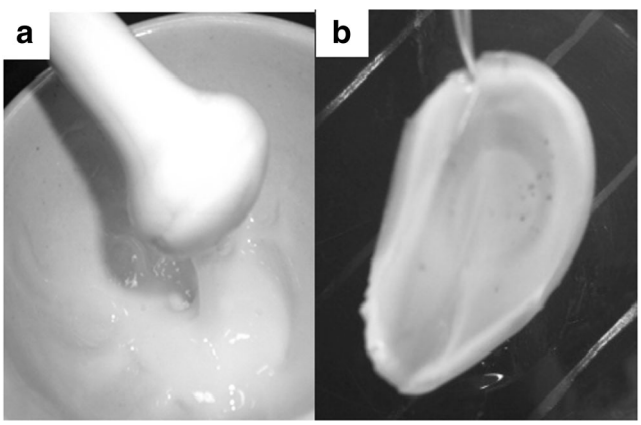

Fig. 5. Chitosan gel (**a**) and film (**b**) containing rose-hip-oil-loaded nanocapsules

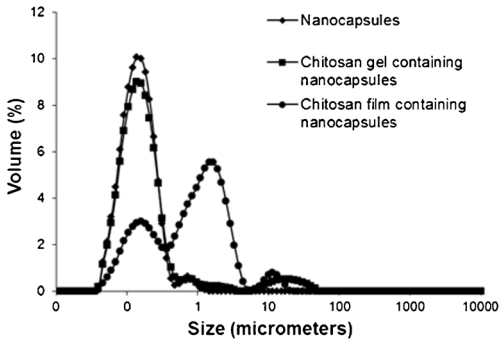

Fig. 6. Size distribution of nanocapsules incorporated into chitosan gel, into chitosan film, and right after production in colloidal dispersion

(Fig. 5b) presented also opaque aspect, besides apparent good mechanical resistance and good adhesion on the skin, the latter especially when the film was wet. It was previously described that the inclusion of polymeric nanoparticles to chitosan films increased their "time to break" and tensile strength (21). The films prepared were of 3 cm of diameter and presented initial weights of 209±8 mg and thickness of 2.78±0.05 mm. The chitosan gel and film containing nanocapsules formulation presented slightly acid pH (gel=4.4±0.1; film=6.1±0.0), due to addition of lactic acid, necessary for the chitosan chain entanglement. The values were considered suitable for cutaneous use, due to acid properties of the *stratum corneum* (34).

Regarding the presence of nanocapsules in the chitosan dermal formulations, size distribution graphs were obtained by laser diffraction (Fig. 6). Analyzing the size distribution, it is possible to see that the gel and the film presented particles in the nanometric range, with similar size to that observed for the improved nanocapsules in aqueous colloidal dispersion. The results indicate that the incorporation of nanocapsules in chitosan formulations was successfully performed, and no damage is caused to the particles due to incorporation in the hydrogel, as previously described (8) or in the films. Although both of the chitosan formulations presented a population in the nanometric range, the particle size distribution of the chitosan gel and chitosan film containing nanocapsules presented distinct profiles. The chitosan film presented an additional population of close to 1 μm of average diameter. Such larger particles can be agglomerated nanocapsules within the physically cross-linked chitosan. The chitosan chains are entangled in the gel, while they are physically cross-linked by tripolyphosfate in the film formulation. Such cross-linked chitosan presents a very tangled network (35), which can hinder the dispersion of nanocapsules into their primary size in water during the measurement of particle size distribution. The chosen vehicles based on chitosan appear to be suitable alternatives for carrying the rose-hip-oil-loaded polymeric nanocapsules.

The chitosan gel was additionally evaluated according to its rheological profile and its physical stability. The rheological data (shear stress vs. shear rate) were well fitted to the Ostwald flow model (r>0.99), which corresponds to the pseudoplastic flow favoring the cutaneous applications of products (36). The consistence index was 31.75±1.73 and the flow index was 0.72±0.01. The average consistence value observed is higher than the values previously observed for chitosan gels containing polymeric nanocapsules and the adjuvants (PCA-Na and volatile silicone) (28), which were around 20. This difference is probably due the fact that the number of particles in the original aqueous suspension and posterior in the hydrogel is higher, when compared to the previous investigation. Regarding the physical stability of the formulation, Fig. 7 shows the delta backscattering obtained as mentioned in the "Nanoparticle Properties" section. No specific instability phenomena are detected for the chitosan hydrogel formulation, since the variation in the delta backscattering is under 10% (37). The incorporation of the rose-hip-oil-loaded nanocapsules in a chitosan hydrogel reduced the creaming of the particles observed when these were in aqueous suspension, due to increase in viscosity of the system. Although the creaming did not interfere in the nanocapsules properties, its reduction was considered positive, since the creaming can interfere in the homogeneity of doses.

The chitosan film containing rose-hip-oil-loaded polymeric nanocapsules was additionally evaluated according to water absorption capacity. It was observed that the films decreased their weights in 23±2% after 30 min and 31±4% after 1 h in water immersion. After 3 h at the same condition, the weight decrease was kept at 32±2%. The weight decrease observed in the first hour of immersion was probably due to residual substances, such as tripolyphosphate, being released from the films to the water, decreasing the weights of the films. After 3 h, it was observed that the films kept their weights,

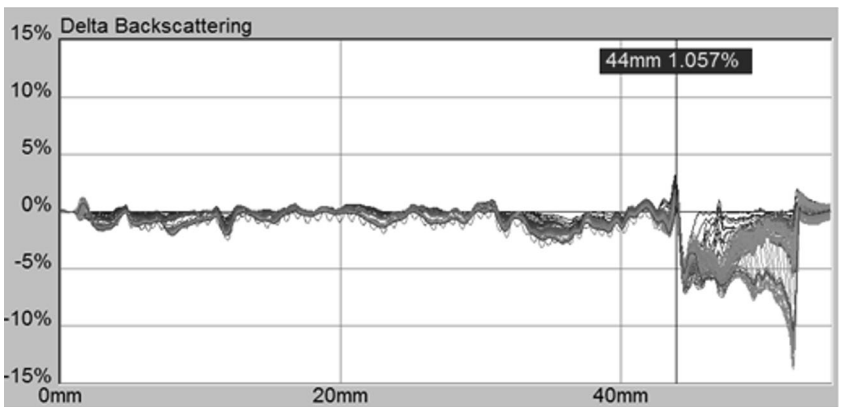

Fig. 7. Delta backscattering analysis of chitosan gel containing rose-hip-oil-loaded nanocapsules

indicating stability of the films in water. No water absorption was observed, oppositely as described for previously reported chitosan films (17,32,22). In the present investigation, the network formed by cross-linked chitosan and nanocapsules might have influenced the water absorption capacity of chitosan. Considering that the formulations are expected to be applied at an injured skin area, the absence of water absorption can be positive. Formulations that completely absorb the wound fluid, preventing a certain moisture level, may hinder the wound healing process (32).

CONCLUSION

The rose-hip-oil-loaded nanocapsules were improved by increasing the acetone and decreasing the rose-hip oil content during particle formation. It was possible to nanoencapsulate an effective dose of rose-hip oil and to protect the oil from UV oxidation. The nanoencapsulation of rose-hip oil allows its incorporation into aqueous formulations. The innovative final formulations of chitosan gel and chitosan film containing rose-hip-oil-loaded nanocapsules presented suitable properties for the application on the skin, combining the advantages of the nanocapsules to the advantages of chitosan.

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Conflict of interest The authors declare no conflict of interests.

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