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Development of Semi-Solid Cosmetic Formulations Containing Coenzyme Q10-Loaded Nanocapsules

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SUMMARY. Nanocapsule suspensions containing coenzyme Q10 were prepared by interfacial deposition. The nanocapsules showed characteristics compatible with dermal application: slightly acid pH, drug content close to 100%, particle size between 213 and 248 nm with low polydispersity and negative zeta potential. Three cosmetic formulations for skin application were developed, one with the free-coenzyme Q10, a second with a suspension of coenzyme Q10-loaded nanocapsules and a third containing dried coenzyme Q10-loaded nanocapsules. The dried nanocapsules were obtained by spray-drying of the suspension. No significant differences in the diameters of the particles after their incorporation in the semi-solid formulations were observed in comparison with those of nanocapsules in the aqueous suspension. The rheological characterization showed that the formulations containing coenzyme Q10-loaded nanocapsules had a pseudoplastic flow, while the formulation containing free-coenzyme Q10 had a yield-pseudoplastic flow. The semi-solid formulations containing coenzyme Q10-loaded nanocapsules suspension or powder of nanocapsules of coenzyme Q10 redispersed in water are promising cosmetic formulations for topical application.

INTRODUCTION

Both organic and inorganic molecules and atoms containing one or more unpaired electrons can be classified as free radicals [1]. This configuration makes the free radicals molecules very reactive, highly unstable and presenting short half-life. Their presence is classified as critical to the maintenance of many physiological functions. The *in vivo* formation of the free radicals occurs naturally as a consequence of the process of transferring electrons during the cell metabolism [2].

Antioxidants are substances that, when present in low concentrations, in relation to the oxidizable substrate, effectively delay or inhibit the oxidation, repressing the formation of free radicals [3]. The imbalance of nutritional conditions, smoking, alcohol consumption, air pollution and other factors may reduce the levels of cell antioxidants [4]. The levels of antioxidant enzymes naturally present in the epidermis and stratum corneum are also decreased by acute and chronic exposure to ultraviolet radiation [5].

Oxidative stress is defined as an imbalance between the production of reactive oxygen species and antioxidant defenses [6,7]. The theory of radicals of oxygen, developed by Harman [8] suggested that aging could be secondary to oxidative stress and Sander *et al.* [5] described that oxidative stress can cause oxidation of proteins in the skin and it is directly related to aging. Thus, the topical administration of antioxidant substances that occur naturally in human skin can be quite advantageous in preventing oxidative damages [9].

The coenzyme Q10 (CoQ10), 2,3-dimethoxy-5-methyl-6-decaprenil-benzoquinone or ubiquinone, is known as cellular endogenous antioxidant. It comprises a long side chain containing ten units of isoprene. Due to its structure (Fig. 1) this coenzyme has very low water solubility. A set of functions for coenzyme Q10 was

KEY-WORDS: Coenzyme Q10, Nanocapsules, Rheological characterization, Semi-solid formulations.

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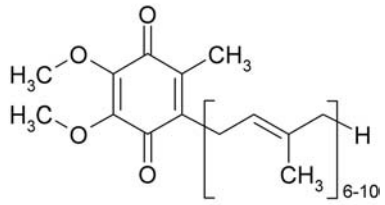

Figure 1. Structure of Coenzyme Q10.

established over the years, but their role as effective antioxidant cellular membranes are still under investigation. The protective effect of coenzyme Q10 has been observed on lipids, proteins and DNA. The coenzyme Q10 is involved in obtaining ATP, which is the major source of energy that drives a range of biological processes [10] and it acts as a stabilizing agent in cell membranes [11]. In the human body, coenzyme Q10 is found in relatively high concentrations in cells with high energy needs, such as heart, liver, muscles and pancreas. Investigations have also showed that coenzyme Q10 plays a central role in mitochondrial oxidative phosphorylation [12,13] as carrier of electrons from complex I and II to the complex III of respiratory chain function and as an antioxidant able to combat free radicals [14-16].

Modern cosmetics are designed to reduce the signs of skin aging, where oxidative stress plays an important role. In terms of skin administration, the coenzyme Q10 has shown the ability to reduce photoaging in vivo with a corresponding decrease in the depth of wrinkles. In addition, its physiological levels showed a decrease with increasing age [17].

During the last two decades, several new pharmaceutical carriers of substances based on nanotechnologies have been widely researched and developed. Nanocarriers are able to protect the encapsulated drug against degradation and facilitate its transport across critical and specific barriers [18]. For those reasons, nanoemulsions, nanosuspensions, liposomes, solid lipid nanoparticles and polymeric nanoparticles have been extensively studied [19-22]. Polymeric nanoparticles can be classified as nanocapsules (vesicular structures) or nanospheres (matricial structures), according to their composition and molecular organization. Nanocapsules can serve as reservoirs of lipophilic substances which are able to gradually release the drug in the stratum corneum, being an efficient way to control the drug penetration in the skin. They are also able to increase the adhesiveness of the active substance during the time of contact with skin [23].

Nanoparticulated systems can also be used as an alternative to improve the penetration through the skin barrier of substances that, in its free form, are poorly permeable [24]. Additionally, those systems can increase the availability of active substances or drugs applied topically [25]. The nanoencapsulation can increase the stability of labile substances during storage and in biological fluid [19,22] and protect substances from photodegradation [26]. Moreover, the nanoencapsulation increased the antioxidant activity of substances. This effect was demonstrated for idebenone [27] and melatonin [28].

The coenzyme Q10 is an attractive active ingredient for the development of cosmetic formulations [29]. Additionally, its reduced form, the ubiquinol, is a potent antioxidant, with the ability to reduce oxidative damage caused by lipid peroxidation of membranes [30]. However, it is essentially photo-unstable when exposed to light. The lack of chemical stability hampers their incorporation into a cosmetic formulation suitable for topical administration [17]. Another limitation stems from the lack of control of the skin permeation of coenzyme Q10. Hoppe and colleagues [17] observed a penetration of 20% in pig epidermis, while only 2% of coenzyme Q10 reached deeper layers of skin.

Some authors have studied and developed alternatives to resolve the instability of coenzyme Q10, including its complexation with cyclodextrins [31] and the preparation of a dry redispersible emulsion [32]. Besides, formulations self-nanoemulsified containing coenzyme Q10 were able to increase its dissolution [33] and solid lipid nanoparticles for topical release [20] showed biphasic release profile of coenzyme Q10. Nehilla *et al.* [34] developed surfactant-free nanoparticles and obtained stable release of Coenzyme Q10 for two months. Regarding the oral route of administration, it was noted that nanoemulsions and dried nanoemulsions containing coenzyme Q10 [35] increased its oral absorption.

Another possible strategy to stabilize the coenzyme Q10 not yet fully exploited is the use of polymeric nanocapsules. Comparative studies of stability were performed by Kwon *et al.* [36], which prepared polymeric nanoparticles using a method based on microfluidization and showed that the inactivation of coenzyme Q10 at high temperatures and UV radiation was effectively reduced when this active was encapsulated in polymeric nanoparticles.

Nevertheless, the full applications of polymeric nanoparticles have not been exploited

due to the lack of stability of formulations when conserved in aqueous medium for a long period. During the storage, microbiological growth, polymer hydrolysis and physicochemical instability as a consequence of particle aggregation can take place [37]. Considering that, our research group and others have been involved in the study of the spray drying technique to stabilize polymeric nanoparticle aqueous suspensions converting them into powders [21]. However, up to now, there is no report in the literature focused on the preparation of semi-solid cosmetic formulations containing redispersible nanocapsule powder. Taking into account the potentiality of cosmetic use of coenzyme Q10 and the useful of nanocapsules as stabilizers of labile substances, such is the case of this active, the objective of this work was to study the feasibility of semi-solid formulations containing nanocapsules of coenzyme Q10, previously dried by spray-drying. Our interest was also directed to the rheological characterization of semi-solid formulations. Specifically, the aim was to verify if the incorporation of coenzyme Q10-loaded nanocapsules could alter the rheological properties of vehicles. It is worth emphasizing that, from the technological point of view, the knowledge of rheological behavior is of fundamental importance in order to ensure an adequate flow of the systems, characteristic required for the therapeutic activity or the cosmetic features of the product [38].

MATERIALS AND METHODS

Materials

Poly(ε-caprolactone) (PCL) (MW = 65,000 $g.mol^{-1}$) and sorbitan monostearate were supplied by Aldrich (France). Caprylic/capric triglyceride was delivered from Brasquim (Brazil). Polysorbate 80, Carbomer (Carbopol 940®) and lactose were obtained from Henrifarma (Brazil). Coenzyme Q10 was purchased from Deg (Brazil) and diazolidinyl urea was obtained from Sarfam (Brazil). Oil nut was delivered from Inovam (Brazil); grape seed oil and sunflower oil from Embacaps, Brazil. All other chemicals and solvents used were of analytical or pharmaceutical grade. All reagents were used as received.

Selection of the oily core of nanocapsules

Prior to prepare the nanocapsules, the solubility of coenzyme Q10 at a concentration of 0.5 mg/mL in different oils (nut oil, grape seed oil, sunflower oil and mixture of triglycerides of capric and caprylic acid) was checked, after magnetic stirring during 1 h at 25 °C.

Preparation of coenzyme Q10–loaded nanocapsules

The nanocapsules were prepared by interfacial deposition of pre-formed polymer technique [39]. Three formulations, called A, B and C containing coenzyme Q10 in concentrations of 0.5, 1.0, and 2.0 mg/mL, respectively, were prepared. The coenzyme Q10 was dissolved in the organic phase composed of triglycerides of caprylic and capric acid (0.33 mL), sorbitan monostearate (76.6 mg), poly(ε-caprolactone) (100 mg) and acetone (26.7 mL). The organic phase was poured on an aqueous phase containing polysorbate 80 (76.6 mg), diazolidinyl urea (0.01 g) and Milli-Q® water (53.3 mL) through a funnel and maintained under magnetic stirring for 10 min. The formulations were prepared in the dark and evaporated in a rotative evaporator Büchi R-114 at 30 °C until a final volume of 10 mL. The macroscopic aspects of suspensions A, B and C were evaluated just after preparation in order to select the best one for further physicochemical characterization. In parallel, a placebo containing all components of the formulation, excepting coenzyme Q10, called NB, was also prepared.

Physical-chemical characterization of coenzyme Q10-loaded nanocapsules

The physical-chemical characterization of the suspensions was performed immediately after preparation. The formulations A, B and C were evaluated for macroscopic aspects. Formulation A (selected by the macroscopic aspects) was also evaluated in terms of particle size, polydispersion index, zeta potential, pH and drug content of coenzyme Q10. NB (placebo formulation) was characterized by the particle size, polydispersion index and zeta potential.

Macroscopic Analysis

The color and presence of precipitates or separation of phases under natural light of each formulation (2.5 mL) packaged in a glass tube were observed.

pH Analysis

Measures of pH were performed directly in the samples using a potentiometer Denver Instrument VB-10, previously calibrated with buffer solutions for calibration pH 4 and 7 (Merck, Germany).

Validation of the spectrophotometric method for the coenzyme Q10 quantification

The determination of coenzyme Q10 in the samples was performed by UV spectrophotometer at 280 nm. The validation followed the parameters outlined in the Brazilian official guide for validation [40]. A stock solution containing 1000 µg/mL of coenzyme Q10 was prepared, from which aliquots were withdrawn and diluted in the range 10 to 50 µg/mL for the standard curve. The repeatability was evaluated from the determination of 6 dilutions of the suspension of coenzyme Q10-loaded nanocapsules in a concentration of 0.5 mg/mL. To analyze the accuracy was adopted the method of addition of the standard with final concentrations of 25, 35, and 45 µg/mL. The specificity of the method was evaluated by analysis of nanocapsules samples without coenzyme Q10 prepared and diluted in the same way that the formulation tested.

Determination of the coenzyme Q10 content in the suspension of nanocapsules

To determinate the content of coenzyme Q10 in the nanocapsules, a sample was diluted in order to get the final theoretical concentration of 15 µg/mL. Thus, aliquots of 300 µL were removed from the formulations A and diluted to 10 mL with a solution of acetonitrile and ethyl acetate (1:1). The absorbance of samples were measured at 280 nm.

Determination of particle size, polydispersity index and zeta potential

The average diameter and the polydispersity indexes of the particles in suspension were determined by dynamic light scattering using a Zetasizer Nano series equipment Nano-Zs (Malvern Instruments). A and NB samples were diluted at a ratio of 1:500 (v/v) in Milli-Q® water and the distribution of size (measured in intensity). The measurements were carried out three times for each sample. The measures of average diameters were also performed after 20 days of storage at 25 °C, protected from light. Using the same equipment, the zeta potential was measured by electrophoretic mobility. The samples were diluted at a ratio of 1:500 (v/v) of 10 mM NaCl and the measurements were performed in triplicate.

Spray dryed nanocapsules

The nanocapsule aqueous suspensions were dried in a mini-spray-dryer MSD 1.0 (Lab machine, Brazil), using lactose as adjuvant (3.0% w/v). The efficiency (yield) of drying was calculated as the sum of the masses of the suspension components (except the water) added the weight of lactose. The drying parameters were: pressure of 2 bar, flow 3 mL/min and temperature of input air of 146 °C.

Microscopic analysis of powders

The samples were metalized with gold (Jeol Jee 4BSVG-IN) and subjected to analysis by scanning electron microscopy (Jeol Scanning Microscope JSM-5800 – Centro de Microscopia Eletrônica, UFRGS), using a voltage of 20 kV and increases between 500 and 25,000 times.

Preparation of hydrogel containing coenzyme Q10-loaded nanocapsules

Suspensions A and NB were thickened with Carbopol® 940 (0.2% w/V) and neutralized with triethanolamine (pH 7); the semi-solid formulations were called FNCo and FNB, respectively. A placebo formulation containing free-coenzyme Q10 (not encapsulated), called FCO was also prepared. In parallel, the nanocapsule-spray-dried powders was recovered by dispersion in aqueous solution, followed by thickening with Carbopol® 940 (0.2% w/V) and neutralization with triethanolamine (pH 7). This formulation was named FPCo.

Physical-chemical characterization of semi-solid formulations

The average diameters of FNCo and FNB were evaluated and the macroscopic analysis was performed according to the techniques cited above for the characterization of suspensions. The size and polydispersity indexes of the particles in the formulations FNCo and FNB were determined in samples diluted at a ratio of 1:500 (v/v) in Milli-Q® water by the same technique described above, in order to compare these results to those obtained with the nanocapsule suspensions (A and NB). The rheological characterization (FNCo, FPCo and FCO hydrogels) was performed using a Brookfield viscometer DV-II + Pro Viscometer, mode LVF, spindle SC4-25, with a range of shear rate ranging from 40 to 56 rpm. Samples (20 g) were kept at 25 ± 1 by using a circulating water bath. Data were analyzed using the equations of flow of Bingham, Casson, Ostwald and Herschel-Bulkley, which describe the rheological models perfect plastic, plastic, pseudoplastic and pseudoplastic with transfer of value, respectively.

RESULTS AND DISCUSSION

Preparation and physical-chemical characterization of the nanocapsule suspensions

Selection of the oily core

Coenzyme Q10 was practically insoluble in the nut oil, grape seed oil and sunflower oil at a concentration of 0.5 mg/ml. On the other hand, coenzyme Q10 was soluble in the mixture of triglycerides of caprylic and capric acid (0.5 mg/mL). So, this oil was selected for the preparation of nanocapsule. Indeed, the solubility of the drug in the oily core is the key parameter to obtain formulations of nanocapsules prepared by the method of nanoprecipitation [21].

Macroscopic Analysis

After preparation, the suspensions of nanocapsules (A and NB) resulted in formulations which presented uniform macroscopic appearances, slightly yellow and white colors (respectively) and bluish opalescent aspects, due to Brownian motion of nanoparticles in suspension (Tyndall effect). The suspensions B and C visually showed the presence of precipitates after the process of evaporation of solvents and, thus, they were not characterized and used in further experiments.

Analysis of pH

Formulation A presented slightly acid pH (Table 1, triplicate of batches) due to the characteristics of its components. The presence of the polymer poly(ε-caprolactone) in the formulations contributes to obtain acid suspensions [41,42]. Besides, this pH value (close to 5.0) is appropriate for products intended for cutaneous administration.

Validation of analytical method and determination of coenzyme Q10 content in the nanocapsules

The method was validated in the range 10 to 50 µg/mL, showing linearity (r^2 = 0.9927 ± 0.001069), precision (repeatability, RSD = 1.23%) and accuracy (90.15%). The specificity of the method was determined by subtracting the absorbance of samples of placebo nanocapsules (without coenzyme Q10). The nanocapsule formulation (A) (Table 1, triplicate of batches) showed a recovery of coenzyme Q10 in the range of 101.83 to 105.23%.

Determination of particle size, polydispersity and zeta potential

Coenzyme Q10-loaded nanocapsules (formulation A) presented sub-micrometric particles with diameters between 213 and 248 nm (Table 1). The average diameters obtained are in agreement with data reported in the literature for nanocapsules prepared by the method of interfacial deposition of pre-formed polymer [28,42]. Moreover, the nanocapsules are within the range of diameters suitable for dermal application [23]. For all formulations was observed an index of polydispersity (Table 1) close to 0.15, which corresponds to a variation in size of approximately 32 nm around the average diameter of 231 nm, indicating homogeneity of the systems. The polymeric nanocapsules of coenzyme Q10 showed similar size distribution of the solid lipid nanoparticles containing coenzyme Q10 developed by Teeranachaideekul *et al.* [20]. There was no significant increase in the average diameter of particles in suspensions within the 20 days of storage at room temperature (Fig. 2). The zeta potential values are related to the potential surface of the particles and were negative, close to -10 mV (Table 1). These values are similar to those reported in the literature for nanocapsules of poly(ε-caprolactone) coated with polysorbate 80 [19].

Spray drying the suspension of nanocapsules

Coenzyme Q10-loaded nanocapsules were sprayed in a drying chamber using lactose as drying adjuvant. Due to the large specific surface of the droplets and the flow of hot air, the dehydration occurs in a few seconds. Lactose was selected as an adjuvant for drying based on the studies of Tewa-Tagne and collegues [43] that showed better results for this excipient in com-

Formulation	Diameter (nm)	Polydispersity index (PDI)	ζ Potential (mV)	Drug content (%)	pH
A1	246 ± 1	0,15 ± 0,04	-9,5 ± 0,80	105,23 ± 1,32	5,18 ± 0,02
A2	229 ± 6	0,16 ± 0,02	-10,31 ± 0,52	101,91 ± 4,15	5,32 ± 0,01
A3	218 ± 7	0,15 ± 0,02	-11,99 ± 0,46	101,83 ± 2,23	5,34 ± 0,04

Table 1. Characterization of suspensions of nanocapsules containing coenzyme Q10. Values represent the mean ± standard deviation of three measures of the same batch. A1, A2 and A3 correspond to three different batches of the suspension of nanocapsules.

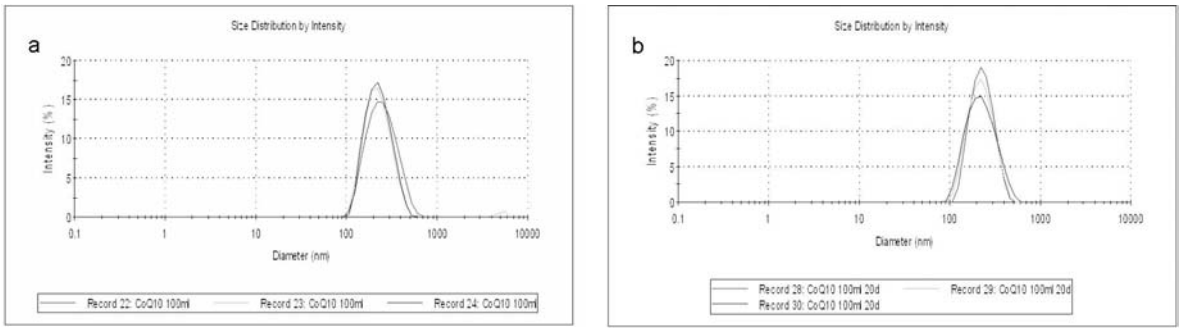

Figure 2. Average diameter of particles in coenzyme Q10-loaded nanocapsule suspension just after preparation (**a**) and after 20 days of storage (**b**).

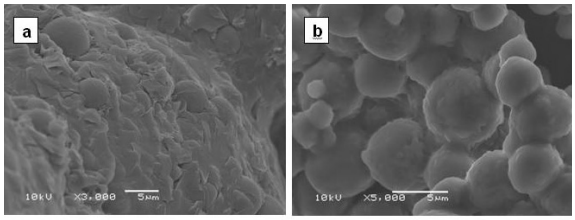

Figure 3. Scanning electron microscopy of lactose powder (3,000 x of magnification) (**a**) and clusters of lactose containing coenzyme Q10-loaded nanocapsules (6,000 x of magnification) (**b**).

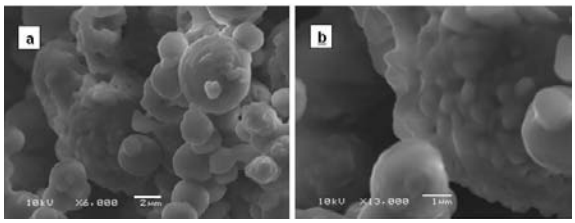

Figure 4. Scanning electron microscopy of lactose containing coenzyme Q10-loaded nanocapsules 6,000 x of magnification (**a**) and 13,000 x of magnification (**b**).

parison with maltodextrin and mannitol, resulting in better morphology and more appropriate rebuilding of the suspension (empty nanocapsules). Three batches of coenzyme Q10 loaded-nanocapsules (formulation A) were dried with yields of 64, 52 and 52%. Converting aqueous suspensions in powders is suitable, because despite the biopharmaceutical benefits of aqueous suspensions, they have disadvantages as a consequence of physical and chemical instabilities during the storage, which limits their industrial applicability [44-46].

Microscopic analysis of powders

SEM analyses showed differences between the lactose spray-dried powder and the clusters of lactose and coenzyme Q10-loaded nanocapsules (Fig. 3). On the surface of dry coenzyme Q10-nanocapsules were observed nanostructures presenting similar diameters than those measured by PCS in the nanocapsule aqueous suspension (formulation A) before drying (Fig. 4). The powder of coenzyme Q10 nanocapsules was easily redispersible in water.

Preparation and physical-chemical characterization of semi-solid formulations containing nanocapsules of coenzyme Q10

All the semi-solid formulations, FNCo, FNB, FPCo and FCO showed bright and homogeneous appearance and semi-solid consistency. The colors were yellowish for FCO, slightly yellowish for FNCo and FPCo and white for FNB. No coalescence or aggregation of the particles were observed in semi-solid formulations after thickening with Carbopol® 940 the dried polymeric nanocapsules redispersed in water (FPCO), the nanocapsule suspensions (empty or containing coenzyme Q10) (FNB and FCO). The average diameter (measured by dynamic light scattering) of semi-solid samples (FNCo and FNB) after aqueous dilution (1:500) were similar than those measured in the original suspension (A) and the NB (Fig. 5) before drying. This finding means that there was maintenance of the size of nanocapsules (empty or containing coenzymeQ10-nanocapsules) before and after their incorporation into hydrogels.

The rheological properties of hydrogels were analyzed considering the average coefficients of determination (r^2) obtained from the curves of shear stress versus shear rate for different types of flow. For the formulation FCO, the coefficients of determination (Table 2) were close to 1 when the results were adjusted to the flow model of Herschel-Bulkley, which corresponds to yield- pseudoplastic behavior [47]. In contrast, the formulations FNCo and FPCo best fits to the

Formulation	Bingham	Casson	Ostwald	Herschel-Bulkley
FCO	0,977 + 0,017	0,976 + 0,021	0,990 + 0,001	0,999 + 0,001
FNCo	0,978 + 0,004	0,981 + 0,003	0,987 + 0,003	0,977 + 0,004
FPCo	0,982+ 0,011	0,987 + 0,014	0,995 + 0,008	0,983 + 0,013

Table 2. Average regression coefficients (r^2) for the models of the flow curves of shear stress versus shear rate (formulations FCO, FNCo and FPCo).

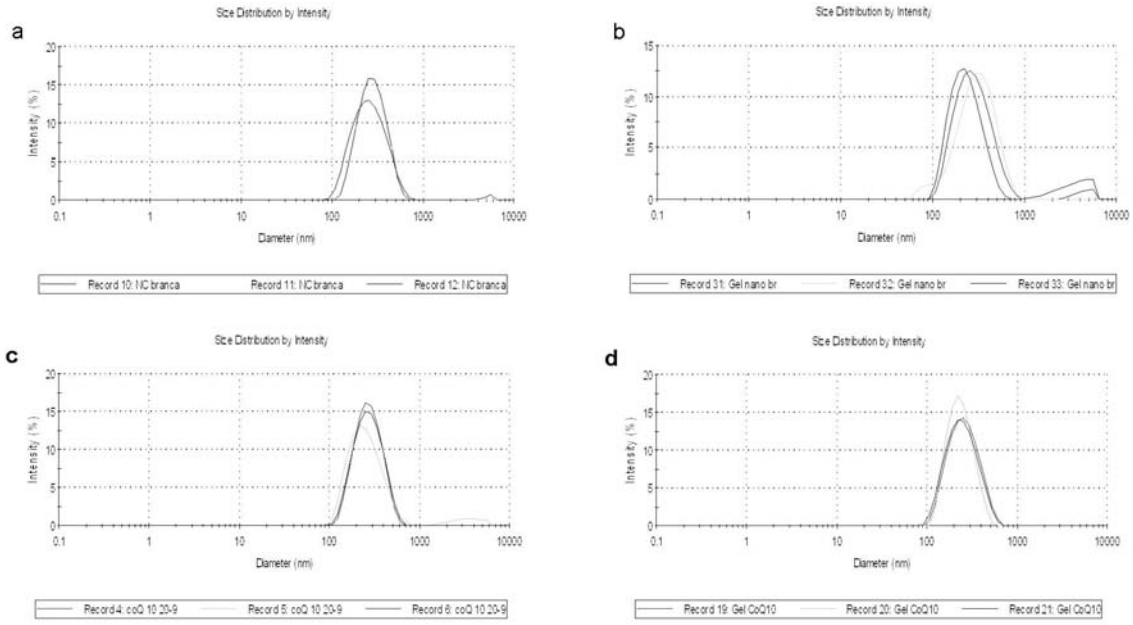

Figure 5. Average diameter of particles in placebo-nanocapsules (**a**) nanocapsules containing coenzyme Q10 (**b**), the gel containing placebo-nanocapsules (**c**) and the gel containing nanocapsules of coenzyme Q10 (**d**).

model of Ostwald, which corresponds to pseudoplastic flow. Indeed, the rheological behavior of gels prepared with acrylic acid polymers has been studied because they are widely used in formulations for dermal application [48], showing in general, flow curves described according to the flow models of Ostwald and Herschel-Bulkley [49]. In the present work, we clearly demonstrated that the incorporation of coenzyme Q10-nanocapsules in the hydrogels altered the rheological pattern of flow of formulations from the yield- pseudoplastic to pseudoplastic model.

CONCLUSION

The nanoencapsulation of coenzyme Q10 was shown to be feasible through the use of poly(ε-caprolactone) as polymer constituent of the wall of the vesicles and medium-chain triglycerides as a core of nanocapsules, which were stabilized by surfactants and prepared by the technique of interfacial deposition of polymer.

The conversion of the suspension of nanocapsules containing coenzyme Q10 into a powder was effectively performed by spray drying technique using lactose as a hydrophilic adjuvant. In this sense, this powder, potentially stable for long periods of time, can be considered an interesting material for the production of innovative cosmetics. Additionally, both the coenzyme Q10-loaded nanocapsules (in aqueous suspension or a water-dispersed powder) were successfully formulated in semi-solid formulations prepared by using carbomer. The presence of nanocapsules in the hydrophilic gel caused an alteration of the rheological behavior, from yeld-pseudoplastic to pseudoplastic.

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REFERENCES

1. Halliwell, B. (1994) *Lancet* **344**: 721-4.
2. Pompella, A. (1997) *Int. J. Vitam. Nutr. Res.* **67**: 289-97.
3. Sies, H. & W. Stahl (1995) *Am. J. Clin. Nutr.* **62**: 1315S-21S.
4. Machlin, L.J. (1992) *Ann. N. . Acad. Sci.* **669**: 1-6.
5. Sander, C.S., H. Chang, S. Salzmann, C.S. Muller, S. Ekanayake- Mudiyanselage, P. Elsner & J.J. Thiele (2002) *J. Invest. Dermatol.* **118**: 618-25.
6. Halliwell, B. (1999) *Free. Radic. Res.* **31**: 261-72.
7. Gutteridge, J. M. & J. Mitchell (1999) *Br. Med. Bull.* **55**: 49-75.
8. Harman, D. (1956) *J. Gerontol.* **11**: 298-300.
9. Trommer, H. & R.H. Neubert (2005) *J. Pharm. Sci.* **8**: 494-506.
10. Crane, F.L. & J.L. Glenn, (1957) *Biochim. Biophys. Acta* **24**: 100-7.
11. Grossi, G., A.M. Bargossi, P.L. Fiorella, S. Piazzi, M. Battino & G.P. Bianchi (1992) *J. Chromatogr.* **593**: 217-26.
12. Crane, F.L. (1977) *Annu. Rev. Biochem.* **46**: 439-69.
13. Ernster, L. & G. Dallner (1995) *Biochim. Biophys. Acta* **1271**: 195-204.
14. Crane, F.L. & P. Navas (1997) *Mol. Aspects Med.* **18**: S1-6.
15. James, A.M., R.A. Smith & M.P. Murphy (2004) *Arch. Biochem. Biophys.* **423**: 47-56.
16. Turunen, M., J. Olsson & G. Dallner (2004) *Biochim. Biophys. Acta* **1660**: 171-99.
17. Hoppe, U., J. Bergemann, W. Diembeck, J. Ennen, S. Gohla, I. Harris, J. Jacob, J. Kielholz, W. Mei, D. Pollet, D. Schachtschabel, G. Sauermann, V. Schreiner, F. Stab & F. Steckel (1999) *Biofactors* **9**: 371-378.
18. Alonso, M.J.D. (2004) *Biomed. Pharmacother.* **58**: 168-72.
19. Schaffazick, S. R., A.R. Pohlmann, T. Dalla-Costa & S. S Guterres. (2003) *Eur. J. Pharm. Biopharm.* **56**: 501-5.
20. Teeranachaideekul, V., E. B. Souto, V. B. Junyaprasert, & R. H. Muller (2007) *Eur. J. Pharm. Biopharm.* **67**: 141-8.
21. Schaffazick, S.R., S.Guterres, L.L Freitas & A.R. Pohlmann (2003) *Quím. Nova* **26**: 726-37.
22. Schaffazick, S.R., A.R. Pohlmann, C. A. de Cordova, T.B. Creczynski-Pasa, & S.S. Guterres (2005) *Int. J. Pharm.* **289**: 209-13.
23. Guterres, S.S., M.P. Alves & A.R. Pohlmann (2007) *Drug Target Insights* **2**: 147-57.
24. Jenning, V., A. Gysler, M. Schafer-Korting & S.H. Gohla (2000) *Eur. J. Pharm. Biopharm.* **49**: 211-8.
25. Jenning, V., M. Schafer-Korting & S. Gohla (2000) *J. Control. Release* **66**: 115-26.
26. Müller, C.R., S.E. Haas, V.L Bassani, S.S Guterres, H. Fessi, M.R. Peralba & A.R. Pohlmann (2004) *Quim. Nova* **27**: 555.
27. Palumbo, M., A. Russo, V. Cardile, M. Renis, D. Paolino, G. Puglisi & M. Fresta (2002) *Pharm. Res.* **19**: 71-8.
28. Schaffazick, S.R., I.R. Siqueira, A.S. Badejo, D.S. Jornada, A.R. Pohlmann, C.A. Netto & S.S. Guterres (2008) *Eur. J. Pharm. Biopharm.* **69**: 64-71.
29. Rona, C., F. Vailati & E. Berardesca (2004) *J. Cosmet. Dermatol.* **3**: 26-34.
30. Frei, B., M. C. Kim & B. N. Ames (1990) *Proc. Natl. Acad. Sc.i U.S.A.* **87**: 4879-83.
31. Lutka & J. Pawlaczyk (1995) *Acta Pol. Pharm.* **52**: 379–86
32. Takeuchi, H., H. Sasaki, T. Niwa, T. Hino, Y. Kawashima, K. Uesugi & H. Ozawa (1992) *Int. J. Pharm.* **86**: 25-33.
33. Nazzal, S., II. Smalyukh, O.D. Lavrentovich & M.A. Khan (2002) *Int. J. Pharm.* **235**: 247-65.
34. Nehilla, B.J., M. Bergkvist, K.C. Popat & T.A. Desai (2008) *Int. J. Pharm.* **348**: 107-14.
35. Hatanaka, J., Y. Kimura, Z. Lai-Fu, S. Onoue & S. Yamada (2008) *Int. J. Pharm.* **363**: 112-7.
36. Kwon, S.S., Y.S. Nam, J.S. Lee, B.S. Ku, S.H. Han, J.Y. Lee & I.S. Chang (2002) *Eng. Asp.* **210**: 95-104.
37. Coffin M.D. & J.W. Mcginity (1992) *Pharm. Res.* **9**: 200.
38. Woolfson, A.D., R.K. Malcolm, K. Campbell, D.S. Jones, J.A. Russell (2000) *J. Control. Release* **67**: 395-408.
39. Fessi, H., F. Puisieux, J. Ph. Devissaguet, N. Ammoury & S. Benita (1989) *Int. J. Pharm.* **55**: R1-R4.
40. ANVISA, Agência Nacional de Vigilância Sanitária (2003) Resolução RE N° 899, de 29 de Maio de 2003. Diário Oficial da República Federativa do Brasil, Brasília DOU de 02/06/2003.
41. Cruz, L., S.R. Schaffazick, T. Dalla Costa, L.U. Soares, G. Mezzalira, N.P. da Silveira, E.E. Schapoval, A.R. Pohlmann, & S.S. Guterres (2006) *J. Nanosci. Nanotechnol.* **6**: 3154-62.
42. Michalowski, C.B., S.S. Guterres & T. Dalla Costa (2004) *J. Pharm. Biomed. Anal.* **35**: 1093-100.
43. Tewa-Tagne, P., S. Briancon & H. Fessi (2006) *Int. J. Pharm.* **325**: 63-74.
44. Franks, F. (1998) *Eur. J. Pharm. Biopharm.* **45**: 221-9.
45. Schmidt C. & R. Bodmeier (1999) *J. Control. Release* **57**: 115-25.
46. Müller C.R., V.L. Bassani, A.R. Pohlmann, C.B. Michalowski, P.R. Petrovick & S.S. Guterres (2000) *Drug Dev. Ind. Pharm.* **26**: 343-7.
47. Alves, P.M., A.R. Pohlmann & S.S. Guterres (2005) *Pharmazie* **60**: 900-4.
48. Lucero M.J., M.J. León, J. Vigo & A.M. Rabasco (1991) *Ind. Farm.* **6**: 62-8.
49. Gungor, S. & N. Bergisadi (2003) *Pharmazie* **58**: 155-6.