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THE ANTIOXIDANT EFFECTS OF GRAPE SEED OIL ON RETINYL PALMITATE STABILITY

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Abstract:

Retinyl palmitate (RP) is recognized as a potential substance for the prevention and treatment of photoaging and skin inflammation. However it is also associated with skin irritations and extreme photo instability. In order to improve its skin delivery, photostability and to avoid undesirable topical side effects the RP was nanoencapsulated using the interfacial deposition of preformed polymer using the grape seed oil (GSO) a potential antioxidant as oil phase. Dynamic light scattering showed droplet size close to 214 nm, polydispersity index close to 0.18 and zeta potential with negative charge. Scanning electron microscopy exhibits spherical shape nanocapsules with a diameter of 205.6 ± 62.2 nm in good agreement with hydrodynamic diameter of nanocapsules in the dispersions. Presence of GSO as oily phase in nanocapsules increases the RP stability as longer than 180 days. It was observed a sustained and controlled RP dermal release by the analysis of RP in the donor and receptor phase by using vertical Franz diffusion cells.

Key word: *Retinoid, Polymeric nanocapsules, Stability, Skin permeation, Antioxidant.*

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INTRODUCTION:

In dermatology and cosmetic sciences, the concept of skin aging differentiates in chronological and photo ageing [1]. Photoaged skin superficially results in dark pigmentation patches, coarse wrinkles and rough epidermis texture, these changes are induced by alterations in deeper dermal connective tissues [2, 3]. Photoaging damages are mainly caused by UV-induced light causing significant down regulation of procollagen synthesis and induction of matrix metalloproteinases expression in human skin, leading to wrinkle formation [4]. Retinoids are frequently used in the topical treatment of acne and added in anti-aging products. As reported in the medical literature the major side effects for retinoid therapy is dryness, peeling and skin irritation [5-7].

Skin irritation side effects have been mitigated by nanoparticle encapsulation, where the mechanism of action is likely to be controlled release [8-13]. Retinol [14, 15], and vitamin A palmitate [16], have also been delivered to skin with solid lipid nanoparticles (SLN). Retinyl palmitate take part in the regulation of epidermal cell growth, inhibit the final step of keratinization, participate in the collagen synthesis process, prevent atrophy of connective tissue, enhance glycosaminoglycane synthesis, and are essential in the reproduction of basal membrane cells [17]. Recently the Scientific Committee of Consumer Safety considers that the use of Vitamin A in body lotions at the maximum concentration of 0.05% per se is safe [18]. In order to increase retinol stability antioxidants has been added to formulations [19]. Improved stability and controlled release have been the primary focal points of nanoparticles for retinoid delivery [8, 9, 11-12, 15, 27].

Grape seed oil (GSO) is a natural product rich in unsaturated fatty acids. GSO is also rich in antioxidants such as tocopherols and phytosterols that may exert a superior anti- oxidant activity [20-22] and should be an eligible product to increase RP stability.

Research reported here includes the ability to fabricate retinyl palmitate loaded polymeric nanocapsules carriers containing grape seed oil that improves the RP stability over time. Research also includes the development of a reliable and efficient electroanalytical method for the estimation of RP concentration in the dispersion. The analysis of the suitability of the nanocapsules as drug delivery systems was performed by using plastic surgery human skin in Franz diffusion cells, and the diffusion profile of free and nanoencapsulated RP dispersed in a hydrogel was compared.

EXPERIMENTAL:

Retinyl palmitate (RP, $C_{36}H_{60}O_2$, molar mass 524.86 g mol^{-1}), Poly(ε-caprolactone) (PCL) 70000 g /mol, sorbitan monostearate (Span 60®), polysorbate 80 (Tween 80®), were purchased from Sigma-Aldrich (St. Louis, MO, USA). Grape seed oil (GSO, Ferquima, Brasil), Pentaerythrityl Tetra-di-t-butyl Hydroxyhydrocinnamate (Tinogard TT, BASF). Acetonitrile (ACN), acetic acid were acquired from Tedia (São Paulo, Brazil) and acetone obtained from Synth (Rio de Janeiro, Brazil). Carbopol Ultrez 10 NF and triethanolamine were obtained from DEG (São Paulo, Brazil) and imidazolidinyl urea (Germall 115) was acquired from Alpha Química (São Paulo, Brazil). All solvents used were of analytical grade. Ultrapure water was obtained from a Millipore Gradient 10.

All nanocapsules (NC) suspensions were prepared by interfacial deposition and the nanoemulsion by spontaneous emulsification as previously described [24]. Briefly, an organic solution composed of poly (ε-caprolactone) (0.25 g), the sorbitan monostearate (0.172 g), retinyl palmitate (0.0012 g), grape seed oil (0.75 g) and polysorbate 80 (0.018 g) were dissolved in acetone (67 mL) at 40 °C. In a previous experimental design the mixture of sorbitan monostearate and polysorbate 80 used in the organic phase gives a hydrophile-lipophile balance (HLB) number of 5.37, producing the smaller and more stable NC. In a separate flask, polysorbate 80 (0.19 g) was added into 134 mL of water at room temperature. In order to compare the role of synthetic antioxidant organic phases was prepared as above described including 0.5 % m/m of Tinogard TT. Three sets of formulations were prepared nominated as A (with GSO and Tinogard TT), B (with GSO) and C (without GSO and Tinogard TT). The organic solution was poured into the aqueous phase under magnetic stirring at room temperature. After 10 min, the acetone was eliminated and the aqueous phase concentrated under reduced pressure at 50 °C using a rotatory evaporator. Final volume was adjusted to 25 mL, all the formulations were made in triplicate.

Measurements of particle sizes and zeta potentials were made at 25 °C using a NanoSizer ZS (Malvern, UK). The particle sizes, size distributions and polydispersity were determined in diluted samples using reverse osmosis purified water, only the dilution medium was filtered in order to avoid any sample selection. In parallel, the zeta potential measurements were performed after diluting the samples with 10 mM NaCl aqueous solution. For size, polydispersivity index and zeta potential

measurements the NC dispersion were diluted by 1:500.

The photomicrographs were taken using a scanning electron microscope (SEM) FEI Inspect F50 model. The dispersions was diluted in isopropanol in the ratio 1: 20.000 were dropped onto a silicon wafer and allowed to evaporate in contact with air for 6 h.

Quantification of the total amount of RP present in NC suspension was determined by differential pulse voltammetry. The electrochemical experiments were carried out on an Autolab Metrohm Eco Chemie PGSTAT30 Potentiostat-Galvanostat Electrochemical System managed by GPES software (Utrecht, Netherlands) for data collection and analysis. Electrochemical experiments were performed in a one-compartment glass cell (30 mL) with three electrodes. An Ag/AgCl (3.0 mol L^{-1} KCl) electrode was used as reference electrode while a 2.0 cm^2 platinum foil and a glassy carbon electrode (GCE) with a diameter of 3.0 mm were used as a counter and working electrodes, respectively. Prior the measurements the GCE electrode surface was polished in a felt pad with an alumina suspension (0.25 mm particle size). Methodology for quantification was modified from literature [25, 26] by using 25 mL of a mixture of 95 % methanol, 5% of aqueous 0.1 mol L^{-1} Briton and Robinson buffer solution (pH 6), 0.1 mol L^{-1} of tetrabutylammonium perchlorate as supporting electrolyte and 2 mL of polysorbate 80. Electrolyte was deaerated during 2 min before each electrochemical measurement with a nitrogen flux that was maintained over the solution during measurements. Oxidation peak at 0.68 V was selected to analytical determination of RP with a good linear relationship between peak current and RP concentration as shown in Figure 1 for the calibration curve in the range of 5 to 50 µmol L^{-1} obtained by the standard addition method. Optimized conditions for differential pulse voltammetry (DPV) were a scan rate of 30 mVs^{-1}, pulse amplitude height of 75 mV and pulse width of 0.1 s. Determination of RP loaded into the nanocapsules was done by first diluting the dispersion in 5 mL of ACN and afterwards in the supporting electrolyte solution.

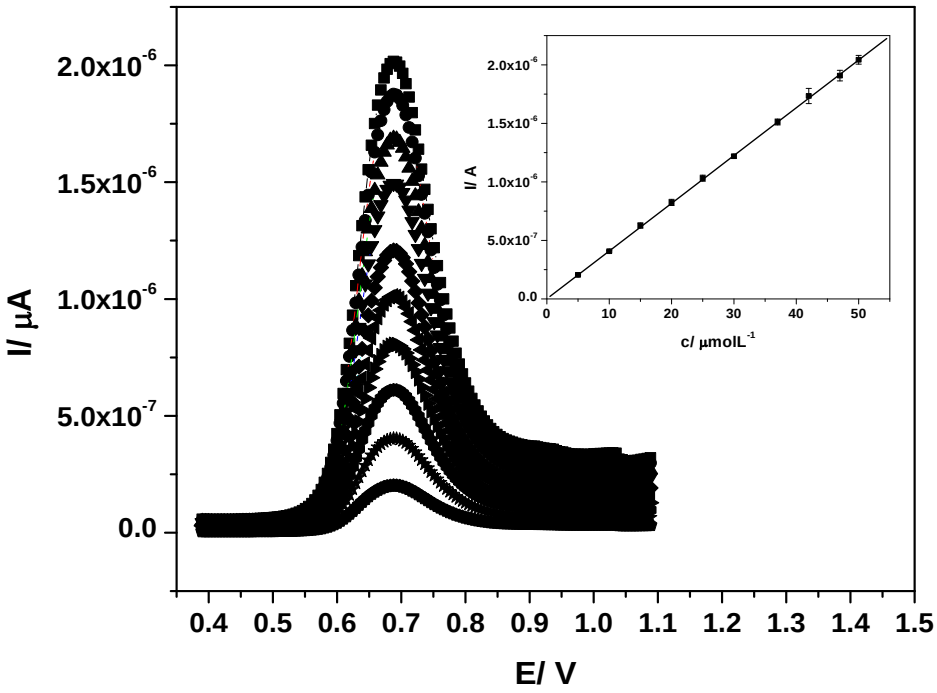

Figure 1. Differential pulse voltammograms of RP in supporting electrolyte solution with different RP concentrations registred with scan rate of 30 mV s^{-1}, width pulse 0.1 s and pulse amplitude height of 75 mV. Insert anodic peak current (I_p) *versus* RP concentration plot (calibration curve, y= 1.12×10^{-9} + 4.08×10^{-8} x, R^2=0.9998).

Concentration of RP associated with the NC were evaluated considering the total concentration of RP in the formulation and the concentration of free RP present in the aqueous phase of the dispersion. Amount of free RP in dispersion was determined by centrifugation/filtration method in which 300 µL of the NC dispersion was centrifuged at 4000 g during 10 min using a 100 kDa (Millipore Microcon) membrane. Nanocapsules are retained on the membrane filter while the aqueous phase containing the free RP cross the membrane into the receptor phase. The RP content on filtrate was measured by differential pulse voltammetry leading to the encapsulation efficiency. This centrifugation/filtration methodology was used for the stability analysis of RP. RP retained on the membrane was maintained under agitation during 3h, enough to solubilize the polymer, in 5 mL of acetonitrile with 0.2 mL of Tween 80 at 40 °C. This solution was added to 20 mL of supporting electrolyte solution for the analysis. The stability test of NC containing RP was performed during 180 days in order to verify the effect of grape seed oil in RP stability.

For diffusion test Carbopol Ultrez 10 NF (polymer of acrylic acid) at 0.5% (w/w) was dispersed in the free RP and RP nanocapsules always resulting in a concentration of 0.05% (w/w) of the drug. Polymeric dispersion was neutralized with triethanolamine (0.2%, w/w) to a suitable pH 6.0 to obtain a proper consistency of the hydrogel and to be applied onto the skin. Imidazolidinyl urea was added as a preservative (0.6%, w/w).

Human skin discarded from facial plastic surgery of female healthy patients was used as permeation membranes. Skin was cleaned and stored at -30 °C for few days until further use. For the experiment, on the vertical Franz diffusion cell the adipose tissue in the skin had been removed and a 400 µm thick piece of 1.79 cm^2 in area was positioned at donor chamber. Stratum corneum side of the skin was exposed to the donor chamber while the dermis was bathed with 13.0 mL of degassed phosphate buffer pH 7.4 solution containing 1 % polysorbate 80 in receptor chamber, constantly stirred during the experiment and maintained at 37 °C. Experiment was initiated by dropping 1.0 mL of the RP-loaded NC suspension (RP concentration = 1200 µmol L^{-1}) into the upper compartment, directly onto the mounted skin (RP final coverage = 1.2 mg cm^{-2}). At 1, 3, 6, 9, 21 and 24 h later, 1.0 mL of the receptor fluid was withdrawn and replaced with fresh receptor medium. Penetration of each sample was compared to the penetration of the free RP solution derived from using skin from the same people with the same thickness to standardize the results, in order to eliminate variances due to differences in the permeability of skin from different people. Secondly, in order to determine the amount of unabsorbed RP on the skin at different time intervals post application, 5.0 mL of the fresh receptor medium was dropped into the upper compartment of the diffusion cell, directly onto the mounted skin. The Franz cell was gently agitated and then the liquid was quickly withdrawn and subjected to RP quantification. Concentration of RP in each aliquot of the withdrawn receptor and donor fluid was determined by pulse differential polarography, with the aid of a calibration curve. Experiment was performed with triplicate replications under light-proof conditions.

RESULTS AND DISCUSSION:

All formulations as prepared exhibits Tyndall effect and were milky bluish in aspect. Table 1 shows the results of physicochemical characterization in terms of apparent hydrodynamic diameter, zeta potential, polydispersity index (PDI) and drug loading for the freshly prepared A, B and C formulations.

Table 1. Main physical and chemical properties of as prepared nanoencapsulated retinyl palmitate formulations.

Formulation	Diameter (nm)	Zeta potential (mV)	PDI	Drug loading (%)
A - with GSO and Tinogard TT	215 ± 10	-15.0 ± 1.4	0.15± 0.08	99.4 ± 2.7
B - with GSO	214 ± 8	-12.3 ± 0.9	0.18± 0.06	99.2 ± 2.3
C - without GSO and Tinogard TT	176 ± 6	-11.7± 1.8	0.12± 0.04	86.1 ± 2.1

All formulations presented size in the nanometric range, PDI values < 0.2 exhibiting a monomodal distribution and negative zeta potential values indicating a stable dispersion. RP characteristically have low water solubility, however with the association values obtained after the preparation the stability were higher than 85%. Presence of GSO increases the solubility of RP in oily core, increasing the association efficiencies. Nanocapsules were prepared using GSO in oil phase, a non-enzymatic antioxidant, as oil component due to its important antioxidant activity [23]. SEM based image reveled spherical nanoparticles as shown in Figure 2 where the dry diameter was evaluated as 205.6 ± 62.2 nm in good agreement with the hydrodynamic diameter for three formulations. Results clearly indicated that the higher loading capacity afforded by the presence of GSO on oily phase during the encapsulation increases the nanocapsules size. Negative zeta potential is related to polymer shell and due to the hydrolysis of the RP as proposed previously [27].

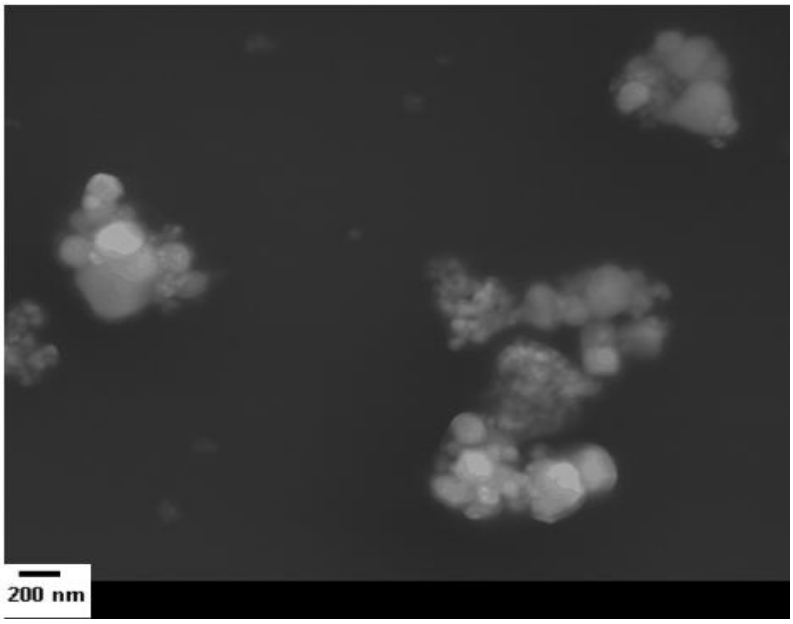

Figure 2. SEM micrography of polymeric nanocapsules containing RP obtained from formulation B.

As depicted in Figure 3, in the stability profile for all formulations, 0.0012 g of free RP mixed in water with surfactants (0.17 g sorbitan monostearate and 0.018 g polysorbate 80) exhibited an extreme instability. Additionally, a decrease of 50% of initial concentration during the first 24 h is observed. Moreover, an increase on RP stability is observed for encapsulated formulations (A, B and C).

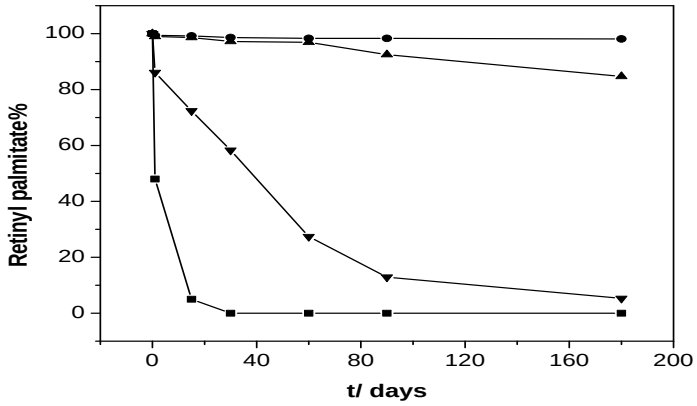

Figure 3. Stability profiles of free RP (■) and RP encapsulated in formulations A (●), B (▲) and C (▼).

Table 2 -Stability of encapsulated RP (Formulation A)

Time (day)	Diameter (nm)	PDI	Zeta potential (mV)	Drug loading (%)
1	215 ± 10	0.15 ± 0.08	-15 ± 3.4	99.4 ± 2.7
15	215 ± 7	0,12 ± 0.03	-18 ± 3.2	99.2± 2.6
30	215 ± 15	0,10 ± 0.05	-18 ± 2.1	98.6± 3.4
60	225 ± 3	0,11 ± 001	-20 ± 1.2	98.3± 3.3
90	227 ± 13.4	0,10 ± 0.01	-17 ± 0.02	96.8± 2.2
180	227± 5.2	0,11 ± 002	-19 ± 2.1	96.8 ± 1.7

Stability results indicate that the presence of GSO leads to a stable system for up to 180 days as shown in Figure 3. Results with Tinogard TT are comparable to GSO in antioxidant effect. Long time stability could be associated to two factors: 1) Presence of GSO, and; as pointed out previously due to the 2) Low load of RP in nanocapsules. These two factors combined increased the polymeric chains that shielded the RP molecules from being attacked by the surrounding water and oxidizing agents. Oxidizing agents can potentially cause chemical degradation of the RP molecules [28]. Average stability for encapsulated retinoids reported in the literature is 30 days[11, 28]. However, results reported here indicate a 6 times higher stable retinoids system which remained stable for up to 180 days. Physico-chemical stability of nanocapsules was evaluated periodically for a period of up to180 days shortly after the synthesis and the results found are set forth in Table 2.

Tests for transporting the RP were performed using Franz cell with facial skin. Skin was fixed on the donor recipient of Franz diffusion cell in order to compare the transdermal transport of free RP with poly(ε-caprolactone) encapsulated RP, through the facial skin (composed of both dermis and epidermis). Surface of the skin was washed with fresh receptor medium at different times after the application, and the wash liquid was analysed for RP. Values of the release profiles were obtained from the peak current of the differential pulse voltammetry. Results of RP diffusion through the skin are shown in Figure 4, indicating that for the free RP, the RP concentration profile on the surface of the epidermis was found to decrease in the washes at 3 h after application. Results also indicate that with a 1.2 mg cm^{-2} of skin coverage, neither the free RP nor the RP in polymeric nanocapsules could significantly, if at all, penetrate fully across this full thickness skin, up to 9 h and 20 h respectively post application. Additionally, in Figure 4 a delay of 6h is observed for the diffusion of RP from epidermis through the dermis and after 12 h the amount of RP in receptor is constant.

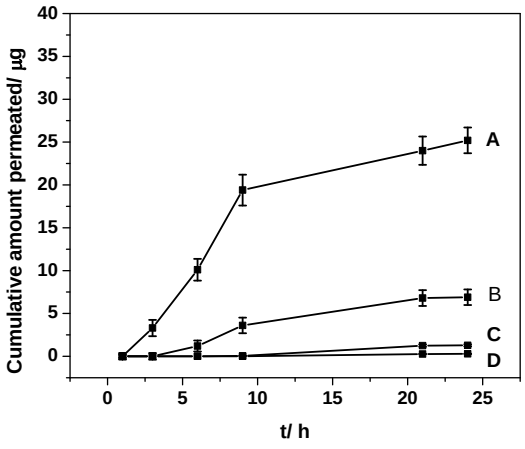

Figure 4. Release profile of the free PR accumulated on (A) dermis, (B) permeated across human skin and nanoencapsulated RP accumulated on (C) dermis, (D) permeated across human skin using the Franz cell.

For the RP-loaded polymeric nanocapsules, 100 % of the applied amount of RP could be detected at 3 h after application but after 24 h 99.8 % RP could be detected. From the experiments on RP stability discussed previously, in the same solution as those used in transdermal diffusion test the RP degradation could be excluded and so it was concluded that by 24 h after application, most of the encapsulated RP was in the skin tissue. Generally, drug release from nanocapsules depends on four processes: 1. Desorption of the drug present on the surface of the nanoparticles; 2. Diffusion of the drug through the polymer wall of the nanocapsules; 3. Erosion of the polymer matrix; 4. Combination of diffusion / erosion processes. Research results indicate that no degradation of RP occurs on skin for the nanoencapsulated systems in the presence of GSO. At the donor the profile of cumulative RP amount permeated through the skin decreases faster in free RP than nanoencapsulated RP, indicating a faster transport through the epidermis than the encapsulated RP. Probably the RP diffuses through the polymer wall and then penetrates in dermis. A delay of 9h was observed between the first nanoencapsulated RP amount to be detected in the receptor chamber as compared with free RP.

CONCLUSIONS:

Nanocapsules containing a poly(ε-caprolactone) shell and a retinyl palmitate (RP) core were prepared by pre-formed polymer interfacial deposition method and their stability compared to the equivalent system without grape seed oil (GSO). The nanocapsules containing RP and GSO were larger than the RP nanocapsules without GSO being 220 nm against 176 nm diameter, respectively). SEM images revealed a poly(ε-caprolactone) spherical nanoparticules with diameters ranging between 143 and 267 nm. Electroanalytical method used for the RP analysis showed linearity, selectivity, efficiency and utilizes direct sample injection, thereby eliminating elaborate sample preparation prior to analysis. Polymeric nanocapsules were able to encapsulate more than 99% of RP in oily phase containing GSO. Research results have shown that the improvement on RP stability is associated to the nanoencapsulation and the presence of GSO an efficient antioxidant. Nanoencapsulated RP in the presence of GSO exhibited a higher stability than the others retinoid compounds described previously. This stability extended over 190 days and a small decay of 8% is observed. Franz cell experiment indicated that the RP-loaded polymeric nanocapsules could be absorbed into the facial human skin, although at a slower rate than the free RP. Furthermore, nanocapsules containing RP were able to deliver it into the skin and in particular reach the dermis.

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REFERENCES:

1. Rabe, J. H., Mamelak, A. J., McElgunn, P. J. S., Morison, W. L., Sauder, D. N. (2006) J. Am. Acad. Dermatol., 55: 1-19.
2. Naylor, E. C., Watson, R. E. B., Sherratt, J. (2011) Maturitas, 69: 249– 256.
3. Jenkins, G. (2002) Mechanisms of Ageing and Development, 123: 801–810.
4. Fisher, G. J., Wang, Z. Q., Datta, S. C., Varani, J., Kang, S., Voorhees, J. J. (1967) The New England Journal of Medicine, 13: 1419-1428.
5. Tirado-Sanchez, A., Espındola, Y. S., Ponce-Olivera, R. M., Bonifaz, A. (2013) Journal of Cosmetic Dermatology, 12: 103-107.
6. Thielitz, A., Gollnick, H. (2008) Am. J. Clin. Dermatol. 9: 369–381.
7. Kafi, R., Kwak, H. S., Schumacher, W. E., Cho, S., Hanft, V. N., Hamilton, T. A., King, A. L., Neal, J. D., Varani, J., Fisher, G. J., Voorhees, J. J., Kang, S. (2007) Arch. Dermatol. 143: 606–612.
8. Eskandar, N. G., Simovic, S., Prestidge, C. A. (2009) Pharm. Res. 26: 1764–1775.
9. Castro, G. A., Coelho, A. L., Oliveira, C. A., Mahecha, G. A., Orefice, R. L., Ferreira, L. A. (2009) Int. J. Pharm. 381: 77–83.
10. Taepaiboon, P., Rungsardthong, U., Supaphol, P. (2007) Eur. J. Pharm. Biopharm. 67: 387–397.
11. Ourique, A. F., Pohlmann, A. R., Guterres, S. S., Beck, R. C. R. (2008) International Journal of Pharmaceutics 352: 1–4.
12. Teixeira, Z., Dreiss, C. A., Lawrence, M. J., Heenan, R. K., Machado, D., Justo, G. Z., Guterres, S. S., Durán N. (2012) Journal of Colloid and Interface Science 382: 36–47.
13. Ascenso, A., Salgado, A., Euletério, C., Praça, F. G., Bentley, M. V. L. B., Marques, H. C., Oliveira, H., Santos, C., Simões, S. (2014) Eur J Pharm Biopharm. 88: 48-55.
14. Jenning, V., Schäfer-Korting, M., Gohla, S. (2000) J. Control. Release 66: 115–126.
15. Jenning, V., Gysler, A., Schäfer-Korting, M., Gohla, S. H. (2000) Eur. J. Pharm. Biopharm. 49: 211–218.

16. Pople, P. V., Singh, K. K. (2006) AAPS PharmSciTech 7: 91.
17. Arsic, I., Vidovic, S., Vuleta, G. (1999) Int. J. Cosmet. Sci., 21: 219-225.
18. Rousselle, C. (2017) Regulatory Toxicology and Pharmacology 84: 102-104.
19. Gaspar, L. R., Maia Campos, P. M. B. G. (2007) International Journal of Pharmaceutics 343, 181–189.
20. Jayaprakasha, G. K., Singh, R. P., Sakariah, K. K. (2001) Food Chemistry 73: 285– 290.
21. Pardo, G. D., McClements, D. J. (2015) Food Chemistry 167: 205–212.
22. Yu, J., Ahmedna, M. (2013) International Journal of Food Science & Technology, 48: 221–237.
23. Garavaglia, J., Markoski, M. M., Oliveira, A., Marcadenti, A. (2016) Nutr. Metab. Insights 9: 59- 64.
24. Fessi, H., Puisieux, F., Devissaguet, J. Ph., Ammoury, N., Benita, S. (1989) Int. J. Pharm. 55: R1-R4.
25. Wang, L. H. (2000) Analytica Chimica Acta 415: 193–200.
26. Silva, F. L. O., Tinoco, L. M. S., Ferreira, L. A. M., Malagutti, A. R., Carneiro, G. (2015) Electrochim. Acta, 182: 929-934.
27. Arayachukeat, S., Wanichwecharungruang, S. P., Tree-Udom, T. (2011) International Journal of Pharmaceutics 404: 281–288.