RESEARCH ARTICLE

Nanoemulgel based on Guar Gum and Pluronic® F127 containing Encapsulated Hesperidin with Antioxidant Potential

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Abstract

Introduction: The development of antioxidant cosmetic products based on natural products added to nanotechnology is gaining more and more prominence. However, some of these actives are hydrophobic, which makes their placement in aqueous systems impossible. As a strategy to deliver hydrophobic actives in hydrophilic gels, nanoemulgels have been presented.

Materials and Methods: The present study aimed to prepare an aqueous nanoemulgel based on guar gum and Pluronic® F127 as a strategy for the delivery of hesperidin, a hydrophobic flavonoid. A nanoemulsion was prepared and characterized to be incorporated into the gel. The formed nanoemulgel was evaluated for rheology and antioxidant activity.

Results and Discussion: The nanoemulsion had a particle size of 192 ± 4 nm, a polydispersity index of 0.11 ± 0.01 and a zeta potential of -32.1 ± 0.9 mV. The encapsulation efficiency obtained was 99.35% and the rheological study of the nanoemulgel showed satisfactory gel characteristics for topical use. The antioxidant potential of nanoemulsions by the DPPH method presented an IC_{50} value of 14.4 ± 1.3 µg mL^{-1} expressed in hesperidin equivalent to 17.4 mg mL^{-1} of the nanoemulgel. The formulated nanoemulgel delivered hesperidin successfully without affecting its antioxidant properties, showing promise as a cosmetic for topical anti-aging use.

Keywords: Guar gum, Hesperidin, Nanoemugel, Pluronic.

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Introduction

With the development of new formulas, technologies and products, cosmetics have been expanding their market and becoming increasingly present in the business world.[1] In the composition of cosmetics, there are several chemical substances of synthetic origin, even though they are considered safe by industries that use them and their quantities are within the requested parameters, the periodic use of these products can cause a chemical accumulation causing adverse reactions such as allergies.[2] In this way, researchers seek to develop new products made from natural sources that can make the product safer and less likely to have adverse reactions. In view of the increase in skin products of natural origin, there was a need to develop new technologies that can increase the methods used, as well as the use of antioxidant substances, for example, hesperidin.[3]

Hesperidin (3',5-dihydroxy-4'-methoxy-7-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyloxy]flavan-4-one) has anti-inflammatory, antifungal and anticancer however, it has low solubility, which restricts its application in cosmetics. Hesperidin (Figure 1) is a flavonoid usually found in the peel of citrus fruits, such as tangerine, orange and lemon, which in its composition contain polyphenolic substances that are responsible for the antioxidant activity.[4]

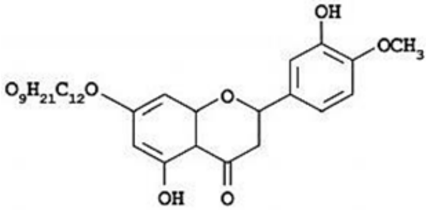

Figure 1 – hesperidin

However, its effectiveness can be minimized due to the influence of pH, easy oxidation and low solubility in water.[5] Nanotechnology is used as a strategy for the release of hydrophobic actives.

Nanomaterials that have been standing out in the production of cosmetics due to the particle size being smaller than 1000 nm, which facilitates the penetration of the product into the skin and has been presented in the literature as a solution for the delivery of hydrophobic actives.[1] Among the most used forms of nanotechnology in cosmetics are polymeric

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nanoparticles, solid lipid nanoparticles, nanogels and nanoemulsions. In the literature, Costa (2018) and Rebouças (2022) highlights the use of nanotechnology to carry hydrophobic actives for the treatment of cancer.[6,7]

Nanoemulsions are colloidal dispersions with an average size between 20 and 500 nm and a physical stability that can be applied in several segments.[8] Nanocosmetics, especially nanoemulgels, which are nanoemulsions incorporated into polymeric gels, can provide the delivery of actives hydrophobic natural substances generating greater efficiency and reducing adverse effects. Nagaraja *et al.* (2021) successfully encapsulated the lipophilic flavonoid naringin in a nanoemulgel.[9]

Pluronic® F127 is a triblock copolymer, non-ionic, biocompatible and non-toxic and forms thermoresponsible gels. Successfully used in association with polysaccharide for the delivery of drugs such as metropolol succinate for dermal application.[10] Additionally, pluronic gel F-127 has been proven to have a mild inflammatory nature and stimulate the expression of vascular endothelial growth factor (VEGF) when applied on the skin.[11]

A polymer used to form gels is guar gum, a galactomannan extracted from the endosperm of seeds of Cyamopsis tetragonolobus, which has several applications: as a dispersing, emulsifying and gelling agent, being hydrophilic. In addition, it has stabilizing properties; therefore, its association with nanoemulsions or nanogels with a high water content in their composition is interesting.[12]

In view of the above, the present work aims to prepare a nanoemulgel based on guar gum and Pluronic® F127 for loading hesperidin as a strategy for the delivery of the hydrophobic active in a hydrophilic gel.

MATERIAL AND METHODS

Materials

Guar gum Polymer molecular weight (Mw= 11.2 x 106, M/G ratio = 1.60) was purchased from Êxodo Científica (São Paulo, Brazil), hesperidin 95% was purchased from Xi'an Quanao Biotec (Xi'an, China), Pluronic® F-127 was purchased from Sigma-Aldrich (Germany), Methanol purchased from Êxodo Científica (São Paulo, Brazil), Oleic acid PA purchased from Êxodo Científica (São Paulo, Brazil), DPPH (2, 2-Diphenyl-1-picryl-hydrazyl) (Sigma-Aldrich, Germany) and used ultrapure water was obtained in a MiliQ purifier.

Preparation and characterization of the nanoemulsion

Initially, 100 mg of hesperidin was solubilized in 3.00 g of oleic acid and added to 17 g of an aqueous solution containing 1.00 g of Pluronic® F-127. The mixture was subjected to ultrasonication in a Brason Sonifer W-450 ultrasonic processor with probe, with 70% amplitude, 100-105 W power, for 2 minutes, in cycles of 10 seconds on and 10 seconds off in an ice bath.[7]

The nanoemulsion was characterized, in triplicate, for zeta potential, droplet size, polydispersity index, mechanical stress resistance and encapsulation efficiency. To determine the zeta potential of the nanoemulsion, the laser doppler microelectrophoresis technique was used. The droplet size and the polydispersity index (PDI) were used by the dynamic light scattering (DLS) method using the Malvern Zetasizer Nano meter, model ZS ZEN 90 with sample dilution 1000x MiliQ in water.

The mechanical stress resistance test was performed by submitting 5 mL of the nanoemulsion sample to centrifugation using a Centribio 80-2B centrifuge at 3000 rpm for 30 minutes. The occurrence of creaming, precipitation and phase separation were evaluated.[7]

The encapsulation efficiency (EE) was determined using a Turbo 15 Vivaspin ultrafiltration tube (Sartorius, Göttingen, Germany) at 3000 rpm for 30 min, with a cut of 3000 Daltons.[7] After centrifugation, the centrifuged material was collected to be quantified. Thus, the encapsulation efficiency of hesperidin was calculated by the ratio between the encapsulated amount of the bioactive after ultrafiltration and the amount added to the nanoemulsion formulation. The quantification of the active was performed by UV-VIS spectrophotometry at 285 nm using methanol as solvent in a Varian 1E Spectrophotometer.[4]

Nanoemulgel preparation

To prepare the nanoemulgel, 2.00 g of guar gum was solubilized in 98 g of water at 70-72 °C, stirring in a mechanical shaker for 30 minutes. After the formation of the gel, 20 g of the previously prepared nanoemulsion was added under slow and constant stirring until total incorporation into the gel.

Rheological study

Rheology is related to the product's stability and package design, which depends on viscosity. For the rheological study of the nanoemulgel, we used a TA Instruments rheometer, Rheometer AR 2000, with a gap of 27 µm, cone-plate geometry with a diameter of 40 mm and an angle of 1º. Rheological experiments of viscosity as a function of shear rate and determination of elastic modulus and viscosity modulus as a function of oscillation stress and time were carried out.

Antioxidant activity test using DPPH assay

Approximately 240.3 mg of gel sample (containing 200 µg of hesperidin) was accurately weighed and placed in a 10.0 mL vial volumetric flask, dissolved and made up with methanol.

The sample solution was then sonicated for 10 min and centrifuged at 4000 rpm for 10 minutes. The supernatant of ample was filtered, the sample solution 20 $\mu g\ mL^{-1}$ (hesperidin) was then diluted to obtain sample concentrations of 1, 5, 10, 15 $\mu g\ mL^{-1}$. A 3.0 mL aliquot of diphenyl picrylhydrazyl (DPPH) was added to 1.0 mL of each sample solution. Methanol (1.0 mL) and DPPH 50 $\mu g\ mL^{-1}$ solutions (3.0 mL) were used as a blank. The mixture was shaken for 20 s and incubated in a dark room at room temperature (25°C ± 2°C) for 30 min.[13]

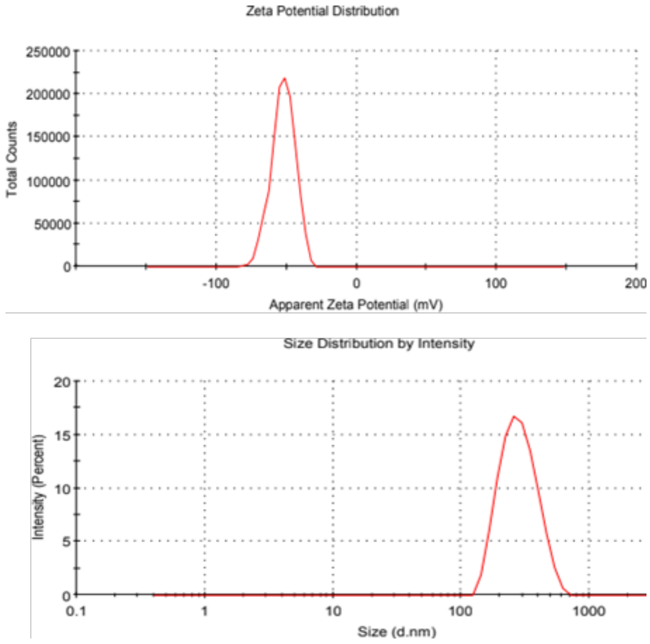

Figure 2: Zeta potential distribution and size distribution

The antioxidant activity was determined by measuring the absorbance of each sample by UV-visible spectrophotometry in a wavelength of 515 nm. The result obtained was used to calculate the percentage of DPPH inhibition of hesperidin in cream samples. The percentage of DPPH radical inhibition was calculated by comparing the sample and the results blank.

Statistical analysis

All measurements were performed in triplicate. The nanoemulsion characterization results were expressed with the standard deviation. For comparison of several groups, analysis of variance (ANOVA) was applied, considering $p < 0.05$ as statistically significant. Linear regression analysis for the antioxidant test was performed by GraphPad Prism 5.0.

RESULTS AND DISCUSSION

Characterization of the nanoemulsion

The nanoemulsion had a size of 192 ± 4 nm. PDI and zeta potential showed characteristic stability values of 0.11 ± 0.01 and -32.1 ± 0.9 mV, respectively. In Figure 2 the distribution of zeta potential and size by intensity is shown.

The zeta potential was negative and different from zero, being desirable characteristics because the farther from zero the surface charge allows a repulsion of the particles, which guarantees stability. Nanoemulsions stabilized with polymeric surfactants such as Pluronic F-127, which have a high molecular weight, are easily stabilized and have zeta potentials close to -20 mV by steric effect.[14]

In the mechanical stress resistance test, no instability or phase separation phenomena were observed, indicating that the prepared nanoemulsion was stable.

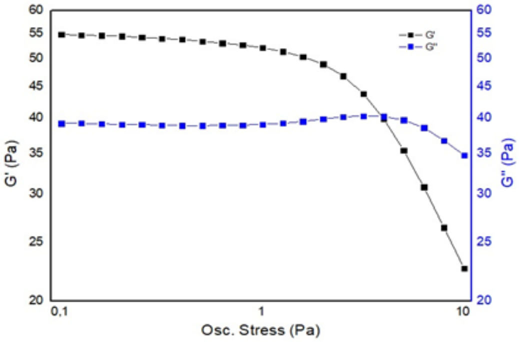

Figure 3: G' and G" as a function of stress oscillation

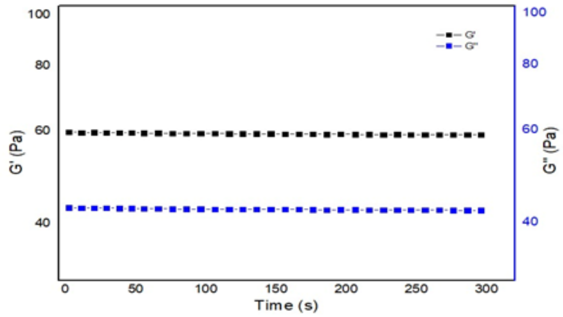

Figure 4: G' and G" as a function of time

The Encapsulation Efficiency obtained was 99.35% ± 0.51, this high value indicates that the active was successfully encapsulated and can also be explained by the intermolecular forces existing between the hesperidin molecule and the oleic acid that guarantee the solubility and thus they keep the active in the oil. In the literature Rebouças (2022) reports encapsulation efficiency greater than 99.99% when encapsulating betulinic acid, a hydrophobic triterpene in linseed oil.[7]

Rheological study

In the rheological study we obtain information about the viscoelastic behavior of the nanoemulgel. The relationship between G' and G" with the frequency of oscillation can be explained through the mechanics of the spectrum, which allows its characterization.[15] Viscoelasticity occurs when a material has both solid and liquid characteristics.[16] When a fluid has a certain resistance in its flow, indicating that it is viscous, viscoelasticity depends on two parameters, the elastic modulus (G'), which is the ability of a material to accumulate energy, and the viscous modulus (G"), which is the ability of the fluid to lose energy.[16] As shown in Figure 3, we observed the G', which refers to the elastic modulus, which ranged from 40 to 35 Pa, and the G" that refers to the viscous modulus, which ranged from 60 to 20 Pa.

Thus in the graph they remain constant during a period of stress from 0.1 to 4 and undergo a decay after greater exposure to stress. In the time interval from 0 to 300 seconds, we observed in Figure 4 that the elastic modulus and viscous modulus remained constant and did not change.

The rheological characteristics presented by the nanoemulgel show stability and gel consistency, being therefore satisfactory for use as a topical gel.

Antioxidant activity

DPPH is a stable free radical molecule that has one free electron on its nitrogen atom. This test is based on the principle that DPPH accepts hydrogen atoms from antioxidant molecules such as hesperidin, which results in a more stable reduced form of DPPH-H.

The equation obtained by linear regression was $y = 3.32x + 2.11$ with coefficient of determination 0.9965 for the sample and $y = 3.82x + 1.80$ with coefficient of determination 0.9921 for hesperidin. The 50% inhibition of the concentration (IC50) of nanoemulgel was 14.4 ± 1.3 µg mL^{-1} which corresponds to 17.3 mg of nanemulgel per 1 mL. The IC50 of pure hesperidin was also determined, showing a value of 12.3 ± 0.9 µg mL^{-1}, not showing significant differences with the nanoemulgel when expressed as hesperidin ($p > 0.05$), which means that the other components of the formulation did not interfere with the antioxidant activity.

The compound is said to have strong antioxidant activity if the IC^{50} is between 10 µg mL^{-1} and 50 µg mL^{-1}.[13] Based on the results obtained, hesperidin in the nanoemulgel showed strong antioxidant activity. The IC^{50} value is the concentration of antioxidant compounds needed to inhibit 50% of existing DPPH activities. The antioxidant properties of hesperidin are related to its chemical structure as it is formed by phenolic groups, where the activity increases due to the high concentration of polyphenolic content.[13] In addition, we have the relationship between antioxidant activity and anti-aging activity, where antioxidant activity assists in the anti-aging process as free radicals, which are responsible for the oxidation of cells, are captured by the active antioxidant.[18]

CONCLUSION

The preparation of the nanoemulgel was carried out successfully, which showed characteristic stability results, satisfactory rheology for topical use and preserved hesperidin antioxidant activity. Therefore, the nanoemulgel proposed in this study is promising as an anti-aging cosmetic due to its antioxidant action, being a new alternative to transport the hydrophobic active hesperidin in a hydrophilic matrix of easy absorption by the nanostructuring strategy. Proof of its effectiveness as an anti-aging cosmetic can be carried out in future clinical trials.

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REFERENCES

1. Aziz ZAA, Mohd-Nasir H, Ahmad A, Mohd Setapar SH, Peng WL, Chuo SC, Khatoon A, Umar K, Yaqoob AA, Mohamad Ibrahim MN. Role of Nanotechnology for Design and Development of Cosmeceutical: Application in Makeup and Skin Care. Frontiers in Chemistry. 2019; 7: 739. Avaiable from: doi.org/10.3389%2Ffchem.2019.00739
2. Mulia K, Ramadhan RA, Krisanti EA. Formulation and characterization of nanoemulgel mangosteen extract in virgin coconut oil for topical formulation. MATEC Web of Conferences. EDP Sciences. 2018; 01013. Avaiable from: doi.org/10.1051/matecconf/201815601013
3. Rohman A, Rafi M, Alam G, Muchtaridi M, Windarsih A. Chemical composition and antioxidant studies of underutilized part of mangosteen (Garcinia mangostana L.) fruit. Journal of Applied Pharmaceutical Science. 2019; 9(08): 047–052. Avaiable from: doi.org/10.7324/JAPS.2019.90807
4. Kuntić V, Pejić N, Mićić S. Direct spectrophotometric determination of hesperidin in pharmaceutical preparations. Acta chimica Slovenica. 2012; 59(2): 436-441. Avaiable from: pubmed.ncbi.nlm.nih.gov/24061264/
5. Anwar A, Masri A, Rao K, Rajendran K, Khan N, Raza M, Siddiqui R. Antimicrobial activities of green synthesized gums-stabilized nanoparticles loaded with flavonoids. Scientific Reports. 2019; 9(1): 3122. Avaiable from: doi.org/10.1038/s41598-019-39528-0
6. Magura J, Hassan D, Moodley R, Mackraj I. Hesperidin-loaded nanoemulsions improve cytotoxicity, induce apoptosis, and downregulate miR-21 and miR-155 expression in MCF-7. Journal of Microencapsulation. 2021; 38(7-8): 486-495. Avaiable from: doi.org/10.1080/02652048.2021.1979673
7. Rebouças LM, Sousa AC, Gramosa NV, Araújo TG, Oliveira FC, Pessoa ÓC, Araújo RS, Santos EMA, Ricardo NM. Linseed Oil Nanoemulsion with Pluronic® F127 Loaded with Betulinic Acid: Preparation, Rheology, MTT Assay and in vitro Release Kinetics. Journal of the Brazilian Chemical Society. 2022; 1-13. Avaiable from: doi.org/10.21577/0103-5053.20220063
8. Sungpud C, Panpipat W, Chaijan M, Sae YA. Techno-biofunctionality of mangostin extract-loaded virgin coconut oil nanoemulsion and nanoemulgel. PLoS One. 2020; 15(1): e0227979. Avaiable from: doi.org/10.1371/journal.pone.0227979
9. Nagaraja S, Basavarajappa GM, Karnati RK, Bakir EM, Pund S. Ion-Triggered In Situ Gelling Nanoemulgel as a Platform for Nose-to-Brain Delivery of Small Lipophilic Molecules. Pharmaceutics. 2021; 13(8): 1216. Avaiable from: doi.org/10.3390/pharmaceutics13081216
10. Parhi R. Development and optimization of pluronic® F127 and HPMC based thermosensitive gel for the skin delivery of metoprolol succinate. Journal of Drug Delivery Science and Technology. 2016; 36: 23-33. Avaiable from: doi.org/10.1016/j.jddst.2016.09.004
11. Kant V, Gopal A, Kumar D, Gopalkrishnan A, Pathak N, Kurade N, Tandan S, Kumar D. Topical pluronic F-127 gel

application enhances cutaneous wound healing in rats. Acta histochemica. 2013; 116(1): 5-13. Avaiable from: doi.org/10.1016/j.acthis.2013.04.010

12. Shen Y, Li Y. Acylation modification and/or guar gum conjugation enhanced functional properties of pea protein isolate. Food Hydrocolloids. 2021; 117: 06686. Avaiable from: doi.org/10.1016/J.FOODHYD.2021.106686
13. Harmita H, Iskandarsyah I, Afifah SF. Effect of transfersome formulation on the stability and antioxidant activity of n-acetylcysteine in anti-aging cream. International Journal of Applied Pharmaceutics. 2020; 12(1): 156–162. Avaiable from: doi.org/10.22159/ijap.2020.v12s1.ff034
14. Honary S, Zahir F. Effect of zeta potential on the properties of nano-drug delivery systems-a review (Part 2). Tropical Journal of Pharmaceutical Research. 2013;12(2);265-273. Avaiable from: doi.org/10.4314/tjpr.v12i2.20
15. Antunes FE, Gentile L, Rossi CO, Tavano L, Ranieri GA. Gels of Pluronic F127 and nonionic surfactants from rheological characterization to controlled drug permeation. Colloids and Surfaces B: Biointerfaces. 2011; 87(1): 42-48. Avaiable from: doi.org/10.1016/j.colsurfb.2011.04.033
16. Kavanagh GM, Ross-Murphy SB. Rheological characterisation of polymer gels. Progress in Polymer Science. 1998; 23(3); 533-562. Avaiable from: doi.org/10.1016/S0079-6700%2897%2900047-6
17. Nabil M, Donia, T, Mohamed T. Antioxidant effect of hesperidin isolated from orange peels. Delta Journal of Science. 2016; 35: 1-5. Avaiable from: https://www.researchgate.net/publication/314245524_Antioxidant_effect_of_hesperidin_isolated_from_orange_peels_Antioxidant_effect_of_hesperidin_isolated_from_orange_peels
18. Hirata LL, Sato MEO; Santos CAM. Radicais livres e o envelhecimento cutâneo. Acta Farm. Bonaerense. 2004; 23(3): 418-24. Avaiable from: http://www.latamjpharm.org/trabajos/23/3/LAJOP_23_3_6_1_7IT93QRE42.pdf