Vol.66(spe): e23230300, 2023
https://doi.org/10.1590/1678-4324-ssbfar-2023230300
ISSN 1678-4324 Online Edition
II South-Brazilian Symposium on Pharmacognosy

Article - Health Science/Pharmacognosy

Preparation, Characterization and Stability Study of Eugenol-Loaded Eudragit RS100 Nanocapsules for Dental Sensitivity Reduction

Ana Paula Vilela[1]
https://orcid.org/0000-0002-7127-1276

Leandro Ferreira[1]
https://orcid.org/0000-0003-1423-0512

Patrícia Bubna Biscaia[1]
https://orcid.org/0000-0003-3011-2321

Karine Letícia da Silva[2]
https://orcid.org/0000-0001-8889-7278

Flávio Luís Beltrame[1]
https://orcid.org/0000-0001-7067-5802

Guilherme dos Anjos Camargo[1*]
https://orcid.org/0000-0002-3440-2791

Marcia Rezende[3]
https://orcid.org/0000-0001-8474-5656

Jessica Mendes Nadal[1]
https://orcid.org/0000-0002-2419-2110

Andressa Novatski[4]
https://orcid.org/0000-0002-8327-6285

Jane Manfron[1,4]
https://orcid.org/0000-0003-1873-2253

Alessandro Dourado Loguercio[1,2]
https://orcid.org/0000-0001-9880-4856

Paulo Vitor Farago[1,2,4]
https://orcid.org/0000-0002-9934-4027

[1]Universidade Estadual de Ponta Grossa, Programa de Pós-graduação em Ciências Farmacêuticas, Ponta Grossa, Paraná, Brasil; [2]Universidade Estadual de Ponta Grossa, Programa de Pós-graduação em Odontologia, Ponta Grossa, Paraná, Brasil; [3]Faculdade Paulo Picanço, Escola de Odontologia, Fortaleza, Ceará, Brasil; [4]Universidade Estadual de Ponta Grossa, Programa de Pós-graduação em Ciências da Saúde, Ponta Grossa, Paraná, Brasil.

Editor-in-Chief: Yasmine Mendes Pupo
Associate Editor: Najeh Maissar Khalil

Received: 21-Mar-2023; Accepted: 05-May-2023

*Correspondence: guuicamargo.gc@gmail.com; Tel.: + 55 (42) 999124063 (G.A.C.).

HIGHLIGHTS

- Eugenol-loaded Eudragit RS100 nanocapsules were suitably obtained and characterized.
- A simple and fast UPLC/MS-MS method for determining eugenol in Eudragit RS100 nanocapsules was developed and validated.
- Eugenol-loaded Eudragit RS100 nanocapsules showed physical stability after 60 days of preparation.
- Eugenol-loaded Eudragit RS100 nanocapsules may be further used for reducing dental sensitivity.

Abstract: Eugenol is a phenolic compound with numerous biological activities. It is widely used in dentistry to treat toothache and pulpitis. In particular, eugenol may be used for tooth whitening procedures to minimize dental sensitivity in patients. However, eugenol has some disadvantages such as its volatility, its photosensitivity, and immediate effect, which can be avoided by using pharmaceutical nanotechnology. The

aim of the present study was to obtain, characterize, quantify, and evaluate the physicochemical stability of eugenol-loaded Eudragit RS100 nanocapsules. The nanocapsules (NCs) were prepared by interfacial deposition of the preformed polymer method. The NCs were characterized through morphological and spectroscopic studies. The encapsulation efficiency was achieved by quantifying the non-encapsulated eugenol using a previously developed and validated analytical method. The physicochemical stability of NCs was assessed at predetermined time intervals for 90 days after preparation. The nanocapsules were successfully prepared by the chosen method and had a predominantly spherical shape with a smooth surface. The mean size, the polydispersion index, and the zeta potential were in agreement to literature data. Infrared spectra ensured that the nanoencapsulation process did not result in chemical reactions between the drug and the polymer. The formulations showed encapsulation efficiency higher than 90% and remained stable after 60 days of preparation. Thus, eugenol-loaded Eudragit RS100 nanocapsules may be further considered as an alternative formulation for dental sensitivity in order to provide a controlled release, a decreased toxicity, and a better dental sensitivity relief.

Keywords: clove; essential oil; polymeric nanoparticles; Syzygium aromaticum; tooth whitening.

INTRODUCTION

The tooth whitening is one of the most common procedures in aesthetic dentistry. The in-office bleaching technique using hydrogen peroxide is highly requested by patients since most of them do not adapt to the tray use and desire faster results. However, the In-office bleaching may have a high rate of dental sensitivity (DS) and, for this reason, drugs such as anti-inflammatory, corticosteroids, and analgesics are often tested to reduce or eliminate this adverse effect [1].

Eugenol or 4-allyl-2-methoxyphenol, $C_{10}H_{12}O_2$, is the main component of clove essential oil [Syzygium aromaticum (L.) Merr. & L. M. Perry]. It is a liquid phenolic compound with light yellow or colorless color [2,3]. Eugenol has numerous biological activities previously reported in the literature such as: antimicrobial, anti-inflammatory, antioxidant, analgesic, and anesthetic properties [2,4,5]. Eugenol has anti-inflammatory and anesthetic effects by blocking the release of inflammatory mediators and cytokines, and it is used in dentistry in patients who undergo tooth whitening due to DS caused by the procedure [3,6].

Studies have shown that eugenol is used in different areas safely, as it has no carcinogenic or mutagenic effects, and the accepted daily intake is 2.5 mg of eugenol per body weight in humans [7]. However, eugenol has disadvantages such as volatility, photosensitivity, and immediate action. In that sense, it is imperative to obtain a novel technology in order to provide a better therapeutic efficacy, a prolonged drug release, a reduced toxicity, and a longer time of action [8-10].

Nanotechnology turns out to be an interesting area to improve the therapeutic action of numerous substances. This technology refers to the controlled production of structures and systems on the nanometer scale that is in one billionth of a meter (10-9 m) from the use of molecules and materials of the most diverse origins [11]. Polymeric nanocapsules are one of the most studied nanocarriers and are used to control the drug release. These nanocapsules (NCs) are core-shell structures, which present sizes between 100 and 300 nm. The oily core can dissolve lipophilic drugs and can provide a controlled release. Trus, nanoencapsulation can diminish the problems related to eugenol use [12].

Eudragit RS100 is a copolymer of poly(ethyl acrylate-co-methyl methacrylate-co-trimethylammoniethyl methacrylate chloride) and contains between 4.5 to 6.8% of its quaternary ammonium groups. These chemical groups provide a positive surface charge to the polymer, which makes it interact with negatively charged drugs or the cell surface of target tissues. This feature can subsequently maximize cellular uptake of the drug-polymer complex and also underscores its mucoadhesive feature [13-15]. Eudragit RS100 is insoluble in water and has low permeability, but it is permeable to digestive fluids. Its application is widely associated with the production of oral and topical formulations. Previous studies have reported an adequate biocompatibility in different mucous membranes. It is also insoluble at physiological pH, but swells in the presence of water [14,15].

In this sense, the aim of this study was to obtain a formulation based on Eudragit RS100 nanocapsules containing eugenol for buccal application. In addition, this formulation was characterized through morphological and spectroscopic methods, the eugenol encapsulation efficiency was achieved, and the physicochemical stability was investigated for 90 days after preparation.

MATERIAL AND METHODS

Materials

Eugenol 99% pure (Sigma-Aldrich, St. Louis, MO, USA), Eudragit RS100 (Mw = 150,000 g/mol, Röhm, Darmstadt, Alemanha), sorbitan monooleate (Span 80, Oxiteno, Mauá, Brazil), polysorbate 80 (Tween 80, Delaware, Porto Alegre, Brazil), medium chain triglycerides (MCT, 99% pure, Focus Química, São Paulo, Brazil), lactose monohydrate (LAC, Biotec Produtos Químicos, São José dos Pinhais, Brazil), acetone (99.9% pure, Vetec Química, Rio de Janeiro, Brazil), acetonitrile HPLC grade (J.T. Baker, Phillipsburg, NJ, USA), Formic acid (Biotec Produtos Químicos, São José dos Pinhais, Brazil) were used as received. Water was purified in a Milli-Q Plus water purification system (Millipore, Bedford, MA, USA).

Preparation of eugenol-loaded Eudragit RS100 nanocapsules

The polymeric nanocapsules (NC-E) were prepared by the method of interfacial deposition of preformed polymer [16,17]. Briefly, Eudragit RS 100 (0.100 g) was solvated in acetone (27 mL) in the presence of Span 80 (0.0770 g), eugenol (0.150 g), and medium chain triglycerides (MCT) (0.150 g) under mechanical stirring at 40ºC until complete dissolution. Then, the organic phase was poured slowly over the aqueous phase (53 mL) containing Tween 80 (0.0770 g). This emulsion was maintained under magnetic stirring at 40°C for 10 minutes. Organic solvent was then eliminated by evaporation in a rotary evaporator (Quimis, São Paulo, Brazil) to a final volume of 10 mL (1.5 mg/mL). A sample with no drug (NC-C) was prepared as control.

Characterization of eugenol-loaded Eudragit RS100 nanocapsules

pH Determination

The pH values were obtained by using a digital potentiometer, previously calibrated with pH 4.0 and 7.0 buffer solutions. The pH was measured directly in each colloidal suspension after preparation. The results were expressed as the mean of six different samples.

Determination of Mean Diameter, Polydispersity Index (PDI), and Zeta Potential of NCs

Mean particle size, polydispersity index (PDI), and zeta potential were measured (n = 3) after diluting an aliquot of the nanocapsule suspension in ultrapurified water (1:500). All analyses were carried out on the Zetasizer Nanoseries (Malvern Instruments, Malvern, United Kingdom). A one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was performed to verify the statistical difference between the mean values.

Field Emission Scanning Electron Microscopy (FESEM)

Morphological and surface evaluation of NCs were performed by Field Emission Scanning Electron Microscopy (FESEM) (Tescan, Mira 3 model, Brno, Czech Republic) at an acceleration voltage of 8 to 10 kV [18,19]. Samples were previously subjected to metallization with gold in an IC-50 Ion metallizer Coater (Shimadzu, Kyoto, Japan) [20].

Fourier-Transform Infrared Spectroscopy (FTIR)

The NC-E formulation was assessed by Fourier-transform infrared spectroscopy (FTIR) using potassium bromide (KBr) pellets with 4 mg of the sample and 196 mg of spectroscopic grade KBr (2%, m/m) at the IR Prestige-21 equipment (SHIMADZU, Quito, Japan) in the range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1} and 32 scan/min. The obtained spectrum was evaluated against the pure drug, the Eudragit RS100 polymer, and the control formulation (NC-C) [21]

UPLC-MS/MS conditions

Experiments were performed in a Acquity Waters® HClass UPLC, equipped with a refrigerated autoinjector (Sample Manager FTN) and a quaternary pump (Quaternary Solvent Manager) to obtain the chromatograms. A C_{18} column [Acquity BEH HSS T3 (1.8 µm, 2.1 x 50 mm)] and an isocratic elution condition with acetonitrile:acidified water containing 0.1% formic acid (80:20 V/V) were used. The injection volume was 5 µL, the flow rate was 0.3 mL/min, the column temperature was 25 ºC and the run time analysis was 2 min. Detection was performed by electrospray ionization (ESI) in positive mode; for which the following probe

conditions were used: source temperature of 150 °C, capillary of 3.7 KV, cone of 25V, collision energy of 68 V, desolavation gas flow of 60 L/h and desolavation temperature 400 °C. The multiple reaction monitoring (MRM) analysis allowed monitoring: *m/z* 165.2 → 137 (quantitative ion); 165.2 → 124 (qualitative ion). All operations were performed using the MassLynx program (Waters).

Preparations of standard solutions and samples

The standard eugenol solution (500.0 µg/mL) was prepared in acetonitrile:water (1:1, v/v), at. Dilutions were performed in order to obtain solutions with a concentration between 2.4 – 240.0 µg/mL. Before injection the solutions were filtered through a polytetrafluoroethylene filter (PTFE, Cromafil® Xtra, 0.2 µm x 13 mm, Macherey-Nagel GNBH & Co. KG, Düren, Germany), and added in a vial.

Method validation

The validation of the analytical method by UPLC-MS/MS was carried out according to the criteria proposed by the International Conference on Harmonization guidelines [22]. The following parameters were evaluated: linearity, limits of detection and quantification, precision, accuracy and robustness.

The linearity of the method was evaluated by linear regression using the method of least squares, by averaging the points of three authentic analytical curves, at concentrations of: 2.4, 4, 12, 24, 48, 72, 96, 192 and 240 µg/mL. The slope and other parameters of the analytical curves were calculated by linear regression and analysis of variance (ANOVA). Assessment of each point was performed in triplicate.

The limits of detection (LD) and quantification (LQ) were obtained experimentally by injecting solutions with concentrations lower than the working range. Repeatability was evaluated through the analysis of three different concentrations (12.8, 48 and 192 ng/mL), totaling nine determinations in different periods of the same day (morning, afternoon and evening), by the same analyst, using the same instrument. Intermediate precision was determined by analysis at the same concentrations used for repeatability, but measurements were performed on different days, with different analysts, still using the same instrument. The results of these analyzes were expressed in the form of relative standard deviation (RSD).

The accuracy value was determined by calculating the percent recovery of eugenol for these three concentration levels and then determining the relative standard deviation. The robustness was evaluated in the samples at 30.0 µg/mL, by variations in the flow rate to 0.33 mL/min, the concentration of the acetonitrile mobile phase and water acidified with formic acid 82:18 (v/v) and oven temperature to 25,5 °C. The results were analyzed using the RSD comparing with the values obtained for the standard condition.

Method applicability: Evaluation of Encapsulation Efficiency

Encapsulation efficiency (EE) was determined by an indirect method, quantifying the concentration of non-encapsulated drug. Therefore, 500 µL of the nanosuspension were subjected to ultrafiltration/centrifugation using a device (Amicon® 10,000 Mw, Milipore) at 6000 rcf for 30 minutes. Free eugenol was determined in the ultrafiltrate, using the validated UPLC-MS/MS method. The EE (%) was calculated from the difference between the mass of the drug initially added in each formulation and the mass present in the formulations [23,24] according to Equation 1.

$$EE(\%) = \frac{theoretical\ drug\ content - free\ drug\ content}{theoretical\ drug\ content} x\ 100 \quad (1)$$

Physicochemical stability

NC-C and NC-E suspensions were stored in a refrigerator and protected from light in an amber bottle. Mean particle size, polydispersity index, and zeta potential were measured (n=3) at times of 0, 30, 60, and 90 days. All resulting stability data were evaluated using the GraphPad Prism program, version 6.01 for Windows. Data were expressed as mean ± standard deviation. ANOVA with Tukey's post-test, followed by Student's t-test was used for statistical comparisons, with a significance level of 5% (α = 0.05).

RESULTS

Preparation of eugenol-loaded Eudragit RS100 nanocapsules

NC-E and NC-C were suitably obtained by the interfacial deposition of the preformed polymer method, and these formulations resulted in colloidal nanodispersion with bluish opalescence as previously reported [25].

Characterization of eugenol-loaded Eudragit RS100 nanocapsules

pH determination

Table 1 shows the values achieved for the pH of the recently obtained nanosuspensions.

Table 1. pH values obtained after preparing the Eudragit RS100 nanocapsules

Formulations	pH	SD*
NC-E	5.40	0.09
NC-C	5.60	0.15

* SD = standard deviation (n = 3)

Determination of Mean Diameter, Polydispersity Index (PDI), and Zeta Potential of NCs

The results of mean diameter, PDI and zeta potential for NC-E and NC-C are summarized in Table 2.

Table 2. Values of mean diameter, PDI, and zeta potential for NC-E and NC-C

Formulations	Mean diameter (nm)		PDI		Zeta potential (mV)	
	Mean	SD*	Mean	SD*	Mean	SD*
NC-E	121.80	4.81	0.25	0.02	25.89	2.84
NC-C	122.95	0.64	0.19	0.03	20.25	6.61

* SD = standard deviation (n = 3)

Field Emission Scanning Electron Microscopy (FESEM)

Photomicrographs performed for NC-E and NC-C after freeze-drying using 1% lactose are depicted in Figure 1 (A and B, respectively). These images confirmed the nanocapsule formation through the aforementioned method as well as the nano-sized dimensions of these formulations. The Eudragit RS100 nanocapsules mainly presented particles with spherical shape and smooth surface. No pore was observed.

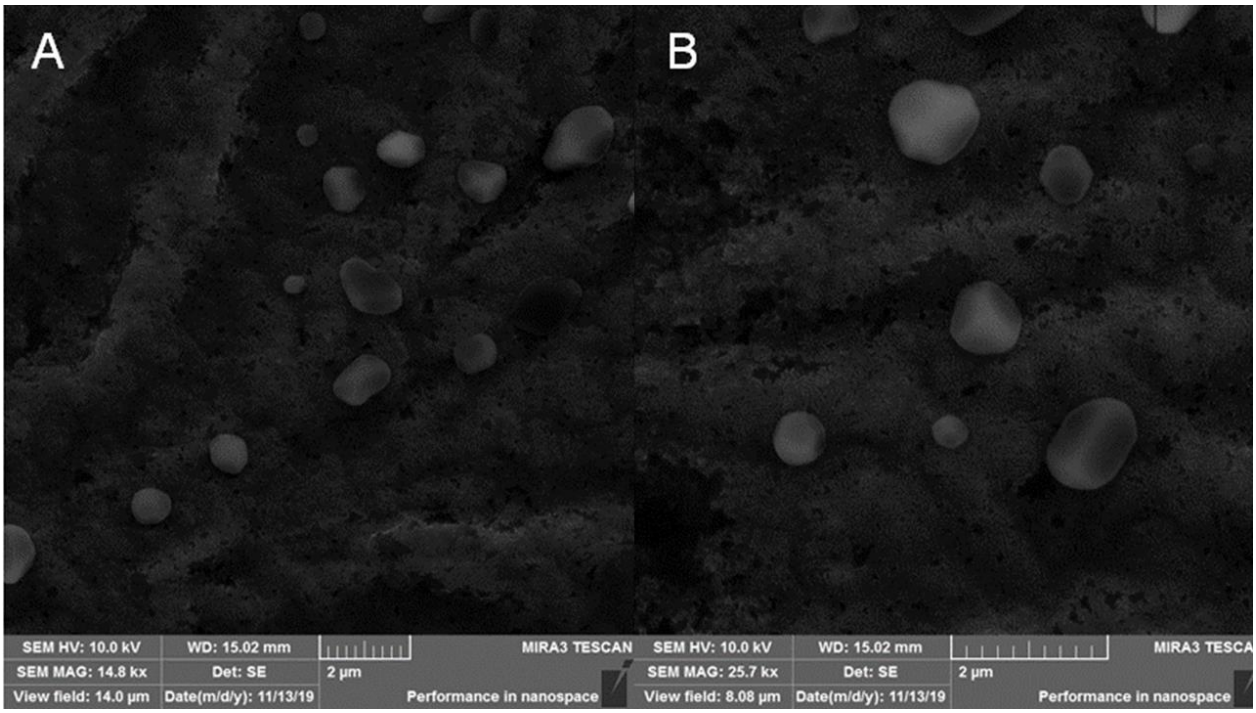

Figure 1. Photomicrographs of freeze-dried Eudragit RS100 nanocapsules NC-E (A) and NC-C (B) by FESEM, magnification from 14,800 (A) to 25,700 Kx (B)

Fourier-Transform Infrared Spectroscopy (FTIR)

Figure 2 shows the FTIR spectra of pure eugenol, pure polymer (Eudragit RS100), lactose monohydrate, and nanocapsule formulations. Regarding to eugenol, a stretching bands of OH group at 3,515 cm^{-1}, a C-H signal of CH_3 group at 2,968 cm^{-1}, a C=C stretching band at 1,607 cm^{-1}, a CH_2 group signal at 1,439 cm^{-1}, and C-O bond signals at 1,269 and 1,032 cm^{-1} were achieved.

For Eudragit RS100, a C=O stretching vibration band of the carbonyl group at 1,741 cm^{-1} was assigned. The same signal was found in the FTIR spectra for the nanoformulations. Considering the lactose, stretching vibration bands of OH group at 3,322 cm^{-1} and C-H group at 2,980; 2,943; and 2,879 cm^{-1} were observed. Nanocapsules NC-E and NC-C showed an overlap of the raw materials signals and no novel signal was evidenced for these formulations. In that sense, no chemical reactions occurred during the nanoencapsulation process.

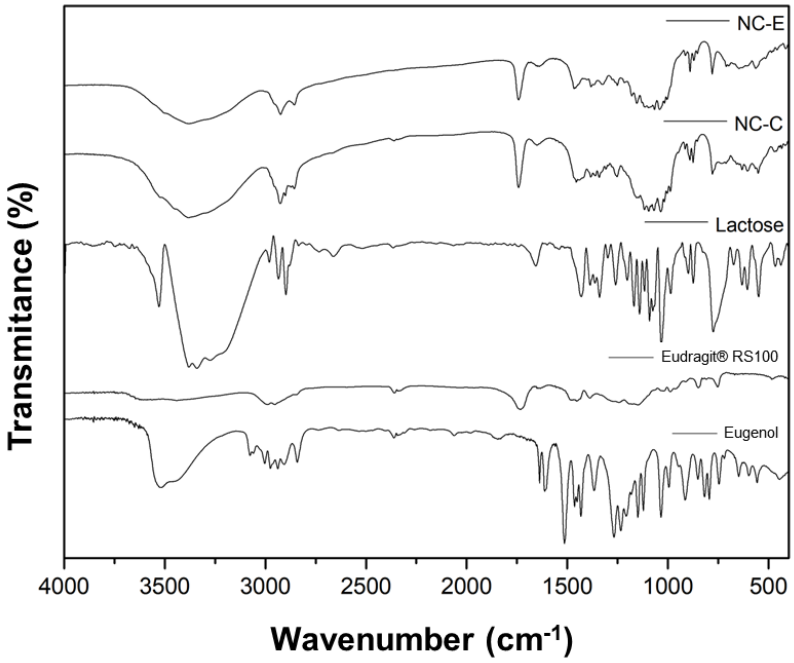

Figure 2. FTIR spectra of pure eugenol, Eudragit RS100, lactose, and nanoformulations NC-E and NC-C.

Method validation

Figure 3 presents the chromatogram of the proposed analytical method for eugenol quantification in the loaded nanoformulation NC-E. Eugenol showed a retention time of 0.72 minutes, which is considered an optimal time for routine analyses. Eugenol identification was possible by its fragmentation as major ions, a qualitative one at *m/z* = 124 and a quantitative one at *m/z* = 137.

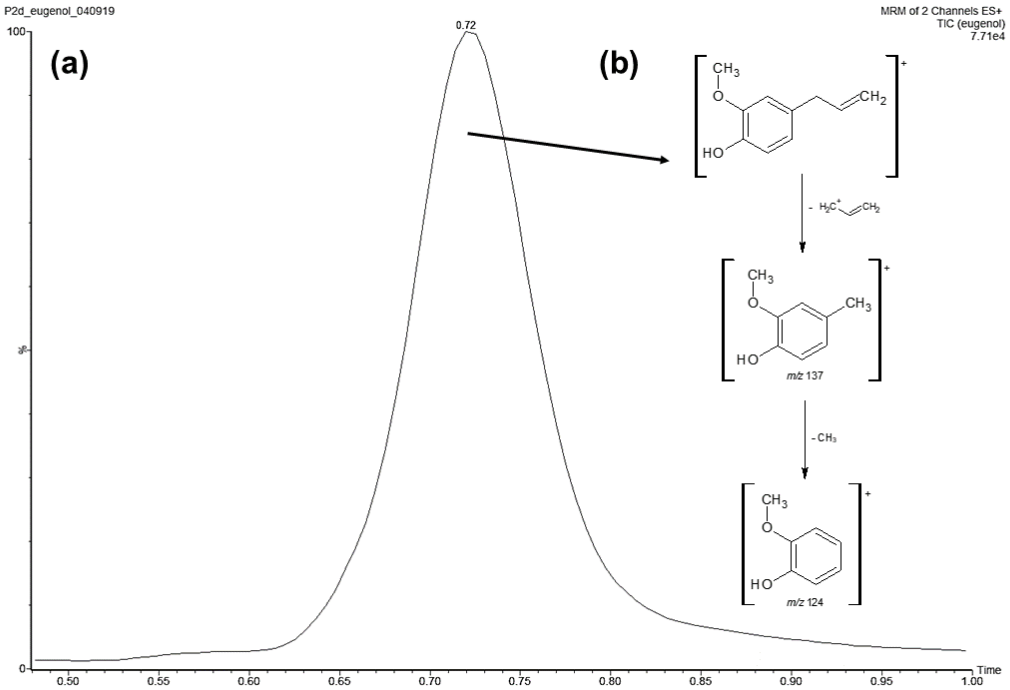

Figure 3. Representative UPLC/MS-MS chromatogram of eugenol standard. Mobile phase: acetonitrile:acidified water with 0.1% formic acid (80:20 V/V); flow rate: 0.3 mL/min; column temperature: 25ºC; and injection volume: 5 µL. Figure detail: eugenol fragmentation at *m/z* = 165.2 → 137; 165.2 → 124.

Linearity was carried out in nine different concentrations of the analyte and in triplicate. The analytical curve is represented in Figure 4.

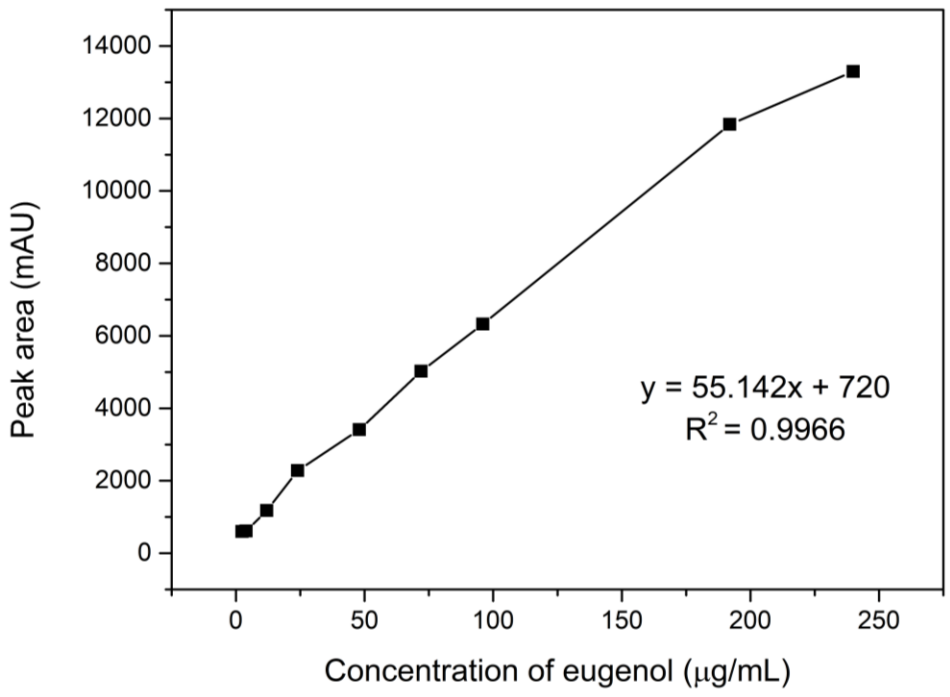

Figure 4. Mean analytical curve for the eugenol determination by UPLC/MS-MS at the concentration range of 2.4 to 240 µg/mL (n = 3)

Considering the linear regression analysis, the angular coefficient, the linear coefficient, and the determination coefficient (r^2) values were obtained. These results are described in Table 3. The linear regression analysis showed no lack of fit by ANOVA.

Table 3. Data obtained by linear regression analysis for the eugenol quantification in nanoformulations by UPLC/MS-MS

Parameters	Results
Range	2.4-240 µg/mL
linear equation	y = 55.142 x + 720
slope (a)	55.142
y-axis intercept (b)	720
determination coefficient (r^2)	0.9966
correlation coefficient (r)	0.9983

LD and LQ values of the analytical method were experimentally determined and resulted in LD = 48 ng/mL and LQ = 480 ng/mL. Precision values are summarized in Table 4. The RSD values obtained were less than 5.0% and the statistical analysis showed that the results do not have a significant difference ($p > 0.05$).

Table 4. Experimental values obtained from the repeatability and the intermediate precision assays

Parameters	Concentration (µg/mL)	RSD* (%)
repeatability (n = 5)	12.8	3.07
	48	3.38
	192	2.71
intermediate precision (n = 9)	12.8	3.89
	48	3.83
	192	4.69

* RSD = relative standard deviation

Accuracy was evaluated using the recovery method and demonstrated recovery percentage values between 97.95 and 98.89% for the three concentration levels evaluated as described in Table 5.

Table 5. Experimental values obtained from the accuracy assay

Theoretical concentration of eugenol (µg/mL)	RSD* (%)	Recovery (%)
12.8 (n = 3)	3.07	97.95
48 (n = 3)	3.38	98.53
192 (n = 3)	2.71	98.89

* RSD = relative standard deviation

The robustness of the analytical method was based on the RSD values obtained from changing of the analysis parameters, such as the analytical column temperature (25.5 ºC), the isocratic flow (0.33 mL/min), and composition of the mobile phase (acetonitrile: acidified water 82:18 V/V). Regarding these values, RSD values were less than 5%, which characterizes the method as robust for all parameters as summarized in Table 6.

Table 6. Experimental values obtained from the robustness assay

Parameters	RSD* (%)
Temperature (25.5 ºC)	3.41
Flow (0.33 mL/min)	3.06
Mobile phase (acetonitrile: acidified water 82:18)	4.62

* RSD = relative standard deviation

Method applicability: Evaluation of Encapsulation Efficiency (EE)

The EE (%) was performed using the previously developed and validated UPLC-MS/MS method and resulted in a mean encapsulation of 93.6% from the initial eugenol used.

Physicochemical stability

The stability testing of eugenol-loaded Eudragit RS100 nanocapsules are described in Figure 5. In brief, the pH and the zeta potential values changed after 90 days of storage. The particle size and the PDI values showed no significant difference during the stability study.

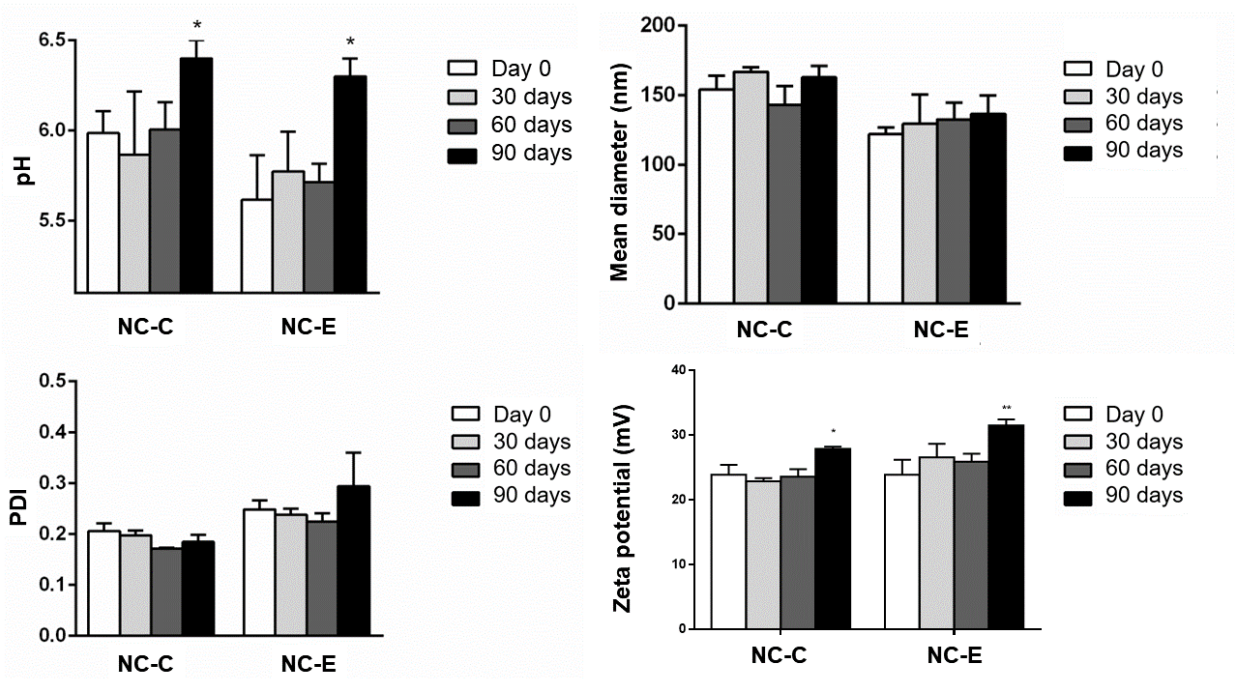

Figure 5. Values of pH, mean diameter, PDI, and zeta potential immediately after nanoencapsulation and after 30, 60, and 90 days of preparation. NC-E: eugenol-loaded Eudragit RS100 nanoparticles; NC-C: negative control. Student's t-test with Tukey's post-hoc test, with significance of *p < 0.05; **p < 0.01

DISCUSSION

Regarding the preparation, the bluish opalescence observed for the nanoformulations is due to the brownian movement of polymeric nanoparticles known as the Tyndall effect as previously reported in the literature [16,26,27]. The eugenol concentration of 1.5 mg/mL was chosen because it is commonly used for formulations containing essential oils and volatile compounds [28, 29].

Considering the characterization, the nanoformulations showed pH values in accordance with other Eudragit RS100 nanocapsule suspensions. NC-E and NC-C presented the expected acid character due to the ester group found in the chemical structure of the (meth)acrylic polymer used [16,30]. The mean diameter values are consistent with polymeric nanocapsules, which generally have a diameter between 100 and 300 nm. Different factors, such as the formulation, the preparation method, the oily nature of the core, the polymer shell, and the presence of the drug [13,16] may influence the particle size.

In particular, the oily nature of the core may change some relevant physicochemical properties, such as viscosity, hydrophobicity, and surface tension. The presence of the drug in the organic phase, before polymer precipitation in the aqueous medium, may also influence the nucleation process, resulting in nanoparticles with a larger diameter and size distribution [16,25].

Polydispersion index (PDI) values close to 0 are considered monodisperse and greater than 0.5 indicate heterogeneous dispersion [16,31,32]. Values similar to those of the present study were found by Adibkia and coauthors (2011) [14] in a stability analysis of Eudragit RS100 nanocapsules containing naproxen, which achieved PDI from 0.12 to 0.29. Zeta potential analysis allows identifying the electrical charges observed on the surface of nanocapsules, which are considered stable when the zeta potential value is close to 30 mV in module [16]. The nanocapsules presented positive zeta potential higher than + 20 mV due to the positive charge of the quaternary ammonium groups from Eudragit RS100 [33,34], which is a polymer of cationic nature [14,35,36]. These values, although not ideal, may provide an acceptable surface stability over days.

The images of FESEM confirmed the nanoencapsulation by the proposed method as well as the suitable nano-sized dimensions of the formulations [37]. The FTIR data demonstrated no chemical interaction between eugenol and Eudragit RS100 in NC-E [38]. Thus, these results indicate that eugenol may be properly encapsulated in the oily core and the polymer is acting only like a drug carrier as expected [17,39].

The retention time achieved for the UPLC-MS/MS experiments was considered ideal for routine analyses of quality control. The chosen method was selective for the analyte detection after the eugenol fragmentation. After this procedure, two major ions were identified. The quantitative ion was set since it presented a larger area under the curve and a higher concentration in relation to the first qualitative ion.

Considering the results observed during the validation process, the developed analytical method was selective, linear, precise, accurate, and robust for the quantification of eugenol in nanocapsules of Eudragit RS100 according to the validation requirements [40,41]. In addition, this method is an alternative to the use of gas chromatography apparatus for the quantification of the volatile compound in a pharmaceutical industry environment.

Concerning the drug encapsulation efficiency (EE), Contri and coauthors (2013) [42] reported an EE higher than 80% for eugenol. Sebaaly; Haydar, and Greige-Gerges (2022) [43] obtained an EE for eugenol of about 68% using conventional liposomes and chitosan-coated liposomes. Talón and coauthors (2019) [44] achieved an EE for eugenol between 95 and 98% using whey protein isolate or lecithin by spray-drying. In this regard, the present study showed an EE equal or better than the other studies previously described in the literature for the encapsulation of eugenol.

The pH values after 90 days of preparation showed a significant change, probably due to the hydrolysis of the medium chain triglycerides that make up the core or the surfactant that are released from the interface when the polymer aggregates [45]. The size distribution and the particle diameters result in particles around 130-200 nm and low polydispersity index, which demonstrates the suitable homogeneity of nanoformulations [13,16]. In addition, the unimodal distribution of the particles indicates that NC-E and NC-C remained dispersed over the time with no aggregation and no flocculation [16,31]. Zeta potential of these samples underwent significant changes after 90 days of storage, which can be due to the polymer degradation and to the changed exposure of positive charges [45]. Based on these data, it can be concluded that eugenol-loaded Eudragit RS100 nanocapsules can be used up to 60 days after preparation with reliable features during this time period.

CONCLUSION

In the present paper, eugenol-loaded Eudragit RS100 nanocapsules (NC-E) were successfully obtained by the interfacial deposition of preformed polymer. Physicochemical analyses confirmed that NC-E and control (NC-C) presented pH, mean diameter, PDI, and zeta potential within the standards for nanocapsules. Morphological and surface analysis by FESEM showed that NC-E and NC-C had a spherical shape with a smooth surface. Structural analysis by FTIR confirmed that there was no chemical reaction between the drug and the polymer. The method developed by UPLC/MS-MS was selective, linear, precise, accurate, and robust for a fast drug determination, and can be used for eugenol quantification and stability studies. The formulation showed high encapsulation efficiency, and the validated analytical method was able to quantify the loaded eugenol in the nanoformulation. The physicochemical stability assays showed that both nanoformulations remained stable for up to 60 days.

Thus, it was concluded that the eugenol-loaded Eudragit RS100 nanocapsules developed can be further used in a pharmaceutical dosage form for the topical application of eugenol in order to decrease dental sensitivity during in-office tooth whitening. In addition, the loaded nanoformulation is suitable for reducing the eugenol problems, such as its volatility and immediate effect.

Funding: The authors are grateful to CNPq (grant numbers 313704/2019-8, 402043/2022-7 and 168149/2022-2) and to Araucaria Foundation for financial support.
Acknowledgments: The authors would like to thank the Multi-user Laboratory Complex at the State University of Ponta Grossa for lab facilities.
Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

1. Paula EA, Kossatz S, Fernandes D, Loguercio AD, Reis A. Administration of Ascorbic Acid to Prevent Bleaching-induced Tooth Sensitivity: A Randomized Triple-blind Clinical Trial. Oper. Dent. 2014;39:128-35.
2. Esmaeili F, Rajabnejhad S, Partoazar AR, Mehr SE, Faridi-Majidi R, Sahebgharani M, et al. A. Anti-inflammatory effects of eugenol nanoemulsion as a topical delivery system. Pharm. Dev. Technol. 2016;21:887-93.
3. Pramod K, Ansari S, Ali J. Eugenol: a natural compound with versatile pharmacological actions. Nat. Prod. Commun. 2010;5:1999-2006.
4. Daniel AN, Sartoretto SM, Schmidt G, Caparroz-Assef SM. Anti-inflammatory and antinociceptive activities of eugenol essential oil in experimental animal models. Rev. Bras. Farmacogn. 2009;19:212-7.
5. Tsuchiya H. Anesthetic agents of plant origin: A review of phytochemicals with anesthetic activity. Molecules. 2017;22:1-34.
6. Moura IR, Rabello TB, Pereira KF. [The influence of eugenol in adhesive procedures]. Rev. Bras. Odon. 2013;70:28-32.
7. Food and Drug Administration. Guidance for industry: Considering Whether and FDA-Regulated Product Involves the Application of Nanotechnology, 2014. Available from: http://www.fda.gov/RegulatoryInformation/Guidances/ucm257698.htm.
8. Pramod K, Aji Alex MR, Singh M, Dang S, Ansari SH, Ali J. Eugenol nanocapsule for enhanced therapeutic activity against periodontal infections. J. Drug Target. 2016;24:24-33.
9. Ahmad N, Ahmad R, Alam MA, Ahmad FJ. Quantification and Brain Targeting of Eugenol-Loaded Surface Modified Nanoparticles Through Intranasal Route in the Treatment of Cerebral Ischemia. Drug Res. 2018; 68:584-95.
10. Hu Q, Gerhard H, Upadhyaya I, Venkitanarayanan K, Luo Y. Antimicrobial eugenol nanoemulsion prepared by gum arabic and lecithin and evaluation of drying technologies. Int. J. Biol. Macromol. 2016;87:130-40.
11. Lal R, Pandey G, Sharma P, Kumari K, Malhotra S, Pandey R, et al. Biochemistry of microbial degradation of hexachlorocyclohexane and prospects for bioremediation. MMBR. 2010;74:58-80.
12. Neckel GL, Lemos-Senna E. [Preparation and characterization of nanocapsules containing camptothecin from poly (D,L-lactic acid) and diblock copolymers of poly (D,L-lactic acid) and polyethylene glycol]. Acta Farm. Bonaer. 2005;24:504-11.
13. Dalcin AJF, Ourique AF, Gomes P. Cationic Nanocapsules Containing Eudragit RS100® and Its Potential for Application in Nanomedicine 1. Discip. Sci. 2017;18:545-66.
14. Adibkia K, Javadzadeh Y, Dastmalchi S, Mohammadi G, Niri FK, Alaei-Beirami M. Naproxen-eudragit® RS100 nanoparticles: Preparation and physicochemical characterization. Colloids Surf. B. 2011;83:155-9.
15. Pignatello R, Amico D, Chiechio S, Spadaro C, Puglisi G, Giunchedi P. Preparation and analgesic activity of eudragit RS100® microparticles containing diflunisal. JDDT. 2001;8:35-45.
16. Schaffazick SR, Pohlmann AR. Caracterização e Estudo de Estabilidade de Suspensões de Nanocápsulas e de Nanoesferas Poliméricas Contendo Diclofenaco [Characterization and Stability Study of Suspensions of Nanocapsules and Polymeric Nanospheres Containing Diclofenac]. Acta Farm. Bonaer. 2002;21:99-106.
17. Rudnik LAC, Lyra AM, Barboza FM, Klein T, Kanufre CC, Farago PV, et al. PEG-PCL Nanocapsules Containing Curcumin: Validation of HPLC Method for Analyzing Drug-loading Efficiency, Stability Testing and Cytotoxicity on NIH-3T3 Cell Line. Braz. Arch. Biol. Technol. 2020;63:1-12.
18. Machado CD, Raman V, Rehman JU, Maia BHLNS, Meneghetti EK, Almeida VP, et al. Schinus molle: anatomy of leaves and stems, chemical composition and insecticidal activities of volatile oil against bed bug (Cimex lectularius). Braz Arch Biol Technol. 2019;29(1):1-10.
19. Oberg C, Pochapski MT, Farago PV, Granado CJF, Pilatti GL, Santos FA. Evaluation of desensitizing agents on dentin permeability and dentinal tubule occlusion: an in vitro study. Gen Dent. 2009;57(5):496-501.
20. Budel JM, Duarte MR, Farago PV, Takeda IJM. Caracteres anatômicos de folha e caule de Calea uniflora Less., Asteraceae [Anatomical characters of leaf and stem of Calea uniflora Less., Asteraceae]. Braz. Arch. Biol. Technol. 2006;16(1):53-60.
21. Almeida MA, Nadal JM, Grassiolli S, Paludo KS, Zawadzki SF, Cruz L, et al. Enhanced gastric tolerability and improved anti-obesity effect of capsaicinoids-loaded PCL microparticles. Mater Sci. Eng. C Mater. Biol. Appl. 2014;40:345-56.
22. ICH (2005) Validation of analytical procedures: text and methodology Q2 (R1). International Conference of Harmonization.
23. Nadal JM, Toledo MdG, Pupo YM, de Paula JP, Farago PV, Zanin SMW. A stability-indicating HPLC-DAD method for determination of ferulic acid into microparticles: Development, validation, forced degradation, and encapsulation efficiency. J. Anal. Methods Chem. 2015;2015:7-11.

24. Camargo GA, Lyra AM, Barboza FM, Fiorin BC, Beltrame FL, Nadal JM, et al. Validation of Analytical Methods for Tacrolimus Determination in Poly(ε-caprolactone) Nanocapsules and Identification of Drug Degradation Products. J. Nanosci. Nanotechnol. 2021;21:1-9.
25. Chassot JM, Ribas D, Silveira EF, Grünspan LD, Pires CC, Farago PV, et al. Beclomethasone dipropionate-loaded polymeric nanocapsules: development, in vitro cytotoxicity, and in vivo evaluation of acute lung injury. J Nanosci. Nanotechnol. 2015;15(1): 855-64.
26. Camargo GA, da Costa Filha ARC, Lyra AM, Novatski A, Nadal JM, de Lara LS, et al. Stability testing of tacrolimus-loaded poly(ε-caprolactone) nanoparticles by physicochemical assays and Raman spectroscopy. Vib. Spectrosc. 2020;110:1-11.
27. Gomes MLS, Nascimento NdS, Borsato DM, Pretes AP, Nadal JM, Novatski A, et al. Long-lasting anti-platelet activity of cilostazol from poly(ε-caprolactone)-poly(ethylene glycol) blend nanocapsules. Mater. Sci. Eng. C. 2019;94:694–702.
28. Ali B, Al-Wabel NA, Shams S, Ahamad A, Khan SA, Anwar F. Essential oils used in aromatherapy: A systemic review. Asian Pac. J. Trop. Biomed. 2015;5:589-98.
29. Budel JM, Wang M, Raman V, Zhao J, Khan SI, Rehman JU, et al. Essential Oils of Five Baccharis Species: Investigations on the Chemical Composition and Biological Activities. Molecules. 2018;23(10):1-19.
30. Patra CN, Richa P, Swain S, Jena GK, Panigrahi KC, Ghose D. Pharmaceutical significance of Eudragit: A review. FJPS. 2017;1:1-52.
31. Markwalter CE, Pagels RF, Wilson BK, Ristroph KD, Prud'homme RK. Flash nanoprecipitation for the encapsulation of hydrophobic and hydrophilic compounds in polymeric nanoparticles. JoVE. 2019;2019:1-13.
32. Lino RC, de Carvalho SM, Noronha CM, Sganzerla WG, da Rosa CG, Nunes MR, et al. Development and Characterization of Poly-ɛcaprolactone Nanocapsules Containing β-carotene Using the Nanoprecipitation Method and Optimized by Response Surface Methodology. Braz. Arch. Biol. Technol. 2020;63:1-12.
33. Pupo YM, Farago PV, Nadal JM, Simão LC, Esmerino LA, Gomes OMM, et al. Effect of a novel quaternary ammonium methacrylate polymer (QAMP) on adhesion and antibacterial properties of dental adhesives. Int. J. Mol. Sci. 2014;15(5):8998-9015.
34. Englert AV, Verdi CM, Santos RCV, Cruz L, Sari MHM. Diphenyl Diselenide and Clotrimazole Co-loaded into Eudragit® RS 100 Nanocapsules Formulation Has Superior Antioxidant Potential and Promising Anticandida Activity. Braz. Arch. Biol. Technol. 2020;63:1-12.
35. Chaves PdS, Ourique AF, Frank LA, Pohlmann AR, Guterres SS, Beck RCR. Carvedilol-loaded nanocapsules: Mucoadhesive properties and permeability across the sublingual mucosa. Eur J Pharm Biopharm. 2017;114:88-95.
36. Domingues GS, Guterres SS, Beck RCR, Pohlmann AR. [Nanocoated microparticles containing a hydrophobic model drug: one-step preparation and biopharmaceutical characterization]. Quím. Nova. 2008; 31:1966-1972.
37. Gaspar MDdR, Lyra AM, Camargo GdA, Nadal JM, Teitelbaum AP, dos Santos FA, et al. Development and Validation of Reversed-Phase UHPLC-PDA Method for Determination of Chlorhexidine in Meth(Acrylic) Nanocapsules. Lat. Am. J. Pharm. 2020;39:792-8.
38. Gomes MLS, Nascimento NdS, Borsato DM, Pretes AP, Nadal JM, Novatski A, et al. Long-lasting anti-platelet activity of cilostazol from poly (ε-caprolactone)-poly (ethylene glycol) blend nanocapsules. Mater Sci Eng C Mater Biol Appl. 2019;94(1):694-702.
39. Camargo G dos A, Ferreira L, Schebelski DJ, Lyra AM, Barboza FM, Carletto B, et al. Characterization and In Vitro and In Vivo Evaluation of Tacrolimus-Loaded Poly(ε-Caprolactone) Nanocapsules for the Management of Atopic Dermatitis. Pharmaceutics. 2021;13(21):1-19.
40. Brasil. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. [Provides for the validation of analytical methods and other measures]. Resolução nº 166, de 24 de julho de 2017. Diário Oficial da União, Brasília, 25 jul. 2017: 22.
41. Ribani M, Bottoli CBG, Collins CH, Jardim ICSF, Melo LFC. [Validation in Chromatographic and Electrophoretic Methods]. Quím. Nova. 2004;27:771-80.
42. Contri RV, Ribeiro KLF, Fiel LA, Pohlmann AR, Guterres SS. Vegetable oils as core of cationic polymeric nanocapsules: influence on the physicochemical properties. J. Exp. Nanosci. 2013;8:913-24.
43. Sebaaly C, Haydar S, Greige-Gerges H. Eugenol encapsulation into conventional liposomes and chitosan-coated liposomes: A comparative study. J Drug Deliv Sci Technol. 2022;67:102942.
44. Talon E, Lampi A-M, Vargas M, Chiralt A, Jouppila K, González-Martínez C. Encapsulation of eugenol by spray-drying using whey protein isolate or lecithin: release kinetics, antioxidant and antimicrobial properties. Food Chem. 2019;295:588-98.
45. Melo NFSd, Grillo R, Fraceto LF, Dias Filho NL, Paula Ed, Araújo DRd, et al. [Development and characterization of poly (L-lactide) nanocapsules containing benzocaine]. Quim. Nova. 2010;33:65-9.