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Article

Preparation of mupirocin-loaded polymeric nanocapsules using essential oil of rosemary

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The purpose of this study was to prepare and characterize mupirocin-loaded polymeric nanocapsules using two different oils and to develop and validate an analytical method for quantitative determination by high performance liquid chromatography. The mean size of the nanoparticles was 233.05 nm and 275.03 nm for nanocapsules with a rosemary oil like oily core and caprylic/capric triglyceride, respectively, and a good polydispersity index below 0.25 for both formulations. The nanocapsules showed good stability when stored at 40 ºC and room temperature for 30 days. The quantitative method was performed with a mobile phase consisting of ammonium ammonium acetate (0.05 M adjusted to pH 5.0 with acetic acid) and acetonitrile 60:40 (v/v); the flow rate was 0.8 mL/min, UV detection at 230 nm. The analytical method was linear in the range of 5.0-15.0 µg/mL, specific for both oils, accurate, precise (intermediate precision RSD = 1.68% and repeatability RSD = 0.81%) and robust under the evaluated conditions. Therefore, this method can be performed for quantification of mupirocin in polymeric nanocapsules containing both oils.

Uniterms: Mupirocin/High Performance Liquid Chromatography. Mupirocin/Polymeric nanocapsules. Rosemary oil.

INTRODUCTION

Mupirocin (MUP) is an antibiotic used to treat skin surface infections. It is highly effective against *Staphylococcus* and *Streptococcus,* and is utilized to control methicillin-resistant *Staphylococcus aureus*. The chemical structure consists of a chain of fatty acids loaded on monic acid by an ester type linkage that mimics the carbon skeleton of isoleucine, competing with this aminoacid for the active site of isoleucyl-tRNA bacterial synthetase, inhibiting bacterial protein synthesis. However, reports of increased resistance to mupirocin are a matter of concern (Martindale, 2011; Poovelikunnel, Gethin, Humphreys, 2015; Sutherland *et al.*, 1985; Thomas *et al.,* 2010).

MUP is a crystalline white powder, slightly soluble in water, freely soluble in acetone and dichloromethane. It is a strong acid with a pKa value 4.83, presenting a molecular formula $C_{26}H_{44}O_9$, melting point about 77–78 ºC and a partition coefficient of 2.25 (British Pharmacopeia, 2012; Martindale, 2011; USP, 2012).

Recently, several studies have reported utilizing molecules with activity against microorganisms that are capable of forming biofilm in different places (Comin *et al.*, 2016; Iannitelli *et al.,* 2011; Jain *et al.,* 2009; Maryam *et al.,* 2015). Along these lines, MUP-loaded polymeric nanocapsules were developed by our research group in an attempt to increase the activity of MUP against *Staphylococcus aureus*.

Nanocapsules are vesicular systems in which the drug is confined to a cavity surrounded by a single polymer. These systems present a particle size less than 1 µm. They have the capacity to control the drug release profile, increase the stability of drugs during storage and provide vectoring through organs and cells (Couvreur *et al.*, 2002; Schaffazick *et al.*, 2003, Zili, Sfar, Fessi, 2005).

In order to ensure reliable and interpretable information about a sample, the analytical methods must first be validated. The validation of the analytical method is a continuous process that starts by planning an analytical

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and continuous strategy throughout the development period (Ribani *et al.*, 2004).

Some analytical methods have been described in the literature for the determination of MUP in pharmaceutical formulations, liposomal hydrogels, and penetrations studies (Berg, 2011; Echevarría *et al.,* 2003; Jagota *et al.,* 1992; Shailesh, Kulkarni, 2014). Official techniques for quality control of MUP in raw material, cream, ointment and, nasal ointment are described in the United States Pharmacopeia (USP, 2012) and British Pharmacopeia (2012).

Due to the lack of a methodology for the determination of MUP in polymeric nanocapsules, this study aimed to develop and validate a method for the determination of MUP in polymeric nanocapsules containing essential oil of rosemary and caprylic/capric triglyceride, as well as to study the stability and the physical-chemical properties of the different formulations).

MATERIAL AND METHODS

Reagents and materials

MUP with 100.0% purity standard substance was obtained from The United States Pharmacopeia, batch G0M003. The MUP raw material batch: 15537/2014 (purity 96.9%) was kindly supplied by Cristália®. The polymeric nanocapsule excipients were: poly(ε-caprolactone) (PCL, MW: 70000-90000) and sorbitan monostearate 60 (Span®60) which were purchased from Sigma-Aldrich® (São Paulo, Brazil), essential oil of rosemary was purchased from Petite Marie® (Itaquaquecetuba, Brazil), caprylic/capric triglyceride (Crodamol® GTCC) from Alpha Química® (Porto Alegre, Brazil), polysorbate 80 (Tween® 80) purchase from Synth® and acetone from Synth®. All other reagents and solvents used were of analytical grade.

Instrumentation and chromatography conditions

The apparatus used for the LC analysis was a Shimadzu® system (Kyoto, Japan), equipped with an LC-20AT pump, SIL-20A ht auto sampler, CTO-20AC column oven, SPD-M20A PDA detector, CBM-20A system controller, and LC solution software was used to quantify the samples. The Ultra Basic Denver potentiometer was used to determine the pH of all solutions. Chromatography separations were achieved using the modified method of the British Pharmacopeia (2012). The separations were performed with a Merck® C18 column (250 mm x 4.6 mm, 5 μm) at 25 °C. The mobile phase composition was ammonium acetate (0.05 M adjusted to pH 5.0 with acetic acid) and acetonitrile 60:40 (v/v). The flow rate was 0.8 mL/min, the UV detection was set at 230 nm and the injected volume was 10 μL.

Development and characterization of polymeric nanocapsules containing mupirocin

Before polymeric nanocapsules were developed, drug solubility had to be studied in two different oils. Briefly, an excess amount of MUP was transferred to an individual erlenmeyer containing 2 mL of each oil. The flasks were covered and shaken at 120 rpm in an orbital incubator (Novatecnica®, NT712) for 24 hours at 37 ± 0.5 ºC. After equilibrium, samples were centrifuged at 4000 rpm for 10 minutes, and then the concentration of MUP in oil was determined by the HPLC method described above.

Polymeric nanocapsules containing MUP were prepared by nanoprecipitation of pre-formed polymers (Fessi *et al.*, 1989). The organic phase was constituted by PCL (100.0 mg), sorbitan monostearate 60 (76.6 mg), essential oil of rosemary (330.0 mg) and mupirocin (10.0 mg) dissolved in acetone (27.0 mL). This organic phase was added with moderate magnetic stirring into an aqueous phase constituted by the polysorbate 80 (76.6 mg) dissolved in water (55.0 mL). The aqueous phase quickly turned milky with bluish opalescence due to the formation of the nanoparticles. After nanoprecipitation, the acetone and a part of water were removed in a rotary evaporator and the nanoparticles were concentrated to a final volume of 10 mL (1.0 mg/mL of MUP). This formulation was called polymeric nanocapsules of mupirocin and rosemary (NCMR). The polymeric nanocapsules containing caprylic/capric triglyceride were prepared using the same procedure, but the core oil was formed by caprylic/capric triglyceride (330.0 mg). Thus, the given name of this formulation was polymeric nanocapsules of mupirocin and caprylic/capric triglyceride (NCMT).

The unloaded nanocapsules of rosemary oil and caprylic/capric triglyceride were called BNCR and BNCT, respectively. These formulations were prepared by the same method but without adding the drug.

Characterization of polymeric nanocapsules

Particle-size, zeta potential and pH: the zeta potential and particle-size distribution of the formulations were determined by Zetasizer Nano ZS® (Nanoseries, Malvern, UK). In both determinations, samples were

diluted in Milli-Q water. The pH values were determined by an Ultra Basic Denver® potentiometer calibrated with pH 4.0 and 7.0 solutions. The nanoparticles were analyzed directly on the electrode. The reported results are the mean values obtained after analyzing three batches of each formulation.

Determination of drug content and encapsulation efficiency

For the analysis of drug from nanocapsules, a quantity equivalent to 1000 µg of drug was transferred to a 10 mL volumetric flask and dispersed in acetonitrile. To optimize the extraction method the volumetric flasks were kept under sonication for 15 min. Then a 1.0 mL aliquot was pipetted into a 10 mL volumetric flask and diluted with the same solvent. The samples were then filtered using a nylon membrane with 0.45 µm porosity. The analysis was performed using the previously developed HPLC method.

To determine the encapsulation efficiency 300 µL of each formulation were placed in Amicon Ultra® filters and centrifuged at 10000 rpm for 15 minutes. The encapsulation efficiency was calculated by the difference between the total drug content and the drug content found in the ultrafiltrate.

Stability evaluation

The different nanoparticles were kept at room temperature and at 40 ºC for 30 days. The effects of storage time (0, 15 and 30 days) on the pH, particle size, zeta potential, drug content and encapsulation efficiency were determined. The stability studies were performed in three different batches of each formulation.

Development and validation of the HPLC method

The method was validated according to the official guidelines (ANVISA, 2003; ICH, 2005; INMETRO, 2007). The parameters evaluated were: specificity, linearity, precision, accuracy, robustness, detection and quantification limits. The polymeric nanocapsules (NCMR) were used to develop this method.

Specificity

Method specificity was assessed using a standard solution containing MUP, samples of MUP-loaded polymeric nanocapsules (NCMR and NCMT) and nanoparticles without MUP (BNCR and BNCT). Moreover, the specificity was evaluated for stress testing (ICH, 2005). All the samples were analyzed in triplicate. The stress conditions follow:

Hydrolytic conditions: 10 mg of MUP reference substance were dissolved in a 10 mL volumetric flask with acetonitrile. Then, a 1 mL aliquot was transferred to a 10 mL volumetric flask and dissolved in HCl 0.1 M and NaOH 0.1 M. After 24 hours, the samples were neutralized and analyzed.

Photolytic degradation: 10 mg of MUP reference substance were dissolved in a 10 mL volumetric flask with acetonitrile. Next, a 1 mL aliquot was transferred to a 10 mL volumetric flask and dissolved with acetonitrile. This solution was exposed to UV light (λ = 254 nm) for 24 hours. Then, a 1 mL aliquot was transferred to a 10 mL volumetric flask and dissolved with acetonitrile.

Oxidative condition: 10.0 mg of MUP reference substance were dissolved in a 10 mL volumetric flask with acetonitrile. After that, a 1 mL aliquot was transferred to a 10 mL volumetric flask and dissolved in hydrogen peroxide 3%. After, 24 hours, a 1 mL aliquot was transferred to a 10 mL volumetric flask and dissolved in acetonitrile.

Temperature condition: 10 mg of MUP reference substance were dissolved in a 10 mL volumetric flask with acetonitrile. Then, a 1 mL aliquot was transferred to a 10 mL volumetric flask and dissolved with acetonitrile. This solution was exposed at 40 ºC. After 24 hours, a 1 mL aliquot was transferred to a 10 mL volumetric flask and dissolved in acetonitrile.

Linearity and detection and quantification limits

Linearity was determined by constructing three independent analytical curves (n=3) with five linear concentrations of MUP (5.0, 8.0, 10.0, 12.0 and 15.0 µg/mL). All concentrations were diluted in acetonitrile and analyzed in triplicate on three consecutive days. The results were assessed by regression analysis using the least squares method to calculate the calibration curves. The detection and quantification limits were determined using an average standard curve, considering the intercept standard deviation and slope.

Precision

Precision was investigated with respect to repeatability (intra-day) and intermediate precision (inter-day). Repeatability was assessed by assaying the concentrations of 5.0, 10.0 and 15.0 µg/mL, in triplicate during the same day, by the same analyst and using

the same instrumentation. Intermediate precision was assessed by carrying out the same analysis on 3 different days, with different instrumentation and analyst. This parameter was expressed as % of relative standard deviation (RSD).

Accuracy (Recovery method)

Accuracy was evaluated at concentration levels of 80, 100 and 120% where a known amount of MUP standard solution was added to sample solution. The theoretical and the measured concentration were then compared. The experiments were repeated three times.

Robustness

Robustness was evaluated using the method proposed by Youden, Steiner (1975). Small variations were induced in the nominal values of the method. The four parameters and the variations variations introduced are shown in Table I. Then, eight runs were performed to determine the influence of each parameter on the final results. According to Nogueira *et al.* (2011), this method will be robust if conditions 1 and 2 are met:

Condition 1: (for factor A and other factors) content of MUP – 5% ≤ A ≤ content of MUP + 5%.

Condition 2: (for factor A and other factors) A – a ≤ 3% involving the MUP content.

An aliquot of each sample was transferred into an individual 10 mL volumetric flask, diluted to volume with acetonitrile, and filtered through a nylon membrane with 0.45 µm porosity, obtaining the final MUP concentration of 10.0 µg/mL. The concentrations of MUP presented in samples were determined from the analytical curve.

System suitability

System suitability was evaluated by five replicate analyses of MUP reference substance at a concentration of 10 µg/mL. The parameters assessed were: number of theoretical plates and tailing factor.

Statistical analysis

All tests were performed in 3 replicates and the results were expressed as average ± standard deviation. The results were submitted to (ANOVA) followed by Tukey test at a significance level of 5%.

RESULTS AND DISCUSSION

Development and optimization of the method

The development of safe, reliable analytical methods is a very important tool for the quality control of pharmaceutical products and raw material. The chromatography technique is a good alternative for performing analysis of drug from nano-based formulations, since it is necessary to have a total separation of the drug from the formulation components (Harter *et al.*, 2014).

Based on the official method (British Pharmacopoeia, 2012), some chromatography conditions were evaluated to optimize this method. Firstly, a mobile phase was tested containing a mixture of methanol and water (75:25 v/v), however, it was not stable at baseline. Another mixture tested was methanol and water pH 5.0 (60:40 v/v), but this condition affected the retention time and shape of the MUP peak. Similar results were shown by Amrutiya, Madan, Bajaj (2010) when researching the simultaneous quantification of prednicarbate, MUP and ketoconazole in topical dosage form. In this work several eluent mixtures and pH values were evaluated to obtain a good separation.

System suitability

To obtain the best chromatographic method, the mobile phase was composed by ammonium acetate (0.05 M, adjusted to pH 5.0 with acetic acid) and acetonitrile 60:40 (v/v), with a flow rate of 0.8 mL/min. These conditions were utilized to provide an adequate peak and satisfactory results according to criteria evaluated. Five replicates of MUP reference substance were evaluated at a concentration of 10 µg/mL. After chromatogram analysis, the number of

TABLE I - Parameters and variations to evaluate the robustness of the chromatography method

Parameter	Variations	
Mobile phase rate (mL/min)	A - 1.0	a - 0.6
Acetonitrile concentration in mobile phase (%)	B - 42	b – 38
Sonication time for extraction of drug (minutes)	C - 20	c - 10
Column supplier	D - Phenomenex	d – Sunfire

theoretical plates and tailing factor were determined. The average values obtained were 4628 and 1.19, respectively.

Specificity

The chromatograms (Figure 1) obtained during the specificity test showed that none of the formulation excipients were eluted in the same retention time as the MUP peak.

Furthermore, the interference of potential degradation products was investigated through a forced degradation test. These studies were performed to identify the factors that would affect the drug stability. Usually the range of degradation is 10% to 30% (Chan *et al.*, 2004).

After evaluation of stress conditions (temperature 40 ºC and oxidative 3%), the concentration remained constant and no possible degradation product of the MUP reference substance was found. In acid and basic hydrolysis, as well as under photolytic conditions (Figure 2) the residual drug content after 24 hours was 10.23%, 6.76% and 16.93%, respectively in relation to the value obtained in the initial analysis.

In the chromatograms obtained after acid and basic hydrolysis, the formation of possible degradation products can be seen at about 3 min and 7 min, respectively.

Linearity

According to Figure 3, the linearity was observed over the concentration range of 5.0 to 15.0 µg/mL, and the analytical curve equation obtained was y = 18386x – 9906 (where, x is concentration and y is the peak absolute area). The statistical analysis showed significant linear regression ($F_{cal} = 6171.664 > F_{tab} = 4.96$) and no significant deviation from linearity ($F_{cal} = 2.0031 < F_{tab} = 3.71$).

The quantification and detection limits were 1.68 µg/mL and 0.56 µg/mL, respectively demonstrating the sensibility of the method for low concentrations.

Precision

This parameter was evaluated as intermediate precision and repeatability and was expressed as relative standard deviation (RSD %). Accordingly, Tables II and III show the mean RSD values of 1.68% and 0.69%, respectively, indicating the method precision over the concentration range. The limit recommended for this evaluation is RSD less than 5% (ANVISA, 2003).

Accuracy (Recovery method)

Three concentration levels and three replicates of each concentration were used to determine accuracy. The average percentage obtained was 98.85%–101.39% satisfying the acceptance criteria between 98.0% and 102.0% for this study (Shabir, 2003).

Robustness

The method described by Youden, Steiner (1975), makes it possible not only to evaluate method robustness, but also to sort the variation of each parameter in the final results. According to Tables IV and V, the values showed satisfactory robustness of the method.

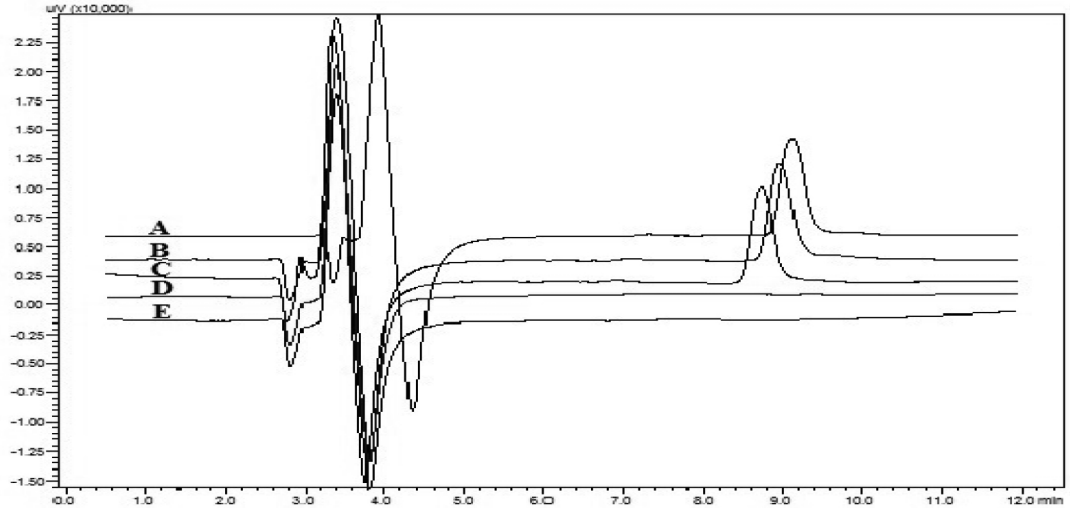

FIGURE 1 - Chromatograms corresponding to: (A) solution of MUP reference substance, (B) Nanocapsules of MUP and caprylic/capric triglyceride oil, (C) Nanocapsules of MUP and rosemary oil, (D) Unloaded nanocapsules of rosemary oil and (E) unloaded nanocapsules of caprylic/capric triglyceride oil.

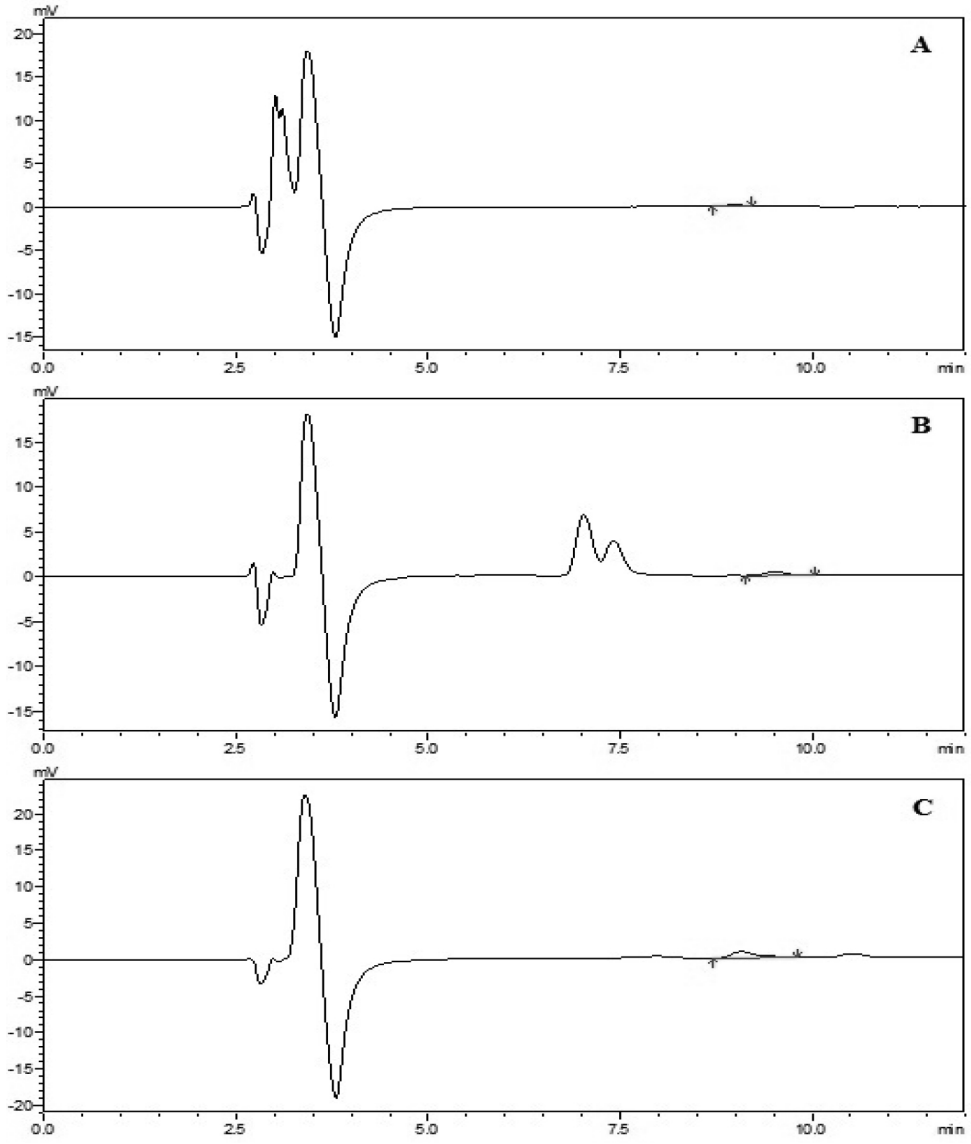

FIGURE 2 - Chromatograms corresponding to: (A) solution of MUP time 24 hours after acid hydrolysis, (B) solution of MUP time 24 hours after basic hydrolysis and (C) solution of MUP time 24 hours after photolytic conditions.

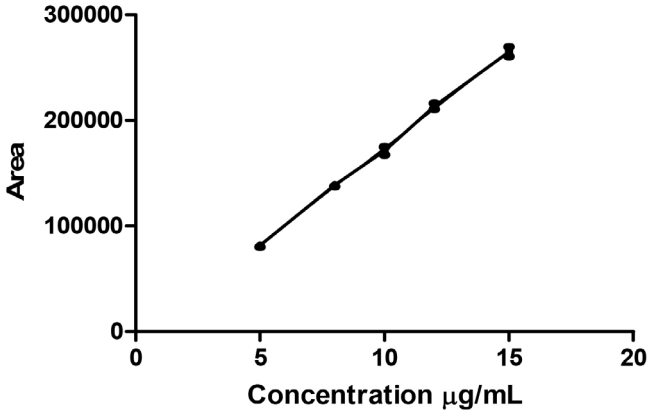

FIGURE 3 - Analytical curve of MUP.

The variation of some method parameters did not change the MUP content in the sample significantly. The results meet conditions 1 and 2, and were therefore considered a robust method for the determination of MUP-loaded polymeric nanocapsules.

Preparation and characterization of polymeric nanocapsules

Blank formulations and MUP-loaded polymeric nanocapsules were prepared through a nanoprecipitation technique. Table VI summarizes some physical-chemical characteristics after preparation of formulations containing the different oils.

TABLE II - Values obtained for the intermediate precision determination for MUP-loaded polymeric nanocapsules with rosemary oil

Day	N	Analyst 1	Analyst 2	Average ± SD	RSD (%)
1	1	98.89	96.16	98.69 ± 1.371	1.39
	2	99.98	99.68		
	3	99.10	98.33		
2	1	99.13	97.71	99.92 ± 1.858	1.86
	2	103.20	100.06		
	3	100.38	99.04		
3	1	100.59	101.24	100.59 ± 1.791	1.78
	2	100.80	102.48		
	3	97.19	101.22		
Average content (%)				99.73	
Average RSD (%)					1.68

RSD – relative standard deviation. SD – standard deviation.

TABLE III - Values obtained for the repeatability determination for MUP-loaded polymeric nanocapsules with rosemary oil

Sample concentration µg/mL	Drug content (%)	Average ± SD	RSD (%)
5	98.38	98.49 ± 0.1629	0.17
	98.42		
	98.68		
10	97.98	97.92 ± 0.9063	0.93
	98.80		
	96.99		
15	98.56	98.94 ± 0.9640	0.97
	100.04		
	98.23		
Average content (%)		98.45 ± 0.5112	
Average RSD (%)			0.69

RSD – relative standard deviation. SD – standard deviation.

TABLE IV - Combinations tested to evaluate the robustness, evaluating condition 1

	Combination assay							
Results	**s**	**t**	**u**	**v**	**w**	**x**	**y**	**z**
Content[a] (%)	98.73	99.04	99.61	100.02	99.51	98.52	99.19	99.05
Average content (%)	99.21							
RSD (%)	0.49							
Acceptable range for content of mupirocin (%)	$94.74\% \leq 99.73^{b} \leq 104.72\%$							

[a]average of three determinations. [b]value obtained of intermediate precision. RSD - relative standard deviation.

Before preparing the nanocapsules, the drug solubility was determined between different oils. The solubilities of MUP in rosemary oil and caprylic/capric triglyceride were 263.8 mg/mL and 54.3 mg/mL respectively. All the formulations presented particle sizes lower than 300 nm with a good monomodal distribution profile. The particle sizes were 233.05 ± 1.94 nm and 280.13 ± 2.91 nm for the nanocapsules with rosemary oil and caprylic/capric

TABLE V - Combinations tested to evaluate the robustness, evaluating condition 2

Content of mupirocin (%)		Difference (%)	Limits for the difference (3%*99.73[a]%)	
A = 99.35	a = 99.07	A – a = 0.28	2.99	
B = 98.95	b = 99.47	B – b = - 0.52	2.99	
C = 99.26	c = 99.16	C – c = 0.10	2.99	Robust
D = 99.00	d = 99.42	D – d = - 0.42	2.99	
E = 98.98	e = 99.44	E – e = - 0.46	2.99	
Average content (%): 99.21				
SD: 0.2031				
RSD (%): 0.2				

[a] value of intermediate precision obtained. SD – standard deviation. RSD - relative standard deviation.

TABLE VI - Physical-chemical characteristics of nanocapsules of MUP and rosemary oil (NCMR), nanocapsules of MUP and caprylic/capric triglyceride oil (NCMT), unloaded nanocapsules of rosemary (BNCR) and unloaded nanocapsules of caprylic/capric triglyceride (BNCT)

Samples	Mean size[b] ± S.D. (nm)	ζ-Potential[b] (mV)	pH[b]	Encapsulation Efficiency[b] (% w/w)	Drug content[b] ± S.D. (%)
NCMR	233.05 ± 1.94 (0.25)[a]	-33.44	4.5 ± 0.78	97.46	100.50 ± 0.15
BNCR	213.00 ± 2.66 (0.22)[a]	- 33.97	5.6 ± 1.08	------	------
NCMT	275.03 ± 2.91 (0.22)[a]	-32.21	4.4 ± 0.84	84.61	99.20 ± 0.10
BNCT	229.77 ± 1.72 (0.19)[a]	- 31.10	5.8 ± 0.19	------	------

[a] polydispersity index. [b] values represent the average between the 3 batches.

triglyceride oil, respectively. The polydispersity index (PDI) found was 0.25 and 0.22 indicating a uniform system size for both formulations. Similar results have been reported for essential oil of rosemary when prepared by the nanoprecipitation method (Ephrem *et al.*, 2014). A PDI value lower than 0.5 is appropriate for colloidal suspension, indicating that the distribution size of the nanocapsules is homogeneous (Kumar *et al.*, 2015; Wu, Zhang, Watanabe, 2011).

The pH values for NCMR and NCMT were 4.5 and 4.4, however for BNCR and BNCT the pH values were 5.6 and 5.8. These values can be justified by the acidic nature of the drug and due to the utilization of PCL around the core oil. In general, the pH values of the polymeric nanocapsules can vary from 3.0 to 7.5 when prepared according to the nanoprecipitation method (Mora-Huertas, Fessi, Elaissari, 2010).

Mupirocin was quantified after 15 min of extraction using acetonitrile as solvent. The average drug content in different formulations was 100.05 ± 0.15% (NCMR) and 99.2 ± 0.10 % (NCMT).

Stability evaluation

In order to evaluate the stability of MUP-loaded polymeric nanocapsules, the formulations containing the drug and unloaded nanocapsules were stored at room temperature and at 40 ºC for 30 days. The stability was evaluated by comparing the initial particle size, zeta potential, pH, encapsulation efficiency and drug content with those obtained after 30 days (Table VII).

After 30 days of storage at room temperature and 40 ºC, considering the average particle size, all formulation exhibited a similar characteristic when compared to the formulation at the initial time ($p > 0.05$). After 30 days of storage at 40 ºC, the pH values were significantly reduced in all formulations compared to the formulation at the initial time ($p<0.05$). This can be explained by the higher temperature, promoting increased polyester rates of hydrolysis and a reduction of the pH values and acidic characteristic of the drug (Mallin *et al.*, 1996).

The MUP-loaded polymeric nanocapsules and unloaded nanocapsules exhibited a similar zeta potential

TABLE VII - Physical-chemical characteristics after stability test of polymeric nanocapsules at room temperature and 40 ºC

Samples	Mean size [b] ± SD (nm)		ζ-Potential [b] (mV)		pH[b]		Encapsulation efficiency [b] (% w/w)		Drug content [b] ± SD (%)	
15 days	25 ºC	40 ºC	25 ºC	40 ºC	25 ºC	40 ºC	25 ºC	40 ºC	25 ºC	40 ºC
NCMR	238.56 ± 0.21	238.05 ± 1.08	-33.7 ± 1.85	-24.1 ± 1.02	4.4 ± 0.12	4.1 ± 0.11	97.10	95.53	99.51 ± 0.24	97.92 ± 0.14
BNCR	221.02 ± 0.14	210.89 ± 1.58	-30.2 ± 1.08	-20.7 ± 1.77	5.4 ± 0.06	4.4 ± 0.14	------	------	------	------
NCMT	268.8 ± 0.84	279.93 ± 1.26	-30.4 ± 0.88	-28.2 ± 1.08	4.5 ± 0.14	3.8 ± 0.09	83.19	82.48	98.90 ± 0.21	97.50 ± 0.37
BNCT	224.53 ± 0.15	234.21 ± 0.19	-32.8 ± 1.21	-28.5 ± 1.78	5.7 ± 0.17	4.5 ± 0.11	------	------	------	------
30 days										
NCMR	235.42 ± 0.64	241.03 ± 1.29	-31.53 ± 0.78	-25.20 ± 1.02	4.48 ± 0.07	3.67 ± 0.18	98.83	96.02	98.18 ± 1.87	98.44 ± 0.82
BNCR	218.89 ± 0.71	238.18 ± 1.04	-29.57 ± 1.94	-22.58 ± 0.88	5.42 ± 0.07	4.01 ± 0.05	------	------	------	------
NCMT	273.57 ± 1.57	281.03 ± 1.35	-31.95 ± 1.33	-27.48 ± 0.91	4.38 ± 0.08	3.46 ± 0.11	84.11	85.43	99.29 ± 1.66	97.05 ± 1.3
BNCT	232.85 ± 0.85	236.85 ± 0.38	-30.01 ± 0.11	-29.73 ± 1.78	5.67 ± 0.04	4.31 ± 0.09	------	------	------	------

All samples showed a polydispersity index lower than 0.25. [b] Values represent the average between the 3 batches. Nanocapsules of mupirocin and rosemary oil (NCMR). Nanocapsules of mupirocin and caprylic/capric triglyceride oil (NCMT). Unloaded nanocapsules of rosemary oil (BNCR). Unloaded nanocapsules of caprylic/capric triglyceride oil (BNCT).

under both conditions (room temperature and 40 ºC). All values were negative, which confirms the stability of nanocapsules. A zeta potential value around ±30 mV is assumed to be good for formulations (Kumar *et al.*, 2015). The negative potential values of the samples are related to the presence of polysorbate 80, presenting a negative surface density of charge (Marchiori *et al*., 2010).

CONCLUSIONS

The rosemary and caprylic/capric triglyceride oils were used as oily core for the formulations. The nanoparticles showed adequate particle size, monomodal size distributions and low polydispersity index. All samples showed a reduction of pH value after storage at 40 ºC. On the other hand, the formulations showed adequate zeta potential according to the literature and appropriate quantity of mupirocin. The proposed analytical method was linear, selective for both oils, precise, accurate and robust for the determination of MUP-loaded polymeric nanocapsules, showing that it is a useful method for quality control of the proposed delivery system. The results of the present study showed promising data for the development of a new formulation containing MUP and rosemary oil in an attempt to increase the activity of MUP against *Staphylococcus aureus.*

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