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ORIGINAL ARTICLE

A simple reversed phase high-performance liquid chromatography (HPLC) method for determination of *in situ* gelling curcumin-loaded liquid crystals in *in vitro* performance tests

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Abstract A simple, rapid, and sensitive analytical procedure has been developed and validated for the *in vitro* measurement of curcumin in samples from mucosae retention studies. Curcumin was analyzed by HPLC using a C_{18} column with UV detection at 425 nm. The mobile phase was acetonitrile and water (50:50 *v*/*v*) acidified with 2% acetic acid at a flow rate of 1.2 mL min^{-1}. The curve range was linear for the receptor solution concentration range 0.5–75 μg mL^{-1}. The specificity showed no interference with the biological matrix and excipients of the acceptor media. Intra and inter-day accuracy, and precision values were lower than 5% and were not statically different ($P < 0.05$). Recoveries ranged from 99.7% to 108%. The limits of detection and quantitation were 11.61 and 500 ng mL^{-1}, respectively. The method is adequate to assay curcumin from esophageal porcine samples, enabling the determination of penetration profiles for *in situ* gelling curcumin-loaded liquid crystals by *in vitro* studies, and fulfilled the requirements for reliability and feasibility for application to the quantitative analysis of curcumin in porcine esophageal mucosae.

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1. Introduction

Curcumin is a natural product isolated as a yellow pigment from the rhizome of *Curcuma longa* L. (Sharma et al., 1990). This drug has shown potential for use in treating cancer (Anand et al., 2008; Goel et al., 2008; Zlotogorski et al., 2013) because it inhibits the nuclear factor kappa beta (NF-kB), especially in oral squamous cancer cells (OSCC) (Aggarwal et al., 2004), that is directly involved in tumorigenesis, (Richmond, 2002) angiogenesis, invasion tumors, and metastasis (Ben-Neriah and Karin, 2011). Curcumin also has poor water solubility and low bioavailability, thus representing an interesting drug model

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to be improved by nanotechnology (Ghalandarlaki et al., 2014; Sun et al., 2012; Ahmad et al., 2015) for the non-invasive administration of drugs (Mathias and Hussain, 2010), especially *via* the buccal route (Calixto et al., 2014; Shojaei, 1998; Campisi et al., 2010).

The buccal route is attractive for the administration of drugs in delivery systems, such as loaded-liquid crystalline systems (Calixto et al., 2014; Salmazi et al., 2015), prolonging the residence time of the dosage in the oral mucosa (Shojaei, 1998; Grabovac et al., 2005) and enabling local treatment of malignant lesions (Nielsen et al., 1998; Bhardwaj and Kumar, 2012; Hearnden et al., 2012). Liquid crystals (LCs) are matter in a state that has properties between those of conventional liquids and solid crystals (Malmsten, 2002). In other words, LCs have the structural behavior and rigidity of a solid, combined with the mobility, disorder, and fluidity of an isotropic liquid (Kato, 2008). By increasing the concentration of solvents, such as water, in these systems, lamellar, hexagonal, and cubic liquid-crystalline forms can be generated (Malmsten, 2007; Mezzenga, 2012).

LCs can be used as drug delivery systems because of the high ability of poorly water-soluble drug solubilization (Malmsten, 2002), like curcumin, besides showing interesting mucoadhesive properties being very interesting to buccal administration to increasing the time retention of LCs formulations.

Self-assembly systems display phase transformations and notable *in situ* thickening after administration to body cavities, such as the mouth. When in contact with the oral environment, LCs have the ability to incorporate saliva, becoming a more viscous liquid-crystalline mesophase (Malmsten, 2007) due to its transitional from a lamellar phase structure to a hexagonal or cubic mesophase (Lee et al., 2001; Boyd et al., 2006; Bruschi et al., 2008; Carvalho et al., 2013) that can promote controlled release and, consequently, result in the greatest mucoadhesion pharmaceutical form in the oral environment (Malmsten, 2002, 2007; Bruschi and de Freitas, 2005; Gilhotra et al., 2014). Due to their high viscosity, hexagonal and cubic phases have been suggested as mucoadhesives (Carvalho et al., 2013; Fonseca-Santos et al., 2015; Oyafuso et al., 2015), as well as the incorporation of (muco)adhesive polymers such as chitosan (Dhawan et al., 2004; Pendekal and Tegginamat, 2012) that can also interact locally with oral tumor cells (He et al., 2010; Wu et al., 2012; Yu et al., 2012).

The vertical diffusion cell, also known as the Franz cell, is widely used for the drug release-rate testing of semisolids and other topical formulations (Hanson and Heaney, 2013; Shah, 2005; Ng et al., 2010). The dosage forms are placed in a donor area that then permeates and/or diffuses through a membrane into the receptor solution, from which samples are withdrawn periodically and analyzed for drug concentrations (Hanson and Heaney, 2013). The experimental conditions for drug release testing, such as acceptor media, types of membrane, and usage of different animal tissue as models, depend on the purpose of the experiments, i.e., whether the aim is quality control (Ng et al., 2010), biopharmaceutical characterization (Siewert et al., 2003), or bioavailability testing in order to decrease or eliminate animal tests (Shah, 2005, 2001; Ng et al., 2010; Shah et al., 2002).

Analytical methods, employed for the quantitative determination of drugs in biological samples and pharmaceutical dosage forms (Silva et al., 2014; Mattos et al., 2013; Chorilli et al., 2011a,b; Jain et al., 2015; Dewani et al., 2015; Chiva Carvalho et al., 2013; Carvalho et al., 2009), can influence the evaluation and interpretation of bioavailability, bioequivalence, and pharmacokinetic data. It is, therefore, essential to employ well-characterized and fully validated analytical methods to give reliable results that can be interpreted with satisfaction (Shah et al., 1992).

A variety of analysis techniques for the quantification of total and isolated curcuminoids in different matrices have been reported, especially spectrophotometric methods for the determination of total curcuminoids (Kadam et al., 2013; Silva-Buzanello et al., 2015; Ahmed et al., 2012). However, this approach cannot be used to quantify individual curcuminoids, so various liquid chromatographic methods have been developed for this purpose. High-pressure liquid chromatography with UV detection (HPLC–UV) is the most common method for the determination of curcuminoids and curcumin in turmeric samples, biological samples, or dosage forms (Jadhav et al., 2007; Li et al., 2009; Thorat and Jangle, 2013; Nascimento et al., 2012; Wichitnithad et al., 2009; Jayaprakasha et al., 2002; Syed et al., 2015; Koop et al., 2013). Due to the very labile characteristics of curcuminoids, C_{18} columns are preferred for HPLC analysis (Khurana and Ho, 1988). These methods reported by HPLC–UV for curcuminoids, especially those in older literature, have several disadvantages, including unsatisfactory separation times, poor resolution, complicated solvent mixtures with gradient elution, and long analysis times.

The aim of this study was to develop and validate an analytical method for evaluating the performance test of *in situ* gelling curcumin-loaded liquid crystals.

2. Experimental

2.1. Chemicals

Polyoxypropylene-(5)-polyoxyethylene-(20)-cetyl alcohol (PPG-5-Ceteth-20) was purchased from Croda (Campinas, Brazil). Oleic acid and sodium dodecyl sulfate were purchased from Synth (Diadema, Brazil). Curcumin (77% purity), low molecular weight chitosan (Ch), and poloxamer 407 (Po) were purchased from Sigma Aldrich (St Louis, USA). HPLC grade acetonitrile and methanol were purchased from J. T. Baker (Mexico City, Mexico). Glacial acetic acid, sodium dihydrogen phosphate, and sodium hydrogen phosphate were purchased from Qhemis Hexis (Indaiatuba, Brazil). Ultrapure water was obtained from a Milli-Q® Direct Water Purification System.

2.2. Chromatographic system and instrumentation

A Varian® ProStar HPLC system was used for analyses, consisting of two pumps (Solvent Delivery Module, model 210), a ProStar 330 UV–vis PDA spectrophotometric detector (set at 425 nm), a ProStar 410 auto sampler (sample injection volume, 20 μL) and Timerline 101 column oven (set at 33 °C). A reverse-phase C_{18} column Luna® (250 mm × 4.6 mm i.d. 5 μm particle size) was used. The mobile phase was a 50:50 (*v*/*v*) mixture of acetonitrile and 2% acetic acid in water at a flow rate of 1.2 mL min^{-1}.

2.3. Standard and working solutions

Curcumin was dissolved in methanol in a 20.0 mL volumetric flask to a final concentration of 1.0 mg mL^{-1}. The solution was sonicated for 5 min in an ultrasonic bath and completed to the final volume. Working solutions (0.5–75.0 μg mL^{-1}) were prepared by dilution of aliquots of the standard solution with methanol (1.0 mg mL^{-1}) in a 5.0 mL volumetric flask.

2.4. Validation methods

2.4.1. Linearity

To determine linearity, curcumin standard solution was diluted in triplicate at concentrations ranging from 0.5 to 75.0 μg mL^{-1} (working solutions). Linearity was evaluated

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by linear regression analysis, which was calculated by least-squares regression analysis and ANOVA test ($\alpha = 0.05$).

2.4.2. Specificity and selectivity

The specificity of the method was determined by curcumin, the acceptor media (buffer phosphate, pH 6.8 containing 0.5% sodium dodecyl sulfate, SDS), and methanol extract mucosae esophageal porcine (biological membrane used in permeation and retention studies) to evaluate interference in the quantitation of the drug.

2.4.3. Accuracy

To evaluate the accuracy of the method, a recovery experiment was performed. Curcumin concentrations of 1.0, 50.0 and 70 μg mL^{-1} were added to the matrix samples: phosphate buffer (pH 6.8 containing SDS; acceptor media) and mucosae esophageal methanolic extract. These results were expressed as recovery data and values should be within the interval 80–120% (Resolução RE, 2003).

2.4.4. Precision

Intra and inter-day precision was analyzed by injecting curcumin working solutions in triplicate ($n = 3$) at three concentrations (1.0, 50.0 and 70.0 μg mL^{-1}) over an interval of two days between repeatability and intermediate precision tests (Validation of Analytical Procedures, 2005). The results were expressed as percentage of RSD and inter-day averages were evaluated statically by Student's *t*-test (tailed test, $P < 0.05$).

2.4.5. Limits of detection and quantitation

Limits of detection (LOD) and quantitation (LOQ) were determined using a calibration curve method according to ICH recommendations (Validation of Analytical Procedures, 2005). The response standard deviation (RSD) was taken as the SD of *y*-intercepts of the regression lines of the three calibration curves. The LOD were calculated as follows:

$$\mathrm{LOD} = 10 \times \sigma / S \quad (1)$$

where σ is the standard deviation of the response, and S is the slope of the calibration curve. Limit of quantitation (LOQ) was determined as the first concentration of calibration curves.

2.5. Liquid crystal preparation and characterization

LCs were prepared at room temperature by mixing oleic acid as the oily phase (O), PPG-5-Ceteth-20 as the surfactant (S), and water (W) containing 0.5% Ch and Po as the aqueous phase. The samples contained the following O/S/W proportions: (LC1) 3:4:3, (LC2) 1:2:2 and (LC3) 1:4:5. Sample LC1 was diluted with artificial saliva (10:3) to generate the LC1d formulation. These formulations were analyzed by polarized light microscopy (PLM) to confirm liquid crystal mesophases at 20× magnification. The artificial saliva is composed of sodium phosphate dibasic anhydrous (802.9 mg), sodium phosphate monobasic anhydrous (362.6 mg), 70% sorbitol solution (42.7 g), potassium chloride (625.0 mg), sodium chloride (865.0 mg), magnesium chloride hexahydrate (125.0 mg), calcium chloride dehydrate (72.0 mg), methylparaben (1.8 g), propylene glycol (10 mL) and purified water to 1000 mL (Nakamoto, 1979).

2.5.1. Method applicability

The proposed analytical method was used to evaluate the content of curcumin retained in the porcine mucosa when curcumin-loaded LCs were applied to the mucosa. A Hanson MicroettePlus® Franz diffusion cell (Chatsworth, USA) was filled with a media volume of 7.0 mL (phosphate buffer, pH 6.8 containing 0.5% SDS) and stirred at 300 rpm at a temperature of 37 ± 0.5 °C.

Porcine mucosa esophageal tissue was treated with buffer phosphate for 5 min, then fixed to the diffusion cell. Formulations were placed (600 mg) above the membrane. The experiment was carried out with curcumin-loaded LC1 (5.0 mg g^{-1}) and a formulation diluted to 10% with artificial saliva (LC1d), and the results were compared to the release profile of curcumin dissolved in oleic acid as a control. During the test, media aliquots of 1.0 mL were withdrawn at intervals (0.5, 1, 2, 4, 8 and 12 h) using a Hanson AutoPlus™ Multifill™ autosampler (Chatsworth, USA). The aliquots were filtered through 0.45 μm PTFE disk filters and then injected into HPLC.

The extraction of curcumin from the retained pig esophageal mucosa was carried out according to an adapted method (Mazzarino et al., 2015). After 12 h of *ex vivo* permeation, the mucosa was removed from the Franz cell and the area of tissue exposed to permeation was cleaned with cotton dipped in methanol, and then the tissue was cut and perforated. The fragments obtained were triturated with 4 mL of methanol in a tissue homogenizer at 10,000 rpm for 2 min to achieve total dispersion of the mucosa. The resulting suspension was sonicated in an ultrasonic bath for 50 min to break the cells.

The homogenate was centrifuged at 4000 rpm for 10 min. The obtained supernatant solution was filtered into a 5 mL volumetric flask and the volume completed with methanol. The solution was filtered through 0.45 μm PTFE disk filters and injected into HPLC for analysis. Extractions were performed from the mucosa of each diffusion cell ($n = 6$) and are expressed as percentages. All average values of retention were tested by ANOVA following Tukey's post hoc test ($\alpha = 0.05$).

3. Results and discussion

3.1. Validation studies

3.1.1. Linearity

Statistical analysis, using least-squares regression analysis of curcumin assay results in the range 0.5–75 μg mL^{-1}, gave a linear regression equation for the three calibration curves for curcumin of: $y = 0.002027x - 0.000228$, $r = 0.999859$ (Fig. 1). ANOVA of regression showed an *F*-critical value of 6.0993×10^{-36} and an *F*-tabulated value of 80768.6, which indicated a significant regression (because *F*-critical value < *F*-tabulated value). According to the validation criteria adopted, this method was linear over the whole tested concentration range. The calibration curves are shown in Fig. 1 and ANOVA is shown in Table 1. The validity of the assay was verified by means of ANOVA, which showed that there is linear regression with no deviation from linearity ($P < 0.05$).

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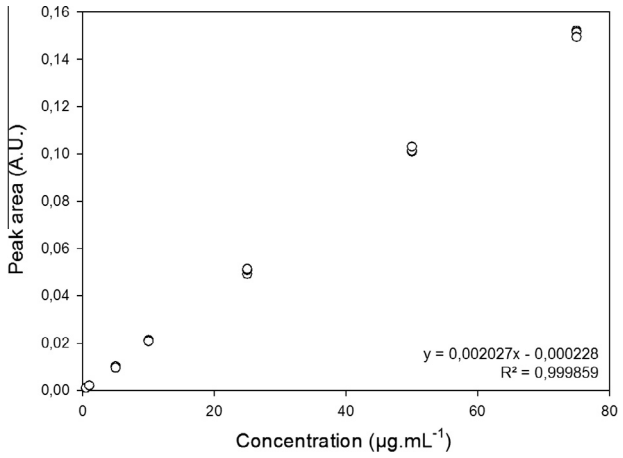

Figure 1 Calibration curves of curcumin at concentrations of 0.5–75 μg mL^{-1} in methanol.

3.1.2. Specificity and selectivity

The chromatograms in Fig. 2 show good peak resolutions, indicating the high specificity and selectivity of this method. The resolutions (R) of peaks were 2.2 and 3.0 between peaks 2/1 and 3/2, respectively, indicating a high degree of peak separation ($R > 2$). The chromatogram showed three peaks and was reported by Jayaprakasha et al. (2002), where the major peak was attributed to curcumin (Goel et al., 2008) and two minor peaks were identified as demethoxycurcumin (Anand et al., 2008) and bisdemethoxycurcumin (Sharma et al., 1990) by co-injection of standards. These authors confirmed the compounds associated with elution peaks by ^{1}H and ^{13}C NMR analysis.

The method developed by these authors was similar to that developed in this work; however, the chromatographic conditions were slightly different with regard to mobile phase proportions (acetonitrile/water acidified with 2% acetic acid; 60:40 vs. 50:50 *v/v* in this work), the length of the C_{18} column (150 mm vs. 300 mm in this work), and the solvent flow rate (2 mL min^{-1} vs. 1.2 mL min^{-1} in this work). The total chromatographic analysis time was 16 min per sample, with curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin eluting at retention times of 13.6, 12.1 and 10.8 min, respectively (Wichitnithad et al., 2009). However, chromatographic analysis with a C_{18} column of 200 mm length would generate an analysis time greater than 15 min. Therefore, our method used the small changes mentioned above to achieve the best peak resolution and a shorter analysis time.

The chromatogram of curcumin exhibited a characteristic peak (Goel et al., 2008) at a retention time (t_R) of 11.5 min, and two minor peaks at a t_R of 9.5 and 10.5 min, reported in the literature as bisdesmethoxycurcumin and desmethoxycurcumin, respectively (Wichitnithad et al., 2009) (Fig. 2A). The short retention times were very convenient for routine procedures in quality control. The methodology was selective, because no interfering signals were detected at the t_R of acceptor media (phosphate buffer, pH 6.8) and mucosae methanolic extract, and none interfered with curcumin detection (Fig. 2B).

3.1.3. Accuracy

Curcumin working solution and spiked samples (methanol, phosphate buffer with 0.5 DSD and mucosae extract) were fortified at three concentrations (1.0, 50.0 and 70.0 μg mL^{-1}) by diluting aliquots of the standard solution (1.0 mg mL^{-1}). Each replicate was injected into the C_{18} column and peak areas were obtained. These values were substituted into the regression line equation to calculate the recovered concentrations of the samples. Accuracy is reported in Table 2 as a percentage recovery relative to the known amount of curcumin in the samples. The recovery values were within the confidence interval of 80–120%.

3.1.4. Precision

The intra-day precision was analyzed by injecting curcumin working solutions in triplicate at 1.0, 50.0 and 70.0 μg mL^{-1}, corresponding to low, average, and high concentrations in the calibration curve range, respectively. The inter-day precision was analyzed by injecting each concentration three times ($n = 3$) on two consecutive days. The standard deviation (SD) and RSD were calculated for each sample (Table 3). The low values of RSD ($< 2\%$) reflected the high precision of the method and the P-value (< 0.05) showed no mean significance.

3.1.5. LOD and LOQ

The LOQ value was taken as the lowest concentration of curcumin in the acceptor media that could be quantitatively measured. The LOD was 11.61 ng mL^{-1} and LOQ was 0.5 μg mL^{-1}.

3.2. Liquid crystal preparations and characterization

Fig. 3A shows that it was possible to obtain an optically transparent liquid system (OPCLS) when the concentrations of S, O, and W were 40%, 30–70%, and above 50–70%, respectively, and a minor region with 30%, 50–70% and 70%, respectively. The transparent semisolid system (OPCSS) was formed when concentrations of S, O, and W were 25–40–30%, 5–25%, and 45–55%, respectively. Emulsion systems were obtained in regions where concentrations of S, O, and W were below 5–20%, 5–70%, and 85-95%, respectively. Phase separation occurred in a large proportion of the formulations, in two distinct regions: the first region was formed when the concentrations of S, O, and W were greater than 55–100%, 5–40%, and below 5–40%, respectively; the second

Table 1 ANOVA for simple linear regression.

Model	d.f.	Sum of squares	Mean squares	*F*-tabulated	*F*-critical
Regression	1	0.060102483	0.060102	80768.6	6.1×10^{-36}
Residue	19	1.41385×10^{-05}	7.44×10^{-07}		
Total	20	0.060116621			

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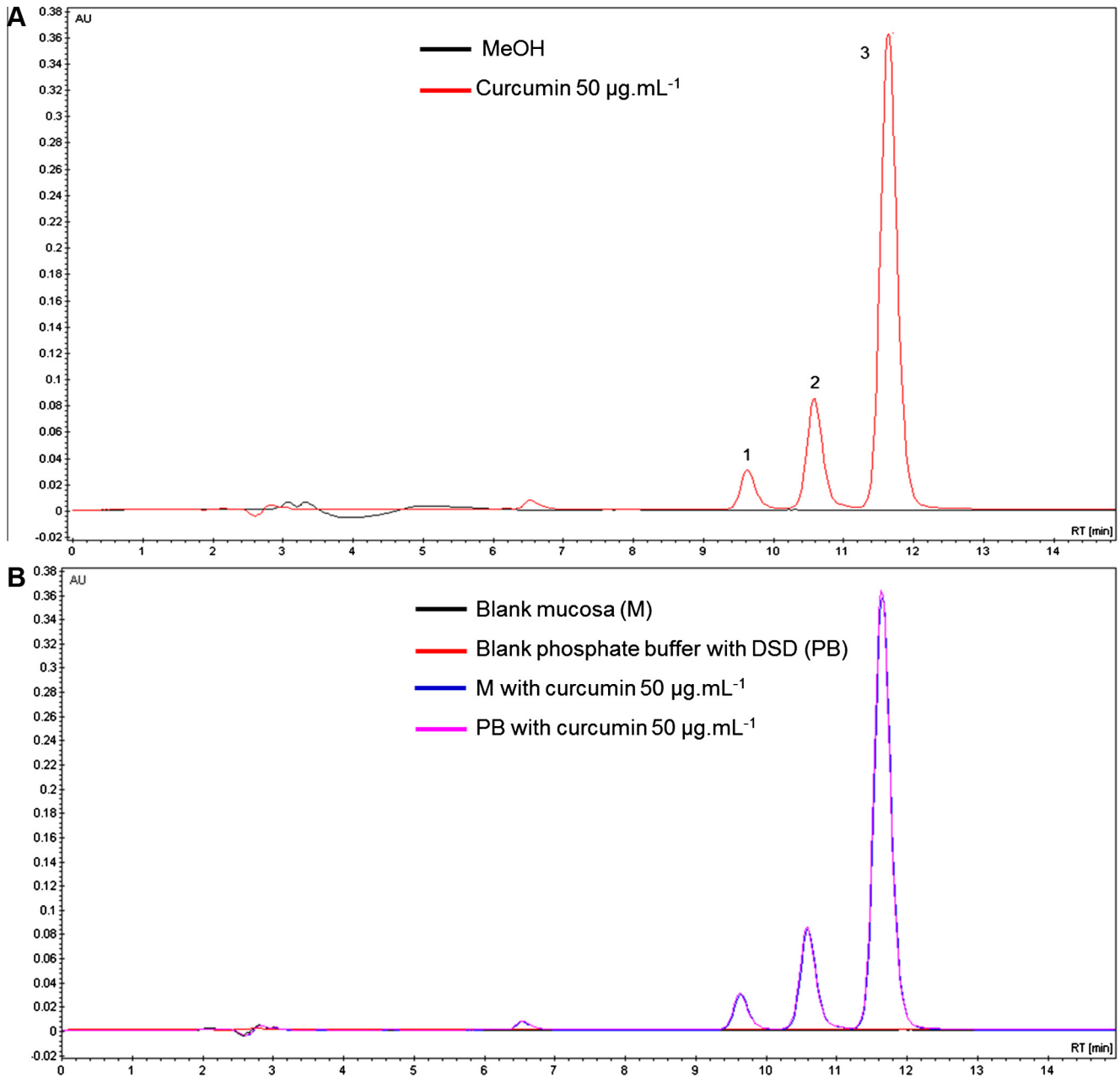

Figure 2 Chromatograms of curcumin (50 µg mL^{-1}), blank methanol, and buffer phosphate as acceptor media and mucosae methanolic extract, fortified or unfortified.

Table 2 Accuracy of curcumin at different concentrations (1.0, 50.0 and 70.0 µg mL^{-1}) in matrices (methanol, phosphate buffer with 0.5 DSD, and mucosae extract).

Matrix	Theoretical concentration (µg mL^{-1})	Experimental concentration (µg mL^{-1})	RSD (%)	Recovery (%)
Methanol	1.0	1.01 ± 0.02	1.98	101.0 ± 2.0
	50.0	50.3 ± 0.05	0.10	100.7 ± 0.1
	70.0	70.4 ± 0.7	0.30	100.0 ± 0.1
Phosphate buffer w/DSD	1.0	1.02 ± 0.03	2.94	102.0 ± 3.0
	50.0	50.12 ± 0.11	0.22	100.2 ± 0.2
	70.0	69.80 ± 0.09	0.13	99.7 ± 0.13
Mucosae extract	1.0	1.08 ± 0.03	2.78	108.0 ± 3.0
	50.0	50.10 ± 0.59	1.18	100.4 ± 1.18
	70.0	71.00 ± 0.9	1.41	101.4 ± 1.28

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Table 3 Intra- and inter-day of curcumin at different concentrations (1.0, 50.0 and 70.0 μg mL^{-1}).

Theoretical concentration (μg mL^{-1})	Intra- and inter-day precision						*P*-value[c]
	Experimental concentration (μg mL^{-1})[a]		RSD (%)[b]		Recovery (%)[a]		
	Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day	
1.0	1.01 ± 0.02	1.01 ± 0.01	1.98	1.25	101.0 ± 2.0	100.8 ± 1.3	0.5000
50.0	50.3 ± 0.05	50.7 ± 0.61	0.1	1.20	100.7 ± 0.1	101.4 ± 1.22	0.2576
70.0	70.4 ± 0.7	70.03 ± 0.208	0.3	0.99	100.0 ± 1.0	100.6 ± 0.3	0.2168

[a] Mean and SD of samples.
[b] Triplicate in two different days.
[c] Student's *t*-test with significance level of 95%.

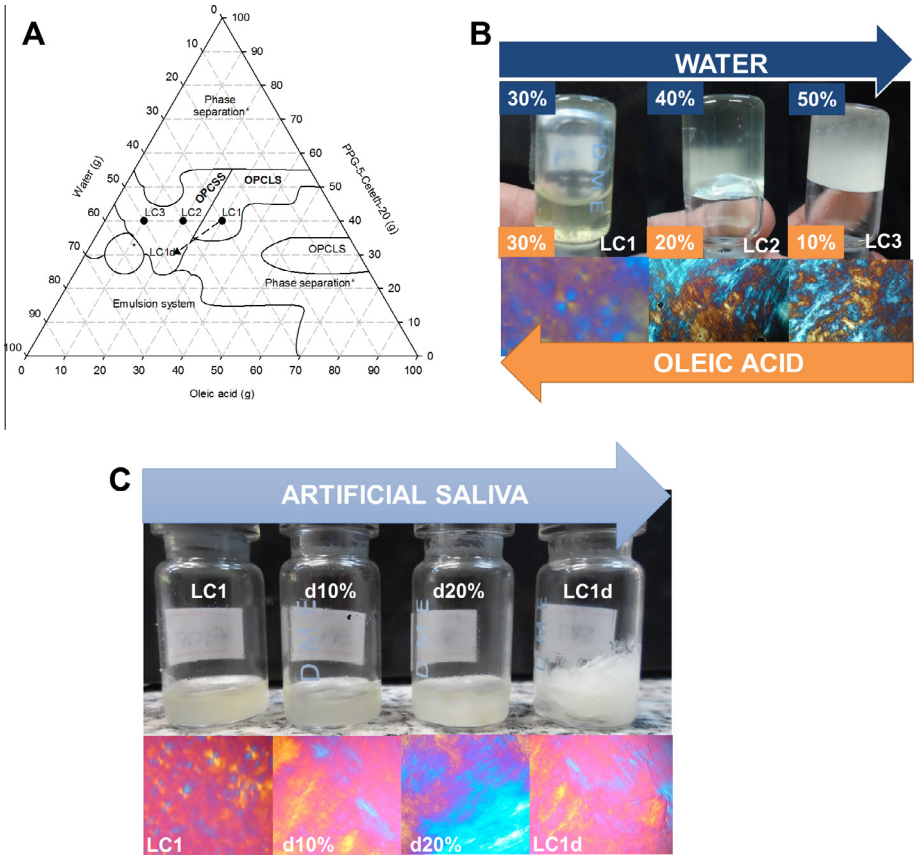

Figure 3 (A) Ternary phase diagram of PPG-5-CETETH-20, oleic acid, and aqueous phase (0.5% chitosan and poloxamer 407); (B) macroscopic appearance and MPL of LC1, LC2, and LC3 formulations; and (C) photomicrographs of LC1 diluted with artificial saliva to 10% (d10), 20% (d20), and 30% (LC1d).

region was formed when the concentrations of S, O, and W were below 40%, 50–100%, and 10–100%, respectively.

Based on the ternary phase diagram, formulation LC1, a liquid formulation composed of 40% (*w*/*w*) PPG-5-CETETH-20, 30% (*w*/*w*) oleic acid, and 30% (*w*/*w*) aqueous phase (chitosan and PO 407 at 0.5%), was selected as the liquid crystal precursor system because its liquid phase facilitated buccal administration (e.g., by syringe) (Bruschi et al., 2008). Both LC2 and LC3 were semisolid formulations composed of 40% PPG-5-CETETH-20, 20% and 10% oleic acid, and 40% and 50% of aqueous phase (chitosan and PO 407 at 0.5%), respectively. These formulations were not adequate for buccal administration due to their poor syringeability.

Moreover, LC1 is located in a phase transition region. Thus, when diluted with artificial saliva, its viscosity increased *in situ*. This can be observed in the dilution line in the diagram in Fig. 3A.

Carvalho et al. (2013) previously developed a phase diagram consisting of oleic acid as the oil phase, PPG-5-CETETH-20 as the surfactant, and water as the aqueous phase. This study also resulted in different regions, but reported a lower region of transparent viscous systems,

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perhaps due to the absence of mucoadhesive polymers such as chitosan and poloxamer, which are present in the diagram in Fig. 3A. Such polymers may have thickening properties (Chattopadhyay and Inamdar, 2010), changing the rheological properties of these systems and leading to an increase in the regions of viscous systems in the diagram.

Using the MPL technique, Malta crosses were observed in the photomicrograph of LC1, suggesting that a lamellar liquid crystalline mesophase had formed (Chorilli et al., 2011c). Thus, the LC2 and LC3 photomicrographs showed striated structures that may be more organized structures typical of a hexagonal liquid crystalline mesophase (Carvalho et al., 2013, 2010), as shown in Fig. 3B. When, LC1 was diluted with artificial saliva in increasing proportions (10%, 20%, and 30%), resulting in LC1d10%, LC1d20% and LC1d, respectively, the photomicrographs showed striated structures that might be the more organized structures typical of a hexagonal liquid crystalline mesophase (Salmazi et al., 2015). The results for these phase transitions are shown in Fig. 3C.

Therefore, these results demonstrated that LC1 behaved as a precursor of a LC system and was able to form a strong LC mesophase with the incorporation of artificial saliva. This transition could be attributed to an increase in the packing constraint in the hydrophilic core of PPG-5-CETETH-20, which reduced the interfacial curvature of the aggregate. Moreover, the subsequent hydration of the surfactant generated a large repulsive force between the head groups of the surfactant, increasing the distances between lamellae (Carvalho et al., 2010). This resulted in the formation of different liquid crystalline structures (Mezzenga, 2012).

3.2.1. Method applicability

During pharmaceutical development, it is appropriate to employ *in vitro* release or permeation studies to select formulations that can provide adequate therapeutic activity. Drug release studies provide valuable data on the structural characteristics of the vehicle and its ability to release the drug.

LC phases have received considerable attention due to their potential as drug delivery systems. Cubic and hexagonal phases provide a slow drug release matrix (Mezzenga et al., 2005; Ubbink et al., 2008) when compared with lamellae phase, which has a rapid drug release matrix (Farkas et al., 2000; Makai et al., 2003).

Recently, Martiel et al. (2015) published work that explained controlled release from LCs, detailing the influence of drug hydrophobicity and oil-surfactant type on this property. The authors summarized that partition coefficients of drugs and the ability of the drug to diffuse through the hydrocarbon tail region were important. This phenomenon was influenced by the bulkiness of the drug, the oil-type, and by either chain stiffening (Martiel et al., 2015). The optimal structural control was obtained by combining a drug with high hydrophobicity with LCs (Martiel et al., 2015), which may not be true for curcumin due to its poor-water solubility.

Fig. 4 shows the cumulative percentage in the esophageal mucosal tissue after a 12 h *ex vivo* test. Statistical analysis showed that there were differences between the mean ($P > 0.05$) percentages of retention for the formulations. The retentions were approximately 3%, 15%, and 10% for the control, LC1, and LC1d, respectively. The lamellar phase retention values were greater than hexagonal systems, which could be related to the similarity of the lamellar liquid-crystalline structure with the cell membrane leading to enhanced interactions between them and causing the release of tissue target from this interaction bio-nanodevice (Nel et al., 2009; Liu and Wang, 2014). In addition, slow release and high-capacity carrying of hydrophobic drugs, such as antitumor drugs, into liquid-crystal systems, is particularly interesting for cancer therapy, where the cytotoxicity of anticancer drugs can be improved.

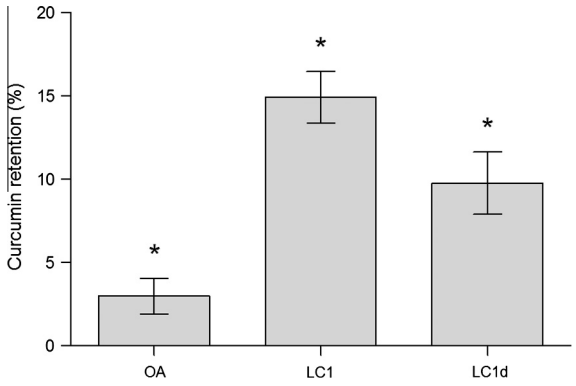

Figure 4 Bar chart of curcumin retained (%) in mucosae esophageal tissue obtained from retention studies after 12 h in oleic acid, lamellar liquid crystal (LC1), and *in situ* gelling liquid crystal (LC1d) $^{*}P > 0.05$.

4. Conclusions

The HPLC method presented here can be considered suitable for analytical determination of curcumin concentrations for *in vitro* performance tests. The method was capable of detecting low concentrations of curcumin in the diffusion cell acceptor media, and the calibration curve was linear over the concentration range. The validation process also showed high selectivity and specificity, since there was no interference between the curcumin peak and media or mucosae components. The methodology was accurate and precise as observed from the recovery and RSD values. The analytical procedure had a 15 min chromatographic run time, which allowed the analysis of a large number of samples in a short period of time. Retention studies showed higher concentrations on mucosae tissue to lamellar mesophase than hexagonal mesophase.

Designed LCs showed *in situ* gelling properties caused by the addition of artificial saliva. This artificial saliva caused the formation of a lamellae mesophase, transforming into hexagonal liquid crystalline formulations with mucoadhesive properties (data not shown).

This paper proposes a HPLC method to quantify curcumin in performance *in vitro* assays for LCs containing curcumin; however, this methodology can be applied to other pharmaceutical dosage forms intended for buccal release of curcumin.

Disclosure

The authors report no conflict of interests in this work.

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