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Original Article

Nanoparticles containing curcuminoids (*Curcuma longa*): development of topical delivery formulation

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ABSTRACT

Solid lipid nanoparticles incorporating *Curcuma longa* L., Zingiberaceae, curcuminoids were produced by the hot melt emulsion method. A Box–Behnken factorial design was adopted to study the nanoparticles production at different levels of factors such as the percentage of curcuminoids, time of homogenization and surfactant ratio. The optimized nanoparticles were incorporated into hydrogels for stability, drug release and skin permeation tests. The average nanoparticle sizes were 210.4 nm; the zeta potential of -30.40 ± 4.16; the polydispersivity was 0.222 ± 0.125. The average encapsulation efficiency of curcumin and curcuminoids was $52.92 \pm 5.41\%$ and $48.39 \pm 6.62\%$, respectively. Solid lipid nanocapsules were obtained with curcumin load varying from 14.2 to 33.6% and total curcuminoids load as high as 47.7%. The topical formulation containing SLN-Curcuminoids showed good spreadability and stability when subjected to mechanical stress test remained with characteristic color, showed no phase separation and no significant change in pH. As a result of slow release, the nanoparticles were able to avoid permeation or penetration in the pig ear epidermis/dermis during 18 h. The topical formulation is stable and can be used in further *in vivo* studies for the treatment of inflammatory reactions, in special for radiodermitis.

Introduction

In the last decade the curcuminoids from *Curcuma longa* L., Zingiberaceae, were reported in several molecular signaling pathways of clinical importance (Goel et al., 2008; Das et al., 2010; Mourtas et al., 2011). They have anti-inflammatory, antifungal, antimicrobial, antioxidant and antiproliferative properties (Aggarwal and Harikumar, 2009; Augustyniak et al., 2010; Irving et al., 2011; Zhou et al., 2011), among others. The main curcuminoids from *Curcuma* are curcumin, desmethoxycurcumin and bisdesmethoxycurcumin, three bioactive substances commonly used as spices and coloring agents in food. However, these molecules have poor bioactivity and instability in neutral and alkaline aqueous solutions (Tønnesen and Karlsen, 1985; Yallapu et al., 2012) and hydrophilic topical formulations. Besides, when curcuminoids were subjected to light exposure there was 30% degradation and 1.8% loss under oxygen action in 30 days. Despite their several utilities and low toxicity (Jayaprakasha et al., 2006), the curcuminoids low bioavailability (Gao et al., 2011) contributes to recent growth of interest in developing new carrier systems (Nair et al., 2010).

Due to anatomical and physiological characteristics of the skin, some ingredients may not acquire the desired activity topically and new drug carrier systems have been used to modify the permeation/penetration. According to Mehnert and Mader (2001), it is becoming evident that the development of a single drug is no longer sufficient for the evolution of therapy, given the insufficient concentration of the drug due to its low absorption, rapid metabolism and elimination; high toxicity in combination with other fabrics and high fluctuation in plasma levels due to unpredictable bioavailability after perioral administration.

Lipid-based nanoparticle systems are commonly used in topical applications (Contri et al., 2011) and are prepared with solid structures at room temperature or at body temperature associated with surface-active agents and water lipids. According to Oliveira (2008), lipophilic drugs will have an easier penetration, given the characteristics of the plasma membrane. In this regard, skin permeation enhancing agents can be used in the formulations and can reduce skin resistance (Martins and Veiga, 2002).

Over the past 10 years solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) through mixtures of lipids that

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acquire a specific molecular arrangement were produced, suffering a major effect of surfactants added to these formulations (Serra et al., 2009). This combination of surfactants is considered to improve stability by preventing agglomeration of the particles and retarding the time of polymorphic transition of lipids to encapsulate the active ingredient (Serra et al., 2009).

Hydrophilic matrix systems are commonly used due to low production costs, acceptance by regulatory organ, simplicity of formulation, production facility, capacity to incorporate a range of substances with extended solubility range (Lyra et al., 2007), and in general, have a thixotropic or pseudoplastic rheological behavior, during application becoming more fluid, facilitating the spreading and recovering the initial viscosity upon ending the application, which prevents the product drips (Martin, 1993). These products tends to have a longer shelf life, because during storage (during which time the product remains at rest), presents constant viscosity, which makes difficult to separating the formulation constituents.

Hydrophilic gels have been demonstrated to be adequate for incorporation of SLN (Jenning et al., 2000). By reducing transepidermal loss of water and improving skin hydration, an increase in penetration and percutaneous absorption has been reported (Jenning et al., 2000; Wissing et al., 2001). Other advantage of this system is to promote the controlled release of substances, reducing their systemic absorption and acting as a physical sunscreen (Attama and Muller-Goymann, 2008). These properties are of great importance for the protection of skin from patients undergoing radiotherapy treatment.

The aim of this research was to develop solid lipid nanoparticles (SLN) containing curcuminoids using a natural wax as encapsulating matrix and to develop and characterize a hydrophilic gel, for topical application. The nanoparticle preparation was studied applying a Box–Behnken design and the hydro gel stability and *ex vivo* skin curcuminoid permeation/penetration was evaluated.

Materials and methods

Plant and other materials

The curcuminoid concentrate was purchased from CHR (Christian Hansen A/S, Denmark) and pharmacognostically characterized, and a sample counterproof was deposited at the "Herboteca LADIFARP" from the FCFRP/USP with Registry number CP01/14-S. The powder moisture content, total ash content, swelling index and size distribution were determined according to the methodologies described in Farmacopeia Brasileira (2010). The results were expressed as mean ± standard deviation of three replicates. Analytical grade curcumin as a standard for quantification purpose was purchased from Sigma (Sigma–Aldrich, St. Louis, MO, USA).

Quantification of curcumin and curcuminoids

For curcumin and total curcuminoid content determination a HPLC method was developed. Briefly, an Ultimate 3000 (Thermo Fisher Brasil Ltda, São Paulo, Brazil) HPLC was used with a C18 Alcrom column (Phenomenex Ltda, São Paulo, Brazil). The mobile phase was 50:50 acetonitrile and acidified water (1% citric acid) at pH 3.5, flow rate of 1.0 ml/min. The UV–vis detector was set up to 425 nm. 50 μl samples were injected (Paulucci et al., 2013).

The curcumin standard (Sigma–Aldrich, St. Louis, USA) solution was prepared with 1 mg of curcumin in 5 ml of 50:50 acetronitrile:water (1% citric acid). Consecutive dilutions were made to obtain seven solutions at concentrations from 25 to 2500 ng/ml, which were injected to prepare the analytical curve. The method linearity and selectivity were also evaluated. Samples of drug (curcuminoids), unloaded nanocapsules, curcumin loaded nanocapsules, pig ear extract, hydrophilic gel, and gel containing nanocapsules were tested for selectivity.

Preparation of solid lipid nanoparticles SLN

SLN were prepared by hot melt emulsion technique (Martins, 2014; Zamarioli, 2014) employing a high-shear homogenizer. Briefly, water was heated at 70 °C with Tween 80 (polyethylene glycol sorbitan monooleate) and lecithin (phosphatidylcholine), prior to addition of the oily phase. The beeswax and curcuminoids were heated and homogenized at same temperature. The microemulsion was produced by dropping the oily phase in aqueous solution with the aid of a glass syringe. The dispersion was submitted to *high shear* homogenization at 70 °C using an Ultra-Turrax T25 (IKA®, Wilmington, USA) at 20,000 rpm during 20 min. The lipid nanoparticles were solidified by cooling down the dispersion. All experiments were kept in an ice bath with magnetic stirring after homogenization for 10 min.

Factorial design

The SLN production was studied using a Box–Behnken factorial design (Boax et al., 1978) for three factors at three levels, resulting in fifteen experiments. Table 1 shows the studied factors and the ranges of each one: the percentage of curcuminoids in beeswax nanoparticles, %C:Bw; the homogenization time using Turrax, HT and the weight proportion of lecithin to Tween 80, L:T. The choices of the ranges of the factors shown in Table 1 were based on preliminary experiments and the range of proportion of lecithin to Tween 80 was based on the hydrophilic–lipophilic balance (HLB).

Characterization of SLN

The particle size (Dp) distribution and polydispersivity index (PdI) were obtained by photon spectroscopy (PCS) employing a Zetasizer Nano Series (Malvern Instruments Limited, Worcs, UK). Zeta potential was assessed by determining the particle electrophoretic mobility using the same apparatus. These measurements were performed at 25 °C using water as the dispersing medium and dilution of suspensions of nanoparticles at a ratio of 1:100 in ultrapure water (Milli Q, Millipore Inc., USA).

Morphology

The surface appearance and shape of SLN were determined by an atomic force microscope (AFM) model SPM-9600 (Shimadzu Co., Kyoto, Japan). The dispersion of SLN was diluted (1:100) using ultrapure water (Milli-Q, Millipore Inc., USA) and spread onto thin mica plates.

Encapsulation efficiency and drug load

Aliquots of 1.5 ml of the SLN dispersions were placed in Eppendorf and subjected to centrifugation in a HT – MCD 2000 (Biosystems Ltd., Curitiba, Brazil) at 9300 × *g* for 20 min; the supernatant was extracted, filtered in 45 μM filter (Millipore Inc., USA) and stored in vials for further HPLC quantification. In each Eppendorf 1.5 ml of Milli-Q water was added to the remaining precipitated material. This solution was subject to new cycles of centrifugation and supernatant collection under the same conditions. The supernatant became colorless after three extraction cycles. The curcumin or curcuminoid content in the three supernatant samples was quantified by HPLC and was assumed as being outside the SLN, or non-encapsulated. 1.5 ml of ethanol 70°GL was added to the precipitate obtained after the third centrifugation. The solution was sonicated for 60 min in ultrasound bath and thereafter

Table 1
Levels and codification of variables studied.

Factors	Level (−1)	Level (0)	Level (+1)
X_1 – percentage of curcuminoids/beeswax	5%	10%	15%
X_2 – time in Turrax* in min	10	15	20
X_3 – proportion of Lecithin/Tween 80	0.5	1.0	1.5

to a cycle of centrifugation at 9300 × *g* for 20 min. The supernatant was aspirated, filtered and placed in vials for curcumin and curcuminoid quantification. The latter were considered to be the curcuminoids that were encapsulated in the SLN. The encapsulation efficiency, %EE, was calculated by Eq. (1).

$$\%EE = (CC/CT + CC) \times 100 \quad (1)$$

where CEnc is the amount of curcuminoids inside the SLN and CT is the total amount of curcuminoids added to the formulation.

Preparation of topical formulation

In order to contemplate the objective of this project, we chose to prepare a known base formulation, the gel Natrosol® (Via Pharma Ltda, São Paulo, Brazil) at a concentration of 1.5%. The incorporation of SLN dispersions into gel components was performed at room temperature, as beeswax becomes softened at around 50 °C.

In vitro release profile

Briefly, an aliquot of 20 μl of the SLN dispersion corresponding to 59.2 mg of curcuminoids was dispersed in phosphate buffer, distributed in five tubes and kept at room temperature. At the predetermined intervals, the tubes were centrifuged at 200 × *g* for 5 min (Bisht et al., 2007) to separate the curcuminoids crystals precipitated after release from SLN. After centrifugation, the dispersion was aspirated and placed into new tubes for subsequent centrifugation, and again the curcuminoid remaining crystals dissolved in 1 ml of methanol. This volume was filtered (0.45 μM) and analyzed in HPLC for quantification.

Ex vivo permeation study

The permeation tests were performed using Franz cell diffusion (1.77 cm^2 of area) with pig ear skin. The pig ear skin was obtained from slaughterhouse overseen by the Ministry of Agriculture, located in the municipality of Ipuã (Olhos D'Água Indústria e Comércio de Carnes Ltda, Ipuã, SP, Brasil).

The experiments were performed with six cells: (1) one cell for the control group with curcuminoids solution; (2) two cells with Natrosol gel containing curcuminoids and (3) three cells with the gel containing nanoencapsulated curcuminoids (0.5 g of gel in each). For the receptor solution 0.2 M phosphate buffer was used with 20% ethanol (Suwantong et al., 2007), pH 7.5, maintained at 37 ± 0.5 °C in thermostatic bath with continuous stirring for 18 h. Samples were withdrawn from the receptor compartment after 0, 15, 30 and 60 min and at 2, 4, 6, 8, 10, 12, 14, 16 and 18 h of assay and immediately quantified by HPLC.

After the 18 h, the skin was removed from Franz apparatus, dried with a fine cloth and repetitive (n = 15) removal of the stratum corneum was performed with adhesive tape (3 M, Sumaré, São Paulo, Brazil) 19 mm × 50 m. The tapes were placed in Falcon tubes (50 ml) containing 0.2 M phosphate buffer with 20% ethanol (Suwantong et al., 2007), agitated in a vortex model AT 56 (Phoenix) for 3 min, filtered through a 0.45 μM membrane (Millipore Corporation, Billerica, MA, USA) and quantified in HPLC.

Retention in the epidermis and dermis

The epidermis and dermis were minced and placed in Falcon tube (50 ml) containing 0.2 M phosphate buffer with 20% ethanol (Suwantong et al., 2007), subjected to high shear agitation (Turrax) at 3000 rpm for 1 min; sonicated in an ultrasound bath Model USC 1400 (Ultrasonic Cleaner) for 30 min and centrifuged for 10 min at 2790 × *g* in a centrifuge model HT – MCD 2000 (Biosystems Ltd., Curitiba, Brazil). The solution obtained was filtered (0.45 μM) and quantified in HPLC.

Stability of gel formulation with SLN

Preliminarily, the Natrosol® gel containing SLN-Cur was submitted to centrifugation at 2790 × *g* for 30 min (Anvisa, 2004). Following, the formulation was subjected to thermal stress cycles alternating 24 h in the oven at 40 ± 2 °C and 24 h in the refrigerator at 5 ± 2 °C. At the end of each cycle, organoleptic and physicochemical characteristics of the gel were evaluated. The pH of the samples was obtained using a DM 20 pHmeter (Digimed, São Paulo, SP, Brazil). This study was performed in six complete cycles. Also, organoleptic and pH evaluation of gel containing the SLN-Cur was performed after three months of storage. Samples were kept at two test temperatures (25 ± 2 °C and 5 ± 2 °C) both in the absence and presence of light.

Statistics

To examine this plan Minitab 16 software (Minitab Inc., State College, PA) was used, with its features of descriptive statistics, experimental design (DOE) and response surface; to generate the response surface graphs, Statistica version 12 (StatSoft, South America, São Caetano do Sul, São Paulo, Brazil) was used. Descriptive analyses were mean, standard deviation, minimum, and maximum. Confidence interval was established for the analysis of the release profile of curcuminoids from SLN taken and variance analysis in all experimental designs, with $p < 0.05$.

Results and discussion

The drug raw material was characterized according to Farmacopeia Brasileira (2010) with all assays made in triplicate. The total ash content was 0.10 ± 0.01%, and moisture content was 0.75 ± 0.02%. Mean particle size D50 was 30.8 μm, while D10 and D90 were 13.7 and 168.2 μm, respectively. Size distribution span was 0.20. The contents of curcumin, demethoxycurcumin and bisdemethoxycurcumin in the drug were determined by HPLC using the curcumin standard from Sigma and showed to be 76.7, 19.4 and 3.9%, respectively.

The HPLC method developed showed to be selective for curcuminoids in samples of nanocapsules containing curcuminoids and pig ear skin used in penetration/permeation assays. The chromatograms for curcumin analytical standard (Sigma–Aldrich, St. Louis, USA), drug containing the three curcuminoids used as raw material in this work and a sample extracted from pig skin after penetration/permeation test are shown in Fig. 1A–C, respectively. The analytical curve showed a squared correlation coefficient, R^2, of 0.999.

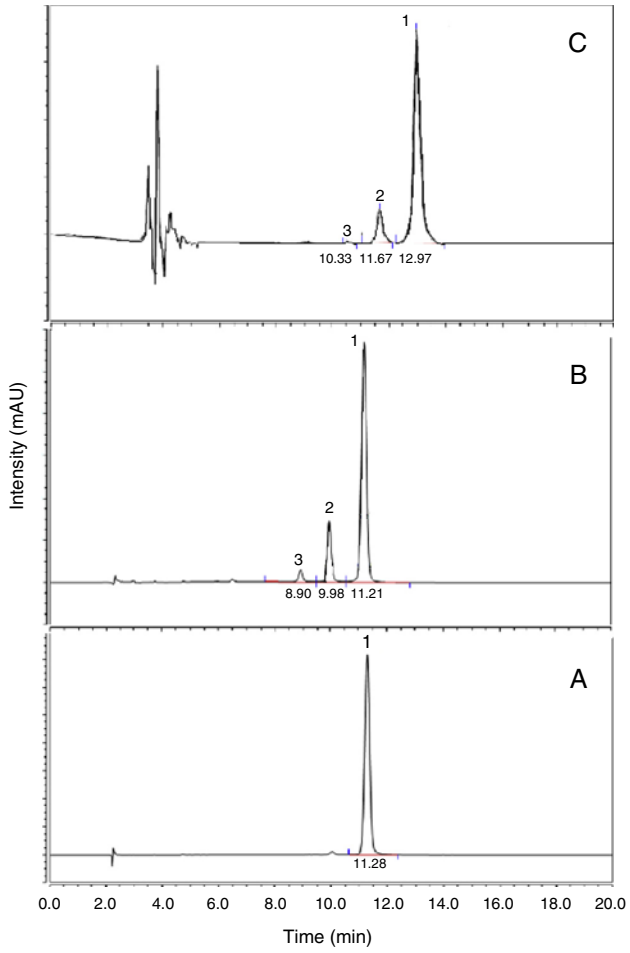

Fig. 1. Chromatograms obtained for: (A) Curcumin analytical standard (Sigma–Aldrich); (B) drug containing the three curcuminoids used as raw material; (C) sample extracted from pig skin after penetration/permeation test.

The Box–Behnken design was idealized after a series of previous experiments, varying the concentrations of beeswax, curcuminoids, surfactants, temperature, duration of shearing, mode of curcuminoid addition and others. The results showed that the HLB (hydrophilic/lipophillic balance) is very important during the nanocapsules preparation and was considered in the further work. The Box–Behnken design applied can be seen in Table 1, which shows the factors studied and their levels. The results of nanoparticle sizes, polydispersivity (PdI) and zeta potential for all the fifteen experimental conditions are shown in Table 2. As can be seen in Table 2, the PdI varied widely from 0.128 to 1.000. High values of PdI may interfere with the reliability of the estimated size of particles (Zetasizer, 2009). The Malvern Zetasizer (2009) software applies the cumulative analysis method, and calculates the zeta-average (size) and polydispersivity from the coefficients of the virial equation. When cumulative analysis is not applicable, the PdI values are above 0.100 and neither zeta-average nor PdI values are reliable. Based on this, the diameters were extracted directly from the frequency distribution of sizes, *i.e.*, from the center value of the peak with higher intensity.

Three examples of the size distributions can be seen in Fig. 2. Fig. 2A presents the signal intensity distribution for experiment 2 from Table 1, where the SLN was prepared with 15% of curcuminoids, 20 min at high shear homogenizer and ratio of lecithin to Tween 80 equal to 1.0. As shown in this plot, there is only one peak with a reasonable narrow distribution and sizes varying from 100 to 200 nm. Depending on the conditions of preparation the profile of the distribution can change considerably, as seen in Fig. 2B, which shows the size distribution for experiment 5 from Table 1. In this processing condition there are two well defined peaks, or a bimodal distribution: the first peak with size from 20 to 80 nm and the second and larger peak with size from 100 to 600 nm. In this condition, there is also a very small peak appearing at sizes above 7000 nm. Fig. 2C shows a third interesting result in a unimodal distribution showing sizes from 150 to 550 nm (experiment 11 in Table 1). These three plots are very illustrative of the strong effect of experimental conditions on the nanoparticles size distributions, and the importance of using multivariate tools for effects elucidation. Since our analysis must be based on the intensity distribution, only the largest peaks were taken into consideration for mean size determination at the situations where there are two or more peaks.

The values of mean sizes, polydispersivity and zeta potential for all fifteen experiments are shown in Table 2. Considering all experiments in Table 2, an averaged particle size was 210.4 ± 146.6 nm, with minimum and maximum of 32.7 nm and 481.9 nm, respectively. The averaged zeta potential was -29.0 ± 4.2 mV, varying from a minimum of -35.3 mV to a maximum of -21.2 mV. The averaged PdI was 0.546 ± 0.300, with a maximum of 1.000 and minimum of the 0.128, but one must remember that this PdI was calculated by Malvern cumulative analysis and can only be used for qualitative purposes (Zetasizer, 2009) and not for quantitative comparison.

The analysis of variance, ANOVA, by response surface methodology resulted in a few significant effects of the factors studied on Dp, PdI and ZP. The Pareto plots in Fig. 3 bring the probabilities p for all the linear, quadratic and interaction effects for the Box–Behnken designs. Fig. 3A shows the Pareto plot for Dp, which indicates that only the linear effect of the ratio of lecithin/Tween, L:T was significant to SLN sizes at 5% ($p < 0.05$). The other factors and their interactions were not significant within the confidence interval of 95%. The polynomial equation to fit the surface response has only X3 as a variable, as presented in Eq. (1).

$$Dp = 144.5 + 143.6(X3) \quad (1)$$

The effect of surfactants ratio, L:T, on Dp was the unique significance in curcuminoids/beeswax SLN. Other authors (yet for Jebors et al., 2010) reported no influence of agitation speed on the particle size, as observed in this work, and this can be attributed to the thermodynamic effect of surfactant addition as being primordial in emulsion formation. Besides, the use of small glassware in the lab scale is commonly associated to strong wall effects, which may cause the multimodal size distributions, which was observed in Fig. 2B, and four other experimental conditions in this work. A common practice is to filter the samples to cut off undesired sizes using 0.22 and 0.45 mm filters. In this work, however, the decision for no filtration aimed to study the actual implications of the method of production and the real interference of the factors analyzed.

For the PdI only the linear effects of %C:Bw (X1) and L:T (X3) were significant at the adopted significance level ($p < 0.05$). The other factors were not significant within the confidence interval of 95%, so they were not kept in the polynomial fitting presented in Eq. (2).

$$PdI = 0.398 + 0.201(X1) - 0.309(X3) \quad (2)$$

The changes in lecithin concentration when L:T is varied may be related to increased polydispersity of the particles, since there is a possible formation of multiple layers of lecithin on the surface of the SLN or the formation of other colloidal structures (Jebors et al., 2010).

Fig. 3B shows that for zeta potential, ZP, only the linear effect of homogenization time (X2) and the quadratic effect of the ratio of L:T (X3) were significant. The other factors were not significant

Table 2
Size, PdI and zeta potential of SLN-Cur.

Exp.	% Curcuminoids/beeswax	Time in Turrax	Proportion lecithin/Tween 80	Size (nm)	Peak intensity (%)	PdI	Zeta (mV)
1	5	20	1.0	405.5	95.1	0.429	−21.2
2	15	20	1.0	128.9	100.0	0.627	−21.4
3	5	10	1.0	201.2	91.5	0.373	−28.7
4	15	10	1.0	62.2	100.0	1.000	−33.4
5	5	15	0.5	247.6	82.7	0.543	−33.3
6	15	15	0.5	45.0	100.0	1.000	−31.3
7	5	15	1.5	374.9	100.0	0.179	−28.9
8	15	15	1.5	481.9	100.0	0.504	−27.1
9	10	20	0.5	32.7	100.0	1.000	−29.1
10	10	10	0.5	62.4	100.0	0.972	−34.0
11	10	20	1.5	297.0	99.0	0.236	−28.0
12	10	10	1.5	382.9	100.0	0.128	−35.3
13	10	15	1.0	159.4	100.0	0.461	−29.3
14	10	15	1.0	150.2	100.0	0.361	−25.0
15	10	15	1.0	123.9	100.0	0.371	−29.1

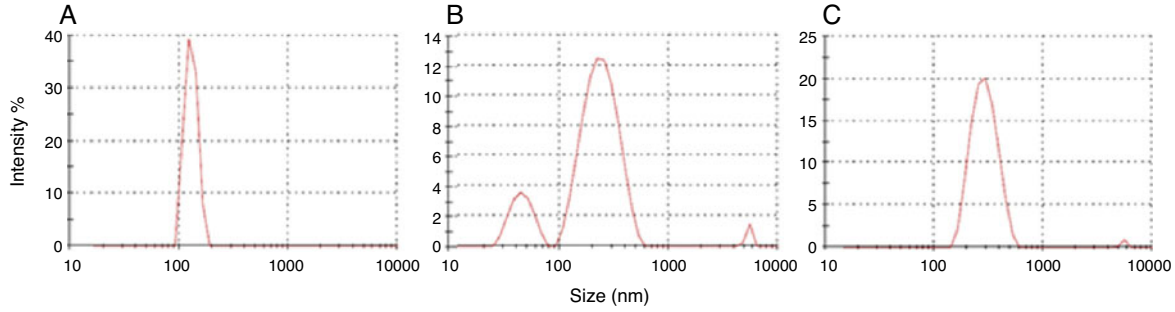

Fig. 2. Size distribution by intensity of experiments 2, 5 and 11 (A–C).

within the confidence interval of 95%, so they were not kept in the polynomial fit presented in equation,

$$ZP = -27.80 + 3.96(X2) - 3.89(X3)^2 \quad (3)$$

The magnitude of the ZP can be associated with the ratio of lecithin used in the formulations and interaction with other components such as the Tween, which is a non-ionic surfactant.

Encapsulation efficiency

The encapsulation efficiency, EE, varied from 41.4 to 62.0% for curcumin and from 31.0 to 58.78% for total curcuminoids. The averaged curcumin encapsulation efficiency for the fifteen experiments was 52.92 ± 5.41% and for total curcuminoids, 48.39 ± 6.62%. The ANOVA by response surface methodology showed that only %C:Bw affected EE at the significance level of 5%. Fig. 4 shows the contour plot of curcumin encapsulation efficiency in beeswax nanoparticles as a function of homogenization time, HT, and percent curcumin content, %C:Bw. Although literature data (Yallapu et al., 2013) indicate that curcumin encapsulation can be higher for other carriers, EE above 50% may be considered good for a lipophilic drug and the EE varies strongly depending on different systems and processes. A polynomial equation was fitted to the date of %EE for curcumin and is given in Eq. (4).

$$\%EE = 53.30 + 5.60(X1) \quad (4)$$

Morphological analysis

Fig. 5 shows the morphology of the SLN by atomic force microscopy, AFM. Fig. 5A presents the topographic view, while the frontal view is shown in Fig. 5B. It was evident that particles were spherical in shape and homogeneously distributed with size ranged between 52 nm and 101 nm, smaller than that measured by dynamic light scattering.

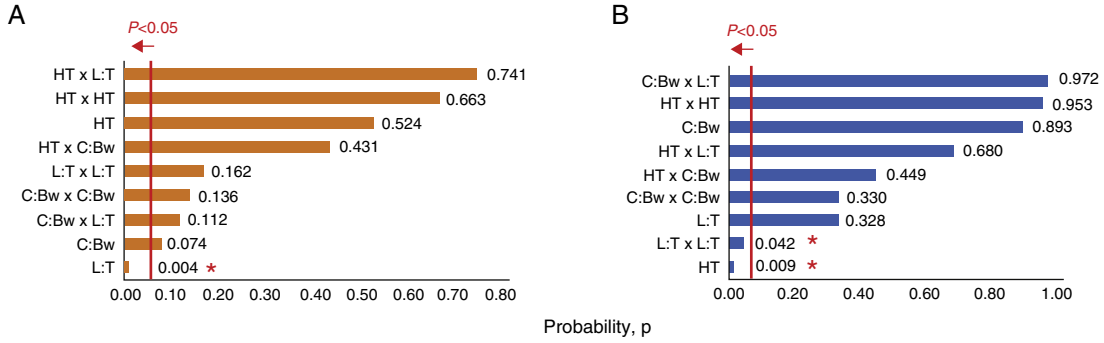

Fig. 3. Pareto diagrams showing the significance of factors, HT, C:Bw and L:T, for particle size (A) and zeta potential (2B). *Significant at 5% ($p < 0.05$).

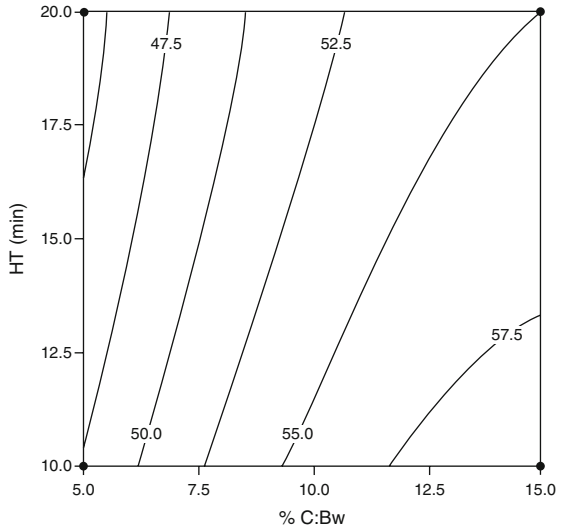

Fig. 4. Contour plot of encapsulation efficiency, EE%, of curcumin in beeswax nanoparticles as a function of homogenization time, HT, and percent curcumin content, %C:Bw. Only the effect of %C:Bw is significant at $p < 0.05$.

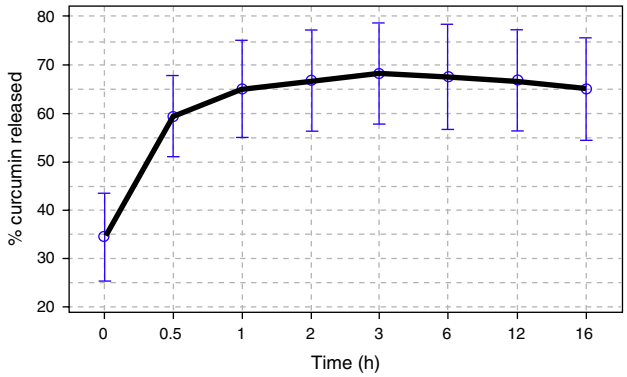

Fig. 6. Curcuminoid release in phosphate buffer (pH 7.4) solution after 16-h test. Experiments made in sextuplicate.

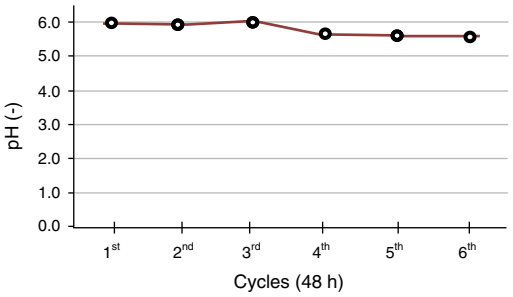

Fig. 7. pH of hydrogel containing the curcumin SLN based on pH values after six 48 h stress cycles of heating (40 °C for 24 h) and cooling (5 °C for 24 h).

Release profile

All the samples of nanocapsules tested showed a sustained but not linear release (Fig. 6), as seen in the literature as a first order kinetics (Nayak et al., 2010; Puglia et al., 2012). Approximately 50–60% of curcumin was released after 2 h of experiment. However, around 40% of curcuminoids remained unreleased after 10 h of experiment. Nayak et al. (2010) found, using the same methodology, that after 12 h of experiment 70% of curcuminoids had been released, and within 24 h of release was around 80%. The extended-release profile may indicate interactions between the lipid and the drug and a more central location of this drug in the SLN (Küchler et al., 2009), beyond the influence of the surfactants used.

Preliminary stability

The formulation did not undergo phase separation when subjected to mechanical stress test by centrifugation (Anvisa, 2004). The gel remained homogeneous, with bright and light yellow color, characteristic odor and no phase separation during the mechanical stress at 3000 rpm for 30 min. Fig. 7 shows the pH values measured at the end of each thermal stress cycle of 24 h at 40 °C and 24 h at 5 °C. The pH remained pretty stable over the test period (Fig. 8) and it has undergone minor changes that were not significantly different than the pH of the gel initially made. The pH of the gel, slightly below 6.0, is physiologically adequate for topical use and also adequate for chemical stability, since curcuminoids are unstable at pH above 7 (Tønnesen and Karlsen, 1985; Rusig and Martins, 1992). Another stability test was run for 90 days, with gel samples stored at test temperatures of 25 ± 2 °C and 5 ± 2 °C, in the absence or presence of light. The organoleptic evaluation of the gel showed no significant changes in appearances and no phase separation. The pH of the gel containing the SLN-Cur did not change significantly after three months if compared to the beginning of the tests. The pH was 5.48 for the gel kept at 25 ± 2 °C in absence of light, 5.13 for the sample at the same temperature but under daylight and 5.66 for the gel stored at 5 ± 2 °C. The centrifugation test was also performed and the three samples did not undergo phase separation.

Ex vivo penetration and permeation study

The HPLC analyses of extratum corneum and epidermis + dermis after the 18 h experiments in Franz cells are shown in Figs. 8 and 9, respectively. There was no permeation of curcuminoids in pig ear skin during any of the sixplicates, which indicate a controlled release from SLN in gel with release time longer than 18 h. Due

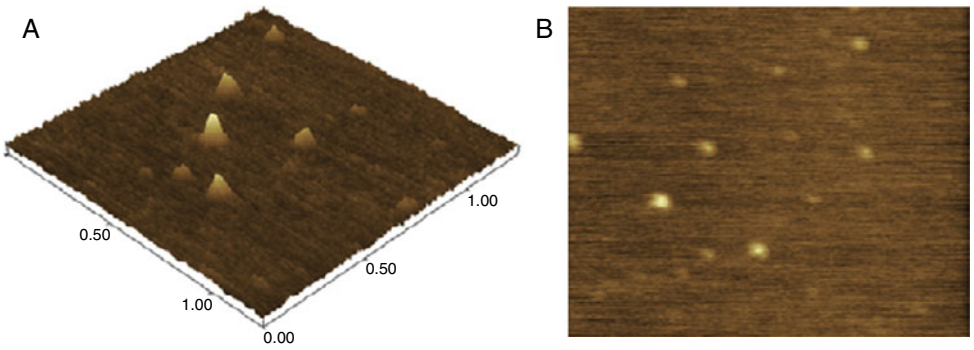

Fig. 5. Atomic force microscopy. Topographic (A) and frontal view (B).

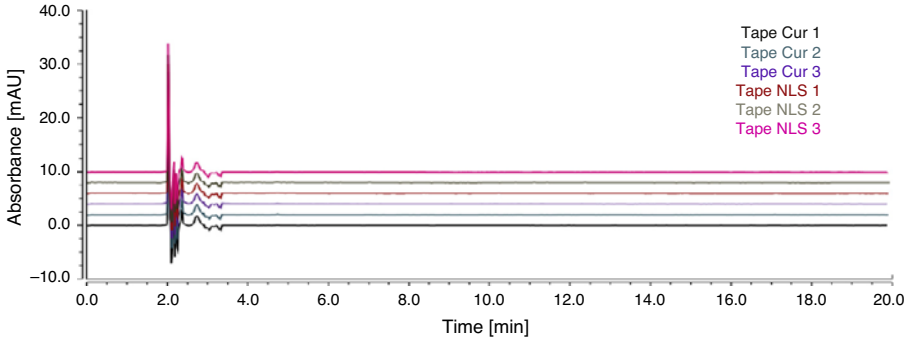

Fig. 8. Chromatograms obtained from samples of extratum corneum after the penetration/permeation tests. Aiming for quantification of curcuminoids after tape stripping. No peaks of curcuminoids were detected. (*Note*: Triplicate tapes of the skin exposed to the gel with free curcuminoids and tape of the skin exposed to gel with SLN-curcuminoids.)

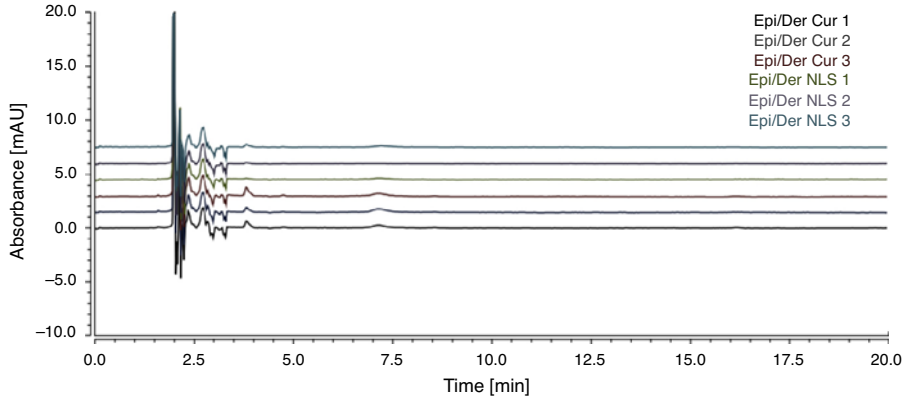

Fig. 9. Chromatograms obtained from samples of pig ear skin after the penetration/permeation tests. Aiming for quantification of curcuminoids in epidermis and dermis. No peaks of curcuminoids were detected. (*Note*: Triplicate tapes Epidermis + dermis exposed to the gel with free curcuminoids and epidermis + dermis exposed to gel with SLN-curcuminoids.).

to limitations imposed by the use of *ex vivo* biological material, the permeation experiment cannot be longer than 18 h, since the pig ear skin undergoes strong changes after this period at 37 °C (Sartorelli et al., 2000).

Penetration of curcumin or curcuminoids was not observed for any formulation in triplicate, *e.g.*, gel containing the SLN-Cur and gel with free curcuminoids, which corroborates with the idea of very slow release from nanocapsules. The results corroborate the statement that nanotechnology can modify the penetration/permeation of substance and control their release, increasing the residence time on the skin surface (Luengo et al., 2006; Guterres et al., 2007; Cevc and Vieri, 2010).

Since the goal of this work was to develop an anti-inflammatory topical formulation for radiodermitis, it is adequate that curcuminoids show a slow release and that SLN-Cur does not penetrate in normal skin, behavior that can be significantly changed in skin damaged by radiotherapy buns. The result is stimulating and should be confirmed in future *in vivo* tests for normal and injured skin, since penetration/permeation *in vivo* can be considerably different from *ex vivo* because of shear caused by body movement and also by skin injures caused by radiation (radiodermitis), which may favor the penetration of curcuminoids.

Conclusions

Beeswax showed to be an excellent natural carrier for curcuminoids nanoencapsulation, resulting in nanocapsules with adequate size and zeta potential. Encapsulation efficiencies and drug load in nanocapsules also demonstrate that the process developed is promising. The application of factorial design to study the preparation process for nanocapsules showed to be a very important tool, allowing establishing the relationships among the factors and quality attributes. Hydrophilic gel containing solid lipid nanocapsules showed to be stable in preliminary tests and that penetration/permeation in pig skin could not be detected in 18 h. Assay in Franz cells using pig ear skin showed no penetration/permeation of nanoencapsulated curcuminoids. The results are stimulating for future *in vivo* tests of anti-inflammatory activity in radiodermitis treatment.

Authors' contributions

CMZ (MSc student) was responsible for most of experimental work (nanocapsules, gel and characterization); RMM developed the nanoencapsulation technique and contributed in all laboratory work and chromatographic analysis. ECC idealized the work and contributed to critical reading of the manuscript. LAPF designed the study, supervised the experiments, critically read and defined the final version of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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