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Curcumin encapsulated in poly-L-lactic acid improves its anti-inflammatory efficacy *in vivo*

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ABSTRACT

Curcuma longa L., commonly known as turmeric, has been used to treat some diseases such as gastric ulcers and rheumatoid arthritis. Studies showed that curcumin (Cur) has low water solubility and impaired bioavailability as a consequence of poor absorption, rapid hepatic and intestinal metabolism and also systemic elimination, justifying the development of new formulations to improve its bioavailability. The aim of this study was to compare the effect of Cur and nanoencapsulated curcumin (CurNano) on acute inflammatory response in rats. For this, we used the miniemulsification/evaporation technique to obtain nanoparticles of poly(L-lactic acid) containing curcumin at different concentrations (1, 3, 9, and 15%). The results of the encapsulation efficiency, scanning electron microscopy, calorimetry, and infrared showed that curcumin was efficiently encapsulated, but there was an upper limit of the concentration of curcumin that can be entrapped in the nanoparticles (around 2.6%). The biological efficacy was evaluated by experimental model of carrageenan-induced paw edema. The data showed an improvement in the anti-inflammatory activity of curcumin because CurNano at dose 50 mg/kg produced an inhibitory effect similar to that of Cur 400 mg/kg. This probably occurred because of an increase in the bioavailability of curcumin after its encapsulation.

Keywords: Curcumin, nanoparticles, inflammation, nanoencapsulation, bioavailability.

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INTRODUCTION

Curcuma longa L. is a plant native to tropical South and Southeast Asia, popularly known as "saffron," a member of the ginger family (Zingiberaceae). This plant is traditionally used in Chinese and Indian traditional and folk medicine, respectively, for the treatment of gastric ulcers, skin and eye infections, and inflammatory diseases (Singh, 2007; Liju et al., 2011).

The anti-inflammatory activity of the plant should be due to the group of pigments called curcuminoids, which are composed of curcumin, dimetoxicurcumin, bis-dimetoxicurcumin, and cyclocurcumin (Kiuchi et al., 1993; Aggarwal et al., 2003; Zeng et al., 2007), with curcumin being the major component of this group and thus the most studied (Asakawa et al., 1981; Jurenka, 2009).

It was reported that the anti-inflammatory property of curcumin can be attributed to inhibition of the expression of the enzyme cyclooxygenase-2 (COX2) and suppression of prostaglandin synthesis (Goel et al., 2001; Aggarwal and Harikumar, 2009). However, other studies indicate that the mechanism of action of curcumin is not

limited to COX2 inhibition and may involve other pathways including a protein kinase downregulation (Dorai et al., 2000); inhibiting the activation of nuclear factor kappa B (NF-kB) (Surh et al., 2002); and release of cytokines (tumor necrosis factor (TNF), interleukin-1β (IL-1β), interleukin-8 (IL-8), and nitric oxide synthase (Singh and Vinayak, 2014; Jobin et al., 1999; Singh and Aggarwal, 1995). Furthermore, it was observed that curcumin inhibits the production of metalloproteinase-3 from chondrocytes (Mathy-Hartert et al., 2009) and induces apoptosis and also inhibits the production of prostaglandin E_2 in synovial fibroblasts of patients with rheumatoid arthritis (Park et al., 2007).

However, some authors have shown that curcumin has low water solubility (Roughley and Whiting, 1973; Anand et al., 2007) and impaired bioavailability after being administered orally (Balaji and Chempakam, 2010), due to poor absorption, rapid hepatic and intestinal metabolism, rapid systemic elimination, and absence of pharmacological activity of metabolites (Aggarwal and Harikumar, 2009). Therefore, there is a need to improve the solubility and bioavailability of curcumin.

The development of drug delivery systems, particularly nanoparticles, has been relevant in the pharmaceutical area because such systems can provide modern therapeutic alternatives, are pharmacologically more effective, and have reduced side effects (Oliveira et al., 2004).

The polymeric nanoparticles are drug carrier systems with diameter less than 1 µm that have been developed for numerous therapeutic applications by oral, parenteral, or local administration (Musyanovych et al., 2008). Particularly, administration of nanoparticles orally is directed to increase the bioavailability of certain drugs such as nonsteroidal anti-inflammatory drugs (Schaffazick et al., 2003) and to reduce the adverse effect irritation of the gastrointestinal mucosa (Guterres et al., 2001).

The use of poly(L-lactic acid) (PLLA) and its derivatives for the development of polymeric encapsulation systems has been interesting because of its non-toxicity, biodegradability, and bioresorbability. Nanoencapsulation can overcome physical-chemical limiting properties of the encapsulated drugs, such as low water solubility, membrane solubility, and degradation at extreme pH. It results in the improvement of pharmacodynamic, pharmacokinetic, and toxicological aspects, such as the control of absorption and tissue distribution, and the reduction of local and systemic toxicity (Motta and Duek, 2006; des Rieux et al., 2006; Ravivarapu et al., 2006).

Many studies have been conducted with the aim of making curcumin as an applicable therapeutic product. However, there are few studies that employ systems release drugs developed for curcumin *in vivo* experimental models, mainly on the study of the acute inflammatory process.

Thus, the aim of this study was to obtain a new formulation of curcumin using biodegradable polymers (CurNano) and compare their effectiveness with that of curcumin *in nature* (Cur) on acute inflammatory response in rats.

MATERIALS AND METHODS

Materials

The poly (L-lactic acid) (PLLA) (Mw = 3.780 g/mol) was ceded by the Graduate Program in Food Technology, Federal Technological University of Paraná. Curcumin was obtained from Sigma-Aldrich (99.5% purity). Dichloromethane (Vetec, Brazil, 99.5% purity) was used as the solvent of the organic phase to obtain the nanoparticles in determining the encapsulation efficiency and percentage recovery. Methanol (PróQuímicos, Brazil, 99.8% purity) was also used to determine the encapsulation efficiency and percentage recovery. Distilled water was used as the continuous medium miniemulsion. Soy lecithin (Alfa Aesar, MA, USA, 90% purity) was used as surfactant in obtaining the nanoparticles. The λ-carrageenan (Sigma-Aldrich) used as a phlogistic agent; hydrogen peroxide, O-dianidisine dihydrochloride, and sodium acetate used as reagents for determination of myeloperoxidase activity; and dihydrochloride sulfanilamide and N-(1-naphthyl)ethylenediamine dihydrochloride reagents used for determination of nitric oxide were obtained from Sigma-Aldrich. The ortho-phosphoric acid was obtained from the Nuclear, Brazil, (85% purity) was also used as a reagent for the determination of nitric oxide.

Developed and characterization of nanoparticles

Synthesis of PLLA nanoparticles containing curcumin

The PLLA nanoparticles were produced by the miniemulsification/solvent evaporation technique (Leimann et al., 2013; Silva-Buzanello et al., 2011). The organic phase was prepared by dissolving lecithin, PLLA and curcumin (1, 3, 9 or 15%wt) in dichloromethane for 10 min. In all experiments, the aqueous phase consisted of distilled water added under stirring (60 rpm) yielding a macroemulsion. The miniemulsification was carried out using a dispersor (Ultraturrax IKA, model T25) at 14,000 rpm in an ice bath for 6 min. The resulting miniemulsion was sonicated (Fisher Scientific - Ultrasonic Dismembrator 120 W, 1/8" tip) with sonication time of 360 s and 100% amplitude in a pulse regime (30 s of sonication followed by 10 s paused). The miniemulsion was gentle stirred for 18 h at 40°C to allow solvent evaporation. No additional co-surfactant was used because it is known that preformed polymers added to the dispersed phase can inhibit diffusional degradation of the miniemulsion during the preparation of nanoparticles (Reimers and Schork, 1996; Wang et al., 1998). It is known that the nanoparticle size is influenced by the position of the ultrasound probe (Mujumdar et al., 1997); therefore the experimental system was designed to maintain the height, the diameter of the vessel and the position of the probe within the container constant where the miniemulsion was prepared.

Determination of curcumin recovery

Curcumin recovery represents the actual amount of curcumin present in the nanoparticles dispersion. Aliquots of 1 ml of the nanoparticles dispersion were dried in a circulation oven at 70°C for

2 h and then 1 ml of dichloromethane and 1 ml of methanol were added. An aliquot of 1 ml of the filtrate (Millipore membrane filter, 0.45 µm) was transferred to a 10 ml volumetric flask. Absorbances were determined by ultraviolet-visible spectroscopy (OceanOptics, USB650 UV-Vis) at 465 nm (Silva-Buzanello et al., 2015) and the curcumin concentration was determined by Equation 1.

$$Rec(\%) = 100\, x \frac{[cur]_{actual}}{[cur]_{added}} \quad (1)$$

Where: Curcumin recovery ($[cur]_{actual}$ = total curcumin concentration present at the end of the nanoencapsulation process; $[cur]_{added}$ = curcumin concentration initially added).

Determination of encapsulation efficiency (EE)

5 ml of nanoparticle dispersion was centrifuged at 1000 rpm for 10 min in order to precipitate the non-encapsulated curcumin crystals. Then, determination followed as described above. To make sure that all non-encapsulated curcumin was precipitated in the centrifugation, 0.5 ml of the supernatant was transferred to an eppendorf tube with an Amicon® filter (100 kDa) and centrifuged at 14,500 rpm for 30 min using (MiniSpin Plus Eppendorf). The filtrate was dried in an oven forced air circulation at 70°C for 24 h. The dried samples were diluted in 1 ml of dichloromethane and 1 ml of methanol, and 1 ml aliquot was transferred to a 10 ml volumetric flask. Absorbances were determined by UV-Vis at 465 nm (Silva-Buzanello et al., 2015). The calculation of the encapsulation efficiency was performed according to Equation 2. The determination of curcumin recovery and encapsulation efficiency, were carried out in duplicate and the data were analyzed using an appropriate statistical software.

$$EE(\%) = 100\, x \frac{[cur]_{encapsulated} - [cur]_{non-encapsulated}}{[cur]_{actual}} \quad (2)$$

Where: Curcumin encapsulation efficiency ($[cur]_{actual}$ = total curcumin concentration present at the end of the nanoencapsulation process; $[cur]_{non\text{-}encapsulated}$ = concentration of non-encapsulated curcumin (amount that crossed the filter during centrifugation); $[cur]_{encapsulated}$ = concentration of encapsulated curcumin).

Characterization of the nanoparticles

The average diameter (Dz) and polydispersity index (PDI) of the nanoparticles were determined by Dynamic Light Scattering (Malvern Zetasizer, NanoSeries) with detector at 173° in samples without dilution (International Standard ISO13321, 1996). PDI (Equation 3, ISO13321) is often used as an indirect measure of how broad the particles size distribution is.

$$PDI = \frac{\sigma^2}{D_Z^2} \quad (3)$$

Where: Polydispersity index (σ = standard deviation; Dz = average diameter)

Differential scanning calorimetry (DSC) was used to evaluate the physical state of curcumin after the encapsulation procedure (Perkin Elmer, STA 6000). The samples were lyophilized and 3 mg were used for analysis in an aluminum sample holder. The samples were heated from 20 to 390°C at 10°C/min and a nitrogen flow of 50 ml/min. To identify the existence of possible chemical interactions between the compounds used in the nanoencapsulation curcumin, Fourier Tranform Infrared Spectroscopy were carried out (FTIR-UATR, Frontier PerkinElmer) with a resolution of 4 cm^{-1} from 4000 to 600 cm^{-1} with attenuated reflectance (UATR). The analysis was performed in triplicate for each sample and the peaks were normalized in order to allow comparison between each sample. The morphology of nanoparticles was evaluated in a scanning electron microscope (SEM, Shimadzu, SS550) using a secondary electrons detector. The non-lyophilized samples were added dropwise on glass sheets, dried for 24 h and gold coated.

Evaluation of the effect of CurNano compared with Cur on the inflammatory response

Animals

Male Wistar rats weighing between 180 and 200 g were used. The animals were kept under controlled temperature of 22°C and in dark/light cycle of 12 h, with water and food *ad libitum* maintained in the sectoral biotery of the Department of Pharmacology and Therapeutics, State University of Maringá (UEM). The experimental protocols were approved by the Ethics Committee on Animal Experimentation of the State University of Maringá (CEAE/UEM 025/2013).

Animal treatment

The treatment of rats with Cur (*in nature* curcumin) and CurNano (nanoencapsulated curcumin), suspended in water at 37°C, respectively, was carried in a single daily oral dose one hour before the induction of the inflammatory response. Rats were treated with Cur at doses of 50, 100, 200, and 400 mg/kg body weight or CurNano at doses of 25 and 50 mg/kg body weight. The drug indomethacin (Indo-5 mg/kg) was used as a positive control.

Induction of paw edema

The rats (n = 6 per group) received intraplantar injection (intradermally) at 100 µl carrageenan (200 µg/paw) solution, dissolved in 0.9% saline into a hind paws. The same volume of vehicle (0.9% saline) was injected into the contralateral paw. The volume of both paws was determined at times of 60, 120, and 240 min after application of carrageenan, with a plethysmograph (Winter et al., 1962). The increase of final volume of the paw was calculated by subtracting the volume of the paw injected with saline (control paw) by volume of the paw injected with carrageenan.

Preparation of plantar tissue

After a 4-h induction of paw edema by carrageenan injection, the rats were euthanized; the plantar tissue was removed and placed in an Eppendorf tube containing 0.6 ml of PBS 4 mM, pH 5.4. Then, the sample was homogenized in a Potter homogenizer. The homogenate was centrifuged at 6000×G at 4°C for 15 min, and the

Table 1. Curcumin recovery and encapsulation efficiency (EE) when 1, 3, 9 and 15 wt% of curcumin were added to the formulation.

Added curcumin concentration (wt%)	Recovery (%)	E.E. (%)*
1	100.9 ± 11.6	98.9 ± 2.2[a]
3	90.5 ± 1.4	89.2 ± 2.8[b]
9	90.4 ± 6.5	51.7 ± 0.5[c]
15	96.0 ± 12.4	18.2 ± 0.5[d]

* Different letters in each row indicate statistically significant differences at $p < 0.05$ (ANOVA).

supernatant was immediately separated and stored at -70°C for later analysis.

Myeloperoxidase activity (MPO)

The activity of MPO was measured in the supernatant of plantar tissue homogenate (Bani et al., 1998). The supernatant of the plantar tissue homogenate (10 µl) was placed in a 96-well microplate in triplicate and then added a solution of 2.9 ml of PBS 50 mM, pH 6, containing 0.19 mg/ml O-dianidisina-hydrochloride and 0.0005% H_2O_2. The reaction was stopped with sodium acetate solution 1.46 M (pH = 3.0), and enzyme activity was determined by end-point technique by measuring the absorbance at a wavelength of 460 nm.

Nitric oxide concentration (NO)

The concentration of nitric oxide was determined by the Griess method, which determines the nitrite production as a measure of the gas production (Green et al., 1982). The supernatant of the plantar tissue homogenate (50 µl) was placed in a 96-well microplate in quadruplicate, and then, Griess solution was added (1% sulfanilamide in 5% phosphoric acid and 0.1% dihydrochloride N-1-naftiletilonodiamine in water) at room temperature. After 10 min, reading was performed using an ELISA plate reader at a wavelength of 550 nm (Saleh et al., 1999). NO concentrations were calculated from a standard curve of sodium nitrite. The results were expressed as µM.

Statistical analysis

The results are expressed as mean ± standard error of the mean (SEM). The data were subjected to analysis of variance (ANOVA), followed by Tukey test. $P < 0.05$ was considered as level of significance.

RESULTS AND DISCUSSION

Curcumin recovery, encapsulation efficiency and scanning electron microscopy

Table 1 shows the results of encapsulation efficiency (EE) and curcumin recovery. Figure 1 presents the images of Scanning Electron Microscopy (SEM) of nanoparticles containing 3 to 15 wt% curcumin added. The results of percentage recovery for all concentrations of curcumin employed during the encapsulation process showed values above 90%; there was no significant difference between groups ($p > 0.05$). On the other hand, encapsulation efficiency decreased significantly with increasing the added amount of curcumin, ranging from 18.2 to 98.9%.

The results demonstrated that because the concentration of curcumin is increased relative to the polymer concentration, a decrease of the encapsulation efficiency occurred, as an indication that there is a maximum curcumin concentration that can be effectively incorporated in the PLLA nanoparticles. This was corroborated by microscopy images, showing that there was no crystals of nonencapsulated curcumin when the concentration used was 3 wt% (Figure 1A and B). However, at a concentration of 15 wt%, a great amount of micron-sized crystals of curcumin can be observed (Figure 1C and D).

Similar data were found by other authors that encapsulated curcumin in starch and gelatin by spray-drying technique, and the results show that the encapsulation efficiency decreased with the increasing added curcumin concentration (Wang et al., 2009). Indeed, the encapsulation was evaluated under different concentrations of curcumin using yeast cells (*Saccharomyces cerevisiae*) as encapsulating agents, and modified starch was described (Paramera et al., 2011). In this study, a decrease in the encapsulation efficiency with increasing proportions of curcumin: yeast cells was observed. In another work, Patel et al. (2010) showed that different concentrations of curcumin was encapsulated in zein nanoparticles (corn protein) using the technique of precipitation in antisolvent, varying the curcumin concentration. The gradual increase in the curcumin concentration led to a decrease in encapsulation efficiency, suggesting that higher proportions of curcumin may result in precipitates that are not incorporated into the polymeric matrix. Together, these studies demonstrated the importance of setting ratios between the encapsulating agent and the encapsulated compound to achieve more satisfactory encapsulation efficiencies.

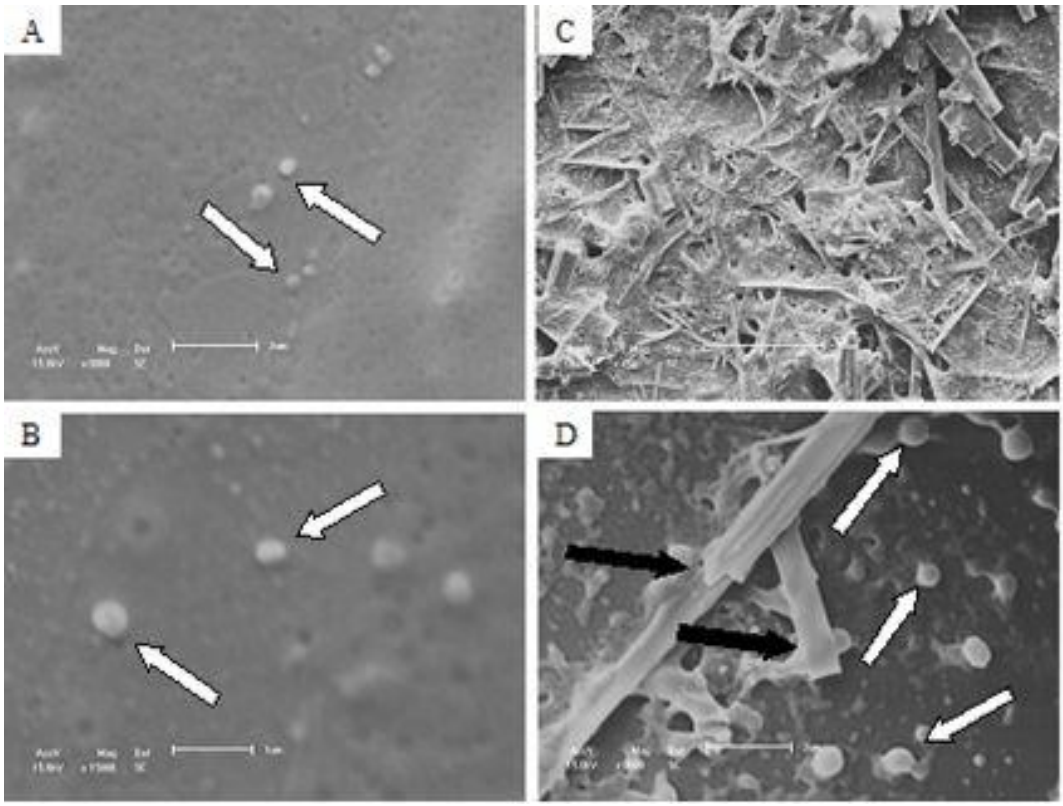

Figure 1. PLLA nanoparticles containing encapsulated curcumin (indicative white arrows) and free curcumin crystals (black arrows indicative). Samples obtained with addition of 3 wt% curcumin. (A) Image with 8000X amplification. (B) Image with 15000X amplification. Samples obtained with addition of 15 wt% curcumin. (C) Image with 450X amplification. (D) Image with 8000X amplification.

Nanoparticles sizes

The results of average nanoparticles size (Dz) showed that particle sizes varied between 275 and 340 nm (Figure 2A), confirmed by the SEM images (Figure 1). The results obtained by PDI show that the size distribution was monodisperse for all evaluated curcumin concentrations (Figure 2B).

Particles presenting narrow distributions and nanometric diameters are often obtained when ultrasound is used as the dispersion system in the miniemulsification process (Musyanovych et al., 2008; Shaikh et al., 2009; Gaumet et al., 2008; Banerjee et al., 2003; Asua, 2002). Some authors have demonstrated that the ability of nanoparticles to cross biological barriers and reach the site of action may be influenced by its size (Gaumet et al., 2008; Banerjee et al., 2003).

Basniwal et al. (2011) attributed the increased antibacterial efficacy of the curcumin nanoparticle of the particle size. Shaikh et al. (2009) obtained curcumin-loaded nanoparticles using poly(L-lactide-co-glycolide) as encapsulant polymer and observed an improved bioavailability of curcumin when administered orally.

Differential scanning calorimetry (DSC) and infrared spectroscopy (IR)

To evaluate the physical state of curcumin after the encapsulation process, thermograms were obtained of samples of *in nature* (pure) curcumin, blank PLLA nanoparticles (without curcumin), nanoparticles containing 3 wt% of added curcumin and a physical mixture of curcumin and PLLA (Figure 3). To identify the existence of possible chemical interactions among reagents used in the nanoencapsulation process, IR spectra were obtained from samples of *in nature* curcumin; blank PLLA nanoparticles (without curcumin added); ad nanoparticles containing 3, 9 and 15wt% of curcumin (Figure 4A and B).

In the thermogram show in Figure 3, we observed that the melting peak of curcumin appears in 173°C which is in agreement with literature (Yallapu et al., 2010a; Yallapu et al., 2010b; Dandekar et al., 2010b). Also noted for PLLA nanoparticles without curcumin added a peak at about 65°C, which corresponds to the relaxation peak after PLLA glass transition, as a result of the sample thermal history. PLLA glass transition temperature was

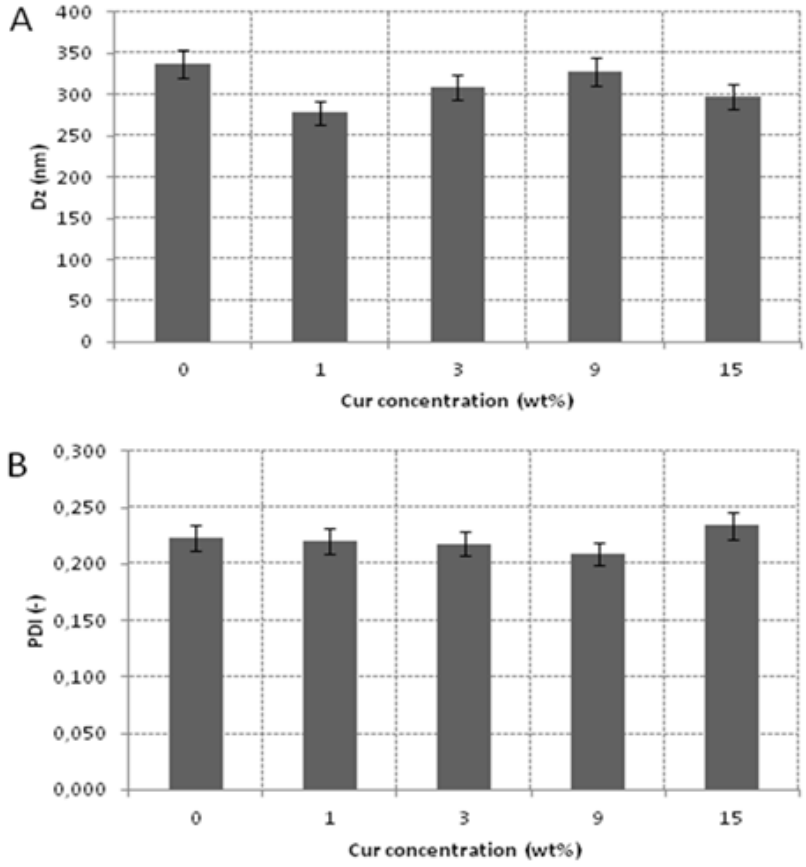

Figure 2. (A) Average nanoparticles diameter (Dz) for different curcumin concentrations added. (B) Polydispersity index (PDI) of the nanoparticles size for different curcumin concentrations added. Error bars indicate 5% of the measured value.

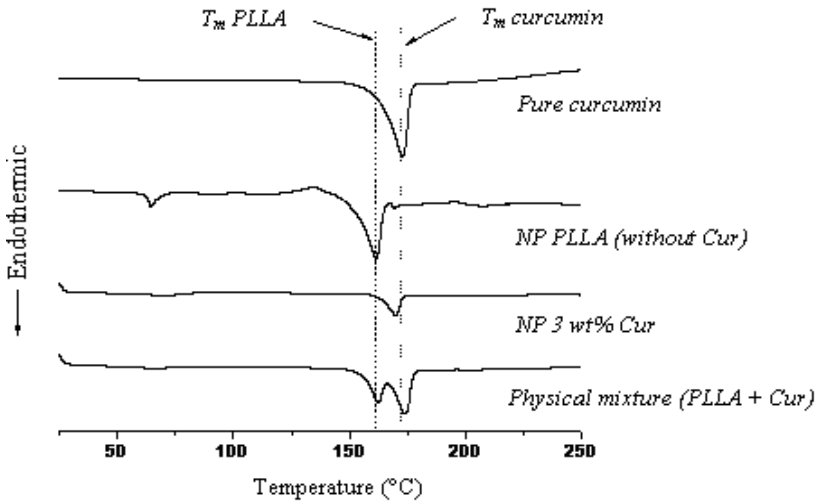

Figure 3. Thermograms of samples of *in nature* curcumin, blank PLLA nanoparticles (NP PLLA without cur), PLLA nanoparticles containing 3 wt% curcumin (NP 3 wt% Cur) and a physical mixture of PLLA and curcumin (PLLA + Cur).

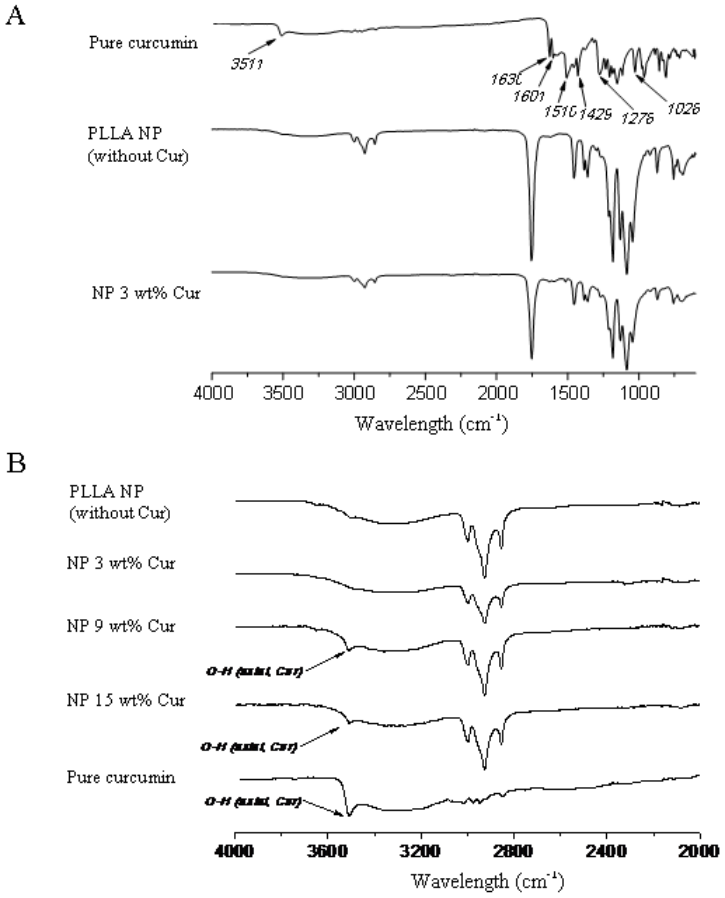

Figure 4. (A) IR spectra of *in nature* curcumin, blank PLLA nanoparticles (without curcumin, NP PLLA) and nanoparticles containing 3 wt% curcumin (NP 3 wt% curcumin). (B) IR spectra of *in nature* curcumin, nanoparticles without curcumin (NP PLLA) and nanoparticles with different concentrations of curcumin (NP 3 wt% Cur, NP 9 wt% Cur and NP 15 wt% Cur).

also observed at about 65°C less evident for the PLLA Cur 3 wt% nanoparticles and where the sample was taken only with physical mixture of curcumin and PLLA nanoparticles. The melting peak of the crystalline structure of the PLLA was recorded around 162°C for PLLA nanoparticles without curcumin added and the physical mixture. In physical mixture of PLLA and curcumin, it is possible to observe the melting peaks of both compounds. However, the melting peak of curcumin disappeared in the sample when it was encapsulated, suggesting that it is in amorphous form distributed within the encapsulant matrix, that is, forming a homogeneous solid mixture with PLLA. The disappearance of the compound peak crystalline melting during its encapsulation is used by several authors as a strong indicative that this compound is within the particles and not merely adsorbed on its surface in the form of crystals (Yallapu et al., 2010a; Yallapu et al., 2010b; Dandekar et al., 2010a; Sharma et al., 2011; Maghsoodi, 2009).

When analyzing the IR spectra, it is worth noting that the axial stretching band of the curcumin hydroxyl group (3510 cm^{-1}) is often used to evaluate the interactions between curcumin and the encapsulants (Paramera et al., 2011; Yallapu et al., 2010b). In the case of encapsulation of curcumin in PLLA, this band is important because this polymer has no infrared absorption in this region. The results in Figure 4 showed that the O-H spectra absorption band was detected for *in nature* curcumin, however, was not detected in the case of nanoparticles 3 wt% curcumin added. This effect can be explained by the fact that curcumin is located inside the nanoparticles, suggesting that the encapsulation actually occurred. However, when larger amounts of curcumin (9 wt% and 15 wt%) were added, the band 3510 cm^{-1} was detected, indicating that at these curcumin concentrations, it was not totally encapsulated and is

present in its free crystalline form. Again, these results confirm the electron microscopy images of the nanoparticles (Figure 1).

Overall, the results demonstrated that curcumin was successfully encapsulated in the PLLA nanoparticles. This can be explained by the high hydrophobe character of curcumin and also by the fact that the miniemulsification/solvent evaporation technique favors the encapsulation of hydrophobic compounds (Alexandrino et al., 2014).

Thus, the CurNano formulation presenting 3 wt% curcumin added was chosen for the biological assays because it presented high encapsulation efficiency and the absence of non-encapsulated curcumin crystals.

Effect of Cur and CurNano on carrageenan-induced paw edema in rats, the myeloperoxidase activity (MPO), and nitric oxide concentration (NO) in plant tissue

The intradermal Cg injection in one of the hind paws of rats is characterized by a local inflammatory response, with maximum intensity of edema in the fourth hour after application of the phlogistic agent with appearing of the erythema, hyperalgesia, edema and neutrophilic infiltrate resulting from release of many inflammatory mediators (histamine, serotonin, bradykinin, prostaglandins, cytokines, and reactive oxygen and nitrogen species) (Moore, 2003; Morris, 2003; Iwata et al., 2010). Treatment with Cur (100 and 200 mg/kg) caused a significant reduction in the inflammatory process, in fourth hour after Cg injection (23.66 and 30.8%, respectively). However, at the higher Cur dose (400 mg/kg), it was possible to identify a significant reduction at the second and fourth hours after Cg injection (25.57 and 36.19%, respectively). On the other hand, Cur at lowest dose (50 mg/kg) was unable to reduce significantly the intensity of the inflammatory response (Figure 5A).

The treatment of rats with CurNano (25 and 50 mg/Kg) promoted a decrease in the inflammation, at the fourth hour after Cg injection (34.9 and 50.2%, respectively). Rats treated with indomethacin, reference non-steroidal anti-inflammatory, showed a significant reduction at second and fourth hours after Cg injection (Figure 5B).

The myeloperoxidase enzyme is found in the neutrophil intracellular granules and is considered as a marker of the influx of these cells into the inflamed site (Klebanoff, 2005). Nitric oxide is involved in the acute inflammatory process by its ability to increase vascular permeability and leukocyte recruitment into the inflamed site (Moncada et al, 1991; Schinella et al., 2014).

It was observed that MPO activity (Figure 6A) and the NO levels (Figure 6B) were reduced in the plantar tissue of rats treated with Cur 400 mg/Kg, CurNano 50 mg/kg, and Indomethacin 5 mg/kg. The percentage reduction in the MPO activity was 38.4, 38.7 and 48.5%, respectively, and the NO concentration was 55.9, 57.7 and 69.7%, respectively. Our data indicated that some treatments reduce the MPO activity, and the NO concentration could be effective in the resolution of inflammation.

Our results show that an improvement in the biological efficacy of curcumin at dose of 50 mg/kg CurNano produced similar inhibitory effect on the inflammatory process, when compared with 400 mg/kg Cur treatment.

The anti-inflammatory effects of curcumin could be due to the ability to inhibit NF-kB (Surh, 2002), as well as inhibiting of the COX-2 expression, resulting in the suppression of prostaglandin synthesis (Goel et al., 2001; Aggarwal and Harikumar, 2009). Additionally, the curcumin ability in reducing leukocyte migration by inhibiting the enzyme lipoxygenase and the synthesis of pro-inflammatory leukotrienes (LTB_4) (Huang et al., 1991; Hong et al., 2004) were demonstrated. Recent work demonstrated that curcumin reduced levels of Intercellular adhesion molecule 1 (ICAM-1) and thereby reduced the number of adherent leukocytes to the endothelium (Thong-Ngam et al., 2012). Another important biological activity of curcumin is its ability to reduce levels of pro-inflammatory mediators such as NO (Brouet and Ohshima, 1995; Jung et al., 2006). Also observed in our results, this is the most effective to nanoencapsulated curcumin.

Overall, our results indicate that the curcumin nanoencapsulation enhances its effectiveness. This result may be explained to the use of polymeric nanoparticles as drug delivery systems because they have been widely used to enhance the effectiveness of many drugs (Ogiso et al., 2001). Other authors attribute the improvement in the biological efficacy, by increasing water solubility of Cur (Basnet et al., 2011; Yadav et al., 2010). The improved water solubility of Cur was observed through the preparation of dispersion in distilled water of CurNano and Cur. It was demonstrated that the CurNano dispersed in water and that this did not occur with Cur, observing the presence of precipitates (data not shown).

Additionally, an improvement in the biological effectiveness nanoencapsulated Cur in PLGA polymer was shown in another study. In this work, a plasmatic peak of 2 h and plasmatic concentrations up to 48 h for nanoencapsulated curcumin was observed, whereas that for curcumin *in nature*, plasmatic peak of only 0.5 h, decreasing rapidly, was observed (Shaikh et al., 2009). Another study showed an increase of serum curcumin 1749-fold when in nanoparticles in comparison to solvent-solubilized curcumin (Zou et al., 2013). These studies attributed this improvement in bioavailability to the protection of Cur forward to its rapid metabolism.

Another plus that should be emphasized in our work is the use of a polymer (PLLA) biodegradable, biocompatible, easily processed, besides being produced from renewable resources such as starch, molasses,

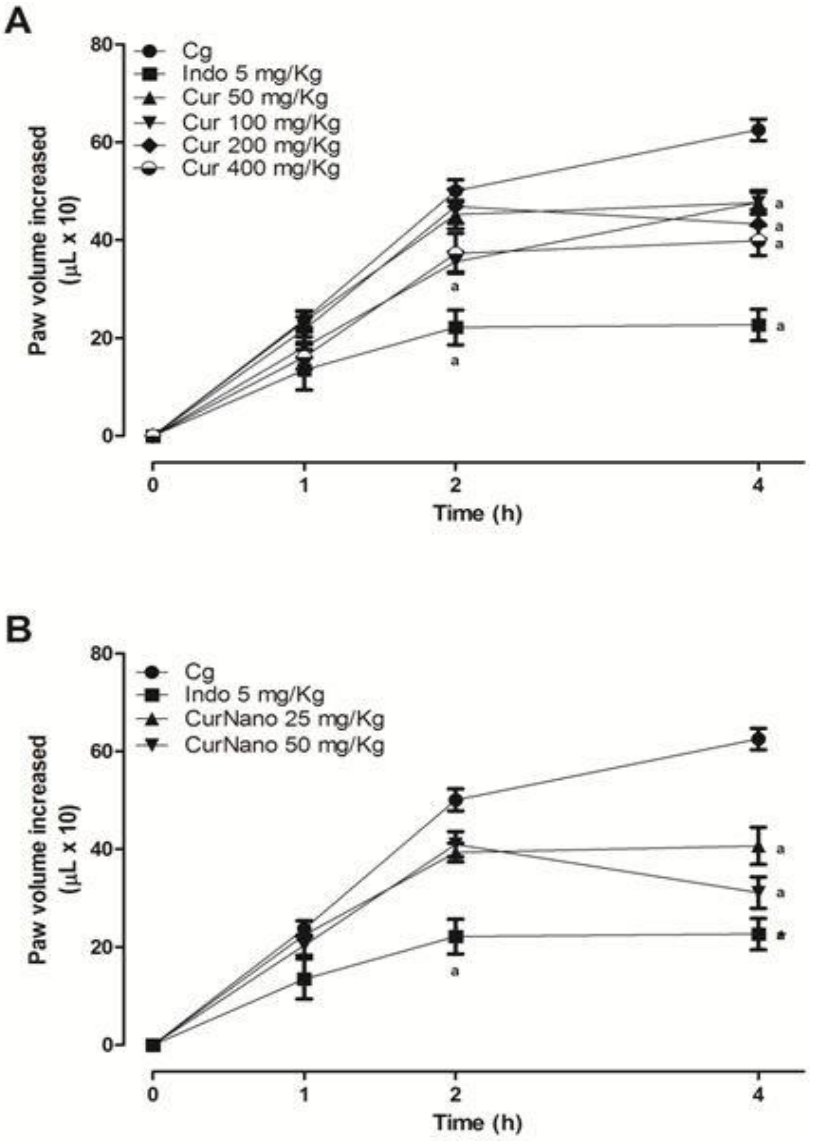

Figure 5. Effect of Curcumin *in nature* (Cur) and Curcumin Nanoparticles (CurNano) on the development of paw edema induced by intraplantar carrageenan injection (Cg 200 µg/paw) in rats (180 to 200 g). **(A)** animals (n = 6 per group) treated with Cur orally at doses of 50, 100, 200, and 400 mg/kg; **(B)** animals (n = 6 per group) treated with CurNano orally at doses of 25 and 50 mg/kg. Positive control rats were treated with indomethacin (Indo) orally at a dose of 5 mg/Kg. Each point represents the mean ± SEM paw volume 1, 2, and 4 h after Cg injection. a = $p < 0.01$ compared to Cg group. (ANOVA, Tukey test).

whey, sugar, and carbohydrate sources (Moon et al., 2001; John et al., 2006).

CONCLUSION

Our results demonstrate that curcumin was successfully encapsulated in PLLA nanoparticles obtained by the miniemulsification/solvent evaporation technique. The curcumin-loaded PLLA nanoparticles showed greater biological effectiveness when dose 8-fold smaller produced an inhibitory effect in the inflammatory process similar to that of the curcumin *in nature.*

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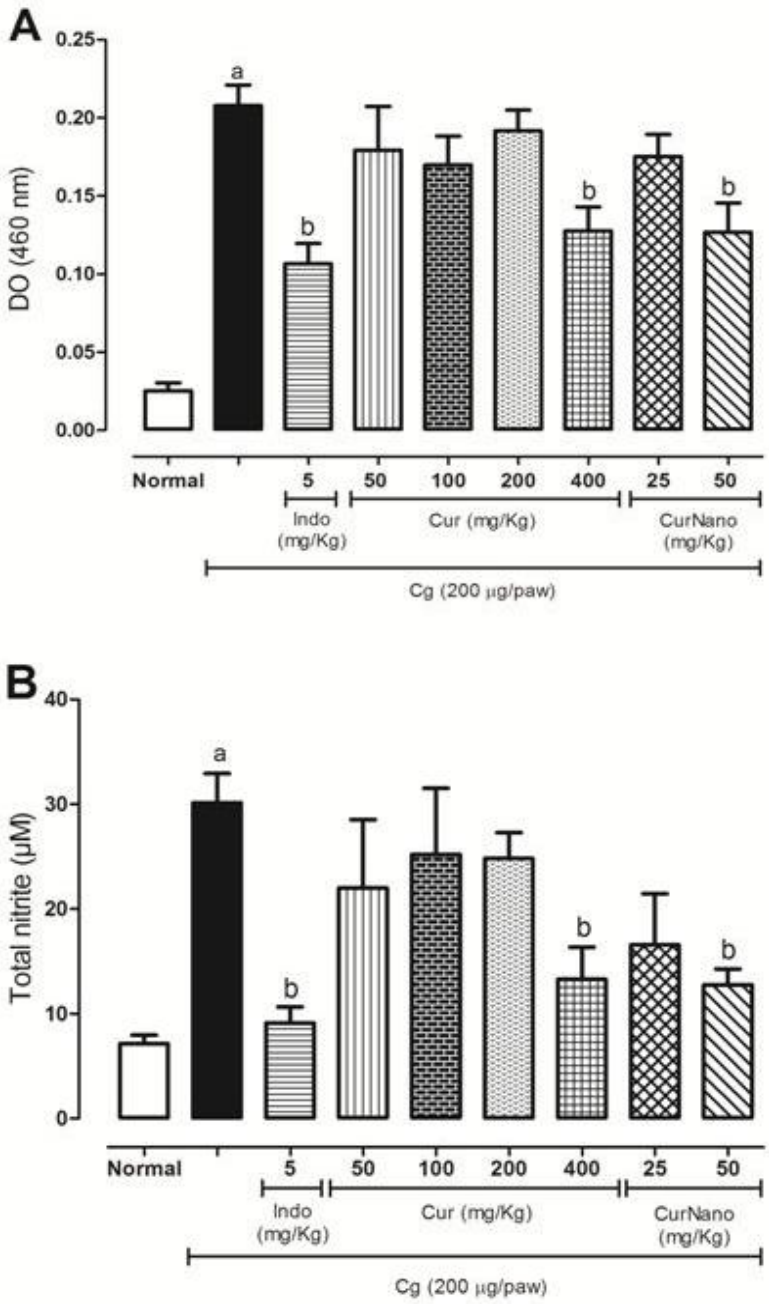

Figure 6. Effect of Curcumin *in nature* (Cur) and Curcumin Nanoparticles (CurNano) on MPO activity **(A)** and the NO concentration **(B)** in the plantar tissue of rats. Rats (n=6 per group) were treated with Cur orally at doses of 50, 100, 200, and 400 mg/kg, and CurNano at doses of 25 and 50 mg/kg one hour before intraplantar Cg injection (200 µg/paw). Positive control rats were treated orally at a dose of 5 mg/kg indomethacin (Indo). Each column represents the mean of MPO activity ± SEM, and the NO concentration ± SEM, average 4 hours after agent phlogistic injection. a = $p < 0.001$ compared to normal group. b = $p < 0.01$ compared with Cg group (ANOVA, Tukey test).

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REFERENCES

Aggarwal BB, **Harikumar** KB, **2009**. Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. Int J Biochem Cell Biol, 41(1):40-59.

Aggarwal BB, Kumar A, Bharti AC, **2003**. Anticancer potential of curcumin: preclinical and clinical studies. Anticancer Res, 23(1A):363-398.

Alexandrino EM, Ritz S, Marsico F, Baier G, Mailänder V, Landfester K, Wurm FR, **2014**. Paclitaxel-loaded polyphosphate nanoparticles: a potential strategy for bone cancer treatment. J Mater Chem B, 2:1298-1306.

Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB, **2007**. Bioavailability of curcumin: problems and promises. Mol Pharm, 4(6):807-818.

Asakawa N, Tsuno M, Hattori T, Ueyama M, Shinoda A, Miyake Y, **1981**. Determination of curcumin content of tumeric by high perfomance liquid chromatography. Yakugaku Zasshi, 101:374-377.

Asua JM, **2002**. Miniemulsion polymerization. Prog Polym Sci, 27:1283-1346.

Balaji S, **Chempakam** B, **2010**. Toxicity prediction of compounds from turmeric (*Curcuma longa* L). Food Chem Toxicol, 48(10):2951-2959.

Banerjee M, Tripathi LM, Srivastava VM, Puri A, Shukla R, **2003**. Modulation of inflammatory mediators by ibuprofen and curcumin treatment during chronic inflammation in rat. Immunopharmacol Immunotoxicol, 25(2):213-224.

Bani D, Masini E, Bello MG, Bigazzi M, Sacchi TB, **1998**. Relaxin protects against myocardial injury caused by ischemia and reperfusion in rat heart. Am J Pathol, 152(5):1367-1376.

Basnet P, Hussain H, Tho I, Basnet-Skalko N, **2011**. Lipossomal Delivery System Enhances Anti-inflammatory Properties of Curcumin. J Pharm Sci, 101(2):598-609.

Basniwal RK, Buttar HS, Jain VK, Jain N, **2011**. Curcumin nanoparticles: Preparation, characterization, and antimicrobial Study. J Agric Food Chem, 59:2056-2061.

Brouet I, **Ohshima** H, **1995**. Curcumin, an anti-tumor promoter and anti-inflammatory agent, inhibits induction of nitric oxide synthase in activated macrophages. Biochem Biophys Res Commun, 206(2):533-540.

Dandekar PP, Dhumal R, Jain R, Tiwari D, Vanage G, Patravale V, **2010a**. Toxicological evaluation of pH-sensitive nanoparticles of curcumin: Acute, sub-acute and genotoxicity studies. Food Chem Toxicol, 48:2073-2089.

Dandekar PP, Jain R, Patil S, Dhumal R, Tiwari D, Sharma S, Vanage G, Patravale V, **2010b**. Curcumin-loaded hydrogel nanoparticles: Application in anti-malarial therapy and toxicological evaluation. Pharmaceut Nanotechnol, 99(12):4992-5010.

des Rieux A, Fievez V, Garinot M, Schneider YJ, Préat V, **2006**. Nanoparticles as potential oral delivery systems of proteins and vaccines: a mechanistic approach. J Control Release, 116(1):1-27.

Dorai T, Gehani N, Katz A, **2000**. Therapeutic potential of curcumin in human prostate cancer. II. Curcumin inhibits tyrosine kinase activity of epidermal growth factor receptor and depletes the protein. Mol Urol, 4(1):1-6.

Gaumet M, Vargas A, Gurny R, Delie F, **2008**. Nanoparticles for drug delivery: The need for precision in reporting particle size parameters. Eur J Pharm Biopharm, 69:1-9.

Goel A, Boland CR, Chauhan DP, **2001**. Specific inhibition of cyclooxygenase-2 (COX-2) expression by dietary curcumin in HT-29 human colon cancer cells. Cancer Lett, 172(2):111-118.

Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR, **1982**. Analysis of nitrate, nitrite, and [^{15}N]nitrate in biological fluids. Anal Biochem, 126:131-138.

Guterres SS, Muller CB, Michalowski CB, Pohlmann AR, Dalla Costa T, **2001**. Gastro-intestinal tolerance after oral administration of spray-dried diclofenac-loaded nanocapsules and nanospheres. STP Pharma Sci, 11:229-233.

Hong J, Bose M, Ju J, Ryu JH, Chen X, Sang S, Lee MJ, Yang CS, **2004**. Modulation of arachidonic acid metabolism by curcumin and related β-diketone derivaties: effects on cytosolic phospholipase A_2, cyclooxygenases and 5-lipoxygenase. Carcinogenesis, 25(9):1671-1679.

Huang MT, Lysz T, Ferraro T, Abidi TF, Laskin JD, Conney AH, **1991**. Inhibitory effects of Curcumin on *in vitro* lipoxygenase and cyclooxygenase activities in mouse epidermis. Cancer Res, 51:813-819.

International Standard ISO13321, **1996**. Methods for Determination of Particle Size Distribution Part 8: Photon Correlation Spectroscopy, International Organization for Standardisation (ISO).

Iwata M, Suzuki S, Asai Y, Inoue T, Takagi K, **2010**. Involvement of nitric oxide in rat model of carrageenan-induced pleurisy. Mediators Inflamm, 1-11.

Jobin C, Bradham CA, Russo MP, Juma B, Narula AS, Brenner DA, **1999**. Curcumin blocks cytokine-mediated NF-kappa B activation and proinflammatory gene expression by inhibiting inhibitory factor I-kappa B kinase activity. J Immunol, 163(6):3474-3483.

John RP, Nampoothiri KM, Pandey A, **2006**. Solid-state fermentation for l-lactic acid production from agrowastes using Lactobacillus delbrueckii. Proc Biochem, 41:759-763.

Jung KK, Lee HS, Cho JY, Shin WC, Rhee MH, Kim TG, Kang JH, Kim SH, Hong S, Kang SY, **2006**. Inhibitory effect of curcumin on nitric oxide production from lipopolysaccharide-activated primary microglia. Life Sci, 79(21):2022-2031.

Jurenka JS, **2009**. Anti-inflammatory properties of curcumin, a major constituent of Curcuma longa: a review of preclinical and clinical research. Altern Med Rev, 14:141-153.

Kiuchi F, Goto Y, Sugimoto N, Akao N, Kondo K, Tsuda Y, **1993**. Nematocidal activity of turmeric: synergistic action of curcuminoids. Chem Pharm Bull (Tokyo) 41(9):1640-1643.

Klebanoff SJ, **2005**. Myeloperoxidase: friend and foe. J Leukoc Biol, 77:598-625.

Leimann FV, Cardozo Filho L, Sayer C, Araujo PHH, **2013**. Poly(3-hydroxybutyrate-co-3- hydroxyvalerate) nanoparticles prepared by a miniemulsion/solvent evaporation technique: effect of PHBV molar mass and concentration. Braz J Chem Eng (Impresso), 30:369-377.

Liju VB, Jeena K, Kuttan R, **2011**. An evaluation of antioxidant, anti-inflammatory, and antinociceptive activities of essencial oil from Curcuma longa. L. Indian J Pharmacol 43:526-31.

Maghsoodi M, **2009**. Physicomechanical Properties of Naproxen-Loaded Microparticles Prepared from Eudragit L100. Am Assoc Pharmaceut Sci, 10(1):120-128.

Mathy-Hartert M, Jacquemond-Collet I, Priem F, Sanchez C, Lambert C, Henrotin Y, **2009**. Curcumin inhibits pro-inflammatory mediators and metalloproteinase-3 production by chondrocytes. Inflamm Res, 58(12):899-908.

Moncada S, Palmer RMJ, Higgs EA, **1991**. Nitric Oxide: physiology, pathophysiology and pharmacology. Pharmacol Rev, 43:109.

Moon S, Taniguchi I, Miyamoto M, Kimura Y, Lee CW, **2001**. Synthesis and properties of high-molecular-weight poly(L-lactic acid) by melt/solid polycondensation under different reaction conditions. High Perform Polym, 13:S189-S196.

Moore AR, **2003**. Pleural models of inflammation: immune and nonimmune. Methods Mol Biol, 225:123-128.

Morris CJ, **2003**. Carrageenan-Induced Paw Edema in the Rat and Mouse. Methods Mol Biol, 225:115-121.

Motta AC, **Duek** EAR, **2006**. Síntese, Caracterização e Degradação "in vitro" do Poli (L-ácido láctico). Polímeros, 16(1):26-32.

Mujumdar S, Senthil KP, Pandit AB, **1997**. Emulsification by ultrasound: Relation between intensity and emulsion quality. Indian J Chem Technol, 4(6):277-284.

Musyanovych A, Schmitz-Wienke J, Mailänder V, Walther P, Landfester K, **2008**. Preparation of biodegradable polymer nanoparticles by miniemulsion technique and their cell interactions. Macromol Biosci, 8(2):127-139.
Ogiso TYT, Iwaki M, Tanino T, Miyake Y, **2001**. Effect of positively and negatively cherged liposomes on skin permeation of drugs. J Drug Target, 9:49-59.
Oliveira AG, Scarpa MV, Correa MA, Cera LFR, Dormariz TP, **2004**. Microemulsões: Estrutura e aplicações como sistema de liberação de fármacos. Quim Nov, 27(1):131-138.
Paramera EL, Konteles SJ, Karathanos VT, **2011**. Stability and release properties of curcumin encapsulated in Saccharomyces cerevisiae, β-cyclodextrin and modified starch. Food Chem, 125:913–922.
Park C, Moon DO, Choi IW, Choi BT, Nam TJ, Rhu CH, **2007**. Curcumin induces apoptosis and inhibits prostaglandin E(2) production in synovial fibroblasts of patients with rheumatoid arthritis. Int J Mol Med, 20(3):365-372.
Patel A, Hu H, Tiwari JK, Velikov KP, **2010**. Synthesis and characterisation of zein–curcumin colloidal particles. Soft Matter, 6:6192-6199.
Ravivarapu H, Mahalingam R, Jasti BR, **2006**. Biodegradable Polymeric Delivery Systems in Design of Controlled Release Drug Delivery Systems. Ed: Xiaoling Li and Bhaskara R. Jasti.
Reimers JL, **Schork** FJ, **1996**. Predominant droplet nucleation in emulsion polymerization. J Appl Polym Sci, 60:251-262.
Roughley PJ, **Whiting** DA, **1973**. Experiments in the biosynthesis of curcumin. J Chem Soc, Perkin Trans 1. 2379:88.
Saleh TS, Calixto JB, Medeiros YS, **1999**. Effects of anti-inflammatory drugs upon nitrate and myeloperoxidase levels in the mouse pleurisy induced by carrageenan. Peptides, 20(8):949-956.
Schaffazick SR, Guterres SS, Freitas LL, Pohlmann AR, **2003**. Caracterização e estabilidade físico-química de sistemas poliméricos nanoparticulados para administração de fármacos. Quim Nova, 26(5):726-737.
Schinella G, Neyret E, Cónsole G, Tournier H, Prieto JM, Ríos JL, Giner RM, **2014**. An aqueous extract of *Ilex paraguariensis* reduces carrageenan-induced edema and inhibits the expression of cyclooxygenase-2 and inducible nitric oxide synthase in animal models of inflammation. Planta Med, 80:961-968.
Shaikh J, Ankola DD, Beniwal V, Singh D, Ravi Kumar MNV, **2009**. Nanoparticle encapsulation improves oral bioavailability of curcumin by at least 9-fold when compared to curcumin administered with piperine as absorption enhancer. Eur J Pharm Sci, 37:223-230.
Sharma M, Sharma V, Panda AK, Majumdar DK, **2011**. Development of enteric submicron particle formulation of papain for oral delivery. Int J Nanomed, 6:2097-2111.
Silva-Buzanello RA, Esperança ES, Souza MF, Leimann LV, Gonçalves OH, **2011**. Influência de condições operacionais na obtenção de nanopartículas de poliestireno. Rebrapa, 2(1):33-37.
Silva-Buzanello RA, Ferro AC, Bona E, Cardozo-Filho L, Araújo PHH, Leimann FV, Gonçalves OH, **2015**. Validation of an Ultraviolet–visible (UV–Vis) technique for the quantitative determination of curcumin in poly(l-lactic acid) nanoparticles. Food Chem, 172:99-104.
Singh AK, **Vinayak** M, **2014**. Curcumin attenuates CFA induced thermal hyperalgesia by modulation of antioxidant enzymes and down regulation of TNF-α, IL-1β and IL-6. Neurochem Res. DOI 10.1007/s11064-014-1489-6.
Singh S, **2007**. From exotic spice to modern drug? Cell, 130(5):765-768.
Singh S, **Aggarwal** BB, **1995**. Activation of transcription factor NF-kappa B is suppressed by curcumin (diferuloylmethane) [corrected]. J Biol Chem, 270(42):4995-5000.
Surh YJ, **2002**. Anti-tumor promoting potential of selected spice ingredients with antioxidative and anti-inflammatory activities: a short review. Food Chem Toxicol, 40:1091-1097.
Thong-Ngam D, Choochuai S, Patumraj S, Chayanupatkul M, Klaikeaw N, **2012**. Curcumin prevents indomethacin-induced gastropathy in rats. World J Gastroenterol, 18(13):1479-1484.
Wang ST, Schork FJ, Poehlein CW, Cooch JW, **1998**. Emulsion and miniemulsion copolymerization of acrylic monomers in the presence of alkyd resin. J Appl Polym Sci, 60(12):2069-2076.
Wang Y, Lu Z, Lv F, Bie X, **2009**. Study on microencapsulation of curcumin pigments by spray drying. Eur Food Res Technol, 229:391-396.
Winter CA, Risley EA, Nuss GW, **1962**. Carrageenan-induced oedema in hind paw on the rat as an assay for anti-inflammatory drugs. P Soc Exp Biol Med, 111:544-547.
Yadav VR, Prasad S, Kannappan R, Ravindran J, Chaturvedi MM, Vaahtera L, Parkkinen J, Aggarwal BB, **2010**. Cyclodextrin-complexed curcumin exhibits anti-inflammatory and antiproliferative activities superior to those of curcumin through higher cellular uptake. Biochem Pharmacol, 80:1021-1032.
Yallapu MM, Gupta BK, Jaggi M, Chauhan SC, **2010b**. Fabrication of curcumin encapsulated PLGA nanoparticles for improved therapeutic effects in metastatic cancer cells. J Colloid Interf Sci, 351:19-29.
Yallapu MM, Jaggi M, Chauhan SC, **2010a**. β-Cyclodextrin-curcumin self-assembly enhances curcumin delivery in prostate cancer cells. Colloid Surface B, 79:113-125.
Zeng Y, Qiu F, Liu Y, Qu G, Yao X, **2007**. Isolation and identification os phase 1 metabolites of demethoxycurcumin in rats. Drug Metab Dispos, 35:1564-1573.
Zou P, Helson L, Maitra A, Stern ST, McNeil SE, **2013**. Polymeric curcumin nanoparticle pharmacokinetics and metabolism in bile duct cannulated rats. Mol Pharmaceut, 10:1977-1987.

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