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Development of calcium alginate beads containing paclitaxel-loaded lipid-core nanocapsules intended for oral administration

Taiane Medeiro Ciocheta*[a], Aline de Cristo Soares Alves[a], Danieli Rosane Dallemole[a], Rodrigo Cé[a], Silvia Stanisçuaski Guterres[a], Adriana Raffin Pohlmann[a]

[a]*Programa de Pós-Graduação em Ciências Farmacêuticas, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.*
**Corresponding author: taianeciocheta@ufrgs.br*

We developed a solid formulation of calcium alginate beads containing paclitaxel-loaded lipid-core nanocapsules (PTX-LNC-Bead) intended for oral administration. The PTX-LNC liquid formulation was prepared by interfacial deposition and trapped into calcium alginate beads. These beads were characterized in terms of size, morphology, swelling rate, encapsulation efficiency, and release of PTX and LNC in simulated gastrointestinal fluids. Results showed that the beads were gastro-resistant with low swelling rate and drug release lower than3.5% at pH 1.2 (2h). At pH 6.8, the beads showed high swelling rate and disintegration after 80 min. Drug release was60% after 600 min. Particle sizing as a function of time confirmed that LNC were released intact from the beads at pH 6.8 showing that PTX-LNC-Bead is a promising product for PTX oral administration. Our results pave the way for novel formulations intended for drug targeting by the oral route.

Keywords: Calcium alginate beads; lipid-core nanocapsules; paclitaxel; cancer.

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Introduction

Paclitaxel (PTX) is a powerful antineoplastic agent that is commonly used as first-line therapy for solid tumors, such as those in breast, colon, and prostate cancers, and melanoma [1,2]. PTX inhibits mitosis by microtubule stabilization and blocks the cell cycle in the G2/M phase, resulting in cell death [3,4,5]. Despite its therapeutic efficacy, PTX may cause side effects, such as myelosuppression, myalgia, arthralgia, cardiotoxicity, hypersensitivity reactions, hematological toxicity, and peripheral sensory neuropathy [6,7]. The first PTX formulation (Taxol®) was a viscous solution containing polyethoxylated castor oil (Cremophor EL®) and ethanol. Taxol® is diluted with a parenteral fluid prior to intravenous administration. However, besides the toxicity of PTX, the observed clinical side effects of the product are also related to the adjuvant, Cremophor EL® [4,8]. As an alternative, nanotechnology has been used to improve PTX apparent solubility in water, thereby decreasing the toxicity of the formulation. As alternatives to Taxol®, Abraxane® (PTX-albumin-conjugate nanoparticles), Genexol-PM® (PTX-loaded polymeric micelles), and Lipusu® (PTX liposomes) are Cremophor EL®-free products approved showing high tolerable dosage [9].

Oral administration can maintain an optimal PTX concentration in the blood, thereby improving the efficacy and decreasing the corresponding side effects of the drug; hence, oral PTX administration is more advantageous than the intravenous route [10]. In addition, patient acceptability shortened hospital stay, minimal infection risk, and medical supervision are improved [11]. Liporaxel® is the first oral PTX approved for gastric cancer in Korea in 2016 and is currently under study as a first-line therapy for recurrent or metastatic breast cancer [12]. Oraxol® is another PTX product, which contains HM30181 (Encequidar), a new oral inhibitor of intestinal P-glycoprotein allowing oral administration. This formulation yielded a higher overall response rate and lesser neuropathy compared with the standard intravenous PTX [13]. To date, no oral PTX nanoformulations are commercially available despite numerous advances. Formulations based on biodegradable nanoparticles can remarkably address the problems with the safety and effectiveness of PTX delivery.

Preclinical studies have reported satisfactory results for PTX in oral nanocarriers; these results were related to improved oral bioavailability, reduced toxicity, and inhibited tumor growth [14,15]. PTX-loaded *N*-deoxycholic acid-*N* and *O*-hydroxyethyl chitosan micelles were obtained with high PTX loading capacity and trapping efficiency [16]. The oral bioavailability of PTX increased up to three times that of the oral Taxol® formulation in rats. In another study, PTX-loaded lipid nanoparticles prepared by melt-emulsification, where glyceryl monostearate was used as the solid lipid and soybean oil as the liquid lipid orally administered to rats, showed an increase of up to 6.8 times the area on the curve and maximum serum drug concentration when compared to the commercial formulation (Intaxel®) [17]. Thus, lipid nanocarriers are good candidates for transporting PTX orally; such nanocarriers exhibit stability against intestinal mucus, without altering the rheological properties of mucus, and improve the diffusion of PTX at low concentrations [18].

Lipid-core nanocapsules (LNCs) are polymeric nanocapsules with a modified core. This core is composed of an organogel of solid and liquid lipids, such as sorbitan

monostearate (SM) and caprylic/capric triglyceride (CCT). The lipid-core is surrounded by a biodegradable polyester, such as poly(ε-caprolactone) (PCL). These nanocapsules are stabilized in water by polysorbate 80 (P80) micelles, forming a hydrophilic corona [19]. This innovative and complex supramolecular structure increases the drug loading capacity to over 40 times compared tonanocapsules containing an oil-core [20]. LNCs show controlled and sustained drug release, which diffusional barriers are related to the viscosity of the organogel and the polymer wall [21]. A liquid formulation of resveratrol-loaded LNC improved the anti-inflammatory effect of resveratrol after oral administration toa murine model of lipopolysaccharide-induced acute respiratory distress syndrome [22]. Furthermore, acetyl eugenol-loaded LNC (or blank-LNCs) reduced the melanoma volume after daily oral to mouse, which effectivity was explained by SK-Mel-28 cellular uptake as visualized in vitro by CytoViva® microscopy images [23]. Furthermore, *trans*-resveratrol-loaded LNC showed drug brain targeting after oral administration to healthy male Wistar rats [24]. In addition, intravital microscopy analysis demonstrated that LNC can cross the blood brain barrier (BBB) since the fluorescence in the brain tissue of mice increased after oral administration of fluorescent-labeled LNC (prepared with dye-polymer conjugate) [25]. This study also demonstrated that indomethacin-loaded LNCs can cross the intestinal barrier, they are absorbed, distributed and, then, they target glioblastoma in brain tissue decreasing the tumor size after oral administration [25]. We developed LNC formulations, and we have been studying their pharmacological applications. LNCs targeting after oral absorption is an excellent advantage of this type of nanocarrier to reduce inflammation and the size of solid tumors.

In the development of oral formulations, the physiological characteristics of the gastrointestinal tract must be considered. These characteristics such as type of buffer, pH values, and bile salts can hinder the oral absorption of drugs [26]. Combining micro- and nanometric drug delivery systems with calcium alginate beads is an interesting strategy for transporting drugs orally [27,28,29]. Biodegradable beads can be designed to protect drugs from the highly acidic upper region of the gastric tract by forming an egg-carton structure due to the crosslinking of sodium alginate withCa^{2+}[30]. These properties enable the encapsulation of a diversity of drugs and their release in a pH-controlled manner [31]. Calcium alginate beads with PTX-loaded polymeric microspheres have been previously developed [32] showing a uniform distribution of the microspheres within the gel matrix with preserved morphology [32]. Furthermore, the oral administration of calcium alginate beads containing PTX-loaded liposomes improved the physical stability of the liposomes against processing stress, increased the transport capacity of the drug, and improved the structural integrity of the liposomes in simulated gastric and intestinal fluids [33].

Considering that nanocarriers can be embedded in calcium alginate beads and that LNCs can cross the intestinal barrier of absorption, we proposed to develop calcium alginate beads containing PTX-loaded LNCs as a solid dosage form, which was design to carry the nanocapsules through the gastrointestinal tract, at which the nanocapsules could be released to absorption, distribution and targeting to tumoral tissues. The present study was dedicated to prepare and characterize PTX-LNC-beads in terms of size, morphology, swelling rate, encapsulation efficiency, and release of PTX and LNC in simulated gastrointestinal fluids.

Experimental section

Materials

PCL (α,ω-dihydroxyl polymer, M_w = 14,000 g mol^{-1}), SM (Span 60®), sodium alginate, P80 (Tween 80®), and PTX semi-synthetic (98% purity) were purchased from Sigma-Aldrich (Steinheim, Germany). CCT was purchased from Henrifarma (São Paulo, Brazil). Calcium chloride and acetone were supplied by Dinâmica (São Paulo, Brazil). HPLC-grade acetonitrile was purchased from Merck (Darmstadt, Germany). All solvents and reagents were of analytical or pharmaceutical grade.

Preparation and characterization of PTX-loaded LNCs

PTX-loaded LNCs were obtained by the interfacial deposition of preformed polymers [34]. The organic phase was composed of PCL (0.1 g), CCT (160 µL), and SM (0.038 g) dissolved in acetone (25 mL). An ethanol solution (2 mL) of PTX (0.001 g) was added to the organic phase, and the resulting mixture was stirred for 60 min at 40 °C. The organic phase was injected into an aqueous phase (53 mL) containing P80 (0.078 g). After 10 min of stirring, the organic solvents were evaporated, and the formulation was concentrated under reduced pressure using a water bath and a rotary evaporator (Büchi, Switzerland) to approximately 9 mL. The volume was adjusted to 10 mL in a volumetric flask. The theoretical drug concentration in the formulation was 100 µg mL^{-1}. This formulation was named PTX-LNC. Blank LNCs (B-LNC) were prepared without adding the drug to the formulation.

The PTX-LNC and B-LNC suspension was characterized by laser diffraction, dynamic light scattering (size distribution, the hydrodynamic mean diameter, and polydispersity index (PDI)), potentiometry, zeta potential, transmission electron microscopy (TEM) and HPLC.

Preparation of calcium alginate beads containing PTX-LNCs

The beads were prepared by adding sodium alginate to 8 mL of the nanocapsule formulation (PTX-LNC) under constant stirring for 2 h. The final concentration of the solution was 2% (w/v) sodium alginate. This solution was

dripped (1 mL min^{-1}) in a calcium chloride solution (100 mL, 2% w/v) using a peristaltic pump (MINIPULS® 3, Gilson Inc., USA) dotted of a nozzle (ϕ= 0.5 mm) at a drip distance of 6 cm with slow stirring for 60 min to cure the calcium alginate beads. After crosslinking, the beads formed were removed from the calcium chloride solution and washed with distilled water. Subsequently, the beads were passed through a funnel with filter paper to remove excess water and oven-dried at 37 °C for 12 h. These beads containing PTX-LNCs were named PTX-LNC-Bead. Beads with the drug dispersed in the matrix (PTX-Bead) were prepared for comparison. To manufacture PTX-Bead, a PTX solution was prepared. Approximately 0.001 g PTX was solubilized in 25 mL of ethanol under stirring for 60 min at 40 °C. Subsequently, the resulting mixture was poured into an aqueous phase (53 mL) containing P80 (0.078 g), and the PTX solution was concentrated on a rotary evaporator (Büchi, Switzerland) to approximately 10 mL. The beads were prepared by adding sodium alginate (2%) to the prepared solution, the PTX-Beads followed the same preparation process as PTX-LNCs.

Mean diameter of calcium alginate beads

The size of the calcium alginate beads was determined using an optical microscope (BX41, Olympus Corporation, Japan). Fifty beads from each batch (n = 3) were placed on a slide individually and at a given size using image analysis software (OLYMPUS Stream™, Olympus Corporation, USA). The results are expressed as mean diameter (mm) ± standard deviation (SD).

Scanning electron microscopy (SEM) of calcium alginate beads

The surface and cross-sectional morphological properties of the prepared dried beads with and without nanocapsules were analyzed via SEM (JSM-6060, JEOL Ltd., Japan) at an accelerating voltage of 5 kV at different magnifications. Cross-section samples were prepared by manual cutting using a scalpel. The samples were placed on carbon-double-sided metal stubs and coated with a layer of gold for observation.

Determination of the drug content of calcium alginate beads

The drug content was determined by liquid chromatography (HPLC). HPLC system (Shimadzu, Kyoto, Japan) is dotted of an LC-20AT pump, an SPD-M20AV detector, and a SIL-20A auto-sampler. A column (4.6 mm× 250 mm, 5 µm; Zorbax Extend-C18, Agilent Technologies, USA) was used as the stationary phase. The mobile phase was composed of acetonitrile:water (60:40, % v/v), which was pumped at an isocratic flow rate of 1.0 mL min^{-1}.
The samples were prepared by adding 40 mg of beads to a volumetric flask containing 10 mL of acetonitrile. The resulting mixture was magnetically stirred for 2 h. Subsequently, the samples were filtered and quantified by HPLC. The drug content (DC%) was determined using Eq. (2)

$$DC\% = \frac{C_{experimental}}{C_{theoretical}} \times 100 \qquad (2)$$

where $C_{experimental}$ is the experimental concentration of PTX after extracting the drug from PTX-LNC-Beads, and $C_{theoretical}$ is the concentration of PTX considering the weighed mass of drug used to obtain the PTX-LNC-Beads. All measurements were performed in triplicate, and the variability was expressed as SD.

Swelling tests

The swelling capacity of the PTX-LNC-Bead was determined gravimetrically on simulated gastric fluid at pH 1.2 and intestinal fluid at pH 6.8 at 37 °C under moderate stirring. Dry samples of PTX-LNC-Bead were incubated in the corresponding medium and collected at specific time intervals. The excess of medium was removed using filter paper and the beads were immediately weighed. Swelling (%) was calculated as follows using Eq. (3)[36]:

$$Swelling\ (\%) = \left[\frac{W_e - W_i}{W_i}\right] \times 100 \qquad (3)$$

where W_i is the dry bead weight, and W_e is the weight of the incubated bead at a certain time after incubation. All measurements were performed in triplicate, and the variability was expressed as SD.

In-vitro drug release assay of calcium alginate beads

The release profile of the samples was evaluated from the direct addition of beads in the medium at 37 ± 0.5 °C under moderate agitation. Two independent experiments were performed at different gastrointestinal conditions: using pH 1.2 for 2 h and pH 6.8 for 10 h to simulate gastric and intestinal media, respectively. The media were prepared as described in the Brazilian Pharmacopoeia VI Edition for simulated media without enzyme. The aliquots were collected in predefined times and centrifuged at 1844 *g* for 20 min. The supernatant was analyzed via HPLC using the methodology described above. The centrifugation residue was extracted with acetonitrile and sonicated for 30min and quantified by HPLC. In this study, the *sink* condition was guaranteed.

In-vitro assay of nanocapsule release

The *in-vitro* nanocapsule release assay was performed simultaneously with the *in-vitro* drug release study of the beads. An aliquot of the release medium at pH 6.8 was collected at predetermined times (1, 4, 6, and 10 h) and diluted in pre-filtered ultrapure water (1:20, v/v). Subsequently, the diluted aliquot was analyzed at 25 °C using a DLS analyzer (Zetasizer Nano ZS, Malvern

Instruments Ltd., UK) to determine the presence of nanocapsules in the release medium.

Statistical analyses

Statistical analyses were performed at $p \leq 0.05$ using software (GraphPad Prism 6.0, GraphPad Software, USA). Analysis of variance (one- or two-way) and Tukey test were performed as appropriate for *post hoc* comparisons. Values are presented as mean ± SD.

Results and Discussion

Preparation and characterization of PTX-LNC

The liquid formulations containing or not PTX (PTX-LNC and B-LNC) showed a macroscopic homogeneous white-opalescent aspect. TEM analysis showed PTX-LNC spherically shaped (Figures 1A and 1B). LD analysis exhibited unimodal size distribution curves in the nanoscale confirming that no aggregation occurred. PTX-LNC and B-LNC presented D[4,3]v values of 161 ± 3 nm and 170 ± 2 nm with polydispersity of 1.42 ± 0.02 and 1.47 ± 0.02, respectively (Table 1). The low span values indicate narrow particle size distributions. LNCs are effective carriers for the delivery of lipophilic drugs, such as PTX. The physicochemical characteristics of the nanocapsules in the developed PTX-LNC and B-LNC were similar($p<0.05$). The LNC formulations prepared by self-assembly using the solvent displacement method (interfacial deposition of polymer) present nanometric particle with unimodal size distributions [34,37,38].

Table 1. Physicochemical properties of PTX-LNC and B-LNC (n = 3). Results are expressed as mean ± SD.

Parameter	PTX-LNC	B-LNC
D[4,3]v (nm)	161 ± 3	170 ± 1
Span	1.42 ± 0.02	1.47 ± 0.02
z-Average diameter (nm)	175 ± 2	177 ± 4
PDI	0.09 ± 0.01	0.10 ± 0.01
ζ potential (mV)	−8.96 ± 0.98	−7.99 ± 0.38
pH	5.73 ± 0.16	5.96 ± 0.06
Experimental drug concentration	96.81 ± 2.25	-

PTX-LNC: paclitaxel-loaded lipid-core nanocapsules.; LNC: lipid-core nanocapsule; D[4,3]v: volume-weighted mean diameter; PDI: polydispersity index; Span: polydispersity.

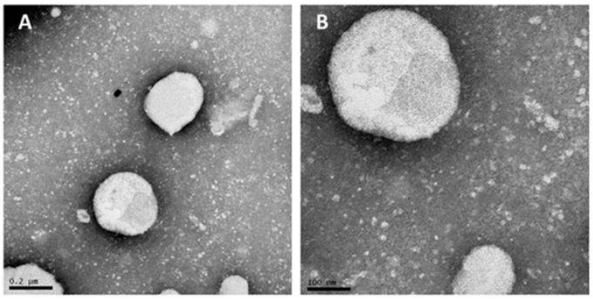


Figure 1. TEM images of PTX-LNC. (A) magnification = 100,000× (bar = 200 nm) and (B) magnification = 200,000× (bar = 200 nm).

Zeta potential is a characteristic of colloids closely related to the kinetic stability of the dispersion. Hence, this parameter is important to estimate the physical stability of nanocarrier formulations [39] when the mechanism of stabilization is based on electrostatic repulsion. However, when the mechanism of stabilization is based on steric hindrance, such as in the case of P80 (a nonionic surfactant), zeta potential cannot predict the stability of the nanoformulation. In those cases, the aggregation of the nanoparticles considering the particle size distribution must be evaluated as a function of time. PTX-LNC showed mean zeta potential slightly negative (Table 1) due to the polyoxygenated materials at the nanocapsule interface PCL and P80-coating. PCL used in this study is a diblock copolymer havingα,ω-dihydroxyl terminal groups. In this case, the LNCs are neutral nanocapsules, and the double layer (after diluting a sample of the formulation) is established by NaCl ionized in the outer phase.

The size of LNCs, expressed in terms of [D4,3]v or z-average diameter, is widely studied constituting an important parameter for determining the stability of LNCs. In previous studies, the LNCs developed using the same α,ω-dihydroxyl PCL (M_w = 14,000 g mol^{-1}) showed size stability after 60 days of storage at 5 °C [40]. Lycopene-loaded LNCs showed stability over 20 d of storage at 25 °C without any change in the granulometric profile [41]. This phenomenon indicates physical stability without showing evidence of flocculation [41]. Furthermore, the stability of lutein-loaded P80-coated LNCs showed stability of z-average diameter at 4 °C and 25 °C for 90 d [42]. These studies provide relevant data to confirm the kinetic stability of LNCs under different storage conditions. The high stability can be attributed to the ability of the nonionic surfactant used (P80) to generate a strong steric hindrance between nanoparticles [34,42].

Development and physicochemical characterization of PTX-Bead and PTX-LNC-Bead.

PTX-LNC-Bead and PTX-Bead were prepared by ionic gelation. The average diameters of PTX-LNC-Bead and PTX-Bead were 1.11 ± 18 mm and 0.866 ± 12 mm, respectively.

The analytical quantification of PTX in the calcium alginate beads was determined by liquid chromatography (HPLC) according to a previously validated method [35].

The method was validated in compliance with guidelines and was found to be linear in the evaluated concentration range within 0.5 to 10µg mL−1 ($r^2 = 0.9998$), non-significant linear deviation ($p>0.05$) (ANOVA). The representative chromatograms of formulations are shown in (Figure 2).

Repeatability was determined at three concentration levels, obtaining an RSD (%) <0.9%, and the accuracy of

the method was >97%. The low values of LOD (0.12 $\mu g.mL^{-1}$) and LOQ (0.36 $\mu g.mL^{-1}$) demonstrated the high sensitivity of the method.

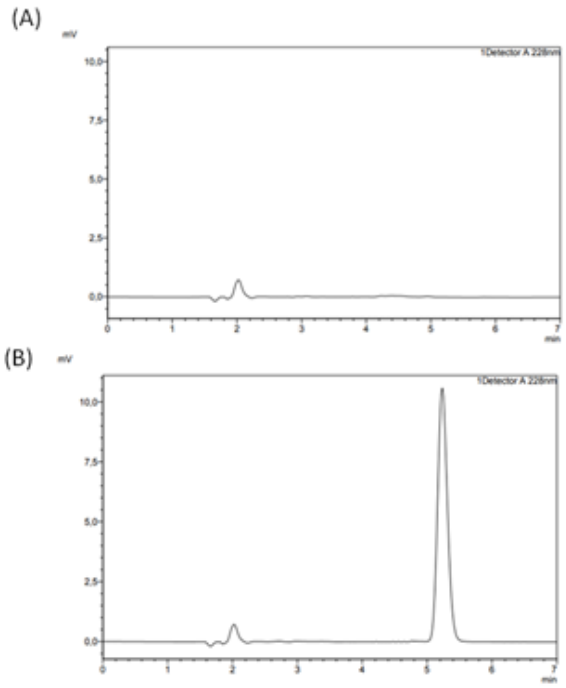


Figure 2. Representative chromatogram of formulations. (A) Blank formulation (LNC-Bead) and (B) PTX-LNC-Bead.

The drug content (Eq. 2) in PTX-LNC-Bead and PTX-Bead were 62.5% ± 3.43% and 31.25% ± 6.2%, respectively. Macroscopically, the PTX-LNC-Bead was spherical and had a smooth surface. SEM analyses showed a remarkable difference between the surface and interior of the spheres because the outer surface of the PTX-LNC-Bead was more porous than that of the PTX-Bead (Figures 2A and 2D). The SEM images (Figures 2A–2F) showed the microscopic aspect of the calcium alginate beads; the external and cutting surfaces of the PTX-Bead were smoother and more uniform than those of the PTX-LNC-Bead (Figures 2B – C and 2E - F).

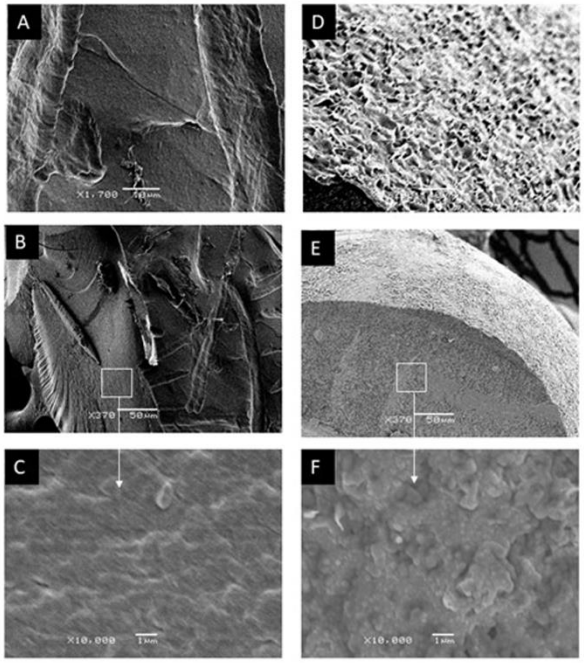


Figure 3. SEM images. (A) Surface of PTX-Bead at magnification = 1.700×. Cross-sectional images of PTX-Bead at (B) magnification = 370× and (C) magnification = 10.000×. (D) Surface of PTX-LNC-Bead at magnification = 1.700×. Cross-sectional images of PTX-LNC-Bead at (E) magnification = 370× and (F) magnification = 10.000×.

In general, beads have been presented as an efficient mechanism for transporting nanoparticles to the intestinal region [27, 28, 43]. The composition of the samples made of alginate presents resistance to severe environments such as gastric medium and an effective release in the intestinal region. The beads were prepared by ionotropic gelation and were form instantly by dropwise addition of the alginate and PTX-LNC solutions in the calcium chloride solution. The methodology was standardized considering the drip and drip distance, alginate and calcium chloride concentrations used to manufacture the samples, based on published articles [28, 29, 44, 45].

The beads with nanocapsules were larger than the PTX-Bead ($p < 0.05$). In addition, the PTX-Bead exhibited a drug loading lower than that of the PTX-LNC-Bead ($p < 0.05$). The size (1.11 ± 18 mm) of the PTX-LNC-Bead was in the same range as the other beads described in the literature. Giri et al. 2017 [29] reported the development of granules containing liposomes loaded with capsaicin, coated, or not coated, with Eudragit® S100; the uncoated bead granules had sizes of 1.213 ± 0.218 mm and drug DC% of 85% ± 1.15%. The cross-sectional cuts of the PTX-LNC-Bead samples showed that the surface and interior were different; in addition, with this test, we can confirm the presence of LNCs distributed homogeneously throughout the mass of the PTX-LNC-Bead (Figure 2F).

Swelling tests

The swelling tests of the nanocapsules were independently conducted in simulated gastric and intestinal fluids. Different beads were subjected to media at pH 1.2 and 6.8. Figure 3 shows the swelling profiles of PTX-LNC-Bead at both pH values for 1 h and 20 min. In the simulated intestinal fluid (pH 6.8), the swelling proportions of PTX-LNC-Bead increased significantly ($p < 0.05$) compared to those in the simulated gastric fluid (pH 1.2). The beads disintegrated, thereby limiting the sample collection for weight determination. Hence, the test was completed at the time described.

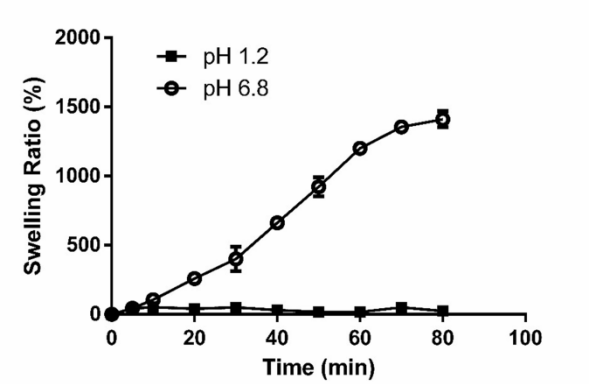


Figure 4. Swelling profiles (w/w) of PTX-loaded beads. Simulated gastric fluid (pH = 1.2, -○-) () and simulated intestinal fluid (pH 6.8; -■-). The values are mean ± SD (n = 3).

The swelling of beads is an important parameter because of the reported ability of the beads to expand and consequently release drugs. In the swelling test, the resistance of the PTX-LNC-Bead was observed in the gastric medium at pH 1.2. Furthermore, the sample exhibited a high ability to absorb the medium, expanded and initiated disintegration at pH 6.8 after 80 min. These characteristics of pH sensitivity are attributed to the presence of carboxylic groups in the alginate structure. The carboxylic group ionizes at a pH above 4.4 and increases the electrostatic repulsion of negative charges, resulting in the expansion of the polymeric chain and swelling of the matrix. By contrast, for a pH below the pKa (pH < 3.4), the carboxylic groups are in their nonionized form, leading to an insoluble structure [46].
Feng et al. 2014 [27] developed multilayer sodium alginate beads containing doxorubicin-loaded microcapsules and reported the use of a coating to deliver the drug in the colon region. The disintegration time increased with the increase in the number of layers added to the beads. When performing the swelling test, formulations with four layers of coating started to disintegrate within 2 h at pH 7. For the uncoated formulations, the disintegration times were lower than those of the coated formulations. These results corroborate the results of our study, where the beads started to disintegrate after 80 min during the experiment.

In-vitro release of PTX

The *in-vitro* release of PTX from PTX-LNC-Bead and PTX-Bead was studied in simulated gastrointestinal fluids (pH 1.2 and 6.8). Aliquots were collected at predetermined times and analyzed by HPLC. The PTX-LNC-Bead and PTX-Bead released small amounts of PTX at pH 1.2 (Figure 4A). The release profiles of PTX-LNC-Bead and PTX-Bead in the simulated intestinal medium at pH 6.8 were significantly different ($p < 0.05$). The PTX-LNC-Bead showed a considerable sustained release compared to the PTX-Bead. The PTX-LNC-Bead released approximately 60% of the drug in 600 min, where as the PTX-Bead released 100% of the drug after 360 min (Figure 4B). In addition, the residue from each collection of the release medium was extracted and quantified (Figure 4C). In this test, we quantified a significant amount of PTX in the residue of PTX-LNC-Bead from the release at pH 6.8. After 600 min, the percentage of drug accumulated in the residue was approximately 20%. This value was added to that quantified in the release assay, resulting in a profile of approximately 80% recovered drug.

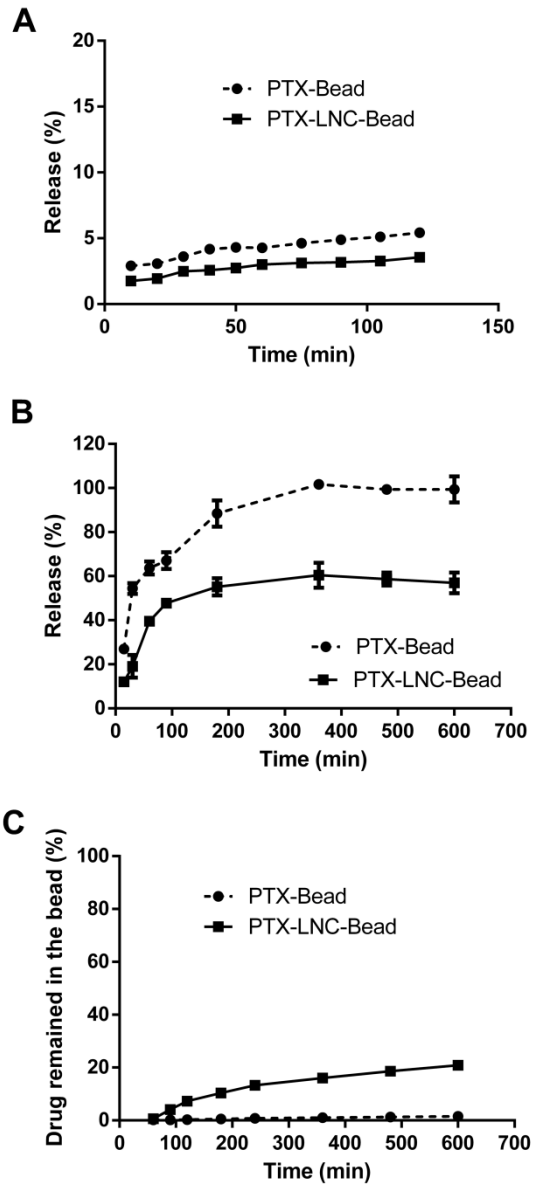


Figure 5. Release profiles of PTX-LNC-Bead and PTX-Bead at (A) pH 1.2 and (B) pH 6.8. (C) PTX remaining in the bead vs time based on the analysis of the residues from the release profiles at pH 6.8.

The results of the *in-vitro* release at pH 1.2 showed a negligible drug loss in the simulated gastric fluid, developed bead, and premature release of PTX (Figure 4A). By contrast, at pH 6.8, PTX-LNC-Bead only slightly released the PTX. However, high PTX concentrations were released. The PTX beads showed drug release values higher than those of the PTX-LNC-Bead at the end of the experiment (Figure 4B). The presence of nanoparticles in the PTX-LNC-Bead may have delayed PTX release. In addition, as shown in Figure 4C, quantification of the centrifugation residue via HPLC during the drug release assay demonstrated high PTX concentrations for the PTX-LNC-Bead. This result was different from the PTX-Bead where the drug was completely released (100%). This result suggests that all drug-containing nanocapsules were released in the medium. To support our hypothesis, we investigated the simultaneous release of nanocapsules and PTX. A portion of the collected aliquot was diluted and analyzed via DLS. Moreover, SEM analysis confirmed the presence of LNCs in the entire mass of beads developed (Figure 2C).

In-vitro release of nanocapsules

The nanocapsule release was performed to verify whether the nanocapsules were also being released from the beads. The experiment was conducted based on the particle size profile at pH 6.8 in the release medium after 1, 4, 6, and 10 h (Figure 5). After 1 h of contact of the PTX-LNC-Bead with the pH 6.8 release medium, the LNCs were identified by DLS. After 10 h, we confirmed the presence of nanoparticles in the release medium. Compared to a nanocapsule suspension, the intensity (%) of the size distribution in the medium was relatively low. This phenomenon may be related to the small number of particles resulting from the sample dilution in the medium.

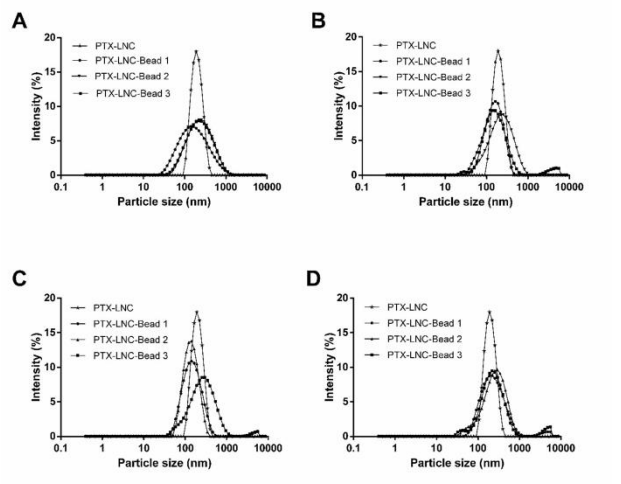


Figure 6. Particle size distribution via DLS of PTX-LNC and PTX-LNC-Bead after (A) 1, (B) 4, (C) 6, and (D) 10 h of immersion in simulated intestinal fluid (pH = 6.8). PTX-LNC is the original particle size distribution of the liquid suspension. PTX-LNC-Bead is in triplicate at pH 6.8 (n = 3).

The results (Figures 5A–5D) demonstrate the presence of LNCs in the release medium at all evaluation times. Thus, we demonstrated that the LNCs loaded with the PTX were released intact from the beads. Therefore, based on previous studies published by the group, we can suggest that these nanocapsules with lipid nucleus will be absorbed in the intestinal region and delivered to different tissues. Rodrigues et al. 2016 [25], in their study conducted *in-vivo*, reported that orally administered LNCs crossed the intestinal barrier without releasing the drug and reached the brain tissue after 240 min. In addition, Peltier et al. 2006 [1] evaluated the oral administration of LNCs loaded with PTX or PTX associated with verapamil, and the group reported that the area-under-the=curved-plasma concentration significantly increased

(about three times) compared with the control group ($p < 0.05$). These results suggest that the LNCs in beads can be an innovative and effective carrier system for a controlled and sustained release of PTX for oral administration to target tumors.

Conclusions

Beads containing PTX-loaded LNCs were prepared. The presence of nanocapsules was confirmed in the entire mass of the beads. The beads showed *in-vitro* resistance to PTX release in acidic pH carried out in simulated gastric fluid. A high PTX concentration and intact PTX-loaded LNC were released in simulated intestinal fluid. The formulation proposed in this study is a promising strategy for oral administration of PTX targeting tumoral tissue. *In-vitro* and *in-vivo* studies are necessary to confirm the stability and effectiveness of the solid product.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Peltier S, Oger JM,Lagarce F, CouetW,Benoît JP. Enhanced Oral Paclitaxel Bioavailability After Administration of Paclitaxel-Loaded Lipid Nanocapsules, Pharmaceutical Research.2006;23 (6): 1243-1250.
2. Ding S, et al. Improved antitumor efficacy of paclitaxel with nano-formulation in breast cancer, Nanotechnol Rev. 2017; 6 (3): 291-299.
3. Yardley DA.Nab-Paclitaxel mechanisms of action and delivery, Journal of Controlled Release. 2013;170: 365-372.
4. Bakrania AK, Variya BC, Patel SS. Novel targets for paclitaxel nano formulations: hopes and hypes in triple negative breast cancer, Pharmacological Research. 2016;111: 577-591.
5. Gorain B, et al. Paclitaxel loaded vitamin E-TPGS nanoparticles for cancer therapy, Materials Science & Engineering C.2018;91: 868-880.
6. Leiva MC, Ortiz R, Cáceres RC, Perazzoli G, Mayevych I, López-Romero JM, et al.Tripalmitin nanoparticle formulations significantly enhance paclitaxel antitumor activity against breast and lung cancer cells in vitro, Scientific Reports.2017; 7:13506.
7. Chou PL, et al. Improvement of Paclitaxel-Associated Adverse Reactions (ADRs) via the Use of Nano-Based Drug Delivery Systems: A Systematic Review and Network Meta-Analysis, International Journal of Nanomedicine.2020; 15: 1731–1743.
8. Liu Y, Zhang B, Yan B. Enabling Anticancer Therapeutics by Nanoparticle Carriers: The Delivery of Paclitaxel, International Journal of Molecular Sciences.2011;12: 4395-4413.
9. Zhang Z, Mei L, Feng SS.Paclitaxel drug delivery systems, Expert Opin. Drug Deliv. DOI: 10.1517/17425247.2013.752354, 2013.
10. Feng C, Li J, Mu Y, Kong M, Li Y, Raja MA, et al. Multilayer micro-dispersing system as oral carriers for co-delivery of doxorubicin hydrochloride and P-gp inhibitor, International Journal of Biological Macromolecules.2017; 94: 170–180.
11. Hahn HS, Lee KH, Lee IH, Lee JH, Whang CS, Jo YW, Kim TJ. Metronomic oral paclitaxel shows anti-tumor effects in an orthotopic mouse model of ovarian cancer, Journal Gynecologic Oncology.2017; 25: 130-135. (NCT03326102) In: Cancer Res. 80 (4 Suppl):Abstract nr OT1-05-02.
12. Pluard TJ., et al. A phase II study to evaluate the efficacy, safety and pharmacokinetics of DHP107 (Liporaxel®, oral paclitaxel) compared to IV paclitaxel in patients with recurrent or metastatic breast cancer: OPERA (NCT03326102), In: Cancer Res.2020;80 (4 Suppl):Abstract nr OT1-05-02.
13. Umanzor G, et al. Oral paclitaxel with encequidar: The first orally administered paclitaxel shown to be superior to IV paclitaxel on confirmed response and survival with less neuropathy: A phase III clinical study in metastatic breast cancer. In: San Antonio Breast Cancer Symposium. Abstract GS6-01, 2019.
14. Baek JS, So JW, Shin SC, Cho CW. Solid lipid nanoparticles of paclitaxel strengthened by hydroxypropyl-β-cyclodextrin as an oral delivery system, International journal of molecular medicine.2012;30: 953-959.
15. Choudhury H,Gorain B, Tekade RK, Pandey M, Karmakar S, Pal TK. Safety against nephrotoxicity in paclitaxel treatment: Oral nanocarrier as an effective tool in preclinical evaluation with marked in vivo

antitumor activity, Regulatory Toxicology and Pharmacology.2017; 91: 179–189.
16. Li H, Huo M, Zhou J, Dai Y, Deng Y, Shi X, Masoud J. Enhanced oral absorption of paclitaxel in N-deoxycholic acid-N, O-hydroxyethyl chitosan micellar system. Journal Pharm. Sci. 2010; 99(11):4543-53.
17. Harshita, Barkat MA, Rizwanullah M, Beg S, Pottoo FH, Siddiqui S, Ahmad FJ. Nanolipidic Carriers with Improved Oral Bioavailability and Anticancer Activity against Human Liver Carcinoma, AAPSPharmSciTech.2019; 20-87.
18. Groo AC, SaulnierP, Gimel JC, Gravier J, Ailhas C, Benoit JP, Lagarce F.Fate of paclitaxel lipid nanocapsules in intestinal mucus in view of their oral delivery, International Journal of Nanomedicine.2013; 8:4291–4302.
19. Jornada DS,et al. Lipid-core nanocapsules: mechanism of self-assembly, control of size and loading capacity. Soft Matter.2012;8: 6646-6655.
20. Poletto FS, et al. How Sorbitan Monostearate Can Increase Drug-Loading Capacity of Lipid-Core Polymeric Nanocapsules, Journal of Nanoscience and Nanotechnology.2015;15: 827–837.
21. Jäger E, et al. Sustained release from lipid-core nanocapsules by varying the core viscosity and the particle surface area. Journal of Biomedical Nanotechnology.2009; 5: 130-140.
22. Oliveira MTP, et al. Orally delivered resveratrol-loaded lipid-core nanocapsules ameliorate LPS-induced acute lung injury via the ERK and PI3K/Akt pathways, International Journal of Nanomedicine.2019; 14: 5215–5228.
23. Drewes CC, et al. Role of poly(ε-caprolactone) lipid-core nanocapsules on melanoma–neutrophil crosstalk, International Journal of Nanomedicine.2017;12: 7153-7163.
24. Frozza RL, et al. Characterization of trans-Resveratrol-Loaded Lipid-Core Nanocapsules and Tissue Distribution Studies in Rats. Journal of Biomedical Nanotechnology.2010; 6: 694–703.
25. Rodrigues SF, et al. Lipid-Core Nanocapsules Act as a Drug Shuttle Through the Blood Brain Barrier and Reduce Glioblastoma After Intravenous or Oral Administration, Journal of Biomedical Nanotechnology.2016;12: 986–1000.
26. Kang YK, et al. Efficacy and safety findings from DREAM: a phase III study of DHP107 (oral paclitaxel) versus i.v. paclitaxel in patients with advanced gastric cancer after failure of first-line chemotherapy. In: Annals of Oncology.2018;29 (5):1220–1226,
27. Feng C, et al. Immobilization of Coacervate Microcapsules in Multilayer Sodium Alginate Beads for Efficient Oral Anticancer Drug Delivery, Biomacromolecules.2014; 15: 985-96.
28. Bansal D, et al. Development of liposomes entrapped in alginate beads for thetreatment of colorectal cancer, International Journal of Biological Macromolecules.2016;82: 687–695.
29. Giri TK, et al. Entrapment of capsaicin loaded nanoliposome in pH responsive hydrogel beads for colonic delivery, Journal of Drug Delivery Science and Technology.2017; 39: 417-422.
30. Benfattoum K, et al. Formulation characterization and in vitro evaluation of acacia gum–calcium alginate beads for oral drug delivery systems. Polym Adv. Technol.2018; 29: 884– 895.
31. Patel N, et al. Development and evaluation of a calcium alginate based oral ceftriaxone sodium formulation, ProgBiomater.2016; 5: 117-133.
32. Ranganath SH, et al. Hydrogel Matrix Entrapping PLGA-Paclitaxel Microspheres: Drug Delivery with Near Zero-Order Release and Implantability Advantages for Malignant Brain Tumour Chemotherapy, Pharmaceutical Research.2009;26 (9).
33. Wang L, et al. Enhanced stability of liposomes against solidification stress during freeze-drying and spray-drying by coating with calcium alginate, Journal of Drug Delivery Science and Technology.2015;30: 163-170.
34. Venturini CG, et al. Formulation of lipid core nanocapsules. Colloids and Surfaces. A, Physicochemical and Engineering Aspects.2011; 375: 200.
35. Zhang M, et al. Paclitaxel and etoposide-loaded Poly (lactic-coglycolic acid) microspheres fabricated by coaxial electrospraying for dual drug delivery, Journal of Biomaterials Science.2018;29 (16): 1949-1963.
36. Mei L, et al. Pharmaceutical nanotechnology for oral delivery of anticancer drugs, Advanced Drug Delivery Reviews.2013; 65 (6): 880-890.
37. Chaves PS, et al. Carvedilol-loaded nanocapsules: Mucoadhesive properties and permeability across the sublingual mucosa, European Journal of Pharmaceutics and Biopharmaceutics.2017; 114: 88-95.
38. Frank LA, Gazzi RP, de Andrade Mello P, Buffon A, Pohlmann AR, Guterres SS. Imiquimod-loaded nanocapsules improve cytotoxicity in cervical cancer cell line. Eur J Pharm Biopharm. 2019; 136:9-17.
39. Xu R, Progress in nanoparticles characterization: Sizing and zeta potential measurement, Particuology.2008;6: 112-115.
40. Calgaroto S, et al. Chemical stability, mass loss and hydrolysis mechanism of sterile and nonsterile lipid-core nanocapsules: The influence of the molar mass of the polymer wall, Reactive and Functional Polymers.2018;133: 161–172.
41. Santos PP, et al. Development of lycopene-loaded lipid-core nanocapsules: physicochemical characterization and stability study, Journal Nanoparticle Research.2015; 17: 107.
42. Brum AAS, et al. Lutein-loaded lipid-core nanocapsules: physicochemical characterization and stability evaluation, Colloids and Surfaces A: Physicochemical and Engineering Aspects.2017;522: 477–484.
43. Reynaud F, et al. Pectin beads loaded with chitosan-iron microspheres for specific colonic adsorption of

ciprofloxacin, Journal of Drug Delivery Science and Technology.2015; 1-7.
44. Fundueanu G, et al. Physico-chemical characterization of Ca-alginate microparticles produced with different methods. Biomaterials.1999; 20: 1427-1435.
45. Lim, HP, et al. Controlled delivery of oral insulin aspart using pH-responsive alginate/κ-carrageenan composite hydrogel beads. Reactive and Functional Polymers.2017; 120: 20-29.
46. Aguero L, et al. Alginate microparticles as oral colon drug delivery device: A review, Carbohydrate Polymers.2017; 15 (168): 32-43.