Sustained Release of an Antitumoral Drug from Alginate-Chitosan Hydrogel Beads and Its Potential Use as Colonic Drug Delivery

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ABSTRACT: Few researches are directed at drug delivery systems for β-lapachone (β-lap), a powerful anticancer agent but with limited pharmaceutical use. To overcome its limitations, we investigated controlled delivery systems of β-lap in simulated gastric fluids *in vitro* from chitosan (CS) and alginate (AL) hydrogel beads with purpose for oral administration. The AL-CS hydrogel beads were formed by coacervation and were characterized by morphology, swelling ratio, and their physicochemical properties. The hydrogel beads, with sizes of roughly 1 mm, presented good stability, and low porosity. The *in vitro* drug release profile was in good agreement with kinetics profiles and the Fickian model indicating diffusion as the release mechanism, with low burst effect, especially in an acid medium and allowing a prolonged release of ~ 72 h (pH 1.2; $k_2 = 0.19 \pm 0.04$) and (pH 7.4; $k_2 = 0.20 \pm 0.01$). The beads were resistant to the acid medium and may be an alternative for β-lap therapy of colorectal cancer. © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 000: 000–000, 2012

Key words: chitosan; alginate; hydrogel; **β**-lapachone; colonic delivery

INTRODUCTION

Oral delivery systems have received great attention due to their noninvasive nature and suitablity for managed release of various medicinal agents, including anticancer drugs, and proteins. Different from conventional methods of drug administration, drug delivery systems allow reduction in release rate, and controlled diffusion. Polymeric materials can avoid both toxic and sub-therapeutic doses in a wide range of administrations, and at the same time expand the possibility for cell targeting.[1,2] This advantages may be useful with anticancer agents like β-lapachone (β-lap) to achieve controlled drug delivery and reduce the side effects associated with the systemic delivery.[3–5]

β-Lap ($C_{15}H_{14}O_3$; PM 242.3) is an *o*-naphthoquinone (Fig. 1) that can be obtained through semisynthesis of lapachol.[6] Lapachol is a substance extracted from the core of the Ipe-Roxo (*Tabebuia avellanedae*) tree native to South America, with high frequency in Brazil, known as Pau d'Arco.[7,8] It is increasingly important to develop studies using this drug because its pharmacological properties make it a promising compound for the treatment of cancer. It is known however for its high toxicity and low solubility in water. The literature shows that acute toxicity (DL_{50}) in albino mice treated intraperitoneally is 1600 mg/Kg for lapachol, for α-lapachone it is 350 mg/Kg, and for β-lapachone it is 80 mg/Kg as determined by Santana et al.[9] Despite isomer β-lap's anticancer and antibiotic activities being higher than that of lapachol, it is 20 times more toxic than it.[10] Nasongkla et al.[11] showed β-lap's low solubility in water (0.038 mg/mL and 0.16 m*M*), which limits its therapeutic applications.

β-Lap has been one of the most extensively studied naphthoquinones due to its remarkable anticancer activity against various cancer cell lines, especially human prostate cancer, breast, lung, melanoma, and leukemia.[12] Studies suggest that the activity of β-lap is tumor-specific, and depends mainly on the overexpression of

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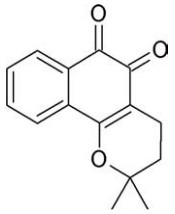

Figure 1 Chemical structure of β-lapachone.

quinone oxidoreductase (NQO1), which is over expressed in some tumor tissues.[13] Moreover, the action of β-lap is to induce free radical formation, and inhibition of topoisomerases I and II, triggering apoptosis of cancer cells.[14–16]

Most drug delivery systems use biodegradable, biocompatible, and natural biopolymers, and are capable of rate and/or time controlled drug release, such as AL and CS.[17] CS is a polysaccharide defined as a linear copolymer of 2-amino-2-deoxy-D-glucopyranose (D-glucosamine) and 2-acetamide-2-deoxy-D-glucopyranose (*N*-acetyl-D-glucosamine), of variable composition depending on the degree of residual acetylation.[17,18] CS can be obtained from chitin by deacetylation reaction in alkaline solutions,[19] and is naturally present in the cell walls of some fungi (*Mucor e Zygomycetes*).[20] Chitin, a polysaccharide of linear chain composed of units of *N*-acetyl-2-dioxo-D-glucopyranose is the main component of the exoskeleton of crustaceans and insects and is also present in the cell walls of fungi and yeast.[19]

AL is a natural water-soluble polymer extracted from brown algae; it is a linear unbranched polysaccharide with links (1–4) and varying residue amounts of α-L-guluronic (G), and β-D-mannuronic acids (M).[4,21] Oppositely charged polysaccharides in aqueous solutions interact spontaneously to form polyelectrolyte complexes (PECs) when mixed. Interactions between cationic CS, and anionic AL leads to PEC formations, and these PECs have potential applications in drug and gene delivery systems in biomedicine.[22–24] These hydrogels based on polyelectrolyte complexes have been shown to be resistant to attack by enzymes and the impact of changes in pH during gastrointestinal transit, protecting the body from the toxic effects of therapeutic compounds.[25]

The aim of this work was develop a β-lap delivery system using AL-CS hydrogel beads assayed *in vitro* with simulated gastric fluid (SGF) for purposes of oral administration. The influence of the β-lap loading conditions on hydrogel beads in the release kinetics studies were carried out. The hydrogel beads were characterized with regard to their morphology, swelling ratio, and their physicochemical properties. FTIR, DSC, and X-ray analysis reveal the presence of interation between AL and CS, but DSC and X-ray studies did not show any significant drug interaction with the biopolymers used. Effective release of β-lap occurred in simulated colonic fluid (SCF) with retanined release in simulated gastric fluid (SGF). This retention of the drug in acid medium, occured especially in the early hours, while there was a more pronounced release of the drug in alkaline medium, which simulates the phase of drug absorption. Therefore, the beads are stable in acid medium, important criterion to oral delivery of bioactive compounds.

EXPERIMENTAL

Materials

CS from shrimp shell-low M_w and AL were purchased from Sigma-Aldrich (St. Louis, MO). Insulin syringe 100 U (BD Ultra-Fine™), acetic acid, $CaCl_2$, SGF at pH 1.2, and SCF at pH 7.4. All other chemicals were analytic-grade and used directly without further purification.

Synthesis of β-lapachone

The β-lap was synthesized with a 76% yield by the use of sulfuric acid on natural lapachol derived from *Tabebuia avellanedae* (Bignoneaceae), following methodology adapted from Cavalcante et al.[26] The compound was purified by silica-gel column chromatography with an *n*-hexane-dichloromethane (8 : 2, v/v) eluent system. The product was characterized by the usual spectroscopic methods, including ^{1}H-NMR (hydrogen nuclear magnetic resonance) and IR (infrared spectroscopy). The physico-chemical properties and melting point were evaluated chromatographically through comparison of the product profile against a pure sample.

Preparation of AL-CS hydrogel beads and β-lap loading

The process of beads formation was done in accordance with Yu et al.,[3] with some modifications. Here, we used 2% AL and 0.5% CS solutions dissolved in distilled water and 1% acetic acid v/v, respectively, which remained under magnetic stirring until the formation of homogeneous gels. β-Lap 0.1% in ethanol 70% was associated with the AL and CS gel in preparation of the beads and the formation of hydrogels, and differing in accordance with the two conditions set out in A_1 and A_2. In the A_1 condition, the β-lap was added only in the AL gel with stirring until reaching a uniform suspension. In the A_2 condition, the β-lap was added to both gels. The resulting beads were kept 24 h in this medium to promote the stabilization of the polyelectrolyte complex,

washed three times with distilled water, and frozen to −80°C, and then freeze dried (IP21 Liobrás).

Swelling determination of β-lap loaded AL-CS hydrogel beads

The swelling degree was estimated by placing 0.1 g of freeze-dried β-lap loaded AL-CS hydrogel beads in a centrifuge tube with 15 mL of SGF at pH 1.2 and SCF at pH 7.4. The tubes were turned upside down to wet the hydrogels and maintained for 24 h at 37°C. At predetermined time intervals, the swollen samples were removed from the solution, quickly wiped with filter paper to remove droplets on the surface, and weighed. The study was performed in duplicate. The swelling degree (μ) was estimeted via eq. (1).

$$\mu = \frac{\text{weight (hydrated)} - \text{weight (dry)}}{\text{weight (dry)}} \times 100 \qquad (1)$$

Characterization of hydrogel beads

Morphological characterization

The morphological characterization of the AL-CS hydrogel beads was performed by scanning electron microscopy (SEM). The hydrogel beads were freeze-dried and fixed on an aluminum sample holder with a carbon-based adhesive tape. The samples (whole and cut beads) were coated with carbon and observed on a SEM (LEO-1430) apparatus. The beam current used was 500 pA, and the beam power was 20 KVA.

FTIR spectra analysis

FTIR spectra were measured to evaluate the chemical interaction between CS and AL in a UV-1650 PC spectrophotometer, from 400 to 4000 cm^{-1}. The AL-CS hydrogel beads were frozen to −80°C and lyophilized by freeze-dryer, then the samples were thoroughly grounded with exhaustively dried KBr, and pellets were prepared by compression under vacuum for further measurement.

Differential scanning calorimetry

DSC studies were performed using a DSC Mettler Toledo model 30TC 15 (Mettler, Zurich, Switzerland). The AL-CS hydrogel beads (5 mg) were scanned in sealed aluminium pans in nitrogen atmosphere (30 mL/min). DCS thermograms were scanned in the first heating run at a constant rate of 10°C/min, and a temperature range of 25 to 325°C. DSC thermograms of pure biopolymers, blank, and drug (20%) loaded AL-CS hydrogel beads were recorded.

X-ray diffraction studies

The measures for X-ray diffraction (XRD) of blank- and drug (20%)-loaded CS-AL Hydrogel beads were performed using the X-ray diffractometer—SHIMADZU—model XRD 7000, operating in θ-θ geometry. Copper pipe (λ1 = λ2 = 1.54060 and 1.54439 Å) was used at a voltage of 40 kV and 30 mA, with θ ranging from 3° to 90°, 0.02° step, and speed of 1° min.

In vitro β-lapachone release kinetics studies

The release studies of all AL-CS hydrogel bead formulations were performed by using an orbital shaker, at a rotation speed of 100 rpm at 37°C for 168 h, using the dissolution media of SGF at pH 1.2 and SCF at pH 7.4. We used 30 mL of dissolution medium for each 0.1 g of dried beads stored in Erlenmeyer flasks (50 mL). At predetermined time points, a 1000 μL aliquot was withdrawn, the same quantities were replaced by fresh medium. The concentration of β-lap was determined in a DU 640 Spectrophotometer at 257 nm. The β-lap released from the AL-CS hydrogel beads was quantified from the appropriate calibration curves. The study was performed in duplicate.

The kinetic data of β-lap for AL-CS hydrogel beads was fitted according to an exponential model (Fickian diffusion) using the following equation:

$$M_t/M_\infty = (1 - k_1\, e^{-k_2 t}) \qquad (2)$$

where, M_t and M_∞ are the mass of the drug released at a determined time (t) and at an infinite time (t_∞) of the kinetic process, respectively; k_1 is a fitting constant and k_2 is the kinetic rate constant.

Statistical analysis

Results were expressed as mean ± standard deviation. Factorial ANOVA was used to test data for β-lap release, and the swelling studies. The data were statistically analyzed by Tukey's test at a significance level of 5% ($P < 0.05$).

RESULTS

AL-CS hydrogel beads preparation and characterization

CS, one of the most common polymers used in pharmaceutical research, is a cationic biopolymer; it was associated with AL, of opposite charge to form hydrogel in the form of beads. AL in concentrations of <2%, did not favor the formation of beads when it was dripped in a coacervate solution of CS and CaCl2. In Figure 2, representative digital images of AL-CS

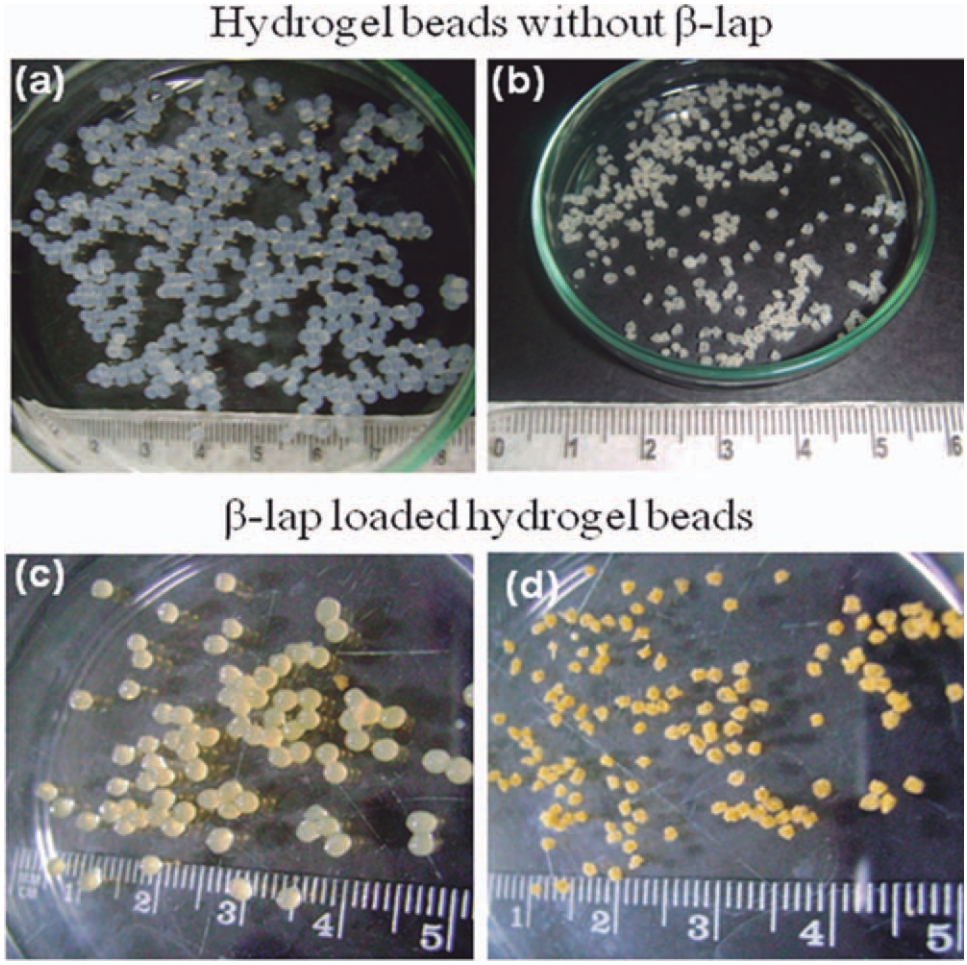

Figure 2 Digital images of AL-CS hydrogel beads: (a) Blank beads not freeze dried; (b) Blank beads freeze-dried; (c) β-lap loaded beads not freeze dried; (d) β-lap loaded beads freeze dried. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

hydrogel beads (blank beads and β-lap loaded beads), in two different states, swollen and dry are shown. It can be seen that at concentrations of AL 2%, compact beads were obtained, with a semitransparent surface and an almost perfect spherical shape, the diameter reached roughly 2 mm in the fully swollen state [Fig. 2(a,c)]. After lyophilization, the dry beads showed a surface with wrinkles, and diameter of roughly 1 mm, with a less spherical shape when compared to the fully swollen state [Fig. 2(b,d)]. In the formulation of beads where β-lap was associated with both CS and AL gels, before the process of bead formation, they were of an orange color in addition to the features already mentioned [Fig. 2(c,d)].

The morphological characterization of the beads, surface area and matrix, was performed by SEM. As can be seen in Figure 3, the CS beads showed a characteristic surface [Fig. 3(a1,3a2)] and a porous matrix [Fig. 3(a3)] or sponge-like structure. Compared with CS beads, AL-CS hydrogel beads displayed a rough surface, with lower porosity [Fig. 3(b1,b2)] and a uniform matrix [Fig. 3(b3)].

IR analysis allows the observation of vibration modes for the specific groups of compounds analyzed. Thus, we obtained infrared spectra of the AL, CS, and AL-CS hydrogel beads, as shown in Figure 4. In the CS IR spectrum, we observed peaks at 1620, 1596, and 1421 cm^{-1}, characterizing the amino groups ($—NH_3^+$). Moreover, the IR spectrum of AL shows modes of vibration in the band of 1610 to 1418 cm^{-1} related to carboxyl group (COO^-). A wide absorption band was observed around 1030 cm^{-1}, which can be attributed to the COH stretch. By analyzing the profile of the absorption spectrum of blank AL-CS hydrogel beads, there were differences in the modes of vibration of their peaks when compared to the spectra of isolated polymers, characterizing the molecular interaction between them. The appearance of the bands 1635 and 1624 cm^{-1} as well as the disappearance of the band at 1596 cm^{-1} characteristic of the CS amino group, suggested the formation of a polyelectrolyte complex between AL and CS. The presence of the drug in the hydrogel was not observed in the infrared spectra (data not shown).

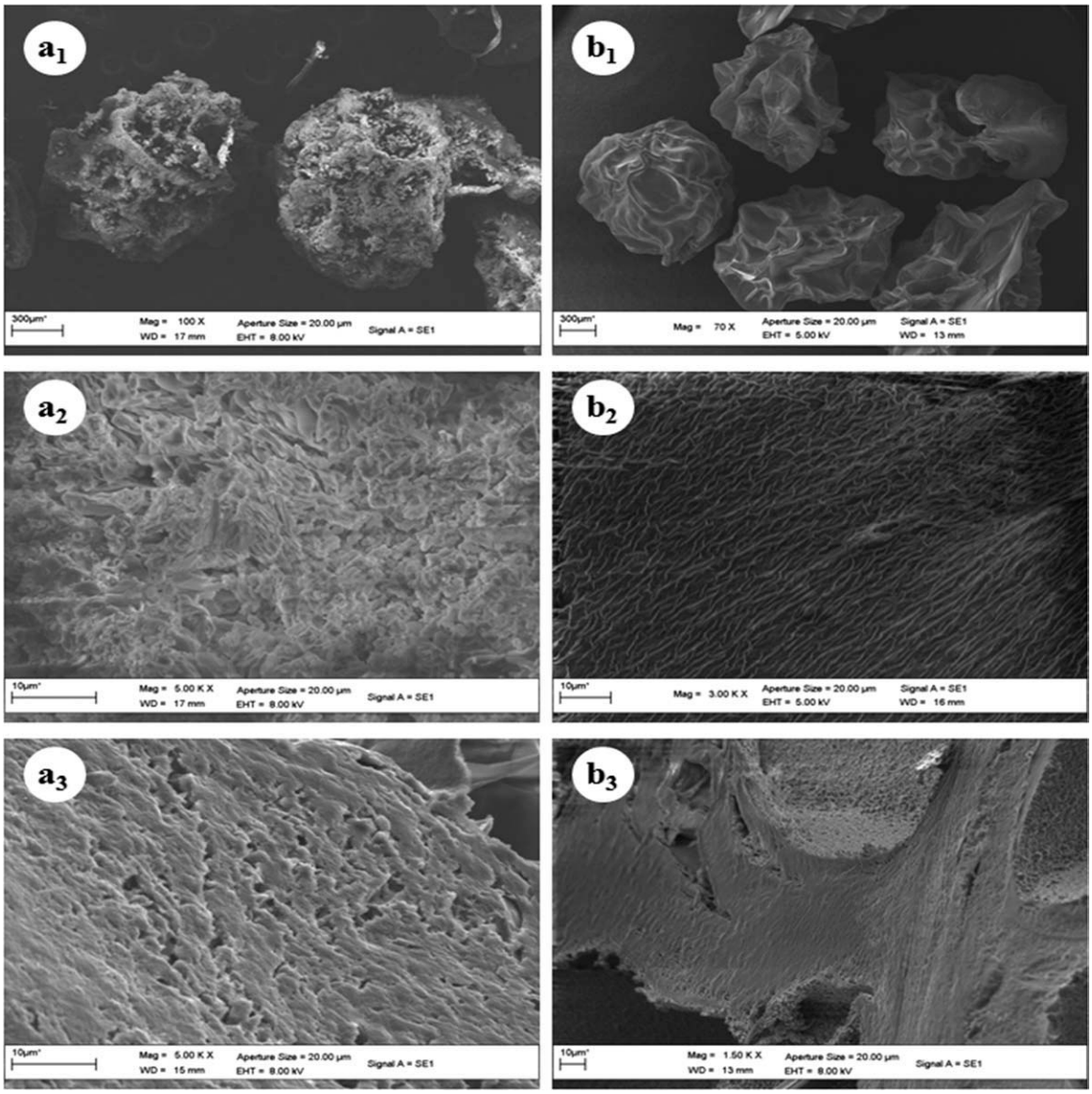

Figure 3 Scanning electron micrographs SEM of CS and AL-CS hydrogel beads: (a_1) CS beads 100x; (a_2) CS surface details 5000x; (a_3) CS matrix details 5000×; and (b_1) AL-CS hydrogel beads 100×; (b_2) AL-CS hydrogel surface details 1000×; and (b_3) AL-CS hydrogel matrix details 5000×.

The Figure 5 shows the DSC thermo analysis of AL, CS, β-lap, blank AL-CS hydrogel beads, and drug loaded AL-CS hydrogel beads. In the calorimetric studies, the thermogram of AL is characterized by an endothermic peak around 60°C and exothermic peaks at 215, 245, and 256°C [Fig. 5(a)]. The endothermic peak is attributed to a dehydration process associated to hydrophilic groups of polymers while the exothermic peaks result from polymer degradation, and denotes the AL degradation temperature. The endothermic peak in the thermogram of AL at around 58°C exists in the thermograms of blank hydrogel beads [Fig. 5(c)], and β-lap- loaded hydrogel beads [Fig. 5(d)], pointing to AL degradation. The DSC scans of CS polymer exhibited an endothermic peak at about 52°C that was attributed to water evaporation. The exothermic baseline deviation beginning around 250°C indicates the onset of CS degradation [Fig. 5(b)]. This exothermic exists in the thermogram of blank hydrogel beads and β-lap loaded hydrogel beads, which is transformed in a significantly broad band at a range of 251–317°C [Fig. 5(c,d)]. It can be seen that the β-lap DSC curve displays a sharp endothermic event at 155.7°C, which is due to drug melting, characteristic of a crystalline substance. In the thermogram of β-lap loaded hydrogel beads, this endothermic peak at 155.78°C is evident [Fig. 5(e)].

Figure 6 shows XRD studies of AL, CS, β-lap, blank AL-CS hydrogel beads and β-lap loaded AL-CS hydrogel beads, complementing the results obtained by DSC analysis. The diffractogram of

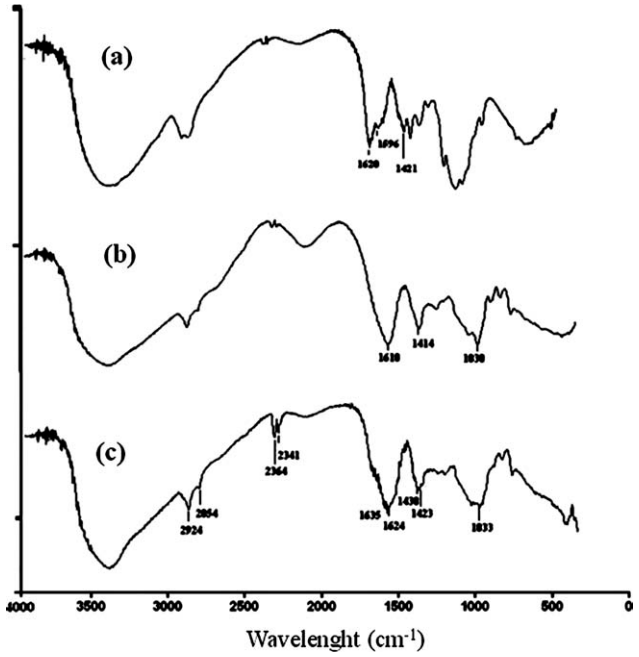

Figure 4 FTIR spectra of CS, AL and AL-CS hydrogel beads.

blank AL-CS hydrogel beads showed changes in the peaks compared with AL and CS diffractograms, confirming the formation of the polyelectrolyte complex between AL and CS. The diffractogram of β-lap exhibit a main sharp peak at 9.4°2θ, and other secondary peaks like 12.08, 15.4 and 19.6°2θ (Fig. 7). The diffractogram remained unchanged in the β-lap-loaded AL-CS hydrogel beads (Fig. 6).

Hydrogel swelling properties

The equilibrium swelling of the AL-CS hydrogel beads was measured in a pH of 1.2 (SGF) and in a pH of 7.4 (SCF) at 37°C for 1440 min. The A_2 condition showed highest swelling ratio, differing from its swelling profile according to pH ($P < 0.05$). Moreover, it appears that the A_1 condition did not vary with pH, showing no significant difference ($P > 0.05$), as shown in Figure 8(a,b). The AL-CS hydrogel beads in the A_2 condition at pH 7.4, showed an swelling average approaching 17.5%, a value seven times greater than that found for the condition A_1, at the same pH, but also proved to be significantly different in the swelling degree at pH 1.2 in same A_2 condition, during the period of 30 min to 24 h ($P < 0.05$). Higher swelling degrees were observed for prolonged assays (after 24 h) in both A_1 and A_2 condition (data not shown).

In vitro β-lap release kinetics studies

The release profiles of β-lap from AL-CS hydrogel beads at pH 1.2 and pH 7.4 are illustrated in Figures 9 and 10, respectively. The symbols represent the data experimentally measured, and the curves represent the fit to the mathematical modeling. The release kinetics of β-lap from AL-CS hydrogel beads was fitted according to the exponential model $M_t/M_\infty = (1 - k_1\, e^{-k_2 t})$ and the calculated kinetic parameters of β-lap loaded-AL-CS hydrogel beads are summarized in Table I.

A higher release pattern of β-lap from the AL-CS hydrogel beads in the A_1 condition was observed when compared to the A_2 condition at pH 1.2 (Fig. 9) and pH 7.4 (Fig. 10) from 6 h of drug released, showing a significant difference between the conditions when analyzed at pH 1.2 or pH 7.4 ($P < 0.05$). The kinetic profile of the drug released from β-lap loaded-AL-CS hydrogel beads at pH 1.2 in the A_1 condition ($k_2 = 0.19 \pm 0.04$ and release rate $= 8.46 \pm 0.09$ μg/h) and A_2 condition ($k_2 = 0.99 \pm 0.06$ and release rate $= 10.65 \pm 0.19$ μg/h) showed burst effects of 7 and 12%, respectively, after the first 0.75 h of the kinetic process. At pH 7.4, the kinetic profile of β-lap showed burst effects of 24% after 3.25 h of the kinetic process in the A_1 condition ($k_2 = 0.20 \pm 0.01$ and a release rate equaling 7.53 ± 0.07 μg/h), and 22% after 0.75 h of kinetic process in the A_2 condition ($k_2 = 3.17 \pm 3.88$ and a release rate equal to 24.94 ± 0.93 μg/h), Table I. The maximum releases of β-lap at pH 1.2 were ~ 47% (A_1 condition) and 21% (A_2 condition) at 72 and 24 h, respectively. While at pH 7.4, the maximum releases of β-lap were ~ 50% (A_1 condition), and 28% (A_2 condition) at 72 and 2.25 h, respectively.

DISCUSSION

AL-CS hydrogel beads were formed by coacervation, in which the carboxyl groups of AL electrostatically interact with CS amino groups. CS has a tendency to ionize in solutions with acidic pH, since it presents a pKa of around 6.5. However, when AL is dissolved in a neutral pH solution, the carboxyl groups become negatively charged. In aqueous solutions with a pH between 3.5 and 6.5, the amino groups of CS interacted with the carboxyl groups of AL, probably by electrostatic interaction or hydrogen bonds to form the polyelectrolyte complex.[27–29] According to George and Abraham,[4] the formation of a polyelectrolyte complex, reduces the porosity of the matrix of polymers contributing to a better encapsulation of bioactive compounds. In this study, we formed these hydrogels for biotechnological application studies as carriers in controlled delivery systems for β-lap, an antitumor drug.

The morphological characteristics of the beads showed size of ~ 1 mm, and low porosity of both surface and matrix, which may be assigned to the formation of a polyelectrolyte complex between CS and AL, which allows the formation of a less porous

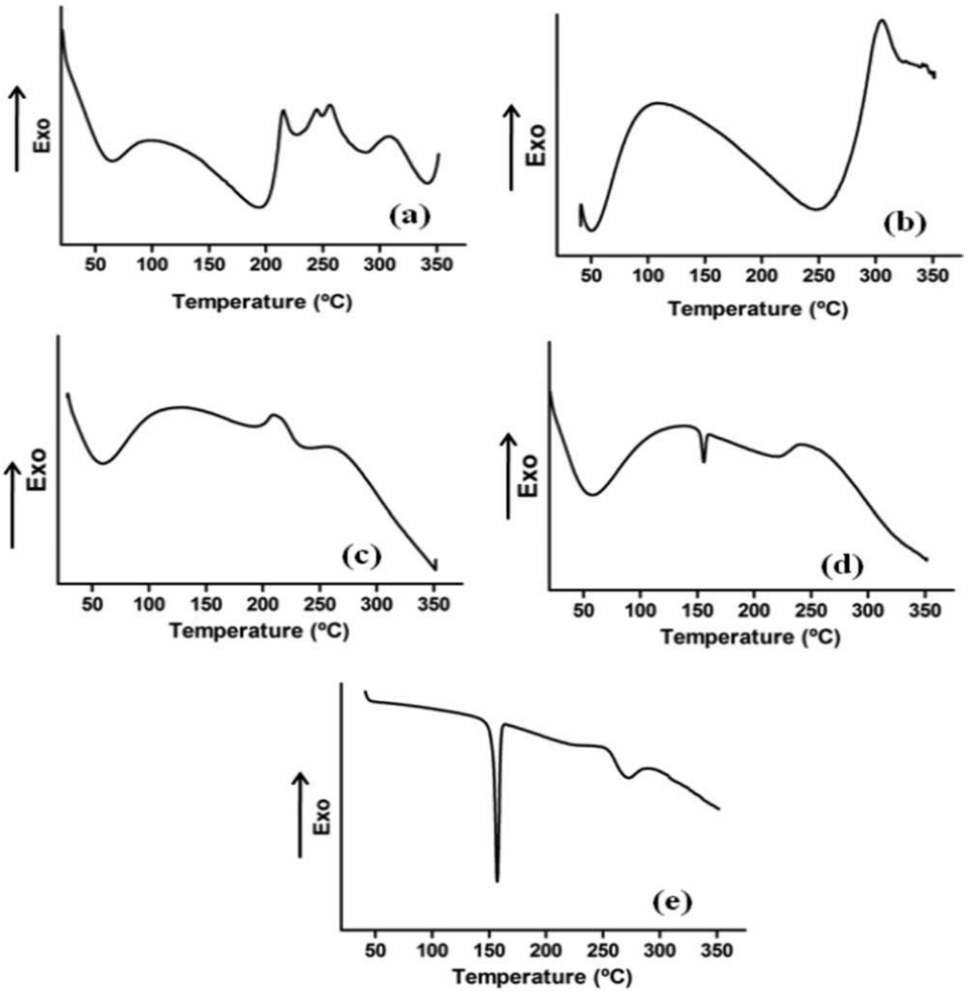

Figure 5 DSC thermograms of (a) Alginate; (b) Chitosan; (c) Blank AL-CS hydrogel beads; (d) β-lap loaded AL-CS hydrogel beads; (e) β-lap.

surface, and matrix. Analyzing the endothermic peak in the thermogram of AL and the exotherm peak of CS denotes the polyelectrolyte interaction of both COO^-/NH_3^+ of the AL and CS, respectively, like those reported in the literature.[29–32] The endothermic event at 155.78°C seen in the β-lap-loaded hydrogel beads DSC curve relates to the endothermic peak of the β-lap DSC curve, and suggests no significant molecular interactions between the drug and the excipients. Rastogi et al.[33] reported similar results for encapsulated isoniazid in AL microspheres. X-ray studies confirmed no physico-chemical interaction (drug/polymer) due to the presence of characteristic peaks of β-lap in the crystalline form, which is also observed by other authors.[34,35] However, the diffractogram of blank AL-CS hydrogel beads showed changes in the peaks comparing AL and CS diffractograms, characterizing the interaction between them, and confirming the results obtained by IR and DSC analysis.

Hydrogels, which are hydrophilic polymer chains in dried form, have the ability to release the

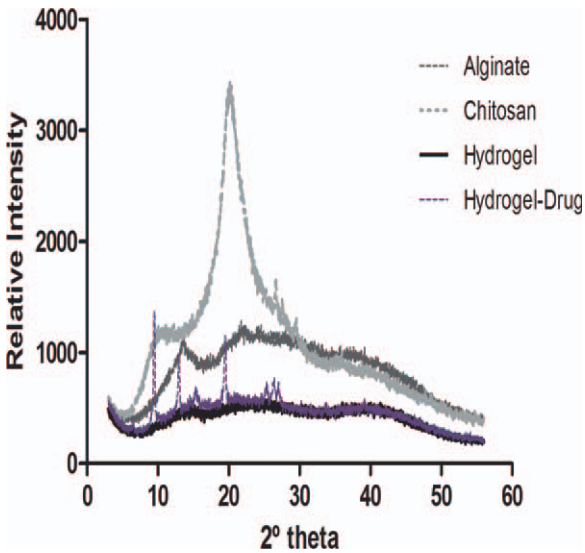

Figure 6 X-ray diffractograms of Alginate; Chitosan, Blank AL-CS hydrogel beads and β-lap loaded AL-CS hydrogel beads. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

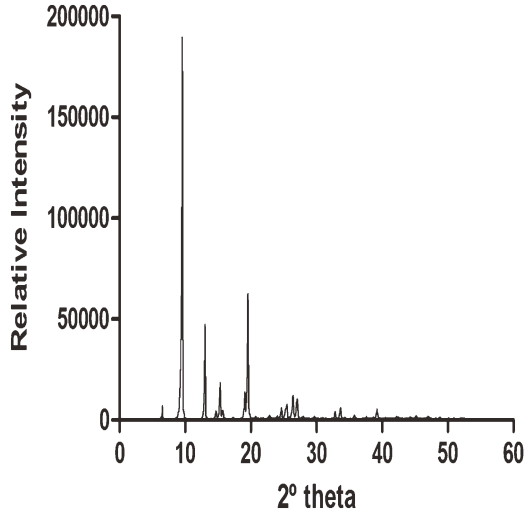

Figure 7 X-ray diffractograms of β-lapachone.

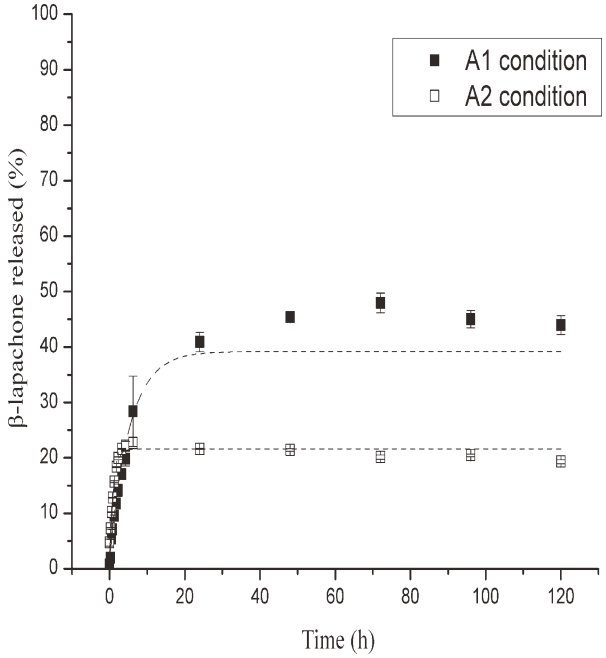

Figure 9 *In vitro* release of β-LAP from AL-CS hydrogel beads. β-LAP-loaded AL-CS hydrogel beads in the A_1 Condition (black squares) and A_2 Condition (white squares) in pH 1.2 under orbital shaker, at a rotation speed of 100 rpm and 37°C. Data are expressed as mean ± S.D. (n = 2). Lines represent the non-linear fitting of Fickian diffusion model.

bioactive compound contained in them (in the presence of water) for controlled diffusion. According to Pasparakis et al.[36] by studying the swelling kinetics of these materials, one can make a good prediction of the behavior of the diffusion, and the ability of the hydrogel to be used as a carrier system for controlled release. Thus, the swelling degree is the ability of the hydrogel to absorb water over time, showing its stability in different mediums. Drug release from hydrogels occurs by different processes including swelling due to water entering the polymer matrix, resulting in the relaxation of polymer chains, and allowing the outflow of the drug.[4,22,27,37]

β-lap associates with AL-CS hydrogel beads but probably does not interact physically or chemically. Nevertheless, the drug release results of hydrogel beads show a release profile correlated to changes in pH.[33,37] The results obtained for swelling ratio

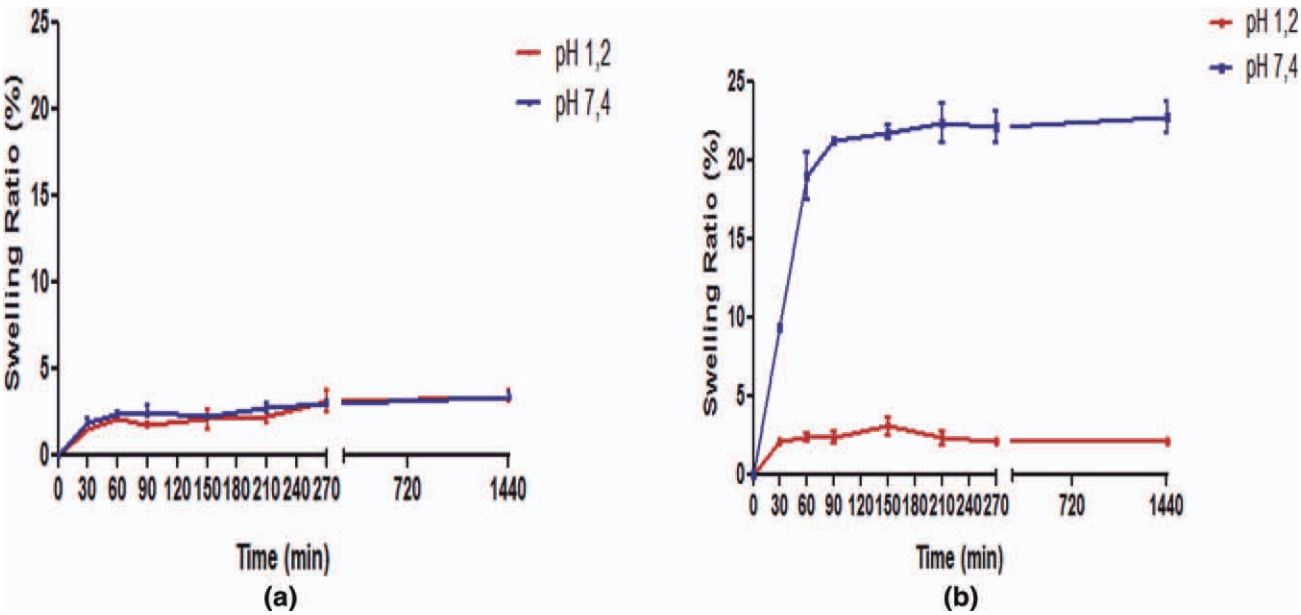

Figure 8 Swelling behavior of β-lap loaded AL-CS hydrogel beads: (a) A_1 Condition and (b) A_2 Condition in differents pHs under a orbital shaker, at a rotation speed of 100 rpm and 37°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

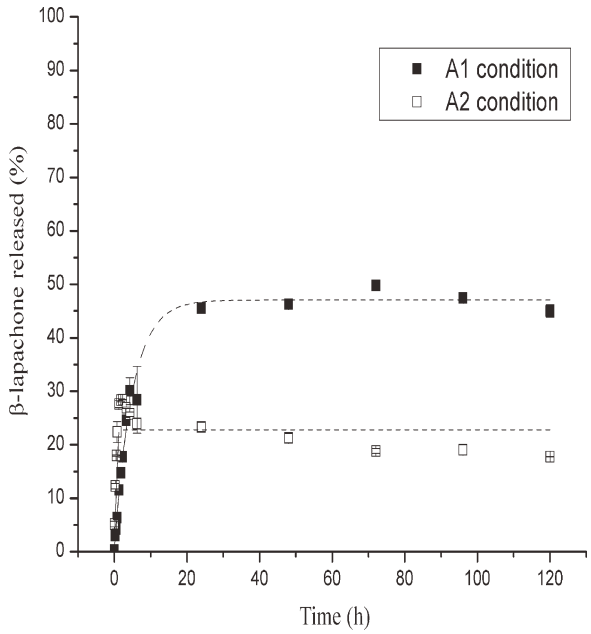

Figure 10 *In vitro* release of β-lap from AL-CS hydrogel beads. β-LAP-loaded AL-CS hydrogel beads in the A_1 Condition (black squares) and A_2 Condition (white squares) in pH 7.4 under orbital shaker, at a rotation speed of 100 rpm and 37°C. Data are expressed as mean ± S.D. (n = 2). Lines represent the non-linear fitting of Fickian diffusion model.

analysis showed that the A_2 condition was significantly different than the A_1 condition at a pH of 7.4. This behavior may be the result of a greater neutralization of the amino groups in CS when compared with the A_1 condition. Thus, the carboxyl groups of AL not associated with CS is free to be ionized at this pH, favoring the swelling of the beads.[4]

The development of a β-lap delivery system from AL-CS hydrogel beads is an important pharmaceutical objective, due to the high toxicity of the drug and its low solubility in water.[11] The release of the drug from these hydrogels is affected by the pH medium, which can influence the ionization of amino groups in CS and carboxyl groups in AL. The complexation of AL to CS makes these polymers stable in the face of changes in pH, and reduces the porosity of the matrixes, or the unwanted spread of the drug.[2,4] To use this system in delivering drugs orally, the formulation needs to take into consideration some of necessary factors. The natural pH of the gastrointestinal tract undergoes changes from acidic (pH = 1.2) in the stomach to slightly alkaline (pH = 7.4) in the intestine,[4,25,27] as well as gastrointestinal tract passage time from the mouth to the stomach cecum, which varies from 3 to 16 h. Therefore, resistance to acid, enzymes, and a time-controlled release are necessary for oral drug vehicles.[27]

Regarding the *in vitro* release kinetics of β-lap from CS-AL hydrogel beads, we observed that the release profile of β-lap at pH 1.2 had a lower burst effect (7 and 12%, A_1 condition and A_2 condition, respectively, in the first 0.75 h) while, at pH 7.4, we observed that the initially faster drug release achieved higher values. These results are satisfactory for oral route drug delivery systems, because they show a lower drug release in acid medium, which represents the gastric medium. A more pronounced release of the drug in an alkaline medium is expected, which simulates the phase of drug absorption into the *in vivo* system.[3,27,38] However, we observed that the A_2 condition allowed a faster release of the drug. The data recorded values of k_2 constant, reaching maximum release of β-lap in 24 h (pH 1.2), and 2.25 h (pH 7.4), while the A_1 condition showed a controlled release profile for 72 h.

For hydrogel, the larger swelling ratio usually accelerates loaded drug release, but the opposite results were found in this study, at least in the essays of swelling up to 24 h. According to the molecular size of β-lap (242 g/mol), the swelling properties, related to polymer relaxation during gel swelling, was not the main determining factor that influenced drug delivery, that is, the swelling of the hydrogel was not decisive for release, but the kinetic data of the release studies indicates that drug release from the polymer was due to Fickian diffusion.

These results showed that the A_1 condition would be favorable for development of a β-lap delivery

TABLE I
Calculated release parameters from AL-CS hydrogel beads containing β-lap by using exponential and linear models.

pH 1.2				
β-Lap loading AL-CS hydrogel beads	% Burst effect	Exponential model $M_t/M_\infty = (1 - k_1e^{-k2t})$		Linear model
		k_1	k_2	Release rate (μg/h)
A_1 condition	7.11 ± 0.01 (0.75 h)	0.94 ± 0.01	0.19 ± 0.04	8.46 ± 0.09
A_2 condition	12.80 ± 0.04 (0.75 h)	0.86 ± 0.03	0.99 ± 0.05	10.65 ± 0.19
pH 7.4				
β-Lap loading AL-CS hydrogel beads	% Burst effect	Exponential model $M_t/M_\infty = (1 - k_1e^{-k2t})$		Linear model
		k_1	k_2	Release rate (μg/h)
A_1 condition	24.54 ± 0.58 (3.25 h)	0.99 ± 0.01	0.20 ± 0.01	7.53 ± 0.07
A_2 condition	22.38 ± 1.95 (0.75 h)	0.91 ± 1.26	3.17 ± 3.88	24.94 ± 0.93

system, because it showed a lower burst effect (followed by the controlled and sustained release of the drug). We suggest that the A_2 condition presents a higher concentration of β-lap on the surface of the particles due to the particle formulation methodology.

CONCLUSIONS

In this work, was prepared β-lap-loaded AL-CS hydrogel beads by coacervation for prolonged simulated gastrointestinal release, with good stability in acid medium. FTIR, DSC, and X-ray analysis revealed the presence of interaction between AL and CS, but DSC and X-ray studies did not show any significant molecular drug interactions with the biopolymers used, and in the assay conditions. The A_1 condition, with respected to the β-lap loading was effective for drug delivery system. The A_1 condition may be developed for release of drugs such as β-lap, for sustainable oral delivery. This strategic innovation for development of a delivery system for β-lap could be efficient for *in situ* cancer therapies, such as colorectal therapy, avoiding the limitations found in conventional oral administration, and providing a new perspective for the use of this antitumoral drug.

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