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Alginate–chitosan systems: *In vitro* controlled release of triamcinolone and *in vivo* gastrointestinal transit

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ABSTRACT

The aim of this study was to develop multiparticulate therapeutic systems of alginate (AL) and chitosan (CS) containing triamcinolone (TC) to colonic drug delivery. Multiparticulate systems of AL–CS, prepared by a complex coacervation/ionotropic gelation method, were characterized for morphological and size aspects, swelling degree, encapsulation content and efficiency, *in vitro* release profile in different environments simulating the gastrointestinal tract (GIT) and *in vivo* gastrointestinal transit. The systems showed suitable morphological characteristics with particle diameters of approximately 1.6 mm. In simulated gastric environment, at pH 1.2, the capsules presented low degree of swelling and *in vitro* release of drug. A higher swelling degree was observed in simulated enteric environment, pH 7.5, followed by erosion. Practically all the drug was released after 6 h of *in vitro* assay. The *in vivo* analysis of gastrointestinal transit, carried out in rats, showed that the systems passed practically intact through the stomach and did not show the same profile of swelling observed in the *in vitro* tests. It was possible to verify the presence of capsules in the colonic region of GIT. The results indicate that AL–CS multiparticulate systems can be used as an adjuvant for the preparation of therapeutic systems to colonic delivery of drugs.

1. Introduction

Colonic drug delivery systems have been subject of great interest, due to the recently recognized importance of this region of the gastrointestinal tract (GIT) not only for local therapy but also for systemic drug delivery. The release of drugs specifically into the colon is considered an important alternative for the treatment of inflammatory processes, like Crohn's disease and ulcerative colitis, infectious diseases, like spasmodic, amebic, bacterial and viral colitis, as well as carcinomas (Rijnierse, Nijkamp, & Kraneveld, 2007). Moreover, the specific systemic absorption in the colonic region offers interesting possibilities for the treatment of diseases susceptible to diurnal rhythm, such as asthma, arthritis or inflammations (Friend & Sellin, 2005). The colon has also been reported as an ideal site for the absorption of proteins and peptides, like insulin (Sinha & Kumria, 2001; Yamamoto, Tozaki, Okada, & Fujita, 2000), calcitonin (Antonin et al., 1996), leuprolide (Zheng, Qiu, Fu Lu, Hoffman, & Reiland, 1999), and albumin (Sriamornsak, 1999).

Naturally occurring polymers, such as cellulose, starch, chitosan (CS), pectin (PC) and alginate (AL), are biodegradable materials with low toxicity and low cost. They have been widely used as excipients in several pharmaceutical formulations for several decades (Bajpai, Saxena, & Sharma, 2006). The possibility of decomposition by colonic enzymes makes possible the use of such polymers as reliable support for colonic drug delivery pharmaceutical dosage forms (Vandamme, Lenourry, Charrueau, & Chaumeil, 2002).

Alginic acid is a polysaccharide found in several species of brown algae (Phaeophyceae). It is a linear copolymer of uronic acids, namely D-mannuronic and L-mannuronic, linked through 1,4 bonds and arranged in blocks of one type of residue (M or G blocks) or of both residues (MG blocks) (Dentini et al., 2007; George & Abraham, 2006).

Microcapsules with a core of calcium–AL and a polyanion–polycation membrane have been researched for different applications. Polylisine and CS are the polycations the most commonly used for the production of capsules (Ferreiro, Tillman, Hardee, & Bodmeier, 2002; Lucinda-Silva & Evangelista, 2003) and the most common methods used are complex coacervation and some kinds of gelation (Gåserød, Smidsrød, & Skjåk-bræk, 1998).

AL–CS systems have been used as carrier for the controlled release of proteins and drugs. They are biocompatible, biodegradable and they possess mucoadhesive properties (Wittaya-Areekul, Kruenate, & Prahsarn, 2006). The formation of AL–CS polyelectrolytic complex alters the permeability of the AL–calcium particles

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Table 1
Composition of the TC containing AL–CS particles.

Sample	AL (%)	CS (%)	$CaCl_2$ (%)	TC (mg/mL)	Drug contents (%)
ALT1				1.0	5.53 ± 0.35
ALT2				2.0	11.44 ± 0.45
ALT3				3.0	16.73 ± 1.76
ALT4			1.50	4.0	21.32 ± 0.59
ALT5	1.00	0.50		5.0	28.22 ± 2.73
ALT6				6.0	33.08 ± 1.00
ALT7				7.0	39.39 ± 0.76
ALT8			2.00		32.80 ± 0.31
ALT9			3.00	5.0	34.60 ± 0.52
ALT10	1.50	0.75	2.00		30.49 ± 1.41

in the GIT. This complex is insoluble in acidic medium, decreasing the dispersion of CS, and resulting in particles with low porosity and low drug diffusion rate (George & Abraham, 2006; Prashanth & Tharanathan, 2007).

Multiparticulate dosage forms (e.g. pellets, granules or beads) have several advantages over unitary forms, such as ease of dispersion, possibility of releasing the drug more uniformly throughout the gastrointestinal tract, and greater flexibility in formulation (Zhang, Alsarra, & Neau, 2002). As the size of multiparticulate systems is small (usually between 0.7 and 2.00 mm), they traverse more easily through the upper GI tract, quickly reaching the colon. Here, they show a slower transit through its ascending portion, increasing their residence time in colonic region (Friend & Sellin, 2005).

The present work aimed the development of multiparticulate systems based on AL–CS complexes containing triamcinolone (TC) for colonic drug delivery and, consequently, to serve as an aid to inflammatory disease therapy. The systems were prepared by complex coacervation/ionotropic gelation method in aqueous environment. These hydrophilic systems allow the incorporation of large amounts of lipophilic drugs, such as anti-inflammatory and anticancer agents, to be specifically released in the colon. Triamcinolone (TC) is a steroidal anti-inflammatory and immunosuppressive drug, more potent than sulfasalazine for treating locally inflammatory processes in the colon (López, Reyes, Igea, Espinar, & Méndez, 1999). TC is a very fine crystalline white powder, odorless, slightly hygroscopic, and very slightly soluble in water (in about 5000 parts).

2. Materials and methods

2.1. Materials

Chitosan (commercial grade), MW 1.4×10^5 Da, after purification, was purchased from Sigma (São Paulo, Brazil), sodium alginate, MW 3.27×10^5 Da (pharmaceutical grade) from Henrifarma (São Paulo, Brazil), triamcinolone (pharmaceutical grade) from Galena (São Paulo, Brazil). Other substances and solvents used were of analytical grade.

2.2. Methods

2.2.1. Preparation of AL–CS capsules

The capsules were prepared by complex coacervation/ionotropic gelation, following methodology previously described in other works of our group (Lucinda-Silva, Monteiro, Carvalho, & Evangelista, 2006). Initially, TC was added to the alginate dispersion and maintained under stirring until obtaining a homogeneous suspension of drug in the polymeric dispersion. The dispersion was dropped into the CS dispersion containing calcium chloride. In order to optimize the encapsulation efficiency, different drug concentrations were tested (Table 1).

2.2.2. Morphological and size distribution analyses

The particles morphology was studied by stereoscopy and scanning electron microscopy (Jeol mod. T330A). Size distribution, area and roundness were assessed by Leica MZ APO stereoscope and Leica Qwin Image Analysis Systems software. Approximately 150 particles per sample were analyzed and their size was determined using the diameter according to Feret at 0°.

2.2.3. Swelling behavior

Swelling ratio was verified in different media simulating the different pH conditions of the GIT, simulated gastric (pH 1.2) and enteric (phosphate buffer, pH 7.4) environments. The swelling ratio was assessed by the increasing of particles diameter (Leopold & Eikeler, 1998), using a Leica MZ APO stereoscope and Leica Qwin software. The diameter measured was the Feret's at 0° (Barber, 1993).

2.2.4. Determination of drug content and encapsulation efficiency

For drug content determination, about 4 mg of the samples were accurately weighed and kept in contact with 20 mL of 50 mM phosphate buffer pH 7.5 for 2 h under stirring. The samples were then filtered and the drug was analyzed at 239 nm (spectrophotometer HITACH®, mod. U-2000). Drug contents (DC) was given by the following equation, in which m_{TC} is the mass of TC in the sample and m_s is the sample mass:

$$\mathrm{DC}\,\% = \frac{m_{TC}}{m_s} \times 100$$

The encapsulation efficiency (EE) was given by the following equation, in which Y is the yield of the batch and M_{TC} is the mass of TC used in the preparation of the batch:

$$\mathrm{EE}\,\% = \frac{\mathrm{DC}\% \times Y}{m_{TC}}$$

2.2.5. In vitro drug release

The test was performed on a Dissolution Station (Hanson, model SR8-Plus) using the basket method under the following conditions: volume of receptor medium (simulated gastric or enteric media without enzymes) 400 mL, stirring rate 50 rpm, temperature 37 ± 0.5 °C, length of assay 6 h, aliquots withdrawn at 10, 20, 30, 45, 60, 90, 120, 180, 240, 300, and 360 min, samples 40 mg of capsules. The drug was spectrophotometrically quantified at 239 nm.

In order to evaluate the *in vitro* drug release kinetics, first order, Higuchi, Hixson–Crowell, Korsmeyer–Peppas and Baker–Lonsdale mathematical models were used. The adequacy of the delivery profiles to the mathematical models was based on the correlation coefficient value (r^2). The study was conducted using the software Sigma-Plot® version 10.0.

2.2.6. Visual evaluation of in vivo gastrointestinal transit

The gastrointestinal transit of the AL–CS capsules was evaluated after oral administration in male Wistar rats weighing 200–250 g.

All animals were housed at the Biologic Control Laboratory of São Paulo State University, and kept at 23–25 °C. Animals had free access to water and food. The experiments were performed to minimize animal suffering by guidelines of animal laboratory of Universidade do Vale do Itajaí (Brazil).

The animals were randomly separated in two groups: control group (C, $n = 21$), to which a solution of the drug was administered; test group (T, $n = 28$), to which AL–CS capsules were administered. This group was further divided into seven subgroups of four animals (A, B, C and D).

After 12 h of fasting, 10 AL–CS capsules were orally administered with 1 mL of distilled water by means of a 2 mm wide spatula to the animals under light anesthesia. The GI transit was determined by counting the capsules present in the gastrointestinal lumen. The animals were sacrificed in a CO_2 chamber after preestablished time periods (0.5, 1, 2, 4, 6, 8, and 10 h) to open their abdomens.

The GI tract was sectioned in stomach (I), three different regions of the small bowel of around 40 cm each (II, III and IV), and two portions of the large bowel of around 12 cm each (V and VI).

The different sections of the GI tract were visually analyzed for the localization of capsules and were photographed by means of a stereoscope connected to a camera for image capture and further evaluation by Leica Qwin software.

2.2.7. Statistical analysis

The results were expressed as mean ± S.D. For the group comparisons, one-way analysis of variance was applied. The data were statistically analyzed using variance analysis followed *T*-test. *p* value less than 0.05 were considered as indicative of significance.

3. Results and discussion

3.1. Morphology and size distribution of AL–CS capsules

Early studies showed that the formation of polyelectrolytic complex between AL and CS was optimal when the dispersions were maintained at pH 4.8, the adequate mass ratio between AL and CS being 2 to 1 or 0.86 mole of AL to each mole of CS (Lucinda-Silva & Evangelista, 2005). During the development of AL–CS capsules, different drug to polymer ratios were tested, as well as different concentrations of calcium chloride (Table 1), since calcium is responsible for the formation of the gel structure with AL.

Calcium is an important element for building and maintenance of AL–CS capsules structure. When AL dispersion is placed in contact with the calcium containing CS dispersion, calcium ions bind to the carboxylic groups of the polymer chain, building a gelled net called "egg box" structure. CS, probably due to its high molecular weight, complex with AL rather at the surface of the gelled Ca–AL structure and builds a kind of membrane.

Fig. 1 shows photomicrographs of AL–CS capsules containing the model drug TC. The presence of the drug suspended within the hydrophilic matrix resulted in a more intense whitish aspect to the capsules. In a general way under the morphological aspects, the drug containing capsules were more homogeneous, presenting relative sphericity, with roundness mean value of 1.31 ± 0.151, and a slightly rough surface.

The size distribution analysis showed that an increase in the drug concentration to 5.0 mg/mL in the AL dispersion (sample ALT5) produced particles slightly grater than those prepared with 1 mg/mL of drug (sample ALT1), the mean values being, respectively, 1.68 mm ± 8.87% and 1.64 mm ± 10.98%.

3.2. Drug content and encapsulation efficiency

Since TC is very slightly soluble in the AL dispersion, the drug in a micronized form was used. In the optimization of TC incorporation

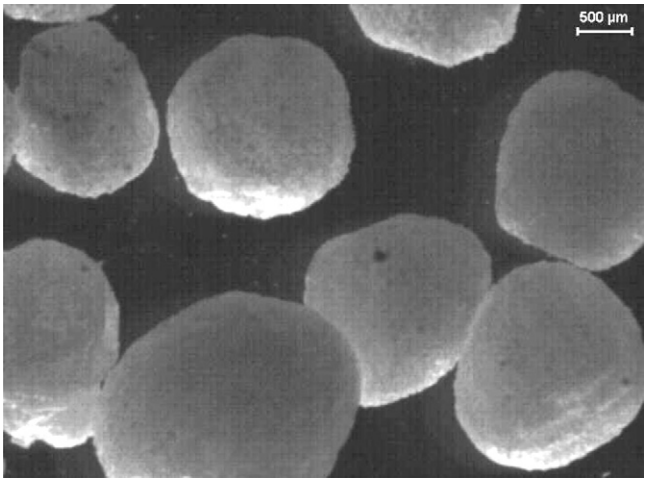

Fig. 1. Photomicrograph of TC containing AL–CS capsules. Magnification of 32×.

to the AL–CS capsules, increased drug concentrations (from 1.0 to 7.0 mg/mL, samples ALT1 to ALT7) in the AL dispersion were tested, followed by the assessment of drug contents and encapsulation efficiency. It was observed that an increasing of the initial drug concentration caused the increasing of drug contents from 5.53 to 39.39% (drug concentration of 1.0 and 7.0 mg/mL, respectively).

Encapsulation efficiency was dependent on initial drug concentration. For drug concentrations from 1 mg/mL until 4.0 mg/mL an increasing of encapsulation efficiency was observed, staying constant for 5.0 mg/mL and decreasing at 6.0 and 7.0 mg/mL. This behavior is probably more related to the technological difficulty of preparing a stable suspension at concentrations above of 5.0 mg/mL than to the ability of drug incorporation by the AL–CS capsules.

3.3. Swelling behavior

In order to verify the influence of the drug on the swelling behavior, AL–CS capsules without drug or containing 5 mg/mL of drug were analyzed for this physical parameter in both simulated gastric and enteric media.

Fig. 2 allows the comparison of the swelling behavior of different capsules in simulated gastric medium. It was observed that the swelling degree in this medium was lower than in enteric environ-

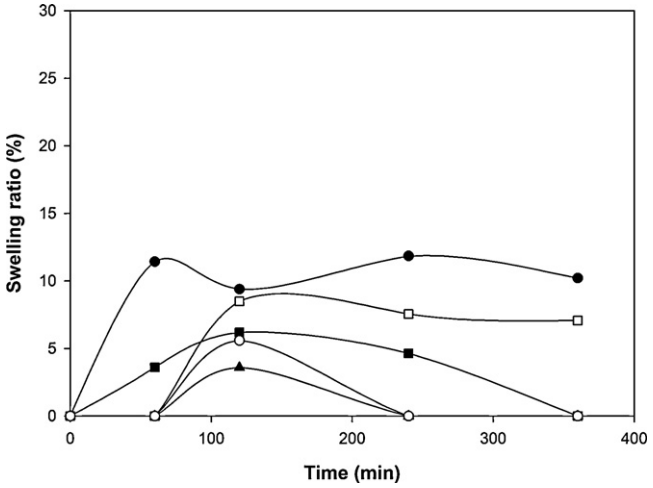

Fig. 2. Swelling profile of AL–CS capsules with and without TC prepared with different calcium chloride concentrations in simulate gastric medium: (■) 1.5% Ca without drug; (□) 2.0% Ca without drug; (▲) 3.0% Ca without drug; (●) 1.5% Ca + 5 mg/mL TC; (○) 1.5% Ca + 1 mg/mL TC.

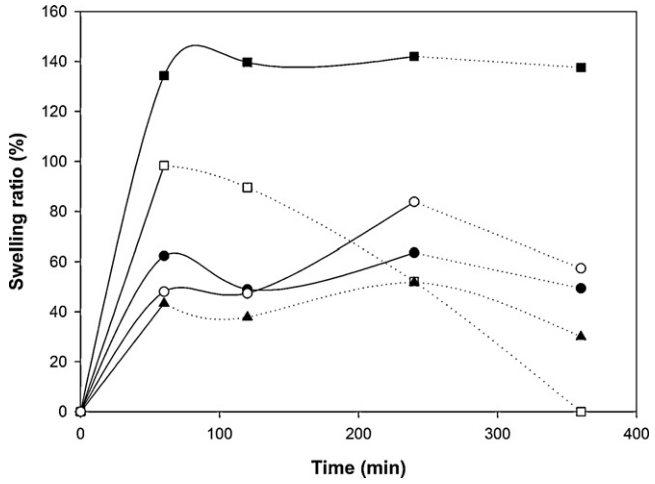

Fig. 3. Swelling profile of AL–CS capsules with and without TC prepared with different calcium chloride concentrations in phosphate buffer pH 7.5: (■) 1.5% Ca without drug; (□) 2.0% Ca without drug; (▲) 3.0% Ca without drug; (●) 1.5% Ca + 5 mg/mL TC; (○) 1.5% Ca + 1 mg/mL TC. (—) swelling; (···) swelling plus erosion.

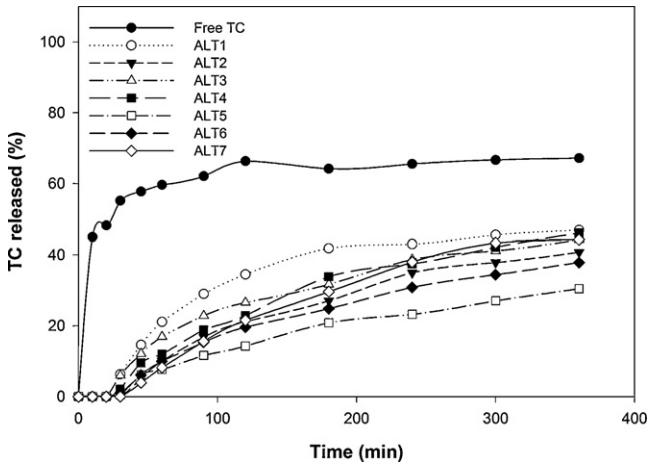

Fig. 5. Influence of initial TC concentration on the *in vitro* drug release from AL–CS capsules. For samples composition see Table 1.

ments, the highest swelling rate being 12% after 360 min of assay for capsules prepared with 1.5% of calcium chloride and containing TC. Such size alterations and the low swelling rate are probably related to the constricted ionization of AL at pH values near to 1, reducing the repulsion between the polymer chains and allowing the capsule gelled structure to be closed (Kim, Chung, Shin, Yam, & Chung, 2008).

In phosphate buffer pH 7.5 the swelling was faster than in the gastric medium tested (Fig. 3). In this case a fast swelling is followed by erosion of the capsules. Capsules without drug but containing 1.5% Ca shown higher swelling rate than capsules with 2 and 3% Ca or containing 5 and 1 mg/g TC ($p < 0.056$, 0.017, 0.017 and 0.020, respectively). The capsules swelled weakly in simulated gastric medium and showed a slight size decrease along the assay. In enteric medium, the capsules showed a fast swelling during the first hour of assay followed by swelling plus erosion after that.

When weak ions, such as AL and CS, are involved in polyelectrolytic complexes, the number of electrostatic linkages is a function of pH, due to changes in the dissociation degree of the polyelectrolytes. This fact can explain the dependence of the swelling degree of such complexes upon pH values. According to this mechanism, the gelled complex swells less at acidic pH and more at pH values above 6.3, the pK_a of CS.

Fig. 4 shows the swelling behavior of AL–CS capsules containing TC in both gastric and enteric environments. The photomicrographs allow the visualization of different swelling behaviors in different media. It is possible to observe some insoluble drug as crystals on the particles surface after 2 and 4 h (Fig. 4B).

3.4. In vitro release

The drug targeting to the colon via the oral route includes reservoir and matrix systems, such as tablets and multiparticulate systems, for which the drug release can be more effectively controlled by enzymatic action of the colonic flora (Sinha, Mittal, Bhutani, & Kumria, 2004; Yang, 2008). The development of therapeutic formulations specific to the large bowel may be seen as a technological challenge, since the therapeutic system needs to pass intact through the stomach and the small intestine, before it can release the drug into the large intestine (Friend & Sellin, 2005).

AL–CS capsules were submitted to the *in vitro* release assay in media simulating the pH values of the GI tract.

Initially, the capsules were evaluated in simulate gastric medium. Fig. 5 shows the drug release profile from AL–CS capsules containing different drug concentrations. The release profiles of drug incorporated in the polymeric systems were statistically different from the dissolution profile of the nonencapsulated drug ($p < 0.00001$). Drug concentration in the polymer matrix influenced the behavior of drug release along the first hour of analysis. The

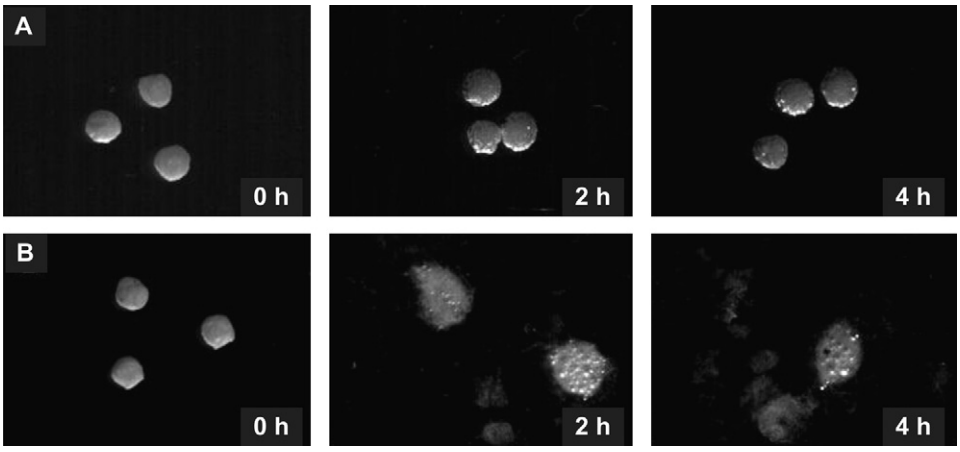

Fig. 4. Photomicrographs of AL–CS capsules during the swelling assay in simulated gastric (A-row) and enteric (B-row) media (magnification 8×).

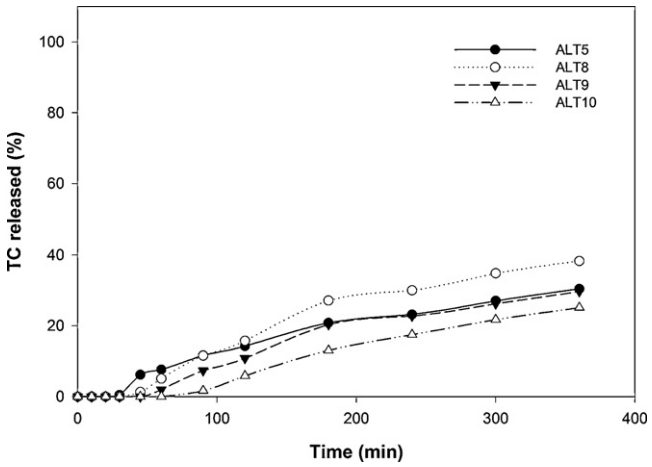

Fig. 6. Influence of polymers concentration on the *in vitro* TC release from AL–CS capsules in simulated gastric medium. For samples compositions see Table 1.

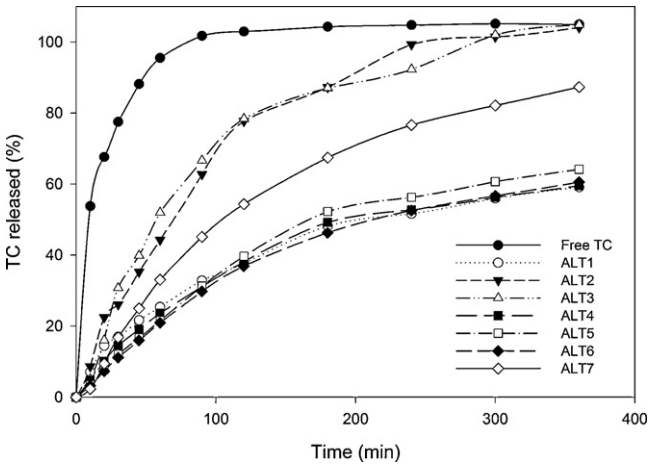

Fig. 7. Influence of initial TC concentration on the *in vitro* drug release from AL–CS capsules in simulated enteric medium. For samples composition see Table 1.

amount of drug release was inversely proportional to the initially drug added, presenting correlation between the initial drug concentration and the amount released ($r^2 = 0.9658$). This behavior is probably related with the low solubility of the drug in aqueous medium, reducing the proportion of drug dissolved when its concentration is increased in the polymer matrix. At the end of the dissolution test (360 min), the amount of drug released from the capsules present statistically no significant difference among the batches.

As the swelling studies showed, AL–CS capsules presented low swelling degree and no erosion in the simulated gastric medium. Such behavior is related to the ionization of the polyelectrolytes CS and AL. Near pH 1, CS is protonated, its amino groups staying as $–NH_3^+$, and AL has its carboxylic groups non-ionized, as –COOH, and shows the tendency to precipitate. This behavior can lead the structure to be closed, resulting in a stronger control over the drug release in acidic medium.

In order to improve the control of the drug release in acidic medium, some modifications were performed on the AL–CS capsules. Fig. 6 shows the drug release from capsules prepared with the same initial drug concentration but different ratios between polymer and calcium. Sample ALT5 was used as reference to the modifications done, because, as presented in Fig. 5, this was the batch that released smaller amount of drug. Increasing the calcium concentration to 2 and 3% (ALT8 and ALT9, respectively) allowed to a more effective control on drug release during the first 1 h. Sample ALT10, in which the capsules were prepared with higher AL and CS concentrations, shows a reduced release rate. The constricted ionization of AL in an extremely acidic medium probably has influenced the release behavior observed for these capsules. In general, tablets and pellets leave the empty stomach after 60–90 min (Friend & Sellin, 2005) and they should release in this GIT section no drug or only a small amount of it, since the objective is that the drug begins to be released just into the large bowel. The AL–CS capsules developed guaranteed an efficient control over the drug release, as they released only 5% of drug after the initial 2 h.

Since AL and CS are ionic polysaccharides, they show pH-dependent solubility and capacity of forming gels. Furthermore, the polymers interactions as well as the swelling of the capsules containing them vary when the pH of the medium is modified.

The drug release from AL–CS into simulated enteric medium was faster (Fig. 7) than in simulated gastric medium. Capsules of samples ALT2 and ALT3 released the whole amount of the drug until 6 h of assay, while the other samples, except for ALT7, released between 55 and 60% of the drug in the same time period. Samples with higher drug contents showed lower drug release rate. This may be related to the drug distribution in the system and its solubility in water. Higher amounts of drug, when dispersed within the polymeric matrix, resulted in particles more agglomerated and, consequently, reduced surface area to solvation and dissolution, such condition being reinforced by the low drug solubility.

As the swelling tests at pH 7.5 showed, the particles presented a significant higher swelling degree in this medium than in the gastric one. Additionally, in this pH condition, the particles underwent erosion after the swelling process reached a critical step. This behavior favors a greater amount of drug to be released, because the drug will be more exposed to the dissolution medium after the matrix erosion. Thus, in this case, swelling and erosion contribute simultaneously to the drug diffusion.

Comparing the drug release profiles from AL–CS capsules both in gastric and in enteric media, it can be observed that the difference of release rates for samples containing different amounts of drug was maintained at the almost same level. In other words, samples containing greater initial amount of drug released more TC and *vice versa*.

Fig. 8 presents the drug release from AL–CS capsules in which changes on the formulation were made. It was observed that the increase of calcium concentration (samples ALT8 and ALT9) did not decrease the drug release rate, as compared with the reference sample (ALT5). On the other hand, in contrary to the behavior observed

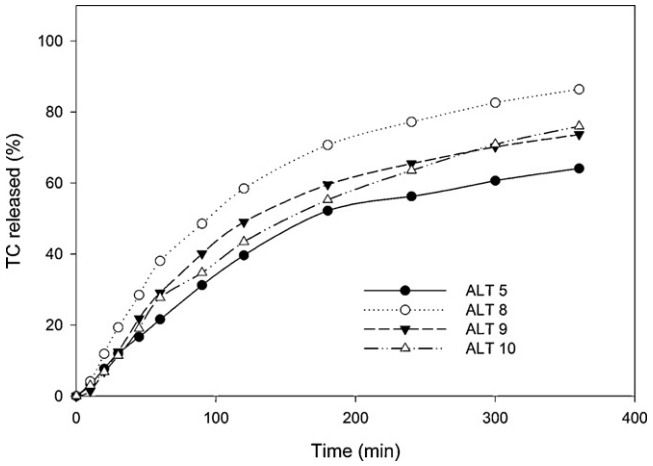

Fig. 8. Influence of polymers concentration on the *in vitro* TC release from AL–CS capsules in simulated enteric medium. For samples composition see Table 1.

Table 2
Correlation coefficients for different mathematical models applied on the release kinetics from AL:CS systems.

Sample	Correlation coefficients (r^2)[a]			
	SGM[b]		SEM[c]	
	First order	Korsmeyer–Peppas (n)[d]	First order	Korsmeyer–Peppas (n)[d]
ALT1	0.9765	**0.9133** (*0.59*)	0.8826	**0.9892** (*0.49*)
ALT2	**0.9693**	0.9610 (*0.83*)	**0.9893**	0.9868 (*0.76*)
ALT3	**0.9347**	0.9563 (*0.66*)	**0.9908**	0.9828 (*1.02*)
ALT4	**0.9745**	0.9678 (*0.78*)	0.9323	**0.9798** (*0.55*)
ALT5	0.9715	**0.9719** (*0.81*)	0.9724	**0.9783** (*0.67*)
ALT6	**0.9733**	0.9697 (*0.80*)	0.9683	**0.9805** (*0.62*)
ALT7	**0.9727**	0.9636 (*0.89*)	**0.9962**	0.9876 (*0.88*)
ALT8	**0.9654**	0.9611 (*0.97*)	**0.9906**	0.9873 (*0.82*)
ALT9	0.9550	**0.9576** (*1.05*)	**0.9746**	0.9746 (*0.80*)
ALT10	0.9257	**0.9709** (*1.36*)	**0.9930**	0.9835 (*0.80*)

[a] Bold values indicate the best fits.
[b] Simulated gastric medium.
[c] Simulated enteric medium.
[d] Release exponent (n) evaluated for <60% of drug released.

in gastric environment, capsules prepared with higher polymer concentration did not present higher drug retention than the reference capsules. This can be related with the AL ionization in both release media. In the acidic media, AL is unionized, presents almost no repulsion among chains, and the swelling and, consequently, the release are not favored. Thus, an increased polymer concentration can lead the polymer matrix to have more effective control over the drug release. In simulated enteric medium, pH 7.5, AL is in the ionized form, allowing the particle to swell. When the particle swells more, not only the drug diffuses more easily the matrix outwards, but also the matrix erosion occurs. Thus, an increase in polymer concentration not necessarily causes the same effect in different release media.

Generally, the transit time of the most common pharmaceutical dosage forms through the thin intestine is relatively constant, even when they are administered with meals. The mean transit time for pellets as well as for tablets is around 3–4 h (Friend & Sellin, 2005). The AL:CS capsules showed gradual release, being less than 50% of the drug released in 3 h of analysis.

Drug release from hydrophilic matrices is controlled by the rate of the matrix hydration and by the ability of the matrix to build gels after that, both properties influencing the drug diffusion and the gel formation and erosion. A fast hydration is desired to build a gel layer and to avoid an excessive amount of drug to be released during the initial time periods, the so-called burst effect. The thickness of such gel layer plays an important role, since as larger it is then longer will be the pathway needed to be followed by the drug until the elution medium can be reached and weaker will be the susceptibility of the matrix to undergo erosion (Wakerly, Fell, Attwood, & Parkins, 1997). The drug release profiles presented show that the AL–CS capsules swell quickly in enteric medium and the gel built allowed the drug retention, leading to a gradual release.

Table 2 shows the correlation coefficients of the release profiles when different mathematical models for the analysis of the release kinetics were applied. For the models studied, the release profiles showed higher correlation coefficients for the first order and Korsmeyer–Peppas models. For others models the correlation coefficients were smaller than 0.97. Therapeutic systems, that follow a first order profile, release the drug in a proportional way to the remaining amount in the system. The amount of drug released by unit of time decreases (Costa & Lobo, 2001).

The kinetics analysis of the release profiles in simulated gastric and enteric media showed that no alteration of the kinetics release was observed with increased drug present in the polymeric matrix. The release profiles in simulated enteric medium presented larger adjustment to the first order kinetic model than when analyzed in simulated gastric medium. As discussed above, in simulated gastric medium the systems present higher capacity of drug retention due to the closing of the matrix, while in enteric medium a higher swelling degree was observed, enabling a faster drug release.

The Korsmeyer–Peppas model is used to examine the release of polymeric dosage forms, when the release mechanism is not well known or when more than one type of apparently unrelated release mechanism may be involved: one due to drug transport (Fickian transport) and the other related to the phenomena of swelling and matrix relaxation (Costa & Lobo, 2001; Colombo, Bettini, Santi, & Peppas, 2000).

For the models derived from the Korsmeyer–Peppas equation, it is the n value that characterizes the drug release mechanism, depending on the geometric shape of the particle (Colombo et al., 2000). Table 2 shows the n values obtained for the systems. According to the n values, the TC transport mechanism from the systems can be characterized by anomalous transport and super case II transport, and the mechanism that predominated was anomalous transport, which is characterized by a speed of the same magnitude order for both solvent diffusion and polymer relaxation.

3.5. Gastrointestinal transit of AL–CS capsules

The transit of AL–CS capsules within the GI tract of rats was evaluated by means of oral administration of the samples and further sacrifice of the animals for macro- and microscopic assessment of presence/absence of the capsules.

Fig. 9 shows the percent frequency of AL–CS capsules found in different GIT sections after various time periods. In the groups of animals sacrificed up to 2 h after the administration, it can be observed a decrease in the amount of capsules present in the stomach, from 72.50% after 30 min to 37.50% at 2 h after the administration. At this time, it was also possible to confirm the presence of capsules in the distal portion of the small intestine and in the proximal portion of the large bowel. Unexpectedly, at 4 h the majority of the capsules could be found in the stomach. Such delay in the stomach emptying can be related to the great amount of meal present in the gastric cavity, as can be seen in Fig. 10, since this is one of the main influencing factors of gastric emptying (Corá et al., 2005). Although all animals were submitted to the same fasting period, the interindividual variation concerning to meal digestion can explain these results.

For the group of animals sacrificed after 6 h from the capsules administration, although an expressive number of capsules also could be found in the stomach (45%), a greater amount of them could be seen in the large intestine (around 7.5%).

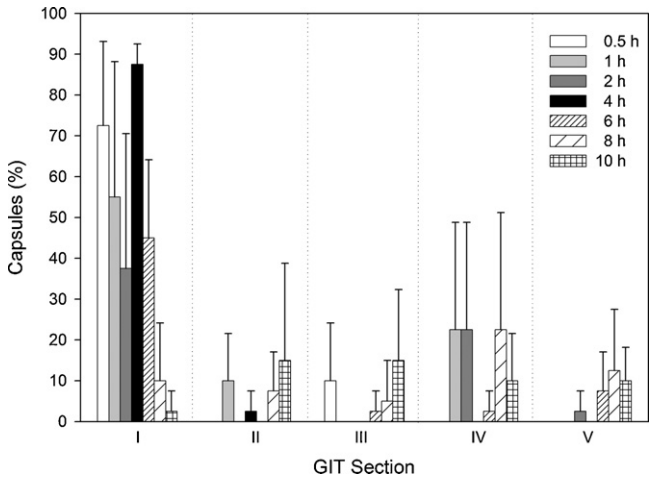

Fig. 9. Distribution of AL:CS capsules in the GIT of rats at different time periods after oral administration. GIT regions: I – stomach; II, III and IV – different regions of the small bowel and V – large bowel.

After 8 and 10 h an almost total absence of capsules could be observed in the stomach, mainly after 10 h, and numerous capsules could be found both in the final portion of the thin intestine and in the initial part of the colon.

The photomicrographs (Fig. 10) show that, regardless of the time, the AL–CS capsules present in the stomach maintained intact with practically no swelling. These results agree well with the *in vitro* swelling behavior in simulated gastric medium.

AL–CS capsules found in different thin intestine sections presented intact or with just a slight swelling, as can be seen in Fig. 11. It was also possible to verify that the capsules migrated rapidly to the final portion of the thin intestine after the stomach emptying, since only few capsules could be seen in the initial portion of this organ (section II). In the majority of the animals no capsules could be found in the median portion (section III), however, higher amounts of capsules could be seen in the distal portion of the thin intestine (section IV).

Fig. 12 shows structurally unaltered AL–CS capsules found in the large bowel (section V), proving that the delivery systems produced are able to reach the colon.

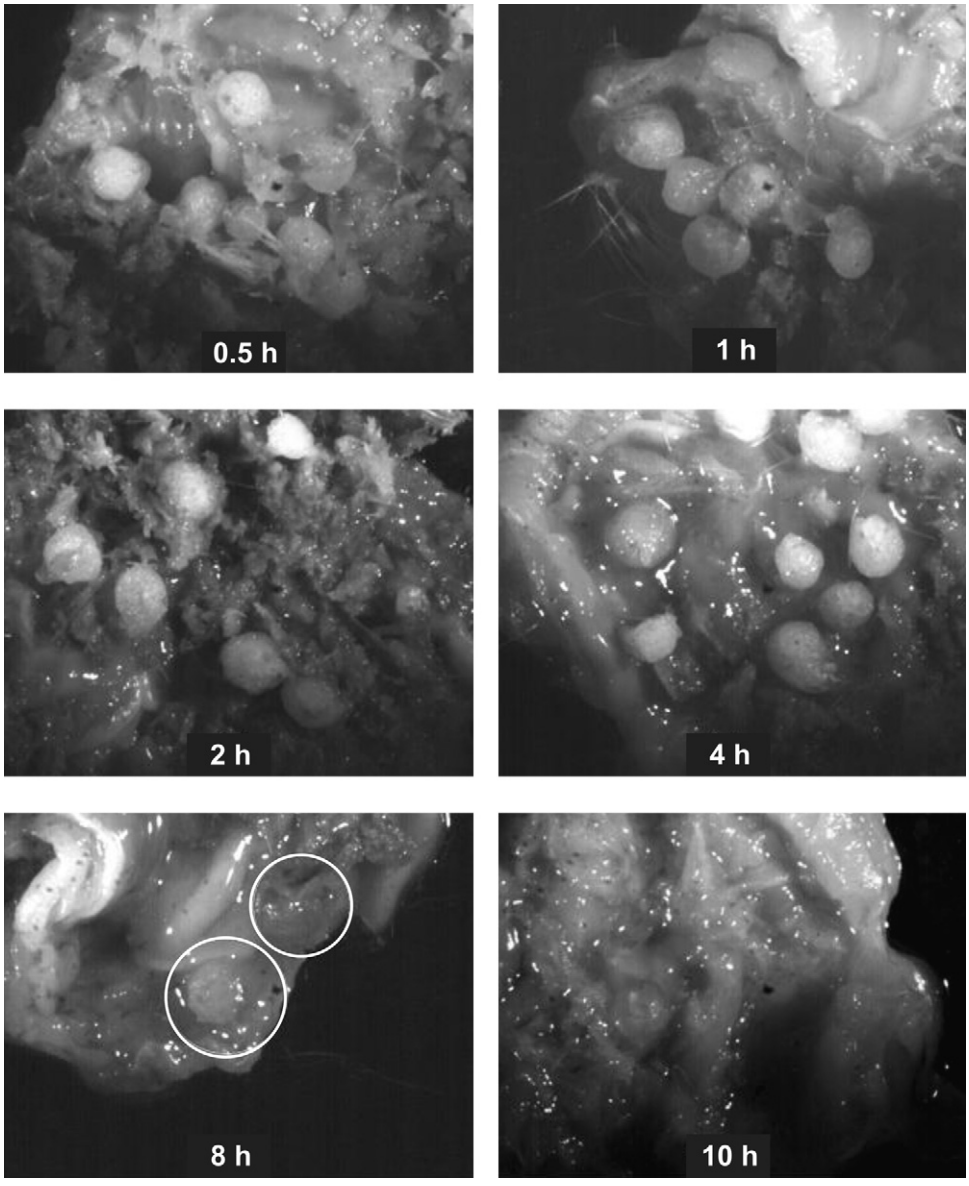

Fig. 10. Photomicrographs of the internal region of stomach after different time periods of administration of AL:CS capsules.

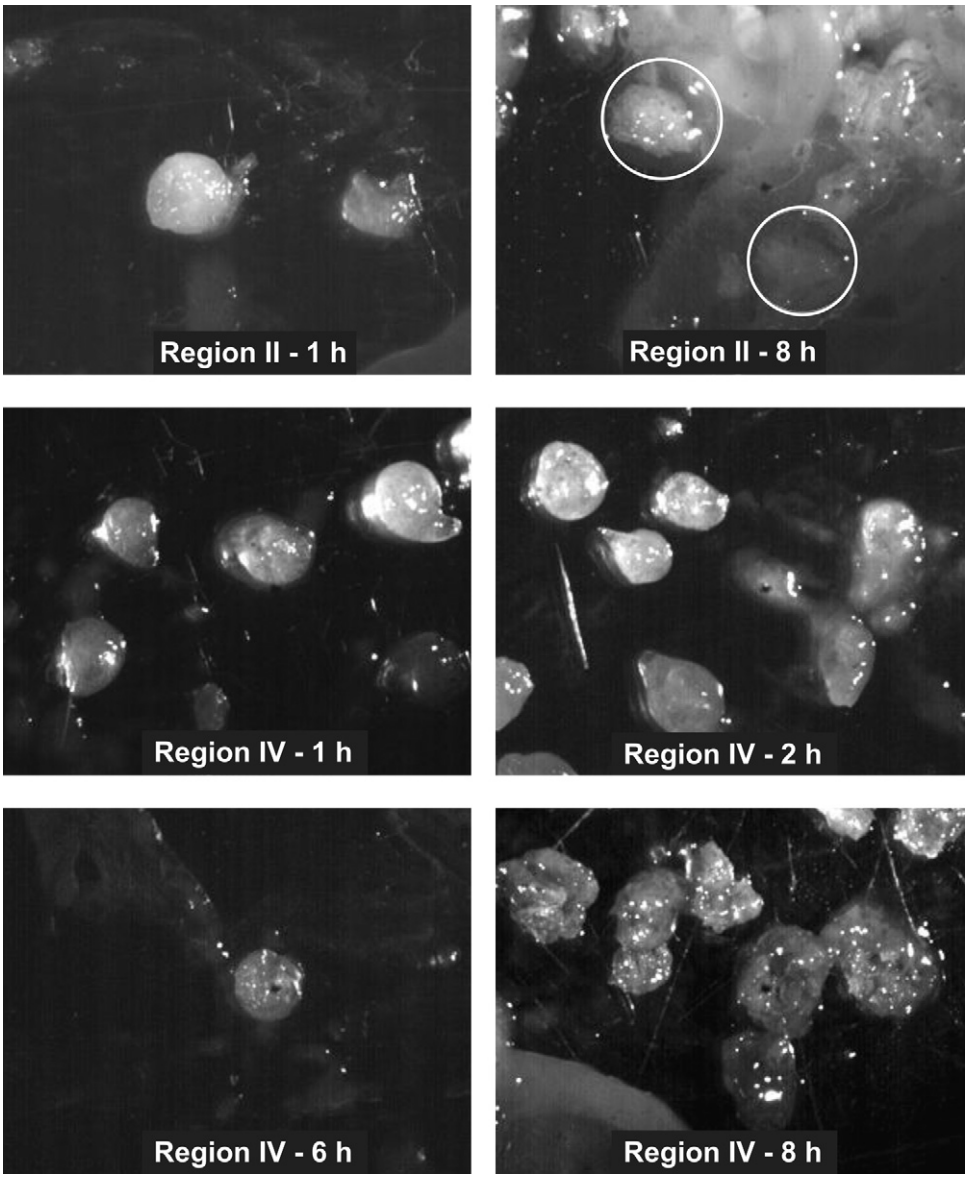

Fig. 11. Photomicrographs of the different regions of small bowel after different time periods of administration of AL:CS capsules.

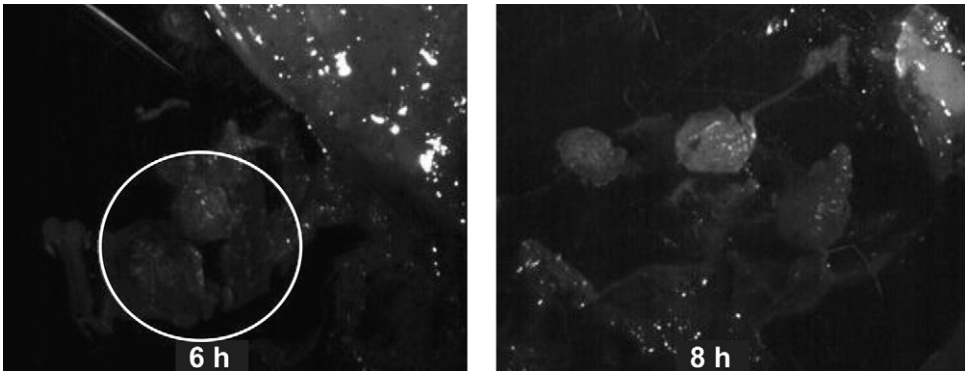

Fig. 12. Photomicrographs of the AL:CS capsules in the initial region of large bowel after different time periods of administration.

4. Conclusions

The AL–CS multiparticulate systems obtained showed spherical shape and slightly rough surface. Size and morphology of the systems were not altered by incorporation of the drug in different concentrations. Drug content was dependent on initial drug concentration and maximal encapsulation efficiency was obtained by addition of 5 mg/g drug. Higher drug concentrations resulted

in a decrease on encapsulation efficiency. Capsules presenting an efficient control over the drug release in simulated gastric medium were obtained, allowing the release of only about 5% of the drug until 2 h. The drug release was in accordance with swelling behavior. In simulated gastric medium the capsules presented low swelling degree, while in simulated enteric media a higher swelling rate followed by erosion could be observed. Drug release in simulated enteric medium was faster than in simulated gastric medium. The release profile in both media presented a first order release kinetics. By application of Korsmeyer–Peppas mathematical model, it was observed that the systems presented an anomalous mechanism of drug release, the diffusion and swelling mechanisms probably occurring simultaneously. Evaluating the *in vivo* gastrointestinal transit, it was found that the AL–CS systems stayed intact in the stomach, presented a faster transit within the small bowel and a lower swelling rate than that observed in the *in vitro* tests.

References

Antonin, K. H., Rak, R., Bieck, P. R., Preiss, R., Schenker, U., Hastewell, J., et al. (1996). The absorption of human calcitonin from the transverse colon of man. *International Journal of Pharmaceutics*, *130*, 33–39.

Bajpai, K. S., Saxena, K. S., & Sharma, S. (2006). Swelling behavior of barium ions crosslinked biopolymeric sodium alginate–carboxymethyl guar gum blend beads. *Reactive and Functional Polymers*, *66*, 659–666.

Barber, T. A. (1993). Particle population analysis. In T. A. Barber (Ed.), *Pharmaceutical particulate matter analysis and control* (pp. 266–349). Bufalo Grove: Interpharm Press Inc.

Colombo, P., Bettini, R., Santi, P., & Peppas, N. A. (2000). Swellable matrices for controlled drug delivery: Gel-layer behaviour, mechanisms and optimal performance. *PSTT*, *3*, 198–204.

Corá, L. A., Romeiro, F. G., Stelzer, M., Américo, M. F., Oliveira, R. B., Baffa, O., et al. (2005). AC biosusceptometry in the study of drug delivery. *Advanced Drug Delivery Reviews*, *57*, 1223–1241.

Costa, P., & Lobo, J. M. S. (2001). Modeling and comparison of dissolution profiles. *European Journal of Pharmaceutical Sciences*, *13*, 123–133.

Dentini, M., Rinaldi, G., Barbetta, A., Risica, D., Anselmi, C., & Skjak-Braek, G. (2007). Ionic gel formation of a (pseudo)alginate characterised by an alternating MG sequence produced by epimerising mannuronan with AlgE4. *Carbohydrate Polymers*, *67*, 465–473.

Ferreiro, M. G., Tillman, L., Hardee, G., & Bodmeier, R. (2002). Characterization of alginate/poly-L-lysine particles as antisense oligonucleotide carriers. *International Journal of Pharmaceutics*, *239*, 47–59.

Friend, D. R., & Sellin, J. (2005). Drug delivery in advancing the treatment of inflammatory bowel disease. *Advanced Drug Delivery Reviews*, *57*, 215–216.

Gåserød, O., Smidsrød, O., & Skjåk-bræk, G. (1998). Microcapsules of alginate–chitosan. I. A quantitative study of the interaction between alginate and chitosan. *Biomaterials*, *19*, 1815–1825.

George, M., & Abraham, E. T. (2006). Polyionic hydrocolloids for the intestinal delivery of protein drugs: Alginate and chitosan—a review. *Journal of Controlled Release*, *114*, 1–14.

Kim, W.-T., Chung, H., Shin, I. I.-S., Yam, K. L., & Chung, D. (2008). Characterization of calcium alginate and chitosan-treated calcium alginate gel beads entrapping allyl isothiocyanate. *Carbohydrate Polymers*, *71*, 566–573.

Leopold, C. S., & Eikeler, D. (1998). Eudragit® E as coating material for the pH-controlled drug release in the topical treatment of inflammatory bowel disease (IBD). *Journal of Drug Targeting*, *6*, 85–94.

López, M. E. V., Reyes, L. N., Igea, S. A., Espinar, F. J. O., & Méndez, J. B. (1999). Formulation of triamcinolone acetonide pellets suitable for coating and colon targeting. *International Journal of Pharmaceutics*, *179*, 229–235.

Lucinda-Silva, R. M., & Evangelista, R. C. (2003). Microspheres of alginate–chitosan containing isoniazid. *Journal of Microencapsulation*, *20*, 145–152.

Lucinda-Silva, R. M., & Evangelista, R. C. (2005). Studies on the formation of complex coacervates between chitosan and alginate during microparticles preparation. *Acta Farmaceutica Bonaerense*, *24*, 366–370.

Lucinda-Silva, R. M., Monteiro, K. C. M., Carvalho, L. Q., & Evangelista, R. C. (2006). Multiparticulate systems of pectin–chitosan: Study of swelling and drug release. *Acta Farmaceutica Bonaerense*, *25*, 538–543.

Prashanth, K. V. H., & Tharanathan, R. N. (2007). Chitin/chitosan: Modifications and their unlimited application potential an overview. *Trends in Food Science & Technology*, *18*, 117–131.

Rijnierse, A., Nijkamp, F. P., & Kraneveld, A. D. (2007). Mast cells and nerves tickle in the tummy implications for inflammatory bowel disease and irritable bowel syndrome. *Pharmacology & Therapeutics*, *116*, 207–235.

Sinha, V. R., & Kumria, R. (2001). Polysaccharides in colon-specific drug delivery. *International Journal of Pharmaceutics*, *224*, 19–38.

Sinha, V. R., Mittal, B. R., Bhutani, K. K., & Kumria, R. (2004). Colonic drug delivery of 5-fluorouracil: An in vitro evaluation. *International Journal of Pharmaceutics*, *269*, 101–108.

Sriamornsak, P. (1999). Effect of calcium concentration, hardening agent and drying condition on release characteristics of oral proteins from calcium pectinate gel beads. *European Journal of Pharmaceutical Sciences*, *8*, 221–227.

Vandamme, Th. F., Lenourry, A., Charrueau, C., & Chaumeil, J.-C. (2002). The use of polysaccharides to target drugs to the colon. *Carbohydrate Polymers*, *48*, 219–231.

Wakerly, Z., Fell, J. T., Attwood, D., & Parkins, D. (1997). Studies on amidated pectins as potential carriers in colonic drug delivery. *Journal of Pharmacy and Pharmacology*, *49*, 622–625.

Wittaya-Areekul, S., Kruenate, J., & Prahsarn, C. (2006). Preparation and in vitro evaluation of mucoadhesive properties of alginate/chitosan microparticles containing prednisolone. *International Journal of Pharmaceutics*, *312*, 113–118.

Yamamoto, A., Tozaki, H., Okada, N., & Fujita, T. (2000). Colon-specific delivery of peptide drugs and anti-inflammatory drugs using chitosan capsules. *S.T.P. Pharmaceutical Sciences*, *10*, 23–34.

Yang, L. (2008). Biorelevant dissolution testing of colon-specific delivery systems activated by colonic microflora. *Journal of Controlled Release*, *125*, 77–86.

Zhang, H., Alsarra, A. I., & Neau, H. S. (2002). An in vitro evaluation of a chitosan containing multiparticulate system for macromolecule delivery to the colon. *International Journal of Pharmaceutics*, *239*, 197–205.

Zheng, Y., Qiu, Y., Fu Lu, M.-Y., Hoffman, D., & Reiland, T. L. (1999). Permeability and absorption of leuprolide from various intestinal regions in rabbits and rats. *International Journal of Pharmaceutics*, *185*, 83–92.