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Chitosan–pectin multiparticulate systems associated with enteric polymers for colonic drug delivery

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ABSTRACT

The great challenge in using native degradable polysaccharides for the development of drug delivery systems is their high aqueous solubility, which may contribute to the undesirable premature and localized release of the drug. Multiparticulate systems showing simultaneously specific biodegradability and pH-dependent drug release were prepared based on chitosan (CS), amidated pectin (PC), and calcium ions, using triamcinolone (TC) as model drug. Hidroxypropylmethyl cellulose phthalate (HPMCP) and cellulose acetate phthalate (CAP) were successfully incorporated into the system and aided the target action of the carbohydrates. Particles were characterized for size distribution, morphology, swelling behavior and dissolution tests in media simulating the gastrointestinal tract. The addition of CAP and HPMCP resulted in the highest control over the drug release in all media. CAP:TC formulation presented the slowest drug release rate, of only 1.33%, in acidic medium after 2 h, while the control formulation released 45.52% after the same time.

1. Introduction

Colon-specific drug delivery systems have been the focus of increasing interest for the last decades, due to the recognized importance of this region of the gastrointestinal tract (GIT), for both local and systemic therapy (Maestrelli, Cirri, Corti, Mennini, & Mura, 2008; Rijnierse, Nijkamp, & Kraneveld, 2007; Yang, Chu, & Fix, 2002). At present, the specific drug delivery to the colon is considered an important alternative for the treatment of serious local diseases, such as Crohn's disease, ulcerative colitis, carcinomas and infections (Friend & Sellin, 2005; Oosegi, Onishi, & Machida, 2008; Yamamoto, Tozaki, Okada, & Fujita, 2000). Additionally, specific systemic absorption at colonic region offers interesting possibilities for the treatment of diseases susceptible to the diurnal rhythm, such as asthma, arthritis or inflammation (Crcarevska, Dodov, & Goracinova, 2008; Mastiholimath, Dandagi, Jain, Gadad, & Kulkarni, 2007).

Oral drug delivery systems designed to target the colon need to protect the drug during its transit through the stomach and small intestine, before allowing its fast release on entry into the colon, triggered by specific enzymatic reactions by colonic flora (Maestrelli et al., 2008). Different systems have been developed for colon specific drug delivery. They include systems using pH-sensitive polymers as enteric coatings (Leopold & Eikeler, 1998; Maestrelli et al., 2008), those based either on transit time (Zou et al., 2009) or increasing of luminal pressure within the GIT (Mastiholimath et al., 2007), and enzymatically controlled delivery systems (Lucinda-Silva, Salgado, & Evangelista, 2010). The approach by which the drug release will be accomplished by the colonic flora seems to be more interesting with regard to selectivity (Hejazi & Amiji, 2003; Yang et al., 2002). In comparison to conventional single unit types, multiparticulate systems present several advantages, such as more predictable rate of gastric emptying and fewer localized adverse effects (Rahman et al., 2008). The use of naturally occurring polysaccharides attracts a lot of attention for drug targeting to the colon, since these polymers are found in abundance, are inexpensive and are available in a variety of structures with different properties (Vandamme, Lenourry, Charrueau, & Chaumeil, 2002). They can be easily chemically and biochemically modified and are highly stable, safe, non-toxic, hydrophilic and gel forming, and, in addition, biodegradable (Li, Xie, Lin, Xie, & Ma, 2009; Sinha & Kumria, 2001). They include naturally occurring polysaccharides obtained from superior plants (guar gum, inulin, pectin), animals (chitosan, chondroitin sulphate), algae (alginates) or microbes (dextran), and can be broken down by the colonic microflora to simple saccharides (Vandamme et al., 2002).

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Chitosan (CS) is a cationic polysaccharide obtained from the alkaline *N*-deacetylation of chitin, the second most abundant natural polymer on earth and its polymer chains consist of *N*-acetyl-glucosamine and glucosamine residues (George & Abraham, 2006; Prashanth & Tharanathan, 2007). Pectin (PC) is an anionic polysaccharide able to provide colon-specific delivery for several drugs (Bernkop-Schnürch, 2000; Biguccia, Luppia, Monacoa, Cerchiarab, & Zecchia, 2009; Liu, Fishman, Kost, & Hicks, 2003; Lucinda-Silva & Evangelista, 2005). The polymeric chain of PC contains galacturonic acid, rhamnose, arabinose and galactose. Amidated PC is a low-methoxyl PC in which some of the carboxylic acid groups are amidated. It is more tolerant of pH variations and calcium level than conventional PC, making it useful for the design of colonic delivery systems. Gelation of droplets in the presence of calcium may provide a valuable approach to the formation of a multiparticulate system for colonic delivery (Lootens et al., 2003). The properties of such amidated PC particles may be altered by the formation of polyelectrolyte complex membrane around the particle using cationic polymers, such as CS (Maestrelli et al., 2008; Munjeri, Collett, & Fell, 1997). However, a disadvantage of such systems is that a substantial amount of drug may be released, mainly by erosion of the coating and diffusion, in the upper part of the GIT (Lucinda-Silva et al., 2010).

Previous findings from our research group have demonstrated the reliability of such systems. For example, microparticles with CS and alginate or PC, cross-linked or not with glutaraldehyde, were obtained by complex coacervation (Lucinda-Silva & Evangelista, 2003, 2005). Although the particles could reach the colon, as verified by in vivo assays with rats, a great amount of the incorporated drug, isoniazid, was rashly released, in vitro, into gastric and enteric simulated juices (Lucinda-Silva et al., 2010). This inadequate release resulted from the swelling process the particles underwent. Other results showed that the presence of gastric resistant polymers, among them cellulose acetate phtalate (CAP) and hidroxypropilmethylcelulose phtalate (HPMCP), just in simple physical mixtures obtained by coevaporation, was able to decrease considerably the swelling degree and, consequently, the fast drug release in acid medium (Ferrari, Oliveira, Chibebe, & Evangelista, 2009).

Therefore, based on previous interesting results, the aim of this study was the preparation and characterization of multiparticulate CS:PC-based colonic delivery systems containing pH-sensitive polymers, namely HPMCP and CAP, by a complex coacervation method, using triamcinolone (TC) as model drug.

2. Materials and methods

2.1. Raw materials

Chitosan (CS), hydroxypropylmethylcellulose phthalate (HPMCP) and cellulose acetate phthalate (CAP) were obtained from Sigma (São Paulo, Brazil). Low methoxyl pectin (PC) (Pectin 8003) was obtained from CPKelco (Campinas, Brazil). Pectinase enzyme (Pectinex® Ultra SP-L, Curitiba-PR, Brazil). Other chemicals used were of reagent grade mainly supplied by Merck (São Paulo, Brazil).

2.2. Particles preparation

The particles were prepared by complex coacervation from CS (0.5%) and PC (1.5%) dispersions. The CS dispersion (pH 4.8) was prepared by dispersing 0.5% of CS in 0.1 M acetic acid under magnetic stirring. Then, 2% calcium chloride was added to the CS dispersion. The 1.5% PC dispersion (pH 5.0) was prepared in deionized water. To this dispersion were added TC (100 mg) and, for some samples, 0.5% of pH-sensitive polymer (HPMCP or CAP). For the complex coacervation, 20 ml of the PC dispersion (with TC and with or without CAP or HPMCP) was dropped through a needle with an internal diameter of 2 mm into 40 ml of CS dispersion containing 2% of calcium (Lucinda-Silva & Evangelista, 2005). The contact of PC with Ca^{2+} caused an immediate gelation and droplets formation. Then CS neutralized the free carboxylic groups, hardening the droplets. After 3 h under stirring, the particles were filtered and freeze-dried. Table 1 shows the composition of the different samples prepared.

Table 1
Composition of the samples.

Sample	PC (%)	CS (%)	Ca^{2+} (%)	HPMCP (%)	CAP (%)	TC[a] (mg)
PC:CS:TC	1.5	0.5	2.0	–	–	100
HPMCP:TC	1.5	0.5	2.0	0.5	–	100
CAP:TC	1.5	0.5	2.0	–	0.5	100

[a] Corresponding to 33.33% and 50% in relation to the mass of PC and CS, respectively.

2.3. Morphological analyses

Morphological analyses were performed on images captured by a Leica MZ APO stereoscope and processed by Leica Qwin software and scanning electron microscope (Jeol JSM-T330A). For the stereoscopic analyses, the samples were placed and analyzed directly on Petri plates. For the assay by scanning electron microscopy, dry samples were placed on a double ribbon face adhered to a metal support and coated under vacuum with colloidal gold.

2.4. Granulometric analyses

Granulometric analyses were performed by observations with the aid of both an optical microscope and a stereoscope (Leica MZ APO with Leica Qwin software). The particles were placed on Petri plates and the size distribution of about 300 particles was assessed according the Feret's diameter at 0° (Barber, 1993).

2.5. Swelling behavior

The swelling behavior was evaluated by image analysis of particles immersed in media with pH values of 1.2 and 7.4, simulating the different environments of the GIT. The Feret's diameters (at 0°) of 15 particles/sample were evaluated on a Leica MZ APO stereoscope managed by Leica Qwin software for image capture. The variation of particles diameters was verified after 0, 30, 60, 90 and 120 min. The swelling degree (SD, in %) was calculated using the following equation:

$$SD = \frac{D_1 - D_0}{D_0} \times 100$$

where D_1 is the diameter of the particle after a determined contact time with the liquid and D_0 is the initial diameter.

2.6. In vitro drug release studies

The in vitro drug release from the particles was performed on a Hanson SR8 dissolution station, using the basket apparatus (USP XXV, 2006). In order to simulate, respectively, gastric or enteric media, 900 ml of either 0.1 M HCl (pH 1.2) or 0.5 M phosphate buffer (pH 7.4) were used. The temperature was kept at 37 °C and the stirring rate at 50 rpm. About 40 mg of TC-containing particles (corresponding to ca. 5 mg of TC), precisely weighed, were placed in the baskets, samples of 2.5 ml were withdrawn after appropriated times and the drug released was spectrophometrically assessed at 242 nm. CS:PC:TC particles were taken as reference. Each experiment was repeated at least three times. After 2 h in gastric media,

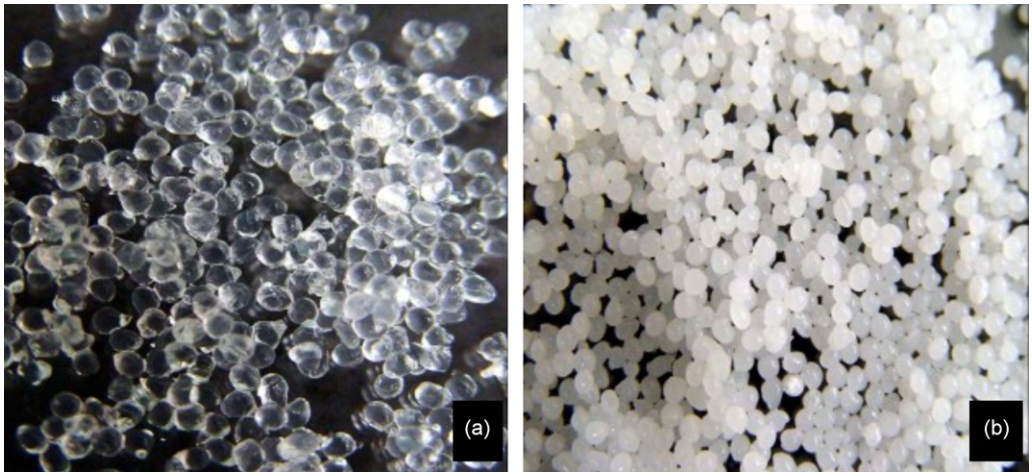

Fig. 1. Photomicrographs of PC:CS:CAP particles before drying: (a) without TC and (b) containing TC.

the baskets were removed and placed quickly in 900 ml of the subsequent receptor medium, where they were kept for further 4 h.

In order to verify the TC release profile in colonic media, 10 mM phosphate buffer pH 5.0 containing 2% of pectinase enzyme (Pectinex® Ultra SP-L, Curitiba-PR, Brazil) was used as dissolution medium and the assay was performed as described above.

2.7. Statistical analysis

All experiments were carried out in triplicate. Results were reported as means ± standard deviation, unless otherwise stated. Statistical significance was analyzed using Student's *t*-test, the differences between experimental groups being considered significant when *p*-value was less than 0.05.

3. Results and discussion

3.1. Morphological analyses

Quasi-spherical particles were obtained, being bright and translucent when without TC and opaque and white when containing drug, as can be seen in Fig. 1 for particles not yet dried. Under the scanning electron microscope, the practically spherical structure of the particles can be seen and the rough aspect of the surface is more evident (Fig. 2). It can be observed that there are

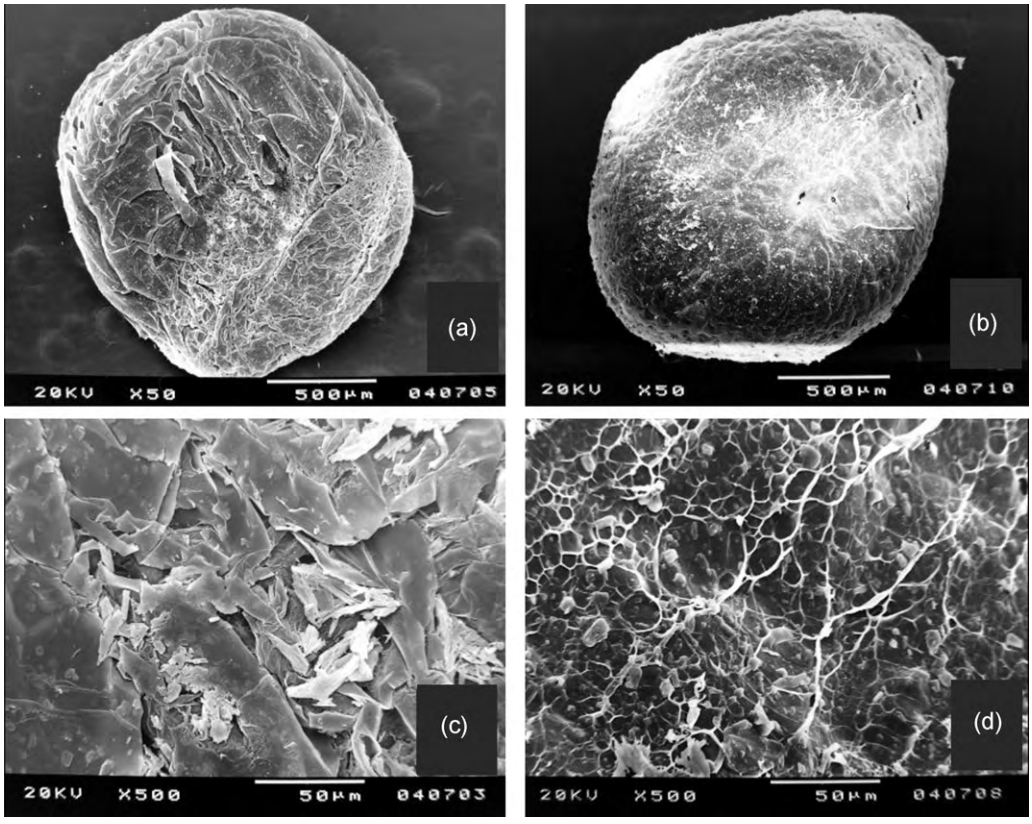

Fig. 2. Scanning electron photomicrographs of particles of formulations: (a) CAP:TC magnification 50×, (b) HPMCP:TC magnification 50×, (c) CAP:TC magnification of 500× and (d) HPMCP:TC magnification of 500×.

Table 2
Mean diameters of the particles.

Samples	Mean diameter (mm ± SD)
CAP:TC	2.42 ± 0.53
HPMCP:TC	2.01 ± 0.41
PC:CS:TC	1.83 ± 0.65

small interstitial spaces on the surface of the particles, able to further generate channels, which would facilitate the biological fluids to diffuse the matrix inwards and thus contributing to accelerate the drug release. At the highest magnification (500×), the difference between the two formulations becomes more clear, showing that the CAP:TC particles have a denser surface, presenting narrow interstitial spaces, while the surface of HPMC:TC particles is more porous. Under a first analysis, this may suggest that the CAP-containing PC:CS formulation could be more resistant against the influence of the media where they would be analyzed, promoting a stronger control over drug release.

3.2. Granulometric analysis

Table 2 shows the mean diameter of the particles. There was no significant variation in particle size due to changes in the composition of the samples, the mean diameter being 1.8–2.5 mm ($n = 230$). The results showed that the enteric polymers added did not present direct relation with the particles diameter, suggesting that the variation observed should be related with the preparation method used.

3.3. Swelling behavior

Capsules of both HPMCP:TC and CAP:TC presented similar swelling profiles, which showed lower swelling degrees than those presented by particles without pH-sensitive polymers both in gastric and in enteric medium (Figs. 3 and 4, respectively), thus confirming that the enteric polymers added were able to protect the particles against swelling.

The swelling ratio is influenced by the gel dimensions (swelling front) and by the rate in which water can diffuse into the polymer chains. At low pH values, the interaction between PC and CS is favored by limiting the PC ionization, due to the protonation of acidic groups, which reduces the repulsion and favors the polymer chains to be more close together, making the gel insoluble in the environment.

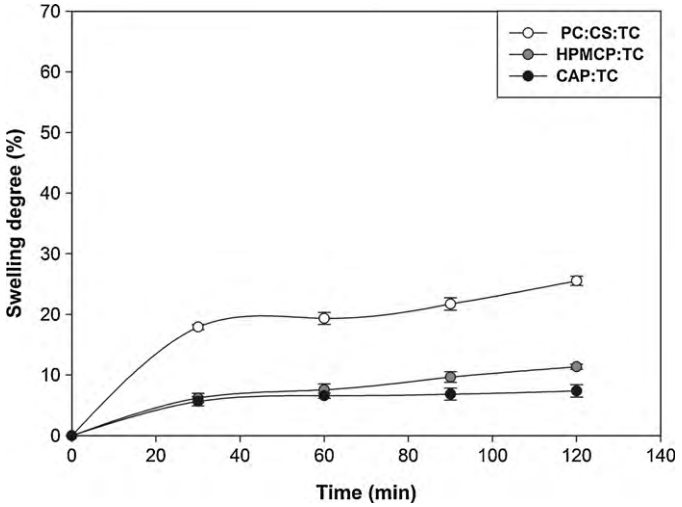

Fig. 3. Particles swelling behavior in acid medium.

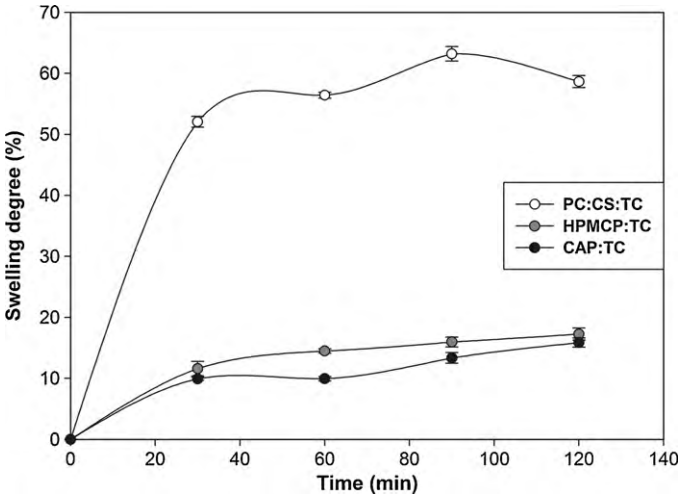

Fig. 4. Particles swelling behavior in enteric medium.

Comparing the swelling profile of control samples (PC:CS:TC) with those of samples containing enteric polymers (CAP:TC; HPMCP:TC), it is evident that the incorporation of both enteric polymers significantly decreased the swelling degree in the simulated gastric juice, promoting a greater protection of the particles. In contrast to the swelling profile observed in simulated gastric medium (pH 1.2), PC:CS particles can intensely swell at pH 7.4 (Fig. 4), due to the negative charges of carboxylic groups ($-COO^-$) of the PC, causing mutual repulsion (Chang & Lin, 2000). Apparently, this repulsion force is stronger than the attraction force, leading the particles volume to increase.

Additionally, deionization of amino groups of CS takes place in alkaline solutions, causing the bridge between the two groups to disappear. At this pH range, erosion of the particles can also occur and the resulting particle size is given by a balance between swelling and erosion (Munjeri et al., 1997). As shown in Fig. 4, the addition of enteric polymers promoted a significantly stronger resistance of the particles against swelling also in phosphate buffer pH 7.4, leading the particles to show a swelling of about 25% and no erosion during the 120 min of analysis. On the other hand, at the same conditions, PC:CS:TC (control) samples underwent erosion and showed about 84.7% of swelling after 2 h of contact.

3.4. Dissolution test

With the purpose to simulate the initial gastrointestinal transit, the same samples (40 mg) assayed in simulated enteric medium have been prior submitted to simulated gastric medium for 2 h.

The drug release profiles from the formulations containing enteric polymers (HPMC:TC and CAP:TC) were compared with that from PC:CS:TC, a formulation of particles without enteric polymers, used as control.

In general, the particles of the formulations containing enteric polymers presented no drug release during the first hour of assay (see Fig. 5). The CAP:TC formulation presented the strongest control over the drug release, allowing almost no drug (1.33%) to be dissolved during 120 min of analysis in gastric medium and only 7.2% during the first hour of the dissolution test in enteric medium.

The samples containing enteric polymers released more drug in enteric medium than in gastric medium (see Fig. 5), however with a more effective release control. These samples allowed the delivery of less than 40% of drug within 4 h of assay, while the PC:CS:TC particles released about 51% of the drug after only 1 h of assay in enteric medium. These results agree well with those obtained at pH 7.4, in which the particles containing CAP or HPMCP pre-

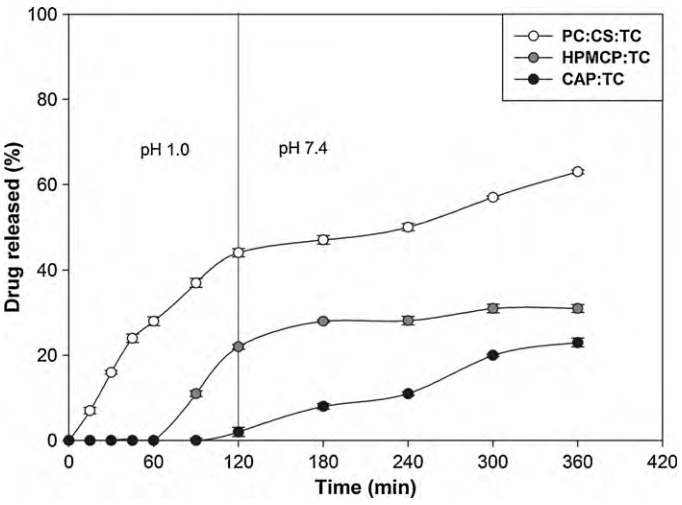

Fig. 5. In vitro TC release in simulated gastric–enteric medium.

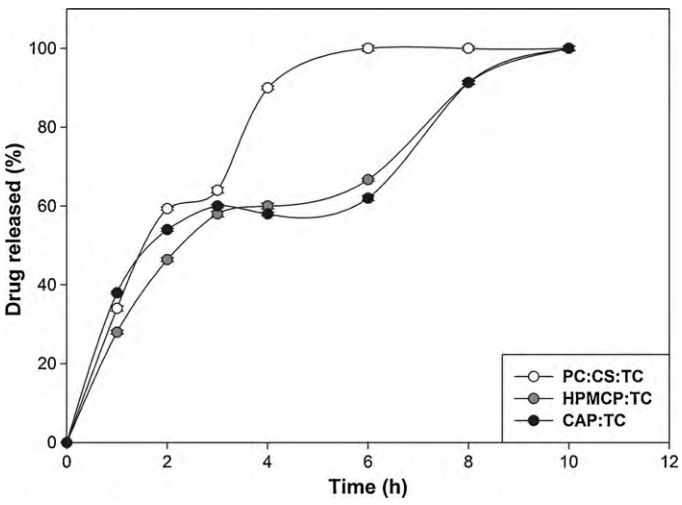

Fig. 6. In vitro TC release in simulated colonic medium.

sented lower swelling degree than those particles without enteric polymers. Besides the reduction in the swelling ratio, the particles did not undergo erosion in this medium, this fact contributing for decreasing the release rate.

This evidences that the incorporation of both CAP and HPMCP causes the decrease of the swelling ratio also at pH 7.4, a pH level at which PC can be found in its highly ionized form, what would favor a high swelling ratio and, consequently, a higher drug release rate (Lucinda-Silva & Evangelista, 2003).

The particles of CAP:TC formulation are those that presented the lowest swelling degree and, consequently, the slowest drug release rate, releasing only 23.70% of the drug after 4 h of analysis.

In order to simulate the enzymatic action found in the colonic region, Pectinex® SP-L was added to the medium. This product is an enzymatic preparation produced by selected strains of *Aspergillus aculeates* with high pectinolytic activity caused by enzymes, such as pectintranseliminase, polygalacturonase and pectinesterase, and also small amounts of hemicelulases and celulases (Ahrabi, Madsen, Dyrstad, Sande, & Graffner, 2000).

The analysis was carried out after adding the enzymes to 10 mM phosphate buffer pH 5.0. This value was chosen because it is the average between the pH value of maximal enzymatic activity (pH 3.5) and the typical colonic pH value, referred as being between 6.4 and 7.0, respectively from the ascending to the descending colon (Vandamme et al., 2002). Fig. 6 presents the drug release profile from particles of PC:CS:TC; CAP:TC and HPMCP:TC formulations in colonic medium.

According to Munjeri et al. (1997), calcium ions can contribute to a more effective action of these enzymes. Comparing the release profile of the drug from both CAP:TC and HPMC:TC formulations with that of PC:CS:TC formulation, it is observed that the particles containing enteric polymers sustained the drug release until 10 h of analysis, while for the particles of PC:CS:TC formulation 100% of the drug were released in 5 h of dissolution test.

It is important to consider that the reduction of pH from 7.4 to 5.0 significantly modifies the PC ionization. At pH 7.4, PC is in a highly ionized state, presenting high swelling ratio, with consequent increase of the drug release; at pH 5.0, most amino groups in CS are protonated while most carboxyl groups in PC are deprotonated. Thus, the electrostatic interaction between $-NH_3^+$ and $-COO^-$ is stronger as compared with the conditions at pH 1.2 and pH 7.4. Although the ionization of carboxyl groups causes the swelling of PC network, the increased electrostatic interaction between $-NH^{3+}$ and $-COO^-$ retards the drug release from the particles (Yu et al., 2009). This ionization profile at pH 5.0 influences the interaction between the polymers and, consequently, it modifies the swelling capacity. This reduction of the swelling ratio could reduce the amount of the drug released. However, the lowering of the pH value from 7.4 to 5.0 in simulated colonic medium did not hinders the drug release. The drug release rate is significantly higher in this medium than in enteric medium. These results confirm that the incorporation of the enteric polymers CAP and HPMCP to the PC:CS particles allowed the drug release to be driven by both pH variation and enzymatic action, making the systems more appropriate for colonic targeting.

The CAP:TC particles showed to be the most adequate system, since they presented lower swelling degree and stronger control over the drug release even before the particles reached the colonic conditions. This behavior can be related with the molecular structure of CAP, which presents more substituents with hydrophobic character, increasing its resistance against the alkaline medium. This fact can be further confirmed by the relative difficulty in preparing a dispersion of this polymer by dissolution in alkaline conditions and also by the morphological differences of the particles' surfaces of CAP:TC and HPMCP:TC formulations observed by scanning electron microscopy (see Fig. 2).

Fig. 5 shows that in gastric medium the control sample (without enteric polymers) released about 50% of TC until 2 h of assay. Thus, in enteric medium, the release curve shows an ascending sigmoid shape. Even so, TC was released just up to about 60% in 7 h of assay.

On the other hand, the samples containing enteric polymers released significantly less TC in the same time period. The HPMCP-containing sample shows a lag time of about 60 min, during which no TC was released. After this point, the release profile turns into a S-shaped pattern, but with an almost constant release rate. Only 28% of TC was released after 7 h of assay. CAP:TC samples showed a similar behavior, but with a longer lag time (without drug release) of almost 2 h. The release continues in a smooth ascending fashion, almost parallel to the HPMCP curve, and the maximal amount of TC released reaches 23%.

For all samples, drug release in colonic medium follows first order kinetics until about 2 h of assay and no significant difference was found among them, since $k = 0.4368$; 0.3171; and 0.4199 (for PC:CS:TC, HPMCP:TC, and CAP:TC, respectively) with $R^2 > 0.99$. At this point, about 50–60% of TC was released. After that, when the samples reached the enteric conditions, several release mechanisms should be simultaneously involved and a significant difference can be observed between the release rate from the samples containing enteric polymers or not. The former samples showed an almost constant release rate for almost 4 h, followed

by a light increase, the profile presenting a smooth sigmoidal shape. The total amount of TC was released only after 10 h of assay following a controlled dissolution pattern. On the other hand, in this same 4 h period, the release profile of the control samples (without enteric polymers) were also S-shaped, but with an upward curvature, showing that the drug was released very faster up to 100%, this maximal point been reached significantly earlier.

4. Conclusions

The in vitro drug release studies showed that the addition of both enteric polymers, CAP and HPMCP, to the PC:CS:TC particles resulted in higher control over drug release in all media analyzed. Particles from all charges also exhibited enzyme-controlled drug release properties in simulated colonic medium. In acid medium, CAP:TC and HPMCP:TC samples were those that released lesser amounts of TC, about 1.3% and 14.3%, respectively, after 2 h of analysis, while the samples of particles used as control (PC: CS:TC) released about 45.5% after only 2 h of analysis. The incorporation of CAP and HPMCP reduced the swelling of the particles in both gastric and enteric media. This improvement and the variety of drug transport mechanisms involved clearly suggest that this multiparticulate system has great potential for site-specific drug delivery through oral administration. Although enteric polymers can act as useful aid agents, the crucial condition to allow drug release in colon remains on the dependence of enzymatic degradation of polysaccharides by the microflora.

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