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Multiparticulate Systems of Pectin-Chitosan: Study of Swelling and Drug Release

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SUMMARY. The aim of this study was to prepare multiparticulate systems of pectin:chitosan (PC:CS) and to evaluate their swelling ratio and the drug release in different environments. PC:CS particles containing triamcinolone were prepared by a complex coacervation/ionotropic gelation method in aqueous environment. The polymer ratio, the calcium concentration and the contact time of the capsules with chitosan dispersion for particles formation and the structures obtained were analyzed. The systems were characterized in relation to morphology, size, swelling, and drug release behavior. The methodology used allowed the production of spherical particles with narrow range of size distribution. The entrapment efficiency for triamcinolone was 84.31 ± 4.39. It was observed that the particles present a relatively low swelling ratio in acidic medium and a larger swelling ratio in enteric medium. The release profile was dependent on pH and can be related with the swelling ratio.

RESUMEN. "Sistemas Multiparticulados de Pectina-Quitosano: Estudio de Hinchamiento y de Liberación de Fármacos". El objetivo de este estudio fue preparar sistemas multiparticulados de pectina:quitosano (PC:CS) y evaluar el comportamiento del hinchamiento y la liberación de la droga en diversos ambientes. Las partículas de PC:CS contiendo triamcinolona fueron preparadas mediante el método de coacervación compleja en un ambiente acuoso. La proporción del polímero, la concentración del calcio y el tiempo del contacto de las cápsulas con la dispersión del quitosano para la formación de las partículas y las estructuras obtenidas fueron analizados. Los sistemas fueron caracterizados en lo referente a morfología, a tamaño, al hinchamiento y al comportamiento de liberación de la droga. La metodología usada permitió la producción de partículas esféricas con la gama estrecha de la distribución de tamaño. La eficacia del encapsulación para la triamcinolona fue 84,31 ± 4,39. Fue observado que las partículas presentan un cociente relativamente bajo de hinchamiento en medio ácido y un cociente mayor de hinchamiento en medio entérico. El perfil del liberación fue dependiente del pH y se puede relacionar con el cociente de hinchamiento.

INTRODUCTION

Biodegradable polymers, such as cellulose, starch, chitosan (CS), pectin (PC) and sodium alginate (AL) are materials of low toxicity and low manufacturing costs. They have been constantly used as adjuvants in pharmaceutical formulations [1,2]. The possibility of enzymatic decomposition of these polymers by colonic enzymes, mainly by glycosidase, makes their use possible as a support for dosage forms intending colonic drug release [3,4].

Pectin (PC) has been used as agglutinant for tablets and in the preparation of pellets [5,6]. Recently, PC has been investigated in the preparation of microparticles [7], in sustained release matrix tablets and as potential adjuvant for the colonic delivery of drugs [8-10]. Humans and animals do not have in their superior GIT enzymes able to degrade PC. However, PC can be hydrolized to short chain fatty acids and carbon dioxide by enzymes produced by the normal colonic flora [4,11,12].

Since biodegradable polymers are soluble in aqueous environment, they can be used in the form of hydrogels and pellets. Hydrogels, constituted of crosslinked polymeric chains of acrylates, polyesters and polysaccharides were de-

KEY WORDS: Chitosan, Pectin, Multiparticulate systems, Swelling, Triamcinolone.
PALABRAS CLAVE: Hinchamiento, Quitosano, Pectina, Microencapsulación, Triamcinolona.

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veloped. The larger the degree of crosslinking of the polymers, the least is the tendency to swell and slower is its enzymatic decomposition, because swelling exposes the degradable link of the polymer to the enzyme action and consequent degradation in the colon [13,14,15]. Crosslinked polysaccharide gels have been prepared with azo-inulin and azo-dextran and it was observed that they could be degraded by reduction of the azo-linkages and by degradation of the polysaccharide polymeric chain [16,17].

The present work studied the *in vitro* swelling of PC:CS particles containing triamcinolone in order to evaluate a possible application of such systems as carriers for the intestinal delivery of drugs, mainly to the colonic region.

MATERIALS AND METHODS

Materials

PC of low methoxyl grade was kindly supplied by Braspectina (Limeira - SP - Brazil). CS of practical grade was purified before use. Triamcinolone of pharmaceutical grade was obtained from Galena (São Paulo - SP - Brazil). Pectinex™ Ultra SP-L (pectinase enzymes) was purchased from Novozymes (Curitiba - PR - Brazil). All other reagents were of analytical grade.

Studies of polyelectrolytic complex formation between PC and CS

The mass proportion of each polymer to be used in the PC:CS complex formation and the most appropriate pH (2.8, 3.8 or 4.8) for particles preparation were determined by means of viscosimetric assay.

For these viscosimetric analyses, a 0.5% CS dispersion was added into a 1.0% PC dispersion under stirring. After 30 minutes of stirring, the complex built was separated by filtration and the viscosity of the supernatant was measured in a rotating viscometer (Selecta, mod. Visco Star L). The polyelectrolytic complexes were prepared at different ratios between the two polyelectrolytes and the pH was adjusted to different values: 2.8, 3.8 and 4.8.

Preparation of PC:CS particles

The multiparticulate systems were prepared by a complex coacervation/ionotropic gelation method in aqueous environment. The polyanionic (PC) dispersion was dropped by a syringe through a 26 $^{1/2}$ gauge needle into the polycation (CS) dispersion containing calcium chloride, using different proportions of the polymers and of calcium ions (Table 1). The particles were left in contact with the CS dispersion for different time periods and then washed with water and freeze-dried.

For the preparation of drug-containing systems, triancinolone at a concentration of 5 mg/mL was suspended in the PC dispersion before the formation of the particles.

Determination of the swelling ratio

The swelling ratio was determined in environments with different pH values, simulating the different environments of GIT: simulated gastric medium (pH 1.2), acidic medium pH 3.0, and simulated enteric medium (phosphate buffer pH 7.4). The swelling capacity was related to the increasing of particles diameter [8,18], measured before the contact of the particles with the different media and after 1, 2 and 3 h of contact. For these analyses, the diameter according to Feret at 0º was measured by *Leica® Qwin* software through a *Leica® MZ APO* stereoscope. The swelling ratio was calculated by equation [**1**], where S.R. is the % swelling ratio, d1 is the diameter after swelling and d0 is the diameter before contact with the simulated medium.

Formulation	PC (%)	CS (%)	Ca^{+2} (%)	Time of crosslinking (h)
PC1	0.75	0.25	2.00	1
PC2	1.50	0.50	0.10	1
PC3	1.50	0.50	0.50	1
PC4	1.50	0.50	1.00	1
PC5	1.50	0.50	1.50	1
PC6	1.50	0.50	2.00	1
PC7	1.50	0.50	2.00	1.5

Table 1. Formulations of PC:CS particles tested. PC: pectin; QS: chitosan; Ca^{+2}: $CaCl_2$.

$$S.R. = \frac{d_1 - d_0}{d_0} \times 100 \qquad [1]$$

Drug release profile

This test was accomplished for verification of the drug release profile and analysis of the release mechanisms *in vitro*. The assay was performed in a dissolution station (Hanson SR8-plus), using the basket method, under the following conditions: medium volume of 400 mL; agitation speed of 50 rpm; temperature of 37 ± 0.5 °C; sampling times at 10; 20; 30; 45; 60; 90; 120; 180; 240; 300 and 360 min. The release media used were: simulated gastric and enteric juices without enzymes and phosphate buffer 10 mM pH 5,0 containing 2% of pectinase enzyme (Pectinex™ Ultra SP-L). The drug was quantified by UV spectrophotometry at 239 nm.

Determination of triancinolone contents and entrapment efficiency

For determination of the drug contents, about 4 mg of capsules were placed in contact with 20 mL of phosphate buffer 50 mM pH 7.5 for 2 h, under stirring. The samples were filtered and the drug quantified at 239 nm. Drug contents was calculated using equation [**2**], where *TE* is the drug contents, m_{TC} is the TC weight quantified in the sample, m_{cap} is the weight of the capsules in the sample.

$$TE(\%) = \frac{m_{TC}}{m_{cap}} \times 100 \qquad [2]$$

The entrapment efficiency was calculated from the drug contents and of the capsules yield using equation [**3**], where *EE* is the entrapment efficiency, *TE* is the drug contents, *R* is the capsules yield in mass, MTC_{total} is the total mass of drug used in the formulation analyzed.

$$EE(\%) = \frac{TE \times R}{m_{TCtotal}} \qquad [3]$$

Morphological and granulometric analyses

Morphological and granulometric analyses of the particles were accomplished through the *Leica®MZ APO* stereoscope and *Leica® Qwin Image Analysis Systems* software for particles size assessment.

The particles were put on a glass slide and the size distribution of the particles for each batch was analyzed, using the diameter according to Feret at 0° as parameter for size determination.

Determination of calcium concentration in PC:CS systems

The amount of calcium present in systems containing different initial calcium chloride concentrations in relation to CS dispersion was determined by a method described elsewhere [19].

Fifty mg of PC:CS capsules and 3 mL of concentrated nitric acid were added to digestion tubes. The mixture was submitted to a digestion process at 120-130 °C for 2h.

After digestion, the volume of the mineralized sample was completed to 100 mL with deionized water in a volumetric flask and diluted 50 times with 1% lantanium solution. The samples diluted with lantanium solution were quantified by atomic absorption spectrophotometry (Perkin Elmer, mod. 3110), under the following operational conditions: calcium lamp, wavelength 422.7 nm, current of 8 mA, 4.5:1.7 air:acetylene mixture. The method validation was performed by determination of linearity, detection and quantification limits and accuracy.

RESULTS AND DISCUSSION

CS and PC are polymers that originate viscous dispersions at relatively low concentrations. Besides, since the product of the complex coacervation process is an insoluble coacervate, the larger the degree of complex formation, the smaller is the viscosity of the supernatant (colloid poor phase) after phase separation. This property was analyzed measuring the viscosity of the supernatant resulted from the complex formation between the polyelectrolytes mixed at different proportions (Fig. 1).

The pH of the CS dispersion was adjusted to 2.8, 3.8 or 4.8. Lowest pH values allow amino groups to be ionized, favoring the complex formation with the PC anions. The ideal ratio for the complex formation between the polyelectrolytes PC:CS was around 2.3 for the formulation with low methoxyl PC.

The resulting PC:CS particles were practically spherical and white (Fig. 2). The granulometric analyses demonstrated narrow size distributions and most of the particles presented sizes between 1.2 and 1.8 mm (Fig. 3). The size distribution can be related with the pressure and the speed during dropping and with the viscosity of the polymeric dispersions. It was observed that the increase of the pectin concentration caused the increase of particles size.

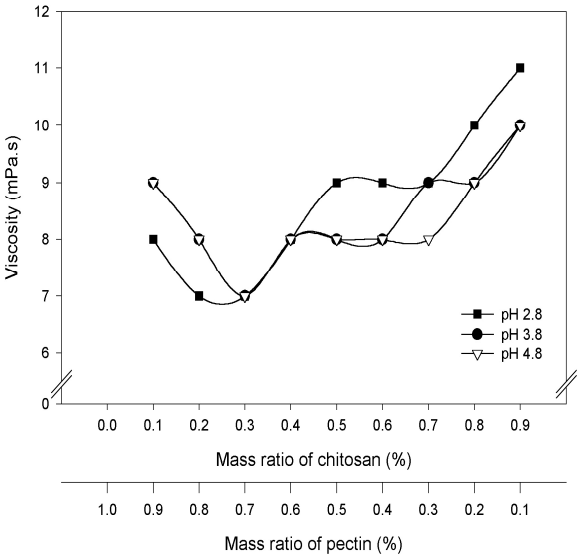

Figure 1. Viscosity of the CS and PC dispersions mixtures at different pH values.

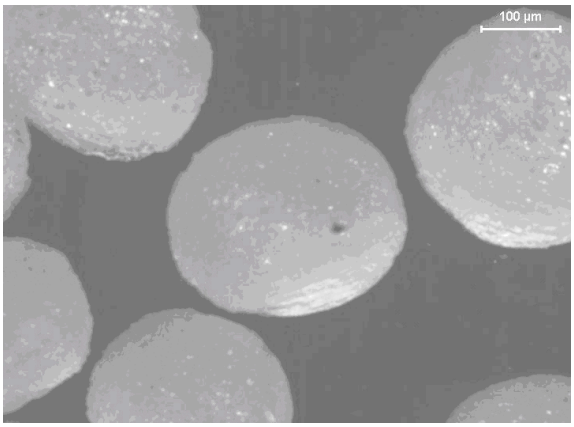

Figure 2. Photomicrography of PC:CS capsules containing triamcinolone.

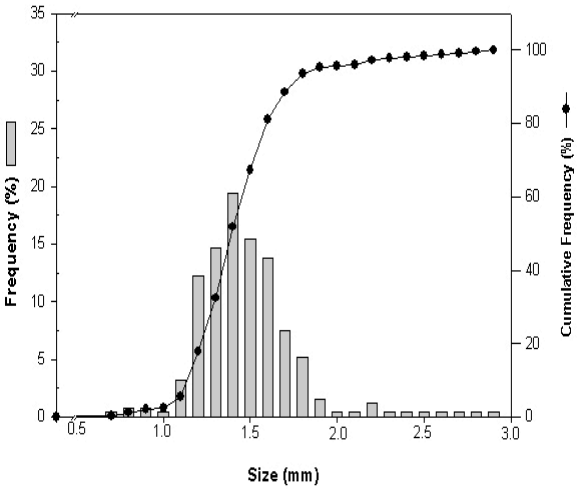

Figure 3. Size distribuition of PC:CS multiparticulate systems.

Initial $CaCl_2$ (%)	Calcium linked (mg)	Calcium linked (%)
0.1	1.8486	3.68
0.5	3.6678	7.25
1	5.0672	9.96
2	5.3470	10.84
3	5.2071	10.56

Table 2. Calcium amount in the PC:CS multiparticulate systems.

Calcium is an important component for formation of the PC:CS capsules. When the PC dispersion enters into contact with the calcium-containing CS dispersion, calcium links to the carboxyl groups of the polymeric chain building a gelified net called "egg box" structure [9,20]. During system development, the calcium amount in the capsules was quantified in order to verify its influence on the further swelling process of the systems.

The methodology used for calcium quantification in the capsules presented the following analytical parameters: correlation coefficient 0.9997; detection limit 0.0472 µg/mL; quantification limit 0.1572 µg/mL; variance of 1.64% and accuracy of 109.43%. As presented in Table 2, it was verified that there was an increase of the calcium concentration linked with the increase of the concentration of calcium added to the system. It was observed that for concentrations greater than 2% the amount of calcium linked does not alter in a significant way.

The swelling ratio is influenced by the gel dimensions and by the rate in which the water can diffuse the polymeric structure inwards. The interaction between PC and CS is favored at low pH values because there is a limitation of PC ionization, which reduces the repulsion and favors the closing of the gel chains, as can be observed in Fig. 4, where the maximum swelling ratio was about 25%.

As showed in Fig. 4, the amount of calcium in the system influences the swelling ratio, being larger for smaller calcium concentrations (0.1%, 0.5% and 1.0%, PC2, PC3 and PC4, respectively) and lower for higher calcium concentrations (1.5% and 2.0%, PC5 and PC6, respectively). Such a behavior is probably related with the reticulation of the PC polymeric chains by calcium. At low calcium concentrations there is an insufficient amount of this ion for maximum reticulation of the polymer, leading to the formation of a fragile and swellable matrix.

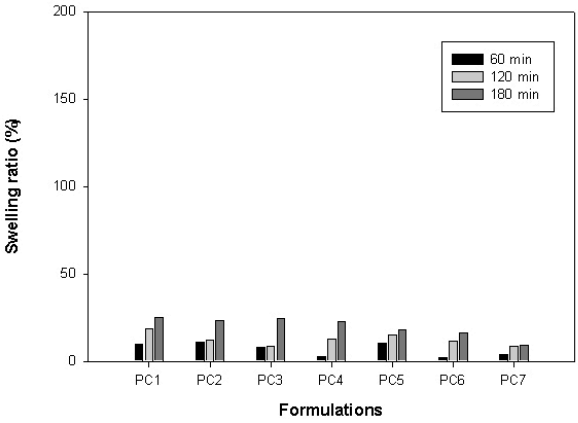

Figure 4. Swelling ratio of PC:CS particles from different formulations in simulated gastric medium, pH 1.2.

Comparing formulations PC1 and PC7, it was observed that the PC1 particles presented a swelling ratio larger than that of PC7 particles. This difference is probably related with the polymeric concentration in the systems (0.75% PC and 0.25% CS for PC1 and 1.5% PC and 0.5% CS for PC7, respectively).

The general tendency for the swelling process of polymers possessing acidic groups in their structure is to decrease in extremely acid pH values, due to protonation and consequent decreasing solubilization in this medium. A higher swelling ratio was observed at pH 3.0, where polymer reticulation was minimal, because the pH is near the pKa of carboxylic groups of D-galacturonic acid, 3.23, and the ionization rate of these groups is approximately 50%. This partial ionization of the polymer causes the appearance of the repulsion forces among the polymer chains, facilitating the relaxation of the polymeric matrix and consequent swelling. As observed in Fig. 5, at pH 3.0 the

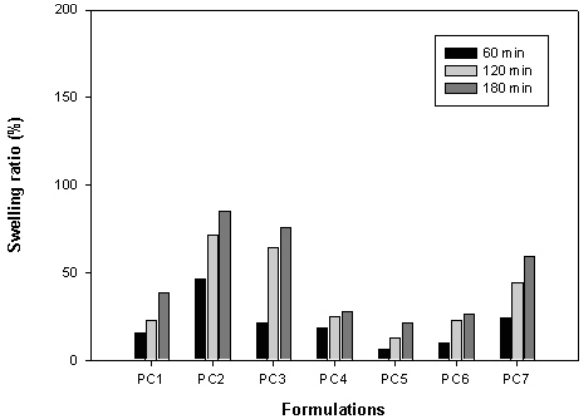

Figure 5. Swelling profile of PC:CS particles from different formulations in acid environment, pH 3.0.

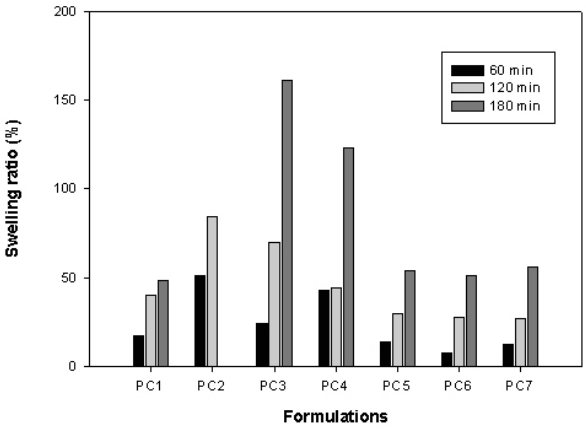

Figure 6. Swelling profile of PC:CS particles from different formulations in simulated enteric environment, phosphate buffer solutions, pH 7.4.

particles swell up to 70% of the initial value, 40% more than at pH 1.2. The particles prepared with lower calcium concentration, 0.1% and 0.5% (PC2 and PC3), presented a swelling ratio of 85.0% and 75.0%, respectively.

PC:CS particles can swell intensely at pH 7.4, due to the mutual repulsion induced by the negative charges of the PC carboxylic groups. It can be said that, in this case, the repulsion force is greater than the attraction force, which tends to reduce the volume of the particles. Besides, there is deionization of CS amino groups in alkaline solutions, and the consequent disappearance of the bridge between the two groups. At such pH value the erosion of the particles can also take place, that is, the increase of the particle size can be the result of a balance between the swelling and the erosion processes [8], as shown in Fig. 6. The particles prepared with 1.5% and 2.0% of calcium (PC1, PC5, PC6 and PC7) presented no great differences in swelling ratio, not depending on crosslinking time. The PC2 particles (0.1% calcium chloride) suffered erosion after 2 h of contact with alkaline environment.

The systems presented TC contents of 22.58 ± 0.01% and entrapment efficiency of 84.31 (4.39. Since they are systems of hydrophylic and polymeric nature, the incorporation of a high amount of a poorly soluble drug into the polymeric structure is facilitated.

As shown in Fig. 7, the drug release profile was dependent on the pH of the dissolution medium. These results are in agreement with the results obtained in the swelling study. In acidic pH the system presents low swelling ratio and a more controlled drug release, about only 5% of the drug being released in 2 h. In simulat-

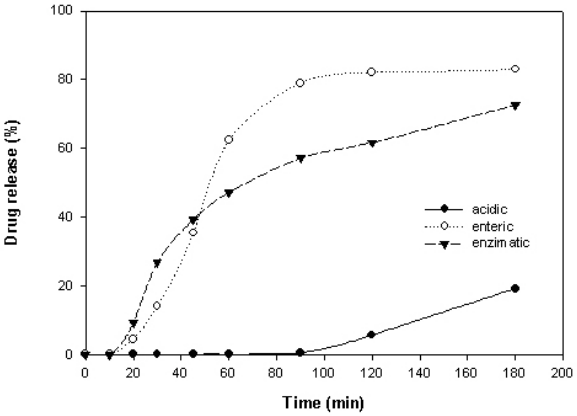

Figure 7. Release profiles of triamcinolone in simulated gastric (pH 1.2), enteric (pH 7.4) and colonic media (pectinase enzymes).

ed enteric and colonic media more than 60% of the drug was released in 3 h of assay.

The results obtained in the present work allowed to evaluate important aspects of the behavior of PC:CS multiparticulate systems in the different simulated GIT regions. The results showed that the PC:CS particles present relatively low swelling ratio in acidic medium but higher swelling ratio in enteric medium. It was also observed that the drug release profile is dependent upon the swelling behavior of the systems. To target the drug to the colonic region it will be necessary to reduce the swelling ratio in enteric medium by means of, for example, pH dependent coatings or crosslinking of the systems.

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