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Colonic Drug Delivery: Influence of Cross-linking Agent on Pectin Beads Properties and Role of the Shell Capsule type

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ABSTRACT For colonic delivery, pectin beads obtained by ionotropic gelation method have been already reported as an interesting approach. This study investigated the influence of the cross-linking agent (calcium or zinc) and the type of shell capsule used (classical or enteric capsules) on pectin beads properties and on their performance to target the colon (in vitro dissolution studies with subsequent pH change to mimic overall gastro-intestinal tract). Zinc pectinate beads seemed to be relatively similar to calcium's ones in morphological point, except on the surface aspect. When beads were introduced in classical hard capsules, ketoprofen release was not significantly different between CPG and ZPG beads, and it was too premature and too quick due to a chemical erosion of the pectinate matrix (acid + basic attacks). However, zinc pectinate beads showed slower ketoprofen release compared with calcium pectinate beads when enteric hard capsules were used. This interesting finding could be due to the strength of the network formed during the process between the zinc cations and the LM-pectin following the "egg-box" model. This network was stronger and induced a reduction of swelling and hydration when contact with dissolution medium, then subsequently a decrease of drug release. Thus, the zinc pectinate beads could protect sufficiently drug entrapped from the upper gastro-intestinal conditions and drug release will be controlled by pectin degradation with colonic microflora. Finally, these zinc pectinate beads in enteric hard capsules are promising as a carrier for specific colonic delivery of drugs after oral administration.

KEYWORDS Colonic delivery, Low-methoxy Pectin, Ionotropic gelation, Egg-box model, Ketoprofen, Cross-linking agent, Calcium, Zinc, Enteric hard capsule

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INTRODUCTION

Delivery systems intended for colon specific delivery, also called "smart systems," are oral dosage forms containing a triggering mechanism, which only respond to the physiological conditions particular to the colon.

The necessity and advantages of colon-specific drug delivery systems have been well recognized and documented. Targeting pharmaceutical drugs to the colon makes it possible to guarantee local or systemic drug delivery to this site (Lee & Mukkerjee, 2002). In addition to provide more effective therapy of colon related diseases such as irritable bowel syndrome, inflammatory bowel disease including Crohn's disease and ulcerative colitis, colonic delivery has the potential to address important therapeutic needs including oral delivery of macromolecular drugs (Yang et al., 2002). Because of relatively low proteolytic enzyme activities, the colon is viewed as the preferred absorption site for oral administration of protein and peptides drugs, for example insulin (Wakerly et al., 1996).

Due to the distal location of colon in the gastrointestinal (GI) tract, a colonic drug delivery system should prevent drug release in the stomach and small intestine, and involve a rapid onset of the drug release as soon as it will enter into the colon (Yang et al., 2002).

The challenge in the design of oral drug delivery systems, which effectively carry drugs to the colon site, is to meet certain criteria. Firstly, they need to remain intact when travelling through the upper GI tract in order to protect the entrapped drug from chemical and enzymatic degradation. Secondly, they should be able to release this drug immediately once it reaches the colon segment of the lower GI tract. Then, the released drug needs to be absorbed at an efficient rate in order to be therapeutically effective (Liu et al., 2003).

The physiological changes along the GI tract can be generally characterized as a continuum, with, on one hand, decreases in enzymatic activity, in motility, and in fluid content and, on another hand, an increase in pH. These gradual changes in physiological parameters are unfavorable to obtain an efficient colonic delivery (Vandamme et al., 2002). However, colon microflora is increasingly recognized as a preferable triggering component in the design of colonic drug delivery systems since the great increase in bacteria population and corresponding activities in the colon represent an event independent of GI transit time and of fed or fasted state. The primary sources of carbon and energy for these bacteria are polysaccharides present in dietary residues and host-produced secretions (Wakerly et al., 1996).

Thus, a large number of polysaccharides (i.e., pectin, chitosan, cyclodextrin, dextrans) provide promising drug carriers in a biomimetic approach, for colon-specific drug delivery (Sinha & Kumria, 2001).

Pectin is a water-soluble polysaccharide found in the cell wall of most plants (Sinha & Kumria, 2001). Though it is a heterogeneous polysaccharide, pectin contains linear chains of (1→4)-linked α-D-galacturonic acid residues. These uronic acids have carboxyl groups, some of which are naturally presented as methyl esters and others as carboxamide groups. The degree of esterification (DE) and degree of amidation (DA), which are both expressed as a percentage of carboxyl groups (esterified or amidated), are important means to classify pectin. Pectin is used to provide drug delivery systems for colonic delivery by different means (matrices, coated forms, combination of pectin with other polymers, gelation as calcium pectinate) (Liu et al., 2003). Major efforts have been focused on looking for pectin derivatives, which are more water resistant, while still enzymatically degradable. The ability of amidated low-methoxy (LM) pectin (with DE $< 50\%$) to form rigid gels with divalent cations has been used, for this purpose, in the production of calcium pectinate gel beads by ionotropic gelation method (Bourgeois et al., 2002 ; Dupuis et al., 2004).

The relative advantages of multiple unit dosage forms (in terms of bioavailability, more consistent blood levels, predictable gastrointestinal transit time, less localized gastrointestinal disturbances and greater product safety) over single unit product are well established (Pillay & Fassihi, 1999) and promote the development of such drug delivery systems.

The aims of this study were to investigate the influence of two formulation parameters, the type of cross-linking agent (calcium, zinc) and the type of shell capsule used (classical or enteric hard capsule) upon pectin beads characteristics and upon their performance to target the colon.

For this purpose, pectinate gel beads were prepared by a cross-linking reaction between a low-methoxy amidated pectin with ketoprofen as a model drug and two cross-linking agents (calcium or zinc ions). The calcium pectinate beads (CPG) and the zinc pectinate beads (ZPG) were characterized by their morphological aspect and their drug content. Then, these beads were introduced in classical or enteric hard capsules and tested in vitro to investigate their dissolution performances.

MATERIALS AND METHODS

Materials

Amidated low methoxy pectin (Unipectine OF305C, DE ≈ 25% and DA ≈ 21%) was a gift from Degussa Texturant Systems (France).

Ketoprofen was used as received (Nordic Synthesis, Sochibo Francochim). It was chosen as drug model due to its poor water solubility (1 g in more than 10 L, at 20°C). It is a weak acid with a pKa = 4.55 and has a melting point of 94.5°C (Vergote et al., 2001). This drug is also a good candidate for the development of enteric products due to its well-known gastrotoxicity and its anti-inflammatory effect, interesting for several local colonic diseases (Xi et al., 2005).

Calcium chloride dihydrate ($CaCl_2$), Zinc acetate ($Zn(CH_3COO)_2$) were purchased from Sigma Aldrich.

The enteric coating agent, HP55, HydroxyPropylMethylCellulose Phtalate (HPMCP) was a generous gift from Shin-Etsu Chemical Co, Ltd.

All other materials used in the dissolution studies were of analytical reagent grade and were used as received.

Methods

Preparation of Pectinate Gel Beads

The ionotropic gelation technique, previously described by several authors (Aydin & Akbuga, 1996; Sriamornsak & Nunthanid, 1999; Bourgeois et al., 2002; El-Gibaly, 2002), was modified as following:

Pectin aqueous solution at a concentration of 4% (w/v), determined by preliminary tests, was prepared overnight. Then, an appropriate amount of the model drug ketoprofen (2% w/v) was dispersed in the solution until a uniform dispersion was obtained. This bubble-free dispersion was added drop-wise, at an average rate of 2 mL/min, using a nozzle of 0.8 mm inner diameter, into a gently agitated solution of the cross-linking agent ($CaCl_2$ or $Zn(CH_3COO)_2$ at 10%). The falling distance was 3 cm. The gelled beads, instantaneously formed, were allowed to cure in the cross-linking solution for 20 min, and were then separated by filtration, washed with deionized water, and dried at 37°C for 48 h in a drying-room.

All batches were prepared in triplicate.

Beads Characteristics

Morphological Studies

Morphological examination of the pectinate gel microparticles was conducted by scanning electron microscopy (SEM) using a JEOL scanning electron microscope (JSM-6400F) at 20 kV. Pectinate beads were coated with nickel under vacuum by SPI Sputter coating unit. The examinations were performed at two magnifications (X 20, X 350). Size, shape, and surface of pectinate beads were evaluated in this manner (Dupuis et al., 2004).

Determination of Drug Content

The ketoprofen content of the beads was determined through Eq. 1

$$\text{Entrapment efficiency (drug entrapment ability in \%)} = AQ / TQ \cdot 100 \quad (1)$$

in which AQ is the actual quantity of drug present in the matrices (drug content) and TQ is the theoretical quantity of drug (initial ketoprofen loading dose during the preparation of the beads).

Drug Release Studies

The aim of these studies was to investigate the resistance of the beads in the upper part of the GI tract and so their ability to release ketoprofen in a colonic medium.

Release studies were performed using an in vitro rotating paddle dissolution apparatus (Model Erweka DT-6) at 50 rpm and 37 ± 0.2°C.

The different bead samples (accurate weight of approximately 200 mg) were introduced into classical or enteric (coated with HPMPC) hard capsules.

The coating was performed by dipping in an ethanol/acetone solution (50/50) at 10% (w/w) of HPMPC and then by drying with warm air (n = 20 capsules). Dipping was repeated at least five times. Then, the efficacy of the enteric coating was checked by a disintegration test following European Pharmacopeia recommendations (capsules undamaged after 2 h in HCl 0.1 M, n = 3). If this test failed, the batch was rejected.

These hard capsules were then tested in triplicate (n = 3) in three different media under the following conditions to mimic the gastro-intestinal tract:

simulated gastric fluid (pH = 1.2 buffer, NaCl/HCl N)2 h
simulated intestinal fluid (pH = 7.4 buffer, KH_2PO_4/NaOH N)3 h
and (pH = 6, deionized water)2 h

This pH cascade was found upon physiological data (Vandamme et al., 2002). Before reaching the colon, the oral dosage forms must pass through the stomach (pH ~ 1.5–3.5), the duodenum (pH ~ 6), the small intestine (pH ~ 5.5–6.8) and the ileum (pH ~ 7–8). So, we chose to evaluate an acid medium (pH = 1.2), a basic medium (pH = 7.4) before the entrance in the colon (pH = 6).

Dissolution medium samples were withdrawn at various time intervals up to 400 min and ketoprofen released was analyzed spectrophotometrically at 260 nm.

RESULTS AND DISCUSSION

Concerning the ionotropic gelation method, the key parameters, as well as formulation or processing factors, having an influence on the bead properties have been described by several authors (Sriamornsak, 1999; Sriamornsak & Nunthanid, 1999; Dupuis et al., 2004). They are mainly the type of pectin used (DE, DA, molecular weight) and its concentration, the type of drug and its concentration, the cross-linking conditions (concentration, time) and the drying conditions.

Ionotropic gelation method has been also described previously with alginic acid and microspheres obtained from two salts (calcium and zinc) showed different morphology and different drug release (Chan et al., 2002). Thus, the influence of cross-linking agent during beads manufacture was evaluated in this study with LM-pectin.

Beads Characteristics

When the aqueous solution of LM-pectin containing ketoprofen was dropped into cross-linking agent solutions (calcium or zinc), gelled pectinate beads were produced instantaneously. Intermolecular cross-links take place between the negatively charged carboxyl groups of LM-pectin and the positively charged counter-ions. An "egg-box" model (Fig. 1) has been suggested for the building of gel network (Cardoso et al., 2003). Calcium binding to pectin reduces the solubility and induces non-covalent associations of the carbohydrate chains.

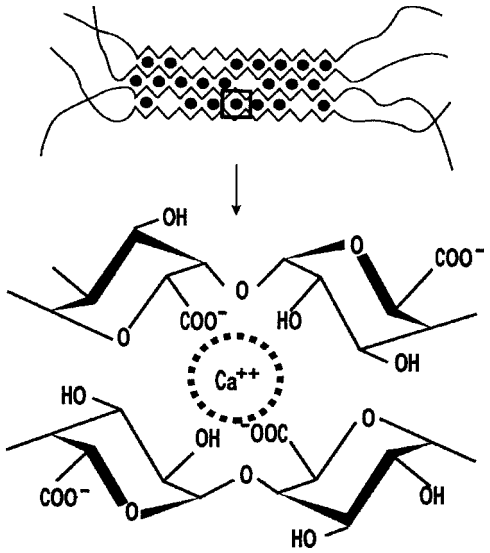

FIGURE 1 The "Egg Box" model.

TABLE 1 CPG and ZPG Bead Properties

	CPG beads	ZPG beads
Bead sizes (mm)	$(2.09 \pm 0.10) \times (1.84 \pm 0.13)$	$(1.92 \pm 0.14) \times (1.69 \pm 0.11)$
Entrapment efficiency (%)	90–93%	59–70%

The obtained beads were spherical with mean diameters of 4–5 mm before drying. After drying, the bead sizes (Table 1) were determined by MEB studies (Fig. 2 and Fig. 3). As for alginate beads, ZPG beads seemed to be smaller than CPG beads (Chan et al., 2002). However, the difference was not significant. The bead sizes depended essentially on the drying method and on the nozzle diameter during the process. For similar conditions, others authors have found similar bead sizes (Sriamornsak & Nunthanid, 1999).

From MEB photographs, morphological characteristics and bead surfaces obtained from a 10% cross-linking solution of $CaCl_2$ (Fig. 2a and b) or $Zn(CH_3COO)_2$ (Fig. 3a and b) were studied. Even if the size and the shape were very similar, the surface beads differed according to counter-ion used. The CPG beads (Fig. 2) were characterized by a granular surface, which was already obvious at the magnification X 20 (Fig. 2a). At the magnification X 350 (Fig. 2b), roughness (asperities from 20 to 50 μm) covered by a uniform film could be observed. The ZPG beads were characterized by a globulous surface (X 20 – Fig. 3a). At the magnification X 350 (Fig. 3b), a

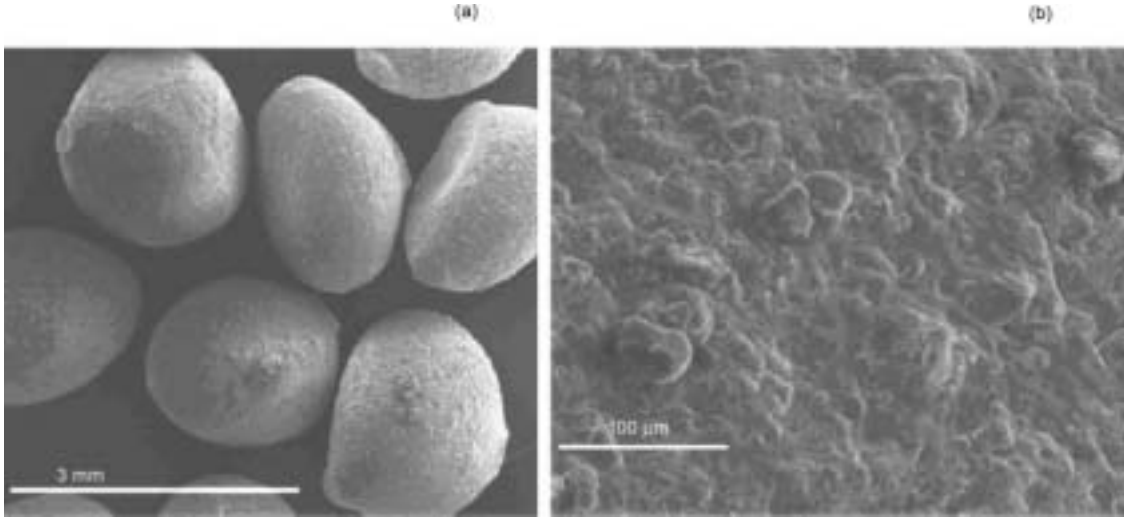

FIGURE 2 **Scanning Electron Micrographs of CPG Beads. (a) Magnification X 20. (b) Magnification X 350.**

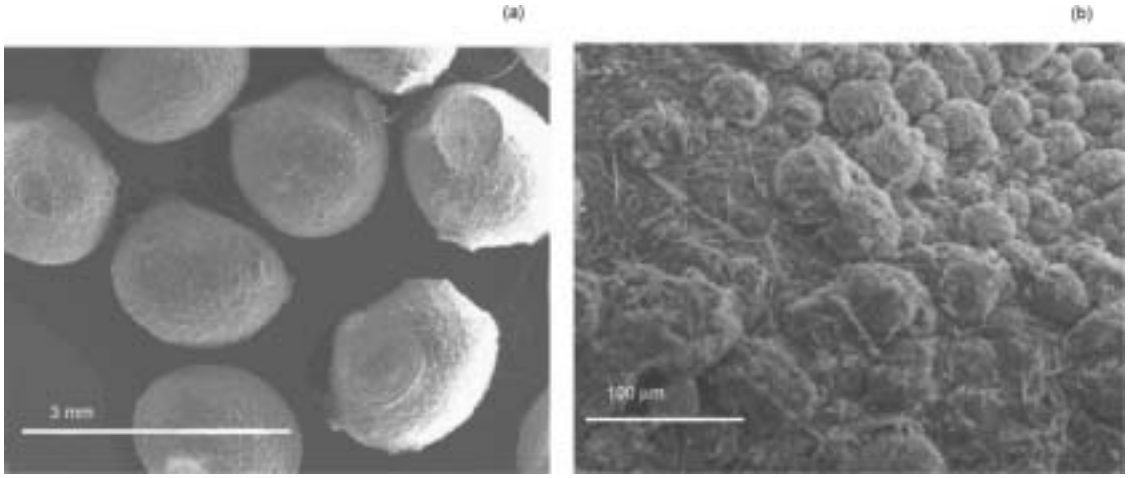

FIGURE 3 **Scanning Electron Micrographs of ZPG Beads. (a) Magnification X 20. (b) Magnification X 350.**

rough surface with distinct globules (10 to 40 µm) and needle-like structures could be noted.

Pectinate beads had efficiently encapsulated ketoprofen. Entrapment efficiency was presented in Table 1. The ZPG beads (60–70%) had entrapped less ketoprofen during the reticulation process than the CPG beads (90–93%) but it was the same order of entrapment efficiency than other authors (El Gibaly, 2002). Moreover, our data were confirmed by ketoprofen determination in the reticulation solution at the end of the manufacturing process. 30% of the total initial amount of ketoprofen was found in the reticulation solution of ZPG beads versus 2% for CPG beads. This fact may be explained by the development of a strong network of zinc pectinate during beads preparation (Chang et al., 2002), responsible of ketoprofen ejection in the outer part of the beads and then a drug leakage in the reticulation solution.

Drug Release Studies

In vitro dissolution studies were carried out to check the suitability of using the CPG beads or the ZPG beads to protect the active substance in the upper part of the gastro-intestinal tract and then to release it specifically in the colon. Moreover, these studies provided global information, particularly about bead structures with their ability to swell in dissolution medium and release ketoprofen more or less rapidly.

Dissolution profiles for beads introduced in classical hard capsules were similar whatever the beads type (CPG, ZPG) under gastro-intestinal conditions

(sequences of pH = 1.2, pH = 7.4 and pH = 6). Three steps in the dissolution process could be described (Fig. 4). The first step was characterized by the presence of the beads in the gastric medium. The release of ketoprofen was slight but different for CPG beads and ZPG beads. That difference in percentage corresponded, however, to the same quantity of ketoprofen released and was due to variations in entrapment efficiency (Table 1). The second step began when the beads were dipped in the intestinal medium. At this point, the release of ketoprofen from the beads was very fast and an erosion of the matrices was responsible for the break down of the beads and their total disappearance. The rate of this erosion process was not significantly different for the two kinds of beads (CPG = 4%/min and ZPG = 3.3%/min). The last step of the dissolution profiles corresponded to the 100% level of dissolution.

These findings can be explained by two mechanisms. Firstly, after release of the beads from the capsule in the gastric medium, they were attacked by acid ions (H+) and consequently they released few ketoprofen. In fact, at low pH values, pectin depolymerization occurs and pectins are split up spontaneously by deesterification (Norziah et al., 2001). This hydrolytic process led to a slight attack at the surface of the beads but no damage of the matrix occurs. When beads get in the intestinal medium, they rapidly disappeared and released all the entrapped ketoprofen. The literature reported indeed that pectin has a good stability in aqueous solutions at pH around 3 or 4 (Pillay & Fassihi, 1999). Above this pH, the galacturonan chain of pectin depolymerises by a mechanism known as β-elimination. The β-elimination occurs at glycosidic bonds to the C-4 position of anhydrogalacturonic moieties that are methyl-esterified at their C-6 carboxylate group (Jarvis, 2002). This double mechanism of depolymerisation (acid + basic attacks) induced disappearance of the beads by chemical erosion of the matrix. This erosion needs a succession of gastric and intestinal medium contact at 37°C to produce the depolymerisation of the pectinate matrix.

However, some authors (El Gibaly, 2002) showed significantly different drug release kinetics between CPG and ZPG beads but in vitro dissolution studies were performed only at pH = 7.4 and they never tested the overall gastro-intestinal conditions. To overcome the acid attack, enteric hard capsules were used in order to protect the beads from the contact with the gastric medium. When beads were introduced in an enteric hard capsule, the beads release from the capsule took place later only in the intestinal medium. Then, two sharply different dissolution profiles were noticed, depending on cross-linking agent (Fig. 5). Use of enteric polymers as an enteric capsule has not been reported in the literature for beads obtained by

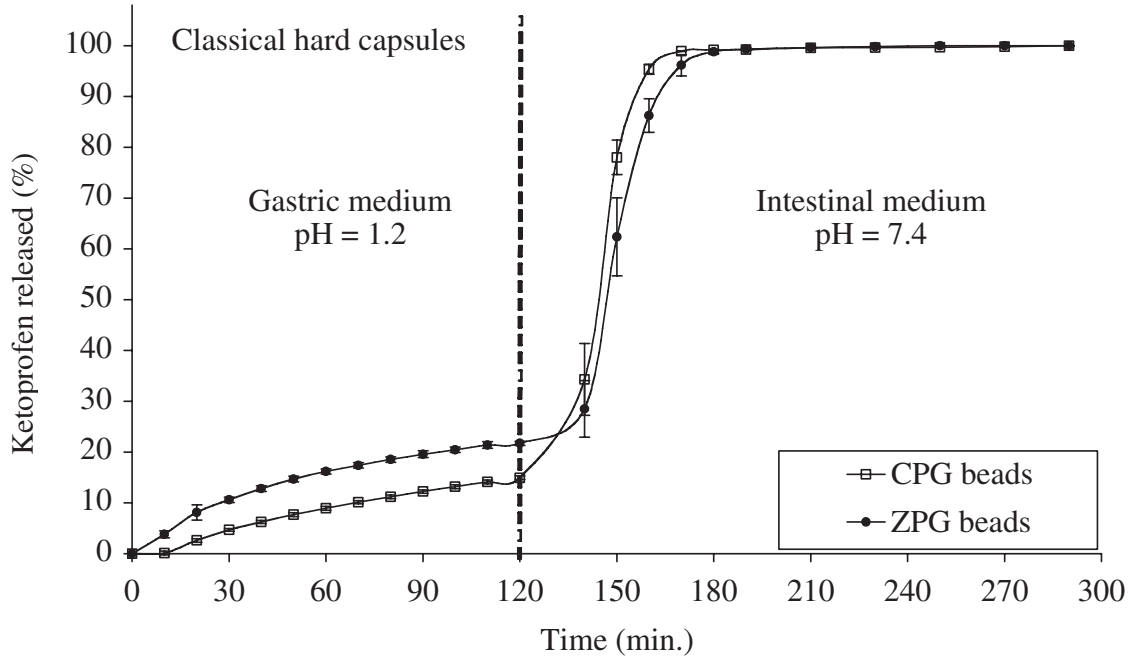

FIGURE 4 **Dissolution Profiles of CPG and ZPG Beads Under Gastro-Intestinal Conditions from Classical Hard Capsules (n = 3).**

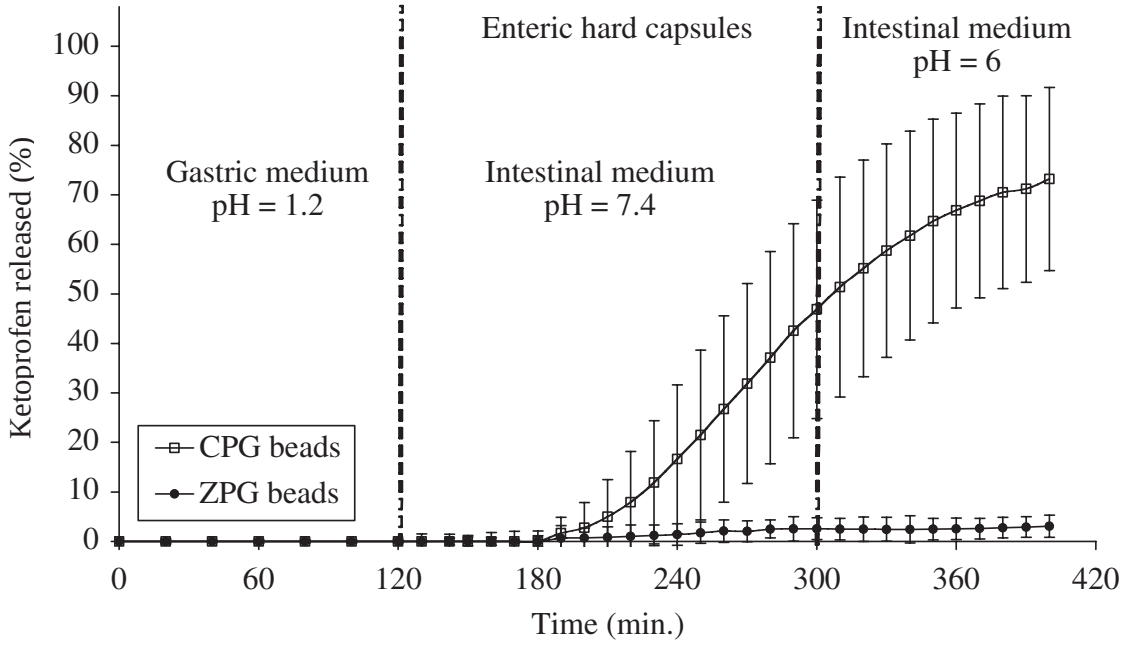

FIGURE 5 **Dissolution Profiles of CPG and ZPG Beads Under Gastro-Intestinal Conditions from Enteric Hard Capsules (n = 3).**

ionotropic gelation. Some authors have used these polymers to coat monolithic dosage forms or pellets as matrix made of pectin and other excipients and have shown their great interest (Rubinstein et al., 1993; Ashford et al., 1994; Wakerly et al., 1997). For example, the acrylic polymers Eudragit® S100 and L100 were tested to coat pectin-based matrices and lowered in a great extent the release of the active substance in the gastric medium (Ahrabi et al., 2000; Mura et al., 2003). The strategy of using an enteric hard capsule was therefore a new application of this system in order to stabilize the beads and to improve their strength in the upper gastro-intestinal tract by reducing pectin solubilization (Xu et al., 2005). Most authors carried out dissolution studies on calcium pectinate beads but only with intestinal conditions (i.e., pH $>$ 6) (Sriamornsak, 1998; Bourgeois et al., 2002). In this way, El Gibaly (2002) reported that zinc pectinate gel (ZPG) beads were more prone than CPG, to resist in the upper GI fluids and to protect ketoprofen from the release in this medium. Here same findings were obtained but with overall conditions of the GI tract. In this case, pectin beads were not chemically eroded but the drug release was controlled by pectinate matrix properties. CPG matrix hydrated and swelled as soon as contact with dissolution medium, whereas ZPG matrix did not seem to be changed. For CPG beads, the drug release started after a lag time (60 min) and the matrix followed the Fick's first diffusion law, where dissolution rate could be estimated from the slope of the kinetic profile related to density of the matrix and viscosity of the hydrated gel. Drug diffusion from this hydrophilic matrix stopped as soon as concentration equilibrium was reached even if a small quantity of ketoprofen was still entrapped inside the matrix (75% released at 400 mins).

For ZPG beads, the dissolution profile was fully different from that of CPG beads. Ketoprofen release was almost not obvious and characterized by a steady state of 3% at 400 min. In connection with beads structure, no swelling of the matrix was noticed. Zinc pectinate gel network was too dense and too thick to allow the diffusion of the medium into the beads and the subsequent release of ketoprofen. These findings were supported by sorption isotherms: CPG beads showed a higher ability to take in water of the atmosphere (water content of 45% for CPG beads in a 75% HR air ambient) than ZPG beads (water content of 15% for ZPG beads in a 75% HR air ambient).

Therefore, it appears that differences in release rate could be explained by the difference in degree of cross-linking of the two pectinate gel types, which could affect the swelling rate of the beads during drug release and consequently the penetration of the dissolution medium into the beads (El Gibaly, 2002). The extent of bead swelling in pH = 7.4 depended on the cross-linking agent type. The CPG beads swelled as soon as contact with medium and completely disappeared when the

ZPG beads seemed to remain undamaged with no disintegration during a period of more than 6 h. This result was consistent with those of Sriamornsak (1998) who showed that the calcium ions form the loose linkage with carboxyl groups in the chains of LM-pectins during "egg-box" formation. A similar tendency has been reported for alginate beads (Aslani & Kennedy, 1996; Chan et al., 2002). Zinc cations produced a more extensively cross-linked and less permeable alginate matrix than calcium cations. Thus, it can be assumed that zinc forms stronger network due to its interaction with LM-pectins, moreover with higher binding affinity and selectivity than calcium (Dronnet et al., 1996). This induces a decrease both in the extent of rehydration and in the molecular porosity (El Gibaly, 2002). It was previously reported (Liu et al., 2003) that gelation of LM-pectin in the presence of counter-ion (calcium) may provide a valuable approach to the formation of a multiparticulate system for colonic delivery. This may be true for a large molecule (i.e., protein), which cannot diffuse through the pores of the matrix beads but can be released due to enzymatic degradation of the matrix. In the opposite, smaller molecules (ketoprofen) can go through the matrix pores. Therefore, zinc pectinate beads are more suitable than calcium pectinate beads for use as a colonic delivery carrier because they resist in the upper part of the GI tract and they go into the colon with a high level of drug.

CONCLUSIONS

The objectives of this study were to investigate the influence of the cross-linking agent (calcium or zinc) used during ionotropic gelation process upon amidated-LM pectin beads characteristics and upon their performances to target the colon after oral administration in a different type of hard capsules. Beads obtained were relatively similar in shape and size but calcium-pectinate (CPG) beads entrapped more ketoprofen than zinc-pectinate (ZPG) beads and their surface texture were completely different. When beads were introduced in classical hard capsules and were evaluated in gastro-intestinal conditions, no difference was shown in ketoprofen release between CPG and ZPG beads, which delivered drug too quickly to reach the colon due to a chemical erosion of the pectinate matrix by an acid and basic attack. However, when enteric hard capsules were used, ZPG beads released few ketoprofen in comparison with CPG beads. ZPG beads showed the higher ability to resist in the upper gastro-intestinal tract. This finding could be explained by a higher binding ability and a higher gel reticulation strength of zinc with LM-pectin in comparison to calcium ions, reducing both extent of swelling and entrance of dissolution medium and then subsequent drug release. In this regard, ZPG beads offered a greater degree of protection from early ketoprofen release in the upper gastro-intestinal tract and provided an interesting approach to colon-specific drug delivery.

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