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Colon-specific drug delivery: Influence of solution reticulation properties upon pectin beads performance

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Abstract

In this study, pectinate gel beads were produced by ionotropic gelation method with different solutions of cross-linking agents and ketoprofen was entrapped as model drug. The influence of these formulation parameters was investigated upon bead properties and upon their performance to target the colon. Zinc pectinate beads obtained with 10% of counter-ions solution at pH 1.6 exhibited the strongest gel network due to "egg-box" dimmer formation helped by hydrogen bonding. Furthermore the gel network formed at low pH was arranged in a compact three-fold conformation. Thus, this matrix structure in enteric capsules induced the lowest drug release in the upper gastro-intestinal tract (pH 1.2 following by pH 7.4). However ketoprofen release occurred specifically in the colon thanks to the presence of pectinolytic enzymes and the release rate can be modulated by the counter-ion concentration during the reticulation process. Therefore this approach using pectinate beads is very promising as efficient carrier for specific delivery of drug into the colon, after oral administration.

Keywords: Colon targeting; Pectinate beads; Low-methoxy pectin; Ionotropic gelation; Cross-linking agents; Cross-linking conditions; Drug release; Ketoprofen

1. Introduction

Colonic drug delivery is intended for the local treatment of ulcerative colitis, irritable bowel syndrome and can potentially be used for colon cancer or the systemic administration of drugs that are adversely affected by the upper gastro-intestinal (GI) tract (Yang et al., 2002). The advantages of local treatment in the colon have been described: reduced incidence of systemic side effects, administration of lower doses of drug, and maintenance of the drug in its intact form as close as possible to the target site. The colon has also been mentioned as an ideal site for protein and peptide absorption (Lee and Mukkerjee, 2002). Acidic and enzymatic degradation are major obstacles in the oral administration of peptide drugs, but by targeting delivery to the colon it is assumed that proteolysis can be minimized.

There has been considerable research into the design of colonic delivery systems and targeting has been achieved by several ways (Vandamme et al., 2002). The primary approaches included prodrugs, pH-sensitive and time-dependent systems. Nevertheless, these parameters (pH, time) can vary from one individual to the next and also according to the pathological and dietary conditions. So these systems can lead to premature and non-specific drug delivery in the colon and they had limited success. Precise colon drug delivery requires that the triggering mechanism in the delivery system only respond to the physiological conditions particular to the colon (Yang et al., 2002).

Natural polysaccharides are now extensively used for the development of solid dosage forms for delivery of drug to the colon (i.e. pectin, chitosan, cyclodextrin, dextran,...). The rationale for the development of a polysaccharide based delivery system for colon is the ability of the colonic microflora to degrade various types of polysaccharides that escape small bowel digestion (Vandamme et al., 2002). Pectins are polysaccharides components of plant cell walls and consist of linear polymers of D-galacturonic acid residues with varying degrees of methyl ester substituents (Sinha and Kumria, 2001). The degree of esterification (DE) and degree of amidation (DA), which are both expressed as a percentage of carboxyl groups (esterified

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or amidated), are important means to classify pectins. Pectins are broken down by various microbial sources including human colonic bacteria and may therefore be utilized as colonic delivery systems if their solubility is reduced (Liu et al., 2003). Therefore, major efforts have been focused on looking for pectin derivatives, which are more water resistant, while still enzymatically degradable (Liu et al., 2003).

One interesting approach is to use calcium salts of pectins because calcium binding reduces the solubility and induces non-covalent associations of carbohydrate chains (Sriamornsak, 1998) through "egg-box" complexes.

The aims of this paper were to investigate the influence of the solution reticulation properties when using the ionotropic gelation method, i.e. type and concentration of counter-ions (calcium/zinc; 5%/10%) and pH of the solution (pH 1.6 or 6), upon pectin beads properties and upon their performance to target the colon.

For this purpose, pectinate gel beads were prepared by a cross-linking reaction between amidated low-methoxy pectin with ketoprofen and different solutions of cross-linking agents (calcium or zinc ions). Calcium pectinate beads (CPG) and zinc pectinate beads (ZPG) were characterized by their morphological aspect and their drug content. Then, the beads were tested *in vitro* through dissolution conditions mimicking gastric to colon transit in order to check their efficacy to target the colon.

2. Materials and methods

2.1. 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 $pK_a = 4.55$ and a melting point of 94.5 °C (Vergote et al., 2001). Ketoprofen is a good candidate for the development of colonic delivery system: it is a potent non-steroidal anti-inflammatory agent and it has a serious adverse effect on the gastro-intestinal tract (Xi et al., 2005).

Calcium chloride dihydrate ($CaCl_2$), Zinc acetate ($Zn(CH_3COO)_2$) and pectinolytic enzymes from *Aspergillus aculeatus* (Novozyme Corp., 26000 PG/mL at pH 3.5) 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.

2.2. Methods

2.2.1. Preparation of pectinate gel beads

The ionotropic gelation technique, previously described by several authors (Aydin and Akbuga, 1996; Sriamornsak and Nunthanid, 1998; Sriamornsak, 1999; Bourgeois et al., 2002) was used as following: pectin aqueous solution at a concentration of 4% (w/v) was prepared overnight. Then, an appropriate amount of the model drug ketoprofen (2% w/v) was dispersed into 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 $Zn(CH_3COO)_2$) with two concentrations (5 or 10%) and two different pH (1.6 or 6 adjusted by using an adequate amount of HCl N). 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 deionised water and dried at 37 °C for 48 h in a drying-room.

All batches were prepared in triplicate.

2.2.2. Bead characteristics

2.2.2.1. Morphological studies. Morphological examination of the pectinate gel beads 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 (20× and 350×). Size, shape and surface of pectinate beads were evaluated in this manner (Dupuis et al., 2004).

2.2.2.2. Determination of drug content. The ketoprofen content of the beads was determined through Eqs. (1) and (2):

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

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):

$$\text{entrapment percentage} = [\text{AQ}/(\text{total weight of beads by batch} - \text{AQ})] \times 100 \quad (2)$$

AQ was determined during release studies after whole beads disappearance in the media.

2.2.2.3. Drug release studies. In order to mimic mouth to colon transit, dissolution studies were performed using an *in vitro* USP rotating paddle 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 enteric capsules (coated with HPMPC). The coating of capsules 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

capsules batch was rejected. These capsules were then tested in triplicate ($n = 3$) in three different media (one after another, volume = 1000 mL) under the following conditions:

- 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
- simulated colonic fluid without or with pectinolytic enzymes (pH 6, water deionised): 3 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, these experiments tested an acid medium (pH 1.2), a basic one (pH 7.4) before the entrance in the colon (pH 6). This pH 6 was also set because it is the pH of the maximum activity of the pectinolytic enzymes.

Dissolution media samples were withdrawn at various time intervals up to 480 min and ketoprofen released was assayed spectrophotometrically (Uvikon 930–Kontron Instrument) at 260 nm using a specific calibration curve for each medium. All UV measurements were performed with a double beam apparatus against a blank made with the medium tested. Cumulated released amounts (in mass units and in percentage of the initial amounts) were plotted versus time. Times corresponding to 20, 50 and 80% ketoprofen release (T20, T50 and T80) were also determined as dissolution specifications of controlled release dosage forms (Pillay and Fassihi, 1998).

3. Results

3.1. Bead characteristics

When the aqueous solution of amidated LM-pectin containing ketoprofen was dropped into counter-ions solutions (calcium or zinc), gelled beads were produced instantaneously by ionotropic gelation. In this process, intermolecular cross-links were formed between the negatively charged carboxyl groups of LM pectin and the positively charged counter-ions, as previously described in the "Egg-box Model" (Grant et al., 1973). The resulting beads were spherical, with mean diameters of 4–5 mm before drying. After drying, water content dropped from 90% to 7–15% and bead sizes sharply decreased (Table 1) as seeing in scanning electron microscopy pictures (Figs. 1 and 2). As for alginate beads, ZPG beads seemed to be smaller than CPG beads (Chan et al., 2002). However these differences were not significant between the different formulations because bead sizes depended mainly on the drying method and on the nozzle diameter from which the drop was formed during the process. Some authors have found similar bead sizes with a nozzle of 0.8 mm and amidated pectin (Sriamornsak and Nunthanid, 1999).

Table 1
Bead characteristics: CPG and ZPG bead sizes measured on SEM pictures

Type of beads	Length (mm)	Width (mm)
CPG 10% pH 1.6	2.09 ± 0.10	1.84 ± 0.13
CPG 10% pH 6	2.11 ± 0.11	1.71 ± 0.10
CPG 5% pH 1.6	1.94 ± 0.22	1.62 ± 0.16
ZPG 10% pH 1.6	1.92 ± 0.14	1.69 ± 0.11
ZPG 10% pH 6	1.90 ± 0.16	1.67 ± 0.07
ZPG 5% pH 1.6	1.84 ± 0.10	1.68 ± 0.13

Codes for type of beads: CPG, calcium pectinate gel beads; ZPG, zinc pectinate gel beads; 5 or 10%: concentration of cross-linking agent in the reticulation solution used; pH 1.6 or 6: pH of the reticulation solution.

From MEB photographs, bead textures were studied for beads obtained from a 10% counter-ion solution of $CaCl_2$ or $Zn(CH_3COO)_2$ at various pH. Even if size and shape were very similar, the bead surfaces showed different aspects. The CPG beads were characterised by a rough surface which was already obvious on the magnification 20× (Fig. 1a). On the magnification 350×, roughness (asperities of 20–50 μm) covered by a uniform film could be observed (Fig. 1b). However, pH of the solution reticulation did not influence the appearance of these CPG beads.

The ZPG beads were characterised by a globulous surface, which was already noticeable on the magnification 20× (Fig. 2a). On the magnification 350× (Fig. 2b), a rough surface with globules (10–40 μm) and needle-like structures (3 μm × 33 μm) could be observed. The size of these globules

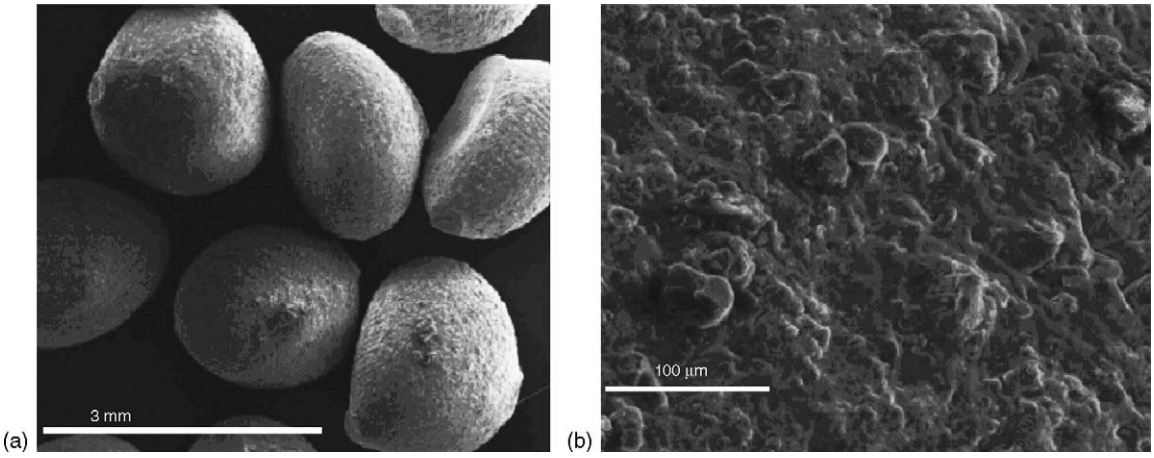

Fig. 1. Scanning electron micrographs of CPG 10% beads at pH 1.6. (a) Magnification 20×. (b) Magnification 350×.

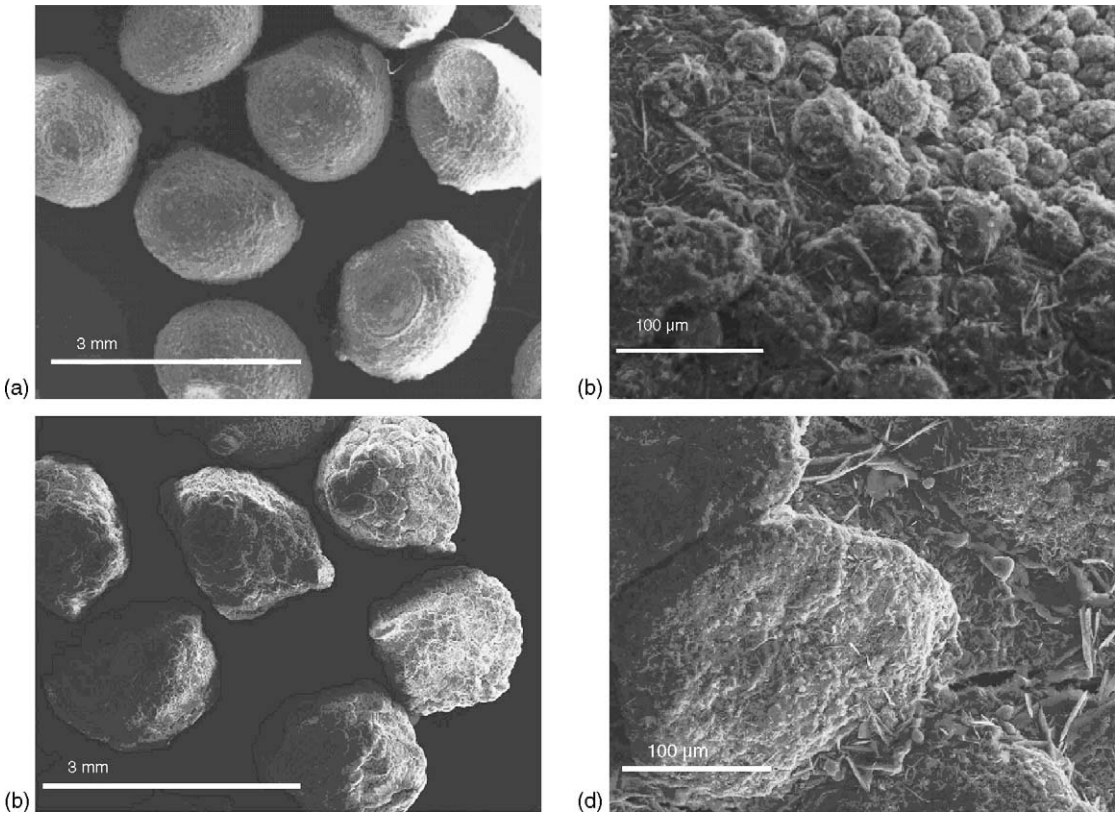

Fig. 2. Scanning electron micrographs of ZPG 10% beads at pH 1.6. (a) Magnification 20×. (b) Magnification 350×. Scanning electron micrographs of ZPG 10% beads at pH 6. (c) Magnification 20×. (d) Magnification 350×.

differed for ZPG beads obtained from the acidic counter-ion solution (pH 1.6) (Fig. 2a and b) and for ZPG beads obtained from the pH 6 counter-ion solution (Fig. 2c and d): the globules were ten fold bigger at pH 6 than at pH 1.6.

Entrapment efficiency and entrapment percentage were presented in Table 2. ZPG beads had entrapped less ketoprofen during the reticulation process than CPG beads and beads obtained from the pH 1.6 counter-ion solution had entrapped less ketoprofen than the ones obtained from the pH 6 solution. Moreover, our data were confirmed by ketoprofen determination in the reticulation solution, at the end of the manufacturing process. Thirty percent 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 (Chan et al., 2002) responsible of ketoprofen ejection in the outer part of the beads and then a drug leakage in the reticulation solution. Entrapment efficiency was also in the same range of values than others authors (El-Gibaly, 2002).

Table 2
Drug content of beads: entrapment efficiency and entrapment percentage for the different pectin beads

Type of beads	Entrapment efficiency (%)	Entrapment percentage (%)
CPG 10% pH 1.6	90–93	27–28.5
CPG 10% pH 6	98–99	24–25
CPG 5% pH 1.6	98	47
ZPG 10% pH 1.6	59–70	16–19
ZPG 10% pH 6	63–73	16–18
ZPG 5% pH 1.6	57	21

Beads obtained from a 5% counter-ion solution had a higher percentage of encapsulation because the quantity of matrix (pectinate) in these beads was smaller than for 10% counter-ion pectinate matrices.

3.2. Drug release studies

For *in vitro* evaluation of colon-specific drug delivery systems, the ideal dissolution testing should closely mimic the *in vivo* conditions with regard to pH, types of enzymes and enzymes activity, fluid volume and mixing intensity. Such dissolution specifications will be very difficult to be validated. Nonetheless, several methodologies were reported in the literature (Yang et al., 2002). Conventional methods could be used and provide essential information on the processing specifications and on the functionality of the system design rather than in vivo performance. To improve the relevance of the findings obtained, bacterial cultures or rat caecal contents were commonly added in dissolution media as well as isolated enzymes (Wakerly et al., 1996). Therefore, we added pectinolytic enzymes after a period in pH 1.2 (simulated gastric fluid) and in pH 7.4 (simulated intestinal fluid) to be close to the colon medium.

Table 3
Dissolution specifications of the different pectin beads under gastro-intestinal conditions without or with pectinolytic enzymes ($n = 3$)

Type of beads	T20 (min)	T50 (min)	T80 (min)
CPG 10% pH 6	221 ± 39	295 ± 15	>400
CPG 10% pH 1.6 – enzymes	248 ± 18	309 ± 35	>400
CPG 10% pH 1.6 + enzymes	253 ± 13	299 ± 24	419 ± 66
CPG 5% pH 1.6	203 ± 4	245 ± 5	294 ± 61
ZPG 10% pH 6	265 ± 23	>400	>400
ZPG 10% pH 1.6 – enzymes	>400	>400	>400
ZPG 10% pH 1.6 + enzymes	>400	>400	>400
ZPG 5% pH 1.6	425 ± 6	>400	>400

In a previous work (Dupuis et al., 2006) we have shown promising findings when ZPG beads were tested after administration in enteric capsules. In this study, we focused on the influence of the solution reticulation properties (type and concentration of counter-ions (calcium/zinc; 5%/10%) and pH of the solution (pH 1.6 or pH 6) upon the ketoprofen release and the efficacy to target the colon. All the dissolution specifications (T20, T50 and T80) of the different kinetic profiles with the different pectin beads are stated in Table 3.

3.2.1. Influence of the counter-ion type

The use of an enteric capsule enabled to observe differences in beads profiles according to the type of counter-ions (Dupuis et al., 2006) because they protect beads from the contact with the gastric medium. In the intestinal medium, the dissolution profiles were very different from CPG and ZPG beads, as previously described (El-Gibaly, 2002) and shown in Fig. 3 and Table 4. For CPG beads, the ketoprofen release became steady (linear part) after a lag time (30–60 min). This could be linked to bead appearance: swelling and gradual disappearance of bead opacity were noticed as beads were hydrated by the medium. So, these CPG beads showed behaviour of hydrophilic matrices (Sriamornsak and Nunthanid, 1998). For ZPG beads, ketoprofen release was lower and characterised at the end by equilibrium. In connection with beads structure, no swelling was visible to the naked eye during all the experiment.

3.2.2. Influence of the pH of the counter-ion solution

The influence of the reticulation solution pH was shown in Fig. 3. It was not obvious for CPG beads but it was significant for ZPG beads as confirming by dissolution data (Tables 3 and 4). Beads reticulated with a pH 1.6 solution released very little ketoprofen in the upper gastro-intestinal tract and their dissolution

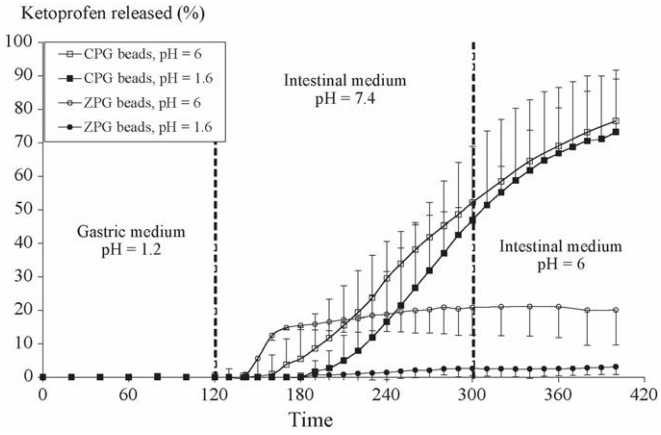

Fig. 3. Dissolution profiles of CPG and ZPG 10% beads at pH 1.6 or at pH 6 under gastro-intestinal conditions ($n = 3$).

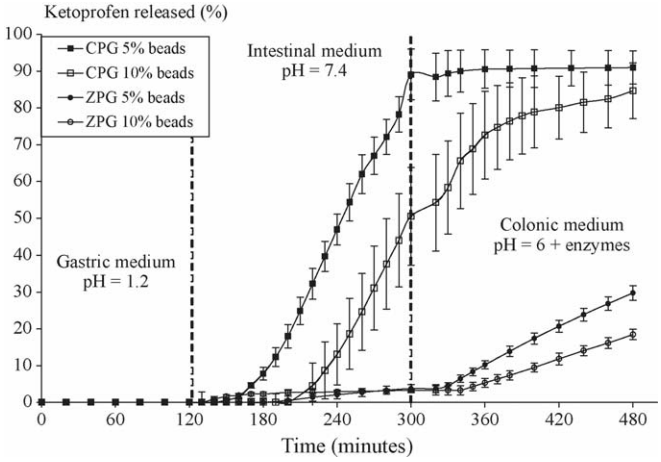

Fig. 4. Dissolution profiles of CPG and ZPG, 5 or 10%, beads (pH 1.6) under gastro-intestinal conditions with pectinolytic enzymes ($n = 3$).

profile reached a steady state at 3% in 250 min. Whereas, beads reticulated with a pH 6 solution released more entrapped ketoprofen and their kinetic profile reached a steady state at 20% since 260 min and before entrance in the colon.

3.2.3. Influence of the counter-ion concentration

In this part of the release studies, only the beads that showed the best resistance in the upper gastro-intestinal tract were tested, i.e. ZPG beads, 10 and 5% reticulated at pH 1.6 and CPG beads obtained under the same conditions.

Fig. 4 illustrates dissolution profiles of CPG and ZPG beads in presence of pectinolytic enzymes to better mimic the overall

Table 4
Amounts of ketoprofen released (mg) of CPG and ZPG 10% beads at pH 1.6 or at pH 6 under gastro-intestinal conditions ($n = 3$)

Dissolution time (min)	CPG 10% pH 6 beads	CPG 10% pH 1.6 beads	ZPG 10% pH 6 beads	ZPG 10% pH 1.6 beads
120	0	0	0	0
180	4.72 ± 3.10	3.67 ± 0.52	10.64 ± 0.32	3.43 ± 0.55
240	18.09 ± 8.04	12.07 ± 6.66	11.57 ± 1.38	3.78 ± 0.57
300	28.87 ± 11.12	25.47 ± 9.83	11.29 ± 2.18	4.09 ± 0.59
360	34.80 ± 12.84	34.30 ± 8.87	11.16 ± 2.44	4.08 ± 0.57
400	37.67 ± 13.05	37.10 ± 8.40	10.91 ± 2.78	4.13 ± 0.58

Table 5
Amounts of ketoprofen released (mg) of CPG and ZPG, 5 or 10% beads at pH 1.6 under gastro-intestinal conditions with pectinolytic enzymes ($n = 3$)

Dissolution time (min)	CPG 5% pH 1.6 beads	CPG 10% pH 1.6 beads	ZPG 5% pH 1.6 beads	ZPG 10% pH 1.6 beads
120	0	0	0	0
180	9.25 ± 1.10	3.20 ± 0.53	6.59 ± 0.40	5.01 ± 0.12
240	34.23 ± 2.74	10.41 ± 3.69	7.56 ± 0.37	5.18 ± 0.18
300	55.91 ± 4.62	26.77 ± 5.87	8.13 ± 0.37	5.29 ± 0.12
360	56.84 ± 3.59	36.41 ± 5.28	10.30 ± 0.38	5.60 ± 0.30
420	56.98 ± 3.34	39.68 ± 3.77	13.83 ± 0.54	6.99 ± 0.34
480	57.11 ± 3.13	41.66 ± 2.24	16.87 ± 0.66	8.75 ± 0.43

GI tract and to check the suitability of pectin beads to release specifically ketoprofen in the colon (Wakerly et al., 1996). Their dissolution specifications were in Table 3 and data in Table 5.

For CPG beads at 10%, the ketoprofen release with pectinolytic enzymes in colonic medium was not statistically different from the release in absence of these enzymes (Tables 3–5; Figs. 3 and 4): unfortunately, the major part of the encapsulated ketoprofen (up to 50%) was already released in the intestinal medium. For ZPG beads at 10%, pectinolytic enzymes induced a notable release of ketoprofen as soon as the addition of enzymes. At the end of the experiment (480 min), the percentage of ketoprofen released was 3% without enzymes and 18.5% with enzymes.

The influence of the counter-ion concentration was also shown in Fig. 4 and Tables 3 and 5. It was different according to the type of the counter-ion. For CPG beads, the ketoprofen release happened significantly sooner with CPG 5% than with CPG 10% and the kinetic profiles had the same feature but shifted to higher amounts with CPG 5% (Table 5). For ZPG beads, little release took place in gastric and then intestinal medium (pH 7.4) whatever was the concentration of the counter-ion. However in colonic medium, ketoprofen release occurred in a larger extent from ZPG 5% beads than from ZPG 10% beads and reached, respectively, 30 and 18.5% at 480 min. The enzymatic erosion of the beads led to drug release at a rate of 0.15%/min for ZPG 5% beads and 0.10%/min for ZPG 10% beads.

4. Discussion

By ionotropic gelation method, pectin beads could be obtained and some authors have shown their suitability for colonic delivery (Aydin and Akbuga, 1996; Sriamornsak, 1998). The type of pectin used (DE, DA, molecular weight) and its concentration, the type of drug and its concentration, the cross-linking and the drying conditions have already been described as parameters influencing the performance of the beads (Sriamornsak, 1999; Sriamornsak and Nunthanid, 1999).

In a previous work (Dupuis et al., 2006) we showed that the use of zinc as counter-ion led to pectin beads stronger in the upper GI than calcium counter-ion due to a higher binding ability with a higher affinity (Dronnet et al., 1996) and a higher pectinate gel strength (El-Gibaly, 2002). These properties resulted to a decrease of swelling in dissolution media and consequently a decrease of drug release (El-Gibaly, 2002). However enteric capsules were needed to avoid chemical erosion of pectin matrix by acid and basic attack in pH 1.2 and 7.4 media (Dupuis et al., 2006). In this paper, the influence of the pH during the reticulation process of the beads was evaluated as well as the concentration of both counter-ion all along the GI tract until the colon.

The influence of the pH of the counter-ion solution was not the same for CPG beads and ZPG beads. For CPG beads, no significant effect was noticed upon morphological and texture aspects and upon drug release. At the opposite for ZPG beads, the pH influence was more obvious because at pH 1.6 beads released very little ketoprofen (3%) whereas at pH 6 beads released 20% of ketoprofen. pH seemed to influence the structure of the pectinate network, as it was also established for zinc beads by considering MEB photographs with different bead surfaces. The acidification of the counter-ion solution during the reticulation process was responsible for the creation of a strong pectinate counter-ions network, leading to a very strength matrix (Pillay and Fassihi, 1999). Indeed, polymeric chains of pectin were not only linked together by dimmer associations described in the "egg-box" model (interaction between cations and carboxylate groups), but also by a combination of hydrogen bonding and hydrophobic interactions. This latter mechanism of gelation has been studied by several authors with calcium counter-ion (Gilsenan et al., 2000; Cardoso et al., 2003; Lootens et al., 2003). The non-ionic associations, analogous to those in conventional high-methoxyl pectin gels, became progressively more significant as the pH decreased (Gilsenan et al., 2000). Lootens (Lootens et al., 2003) reported by rheological studies that pH below 3 (lower than pectin intrinsic pK_a value) led to the formation of stronger gels for amidated pectin. Low pH probably assisted intermolecular association by reducing the charges on the polymer, thus lowering intermolecular charge–charge repulsion and also reducing the solubility of the polymer chain. Protonation of carboxyl groups appeared to promote conformational ordering and association by suppression of electrostatic repulsion and by allowing the carbonyl groups to act as hydrogen-bond donors (Lootens et al., 2003). Besides the influence of the charge density of the polygalacturonate chain, the distribution pattern of free and esterified carboxyl groups had also an important effect on the strength of ion binding. In fact, the affinity of pectin chains towards cation is known to increase with decreasing degree of esterification (Cardoso et al., 2003). On the basis of evidence from potentiometry, viscosimetry, isothermal calorimetry and chiroptical measurements, a change in conformation from an extended (two-fold) structure to a more compact

(three fold) form has been proposed for polygalacturonate chains in dilute solution, in response to reduction in pH at fixed temperature (Gilsenan et al., 2000). At pH values where most of the carbonyl groups were ionised, the chain would be stiffened and extended by intermolecular electrostatic repulsion, giving local conformations close to the extended two-fold structure. Reduction in charge-density by lowering pH would allow the chain to adopt a more compact arrangement close to the three fold structure as found in the solid state (Gilsenan et al., 2000). It is conventional to define two components in a polysaccharide gel: "inter-junction segments" where the polymer chains are in a solution-like state and "junction zones" where parts of two or more chains are bound together. In the "egg-box" model, the inter-junction segments are single chains and the junction zones are pairs of galacturonan chains in the two-fold helical conformation (two residues per turn of the helix) with ions fitted between them like eggs in an egg-box (Grant et al., 1973). A conformational transition took place when lowering the pH of pectin solution with a transition being from the two-fold to the three-fold helical form. The same transition occurred between gel and solid forms of calcium pectinate and has been observed by changes in ^{13}C NMR spectrum (Jarvis and Apperley, 1995). These findings with calcium could be applied in this study. No significant effect was obvious with CPG beads but a same trend was obtained for ketoprofen release which started sooner with CPG at pH 6 than with CPG at pH 1.6. Likewise, similar effect was reported by Pillay and Fassihi (1999) with cross-linking pellets of diclofenac. Negligible drug release occurred at pH 1–4 but it happened at pH 6.6 and drug entrapment was enhanced at pH 1.6 as it was shown with data in Table 2.

All these studies, about connections between pectin chain conformations alone and with ions in neutral or acid pH, underlined the importance of the knowledge of the reticulation process to better characterise pectin beads. In this study, it was shown that counter-ion type and pH of the counter-ion solution had a great influence upon the bead ability to resist in the upper gastro-intestinal tract.

ZPG beads, obtained from an acid counter-ion solution (pH 1.6), showed the best ability to reach the colon with enough amount of ketoprofen, whereas CPG beads did not succeed in (Table 4).

In the next part of this work, the ability of the beads (ZPG and CPG) to release specifically ketoprofen in the colonic medium was investigated. Only beads obtained at pH 1.6 were studied because they were stronger in the upper gastro-intestinal tract.

The effect of pectinolytic enzymes on CPG beads was not very obvious because CPG beads had released the major part of the entrapped ketoprofen in the intestinal medium by diffusion through the hydrated matrix. However, at the naked eye, it was noted that enzymes had led to a rapid erosion of the swollen matrix with the subsequent bead disappearance.

For ZPG beads, the enzymatic erosion of the beads had led to a gradual release of ketoprofen depending on enzymes concentration in the medium and density of the zinc pectinate network when a steady state of 3% was recorded without enzyme (desionised water, pH 6). The rate of enzymatic erosion helped us to estimate that the beads would release the totality of the entrapped ketoprofen within about 17 h (ZPG 10%). This finding was consistent with colon specific delivery since Semdé (Semdé et al., 2000) determined that the transit time in the part of the colon favourable to absorption was about 19 h. So, these ZPG beads were suitable for colonic delivery with a drug protection in the upper GI and a specific delivery in the contact of the colon medium.

Then, the findings obtained for CPG 5% and ZPG 5% beads were reported in comparison with CPG 10% and ZPG 10% (Table 5 and Fig. 4). For CPG beads, the counter-ion concentration changed the bead behaviour. Other authors have clearly shown that the pectinate gel bead characteristics were influenced by the calcium chloride concentration. Especially, increasing calcium concentration led to a greater degree of cross-linking and aggregation of the pectin chains inducing higher gel strength and limiting swelling patterns and subsequent drug release (Sriamornsak, 1999). An optimal concentration for calcium ions could be determined and was set at 8% to get the best reticulation strength with a 6% pectin solution (Bourgeois et al., 2002). It was just a point in the range of concentration tested and could well explained our data. For ZPG beads, the effect of counter-ion concentration was only seen in the colonic medium, probably due to a stronger network of gel in comparison with CPG beads. In colon, the rate of drug release was increased with ZPG 5%. Pectinolytic enzymes could attack more easily ZPG 5% beads and consequently, these beads would release the totality of the entrapped ketoprofen more quickly than ZPG 10% (Table 5). May be an optimal concentration for zinc ions toward pectin during the reticulation process could be also found as for calcium in order to adjust the kinetic profile to an efficient therapeutic level.

5. Conclusions

The approach presented in this study focused on the influence of the solution reticulation properties upon pectin beads obtained by ionotropic gelation method in order to target the colon.

In this regard, zinc counter-ions provided the pectin beads with the strongest network matrix in comparison with calcium counter-ions. The acidification of the counter-ion solution grew up the strength of the pectinate gel involving two mechanisms: intermolecular cross-linking following the "egg-box" model and non-ionic hydrogen bonding or hydrophobic interactions. Reduction in pH promoted also a conformational transition to a more compact three-fold structure. Increasing concentration of the counter-ion led to a greater degree of cross-linking of pectin chains inducing stronger gel and reducing swelling and drug release. Nevertheless, a maximum of counter-ion concentration has been described in literature and may be determined for each case of reticulation.

Finally, pectinate beads were evaluated for drug release in conditions mimicking the overall gastro-intestinal tract (pH 1.2 then 7.4) until the colon (pH 6 with pectinolytic enzymes). The strongest matrix obtained with 10% zinc counter-ions at pH 1.6 reached the colon medium with almost all the drug loading dose and was attacked gradually by enzymes with subsequent drug release.

Therefore, this approach suggested that zinc pectinate beads are promising as efficient carriers for specific drug delivery to the colon.

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