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Zinc-pectinate beads as an in vivo self-assembling system for pulsatile drug delivery

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ABSTRACT

Zinc-pectinate beads are interesting drug carriers for oral delivery. In order to investigate their in vitro and in vivo release behaviour, ionotropic gelation was used to entrap theophylline into calcium- or zinc-pectinate beads. Beads were investigated in vitro for their particle properties, especially the release kinetic in different media, and their in vivo pharmacokinetic parameters were tested in rats. Particle size varied between 1.8 and 2.8 mm and encapsulation rates between 27 and 30% for Ca- and Zn-pectinate beads, respectively. While Ca-pectinate beads revealed a relative fast disintegration, drug release profiles from Zn-pectinate beads were very much release medium-dependent. Especially, in the presence of phosphate ions, the release from Zn-pectinate beads was blocked at 20% and 40% of the total drug load when tested in phosphate buffer or simulated colonic medium. In vivo Zn-pectinate beads (t_{max}: 12.0 ± 0.1 h) led to a significant lag time for the theophylline absorption compared to Ca-pectinate (t_{max}: 6.0 ± 2.8 h) or free theophylline (t_{max}: 2.5 ± 2.1 h). This delayed release was attributed to the formation of a zinc phosphate coating in vitro and in vivo inducing the retention of theophylline release. Zn-pectinate beads exhibit interesting properties due to its potential as pulsatile delivery system induced by the in situ formation of Zn phosphate, while Ca-pectinate was found to be of limited suitability for controlled release of theophylline.

1. Introduction

Oral colon specific drug delivery systems have recently gained importance for systemic delivery of drug (such as peptide delivery) (Luppi et al., 2008) and also for the treatment of local diseases (inflammatory bowel diseases) (El-Kamel et al., 2008). The major obstacles to drug delivery to the colon are the absorption and degradation pathways in the upper gastro-intestinal tract (GIT).

Many approaches have been developed to achieve colonic targeting included prodrugs, pH-dependent systems, time-dependent systems and biodegradable systems. However, efficient colon drug delivery needs that the system only responds to the physiological conditions particular to the colon (Yang et al., 2002).

Several carbohydrate polymers are able to satisfy these requirements to some extent because the colonic microflora can degrade various polysaccharides that escape small bowel digestion (Vandamme et al., 2002).

A large diversity of polysaccharide-based drug delivery systems has been developed, among them single-unit forms such as coated tablets or matrices and multiparticulate forms such as beads or microparticles (Liu et al., 2003). One major formulation approach for formulating polysaccharides is the ionotropic gelation method where cations build insoluble associates with carbohydrate chains resulting in the so-called "egg-box" complexes (Bourgeois et al., 2006).

These pectinate beads are considered as pulsatile drug delivery systems as they release a drug following a programmed lag phase (Roy and Shahiwala, 2009). These multiparticulate dosage forms are gaining much favour over single-unit dosage forms because of their potential benefits like predictable gastric emptying, reduced risk of local irritation, or less inter- and intra-subject variability (Chambin et al., 2005). These multi-unit systems based on hydrophilic polymers (so biodegradable) have been shown to quickly spread out all along the gastro-intestinal tract, particularly in the colon with an increase of surface area exposed to bacterial breakdown that produces a rapid drug release and hereby improves drug absorption (Maestrelli et al., 2008).

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Beside the most commonly encountered polysaccharide, which is alginate, pectinate is cited in literature to be useful for bead formation (Sinha and Kumria, 2001). Pectinates have been complexed with calcium or zinc ions and their resistance to proteases and amylases in the upper GIT combined with their digestion by the microflora in the colon make them an interesting approach for colonic delivery (Liu et al., 2003; Das et al., 2010).

The major drawback of these polysaccharide-based carriers is the failure of retaining the drug from being available in the upper parts of the gastro-intestinal tract (Dupuis et al., 2006).

While many studies deal with delaying the release by the integration of additional excipients such as additional coatings or cross-linking, for example by ethylcellulose or chitosan (Liu et al., 2003; Bigucci et al., 2008; Oliveira et al., 2010), only few studies focus on the choice of the appropriate cation. Until now calcium is generally favoured and other cations like magnesium or zinc are hardly mentioned (Das et al., 2010), although it was reported that zinc-pectinate beads were more appropriate to resist in the upper GIT and to refrain drug from premature release (El-Gibaly, 2002). The mechanism of the sustained release obtained by these zinc-pectinate beads has not been analyzed in detail and was suggested to be related to the strong gel network during "egg-box" formation (Chambin et al., 2006; Khoder et al., 2009).

Subsequently, we focus here on the release behaviour of Zn-pectinate beads in vitro and the related mechanisms of sustaining the release. These results were completed with a pharmacokinetic study in rats in order to draw conclusions on the significance of the outcome in vivo. Theophylline was used as a model drug, since it is well absorbed in the large intestine in humans (Maestrelli et al., 2008) and both its anti-asthma activity and pharmacokinetic properties make it an interesting candidate for pulsatile drug release triggered by the colonic microflora (Mastiholimath et al., 2007). All experiments were done in comparison with calcium-pectinate beads as a reference.

2. Materials and methods

2.1. Materials

Amidated low methoxy pectin (LM pectin called Unipectine OF305C, DE 25% and DA 21%) was a gift from Cargill (France).

Theophylline was received from Sigma–Aldrich. Its water solubility is 8.3 g/l at 25 °C and it has a melting point at 271 °C.

Calcium chloride dihydrate ($CaCl_2{\cdot}2H_2O$), zinc acetate dihydrate ($Zn(CH_3COO)_2{\cdot}2H_2O$) and pectinolytic enzymes from *Aspergillus aculeatus* (Novozyme Corp., 26,000 PG/ml at pH 3.5) were purchased from Sigma–Aldrich.

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

2.2. Pectinate gel beads preparation

Pectinate gel beads were manufactured by ionotropic gelation method as described previously (Dupuis et al., 2006): a dispersion of LM pectin solution at 4% (w/v) containing theophylline (2%, w/v) as model drug was added drop-wise (average rate of 2 ml/min, nozzle diameter of 0.8 mm) into a gently agitated solution of the cross-linking agent (calcium chloride or zinc acetate at 10%, w/v) which was pre-adjusted at pH = 1.2 using 1 M HCl solution. The falling distance was set to 3 cm. The gelled beads, instantaneously formed were allowed to cure in the cross-linking solution for 20 min; then beads were separated by filtration, washed with deionized water and dried at 37 °C for 48 h in an oven-drying.

2.3. Beads characterisation

2.3.1. Morphological studies

The pectinate beads were analyzed for their size distribution by optical microscopy. For the imaging by scanning electron microscopy, beads were dried on supports, and coated with gold and palladium under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. Samples were then observed with the scanning electron microscope (JEOL JSM-5600 SEM) at 25 kV.

SEM pictures were also performed during dissolution experiments, particularly after 1 h in each dissolution medium in order to observe the surface change of these particles during contact with the medium.

2.3.2. Determination of drug content

Drug content of the beads was determined by introducing accurately weighed beads into a pH 1.2 medium and after in pH 7.4 phosphate buffer. This succession of conditions ensured whole disappearence of the beads in the media. The entrapment efficiency was obtained through Eq. (1).

$$\text{Entrapment efficiency} = \frac{AQ}{TQ} \times 100 \quad (1)$$

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

2.3.3. Determination of water uptake

Water uptake of beads was recorded with a dynamic sorption apparatus (Autosorp, Biosystems). Beads were equilibrated at 10% relative humidity during 100 h, then the relative humidity was raised to 70% and the water uptake was measured by the weight variation of the bead samples.

2.4. In vitro dissolution tests

Release studies were performed using an in vitro rotating paddle dissolution apparatus (USP apparatus II, Erweka DT-6) where theophylline loaded calcium- or zinc-pectinate beads were tested in three different media: a simulated intestinal fluid (buffer, KH_2PO_4/NaOH 1 M), a distilled water (pH was pre-adjusted with 1 M NaOH) and a Tris-buffer medium (without phosphate), all media were at pH = 7.4. Then an experiment was also carried out with the simulated intestinal fluid (phosphate buffer at pH = 7.4) with pectinolytic enzymes from *A. aculeatus* (Pectinase®, Sigma–Aldrich at 26,000 PG/ml).

Drug release experiments were performed as follows: 200 mg of dry beads were suspended in the dissolution media (1000 ml) under gentle stirring at 50 rpm and 37 ± 0.2 °C. At appropriate intervals up to 24 h, 3 ml samples were withdrawn and assayed for drug release and replaced by 3 ml of fresh medium. The amount of theophylline in the release medium was determined by the UV spectrophotometry at 270 nm (Uvikon 930-Kontron Instrument) using a specific calibration curve for each medium. Cumulated release amounts (in percentage of the initial amounts) were plotted versus time.

2.5. X-ray diffraction studies

At the end of the dissolution test, zinc-pectinate beads were collected, crushed and dried at 105 °C for 24 h. They were then studied by X-ray diffractions (XRD) through a Siemens D5000 diffractometer, using Cu Kα (λ = 1.5406 Å) radiation and an INEL CPS 120 curved detector. The X-ray patterns were recorded in the 2θ range 5–60° with a scan rate of 0.5° min^{-1}.

Table 1
Ca- and Zn-pectinate beads characteristics.

	Ca-pectinate beads	Zn-pectinate beads
Bead sizes in mm	2.4 ± 0.4	2.0 ± 0.3
Water content (%)	12.0 ± 2.3	5.2 ± 1.8
Entrapment efficiency (%)	30.2 ± 0.5	27.2 ± 0.3
Water loss after equilibrium at 10% RH (%)	−11.7 ± 0.05	−3.5 ± 0.15
Water uptake after equilibrium at 70% RH (%)	44.8 ± 0.18	7.1 ± 0.33

2.6. Pharmacokinetic studies

All animal experiments were carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council, National Academy of Sciences, US). Sprague-Dawley rats (250–320 g) were received from Janvier, France and were housed in groups of three and acclimatized to laboratory conditions a week before the experiments, with food and water ad libitum. 24 h before the experiment, the food was withdrawn but free access to water was allowed. Rats received orally by gavage either various theophylline formulations as dry particles at a theophylline dose of 30 mg/kg or pure drug suspension in 0.5% sodium carboxymethyl cellulose (NaCMC).

Blood specimens of 0.5 ml were collected from the jugular vein sampling at different predetermined time points in tubes containing 20 μl of heparin as anticoagulant. 0.3 ml of plasma was mixed with 1.2 ml of acetonitrile and precipitated plasma protein were eliminated by centrifugation at 10,000 × *g* for 10 min. 50 μl aliquots were injected on to the HPLC. All HPLC analyses were run under the following set-up: RP18 column, eluent acetonitrile:H_2O 1:15 at 1 ml/min detected at 273 nm.

2.7. Statistical analysis

The results were expressed as mean values ± S.D. The statistical analysis of treatments with more than two groups was performed with Kruskal–Wallis test followed by Dunn's test, except when normality and equal variance were passed, it was followed by the Tukey test. In all cases, $P < 0.05$ was considered to be statistically significant.

3. Results

Calcium (Ca-) and Zinc (Zn-) pectinates allowed the instantaneous formation of theophylline loaded beads during the manufacturing process. The carboxyl groups of pectin are known to engage in coordination bonds with divalent cations by "egg-box" structure. Amidation of pectin is known to increase the sensitivity towards gelation by calcium (Hagesaether et al., 2008) and to reduce the bead diameter due to reduced hydrohilicity and internal hydrogen bonding between amide groups. Ca- and Zn-pectinate beads characteristics are presented in Table 1.

During equilibrium at 10% of relative humidity, both beads lost water (or weight) until a plateau and this weight loss was higher for Ca-pectinate (−11.7 ± 0.05%) than Zn-pectinate (−3.5 ± 0.15%). When relative humidity was raised to 70%, Ca-pectinate beads adsorbed hugely more water (44.8 ± 0.18%) than Zn-pectinate beads (7.1 ± 0.33%) (Table 1).

Both bead types exhibited an irregular shape after drying (data not shown). The entrapment efficiency for both bead types was varying between 27 and 30% of theophylline (Table 1).

Dissolution profiles of Ca-pectinate beads were similar whatever the dissolution media (Fig. 1). Apparently, drug diffusion was simply controlled by theophylline solubility in aqueous media and the swelling of the polymer. However, it could be noted that Ca-pectinate beads were completely disintegrated in phosphate buffer and not swelled and dissolved as in water or in Tris-buffer.

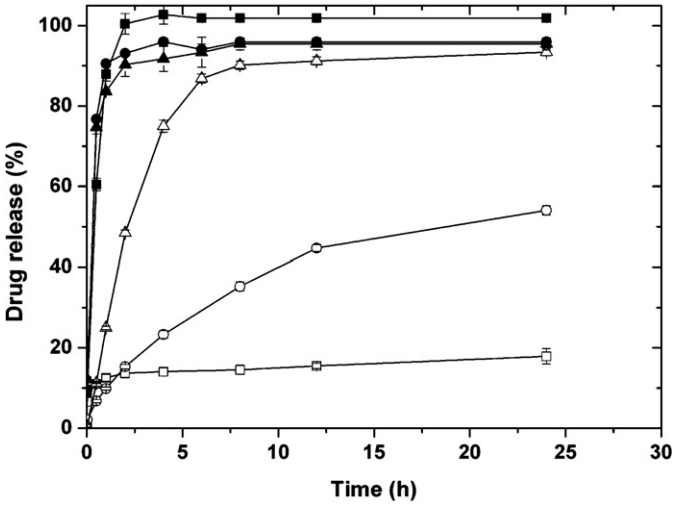

Fig. 1. In vitro dissolution profiles of theophylline from Ca-pectinate (full symbols) and Zn-pectinate (empty symbols) beads in three different media: phosphate buffer (square), water (circle), and Tris-buffer (triangle) at pH7.4.

On the contrary, Zn-pectinate beads exhibited completely different behaviour in drug release depending on the dissolution medium (Fig. 1). In water, drug release increased as a function of time and achieved 54% at 24 h. In Tris-buffer, theophylline release was quicker and achieved 90% at 8 h. Finally in phosphate buffer, after an initial burst, release stagnated below 20% over the entire period.

The release profiles remained unchanged for Ca-pectinate beads when dissolution tests were performed in simulated colonic containing pectinases (Fig. 2). For Zn-pectinate beads, the burst release was higher giving then a higher plateau (around 40%). However, the profiles looked similar with or without enzymes but were completely different with Ca-pectinate or Zn-pectinate beads.

In order to understand the difference of behaviour between these two different media, the surfaces of calcium- and zinc-pectinate microparticles were analyzed by SEM after 1 h of dissolution (Fig. 3). In water, Zn-pectinate beads exhibited slight

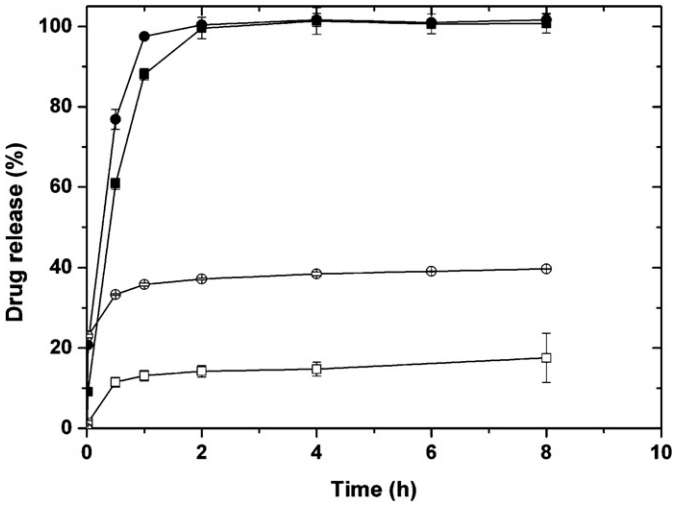

Fig. 2. In vitro dissolution profiles of theophylline from Ca-pectinate (full symbols) and Zn-pectinate (empty symbols) beads in phosphate buffer (square) and phosphate buffer containing pectinolytic enzymes (circle).

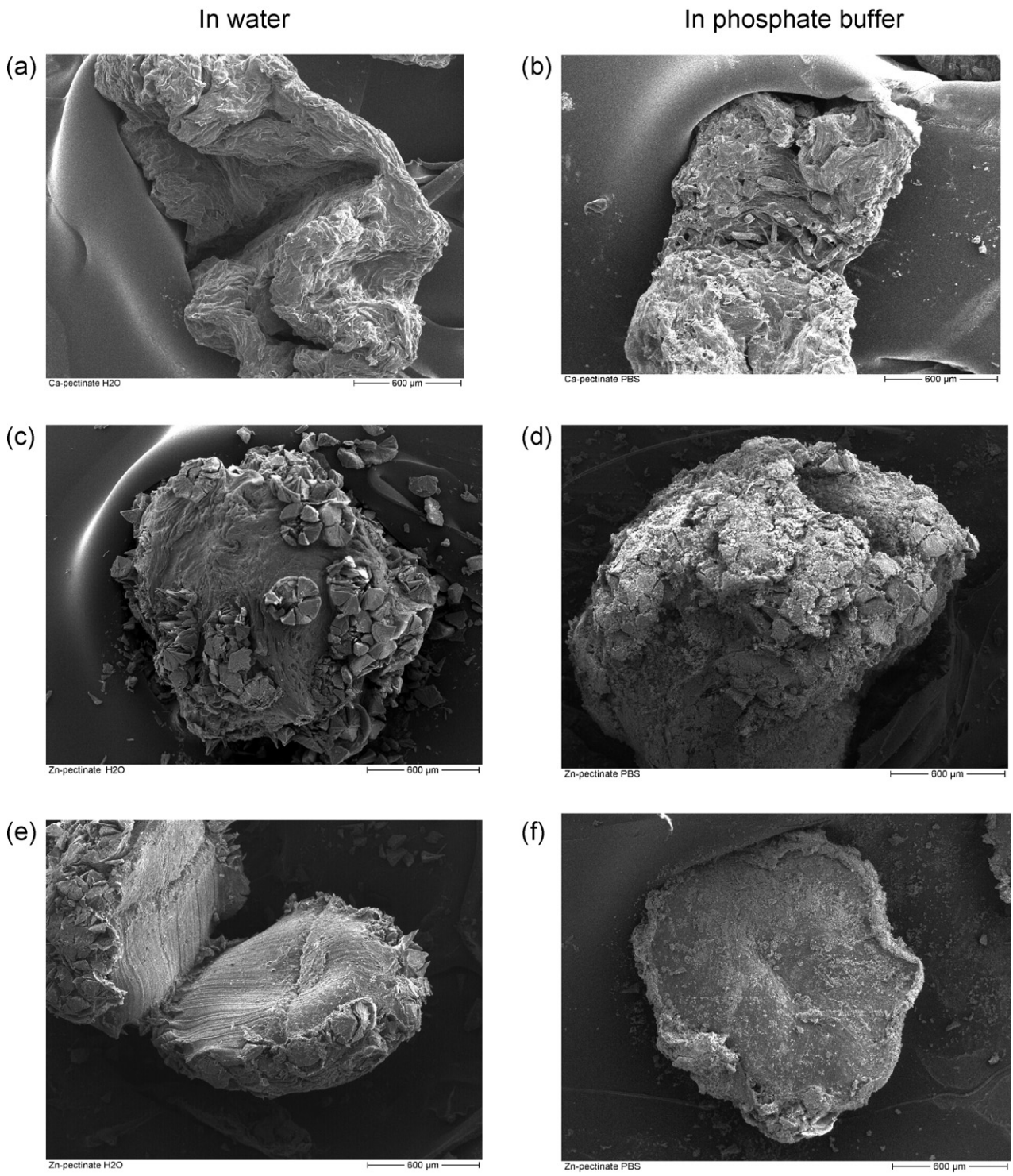

Fig. 3. Scanning Electron Micrographs (SEM) of Ca-pectinate and Zn-pectinate beads after 1 h of dissolution (section). (a) Ca-pectinate beads in water, (b) Ca-pectinate beads in phosphate buffer, (c) Zn-pectinate beads in water, (d) Zn-pectinate beads in phosphate buffer, (e) cross-section of Zn-pectinate beads in water, and (f) cross-section of Zn-pectinate beads in phosphate buffer.

signs of surface degradation (Fig. 3c and e), which were absent in Ca-pectinate beads (Fig. 3a). In phosphate buffer, no changes in surface morphology were observed in Ca-pectinate (Fig. 3b). On the opposite, drastic surface modifications were observed with Zn-pectinate beads after 1 h in the phosphate buffer (Fig. 3d). A continuous layer was found to be precipitated on the particle surfaces (Fig. 3f), making as a coating layer (Figs. 3f and 4a).

Dissolution experiments carried out in Tris-buffer confirmed this hypothesis of interaction between Zn^{2+} and phosphate buffer, since in absence of phosphate ions in the buffer complete theophylline release was achieved after around 8 h (Fig. 1). Ca-pectinate beads exhibited also the same kinetic as in other media (phosphate buffer and water at pH = 7.4) with a complete release after 2 h (Fig. 1).

To be prognostic of the in vivo performance, the dissolution testing should be able to discriminate the impact of upper gastrointestinal tract transit on the drug delivery system and yet closely mimic the in vivo colonic environment with regard to pH, the

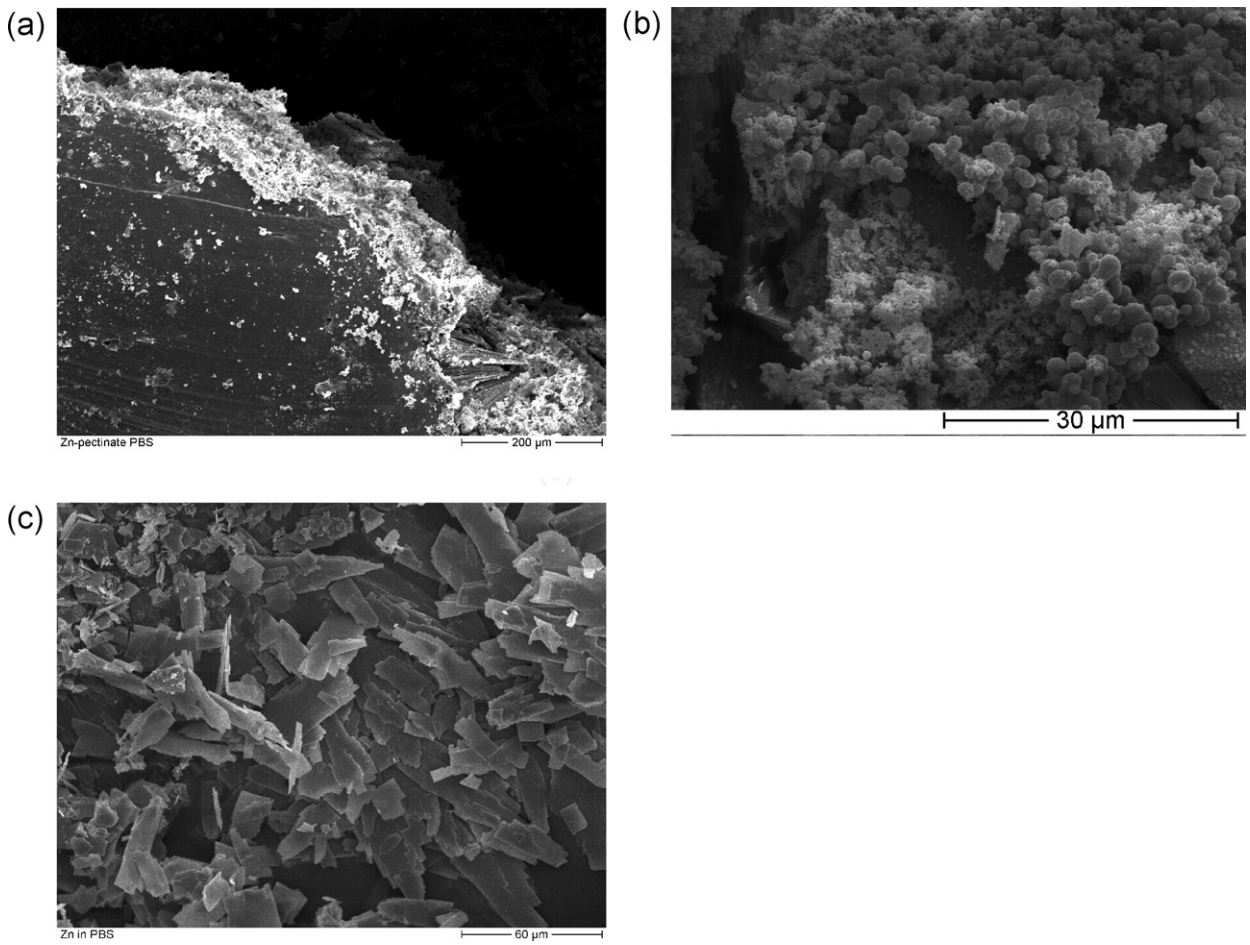

Fig. 4. Scanning Electron Micrographs (SEM) of (a) magnification of cross-section of Zn-pectinate beads in phosphate buffer (200×), (b) Zn-pectinate bead surface after 1 h in phosphate buffer, and (c) Zn crystals obtained directly after precipitation in phosphate buffer.

volume and distribution of liquids but also to bacteria and the composition and activity of enzymes. Apparently, the development of such a dissolution method will be highly challenging, if not impossible (Yang, 2008). Therefore, in vivo studies are essential in this field.

A preliminary in vivo study in rats revealed significant differences in pharmacokinetic parameters between bead formulations and oral theophylline as a suspension (Fig. 5). Free theophylline was readily absorbed while pectinate formulations showed a variable delay in absorption. While Ca-pectinate only led to a slight delay of absorption compared to free theophylline, Zn-pectinate beads showed a lag time of around 4 h with no drug availability. The observed t_{max} was delayed accordingly with values of 6.0 ± 2.8 h and 12.0 ± 0.1 h, respectively for Ca-pectinate and Zn-pectinate beads. It was remarkable that the relative bioavailability was lower when theophylline was entrapped into beads with lowest values with Zn-pectinate beads at around 35% (Table 2).

4. Discussion

Zn-pectinate beads have been studied recently for their formulation particularities and formation mechanisms (Khoder et al., 2009; Assifaoui et al., 2010). Similar to what has been found for the alginate bead formation with calcium, it is reasonable to consider that two populations of Zn ions are present in pectinate gels: those ions within the buckled "egg-box" junction and those freely interacting with other anions (Kikuchi et al., 1999).

The entrapment efficiency was relatively low related to the high aqueous solubility of theophylline. These findings are in line with other reports in literature (Maestrelli et al., 2008) and could be explained by a diffusion of drug in the cross-linking solution, during the curing time.

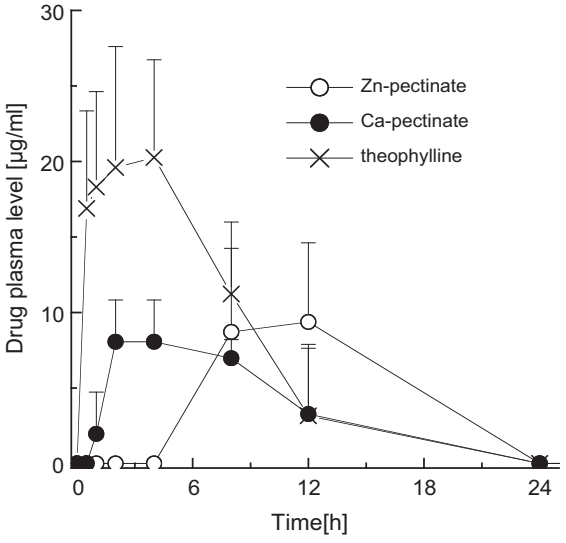

Fig. 5. Drug plasma levels versus time for theophylline suspension (cross symbol) as reference product, Ca-pectinate (full symbol) or Zn-pectinate (empty symbol) beads for $n = 3$ rats.

Table 2
Main theophylline pharmacokinetic parameters after oral administration to rats of different formulations: theophylline suspension, Ca-pectinate beads, Zn-pectinate beads ($n = 3$; data are given as mean ± SD).

	Theophylline suspension	Ca-pectinate beads	Zn-pectinate beads
t_{max} [h]	2.5 ± 2.1	6.0 ± 2.8[a]	12.0 ± 0.0[a,b]
C_{max} [μg/ml]	21.4 ± 8.0	8.9 ± 1.5	9.4 ± 5.2
AUC_{0-24} [μg/ml h]	151.3 ± 9.8	64.0 ± 25.3[a]	53.6 ± 32.6[a]
F rel [%]	100.0 ± 3.2	52.3 ± 16.7[a]	35.4 ± 11.6[a]

[a] Significantly different from theophylline suspension group.
[b] Significantly different from Ca-pectinate beads suspension group.

The higher water content in Ca-pectinate compared to Zn-pectinate beads was attributable to the higher hygroscopicity of Ca^{2+} ions (Bourgeois et al., 2002) and could therefore be extrapolated as a higher water affinity of the entire matrix structure. This water affinity could influence bead swelling during dissolution (Assifaoui et al., 2011) and also bead stability during storage.

In release studies Ca^{2+} ions undergo an exchange mechanism, which might be a dominant factor for pectinate bead disintegration. When calcium beads are placed in the phosphate buffer, Na^+ ions replace Ca^{2+} ions in the binding with carboxyl groups and as electrostatic repulsion among carboxyl groups increases, an enhanced chain relaxation followed by a stronger gel swelling is observed (Bajpai and Sharma, 2004).

In addition to that the capture of the Ca^{2+} ions by phosphate ions present in the dissolution medium change the equilibrium towards this medium which further accelerates the disintegration of the alginate and pectinate bead structure, respectively (Del Gaudio et al., 2005; Sriamornsak and Kennedy, 2008).

In opposite, Zn-pectinate interaction is apparently stronger and subsequently, beads remain stable in dissolution medium. Similar to our observation Zn-pectinate beads has already been mentioned to delay drug release in comparison with Ca-pectinate beads (El-Gibaly, 2002; Atyabi et al., 2005). Although Zn^{2+} is often described as a stronger cross-linker than Ca^{2+} (Atyabi et al., 2005), other possible explanations for the stronger intra-network interactions have been discussed. Since Zn^{2+} possesses a lower coordination number than Ca^{2+}, zinc ions did not participate in the formation of pectinate gel according to the "egg-box" model but the gel formation seemed to be due to strong hydrogen bond interactions and hydrophobic interactions between pectin chains (Assifaoui et al., 2010).

When Zn-pectinate beads were tested in phosphate buffer the formation of crystals are observed (Fig. 4). This phenomenon has been reported recently however without any further analysis or explanation (Khoder et al., 2009). So, the Zn-pectinate bead surface was analyzed with X-ray diffractions at the end of the dissolution experiment. The diffractogram data (Fig. 6) showed the presence of peaks associated to zinc phosphate crystals ($Zn_3(PO_4)_2{\cdot}2H_2O$) and $ZnHPO_4$ which come from the interaction between Zn^{2+} present on the bead surface and $H_2PO_4^-$ into the dissolution medium according to the following reactions (I) and (II):

$$3Zn^{2+} + 2H_2PO_4^- + 2H_2O \rightarrow (Zn_3(PO_4)_2{\cdot}2H_2O) + 4H^+ \quad \text{(I)}$$

$$Zn^{2+} + HPO_4^{2-} \rightarrow ZnHPO_4 \quad \text{(II)}$$

The observed precipitates (Fig. 4b) were able to completely block further drug release in phosphate buffer or in pectinolytic buffer (Fig. 2), which indicates that the precipitate also efficiently blocked the enzyme's access to the bead surface and degrading the pectin network.

The formation of $Zn_3(PO_4)_2{\cdot}2H_2O$ is enhanced because of its lower solubility (Singh et al., 2002) and has been also described to have coating properties by some authors (Zhang et al., 2008). The unusual crystal shape compared to the needle-like appearance obtained after precipitation in solution (Fig. 4c) is probably related to the presence of pectinate, which may influence the crystallisation of Zn phosphate. It seems however that according to the spherical shape and the high packing density this coating is responsible for the lower water uptake into Zn-pectinate beads and the subsequent decrease of drug release rate as confirming by Assifaoui et al. (2011).

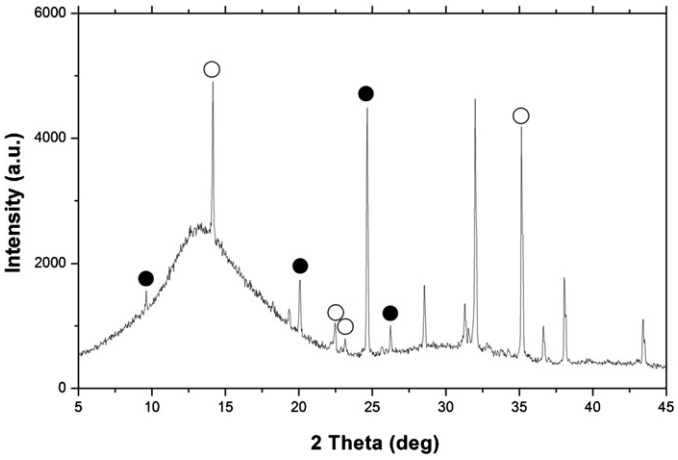

Fig. 6. X-ray diffractions of Zn-pectinate bead surface at the end of the dissolution tests in phosphate buffer. The identification of different peaks was performed by comparing the diffraction patterns with JCPDS reference patterns. Circles and black circles indicated $ZnHPO_4$ (JCPDS file 37-0315) and $Zn_3(PO_4)_2{\cdot}2H_2O$ (JCPDS file 33-1474).

When looking at the in vivo experiments, Ca-pectinate beads undergo several degradation processes during their intestinal passage. Firstly, the carriers hardly resist swelling in the presence of aqueous liquids; especially at elevated pHs a rather accelerated disintegration is observed. Besides, pectinate is substrate of enzymatic degradation of the colonic flora which may complete the bead matrix degradation. With a look to the in vitro release kinetics, it appears questionable whether this effect contributes to the release in vivo especially since t_{max} is reached too early to be in line with the intestinal transit times usually reported from studies with rats (Lamprecht et al., 2004). Zn-pectinate beads showed a more delayed drug release than Ca-pectinate beads with no bioavailability of theophylline within the first 4 h (Fig. 5). This is assumed that bead surface coating by the formation of zinc phosphate also occurs in vivo, thus being responsible for the delayed drug release. This zinc phosphate based "protective" layer around the pectinate beads is apparently maintained until a significant motility omits the coating. The subsequent bead degradation leads to a substantial drug release resulting in a plasma peak after around 12 h, which corresponds to the transit time to colonic tissue in rats.

This phenomenon is however depending on a sufficiently high phosphate ion concentration in lumen in order to allow a reliable and reproducible coating on the beads.

5. Conclusions

Zn-pectinate beads obtained by ionotropic gelation method were evaluated in in vitro dissolution experiments and in vivo pharmacokinetics studies in comparison with Ca-pectinate beads. The in vitro drug release from Zn-pectinate beads was depending on the dissolution media, especially related to the presence of phosphate ions. In phosphate buffer, Zn^{2+} ions form an insoluble zinc phosphate "coating" blocking further theophylline release, even when pectinases were present. Pharmacokinetic data suggested that the zinc phosphate coating also delays the release in vivo by the self-assembling mechanism of the zinc phosphate. Zn-pectinate beads can be proposed as a promising approach for the design of pul-

satile drug delivery systems at the opposite of Ca-pectinate beads showing a too premature release.

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References

Assifaoui, A., Loupiac, C., Chambin, O., Cayot, Ph., 2010. Structure of calcium and zinc pectinate films investigated by FTIR spectroscopy. Carbohydr. Res. 345, 929–933.

Assifaoui, A., Chambin, O., Cayot, Ph., 2011. Drug release from calcium and zinc pectinate beads: impact of dissolution medium composition. Carbohydr. Polym. 85, 388–393.

Atyabi, F., Majzoob, S., Iman, M., Salehi, M., Dorkoosh, F., 2005. In vitro evaluation and modification of pectinate gel beads containing trimethyl chitosan, as a multi-particulate system for delivery of water-soluble macromolecules to colon. Carbohydr. Polym. 61, 39–51.

Bajpai, S.K., Sharma, S., 2004. Investigation of swelling/degradation behaviour of alginate beads crosslinked with Ca^{2+} and Ba^{2+} ions. React. Funct. Polym. 59, 129–140.

Bigucci, F., Luppi, B., Cerchiara, T., Sorrenti, M., Bettinetti, G., Rodriguez, L., Zecchi, V., 2008. Chitosan/pectin polyelectrolyte complexes: selection of suitable preparative conditions for colon-specific delivery of vancomycin. Eur. J. Pharm. Sci. 35, 435–441.

Bourgeois, S., Gernet, M., Andremont, A., Fattal, E., 2002. Design and characterization of pectin beads for the colon delivery. In: Proceedings of the Fourth World Meeting ADRITELF/APGI/APV Florence , pp. 805–806.

Bourgeois, S., Gernet, M., Pradeau, D., Andremont, A., Fattal, E., 2006. Evaluation of critical formulation parameters influencing the bioactivity of [beta]-lactamases entrapped in pectin beads. Int. J. Pharm. 324, 2–9.

Chambin, O., Rota, A., Rochat-Gonthier, M.H., Pourcelot, Y., 2005. Performance of multilayered particles: influence of a thin cushioning layer. Drug Dev. Ind. Pharm. 31, 739–746.

Chambin, O., Dupuis, G., Champion, D., Voilley, A., Pourcelot, Y., 2006. Colon-specific drug delivery: influence of solution reticulation properties upon pectin beads performance. Int. J. Pharm. 321, 86–93.

Das, S., Ng, K.-Y., Ho, P.C., 2010. Formulation and optimization of zinc-pectinate beads for the controlled delivery of resveratrol. AAPS Pharm. Sci. Technol. 11, 729–742.

Del Gaudio, P., Colombo, P., Colombo, G., Russo, P., Sonico, F., 2005. Mechanisms of formation and disintegration of alginate beads obtained by prilling. Int. J. Pharm. 302, 1–9.

Dupuis, G., Chambin, O., Génelot, C., Champion, D., Pourcelot, Y., 2006. Colonic drug delivery: influence of cross-linking agent on pectin beads properties and role of the shell capsule type. Drug Dev. Ind. Pharm. 32, 847–855.

El-Gibaly, I., 2002. Oral delayed-release system based on Zn-pectinate gel (ZPG) microparticles as an alternative carrier to calcium pectinate beads for colonic delivery. Int. J. Pharm. 232, 199–211.

El-Kamel, A.H., Abdel-Aziz, A.A.M., Fatani, A.J., El-Subbagh, H.I., 2008. Oral colon targeted delivery systems for treatment of inflammatory bowel diseases: synthesis, in vitro and in vivo assessment. Int. J. Pharm. 358, 248–255.

Hagesaether, E., Bye, R., Sande, S.A., 2008. Ex vivo mucoadhesion of different zinc-pectinate hydrogel beads. Int. J. Pharm. 347, 9–15.

Khoder, M., Tsapis, N., Huguet, H., Besnard, M., Guentin, C., Fattal, E., 2009. Removal of ciprofloxacin in simulated digestive media by activated charcoal entrapped within zinc-pectinate beads. Int. J. Pharm. 379, 251–259.

Kikuchi, A., Kawabuchi, M., Watanabe, A., Sugihara, M., Sakurai, Y., Okano, T., 1999. Effect of Ca^{2+}-alginate gel dissolution on release of dextran with different molecular weights. J. Control. Release 58, 21–28.

Lamprecht, A., Yamamoto, H., Takeuchi, H., Kawashima, Y., 2004. pH-sensitive microsphere delivery increases oral bioavailability of calcitonin. J. Control. Release 98, 1–9.

Liu, L., Fishman, M.L., Kost, J., Hicks, K.B., 2003. Pectin-based systems for colon-specific drug delivery via oral routes. Biomaterials 24, 3333–3343.

Luppi, B., Bigucci, F., Cerchiara, T., Mandrioli, R., Di Pietra, A.M., Zecchi, V., 2008. New environmental sensitive system for colon-specific delivery of peptidic drugs. Int. J. Pharm. 358, 44–49.

Maestrelli, F., Cirri, M., Corti, G., Mennini, N., Mura, P., 2008. Development of enteric-coated calcium pectinate microspheres intended for colonic drug delivery. Eur. J. Pharm. Biopharm. 69, 508–518.

Mastiholimath, V.S., Dandagi, P.M., Samata Jain, S., Gadad, A.P., Kulkarni, A.R., 2007. Time and pH dependent colon specific, pulsatile delivery of theophylline for nocturnal asthma. Int. J. Pharm. 328, 49–56.

Oliveira, G.F., Ferrari, P.C., Carvalho, L.Q., Evangelista, R.C., 2010. Chitosan-pectin multiparticulate systems associated with enteric polymers for colonic drug delivery. Carbohydr. Polym. 82, 1004–1009.

Roy, P., Shahiwala, A., 2009. Multiparticulate formulation approach to pulsatile drug delivery: current perspectives. J. Control. Release 134, 74–80.

Singh, R., Doolitle, J., Dutta, P.K., 2002. Aqueous transformations of zincophosphate microporous materials: influence of framework topology. J. Phys. Chem. B 106, 2146–2152.

Sinha, V.R., Kumria, R., 2001. Polysaccharides in colon-specific drug delivery. Int. J. Pharm. 224, 19–38.

Sriamornsak, P., Kennedy, R.A., 2008. Swelling and diffusion studies of calcium polysaccharide gels intended for film coating. Int. J. Pharm. 358, 205–213.

Vandamme, T.F., Lenourry, A., Charrueau, C., Chaumeil, J.C., 2002. The use of polysaccharides to target drugs to the colon. Carbohydr. Polym. 48, 219–231.

Yang, L., Chu, J., Fix, J., 2002. Colon-specific drug delivery: new approaches and in vitro/in vivo evaluation. Int. J. Pharm. 235, 1–15.

Yang, L., 2008. Biorelevant dissolution testing of colon-specific delivery systems activated by colonic microflora. J. Control. Release 125, 77–86.

Zhang, S.I, Chen, H.h., Zhang, M.M., 2008. The growth of zinc phosphate coatings on 6061-Al alloy. Surf. Coat. Technol. 202, 1674–1680.