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Drug release from calcium and zinc pectinate beads: Impact of dissolution medium composition

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ABSTRACT

The aim of this study was to investigate drug release from calcium and zinc pectinate beads and to understand the impact of medium electrolytes during drug transfer. A potential drug carrier for colonic drug delivery (rutin) was prepared with calcium and zinc pectinate beads and was tested in three different simulated intestinal fluids (pH 7.3) with phosphates (Sorensen's and Mc Ilvaine's buffers) and without phosphates (Tris-buffer). According to swelling studies and zinc ions release, it was showed that zinc ions keep adhering to the bead surface. Drug release and swelling behaviour from the two dosage forms depend not only on pH and ionic strength but also on the electrolytes there were in the dissolution medium. In calcium pectinate beads, rutin release was faster when phosphate buffers were used because precipitates ($CaHPO_4$) were formed. This precipitate has a pumping effect on the calcium ions, destabilizing the gel structure and enhancing rutin release. In the case of zinc pectinate beads, two kinds of precipitate can be developed depending on the electrolytes composition. The development of $Zn_3(PO_4)_2$ with a coating property reduced rutin release (Sorensen's buffer). On the other hand, development of $ZnHPO_4$ has the pumping effect of zinc ions coming from the beads which increased rutin release (Mc Ilvaine's buffer).

1. Introduction

Targeting drugs to the colon by the oral route can be achieved through different approaches including time-, pH-, enzyme-, or pressure-controlled systems (Sinha & Kumria, 2001; Vandamme, Lenourry, Charrueau, & Chaumeil, 2002; Yang, Chu, & Fix, 2002). Before their local or systemic absorption in the colon, drugs must first of all pass through the stomach (pH ~ 1–2.5), duodenum (pH ~ 6), jejunum (pH ~ 5.5–6.8) and caecum (pH ~ 6.8–7.3) (Vandamme et al., 2002). To ensure specified delivery and good absorption of the drug in the colon site, the dosage form must be formulated by taking into consideration the gastrointestinal tract (large pH variations, enzymes, biliary salts). Low methoxy pectin, a polysaccharide, has the possibility to form gels in presence of some divalent cations (calcium or zinc). It delays drug release in the upper gastrointestinal tract because of its insolubility (Sriamornsak & Nunthanid, 1998). Pectin is a hydrophilic linear polysaccharide extracted from plant cell walls, composed of long sequences of partially methyl-esterified (1–4)-linked α-D-galacturonic residues interrupted by defects of other neutral sugars such as D-xylose, D-glucose, L-rhamnose, L-arabinose and D-galactose. Pectin is usually characterized by its degree of esterification (DE) and in some case by its degree of amidation (DA) which are both expressed as a percentage of esterified and amidated carboxyl groups respectively (Capel, Nicolai, Durand, Boulenguer, & Langendorff, 2006).

In recent years, pectinate beads obtained by ionotropic gelation using divalent cations such as calcium or zinc ions have shown promising forms for colonic drug delivery (Atyabi, Majzoob, Iman, Salehi, & Dorkoosh, 2005; Bourgeois, Gernet, Pradeau, Andremont, & Fattal, 2006; Chambin, Dupuis, Champion, Voilley, & Pourcelot, 2006; El-Gibaly, 2002; Sriamornsak, 1998; Sriamornsak & Kennedy, 2007). It was demonstrated that pectinate beads are less sensitive to simulated gastric fluid pH and they are completely degraded by colonic pectinolytic enzymes (Chambin et al., 2006). A previous study (Chambin et al., 2006) showed that ketoprofen release from calcium beads (CPG) and zinc beads (ZPG) occurred in free enzyme simulated intestinal fluid and that the release from zinc pectinate beads was lower, which concurs with El-Gibaly's work (El-Gibaly, 2002). Atyabi et al. (2005) also showed that the release of Coomassie Brilliant Blue from CPG and ZPG in phosphate buffer (pH 7.3) without enzymes was lower and delayed for zinc pectinate beads. Previous studies of pectinate films (Assifaoui, Loupiac, Chambin, & Cayot, 2010; Wellner, Kacuráková, Malovíková, Wilson, & Belton, 1998) showed that the nature of divalent cations used as cross-linking agents can affect the molecular structure of pectin

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network. This may explain the differences in the dissolution profiles between calcium and zinc pectinate beads as observed by several authors (Atyabi et al., 2005; Chambin et al., 2006; El-Gibaly, 2002). The difference in structure between calcium and zinc pectinate networks could be responsible of the mucoadhesive properties observed in ZPGR. According to Hagesaether, Bye, and Sande (2008), the mucoadhesive properties of pectinate beads can be related to the amount of zinc in the beads after swelling. Another parameter should be taken into account and may explain the difference in dissolution profiles between calcium and zinc pectinate beads, is the possible interaction between these dosage forms and dissolution medium electrolytes.

To predict in vivo performance, an *in vitro* dissolution test should be carried out in perfect sink condition, using a dissolution medium which is representative of physiological fluids in the human body (McConnell, Short, & Basit, 2008). It has been demonstrated that simulating only gastrointestinal pH was not sufficient to mimic the behaviour of the solid dosage form. The ionic composition and buffer capacity of gastrointestinal fluid must be taken into consideration too, (Boni, Brickl, & Dressman, 2007; Fadda, Merchant, Arafat, & Basit, 2009; Hörter & Dressman, 2001; McConnell et al., 2008; Vertzoni, Symillides, Iliadis, Nicolaides, & Reppas, 2003). Small intestinal luminal fluids are buffered by the bicarbonate secreted by the pancreas and intestinal epithelial cells. There are numerous other electrolytes in the luminal fluids (Na^+, K^+, Mg^{2+}, Ca^{2+} and Cl^-) and they play a role in modifying the water structure and drug solubility (Fadda & Basit, 2005). Bicarbonate buffer medium could be physiologically used as a relevant medium for the fasted small intestine. But its suitability for dissolution testing is limited by a difficulty to use the bicarbonate buffer and a poor capacity to reproduce this medium (Boni et al., 2007) because of the continuous rise in pH through evaporation of CO_2 (Fadda et al., 2009). It was demonstrated (Chan, Boswell, & Zhang, 2001; Fadda & Basit, 2005; Ibekwe, Fadda, Parsons, & Basit, 2006) that physiological buffers such as Hanks or Krebs buffers simulate better the intestinal fluid and can be used in this study. However, these buffers contain additional electrolytes such K^+, Mg^{2+}, SO_4^{2-} and Ca^{2+} which can have an influence on the dissolution of drug release. Despite evidence of the influence of the dissolution medium composition on drug release, a phosphate buffer medium (KH_2PO_4–Na_2HPO_4) is still often used in dissolution testing.

In this study, calcium and zinc pectinate beads containing rutin were prepared by ionotropic gelation method. Rutin is a common dietary flavonoid with a wide range of biological and pharmaceutical properties such as free radical scavenging and suppression of cellular immunity. Because rutin is hydrolyzed to its aglycone quercetin by the β-glysosiase enzymes of the colonic microorganisms, both rutin and quercetin have been shown to inhibit tumour development in animal cancer models (Deschner, Ruperto, Wong, & Newmark, 1991; Kim et al., 2005). The drug release of the rutin molecule entrapped in these two matrices was assessed *in vitro* in three different dissolution media (pH = 7.3) with phosphates (Sorensen's and Mc Ilvaine's buffers) and without phosphates (Tris-buffer). To better understand the drug release mechanisms from these two dosage forms as a function of the dissolution medium, complementary studies such as swelling measurements, dissolution calorimetry and cation content in beads during the dissolution test were carried out.

2. Materials and methods

2.1. Materials

Non amidated low methoxy pectin (Unipectin OF 300) with a degree of esterification ranging from 27% to 33% was a gift from Cargill (France). This kind of pectin contains above 80% w/w of galacturonic acid. Rutin, an antioxidant used in this study as a model drug, was obtained from Sigma–Aldrich (France). Calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) and zinc chloride ($ZnCl_2$) were purchased from Merck and VWR prolabo, respectively. All other chemicals were of analytical reagent grade and used as received.

2.2. Preparation of beads

Before use, low methoxyl pectin was purified according to the Yapo method (Yapo, 2009) which consists in washing the pectin with an ethanol solution (85%, v/v). The alcoholic suspension was then filtered and dried at 40 °C for 4 h. The purified pectin (2 g) was then dispersed in 100 mL of acetate buffer (acetic acid/sodium acetate) at pH = 5 *i.e.* 1.5 pH-unit upper pK_a of pectin carboxylic group so as to deprotonate the pectin carboxylic groups (R–COOH/R–COO^-). An accurate amount of rutin (1 g) was added to this dispersion and stirred until it was homogenized. Pectinate beads were manufactured by an ionotropic gelation method (El-Gibaly, 2002). The dispersion (20 g L^{-1} and 10 g L^{-1} of pectin and rutin, respectively) was added drop-wise into a gently stirred solution of the cross-linking agent (calcium chloride or zinc chloride at 100 g L^{-1}), which was pre-adjusted at pH = 5.5 using hydrochloride acid (1 M). The gelled particles thus formed were then allowed to remain in the cross-linking solution for 10 min. Then, the particles were washed with deionized water and dried at 37 °C for 48 h in a drying-room (relative humidity of 40%). Calcium and zinc pectinate beads containing rutin were called CPGR and ZPGR, respectively.

2.3. Swelling measurements

Both CPGR and ZPGR beads were weighed (25 mg) and then placed in different glass test tubes containing 10 mL of the three dissolution media (Sorensen's, Mc Ilvaine's and Tris buffers). They were allowed to swell for a definite period of time at room temperature. The beads were periodically removed and drained with filter paper to remove excess water. Then the change in weight was measured until mass equilibrium had been achieved. The swelling ratio (SR) was calculated using the following formula:

$$\%SR = \frac{w_t - w_0}{w_0} \times 100$$

where w_t is the weight of the beads at a specific time point and w_0 is the initial weight of the dry beads.

2.4. Dissolution studies

Rutin release from the two different beads were performed *in vitro* using a rotating paddle dissolution apparatus (Erweka DT-6) at 50 rpm and 37 ± 0.2 °C. An accurate amount (200 mg) of CPGR or ZPGR beads was introduced into a container with 1 L of dissolution medium. Rutin release was assessed at pH = 7.3 in three buffers: Sorensen's buffer corresponding to a potassium-sodium hydrogeno- and dihydrogenophosphate buffer, Mc Ilvaine's that is a sodium citrate and hydrogenophosphate buffer and Tris-buffer that is a chloride Tris-(hydroxymethyl) aminomethane buffer. The composition of the different buffers is presented in Table 1. The rutin release from these two forms was assayed at various time intervals through UV spectrophotometry at 268 nm up to 5 h.

2.5. Dissolution calorimetry

In order to understand and quantify the heat of interactions which may occur when pectinate beads (CPGR or ZPGR) are in contact with the three different buffers, dissolution calorimetry measurements were carried out isothermally (37 ± 0.1 °C) in a DSC

Table 1
Composition of the buffer systems studied.

Buffer component (mM)	Sorensen	Mc Ilvaine	Tris
$H_2PO_4^-$	13		
HPO_4^{2-}	53	182	
Na^+	106	362	
K^+	13		
Citric acid: $C_6H_8O_7 \cdot H_2O$		9	
Tris(hydroxymethyl) aminomethane			50
HCl			44

III (Setaram Instrumentation, France) fitted with a two-cell mixing system. In this experiment, the liquid (dissolution medium) and the bead (10 mg) were placed separately in the special cell by a cover. The whole was then introduced in the calorimeter and thermally stabilized for at least 1 h. After getting a stable baseline, measurement was initiated by pushing the lid allowing contact between the beads and the medium. The released or absorbed heat was measured in relation to a reference cell containing the same amount of liquid without the beads. A calorimetric curve was obtained and the data were treated for baseline corrections. The area under the curve was integrated yielding the absorbed or released heat (J/g) during the experiment. All the measurements were done at least in triplicate.

2.6. Calcium and zinc analysis

During dissolution tests, the amount of calcium and zinc released was collected in the dissolution medium, each hour and was determined by atomic absorption spectroscopy (ContrAA 700, Analytik Jean, Germany).

2.7. X-ray diffraction

During the dissolution test, precipitate compounds were obtained from CPGR and ZPGR. These compounds were collected and dried at 105 °C for 24 h. They were then studied by X-ray diffractions (XRD) through a Siemens D5000 diffractometer, using CuK_α ($\lambda = 1.5406$ 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}.

3. Results

The dissolution profiles from CPGR and ZPGR beads in different dissolution media are presented in Fig. 1. The release profiles for both calcium and zinc beads are strongly affected by the dissolution media compositions. In Tris-buffer, rutin release was lower than in the other buffers and no significant difference in CPGR and ZPGR dissolution profiles was observed. In Mc Ilvaine's buffer, the release profiles were different from CPGR and ZPGR; however, in these two forms 60% of rutin release was achieved after 3 h of dissolution test. In Sorensen's buffer, rutin release was slower for ZPGR than it was in CPGR.

The swelling behaviour of the two formulations in different dissolution media was different (Fig. 2). Positive swelling ratio refers to the overall swelling and weight gain of beads with water absorption, while negative swelling percentage and downward trend signals the erosion and/or drug release of beads. In CPGR beads, swelling seems to be higher and buffer-dependent (Fig. 2a), while in ZPGR beads, swelling is lower and almost similar irrespective of dissolution medium (Fig. 2b). When Mc Ilvaine's buffer was used, two phases were observed: a swelling phase followed by rapid erosion, occurring after 15 and 60 min for ZPGR and CPGR, respectively. In Sorensen's buffer, the swelling rate and swell ratio were higher in

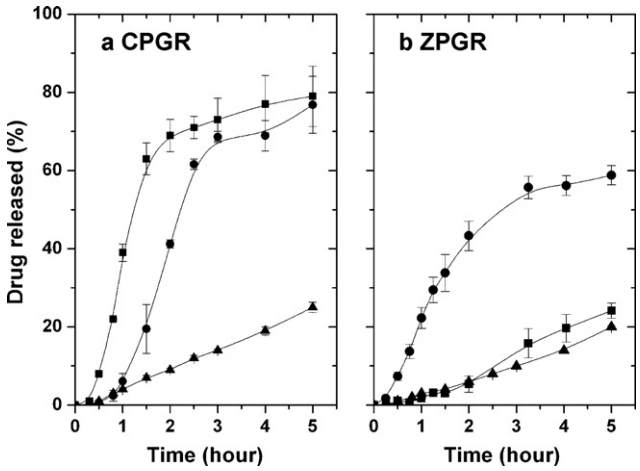

Fig. 1. Release profiles of rutin from calcium and zinc pectinate beads at fixed pH = 7.3 in three different buffers: square: Sorensen's, circle: Mc Ilvaine's and triangle: Tris-buffer.

CPGR than in ZPGR. For Tris-buffer, CPGR beads swelled gradually while the swelling of ZPGR beads was stopped after 15 min.

Calcium and zinc release from both CPGR and ZPGR beads during dissolution tests in different dissolution media were measured (Fig. 3). Results showed that calcium and zinc release were also buffer-dependent. The amount of released calcium was 10 times higher with Tris-buffer than with the other buffers (Fig. 3a). Zinc release from ZPGR was higher in Tris-buffer than it was with Mc Ilvaine's. The amount of released zinc in Sorensen's was the lowest of all (Fig. 3b). The low release of calcium in the two buffers (Sorensen's and Mc Ilvaine's) and zinc in Sorensen's buffer can be explained by the possible interaction with the electrolytes present in the dissolution medium which induce a non-soluble complex development.

It was demonstrated that swelling/dissolution can be investigated using a calorimetric method (Conti, Gaisford, Buckton, & Conte, 2006). The kinetic thermograms for the two dosage forms in three different dissolution media were measured (data not shown). Whatever the dissolution medium and the dosage form, an exothermic peak was observed with different shapes and intensities. This exothermic peak which appears when beads are in contact with the dissolution medium represent the sum of all the events occurring in the vessel during the measurement period. These events include hydration, swelling, interactions with dis-

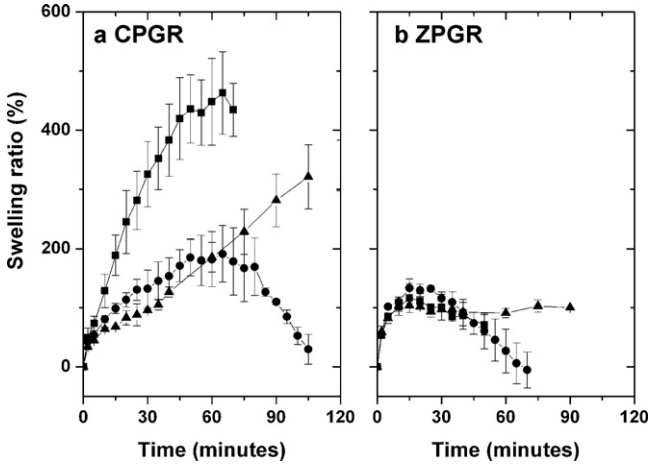

Fig. 2. Swelling behaviour of calcium and zinc pectinate beads in three different buffers: square: Sorensen's, circle: Mc Ilvaine's and triangle: Tris-buffer.

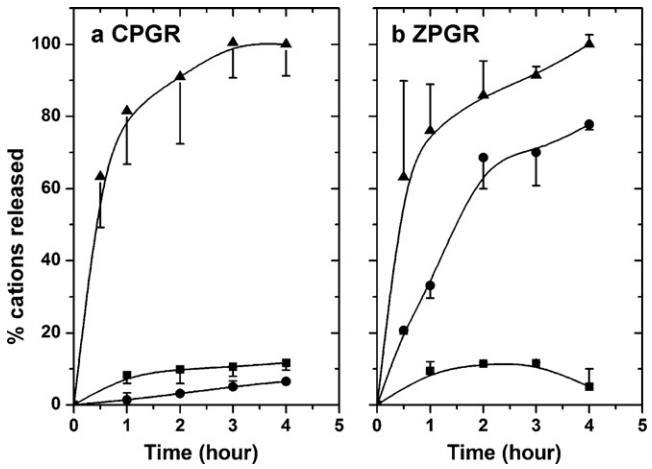

Fig. 3. Calcium and zinc release from both CPGR and ZPGR beads in function of dissolution medium: square: Sorensen's, circle: Mc Ilvaine's and triangle: Tris-buffer.

solution medium, drug dissolution and/or crystallization (Arnot, Minet, Patel, Royall, & Forbes, 2004; Conti et al., 2006; Marabi et al., 2007). The exothermic peak area was calculated and is reported in Fig. 4. For CPGR, the peak area was lower than the ZPGR, whatever the dissolution medium that was used. For Tris-buffer, the peak area was 4 times higher in ZPGR than in CPGR (−46 and −12 J/g, respectively).

4. Discussion

The drug release process from a dosage form can have many causes, such as water penetration into the dosage form, drug and excipient dissolution, changes in drug rate after altered microenvironmental conditions (e.g. pH and ionic strength), diffusion of drugs out of the dosage form in function of the time (Siepmann & Siepmann, 2008). In calcium and zinc pectinate beads, rutin release was buffer-dependent. In Sorensen's buffer, rutin release was slower for ZPGR than it was in CPGR (Fig. 1). This last result is similar to those observed in the literature (Atyabi et al., 2005; Chambin et al., 2006; El-Gibaly, 2002). In phosphate buffer (KH_2PO_4/NaOH, pH 7.3), ketoprofen release from zinc pectinate gel beads was lower in comparison to calcium pectinate beads (Chambin et al., 2006). The authors have attributed these differences to the degree of crosslinking between the two gel types, which could affect the swelling rate of the beads during drug release and consequently the penetration of the solvent into the microparticles.

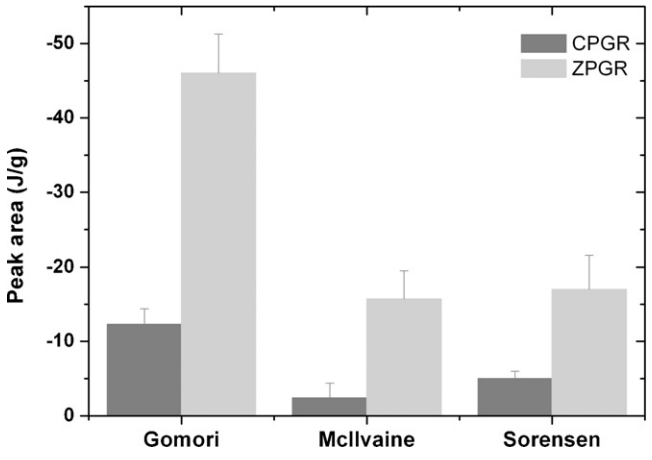

Fig. 4. Peak area (J/g) calculated from the calorimetric dissolution for CPGR and ZPGR in the three dissolution media.

In Tris-buffer, rutin release (Fig. 1) was similar in both CPGR and ZPGR although the swelling behaviour was different (Fig. 2). CPGR beads are still swelling even after 60 min while ZPGR swelling stops after 15 min. Calorimetry studies showed that when ZPGR beads were in contact with Tris-buffer, an exothermic peak was observed; it was 4 times higher than in the CPGR peak (Fig. 4). Since no insoluble complex was observed in Tris-buffer during the dissolution test, it may be assumed that no interactions occurred between cross-linking cations and the buffer constituents. In this case, the differences observed of the swelling could only be attributed to the bead structure and to the calcium and zinc capacities to form different pectin networks. After 3 hours' dissolution, the zinc amount in ZPGR (0.25 mol/100 g of beads) was higher than calcium amount in CPGR (0.1 mol/100 g of beads). The stability of beads mainly depends on the amount of zinc and calcium retained in beads. Beads showed a sufficient stability if they contained a minimal concentration of zinc (0.08 mg/mg beads corresponding to 0.12 mol/100 g of beads (Khoder, Tsapis, Domergue-Dupont, Gueutin, & Fattal, 2010)). Beads were over 33% rutin and 66% (w/w) pectin which has 53% (w/w) of galacturonic units. The molar weight of a galacturonic unit (G) is 183 g/mol and therefore the number of moles in pectin is 0.3 mol/100 g of beads. The cation/G ratios for Ca^{2+} and Zn^{2+} were 0.33 and 0.83, respectively. According to the egg-box model (Grant, Morris, Rees, Smith, & Tho, 1973) 4 galacturonic units in pectin will interact with one divalent cation to form a stable gel which gives a cation galacturonic ratio equal to 0.25 (Braccini & Perez, 2001; Fang et al., 2008). This ratio was higher in the two dosage forms, which showed that all the amounts of cations were not inside the eggbox. The amount of cations which were on the outer layer of the eggbox is higher in zinc pectinate than it is in calcium pectinate. It may be assumed that zinc cations interact with fewer than 4 galacturonic units. Therefore, zinc ions keep located on the bead surface. Cross-linking pectin with zinc is likely to give a structure in which water cannot diffuse inside the network (as the swelling ratio was lower). Calcium ions set up stable interactions with carboxylate groups while the lower coordination of zinc ions prevents a structured gel to develop (Assifaoui et al., 2010). According to the swelling studies, the calcium pectin network could be more stable and more flexible: the swelling ratio keeps going without any degradation of the form as long as one hour (Fig. 2a). The small difference in the calcium galacturonic ratio between the experimental and the theoretical values (0.33 and 0.25, respectively) may be attributed to the presence of some calcium on the bead surface. The number of ions on the surface may be higher in ZPGR than in CPGR. The diffusion of these ions to the dissolution medium may contribute to the exothermic reaction that was observed during dissolution calorimetry. It was demonstrated that hydration, swelling and dissolution of water-soluble substances probably occur at the same time and can contribute to the observed peak (Arnot et al., 2004; Conti et al., 2006; Miyagawa, Ogawa, & Yamano, 1995). The polymer swelling in water is something very complex as it must be considered from the viewpoints of adsorption, absorption, permeation, diffusion, hydration, osmotic pressure, and other factors, (Miyagawa et al., 1995). During the first 5 min, since the drug release profiles were similar to one another, the major difference in the enthalpy values in CPGR and ZPGR (−46 and −12 J/g, respectively) in Tris-buffer may be attributed to the swelling behaviour (Fig. 2), and/or to the high amount of zinc ions present on the surface beads (Fig. 3). A previous study (Miyagawa et al., 1995) showed that the swelling of seaweed food could be endothermic in some cases exothermic. The exothermic reaction triggered by the accompanying hydration of hydrophilic groups in the substance during the endothermic reaction is the sum of the dissolution of some soluble components and of the hydration of

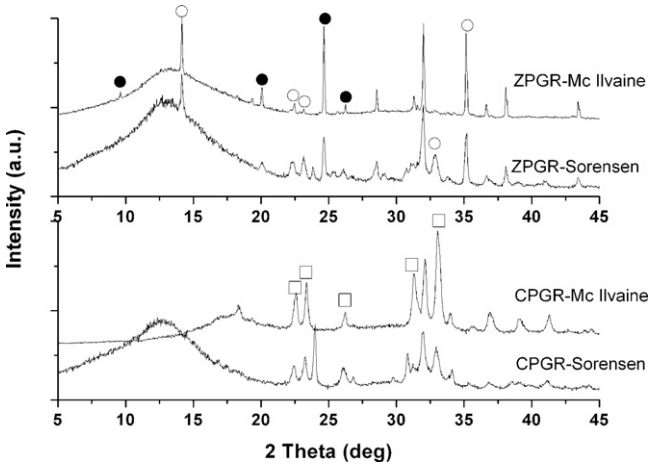

Fig. 5. X-ray diffractions of precipitates which are formed at the end of the dissolution tests in phosphate buffers. The identification of different peaks was performed by comparing the diffraction patterns with JCPDS reference patterns. Empty squares, circles and black circles indicated $CaHPO_4{\cdot}2H_2O$ (JCPDS file 09-0077), $ZnHPO_4$ (JCPDS file 37-0315) and $Zn_3(PO_4)_2{\cdot}2H_2O$ (JCPDS file 33-1474).

the insoluble skeleton of the polysaccharide. More experiments are necessary to determine the processes contributing to the origins of the observed exothermic reaction.

In Sorensen's and Mc Ilvaine's buffers, non-soluble complexes were observed during the dissolution test. In CPGR beads, the calcium release was very low (Fig. 3). Cations diffuse from the bead surface and then interact with the phosphate ions present in the dissolution medium to make a precipitate. In both buffers two types of phosphate ions are present ($H_2PO_4^-$ and HPO_4^{2-}) with a high proportion of HPO_4^{2-} for Mc Ilvaine's buffer (Table 1). Moreover, in both buffers, the released calcium from CPGR got quickly entrapped by phosphate ions inducing the creation of a calcium concentration gradient. Therefore, phosphate ions have a pumping effect on the calcium ions, destabilizing the gel structure and enhancing rutin release (Fig. 3a). However differences in the dissolution profiles and the swelling behaviour in Sorensen's and Mc Ilvaine's buffers came from the difference in the composition and buffer capacity of the dissolution media (Chan et al., 2001; Fadda & Basit, 2005; Hörter & Dressman, 2001; Vertzoni et al., 2003). It is noted that the pK_a of phosphate ($H_2PO_4^-/HPO_4^{2-}$) citrate and Tris are respectively 7.1, 6.4 and 8.2. All these pK_as vary at least by one unit around 7.3, which indicates that their buffer capacities are acceptable. During the dissolution test, rutin release is governed by the swelling ratio and the making of the precipitate. With Mc Ilvaine's buffer, the amount of phosphate ions was higher, then the precipitate formed faster inducing a limitation in the swelling rate (Fig. 2a). This kind of limitation may be responsible for the delayed rutin release as observed in Fig. 1a. In calorimetry studies (Fig. 4) the exothermic peaks are lower when dissolution medium contained phosphate ions (5 and 2.4 J/g for Sorensen's and Mc Ilvaine's buffers respectively). This can be explained by the formation of a non-soluble complex which is thermodynamically unfavoured (endothermic reaction). The precipitate formed during the dissolution test was collected, dried up and characterized using X-ray diffraction (Fig. 5). In the X-ray diffraction picture, most of the peaks were identified as peaks corresponding to formed crystals ($CaHPO_4{\cdot}2H_2O$) following Eq. (1):

$$Ca^{2+} + HPO_4^{2-} + 2H_2O \rightarrow (CaHPO_4{\cdot}2H_2O) \quad (1)$$

$CaHPO_4{\cdot}2H_2O$ can easily be formed in aqueous solutions, using sodium phosphate and potassium phosphate and aqueous solutions containing calcium ions at room temperature, followed by drying at 37 °C (Tas & Bhaduri, 2004). It is noted that the solubility of ($CaHPO_4{\cdot}2H_2O$), Ksp, is about $10^{-6.6}$ (easily soluble in water) (Markich, Brown, & Jeffree, 2001).

In ZPGR beads, the diffractogram data (Fig. 5) 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 $H_2PO_4^-$ according to the following reactions (2) and (3):

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

$$Zn^{2+} + HPO_4^{2-} \rightarrow ZnHPO_4 \quad (3)$$

The development of ($Zn_3(PO_4)_2{\cdot}2H_2O$) is promoted because of its low solubility, Ksp, being reported in the order of 10^{-35} (Singh, Doolittle, & Dutta, 2002; Zhang, Chen, Zhang, & Zhang, 2008). In addition, ($Zn_3(PO_4)_2{\cdot}2H_2O$) has a coating property which accounts for the layer that is visible to the naked eye on the bead surfaces during the dissolution experiment. This coating property can be responsible for the ZPGR beads low swelling ratio (Fig. 2b). In Sorensen's buffer, the amount of zinc released is very low (Fig. 3b), which accounts for the growth of a low solubility precipitate on the bead surface. The development of this layer may explain the low release of rutin in this dissolution medium (Fig. 1b). In Mc Ilvaine's buffer, the amount of HPO_4^{2-} is higher than in Sorensen's, which can favour the development of $ZnHPO_4$ instead of ($Zn_3(PO_4)_2{\cdot}2H_2O$) with no coating properties. It was demonstrated that $ZnHPO_4$ is more soluble than ($Zn_3(PO_4)_2{\cdot}2H_2O$) with Ksp equal to $10^{-6.6}$ (Markich et al., 2001). In this case, the development of $ZnHPO_4$ acts as a pumping effect of zinc ions from the beads and then increases rutin release as $CaHPO_4$.

5. Conclusion

Calcium and zinc pectinate beads containing rutin as a model drug were prepared by ionotropic gelation and the impact of the dissolution composition on drug release, swelling behaviour and calcium or zinc release were investigated. Three dissolution media were used in order to imitate the simulated intestinal fluid (pH = 7.3), with phosphate ions (Sorensen's and Mc Ilvaine's buffers) and without phosphate ions (Tris-buffer). Differences in rutin release and swelling behaviour for both CPGR and ZPGR were observed. These differences can be attributed to the bead structure and to the calcium and zinc capacities to set up different pectin networks. The amount of zinc was 0.25 mol/100 g, whereas the amount of calcium was 0.1 mol/100 g. The higher amount of zinc ions came from interaction with less than 4 galacturonic units according to the 'eggbox' model. Therefore, a significant amount of zinc was still present on the bead surface. The structure of CPGR beads seems to be more stable and more flexible allowing a higher swelling ratio. During the dissolution tests, cations diffused from the bead surface and then interacted with the phosphate ions present in the dissolution medium to make a precipitate. It was noted that the nature of this precipitate can affect the dissolution profile according to its physicochemical properties. Indeed development of ($Zn_3(PO_4)_2{\cdot}2H_2O$) with a coating property reduces rutin release. On the other hand, development of $ZnHPO_4$ has the pumping effect of zinc ions coming from the beads. In this case, rutin release is increased.

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