Carbohydrate Polymers 84 (2011) 1399–1407

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Polyelectrolyte complex of carboxymethyl starch and chitosan as drug carrier for oral administration

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ARTICLE INFO

Article history:
Received 9 November 2010
Received in revised form 20 January 2011
Accepted 25 January 2011
Available online 2 February 2011

Keywords:
Carboxymethyl starch
Chitosan
Polyelectrolyte complex
Coexcipient
Monolithic tablet
Drug delivery

ABSTRACT

A novel polyelectrolyte complex (PEC) of carboxymethyl starch (CMS) and chitosan was prepared, characterized and tested *in vitro* as a carrier for oral drug delivery. This PEC, containing 14% (w/w) of chitosan, showed a polymorphism with a lower order degree than those of CMS and of chitosan. Under conditions simulating the gastrointestinal transit, NMR imaging analysis showed slower fluid diffusion inside PEC monolithic tablets than inside CMS tablets. The PEC seems to be a more suitable drug carrier for colon targeting than CMS, since it can prolong acetaminophen release time from 8 h to 11 h and aspirin release time from 13 h to 30 h. In contrast, chitosan used as a coexcipient accelerated aspirin release from matrices based on a CMS:chitosan physical mixture.

1. Introduction

Since carboxymethyl starch (CMS) was proposed (Mulhbacher, Mateescu, & Calinescu, 2004) as an excipient for controlled drug release from oral solid dosage forms (tablet), several studies have been undertaken in order to investigate the properties and the efficiency of this excipient. The influence of the degree of substitution (DS), of the degree of protonation, and of the formulated drug type and loading on release kinetics of small molecules from CMS matrices has been recently studied (Assaad & Mateescu, 2010; Assaad, Azzouz, & Mateescu, 2008; Ispas-Szabo, De Koninck, Calinescu, & Mateescu, 2007; Lemieux, Gosselin, & Mateescu, 2009). Moreover, the effects of certain formulation parameters, such as compression force and NaCl electrolyte particle size, on drug release rate have been investigated (Brouillet, Bataille, & Cartilier, 2008; Nabais et al., 2007). CMS has also been suggested for the formulation of large size bioactive agents, such as pancreatic enzymes (α-amylase, lipase and trypsin) (Massicotte, Baille, & Mateescu, 2008), *Escherichia coli*, filamentous surface proteins of *Escherichia coli* (F4 *fimbriae*) and *Lactobacillus rhamnosus* probiotic (Calinescu & Mateescu, 2008; Calinescu, Mulhbacher, Nadeau, Fairbrother, & Mateescu, 2005; Calinescu, Nadeau, Mulhbacher, Fairbrother, & Mateescu, 2007). These studies have shown that CMS can reduce the damaging effect of the acidity of gastric medium on bioactive agents and affords a controlled drug release in intestinal medium. In simulated gastric fluid (SGF, pH 1.2), the CMS in the outer layer of tablet is protonated, making the matrix compact. At higher pH (simulated intestinal fluid, SIF, pH 6.8), the carboxyl groups are deprotonated and ionized, thus favoring hydration, swelling and finally solubilisation of tablet. The solubility of CMS in neutral medium (SIF) and its digestion by pancreatic α-amylase can be limiting factors to effect a sustained drug release (Assaad & Mateescu, 2010; Calinescu & Mateescu, 2008). With the aim to ensure a longer time of drug release and targeting to the colon, chitosan dry powder has been used as a coexcipient in such formulations (Calinescu & Mateescu, 2008; Leonida & Mateescu, 2006). Chitosan has been shown to interact with unmodified starch via intermolecular hydrogen bonds, leading to the formation of chitosan–starch complex (Xu, Kim, Hanna, & Nag, 2005).

There is a recent growing interest for polyelectrolyte complexes of chitosan due to its cationic character and biocompatibility (Chen & Fan, 2007): some of them have been proposed for delivery of bioactive agents, such as chitosan–xanthan complexes (Chitoxan TM) for controlled drug delivery (Chellat et al., 2000); chitosan–carboxymethyl konjac glucomannan and

Abbreviations: CMS, carboxymethyl starch; PEC, polyelectrolyte complex; SGF, simulated gastric fluid; SIF, simulated intestinal fluid; DS, degree of substitution; DDA, degree of deacetylation; DC, dry-coated; 50% CMS:50% chitosan, an excipient containing 50% (w/w) of CMS and 50% (w/w) of chitosan; $t_{90\%}$, time (h) for the release of 90% of drug.

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doi:10.1016/j.carbpol.2011.01.048

chitosan–heparin for delivery of albumin (Du et al., 2005; Liu, Jiao, Liu, & Zhang, 2007); chitosan–dextran sulfate and chitosan–alginate for oral delivery of insulin (Sarmento et al., 2006); chitosan–polyaspartate for delivery of 5-fluorouracil (Zheng et al., 2007). A number of polyelectrolyte complexes of chitosan and polyuronans have been prepared and spray-dried as microspheres (Muzzarelli, Stanic, Gobbi, Tosi, & Muzzarelli, 2004).

The objectives of the present study are (i) to prepare CMS–chitosan polyelectrolyte complex (PEC) and to investigate its performance in drug delivery; (ii) to evaluate the influence of chitosan molecular weight on drug release rate; (iii) to compare the drug dissolution from tablets based on anionic water-soluble excipient (CMS) alone, on cationic water-insoluble excipient (chitosan) alone, on physical mixture powder of these two excipients, or on PEC; and (iv) to compare the dissolution profiles of drugs with different charges and solubilities.

2. Materials and methods

2.1. Materials

High amylose corn starch (Hylon VII) was obtained from *National Starch* (Bridgewater, NJ, USA) and crab shell chitosans were from *Marinard Biotech* (Rivière-au-Renard, QC, Canada). Acetaminophen was from *Sigma-Aldrich* (St-Louis, MO, USA). Metformin (1,1-dimethylbiguanide hydrochloride) was from *MP Biomedicals* (Solon, OH, USA). Aspirin (acetylsalicylic acid) and monochloroacetic acid were from *Fisher Scientific* (Fair Lawn, NJ, USA). The other chemicals were of reagent grade and used without further purification. Pepsin-free simulated gastric fluid (SGF, pH 1.2) and pancreatin-free simulated intestinal fluid (SIF, pH 6.8) were prepared following the USP methods (US Pharmacopeia, XXIV, 2000).

2.2. Preparation of CMS and purification of chitosans

Sodium carboxymethyl starch (CMS) was prepared in aqueous medium from high amylose corn starch as previously described (Calinescu et al., 2005; Mulhbacher, Ispas-Szabo, Lenaerts, & Mateescu, 2001), with minor modifications. Briefly, an amount of 70 g of Hylon VII was suspended in 170 mL of distilled water in a Hobart mixer (Vulcan, Canada) at 55 °C. Then, 235 mL of 1.5 M NaOH were added for gelatinization under continuous mixing for 30 min. Subsequently, 55 mL of 10 M NaOH and a freshly prepared solution of monochloroacetic acid (45.5 g in 40 mL of distilled water) were added. After 1 h of reaction, a volume of 130 mL of distilled water was added and the slurry was cooled-down to room temperature and neutralized with acetic acid. The CMS was then precipitated from the slurry by gradually adding 600 mL of acetone. After that, the CMS was washed by repeated dispersion in volumes of 1 L of 70% acetone and filtrations until a final conductivity of filtrate decreased at about 50 μS/cm. The CMS mass was again washed three times with acetone, and then dried at 40 °C for 24 h. The obtained powder of sodium form CMS was sieved with a 300 μm screen and stored at room temperature.

Two chitosans of different molecular weights were each purified by solubilization in acetic acid and by filtration as follows: an amount of 20 g of chitosan was solubilized in 350 mL of 0.35 M acetic acid and the volume was adjusted to 2 L with distilled water. The acidic solution was filtered under vacuum through Whatman filter papers (medium 40). Subsequently, the chitosan was precipitated with 0.1 M NaOH under continuous stirring. The mass was washed with distilled water, then with nanopure water (volumes of 2 L) until conductivity of about 200 μS/cm and finally with acetone. The chitosan was dried at 40 °C for 24 h, ground and sieved on a 300 μm screen.

2.3. Preparation of CMS–chitosan PEC

A CMS–chitosan polyelectrolyte complex (PEC) was prepared by coagulation of CMS and chitosan-700 in aqueous medium at room temperature. Essentially, 1 g of chitosan-700 was solubilized in 44 mL of 0.1 M HCl, and the volume was adjusted to 150 mL with distilled water. A 1% solution of CMS was prepared by solubilizing 6 g of CMS in 600 mL of distilled water. The precipitation occurred under vigorous mixing by adding the solution of polycation (chitosan-700) to that of polyanion (CMS) at 1:1 ratio ($-NH_3^+$:$-COO^-$), with a final pH of about 5. The PEC, containing 14% (w/w) of chitosan-700, was washed and dried with acetone by the same procedure as for CMS.

2.4. Physical and chemical characterizations of excipients

2.4.1. The degree of substitution

The degree of substitution (DS) of CMS was determined by back-titration as previously described (Assaad & Mateescu, 2010). Briefly, 300 mg of protonated CMS ($n = 3$) were solubilized in 20 mL of 0.05 M NaOH and then the excess of NaOH was titrated with 0.05 M HCl using phenolphthalein as indicator. The blank (20 mL of NaOH) was also titrated by the same method. The amount of –COOH groups and the DS were calculated by using the following equations (Stojanovic, Jeremic, Jovanovic, & Lechner, 2005):

$$n_{COOH} = (V_b - V) \times C_{HCl} \quad (1)$$

$$DS = \frac{162 \times n_{COOH}}{m - 58 \times n_{COOH}} \quad (2)$$

where V_b (mL) is the volume of HCl used for the titration of the blank; V (mL) is the volume of HCl used for the titration of the sample; C_{HCl} (mol/L) is the concentration of HCl; 162 (g/mol) is the molar mass of glucose unit; 58 (g/mol) is the increase in the mass of glucose unit by substitution with one carboxymethyl group, and m (g) is the mass of dry sample.

2.4.2. The degree of deacetylation

The degree of deacetylation (DDA) of each chitosan was determined by acid-base titration. An amount of 150 mg of chitosan was solubilized in 20 mL of 0.1 M HCl and the volume was completed to 200 mL with distilled water. A titration was done with 0.1 M NaOH and the pH and the conductivity were recorded. The DDA was calculated following the method and the equation given by Broussignac (1968) and Muzzarelli (1977):

$$DDA(\%) = \frac{203 \times (v_2 - v_1) \times M \times 100}{m + 42 \times (v_2 - v_1) \times M} \quad (3)$$

where V_1 and V_2 are the volumes of NaOH solutions corresponding to the two inflexion points of the curve obtained by titration; M is the concentration of NaOH (mol/L); m is the weight of chitosan (g); 203 (g/mol) is the molar mass of acetylated unit, and 42 (g/mol) is the difference between molar mass of acetylated unit and that of deacetylated unit.

2.4.3. The molecular weights

The molecular weights of chitosans were determined by viscometric method, using experimental reported viscometric constants data (Kasaai, 2007; Knaul, Kasaai, Bui, & Creber, 1998). Samples were dissolved in a solution containing 0.1 M acetic acid and 0.2 M sodium chloride for chitosan-400 and in a solution containing 0.2 M acetic acid and 0.1 M sodium acetate for chitosan-700. The viscosities of chitosan solutions with different concentrations (0.07–0.7%)

were measured by using an electronic viscometer (*Viscosity Monitoring and Control Electronics*, Medford, MA, USA). The temperature was adjusted at 25 °C for chitosan-400 and at 30 °C for chitosan-700 as reported elsewhere (Roberts & Domszy, 1982; Wang, Bo, Li, & Qin, 1991).

The data on viscosities and concentrations were used to calculate the reduced viscosities. Plotting reduced viscosities against chitosan concentrations gives the intrinsic viscosity ($[\eta]$) by extrapolation of the straight line obtained by linear regression to zero concentration. The average molecular weight (M) of chitosan was calculated from the intrinsic viscosity by Mark–Houwink–Sakurada's empirical equation:

$$[\eta] = kM^{\alpha} \quad (4)$$

where k (dL/g) and α (dimensionless) are constants that depend on the solvent-polymer system.

2.4.4. The Fourier transform infrared spectra

The Fourier transform infrared spectra (FTIR) of the samples were recorded from 4000 to 400 cm^{-1} at 2 cm^{-1} resolution with a total of 32 scans by using a *Nicolet* 4700 spectroscopy (Madison, WI, USA). To prepare the pellets, homogenous mixtures of dried KBr (67 mg) and of polymer powders (3 mg) were compressed at 3 tonnes (*Carver*, Wabash, IN, USA) in flat-faced punches with 12 mm diameter.

2.4.5. The polymorphism

The polymorphism of samples was evaluated by X-ray diffractometer (XRD, *Siemens* D5000, Munich, Germany) at 1.789 Å wavelength. The original XRD spectra, recorded between 5° and 50° (2θ), were treated using Excel software (regression type: moving average, period 10).

2.4.6. The thermogravimetric analyses

The thermogravimetric analyses were carried out in platinum crucible at a heating rate of 10 °C/min between 25 and 900 °C under nitrogen atmosphere (flow rate 100 mL/min). A *Seiko* TG/DTA 6200 (Japan) instrument was used and the alumina was taken as reference material.

2.4.7. The morphology

The morphology of the sample particles was examined by a *Hitachi* (S-4300SE/N) scanning electron microscopy with variable pressure (Hitachi High Technologies America, Pleasanton, CA, USA) at voltage of 15 kV and magnifications of 100× and 500×. Samples were mounted on metal stubs and sputter-coated with gold.

2.4.8. The density

The density of the polymer powders was determined according to the (616) USP method, using a Vankel tapped density tester (*Varian*, NC, USA).

2.5. Preparation of tablets

Monolithic tablets (200 mg, 20% (w/w) loading) were obtained by direct compression (2.5 tonnes) of a homogenous mixture of excipient and drug (acetaminophen, metformin or aspirin) powders. The unloaded (drug-free) tablets of 200 mg were prepared with excipient only. Flat-faced punches with 9.6 mm diameter and a *Carver* hydraulic press were used.

Dry-coated (DC) tablets (200 mg, 20% (w/w) loading) were prepared with a core consisting in a homogenous mixture of drug (40 mg) and excipient (40 mg) and compressed in a 7 mm cylinder outfit. This core was then dry coated with 120 mg of excipient, giving tablet of about 9.6 mm diameter and 2.1 mm thickness after compression.

2.6. Nuclear magnetic resonance (NMR) imaging analysis

NMR imaging analyses were carried out at 37 °C with a *Bruker* Avance-400 NMR spectrometry (Germany) as previously reported (Baille, Malveau, Zhu, & Marchessault, 2002; Malveau, Baille, Zhu, & Marchessault, 2002; Thérien-Aubin & Zhu, 2006, 2009; Thérien-Aubin, Baille, Zhu, & Marchessault, 2005; Thérien-Aubin, Zhu, Ravenelle, & Marchessault, 2008; Wang, Ravenelle, & Zhu, 2010). A standard spin-echo pulse sequence (90-τ-180-τ-Acquisition) was used to obtain spin density images of the unloaded tablets ($n = 3$) in a NMR tube (20 mm diameter) containing 20 mL of dissolution media (SGF or SIF). A slice of 0.5 mm in thickness was selected either perpendicular or parallel to the main magnetic field (axial axis). Eight scans were accumulated with a field of view of 2 cm and an in-plane resolution of 156 μm. An echo time of 3 ms and a repetition time of 1 s were fixed, leading to an acquisition time of about 17 min for each image. Each tablet was first incubated for 2 h in SGF and then in SIF until the end of the test. The percentage of axial and radial swelling was calculated by comparison to the initial dimension of the tablet.

2.7. In vitro dissolution tests

The *in vitro* dissolution tests were carried out at 100 rpm and 37 °C in an USP dissolution apparatus II (*Distek* 5100, North Brunswick, NJ, USA) coupled with an UV spectrophotometer (*Hewlett Packard* 8452A, USA). The tablets ($n = 3$) were incubated in SGF (1 L) for 2 h and then in SIF (1 L) up to complete release. The drug release from tablets was evaluated by measuring the absorbance at the appropriate wavelength (acetaminophen at 244 nm, metformin at 218 nm, and aspirin at 246 nm).

3. Results and discussion

3.1. Characterization of the excipients

The degree of substitution of carboxymethyl starch (CMS) determined by the back-titration method was about 0.14, representing the average number of carboxymethyl groups per glucose unit. The degrees of deacetylation of chitosans determined by acid-base titration were about 80% and the approximate molecular weights determined by Mark–Houwink–Sakurada method were about 400 kDa for chitosan-400 and 700 kDa for chitosan-700.

The scanning electron microscopy micrographs showed that chitosan particles were compact, whereas those of CMS and PEC were porous (Fig. 1). The morphology of the polyelectrolyte complex (Fig. 1, d1 and d2) appeared homogenous, indicating a uniform distribution and a good compatibility between CMS and chitosan.

Chitosan-400 and chitosan-700 showed the highest tapped densities (0.61 and 0.64 g/mL, respectively) due to their compact morphology, whereas PEC showed the lowest density (0.20 mg/mL) due to its higher granulometry and porosity (Fig. 1). Intermediate density (0.36 mg/L) was found for CMS.

3.2. CMS–chitosan interactions and preparation of PEC

When the chitosan-700 solution was added to the CMS solution, immediate coagulation and precipitation occurred. This suggests effective interactions between functional groups of CMS and of chitosan-700 with possible partial charges neutralization, leading to the formation of a polyelectrolyte complex. To verify this

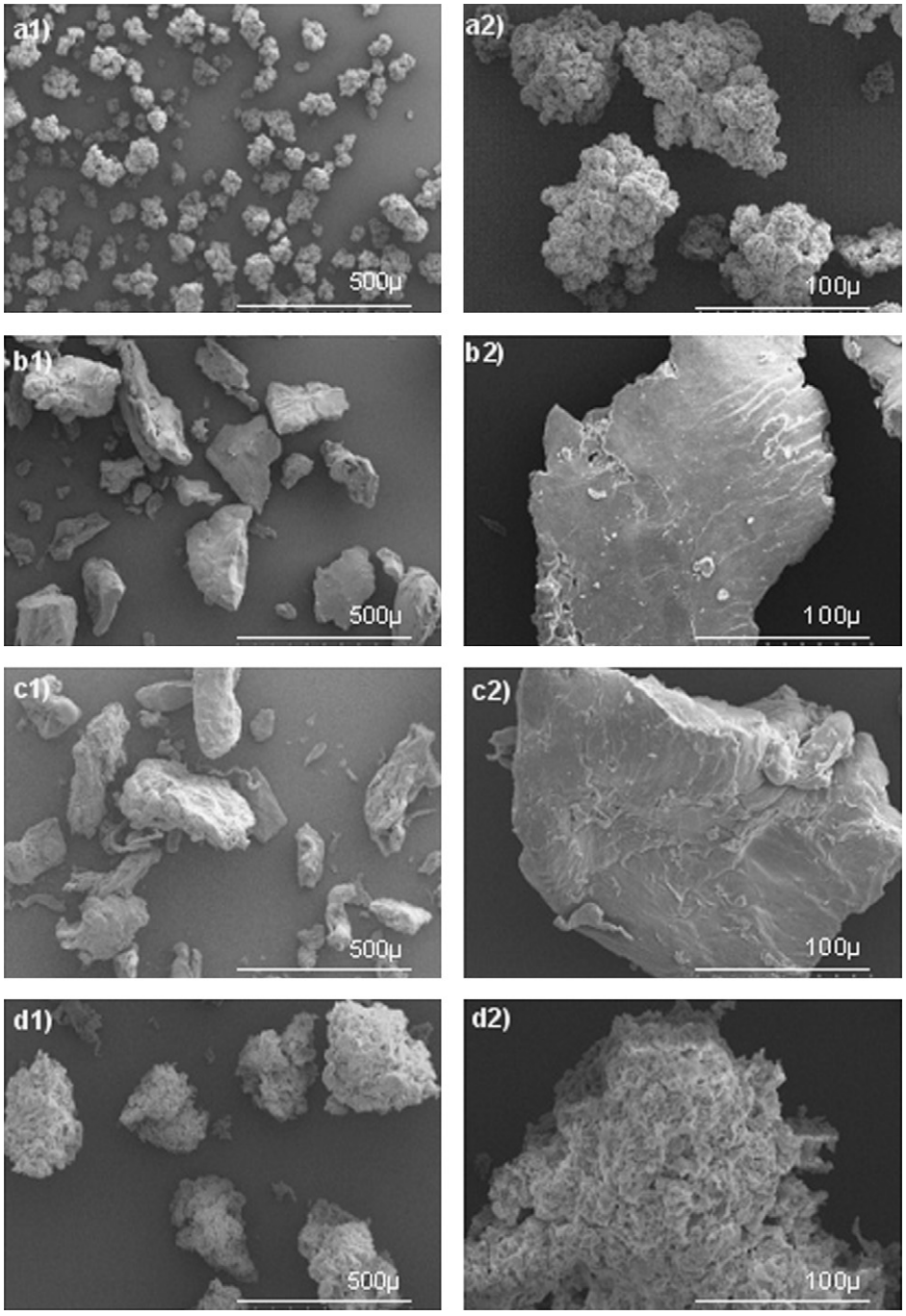

Fig. 1. Scanning electron microscopy micrographs of (a) CMS, (b) chitosan-400, (c) chitosan-700, and (d) PEC at magnifications of 100× and 500× and voltage of 15 kV.

hypothesis, the products were characterized by FTIR spectroscopy, by X-ray diffractometry (XRD) and by thermogravimetry (TGA) (Figs. 2–4).

The FTIR spectrum of CMS (Fig. 2) presents two characteristic bands at 1603 and 1417 cm^{-1}. They were attributed respectively to asymmetrical and symmetrical stretching vibration of $-COO^-$ groups (Silverstein, Webster, & Kiemle, 2005; Zoldakova, Srokova, Sasinkova, Hirsch, & Ebringerova, 2005). The bands at 2930 and 1643 cm^{-1} are assigned respectively to C–H stretching and to O–H groups.

The spectrum of chitosan-700 shows characteristic absorption bands of chitosan at 1653 and 1597 cm^{-1} ascribed to $-CONH_2$ stretching vibrations, and two bands at 2922 and 2876 cm^{-1} due to C–H stretching. The bands at 1417 and 1376 cm^{-1} were assigned to the C–H symmetrical deformation mode as per Mathew and Abraham (2008).

The polyelectrolyte complex (PEC) shows a spectrum similar to that of 50% CMS:50% chitosan-700, with bands at about 2923–2880, 1636, 1600, 1417 and 1376 cm^{-1}. This indicates the presence of both CMS and chitosan in the PEC. Roughly similar spectra of dry blend polymer powders and polyelectrolyte complexes were reported in other studies, as for chitosan and carboxymethyl cellulose polymers (Fukuda, 1980). The weak shoulders at around 1735 and 1540 cm^{-1} for PEC obtained at pH 5 could be assigned respectively to –COOH and $-NH_3^+$ groups. These shoulders suggest that interactions between CMS and chitosan in the PEC may occur via hydrogen bonds (–OH, –COOH) or ionic interactions ($-COO^-$, NH_3^+).

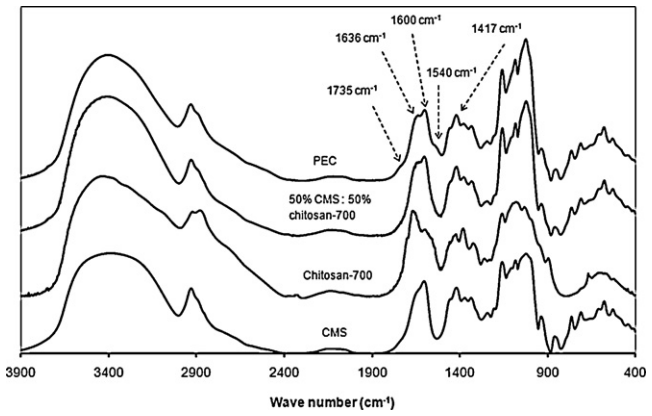

Fig. 2. FTIR spectra of CMS, chitosan-700, 50% CMS:50% chitosan-700, and PEC. Pellets (12 mm diameter) were prepared by compression at 3 tonnes of KBr (67 mg) and sample (3 mg) mixtures.

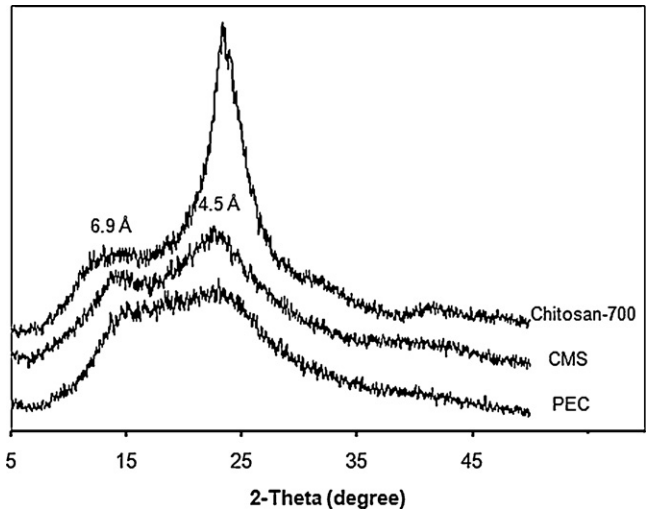

Fig. 3. X-ray diffraction patterns of CMS, chitosan-700, and PEC.

The XRD pattern (Fig. 3) of CMS shows the two characteristic peaks at 6.9 and 4.5 Å, indicating a V-type single helix structure as previously reported (Assaad & Mateescu, 2010). The pattern of chitosan-700 shows characteristic crystalline peaks at around 6.9 and 4.4 Å (major one), fitting well with the typical XRD pattern of chitosan (Choi, Kim, Pak, Yoo, & Chung, 2007; Wang et al., 2005).

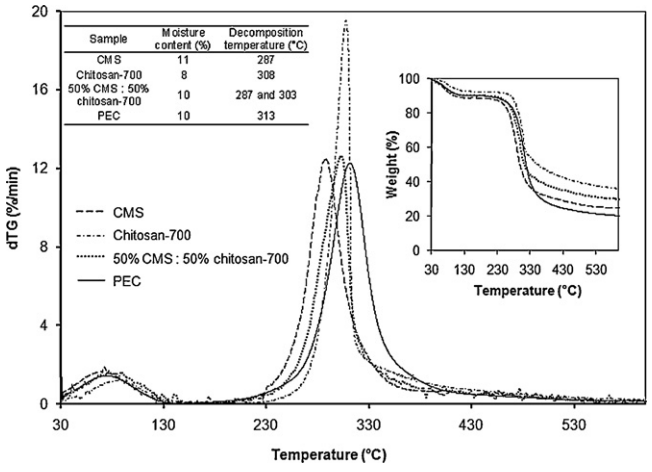

Fig. 4. Thermogravimetric patterns of CMS, chitosan-700, 50% CMS:50% chitosan-700, and PEC at heating rate of 10 °C/min between 25 and 600 °C.

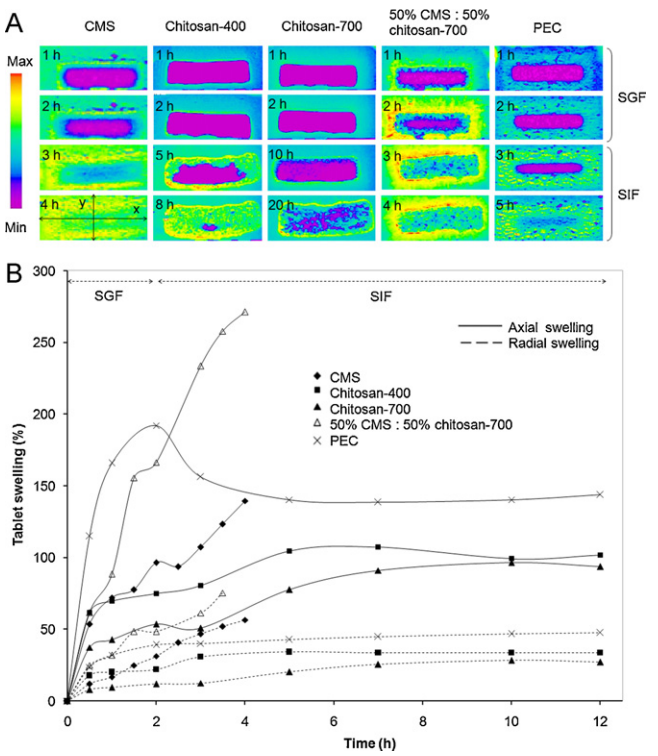

Fig. 5. NMR images at various times of unloaded tablets of (CMS, chitosan-400, chitosan-700, 50% CMS:50% chitosan-700 and PEC) incubated for 2 h in SGF and then transferred to SIF: (A) axial side images where *x* indicates the radial direction and *y* the axial direction, (B) axial and radial swelling. (For interpretation of the references to color in this figure the reader is referred to the web version of the article.)

The order degree of the PEC is definitely lower to those of CMS and chitosan-700. The suppression of crystalline peak of chitosan-700 at 4.4 Å and the broad amorphous pattern of the PEC indicate a good compatibility and strong interactions between CMS and chitosan with a complete dispersion of chitosan chains. These intermolecular interactions could prevent macromolecules to crystallize individually as reported for some interpolymer complexes (Mathew & Abraham, 2008; Sakurai, Maegawa, & Takahashi, 2000; Xu et al., 2005; Yin, Yao, Cheng, & Ma, 1999).

The TGA results (Fig. 4) show relatively lower moisture content for chitosan-700 than for CMS and PEC, maybe due to higher hydrogen association of chitosan chains. The 50% CMS:50% chitosan-700 shows a nonsymmetrical dTG peak with a weak shoulder at around 287 °C and a maximum at 303 °C, indicating the presence of two components. The difference of decomposition temperatures between CMS (287 °C) and chitosan-700 (308 °C) seems not enough to identify two separate peaks for the dry powder mixture of these two polymers. Differing from 50% CMS:50% chitosan-700, the PEC presented a symmetrical dTG peak and the highest decomposition temperature (313 °C).

Overall, these results suggest a good compatibility between CMS and chitosan, a strong interaction between the chains of these two polymers, and the formation of a homogenous polyelectrolyte complex.

3.3. Examination of tablets hydration and swelling by NMR

Water penetration into unloaded tablets (Fig. 5A) and the axial and radial swelling (Fig. 5B) were followed by NMR imaging in SGF for 2 h and then in SIF to simulate the gastrointestinal tran-

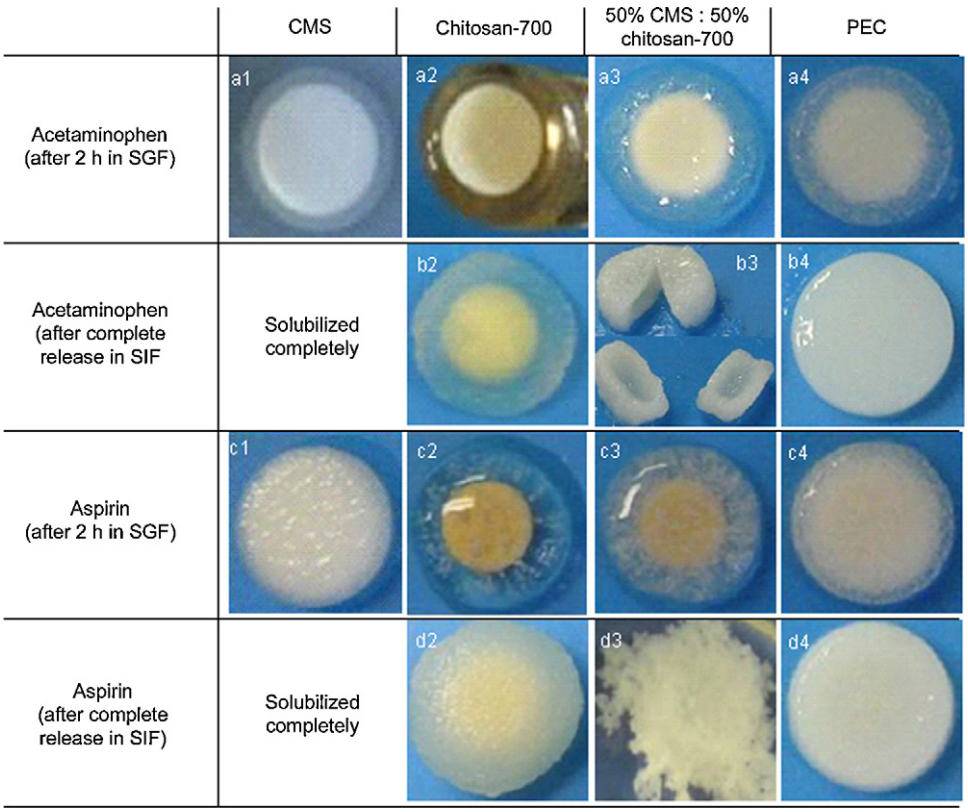

Fig. 6. Photographs of CMS, chitosan-700, 50% CMS:50% chitosan-700 and PEC tablets (200 mg, 20% loading) during dissolution tests (1 L, 37 °C, 100 rpm). Photographs were taken for the tablets, first after 2 h of incubation in SGF and then after the complete drug (acetaminophen or aspirin) release in SIF. The sizes of tablets were not normalized.

sit. The location where the water concentration matches 1/6 of the maximal concentration corresponding to free fluid (SGF or SIF) was considered as the front of fluid diffusion inside the tablets (Baille et al., 2002; Malveau et al., 2002).

For all tablets, the axial swelling was higher than radial swelling (Fig. 5B). This may be explained by the formation of flat oriented particles in tablet after axial compression of polymer powders. Upon tablet hydration, the stress resulting from compression is released, leading to a higher swelling in the direction where compression force was applied (Le Bail, Morin, & Marchessault, 1999; Malveau et al., 2002; Thérien-Aubin & Zhu, 2006, 2009; Thérien-Aubin et al., 2005, 2008; Wang et al., 2010).

After 2 h in SGF, the CMS tablet still showed a dry core (purple) with a partial penetration of SGF and formation of a gel network in the outer layer (blue and green) (Fig. 5A). In acidic medium (SGF, pH 1.2), the carboxylate groups (–COONa) of the outer layer are converted to carboxyl groups (–COOH), thus reducing the solubility of the CMS excipient and limiting the gastric fluid penetration into the tablets. When tablets were transferred to SIF (pH 6.8), the fluid advanced rapidly to the core which became hydrated within 2 h in this neutral medium. The protonation acquired in SGF is lost and the –COOH groups turn into their salt form (–COOK), increasing thus the solubility of the excipient and accelerating intestinal fluid advancement to the core of the tablet. The axial and radial swelling of CMS tablet increase relatively fast, reaching 150% and 60%, respectively, after 4 h of incubation (Fig. 5B).

The diffusion of fluid (SGF or SIF) into the chitosan (chitosan-400 and chitosan-700) tablets was slower than into the CMS tablets (Fig. 5A). A gel network was developed by chitosan in SGF due to the protonation of amino groups exposed to the acid medium. In SIF, the tablet size was stabilized (Fig. 5B) due to chitosan insolubility in neutral medium while an anisotropic fluid diffusion was observed (Fig. 5A). For chitosan-400 the core was almost completely hydrated after 8 h of incubation, whereas for chitosan-700 the core still showed dry regions even after 20 h. Thus, chitosan-700 with a higher molecular weight seems to provide a thicker (more substantial) outer layer gel than chitosan-400.

The tablets of 50% CMS:50% chitosan-700 mixture showed the fastest fluid diffusion (Fig. 5A) and the highest swelling (Fig. 5B). The gel network formed in SGF was less substantial than that formed with chitosan tablets due to close neighboring of CMS in the mixture. In SIF, the chitosan would be deprotonated, whereas the CMS would be converted to the salt form triggering a higher in situ hydration of tablet previously swollen in SGF.

The tablets of PEC presented slower fluid diffusion than CMS and 50% CMS:50% chitosan-700 tablets, particularly in SIF (Fig. 5A). This suggests that association of CMS and chitosan at molecular level as PEC favors more interactions between these two compounds than in physical mixture of powders. A more extensive swelling occurred in the first two hours of incubation in SGF due to the protonation and the hydration of chitosan within the PEC. When SGF was changed to SIF, the size of PEC tablets was reduced due to the deprotonation and dehydration of chitosan chains in neutral medium, indicating a stronger interaction between chitosan and CMS than that between CMS and water. The shape of tablets was after that as stable as those of the chitosans, despite the low ratio (14%) of chitosan in PEC. This is an important aspect and can be related to the insolubility of chitosan in neutral medium and to a lower tendency of CMS to swell when intimately complexed with chitosan.

3.4. *In vitro dissolution tests*

All dissolution tests, except for metformin formulated in monolithic tablets, were followed first in SGF for 2 h and then in SIF until complete drug release, simulating thus the gastrointestinal transit.

The shape of tablets and the dissolution profiles of acetaminophen, metformin and aspirin are presented in Figs. 6 and 7. Unless otherwise specified, the tablets (200 mg) used for dissolution were monolithic.

The release rates of acetaminophen from chitosan-400 and 50% CMS:50% chitosan-400 matrices were higher than from CMS matrix, whereas the release rates from chitosan-700 and 50% CMS:50% chitosan-700 matrices were lower than from CMS matrix (Fig. 7A). That is why the chitosan-700 was chosen to prepare the PEC. In addition, the 75% CMS:25% chitosan-700 matrix showed almost the same release rate as CMS (not shown). It seems that a molecular weight of 700 kDa rather than 400 kDa and an adequate ratio in dry blends are required for chitosan to favor a longer drug release time.

Although the chitosan-700 matrix showed the lowest release rate, chitosan alone does not seem suitable for controlled drug release, because the transformation of the gel developed in SGF (Fig. 6, a2) to a semi-solid form (Fig. 6, b2) that limits the diffusion of SIF into the tablet makes the release slow (Fig. 7A). It is worthwhile to note that the solid core of tablet was still compact and insoluble even after the complete acetaminophen release (Fig. 6, b2).

The faster release from tablets based on CMS:chitosan-700 powder mixture compared to that from those with chitosan-700 as only excipient, indicates that the CMS favors the tablet hydration and accelerates the diffusion of SIF into the tablets. These results are in agreement with those obtained by NMR imaging (Fig. 5A). At the end of the dissolution tests, the tablets based on a mixture of CMS and chitosan powders appeared as a water-insoluble empty shell (Fig. 6, b3) with a crust still containing a mixture of these two polymers as confirmed by FTIR analysis (not shown). This indicates that although the tablets are based on dry blend of polymer powders, physical or chemical interactions can occur between CMS and chitosan during the dissolution.

The release rate of acetaminophen from PEC matrix was lower than that from 50% CMS:50% chitosan-700 matrix (Fig. 7A). This is an interesting advantage for PEC which contains only 14% (w/w) of chitosan-700, considering the higher cost of chitosan compared to that of CMS.

Metformin is a freely soluble drug (US Pharmacopeia, 2000) and its release from hydrophilic excipients is usually fast. Neither CMS nor chitosans, separately or in association, were able to control the release of metformin in monolithic dosage form (Fig. 7B). Dry coated (DC) tablets can delay this release in SGF, especially when the outer part of tablet is based on chitosan-700. However, when the fluid reached the inner core of the tablets the release was accelerated. The dissolution profiles of metformin from tablets based on chitosan-400 were similar to those based on chitosan-700, but with higher release rate (not shown). The dry coating formulation can be of interest, considering the very high hydrosolubility of metformin and the fact that the release is undesired in stomach.

For aspirin, which is slightly soluble in water (US Pharmacopeia, 2000), the higher the molecular weight of chitosan, the lower was the release rate (Fig. 7C). CMS and chitosan-700 provided a low release of aspirin in SGF and a long sustained release in SIF. Probably, this is due to the interactions of carboxyl groups of aspirin with carboxyl groups and hydroxyl groups of CMS, and to the formation of consistent outer layer gel network in tablets based on chitosan (Fig. 6, c2). As with acetaminophen, the dissolution of aspirin from chitosan-700 matrix after 80% of release was reduced probably due to the formation of chitosan insoluble outer layer in SIF (Fig. 6, d2). The matrices based on CMS:chitosan mixture showed an accelerated release of aspirin compared to those based on individual excipient (CMS or chitosan). It seems that the aspirin–CMS and aspirin–chitosan interactions compete and reduce those between these two polymers, favoring a faster

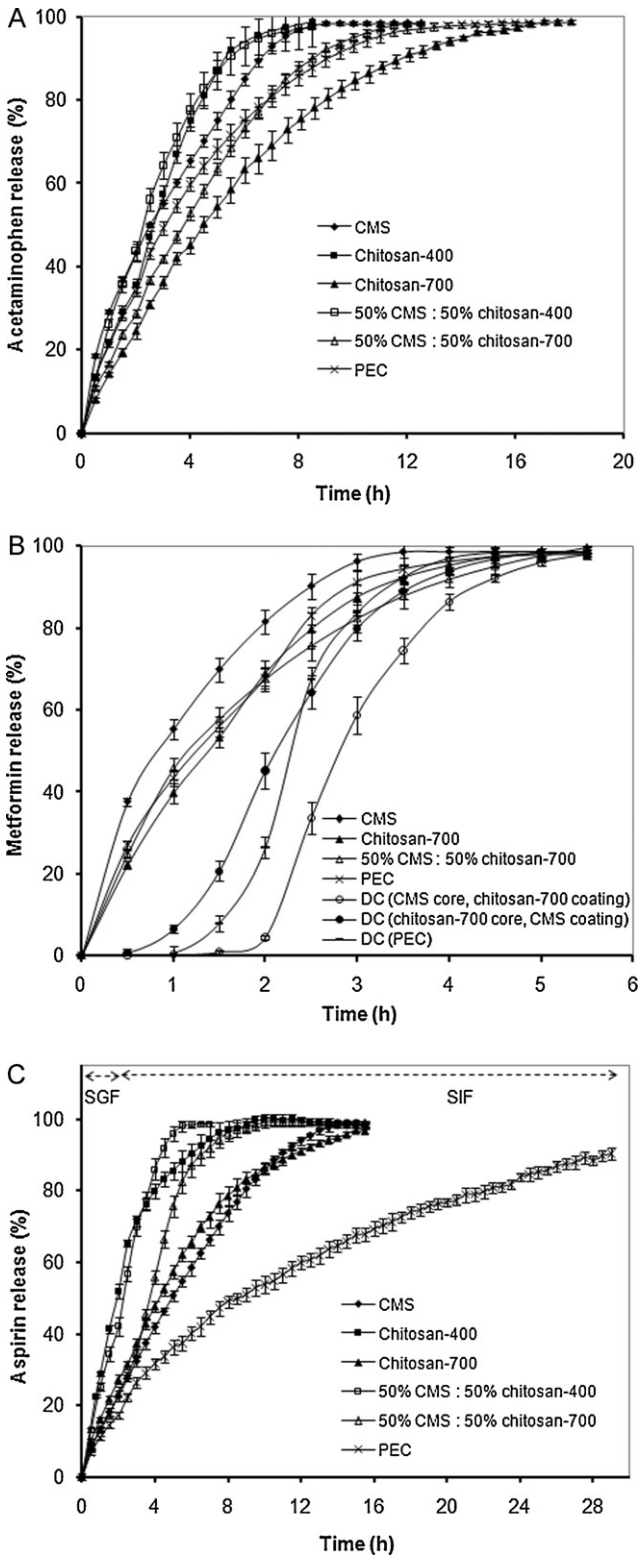

Fig. 7. Kinetics of drug dissolution from tablets (200 mg, 20% loading) of CMS, chitosan-400, chitosan-700, 50% CMS:50% chitosan-400, 50% CMS:50% chitosan-700 and PEC. The tablets were incubated (1 L, 37 °C, 100 rpm) for 2 h in SGF and then transferred to SIF. (A) Acetaminophen; (B) metformin, monolithic tablets were incubated only in SGF; (C) aspirin.

tablet erosion and aspirin release. Similar to what was observed with acetaminophen, a water-insoluble excipient residue was still present after complete release of aspirin (Fig. 6, d3), indicating the presence of CMS–chitosan attractions. A sustained release of aspirin, over more than 30 h, was observed with PEC (Fig. 7C). This time release is markedly longer than $t_{90\%}$ obtained with 50% CMS:50% chitosan-700 (6.5 h), CMS (11 h) or chitosan-700 (11.5 h). This difference is ascribed to the interactions between aspirin, CMS and chitosan within the PEC matrix. These interactions reduce the diffusion of both aqueous fluid and the drug, leading thus to the lower release rates. The remained tablet based on the PEC after the complete release of drug (Fig. 6, d4) further supports the existence of the interpolymer attractions. In this case, aspirin interacts with the hydroxyl, carboxyl and amino groups of the PEC without dissociation of interactions between the chains of CMS and chitosan.

Taken together, these results showed the advantage of PEC for monolithic formulations. The PEC excipients, containing only 14% of chitosan-700, can afford controlled release of acetaminophen and aspirin. Moreover, the tablets of the PEC were homogenous and less swellable than those of 50% CMS:50% chitosan-700 (Figs. 5B and 6).

4. Conclusion

The CMS–chitosan polyelectrolyte complex (PEC) showed a polymorphism with a lower order degree than those of carboxymethyl starch (CMS) and of chitosan-700. The fluid (SGF or SIF) diffusion and the swelling were lower with PEC tablets than with those based on CMS:chitosan-700 powder mixture. The PEC provided a controlled release of acetaminophen and a markedly slower sustained release of aspirin than that provided by CMS or chitosan-700, making this excipient favorable to colon targeting.

Chitosan at molecular weight of about 400 kDa (chitosan-400) did not afford a long release time for any of the three tracer drugs (metformin, acetaminophen and aspirin). CMS and chitosan-700 matrices showed a fast release of metformin, a controlled release of acetaminophen and a sustained release of aspirin. This indicates that the drug solubility has a major influence on release rate irrespective of the charge of drug and of excipient. The low hydration of chitosan and its insolubility in neutral medium can be a limitation for drug delivery with this excipient alone, but it can be an advantage in the case of PEC. Adding an adequate amount of chitosan with an appropriate molecular weight to the formulations based on CMS can prolong the release time of acetaminophen. Contrarily, the aspirin release from CMS matrix was accelerated when chitosan was added as coexcipient.

Acknowledgements

Support from NSERC (Natural Sciences and Engineering Research Council) of Canada granted to MAM and XXZ, from CRIP-FQRNT (Swine Infectious Disease Research Centre – Quebec Nature and Technology Research Fund), and graduate studentships from FQRNT and from MITACS (Canada) awarded to E. Assaad, are gratefully acknowledged.

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