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Research paper

Development of time-, pH-, and enzyme-controlled colonic drug delivery using spray-dried chitosan acetate and hydroxypropyl methylcellulose

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Abstract

A colonic drug delivery with a new concept based on a combination of time-, pH-, and enzyme-controlled system was developed. Spray-dried chitosan acetate (CSA) prepared from low molecular weight chitosan was characterized. A combination of CSA and hydroxypropyl methylcellulose (HPMC) was used as new compression-coats for 5-aminosalicylic acid (5-ASA) tablets. Factors affecting *in-vitro* drug release, i.e. % weight ratio of coating polymers, enzyme activity, pH of media, and excipients in core tablets, were evaluated. The tablets compression-coated with HPMC:CSA at 60:40 and 50:50% weight ratio providing lag times about 5–6 h were able to pass through the stomach (stage I, 0.1 N HCl) and small intestine (stage II, pH 6.8, Tris–HCl). The delayed release was time- and pH-controlled owing to the swelling with gradual dissolving of CSA and HPMC in 0.1 N HCl and the less solubility of CSA at higher pH. After reaching the colon (stage III, pH 5.0, acetate buffer), the dissolution of CSA at low pH triggered the drug release over 90% within 14 h. Furthermore, the degradation of CSA by β-glucosidase in the colonic fluid enhanced the drug release while adding the disintegrant or the osmotic agent in the core tablets would affect the drug release.

Keywords: Chitosan acetate; Colonic drug delivery; Compression-coated tablets; Spray drying; Characterization

1. Introduction

In the treatment of inflammatory bowel disease (IBD), colon specific drug delivery are designed as time-, pH-, enzyme-, or pressure-controlled systems to deliver anti-inflammatory agents to the sites of inflammation and hence systemic drug absorption should be minimized as this leads to unwanted systemic side effects [1–3]. A dual mechanism combining pH- and time-controlled systems has been developed to reduce the variability affecting the drug release such as the shallow pH gradient between the small and large intestine, the pH change in disease states, inter-subject variations and variable gastric residence times depending on the presence or absence of food [1,4–9]. A newer approach is the enzyme-controlled system which local enzymes produced from microflora in the human colon such as amylase, pectinase, and β-D-glucosidase breakdown a prodrug or a formulation containing biodegradable polymers such as pectin, guar gum, and chitosan to release the drug. However, enteric coatings were suggested to protect the polymers until they reached the colon [1,3,10–12]. 5-ASA plays an important role in the treatment of IBD. Pectin-HPMC compression-coating of 5-ASA tablets was developed for colonic delivery. The biodegradation of pectin by the pectinolytic enzyme was reported to

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enhance the drug release in, pH 6.8, phosphate buffers [13]. In the development of colonic drug delivery, the systems must pass through the stomach (pH 1.5–3.5), the duodenum (pH 6), the intestine (pH 5.5–6.8), and the caecum (pH 6.8–7.3) until reached the colon. However, the luminal pH of the distal intestine in patients with IBD was found to be lower than that seen in healthy volunteers and was around 5.3 ± 0.3 in patients with Crohn's disease [1,10]. Therefore, those enteric coated systems as well as the systems designed to release the drugs at high pH media might not be appropriate.

Various chitosan salts such as chitosan aspartate, glutamate, lactate and hydrochloride were reported to enhance the rate of drug release due to enzyme catalyzed hydrolysis by β-glucosidase at pH 7.0 [14,15]. Chitosan succinate and phthalate in matrix tablets were also suggested to be suitable for colonic controlled release system [16]. In our previous study, microparticles of spray-dried chitosan acetate (CSA) produced from high molecular weight chitosan were characterized and applied as a wet granulation binder for sustained release tablets according to its swelling with gradual dissolving property in distilled water and acidic media [17]. The present study was aimed to develop a more specific colon drug delivery system combining time-, enzyme-, and pH-controlled systems using CSA prepared from low molecular weight chitosan as a new compression-coating material in combination with hydroxypropyl methylcellulose (HPMC) for 5-ASA tablets. According to the properties of CSA such as the swelling and forming dissolving gel in acidic media, the low solubility in high pH media and the possibility to be degraded by colonic β-polysaccharidase enzymes, the proposed system would be accomplished. Physicochemical and micromeritic properties of the salt were characterized. *In-vitro* drug release was studied in 0.1 N HCl, pH 6.8, Tris–HCl and, pH 5.0, acetate buffer mimicking the gastric, small intestine and colonic fluid of IBD patients, respectively. Factors affecting the drug release i.e. % weight ratio of the coating polymers, enzyme activity, pH of media, and component of excipients in the core tablets, i.e. diluent, disintegrant, and osmotic agent were evaluated.

2. Materials and methods

2.1. Materials

The materials used in this experiment and the suppliers are as follows: 5-aminosalicylic acid (lot & filling code 400895/1 13799, Fluka), β-glucosidase (lot & filling code 1264252 34706394, Fluka), chitosan, molecular weight of 45 kDa, 87% degree of deacetylation (COA 280604, Sea Fresh Co. Ltd., Thailand), dibasic calcium phosphate (Emcompress®, lot no. 7031X, Rama Production, Thailand), α-lactose monohydrate (Tablettose 80®, lot no. 9928, Meggle, Germany), and sodium starch glycolate (Explotab®, batch no. E9963, Rama Production, Thailand). Hydroxypropyl methycellulose (HPMC K4M, batch no. QC 14012N01) was a gift from Colorcon Asia Pacific Pte. Ltd., Singapore. All excipients were of pharmaceutical grade and other chemicals were of reagent grade.

2.2. Preparation of CSA

Low molecular weight chitosan (CS) was chosen for its better solubility and ease of degradation by colonic enzymes. Chitosan was dissolved in an aqueous acetic acid to make a 3.5% w/w solution. The solution was sprayed at an inlet temperature of 125 ± 2 °C using a spray dryer (Minispray Dryer, Büchi 190, Switzerland). The obtained powder was collected for investigation.

2.3. Physicochemical and powder characterization of CSA

Physicochemical properties of CS and CSA were characterized in the same manner as described in our previous study [17]. Briefly, scanning electron photomicrographs of the gold-coated samples were taken at appropriate magnification under a scanning electron microscope (model MX 2000, Cam Scan, Cambridge, England). Thermal behaviors were determined by using a differential scanning calorimeter (DSC 7, Perkin–Elmer, USA) at a heating rate of 5 °C/min under nitrogen purge and a thermogravimetric analyzer (TGA7, Perkin–Elmer, USA) conducting at a heating rate of 5 °C/min under nitrogen purge. Powder X-ray diffraction patterns were measured by using powder X-ray diffractometer (Diffractometer D8, Bruker AXS, Germany). Transmission infrared spectra of CS and CSA were measured by the KBr method using a Fourier transform infrared spectroscope (model Magna-IR system 750, Nicolet Biomedical Inc., Madison, WI, USA) as well as ^{13}C NMR spectra using a high-resolution solid-state ^{13}C NMR spectrometer (400 MHz, Bruker, Switzerland).

Moisture absorption isotherms were studied by gravimetric analysis. The samples were placed inside a desiccator containing saturated solution of various salts, i.e. lithium chloride (11% RH), magnesium chloride (32% RH), sodium dichromate (54% RH), sodium chloride (75% RH), potassium chloride (84% RH), potassium nitrate (93% RH), and distilled water (100% RH) [17]. After storage at 25 °C for 48 h, the sample weight gain was recorded and calculated as the percentage of moisture sorption. The experiment was conducted in five measurements ($n = 5$).

The interaction between 5-ASA and CSA was investigated by mixing the drug with CSA powder or CSA gel at 1:1 and 1:2 dry weight ratios. The gel mixture was dried into powders at 60 °C for 6 h. DSC thermograms and FTIR spectra of the mixtures were determined under the same conditions as mentioned earlier.

Micromeritic properties of CSA were measured, i.e. true density (helium pycnometer, Multivolume 1305, Micromeritic Instrument Corporation, USA), particle size distribution (laser diffraction particle size analyzer, Coulter, LS 100Q, USA), angle of repose (fixed base method), Carr's

index, Hausner ratio and percentage of porosity (ε) were calculated from true (ρ_{true}), tapped (ρ_{tap}) and bulk density (ρ_{bulk}) using the following equations [18].

$$\text{Carr's index} = [(\rho_{tap} - \rho_{bulk})/\rho_{tap}] \times 100 \quad (1)$$

$$\text{Hausner ratio} = \rho_{tap}/\rho_{bulk} \quad (2)$$

$$\varepsilon = [1 - \rho_{tap}/\rho_{true}] \times 100 \quad (3)$$

2.4. Preparation of compression-coated tablets

Each 5-ASA core tablet of 100 mg was compressed at a fixed compression force using a hydraulic press and a 6-mm diameter die and flat-face punch set. The formulations of different core tablets as A, B, C, D, and E are shown in Table 1. Core A was compression-coated with 200 mg of dry blends of HPMC:CSA at various % weight ratios (100:0, 80:20, 60:40, 50:50, and 40:60) using a hydraulic press and a 10-mm diameter die and flat-face punch set (Fig. 1). The compression force was fixed at 0.5 kN. The other core tablets were coated with HPMC:CSA at 60:40% weight ratio.

2.5. In-vitro drug release study

In-vitro releases of 5-ASA from core A compression-coated with HPMC:CSA at various % weight ratios were studied using USP apparatus 3 (BIO-DIS®, RRT8, Caleva Ltd., UK). This apparatus is the most attractive for the study of modified release formulations since changing the media to simulate passage through the GI tract can be easily and reproducibly achieved [19]. At first, the drug dissolution was determined in 0.1 N HCl for 2 h (stage I). Afterwards, each tablet was transferred to, pH 6.8, Tris–HCl buffer and run for 3 h (stage II) and then in, pH 5.0, acetate buffer until 24 h (stage III, during the 6th to the 24th hour from the beginning). The amount of drug release was analyzed by UV spectrophotometry (Lambda 2, Perkin–Elmer, USA) at maximum wavelengths of 301.5 nm in 0.1 N HCl, 334.5 nm in, pH 6.8, Tris–HCl buffer and 299 nm in pH 5.0, acetate buffer.

The enzyme effect on the drug release from core A coated with HPMC:CSA at 60:40% weight ratio was studied by adding 10 mg of β-glucosidase into, pH 5.0, acetate buffer (250 mL), at the beginning of the stage III.

To study the effect of pH media, the drug release from core A coated with HPMC:CSA at 60:40% weight ratio was studied by using, pH 7.0, Tris–HCl buffer simulating colonic pH of healthy volunteers instead of the acid medium during the stage III.

The effect on the drug release of excipients in various core tablets coated with HPMC:CSA at 60:40% weight ratio was also investigated.

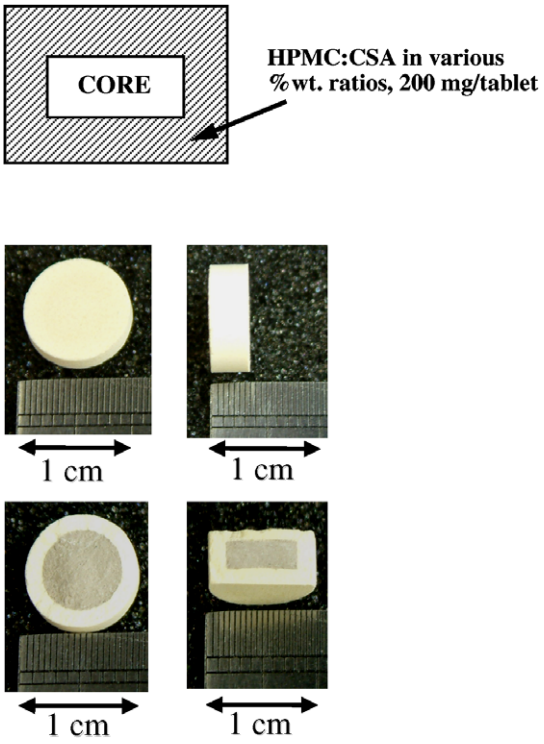

Fig. 1. 5-ASA compression-coated tablets.

2.6. Effect of enzyme on polymer degradation

The effect of β-glucosidase enzyme on the degradation (or erosion) of HPMC and CSA was investigated by using a disintegration apparatus (Sotax DT3, Switzerland). Compacts of HPMC or CSA were compressed into a 300-mg tablet with a 10-mm die and flat-face punch set at the fixed compression force of 0.5 kN. The tablets were placed in disintegration racks and run in, pH 5.0, acetate buffers with and without β-glucosidase enzyme for 8 h. The temperature of the medium was controlled at 37 ± 2 °C. The experiment was done in triplicate. After the test, the remained tablets on the sieves were dried at 60 °C until constant weight.

Table 1
Formulations of core tablets

Components	Core A (mg)	Core B (mg)	Core C (mg)	Core D (mg)	Core E (mg)
5-ASA	50	50	50	50	50
Sodium starch glycolate	–	25	–	5	5
Sodium chloride	–	–	25	–	–
α-Lactose monohydrate	50	25	25	45	–
Dibasic calcium phosphate	–	–	–	–	45
Total weight	100	100	100	100	100

The % erosion of the polymer tablets in each medium was calculated in dry weight following the equation:

$$\% \text{ erosion} = (w_0 - w_1) \times 100/w_0 \quad (4)$$

where w_0 is initial tablet weight (mg); w_1 is dry constant weight of the remained tablet (mg).

3. Results and discussion

3.1. Physicochemical and powder characterizations

Physicochemical properties of CSA prepared from low molecular weight chitosan were in good agreement with that obtained from the high molecular weight chitosan as described in our pervious study [17]. Briefly, CSA was spherical agglomerate particles with a mean diameter of about 14 μm. According to its halo diffraction pattern, CSA was an amorphous solid. Its thermal behavior was similar to that of the high molecular weight chitosan acetate salt. The solid-state ^{13}C NMR spectrum also exhibited the resonance peaks assigned to the acetate functionality of CSA [17,20,21]. The salt was classified as moderately to very hygroscopic according to the moisture absorption isotherms study [22]. All the data mentioned above are not shown.

Chitosan has been reported to interact with salicylic acid and might also interact with other acidic drugs [23]. In our previous study, no change was observed in the DSC thermogram of the physical mixture between CSA prepared from high molecular weight chitosan and salicylic acid [17]. It is necessary to investigate the interaction between CSA and 5-ASA in this study. Figs. 2 and 3 show the DSC thermograms and FTIR spectra of 5-ASA, CSA, physical, and gel mixtures between 5-ASA and CSA at 1:1 and 1:2 weight ratios. The DSC thermograms of all physical and gel mixtures exhibited the broader endothermic melting peaks of 5-ASA than that of the pure drug and the peaks were shifted to the lower temperature. The change in thermal behavior of the physical mixtures led to the following investigation. The physical mixtures were heated up around 280 °C and the samples obtained were subjected to FTIR measurement. The results from the FTIR spectra (data not shown) showed a change similar to that of the gel mixtures (Fig. 3d and e). Thus, the shift of the drug melting peaks observed in the DSC thermograms might be a result of heat-induced drug-polymer interaction during DSC measurements. It is likely that only DSC could not be used to investigate the interaction of the drug-CSA physical mixtures.

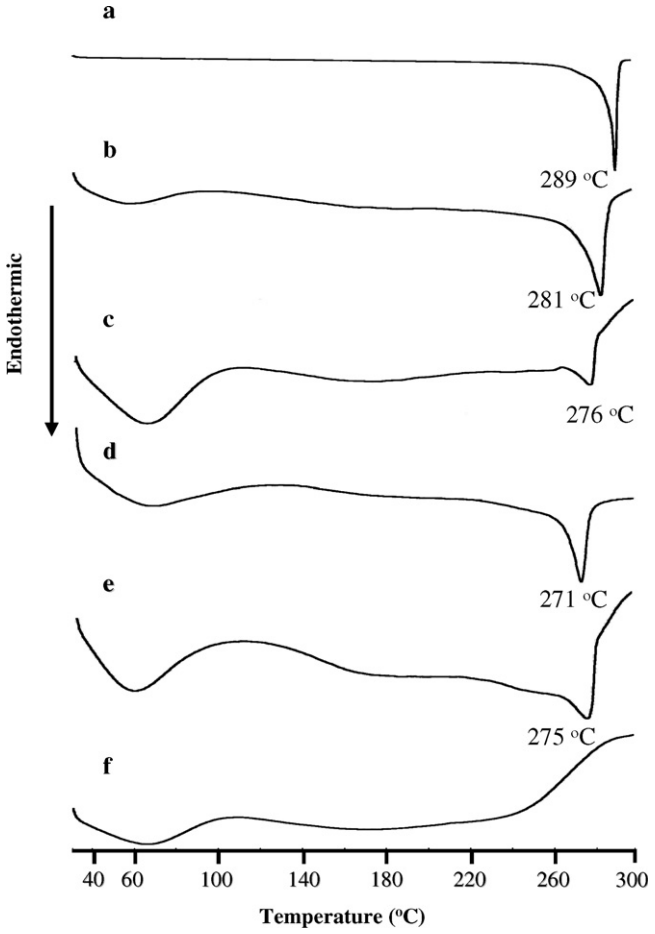

Fig. 2. DSC thermograms of (a) 5-ASA powder, (b) 5-ASA:CSA 1:1 physical mixture, (c) 5-ASA:CSA 1:2 physical mixture, (d) 5-ASA:CSA 1:1 gel mixture, (e) 5-ASA:CSA 1:2 gel mixture, and (f) spray-dried chitosan acetate (CSA).

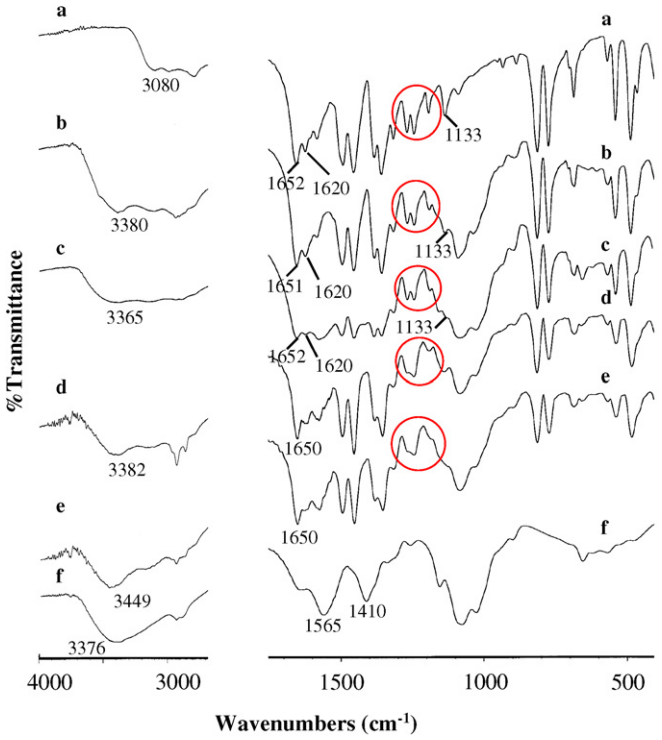

Fig. 3. Transmission infrared spectra of (a) 5-ASA powder, (b) 5-ASA:CSA 1:1 physical mixture, (c) 5-ASA:CSA 1:2 physical mixture, (d) 5-ASA:CSA 1:1 gel mixture, (e) 5-ASA:CSA 1:2 gel mixture, and (f) spray-dried chitosan acetate (CSA).

In the FTIR spectrum of pure 5-ASA, the assignments of the observed absorption bands are as follows: COO—H stretching (hydrogen bonded) associated with the hydroxyl groups at 3080 cm^{-1}, C=O stretching at 1650 cm^{-1}, NH_2 bending at 1620 cm^{-1}, C—O stretching at 1133 cm^{-1} and in plane C—O—H bending at 1190–1267 cm^{-1} (the peaks in the circle) [24,25]. The FTIR spectrum of CSA exhibited the peaks assigned to an asymmetric and a symmetric COO^- stretching at 1565 and 1410 cm^{-1}, respectively. No drug-polymer interaction was observed in the FTIR spectra of the non-heated physical mixtures since the absorption peaks of the drug still could be detected though some peaks were hardly seen in the mixture of higher amount of CSA. In the spectra of the gel mixtures, the peaks assigned to C—O—H bending (in the circles) and NH_2 bending were broadened and nearly disappeared in the mixture with higher amount of CSA. It was suggested that CSA in gel form might interact with 5-ASA. According to the pK_a value of about 6.2–7.0 [26], chitosan will be protonated at low pH during gel formation in an aqueous medium (pH about 5.5). Since the dissociation constants of 5-ASA were reported to be pK_{a1} at 3.0 (carboxylate group), pK_{a2} at 6.0 (amino group) and pK_{a3} at 13.9 (hydroxyl group) [24], the drug would be partly ionized in forms of COO^- and NH_3+ ions at pH of water and the drug-polymer interaction might occur.

The micromeritic properties of CSA are shown in Table 2. According to Wells [22], the value of angle of repose, Carr's index and Hausner ratio of CSA indicated that it possessed the poor to fair flow property. The porosity of CSA was about 73% which was close to the commercial microcrystalline cellulose (Avicel PH101) [27]. In addition, physicochemical and micromeritic characteristics of CSA in this study were in good agreement with those of chitosan acetate prepared from high molecular weight chitosan in our previous study [17].

3.2. In-vitro drug release study

3.2.1. Effect of coating polymer ratios

Fig. 4 shows the release of 5-ASA from the tablets (core A) coated with blends of HPMC:CSA in various % weight ratios. The polymer blends controlled the drug release by providing lag times varying from 4 to 10 h. As the amount of CSA increased, the rate of drug release increased but the lag time decreased. Especially at the ratios of 60:40 and 50:50, the extended lag time during the stage I (0.1 N HCl) and II (pH 6.8, Tris–HCl buffer) was about 5–6 h which was considered to be sufficient for colonic arrival. Macleod's study reported the supportive data that the radiolabelled (^{99m}Tc) tablets coated with a pectin-chitosan-HPMC film with the lag time about 5 h were able to pass through the stomach and small intestine and capable to release the tablet core materials at the colon [28]. The delayed release of 5-ASA during the lag time was mainly due to the time-controlled mechanism of the swelling with gradual dissolving of HPMC. CSA also formed a swollen gel which partly dissolved in 0.1 N HCl during the first 2 h but the gel dissolution was decreased after transferring to the higher pH medium in the stage II. In the stage III (pH 5.0, acetate buffer), the drug was released over 90% within 14 h. The increasing rate of drug release in this stage was attributed to the more rapid dissolving of CSA gel especially in the colonic acid medium. Although the interaction between the drug and CSA gel was observed as already described in section 3.1, no effect on the drug release from the compression-coated tablets was found.

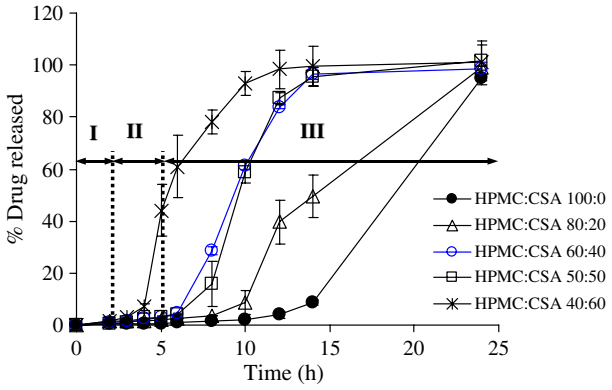

Fig. 4. Dissolution profiles of 5-ASA from core A compression-coated with HPMC:CSA at various % weight ratios during stage I, II, and III ($n = 3$).

Table 2
Micromeritic properties of CSA

Properties	CSA
True density (g cm^{-3}) ($n = 1$)[a]	1.48
Angle of repose (°) ($n = 5$)	33.23 ± 1.82
% Carr's index ($n = 5$)	39.50 ± 1.43
Hausner ratio ($n = 5$)	1.65 ± 0.04
% Porosity ($n = 5$)	73.45 ± 0.35

[a] With 10 repeated measurements.

3.2.2. Effect of enzyme

In this study, core A coated with HPMC:CSA at 60:40% weight ratio was chosen according to providing the appropriate lag time. Fig. 5 demonstrates the effect of β-glucosidase enzyme on the increase of drug release from the compression-coated tablets. After adding the enzyme in the stage III, the rate of drug release was increased and the time for the 50% of drug release ($t_{50\%}$) was about 8 h while the $t_{50\%}$ of those with no enzyme was about 9.3 h. It was suggested that the degradation of CSA by β-glucosidase enzyme enhanced the erosion of the coating materials and hence the rate of drug release was increased. Orienti et al. [14] also reported that the degradation of different chitosan salts by β-glucosidase enzyme enhanced the drug release.

The effect of β-glucosidase enzyme on polymer degradation is shown in Table 3. CSA and HPMC tablets swelled and subsequently formed gel in, pH 5.0, acetate buffer. In the presence of β-glucosidase, the erosion of CSA gel

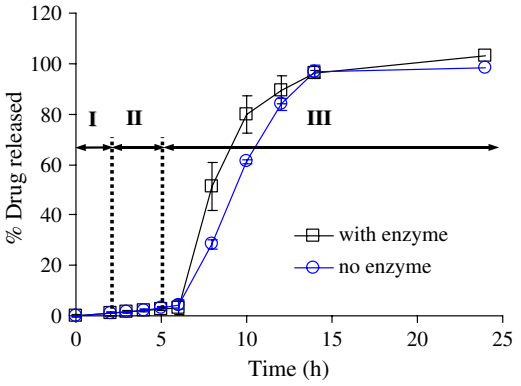

Fig. 5. Dissolution profiles of 5-ASA from core A compression-coated with HPMC:CSA at 60:40% weight ratio with and with out adding β-glucosidase enzyme in the stage III ($n = 3$).

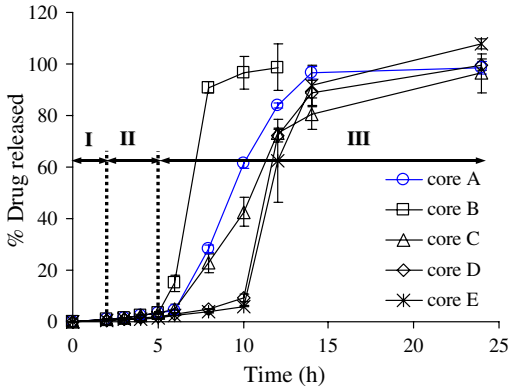

Fig. 7. Dissolution profiles of 5-ASA from different core tablets (core A, B, C, D, and E) compression-coated with HPMC:CSA at 60:40% weight ratio during stage I, II, and III ($n = 3$).

Table 3
Effect of enzymes on polymer degradation in, pH 5.0, acetate buffer

pH 5.0, acetate buffer	% Erosion ($n = 3$)	
	No enzyme	With β-glucosidase
CSA tablets	53.95 ± 2.23	63.32 ± 1.67
HPMC tablets	47.90 ± 2.50	49.39 ± 8.59

within 8 h was about 10% higher than that with no enzyme. On the contrary, no enzyme effect on the degradation of HPMC was observed. It was shown that the activity of β-glucosidase is specific for β-1–4 linkage polymer comprising of D-glucosamine units. This finding supported that the enzyme-controlled system could help increase the rate of drug release in this colon specific delivery system.

3.2.3. Effect of pH of media

The effect on drug release from core A coated with 60:40% weight ratio in different colonic pHs mimicking patients with IBD (pH 5.0, acetate buffer) and healthy volunteers (pH 7.0, Tris–HCl buffer) is shown in Fig. 6. The slower drug release with longer period of lag time was observed in the higher colonic pH. It was due to the less solubility of CSA in the increased pH medium. This result suggested that the pH-dependent solubility of CSA affected the rate of drug release and the promising colonic drug release in the IBD patients would be achieved. In addition, the appropriate pH of media should be considered in the *in-vitro* drug release study.

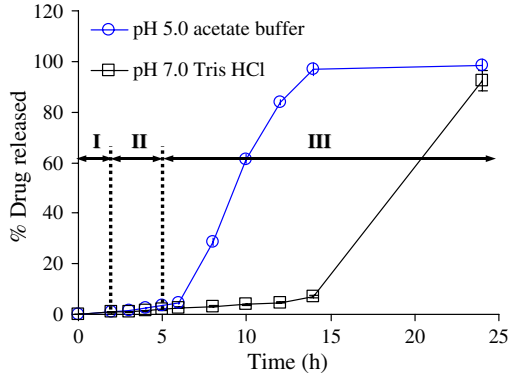

Fig. 6. Dissolution profiles of 5-ASA from core A compression-coated with HPMC:CSA at 60:40% weight ratio in different media in the stage III ($n = 3$).

3.2.4. Effect of disintegrant, osmotic agent and diluents in different core tablets

The release of 5-ASA from different core tablets coated with blends of HPMC:CSA at 60:40% weight ratio is shown in Fig. 7. The tablets containing higher amount of sodium starch glycolate (core B) gave the fastest drug release among the various core tablets. It is generally known that sodium starch glycolate is a superdisintegrant with rapid swelling action that causes bursting effect after water penetration into the tablets. However, the small amount of the disintegrant in core D did not expand enough to burst the tablets but only formed gel that delayed the lag time.

Sodium chloride, an osmotic agent, was incorporated into core C for the purpose of helping increase water penetration and hence the drug release would be increased. However, the rate of drug release was found to delay to some extent. This might be owing to the preferential moisture absorption of sodium chloride that the penetrated water was absorbed to dissolve itself and the drug dissolution was delayed (the aqueous solubility of 5-ASA and sodium chloride is 0.844 and 357 mg/mL at 25 and 20 °C, respectively [24,29]). In addition, the comparison between the drug release from core D and E which was different in the component of α-lactose monohydrate and dibasic calcium phosphate as water soluble and insoluble diluents, respectively, indicated no different rate of drug release.

4. Conclusion

A colonic drug delivery based on a combination of time-, pH-, and enzyme-controlled system was successfully developed. The results from this study clearly show the potential

of a new combination coating material of HPMC and CSA for colonic drug delivery. These compression-coats at the appropriate mixing ratio (HPMC:CSA at 60:40% weight ratio) are capable of retarding the release of 5-ASA until the dosage forms reaching the colon and there the drug is released. The delay release mechanisms during the lag time are time- and pH-controlled by the swelling gel erosion of both polymers in the acidic medium and the less solubility at high pH of CSA, respectively. The solubility of CSA at low pH plays an important role on the trigger of drug release in the acidic colonic fluid. The system is also enzyme-controlled according to the degradation of CSA by the colonic enzyme helping increase the rate of drug release in the colon. Chitosan acetate may interact with the acidic drug but has no effect on the drug release in this system. Finally, the appropriate amount of super disintegrant in the core tablets enhances the rate of drug release while the osmotic agent slightly retards the drug release.

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