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Chitosan-based controlled porosity osmotic pump for colon-specific delivery system: Screening of formulation variables and in vitro investigation

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Abstract

A microbially triggered colon-targeted osmotic pump (MTCT-OP) has been studied. The gelable property at acid condition and colon-specific biodegradation of chitosan were used to: (1) produce the osmotic pressure, (2) form the drug suspension and (3) form the in situ delivery pores for colon-specific drug release, respectively. The scanning electron microscopy (SEM) study and the calculation of membrane permeability were applied to elucidate the mechanism of MTCT-OP. The effects of different formulation variables, including the level of pH-regulating excipient (citric acid) and the amount of chitosan in the core, the weight gain of semipermeable membrane and enteric-coating membrane, and the level of pore former (chitosan) in the semipermeable membrane, have been studied. Results of SEM showed that the in situ delivery pores could be formed in predeterminated time after coming into contact with dissolution medium, and the number of pore was dependent on the initial level of pore former in the membrane. The amount of budesonide release was directly proportional to the initial level of pore former, but inversely related to the weight of semipermeable membrane. The effects of variations in the level of citric acid and chitosan in the core formulation on drug release were studied. The different levels of enteric-coating membrane could prevent cellulose acetate membrane (containing chitosan as pore former) from forming pore or rupture before contact with simulated colonic fluid, but had no effect on the drug release. Budesonide release from the developed formulation was inversely proportional to the osmotic pressure of the release medium, confirming that osmotic pumping was the major mechanism of drug release. These results showed that MTCT-OP based on osmotic technology and microbially triggered mechanism had a high potential for colon-specific drug delivery.

Keywords: Microbially triggered; Colon targeting; Osmotic pump; Chitosan; Budesonide

1. Introduction

There has been considerable exploration in developing colon-specific drug delivery systems during the last few years. Targeting drugs to the colon not only ensure direct treatment of colon diseases, but also are utilized as a means of achieving chronotherapy for diseases that are sensitive to circadian rhythm. Furthermore, delivery of drugs to the systemic circulation through colonic absorption provides a novel peroral route of introducing degraded/poorly absorbed peptides and proteins because of the relatively low proteolytic enzyme activities and longer retention time in the colon.

The various approaches developed for the purpose of achieving colonic targeting include time-controlled delivery systems (Steed et al., 1997; Hebden et al., 1999), pH-dependent delivery systems (Markus et al., 2001; Cole et al., 2002), pressure-controlled delivery systems (Muraoka et al., 1998; Shibata et al., 2001), prodrugs (Ahrabi et al., 2000; Maris et al., 2001) and microflora-triggered delivery systems (Brondsted et al., 1998; Katsuma et al., 2002; Yano et al., 2002). Among these approaches, there appears more interest in microflora-activated systems since the abrupt increase of the bacteria population

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and associated enzyme activity in the colon represent a non-continuous event that is independent of GI transit time.

Chitosan is a high molecular weight cationic polysaccharide (Singla and Chawla, 2001). It has favorable biological properties such as non-toxicity and biocompatibility. Chitosan has been evaluated for its potential as colon-specific drug delivery in several forms such as capsules (Tozaki et al., 1997, 1999, 2002), matrices (Zambito and Colo, 2003), hydrogels (Shu et al., 2001; Zhang et al., 2002), and microspheres (Lorenzo-Lamosa et al., 1998).

Osmotic systems utilize the principle of osmotic pumping for the delivery of drugs. Various types of osmotic pumps and formulation aspects have been reviewed (Verma et al., 2002). Osmotic pump which was patented as OROS-CT has already been established to target the drug locally to the colon for local or systemic therapy (Theeuwes et al., 1990). OROS-CT was designed with a 3–4 h post-gastric delay to prevent drug delivery in the small intestine. Drug release begins when the unit reaches the colon. This system was essentially a time-controlled release system. Even through the transit time in small intestine is rather consistent, high variation of gastric retention time makes this system complicated in predicting the accurate location of drug release.

With all these considerations in mind, we designed a new microbially triggered colon-targeted osmotic pump (MTCT-OP) based on chitosan. Fig. 1 shows schematic diagram of MTCT-OP, which consists of an osmotic core (containing drug and chitosan with organic acid as excipient), an inner semipermeable membrane layer composed of the mixture of cellulose acetate and chitosan powder, and an outer enteric-coating layer. During its transit through the GI tract, MTCT-OP remains intact in the stomach due to the enteric-coating layer, but this layer will dissolve in the small intestine, where pH is above 6, and water is imbibed into the core. The acid aqueous environment produced by dissolution of organic acid in tablet core causes chitosan to swell and to constantly form a flowable gel through which water-insoluble drug dispersed. However, the drug is still not released because the pore former chitosan in the semiperme-

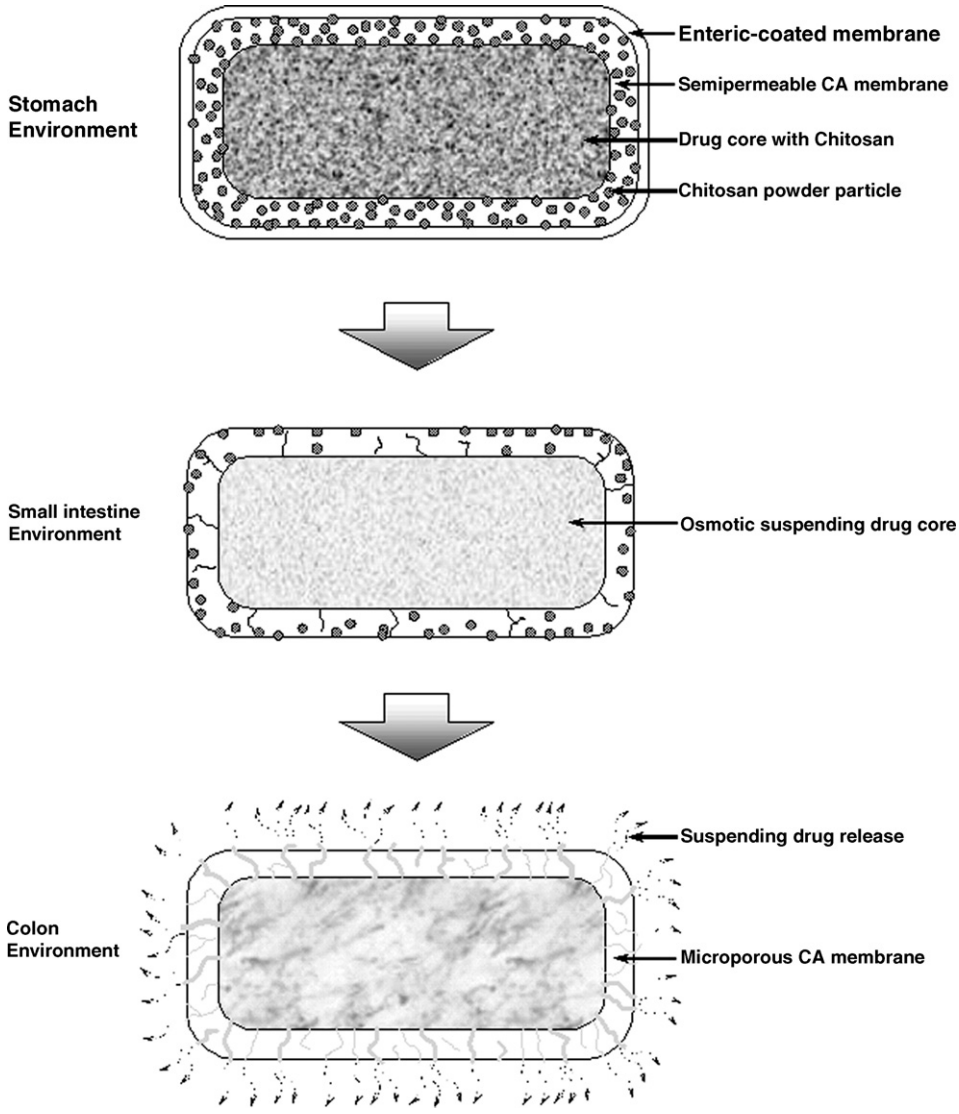

Fig. 1. Schematic diagram of MTCT-OP.

able membrane is scarcely dissolved. When MTCT-OP reaches the colon, chitosan in the semipermeable membrane is specifically degraded by microflora of the colon and thereby results in an in situ formation of a microporous membrane. Swelling of tablet core forces drug gel out through the microporous orifice at a relatively constant release rate for up to 24 h in the colon. Targeting drugs to the colon by microflora-activated mechanism and controlled release procedure at a pre-programmed rate are the main characteristics of chitosan-based MTCT-OP.

Budesonide (BUD) is a very potent corticosteroid with a high ratio of topical to systemic antiinflammatory activity, because of its strong affinity to corticosteroid receptors and its rapid first-pass metabolism in the liver (Edward, 2003). Slow release forms containing BUD have been recommended as a potential method for the treatment of inflammatory bowel. Therefore, it is necessary to deliver BUD specifically to colon in order to maximize the local concentration in inflamed intestinal mucosa.

The purpose of this research was to establish a new microbially triggered osmotic pump system based on chitosan for colonic drug delivery and propose its delivery mechanism. BUD was selected as a model drug for MTCT-OP. The influences of formulation variables on drug release and the drug delivery behavior of optimized MTCT-OP in the simulated in vivo physiological conditions were investigated.

2. Materials and methods

2.1. Materials

Budesonide (BUD) was gift sample from Lunan Pharmaceutical Co. Ltd. (Shandong, China). Following excipients and chemicals were purchased from commercial sources and used as received: Chitosan (deacetylation degree 96%, Haizibei Co. Ltd., Shandong, China), silicified microcrystalline cellulose (SMCC, Pro-Solv® 90, JRS Pharm, Germany), citric acid, acetone-GR and ethanol-GR (Bodi Chemical Co., Tianjin, China), cellulose acetate (CA, 54.5–56 wt.% acetyl content, Shanghai Chemical Co., Shanghai, China), Eudragit® L100-55 (Röhm Pharm, Darmstadt, Germany), triethylcitrate (TEC, Aldrich Chemical, USA), polyethylene glycol (PEG) 400 (Pudong Chemical Co., Shanghai, China), talc and magnesium stearate (Mg-St, Aoda Pharm Co. Ltd., Liaoning, China), HPLC grade acetonitrile (Kemiou Chemical Co., Tianjin, China). Solvents of reagent grade and double distilled deionised water were used in all experiments.

2.2. Preparation of core tablets

BUD was pre-mixed with a small amount of chitosan by spatulation, followed by mixing manually for 10 min with the remaining chitosan and other ingredients. The resultant powder mixture was sieved through 100 mesh screen and directly compressed into tablets using 8.0 mm standard concave punches on a single punch tablet machine (Tianhe, Shanghai, China). The weight of each tablet was determined to be with in the range of 200 ± 5 mg in order to maintain the relatively constant volume and surface area. The core compositions are listed in Table 1.

Table 1
Composition for core tablets

Ingredients (mg/tablet)	Core code							
	F0	F1	F2	F3	F4	F5	F6	F7
Budesonide	9	9	9	9	9	9	9	9
Chitosan	100	50	50	50	50	40	55	60
Citric acid	0	50	80	100	130	80	110	120
SMCC	90	90	60	40	10	70	25	10
Magnesium stearate	1	1	1	1	1	1	1	1

2.3. Microporous semipermeable membrane coating

CA in acetone containing different levels of pore forming agent (chitosan) was used as coating formulation (Table 2). The weight gains of microporous semipermeable membrane were 10%, 12% and 14%, respectively. PEG 400 (25% of total coating materials) acted as a hydrophilic plasticizer and was added to enhance the physical–mechanical property of CA membrane. The coating conditions were as follows: stainless steel pan, 200 mm diameter; 4 baffles; rotation rate of the pan, 40 rpm; nozzle diameter of spray gun, 1 mm; spray rate, 3 mL/min; spray pressure, 2 bar; drying temperature, 40 °C. The surface morphology of the coated tablets was a little bit rough even though the fine micronized chitosan powder (400 mesh 98% pass, mean particle size 35 μm) was used. After coating, the tablets were dried for 12 h at 50 °C to remove residual solvent.

2.4. Enteric coating

Different weight gains (E1: 5%, E2: 6% and E3: 7%, respectively, w/w) of enteric layer's materials (Eudragit® L100-55/TEC/talc/95% ethanol = 6%:0.6%:1.4%:92%, w/w) were coated on the surface of microporous semipermeable membrane. Operating conditions were as follows: stainless steel pan, 200 mm diameter; 4 baffles; rotation rate of the pan, 50 rpm; nozzle diameter of spray gun, 1 mm; spray rate, 1 mL/min; spray pressure, 1.5 bar; drying temperature, 30 °C. The surface of MTCT-OP tablet had a smooth and uniform appearance. Coated tablets were dried for 4 h at 40 °C.

2.5. Preparation of rat caecal medium

It was demonstrated that the extracellular enzymes released from the microflora in rat caecal contents had more pro-

Table 2
Microporous semipermeable membrane coating formulation

Formulation code	Coating composition		
	Chitosan	CA	PEG400
S0	30	45	25
S1	25	50	25
S2	20	55	25
S3	15	60	25

Composition given in terms of coating materials (%, w/w). Total coating materials in the coating solution: 4.0%.

found degradation effects on chitosan than did their membrane enzymes (Zhang and Neau, 2002). So the extracellular enzymes were isolated specifically to mimic colonic conditions. Briefly, Male Wistar rats, weighting 200–300 g and maintained on a normal diet, were lightly anesthetized under ether and then sacrificed by decapitation. The caecum was exteriorized, ligated at two ends (2.0 cm distances), cut loose, and immediately removed from the rat body. The formed caecal bag was then opened, its content weighted, pooled, and suspended in two volumes of cold bicarbonate-buffering saline (BBS, pH 7.0: $NaHCO_3$, 110 mM; $Na_2HPO_4{\cdot}12H_2O$, 20 mM; NaCl, 8.0 mM; KCl, 6.0 mM; $CaCl_2{\cdot}2H_2O$, 0.5 mM; $MgCl_2{\cdot}6H_2O$, 0.4 mM) to give a final caecal dilution of 33% (w/v). The suspension was filtered through 400 mesh grit twice to remove debris. Supernatants were then centrifuged at 15,000 × *g* for 30 min in order to obtain a clear supernatant containing extracellular enzymes (Zhang et al., 2002). Throughout the preparation of rat caecal medium (RCM) and further in vitro dissolution study in simulated colon conditions, both the pH of BBS and anaerobic environment were maintained by bubbling CO_2 gas at a rate of 1 mL/min. The rat study protocol was approved by the Animal Care and Use Committee of Shenyang Pharmaceutical University, Department of Pharmacy.

2.6. In vitro dissolution

In order to verify the validity of MTCT-OP triggered by colon-specific bacteria, following three step-dissolution conditions (Wong et al., 1997) were carried out in a basket apparatus (China Pharmacopoeia 2005) to simulate the physiological conditions of GIT: 2 h in 250 mL of simulated gastric fluid (SGF, 3.2 mg/mL pepsin in 0.05 M hydrochloric acid, pH 1.2), 4 h in 250 mL of simulated intestinal fluid (SIF, 10 mg/mL pancreatin in Sorensen's phosphate buffer, pH 7.4) and finally, 18 h in 100 mL of RCM. The stirring rates in SGF, SIF and RCM were 20, 20, 5 rpm, respectively, and the temperature was maintained at 37 °C. The RCM was replaced at 12 and 18 h to maintain sink conditions because of the low solubility of BUD. Aliquots of dissolution medium were withdrawn at predetermined time intervals and the same volume of corresponding release medium was replenished to maintain a consistent volume. Each test was performed in triplicate.

2.7. HPLC analysis

Sample of 2 mL of dissolution medium was centrifuged at 15,000 × *g* for 10 min. The Supernatant was filtered through a 2 μm filter. Then, 20 μL of the resulting solution was injected in HPLC Apparatus (Agilent 1100, Hewlett-Packard Co., USA) consisting of a photodiode array detector (G1315B), dual liquid chromatography pump (G1312A), on-line degasser (G1322A), and an injector (7725i, Rheodyne Co., USA). The column was a reverse phase column (Nucleosil C_{18}, 250 mm × 4.6 mm i.d., 5 μm, Phenomenex Inc., UK). The mobile phase was acetonitrile/0.0256 mM phosphate buffer (pH 3.2) (30/70, v/v) and the flow rate was 1.5 mL/min (European Pharmacopoeia, 2005). The temperature of system was maintained at 30 °C. The detection limit was 0.4 μg/mL. Commercial BUD is an epimeric mixture of two isomers which have the same pharmacological activity. The two epimers could be separated under the conditions described above.

2.8. Scanning electron microscopy (SEM)

Separated coating membranes (varying in chitosan concentration) obtained before and after the dissolution test, respectively, were examined for their porous morphology by scanning electron microscope (Jeol 6100, JEOL, Japan). Membranes were dried at 45 °C for 12 h and stored between sheets of wax paper in a dessicator before examination. The membrane samples were sputter coated for 5–10 min with gold by using fine coat ion sputter (JFC-1100, Jeol, Japan) and examined under SEM.

2.9. Statistic analysis

All values were expressed as their mean ± S.E. Release profiles of various formulations were compared using model independent pair-wise approach, which included the calculation of 'difference factor' f_1 and 'similarity factor' f_2. The two release profiles were considered to be similar, if f_1 value was lower than 15 (between 0 and 15) and f_2 value was more than 50 (between 50 and 100). For the calculation of f_1 and f_2 values, only one data point at which more than 85% of the drug release had been released was taken into consideration. One-way analysis of variance test (ANOVA) was also performed to check whether there was significant difference among the different formulations and a value of $P < 0.05$ was statistically significant.

3. Results and discussions

3.1. SEM of microporous semipermeable membrane

In order to study the changes in the membrane structure throughout the dissolution procedure and the mechanism of drug release from MTCT-OP, the membranes of coated tablets obtained after three step-dissolution studies, were investigated by SEM. Fig. 2 shows SEM micrographs of membrane surfaces of different formulations (F3/S0/E1 and F3/S2/E1 containing 30% and 20%, w/w of in situ pore forming agent—chitosan, respectively) with the weight gains of 12% (w/w) (inner semipermeable membrane) and 5% (w/w) (outer enteric membrane). In Fig. 2, panels '-1', '-2' and '-3' represent the membrane obtained after SGF dissolution studies, SIF dissolution studies and RCM dissolution studies, respectively.

Before dissolution studies, no porous membrane structure was observed with the presence of different levels of pore former (chitosan). The surfaces of coated tablets were yellowish and glossy and the membrane appeared to be integral and smooth with no visible imperfections. Fig. 2A-1 and B-1 showed SEM micrographs of the surface of membranes after SGF dissolution studies, suggesting that there was no evidence of formation of pores in the membrane. The results demonstrated that the enteric-coating membrane could prevent chitosan incorporated in the CA film from dissolving in the SGF.

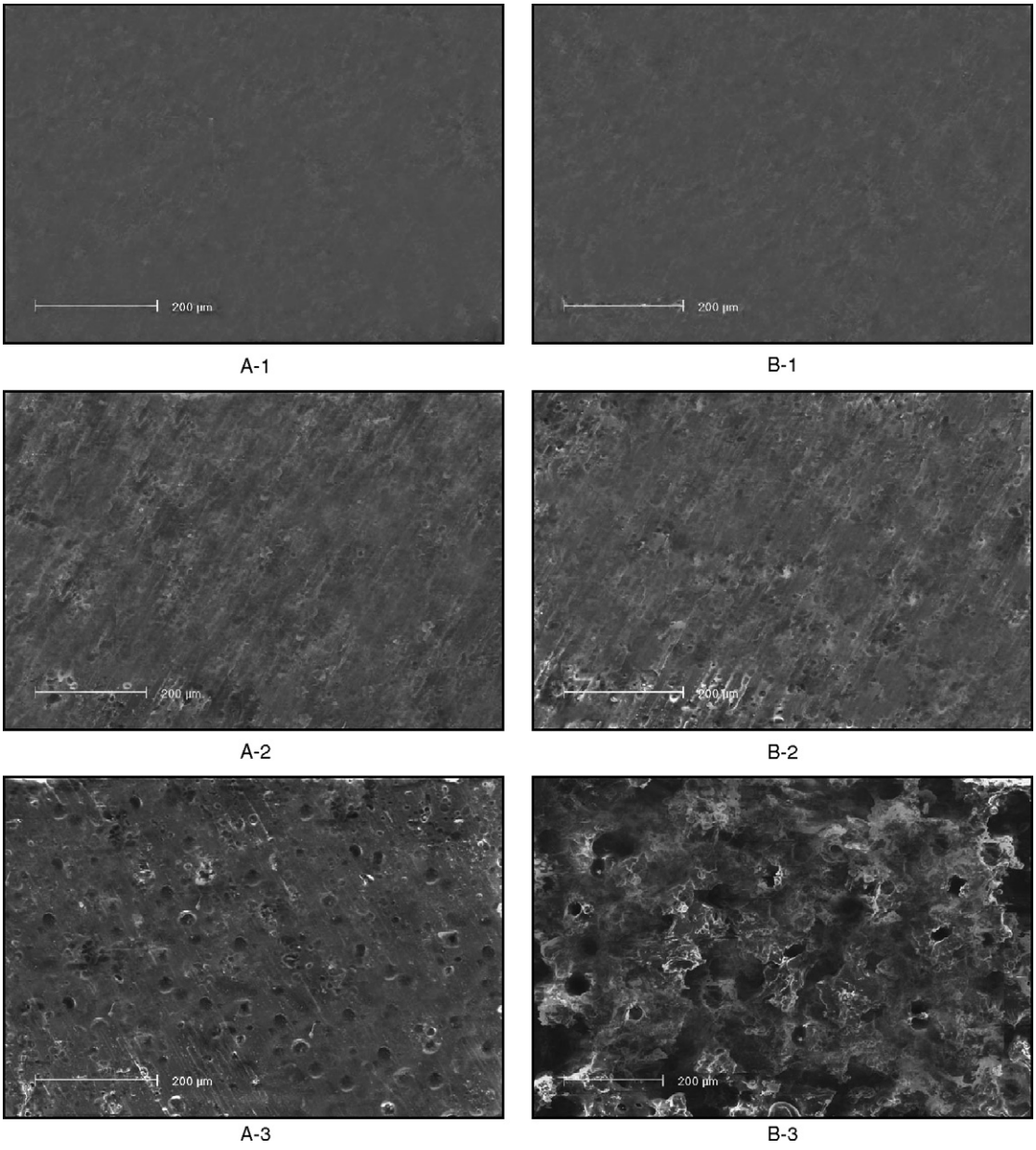

Fig. 2. SEM micrographs showing the membrane structure of formulation F3/S0/E1 (A) and F3/S2/E1 (B) after SGF dissolution (-1), after SIF dissolution (-2) and after RCM dissolution (-3), respectively.

After SIF dissolution studies, the enteric membrane dissolved, but no pore was still not developed in the semipermeable membrane because of insolubility of chitosan in intestinal condition and the absence of bacterial enzymes which might degrade the chitosan. Throughout the SIF dissolution studies, no in situ formation of pores in the semipermeable CA membranes containing chitosan took place as shown in Fig. 2A-2 and B-2.

After exposure to RCM release medium, formation of in situ pores in the membranes was observed as shown in Fig. 2A-3 and B-3, which possibly acted as an exit for the drug release. When comparison was made between the membranes containing 30% and 20% level of chitosan (F3/S0/E1 and F3/S2/E1, respectively), it was found that the former became more porous after RCM dissolution studies. After complete dissolution, the exhausted membrane from F3/S2/E1 showed the smaller pore size and did not exhibit swelling or rupturing. Membrane from F3/S0/E1 showed major pore size and caused rupturing of membrane during dissolution.

The SEM study indicated that in situ formation of pores was possible in the thin structure of the semipermeable CA membrane containing chitosan because of its biodegradability when meeting colonic bacteria. Moreover, it can be concluded that biodegradation of pore former (chitosan) from the membrane (after coming into contact with the RCM environment) left behind the porous membrane for drug release. The numbers of pores were directly proportional to the initial level of pore former in the membrane.

3.2. Formulation aspects of core tablets

3.2.1. Effect of chitosan:citric acid ratio on drug release

It has been demonstrated that polymer with appropriate viscosity and expanding property can be used as osmotic agents for the release of water-insoluble drug (Janicki et al., 1987). Due to its high molecular weight and a linear unbranched structure, chitosan is completely biodegradable, toxicologically harmless and low cost, and exhibits an excellent gelation characteristic (Singla and Chawla, 2001). Hence the potential for chitosan to be used as a polymeric osmotic agent in osmotic pump is obvious. The hydration and gel formation of chitosan are very much dependent on the pH of surroundings. It is insoluble at an alkaline and neutral pH but soluble at acid condition. Upon dissolution, amine groups of the polymer become protonated, forming a resultant viscous and soluble polysaccharide. Inclusion of citric acid as pH-regulating excipient in the developed formulations was expected to decrease the microenvironmental pH of the core to a suitable level at which chitosan could form appropriate viscous gelling solution and hence, to enhance the osmotic pressure of core tablets.

In initial trials, core tablets were coated with microporous membrane and enteric coat (formulation code: F0/S2/E1). However, there was no drug release till 24 h. It was probably because no gel forming action happened to chitosan. Based on this, we thought that it was necessary to add a suitable amount of citric acid into core tablets in order to gelatinize chitosan.

It was clearly evident from Fig. 3 that the concentration of citric acid in the core formulation had a marked effect on drug release after it came with RCM. As the concentration of citric acid increased, viscosity of chitosan solution also increased, which caused the increment of osmotic pressure of core tablets. It could be seen that release amount was higher in batch F3 compared with that in batch F2 and F1 where the concentrations of citric acid were lower. When the amount of citric acid added into the tablets increased, a greater amount of chitosan would be gelled and hydrated, thus the osmotic pressure that pushed the drug suspension solution out of the system would increase accordingly. At the same time, we found as further increase of citric acid, there was no proportionate increase in drug release. Taking the release profile F3 as the reference, the release profile was similar in F3 and F4. This result supported the report that the viscosity and swelling property of chitosan solution would keep the same level when the maximal hydration and solubility occurred at specific concentration of citric acid (Nigalaye et al., 1990).

3.2.2. Effect of amount of chitosan on drug release

Based on above result, the core formulation F3 (chitosan/citric acid ratio 1:2) was selected as optimum for the following studies. However, the amount of drug release was not sufficient. To improve the amount of drug release, four different amounts (F3: 150 mg, F5: 120 mg, F6: 165 mg and F7: 180 mg, respectively) of chitosan–citric acid mixture (1:2) were taken (Table 1) in core tablets to observe the effect on drug release after 24 h dissolution studies.

Fig. 4 illustrates the influence of different added amounts of chitosan–citric acid mixture (1:2) on the amount of drug release from osmotic core tablets with an in situ delivery pores formation. F5 shows that the amount of drug release is less than that in F3, indicating that decrease of chitosan reduced the viscosity of core tablets and thus decreased the osmotic pressure. Both F6 and F7 show considerable increment of drug release, and the former exhibits higher release amount. The surprising decrease of drug release in high chitosan dosed F7 compared with F6 could be due to the interaction between the drug-suspending effect and drug release retarding effect of chitosan. As a suspending agent, the larger the amount of chitosan being used, the higher the viscosity of the core suspension would be, leading to more efficient suspension of water-insoluble budesonide in the tablets. As a consequence, the release amount increased when increasing the added amount of chitosan, because the available surface area for drug dissolution enhanced. However, in the case of F7, large amount of chitosan form a high viscous gel, which on one hand promoted more efficient suspension of budesonide particles, but on the other hand, decreased the amount of drug release because of the retardation on the liquefaction and dissolution of the tablet core. When the retardant property of higher-viscosity chitosan became predominant, the amount of drug release would decrease inversely because only liquefied and dissolved suspension would be delivered through the pores. Hence, core formulation F6 was adopted in further formulation studies.

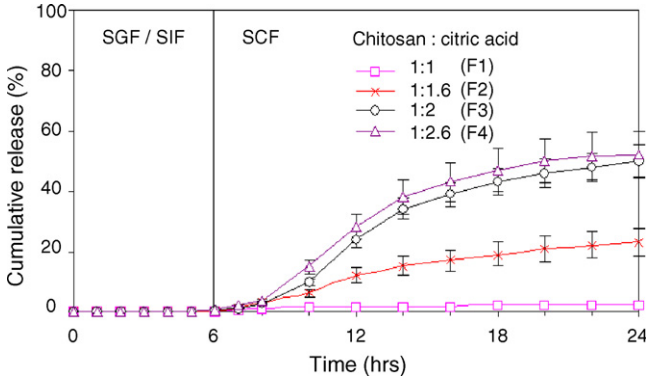

Fig. 3. Effect of chitosan:citric acid ratio on release profiles.

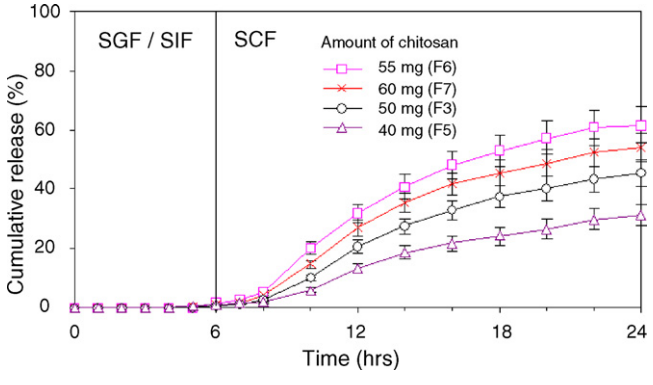

Fig. 4. Effect of amount of chitosan (mixed with citric acid at the same ratio of 1:2) on release profiles.

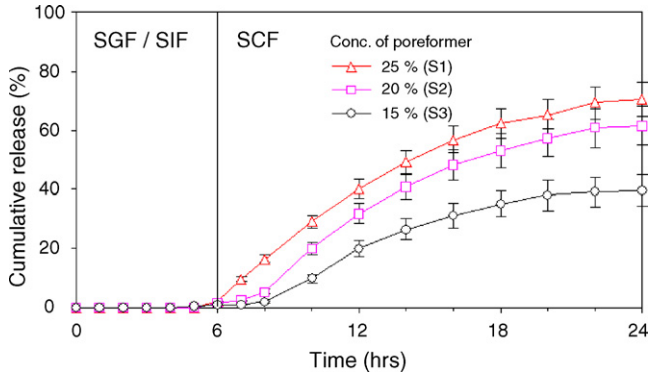

Fig. 5. Effect of concentration of pore former on release profiles.

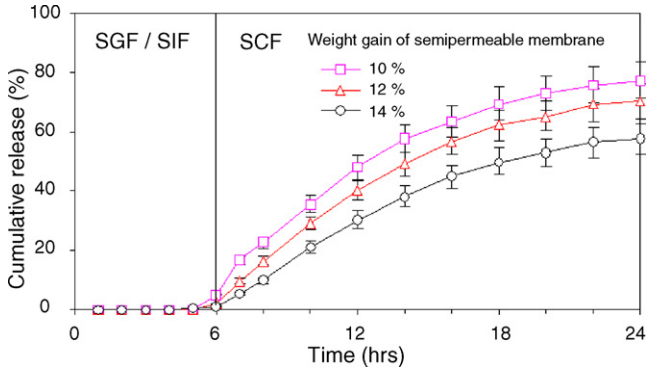

Fig. 6. Effect of weight gain of semipermeable membrane on release profiles.

3.3. Evaluation of membrane variables

3.3.1. Effect of concentration of pore forming agent (chitosan) on drug release

The SEM and further isolated film study have proved that chitosan incorporated in CA films was susceptible to digestion by bacterial enzymes in a simulated colonic environment and allowed drug release to occur. The extent of digestion was directly proportional to the amount of chitosan present in the film.

To study the effect of concentration of pore forming agent (chitosan), core formulation F6 was coated with coating compositions containing 25% and 15%, w/w (of coated membrane weight) concentrations of chitosan (F6/S1/E1 and F6/S3/E1, respectively). Release profiles from these formulations, in comparison with F6/S2/E1 (containing 20%, w/w of chitosan), are shown in Fig. 5. It clearly indicated that the concentration of chitosan had a direct effect on drug release. The formulation of coating composition S1 released more than 70% of budesonide after complete dissolution studies. This result suggested that slightly higher concentration of pore forming agent may be useful to release maximum budesonide content from the systems. Other scholars have also obtained similar results (Zentner et al., 1990).

Another parameter affected by the concentration of pore forming agent was the lag time of drug release. Before RCM dissolution study, it was expected that there was a lag time of 6 h to reach specific colon region, which meant that the release of drug was only activated by RCM containing colonic bacteria. However, in the RCM dissolution study, no lag time should be shown. The concentration of chitosan in the membrane might be the key factor to this lag time. The lag time was inversely related to the initial level of pore forming agent in the membrane. The lower concentration of pore forming agent (15% (w/w), S3) showed longer average lag time (8 ± 0.4 h) and the higher concentration of pore forming agent (25% (w/w), S1) showed shorter average lag time (6 ± 0.2 h) in the whole dissolution set-up.

3.3.2. Effect of weight gain of semipermeable membrane on drug release

The drug release rate from in situ microporous membrane was affected by overall membrane thickness. Optimal tablets (F6/S1/E1) with different weight gain (10%, 12% and 14%, respectively) of microporous membrane were prepared to demonstrate the effect of coating thickness on drug release. In vitro release profiles from these formulations are shown in Fig. 6. It was evident that drug release decreased as the weight gain of the membrane increased. As the thickness of membrane increased, the imbibing water rate of semipermeable membrane and the liquefaction rate of the tablet core decreased correspondingly, resulting in the decrement of drug release. In addition, more weight gain of CA membrane would make the chitosan incorporated less accessible to bacterial degradation, resulting in the slower drug release. In any of the formulations, no bursting of the systems was observed during the dissolution procedure, thus assuring that the formulations can be expected to remain intact in GIT without any incidence of dose dumping. From this study, it could be concluded that membrane thickness had a profound effect on drug release from MTCT-OP system. The release rate was inversely proportional to weight gain of microporous membrane.

3.3.3. Effect of the thickness of enteric-coating membrane on drug release

In all above release studies, the weight gain 5% of enteric-coating membrane was kept constantly. Neither in situ formation of delivery pores nor drug release were observed before RCM dissolution studies with SEM study and dissolution aspect. To further evaluate the role of enteric-coating membrane thickness on the release rate of budesonide, core formulation (F6) coated with microporous semipermeable membrane formulation (S1, weight gain 10%) was coated again with three different thickness enteric-coating membranes (5%, 6% and 7%, respectively). As the film thickness increased, no significant difference in release profiles could be found ($P < 0.05$). This result probably suggested that enteric polymer (Eudragit L100-55) dissolved too quickly in SIF medium to change the release profiles significantly.

3.4. Drug-release mechanism

In a majority of controlled-porosity osmotic system, the most preferred method for the formation of pores was to incorporate water-soluble additives into the membrane wall. In the case of

MTCT-OP, chitosan was substituted for a soluble additive to act as an in situ pore forming agent, which was only degraded by bacteria available in the colon. Microbially triggered colon-targeted osmotic pump (MTCT-OP) contained chitosan in the semipermeable membrane, which was degraded after coming with SIF medium, thereby resulting in an in situ formation of a microporous membrane through which drug release took place. The biodegradable properties of colonic bacteria might play an important role in colon-specific drug release from such system.

For drug release systems that release by osmotic pressure, water influx into device core could be described by the following equation:

$$\frac{dV}{dt} = \frac{A}{h} L_p(\sigma\,\Delta\pi - \Delta p) \quad (1)$$

where dV/dt is the water influx into the core, A and h are the membrane area and membrane thickness, respectively, L_p is the filtration coefficient, σ is the reflection coefficient, and $\Delta\pi$ and Δp are the osmotic and hydrostatic pressure differences, respectively, between the inside and outside of the system. The general expression for the solute release rate, obtained by pumping through the orifice is given by:

$$\frac{dM}{dt} = \frac{dV}{dt} C \quad (2)$$

where dM/dt is the release rate, dV/dt is given by (1), and C is the concentration of solute in suspension being pumped.

As the size of the delivery orifice increases, hydrostatic pressure difference is minimized and $\Delta\pi \geq \Delta p$. By replacing dV/dt in Eq. (1), the following equation is obtained:

$$\frac{dM}{dt} = \left(\frac{A}{h} L_p \sigma C\right) \Delta\pi \quad (3)$$

Eq. (3) indicates that a plot of the release rate versus $\Delta\pi$ should be linear with a slope given by the expression in parentheses.

To study the drug-release mechanism, release studies of the optimized formulation (F6/S1: weight gain 10%/E1: weight gain 5%) were conducted in RCM of different osmotic pressure. The initial portion of the drug profiles were used to calculate the initial drug release rate (dM/dt). The dM/dt as a function of the external osmotic pressure (π_{out}) is shown in Fig. 7. The results of release studies showed that the drug release was highly dependent on osmotic pressure of the release medium. Drug release rate from the formulations decreased with the increment of external osmotic pressure. The osmotic pressure difference could be written as $\Delta\pi = (\pi_{in} - \pi_{out})$. When dM/dt obtained were plotted against π_{out}, a well linear relationship ($r = 0.9653$) was obtained with a slope of $(A/h)L_p\sigma C$. From Fig. 7, the slope of the linear portion fit was 0.0315 mg/h atm. $L_p\sigma$ was calculated to be 7.0×10^{-6} cm^2/h atm based on Eq. (3) by substituting the values of $h = 0.02$ cm, $A = 2.01$ cm^2 and $C = 45$ mg/cm^3. The value complied with the literature data of controlled porosity osmotic pump ($L_p\sigma = 7.9 \times 10^{-6}$ cm^2/h atm) (Zentner et al., 1985). It was concluded that osmotic pumping was the major mechanism governing drug release from MTCT-OP.

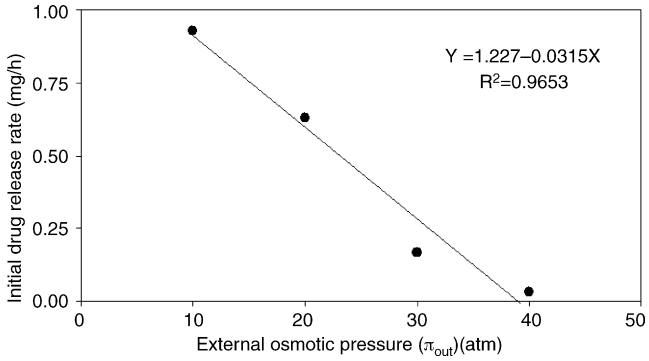

Fig. 7. Linear relationship between the release rate and external osmotic pressure.

Dissolution data of the optimized formulation was fitted to various mathematical models (zero-order, first-order, and Higuchi) in order to describe the kinetics of drug release after in situ formation of delivery pores (i.e. after 6 h). The sum of squares of residues (SSR), the Akaike Information Criterion (AIC), the coefficient of determination (R^2) and the correlation coefficient (r) were taken as criteria for choosing the most appropriate model (Fassihi and Pillay, 1998). Drug release from optimal formulations fitted well into first-order model (Table 3) and apparent lag time was 6 h. The reason for first-order release from the optimal formulations could be that the addition of enzymes degradable chitosan to a semipermeable CA lead to the formation of the in situ delivery orifice in the film due to the polymer degrading and leaching out into the surrounding medium. When chitosan in the semipermeable CA was fully degraded by colonic microflora enzymes it would leave channels through which drug could release, leading to a gradual increase in drug release. This experiment confirmed that chitosan in the mixed film coat was accessible to enzymatic attack. The degradation of chitosan was therefore a rate-limiting factor.

Table 3
Fitting of drug release data of optimized formulation according to various mathematical models

Model	Parameters used to assess the fit of model						
	SSR	AIC	R^2	r	Intercept	Slope	k
Zero-order	515.1981	70.69	0.9223	0.9604	16.6805	3.9694	3.9694
First-order	81.1121	50.35	0.9862	−0.9931	4.4844	−0.0826	−0.0826
Higuchi	102.3079	52.91	0.9846	0.9923	0.7543	18.9523	18.9523

k, release rate constant for respective models (k_0 in %/h, k_1 in h^{-1} and k_H in %/h$^{1/2}$ for zero-order, first-order and Higuchi mathematical models, respectively).

4. Conclusion

A microbially triggered colon-targeted osmotic pump (MTCT-OP) was developed based on both the gelable property at acid conditions and colon-specific biodegradation of chitosan. The SEM indicated that the chitosan was accessible to enzymic degradation which allowed the in situ formation of delivery pores for releasing drug under conditions that may be expected to pertain in the colon, the number of pore being dependent on the initial level of pore former in the membrane. Budesonide release from the developed formulation was inversely proportional to the osmotic pressure of the release medium. The parameter of membrane permeability complied well with literature data, confirming that osmotic pumping mechanism was the major principle of drug release. The effects of different formulation variables were studied to select the optimal formulation. Drug release was directly proportional to the initial level of pore former, but inversely related to the membrane weight. Level of pH modifier (citric acid) affected the viscosity of chitosan solution, resulting in the change of osmotic pressure in the core tablets. The amount of chitosan in core formulation had a profound effect on the amount of drug release. An optimal core formulation that benefited from suspending and osmotic effect of chitosan has been developed. The enteric-coating membrane could prevent CA membrane containing pore former (chitosan) from forming pore or rupture before RCM dissolution procedure, and no significant difference in release profiles could be found when the level of enteric-coating membrane differed. MTCT-OP exhibited gastric and small intestine resistance but were susceptible to bacterial enzymatic attack, and the potential of MTCT-OP as a carrier for drug delivery to the colon was confirmed in simulated physiological GIT conditions. Furthermore, the physicomechanical and digestible property of chitosan-cellulose acetate free films and in vivo/in vitro correlation of MTCT-OP based on these studies are in progress to fully evaluate the system.

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References

Ahrabi, S.F., Madsen, G., Dyrstad, K., Sande, S.A., Graffner, C., 2000. Development of pectin matrix tablets for colonic delivery of model drug ropivacaine. Eur. J. Pharm. Sci. 10, 43–52.

Brondsted, H., Andersen, C., Hovgaard, L., 1998. Crosslinked dextran—a new capsule material for colon targeting of drug. J. Control. Release 53, 7–13.

Cole, E.T., Scott, R.A., Connor, A.L., Wilding, I.R., Petereit, H.U., Schminke, C., Beckert, T., Cade, D., 2002. Enteric coated HPMC capsules designed to achieve intestinal targeting. Int. J. Pharm. 231, 83–95.

Edward, J.O.C., 2003. Review of the unique properties of budesonide. Clin. Ther. 25, C42–C60.

European Pharmacopoeia, 2005. European Directorate for the Quality of Medicines. Strasbourg, France, pp. 1128–1130.

Fassihi, R., Pillay, V., 1998. Evaluation and comparison of dissolution data derived from different modified release dosage forms: an alternative method. J. Control. Release 55, 45–55.

Hebden, J.M., Gilchrist, P.J., Perkings, A.C., Wilson, C.G., Spiller, R.C., 1999. Stool water content and colonic drug absorption: contrasting effects of lactulose and codeine. Pharm. Res. 16, 1254–1259.

Janicki, S., Cichon, R., Jedras, Z., Sawicki, W., 1987. Gastrointesting therapeutic system delivering of a water insoluble drug: isosorbide dinitrate (ISDN). Pharmazie 42, 95–96.

Katsuma, M., Watanabe, S., Kawai, H., Takemura, S., Masuda, Y., Fukui, M., 2002. Studies on lactulose formulations for colon-specific drug delivery. Int. J. Pharm. 249, 33–43.

Lorenzo-Lamosa, M.L., Remunan-Lopez, C., Vila-Jato, J.L., Alonson, M.J., 1998. Design of microencapsulated chitosan microspheres for colonic drug delivery. J. Control. Release 52, 109–118.

Maris, B., Verheyden, L., Van Reeth, K., Samyn, C., Augustijns, P., Kinget, R., Van den Mooter, G., 2001. Synthesis and characterization of inulin–azo hydrogels designed for colon targeting. Int. J. Pharm. 213, 143–152.

Markus, W.R., Klein, S., Beckert, T.E., Petereit, H., Dressman, J.B., 2001. A new 5-aminosalicylic acid multi-unit dosage form for the therapy of ulcerative colitis. Eur. J. Pharm. Biopharm. 51, 183–190.

Muraoka, M., Hu, Z., Shimokawa, T., Sekino, S., Kurogoshi, R., Kuboi, Y., Yoshikawa, Y., Takada, K., 1998. Evaluation of intestinal pressure-controlled colon delivery capsule containing caffeine as a model drug in human volunteers. J. Control. Release 52, 119–129.

Nigalaye, A.G., Adusumilli, P., Bolton, S., 1990. Investigation of prolonged drug release from matrix formulations of chitosan. Drug Dev. Ind. Pharm. 16, 449–467.

Shibata, N., Ohno, T., Shimokawa, T., Hu, Z., Yoshikawa, Y., Koga, K., Murakami, M., Takada, K., 2001. Application of pressure-controlled colon delivery capsules to oral administration of glycyrrizin in dogs. J. Pharm. Pharmacol. 53, 441–447.

Shu, X.Z., Zhu, K.J., Song, W., 2001. Novel pH-sensitive citrate crosslinked chitosan film for drug controlled release. Int. J. Pharm. 212, 19–28.

Singla, A.K., Chawla, M., 2001. Chitosan: some pharmaceutical and biological aspects—an update. J. Pharm. Pharmacol. 53, 1047–1067.

Steed, K.P., Hooper, G., Monti, N., Benedetti, M.S., Fornasini, G., Wilding, I.R., 1997. The use of pharmacoscintigraphy to focus the development for a novel 5-ASA colon targeting system ('TIME CLOCK® ' system). J. Control. Release 49, 115–122.

Theeuwes, F., Guittard, G.V., Wong, P.S.L., 1990. Delivery of drug to colon by oral dosage form. US Patent 4,904,474.

Tozaki, H., Fujita, T., Odoriba, T., Terabe, A., Suzuki, T., Tanaka, C., Okabe, S., Muranishi, S.S., Yamamoto, A., 1999. Colon-specific delivery of R68070, a new thromboxane synthase inhibitor, using chitosan capsules: therapeutic effects against 2,4,6-trinitrobenzene sulfonic acid-induced ulcerative colitis in rats. Life Sci. 64, 1155–1162.

Tozaki, H., Komoike, J., Tada, C., Maruyama, T., Terabe, A., Suzuki, T., Yamamoto, A., Muranishi, S., 1997. Chitosan capsules for colon-specific drug delivery: improvement of insulin absorption from the rat colon. J. Pharm. Sci. 86, 1016–1021.

Tozaki, H., Odoriba, T., Okada, N., Fujita, T., Terabe, A., Suzuki, T., Okabe, S., Murnishi, S., Yamamoto, A., 2002. Chitosan capsules for colon-specific drug delivery: enhanced localization of 5-aminosalicylic acid in the large intestine accelerates healing of TNBS-induced colitis in rats. J. Control. Release 82, 51–61.

Verma, R.K., Krishna, D.M., Garg, S., 2002. Formulation aspects in the development of osmotically controlled oral drug delivery systems. J. Control. Release 79, 7–27.

Wong, D., Larrabee, S., Clifford, K., Tremblay, J., Friend, D.R., 1997. USP dissolution apparatus III (reciprocating cylinder) for screening of guar-based colonic delivery formulations. J. Control. Release 47, 173–179.

Yano, H., Hirayama, F., Kamada, M., Arima, H., Uekama, K., 2002. Colon-specific delivery of prednisolone-appended alpha-cyclodextrin conjugate: alleviation of systemic side effect after oral administration. J. Control. Release 79, 103–112.

Zambito, Y., Colo, G.D., 2003. Preparation and in vitro evaluation of chitosan matrices for colonic controlled drug delivery. J. Pharm. Pharm. Sci. 6, 274–281.

Zentner, G.M., Rork, G.S., Himmelstein, K.J., 1985. The controlled porosity osmotic pump. J. Control. Release 1, 269–282.

Zentner, G.M., Rork, G.S., Himmelstein, K.J., 1990. Controlled porosity osmotic pump. US Patent 4,968,507.

Zhang, H., Ibrahim, A.A., Neau, S.H., 2002. An in vitro evaluation of a chitosan-containing multiparticulate system for macromolecule delivery to the colon. Int. J. Pharm. 239, 197–205.

Zhang, H., Neau, S.H., 2002. In vitro degradation of chitosan by bacterial enzymes from rat cecal and colonic contents. Biomaterials 23, 2761–2766.