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Studies of chitosan/Kollicoat SR 30D film-coated tablets for colonic drug delivery

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ABSTRACT

The aim of the study was to define in vitro and in vivo characteristics of chitosan/Kollicoat SR30D film-coated tablets of theophylline for colonic delivery. The tablet cores were coated to different film thicknesses with blends of Kollicoat SR30D and chitosan (2.5:1, 3.5:1, and 5:1, w/w). Swelling and drug release studies were carried out in simulated gastric fluid, simulated intestinal fluid and simulated colonic fluid, respectively. The mechanism of drug release was determined using the Korsmeyer–Peppas model. The in vivo degradation of the tablets was also studied in rats. The swelling behavior and drug release depended on the composition of the coating, as well as the ratio of Kollicoat SR30D to chitosan. The coating was susceptible to enzymatic action, and more accessible to bacterial enzymes than β-glucosidase enzyme. The extent of swelling and digestion correlated with the amount of chitosan within the coating. The drug release data fit well into the Korsmeyer–Peppas equation, indicating that the drug release was controlled by polymer relaxation. The in vivo pharmacokinetic studies of the coated tablets showed delayed T_{max}, decreased C_{max} and prolonged *MRT*. Chitosan/Kollicoat SR30D coated tablets could deliver the drug to the targeted site for local action.

1. Introduction

Colonic drug delivery is recognized to be advantageous in the treatment of disorders of the large intestine, such as irritable bowel syndrome, colitis, Crohn's disease, colon cancer, and infectious diseases where it is necessary to attain a high concentration level of active agent in the large intestine (Jain et al., 2007). Various strategies are currently available to target the release of drugs to the colon: (1) pH-dependent systems, (2) time-dependent systems, (3) prodrugs, (4) pressure-dependent systems and (5) colonic microbiota-activated systems.

Microbially activated delivery systems for colon targeting are being developed to exploit the potential of the specific nature of diverse and luxuriant microbiota associated with the colon compared to other parts of the gastrointestinal (GI) tract (Kosaraju, 2005). These colonic microbiotas produce a large number of hydrolytic and reductive enzymes (Rowhmd, 1988) which can potentially be utilized for colonic delivery. Prodrugs (Ryde, 1992) and coatings based on azoaromatic polymer (Saffran et al., 1986) and matrices (Brondsted and Kopecek, 1991) containing azoaromatic cross-links are examples of systems that are degradable by reductive enzymes released by colonic bacteria (Jain et al., 2006). Apart from azo reductase enzyme, the colonic bacteria release other polysaccharidases like glucosidases which are responsible for the degradation of polysaccharides (Larsen et al., 1989). Hence, drug delivery systems based on polysaccharide can be used for colonic delivery. Many natural polysaccharides, such as amylose, pectin, guar gum and chitosan, have been investigated for their potential to obtain colonic delivery. Especially, the blend of amylose/ethylcellulose film has been shown to have great potential as colonic targeting carries (Cummings et al., 1996; Milojevic et al., 1996a,b; Basit et al., 2004; McConnell et al., 2008). Currently, the amylose/ethylcellulose coating system for colonic targeting, which is known as COLAL, is in

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late stage clinical trials for the local treatment of ulcerative colitis.

Chitosan, a natural polymer obtained by alkaline deacetylation of chitin, is non-toxic, biocompatible, and biodegradable. It is susceptible to glycosidic hydrolysis by microbial enzymes in the colon since it possesses glycosidic linkages similar to those of other enzymatically depolymerized polysaccharides (Kosaraju, 2005). As a result, this compound could be promising for colonic delivery if its solubility is reduced in gastric acid conditions (Tozaki et al., 1997; Shimono et al., 2002). This could be accomplished by combining water-insoluble polymers to produce an insoluble film coating. In fact, the method of film coating by the use of aqueous dispersion of chitosan–polymer composite appears to be more effective than that of other coating methods (e.g. compression–coating) using a blend of chitosan–polymer composite. However, no suitable hydrophobic polymer is successfully selected to combine with chitosan as a film-coating material for colonic delivery until now. In our previous investigation, the characteristics of chitosan/Kollicoat SR 30D (composed of 27% polyvinyl acetate (PVAc) free films were studied (He et al., 2008c). The results indicated that the chitosan/Kollicoat SR30D films could be successfully prepared by casting/solvent evaporation method. The free films not only had good mechanical properties, but also were susceptible to digestion by colonic bacterial enzymes. Thus, the application of such chitosan/Kollicoat SR30D films as coatings to oral dosage forms could direct solid dosage forms to the large intestine, where the component of the polysaccharide chitosan, incorporated into the blend film, would be digested. This digestion would allow the delivery of the drug(s) present in the dosage form in the colon. Single-unit systems provide an alternative and more common platform for oral modified-release drug delivery because of their ease and cost of manufacture (Wilson and Basit, 2005). The purpose of current work therefore was (i) to prepare chitosan/Kollicoat SR30D film-coated tablets for colonic delivery; (ii) to investigate the effects of the polymer blend ratio and coating level on the resulting drug release; (iii) to study the in vivo degradation of tablets in rats; and (iv) to assess the pharmacokinetics in dogs. Theophylline is useful in the treatment of nocturnal asthma and falls under Class I (high solubility-high permeability) drug according to the Biopharmaceutical Classification System and is well absorbed in the whole GI tract (Han et al., 2008), thus it was chosen as the model drug in our study.

2. Method and materials

2.1. Materials

Kollicoat SR30D was a gift from BASF (Ludwigshafen, Germany). Chitosan (molecular weight of 45 kDa, 85% degree of deacetylation) was obtained from Luyang Chemical Co., Ltd. (Rongcheng, China); β-glucosidase enzyme was from Yusen Bio. Ltd. (Shanghai, China). Theophylline was purchased from Xinhua Pharm. Co., Ltd. (Zibo, China).

2.2. Preparation of chitosan/KollicoatSR30D film-coated tablets

Tablets were prepared by wet granulation to the following formula: 35% theophylline, 40% lactose, 19% microcrystalline cellulose, 5% polyvinyl pyrrolidone and 1% magnesium stearate. The tablets were manufactured using a single punch tabletting machine (Gylongli Co., Ltd., Beijing, China). The tablets were bi-convex in design, 8 mm in diameter and 200 mg in mass.

Chitosan solutions (2.5 wt.%) were prepared by dissolving chitosan in 0.5% acetic acid solution at ambient temperature with stirring for overnight. The pH value of the solution was adjusted to 4.0–4.5 before use. Then, predetermined amounts of Kollicoat SR30D (2.5:1, 3.5:1 and 5:1, Kollicoat SR30D to chitosan, w/w) were added to this solution with stirring and stirred for a further 3 h to produce coating formulations.

The tablets were coated using a rotary tablet machine (Huanghai Machinery Co., Ltd., Shanghai, China). Drying air was introduced into the front of the pan approximately perpendicular to the tablet bed and the extract was located at the top of the coating pan. The coating conditions were as follows: atomizing pressure of 3.0 bar, inlet temperature of 50 °C, a bed temperature of 30 °C, a pan rotation speed of 20 rpm and a spray rate of 10–12 g/min (NO-2B spray gun, Youda Co., Ltd., Shanghai, China). The coated tablets were further dried in a coating pan for 15 min at 40 °C after the coating process was completed. Furthermore, the tablets coated with Kollicoat SR30D (15%, w/v solids content) were also prepared to obtain a predetermined weigh gain. A series of coated products were produced with different film thicknesses and quantified by the total weight gain (%TWG).

2.3. Swelling test

Chitosan/Kollicoat SR30D coated tablets were accurately weighed and immersed in a flask of dissolution test containing 250 ml of different medium at 37 °C. At specific intervals, the swollen tablet was withdrawn from the medium and weighed after removal of excess surface water by light blotting with a filter paper. The swelling behavior of the coated tablets was calculated using Eq. (1)

$$SD = \frac{W_t - W_o}{W_o} \quad (1)$$

where SD is the swelling degreed of tablet, W_t is the weight of tablet at appropriate intervals in buffer saline and W_o is the absolutely dried weight of tablet. Swelling tests were separately carried out in simulated gastric fluid (SGF, 0.1 M HCl, pH 1.2), simulated intestinal fluid (SIF, phosphate buffer, pH 6.8) and simulated colonic fluid (SCF). The SCF was SIF to which was added rat cecal contents (4%, w/w) (He et al., 2008a) or β-glucosidase enzyme (4%, w/w) (Nunthanid et al., 2008). Three parallel measurements were performed in each case.

2.4. In vitro release studies

The drug release from the chitosan/Kollicoat SR30D coated tablets was determined as follows: the coated tablets were placed into conical flask with 100 ml of release medium and incubated at 37 °C under shaking 100 strokes/min. The release media was SGF (pH 1.2), SIF and SCF with adding of rat cecal contents (4%, w/w) (He et al., 2008a) or β-glucosidase enzyme (4%, w/w), prepared according to the Chinese Pharmacopoeia 2005, respectively. At appropriate intervals, five milliliters of the solutions was replaced by fresh medium. The amount of theophylline released from the tablets was measured using an HPLC method described below.

Mean dissolution time (MDT) reflects the time for the drug to dissolve and is the first statistical moment for cumulative dissolution process that provides an accurate drug release rate. It is an accurate expression for drug release rate. A higher MDT value indicates great drug-retarding ability (Tanigawara et al., 1982; Patel and Patel, 2007). Each in vitro release dissolution testing was performed in triplicate. The MDT was estimated by Eq. (2):

$$MDT = \frac{n}{n+1} \cdot k^{-1/n} \quad (2)$$

where n is the release exponent and k is release rate constant (Mockel and Lippold, 1993) derived from exponential or Korsmeyer–Peppas equation.

2.5. Mechanisms and kinetics of drug release

The mechanism of drug release from cylindrical rods during dissolution test in SGF, SIF and SCF was determined using Eq. (3), the Korsmeyer–Peppas model:

$$\frac{M_t}{M_f} = k \cdot t^n \quad (3)$$

where M_t and M_f are the absolute cumulative amount of drug released at time t and infinite time, respectively; k is a constant incorporating structural and geometric characteristics of the drug dosage form, and n is the release exponent, indicative of the mechanism of drug release. Drug release data were employed for determination of the release exponent.

When the exponent n takes a limiting value of 0.45, it is in the case of diffusion-controlled drug release (Fickian release). Case II transport or relaxation controlled delivery, the exponent n is 0.89 for release from cylinders. Values of n between 0.45 and 0.89 can be regarded as an indicator for the non-Fickian release or anomalous transport. The non-Fickian kinetics is regarded as couple diffusion/polymer relaxation. In addition, Super Case II kinetics is regarded when the values of $n > 0.89$ for release from cylinders are observed (Korsmeyer et al., 1983).

2.6. In vivo degradation studies

The coated tablets (TWG-40% 1:1 ratio and TWG-40% 2:1 ratio Kollicoat SR30D: chitosan) were tested for in vivo degradation in the cecum of rats (weighing from 200 to 250 g) using a method described previously by Adkin et al. (1997) and Haupt et al. (2006). Briefly, the tablets were implanted into the cecum of the anaesthetized rats after being mounted in gauze bags. After 24 h, the rats were sacrificed. The collected tablets from the bags were dried at 50 °C for 6 h, and then weighed. The rats treated with antibiotic cocktail (300 ml of intra-cecal administration of chloramphenicol 0.5 mg/ml and cefazoline 500 mg/ml) were used as controls. In the control study, the coated tablets were weighed and implanted, and then the tablets were dried and weighed after 24 h. The antibiotic cocktail was added to the drink water of the control rats for the 24 h of the study.

2.7. In vivo studies in dogs

The pharmacokinetics of uncoated tablets and coated tablets (TWG-25% 3.5:1 ratio Kollicoat SR30D: chitosan) of theophylline were assessed and compared in dogs in a randomized, two-period crossover study. The washout period between administrations was one week. Six male beagle dogs weighing from 8 to 10 kg were used in this study. The dogs were fed standard laboratory chow with water and fasted overnight before the experiments. The animals used in the experiments received care in compliance with the "Principles of Laboratory Animal Care" and "Guide for the Care and Use of Laboratory Animals". Experiments followed an approved protocol from Hebei Medical University Institutional Animal Care and Use Committee.

The uncoated or coated tablets (containing 70 mg of drug) were orally administered in dogs. At time intervals, two milliliters of blood samples were collected from saphenous vein into heparinized tubes and centrifuged at 1000 g for 10 min and stored at −20 °C until assay. Blood sampling time points were 0, 1, 2, 5, 7, 9, 11, 13, 15 and 24 h after administration of the coated tablets; for the uncoated tablets, blood samples (2.0 ml) were at 0, 0.5, 1, 2, 4, 5, 15 and 24 h after administration. Frozen plasma samples were prepared using the procedure reported by Hayashi et al. (2007). The drug concentration of plasma samples was determined using a validated HPLC method described below.

2.8. HPLC assay

The drug concentrations in all samples were determined using an HPLC assay. The HPLC system consisted of a Waters 2487detector (UV) and an Empower workstation. The separations were performed at 25°C using a 250 mm × 4.6 mm column (Diamonsil™ C_{18}). The mobile phase was consisted of a mixture of 0.05 mol/l ammonium acetate/methanol (70:30, v/v), and the mobile phase was filtered and pumped at a flow rate of 1 ml/min (Mo et al., 2005). The paracetamol was used as an internal standard. The column was maintained at a temperature of 25°C. The eluent was detected by UV detector at 273 nm.

2.9. Pharmacokinetics and data analysis

Pharmacokinetic (PK) parameters were calculated by non-compartment analysis based on statistical moment theory using Microsoft Excel software. The PK parameters, such as maximum plasma concentration (C_{max}) and time of maximum concentration (T_{max}), were obtained directly from the plasma concentration–time plots. The area under the plasma concentration–time curve up to the last time (t) (AUC_{0-t}), area under curve extrapolated to infinity ($AUC_{0-\infty}$) and area under the first moment curve extrapolated to infinity ($AUMC_{0-\infty}$) were calculated using the linear trapezoidal rule. The mean residence time (MRT) was calculated as $AUMC/AUC$. Results were expressed as mean ± standard deviation.

Variations in PK parameters were tested using analysis of variance (ANOVA). Difference in mean PK parameters of theophylline between 3.5:1 ratio TWG-25% coated tablets and uncoated tablets was subjected to t-test to find the statistical significance. In all the cases, a value of $P < 0.05$ was considered statistically significant.

3. Result and discussion

3.1. Swelling test

The results of swelling behavior in SGF, SIF and SCF are shown in Fig. 1(A–D). The SD depended on the Kollicoat SR30D/chitosan blend ratio as well as the amount of chitosan in the film. As expected, the SD increased with decreasing the proportion of Kollicoat SR30 or increasing the amount of chitosan in the coating. That could be attributed to the hygroscopic characteristics of polysaccharides chitosan and the high water uptake of the polymer. Compared with the swelling behavior in SIF, the SD was higher in SGF than in SIF since the amino-group in chitosan was prone to be protonated and dissolved in acid environments. The swelling equilibrium time was also decreased with the increasing of mass ratio of chitosan. The SD of the coated tablets in SCF significantly increased when compared with that in SIF and SGF; and the effect of the addition of chitosan on the SD was more profound in SCF. The resulting swelling in SCF with cecal contents was relatively higher than that in presence of β-glucosidase enzyme. An increase in swelling for polysaccharide materials after degradation indicated bulk degradation (Langer and Peppas, 1981). Thus, this is due to the penetrating of rat cecal bacteria or β-glucosidase enzyme into the polymeric chains (Sinha and Kumria, 2003), hydrolyzing the glycosidic linkages within the chitosan, reducing the network density and finally increasing the swelling degreed (Akhgari et al., 2006).

3.2. In vitro release studies

Fig. 2(A–D) shows the theophylline release profiles in SGF, SIF and SCF, respectively. The release rate from the chitosan/Kollicoat SR30D coated tablets is influenced by the amount of chitosan present in the film and the coating levels. It appears that the blend ratio has a more profound effect on the drug release than

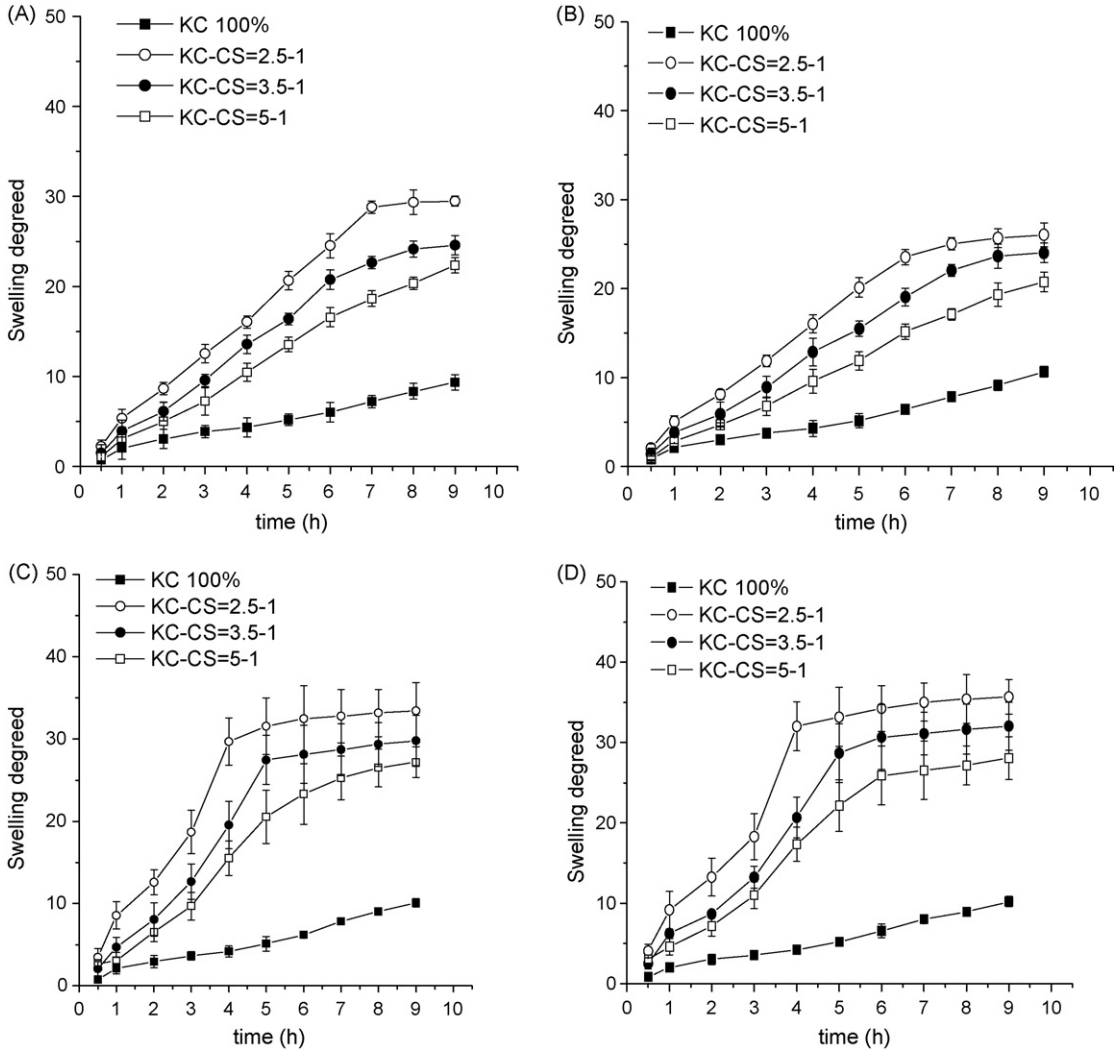

Fig. 1. Swelling behavior of Kollicoat SR30D (KC)/chitosan (CS) film-coated tablets with TWG-20% in SGF (A), SIF (B), and SCF in presence of β-glucosidase enzyme (C) or rat cecal contents (D), respectively ($n = 3$).

the coating levels. With increasing the coating levels the release rate decreased, which could be attributed to the increased diffusion pathway. Similarity to the results of the swelling experiment, the drug release in SGF was faster than that in SIF for the coated tablets since the chitosan was dissolved and leached from the coating in acidic medium. Faster drug release in SCF than in SGF or SIF ($P < 0.05$) demonstrated the susceptibility of chitosan in the coating to bacterial enzymes and degradation. The release rate from Kollicoat SR30D per se coated tablets in SCF was almost the same as that in SGF or SIF. It indicated that PVAc is recalcitrant to bacterial or β-glucosidase enzyme action, which functions in controlling the swelling of the chitosan in the coating. The adding of rat cecal contents or β-glucosidase enzyme to the dissolution medium resulted in an increase in the release of theophylline from the coated tablets; and the MDT was lower than that in SIF or SGF (Table 1). It could be explained that the rat bacterial enzymes or β-glucosidase enzyme increased the rate and extent of the chitosan leaching from the film coatings; the leaching of the large-molecular weight chitosan created aqueous channels or water-filled pores that allow diffusion of drug molecules through the film.

Fig. 3 shows the theophylline release behavior from the 3.5:1 ratio TWG-25% coated tablets in simulated GI tract condition. It was observed that a small amount of drug was released in the simulated gastric and small intestinal conditions, and a considerable amount of drug was released in SCF. Thus, it appeared that the coated tablets may specially deliver the drug to the colon.

To achieve the local therapy for colon-related disease, the chitosan/Kollicoat SR30D film-coated tablets should prevent drug release in the upper GI tract, and the chitosan in the coatings should be sensitive to enzymatic action to ensure that most of the drug is released in the targeted site (Basit et al., 2004). In addition, the rate of enzymatic degradation and drug release in the colon was direct ratio to the amount of chitosan in the coating (He et al., 2008c). Thus, the 3.5:1 ratio TWG-25% coated tablets were considered to be a more suitable formulation when compared with other formulations (2.5:1 and 5:1 ratio Kollicoat SR30D: chitosan). Thus, the 3.5:1 ratio TWG-25% coated tablets were selected for the next in vivo experiments to evaluate its ability to target the drug to the colon.

3.3. Mechanisms and kinetics of drug release

The Korsmeyer–Peppas model is used to analyze drug release from pharmaceutical dosage forms when the release mechanism is not well known or when more than one type of release phenomena is involved (Korsmeyer et al., 1983). The drug release data fitted to the Korsmeyer–Peppas model is shown in Table 1. It is indicated that the release data fit well the model since the correlation coef-

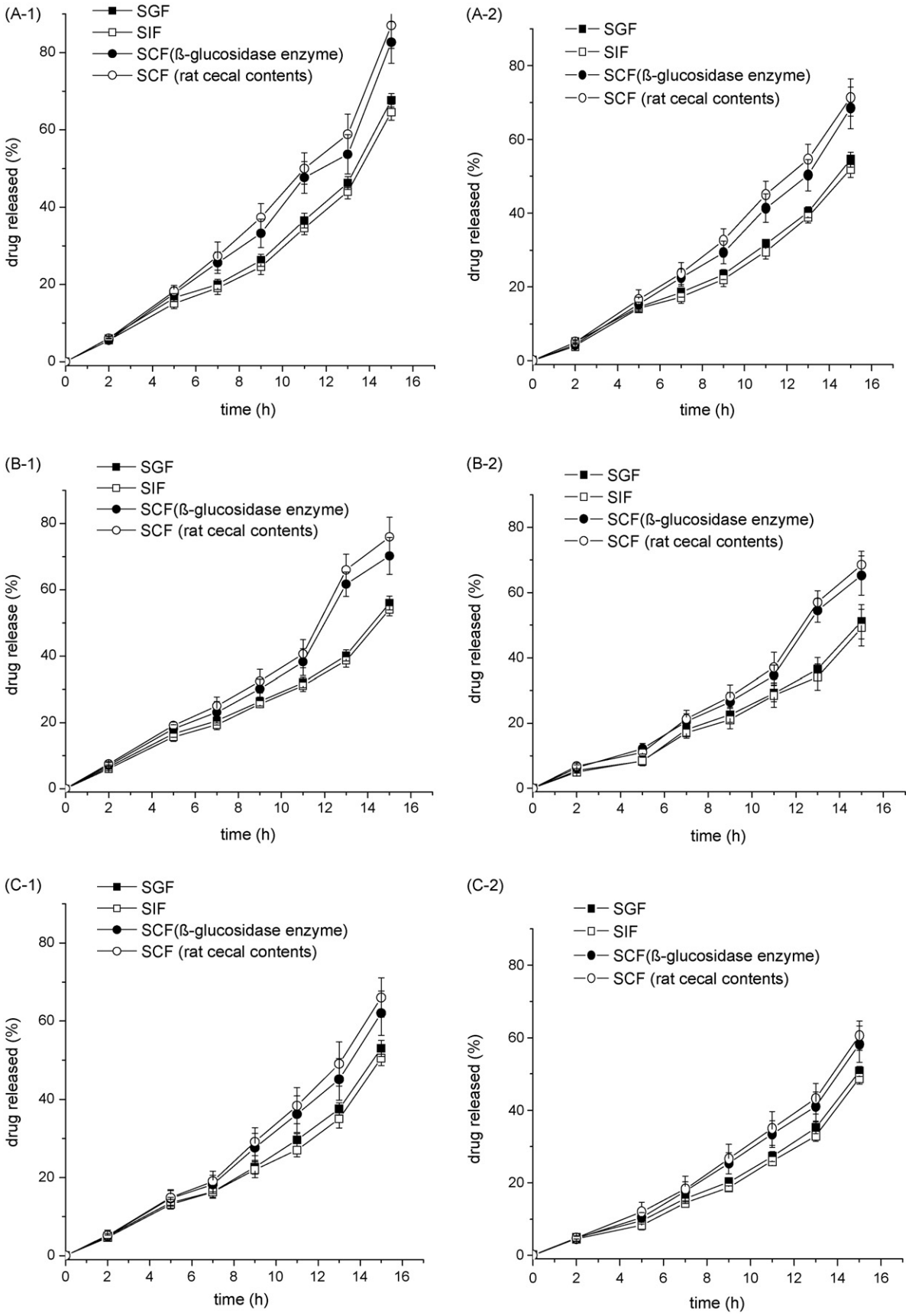

Fig. 2. Effect of Kollicoat SR30D (KC) to chitosan (CS) ratio and coat thickness (%TWG) on theophylline release from the KC/CS film-coated tablets in SGF, SIF, and SCF in presence of β-glucosidase enzyme or rat cecal contents ($n = 3$). A: KC-CS =2.5-1, A-1, TWG-25%; A-2, TWG-30%; B: KC-CS =3.5-1, B-1, TWG-20%; B-2, TWG-25%; C: KC-CS =5-1, C-1, TWG-15%; C-2, TWG-20%.

Table 1
Korsmeyer–Peppas model fitting of release data and mean dissolution time (*MDT*) of Kollicoat SR30D/chitosan coated tablets ($n = 3$).

Formulation	Korsmeyer–Peppas model				Mean dissolution time (h)
	Correlation coefficient, r^2	Kinetic constant, k	Diffusional exponent, n	Order of release	
In simulated gastric fluid (SGF) pH 1.2					
2.5:1 with TWG-25%	0.9911	0.030	1.01	Super case II	16.55 ± 1.36
2.5:1 with TWG-30%	0.9937	0.024	1.05	Super case II	17.49 ± 2.51
3.5:1 with TWG-20%	0.9959	0.035	0.92	Super case II	18.05 ± 0.65
3.5:1 with TWG-25%	0.9955	0.029	0.98	Super case II	18.55 ± 1.87
5:1 with TWG-15%	0.9922	0.025	1.01	Super case II	19.29 ± 3.65
5:1 with TWG-20%	0.9907	0.023	0.99	Super case II	22.04 ± 2.05
In simulated intestinal fluid (SIF) pH 6.8					
2.5:1 with TWG-25%	0.9979	0.028	1.03	Super case II	16.59 ± 1.32
2.5:1 with TWG-30%	0.9963	0.019	1.14	Super case II	16.84 ± 2.35
3.5:1 with TWG-20%	0.9972	0.032	0.95	Super case II	18.48 ± 1.69
3.5:1 with TWG-25%	0.9964	0.025	1.07	Super case II	18.70 ± 0.67
5:1 with TWG-15%	0.9941	0.023	1.04	Super case II	19.24 ± 2.35
5:1 with TWG-20%	0.9901	0.023	0.98	Super case II	23.34 ± 1.87
In simulated colonic fluid (SCF) with β-glucosidase enzyme					
2.5:1 with TWG-25%	0.9979	0.023	1.23	Super case II	11.66 ± 3.54
2.5:1 with TWG-30%	0.9963	0.017	1.33	Super case II	12.23 ± 2.67
3.5:1 with TWG-20%	0.9972	0.018	1.31	Super case II	15.02 ± 3.65
3.5:1 with TWG-25%	0.9891	0.033	0.98	Super case II	16.02 ± 3.04
5:1 with TWG-15%	0.9915	0.022	1.13	Super case II	14.99 ± 2.87
5:1 with TWG-20%	0.9960	0.021	1.14	Super case II	15.86 ± 4.05
In simulated colonic fluid (SCF) with rat cecal contents					
2.5:1 with TWG-25%	0.9982	0.026	1.20	Super case II	11.31 ± 2.65
2.5:1 with TWG-30%	0.9984	0.021	1.26	Super case II	12.01 ± 2.98
3.5:1 with TWG-20%	0.9987	0.039	0.97	Super case II	13.59 ± 5.64
3.5:1 with TWG-25%	0.9907	0.036	0.95	Super case II	15.81 ± 4.05
5:1 with TWG-15%	0.9933	0.023	1.13	Super case II	14.67 ± 2.97
5:1 with TWG-20%	0.9956	0.021	1.15	Super case II	15.15 ± 4.01

ficient (r^2) was greater than 0.99 in all cases. The release exponent ranged from 0.95 to 1.33 in SGF, SIF and SCF for all coated tablets, and the values of kinetic constant ranged from 0.017 to 0.035. The drug release can be ascribed to a 'Super case II' transport, in which the drug release seemed to be controlled by polymer relaxation since the values of exponent was greater than 0.89 (Korsmeyer et al., 1983). The result is consistent with a recent study by Ghaffari et al. (2008), suggesting that pectin–chitosan/Eugragit RS coated pellets exhibited an increased drug release following with an anomalous release (Super case II' transport) in the simulated colonic medium.

MDT value is always used to characterize the rate of drug release from a dosage form and indicates the drug release retarding efficiency of polymer (Kuksal et al., 2006). The coated tablets showed a faster drug release and lower MDT in SCF than that in SIF or SGF. According to the mechanism of drug release, it could be explained that the extent of film relaxation was promoted by colonic bacteria or β-glucosidase enzyme.

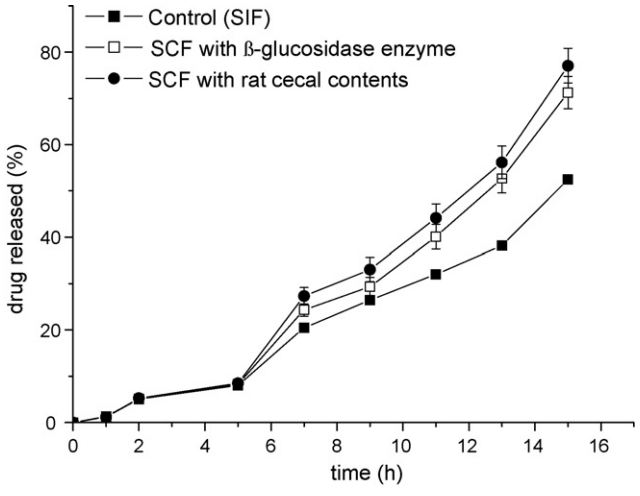

Fig. 3. Mean percentage release of theophyllin from the 3.5:1 ratio TWG-25% coated tablets after 2 h in SGF, 3 h in SIF and 10 h in SCF with and without β-glucosidase enzyme or rat cecal contents, respectively ($n = 3$).

3.4. Comparative degradation in SCF with rat bacterial enzymes or β-glucosidase enzyme

The ability of the enzymes to diffuse into the coating is directly correlated to the extent of enzymatic degradation and the mode of degradation (Zhang et al., 2002). Swelling is indispensable for enzymatic degradation of the azo polymer films in the colon (Van den Mooter et al., 1994). The coated tablets in SCF with rat cecal contents were swollen to a larger extent when compared with the swelling degree in SCF with β-glucosidase enzyme. It was explained that rat cecal contents contained more than one bacterial enzyme (included *bacteroides, bifidobacteria* and *enterobacteria, etc.*) that could degrade the chitosan in the coating (Finegold et al., 1977; Gamelin et al., 1998; Yang, 2008). In fact, Salyers and Leedle (1983) also reported that more than one bacterial enzyme was involved in the digestion of polysaccharides. It meant that the degradation bond (1,4 glycosidic bond) could be recognized and digested by more than one bacterial enzyme. Thus, the rat bacterial enzymes were more effective at penetrating into the coating than β-glucosidase enzyme, promoting the degradation of the coating by the bacterial enzymes. The drug release from the tablets was dependent on the enzyme-catalyzed hydrolysis of those bonds. Thus, the release rate in SCF with cecal contents was faster (lower MDT) than that in presence of β-glucosidase enzyme in the experiment.

3.5. In vivo degradation studies

The simulated colonic fluids in presence of rat cecal contents could not fully represent the real colonic environment contained

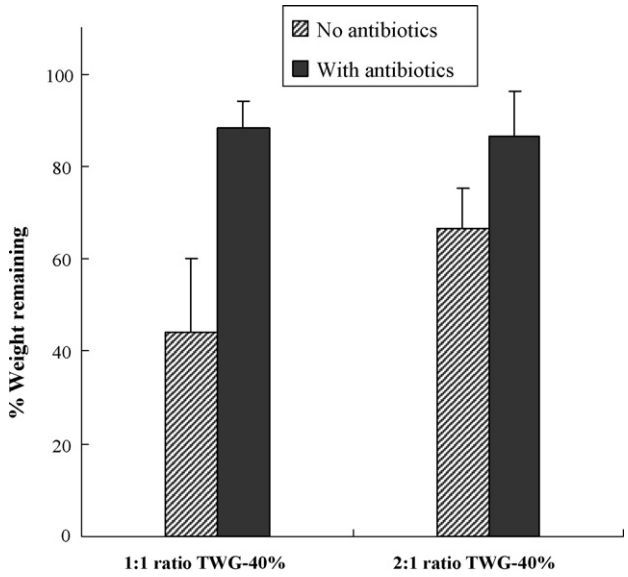

Fig. 4. The degradation of the 1:1 ratio TWG-40% and 2:1 ratio TWG-40% tablets in the rat cecum with and without antibiotic treatment ($n = 3$).

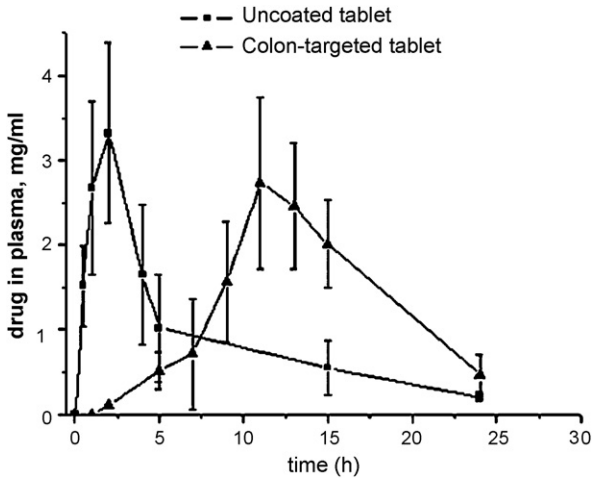

Fig. 5. Plasma concentration profiles of theophyllin after oral administration of uncoated or colon-targeted tablets in dogs ($n = 6$).

bacterial enzymes (Adkin et al., 1997). In order to better mimic the colon conditions, the in vivo degradation model was carried out in the study since the rat cecum possesses similar enzymatic activity to the human colon (Hawksworth et al., 1971).

The in vivo degradation of coated tablets in the rat cecum is shown in Fig. 4, suggesting that increasing the chitosan component of the film coating increased the extent of degradation. With increasing the blend ratio the extent of degradation decreased. After 24 h, the weight loss was 55.7% and 33.9% for the 1:1 ratio TWG-40% and 2:1 ratio TWG-40% formulations, respectively. Compared with control rats treated with antibiotic cocktail, the weight loss was 21.7% and 13.3% for the two formulations, respectively. The weight loss was mainly due to the release of the degradation products. A significant difference, between the weight loss in antibiotic treated and non-treated of the two formulations, was observed. It is because that the enzymatic activity of bacteria was reduced when the rats were treated with antibiotics (Gliko-Kabir et al., 2000). The results also indicated that the degradation of the chitosan in the coating was caused by the cecal bacteria.

3.6. Pharmacokinetics

The pharmacokinetics of the 3.5:1 ratio TWG-25% coated tablets was evaluated in dogs and compared with the uncoated tablets of theophylline. Mean plasma theophylline concentration *vs.* time profiles after a single oral dose of the two formulations are shown in Fig. 5. Mean values of pharmacokinetic parameters are summarized in Table 2.

The chitosan/Kollicoat SR30D coated tablets showed sustained release/absorption characteristics. There was a significant delay in the absorption time and T_{max} of theophylline after oral administration of the coated tablets. Theophylline appeared in the plasma within 0.5 h after oral administration of the uncoated tablets; it took about 5 h for theophylline to appear in plasma when the coated tablets were administered (Fig. 5.). The postponed in vivo drug release in upper GI tract is ascribed to the water-insoluble and resistant enzyme degradation nature of the PVAc which functioned in reducing the swelling of chitosan in the coating. Furthermore, the chitosan within the coating is not digested by human digestive enzymes in the upper GI tract (Chourasia and Jain, 2004). When the coated tablets were transited to the large intestine contained abundant microbiota, the microbiota could degrade the chitosan in the coating. The digested chitosan created aqueous channels or water-filled pores that allowed diffusion of drug molecules through the film, resulting in an increase of drug release.

Table 2
Pharmacokinetic parameters of theophylline after oral administration of uncoated or coated tablet to dogs ($n = 6$).

	Uncoated tablet	Colon-targeted tablet
C_{max} (mg/ml)	3.35 ± 0.86	2.46 ± 0.51*
T_{max} (h)	2.00 ± 0.28	11.00 ± 1.05*
MRT (h)	6.61 ± 1.02	10.69 ± 2.25*
$AUC_{0-\infty}$ (μg h/ml)	25.46 ± 2.35	19.57 ± 3.08*

AUC, area under the plasma–concentration time curve; C_{max}, maximum plasma concentration; T_{max}, time of C_{max}; *MRT*, mean residence time.
* $P < 0.05$.

The T_{max}/C_{max} of theophylline from the coated tablets was 11.00 ± 1.05 h/2.46 ± 0.51 mg/ml and 2.00 ± 0.28 h/3.35 ± 0.86 mg/ml, respectively. In the case of the uncoated tablets, C_{max} was 3.35 ± 0.86 mg/ml and T_{max} was 2.00 ± 0.28 h, which were both significantly different from the values obtained from the coated tablets ($P < 0.001$). The mean C_{max} for the coated tablets was lower than that for the uncoated tablets ($P < 0.05$); it was attributable to the sustained in vitro drug release profile of the coated pellets and tight junctions and few transport carriers of the large intestine (limiting carrier-mediated and paracellular transport) (Jung et al., 2006).

The *MRT*s for the uncoated and coated theophylline tablets were 6.6 and 10.7 h, respectively, which in another aspect confirmed the delayed absorption of the in situ coated tablets as a result of retardation on initial drug release. There was statistically significant difference ($P < 0.05$) in the *AUC* values between the uncoated tablets and the coated tablets. It may be due to the small surface available for absorption within the colon and tight junctions in the large intestine (Jung et al., 2006). The extended release characteristics and prolonged MRT of the chitosan/Kollicoat SR30D coated tablets indicated that drug release occurred during the transiting period in the colon and along its length, which was beneficial to local treatment of the colon-related diseases (Ji et al., 2007; He et al., 2008b; McConnell et al., 2008). It should be noted that the profiles of in vivo drug release was consistent with our previous report (He et al., 2008b) and the study by McConnell et al. (2008). The studies showed that the characteristics of sustained drug release and absorption with a prolonged residence time in the colon were obtained from the pectin or amylose/ethylcellulose film-coated sys-

tems. In fact, these coating systems have similar component in the coating, being composed of water-insoluble polymer and polysaccharides. Thus, they had similar mechanism of in vitro drug release, resulted in a similar nature of in vivo drug release. The results also indicated that the method of combining water-insoluble polymer with polysaccharides as film coatings for site-specific to the colon is promising.

4. Conclusions

The blend of chitosan/PVAc as a film coat may provide the necessary protection to a drug in the upper GI tract while allowing enzymatic breakdown and drug release in the colon. The coating was susceptible to enzymatic action, and better accessible to bacterial enzymes contained in the cecal contents than β-glucosidase enzymes. The SCF with rat cecal bacterial enzymes were more effective than that with β-glucosidase enzyme for the digestion of chitosan within the coating. The extent of swelling and digestion correlated with the amount of chitosan present within the coating. The release rate is influenced by the blend ratio and the coating levels. The in vivo pharmacokinetic studies of the coated tablets showed delayed T_{max}, decreased C_{max} and prolonged *MRT*, indicating that the coated tablets did not release the drug in the stomach or small intestine, but delivered it to the colon for local action.

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