European Journal of Pharmaceutics and Biopharmaceutics 72 (2009) 266–274

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research paper

An investigation into the characteristics of chitosan/Kollicoat SR30D free films for colonic drug delivery

He Wei [a,b,*], Fan Li-Fang [c,d], Xiang Bai [a], Li Chun-Lei [b], Du Qing [a], Chang Yong-Zhen [e], Cao De-Ying [a,*]

[a] Department of Pharmaceutics, Hebei Medical University, ShiJiaZhuang, PR China
[b] CSPC Pharmaceutical Technology Co., Ltd, ShiJiaZhuang, PR China
[c] Department of Pharmaceutical Analysis, Hebei Medical University, ShiJiaZhuang, PR China
[d] Hebei Yiling Pharmaceutical Group, Medicine Institute, Hebei Medical University, Beijing, PR China
[e] Department of Pharmaceutics, XingTai Medical College, Hebei Medical University, XingTai, PR China

ARTICLE INFO

Article history:
Received 2 May 2008
Accepted in revised form 29 October 2008
Available online 8 November 2008

Keywords:
Chitosan
Kollicoat SR30D
Colonic drug delivery
Mixed-film
Permeability

ABSTRACT

The purpose of the study was to establish the physico-mechanical, digestibility, permeability and swelling properties of chitosan/Kollicoat SR30D films as potential coatings for colonic drug delivery. Free films containing different ratios of chitosan to Kollicoat SR30D were prepared by casting/solvent evaporation method. The resultant mixed films were characterized in terms of puncture strength and elongation (%), glass transition temperature, swellability, polymer miscibility, permeability, and digestibility under different media. The mixed films possessed good mechanical properties, which could be used as film-coating materials for drug delivery. The extent of digestion was directly proportional to the amount of chitosan present within the film. No apparent miscibility was detected between the chitosan and Kollicoat SR30D, regardless of the film composition. The films were found to be susceptible to digestion by bacterial or β-glucosidase enzymes in simulated colonic fluid (SCF). The SCF with rat cecal bacterial enzymes had a more profound hydrolytic activity than that with β-glucosidase enzyme for the digestion of chitosan within the mixed films. Overall, the results indicated that such chitosan/Kollicoat SR30D films had potential as a coating system for drug delivery to the colon.

1. Introduction

The colon is a potential site for targeted drug delivery. In terms of local treatment diseases, such as ulcerative colitis, Crohn's disease, colon cancer, and amebiasis, would benefit from targeted drug delivery. Delivery of a drug molecule directly to its site of action may allow a reduction in dose, and consequently a reduction in potential systemic side effects, which are a major issue in the treatment of these conditions [1]. Various approaches used for targeting the drugs to the colon include time-, pressure- and pH-, and enzyme-based systems [2]. Minor variation in pH between the small intestine and the colon makes the pH-dependent systems less specific, in terms of targeted release in the colon [3]. A major limitation with the time-based system is that *in vivo* variation of the small intestinal transit time may lead to drug release in the small intestine or in the terminal part of the colon. Pressure-dependent systems hold some promise, but currently little is known about the luminal pressures of different regions of gastrointestinal (GI) tract, and at present the commercial manufacturing methods have some unresolved issues to be addressed [4]. Microbiota-activated delivery systems are considered to be preferable and promising, since the abrupt increase of the bacteria population and associated enzymatic activities in ascending colon represents a non-continuous event independent of GI transit time and pH [5–7]. Recently, a study by McConnell et al. [8] also showed that microbially triggered drug delivery to the colon was more site-specific than pH-responsive drug delivery. Questions concerning the inter-individual variation, the influence of disease state, diet, age, and concomitant drug administration (particularly antibiotics), may be raised. However, an extensive growth of anaerobic microorganisms is observed in the colon. In contrast, the microbiota of the upper GI tract is not as prominent, and consists mainly of aerobic microorganisms. These colonic microbiota produce a large number of hydrolytic [9] as well as reductive enzymes [10] (such as azoreductase, β-glucosidase, β-galactosidase, β-xylosidase, α-arabinosidase, nitroreductase, azareductase, deaminase, and urea dehydroxylase), which can potentially be utilized for colonic drug delivery. The reductase enzyme releases other polysaccharidases such as glucosidases; glycosidases are released by colonic microbiota, which are responsible for the degradation of polysaccharides

* Corresponding authors. Department of Pharmaceutics, School of Pharmaceutical Science, Hebei Medical University, 361, ZhongShan East Road, ShiJiaZhuang, 050017, PR China. Tel.: +86 311 86265591.
E-mail addresses: hhewwei@126.com (H. Wei), caody3@163.com (C. De-Ying).

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doi:10.1016/j.ejpb.2008.10.017

[11,12]. Hence, drug delivery systems based on polysaccharide can be used for colonic drug delivery.

Chitosan (CS) is a functional linear polymer derived from chitin, the most abundant natural polysaccharide on the earth after cellulose, and it is not digested in the upper GI tract by human digestive enzymes [13,14]. Chitosan is a copolymer consisting of 2-amino-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-glucose units linked with β-(1 → 4) bonds. Chourasia and Jain's report [15] and a recent study by McConnell et al. [16] indicated that chitosan was susceptible to glycosidic hydrolysis by microbial enzymes in the colon since it possesses glycosidic linkages similar to those of other enzymatically depolymerised polysaccharide. The main properties favoring the use of chitosan in various pharmaceutical preparations include its biological inertness [17], biodegradability [18], bioadhesive properties [19], and its good film-forming properties [20,21]. Being dissolved in gastric acid, chitosan is not able to shield its drug load effectively during its passage through the upper GI tract in colonic drug delivery. An ideal approach is to modify the solubility while still retaining its biodegradability. To overcome the problem of dissolution of chitosan in the gastric acid, many approaches have been evaluated with the aim to create an effective colon drug delivery system, such as an enteric layer over the chitosan [22]. Among these approaches, the combination of water-insoluble polymers as film-coating materials appears especially promising [23]. However, no suitable hydrophobic polymer has been selected to combine with chitosan as a film-coating material for colonic drug delivery until now. The reason is that chitosan can only be dissolved in acidic conditions, and most of the water-insoluble film-coating materials are not dissolved or stable in an acidic medium (e.g., ethylcellulose, Surelease®, Aquacoat, Eudragit® RL and RS). Other film-coating materials, such as Eudragit® L, S and FS, will form interpolyelectrolyte complexes with chitosan in an aqueous medium [24–26], which are not suitable to be utilized as film-coating materials for pellets, tablets or other dosage forms.

Kollicoat SR30D is an aqueous dispersion composed of 27% polyvinyl acetate (PVAc), and 2.5% povidone functions as a pore former. With adequate plasticizing, the formed PVAc film has been shown to possess unique physical and mechanical properties such as enormous flexibility, rendering the film-coated pellets compressible without rupture [27]. Secondly, PVAc-based matrix and film-coated products were demonstrated to release drugs in a pH-independent fashion [28,29]. Additionally, Kollicoat SR30D has a lower viscosity, lower minimum film formation temperature (18 °C) and suitable pH value (4.5) when compared to other commercially available aqueous dispersions [30].

Chitosan is a weak base with a pK value of the D-glucosamine residue of about 6.2–7.0 [31], and therefore, it is soluble in the aqueous dispersions of PVAc. Both chitosan and Kollicoat SR30D have a good film-forming ability. Thus, chitosan/Kollicoat SR30D could form a good film-coating material for pellets, tablets or other dosage forms and specifically for delivery of the drug to the colon. To the best of our knowledge, this is the only scientific report describing the water-insoluble polymers blended with the polysaccharide chitosan as film-coating materials for colonic drug delivery, which have no interaction or interpolyelectrolyte between them.

The purpose of the study was to prepare and characterize the properties of the chitosan/kollocoatSR30D mixed films for colonic drug delivery. The mechanical properties, digestibility properties, permeability and swelling characteristics in simulated gastric and intestinal media, and water vapor transmission (WVT) were evaluated. Further, glass transition temperature (T_g) and thermal properties were also investigated. Differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and Fourier transform infrared (FTIR) spectrometry analysis were carried out for this purpose.

2. Materials and methods

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). 5-FU was obtained as a gift sample from Shijiazhuang No. 4 Pharmaceutical Co., Ltd. (ShiJiaZhuang, China). All chemicals were of analytical grade.

2.2. Preparation of free films

Chitosan solutions (2.5 wt.%) were prepared by dissolving chitosan in 0.5% acetic acid solution at ambient temperature with stirring overnight. The pH value of the solution was adjusted to 4.0–4.5 before use. Then, predetermined amounts of Kollicoat SR30D were added to this solution with stirring. The following Kollicoat SR30D (KC): chitosan (CS) blend ratios were investigated: 100:0, 90:10, 80:20, 70:30 and 60:40 (w/w). Final dispersion was stirred by using a magnetic stirrer for 24 h and then poured into Teflon plates. Volume of suspension was 15 ml in each plate, and subsequent controlled drying was done (24 h at 30–35 °C in an oven). The films obtained were carefully removed from the substrate and macroscopically examined for the presence of air bubbles and cracks, transparency, and flexibility. Then, the films were cut with a scalpel to different special pieces for various tests. The thickness of the films was measured at five different places by using a micrometer (Shanghai Precision Instruments Co., Ltd., China), and the average thickness of 130–150 μm was selected.

2.3. Mechanical testing of the films

The mechanical properties of the films in the dry or wet state were evaluated by puncture test using an Instron Model 4201 universal testing apparatus, which was described by Bodmeier and Paeratakul [32]. A stainless puncturing probe with a spherical end (diameter 5 mm) was driven through the dry film with a speed of 4 mm/min. Dry or wet film specimens were positioned in the film holder between the two mounting plates followed by tightening of the holding screws to prevent slippage of the films. The wet films were carefully blotted to remove water from the film surface prior to the mounting. The load at break and the maximum displacement of the film samples were measured, and then converted to puncture strength (MPa) and elongation at puncture (%). The puncture strength and % elongation were calculated using the following equations:

$$\text{Puncture strength} = \frac{F}{A_{cs}} \quad (1)$$

where F is the load required for puncture and A_{cs} is the cross-sectional area of the edge of the dry film located in the path of cylindrical opening of the film holder (A_{cs} = 2rd, where r is the radius of the hole and d is the thickness of the film).

$$\text{Elongation } (\%) = \frac{\sqrt{r^2 + D^2} - r}{r} \times 100 \quad (2)$$

where r is the radius of the film exposed in the cylindrical hole of the film holder and D is the displacement of the probe from the point of contact to the point of film puncture.

2.4. Water vapor transmission test

Water vapor permeation of the free films was determined gravimetrically in triplicate. The permeability cups were 2.0 cm in diam-

eter. The inside of the cup was filled with 10 ml of distilled water, and the film was subsequently attached to the cup with a-cyanoacrylate adhesive Super Glue (Shantou, China). The cup with the film was then weighed and stored in a desiccator filled with silica gel. After 24, 48, 72, 96, and 120 h of storage the cups were reweighed in order to determine the permeated amount of water (mass loss), and the profile of mass change was plotted versus time for each free film. WVT was calculated using the following equation [33]:

$$\mathrm{WVT} = \frac{g \times 24}{t \times A} \tag{3}$$

where g represents mass loss, t is the time (measured in hours during which the weight loss occurred), and A is the exposed area of the film.

2.5. Swelling experiments

Firstly, a piece of 1 cm^2 of each free film was dried in an oven at 50 °C for 24 h. Then, the dried film was accurately weighed and immersed in a flask of dissolution test containing 250 ml of different media at 37 °C. At specific intervals, the swollen sample was withdrawn from the medium and weighed after the removal of excess surface water by light blotting with a filter paper. During the first 10 min, intervals of sampling were 1 min. Sampling time was gradually increased after this period until 3 h. The swelling behavior of the films was calculated as follows [34]:

$$I_s\ (\%) = \frac{M_s - M_i}{M_i} \times 100 \tag{4}$$

where I_s is the swelling index, M_s is the film mass after a certain swelling period, and M_i is the dry film mass. Swelling tests were separately carried out in simulated gastric fluid (SGF), simulated intestinal fluid (SIF) with pH 6.8 and also simulated colonic fluid (SCF) with the addition of rat cecal contents (4%, w/w) [35] or β-glucosidase enzyme (4%, w/w) [36] to the media with pH 6.8 or 5.0, respectively. Three parallel measurements were performed in each case.

2.6. Drug permeability

Permeability of the model drug across the polymeric films was determined in horizontal side-by-side diffusion cell (diffusion area 0.95 cm^2) at 37 °C. Different experimental conditions were set up to examine the permeability of the drug through polymer films. The initial concentration of the drug in the donor compartment was 12 μg/ml (saturated solution). Both the donor and acceptor compartments were composed of SGF, SIF and SCF with rat cecal contents (4%, w/w) or β-glucosidase enzyme (4%, w/w). The drug concentrations in the sample were analyzed by high-performance liquid chromatography (HPLC) assay [35].

When the stationary state was achieved, the permeability coefficient of the studied drug was obtained by the following formula [37]:

$$\frac{2PS}{V}t = -\mathrm{In}\frac{(C_o - 2C_a)}{C_o} \tag{5}$$

where P is the permeability coefficient, S is the surface area of the film through which diffusion takes place, V is the volume of the acceptor or donor compartment, t denotes time, C_0 is the initial concentration of 5-FU in the donor compartment, and C is the concentration of caffeine in the acceptor compartment. P can be calculated from a plot of $-\ln (C_0 - 2C_a)/C_0$ *vs.* time. Three parallel measurements were performed in each case.

2.7. Digestion of free films

Films were cut into strips of 1 × 1 cm size, accurately weighed and placed individually into glass dropping bottle (250 ml). In the presence of a continuous supply of CO_2, these bottles were placed into the control PBS or simulated colonic medium with rat cecal contents (8%, w/w) or β-glucosidase enzyme (8%, w/w) (40 ml medium per bottle) and subsequently sealed with lid, and these were positioned onto a rocking platform operated at 100 rpm/min. After 24 h, the films were removed, washed thoroughly with deionised water and dried in an oven at 50 °C overnight. The percentage film weight loss was calculated according to the following formula [16]:

$$W_d(\%) = \frac{W_0 - W_t}{W_0} \times 100 \tag{6}$$

where W_d is % film digestion, W_0 is the original weight of the film, and W_t is the weight after time t (24 h).

2.8. Glass transition temperature

The T_g's of the films chitosan/Kollicoat SR30D were measured by a differential scanning calorimeter, Perkin-Elmer 7 DSC (Perkin-Elmer, USA), with an intracooler and nitrogen purge. Each film sample consisted of 8 mg of small discs piled into a 50 μl aluminum sample pan with a pierced lid to allow evaporation of volatile materials and, at the same time, to avoid sample expansion and warping of the sample pan. The sample was first heated from 0 to 200 °C at a rate of 10 °C/min, and then cooled back to 0 °C at a rate of 20 °C/min. This step was designed to remove moderately bound moisture and solvent residue so that the endotherm would not obscure the glass transition. The sample was then reheated at a rate of 10 °C/min until the T_g passed. The T_g for each film was determined from the midpoint of a small endothermic rise of the pre and post-transition baselines using six parallel thermograms. The method was similar to the one described by Okhamafe and York [38].

2.9. Thermogravimetric analysis (TGA)

Thermogravimetric analysis of chitosan/Kollicoat SR30D was performed using a thermogravimetric analyzer (Pyris6 TGA, Perkin-Elmer, USA). TGA was performed with a 7–10 mg sample in aluminum pans under a dynamic nitrogen atmosphere. The experiments were run at a heating rate of 10 °C/min.

2.10. Fourier transform infrared (FTIR) spectroscopy

About 2% (w/w) of films of chitosan/Kollicoat SR30D blend, with respect to the potassium bromide (KBr) disc, were mixed with dry KBr (FTIR grade, Aldrich, Germany). The mixture was ground into a fine powder using an agate mortar before compressing into a disc. Each disc was scanned at a resolution of 4 cm^{-1} over a wave number region of 400–4000 cm^{-1} using a FTIR spectrometer (Spectrum 100 FTIR System, Perkin-Elmer, USA) coupled to a personal computer with AssureID software packages. The characteristic peaks of IR transmission spectra were recorded.

2.11. Statistical analysis

All the experiments were done in triplicate. The results were expressed as mean ± standard deviation. One-way analysis of variance was performed to assess the significance of the differences among the data. Tukey–Kramer post-hoc test was used to compare the means of different treatment data. Results with $p < 0.05$ were considered statistically significant.

3. Results and discussions

3.1. Mechanical properties of free films

Polymer films must be mechanically strong such that they do not break or fracture during processing, packaging, shipping, and

Table 1
Mechanical properties of different chitosan (CS)/Kollicoat SR30D(KC) films in the dry and wet state (data represent mean ± SD, $n = 3$).

Film	Puncture strength, MPa		Elongation, %	
	Dry	Wet	Dry	Wet
Kollicoat 100%	6.34 ± 0.37	6.01 ± 0.61	186.35 ± 35.71	234.64 ± 42.35
KC:CS = 90:10	5.67 ± 0.15	5.01 ± 0.23	156.64 ± 30.50	214.32 ± 19.64
KC:CS = 80:20	5.01 ± 0.42	4.64 ± 0.33	134.35 ± 10.22	201.75 ± 31.25
KC:CS = 70:30	4.75 ± 0.31	4.51 ± 0.46	110.32 ± 24.32	196.36 ± 26.54
KC:CS = 60:40	4.67 ± 0.21	4.13 ± 0.43	98.64 ± 43.57	172.31 ± 27.68
KC:CS = 50:50	4.34 ± 0.51	3.89 ± 0.37	86.67 ± 37.35	164.35 ± 33.21
Chitosan 100%	2.07 ± 1.23	1.12 ± 0.95	52.35 ± 22.97	69.57 ± 16.46

storage [39]. The puncture strength and elongation of dry and wet polymeric films were determined by puncture test, and the results are shown in Table 1. In dry state, films prepared from Kollicoat SR30D showed a higher puncture strength and an elongation value when compared to other aqueous colloidal polymethacrylate dispersions (Surelease, Aquacoat, Eudragit RL 30D and RS 30D) [32]. It meant that Kollicoat SR30D had good mechanical properties, which was suitable to be used as film-coating materials for drug delivery. In wet state, the puncture strength of Kollicoat SR30D films decreased and elongation (%) increased due to the hydrophobic character of Kollicoat SR30D; however, the difference in the puncture strength was not significant ($P > 0.05$). The hydration of the polymer and the resulting interference of water with the interchain hydrogen bonding were responsible for the decrease in puncture strength and for the increase in flexibility [32].

In the case of the mixed polymeric films, there was a gradual decrease in the puncture strength and elongation values with an increase in the concentration of chitosan within the film. Macleod and his coworkers [40] reported that polysaccharides pectin could reduce ethylcellulose chain deformation capacity by affecting polymer chain mobility; in addition, the presence of polysaccharides amylose could alter the structure of the ethylcellulose films, resulting them to become weaker and softer [41]; these might be applicable to chitosan too. Furthermore, there were investigations which indicated that HPMC, polysaccharides amylose and pectin appeared as isolated domains in the insoluble polymer films and thus made the film more brittle and decreased the elongation, thereby indicating that the two polymers were incompatible [40–42]. Thus, it is speculated that chitosan would also be distributed in the films as an isolated domain, which decreased the puncture strength and increased the elongation values. In wet state, the elongation (%) of the mixed films increased more rapidly than Kollicoat SR30D films per se. The explanation for it was that the existing of chitosan would increase the water uptake of the mixed films, and then the water functioned as a plasticizer, which caused a decrease in the intermolecular forces along the polymer chains resulting in a decrease in puncture strength, and reduction in the brittleness of polymeric materials [32]. Although the increasing

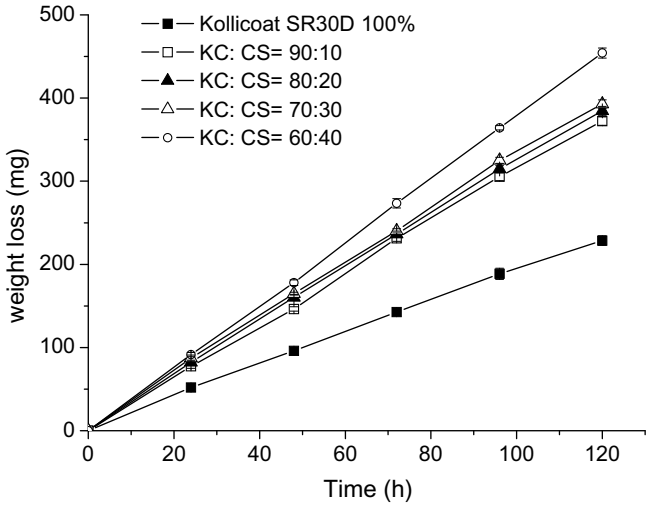

Fig. 1. Profiles of water vapor transmission through free films containing Kollicoat SR30D (KC)/chitosan (CS) Error bars indicate SD ($n = 3$).

Table 2
Water vapor transmission of chitosan(CS)/Kollicoat SR30D(KC) films (data represent mean ± SD, $n = 3$).

Formulation	Mass change (mg/h)	WVT (mean ± SD; $n = 3$, g/24 h m^2)
Kollicoat SR30D 100%	1.91 ± 0.10	1.46 ± 0.08
KC:CS = 90:10	3.10 ± 0.23	2.37 ± 0.17
KC:CS = 80:20	3.21 ± 0.13	2.45 ± 0.10
KC:CS = 70:30	3.27 ± 0.25	2.50 ± 0.19
KC:CS = 60:40	3.78 ± 0.09	2.89 ± 0.07

Table 3
Swelling index (I_s) of chitosan (CS)/Kollicoat SR30D (KC) free films in SGF, SIF, and SCF in presence of rat cecal contents or β-glucosidase enzyme (data represent mean ± SD, $n = 3$).

Formulation	Maximum swelling index (%)			
	SGF	SIF	SCF (rat cecal contents)	SCF (β-glucosidase enzyme)
Kollicoat SR30D 100%	28.83 ± 1.37	26.94 ± 2.03	29.03 ± 1.68	28.55 ± 1.05
KC:CS = 90:10	50.35 ± 2.67	42.50 ± 3.75	57.36 ± 1.69	52.67 ± 2.01
KC:CS = 80:20	71.19 ± 3.75	60.99 ± 3.97	97.18 ± 1.08	85.65 ± 3.57
KC:CS = 70:30	88.23 ± 3.57	74.25 ± 4.29	114.27 ± 2.07	100.59 ± 3.28
KC:CS = 60:40	122.34 ± 6.37	95.68 ± 5.37	153.08 ± 1.24	135.08 ± 1.06

Table 4
Permeability of different chitosan(CS)/Kollicoat SR30D(KC) films to 5-FU (data represent mean ± SD, $n = 3$).

Formulation	Permeability coefficients, P ($\times 10^{-6}$ cm/s)			
	SGF	SIF	SCF (rat cecal contents)	SCF (β-glucosidase enzyme)
Kollicoat SR30D = 100%	1.124 ± 0.027	1.079 ± 0.037	1.141 ± 0.121	1.103 ± 0.101
KC:CS = 90:10	1.519 ± 0.116	1.234 ± 0.037	2.015 ± 0.218	1.722 ± 0.192
KC:CS = 80:20	1.825 ± 0.267	1.343 ± 0.169	2.735 ± 0.128	2.267 ± 0.137
KC:CS = 70:30	2.067 ± 0.354	1.456 ± 0.467	3.328 ± 0.334	2.627 ± 0.345
KC:CS = 60:40	2.279 ± 0.311	1.537 ± 0.269	4.025 ± 0.687	3.256 ± 0.675

chitosan concentration within the films had an adverse effect on the puncture strength and elongation, the mixed films still possessed good flexibility.

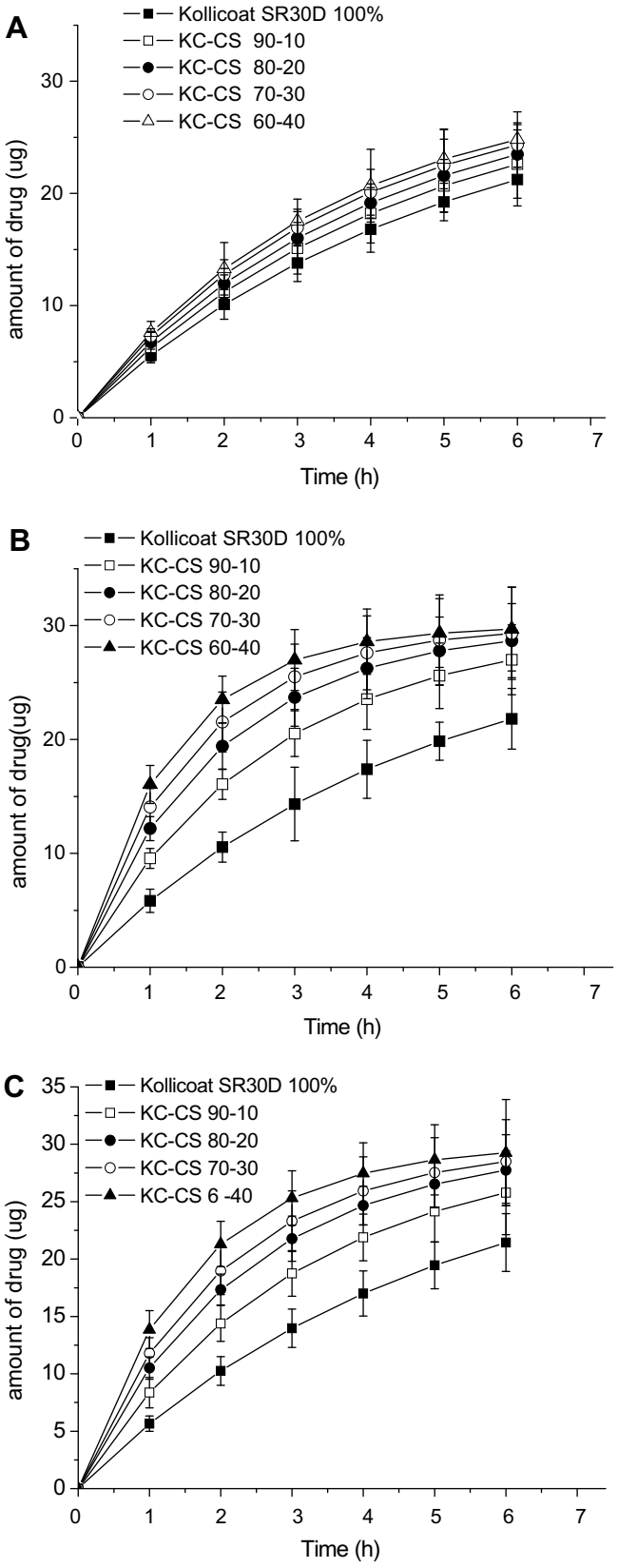

Fig. 2. Permeability profiles of free films containing Kollicoat SR30D (KC)/chitosan (CS) in control media (A) and simulated colonic contents (B, rat cecal contents; C, β-glucosidase enzyme) for 5-FU. Error bars indicate SD ($n = 3$).

3.2. WVT experiments

According to Fig. 1, the rate of water vapor permeation was constant for the free films containing chitosan and Kollicoat SR30D. Table 2 lists the results of WVT experiments for all the formulations. An increase in WVT was found for the following series: KC: CS 50:50 > KC: CS 60:40 > KC: CS 70:30 > KC: CS 80:20 > KC: CS 90:10. The results demonstrated that WVT was affected by the composition of the film. An increase in polysaccharide concentration significantly influences ($P < 0.05$) WVT with the addition of chitosan. It is well known that increasing the hydrophilic nature of a polymer membrane induces water vapor tendency, and as a result increases water vapor permeation. Chitosan has good hydrophilic characteristics. So, the incorporation of chitosan into a Kollicoat SR30D film would engender an increase in hydrophilicity, and then the WVT increased.

3.3. Swelling test

The results of swelling experiments are listed in Table 3. Formulations containing Kollicoat SR30D and chitosan had low swelling index in SIF. Swelling index in SGF and SIF increased with the addition of chitosan ($P < 0.05$), which was due to the hygroscopic characteristics of polysaccharides chitosan and the higher water uptake of polymer. Swelling index was higher in SGF than in SIF, because the amino-group (—NH_2) in chitosan was protonated and dissolved in acid environments. Compared with the swelling index in SIF and SGF, swelling of the free films in SCF significantly increased with the addition of chitosan ($P < 0.05$). Langer and Peppas [43] have shown that an increase in swelling for polysaccharide materials after degradation indicated bulk degradation. Thus, this is due to the presence of rat cecal bacterial or β-glucosidase enzymes con-

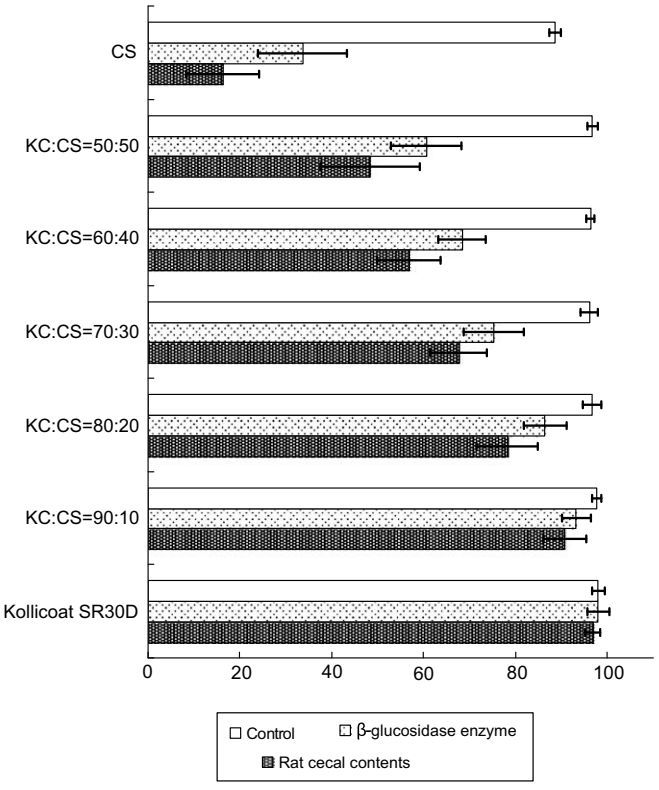

Fig. 3. Percentage remaining of different Kollicoat SR30D (KC)/chitosan (CS) films after 24 h incubation in simulated colonic fluids (β-glucosidase enzyme or rat cecal contents) and control media. Error bars indicate SD ($n = 3$).

tained in the media, which could diffuse into the polymeric chains [5], hydrolyze the glycosidic linkages within the chitosan, and reduce the network density and finally increased swelling. On the other hand, the swelling index in SCF in the presence of rat cecal bacterial enzyme was relatively higher than that obtained in the presence of β-glucosidase enzyme. It was reported that rat cecal contents contained more than one bacterial enzyme (including *bacteroides, bifidobacteria* and *enterobacteria*) that could degrade chitosan [7,44,45]. In fact, even with simple linear one-component polysaccharides, more than one bacterial enzyme is usually involved in its degradation or breakdown [46]. It was reported that increasing swelling index of the crosslinked chitosan film would result in a better accessibility of the β-(1 → 4) bonds in the colon [16]. Thus swelling and hydration of the composite film were necessary and important before enzymatic breakdown occurred. Films containing the highest concentration of the most hydrophilic polysaccharide reached the highest degree of swelling.

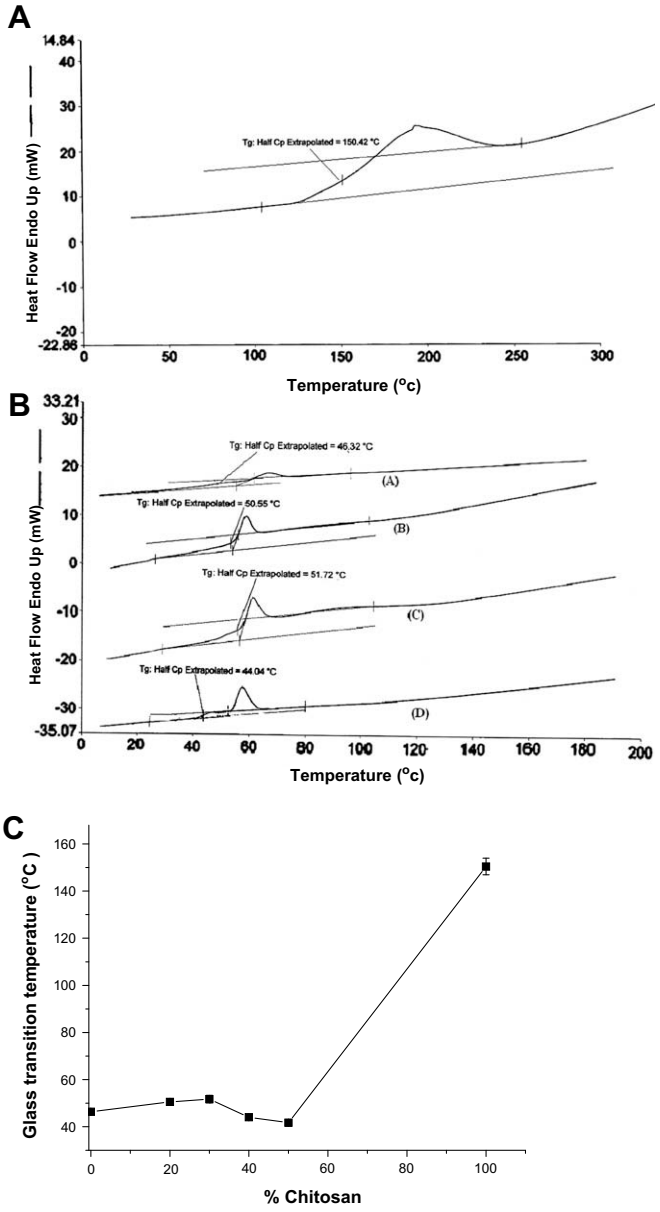

Fig. 4. (A) DSC temperatures scan for chitosan film per se. (B). DSC temperatures scan for Kollicoat SR30D film (A) and Kollicoat SR30D /chitosan mixed films containing 20% (w/w) chitosan (B), 30% (w/w) chitosan (C) and 40% chitosan (D). (C). Glass transition temperatures as a function of chitosan concentration for Kollicoat SR30D films. Error bars indicate SD ($n = 3$).

3.4. Drug permeability

The dates of permeability of the drug in different media are shown in Table 4 and Fig. 2(A–C). The permeability of the mixed films remained relatively very low in SGF, as well as in SIF, as long as the concentration of the chitosan added was low, indicating that the insolubility of the film as given by Kollicoat SR30D was maintained. An increase in the amount of chitosan induced an increase in diffusion. The results of the permeability agreed with the results of the swelling, in which an increase in the chitosan content of the film resulted in an increase in the drug permeability. Similar to the results of the swelling experiment, permeability in SGF was higher than in SIF for the mixed films since the chitosan was dissolved and leached from the mixed films in low pH conditions. Though PVAc is a water-insoluble polymer, the drug molecules were diffused in SIF relatively easily, since povidone exists in Kollicoat SR30D films which functions as a pore former. Lower permeability of the mixed films in SGF and SIF than in SCF ($P < 0.05$) demonstrated the susceptibility of chitosan in these films to bacterial enzymes and degradation in simulated colonic media. The addition of rat bacterial enzymes or β-glucosidase enzyme to the phosphate buffer increased the rate and extent of chitosan leaching from Kollicoat SR30D films. The leaching of the large-molecular weight chitosan creates aqueous channels or water-filled pores that allow the diffusion of the drug molecules through the film. Thus, the permeability in SCF was higher than that in SGF and SIF.

At the 0.05 level, the population means are not significant between rat cecal bacterial enzymes and β-glucosidase enzyme; the drug permeability of the values in SCF with rat bacterial enzymes was higher than that obtained from SCF with β-glucosidase enzyme. Thus, it may be concluded that rat bacterial enzymes were more effective at diffusing into the mixed films than were β-glucosidase enzymes, enabling bulk degradation by the bacterial enzymes.

3.5. Digestion of the free film

The influence of chitosan concentration on the digestibility of the mixed films is shown in Fig. 3. No weight loss of the Kollicoat SR30D film per se was observed in SCF and control medium, indicating that it could not be degraded by the bacterial enzymes in the large intestine; however, more than 80% weight loss of the chitosan films per se was observed, which indicated that chitosan could be digested in SCF. Thus, a greater proportion of the digested film was observed for the blended chitosan/Kollicoat SR30D films with

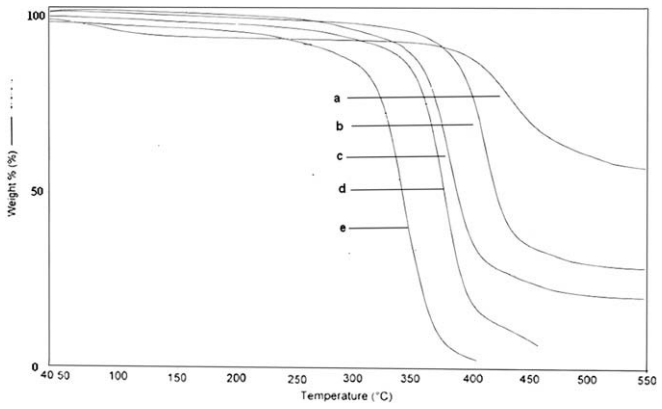

Fig. 5. Thermogravimetric analysis of (a) chitosan, (b) Kollicoat SR30D, and Kollicoat SR30D/chitosan blend (c, 8:2 ratio; d, 7:3 ratio; e, 6:4 ratio).

a higher chitosan content. In contrast, very little weight loss, and hence digestion, was noted in any of the free films after 24 h incubation in the control buffer medium. When chitosan concentration was only 10%, low proportion of the digested film was observed. This was the reason that the presence of the increased levels of insoluble polymers could lead to less accessibility to enzymatic attack as the chitosan no longer forms a continuous network throughout the film.

Similar to the results of swelling and permeability, the proportion of the mixed films being digested in SCF with rat bacterial enzymes was higher than that obtained from SCF with β-glucosidase enzyme due to a better accessibility of degradable bonds of chitosan to rat cecal microbial enzymes.

3.6. Glass transition temperature

One of the most common polymer properties determined for the amorphous polymers is the glass transition temperature (T_g). T_g values for Kollicoat SR30D and chitosan films were 46 and 150 °C, respectively (Figs. 4A–B). The addition of chitosan to Kollicoat SR30D would increase the T_g of the Kollicoat SR30D films (Fig. 4C). However, no significant increase in T_g was observed, which indicated that chitosan in Kollicoat SR30D films was poorly miscible [47]. Poor miscibility of chitosan in Kollicoat SR30D films suggested that chitosan was distributed as isolated domains in the films, and the characteristics of chitosan within the mixed films are likely to remain unchanged. Chitosan within the mixed films should therefore remain recognizable to cecal and colonic bacterial enzymes as a digestion substrate. On the other hand, low T_g was also beneficial to the film-coating process.

3.7. Thermogravimetric analysis (TGA)

Fig. 5 shows the results of thermogravimetric analysis (TGA) of chitosan, Kollicoat SR30D and chitosan/Kollicoat SR30D-blended films. The pure chitosan film showed the first thermal event centered at 50 °C, which was related to the evaporation of an unbound water. The second thermal event for chitosan was observed at about 350 °C. The initial decomposition temperature of chitosan/Kollicoat SR30D-blended films was at about 280 °C, whereas the initial decomposition was at about 300 °C for Kollicoat SR30D film. Although there was no significant effect of chitosan on initial decomposition temperature, the maximum decomposition temperature of Kollicoat SR30D shifted significantly from about 425 °C to about 330 °C after the films were added with chitosan. The wt.% loss increased with the increasing chitosan content in the blended films. From the results shown in Fig. 5, it could be concluded that the thermal stability of the blended films decreases with an increasing chitosan content. The degradation profile of the chitosan/Kollicoat SR30D-blended films consisted of the events typical for Kollicoat SR30D, indicating on their immiscibility in the blend.

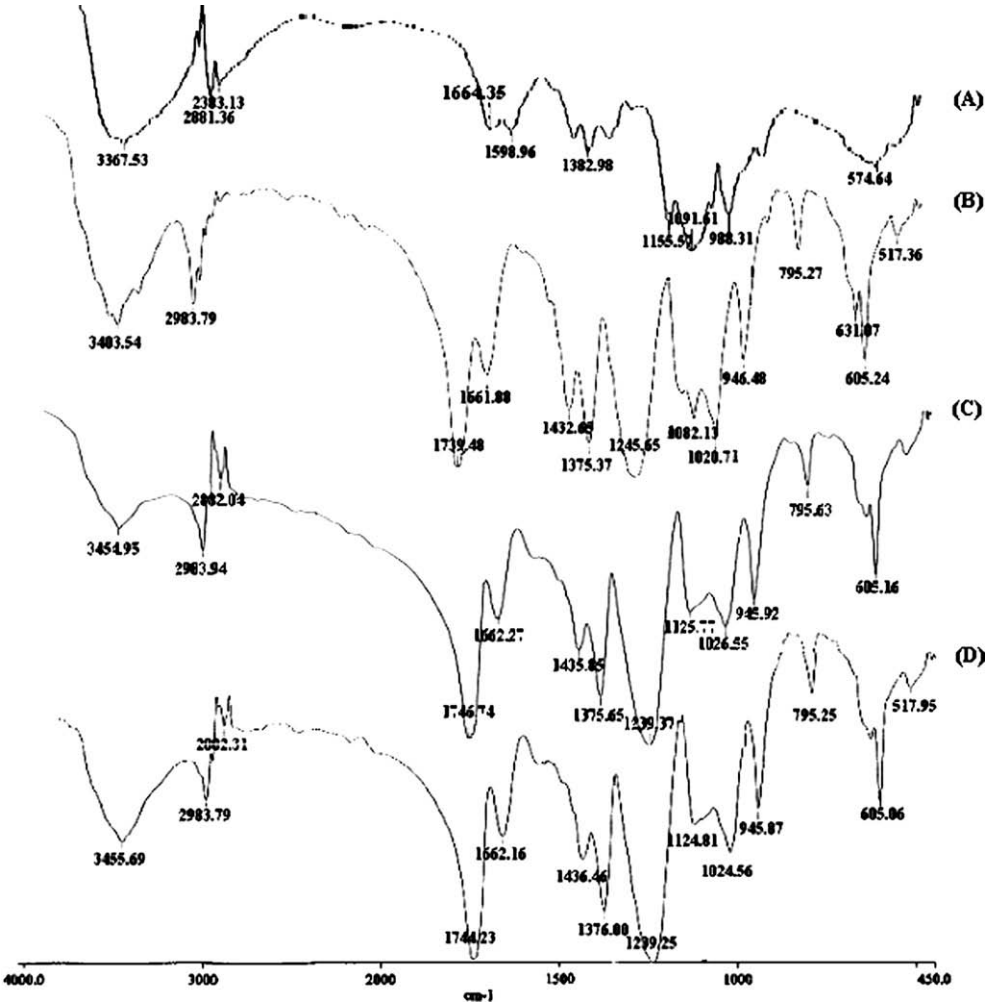

Fig. 6. FTIR spectra of (A) chitosan, (B) PVAc, (C) physical mixture of PVAc and chitosan (7:3 ratio) and (D) PVAc /chitosan blend (7:3 ratio).

3.8. Fourier transform infrared (FTIR) spectroscopy

As seen from Fig. 6, the characteristic absorption peaks of chitosan appeared at 3367.63, 2883.53, 1664.35, 1598.96, 1382.98 and 1091.61 cm^{-1}, respectively, denoting stretching vibration of —OH, —CH_2—, —C=C—, —NH_2 and —C—O. The characteristic absorption peaks of Kollicoat SR30D appeared at 1739.48 and 1245.65 cm^{-1} corresponding to —C=O— and —C—O— stretching vibration. In the physical mixture and blend of chitosan/Kollicoat SR30D film spectrum, the characteristic peaks of both Kollicoat SR30D and chitosan could be observed, and the spectrum could be regarded as a simple superimposition of that of Kollicoat SR30D and chitosan. It indicated that there were no significant interactions between the chitosan and Kollicoat SR30D. Although chitosan was dissolved and —NH^{3+} and —NH_2 would exist in pH values of 4.0–4.5, there was no free COO^- that was dissociated from PVAc. Thus, no interaction was observed between the chitosan and Kollicoat SR30D. Additionally, no interaction between them would make chitosan randomly distributed throughout the structure of the mixed films. Degrading enzymes attacking the polysaccharides are commonly classified according to whether they cleave a susceptible glycosidic bond situated at a terminal residue in a chain and successively release monomer units from the chain end (exo-activity) or they depolymerize polysaccharides by an apparently random splitting of interior glycosidic bonds (endoactivity). Thus, no interaction between them would cause less hindrance to the accessibility of the enzyme to the active site; the characteristics of being digested by cecal and colonic bacterial enzymes were not altered since the proposed hydrolysis site (the β [1,4] link) was not affected.

4. Conclusion

The study showed that chitosan/Kollicoat SR30D films could be successfully prepared by casting/solvent evaporation method, and the mixed films possessed good mechanical properties which could be used as film-coating materials for drug delivery. The examination of the FTIR, T_g and TGA properties of the mixed film revealed that the chitosan and Kollicoat SR30D were not miscible in the blend. The results indicated that the mixed films exhibited a higher swelling ratio in SGF and SIF and its swellability tended to increase in SCF. The mixed films were susceptible to rat bacterial enzymes, and better accessible to bacterial enzymes contained in the cecal contents than to β-glucosidase enzymes. The SCF with rat cecal bacterial enzymes was more effective than that with β-glucosidase enzyme for the digestion of chitosan within the films. The extent of digestion correlated with the amount of chitosan present within the film. 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 mixed film, would be digested. This digestion would allow the delivery of the drug(s) present in the dosage form. Furthermore, chitosan/Kollicoat SR30D film-coated pellets/tablets for the colonic drug delivery are currently in progress; and concerning the effect of osmotic pressure built by contact with water in the core of the dosage form on the drug release; and when a water-soluble drug is incorporated into the coated dosage forms, the osmotic pressure may cause the (micro) rupturing of the polymeric films, therefore further research using in vitro experiments is needed to prove the colonic drug delivery.

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