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Research Paper

Pectin/Kollicoat SR30D isolated films for colonic delivery [I]: a comparison of normal and colitis-induced models to assess the efficiency of microbially triggered drug delivery

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Abstract

Objectives The purpose of the study was to evaluate digestion of pectin/Kollicoat SR30D free films for colonic delivery *in vitro* and *in vivo*.

Methods Free films containing different ratios of pectin to Kollicoat SR30D were prepared by casting/solvent evaporation method. An in-vitro comparison of swelling, degradation and permeability of the free films was carried out in simulated colon fluids containing caecal contents from normal rats with colitis induced by 2,4,6-trinitrobenzene sulfonic acid (TNBS) or oxazolone. A comparative in-vivo evaluation of degradation was also conducted in normal and colitis-induced model rats.

Key findings The pectin within the mixed films was susceptible to rat colonic bacterial enzymes. The extent of digestion correlated with the amount of pectin present within the film. *In vitro*, the swelling index, drug permeability and extent of film digestion in simulated colon fluids with caecal contents obtained from normal rats were higher than from TNBS- or oxazolone-induced model rats, whereas in-vivo degradation was similar in the three groups of rats. The pectin/Kollicoat SR30D free films were completely degraded in the colitis-induced rats.

Conclusions Pectic/Kollicoat SR30D films may be useful as coatings to target delivery of drugs to the colon.

Keywords colonic drug delivery; degradation; Kollicoat SR30D; pectin; permeability; rat

Introduction

The advantages and necessity of colon targeting to provide more effective therapy for colon-related diseases such as irritable bowel syndrome, colon cancer and inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis, are widely recognized.[1] Microbial-triggered drug delivery to the colon exploits the fact that there are over 400 distinct species of bacteria with a population of 10^{11}–10^{12} colony-forming units (CFU)/ml; *Bacteroides*, bifidobacteria, eubacteria, clostridia, enterococci and enterobacteria species greatly outnumber other species.[2–4] This vast microflora fulfils its energy needs by fermenting various types of substrates that have been left undigested in the small intestine (e.g. di-, tri- and polysaccharides).[5,6] For this fermentation, the microflora produce a vast number of enzymes such as amylase, pectinase, xylanase, β-glucuronidase, β-xylosidase, α-arabinosidase and β-galactosidase.[7–9] The reductase enzymes are responsible for the degradation of undigested polysaccharides.[10,11]

To target the colon effectively, the dosage form must first reach the colon intact, having avoided degradation by the pancreatic enzymes in the upper gastrointestinal tract. Polysaccharides that fulfil these criteria have been investigated for colonic delivery. Amylose in combination with ethylcellulose (as a film coating) has been shown to prevent release of drug from solid dosage forms in the stomach and small intestine but allows drug release in the colon *in vitro*[12–14] and *in vivo*.[15–17]

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Pectin is a non-starch linear polysaccharide extracted from plant cell walls. It is refractory to host gastric and small intestinal enzymes but is almost completely degraded by colonic bacterial enzymes.[18] Because it is soluble in water, however, pectin is not able to shield its drug load effectively during passage through the stomach and small intestine. Many approaches have been evaluated in attempts to create an effective pectin-based drug-delivery system. Among these approaches, the combination of pectin with water-insoluble polymers as film coating materials appears particularly promising.[19–22] The method of film coating by the use of aqueous dispersion of pectin–polymer composite appears to be more effective than other coating methods (e.g. compression coating) using a blend of pectin–polymer composite.

Kollicoat SR 30D is an aqueous dispersion composed of 27% polyvinyl acetate (PVAc). 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.[23] Secondly, PVAc-based matrix and film-coated products were demonstrated to release drugs in a pH-independent fashion.[24,25] In addition, Kollicoat SR 30D has a lower viscosity and a lower minimum film formation temperature (18°C) than other commercially available aqueous dispersions.[23]

The composition of colonic bacteria and corresponding enzymes can be influenced by many factors, including age, diet, diseases, medication such as antibiotics, and geographic regions, particularly when the gastrointestinal tract is diseased.[26,27] Metabolic activities of the colonic microflora are reported to be decreased and normal bacteria are disturbed in the patient with IBD.[28,29] A study by Ott and colleagues[30] also showed that bacterial diversity in active IBD was reduced compared with controls. Species lacking in disease were members of the normal anaerobic microflora. In addition, IBD is associated with less variability in the composition of the colonic bacteria.[31,32] Based on the above facts, the suitability of microbially triggered drug delivery for effective therapy for colon-related diseases has been questioned, although this is only speculative without data in support. In fact, as far as we know, there have been no reports of the degradation properties of polysaccharides in IBD.

Our aim was therefore to determine whether pectin is degraded completely by colonic bacterial enzymes in disease states. In the present study, the swelling, degradation and drug permeability of pectin/Kollicoat SR 30D free films was investigated *in vitro* in simulated colon fluid containing colonic bacteria obtained from normal rats and from rats with colitis induced by 2,4,6-trinitrobenzene sulfonic acid (TNBS) or oxazolone (OXA). The TNBS[33] and OXA[34] rat models were chosen as well recognized experimental models that allow induction of colitis at an exact location. Film degradation was also evaluated *in vivo* in normal and colitis-induced model rats. 5-Fluorouracil (5-FU) was used as the model drug.

Materials and Methods

Materials

Kollicoat SR30D was a gift from BASF (Ludwigshafen, Germany); pectin (degree of methylation of approximately 35%) was a gift from Shangyao Fuda Pectin Co. Ltd (Jingxi, China); 5-FU was a gift from Shijiazhuang No. 4 Pharmaceutical Co. Ltd (ShiJiaZhuang, China); TNBS and OXA were purchased from Sigma-Aldrich Chemie GmbH (Beijing, China). All chemicals were of analytical grade.

Colitis models

The study was conducted in accordance with the standards of care and use of laboratory animals established by Hebei Medical University. Before the induction of colitis rats were fasted for 12 h, with access to water ad libitum.

In the first model of colitis, inflammation was induced by TNBS after the following procedure. Animals were catheterized 4 cm intrarectally under light anaesthesia induced with ether; TNBS was applied at a dose of 160 mg/kg body weight in a volume of 100 ml (in 50% ethanol). The rats were housed for a day without treatment to allow complete development of IBD.

The OXA model was set up as follows. The rats were immunized with an ethanol solution containing 3% OXA applied topically to the skin. The challenge was performed a week later by rectal administration of 10 mg haptenating agent; 3% OXA solution was administered per rectum in a total volume of 100 μl of an ethanol/water mixture.

Preparation of free films

Pectin solutions (2.0 w/v) were prepared by dissolving pectin in warmed (40–50°C) distilled water. A predetermined amount of Kollicoat SR30D was then added to this solution with stirring. The following ratios of Kollicoat SR30D to pectin were investigated: 100 : 0, 90 : 10, 80 : 20, 70 : 30 and 60 : 40 (w/w). Final dispersions were stirred using a magnetic stirrer for 12 h, and then poured into Teflon plates (diameter 9 cm). The volume of suspension in each plate was 25 ml. Plates were then dried in an oven at 30–35°C for 24 h. The films obtained were carefully removed from the substrate and inspected for the presence of air bubbles, cracks, transparency and flexibility. Films were then cut with a scalpel for various tests. The thickness of the films was measured at five different places using a micrometer (Shanghai Precision Instruments Co., Ltd, Shanghai, China) and the average thickness of 130–140 μm was selected.

Determination of the mechanical properties of wet and dry films

The mechanical properties of wet and dry films were evaluated by puncture test using an Instron Model 4201 universal testing apparatus (Tianyuan Experiment Machinery Co. Ltd, Tiandu, China).[35] Samples of dry or wet films (3.5 × 4 cm) were positioned in the film holder between the two mounting plates and the holding screws were tightened to prevent slippage of the films. The distance between the grips was 3.5–4 cm. A stainless steel puncturing probe with a spherical end (diameter 5 mm) was driven through the dry film at a speed of 4 mm/min. The wet films were carefully blotted to remove water from the film surface before mounting. The load at break and the maximum displacement of the film samples were measured, and then converted to puncture strength[36] and elongation at puncture. Puncture

strength was calculated from F/A_{cs} 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 the 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). Elongation (%) was calculated using the equation

$$\text{Elongation } (\%) = \frac{\sqrt{r^2 + D^2} - r}{r} \times 100 \quad (1)$$

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.

Water vapour transmission (WVT) test

Water vapour permeation of free films was determined gravimetrically in triplicate. The permeability cups were 2.0 cm in diameter. The inside of the cup was filled with 10 ml distilled water, and the film was subsequently attached to the cup with α-cyanoacrylate adhesive Super Glue (Lida Glue Co. Ltd, Shantou, China). The cup with the film was then weighed and stored in a desiccator filled with silica gel. Cups were weighed after 24, 48, 72, 96 and 120 h 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 from WVT = $(g \times 24)/(tA)$ where g represents mass loss, t is time (measured in hours during which the weight loss occurred), and A is the exposed area of the film.[37]

Swelling experiments

Firstly, a 1 cm^2 piece of each free film was dried in an oven at 50°C for 24 h. The dried film was then weighed accurately and immersed in a dissolution flask containing 250 ml of different media at 37°C. The swollen sample was withdrawn from the medium at specific intervals and weighed after removal of excess surface water by light blotting with filter paper. Samples were weighed every minute for the first 10 min; the sampling time was gradually increased after this period until 3 h.

The swelling behaviour of the films was calculated as I_s (%) = $(M_s - M_i)/M_i \times 100$ 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.[38] Swelling tests were carried out separately 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 caecal contents (8%, w/w) obtained from normal or colitis-induced rats.[39]

Drug permeability

Permeability of the model drug (5-FU) across the polymeric films was determined in a 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 5-FU through polymer films. The initial concentration of drug in the donor compartment was 12 μg/ml (saturated solution). The donor and acceptor compartments were both composed of SGF, SIF or SCF. Concentrations of 5-FU in the samples were determined by HPLC.[39]

When steady state was achieved, the permeability coefficient of the studied drug was determined using the following formula:[40]

$$\frac{2PS}{V}t = -\ln\frac{(C_0 - 2C_a)}{C_0} \quad (2)$$

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_a is the concentration of 5-FU in the acceptor compartment. P can be calculated from a plot of $-\ln (C_0 - 2C_a)/C_0$ against time.

Degradation of free films *in vitro*

Films were cut into 1 × 1 cm pieces, weighed accurately and then placed individually into a glass dropping bottle (100 ml). In the presence of a continuous supply of CO_2, these bottles were placed into the control phosphate buffer (pH 6.8) or SCF (30 ml medium per bottle) and the lid sealed. Bottles were then put on a rocking platform operated at 100 rpm. After 24 h, the films were removed, washed thoroughly with deionized water and dried in an oven at 50°C overnight. The percentage film weight loss was calculated using the formula W_d (%) = $[(W_0 - W_t)/W_0] \times 100$ 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).[41]

Degradation of free films *in vivo*

Pectin and pectin/Kollicoat SR30D free films were implanted in the caecum of anaesthetized normal or colitis-induced rats (200–250 g). The films were inserted in tiny gauze-sponge pouches (to allow debris recovery), which, in turn, were secured to the caecum epithelium by suturing.[42,43] After recovery, the rats were maintained for 12 h or 24 h with free access to rat chow and water. After euthanasia, the gauze-sponge bags were removed and the films' debris weighed.

Measurement of glass transition temperature (T_g)

The T_g of the Kollicoat SR30D/pectin films was measured using a differential scanning calorimeter (PerkinElmer 7 DSC; PerkinElmer, Shanghai, US), with an intracooler and nitrogen purge. Each film sample consisted of 8 mg of small discs piled into a 50-μl aluminium 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 heated from 0 to 200°C at a rate of 10°/min and then cooled back to 0°C at a rate of 20°/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 5°/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.[44]

Statistical analysis

Results are expressed as mean ± SD. Kruskal–Wallis non-parametric one-way analysis of variance with Dunn's post-hoc

test was used to compare the means of different treatment data. Results with $P < 0.05$ were considered statistically significant.

Results

Mechanical properties of free films

Polymer films must be mechanically strong so that they do not break or fracture during processing, packaging, shipping and storage.[45] The puncture strength and elongation of dry and wet polymeric films were determined by puncture test; the results are shown in Table 1. In the dry state, films prepared from Kollicoat SR30D showed high puncture strength and elongation values. In the wet state, the puncture strength of Kollicoat SR30D films decreased and % elongation increased because of the hydrophobic character of Kollicoat SR30D; however, the differences in the puncture strength were not significant. 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 the increase in flexibility.[35]

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 pectin within the film; the difference in both puncture strength and elongation was significant ($P < 0.05$) with addition of pectin up to the ratio of 30%. It has been reported that polysaccharides can reduce ethylcellulose chain deformation capacity by affecting polymer chain mobility, and alter the structure of the films, making the films weaker and softer.[11,46,47] Thus, these might be applicable to Kollicoat SR30D films too. Hydroxypropyl methyl cellulose and the 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 indicated that the two polymers were incompatible.[47,48]

In the wet state, the elongation of mixed films increased rapidly. The explanation for this is that pectin increases the water uptake of the mixed films; the water then functions as a softening agent, causing 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.[49] Although the increasing pectin concentration within the films had adverse effects on the puncture strength and elongation, the mixed films still possessed good mechanical properties.

WVT experiments

As shown in Figure 1, the rate of water vapour permeation was constant for free films containing pectin and Kollicoat SR30D. Table 2 lists the results of WVT experiments for all the formulations and shows that WVT was affected by the composition of the film. An increase in polysaccharide concentration significantly influenced WVT with the addition of pectin up to a ratio of 10% ($P < 0.05$). It is well known that increasing the hydrophilic nature of a polymer membrane induces water vapour tendency and as a result increases water vapour permeation.[50,51] Akhgari *et al.*[52] and Cavalcanti *et al.*[53] also reported that incorporating the polysaccharide inulin into

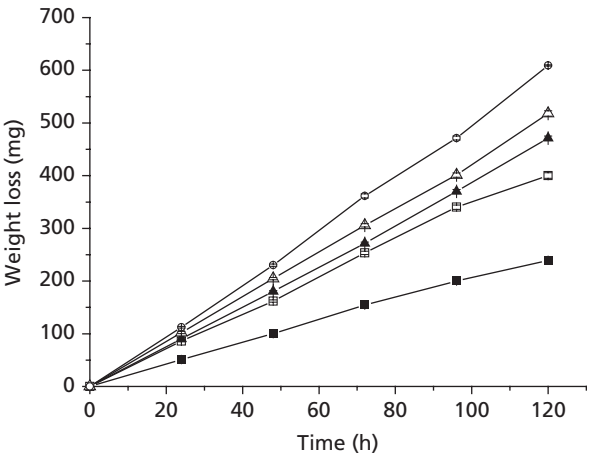

Figure 1 Profiles of water vapour transmission through free films containing Kollicoat SR30D (K) and pectin (P) ($n = 3$). Where (-■-) Kollicoat 100%, (-□-) K:P 90:10, (▲) K:P 80:20, (△) K:P 70:30 and (-○-) K:P 60:40.

Table 2 Water vapour transmission (WVT) of Kollicoat SR30D (K)/pectin (P) free films

Formulation	Mass change(mg/h)	WVT (g/24 h per m^2)
Kollicoat film	2.01 ± 0.16	1.53 ± 0.12
K:P 10:90	3.26 ± 0.36*	2.49 ± 0.25*
K:P 20:80	3.86 ± 0.24*	2.95 ± 0.18*
K:P 30:70	4.19 ± 0.31**	3.20 ± 0.24**
K:P 40:60	5.08 ± 0.42**	3.88 ± 0.32**

Values are mean ± SD, $n = 3$. *$P < 0.05$, **$P < 0.01$ vs Kollicoat film.

Table 1 Mechanical properties of different Kollicoat SR30D (K)/pectin (P) films in the dry and wet state

Formulation	Puncture strength (MPa)		Elongation (%)	
	Dry	Wet	Dry	Wet
Kollicoat film	6.57 ± 0.57	5.74 ± 0.86	197.67 ± 20.64	246.36 ± 25.67
K:P 90:10	5.24 ± 0.87	5.01 ± 0.95	163.54 ± 21.67*	188.59 ± 31.89*
K:P 80:20	4.67 ± 0.76	4.05 ± 0.67	132.67 ± 32.54**	162.48 ± 40.65**
K:P 70:30	4.02 ± 0.32*	3.06 ± 0.58*	127.68 ± 18.69**	144.27 ± 19.67**
K:P 60:40	3.24 ± 0.32**	2.86 ± 0.67**	108.67 ± 32.54**	142.57 ± 27.68**
K:P 50:50	3.11 ± 0.79**	2.76 ± 0.49**	100.27 ± 22.04**	115.09 ± 18.34**

Values are mean ± SD, ($n = 3$). *$P < 0.05$, **$P < 0.01$ vs Kollicoat film.

the water-insoluble polymer Eudragit RS and RL films increased the WVT of the films. Similarly, pectin is soluble in water and has good hydrophilic properties; thus, the incorporation of pectin into a Kollicoa SR30D film would also engender an increase in hydrophilicity, and thus the WVT increased.

Swelling test

The results of swelling experiments are shown in Table 3. As expected, films containing higher concentrations of pectin displayed an increasing degree of swelling. Formulations containing Kollicoat SR30D and pectin had a low swelling index. Swelling index in SGF and SIF increased with the addition of pectin ($P < 0.05$), which is due to the hygroscopic characteristics of pectin and the higher water uptake of the polymer. Swelling index was lower in SGF than that in SIF, since pectin remains as aggregates of macromolecules in acid environments, while at neutral pH pectin aggregates tend to dissociate and expand.[21] Compared with the swelling index in SIF and SGF, swelling of the free films in SCF significantly increased with addition of pectin ($P < 0.05$). It has been reported that enzymatic activity increased the swelling index of inulin/Eudragit RS blended films, since the inulinase enzyme could diffuse into the polymeric chains,[53] hydrolysing the glycosidic linkages within the inulin, reduced the network density and finally increased swelling.[52,54] Similarly, the presence of colonic bacteria, which contain more than one enzyme, would also increase the swelling index of the mixed films. Increasing the swelling index of the film would result in better accessibility of the β-(1→4) bonds in the colon. A study by Van den Mooter *et al.*[37] showed that enzymatic degradation of azo polymer films depended largely on their degree of swelling. 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 displayed the highest degree of swelling.

The swelling in SCF with caecal contents obtained from normal rats was higher than that from colitis-induced rats, although the difference was not significant.

Drug permeability

Data for drug permeability in different media is shown in Table 4 and Figure 2. The permeability of the mixed films remained relatively low in SGF as well as in SIF, as long as the concentration of the pectin added was low, indicating that the insolubility of the film conferred by Kollicoat SR30D was maintained. Films containing higher concentrations of pectin displayed increasingly faster permeation to the drug compared with films with lower pectin content. The permeability results agree with the results of swelling tests: an increase in the pectin content of the film resulted in an increase in the drug permeability. Similar to the results of the swelling experiment, drug permeability in SGF was lower than in SIF; the gelling ability and solubility of pectin depended strongly on the pH of the surrounding medium.[21]

The lower permeability of mixed films in SGF and SIF than in SCF ($P < 0.05$) demonstrated the susceptibility of pectin in these films to bacterial enzymes and degradation in simulated colonic medium. The increased permeability in SCF could be explained firstly as a direct consequence of swelling levels presented by the different films. This hydration level favoured enzyme access to the film components (pectin), permitting enzymatic decomposition and a consequent increase in permeability due to the formation of pores or fissures in the tested membranes. Pectin could be degraded by the bacteria in human colon, particularly *Bacteroides*.[26] Because of the similarity of the intestinal microflora between humans and rats,[55] the rat colonic bacterial enzymes could

Table 3 Swelling of free films containing Kollicoat SR30D (K)/pectin (P) in simulated gastric fluid (SGF), simulated intestinal fluid (SIF) or simulated colonic fluid (SCF) containing caecal contents from normal, TNBS- or OXA- induced rats

Formulation	SGF	SIF	SCF (normal)	SCF (TNBS)	SCF (OXA)
Kollicoat film	29.04 ± 0.57	29.17 ± 1.25	29.27 ± 2.07	29.01 ± 2.14	30.21 ± 2.35
K:P 90:10	35.59 ± 6.57	37.98 ± 3.57	44.07 ± 1.37	40.02 ± 5.68	42.35 ± 6.57
K:P 80:20	51.34 ± 6.87	54.87 ± 2.18	71.26 ± 2.67*	61.05 ± 8.97*	63.54 ± 3.57*
K:P 70:30	65.54 ± 12.68	68.25 ± 4.29	93.18 ± 3.57*	81.35 ± 10.35*	84.35 ± 8.97*
K:P 60:40	73.58 ± 14.97	78.35 ± 5.37	114.13 ± 5.03**	97.67 ± 12.35**	102.35 ± 13.57**
K:P 50:50	88.69 ± 16.57	96.57 ± 13.64	140.35 ± 15.67**	121.35 ± 12.67**	128.67 ± 15.67**

Values are maximum swelling index (%), mean ± SD, $n = 3$. *$P < 0.05$, **$P < 0.01$ vs SIF.

Table 4 Permeability of different free films to 5-fluorouracil

Formulation	SGF	SIF	SCF (normal)	SCF (TNBS)	SCF (OXA)
Kollicoat film	1.12 ± 0.14	1.08 ± 0.03	1.14 ± 0.12	1.10 ± 0.10	1.21 ± 0.21
K:P 90:10	1.33 ± 0.21	1.42 ± 0.12	1.68 ± 0.32	1.51 ± 0.13	1.61 ± 0.20
K:P 80:20	1.51 ± 0.18	1.63 ± 0.22	2.22 ± 0.20*	1.97 ± 0.23*	2.14 ± 0.30*
K:P 70:30	1.89 ± 0.44	1.92 ± 0.40	3.15 ± 0.41*	2.75 ± 0.42*	2.98 ± 0.53*
K:P 60:40	2.01 ± 0.55	2.31 ± 0.50	3.78 ± 0.37**	3.23 ± 0.40**	3.47 ± 0.36**

Values are permeability (P) 10^{-6} cm/s; mean ± SD, $n = 3$. K, Kollicoat SR30D; P, pectin; SGF, simulated gastric fluid; SIF, simulated intestinal fluid; SCF, simulated colonic fluid containing caecal contents from normal or TNBS- or OXA-induced rats. *$P < 0.05$, **$P < 0.01$ vs SIF.

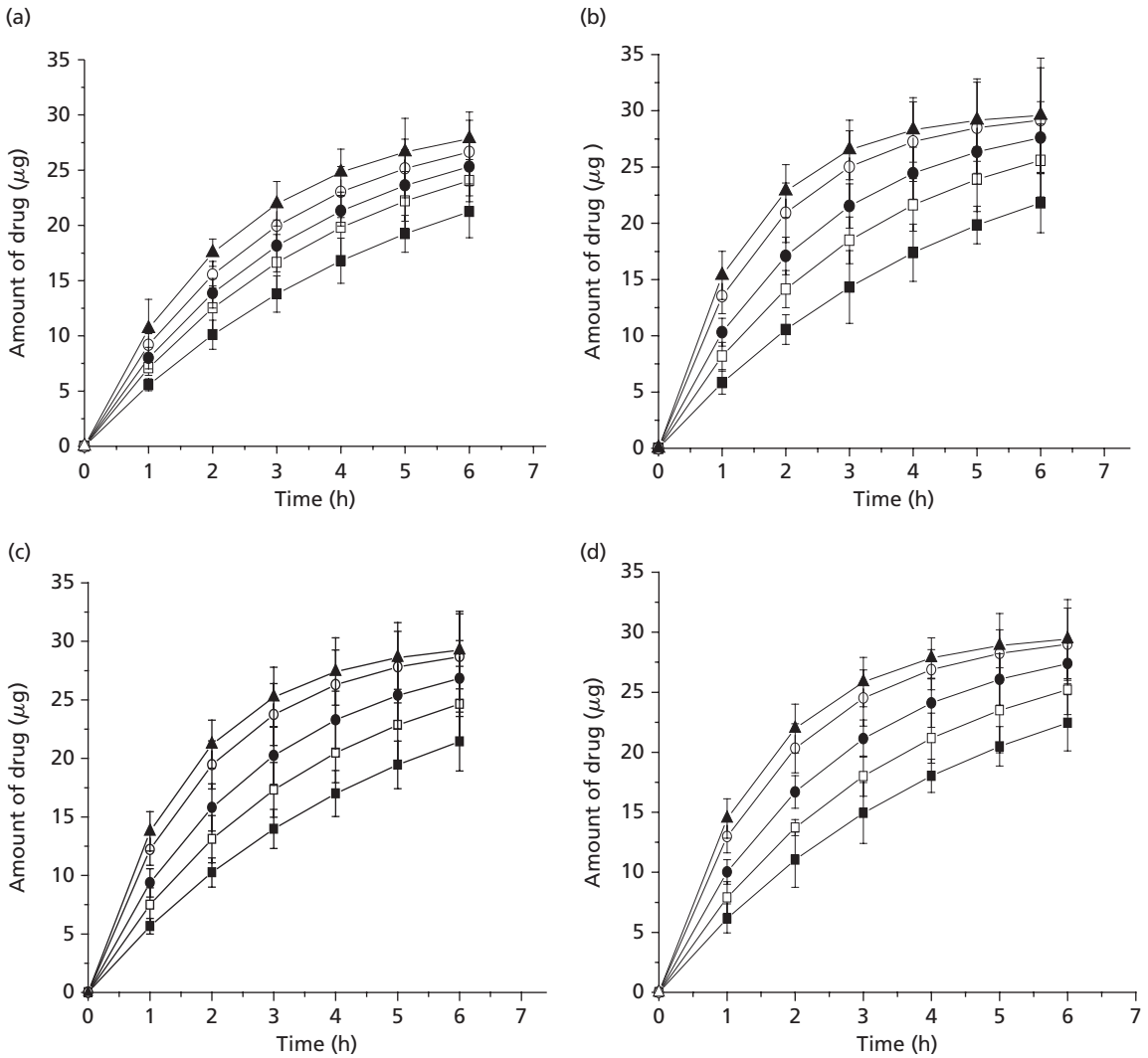

Figure 2 Permeability profiles of free films containing Kollicoat SR30D (K)/pectin (P) to 5-fluorouracil in control media (a) and simulated colonic fluids containing caecal contents from: normal rats (b) TNBS-induced rats (c) and OXA-induced rats (d) (n = 3). Where (-■-) Kollicoat 100%, (-□-) K:P 90:10, (-●-) K:P 80:20, (-○-) K:P 70:30 and (-▲-) K:P 60:40.

degrade the pectin within the films. Thus, the addition of caecal contents to the phosphate buffer increased the rate and extent of pectin leaching from Kollicoat SR30D films, and so the permeability increased.

Similar to the results of the swelling tests, drug permeability in SCF with caecal contents from normal rats was higher than with that from colitis-induced rats, although the difference was not significant.

Degradation of free film *in vitro*

The influence of pectin concentration on the degradation of the mixed films is shown in Table 5. As expected, the films containing the higher pectin concentrations were more prone to enzymatic digestion, and a longer incubation time (24 h) led to greater digestion. In contrast, very little weight loss, and hence digestion, was noted in any of the mixed films after 24 h incubation in the control medium. There was no weigh loss of the Kollicoat SR 30D film *per se* in SCF and control medium, indicating that it could not be degraded by bacterial enzymes. The presence of increased levels of Kollicoat SR30D could reduce accessibility to enzymatic attack, as the pectin no longer forms a continuous network throughout the film. The presence of increasing concentrations of Kollicoat SR30D could result in a denser film network, and greater hindrance in access to the enzymatic active site and a subsequent reduction in digestion. In fact, in an amylose/ethylcellulose system for colonic delivery, the function of the ethylcellulose was to control the swelling of the amylose,[12,46,56] and may thus be applicable to Kollicoat SR30D too. On the other hand, the extent of digestion was directly associated with the swelling of the free films.[57,58] Thus, the ratio of Kollicoat SR30D to pectin should be high enough to control the swelling properties of the films, yet not so high that the digestion of pectin is likely to be hindered.

When compared with the extent of digestion in SCF with caecal contents obtained from normal rats, values obtained from TNBS- or OXA-induced rats were lower, but the difference was not significant.

Degradation of films *in vivo*

The in-vivo degradation of Kollicoat SR30D/pectin-free films in rat caecum is shown in Table 6, which shows that increasing the pectin component of the film increased the extent of film degradation. Compared with in-vitro degradation, the films that were implanted in the rat caecum degraded relatively rapidly, and the extent of digestion increased with the incubation time (12–24 h). The extent of film digestion was similar in normal and TNBS- or OXA-induced rats. This indicates that pectin within the mixed films was also completely degraded in the colitis-induced rats. On the other hand, no weight loss was observed in the Kollicoat SR 30D-alone films, indicating that it could not be degraded by bacterial enzymes, and thus that digestion of the pectin resulted in weight loss of the mixed films.

Glass transition temperature

When the proportion of pectin was less than 30%, no significant increase in the T_g of films was observed (Figure 3), indicating that pectin was poorly miscible in Kollicoat SR30D films.[59] Poor miscibility of pectin in Kollicoat SR30D films suggested that pectin was distributed as isolated domains in the films and that the characteristics of pectin within the mixed films are likely to remain unchanged. Pectin within the mixed films should therefore remain recognizable to colonic bacterial enzymes as a digestion substrate.

Discussion

In in-vitro studies, the swelling index, extent of film degradation and drug permeability in SCF in the presence of caecal contents from normal rats was higher or faster than that from TNBS- or OXA-induced rats, although the difference was not significant. The TNBS and OXA colitis models in rats resemble Crohn's disease (TNBS) or ulcerative colitis (OXA) in humans; therapeutic efficiency can vary significantly in both diseases.[60] It is known that colonic bacterial enzymes, such as glycosidases, which are responsible for the hydrolysis of di- and oligosaccharides, and polysaccharidases, which are capable of hydrolysing various polysaccharides, are produced by the anaerobic bacteria in the human colon,[61] of which *Bacteroides* and bifidobacteria are the predominant species.[62] In TNBS- or OXA-induced model rats, bifidobacteria

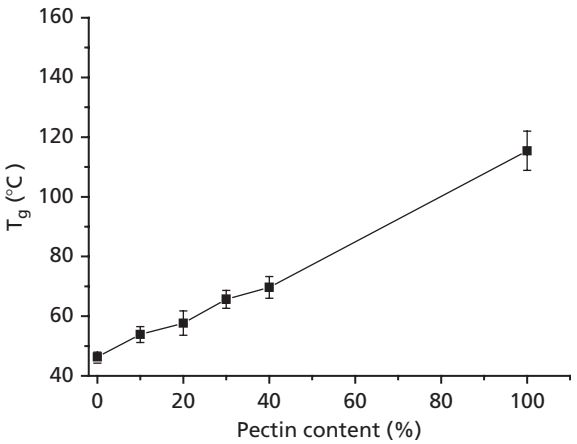

Figure 3 Glass transition temperatures as a function of pectin concentration for Kollicoat SR30D films (n = 3).

Table 5 Percentage remaining of different Kollicoat SR30D (K)/pectin (P) films after 12 h and 24 h incubation in simulated colonic fluids (SCF) (caecal contents from normal, TNBS-induced or OXA-induced rats) and control medium

Formulation	Control (SIF)		SCF (normal)		SCF (TNBS)		SCF (OXA)	
	12 h	24 h	12 h	24 h	12 h	24 h	12 h	24 h
Kollicoat film	98.02 ± 5.35	97.99 ± 2.04	98.04 ± 3.21	97.91 ± 2.35	99.98 ± 2.65	97.98 ± 3.97	97.76 ± 3.05	96.37 ± 3.76
K:P 90:10	99.23 ± 3.67	98.11 ± 0.97	94.34 ± 4.65	90.67 ± 5.64	93.35 ± 3.98	92.27 ± 4.51	97.25 ± 4.01	96.57 ± 5.10
K:P 80:20	98.34 ± 6.65	97.32 ± 2.11	92.67 ± 5.01	82.01 ± 12.35*	93.34 ± 4.35	87.05 ± 6.97*	92.75 ± 5.32	82.66 ± 7.01*
K:P 70:30	97.23 ± 3.57	95.09 ± 1.05	86.23 ± 5.35*	75.31 ± 8.67*	87.35 ± 6.35*	82.36 ± 12.33*	89.34 ± 6.31*	75.67 ± 15.45*
K:P 60:40	95.00 ± 5.62	94.81 ± 1.34	81.24 ± 6.07*	68.24 ± 16.57*	82.24 ± 4.65*	75.67 ± 10.51*	83.64 ± 6.77*	70.65 ± 12.56*
K:P 50:50	93.31 ± 5.08	92.57 ± 2.08	72.05 ± 6.58*	57.25 ± 9.61**	74.87 ± 7.05*	67.67 ± 16.30**	76.65 ± 6.11*	60.35 ± 13.00**
Pectin	68.35 ± 6.02	52.35 ± 3.01	27.24 ± 7.31**	5.25 ± 12.33**	29.34 ± 4.61**	10.24 ± 19.69**	31.25 ± 7.01**	7.57 ± 16.33**

Values are mean ± SD, n = 3. SIF, simulated intestinal fluid; SCF, simulated colonic fluid. *P < 0.05, **P < 0.01 compared with control.

Table 6 In-vivo degradation of the pectin and Kollicoat SR30D (K)/pectin (P) films in the caecum of the normal, TNBS-induced and OXA-induced rats

Formulation	Normal rats (control)		TNBS-induced rats		OXA-induced rats	
	12	24	12	24	12	24
K	97.35 ± 6.11	96.87 ± 3.61	98.11 ± 3.01	95.35 ± 4.25	97.15 ± 3.02	95.33 ± 5.80
K:P 70:30	82.31 ± 11.64	72.35 ± 15.05*	82.28 ± 15.04	69.23 ± 9.66*	81.66 ± 18.32	71.35 ± 16.88*
K:P 60:40	71.53 ± 12.15*	64.28 ± 19.07*	70.66 ± 13.21*	66.12 ± 11.35*	72.05 ± 13.25*	66.31 ± 17.13*
P	26.33 ± 7.04**	1.21 ± 4.02**	25.63 ± 3.99**	1.34 ± 3.55**	24.91 ± 5.59**	1.56 ± 3.52**

Values are weight recovered as a % of the initial implant; mean ± SD, n = 3. *P < 0.05, **P < 0.01 vs Kollicoat film.

numbers are reduced, which results in decreased enzyme activities.[63] Thus, the extent of swelling and degradation, and permeability in SCF in the presence of caecal contents from TNBS- or OXA-induced rats were lower. However, it was surprising that the extent of film degradation *in vivo* in TNBS- or OXA-induced rats was almost the same as in the normal rats. The results do not agree with the hypothesis that the extent of degradation *in vivo* should be lower than that in normal rats. It might be explained by the limitations of the in-vitro model.[43] In in-vitro studies, an 8% (w/w) rat caecal content in buffer could not overcome the reduced enzymatic activities in pathological conditions. In fact, even with simple linear one-component polysaccharides, more than one bacterial enzyme is usually involved in degradation or breakdown.[64] Pectin could be digested by *Bacteroides*, bifidobacteria and eubacteria etc.[1] In the in-vitro model, the low concentration of bacterial enzyme would not result in saturation of degradation. In this situation, the reduced enzymatic activities are responsible for the relatively lower extent of degradation.

Compared with the in-vitro model, the large intestine contains a complex and dynamic microbial ecosystem with high densities of living bacteria, which achieve concentrations of up to 10^{11} or 10^{12} CFU/ml of luminal contents.[65] A large proportion of the faecal mass consists of bacteria (around 60% of faecal solids).[66] The human colon contains approximately 1.5 kg wet weight of bacteria.[67] Many species of bacteria are involved in the digestion of the polysaccharides.[64] Such a high concentration of bacteria enzymes *in vivo* would certainly result in saturation for degradation. The concentration of bacteria enzymes was so high that the reduced enzymatic activity was likely to be overshadowed. Thus, numbers and concentration of bacterial enzymes would be reduced in colitis-induced rats, but saturation for degradation still occurred. Moreover, patients with IBD have higher amounts of bacteria attached to their epithelial surfaces than do healthy people;[68] this may be applicable to TNBS- or OXA-induced rats too. In fact, the process of degradation of pectin is very complex, and many species participate in the degradation. In colitis-induced rats, the concentrations of *Bacterioides,* eubacteria and *Peptostreptococcus* and the number of facultative anaerobes are increased, whereas bifidobacteria numbers are reduced.[69] It means that the increased number of bacteria would compensate for the reduced bacterial enzymes, which ensures that the polysaccharides could be digested completely. In disease states, sufficient enzyme concentrations should be present in most subjects. The amylose/ethylcellulose coating system (known as COLAL) is now in late-stage clinical trials with prednisolone sodium metasulfabenzoate for the local treatment of ulcerative colitis. The successful clinical use of sulfasalazine for the treatment IBD for over 40 years and the more recent development of olsalazine, both of which rely on bacterial azo reductases for their action, also make this a promising avenue for investigation.[70]

Conclusions

The study had shown that pectin/Kollicoat SR30D films could be successfully prepared by a casting/solvent evaporation method. The mixed films possessed good mechanical properties, and could be used as film coating materials for drug delivery. The mixed films were susceptible to bacterial enzymes. The extent of digestion correlated with the amount of pectin present within the film. *In vitro*, the swelling index, drug permeability and extent of film degradation in SCF with caecal contents from normal rats were higher than values obtained with caecal contents from TNBS- or OXA-induced rats; however, similar results were not observed in the in-vivo degradation. The pectin within the mixed films could still be degraded completely in the colitis-induced rats.

The application of such pectin/Kollicoat SR30D films as coatings to oral dosage forms could direct solid dosage forms to the large intestine, where the component of the polysaccharide pectin incorporated into the mixed film would be digested. Utilization of polysaccharides to deliver drugs to the colon is worth investigating further. An in-vivo comparison of the degradation of pectin/Kollicoat SR30D film-coated pellets for colonic delivery in normal and colitis-induced rats is currently in progress.

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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References

1. Jain A *et al*. Perspectives of biodegradable natural polysaccharides for site-specific drug delivery to the colon. *J Pharm Pharm Sci* 2007; 10: 86–128.
2. Gorbach SL. Intestinal flora. *Gastroenterology* 1971; 60: 1110–1129.
3. Cummings JH, Macfarlane GT. The control and consequences of bacterial fermentation in the human colon. *J Appl Bacteriol* 1991; 70: 443–459.
4. Yang L. Biorelevant dissolution testing of colon-specific delivery systems activated by colonic microflora. *J Control Release* 2008; 125: 77–86.
5. Cummings JH, Englyst HN. Fermentation in the human large intestine and the available substrates. *Am J Clin Nutr* 1987; 45: 1243–1255.
6. Rubinstein A. Microbially controlled drug delivery to the colon. *Biopharm Drug Dispos* 1990; 11: 465–475.
7. Hawksworth G *et al*. Intestinal bacteria and hydrolysis of glycosidic bonds. *J Med Microbiol* 1971; 4: 451–459.
8. Scheline RR. Metabolism of foreign compounds by gastrointestinal micro-organisms. *Pharmacol Rev* 1973; 25: 451–523.
9. Englyst HN *et al*. Polysaccharide breakdown by mixed populations of human fecal bacteria. *FEMS Microbiol Ecol* 1987; 95: 163–171.
10. Larsen C *et al.* Macromolecular prodrugs. XVI. Colon-targeted delivery – comparison of the rate of release of naproxen from dextran ester prodrugs in homogenates of various segments of the pig gastrointestinal (GI) tract. *Pharm Res* 1989; 6: 995–999.
11. Macleod GS *et al.* Studies on the physical properties of mixed pectin/ethylcellulose films intended for colonic drug delivery. *Int J Pharm* 1997; 157: 53–60.

12. Milojevic S *et al*. Amylose as coating for drug delivery to the colon: preparation and *in vitro* evaluation using 5-aminosalicylic acid pellets. *J Control Release* 1996a; 38: 75–84.
13. Milojevic S *et al*. Amylose as coating for drug delivery to the colon: preparation and *in vitro* evaluation using glucose pellets. *J Control Release* 1996; 38: 85–94.
14. Wilson PJ, Basit AW. Exploiting gastrointestinal bacteria to target drugs to the colon: an *in vitro* study using amylose coated tablets. *Int J Pharm* 2005; 300: 89–94.
15. Cummings JH *et al*. *In vivo* studies of amylose- and ethylcellulose-coated [^{13}C] glucose microspheres as a model for drug delivery to the colon. *J Control Release* 1996; 40: 123–131.
16. Tuleu C *et al*. Colonic delivery of 4-aminosalicylic acid using amylose-ethylcellulose-coated hydroxypropylmethylcellulose capsules. *Aliment Pharmacol Ther* 2002; 16: 1771–1779.
17. Basit AW *et al*. The use of formulation technology to assess regional gastrointestinal drug absorption in humans. *Eur J Pharm Sci* 2004; 21: 179–189.
18. Chourasia MK, Jain SK. Polysaccharides for colon targeted drug delivery. *Drug Deliv* 2004; 11: 129–148.
19. Wakerly Z *et al*. Pectin/ethylcellulose film coating formulations for colonic drug delivery. *Pharm Res* 1996; 13: 1210–1212.
20. Semde R *et al*. Leaching of pectin from mixed pectin/insoluble polymer films intended for colonic drug delivery. *Int J Pharm* 1998; 174: 233–241.
21. Liu LS *et al*. Pectin-based systems for colon-specific drug delivery via oral route. *Biomaterials* 2003; 24: 3333–3343.
22. He W *et al*. In-vitro and in-vivo studies of pectin/ethylcellulose film-coated pellets of 5-fluorouracil for colonic targeting. *J Pharm Pharmacol* 2008; 60: 35–44.
23. Dashevsky A *et al*. Physicochemical and release properties of pellets coated with Kollicoat SR 30 D, a new aqueous polyvinyl acetate dispersion for extended release. *Int J Pharm* 2005; 290: 15–23.
24. Kolter K, Ruchatz F. Kollicoat SR 30D – a new sustained-release excipient. *Proc Int Symp Control Release Bioact Mater* 1999; 26: 6311.
25. Shao ZJ *et al*. Drug release from Kollicoat SR 30D-coated nonpareil beads: evaluation of coating level, plasticizer type, and curing condition. *AAPS PharmSciTech* 2002; 3: E15.
26. Sinha VR, Kumria R. Microbially triggered drug delivery to the colon. *Eur J Pharm Sci* 2003; 18: 3–18.
27. Swidsinski A *et al*. Biostructure of fecal microbiota in healthy subjects and patients with chronic idiopathic diarrhea. *Gastroenterology* 2008; 135: 568–579.
28. Rowlanmd IR. Factors affecting metabolic activity of the intestinal microflora. *Drug Metab Rev* 1988; 19: 243–261.
29. Carrette O *et al*. Bacterial enzymes used for colon-specific drug delivery are decreased in active Crohn's disease. *Dig Dis Sci* 1995; 40: 2641–2646.
30. Ott SJ *et al*. Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease. *Gut* 2004; 53: 685–693.
31. Vargo D *et al*. Faecal bacterial flora in cancer of the colon. *Gut* 1980; 21: 701–705.
32. Gazzaniga A *et al*. Time-controlled oral delivery systems for colon targeting. *Expert Opin Drug Deliv* 2006; 3: 583–597.
33. Neurath MF *et al*. Antibodies to interleukin 12 abrogate established experimental colitis in mice. *J Exp Med* 1995; 182: 1281–1290.
34. Heller F *et al*. Oxazolone colitis, a Th2 colitis model resembling ulcerative colitis, is mediated by IL-13-producing NK-T cells. *Immunity* 2002; 17: 629–638.
35. Bodmeier R, Paeratakul O. Mechanical properties of dry and wet cellulosic and acrylic films prepared from aqueous colloidal polymer dispersions used in the coating of solid dosage forms. *Pharm Res* 1994; 11: 882–888.
36. Nykanen P *et al*. Citric acid as excipient in multiple-unit enteric-coated tablets for targeting drugs on the colon. *Int J Pharm* 2001; 229: 155–162.
37. Van Den Mooter G *et al*. Characterization of colon-specific azo polymers: a study of the swelling properties and permeability of isolated polymers films. *Int J Pharm* 1994; 111: 127–136.
38. Blanchon S *et al*. Permeability of progesterone and a synthetic progestin through methacrylic films. *Int J Pharm* 1991; 72: 1–10.
39. He W *et al*. Study on colon-specific pectin/ethylcellulose film-coated 5-fluorouracil pellets in rats. *Int J Pharm* 2008; 348: 35–45.
40. Flynn GL *et al*. Mass transport phenomena and models: theoretical concepts. *J Pharm Sci* 1974; 63: 479–509.
41. Mcconnell EL *et al*. An investigation into the digestion of chitosan (noncrosslinked and crosslinked) by human colonic bacteria. *J Pharm Sci* 2008; 97: 3820–3829.
42. Gliko-Kabir I *et al*. Phosphated crosslinked guar for colon-specific drug delivery. II. *In vitro* and *in vivo* evaluation. *J Control Release* 2000; 63: 129–134.
43. Haupt S *et al*. Luminal delivery and dosing considerations of local celecoxib administration to colorectal cancer. *Eur J Pharm Sci* 2006; 28: 204–211.
44. Okhamafe AO, York P. Studies of interaction phenomena in aqueous-based film coatings containing soluble additives using thermal analysis techniques. *J Pharm Sci* 1988; 77: 438–443.
45. Felton LA. Characterization of coating systems. *AAPS PharmSciTech* 2007; 8: E112.
46. Siew LF *et al*. The properties of amylose-ethylcellulose films cast from organic-based solvents as potential coatings for colonic drug delivery. *Eur J Pharm Sci* 2000; 11: 133–139.
47. He W *et al*. Selective drug delivery to the colon using pectin coated pellets. *PDA J Pharm Sci Technol*. (in press).
48. Hjärtstam J, Hjertberg T. Swelling of pellets coated with a composite film containing ethylcellulose and hydroxypropyl methylcellulose. *Int J Pharm* 1998; 161: 23–28.
49. Bodmeier R, Paeratakul O. Dry and wet strengths of polymeric films prepared from an aqueous colloidal polymer dispersion, Eudragit RS 30D. *Int J Pharm* 1993; 96: 129–138.
50. Parra DF *et al*. Mechanical properties and water vapor transmission in some blends of cassava starch edible films. *Carbohydr Polym* 2004; 58: 475–481.
51. Wang ZF *et al*. Effect of temperature and structure on the free volume and water vapor permeability in hydrophilic polyurethanes. *J Membr Sci* 2004; 241: 355–361.
52. Akhgari A *et al*. Permeability and swelling studies on free films containing inulin in combination with different polymethacrylates aimed for colonic drug delivery. *Eur J Pharm Sci* 2006; 28: 307–314.
53. Cavalcanti OA *et al*. Polysaccharides as excipients for colon-specific coatings. permeability and swelling properties of casted films. *Drug Dev Ind Pharm* 2002; 28: 157–164.
54. Vervoort L *et al*. Inulin hydrogels. II. *In vitro* degradation study. *Int J Pharm* 1998; 172: 137–145.
55. Van Den Mooter G *et al*. *In vivo* evaluation of a colon-specific drug delivery system: an absorption study of theophylline from capsules coated with azo polymers in rats. *Pharm Res* 1995; 12: 244–247.
56. Leong CW *et al*. The formation of colonic digestible films of amylose and ethylcellulose from aqueous dispersions at temperatures below 37 degrees C. *Eur J Pharm Biopharm* 2002; 54: 291–297.
57. Langer RS, Peppas NA. Present and future applications of biomaterials in controlled drug delivery systems. *Biomaterials* 1981; 2: 201–214.

58. Rubinstein A. Approaches and opportunities in colon-specific drug delivery. *Crit Rev Ther Drug Carrier Syst* 1995; 12: 101–149.
59. Tarvainen M *et al*. Starch acetate–a novel film-forming polymer for pharmaceutical coatings. *J Pharm Sci* 2002; 91: 282–289.
60. Pellequer Y *et al*. Epithelial heparin delivery via microspheres mitigates experimental colitis in mice. *J Pharmacol Exp Ther* 2007; 321: 726–733.
61. Hovgaard L, Brøndsted H. Current applications of polysaccharides in colon targeting. *Crit Rev Ther Drug Carrier Syst* 1996; 8: 185–223.
62. Salyer A. Energy sources of major intestinal fermentative anaerobes. *Am J Clin Nutr* 1979; 32: 158–163.
63. Favier C *et al*. Fecal beta-D-galactosidase production and Bifidobacteria are decreased in Crohn's disease. *Dig Dis Sci* 1997; 42: 817–822.
64. Salyers AA, Leedle JA. Human intestinal microflora in health and disease. In: Hentge DJ, ed. *Carbohydrate Metabolism in the Human Colon*. New York: Academic Press, 1983: 129–146.
65. Simon GL, Gorbach SL. Intestinal flora in health and disease. *Gastroenterology* 86: 1984; 174–193.
66. Stephen AM, Cummings JH. The microbial contribution to human faecal mass. *J Med Microbiol* 1980; 13: 45–56.
67. Hill MJ, Drasar BS. The normal colonic bacterial flora. *Gut* 1975; 16: 318–323.
68. Swidsinski A *et al*. Mucosal flora in inflammatory bowel disease. *Gastroenterology* 2002; 122: 44–54.
69. Linskens RK *et al*. The bacterial flora in inflammatory bowel disease: current insights in pathogenesis and the influence of antibiotics and probiotics. *Scand J Gastroenterol Suppl*: 2001; 234: 29–40.
70. Ashford M, Fell JT. Targeting drugs to the colon: delivery systems for oral administration. *J Drug Target* 1994; 2: 241–257