International Journal of Pharmaceutics 366 (2009) 140–148

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Studies of chitosan/organic acid/Eudragit® RS/RL-coated system for colonic delivery

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ARTICLE INFO

Article history:
Received 26 May 2008
Received in revised form 4 September 2008
Accepted 6 September 2008
Available online 13 September 2008

Keywords:
Colon-targeted delivery
Chitosan
Succinic acid
Eudragit®
Electrostatic interaction

ABSTRACT

Prednisolone (PDS) beads were coated sequentially with (i) innermost hydrophobic layer of Eudragit® RS/RL, (ii) middle drug release-triggering layer of chitosan, organic acid and Eudragit® RS/RL, and (iii) outermost enteric coating layer. Continuous dissolution studies were carried out in artificial gastric fluid (pH 1.2), followed by intestinal fluid (pH 6.8), and finally in colonic fluid (pH 4 and 6) with and without β-glucosidase. While drug release was prevented in the gastric and small-intestinal fluids, a continuous release was observed in the colonic fluid. Succinic acid provided the fastest rate of release in the colonic fluid compared to citric, tartaric or malic acid. A combined mechanism of drug release is proposed, which considers the swelling of chitosan and Eudragit® RS/RL in the presence of succinic acid possibly via electrostatic interaction between the amine groups of chitosan/quaternary ammonium groups of Eudragit® RS/RL and the carboxyl groups of succinic acid in aqueous medium. The results of plasma pharmacokinetic studies in Sprague–Dawley rats showed that the developed system provided a significant delay (T_{max} 9.3 h) in the absorption profile of PDS compared with simple enteric-coated (T_{max} 4 h) or powder (T_{max} 1 h) formulation that was taken as proof for the colon-targeted delivery.

1. Introduction

Colon-specific drug delivery has gained increasing importance not only just for the treatment of local diseases associated with the colon such as Crohn's disease, ulcerative colitis and colorectal cancer, but also for its potential for the effective delivery of proteins and therapeutic peptides. Current colon-specific technologies have utilized one or two (in combination) of the following primary approaches, with varying degrees of success: (1) pH-dependent systems, (2) time-dependent systems, (3) prodrugs, and (4) colonic microflora-activated systems.

Thus far, the pH-dependent systems prepared with enteric polymers have found practical application in the development of commercial products for the treatment of ulcerative colitis with 5-aminosalicylic acid (Sack and Peppercorn, 1983). However, for such formulations, the large interindividual variability of intestinal pH values poses difficulties in achieving truly colon-targeted delivery (Ashford et al., 1993). Also, in contrast to what was believed in the past, it is now known that the pH of the proximal and transverse colon is more acidic than that in the small intestine, especially in inflammatory bowel disease (IBD) (Press et al., 1988). Thus, the pH-based colon-specific formulations, which are designed to release drug at a higher pH, may fail to release drug completely upon encountering the more acidic colonic pH.

The time-dependent formulations are designed to deliver drugs after a particular time period, which is the time normally required to reach the colon. For this purpose, authors have used polymeric swelling agents such as a hydrogel plug, which would release drug after a particular time period (Wilding et al., 1992). However, the inherent limitation of this approach is the marked inter- and intra-individual variability in gastric emptying, small-intestinal and colonic transit time. This results in a spread of initial release sites in the gastrointestinal tract (GIT) from time-dependent systems (Perkins, 1999).

Two main classes of colonic bacterial enzymes such as azoreductases and polysaccharidases are increasingly recognized as preferable triggering components in the design of colon-specific delivery systems since the abrupt increase of the bacterial population and corresponding enzymatic activities in the colon represent a non-continuous event independent of gastrointestinal transit time (Gorbach, 1971). Based on this idea, different natural and synthetic polymers have been investigated lately for their susceptibility to being cleaved by these bacterial enzymes and thus, for their use as major components of colon-targeted drug delivery systems (Brondsted and Kopecek, 1992; Kopeckova et al., 1994). The natural polysaccharides such as chitosan, pectin, guar

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doi:10.1016/j.ijpharm.2008.09.006

gum, dextrans, amylose and chondroitin sulfate have appeared as more promising alternative polymers, although these polymers undergo the slower process of hydrolysis of their glycoside bonds in the colon. Chitosan has been investigated for colon-specific delivery of drugs because of its biodegradability by colonic bacteria (Tozaki et al., 1997). Chemically, chitosan is a weakly basic copolymer of β-(1,4)-2-acetamido-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose (Roberts, 1992), and thus it dissolves readily in weakly acidic conditions (Chen et al., 1994).

In the present investigation, a combination approach, including the use of a polysaccharide, chitosan and the insoluble acrylic polymers, Eudragit® RS/RL and L100, was pursued to achieve colon-targeted delivery. While the insoluble polymeric barrier was expected to be effective in preventing premature drug release in the upper GIT, the breakdown of chitosan combined with Eudragit® RS/RL in the colon via its dissolution would cause the onset of drug release. However, in the preliminary study, the drug release in the colonic fluid from such a system could be slow such that the performance of the site-specific drug delivery is adversely affected. In order to resolve this potential problem, an attempt was made to include an organic acid, such as succinic acid in the system with a view of facilitating drug release at the colonic site of delivery by enhancing the dissolution process of basic chitosan in the presence of the organic acid. In addition, organic acids have been reported to be capable of interacting with the Eudragit® RS and RL polymers due to the presence of quaternary ammonium groups in these polymers, leading to increase in permeability (Armand et al., 1987; Jenquin et al., 1990).

In this article, we present in vitro release characteristics and corresponding in vivo performance of a novel, multiparticulate delivery system based on a triple-layer coated dosage form design. The innermost layer comprised mainly of Eudragit® RS and Eudragit® RL polymers and it was intended to be a barrier to drug release in the upper GIT, while controlling drug release in the colon (Lehmann, 1997). The next layer was a combination layer comprising chitosan, which is a colon-degradable polysaccharide, and organic acid in a Eudragit® RS/RL film-coating. An outermost enteric layer was deposited with Eudragit® L100 in order to protect the delivery system from the gastric acidic conditions. A multiparticulate dosage form was selected, since such a dosage form was less likely to undergo dose dumping, and also, it may facilitate the spreading of the drug over the inflamed regions of the colonic lumen (Davis et al., 1991). The feasibility of the new chitosan–organic acid triggered multiparticulate delivery system was studied using prednisolone (PDS) as a model anti-inflammatory drug via in vitro evaluation of drug release characteristics and in vivo assessment of pharmacokinetics in Sprague–Dawley rats.

2. Materials and methods

2.1. Materials

PDS was obtained from Spectrum Chemical Mfg. (Gardena, CA). Nonpareil seeds (25–30 mesh) were obtained from Paulaur Corp. (Cranbury, NJ). The following chemicals were obtained from Sigma Chemical Co. (St. Louis, MO) and used as received: polyvinylpyrrolidone, succinic acid, citric acid, tartaric acid, malic acid, methylparaben, talc, triethylcitrate, and trifluoroacetic acid. The Eudragit® polymers (Rohm GmbH & Co., Germany), chitosan (low molecular weight—Aldrich Chemical Co., St. Louis, MO), rodent capsules, no. 9 el (Torpac, Inc., Fairfield, NJ), methyl-*t*-butyl ether (EMD Chemicals, Inc., Gibbstown, NJ), ethyl acetate and 2-propanol (VWR, West Chester, PA) were used as received. β-Glucosidase was obtained from MP Biomedicals (Solon, OH). All other reagents and solvents were of analytical grade.

Table 1
Composition of drug-loaded nonpareil beads (%).

Composition	% (w/w)
Cores	
Nonpareils (25–30 mesh)	82.0
Solids in layering dispersion	
Prednisolone	14.9
Polyvinylpyrrolidone (PVP K 30)	3.1
Water	q.s.
Total	100.0

Table 2
Processing parameters for the preparation of drug-loaded beads in a Uni-Glatt apparatus.

Parameter	Set at value
Charge load (nonpareils) (g)	500
Target quantity of drug to layer (g)	175
Nozzle bore (mm)	1
Atomising pressure (bar)	3
Inlet air temperature (°C)	60–65
Outlet air temperature (°C)	40–45
Suspension spraying time (min)	45–50
Suspension spray rate (g/min)	5–7
Drying in the equipment after layering (min)	10
Final drying in an oven	24 h at 40 °C
Yield calculated after processing (%)	91–94

2.2. Preparation of drug-loaded beads

Drug-loaded beads were prepared by a spray-drying technique. PDS powder was passed through a 177 μm screen and the drug powder was homogeneously dispersed in an aqueous solution of polyvinylpyrrolidone (PVP K 30) while stirring with a magnetic stirrer. The drug dispersion was then sprayed on nonpareil seeds (25–30 mesh) using the Uni-Glatt fluidized bed apparatus (Glatt, Germany) plus a peristaltic pump with a 1 mm nozzle at a feed rate of 5–7 g/min. The spraying process with the drug dispersion was continued to achieve the target drug loading level. The drug-loaded beads were finally dried in an oven at 40 °C for 24 h and the particle size fraction of 0.84–1.00 mm was used for the further coating with acrylic polymers. The composition of the drug-loaded beads and the other processing parameters utilized for the spray-drying method are listed in Tables 1 and 2, respectively.

2.3. Preparation of spraying dispersions for coating

2.3.1. Eudragit® RS/RL dispersion

The coating formula contained methylparaben at 20%, by weight of dry polymer as the plasticizer and talc at 20% level, by weight of polymer dispersion as the glidant (Table 3). The Eudragit® RS and RL dispersions were mixed at 80:20 ratio, and methylparaben was added to the mixture. The polymers were stirred with the plas-

Table 3
Coating dispersion composition for the innermost hydrophobic Eudragit® RS/RL coating layer[a].

Ingredients	Amount (g)	Dry substance (g)
Eudragit® RS30D	133.3	40
Eudragit® RL30D	33.3	10
Methyl Paraben	10	10
Talc	33.3	33.3
Water	467	–
Total	676.9	93.3

[a] Amount of beads to be coated: 500 g; solids content of spraying suspension: 13.8%; polymer applied: 10% (weight gain).

Table 4
Coating dispersion composition for the middle release-triggering coating layer[a].

Ingredients	Amount (g)	Dry substance (g)
Eudragit® RS30D	200	60
Eudragit® RL30D	50.3	15
Methyl Paraben	15	15
Chitosan	7.5	7.5
Succinic acid	1.5% (w/v) solution (7.5 g in 500 ml)	7.5
Talc	50	50
Water	293	–
Total	1123	155

[a] Amount of beads to be coated: 500 g; solids content of spraying suspension: 13.8%; Eudragit® polymers applied: 15% (weight gain).

Table 5
Coating dispersion composition for the outermost enteric coating layer with Eudragit® L100[a].

Ingredients	Amount (g)	Dry substance (g)
Eudragit® L100	100	100
KOH, 1N solution	34	–
Triethyl citrate	50	50
Talc	50	50
Water	1230	–
Total	1464	200

[a] Amount of beads to be coated: 500 g; solids content of spraying suspension: 16%; polymer applied: 20% (weight gain).

ticizer for 16 h, thereafter the talc, after sieving through 177 μm screen, was added, and the dispersion was blended for another 30 min.

The plasticizer was used at 20% level by weight of dry polymer, as Eudragit® RS and RL are hard polymers and hence, require higher amounts of plasticizer for elastic films. Methylparaben was selected as it has been reported to cause greater lowering of Tg of the Eudragit® RS polymer compared to conventional plasticizers (Wu and McGinity, 1999).

2.3.2. Eudragit® RS/RL–chitosan–organic acid dispersion

The method of preparation for the mixed layer dispersion was similar to that for the Eudragit® RS/RL dispersion, except that chitosan dissolved in succinic acid solution in water was added to the Eudragit® RS/RL dispersion before plasticization for 16 h. Talc was added as a last step. The composition of the dispersion is shown in Table 4, respectively.

2.3.3. Eudragit® L100 dispersion

Eudragit® L100 polymer was first dispersed in water, and then potassium hydroxide solution was added dropwise to the polymer dispersion. Triethyl citrate was mixed and stirred with the polymer for 2 h and finally talc, sieved through 177 μm screen, was added and the blending continued for another 30 min. The composition of the enteric polymer dispersion is shown in Table 5, respectively.

2.4. Coating of beads

For the inner coat, the beads were coated in the Uni-Glatt fluidized bed apparatus, and in-process samples were taken to check if the target polymer weight gain was achieved. Coating was continued until complete polymer weight gain was achieved. After the coating, the beads were gently fluidized for about 10 min after which they were cured at 40 °C for 24 h. For the middle coat, the cured beads coated with the inner layer were further coated in the Uni-Glatt apparatus. Again, in-process samples were taken to check for the target weight gain. After the coating, the beads were gently fluidized for 10 min thereafter they were cured in an oven for 24 h at 40 °C. The double-layer coated cured beads were then taken for the final enteric coating, and after the coating, the beads were dried by gentle fluidization in the Uni-Glatt apparatus for 15 min and then, cured for 24 h at 40 °C. The processing parameters used in the fluidized-bed coating procedures are listed in Table 6.

2.5. Product yield and drug loading efficiency

Product yield (%) was calculated by dividing the actual weight of drug-loaded beads after drying with the sum charge load of nonpareils, the solids in the spraying suspension, and multiplying by 100.

Drug loading efficiency was calculated by dividing the actual drug content with theoretical drug content and multiplying by 100. The actual drug content was determined by assay of the drug in beads. Theoretical drug content was calculated by dividing the amount of drug present in the layering suspension with the total of charge load of nonpareils and the amount of drug layering suspension (solids) used. The drug assay determination involved shaking about 200 mg beads with 100 ml of methanol for at least an hour, followed by centrifugation at 2500 rpm for 10 min The supernatant was taken and diluted with the mobile phase, before analysis by high-performance liquid chromatography (HPLC). The HPLC conditions applied are given in Section 2.6.

2.6. Dissolution studies

Dissolution studies were carried out using USP type I method (Basket method, 100 rpm, 37 °C). A sample of 584.5 mg beads, equivalent to 40 mg PDS dose, was taken in 600 ml dissolution medium. As artificial gastric fluid, 0.1N hydrochloric acid (pH 1.2) was used. The artificial intestinal fluid was prepared with 0.05 M potassium phosphate buffer (pH 6.8). Acetate buffer solution with pH 4.0 was used as the artificial colonic fluid. The weak acidic solution was chosen since it would dissolve chitosan readily and would thus simulate the in vivo conditions of chitosan degradation in the colon. Besides, as mentioned earlier, the pH of the colon is more acidic than that of small intestine, especially during chronic colon disease. A β-glucosidase preparation, reported to degrade chitosan, was dissolved in pH 6.0 phosphate buffer and also used as

Table 6
Processing parameters used for the triple-layer coating.

Parameter	Hydrophobic layer (I)	Release triggering layer (II)	Enteric layer (III)
Batch size (g)	500	500	500
Nozzle bore (mm)	1	1	1
Atomising pressure (bar)	2.5	2.5	1.3
Inlet air temperature (°C)	48–50 °C	50–55 °C	45–50
Outlet air temperature (°C)	32–34 °C	34–36 °C	30–32
Spray rate (g/min)	7–10	7	5
Drying in the equipment after coating (min)	5	10	15
Final drying in oven	24 h, 40 °C	24 h, 40 °C	24 h, 40 °C
Yield calculated after processing (%)	80–86	80–86	75–80

artificial colonic medium (Zhang and Neau, 2001). For continuous gradient dissolution, the medium pH was switched by replacing the previous buffer with a preheated next pH buffer solution. PDS concentrations in samples were determined using HPLC. The analysis was carried out on a reversed phase Symmetry® C18 column (3.9 mm × 150 mm; 5 μm); the mobile phase consisting of 60:40 methanol:water was run at a flow rate of 1 ml/min The UV detection wavelength was 254 nm. All the experiments were carried out at least in triplicate.

2.7. Water uptake studies

The water uptake studies were carried out on the double-layer coated beads, i.e. beads with the inner hydrophobic layer and the outer drug release-triggering layer. The coated beads were accurately weighed (approximately 1 g) and immersed in the artificial colonic medium (pH 4) in USP apparatus I, with the stirring speed at 100 rpm. The conditions for the water uptake studies were kept the same as for the dissolution study. At predetermined time intervals, the beads were removed from the release medium, washed twice with distilled water in order to remove the buffer solution from the surface of the beads and then blotted with lint-free tissue paper. The weight of the beads was recorded before and after drying to constant weight in an oven at 50 °C. The water uptake was calculated as follows: water uptake = $(W(t) - W(d))/W(d)$, where $W(t)$ is the weight of the wet beads removed at time t and $W(d)$ is the weight of the beads after drying at time t. The water uptake data are represented as g water/g bead ($n = 3$).

2.8. In vivo studies

Male, Sprague–Dawley rats (400–450 g) were fasted for 16 h (with free access to water), and the dose (10 mg/kg PDS) administered orally in a no. 9 rat capsule via a special capsule-delivering device. The animal was placed in the unrestrained cage during the entire study. Blood samples (0.5 ml) were collected through the tail vein at designated time intervals. The blood samples were prevented from clotting by adding EDTA, and then, centrifuged at 5800 rpm for 15 min and plasma separated out and stored at −20 °C, until analysis.

2.9. Assay method

The PDS concentration in plasma was determined according to the HPLC method reported by Majid et al. (2001). Betamethasone methanolic solution, as internal standard (0.1 ml; 2.5 mg/l), 5% phosphoric acid (0.1 ml) and ethyl acetate/tertiary methyl butyl ether (1:1, v/v; 3 ml) was added to 0.2 ml of plasma. The tubes containing the same were tightly capped and vigorously mixed on a wrist action shaker for 60 min before being centrifuged at 1000 rpm for 10 min. The upper organic layer was then aspirated and transferred to new tubes, and washed with 250 μl 0.1 M NaOH on the wrist action shaker for 15 min followed by centrifugation at 2500 rpm for 10 min. The organic layer was aspirated once again and transferred to glass culture tubes and evaporated to dryness under vacuum at room temperature.

The residue was reconstituted with 200 μl 16% isopropanol in water, vortexed twice for 10 s with a 5-min interval, followed by centrifugation at 5800 × g for 5 min. A 100 μl aliquot of the reconstituted solution was then injected onto the column. The mobile phase used was 16% isopropanol in water containing 0.1% trifluoroacetic acid set at a flow rate of 1.2 ml/min. The HPLC set up consisted of Waters™ system equipped with a Waters™ multisolvent delivery system, a 717 plus autosampler, a 996 photodiode array detector and Empower data management system. Separation was achieved using a Supelcosil® LC-18-DB, 5 μm, 150 mm × 4.6 mm attached to Newguard RP-8, 7 μm, 15 mm × 3.2 mm.

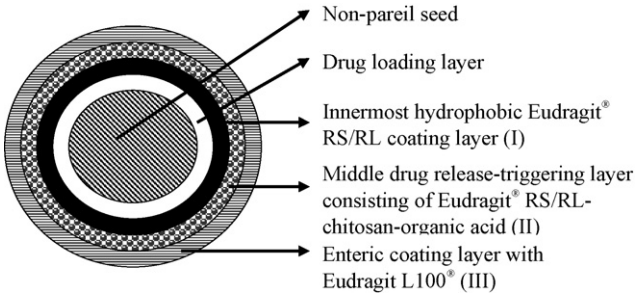

Fig. 1. Schematic representation of a new chitosan–organic acid triggered multiparticulate system for colon-specific drug delivery.

3. Results and discussion

3.1. Fundamental structure of the triple-layer coated beads

The basic structure of the triple-layer film-coated beads has been schematically shown in Fig. 1. The drug-loaded nonpareil beads were coated with three types of polymeric layers successively, using an aqueous coating technique. The innermost layer (I) was the hydrophobic layer consisting primarily of the water-insoluble Eudragit® RS/RL polymers. The purpose of this layer was to act as barrier to any premature drug release from the delivery system prior to reaching the colon. It was expected to be a reasonably hydrophobic layer with drug release being controlled by the thickness of the layer. The middle layer (II) was the combination layer consisting of Eudragit® RS/RL–chitosan–organic acid in the ratio, 0.84:0.08:0.08. The organic acid was expected to remain incorporated in the chitosan–Eudragit® RS/RL combination layer, where it would be held by electrostatic interactions between the numerous amino groups present in the chitosan structure, as well as with the quaternary ammonium groups present in the Eudragit® RS/RL structure and carboxyl groups of organic acid. The dissolution or swelling of the polysaccharide, chitosan in the acidic colonic fluid, would increase the interaction of the organic acid with the underlying polyacrylic film layer of Eudragit® RS/RL, leading to increase in the permeability of the polymeric film and ultimately facilitating drug release. The outermost coating layer (III) was prepared with the enteric polymer, Eudragit® L100 in order to protect the chitosan present in the release-triggering layer from getting dissolved in the acidic stomach. In the entire coating procedures, the prednisolone loading process had an efficiency of approximately 84%. Product yield of the coated beads ranged from 77% to 87%. The loss of the coated product occurred due to the formation of some agglomerates and fines in the product bed, and the loss of coating solids to exhaust.

3.2. Effect of pH on the drug release from the double-layer coated beads

In order to assess the influence of the two coating layers I and II on the release characteristics of PDS, the drug loaded nonpareil beads were coated with the innermost hydrophobic Eudragit® RS/RL (80/20) at a 10% coating level and the middle drug release triggering coating layer consisting of Eudragit® RS/RL (80/20)–chitosan–succinic acid at a 15% coating level using a Uni-Glatt apparatus. From the recent reports in the literature, it is known that the pH of the colon is in fact lower than that of the small intestine due to the acidification of colonic contents by the prod-

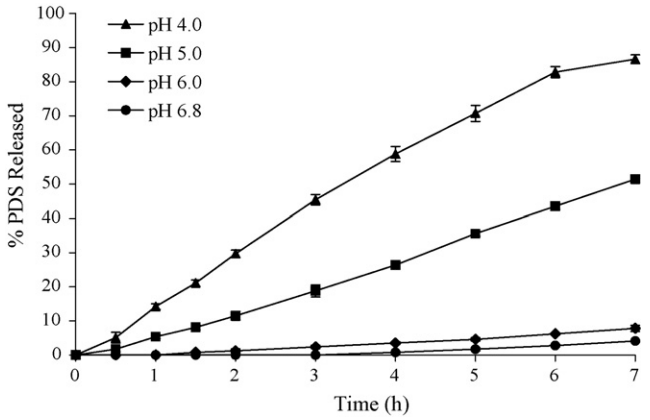

Fig. 2. Influence of pH of dissolution medium on the PDS release (mean ± S.D., $n = 3$) at 37 °C from the drug loaded beads coated with innermost hydrophobic Eudragit® RS/RL (80/20) at a 10% coating level and the middle drug release triggering layer consisting of chitosan–succinic acid–Eudragit® RS/RL (80/20) at a 15% coating level.

ucts of bacterial fermentation (Tozaki et al., 1997; Pye et al., 1990). In case of severe inflammatory bowel disease, the colonic pH often drops down to between 1 and 5 (Evans et al., 1988). Another study showed that the colonic pH went down to 4.7 ± 0.7 in a group of untreated ulcerative colitis and to pH 5.5 ± 0.4 in a group of treated patients (Watts and Illum, 1997). In yet another study, pH values as low as 2–3 were measured in some of the ulcerative colitis patients (Fallingborg et al., 1993). Hence, from point of view of simplification of in vitro evaluation, the in vitro release profiles were determined on the two-layer coated system in the dissolution media with the pH range from colonic pH 4.0 to small intestinal pH 6.8 at 37 °C. As shown in Fig. 2, the dissolution profiles of PDS appear to follow the pseudo zero-order kinetics when determined over the initial 6 and 7 h time period, in which the drug release rates were gradually and appreciably increased with decreasing the pH from 6.8 to 4.0. That is, negligible drug release was observed in the artificial small-intestinal fluid (pH 6.8), whereas in the pathological colonic fluid of pH 4.0, the drug release was markedly enhanced with over 80% drug released in 6 h as compared with only 3% release observed in the small intestinal pH of 6.8 within the same time period.

In order to explain this type of pH-dependent release behavior, it is important to note that chitosan solubility is greatly enhanced at acidic pH. It is known that an acidic medium can cause the dissolution of chitosan, especially if the chitosan is of low molecular weight (Felt et al., 1999). The chitosan solubility increased as the pH of the dissolution medium decreases and a concomitant increase in drug release was observed. It appears that the events taking place in the artificial colon medium (pH 4.0) are leading to greater interaction of the succinic acid with the hydrophobic cationic Eudragit® RS/RL polymer. At pH 4.0, the chitosan dissolved in the dissolution medium, and thus, it allowed the organic acid, which was electrostatically localized with the chitosan in the combination layer, to interact efficiently with the underlying Eudragit® polymeric layer. The interaction of organic acids with the Eudragit® RS polymer has been previously reported (Armand et al., 1987; Jenquin et al., 1990). The organic acids can interact with the quaternary ammonium groups of the Eudragit® RS/RL polymer, resulting in ionic osmosis. Besides, the undissociated organic acid can localize in the hydrophobic parts of the polymer and cause an increase in polymer flexibility, resulting in increased hydration of the polymer. In order to confirm the role played by the organic acid, a control double-layer coated formulation was prepared without the organic acid. In this case, at pH 4.0, the drug release was considerably slower with only approximately 20% being released over a 6 h period.

Thus, it appears that the drug release is being triggered by the dissolution of chitosan and the latter is likely to be dissolved in the colonic fluid, either due to the presence of an acidic pH, as in colonic disease or due to the effect of the bacterial enzymes (Tozaki et al., 1997; Zhang and Neau, 2002). However, the acidic environment of the stomach may dissolve the chitosan, and hence, in order to prevent the breakdown of the delivery system early in the passage through the gastrointestinal tract, an outermost enteric coat is essential. Hence, a triple-layer coated delivery system with an outer enteric coat is likely to meet the colon-targeting characteristics.

3.3. Effect of coating level of middle release-triggering layer on drug release from double-layer coated beads

Keeping the hydrophobic layer (I) thickness at 10% coating level, the middle combination layer (II) was studied at 3 coating levels (10%, 15% and 20%) in the double-layer coated delivery system. As can be seen in Fig. 3, the drug release rate in terms of zero-order kinetics increased appreciably (by approximately 11%) with increase in the coating level of the combination layer from 10% to 15%; thereafter, the drug release rate leveled off at 20% coating level. Thus, increase in the total thickness of the coating barrier that had to be traversed by the drug molecules in the drug release process balanced the effect of the increased level of succinic acid on the swelling of the Eudragit® polymer with increase in the thickness of the combination layer. As mentioned earlier, the organic acid facilitates rapid drug release from the delivery system, and hence, an increase in the amount of succinic acid present in the system is expected to lead to an increase in the drug release rate. But the study reveals that the barrier effects of an increase in the polymer layer thickness that resulted with increased coating levels could counterbalance these effects of the organic acid.

3.4. Water uptake by double-layer coated beads

In order to characterize the hydration phase of the double-layer coated beads, with the innermost Eudragit® RS/RL layer and the middle combination layer containing chitosan, organic acid and Eudragit® RS/RL, when exposed to the artificial colonic medium, water uptake studies were carried out on double-layer coated beads as a function of time. As seen in Fig. 4, a good linear correlation was attained between % hydration and % drug released with a correla-

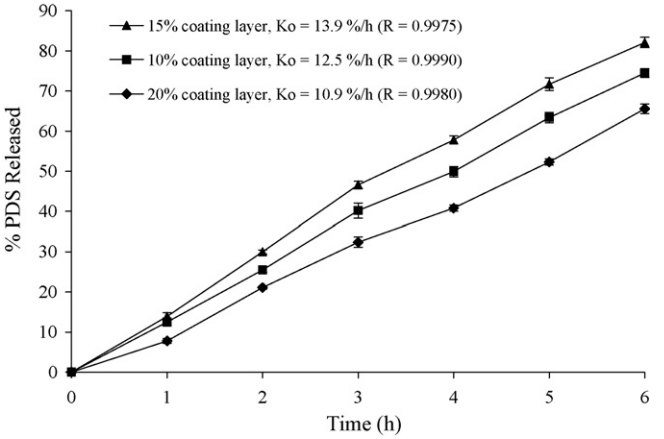

Fig. 3. Influence of the coating level of the middle drug release triggering layer on the PDS release (mean ± S.D., $n = 3$) in artificial colonic fluid (pH 4.0) at 37 °C from the drug loaded beads coated with the innermost hydrophobic Eudragit® RS/RL (80/20) at 10% coating level and various coating levels of the middle coating layer consisting of chitosan–succinic acid–Eudragit® RS/RL.

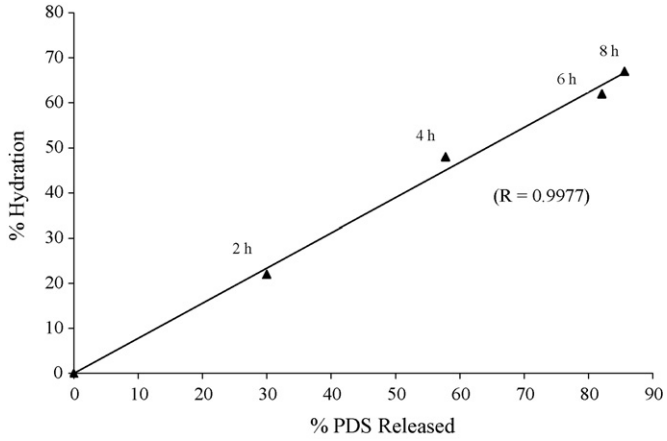

Fig. 4. Correlation between the degree of hydration (%) and % drug released from the PDS beads coated with the innermost hydrophobic Eudragit® RS/RL layer at 10% coating level, and the middle drug release triggering layer of chitosan–Eudragit® RS/RL with succinic acid at 15% coating level, exposed to the artificial colonic fluid (pH 4.0) at 37 °C.

tion coefficient of 0.9973, indicating thereby that the drug release was related to the degree of hydration of the polymeric coating film. The two-layer coated beads without succinic acid did not show any swelling, confirming the role of the organic acid in increasing the hydration of the system during dissolution.

Apparently, the diffusion of dissolution medium and hydration of cationic acrylic polymer coating precedes drug release through hydrated polymer film (Flory, 1953). The Eudragit® RS polymer can be considered similar to an ion exchange resin where ions are bound to an insoluble cross-linked polymer resin carrying oppositely charged functional groups, in this case, quaternary ammonium groups (Okor, 1982). The anionic counterions of quaternary ammonium groups are chloride ions. The degree of hydration and swelling of the resins is affected by the interaction between the cationic groups and the counterions. A stronger interaction will lead to a lesser degree of hydration or swelling and a slower drug release is expected (Helfferich, 1962). Thus, it appears that the interaction of the quaternary ammonium groups of the Eudragit® polymer with the organic acid, compared to that with the chloride ions, is leading to increased hydration of the polymer.

3.5. Effect of type of organic acid incorporated in combination layer on drug release from double-layer coated beads

Table 7 shows the relationship between the pKa_1 values of the di- and tri-carboxylic acids and the drug release rates obtained from the double-layer coated system in artificial colonic fluid of pH 4.0. In this case, the drug loaded nonpareil beads were coated with the innermost hydrophobic Eudragit® RS/RL (80/20) layer at a 10% coating level and the middle release-triggering coating layer comprising chitosan, organic acid and Eudragit® RS/RL (80/20) at a 15% coating level. As shown in Table 7, while the use of succinic acid (pKa_1 4.17) in the combination layer leads to more than 80% drug being released within 6 h, the use of other organic acids with greater acidity such as tartaric acid (pKa_1 3.03), malic acid (pKa_1 3.40), and citric acid (pKa_1 3.13) lead to approximately 2.0–3.3-fold decrease in the drug release rate in artificial colon fluid, pH 4.0. It appears that increase in the acidity of the organic acid is leading to a reduced release-enhancing effect of the organic acid. The study clearly shows that the drug release rate increases significantly with increasing p*K*a from 3.03 to 4.17. In order to explain this effect, one has to look at the mechanism of hydration of the acrylic polymer. As mentioned above, the degree of hydration and swelling of the Eudragit® polymer depends on the interaction between its quaternary ammonium groups and the organic acid counterions. The concentration of reactable anions decreases with increasing p*K*a. If the electrostatic interactions were the major mechanism of the release-enhancing effect, then the drug release rate should have decreased with increasing p*K*a. Hence, it appears that the electrostatic interaction of the organic acid with the cationic groups of the polymer is not necessarily the only factor responsible for the increase in drug release rate. Of the other possible factors, it is important to look at the role played by the undissociated form of the organic acid by distributing to the hydrophobic segment of the Eudragit® RS/RL film. Partitioning of the undissociated compound into the matrix phase of the polymer film can cause an increase in the film flexibility. The relationship between film flexibility and hydration is supported by well-known effects of cross-linking, in which a decrease in film flexibility resulted in a decrease in film hydration (Yasuda and Lamaze, 1971). Thus, the order of drug release rates obtained with various organic acids may be explained better by the apparent role of their lipophilic undissociated forms.

Table 7
Release rates of PDS obtained from the double-layer coated drug loaded beads containing different organic acids in the middle release-triggering layer in artificial colonic fluid (pH 4) at 37 °C[a].

Organic acid	pKa_1	% PDS released at 6 h
Succinic acid	4.17	82.1 ± 1.3
Tartaric acid	3.03	45.6 ± 1.1
Malic acid	3.40	36.5 ± 0.8
Citric acid	3.13	30.7 ± 1.3

[a] Each value represents the mean ± S.D. (n = 3).

3.6. Enteric coating

The objective of applying an enteric coat was to achieve acid resistance to the drug release in the stomach and at the same time the enteric coat should dissolve rapidly in the small intestine, much before the delivery system reaches the colon. Hence, after initial screening, Eudragit® L100, known to dissolve above pH 6.0, was selected. The enteric polymer functionality was studied using different concentrations. A 20% coating level of the Eudragit® L100 provided acid resistance with no drug released for the first 2 h. The lag time for drug release in the simulated gastric conditions of dissolution increased with increasing coating levels of the enteric polymer. However, the 20% coating level was selected as the outermost coat to be used in the final formulation, as it dissolved rapidly under small-intestinal conditions. A delay in the dissolution of the enteric polymer after passing the acidic gastric environment would critically affect the delivery system performance in the colon. The enteric polymer was used as the outermost coat in the triple-layer coated delivery system.

3.7. In vitro evaluation of triple-layer coated colon-targeted delivery system under simulated gastrointestinal conditions

Fig. 5 shows the results of continuous dissolution of PDS from the triple-layer coated colon targeted delivery system (CTDS) in artificial gastric fluid (pH 1.2) for 2 h followed by artificial small-intestinal fluid (pH 6.8) and then, artificial colon fluid (pH 4.0 and 6.0) with or without β-glucosidase. The PDS colon-targeted delivery system was prepared by sequential coating of drug loaded nonpareil beads with the innermost hydrophobic Eudragit® RS/RL: (80/20) at a 10% coating level, with the middle drug release triggering layer comprising chitosan and Eudragit® RS/RL (80/20) with and without succinic acid at a 15% coating level, and with the outer-

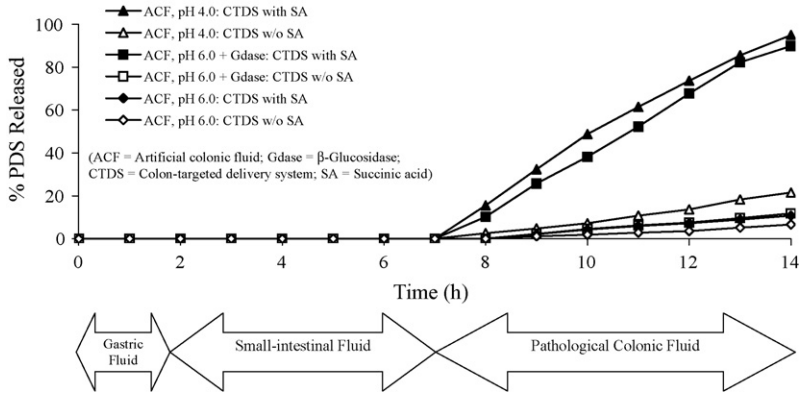

Fig. 5. Continuous release profiles of PDS from the triple-layer coated CTDS beads in artificial gastric fluid (pH 1.2), artificial small-intestinal fluid (pH 6.8) and in artificial colonic fluid (pH 4 and 6) in the present or absence of β-glucosidase at 37 °C.

most enteric coating layer of Eudragit® L100 at a 20% coating level. As shown in Fig. 5, no drug release occurred from the CTDS when exposed to artificial gastric fluid (pH 1.2) for 2 h and after replacement of the dissolution medium to artificial small-intestinal fluid (pH 6.8) for 5 h, the drug release was also practically prevented. Thereafter, when the dissolution medium was changed to the artificial colonic fluids (pH 4 or 6), the drug release occurred in which the rate of drug release was significantly dependent on the presence or absence of succinic acid incorporated into the middle triggering coating layer and pH of dissolution medium with or without β-glucosidase. At the pH 4 colonic condition, the succinic acid containing CTDS (80% released at 6 h) provided a markedly enhanced rate of drug release as compared with the system without succinic acid (10% released at 6 h), thus emphasizing the critical role played by succinic acid in accelerating drug release in the colonic fluids. In contrast, at the pH 6 colonic condition without β-glucosidase, there was only minimal difference in the release rate from the delivery systems with or without succinic acid. However, under the same colonic pH 6 condition with β-glucosidase, the CTDS containing succinic acid showed an appreciably higher rate of drug release when compared with that obtained from the system without succinic acid as observed in the release patterns in the pH 4 colonic fluid. This is apparently because the chitosan incorporated into the triggering layer was not readily dissolved by the aqueous buffer medium of pH 6 whereas the polysaccharide dissolves rapidly in the colonic medium in the presence of β-glucosidase, triggering the mobilization of succinic acid into the underlying hydrophobic layer, eventually facilitating the drug release.

Hence, based on the in vitro results thus obtained, it appears that this triple-layer coated system has met the objectives of colon-specific delivery, mainly, (i) prevention of premature drug release before the delivery system reaches colon on one hand, and (ii) reasonably rapid drug release in the colonic fluids with or without β-glucosidase on the other. In the first 2 h of artificial gastric fluid, the enteric coat was responsible for protecting the delivery system against breakdown by preventing the passage of fluid into it. For the next 5 h, in the artificial small-intestinal fluid, apparently, the enteric coat dissolved; however, no drug release occurred since the chitosan was insoluble at this pH, keeping the succinic acid electrostatically bound in the combination layer itself. The inner hydrophobic layer remained undisturbed and acted as the barrier to drug leaching. In artificial colon fluids, the chitosan dissolved and the succinic acid could interact with the underlying Eudragit® layer and increase its permeability leading to drug release. The results indicate that the bacterial enzymes of the colon should be as effective as acidic pH (during colonic disease) in facilitating drug release from the delivery system.

3.8. In vivo evaluation of triple-layer coated colon-targeted delivery system

The in vivo pharmacokinetic study was performed to see if the colon-targeted release functions of the colon-targeted delivery system (CTDS) could work in the gastrointestinal tract as expected. To minimize the variation of gastric pH, fasted male, Sprague–Dawley rats were used for this study. The average pH in rat stomach in fasted state has been reported to be about 3.9 and the mean intestinal pH to be less than pH 6.6 (McConnell et al., 2008). The enteric polymer used in the delivery system dissolves at and above pH 6.0. Hence, the pH conditions in the rat stomach were not expected to cause the breakdown of the outermost enteric coat. Also, the pH conditions in the small intestine were expected to facilitate latter's dissolution. Finally, the presence of colonic microflora in the relatively acidic conditions of the large intestine was expected to trigger the release of drug from the delivery system.

The PDS-loaded CTDS and enteric-coated delivery system (ECDS), and PDS powder dispersion were orally administered to the Sprague–Dawley rats under fasted condition. Fig. 6 shows the plasma concentration vs. time profiles of prednisolone after the administration. Pharmacokinetic parameters necessary for discussion, calculated from the plasma drug concentration vs. time profiles using WinNonlin software, are listed in Table 8. When the

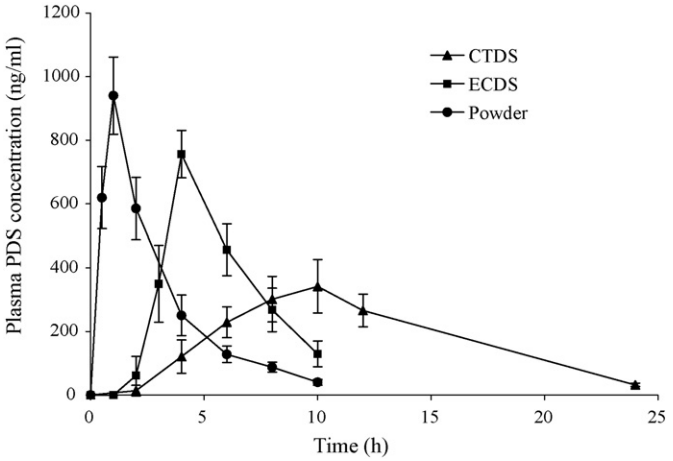

Fig. 6. Mean plasma concentration–time profiles of PDS obtained after peroral administration of the triple-layer coated multiparticulate colon-targeted delivery system (CTDS), simple enteric-coated delivery system (ECDS) and powder dispersion in male Sprague–Dawley rats at a dose of 10 mg/kg. Data shown as mean plasma concentration, and error bars represent the standard deviation ($n = 9$).

Table 8
Pharmacokinetic parameters obtained after peroral administration of three different formulations of PDS in rats at a dose of 10 mg/kg.

PK parameter	PDS formulations[a]		
	CTDS[b]	ECDS[c]	Powder dispersion
$AUC_{0-\infty}$ (ng h/ml)	4250.8 ± 447.3[e]	3548.4 ± 613.5[f]	2998.8 ± 235.7[g]
C_{max} (ng/ml)	363.2 ± 21.2	756.0 ± 26.5	940.4 ± 42.7
T_{max} (h)	9.3 ± 0.4	4.0 ± 0.0	1.0 ± 0.0
T_{lag} (h)	~2[d]	~1[d]	–

$P < 0.001$ (e vs. f; e vs. g).
[a] Each value represents the mean ± S.D. ($n = 9$).
[b] Colon-targeted delivery system.
[c] Enteric-coated delivery system.
[d] Graphically determined by extrapolating the initial linear portion of plasma drug concentration vs. time data to the *X*-axis.

PDS powder was administered, the peak plasma concentration was achieved within 1 h of administration, showing thereby that prednisolone was immediately absorbed from the rat gastrointestinal tract. The time of onset of drug release can therefore be considered close to the time of appearance of drug in the plasma.

A lag time of ~2 h was obtained before plasma concentration could be detected after administration of CTDS. Administration of the ECDS resulted in onset of drug absorption, as manifest in the appearance of drug in the plasma, at ~1 h. From in vitro results, it was expected that the ECDS started releasing the drug after passing the stomach intact. Hence, the time of onset of drug absorption after administration of the ECDS, which is about 1 h, should be approximately the same as the gastric emptying time. The fact that with CTDS, the lag time was greater shows that with latter, the onset of drug release was later than that with ECDS. The transit time of pellets in rats through stomach has been reported to be 1–2 h, and through small intestine to be ~2 h (Tuleu et al., 1999). Thus, the CTDS beads should be able to reach the large intestine in approximately 2–4 h. The present in vivo study suggests based on the observed lag time difference between the ECDS and the CTDS that the CTDS probably began releasing drug at the distal end of small intestine and majority of drug release took place in the large intestine of the rat.

There was significant difference found between the AUC obtained from the CTDS and that from either the ECDS or the powder ($p < 0.001$). Thus, it appears that PDS may be more efficiently absorbed from the large intestine of the rat. As shown in Table 8, considerable differences were also found in other pharmacokinetic parameters. The T_{max} of ECDS was 4 h, whereas that of CTDS extended up to about 9 h. The C_{max} of CTDS was 363.2 ± 21.2 ng/ml, which was almost the half of that of the ECDS. These results imply that the CTDS took a longer time for drug absorption in the GIT, as is expected from in vitro results where the drug was found to be released in a controlled manner. The slower and delayed absorption of PDS from CTDS also showed that the delivery system released PDS only at the lower part of the rat intestine. Since the extent of absorption of PDS, as measured by the AUC, from the CTDS was greater than that from the control formulations, it is obvious that the in vivo conditions in the rat large intestine were able to trigger the release of PDS from the CTDS beads.

It is concluded from the in vivo study that the colon-targeted coated beads did not release any significant amount of drug while passing through the stomach and the small intestine, and when the beads reached the large intestine, chitosan in the release triggering layer was degraded by the bacterial enzymes in the cecum and colon of the rat, facilitating the chitosan/organic acid triggered drug release.

4. Conclusion

A new dosage form intended for specific delivery to the colon was developed. The system is a triple-layer coated multi-unit dosage form, consisting of inner hydrophobic layer, middle, chitosan/succinic acid containing release triggering layer and outer enteric layer. The drug release in the colon could be controlled by manipulation of the coating level of the middle combination layer. In order to protect the delivery system in the acidic gastric conditions, an outer enteric coat was deposited. When the resultant coated beads reached the large intestine of male, Sprague–Dawley rats in 2–4 h after oral administration, the drug release was triggered from the beads in the colonic fluids due to dissolution and degradation of chitosan by the colonic enzymes. The drug release rate was successfully enhanced in colonic conditions by the inclusion of organic acid in the middle polymeric layer of the triple-layer coated delivery system. Thus, a novel way of targeting drug release to the colon was devised based on a combination delivery system design.

References

Armand, J.Y., Magnard, F., Bozon, J., Rollet, J., Taverdet, J.L., Vergnaud, J.M., 1987. Modelling of drug release in gastric liquid from galenic forms with Eudragit matrix. Int. J. Pharm. 40, 33–41.

Ashford, M., Fell, J.T., Attwood, D., Sharma, H., Woodhead, P.J., 1993. An in vivo investigation into the suitability of pH dependent polymers for colonic targeting. Int. J. Pharm. 95, 193–199.

Brondsted, H., Kopecek, J., 1992. Hydrogels for site-specific drug delivery to the colon: in vitro and in vivo degradation. Pharm. Res. 9, 1540–1545.

Chen, R.H., Lin, J.H., Yang, M.H., 1994. Relationships between the chain flexibilities of chitosan molecules and the physical properties of their casted films. Carbohydr. Polym. 24, 41–46.

Davis, S.S., Robertson, C., Wilding, I.R., 1991. Gastrointestinal transit of a multiparticulate tablet formulation in patients with active ulcerative colitis. Int. J. Pharm. 68, 199–204.

Evans, D.F., Pye, G., Branley, R., Clark, A.G., Dyson, T.J., Hardcastle, J.D., 1988. Measurement of gastrointestinal pH profiles in normal ambulant human subjects. Gut 29, 1035–1041.

Fallingborg, J., Christensen, L.A., Jacobson, B.A., Rasmussen, S.N., 1993. Very low intraluminal colonic pH in patients with active ulcerative colitis. Dig. Dis. Sci. 38, 1989–1993.

Felt, O., Furrer, P., Mayer, J.M., Plazonnet, B., Buri, P., Gurny, R., 1999. Topical use of chitosan in ophthalmology: tolerance assessment and evaluation of precorneal retention. Int. J. Pharm. 180, 185–193.

Flory, P.J. (Ed.), 1953. Principles of Polymer Chemistry. Cornell University, Ithaca, New York.

Gorbach, S.L., 1971. Intestinal flora. Gastroenterology 60, 1110–1129.

Helfferich, F., 1962. Ion Exchange. McGraw-Hill Book Co., New York, pp. 168.

Jenquin, M.R., Liebowitz, S.M., Sarabia, R.E., McGinity, J.M., 1990. Physical and chemical factors influencing the release of drugs from acrylic resin films. J. Pharm. Sci. 9, 811–816.

Kopeckova, P., Rathi, R., Takada, S., Rihova, B., Berenson, M.M., Kopecek, J., 1994. Bioadhesive *N*-(2-hydroxypropyl)methacrylamide copolymers for colon-specific drug delivery. J. Control. Release 28, 211–222.

Lehmann, K.O.R., 1997. Chemistry and application properties of polymethacrylate coating systems. In: McGinity, J.W. (Ed.), Aqueous Polymeric Coatings for Pharmaceutical Dosage Forms. Marcel Dekker, New York, pp. 1–76.

Majid, O., Akhlaghi, F., Lee, T., Holt, D.W., Trull, A., 2001. Simultaneous determination of plasma prednisolone, prednisone, and cortisol levels by high-performance liquid chromatography. Ther. Drug Monit. 23, 163–168.

McConnell, E.L., Basit, A.W., Murdan, S., 2008. Measurements of rat and mouse gastrointestinal pH, fluid and lymphoid tissue, and implications for in vivo experiments. J. Pharm. Pharmacol. 60, 63–70.

Okor, R.S., 1982. Effect of polymer cation content on certain film properties. J. Pharm. Pharmacol. 34, 83–86.

Perkins, A.C., 1999. Regional differences in quinine absorption from the undisturbed human colon assessed using a timed release delivery system. Pharm. Res. 16, 1087–1092.

Press, A.G., Hauptmann, I.A., Hauptmann, L., Fuchs, B., Fuchs, M., Ewe, K., Ramadore, G., 1988. Gastrointestinal pH profiles in patients with inflammatory bowel disease. Aliment. Pharmacol. Ther. 12, 673–678.

Pye, G., Evans, D.F., Ledingham, S., Hardcastle, J.D., 1990. Gastrointestinal intraluminal pH in normal subjects and those with colorestal adenoma or carcinoma. Gut 31, 1355–1357.

Roberts, G.A.F., 1992. Structure of chitin and chitosan. In: Roberts, G.A.F. (Ed.), Chitin Chemistry. MacMillan, Houndmills, pp. 1–53.

Sack, D.M., Peppercorn, M.A., 1983. Drug therapy of inflammatory bowel disease. Pharmacotherapy 3, 158–176y.
Tozaki, H., Komoike, J., Tada, C., Maruyama, T., Terabe, A., Suzuki, T., Yamamoto, A., Muranishi, S., 1997. Chitosan capsules for colon-specific drug delivery: improvement of insulin absorption from the rat colon. J. Pharm. Sci. 86, 1016–1021.
Tuleu, C., Andrieux, C., Boy, P., Chaumeil, J.C., 1999. Gastrointestinal transit of pellets in rats: effect of size and density. Int. J. Pharm. 180, 123–131.
Watts, P.J., Illum, L., 1997. Colonic drug delivery. Drug Dev. Ind. Pharm. 23, 893–913.
Wilding, I.R., Davies, S.S., Bakhshaee, M., Stevens, H.N.E., Sparrow, R.A., Brennan, J., 1992. Gastrointestinal transit and systemic absorption of captopril from a pulsed-release formulation. Pharm. Res. 9, 654–657.
Wu, C., McGinity, J.W., 1999. Non-traditional plasticization of polymeric films. Int. J. Pharm. 177, 15–27.
Yasuda, H., Lamaze, C.E., 1971. Permselectivity of solutes in homogeneous water-swollen polymer membranes. J. Macromol. Sci. Phys. B5 11, 111–134.
Zhang, H., Neau, S.H., 2001. In vitro degradation of chitosan by a commercial enzyme preparation: effect of molecular weight and degree of deacetylation. Biomaterials 22, 1653–1658.
Zhang, H., Neau, S.H., 2002. In vitro degradation of chitosan by bacterial enzymes from rat cecal and colonic contents. Biomaterials 23, 2761–2766.