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Research Article

Colon Delivery of Budesonide: Evaluation of Chitosan–Chondroitin Sulfate Interpolymer Complex

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Abstract. The present study was aimed at formulating tablets comprising of coating susceptible to microbial enzyme degradation for releasing budesonide in the colon. Tablets prepared by using Avicel® pH 102 as diluent and Eudragit® L100-55 as binder were coated to a weight gain of 10% *w/w* employing aqueous mixtures containing chitosan (CH) and chondroitin sulfate (CS). The interpolymer complex between CH and CS was characterized using Fourier transform infrared (FTIR) and differential scanning calorimetery (DSC) studies. The tablets were evaluated for release of budesonide through *in vitro in vivo* studies. Formation of bonds between $-COO^-$ and $-OSO_3^-$ groups of CS and $-NH_3^+$ groups of CH was evident in the FTIR spectra of these interpolymer complexed (IPC) films. The DSC thermograms of these films revealed one endothermic transition between 190°C and 205°C, suggesting the formation of new bonds in the IPC. The pH sensitive swelling exhibited by these films was observed to be a function of CH concentration. Tablets coated with aqueous mixtures containing 40:60 or 50:50 ratio of CH/CS totally prevented the release of budesonide in pH 1.2 buffer. The peaks (FTIR) and endothermic transitions (DSC) characteristic of interpolymer complexation were observed to remain unaffected after sequential exposure of the films to pH 1.2 and pH 7.4 buffer IP. This proved the versatility of these IPC films for colon delivery. C_{max} of 1,168.99 and 1,174.2 ng/mL, respectively, at 12 and 8 h post-oral dosing of tablets coated with 40:60 or 50:50 ratio of CH/CS was observed in rats. The aqueous CH/CS (40:60) coating could provide a facile method for delivering budesonide to the colon.

KEY WORDS: budesonide; chitosan; chondroitin sulfate; colon delivery; interpolymer complexation.

INTRODUCTION

Inflammatory bowel disease (IBD) is manifested in the form of localized inflammation of large intestine. The inflammation process is facilitated by defects in both the barrier function of the intestinal epithelium and mucosal immune systems. It may manifest in a variety of forms, the most common being Crohn's disease and ulcerative colitis (1). The treatment comprises of oral administration of anti-inflammatory agents, corticosteroids (2), and/or antibiotics (3). The oral administration of corticosteroids has been effective in patients with active Crohn's disease and ulcerative colitis (4). Budesonide, a nonhalogenated corticosteroid, is highly effective in the treatment of IBD due to its greater topical anti-inflammatory activity than many other glucocorticoids (5). Therefore, a dosage form capable of delivering budesonide to the colon rather than upper GIT can be envisaged to result in high local concentration, thus enhancing the effectiveness of therapy.

Various approaches employed for delivering drugs to the colon include the use of enteric polymers (6), swellable polymers (7), and polysaccharides (8). The pH of colon is lower (6.8) than that of small intestine (7.4) due to secretion of fatty acids (9). Hence, under physiological conditions, the colon release dosage form has to resist drug release at higher pH and subsequently release it at lower pH. Although time-controlled systems have been suggested to satisfy the requirement, the time that dosage form takes to reach the colon is often intractable due to wide variations in gastric emptying time (10). Therefore, dosage forms making use of enzymatically degradable polymers that would release the drug after reaching the colon seem to offer a great promise in this pursuit. These dosage forms can be expected to slowly release budesonide after arrival in the colon due to degradation of the coated polymer by the colonic bacteria. This will provide exposure of the inflamed intestinal mucosa to high local concentrations of budesonide over extended period.

Many researchers have reported the use of natural or modified polysaccharides for sustained or colon delivery of drugs. However, when employed in their putative form, these polysaccharides are required to be used in large quantities (11,12) for achieving colon drug delivery. This is probably due to high solubility of non-cross-linked molecules in the acidic pH.

Natural or modified polysaccharides such as amylase (13), pectin (14), guar gum (15), and konjac glucomannan (16) have been widely investigated for the peroral delivery of drugs to colon. CH carries a net positive charge due to $-NH_3^+$ groups and can be easily cross-linked with other anions, oppositely charged drugs, and polymers (17). CH is easily degraded by lysozyme, by non-specific cellulases, and enzymes secreted by intestinal bacteria (18). CS possesses anionic character due to presence of

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$-COO^-$ and $-OSO_3^-$ groups in its molecular structure. CS also can be degraded by anaerobic bacteria that are resident in the large intestine. However, the highly soluble character of both of these polymers limits their use in their putative form for colon delivery.

In light of the above, it was proposed to formulate tablets of budesonide by coating them with aqueous mixtures containing different ratios of CH–CS in order to yield interpolymer complexed (IPC) film-coated tablets. The characterization of the IPC films was done by Fourier transform infrared (FTIR) and differential scanning calorimetery (DSC). The effect of varying the composition of CH/CS in the coating solution on the *in vitro* release of budesonide from tablets was investigated. Further, the coated budesonide tablets were evaluated for their *in vivo* performance after oral administration to rats.

MATERIALS AND METHODS

Materials

Budesonide was received as a gift sample from Ranbaxy Research Labs, Gurgaon, India. CS (Type C) was received as gift sample from Panacea Biotec, Lalru, India. CH was purchased from Indian Fisheries Institute, Cochin, India. Ammonium acetate and acetic acid were of analytical grade and were purchased from Qualigens Fine Chemicals, India. Acetonitrile and potassium dihydrogen orthophosphate of high-performance liquid chromatography (HPLC) grade were purchased from Merck India, Ltd. All reagents and chemicals were of analytical grade and used as received.

Preparation of CH–CS IPC Films

CH (300 mg) was dissolved in 15-mL solution of 3% *v/v* acetic acid. To this mixture, 8 mL of 5 M ammonium acetate was added. CS (300 mg) was separately dissolved in 7 mL distilled water and slowly added with stirring to CH solution. This mixture was poured in petriplates and dried at 50°C for 48 h. Films with a total polymer content of 2% *w/v* containing 70:30, 60:40, 50:50, 40:60, or 30:70 ratio of CH/CS were prepared using this method. The dried films were stored in a desiccator until use.

Characterization of IPC Films

FTIR Analysis

CH, CH acetate films, CS, and IPC films formed by drying admixtures containing different ratios of CH/CS were subjected to FTIR analysis (Perkin Elmer RXI, USA). The fresh films were sequentially exposed to pH 1.2 buffer IP (19) for 2 h and pH 7.4 buffer IP for 22 h. The exposed films were dried at 50°C for 24 h and subjected to FTIR analysis.

DSC Analysis

CH, CS, and freshly prepared IPC films for FTIR analysis were also subjected to differential scanning calorimetric studies. In addition, a portion of the fresh films was sequentially exposed to pH 1.2 for 2 h and pH 7.4 for 22 h. The sample of each film obtained after each exposure was dried at 50°C for 24 h. These samples were hydrated at 50% RH for 48 h before subjecting to DSC analysis from 0°C to 500°C, employing heating rate of 10°C/min (Mettler Tolelo Star System, Switzerland).

Swelling Index Measurement

The swelling index of the IPC films after exposure to different pH was determined by sequentially immersing the films in pH 1.2 for 2 h and pH 7.4 for 22 h. The swelling index was calculated according to the formula

$$\text{Swelling index} = \frac{W2 - W1}{W1}$$

where W1 is the initial weight of the film, and W2 is the weight of the swollen film. Each experiment was repeated on three films.

Mechanical Properties

The IPC films prepared using different ratios of CH and CS were evaluated for tensile strength and for resilience (Texture Analyser, TAXT, Stable Microsystems, Godalming Surrey, UK). For tensile strength measurements, the pre-test speed was 1.0 mm/s, post-test speed was 10 mm/s, trigger force was 5.0 g, and probe used was A/TG. For resilience measurements, the pre-test speed was 1.0 mm/s, post-test speed was 0.5 mm/s, trigger force was 5.0 g, and probe used was P/5 (5 mm diameter).

Preparation of Core Tablets

Budesonide-loaded tablets. Tablets (average weight 25 mg) containing 3 mg of budesonide were prepared by wet granulation technique. Budesonide and Avicel® pH 102 were granulated using Eudragit® L100-55 (10% *w/w*) as binder employing alcohol as the granulating fluid (20). The granules were passed through #16 and dried at 50±2°C to 2–3% *w/w* residual moisture content. The dried granules were passed through #20 sieve, and fines were retained on #44 sieve; 10% *w/w* of fines was mixed with the granules. Magnesium stearate (1% *w/w*) was added to the granules. Tablets were compressed using biconvex 4-mm punches (D tooling) in a six-station rotary tablet compression machine (A K Industries, M207, Nakodar, India).

Dummy tablets. Dummy tablets (2.5 mm diameter) were compressed (D tooling) using a ten-station rotary compression machine (Clit, CPM, Ahmedabad, India).

Testing of Uncoated tablets

The axial and radial diameters of ten compressed tablets of each batch were determined by using electronic digital vernier calipers.

Hardness of ten tablets was determined with the help of Pfizer hardness tester (Campbell Electronics, Mumbai)

The friability test, weight variation test, and disintegration test were performed in accordance with the method prescribed in USP XXX, NF XXV (21).

Coating of Budesonide Tablets

The solutions used for preparing CH–CS IPC films were prepared freshly for coating budesonide tablets. These solutions containing different CH/CS ratios were coated on a batch comprising of dummy tablets and tablets containing budesonide. Dummy tablets were used along with budesonide-loaded tablets (total tablet load 300 g) for optimizing the tablet load charge in the coating pan. The coating was continued till a weight gain of 10% *w/w* for the budesonide tablets was achieved. The total polymer concentration was kept constant at 2.5% *w/v*. Similarly, a separate batch of compressed tablets was coated with 2.5% *w/v* solution of CH. The coating solution was sprayed at a rate of 5 mL/min with the help of peristaltic pump using a spray gun of 1-mm nozzle (Electrolab, PP201V, Mumbai, India) in a coating pan (12-in. diameter) being rotated at 18 rpm (A K Industries, M 1107, Nakodar, India). Compressed air was introduced at a pressure of 1.5 kg/cm^2. The inlet air temperature was maintained at 60°C. The inner surface of coating pan was modified by attaching inert tubes (8 mm diameter) from the center to the periphery for easy rolling of tablets, thereby ensuring efficient mass transfer of polymer solution.

The coated tablets were also evaluated for weight variation and disintegration time. Further, the axial and radial diameters were measured as described above.

In Vitro Release Kinetics of Budesonide from Coated Tablets

In vitro release of budesonide from coated tablets was evaluated out using USP Dissolution Apparatus 1 (22) utilizing temperature of 37±0.5°C with constant stirring rate of 50 rpm. The dissolution studies were carried out in a pH progression media containing 0.5% *v/v* Tween 20 to maintain sink conditions (23). Buffer pH 1.2 IP (900 ml) was employed for 2 h followed by buffer pH 7.4 IP (900 ml) for 3 h and 100 ml of buffer pH 6.8 containing 2% *w/v* rat cecal contents (24) for further period of 19 h. Drug release studies in rat cecal media were carried out under continuous supply of CO_2. Aliquots (5 mL) were withdrawn at predetermined intervals and immediately analyzed for budesonide using HPLC. An equal volume of respective buffer containing Tween 20 (0.5% *v/v*) was replaced after each sampling. All dissolution studies were repeated on six tablets.

HPLC Analysis

The samples obtained from dissolution studies were filtered through 0.22 μ nitrocellulose filters (Millipore, USA) and manually injected (20 μL) using Rheodyne injector for HPLC analysis. The stationary phase consisted of C8 column (150 mm×4.6 mm, 5 μ). Acetonitrile and 25 mM phosphate buffer (pH 3.2) in the ratio of 60:40 *v/v* at a flow rate of 1 mL/min served as mobile phase (25). Data acquisition and processing were performed by using Empower 2 software (Waters, Austria).

Stability Studies

The tablets coated with 50:50 or 40:60 ratio of CH/CS were sealed in glass vials and stored under 45°C/75% RH for 6 months. Tablets were taken out after every 15 days and evaluated for weight variation, disintegration time, and *in vitro* drug release. The dissolution data of stored tablets was compared with that of freshly prepared tablets by f_1 (*dissimilarity) and f_2 (similarity) analysis.*

Pharmacokinetic Studies

Sprague–Dawley rats of either sex weighing 200–300 g maintained on normal diet were used for this study. Rats were divided into three groups. Each group comprised of four rats for generation of data in one study to ascertain that not more than two blood samples were withdrawn from each rat. Each study was conducted in triplicate. Rats of group I, group II, and group III received oral administration of, respectively, uncoated tablets or tablets coated with admixtures containing 40:60 or 50:50 ratios of CH/CS. A plastic 1-mL insulin syringe was cut at the lower end to form a hollow cylinder of desired length for administering the tablets orally to the rats. This tube was inserted above the tongue by forcibly opening the mouth of the rat. The tablet was inserted into the tube and was made to be swallowed by administration of 1 mL water. Blood samples (1 mL) were collected from retro orbital vein at 0, 1, 4, 8, 12, 16, or 24 h, mixed with 40 μL of heparin, and subjected to centrifugation at 4,000 rpm for 10 min. Upper layer was removed carefully, and to 200 μL of plasma, 20 μL of methanol and 15 μL of internal standard solution (0.005% *w/v* clotrimazole in methanol) were added. Ethyl acetate (2 mL) was added, and the microcentrifugation tubes were vortexed for 10 min. The tubes were then centrifuged at 4,000 rpm for 10 min. The upper layer was aspirated, evaporated, and reconstituted with 1 mL of mobile phase. This solution (20 μL) was injected for HPLC analysis. The protocol for this study was approved by the IAEC of Punjabi University, Patiala, India.

The area under the curve (AUC) was calculated using trapezoidal rule and time lag by extrapolating absorption phase trend line to time axis. The C_{max} was read out from the graph.

RESULTS

The proposed mechanism of interaction between CH and CS during film formation is depicted in Fig. 1.

Spectral Attributes of CH, CS, and CH–CS Films

Figure 2 depicts the FTIR spectrographs of CH (Fig. 2 A), CH acetate film (Fig. 2 B), CS (Fig. 2 C), and IPC films formed by interacting various ratios of CH and CS (Fig. 2 A). CH powder (85% deacetylation) exhibited a peak at 1,560 and 1,422 cm^{-1} (Fig. 2 D–H).

Figure 3 depicts the spectrographs of films containing different ratios of CH and CS after sequential exposure to pH 1.2 for 2 h and buffer pH 7.4 for 22 h.

Thermotropic Attributes of CH, CS, and CH–CS Films

CH powder exhibited one endothermic and one exothermic transition each at, respectively, 80.22°C and 311.30°C (Fig. 4 A). The DSC thermogram of CS revealed one endotherm at 97°C and an exotherm at 239.69°C (Fig. 4 B).

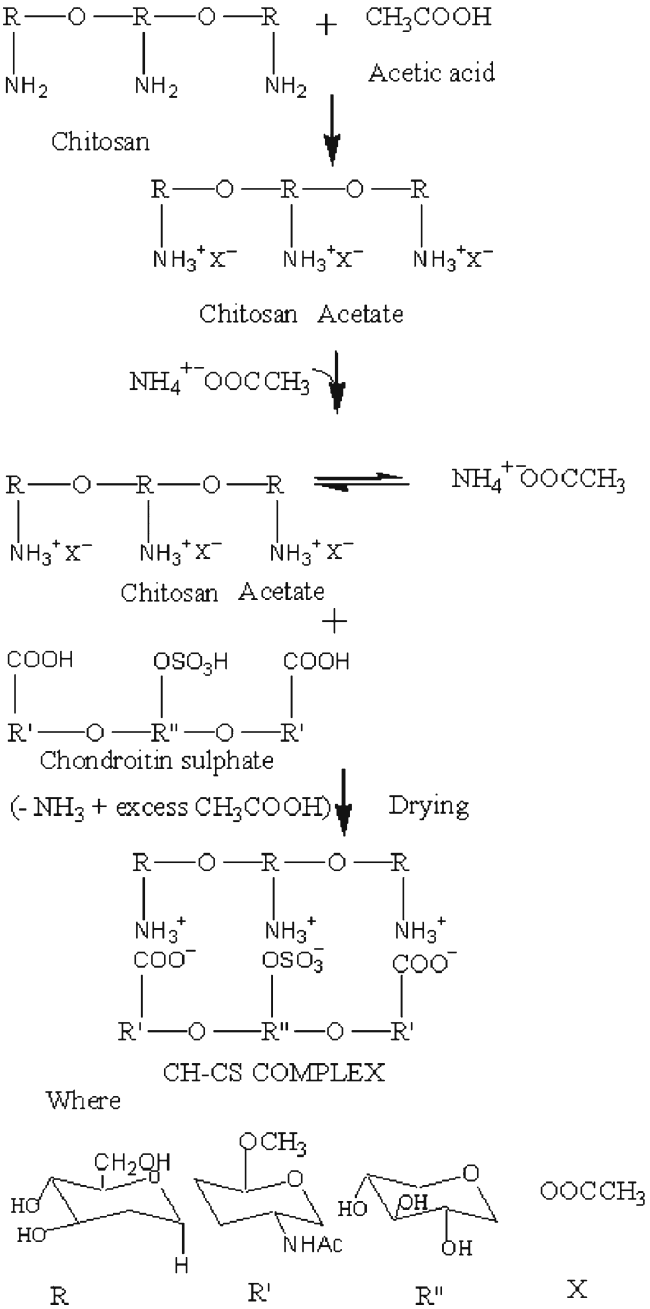

Fig. 1. Proposed mechanism of interaction between CH and CS during preparation of IPC films

The thermograms of IPC films containing any ratio of CH/CS exhibited first endothermic transition with peak temperature ranging between 90°C and 110°C. This was followed by a second endothermic transition with peak temperature ranging between 190°C and 205°C (Fig. 4 C–G). An additional endotherm at peak temperature of 300°C was observed only in the thermogram of IPC films containing 40:60 ratio of CH/CS (Fig. 4 F). Similar endotherm was also evident in the thermogram of CH films prepared in hydrochloric acid (Fig. 4 H).

The thermograms of the films containing any CH/CS ratio except 40:60 exhibited an additional exotherm at 190°C after exposure to pH 1.2 for 2 h (Fig. 5 B–I). Further, an additional endotherm was observed to occur in the temperature range of 270–300°C in the thermograms of IPC films after exposure to pH 1.2 for 2 h (Fig. 5 B, D, F, and H).

The exposure of films containing 70:30 or 60:40 ratio of CH/CS to pH 1.2 for 2 h produced an intense endotherm at 202°C (Fig. 5 D, F) which was reduced considerably after exposure of the films to pH 7.4 for 22 h (Fig. 5 E, G).

Swelling Index Measurements

The swelling of IPC films in pH 1.2 was considerably more as compared to pH 7.4 (Table I).

Mechanical Properties

The tensile strength and resilience properties of CH–CS films are summarized in Table I.

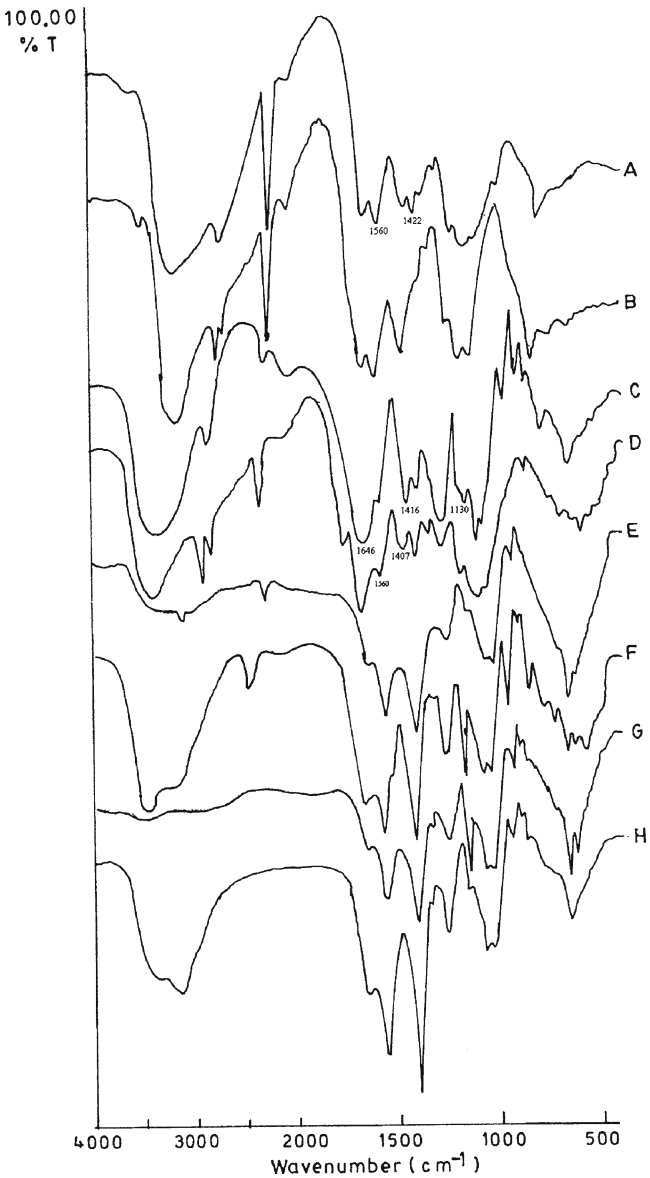

Fig. 2. FTIR spectra of (*A*) CH powder, (*B*) CH acetate film, and (*C*) CS powder; films prepared by interacting CH and CS (*D*) 70:30, (*E*) 60:40, (*F*) 50:50, (*G*) 40:60, and (*H*) 30:70 ratios

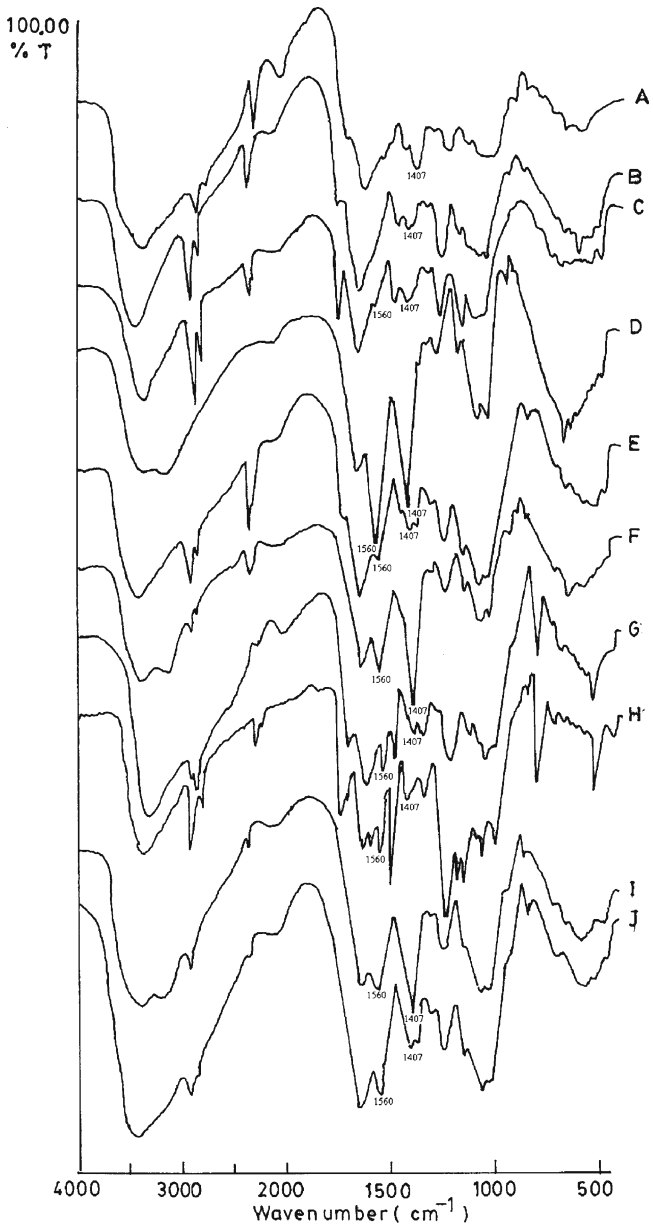

Fig. 3. FTIR spectra of films prepared using various CH/CS ratios and exposed to different pH media: (*A*) 30:70 (pH 1.2), (*B*) 30:70 (pH 7.4), (*C*) 70:30 (pH 1.2), (*D*) 70:30 (pH 7.4), (*E*) 60:40 (pH 1.2), (*F*) 60:40 (pH 7.4), (*G*) 50:50 (pH 1.2), (*H*) 50:50 (pH 7.4), (*I*) 40:60 (pH 1.2), and (*J*) 40:60 (pH 7.4)

Attributes of Core and CH–CS-Coated Tablets (*In Vitro* Studies)

The average weight of uncoated core tablets was 24.67± 1.10 mg. Hardness of the tablets was 4.5±0.5 kg/cm^2, and friability was found to range from 0.36% to 0.46% *w/w*. The axial and radial diameters, respectively, ranged from 1.75 to 1.80 and 3.98 to 4.02 mm. The uncoated tablets prepared by using 10% *w/w* Eudragit L100-55 as a binder started showing signs of cracking within 30 min of exposure to 0.1 M HCl.

The average weight of coated tablets was 27.20±1.25 mg. The axial and radial diameters, respectively, ranged from 2.02 to 2.08 and 4.01 to 4.15 mm. Although these tablets exhibited swelling, they did not soften or crack after exposure to 0.1 M HCl for 2 h.

The *in vitro* release of budesonide from the formulated tablets on sequential exposure to pH 1.2 (2 h), pH 7.4 (3 h) and pH 6.8 (19 h) is shown in Fig. 6. The uncoated tablets containing Eudragit L100-55 released 24% budesonide in 2 h (pH 1.2). The tablets coated with CH alone released 20% budesonide in 2 h (pH 1.2). The tablets coated with 70:30 or 30:70 ratio of CH/CS yielded release profiles similar to those coated with CH alone.

The release of budesonide from tablets coated with 60:40 ratio of CH/CS was 4.8% after exposure to pH 1.2 for 2 h. Additional 12% release was observed in pH 7.4 (3 h). Subsequent exposure of these tablets to pH 6.8 containing

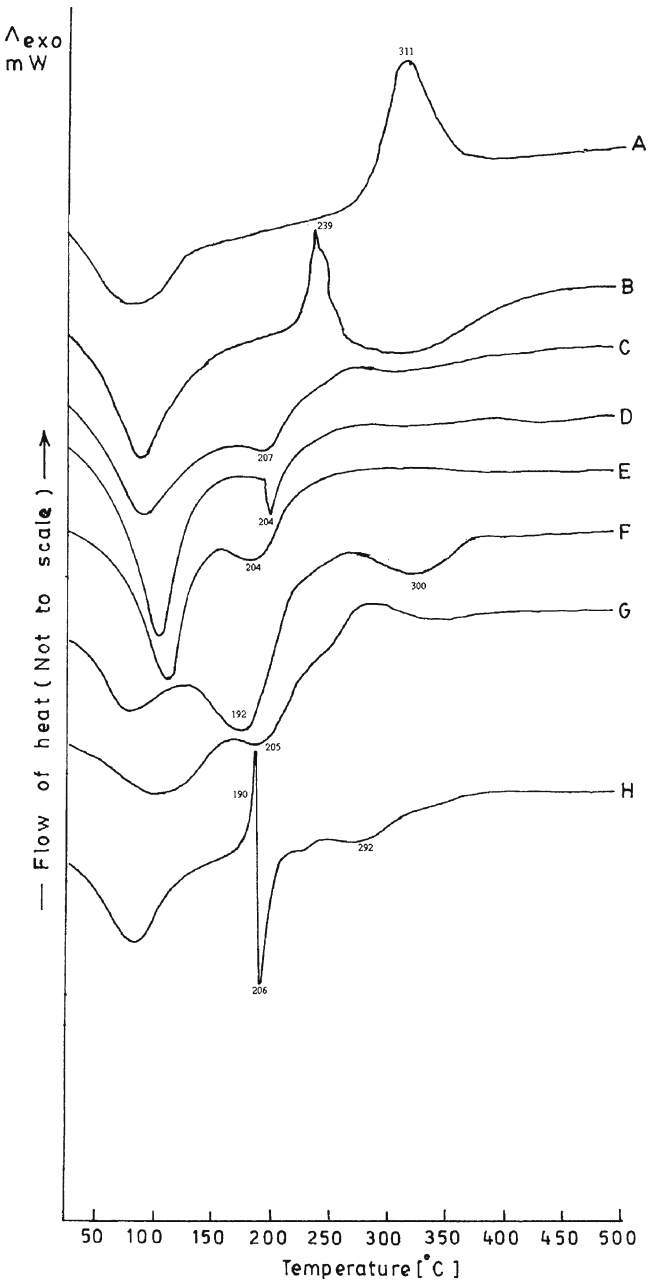

Fig. 4. DSC thermograms of (*A*) CH powder and (*B*) CS powder; films prepared by interacting CH and CS (*C*) 70:30, (*D*) 60:40, (*E*) 50:50, (*F*) 40:60, and (*G*) 30:70 ratios and (*H*) CH HCl film

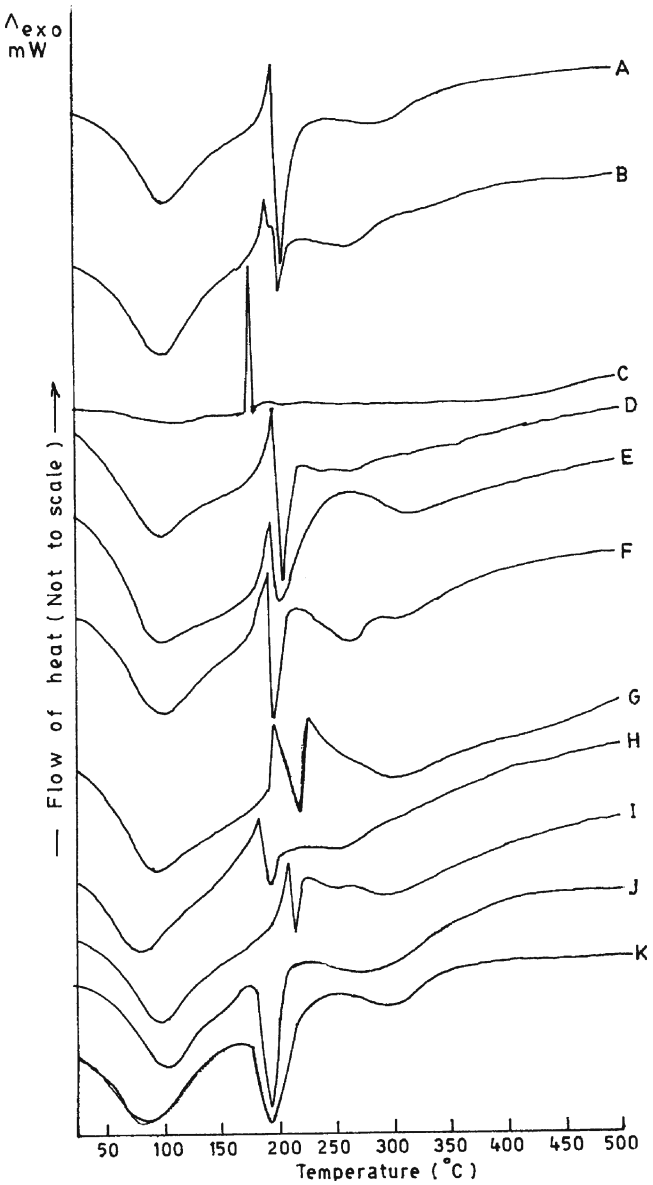

Fig. 5. DSC thermograms of (*A*) CH acetate film exposed to pH 1.2; films prepared using various CH/CS ratios and exposed to different pH media: (*B*) 30:70 (pH 1.2), (*C*) 30:70 (pH 7.4), (*D*) 70:30 (pH 1.2), (*E*) 70:30 (pH 7.4), (*F*) 60:40 (pH 1.2), (*G*) 60:40 (pH 7.4), (*H*) 50:50 (pH 1.2), (*I*) 50:50 (pH 7.4), (*J*) 40:60 (pH 1.2), and (*K*) 40:60 (pH 7.4)

rat cecal contents released the entire drug in 24 h (Fig. 6). The tablets coated with 50:50 or 40:60 ratio of CH/CS did not release budesonide in pH 1.2 (2 h). Subsequent exposure to pH 7.4 for 3 h released ~3% of drug from tablets coated with 50:50 ratio, whereas no drug was released from tablets coated with 40:60 ratio of CH/CS. The final exposure of tablets coated with 50:50 or 40:60 ratio of CH/CS to pH 6.8 containing rat cecal contents for 19 h eventually released, respectively, 50% and 61% budesonide. The f_1 and f_2 values for dissolution of budesonide from fresh and stored tablets (40°C/75% RH) coated with 50:50 ratio of CH/CS were, respectively, 4.98 and 90.40. These values for 40:60 (CH/CS) coated tablets were respectively, 4.36 and 91.43.

Table I. Physicochemical Properties and Thermal Changes of the Freshly Prepared IPC Films

Film composition	Film property				Thermographic attributes					
	Mechanical properties		Swelling index		First endotherm		Second endotherm		Third endotherm	
CH/CS ratio	Tensile strength	% resilience	pH 1.2	pH 7.4	Tm (°C)	ΔH (J/g)	Tm (°C)	ΔH (J/g)	Tm (°C)	ΔH (J/g)
70:30	6.28	21.31	2.28±0.15	0.75±0.03	98.14	313.70	207.82	13.34	*	*
60:40	6.66	23.62	1.60±0.12	0.70±0.02	110.96	65.58	204.75	20.09	*	*
50:50	18.88	25.40	1.11±0.07	0.38±0.03	110.22	46.93	204.52	69.82	*	*
40:60	25.11	30.16	0.58±0.04	0.11±0.02	98.19	75.40	192.27	177.64	300.36	12.67
30:70	11.64	24.78	0.34±0.03	0.00±0.00	110.22	385.64	205.25	35.88	*	*

* No endothermic transition was observed

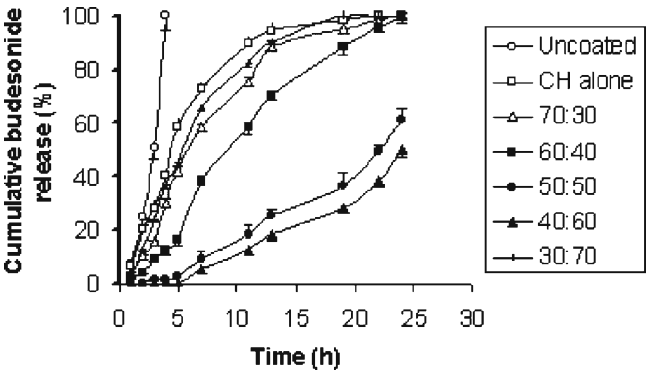

Fig. 6. *In vitro* drug release from uncoated and CH/CS IPC film-coated budesonide tablets

Pharmacokinetic Studies in Rats

Figure 7 depicts the plasma concentration time profiles of budesonide uncoated tablets as well as of tablets coated with 50:50 or 40:60 ratio of CH/CS following their oral administration to rats. The plasma concentration of budesonide was found to rise quickly after administration of uncoated tablets, and C_{max} of 1,091.99 ng/mL was achieved in 2 h. However, the time to achieve C_{max} after oral administration was delayed to 8 h and 12 h, respectively, for tablets coated with 50:50 or 40:60 ratio of CH/CS. The AUC for uncoated, 50:50, or 40:60 CH/CS-coated tablets, respectively, was found to be 5,019.08, 9,768.28, and 10,954.24 ng h/mL.

DISCUSSION

The reaction between $-NH_3^+$ groups of CH and $-COO^-$ or $-OSO_3^-$ groups of CS is reported to be spontaneous and has been investigated for preparing microspheres (26). It is due to this spontaneous reaction that films containing both CH and CS cannot be prepared. In the present investigation, the addition of ammonium acetate to CH was found to mask the reactive $-NH_3^+$ groups. This prevented the reaction between CH and CS, and a homogenous solution was formed when CH and CS solutions were mixed. However, drying this solution at 60–70°C resulted in evaporation of ammonium acetate, which allowed $-NH_3^+$ groups of CH to react with the $-COO^-$ or $-OSO_3^-$ groups of CS. The proposed interaction mechanism between CH and CS is shown in Fig. 1.

The presence of peaks at 1,560 and 1,422 cm^{-1} in the IR spectrograph of CH indicated the presence of $-NH_3^+$ ions (27) and $-COO^-$ ions. These peaks could have arisen due to 15% acetylation of CH powder (Fig. 2 A). The peaks at 1,560 and 1,412 cm^{-1} in the IR spectrograph of CH acetate indicated the presence of $-NH_3^+$ and $-COO^-$ ions, respectively (Fig. 2 B). The peaks observed at 1,646, 1,416, and 1,130 cm^{-1} can be assigned, respectively, to $-CONH_2$, $-COO^-$, and $-OSO_3^-$ groups in CS (27). The peak observed at 1,253 cm^{-1} could be assigned to –COOH present in CS molecule (Fig. 2 C).

The peaks at 1,407 and 1,507 cm^{-1} in the IPC films indicated the presence of $-COO^-$ groups and $-NH_3^+$, thus strongly suggesting the existence of $NH_3^+COO^-$ complex, which resulted in interpolymer complexation between CH and CS. Further, the characteristic peak of $NH_4^+SO_4^-$ is reported to occur in the range of 1,150–1,300 cm^{-1} (27). It is important to note, however, that these peaks were more intense in the spectrographs of the films employing 40:60 (Fig. 2 G) or 50:50 (Fig. 2 F) ratio of CH/CS. Hence, both carboxylate as well as sulfonate linkages can be suggested to be present in all the films prepared using various ratios of CH/CS.

The reduction in the intensity of peak at 1,407 cm^{-1} in 30:70 ratio of CH/CS after exposure to pH 1.2 for 2 h or pH 7.4 (22 h) suggested weakening of carboxylate linkages. Further, the absence of peak at 1,560 cm^{-1} reflected total breakdown of $COO^- NH_3^+$ linkages in these IPC films (Fig. 3 A, B).

The reduced intensities of peaks at 1,560 cm^{-1} (representing N–H stretch of $-NH_3^+$) and 1,407 cm^{-1} (representing C=O stretch of $-COO^-$) in 70:30 or 60:40 ratio of CH/CS after exposure to pH 1.2 for 2 h indicated weakening of carboxylate ($NH_3^+COO^-$) linkages (Fig. 3 C, E). However, exposure of these films to pH 7.4 resulted in reoccurrence of sharp peaks at 1,407 and 1,560 cm^{-1} (Fig. 3 D, F). The opposite findings on exposure to pH 1.2 and pH 7.4 could be attributed to ionic nature of interaction between $-NH_3^+$ of CH and $-COO^-$ of CS. Exposure of the IPC films containing 50:50 or 40:60 ratio of CH/CS did not produce any change in the intensity of the peaks at 1,407 and 1,560 cm^{-1} (Fig. 3 G, I). In addition, the peak at 1,065 cm^{-1} with a shoulder at 1,162 cm^{-1} was observed to remain unaffected after exposure of these films to pH 1.2 and pH 7.4. Hence, it could be suggested that the carboxylate as well as sulfonate linkages in the films containing, respectively, 40:60 or 50:50 ratios of CH/CS were more resistant to the action of pH 1.2 or pH 7.4.

The first broad endotherm in the DSC thermogram of CH (Fig. 4 A), CS (Fig. 4 B), and IPC films prepared by drying admixtures comprising different ratios of CH/CS (Fig. 4 C–G) can be suggested to have arisen due to evaporation of water from the films as all the films were hydrated at 50% RH for 48 h in order to simulate their status during tablet coating. The second endothermic transition occurring in the temperature range of 190–205°C was found to differ in the ΔH value among films containing different ratios of CH/CS. This suggested that the second endothermic transition could be associated with the interaction between CH and CS during formation of IPC (Fig. 4 C–G). The maximum ΔH of the second endotherm was found to be exhibited by IPC films containing 40:60 ratio of CH/CS (Table I). Since the films containing 40:60 ratio of CH/CS

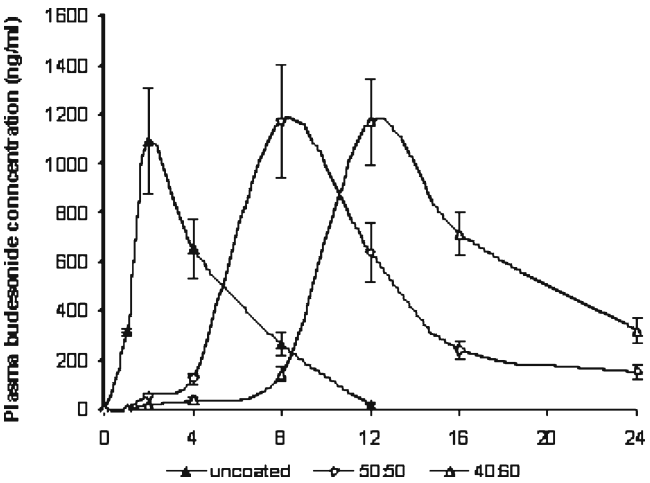

Fig. 7. Pharmacokinetic profile of budesonide following oral administration of uncoated and CH/CS (50:50 and 40:60) film-coated tablets

exhibited two endothermic transitions at 192°C and 300°C (Fig. 4 F) as was obtained in CH HCl films (Fig. 4 H), this suggested a stronger interaction in the IPC films containing 40:60 ratio of CH/CS.

The appearance of an exotherm in all the IPC films and CH acetate film exposed to pH 1.2 for 2 h (Fig. 5 A) except 40:60 ratio of CH/CS seems to have arisen due to ionic interaction of CH (remaining uncomplexed with CS) with acidic buffer components. The fact that the endotherm at 300°C was observed only in thermograms of freshly prepared IPC films containing 40:60 ratio (Fig. 4 F) of CH/CS but occurred in other films after exposure to pH 1.2 (Fig. 5 B, D, F, and H) suggested that CH was predominantly present in complexed state with CS in the former film. As a corollary, some quantity of CH was present in uncomplexed state in other films and hence could interact with the acidic buffer components.

Exposure of the film containing 30:70 ratio of CH/CS to pH 7.4 for 22 h completely obliterated the endotherm observed in the temperature range of 190–205°C (Fig. 5 C), thus suggesting high vulnerability of the complex in this film to be broken down by alkaline pH. The intense endotherm observed at 202°C in films containing 70:30 or 60:40 ratio of CH/CS after exposure to pH 1.2 for 2 h (Fig. 5 D, F) could be attributed to the reaction of excess CH (not complexed with CS present in these films) with the components of the acidic buffer. Further, this endothermic transition was considerably reduced when these films were exposed to pH 7.4 for 22 h (Fig. 5 E, G). Hence, the IPC films containing 70:30 or 60:40 ratio of CH/CS could be suggested to possess weak interaction of CH with CS. The films containing 50:50 or 40:60 ratio of CH/CS were not observed to be influenced by exposure to pH 1.2 (Fig. 5 H, J) or pH 7.4 (Fig. 5 I, K) as the intensity of second endotherm characteristic of interpolymer complexation did not reduce considerably. Hence, the 40:60 or 50:50 (CH/CS) IPC films could be suggested to be most stable in the presence of both pH 1.2 and pH 7.4.

The IPC films were found to exhibit pH sensitive swelling. Further, this swelling was a function of concentration of CH in both acidic and alkaline buffers (Table I). In acidic media, the polyacid is neutralized, and due to the free ammonium groups of CH, excess positive charges appear inside the gel. Their mutual repulsion and the entry of water together with counter ions to neutralize these charges cause excessive swelling (28). However, the IPC films exhibited considerably less swelling in pH 7.4. This can be ascribed to the fact that prolonged immersion in water produced segmental mobility of the interpolymer chains in the swollen state, which allowed the completion of interpolymer reaction eventually leading to shrinkage.

The tensile strength of CH/CS (40:60) IPC film was observed to be highest as compared to other IPC films suggesting its extensibility. In addition, the resilience value of this film was also observed to be highest among other films, indicating its ability to undergo elastic recovery (Table I). These properties indicated suitability of CH/CS (40:60) IPC films for preparing film-coated tablets of budesonide.

The average weight of uncoated core tablets and coated tablets, respectively, was 24.67±1.10 and 27.20±1.25 mg. The acceptance value calculated was 11.98% and 11.31%, respectively, which is well below the maximum 15% USP tolerance limit. Hence, the tablets passed the weight variation test.

The tablets coated with CH alone or 70:30 or 30:70 ratio of CH/CS could not prevent budesonide from being released in acidic pH (Fig. 6). The IR spectrographs (Fig. 3 B) and DSC thermograms (Fig. 5 C) of 30:70 (CH/CS) films revealed complete abolition of the peaks/endothermic transitions characteristic of CH–CS complexation after their exposure to pH 7.4. Similarly, the DSC thermograms (Fig. 5 E) of IPC films containing 70:30 ratio of CH/CS after exposure to pH 7.4 revealed considerable reduction in the intensity of relevant endothermic transitions. Therefore, rapid release of budesonide from tablets coated with 70:30 or 30:70 ratio of CH/CS can be ascribed to weak interaction between $-NH_3^+$ groups of CH and $-COO^-$ groups of CS. Hence, the ratio of CH/CS in the IPC films seems to play a vital role in governing release of budesonide from coated tablets.

The observation of <10% drug released in acidic media (21) in tablets coated with 50:50 or 40:60 CH/CS indicated fulfillment of drug release criteria expected of enteric tablets (Fig. 6). The DSC thermograms (Fig. 5 H, I) and IR spectrographs (Fig. 3 G, H) of 50:50 (CH/CS) as well as DSC thermograms (Fig. 5 J, K) and IR spectrographs (Fig. 3 I, J) of 40:60 CH/CS films after exposure to pH 1.2 and pH 7.4 did not reveal a decrease in the intensity of peaks/endothermic transition characteristic of interpolymer complexation. Hence, the ability of the 50:50 or 40:60 (CH/CS) film coating in totally restraining the release of budesonide from tablets can be attributed to the resistance of the complexation between $-NH_3^+$ of CH and $-OSO_3^-$ or $-COO^-$ of CS to pH 1.2.

Rat cecal contents are widely used in the dissolution media for testing colon-specific drug delivery systems (24). The use of rat cecal contents for this purpose seems to be due to its similarity with the human colonic microflora (29). Hence, exposure of these tablets to dissolution media containing rat cecal contents seems to be responsible for releasing budesonide. This could be attributed to the degradation of the polysaccharides present in the coat by polysaccharidases of the rat cecal content. However, it is worthy to note that the drug was not completely released even after 24 h following sequential exposure to different pH media simulating *in vivo* gastrointestinal transit. Incomplete release of bovine serum albumin (30) and paracetamol (31) has been reported earlier for, respectively, chitosan beads and pectin, chitosan, and HPMC film-coated tablets. This seems to be due to decrease in the activity of polysaccharidases over long intervals of time during *in vitro* testing. Nevertheless, the release of budesonide from tablets coated with 50:50 or 40:60 (CH/CS) was of zero order, indicating slow and continuous release over a period of 19 h in pH 6.8. This can be expected to be of great benefit because budesonide is required to be released slowly during the transit of the tablet through the colon for IBD therapy.

Budesonide was detectable in plasma after 1 h of administration of uncoated tablet, indicating inability of Eudragit L100-55 to delay its release appreciably (Fig. 7). However, there was a time lag of 3.4 and 6.8 h for 50:50 or 40:60 (CH/CS) coated tablets, respectively. The plasma concentrations of budesonide following oral administration of coated tablets were significantly lower ($p<0.05$) as compared to those obtained after oral administration of uncoated tablets till achievement of C_{max} in the latter. This strongly suggested the ability of the IPC films to restrain the

release of budesonide in gastric pH. The C_{max} of budesonide reported in human beings is 1–2 ng/mL after administration of 3 mg gastro-resistant capsules (32). The almost 10^3-fold higher C_{max} observed in rats in the present investigation could be attributed to the presence of 10^3-fold less volume of blood (5 mL) in rats as compared to human beings due to which the drug gets distributed extensively in the latter. It is important to note that the C_{max} of budesonide obtained after administration of coated tablets was not significantly different ($p<0.05$) from that obtained after administration of uncoated tablets. This indicated similar disposition kinetics of budesonide after administration of coated and uncoated tablets. However, it is evident that budesonide was slowly absorbed and excreted after administration of coated tablets. This could have contributed to 1.95- and 2.18-fold greater AUC after administration of, respectively, tablets coated with 50:50 or 40:60 ratio of CH/CS as compared to uncoated tablets. The oral bioavailability of budesonide is only 10% due to extensive and rapid presystemic as well as hepatic first pass metabolism (5). However, Edsbacker *et al.* (33) observed approximately two thirds of dose of budesonide to be absorbed from ileum and colon (33). Hence, although, not anticipated, significant quantity of budesonide released from the coated tablets in the colon could have resulted in its absorption into the systemic circulation as observed in the present study. High plasma concentrations of budesonide are not required for treating inflammation. However, high concentration of budesonide in the plasma indicates its release from the tablet in the colon, which would result in greater exposure of the inflamed mucosa to high local concentration of budesonide.

CONCLUSION

The results of the present investigation revealed distinct advantage of coating tablets with IPC films containing 50:50 or 40:60 ratio of CH/CS as compared to uncoated tablets granulated with Eudragit L100-55 in restraining the *in vitro* release of budesonide in gastric pH. Further, these tablets released budesonide in alkaline media in zero order fashion through 19 h during dissolution studies. Although the release of budesonide in rats was delayed, the peak plasma concentration was comparable to that obtained after oral administration of uncoated tablets. The slow release of budesonide from coated tablets could be attributed to higher AUC as compared to that obtained with uncoated tablets. The ability of the IPC films in providing the observed release characteristics to budesonide core tablets was correlated with the ability of $-NH_3^+$ groups of CH to form complex with $-OSO_3^-$ or $-COO^-$ groups of CS and the stability of these complexes in acidic media. The tablets coated with 40:60 ratio of CH/CS can be envisaged to offer a great promise for colon delivery of budesonide, thereby providing drug concentration for longer durations for effective therapy of IBD.

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REFERENCES

1. Friend DR. New oral delivery systems for treatment of inflammatory bowel disease. Adv Drug Deliv Rev. 2005;57: 247–65.
2. Robinson M. Medical therapy of inflammatory bowel disease for the 21st century. Eur J Surg. 1998;582:90–8.
3. Rahimi R, Nikfar S, Rezaie A, Abdollahi M. A meta-analysis of broadspectrum antibiotic therapy in patients with active Crohn's disease. Clin Ther. 2006;28:1983–8.
4. Klein S, Stein J, Dressman J. Site speific delivery of anti-inflammatory drugs in the gastrointestinal tract: an *in vitro* release model. J Pharm Pharmacol. 2005;57:709–19.
5. Spencer CM, McTavish D. Budesonide: a review of its pharmacological properties and therapeutic efficacy in inflammatory bowel disease. Drugs. 1995;50:854–72.
6. Akhgari A, Sadeghi F, Garekani HA. Combination of time-dependent and pH-dependent polymethacrylates single coating formulation for colonic delivery of indomethacin. Int J Pharm. 2006;320:137–42.
7. Niwa K, Takaya T, Morimoto T, Takada K. Preparation and evaluation of a time-controlled release capsule made of ethylcellulose for colon delivery of drugs. J Drug Target. 1995;3:83–9.
8. Sinha VR, Kumria R. Microbially triggered drug delivery to the colon. Eur J Pharm Sci. 2003;18:3–18.
9. Evans DF, Pye G, Bramley R, Clark AG, Dyson TJ, Hardcastle JD. Measurement of gastrointestinal pH profiles in normal ambulant subjects. Gut. 1988;29:1035–41.
10. Davis SS, Hardy JG, Stockwell A, Taylor MJ, Whalley DR, Wilson CG. The effect of food on the gastrointestinal transit of pellets and an osmotic device (Osmet). Int J Pharm. 1984;21:331–40.
11. Wong D, Larrabee S, Clifford K, Tremblay J, Friend DR. USP dissolution apparatus II (reciprocating cylinder) for screening of guar gum-based colonic delivery formulations. J Control Rel. 1997;47:73–179.
12. Raghavan CV, Muthulingam C, Jenita JAJL, Ravi TK. An *in vitro in vivo* investigation into the suitability of bacterially triggered delivery system for colon targeting. Chem Pharm Bull. 2002;50:892–5.
13. Siew LF, Basit AW, Newton JM. The properties of amylase ethylcellulose films cast from organic-based solvents as potential coatings for colonic drug delivery. Eur J Pharm Sci. 2000;11:133–9.
14. Ahmed IS. Effect of simulated gastrointestinal conditions on drug release from pectin/ethylcellulose as film coating for drug delivery to the colon. Drug Dev Ind Pharm. 2005;31:465–70.
15. Gliko-Kabir I, Yagen B, Penhasi A, Rubinstein A. Low swelling, crosslinked guar gum and its potential use as colon-specific drug carrier. Pharm Res. 1998;15:1019–25.
16. Fan J, Wang K, Liu M, He Z. *In vitro* evaluations of konjac glucomannan and xanthan gum mixture as the sustained release material of matrix tablet. Carbohydr Polym. 2008;73:241–7.
17. Muzzarelli C, Vesna Stanic V, Gobbi L, Tosi G, Muzzarelli R. Spray drying of solutions containing chitosan together with polyuronans and characterisation of the microspheres. Carbohydr Polym. 2004;57:73–82.
18. Xia W, Liu P, Liu J. Advance in chitosan hydrolysis by non-specific cellulases. Biores Technol. 2008;99:6751–62.
19. Indian Pharmacopeia. Indian Pharmacopeia Commission, Ghaziabad; 2007.
20. Marvola M, Nykänen P, Rautio S, Isonen N, Autere A-M. Enteric polymers as binders and coating materials in multiple-unit site specific drug delivery systems. Eur J Pharm Sci. 1999;7:259–67.
21. US Pharmacopeia XXX NF XXV. US Pharmacopeial convention, Rockville; 2007.
22. Zambito Y, Baggiani A, Carelli V, Serafini MF, Colo GD. Matrices for site specific controlled delivery of 5 fluorouracil to descending colon. J Control Release. 2005;102:669–77.
23. Rodríguez M, Vila-Jato J, Torres D. Design of a new multi-particulate system for potential site-specific and controlled drug delivery to the colonic region. J. Control. Rel. 1998;55:67–77.
24. Rubinstein A, Radai R, Ezra M, Pathak S, Rokem JS. *In vitro* evaluation of calcium pectinate: a potential colon specific drug delivery carrier. Pharm Res. 1993;10:258–63.

25. Gupta M, Bhargava HN. Develpoment and validation of high performance liquid chromatographic method for the analysis of budesonide. J Pharm Biomed Anal. 2006;40:423–8.
26. Sui W, Huang L, Wang J, Bo Q. Preparation and properties of chitosan chondroitin sulfate complex microcapsules. Colloids and Surfaces B Biointerfaces. 2008;65:69–73.
27. Kemp W. Infrared spectroscopy, in organic spectroscopy. London: MacMillan; 1991.
28. Berger J, Reist M, Mayer JM, Felt O, Peppas NA, Gurny R. Structure and interactions in covalently and ionically crosslinked chitosan hydrogels for biomedical applications. Eur J Pharm Biopharm. 2004;57:19–34.
29. Hawksworth G, Drasar BS, Hill MJ. Intestinal bacteria and the hydrolysis of glycosidic bonds. J Med Microbiol. 1971;155:451–9.
30. Zhang H, Alsara IA, Neau SH. An *in vitro* evaluation of a chitosan containing multiparticulate system for macromolecular delivery to the colon. Int J Pharm. 2002;239:197–205.
31. Ofori-Kwakye K, Fell JT. Biphasic drug release from film coated tablets. Int J Pharm. 2003;250:431–40.
32. Möllmann HW, Hochhaus G. Pharmacokinetics and pharmacodynamics of budesonide after oral application of 1×3 mg budesonide as Budenofalk 3 mg capsules to healthy subjects with and without breakfast. Data on file, Dr. Falk Pharma, Freiburg/Germany; 1998.
33. Edsbacker S, Larsson P, Wollmer P. Gut delivery of budesonide, a locally active corticosteroid, from plain and controlled-release capsules. Eur J Gastroenterol Hepatol. 2002;14: 1357–62.