AAPS PharmSciTech, Vol. 10, No. 1, March 2009 (© 2009)
DOI: 10.1208/s12249-009-9188-3

Research Article

Optimization of Budesonide Compression-Coated Tablets for Colonic Delivery

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Received 6 September 2008; accepted 31 December 2008; published online 6 February 2009

Abstract. The purpose of this study was to formulate budesonide (BUD) compression-coated tablets for colonic specific delivery. Pectin and guar gum were used as enzyme-dependent polymers. For comparison purposes, both pH- and time-dependent polymers were also tried. *In vitro* release studies were carried out at different pH (1.2, 6.8, and 7.4). Therapeutic efficacy of the prepared tablets compared to commercially available capsules and enema were evaluated in trinitrobenzenesulfonic acid-induced rabbit colitis model. In pH-dependent polymers, Eudragit (EUD) S100/EUD L100 (1:1) released 45.58% in the target area (colon). For time-dependent polymers, decreasing cellulose acetate butyrate (CAB) ratio increased the release in both pH 6.8 and 7.4 till it reached 40.58% and 93.65%, respectively, for 25% CAB. In enzyme-dependent polymers, increasing pectin ratio to 75% retarded the release (4.59% in pH 6.8 and 54.45% in pH 7.4) which was significantly enhanced to 99.31% using pectinolytic enzyme. Formula F14 coated with 75% pectin significantly reduced the inflammatory cells in the connective tissue core of the colon of the treated group and significantly decreased myeloperoxidase activity (3.90 U/g tissue weight). This study proved that BUD compression-coated with 75% pectin may be beneficial in the treatment of inflammatory bowel disease.

KEY WORDS: budesonide; colon-targeting; compression coat; guar gum; pectin; tablets; TNBS-induced rabbit colitis.

INTRODUCTION

Site-specific delivery of drugs to the site of action has the potential to reduce side effects and to increase pharmacological response. One of the seemingly interesting areas to target drugs through oral route is the colon. Various systems have been developed for colon-specific drug delivery: covalent linkage of a drug with a carrier, coating with pH-sensitive polymers, time-dependent release systems, and enzymatically controlled delivery systems (1–3). Enteric-coated systems are the most commonly used for colonic drug delivery, but the pH difference between the small intestine and colon is not being very pronounced leading to poor site specificity. The drawback of the time-dependent release system is its inability to sense any variation in the upper gastrointestinal tract transit time; besides, any variation in gastric emptying time may lead to drug release in the small intestine before arrival to the colon. There is a steep gradient of enzyme activity along the gastrointestinal tract; these enzymes are derived from gut microflora. In humans, the stomach and small intestine contain roughly 103–104 colony forming units (CFU)/mL (4,5). However, the concentration of microflora rises dramatically passing from the terminal ileum to the ascending colon where the numbers reach 1,011–1,012 CFU/mL. These bacteria survive and thrive by fermenting a wide range of substrates (e.g., oligosaccharides, polysaccharides, mucopolysaccharides) left undigested in the small intestine (6). Hence, enzymatically controlled delivery systems is considered a convenient approach for site-specific drug delivery to the colon where no drug release can occur unless the system arrives to the colon (7–9).

BUD is a potent corticosteroid that has important implications in the pharmacotherapy of inflammatory bowel disease, especially in the treatment of ulcerative colitis and Crohn's disease. BUD is approximately twice as active as beclomethasone dipropionate, and it is over 1,000 times more active than either prednisolone or hydrocortisone in inducing intracutaneous vasoconstriction (as a marker of anti-inflammatory activity) (10). BUD is commercially available in the market in the form of enteric-coated preparations mainly for the treatment of small intestine active Crohn's disease. However, these products, similar to other available site-specific dosage forms, are not sufficiently selective to treat colonic inflammatory bowel disease. It was found that less than 5% of the drug was available beyond the ileum and cecum (11), and therefore, colonic delivery still needs to be optimized by a more reliable colon-specific system. Previous workers have developed BUD microparticles for colon delivery (12,13). However, being relatively complex systems, their large-scale manufacturing requires a lot of technological advancement and skills. So, an attempt was made to formulate compression-coated tablets, which could be formulated easily, using the usual tableting techniques. In the

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industrial scale, the one-step dry-coated tablets (OSDRC) manufactured by using a unique punch and die (14) can be applied allowing dry-coated tablets to be assembled in a single turn of a rotary tableting machine without requiring the preparation of core tablets beforehand. Using the OSDRC method, no technical problems are anticipated relating to the supply of the core, such as tablets which have no core or those with an off-center core, which are common problems with conventional dry-coated tablets.

The aim of the present study was to formulate BUD compression-coated tablets to prevent drug release in the stomach, had an additional lag phase to retard drug release in the small intestine, and to deliver drug specifically to the colon. Enzymatically controlled delivery systems were developed using pectin and guar gum in the compression coat. Both pH-dependent polymers, viz EUD L100, EUD S100, and time-dependent polymers, viz hydroxypropyl methyl cellulose (HPMC), cellulose acetate butyrate (CAB), were also tried in an attempt to optimize drug release in the colon. The promising dosage forms were examined for their efficacy using the trinitrobenzenesulfonic acid (TNBS)-induced rabbit colitis model in order to elucidate their usefulness as a specific drug delivery system for the treatment of irritable bowel disease.

MATERIALS AND METHODS

Materials

Budesonide batch no. 5309MO-00905 was supplied by MUP, Cairo, Egypt; anhydrous lactose was obtained from Meggle, Germany; Avicel pH 101 (microcrystalline cellulose) was from J. Rettenmaier and Söhme, Germany; Eudragit S100 and Eudragit L100 were from Rohm GmbH and Co. KG, Pharma Polymer, Germany; hydroxypropyl methyl cellulose K4M (HPMC) was from Aqualon, Wilington, USA; cellulose acetate butyrate (CAB) was from Eastman Chemical, Kingsport, TN, USA; pectin was from Sigma Chemical, St. Louis, USA; guar gum was from Aldrich WI, USA; Pectinex 3XL (3,000 FDU/mL) was from Fluka Chemicals, Switzerland; galactomannanase enzyme from *Aspergillus niger* was from Fluka Chemicals, Denmark; acetonitrile, high-performance liquid chromatography (HPLC) grade, was from Fisher Scientific UK Limited, UK; and TNBS was from Sigma Chemical, St. Louis, USA.

Differential Scanning Calorimetry

In order to investigate the possible interaction between BUD and the polymers used, viz EUD L100, EUD S100, HPMC, CAB, pectin, and guar gum, differential scanning calorimetry (DSC) analysis was carried out on pure substances and their physical mixtures (PM) in equimolar ratios using the Shimadzu DSC-50 instrument equipped with a computerized data station. Samples (3–4 mg) were placed in an aluminum pan and heated at a rate of 10°C/min with indium in the reference pan in an atmosphere of nitrogen to a temperature of 350°C.

Study of the Flow Properties of the Powder Blends Used in the Compression-Coated Tablets

The flow properties of the different powder blends used in the compression-coated tablets was studied using angle of repose (fixed height cone method) (15), Carr's compressibility index (16), and the Hausner ratio methods (17).

Preparation of BUD Compression-Coated Tablets

BUD core tablets were compression-coated with pH-dependent, time-dependent, and enzyme-dependent polymers. Each core tablet consisted of 3 mg BUD and 57 mg anhydrous lactose as direct compression vehicle. BUD and lactose were thoroughly mixed and passed through mesh (149 mm). The mixture was compressed into core tablets with an applied force of 4,000 kg using a single punch tablet machine equipped with concave punch 7 mm in diameter. The core tablets were compression-coated with 400 mg different coat mixtures (Table I). About 50% of the coat formulation was placed in the die cavity (diameter 11 mm). The core tablet was then placed in the center of the die cavity, which was filled with the remainder of the coat formulation. Then, it was compressed around the core tablets at an applied force of 5,000 kg using 11-mm concave punches. The prepared compression-coated BUD tablets were tested for weight variation, hardness, drug content, and friability.

HPLC Analysis of BUD in Tablet Formulations and Dissolution Fluids

The quantitative determination of BUD was performed by HPLC. A Shimadzu HPLC system with UV-Visible detector: SPD-10A, liquid chromatogram: LC-10AD, integrator: C-R6A, injector (20 μL): 6E (Shimadzu, Japan). The analysis was carried out using Hypersil C18 column (Thermo Electron Corporation) with dimensions = 150 × 4.6 mm and particle size = 5 μm at a wavelength of 247 nm. The volume of injection was 20 μL. The mobile phase consisted of acetonitrile–water (40:60). The flow rate was 1.5 mL/min.

In Vitro Drug Release Studies

BUD release from the compression-coated tablets was assessed by dissolution testing using the USP Dissolution Tester, Apparatus I (rotating basket) at a rotation speed of 50 rpm maintained at 37.0±0.5°C (USP Dissolution Tester, Hanson Research Corporation, California, USA). The release study was performed in 250 mL 0.1 N HCl for 2 h, followed by 250 mL phosphate buffer (pH 6.8) for another 3 h, and finally, 250 mL phosphate buffer (pH 7.4) till the end of the 24 h to simulate the pHs pertaining to the stomach, proximal and middle small intestine (duodenum and jejunum), and distal small intestine (ileum), respectively. One milliliter of dissolution medium was withdrawn at 2-, 3-, 5-, 6-, 8-, 12-, 20-, and 24-h time intervals and replaced with an equal volume of media. The collected media was filtered through 0.45 μm membrane, and the concentration of dissolved drug was measured using HPLC as described before. Three replications were made for each tablet batch.

Table I. Compression Coat Composition of the Different Prepared Formulas

	Polymer (%)						
	pH-dependent		Time-dependent		Enzyme-dependent		
Code	Eudragit S100	Eudragit L100	HPMC	CAB	Pectin	Guar gum	Avicel (%)
F1		100					
F2	100						
F3	50	50					
F4	70	30					
F5			100				
F6			50				50
F7			20				80
F8			10				90
F9				100			
F10				50			50
F11				33			67
F12				25			75
F13					100		
F14					75		25
F15					50		50
F16						100	
F17						50	50
F18						15	85
F19						10	90
F20						5	95

In order to evaluate enzyme-triggered drug release of pectin and guar gum compression-coated tablets, 1 mL pectinolytic enzyme for the former (18) and 0.05 mg/mL galactomannanase for the latter (19) were added to the phosphate buffer solution pH 7.4 to simulate the degradation of the polymers by the microflora.

In order to compare between the prepared formulas, the amount released between the fifth and 24th hours for each formula after starting the release was calculated. This amount was taken as a parameter that would be equivalent to the target area (colon).

In order to determine drug release mechanism from the prepared tablets, the release kinetic data (up to 60% release) were analyzed by the following equation (20):

$$\frac{M_t}{M_\infty} = Kt^n$$

where M_t, M_∞, k, and n are the amount of drug released at time t, the total amount of drug in the tablet, a constant, and the exponent for the release kinetics, respectively. The value of n for a tablet was 0.45 for Fickian (case I) release, >0.45 but <0.89 for non-Fickian (anomalous) release, 0.89 for case II (zero order) release, and >0.89 for super case II. A more reliable and informative analysis can be obtained by considering that drug release in swellable matrices depends on two processes: (1) drug diffusion into the swollen polymer and (2) matrix swelling due to the penetration of the dissolution medium. Calculation of the approximate contribution of the diffusional and relaxational mechanisms to the anomalous release process is carried out by fitting the data to the model proposed by Peppas and Sahlin (21) for quantifying the two phenomena controlling the release from a swellable matrix. The equation of the model is:

$$\frac{M_t}{M_\infty} = k_1 t^m + k_2 t^{2m}$$

where the first term of the right-hand side represents the Fickian contribution and the second term is the case II relaxational contribution. In this model, drug release from swellable matrices is described as the result of two transport mechanisms, i.e., diffusion across the gel layer (F) and relaxation of the polymeric chains (R). F is linked to the coefficient of the diffusional contribution (square root dependence on time) and R to the coefficient of the relaxational contribution (linear dependence on time) of the binomial equation describing the time dependence of fractional drug release. For the geometry of our tablets, m of 0.45 was appropriate (22). The K_1 and K_2 are obtained from nonlinear regression curve fitting of the release data. When $K_1 > K_2$, the release is mainly controlled by diffusion, and when $K_2 > K_1$, the release is predominantly controlled by matrix swelling/dissolution, the so-called case II transport kinetic (near zero order). When K_1 is nearly equal to K_2, the release is controlled by a combination of diffusion and polymer relaxation (anomalous transport) (23).

In Vivo Study in Rabbits

Induction of Experimental Colitis

The TNBS-induced experimental rabbit colitis model was selected because the pharmacological responses of rabbit colonic smooth muscle to inflammatory mediators closely resemble those of the human colon (24). Fifteen adult male

rabbits weighing 2.5–3 kg were used throughout the study. The animals were randomly divided into five groups, each consisted of three animals: group I, normal control group; group II, induced colitis group; group III, animals treated with F14 compression-coated with 75% pectin; group IV, animals treated with commercial BUD capsules; and group V, animals treated with commercial BUD enema. Briefly, rabbits were fasted 24 h with free access to water before experimentation. Colitis was induced in all rabbits except the control group following the method described by Depoortere *et al.* (25). The study protocol was reviewed and approved by the ethical committee of the Faculty of Pharmacy, Cairo University. A rubber catheter was inserted approximately 15 cm into the colon and inflated with 3 cm of air. Gentle withdrawal of the catheter caused fecal pellets in the distal colon and rectum to be expelled by muscle action. Colitis was induced by slow intrarectal administration of 2 mL containing 135 mg/kg TNBS in 50% ethanol. The rabbits were housed for 3 days without treatment to maintain the development of a full inflammatory bowel disease model. The animals of groups III and IV received one tablet and one capsule, respectively, each containing 3 mg BUD once daily for five continuous days *via* gastric intubation. The animals of group V received an amount equivalent to 3 mg of commercial enema rectally. Animals were killed 24 h after the last drug administration.

Histopathological Study

Two tissue samples were excised from each colon and maintained in 10% (*v*/*v*) formalin in saline for histopathological evaluation. Sections of 5 μm were stained with hematoxylin and eosin. The histologic damage was evaluated.

Myeloperoxidase Activity

The activity of myeloperoxidase (MPO), which is found in neutrophils, can be used for evaluating the degree of inflammation in the intestine. The distal colon specimen (200 mg) was minced in a beaker containing ten times its amount of water for injection on ice, transferred to a test tube, and homogenized (Potter E1 Vejhem glass homogenizer, Poland) three times for 30 s each. The homogenate (0.5 mL) was mixed with 0.5 mL of HTAB buffer (0.5% HTAB in 50 mM phosphate buffer, pH 6.0) and the mixture was sonicated for 10 s and centrifuged at 10,000 rpm for 15 min. The supernatant was assayed for MPO activity. MPO activity was measured spectrophotometrically (UV-1601 PC spectrophotometer, Shimadzu, Kyoto, Japan): 0.1 mL of supernatant was combined with 2.9 mL containing 0.167 mg/mL *O*-diansidine hydrochloride and 0.0005% hydrogen peroxide. The change in absorbance at 460 nm was measured every minute for 4 min. One unit of MPO activity is defined as the amount which degrades 1 mmol of the peroxide per minute at 25°C (26). The results were expressed as the mean± standard deviation of the mean.

Statistical Analysis

One-way ANOVA test was performed to test the significance of the difference of the obtained results using SPSS.

RESULTS AND DISCUSSION

DSC Study

DSC studies (Fig. 1) revealed that BUD has one main prominent sharp characteristic, endothermic melting peak at 257.38°C. The sharp endothermic peak signifies that BUD used was in a pure crystalline state. The BUD peak was shortened in case of its PM with pectin. However, there was a complete disappearance in the endotherm in the case of PM with EUD S100, EUD L100, CAB, HPMC, and guar gum.

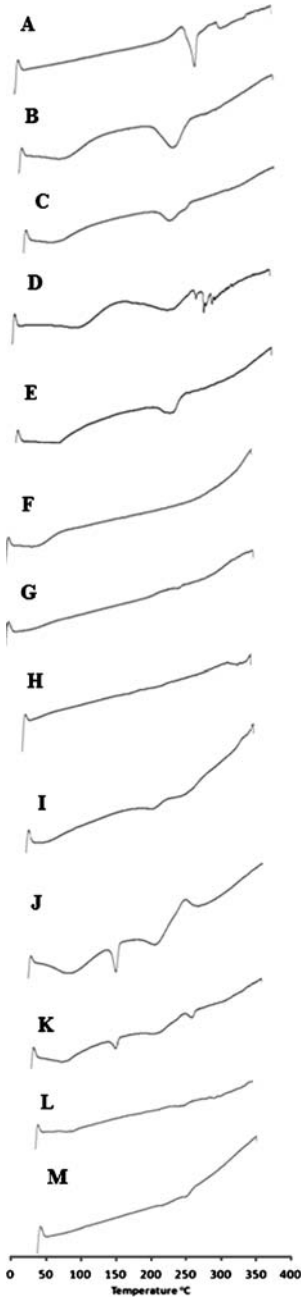

Fig. 1. DSC thermograms of *a* BUD, *b* EUD L100, *c* BUD–EUD L100 PM, *d* EUD S100, *e* BUD–EUD S100 PM, *f* HPMC, *g* BUD–HPMC PM, *h* CAB, *i* BUD–CAB PM, *j* pectin, *k* BUD–pectin PM, *l* guar gum, *m* BUD–guar gum PM

This is indicative of an interaction but not necessarily correspond to an incompatability (27).

Flow Properties of the Powder Blends

Table II shows the values of angle of repose, Carr's index, and Hausner ratio. The values of angle of repose of F1, F2, F3, and F4 were less than 25, indicating very good flowability. The values of other prepared formulas were in the range of 25–40, indicating reasonable flow properties.

Carr's index for powder blends of F5, F6, F16, and F17 were in the range of 23–35, indicating poor flow. Carr's index of F8, F10, F12, F19, and F20 were in the range of 12–16, indicating good flow, while those of other formulas were in the range of 18–21, indicating fair flow. However, Carr's index is a one-point determination and does not reflect the ease or speed with which consolidation occurs. Indeed, some materials have a high index, suggesting poor flow but may consolidate rapidly, which is essential for uniform filling on machine (28).

Hausner ratio is related to interparticle friction. Powders with low interparticle friction have ratios of approximately 1.2, whereas more cohesive, less flowing powders have ratios >1.6 (28). Hausner ratio values for the formulas F5, F6, F16, and F17 were between 1.2 and 1.6, whereas the values for all other formulas were approximately 1.2, indicating low interparticle friction. No formulas showed Hausner ratio >1.6.

Quality Control Tests of Compression-Coated Tablets

All prepared tablet formulations met the USP 27 requirements for weight variation and drug content. The friability of the prepared tablets was within the compendial limits except F16 coated with 100% guar gum which showed high friability percentage (1.46%) that exceeded the pharmacopeial limitation.

Table II. Flow Properties of the Different Powder Blends Used in Compression-Coated Tablets

Formula	Angle of Repose (θ)	Carr's index (%)	Hausner ratio
F1	19.29	19.04	1.23
F2	24.23	20.00	1.25
F3	21.80	20.00	1.25
F4	20.30	20.00	1.25
F5	39.79	25.00	1.33
F6	37.95	25.92	1.35
F7	34.45	20.00	1.25
F8	34.4	16.00	1.19
F9	38.66	20.00	1.25
F10	33.66	15.38	1.18
F11	33.02	17.85	1.21
F12	32.35	15.38	1.18
F13	29.12	19.04	1.23
F14	29.53	20.00	1.25
F15	31.22	19.04	1.23
F16	37.60	31.25	1.45
F17	34.91	25.00	1.33
F18	30.96	20.00	1.25
F19	29.25	16.00	1.19
F20	29.55	15.38	1.18

In Vitro Drug Release

pH-Dependent Polymers

Figure 2 shows that all tablets compression-coated with pH-dependent polymers showed low release percentage in 0.1 N HCl (pH 1.2) after 2 h ranging from 2.98% to 9.78%. Upon replacing 0.1 N HCl with phosphate buffer pH 6.8 for another 3 h, F1 coated with 100% EUD L100 exhibited the fastest release. It released 77.38% of the drug in 3 h. On the other hand, F2 coated with EUD S100 showed the slowest release in this medium where it released only 10.88%. Release of the drug from F3 coated with EUD L100 and EUD S100 in the ratio 1:1 was significantly higher than (44.38%) F4 coated with the same polymers but in the ratio 3:7 (34.8%) in 3 h. Upon completing the release in phosphate buffer pH 7.4 for another 19 h, F1 released most of the drug (97.6%) in the first hour. F2 released 35.99% of the drug after 19 h. The total percentage of the drug released the in case of F3 and F4 were 95.85% and 70.69%, respectively, after the 24 h of the release study.

EUD L100 and S100, used in these formulas, are copolymers of methacrylic acid and methyl methacrylate. The ratio of carboxyl to ester groups is approximately 1:1 in EUD L100 and 1:2 in EUD S100. It is worth noting that F1 coated with EUD L100 exhibited rapid release (88.74%) in the first 5 h. This might be due to the pH-dependent solubility of EUD L100 in pH 6, which, upon addition of phosphate buffer pH 6.8, dissolved rapidly. On the other hand, F2 coated with EUD S100 released only 49.857% in the whole time of the experiment (24 h). Most of the drug release occurred after the addition of phosphate buffer pH 7.4 (35.988%). This might be due to pH-dependent solubility of EUD S100 in pH above 7. The smaller rate and extent of drug release from F4 compared to F3 might be due to the presence of a higher amount of EUD S100 in F4.

The extent of drug release in the target area (from 5 to 24 h) could be arranged in descending order as follows: F3 (45.58%)>F2 (35.988%)>F4 (24.47%)>F1 (10.753%).

By fitting the release data up to 60% of BUD to the Korsmeyer–Peppas model, the values of n for all formulas except F1 were in the range $0.45<n\leq 0.89$, indicating anomalous (non-Fickian) transport. In these formulas, $K_1>K_2$, indicating greater diffusional contribution. As for F1, there was no enough data (more than three points) for fitting to this model.

Time-Dependent Polymers

For HPMC-coated tablets, F5 and F6 coated with 100% and 50% HPMC, respectively, remained intact all over the release time. Further decrease of HPMC percentage in the coat was accompanied by higher rate and extent of drug release where a decrease in the percentage of the polymer coat to 20% in F7 (Fig. 3) led to a slight increase in the release (13.56%) of the drug between the eighth and 24th hours. Also, further decrease in HPMC percentage to 10% in F8 showed the highest rate and extent where it released 11.06% of the drug after 5 h of the release and 43.55% at the end of the release experiment. The decrease in drug release by increasing HPMC concentration in the coat might be due

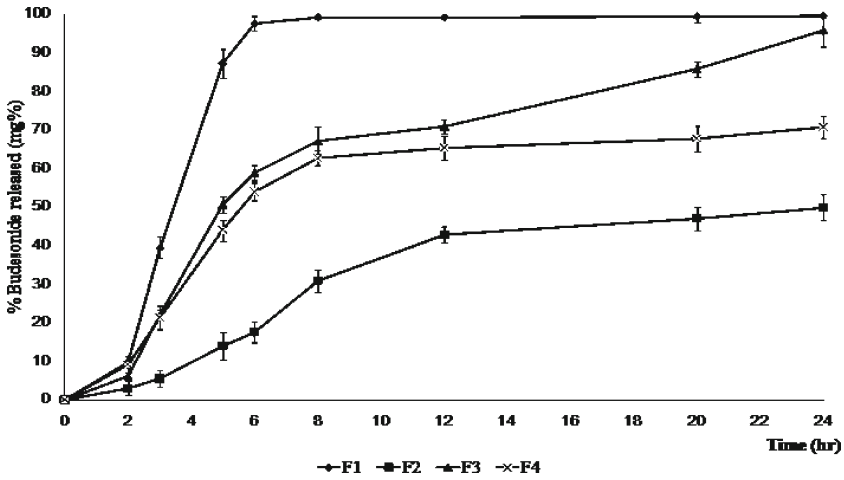

Fig. 2. Release profile of BUD from compression-coated tablets coated with pH-dependent polymers in 0.1 N HCl for 2 h, phosphate buffer (pH 6.8) for another 3 h, and phosphate buffer (pH 7.4) till the end of 24 h

to swelling of the polymer forming a gel layer. This gel layer was sufficiently resistant to withstand extensive erosion even after thorough hydration while allowing the outward diffusion of drug molecules to take place (29).

Coming to the formulas coated with CAB (Fig. 4), F9 coated with 100% polymer showed no drug release in the first 8 h; however, drug release started after that and reached 19.23% after 24 h. Upon decreasing the amount of CAB to 50% in F10, the drug started to be released in the fifth hour in phosphate buffer medium pH 6.8 and the cumulative percentage of the drug released was 45.08% at the end of the release. Further decrease in CAB percentage led to the release of the drug in pH 1.2 where F11 coated with 33% CAB released 4.73% after 2 h while F12 coated with 25% of the polymer released 14.55% of the drug. However, the cumulative percentage of the drug released in phosphate buffer pH 6.8 was 13.79% and 40.58% for F11 and F12, respectively.

The increase in the rate and extent of drug release upon decreasing CAB concentration might be due to the hydrophobic character of BUD which hindered its delivery through the hydrophobic coat. Therefore, the diffusion of BUD through the polymer wall became the controlling step of drug release.

The extent of drug release in the target area of the formulas coated with time-dependent polymers could be arranged in descending order as follows: F11 (51.004%)>F12 (50.141)>F10 (41.974)>F8 (31.984%)>F9 (17.462%)>F7 (12.125%).

By studing the kinetic release data, it was found that the values of n for all formulas coated with time-dependent polymers (except F7 and F9) were in the range $0.45<n\leq 0.89$, indicating anomalous (non-Fickian) transport. In all formulas undergoing anomalous transport, $K_1>K_2$, indicating greater diffusional contribution.

The n values for F7 and F9 were >0.89, indicating super case II transport, and $K_2>K_1$, which reflect greater relaxational contribution. The values for $n>1$ (super case II transport) would be the consequence of a plasticization process in the gel layer arising from a reduction of the attractive forces among polymeric chains that increases the mobility of macromolecules (30).

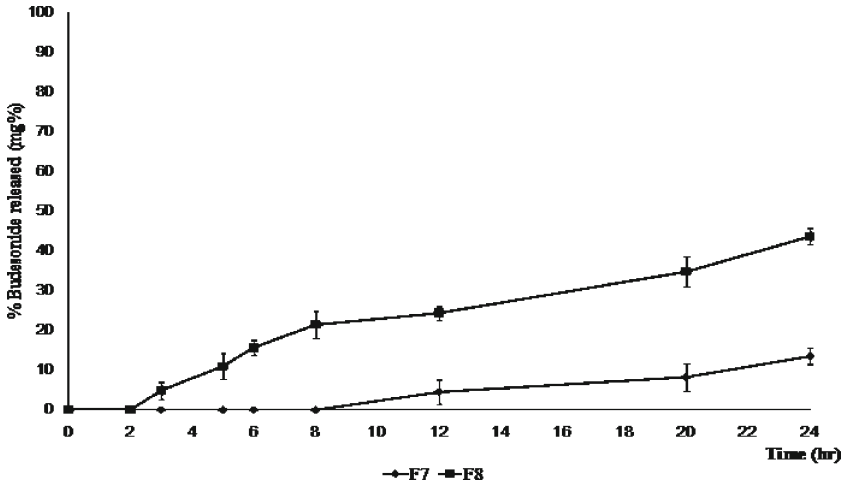

Fig. 3. Release profile of BUD from compression-coated tablets coated with HPMC as time-dependent polymer in 0.1 N HCl for 2 h, phosphate buffer (pH 6.8) for another 3 h, and phosphate buffer (pH 7.4) till the end of 24 h

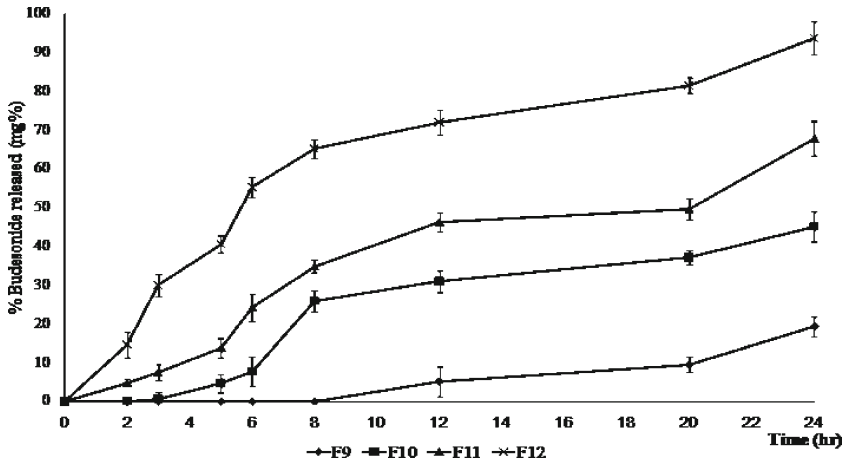

Fig. 4. Release profile of BUD from compression-coated tablets coated with CAB as time-dependent polymer in 0.1 N HCl for 2 h, phosphate buffer (pH 6.8) for another 3 h, and phosphate buffer (pH 7.4) till the end of 24 h

Enzyme-Dependent Polymers

It was found that the release of the tablets coated with pectin was dependent on the polymer concentration (Fig. 5). F13 coated with 100% pectin showed no drug release in the first 6 h of the release. Drug release started after that in pH 7.4 and reached 23.82% at the end of the release experiment. Decreasing the polymer percentage to 75% in F14 showed 4.59% of drug release in pH 6.8. The cumulative percentage of the drug released was 54.45% at the end of the release. F15 coated with 50% pectin released 3.10% of the drug in 0.1 N HCl while it released 23.57% through the subsequent 3 h in phosphate buffer pH 6.8. The cumulative percentage of the drug released was 81.54% at the end of the release.

In case of tablets coated with guar gum, F16 was excluded from the release study due to its high friability percentage (1.46%) which exceeded the pharmacopeial limitation. F17 coated with 50% guar gum was intact and showed no drug release all over the 24 h of the release study. Decreasing the amount of guar gum to 15% in F18 protected the tablets from pH 1.2 and 6.8, however, it caused slight increase in drug release (10.74%) in pH 7.4 till the end of the release experiment (Fig. 6). Further decrease in the polymer percentage to 10% and 5% in F19 and F20, respectively, led to drug release in pH 1.2. Both formulas showed almost similar drug release in the first 5 h. In 0.1 N HCl (pH 1.2), F19 released 4.58% of the drug while F20 released 3.79%. Upon replacing 0.1 N HCl with phosphate buffer (pH 6.8), the cumulative percentage of the drug released was 10.48% and 11.02% in case F19 and F20, respectively.

Pectin is a polysaccharide that consists of α-1,4-D-galacturonic acid and 1,2-D-rhamnose with D-galactose and D-arabinose side chains, while guar gum is a polysaccharide derived from the seeds of *Cyamopsis tetragonolobus*. It consists of linear chains of (1→4)-β-D-manopyranosyl units with α-D-galactopyranosyl units attached by (1→6) linkages (31). It is worth noting that, upon decreasing the concentration of pectin and guar gum, the rate and extent of drug release increased. This might have resulted from the polymer (either pectin or guar gum) becoming hydrated and swelling and forming a viscous gel layer that slows down with further

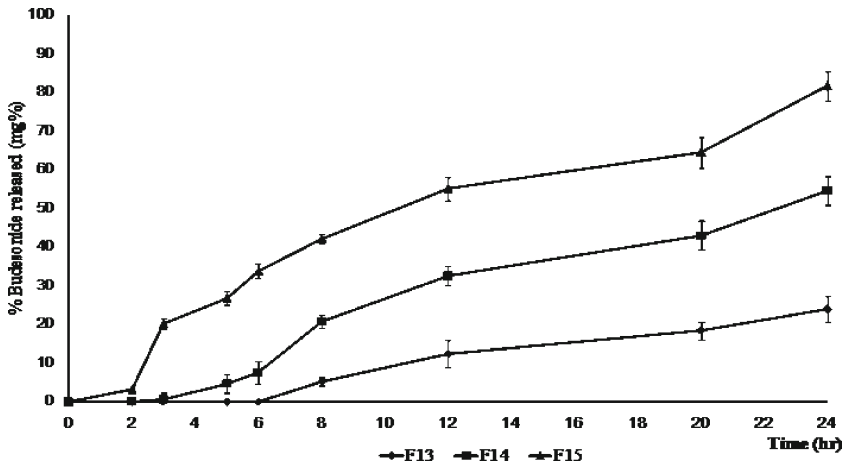

Fig. 5. Release profile of BUD from compression-coated tablets coated with pectin as enzyme-dependent polymer in 0.1 N HCl for 2 h, phosphate buffer (pH 6.8) for another 3 h, and phosphate buffer (pH 7.4) till the end of 24 h

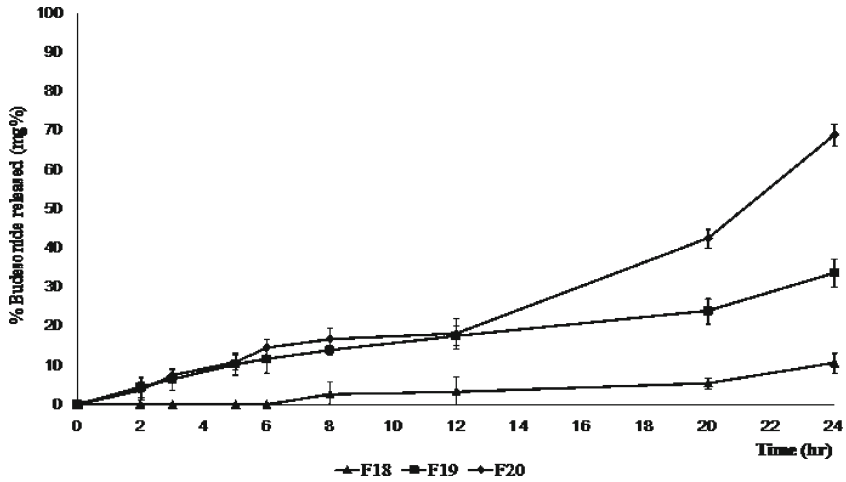

Fig. 6. Release profile of BUD from compression-coated tablets coated with guar gum as enzyme-dependent polymer in 0.1 N HCl for 2 h, phosphate buffer (pH 6.8) for another 3 h, and phosphate buffer (pH 7.4) till the end of 24 h

seeping-in of dissolution fluids toward the core tablets. When water reaches the core tablet, drug release takes place by diffusion which is supported by mechanical erosion of the swollen polymer.

The extent of drug release in the target area for enzyme-dependent polymers could be arranged in descending order as follows: F20 (55.985%)>F15 (53.757%)>F14 (49.725%)> F19 (22.664%)>F13 (22.655%)>F18 (10.487%).

Fitting of BUD data to the Korsmeyer–Peppas model from different compression-coated tablet formulas containing enzyme-dependent polymers showed that the values of n for all formulas except F13 were in the range $0.45<n\leq 0.89$, indicating anomalous (non-Fickian) transport. In case of F14 and F15 undergoing anomalous transport, $K_1>K_2$, indicating greater diffusional contribution, while in the case of F18, F19, and F20, it was found that $K_2>K_1$, indicating greater relaxational contribution. The n values for F13 were >0.89, indicating super case II transport, and $K_2>K_1$, which reflect greater relaxational contribution.

Drug release from pectin- and guar gum-based formulations was repeated in the presence of pectinolytic and galactomannanase enzyme in the dissolution medium of phosphate buffer pH 7.4 for the most promising pectin- and guar gum-coated tablets, respectively.

For pectin-coated tablets, F14 and F15 were considered promising because they released 49.72% and 53.75% of drug, respectively, in the target area, so they were compared by the percentage of drug release before the target area. The percentage of drug released from F14 before target was only 4.735% while it was 27.792% in the case of F15. In other words, drug that missed the target in the case of F14 is less than that in F15. So, F14 (coated with 75% pectin) was more promising and was chosen for repetition of its release. For guar gum-coated tablet, F20 (coated with 5% guar gum) was chosen because it released 55.98% of drug in the target area.

Figure 7 shows the percentage of the drug released from selected formulas containing enzyme-dependent polymers in the presence of enzymes (F14E and F20E) in comparison with release in their absence (F14 and F20). No drug was released in the first 2 h in the case of F14E coated with pectin. This formula released 8.02% of the drug in pH 6.8. The cumulative percentage of the drug released was 99.31% at the end of the release. F20E coated with guar gum released 2.00% of the drug in 0.1 N HCl (pH 1.2) while it released 14.74% through the subsequent 3 h in phosphate buffer pH 6.8. The cumulative percentage of the drug released was 72.13% at the end of the release.

It is worth noting that addition of pectinolytic enzyme during dissolution of F14 significantly increased drug release in the target area from 49.725% to 91.288%. This might be due to increased destruction of pectin chains which might led to faster erosion rate (18). Upon addition of galactomannanase enzyme during dissolution of F20, drug release in the target area increased from 55.985% to 57.39%; however, this difference was not significant. This might be because guar gum and Avicel combine to form a nondisintegrating, swellable matrix; furthermore, there might be physical cross-

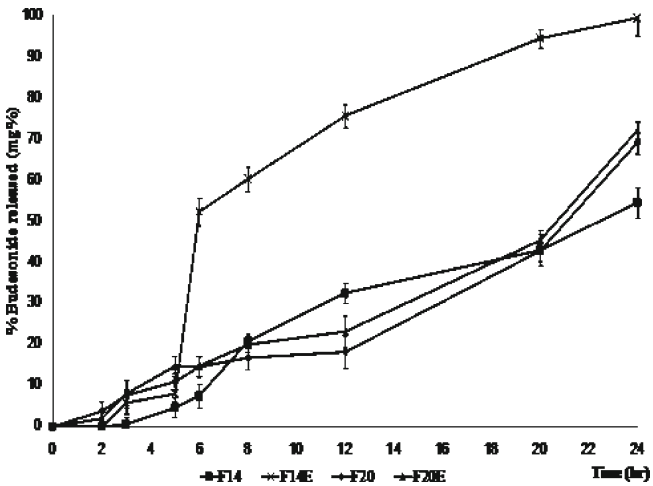

Fig. 7. Release profile of BUD from compression-coated tablets coated with pectin and guar gum in the presence of pectinolytic and galactomannanase enzymes, respectively, in 0.1 N HCl for 2 h, phosphate buffer (pH 6.8) for another 3 h, and phosphate buffer (pH 7.4) till the end of 24 h

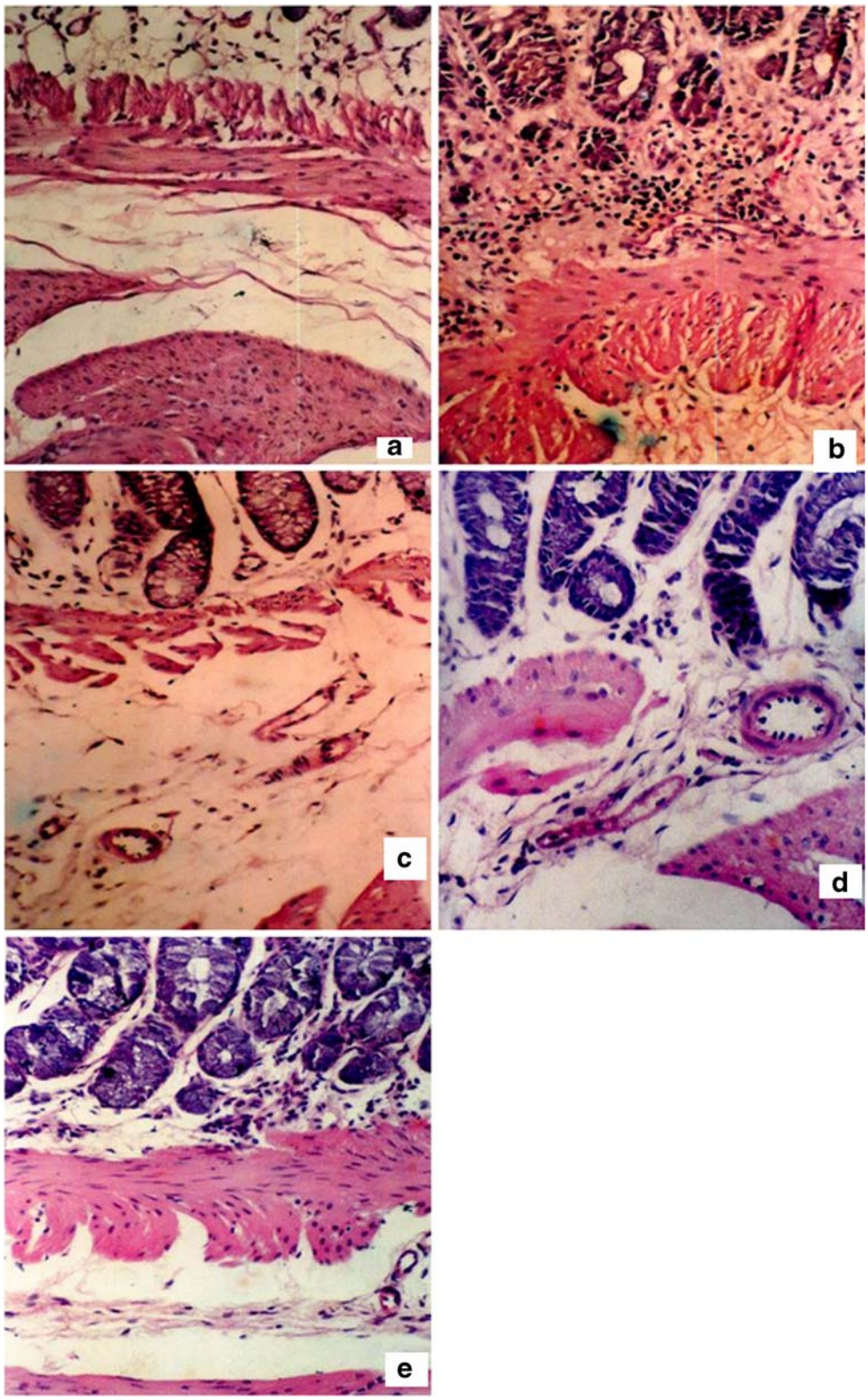

Fig. 8. Histological appearance of colonic tissues **a** normal group, **b** TNBS-induced colitis group, **c** compression-coated tablet-treated group, **d** commercial capsule-treated group, and **e** commercial enema-treated group

linking between Avicel and guar gum that hindered the effect of galactomannanase enzyme [32].

Fitting of BUD release data to the Korsmeyer–Peppas model from the selected formulas containing enzyme-dependent polymers in the presence of enzymes showed that the values of n for F14E and F20E were in the range $0.45 < n \leq 0.89$, indicating anomalous (non-Fickian) transport. In the case of F14E, $K_1 > K_2$, indicating greater diffusional contribu-

tion, while in the case of F20E, $K_2 > K_1$, indicating greater relaxational contribution.

A comparison was made between the most promising formulas from each class of polymers used (pH-, time-, and enzyme-dependent). The parameter of choice was the extent of drug released in the target area. F3 was selected from pH-dependent formulas, F11 and F12 were selected from time-dependent formulas, and F14 was selected from enzyme-dependent formulas. It was found that the amount released from F14 in the presence of pectinolytic enzymes (F14E) gave the most significant highest effect compared to others ($P<0.05$). So, it was chosen for further *in vivo* studies.

In Vivo Study

Histopathological Evaluation

Figure 8a shows the photomicrographs of the colon of the control group. The normal colon is formed of mucosa, submucosa, musculosa, and serosa. The mucosa is differentiated into glands, connective tissue core around the glands, and muscularis mucosa. In the case of the colitis group (Fig. 8b), the glandular epithelium showed edema. The connective tissue core showed mononuclear infiltration till the muscularis mucosa. Vasculitis with perivascular cellular infiltration is evident. Figure (8c, d) revealed that there was marked reduction of inflammatory cells observed in the connective tissue core of groups III and IV treated with F14 (75% pectin compressed-coated BUD tablets) and the commercial capsules, respectively. However, moderate reduction was observed in the case of group V receiving the commercial enema product (Fig. 8e).

Myeloperoxidase Activity

MPO activity, which is an important quantitative index for colonic inflammation, was determined in terms of units per gram tissue weight. MPO activity for normal control group was 3.57±0.26 U/g tissue weight. However, MPO activity of the induced colitis group was found to be 9.16± 1.52 U/g tissue weight. This MPO was significantly decreased to 3.80±0.37 and 3.90±0.62 U/g tissue weight in the case of the commercial capsule-treated group and compression-coated tablet group, respectively. However, the MPO activity of the enema-treated group was slightly higher (4.36 U/g tissue weight).

CONCLUSION

Based on the amount of drug released in the target site (colon), a blend of EUD L100 and EUD S100 in the ratio 1:1 as a pH-dependent polymer, 25% CAB as a time-dependent polymer, and 75% pectin as an enzyme-dependent polymer were considered optimum polymers for the formulation of BUD compression-coated tablets for colonic delivery. The study showed that formula F14 could produce site-specific drug release in the colon with efficient treatment of irritable bowel disease.

REFERENCES

1. M. K. Chourasia, and S. K. Jain. Pharmaceutical approaches to colon targeted drug delivery systems. *J. Pharm. Pharm. Sci.* **6**:33–66 (2003).
2. A. W. Basit. Advances in colonic drug delivery. *Drugs*. **65**14:1991–2007 (2005).
3. A. Jain, Y. Gupta, and S. K. Jain. Azo-chemistry and its potential for colonic delivery. *Crit. Rev. Ther. Drug Carrier Syst.* **25**:349–400 (2006).
4. G. L. Simon, and S. L. Gorbach. The human intestinal microflora. *Dig. Dis. Sci.* **31**9 Suppl:147S–162S (1986).
5. S. L. Gorbach. Intestinal microflora. *Gastroenterology.* **60**6:1110–1129 (1971).
6. A. Rubinstein. Microbially controlled drug delivery to the colon. *Biopharm. Drug Dispos.* **11**6:465–475 (1990).
7. V. R. Sinha, and R. Kumria. Polysaccharides in colon-specific drug Delivery. *Int. J. Pharm.* **224**:19–38 (2001).
8. V. R. Sinha, and R. Kumria. Microbially triggered drug delivery to the colon. *Eur. J. Pharm. Sci.* **18**:3–18 (2003).
9. L. S. Liu, M. L. Fishman, J. Kost, and K. B. Hicks. Pectin-based systems for colon-specific drug delivery *via* oral route. *Biomaterials.* **24**:3333–3343 (2003).
10. S. A. Johansson, K. E. Andersson, R. Brattsand, E. Gruvstad, and P. Hedner. Topical and systemic glucocorticoid potencies of budesonide, beclomethasone dipropionate and prednisolone in man. *Eur. J. Respir. Dis. Suppl.* **122**:74–82 (1982).
11. S. Edsbacker, P. Wollmer, A. Nilsson, and M. Nilsson. Pharmacokinetics and gastrointestinal transit of budesonide controlled ileal release (CIR) capsules. *Gastroenterology.* **104**:695 (1993).
12. Y. Krishnamachari, P. Madan, and S. Lin. Development of pH- and time-dependent oral microparticles to optimize budesonide delivery to ileum and colon. *Int. J. Pharm.* **338**:238–247 (2007).
13. M. Rodriguez, J. A. Antu!nez, C. Taboada, B. Seijo, and D. Torres. Colon-specific delivery of budesonide from microencapsulated cellulosic cores: evaluation of the efficacy against colonic inflammation in rats. *J. Pharm. Pharmacol.* **53**:1207–1215 (2001).
14. Y. Ozeki, Y. Kondo, Y. Watanabe, K. Shimada, K. Harada. Rotary type compression molding machine. PCT WO02/90098, 14 November (2002).
15. P. E. Luner, L. E. Kirsch, S. Majuru, E. Oh, A. B. Joshi, D. E. Wurster, and M. P. Redmon. Preformulation studies on the S-isomer of oxybutynin hydrochloride, an Improved Chemical Entity (ICE). *Drug Dev. Ind. Pharm.* **27**4:321–329 (2001).
16. R. L. Carr. Classifying flow properties of solids. *Chem. Eng.* **72**:69–72 (1964).
17. H. H. Hausner. Friction condition in a mass of metal powders. *Int. J. Powder Metall.* **3**:7–13 (1967).
18. M. Turkoglu, and T. Ugurlu. *In vitro* evaluation of pectin–HPMC compression coated 5-aminosalicylic acid tablets for colonic delivery. *Eur. J. Pharm. Biopharm.* **53**1:65–73 (2002).
19. D. Wong, S. Larrabee, K. Clifford, J. Tremblay, and D. Friend. USP dissolution apparatus III (reciprocating cylinder) for screening of guar-based colonic delivery formulations. *J. Control. Release.* **47**:173–179 (1997).
20. P. L. Ritger, and N. A. Peppas. A simple equation for description of solute release II. Fickian and anomalous release from swellable devices. *J. Control. Release.* **5**:7–42 (1987).
21. N. A. Peppas, and J. J. Sahlin. A simple equation for the description of solute release. III. Coupling of diffusion and relaxation. *Int. J. Pharm.* **57**3:169–172 (1989).
22. J. Siepmann, and N. A. Peppas. Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). *Adv. Drug Deliv. Rev.* **48**2–3:139–157 (2001).
23. H. Kim, and R. Fassihi. Application of a binary polymer system in drug release rate modulation. 1. Characterization of release mechanism. *J. Pharm. Sci.* **86**3:316–322 (1997).
24. W. H. Percy, M. B. Burton, F. Fallick, and R. Burakoff. A comparison *in vitro* of human and rabbit distal colonic muscle responses to inflammatory mediators. *Gastroenterology.* **99**:1324–1332 (1990).
25. I. Depoortere, G. Van Assche, T. Thijs, K. Geboes, and T. L. Peeters. Differential changes in ACh-, motilin-, substance P-, and K(+)-induced contractility in rabbit colitis. *Am. J. Physiol.* **277**1 Pt 1:G61–G68 (1999).

26. N. Cui, D. R. Friend, and R. N. Fedorak. A budesonide prodrug accelerates treatment of colitis in rats. *Gut.* **35**10:1439–1446 (1994).
27. H. Sekizaki, K. Danjo, H. Eguchi, Y. Yonesawa, H. Sunada, and A. Otsuka. Solid-state interaction of ibuprofen with polyvinylpyrrolidone. *Chem. Pharm. Bull.* **43**6:988–993 (1995).
28. J. I. Wells. Pharmaceutical preformulation: the physicochemical properties of drug substances, Elis Horwood, Chichester, UK, 1988, p. 86.
29. M. E. Sangalli, A. Maroni, A. Foppoli, L. Zema, F. Giordano, and A. Gazzaniga. Different HPMC viscosity grades as coating agents for an oral time and/or site-controlled delivery system: a study on process parameters and *in vitro* performances. *Eur. J. Pharm. Sci.* **22**5:469–476 (2004).
30. J. M. Llabot, R. H. Manzo, and D. A. Allemandi. Drug release from carbomer:carbomer sodium salt matrices with potentialnext term use as previous termmucoadhesive drug delivery system. *Int. J. Pharm.* **276**1–2:59–66 (2004).
31. A. M. Goldsten, E. N. Alter, and J. K. Seaman. Guar gum. In R. L. Whistler (ed.), Oral colon specific drug delivery and their derivatives, Academic, Florida, pp. 1–43 (1992).
32. T. Durig, and R. Fassihi. Guar-based monolithic matrix systems: effect of ionizable and non-ionizable substances and excipients on gel dynamics and release kinetics. *J. Control. Release.* **80**:45–56 (2002).