Drug Development and Industrial Pharmacy, 2009; 35(7): 788–795

informa
healthcare

RESEARCH ARTICLE

In vitro drug release and in vivo human X-ray studies of ileo-cecal targeting budesonide fast disintegrating tablet

S.J. Kshirsagar, M.R. Bhalekar and R.P. Pawar

AISSMS College of Pharmacy, Kennedy Road, Pune, Maharashtra, India

Abstract

Crohn's disease is a type of inflammatory bowel disease that frequently affects the ileo-cecal region of the gastrointestinal tract. For effective treatment of this disease, a site-targeting drug in the ileo-cecal region is essential. Budesonide (BD) is a synthetic, non-halogenated glucocorticoid and is the drug of choice for the treatment of Crohn's disease. The present study is an attempt to develop the dosage form of a BD tablet to achieve targeted drug release in the ileo-cecal region. The BD tablets are coated with Eudragit FS 30 D, which is a polymer that specifically dissolves at and above pH 6.8. The in vitro drug release and in vivo tablet disintegration (using X-ray radiography) were carried out. The coating process was optimized successfully. The in vitro performance of the tablet with coating thickness showed that the tablet did not disintegrate till 4.5 hours, which represents the transit time to the ileo-cecal region. In vivo studies also established that the tablet lasted till 4.5 hours. The tablet containing 0.5% superdisintegrant and 10% coating thickness was able to deliver BD effectively to the ileo-cecal region, thus making it a promising drug delivery system for the treatment of Crohn's disease.

Key words: *Crohn's disease; Eudragit FS 30 D; fast disintegrating; ileo-cecal targeting; in vivo X-ray study*

Introduction

The aim of any drug delivery system (DDS) is to provide a therapeutic amount of drug to the proper site in the body, so that the desired drug concentration can be achieved promptly and then maintained[1]. Oral drug delivery is the preferred route of administration, being non-invasive mode of delivery and has good level of patient compliance, and offers flexibility in formulation. Site-specific drug delivery refers to the targeting of a drug directly to a certain biological location, where the target is adjacent to or in the diseased organ or tissue[2]. Crohn's disease is a type of inflammatory bowel disease that occurs frequently in the ileo-cecal region of the gastrointestinal (GI) tract. This disease affects all three layers of the bowel wall (inner lining, muscular layer, and outer lining). The main pathological features of Crohn's disease are ulcers (shallow and deep), connection of the bowel lumen with the surrounding structures (fistulae), and the scar tissue leading to narrowing of the bowel lumen (strictures causing lumen obstruction). Inflammation of the lining of the gut causes symptoms of diarrhea, which may be streaked with blood or mucus if the colon is involved. It is usually associated with the episode of severe abdominal pain. Chronic and extensive Crohn's disease is associated with an increased risk of adenocarcinoma of the colon[3,4].

Budesonide (BD) is a synthetic, non-halogenated glucocorticoid that has higher intrinsic potency than cortisol and prednisolone with a high ratio of topical to systemic anti-inflammatory activity, as a result of its strong affinity for corticosteroid receptors and rapid first-pass metabolism in the liver[5,6]. Site specificity of the drug to the ileo-cecal region and the proximal colon, the most common sites of Crohn's disease, would be very valuable to minimize the proximal drug absorption and allow a high concentration of drug to come in contact with the inflamed intestinal mucosa.

To develop a reliable drug delivery that target a particular site of the GI tract demands thorough understanding of the transit time and pH gradients throughout

Address for correspondence: S.J. Kshirsagar, Assistant Professor, AISSMS College of Pharmacy, Kennedy Road, Near RTO, Pune 411 001, Maharashtra, India. E-mail: sanjayjk@rediffmail.com

(Received 3 Aug 2008; accepted 2 Nov 2008)

ISSN 0363-9045 print/ISSN 1520-5762 online © Informa UK, Ltd.
DOI: 10.1080/03639040802596204

the GI tract. Gastric transit time of single unit non-disintegrating dosage forms has been reported to vary from 15 minutes to more than 3 hours. At the same time, the small intestinal residence time is fairly constant and varies between 3 hours and 4 hours[7]. The values of the pH gradient in the GI tract ranges from 1.2 in the stomach through 6.6 in the proximal small intestine to a peak of about 7.5 in the distal small intestine (ileum). This pH difference between the stomach and the small intestine has historically been exploited to deliver drugs to the small intestine by way of pH-sensitive enteric coating[8]. These polymer coatings are recalcitrant to the acidic condition of the stomach but ionize and dissolve above a certain threshold pH found in the small intestine. For example, methacrylic acid copolymers are the most commonly used enteric polymers, which dissolve at pH 5.5 (stomach), 6.0 (proximal small intestine), and 7.0 (distal small intestine)[9]. The pH in the terminal ileum could rise to 7.5; hence, the delivery devices coated with these polymers have a tendency to release their drug load before reaching the colon, and are more appropriately defined as ileo-colonic delivery systems[10]. A recently developed copolymer of methyl acrylate, methyl methacrylate, and methacrylic acid[11,12] is commercially available as Eudragit® FS 30 D[10]. This polymer has a similar threshold dissolution pH as Eudragit® S, but dissolves in a slower and more controlled manner[13]. A series of in vitro dissolution studies indicated that the tablets[10], or the beads[14], or the pellets[12] coated with this polymer would be more appropriate for drug delivery to the ileo-cecal region in comparison with the more established Eudragit® S. However, the drug carrier performance of the Eudragit® FS 30 D-coated preparations are yet to be investigated completely in view of the complexity of the drug release from enteric-coated preparations[8].

It is very important to correlate the in vitro performance of colon-specific formulation with in vivo studies for ascertaining site specificity due to the diverse conditions in the GI tract of the formulations targeted to the ileo-cecal region for its site specificity. The in vivo performance of Eudragit® FS 30 D-coated preparations is of interest[11,15,16,10]. Recently, the in vivo performance of colon-specific DDS was evaluated successfully using X-ray imaging[17]. On the basis of this study, in vivo X-ray imaging for optimized FS 30 D-coated BD tablets was carried out.

The objective of the present study was to prepare a tablet containing BD, coated with polymer providing immediate release at the ileo-cecal site, the most affected area in Crohn's disease. In this study, the novel Eudragit FS 30 D-coated fast disintegrating tablets were developed for ileo-cecal targeting. BD was used as a model drug because of its therapeutic potential for Crohn's disease. The manufacture and the in vitro release characteristics of the system were described; especially, the in vivo performance in human volunteers was evaluated by examining placebo tablet using X-ray radiography. The present study would also provide the suitable coating thickness for Eudragit FS 30 D, so that the drug would ensure the ileo-cecal delivery efficiency of this system.

Materials and methods

BD was obtained as a gift from Mepro Pharmaceuticals Pvt. Ltd. (unit II) (Wadhawan, India). Eudragit FS 30 D was obtained from Degussa India Pvt. Ltd. (Mumbai, India) as a gift. HPMC 15 cps was a gift from Colorcon India Pvt. Ltd. Other excipients used to prepare tablets and for coating were of standard pharmaceutical grade, and all other chemical reagents used were of analytical grade.

Formulation and evaluation of budesonide tablets

Tablets were prepared by direct compression formula (Table 1) using tablet compression machine (mini press-II, Rimek; Karnavati Engineering Ltd., Mehsana, Gujarat, India). Tablets were biconvex in design, 6 mm in diameter, and 100 mg in mass. The dose of BD in each tablet was 9 mg, which is the recommended daily dose of BD in active Crohn's disease (www.EntocortEC.com). Three different tablet formulations of BD, F1, F2, and F3, were prepared and evaluated. Formulation F1 contained 5% microcrystalline cellulose, formulations F2 and F3 contained sodium starch glycolate as superdisintegrating agent in concentrations 0.5% and 1%, respectively. The tablets were subjected to hardness testing using Monsanto type hardness tester (Magumps), and the friability was tested using Roche friability tester (Veego). The disintegration test was performed using USP disintegration test apparatus (Veego). Drug content was determined on a pooled sample of 20 tablets, the amount equivalent to 9 mg BD was taken from the crushed tablets and dissolved in methanol, the amount of BD in filtrate was determined using the UV spectrophotometer (V-530 Jasco) at 245 nm.

Table 1. Formulation details of budesonide tablet.

Formulation mg/tablet	F1	F2	F3
Budesonide BP	9	9	9
Microcrystalline cellulose	5	5	5
Sodium starch glycolate	–	0.5	1
Magnesium stearate	2	2	2
Talc	2.5	2.5	2.5
Lactose anhydrous	81.5	81	80.5

Preparation of HPMC nonaqueous barrier film-coating system

The purpose of this coating was to form a barrier in between the BD core tablet containing superdisintegrant and outer Eudragit FS 30 D aqueous coating in order to avoid the penetration of dissolution medium into the core of the tablet. The HPMC (15 cps) polymer was gradually added to the ethyl alcohol with continuous agitation. A portion of methylene chloride was added to this suspension to dissolve the polymer. The plasticizer propylene glycol was then added and the volume was made up with methylene chloride (Table 2).

HPMC barrier film coating of tablets

Coating was undertaken in a 12-inch diameter stainless steel coating pan containing baffles (Insta coat; R & D coater, Mumbai). The test tablets (5 g) were "bulked out" with 50 g placebo tablets. Drying air (inlet air) was introduced into the front of the pan approximately perpendicular to the tablet bed. The coating procedure involved in maintaining the inlet temperature at 55°C, spray rate at 1 g/min, nozzle diameter of 0.8 mm, and atomizing pressure at 0.2 bars. The weight gain of 1%–2% was the desired coating level. The average disintegration time of the HPMC-coated tablet was also found to be less than 1 minute, which means that the HPMC coating does not alter the disintegration time of the tablet.

Preparation of aqueous dispersion containing Eudragit FS 30 D

The composition of the coating dispersions containing Eudragit FS 30 D is shown in Table 3. Aqueous Eudragit FS 30 D dispersion as film former, Tween 80 as a wetting agent, and glyceryl monostearate as a glident were added to water, and the mixture was heated at 60°C with stirring for 10 minutes until a fine homogenous dispersion was obtained. After cooling, this dispersion was gently added to Eudragit FS 30 D dispersion and mixed using magnetic stirrer. This coating dispersion does not need a plasticizer because it exhibits a minimum film-forming temperature of 14°C and a low glass transition temperature[18].

Table 2. Formulation details of HPMC non aqueous coating solution.

Sr. no.	Ingredients	Quantity (%)
1	HPMC USP 15 cps	4
2	Colorant (sunset yellow)	1
3	Propylene glycol USP	1.2
4	Ethyl alcohol	45
5	Dichloromethane	q.s. 100

Table 3. Formulation details of Eugragit FS 30 D aqueous coating solution.

Sr. no.	Ingredients	Quantity, w/w
1	Eudragit FS 30D USP	63%
2	Glyceryl monostearate	0.93%
3	Tween 80	1.17%
4	Water	35.65%

Coating of HPMC film-coated tablets with Eudragit FS 30 D aqueous dispersion

The coating dispersion was passed through 0.25-mm sieve before use. HPMC-coated tablets were used for Eudragit FS 30 D aqueous dispersion coating in the same coating pan used for HPMC coating. The spray rate and the bed temperature during the coating process were 2 g/min and 30°C–35°C, respectively (till the end of the process). Before coating, the tablets were preheated to the desired bed temperature for 15 minutes. The tablets were coated to 5%, 10%, 15%, and 20% (w/w) total weight gain.

Drug release studies

The dissolution studies of the coated tablets were carried out using USP XXIII dissolution type II apparatus (DA 6D Veego) at a rotation speed of 100 rpm, at 37°C. The tablets (n = 3) were transferred to the dissolution medium and the samples were taken at selected time intervals, filtered through Whatman filter paper no. 41, and analyzed using an UV spectrophotometer (V-530 Jasco) at 245 nm.

The continuous dissolution method USP XXIII was used for simulating the conditions of the GI tract. Initially, tablets were added in 700 mL of 0.1 N HCl (pH 1.2) for 2 hours. At the end of 2 hours, 233.3 mL tribasic sodium phosphate solution USP was added to all the dissolution vessels, and the pH was adjusted to 6.5 for 1 hour, 6.8 for the next 2 hours, and 7.2 till the end of the study using 2 M NaOH or 2 M HCl[7].

In vivo studies

X-ray imaging was used to monitor the tablets throughout the GI system. Six healthy male volunteers, with a mean age of 29 years (range 22–40) and 50–80 kg body weight, participated in the in vivo studies. They were non-alcoholics, non-smokers, and had not taken any drugs. The purpose of the study was fully explained, and written consent was received from all the volunteers. Each subject orally ingested barium sulfate containing core tablet coated with FS 30 D polymer, three volunteers received tablets with 10% (w/w) coating level and the other three received tablets with 15% (w/w) coating level with 200 mL water, after an overnight fast. Abdominal radiographs were taken at fixed time intervals, and

the tablets were visualized using digital X-ray imaging (CR30X AGFA, MSXML 4.0). Tablets were visualized to determine whether they were located in the terminal ileum or colon for 7 hours. Volunteers were served food 2 hours (breakfast) and 4 hours (lunch) after the administration of the tablets. The Ethics Committee of the B.J. Medical College (Maharashtra University of Health Science), in accordance with the internationally accepted principles, had approved the experimental protocol. Each volunteer received about 0.1 rem of radiation during the GI X-ray radiograph; normally, in a routine abdominal investigation with barium sulfate, a patient receives 0.7 rem of radiation[19]. Therefore, the total radiation dose (about <0.7 rem) received by each volunteer was not found to be higher than that of the standard abdominal radiography.

Scanning electron microscopy

Scanning electron microscopy of optimized formulation (formulation F2 with 10% [w/w] coating level) was carried out to study the morphology of the polymeric films. Transverse section of each sample was scanned using a JEOL JSM-6360 scanning electron microscope at 30× and 1000× magnifications.

Results and discussion

Evaluation of budesonide core tablets

The compressed tablets were subjected to tests, such as thickness, hardness, friability, disintegration time, and percentage of drug content. The results of these tests are given in Table 4. All formulations were within specifications for weight variation, drug content, hardness, and friability. Formulation F1, which contains microcrystalline cellulose, showed mean disintegration time of 8.54 ± 0.5 min. Formulations F2 and F3 contain 0.5% and 1% of superdisintegrant, both the formulations showed almost the same disintegration time periods of 0.28 minute and 0.27 minute; therefore, formulation F2 was selected as optimum formulation containing superdisintegrant for the subsequent study.

In vitro release study

The transit time in the small intestine is relatively constant (approximately 3–5 hours)[7,20]. The simulation of conditions in small intestine was divided into three parts: proximal part of the small intestine with pH 6.5 and residence time 1 hour, lower part of the small intestine with pH 6.8 and residence time 2 hours, and finally, terminal ileum with pH 7.2 till the end of the test. Thus, the continuous dissolution test was carried out for coated BD tablets with pH 1.2, 6.5, 6.8, and 7.2 for 2, 1, 2 hours, and till the end of the study, respectively. The release profiles of BD tablets of different coating weight gain are shown in Figure 1.

At pH 1.2 (simulated stomach) and pH 6.5 (proximal part of the small intestine), none of the formulations released the drug, and after 3 hours the tablets were intact, except the tablet with 5% (w/w) coating level that showed 20% drug release. After 4 hours at pH 6.8, no release was observed except tablets with 5% (w/w) coating level, which shows above 50% drug release. The tablets with 10% (w/w) coating level shows near 15% drug release within 4.5 hours and 100% drug release at pH 7.2 within 30 minutes. This complete release can be attributed to the dissolution of the Eudragit FS 30 D and the immediate release effect of superdisintegrant. It was also observed that the lag time at pH 7.2 increases as the % (w/w) of coating polymer Eudragit FS 30 D increased (formulations with 15% and 20%, w/w), because of increased diffusion path length and tortuosity at higher coating levels[7]. Polymers used for targeting the drug at the most distal part of the GI tract should withstand the lower pH values of the stomach and proximal part of the small intestine, and should also be able to disintegrate at neutral to slightly alkaline pH of the ileum[9]. The coating level of more than 20% (w/w) was not selected for subsequent study because the formulation with lower coating levels has advantages such as lower cost, reduction in processing time, and lower weight and smaller size of final dosage form[7]; hence, it would also result in higher lag time (more than 7 hours) that may correspond to the descending part of the colon where drug absorption is negligible.

Table 4. Core tablets evaluation results ($n = 3$).

Formulations	Weight variation	Thickness (mm)	Hardness (kg/cm^2)	% Friability	Disintegration time (DT) (minute)	% Drug content
F1	0.101 ± 0.002	3.52 ± 0.002	5–6	0.3	8.54 ± 0.5	99.25 ± 0.45
F2	0.10 ± 0.001	3.50 ± 0.002	5–6	0.27	0.28 ± 0.36	98.3 ± 0.35
F3	0.10 ± 0.001	3.52 ± 0.002	5–6	0.25	0.27 ± 0.3	98.5 ± 0.46

$n = 3 \pm$ SD.

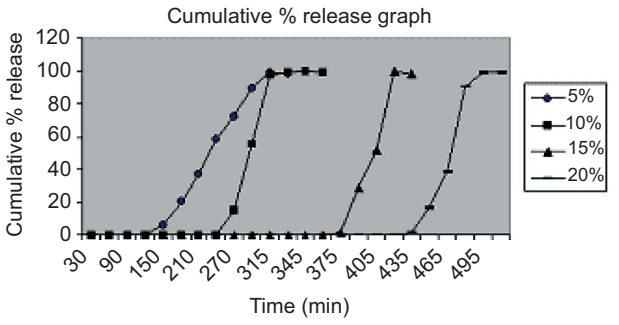

Figure 1. Release profile of Budesonide-coated tablets. Dissolution profile budesonide tablets with 5, 10, 15, and 20% w/w of Eudragit FS 30D at pH 1.2 (2 hours), 6.4 (1 hour), 6.8 (2 hours), and 7.2 till end in 0.2 M phosphate buffer.

Testing the suitability of Eudragit FS 30 D-coated tablets for ileum targeting

It is also possible in some cases, similar to Crohn's disease, that the pH value of small intestine does not approach to 7.0, it may be less than 7.0; therefore, it is important that the drug formulated for ileum targeting to be released at pH 6.8 after a suitable lag time[21,20]. Eudragit FS 30 D is a relatively novel pH-dependent methacrylic acid polymer for colonic delivery purposes. The earlier reported threshold pH values for this polymer was 6.8[7], 7.5[12], 7.2 or above[8]. To test the suitability of Eudragit FS 30 D for ileo-cecal targeting, the pH dissolution profile of the tablet with the 10% coating level was investigated in 0.2 M phosphate buffers at pH 6.8 and 7.2, after 2 hours incubation in 0.1 M HCl (Figure 2).

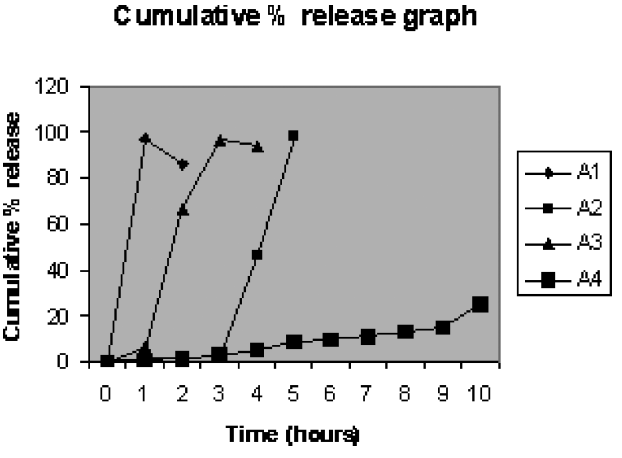

Figure 2. Release profiles of budesonide from tablets with Eudragit® FS 30 D of 10% and 15% (w/w) coating level after 2 hours in 0.1 M HCI (pH 1.2) and subsequently 6 hours in phosphate buffer solution at 6.8 and 7.2 pH. A1 and A2 represents tablets with 10% (w/w) coating level in 7.2 and 6.8 pH, respectively. A3 and A4 represents tables with 15% (w/w) coating level in 7.2 and 6.8 pH, respectively.

The selection criteria of optimum formulation for targeting the drug to the ileo-cecal region in patients with Crohn's disease is the formulation that shows immediate release of the drug at pH 7.2 (pH of distal part of the small intestine) and also shows the release at pH 6.8 after a sufficient lag time.

Dissolution studies of Eudragit FS 30 D-coated polymer in different pH indicates that after 1 hour 100% drug was released from 10% (w/w) tablet at pH 7.2, this can be considered as a critical pH value for this polymer, which is in agreement with Huyghebaert et al.[18] and Gao et al.[8] At pH 6.8 (corresponds to intestinal pH), only 3% of BD was released after 3 hours, which is considered to be the suitable transit time for the dosage form in small intestine. However after 3 hours, 45% drug released from dosage form after suitable lag time at pH 6.8, which has importance in patient having low intestinal pH. Formulation with 15% (w/w) showed 100% drug release after 3 hours at pH 7.2 but showed only 24% drug release after 10 hours at pH 6.8, which indicates that as polymer thickness increase, more time will be taken for polymer dissolution and subsequent drug release.

Scanning electron microscopy

The scanning electron microscopy study of the formulation F7 with Eudragit FS 30 D 10% (w/w) coating level was performed to see the uniformity of the film and to find out the coating thickness of the polymer. The homogeneous film was observed and the average coating thickness of 35.75 µm was observed (Figure 3).

In vivo X-ray results

For this study, formulations coated with 10% and 15% (w/w) of Eudragit FS 30 D were used and compared with each other. The details of the results are shown in Table 5 and Figure 4. From the abdominal radiographs, taken at different time intervals, it is evident that after 2 hours the tablets remained unchanged in the stomach in two subjects (subjects 5 and 6); whereas the tablets had reached the intestinal region in the other four subjects (subjects 1–4). Tablet formulation reached the ileo-cecal region after approximately 4 hours in three subjects (subjects 1–3). At the same time in subject 5, the tablet remains in the stomach, and in subject 6 the tablet reached the jejunum. After 4.5 hours, the tablets in subjects 1–3 disappear. This indicates that the disintegration of the tablet occurred after reaching the ileo-cecal region. At the same time, the tablets in subjects 4 and 6 reached the ascending colon, and in subject 5 the tablet remained in the stomach in intact form. The X-ray image taken after 6 hours shows the disintegration of the tablet in subject 4 in the ascending colon, and in subject 6 the tablet disintegrated in the transverse

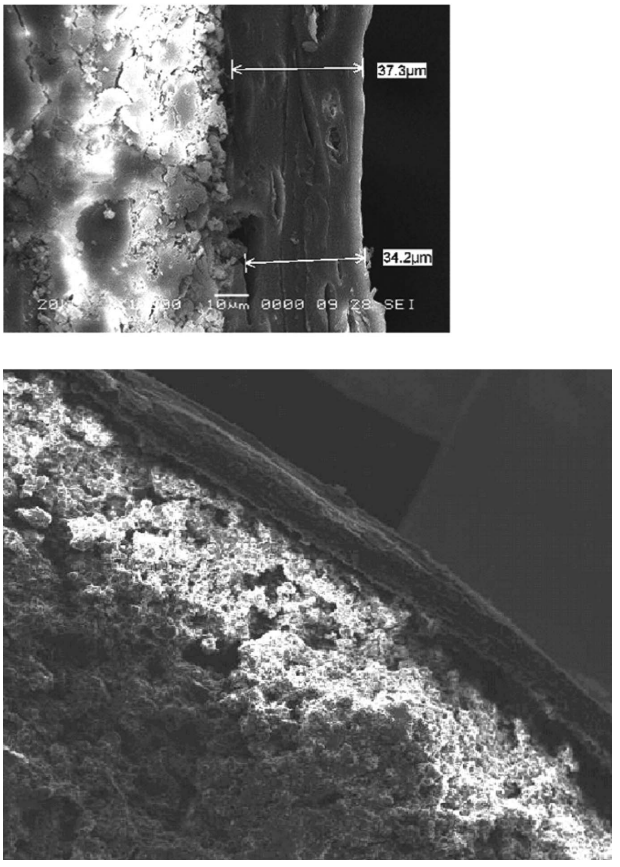

Figure 3. SEM picture of budesonide tablet formulation coated with Eudragit FS 30D 10% w/w coating level (a cross sectional view).

colon. The tablet in subject 5 still remained in the stomach (this may be due to inter-subject variability in transit time) but the tablet was found in intact form; this may be due to the resistance of this polymer to the acidic medium.

These results show similarities with in vitro drug release study. Tablet with 10% (w/w) showed disintegration in the ileo-cecal region after a lag time of 4.5 hours. In vitro dissolution studies of this tablet shows a lag time of about 5 hours, this difference in the lag time may be due to the higher pH sensitivity of the polymer at alkaline pH 7.2, which generally occurs at the ileo-cecal region. Tablets with 15% (w/w) show a lag time of 5–6 hours in subjects 4 and 6, and hence shows similarities with in vitro studies. The present study is in the agreement with the results obtained by Gupta et al.[7], that is, the lag time increases with the increase in the coating level of Eudragit FS 30 D, because at higher coating layer more time is required for diffusion and subsequent erosion.

Data demonstrate that the tablets were discharged from the stomach within 2 hours after dosing. Average small intestinal transit times of 2.5–3 hours were recorded for most of the formulations. Intra- and inter-subject variations were observed in the GI data. No significant stasis was observed at the ileo-cecal junction in any subjects, which is a encouraging finding for ileo-cecal targeting. Formulation with 10% weight gain disintegrated in this ileo-cecal region, whereas tablet with 15% weight gain disintegrated in the ascending and transverse colon.

These results strongly suggest that this novel colon delivery system might be useful for the delivery of drugs to the ileo-cecal region for effective treatment of Crohn's disease.

Conclusions

From both in vitro drug release and in vivo human X-ray studies, it has been found that BD tablet with 10% Eudragit FS 30 D gives the promising results for the ileo-cecal targeting. The key factor that determines the behavior of the polymer is the threshold dissolution pH value. A rapid drug release from the coated tablets occurred only at pH 7.2 after a suitable lag time of 4–5 hours. The in vivo X-ray investigations using human volunteers further demonstrated the ileo-cecal targeting function of Eudragit FS 30 D. This polymer would be useful for the delivery of BD to the lower part of the small intestine for the effective treatment of Crohn's disease.

Table 5. Position of tables throughout the GI tract in the subjects at certain points of time in X-ray study.

Subject	Formulation	2 hours	4 hours	4.30 hours	5 hours	6 hours
1	10% w/w	Ileo-cecal region	Ileo-cecal region	**Disintegrated**	–	–
2	10% w/w	Jejunum	Ileo-cecal region	**Disintegrated**	–	–
3	10% w/w	Proximal intestine	Ileo-cecal region	**Disintegrated**	–	–
4	15% w/w	Jejunum	Ascending colon	Ascending colon	Ascending colon	**Disintegrated**
5	15% w/w	Stomach	Stomach	Stomach	Stomach	Stomach
6	15% w/w	Stomach	Ascending colon	Ascending colon	Clonic flexure	**Disintegrated in transverse colon**

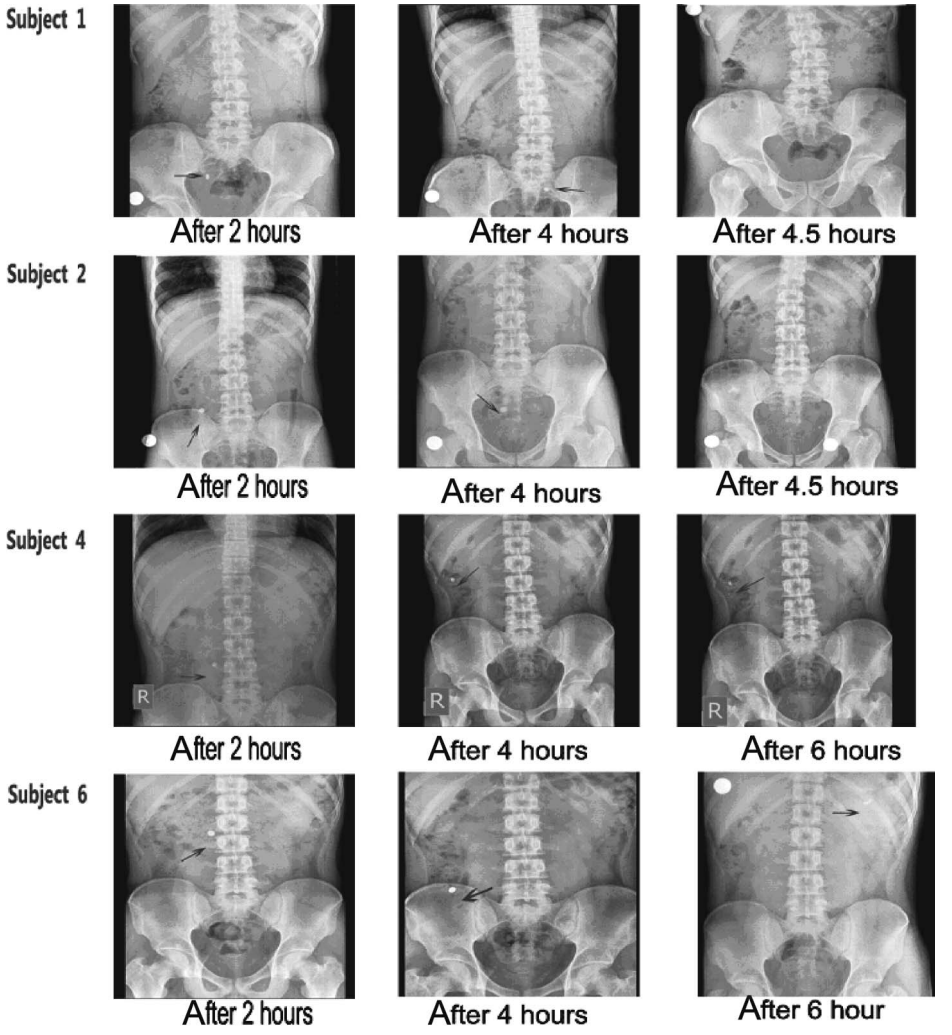

Figure 4. X-ray images of the subjects at different time intervals after administration of tablets.

Acknowledgments

Authors are thankful for the kind support provided by Dr. K. G. Bothara (Principal, AISSMS College of Pharmacy, Pune, India). Mepro Pharmaceutical (Mumbai, India) kindly provided BD and Degussa India Ltd. supplied Eudragit polymers as a gift. Authors also thank B.J. Medical College (Pune, India) for supporting the in vivo study.

Declaration of interest: The authors report no conflicts of interest.

References

1. Ding X, Med B, Alani AWG, Robinson JR. (2006). Extended-release and targeted drug delivery systems. In: Troy DB, ed., Remington: The Science and Practice of Pharmacy, Vol. 2. Easton, PA: Mack Publishing Co., 939–63.
2. Chien YW, Lin S. (1997). Drug delivery: Controlled release. In: Swarbick J, Boylan JC, eds. Encylopedia of Pharmaceutical Technology 2nd ed., Vol. 1. New York: Marcel Dekker, Inc., 811–33.
3. Weathrall DJ, Ledingham JGG, Warrell DA. (1996). Gastroenterology, Oxford Textbook of Medicine. 3rd ed. New York: Oxford Medical Publisher, 1936–43.
4. Lewis C. (1999). Crohn's disease: new drug may help when others fail. U.S.F.D.A. Consumer Magazine.
5. Rodriguez M, Vila-Jato JL, Doloves Jorres. (1998). Design of a new multiparticulate system for potential site specific and controlled drug delivery to the colonic region. J Control Release, 55:67–77.
6. Krishnamacharia Y, Parshotam M, Senshang L. (2007). Development of pH and time-dependent oral microparticles to optimize budesonide delivery to ileum and colon. Int J Pharm, 338:238–47.
7. Gupta VK, Beckert TE, Deusch NJ, Harinaraon M, Price JC. (2001). A novel pH and time based multipotential colonic drug delivery system. I. Development. Int J Pharm, 213:83–91.
8. Gao C, Huang J, Jiao Y, Shan L, Liu Y, Li Y, Mei X. (2006). In vitro release and in vivo absorption in beagles dogs of meloxicam from Eudragit FS30D coated pellets. Int J Pharm, 322:104–12.
9. Chourasia MK, Jain SK. (2003). Pharmaceutical approaches to colon targeted drug delivery system. J Pharm Pharmaceut Sci, 6(1): 33–66.
10. Ibekwe VC, Kendall RA, Basit AW. (2004). *Drug Delivery to the Colon.* The Drug Delivery Companies Report, Spring/Summer, 27–30.

11. Hu Z, Shimokawa T, Ohno T, Kimura G, Mawatari S, Kamitsuna M, Yoshikawa Y, Masuda S, Takada K. (1999). Characterization of norfloxacine release from tablet coated with a new pH sensitive polymer P-4135F. J Drug Target, 7:223–32.
12. Rudolh MW, Klein S, Beckert TFE, Peterieit H, Dressman JB. (2001). A new 5 aminosalicylic acid multi-unit dosage form for the therapy of ulcerative colitis. Eur J Pharm Biopharm, 51:183–90.
13. Basit A, Bloor J. (2003). Perspectives on colonic drug delivery. Business Briefing: Pharmatech, 185–90.
14. Iruin A, Fernandez-Arevalo M, Alvarez-Fuentes J, Fini A, Holgado MA. (2005). Elaboration and "in vitro" characterization of 5-ASA beads. Drug Develop Ind Pharm, 31:231–39.
15. Cole ET, Scott RA, Alyson L, Conner AL. (2003). Enteric coated HPMC capsules designed to achieve intestinal targeting. Int J Pharm, 231: 83–95.
16. Bott C, Rudolph MW, Schneider AR, Schirrmacher S, Skalsky B, Petereit HU, et al. (2004). In vivo evaluation of a novel pH and time based multiunit colonic drug delivery systems. Aliment Pharmacol Ther, 1, 20:347–53.
17. Tugcu-Demiroz F, Acartark F, Takka S, Konus-Bayunaga O. (2007). Evaluation of alginate based mesalazine tablets for intestinal drug delivery. Eur J Pharm Biopharm, 67:491–97.
18. Huyghebaert N, Vermeire An, Remon JP. (2005). In vitro evaluation of coating polymers for enteric coating & human ileal targeting. Int J Pharm, 298:26–37.
19. European Commission. (2000). *Radiation Protecting 118 Referral Guidelines for Imaging*. Luxembourg: Office for Official Publications of the European Commission Directorate-General for the Environment. http://ec.europa.eu/energy/nuclear/radioprotection/publication/doc/118_enpdf.
20. Akhgari A, Garkeni HA, Sadeghi F, Azimaie M. (2005). Statistical optimization of indomethacin pellets coated with pH-dependent methacrylic polymers for possible colonic drug delivery. Int J Pharm, 305:22–30.
21. Nugent SG, Kumar D, Rampton DS, Evans DF. (2001). Intestinal luminal pH in inflammatory bowel disease: possible determinants and implications for therapy with aminosalicylates and other drugs. Gut Online, 48:571–77.