AAPS PharmSciTech, Vol. 10, No. 2, June 2009 (© 2009)
DOI: 10.1208/s12249-009-9223-4

Research Article

Colon Specific Delivery of Indomethacin: Effect of Incorporating pH Sensitive Polymers in Xanthan Gum Matrix Bases

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Received 20 August 2008; accepted 1 March 2009; published online 21 April 2009

Abstract. In the present study, an attempt has been made to design controlled release colon-specific formulations of indomethacin by employing pH responsive polymers Eudragit (L100 or S100) in matrix bases comprised of xanthan gum. The prepared tablets were found to be of acceptable quality with low-weight variation and uniform drug content. *In vitro* release studies indicated rapid swelling and release of significant percentage of drug in the initial period from matrix tablets composed of xanthan gum alone. Addition of pH responsive polymers Eudragit (L100 or S100) to xanthan gum matrix resulted in negligible to very low drug release in the initial period in acidic to weakly acidic medium. Furthermore, with increase in pH of the dissolution medium due to dissolution of Eudragit L100/Eudragit S100 that resulted in the formation of a porous matrix, faster but controlled drug release pattern was observed. Thus, a sigmoidal release pattern was observed from the designed formulations suitable for colonic delivery. Drug release mechanism in all cases was found to be of super case II type, indicating erosion to be the primary cause of drug release. Since the drug release from almost all the matrix bases in the initial phase was negligibly low and followed with controlled release for about 14–16 h, it was concluded that a matrix design of this composition could have potential applications as a colon-specific drug delivery device with additional advantage of easy scale-up and avoidance of all-or-none phenomenon associated with coated colon-specific systems.

KEY WORDS: colon specific delivery; controlled release; Eudragits; matrix; pH sensitive polymers; xanthan gum.

INTRODUCTION

Indomethacin is a non-steroidal anti-inflammatory drug, commonly indicated in the treatment of osteo and rheumatoid arthritis. Recent reports have implicated its use as an anti-cancer agent against various *in vitro* and *in vivo* models of colorectal cancer (1). It has been reported to cause growth inhibition, induction of apoptosis, and reduction in proliferation rates of HT-29 colon cancer cells, along with down-regulation of survivin (an apoptosis inhibitor) (2–4). Oral administration of indomethacin has been reported to cause dose-dependent systemic and local upper gastrointestinal side effects in 35% to 50% patients (5). A formulation of indomethacin with negligible to no release in upper gastrointestinal (GI) tract and controlled release in colonic region would achieve therapeutically effective concentration of drug locally in colon. At the same time, such a formulation would minimize systemic or upper GI tract related side effects of indomethacin.

Various approaches used for targeting drugs to the colon include prodrug-based techniques (including azo polymer and hydrogel systems), time-dependent delivery systems (including osmotic pump, swelling, and coating controlled), pH-sensitive polymer-based coating systems, and bacterial enzyme controlled systems (6,7). Several researchers have reported various colon-targeted formulations of indomethacin. Some of these formulation approaches include using pH-sensitive polymers for coating drug-loaded pellets (8,9), compression coating of tablets using either guar gum (10) and pectin and chitosan mixtures (11) or guar gum and Eudragit FS 30 D coated pellets (12), and drug embedding in HPMC/pectin/calcium chloride matrix bases (13).

pH-based colon-specific drug delivery systems have been developed by coating drug embedded polysaccharide matrices (both single unit and multi unit systems) with pH-dependent polymers (14,15). A summary of some observations and important findings with respect to Eudragit-based coating polymers is presented in Table I. It was shown through *in vitro* release studies that these polymers (either alone or in combination) exhibit excellent protection in gastric pH followed by gradual or sudden release in alkaline environment in different pH conditions (6.0–7.4; Table I). Some coated formulations, based on Eudragit FS 30D, have shown to resist disintegration/dissolution in upper GI tract but have been reported to disintegrate after colonic arrival (12,22–24). However, the use of polymers that release the

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Table I. Use of pH Responsive Polymers in Coating of Single-unit Dosage Forms for Colon Specific Release

pH sensitive polymer	Technique employed	Important findings	Reference
Single polymer coated systems			
Eudragit L100	Matrix tablets of metronidazole prepared with polysaccharides and guar gum grafted with methacrylic acid copolymer and coated with Eudragit L100	In case of guar gum with methacrylic acid graft copolymer, up to 70% drug release occurred during initial 4–5 h from. The release was reduced to 18–24% after enteric coating with EL100	(16)
Eudragit S100	Pectin matrix of theophylline coated with Eudragit S100	Presence of Eudragit coat (27% *w*/*w*) prevented initial drug release (near zero percent release for first 300–340 min) in pH 1.1 (2 h), pH 6.8 (2 h), pH 7.4 (10 h)	(17)
Eudragit S100	Indomethacin pellets coated with Eudragit S100	*In vitro* testing in pH 1.2 (2 h) followed by pH 6.8. 18% drug release in first 3 h with 3% coat	(18)
Eudragit S100	Matrix tablets prepared by direct compression of mixtures of hydroxyethylcellulose and ethylcellulose or micro-crystalline cellulose and coated with Eudragit S100	*In vitro* release studies in simulated gastric fluid pH indicated that the Eudragit S100 enteric-coated matrix tablets showed both gastric resistance and an adequate lag time followed by a controlled-release phase. Tablets coated with 1:0.3:0.7 (*w*/*w*) drug/MCC/HEC tablets, released drug after a 260 min lag time and completed 90% release in 10 h	(19)
Eudragit S100	Tablet cores were coated with Eudragit S dissolved in ethanol (organic), or aqueous dispersion (aqueous), or Eudragit FS aqueous dispersion and administered to human subjects	Tablets coated with Eudragit S (aqueous) disintegrated in all volunteers mainly in the proximal to mid small intestine. Eudragit S (organic) tablets failed to disintegrate in three out of eight volunteers, while disintegration was in the ileocecal junction and ascending colon in all others. Eudragit FS coated tablets disintegrated in 14 out of the 16 administrations with the site of disintegration focused on the ileocecal junction and ascending colon	(20)
Eudragit S100	Tablets (radiolabeled with Technetium 99m) coated with Eudragit S were administered to eight healthy subjects in a three-way crossover study after overnight fasting	Gastrointestinal pH showed variability between and within individuals but no differences were seen between pre-feed and fasted states. Three tablets failed to disintegrate in pre-feed and fed regimens and one in the fasted state; this was attributed to ileocecal pH and ileocecal junction residence time	(21)
Eudragit FS 30 D.	Meloxicam-loaded cores prepared by layering drug-binder (HPMC)-solubilizer (beta-cyclodextrin) solution onto nonpareils and then coated with Eudragit FS 30 D	The *in vitro* drug release from the pellets was pH-dependent with sufficient gastric resistance (pH 1.2: no release; pH 6.8: 6%; pH 7.0: 52%; pH 7.2: 100%; pH 7.4: 100%, after 3 h incubation). The onset of meloxicam absorption from the coated pellets with 15% (*w*/*w*) Eudragit FS 30 D (3.0±0.8 h) was significantly delayed compared to that from the uncoated drug-layered cores (0.6±0.3 h)	(22)
Eudragit FS30D	The combination of Eudragit RL30D and RS30D were used as sustained-release film, and Eudragit FS30D used as enteric film, which was expected to release drug depending on pH and time.	The release profile of tablets was studied in three phosphate buffers with the pH 6.5, 7.0 or 7.4 for 12 h after a simulated gastric presoak for 2 h in 0.1 M HCl. For the *in vitro* study, there was no drug released in 0.1 M HCl for 2 h, and release occurred slowly when pH was above 6.5. For the *in vivo* study, the coated tablets remained intact in the upper gastrointestinal tract, and drug release began after the colonic arrival	(23)

Table I. (continued)

pH sensitive polymer	Technique employed	Important findings	Reference
Eudragit FS30D	A pH- and enzyme-dependent system obtained by coating guar gum and Eudragit FS30D sequentially onto drug-loaded pellets in a fluidized bed coater	Under gradient pH conditions (pH = 1.2, 6.8, 7.4, and 6.5 for 2, 2, 1, and 15 h, respectively), indomethacin was released from Eudragit FS30D-coated pellets quickly after changing pH to 7.4. For guar gum/Eudragit FS30D double-coated pellets, only about 5% of the drug was released after another 1 h, showing retarding effect by guar gum coating. After changing pH to 6.5 and addition of galactomannanase, enzyme-dependent drug release was observed. Pharmacokinetic study in beagle dogs showed good lag times for release of drug	(12)
Eudragit L30 D-55 and Eudragit FS 30 D	Enteric coating of HPMC capsules containing paracetamol with two pH responsive polymers separately	Capsules coated with Eudragit L 30 D-55 were gastro resistant for 2 h at pH 1.2 *in vitro* and disintegrated completely in small intestine *in vivo*. Those coated with Eudragit FS 30 D were resistant for a further 1 h at pH 6.8. and disintegrated in the proximal colon in an average time of 6.9 h post dose	(24)
Eudragit S100, Eudragit FS 30 D, Eudragit P4135	Tablet cores were coated with Eudragit S100 dissolved in ethanol (organic), Eudragit S100 aqueous dispersion (aqueous), or Eudragit FS aqueous dispersion and Eudragit P4135	Dissolution studies done in pH 1.2 and two compendial buffers in the pH range 6.8–7.4. and Hank's physiological buffer (pH 7.4.) All tablets except those coated with P 4135 prevented drug release in acid medium. At pH 7.4, drug release was in the following order: Eudragit S100 (aqueous dispersion)>Eudragit FS>Eudragit S100 (organic solution). Results indicated that tablets coated with Eudragit FS would be appropriate for ileocolonic delivery	(25)
Dual polymer coated systems			
Eudragit L100 and Eudragit S100	5-Amino salicylic acid loaded pellets coated with both polymers in combination	5-ASA release from the coated pellets depended upon both the combination ratio of the Eudragit L100 and ES100 and the thickness of the coating layer. For tablets coated with 1:4 (EL100: ES100) mixture, drug release was <1.0% in pH 1.2 (2 h), <3.0% in pH 6.0 (1 h) and about 80% release in pH 7.2	(26)
Eudragit L100 and Eudragit S100	Indomethacin pellets coated with Eudragit S100: Eudragit L100 (1:4, 1:1 and 1:0) at different level of coating (10%, 15% and 20%, w/w), respectively	Pellets released no indomethacin at pH 1.2 (simulating stomach pH) and pH 6.5 (simulating proximal part of small intestine pH); drug release was slow at pH 6.8 (simulating lower part of small intestine pH), but it was fast at pH 7.2 (simulating terminal ileum pH)	(8)
Eudragit L100 and Eudragit S100	Indomethacin pellets coated with Eudragit S100 and Eudragit L100 as pH-dependent polymers and Eudragit RS was used as a time-dependent polymer as a single coating formulation	Dissolution studies of pellets in the media with different pH (1.2, 6.5, 6.8, and 7.2) showed that drug release in colon could be controlled by addition of Eudragit RS to the pH-dependent polymers. The lag time before drug release could be controlled by coating level	(9)
Eudragit L100 and Eudragit S100	Mesalazine (5-aminosalicylic acid) tablets coated with mixture of both polymers	Initial tablet disintegration occurred in the ileocecal region in three subjects and the ascending colon in five subjects (5.65±0.86 h after dosing)	(27)
Eudragit S100, starch	Tablets coated with a mixture of pH-responsive enteric polymer (Eudragit S100) and biodegradable polysaccharide (resistant starch) in a single layer matrix film	*In vivo* studies involving human subjects showed that the coated tablets were able to resist breakdown in the stomach and small intestine. Consistent disintegration of the dosage form was seen at the ileocecal junction/large intestine	(28)

drug at higher pH values (>7.0) may fail to give reproducible results, since pH in the lower GI lumen (ileum and colon) may fail to exceed the dissolution pH of the polymer in some patients, for example, in case of inflammatory bowel disease (29). Therefore, coated systems, in general, suffer from the drawback of non-reproducible release *in vivo*. Extensive studies carried out by Ibekwe *et al.* in the recent past have shown that tablets coated with Eudragit polymers demonstrated erratic performance *in vivo*, and many tablets failed to disintegrate inside the human body (20,21). This has been previously attributed to the narrow pH gradient between the small and large intestine, intersubject variability in GI pH, residence time of dosage form at ileocecal junction, pH changes that occur in diseased conditions, and fasted or fed states resulting in variable performance of these systems (30,31).

The combination of pH-dependent polymers with time-based polymers could offer a means for achieving controlled release of drug from the coated system (9). Furthermore, pH-based polymers in combination with biodegradable guar gum (12) and starch (28) have also been attempted and proven as better triggers by microbial degradation for colon-specific release. In the present investigation, a novel method of matrix tablet design possessing bimodal release profile was envisaged. The use of pH-dependent polymers (Eudragits L100 or S100) in polymer matrix of hydrophilic polymer (xanthan gum) was envisaged. Previously, our group has reported the use of pH-sensitive polymers alone in matrix (32) and in combination with other polymers ethyl cellulose (32) or polycarbophil and carbopol (33) for potential colon-specific delivery. Application of Eudragits (L100 and S100) in combination with ethyl cellulose as multiunit matrix microsphere for colon-specific delivery has been reported (34). It is expected that a dual polymer matrix embedded system comprising of a combination of swelling controlled and pH-dependent polymers can offer a suitable means of achieving a pH and time-dependent system that releases the drug in a bimodal (sigmoidal fashion). A matrix system, upon exposure to alkaline environment of the colon will result in partial or complete dissolution of pH responsive polymers and will therefore generate a porous system that will facilitate entry of dissolution medium into the pores of the matrix and affect drug release by diffusion and matrix erosion in high pH region. Such a system will result in a release profile suitable for colonic delivery and may help to reduce the improbability in drug release from a coated system, wherein the core is unexposed and drug release can occur only after all the layers of the coat are dissolved.

Xanthan gum, a polysaccharide-based natural gum, has been widely employed as a hydrophilic polymer to prepare controlled release matrices because of its cost effectiveness and regulatory acceptance (35). It has been used as a release retardant polymer alone (36) or in combination with other polymers [galactomannan (37), chitosan (38)] in controlled release matrices. It has also been used as a copolymer with guar gum to form matrix bases (39) and compression coats (10) in colonic delivery. When used as a matrix base, xanthan gum forms a time-dependent swelling-controlled system. The drug release from such matrix is through diffusion from the swollen xanthan gum matrix (40,41).

Therefore, in the present investigation, it was envisaged that the presence of pH-sensitive polymers in a matrix would control the rapid initial swelling of xanthan-gum-based matrices and thereby minimize drug release in the acidic to weakly acidic conditions of the upper GI tract while enhancing the drug release in the neutral to slightly alkaline environment of the colon. The objective of the study was to investigate the effect of pH-responsive polymers Eudragit (L100 or S100) on indomethacin release from xanthan-gum-matrix-based formulations and evaluate their potential for controlled release as well as colon specificity. The effect of varying the polymer proportions (xanthan gum) alone and in combination with EL100 or ES100 was studied. The transit of formulations was investigated in Wistar rat, and percentage drug recovered at different time points was analyzed. The effect of storage on the stability and release profile of selected formulations was also investigated. The stored batches were also evaluated for the absence of physical and chemical interactions.

MATERIALS AND METHODS

Indomethacin (micronized form) was obtained as gift sample from Ajanta Pharma Ltd, Aurangabad, India. Xanthan gum was purchased from Signet Chem, Mumbai, India. Eudragit polymers were obtained from Rohm Pharma, Germany. All other chemicals and reagents used were either of analytical or pharmaceutical grade.

Analytical Method

Indomethacin in pure form and designed formulation was analyzed using in-house developed and validated UV-Visible spectrophotometric method using Jasco V-570 double beam UV-Visible spectrophotometer (Jasco Corporation, Tokyo, Japan) accompanied with Spectra Manager software. The method involved analysis of the drug at 320 nm in phosphate buffer pH 7.4 in the range of 5–50 μg/ml using 1-cm matched quartz cells.

Tablet Manufacturing

Matrix-embedded tablets (each containing 75 mg of indomethacin) using either XG alone or in combination with EL100/ES100 were prepared by wet granulation technique. Batch quantities of drug and polymer(s) pre-sieved through no. 120 mesh (ASTM) and dried at 55°C were mixed. The dry blend was granulated with ethyl alcohol (q.s.) and passed through no. 40 mesh and dried at 55°C on a tray drier. The dried granules were passed through no. 60 mesh and the passings blended with 1% *w*/*w* talc and 0.5% *w*/*w* magnesium stearate and compressed using 7-mm punches on a 16 station rotary tablet compression machine (Cadmach, Ahmedabad, India). Three batches of tablets were prepared for each formulation. Formulas of prepared matrix-embedded tablets containing XG are presented in Table II, respectively.

Physical Characterization of Designed Tablets

The designed formulations were studied for their physical properties like weight variation, thickness, crushing strength, friability, and drug content uniformity. For estimating weight variation, 20 tablets of each formulation were weighed using a Mettler Toledo balance (AG135, Mettler

Table II. Composition, Physical Characterization, and Release Rate Kinetics of XG-based Formulations

	Batches			Drug content[a]			Power law correlation				
	XG (mg)	EL100 (mg)	ES100 (mg)	mg/tablet	Correlation time span (h)	r^f	MSSR[g]	K^h (%/h^n)	n^i	$t_{10\%}{}^j$	$t_{90\%}{}^k$
Effect of XG only											
IXG5	3.75	–	–	73.5±0.2	2–8	0.9504	1.27×10^{-3}	14.277	1.13	0.5	5.1
IXG10	7.5	–	–	74.7±0.1	1–10	0.9204	1.83×10^{-2}	5.220	1.09	1.2	13.7
IXG20	15	–	–	72.6±0.3	2–12	0.9167	1.23×10^{-2}	15.707	0.55	1.1	23.9
Effect of EL100 or ES100 alone on indomethacin matrix											
IEL20	–	15	–	73.0±0.1	2–12	0.9130	3.75×10^{-2}	2.659	1.48	2.1	10.8
IES20	–	–	15	72.6±0.2	2–14	0.9750	1.16×10^{-3}	1.137	1.40	3.5	26.7
Effect of EL100 or ES100 on XG matrix											
IXG5EL5	3.75	3.75	–	76.3±0.4	2–12	0.9942	3.24×10^{-4}	7.327	1.08	2.9	10.2
IXG5EL10	3.75	7.5	–	73.8±0.3	2–14	0.9827	3.42×10^{-3}	1.346	1.61	3.6	13.6
IXG5EL20	3.75	15	–	75.0±0.1	2–12	0.9926	2.67×10^{-4}	1.047	1.55	4.2	17.7
IXG5EL40	3.75	30	–	75.4±0.3	2–14	0.9852	1.05×10^{-3}	1.402	1.27	5.2	26.5
IXG10EL10	7.5	7.5	–	74.1±0.2	4–12	0.9876	2.13×10^{-3}	5.945	1.05	3.7	13.3
IXG10EL20	7.5	15	–	74.2±0.1	4–12	0.9967	7.22×10^{-4}	2.409	1.30	4.6	16.2
IXG5ES5	3.75	–	3.75	74.7±0.2	2–10	0.9950	9.45×10^{-4}	12.159	0.79	2.1	12.6
IXG5ES10	3.75	–	7.5	74.6±0.2	2–12	0.9979	3.57×10^{-4}	5.417	0.78	4.1	36.7
IXG5ES20	3.75	–	15	72.6±0.3	2–14	0.9822	1.30×10^{-2}	4.950	0.79	5.2	39.3
IXG5ES40	3.75	–	30	76.9±0.1	1–12	0.9758	3.53×10^{-3}	0.938	1.26	6.0	37.4
IXG10ES10	7.5	–	7.5	76.3±0.4	1–10	0.9234	1.73×10^{-2}	6.276	0.40	1.0	9.2
IXG10ES20	7.5	–	15	73.5±0.1	1–10	0.9425	1.63×10^{-2}	6.358	0.86	2.0	9.1

Each tablet contains 75 mg of indomethacin. Also contains 1% *w/w* talc and 0.5% *w/w* magnesium stearate as formulation additives. The diameter of the tablets was 0.70±0.01 cm
[a] Mean±SD (n=10)
[b] SD from the mean value (n=20)
[c] Mean±SD (n=10)
[d] Mean of ten tablets
[e] Mean±SD (n=5)
[f] Correlation coefficient
[g] Mean sum of squared residuals
[h] Release rate constant
[i] Diffusional exponent indicative of the release mechanism
[j] Time for 10% of the drug release (in h)
[k] The predicted or calculated time for 90% of the drug release (in h from Eq. 2)

Toledo, GmbH, Greifensee, Switzerland). The crushing strength of ten tablets was measured using Monsanto (standard type) tablet hardness tester. Friability was determined on ten tablets in a Campbell Electronic Friabilator for 4 min at 25 rpm. For estimation of drug content, ten tablets were crushed, and the aliquot of powder equivalent to 10 mg of drug was extracted in methanol/phosphate buffer pH 7.4 (1:9), suitably diluted using phosphate buffer pH 7.4 and analyzed spectrophotometrically at 320 nm.

In vitro Release Studies

In vitro dissolution studies were carried out using USP Type II (paddle method) apparatus (Electrolab TDT-08L with autosampling unit, Mumbai, India) at 75 rpm. The dissolution was carried out for the first 2 h in distilled water (500 ml, pH 6.8–7.0). Then, 200 ml of phosphate buffer concentrate (4.75 g of KH_2PO_4 and 1.07 g of NaOH in distilled water) was added to raise the total media volume to 700 ml and the pH to 7.4 for the remaining period. At predetermined time intervals, a 10-ml sample was withdrawn and replaced with fresh dissolution media. The samples were filtered, suitably diluted using phosphate buffer pH 7.4, and analyzed spectrophotometrically at 320 nm. The release studies were conducted in duplicate per batch for three batches, and the mean values from three batches along with the SD were plotted against time (Figs. 1, 2, and 3) and used for all further calculations. Dissolution of pure indomethacin alone was also recorded and used as a control for *in vitro* release study (shown in Fig. 1). Effect of Eudragit on drug release from XG matrix was compared against an indomethacin matrix formulation prepared with only EL100 or ES100 (20% *w/w* of drug; IEL20 and IES20) as a control (shown in Figs. 2 and 3).

Effect of Simulated GI Fluid pH (Without Enzymes) on Release

Selected formulations from previous study were studied in a medium of changing pH. The initial condition was 350 ml of 0.1 N HCl (pH 1.2) for 0–2 h. From 2–4 h, the pH of the media was raised to 4.5 (for simulation of duodenum), with total dissolution media volume of 600 ml. From the fourth hour onwards, the pH was raised to 7.4 by adding 300 ml phosphate buffer concentrate (2.18 g of KH_2PO_4 and 1.46 g of NaOH in distilled water) to get dissolution volume of 900 ml.

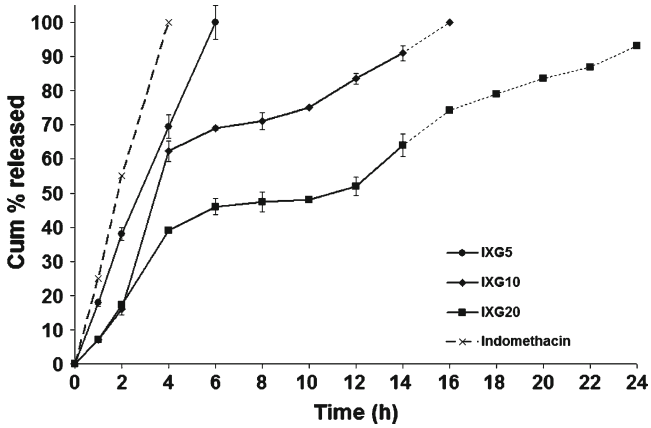

Fig. 1. Release profile of indomethacin from matrices containing varying proportions of xanthan gum. Each data point represents mean±SD (n=6)

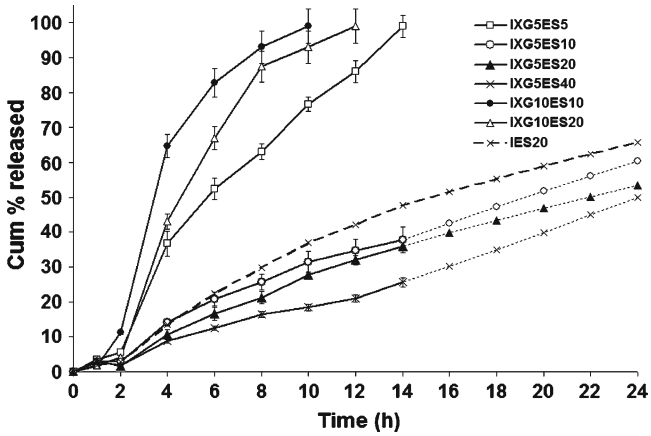

Fig. 3. Release profile of indomethacin from xanthan gum matrix showing effect of varying proportion of ES100. Each data point represents mean ± SD (n=6)

The study was further continued until the end in 900 ml volume. At predetermined time intervals, a 10-ml sample was withdrawn and replaced with fresh dissolution media. After appropriate dilutions, the samples were analyzed by the UV method discussed in previous section. The corresponding release profiles against pure indomethacin dissolution in the respective media are presented in Fig. 4.

Characterization of Sigmoidal Release Profiles by Power Law Equation

In order to understand the mechanism of drug release from these formulations, the cumulative percentage drug release data (after 2 h) was fitted into the power law equation given by Korsemeyer *et al.* (42) and Ritger and Peppas (43).

$$M_t/M_\infty = Kt^n \tag{1}$$

where M_t/M_∞ is percentage of drug released at any time t; K is release rate constant incorporating the structural and geometric characteristics of the polymeric system and the drug; and n is the diffusion exponent indicative of the release mechanism of the drug. The value of n for a cylinder is <0.45 for Fickian release (diffusion controlled), >0.45 and <0.89 for non-Fickian release (diffusion and polymer relaxation), 0.89 for case II release (only relaxation and swelling), and >0.89 for super case II release (relaxation and erosion) for swellable systems. For cylindrical systems like tablets, the n values of 0.45 and 0.89 represent pure diffusion- or erosion-controlled release, respectively. The values of the coefficient were calculated using linear regression analysis between $\log M_t/M_\infty$ and $\log t$ data obtained from drug release studies on MS Office Excel 2003 software. The value of n was obtained as slope of the regression equation, and K was calculated as antilog of the intercept value. The $t_{10\%}$ (time required for 10% drug release) was determined directly from the plot of cumulative percentage drug released *versus* time, while the $t_{90\%}$ (time required for 90% drug release) was calculated as

$$t_{90\%} = \text{anti}\log\{(\log 90 - \log K)/n\} \tag{2}$$

The values of correlation time span, K, n, $t_{10\%}$, and $t_{90\%}$, r (correlation coefficient of the regression analysis), and mean sum of squared residuals (MSSR) as obtained from the dissolution data of designed formulations are given in Table II.

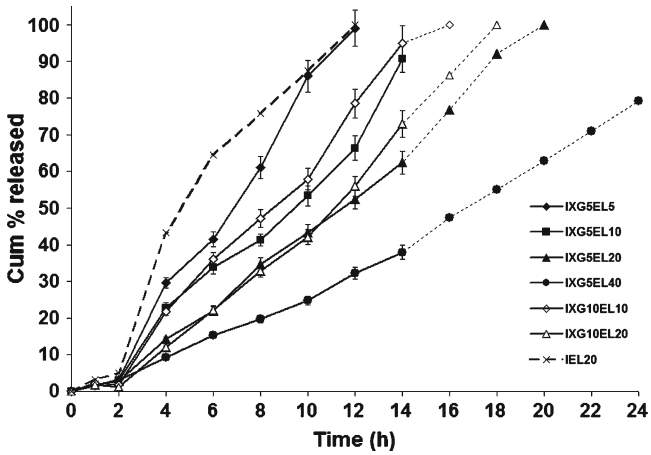

Fig. 2. Release profile of indomethacin from xanthan gum matrix showing effect of varying proportion of EL100. Each data point represents mean±SD (n=6)

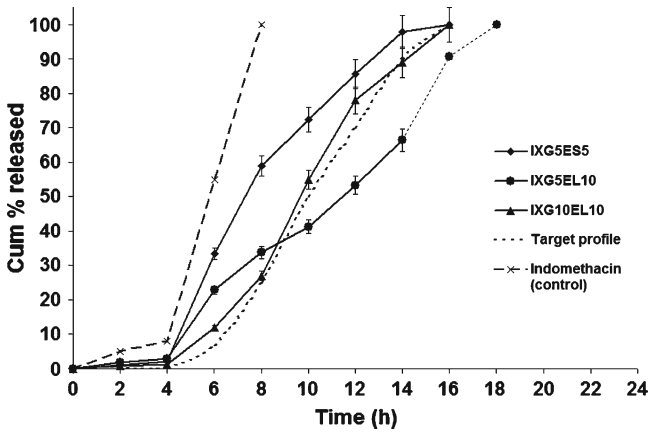

Fig. 4. Release profile of selected formulations in simulated GI fluid pH. Each data point represents mean±SD (n=6)

The correlation time span is the period of drug release phase taken for calculation of release kinetics. Using the calculated values of K and n, the release profiles were predicted beyond 14–24 h for each formulation and are shown as dotted trend line (s) in the respective figures.

The dissolution profiles of selected formulations in changing pH medium (without enzymes) were compared with the target dissolution profile (negligible to no release in first 6 h followed by controlled release up to 14–16 h) using f_1 (dissimilarity) and f_2 (similarity) factor (44) as shown below.

$$f_1 = \left\{ \left[\sum_{t=1}^{n} |R_t - T_t| \right] \Big/ \left[\sum_{t=1}^{n} R_t \right] \right\} \times 100 \quad (3)$$

$$f_2 = 50.\log \left\{ \left[1 + (1/n) \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\} \quad (4)$$

where n is the number of sampling points, R_t and T_t is the drug release from reference and test sample at sampling point t, respectively. The corresponding data are presented in Table III.

Batch Reproducibility and Stability on Storage

Three batches of each formulation were prepared, and the release studies were done using the same conditions for estimating batch reproducibility. In order to assess the long-term stability of the various formulations prepared, selected formulations from each batch were sealed in cellophane packets, placed in hermetically sealed vials and separately stored at ambient conditions (25°C/60% RH) and accelerated stability test conditions (40°C/75% RH) for 6 months. At the end of the study period, the formulations were observed for change in physical appearance, drug content, and *in vitro* drug release characteristics. The initial (zero time) results were compared with post-stability testing period results for statistical differences. The powdered samples of indomethacin matrix tablets were also subjected to differential scanning calorimetry (DSC) study and determination of IR spectrum. For DSC, pure drug and formulation (IX10EL10) equal to 2.5 mg of drug were accurately weighed onto standard aluminum pans, hermetically sealed and thermograms obtained at a scanning rate of 10°C/min over a temperature range of 25–200°C under constant purge of nitrogen gas (flow rate of 30 ml/min) using differential scanning calorimeter (Shimadzu, Japan, Model-DSC-60). For Fourier transform infrared (FTIR), the samples were appropriately diluted with dried potassium bromide, and IR spectra were acquired in the range of 400 to 4,000 cm^{-1} with a resolution of 4 cm^{-1}. The data was processed using Kubelka Munk method for baseline correction.

In vivo Evaluation of Formulations

The GI transit of selected formulation (IXG10EL10) was carried out *in vivo* in rats. The protocol was previously approved by the Institutional Animal Ethics Committee and was in conformance with Principles of Laboratory Animal Care (NIH publication no. 85-23, revised in 1985). Healthy male Wister rats (350–400 g) were selected for the study. Mini tablets were prepared using 4-mm punches, and formulation parameters were optimized suitably to approach as close as possible to the drug release pattern of the actual formulation. Before tablet administration, the animals were kept on overnight fast. The formulation was administered in triplicate for GI transit studies. The tablet was placed in the throat of the animal with a pair of forceps, and about 1.5–2 ml of water was flushed down the throat slowly to facilitate entry of tablet into esophagus with the help of syringe. The animals were killed at fixed time intervals, and the position of tablet was located. The recovered tablets at various time points were analyzed for residual drug content to estimate the amount of drug released at each time point.

Data Analysis

The difference in the release data between the different formulations was compared using paired t test for means and one-way analysis of variance at 5% level of significance using Microsoft Office 2003, Excel package.

RESULTS AND DISCUSSION

Physical Characteristics

Physical appearance, crushing strength, weight variation , and drug content uniformity of different tablet formulations

Table III. Release Kinetics Data from Different Plots for Selected Formulations in Simulated GI Fluid pH

	Power law correlation							Dissimilarity factor[g]	Similarity factor[g]
Batches	Correlation time span (h)	r^a	MSSR[b]	K[c]	n^d	$t_{10\%}{}^e$	$t_{90\%}{}^f$	f_1	f_2
IXG5ES5	4–14	0.9613	3.27×10^{-3}	10.114	0.86	4.2	12.7	13.95	41.84
IXG5EL10	4–14	0.9812	2.21×10^{-3}	4.016	1.12	4.5	15.9	12.95	48.35
IXG10EL10	4–14	0.9012	4.86×10^{-3}	10.972	0.91	4.9	14.1	2.03	71.04

[a] Correlation coefficient
[b] Mean sum of squared residuals
[c] Release rate constant
[d] Diffusional exponent indicative of the release mechanism
[e] Time for 10% of the drug release (in h)
[f] Time for 90% of the drug release (in h)
[g] Comparison with theoretical target release profile. For similarity f_2 should be>50 and f_1<15

were found to be within satisfactory limits. The crushing strength was found to vary between 4.5 and 5.0 kg. The percentage friability in all the formulations was observed to be ≤0.5%. The manufactured tablets showed low weight variation (SD within ±5% of the average weight of the tablet) and high degree of drug content uniformity (within ±7% of the theoretical value) indicating that wet granulation is an acceptable method for good quality matrix tablets of indomethacin for pH-modulated delivery.

In vitro Release of Matrix Tablets

The *in vitro* release profiles of drugs from Eudragit-based systems are normally investigated in buffers like phosphate buffers with pH ranging from 6.5 to 7.5 after a pretest in acid medium (45). Indomethacin, an indole acetic acid derivative with a p*K*a of 4.5, has been reported to have solubility of 0.01 mmol/L (3.66 μg/ml) in pH 1.2 and 5.52 mmol/L (1,975 μg/ml) in pH 7.2 at 37°C (46). Our studies revealed that drug (present in a micronized form) had a solubility of ~53 μg/ml in distilled water at 25°C and ~80 μg/ml at 37°C, which could be due to its ionization at the pH of distilled water (6.8–7.0).

Based on this information, dissolution was carried out in distilled water for the first 2 h. Saturation solubility will not be achieved even when 55–60% of labeled claim is released in the first 2 h in distilled water. Furthermore, Eudragits are insoluble in water (47,48), and their dissolution depends on ionic strength and buffer capacity of the medium (49). Although the pH of distilled water (6.8–7.0) was near the threshold pH for dissolution of Eudragit, the polymer is not expected to dissolve due to its negligible ionic strength.

In addition, previous reports have shown that, as the patient consumes a tablet with a good quantity of water, dissolution of poorly water soluble drugs can be done in distilled water for the initial period (50,51). During preliminary studies, it was observed that drug release from the formulations could be differentiated well in this medium, as release never exceeded the saturation solubility of the drug in any case (Figs. 1, 2, and 3). The release studies were then investigated in phosphate buffer of pH 7.4 (ionic strength of 0.129 and osmolality of 228 mOsm/kg) (25). The drug was freely soluble at this pH, and this medium simulates the alkaline environment of distal small intestine and colon. The dissolution profiles of all the formulations were compared with respect to $t_{10\%}$, $t_{90\%}$, and n values.

A plot of cumulative percentage released *versus* time for matrix tablets of indomethacin prepared using varying proportions of xanthan gum alone (5%, 10%, and 20% *w*/*w* of drug) against pure indomethacin is shown in Fig. 1. It was observed that the initial percentage released from all the formulations was quite high (38% for 5% XG matrix and 15–20% for the others) in the first 2 h followed by a slower and more controlled release during the later stages depending on the proportion of the polymer in the matrix. On the other hand, pure indomethacin, being highly hydrophobic in nature (Log*P* of 4.4) dissolved slowly as a powder. The release kinetics data when fitted to the power equation (Table II) to get the calculated values of $t_{90\%}$ indicated 5.1 h for IXG5, which was extended to 23.9 h for IXG20 when the proportion of xanthan gum was increased from 5 to 20% *w*/*w* of drug, respectively. The use of higher proportions of xanthan gum resulted in the formation of a thick polymeric gel layer, which acted as a barrier to drug diffusion. The values of 1.13 and 0.55 for the diffusional exponent n indicated a change in the release mechanism from super case II ($n>1.0$) to anomalous non-Fickian type ($0.45<n<0.89$). In case of IXG5 and IXG10, where the indomethacin proportion was relatively higher, drug release took place due to erosion of tablet surface, due to limited swelling of xanthan gum in the presence of a hydrophobic drug. On the other hand, due to the relatively higher proportion of xanthan gum in IXG20, the drug release mechanism was elucidated as anomalous due to swelling of xanthan gum and diffusion of drug through the swollen layer (40,41). These results implied that xanthan gum alone in matrix form was not suitable for colonic delivery.

Effect of EL100 on Xanthan Gum Matrices

For the matrix tablets prepared using xanthan gum in 5% *w*/*w* of drug with varying proportions of Eudragit L100 (5%, 10%, 20%, and 40% w/w of drug), the *in vitro* drug release profiles against drug release from indomethacin with 20% EL100 (IEL20) are shown in Fig. 2. The initial percentage of drug release from all the formulations in the first 2 h was almost negligible (less than 5%) in distilled water followed by an linear increase in release rate post 2 h in pH 7.4 that depended on the proportion of EL100. The release kinetics data for the various formulations revealed $t_{10\%}$ (ranging from 2.9 h for IXG5EL5 to 5.2 h for IXG5EL40), implying significant inhibition in the initial drug release (Table II). It was observed that, after 2 h, the release of drug from the formulations was extended from 10.2 h for IXG5EL5 to about 26.5 h for IXG5EL40, indicating extension in duration of release with corresponding increase in relative proportion of EL100. The drug release from these formulations was observed to show strong pH dependency in their release profiles and a sigmoidal character that depended on the relative proportion of EL100. The carboxylic acid group present in Eudragits reacts with the phosphate bases (HPO_4^{2-}) in the buffer resulting in increase in Eudragit dissolution rate in pH 7.4 (52). The release profiles were also significantly different from indomethacin+EL100 (IEL20) matrix, due to the time dependency in release that was conferred by the swelling of xanthan gum (40). Thus, regulating the amount of EL100 in 5% xanthan gum matrix base could confer desired retardation in initial phase followed by controlled release ranging from 12 to 28 h.

With increase in the relative proportion of EL100 from 10% (IXG5EL10) to 20% (IXG5EL20), a proportionate retardation was observed in the corresponding initial release rates resulting in enhanced $t_{10\%}$ values (from 3.6 h for IXG5EL10 to 4.2 h for IXG5EL20). The drug release duration was similarly extended from 13.6 h for IXG5EL10 to 17.7 h for IXG5EL20. This was attributed to increase in total polymer content that resulted in the formation of a relatively strong matrix with decreased porosity and increased tortousity. Similarly, with increase in the relative proportion of EL100 from 10% (IXG10EL10) to 20% (IXG10EL20), a similar effect on the initial drug release rate ($t_{10\%}$ ranging from 3.7 h for IXG10EL10 to 4.6 h for IXG10EL20) was observed. The drug release duration was extended from

13.3 h for IXG10EL10 to 16.2 h for IXG10EL20. Alternately, when the relative proportion of xanthan gum was increased from 5% (IXG5EL10) to 10% *w*/*w* of drug (IXG10EL10), there was insignificant change in the corresponding release kinetics (Table II). This was also true for IXG5EL20 and IXG10EL20, which were statistically similar to each other with respect to the release data. This implied that change in relative proportion of xanthan gum from 5% to 10% in 20% EL100 matrix did not influence matrix properties significantly.

It has been shown previously that high initial swelling of xanthan-gum-based matrices resulted in the release of a significant drug load from formulations during the early drug release phase (41). From the present study, it was inferred that the presence of pH-based polymer EL100 was able to control the initial rapid swelling of xanthan-gum-based matrices and thereby prevent the high percentage of drug release, which was previously observed for formulations prepared with xanthan gum alone (nearly 40% drug release for IXG5 in 2 h). A possible explanation for this could be that, during granulation, the granulating solvent (ethyl alcohol) dissolved a portion of EL100, which not only imparted the necessary adhesion between the matrix components but also formed a layer over the xanthan gum particles. This may have inhibited the swelling of the hydrophilic gum in water. The inhibition of drug release in the initial phase (<2–3% drug release; Fig. 2) is comparable to that reported earlier for EL100 based coated systems in simulated gastric fluid (16).

Secondly, EL100 in a polymeric base could impart a pH-responsive drug release character. With increase in the pH of dissolution medium to 7.4, an increase in the drug release rate was observed on account of matrix erosion due to dissolution of EL100. The formation of a porous matrix then facilitated enhanced diffusion of the drug through the pores. This hypothesis was drawn from a previous report that Eudragit L100 acted as a pore former in a matrix at a higher pH range (53). The values of *n* from Peppas equation for XG+EL100 series ranged from 1.05 to 1.61, indicating release mechanism to be super case II type due to increase in matrix erosion, which can be attributed to dissolution of Eudragit after 2 h in pH 7.4 medium. With the exception of IXG5EL40, all other formulations demonstrated significantly pH- and time-dependent sigmoidal drug release characteristics suitable for colonic delivery. Thus, it was concluded that regulating the relative proportion of EL100 would help attain the desired drug release pattern from a xanthan gum based matrix.

Effect of ES100 on Xanthan Gum Matrices

A plot of cumulative percentage released *versus* time for matrix tablets of indomethacin prepared using xanthan gum in 5% *w*/*w* of drug with varying proportion of Eudragit S 100 (ES100) (5%, 10%, 20%, and 40% *w*/*w* of drug) when compared to indomethacin with ES100 (IES20) is shown in Fig. 3. It was observed that, on increasing the relative proportion of ES100 from 5% to 40% *w*/*w* of drug, there was proportionately greater retardation in the initial release rate as indicated by the $t_{10\%}$ (ranging from 2.1 h for IXG5ES5 to 6.0 h for IXG5ES40). Similarly, drug release was extended from 12.6 h for IXG5ES5 to 37.4 h for IXG5ES40, resulting in a release profile similar to that obtained for indomethacin+ES100 only matrix (IES20) matrix (Table II). The release kinetics calculated for these formulations were significantly different when compared to the matrix base IXG5 (Fig. 1). The $t_{10\%}$ and $t_{90\%}$ values were found to be higher in the case of ES100-based xanthan gum matrices when compared to EL100 probably due to the difference in pH solubility (pH 6.0 for EL100 and 7.0 for ES100) of the two polymers. With increase in relative proportion of hydrophilic gum in matrix, the release profiles differed significantly from indomethacin+ES100 matrix (IES20) matrix as observed for IXG5ES10 and IXG10ES10, and also for IXG5ES20 and IXG10ES20. The hydrophilic component may have aided in the penetration of dissolution fluid that explains the increase in release rate with increase in percentage of hydrophilic polymer, thereby facilitating the release of a highly hydrophobic drug from the matrix.

When the relative proportion of xanthan gum in the matrix was increased from 5% (IXG5ES10) to 10% of drug (IXG10ES10), the release rates were found to increase (Fig. 3). The significantly lowered $t_{10\%}$ (1.0 h) and $t_{90\%}$ (9.2 h) values for IXG10ES10 are indicative of this. Similar release kinetics were observed for IXG10ES20 ($t_{10\%}$ of 2.0 h and $t_{90\%}$ of 9.1 h), which were significantly different from IXG5ES20.

As observed for xanthan gum–EL100 formulations, the retardation in drug release rate was found to depend on the relative proportion of ES100, as shown by the increase in $t_{10\%}$ and $t_{90\%}$ values with increase in proportion of ES100 (Table II). Although good retardation in the initial release phase was observed, there was considerable deviation from the theoretical target of 80–90% release in 14–16 h. As observed in case of EL100-based xanthan gum formulations, ES100-based formulations could successfully retard the initial release, and except for IXG10ES20, all formulations showed less than 3% release in first 2 h, implying that ES100 was as effective in a matrix form in preventing drug release *in vitro* as it is when used as a coating polymer (17–19).

The values of *n* for XG+ES100 series ranged from 0.40 to 1.26, indicating that with an increase in relative proportion of ES100 in the matrix, release mechanism shifted from anomalous (matrix swelling and diffusion) to super case II (erosion type), implying that drug release could have occurred by a combination of several processes like diffusion, swelling of hydrophilic component (polymer relaxation), and erosion of matrix (due to dissolution of Eudragit S100) in alkaline media. Therefore, it was concluded that matrices with 10% xanthan gum with varying proportions of ES100 demonstrated desirable release kinetics *in vitro* and indicate good potential for site specific controlled drug delivery to the colon.

High values of correlation (*r* ranging from 0.9425 to 0.9979) and very low values of MSSR (2.67×10^{-4} to 1.73×10^{-2}) indicate goodness of fit of dissolution data to the power law equation for xanthan gum and Eudragit (EL100 and ES100) matrices (Table II).

Effect of Simulated GI fluid pH (Without Enzymes) on Release

For an ideal colon targeted drug delivery system, the drug release should be prevented in the stomach and small intestine. Release of drugs must be completed within the residence time of the dosage form in the colon. Since colonic residence is highly variable (10 to 30–40 h) (54,55), it was

thought that a drug release program designed for intermediate range of 14–16 h would ensure that maximum drug release would occur even in cases when colonic transit time is on the lower side as is the case in various pathologies of the bowel. Therefore, in the case of the present study, it was assumed that for colon-targeting purpose, a 14- to 16-h extended release formulation with a delay in onset of about 4–6 h would be suitable. This time lag would ensure the passage of the formulation intact through the stomach and small intestine without appreciable drug loss. These two assumptions were used to define a theoretical target release profile shown in Fig. 4.

The *in vitro* release studies conducted in the initial dissolution conditions were intended to characterize and understand the effect of Eudragit on hydrophilic matrix swelling and initial drug release (in distilled water medium for 2 h) and also to investigate the potential of the various formulations to complete drug release in the stipulated time frame of 14–16 h in the alkaline environment of colon (pH 7.4 medium). The performance of selected designed formulations, IXG5ES5, IXG5EL10, and IXG10EL10 was also evaluated in a pH gradient system in order to investigate the suitability of formulations in real-time changing pH situation existing in GI tract (Fig. 4). The choice of pH conditions was pH 1.2 for a duration of 2 h (simulated gastric fluid), pH 4.5 for 2 h (simulated duodenum) followed by pH 7.4 (simulated distal ileum and colon) for the remaining period of study. The drug release from the various formulations was compared with the theoretical target values using f_1 (dissimilarity) and f_2 (similarity) factors (Table III). It was observed that the $t_{10\%}$ and $t_{90\%}$ for IXG10EL10 were 4.9 and 14.1 h, thereby approaching close to target values ($f_2>50$ and $f_1<15$). The drug release from other formulations (IXG5ES5 and IXG5EL10) was also characterized by a sigmoidal pattern and showed only a minor deviation from the target profile (Fig. 4). A significant pH- and time-dependent release pattern was observed for these formulations implying suitability for colon-specific release.

In combination with Eudragit, it was possible to obtain desirable release kinetics from xanthan gum even when employed in very low polymer proportions of 5% and 10%. Furthermore, such matrices can work on the principle of a dual trigger mechanism—pH dependency and time dependent swelling—thereby ensuring bimodality in release. By utilizing a suitable blend of hydrophilic (XG) and slightly hydrophobic (EL100 or ES100) polymers, it was possible to regulate drug release from a matrix to achieve desirable release kinetics (Fig. 4).

Therefore, from the present study, it can be concluded that the use of pH-based polymers in combination with hydrophilic polymer(s) like xanthan gum to form a polymeric matrix base controls the initial swelling of these polymers to a good extent, which could prevent early drug loss from their matrices during upper GI transit. It also confers matrix strength and rigidity to the formulations, thereby enabling lower proportions of these polymers to be used in matrix bases.

Batch Reproducibility and Stability on Storage

No significant difference was observed in the release profile of different batches of each matrix formulation, indicating that the manufacturing process employed was reliable and reproducible. There was no change in the physical appearance or in the drug content of the different formulations at the end of the sixth month storage period at 40°C/75% RH (data not shown). Furthermore, *in vitro* release studies carried out on the formulations stored at accelerated test conditions indicated no statistically significant change in the drug release profiles when compared to formulations stored at ambient conditions (data not shown). These results imply good stability of product on long-term storage. DSC thermograms obtained for pure drug and formulations before and after storage revealed that the melting endotherm and enthalpy of fusion of drug were well preserved in all cases. Furthermore, FTIR studies showed that there was no change in the IR spectrum of drug in formulation (IXG10EL10), and all peaks of pure drug were well preserved. This implied absence of physical and chemical interaction between drug and formulation excipients.

In vivo Evaluation of Formulations

The total length of isolated rat intestine from the stomach was found to be 130±2.5 cm. The formulation IXG10EL10 (administered as mini tablet) was recovered at regular time intervals at a distance of 19.25±2.47 cm (duodenal region) at 2 h, 67.50±10.61 cm (small intestine) at 4 h, 115.50±3.54 cm (cecum) at 6 h and 122.50±3.24 cm (colon) at 8 h. The percentage drug released in GI tract at each time point was calculated by subtracting the percentage drug recovered from each tablet from 100. The percentage tablet at the end of fourth hour was nearly 80%, indicating that drug release was minimal (≈20%) in upper GI tract (Fig. 5). However, release may have been rapid afterwards, as percentage drug recovered at 6 h (from cecal region) was only 25% and that from the colon was less than 15%. The high amount of drug loss in cecal region is attributed to the relatively higher pH of the cecum (6.58±0.4) that could have dissolved the Eudragit polymers and enhanced drug release (56). Thus, it was concluded that, as drug loss during transit through stomach and small intestine was minimal, the formulation could act as a potential colon-specific drug delivery device.

CONCLUSION

Controlled release systems for colon-specific drug were developed successfully and were found to possess acceptable

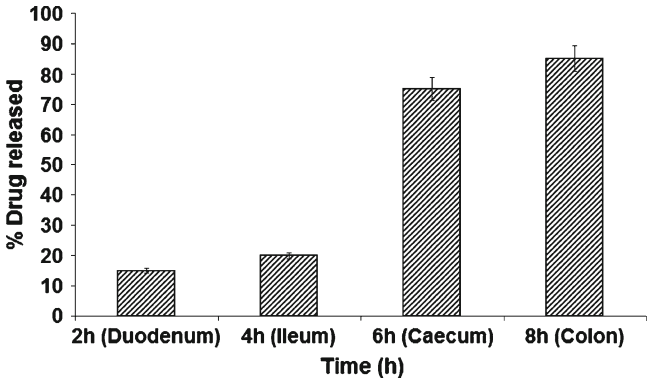

Fig. 5. Percentage drug released from formulation IXG10EL10 at various time points in male Wistar rats ($n=3$)

physical characteristics. Drug release from almost all the matrix bases was characterized by negligible release in the initial phase followed by controlled release for a time period of 14–16 h, which is the normal residence time of a solid dosage form in the colon. Formulations when subjected to stability studies indicated no significant change in physical appearance, drug content, and *in vitro* release pattern. Furthermore, no physical and chemical interaction was evident from DSC and FTIR studies, indicating stability of indomethacin in the prepared matrices. An advantage of such a matrix design that comprises of pH-dependent polymers in polysaccharide matrices is that it can overcome the drawbacks of coated systems wherein there is a possibility of the coat remaining insoluble during its passage through the colon.

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