
Azo Polymers for Colon Targeted Drug Delivery

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Development of a colon specific delivery system bearing flurbiprofen using various azo-aromatic polymers and pH-sensitive polymers are discussed. The azo-aromatic polymers were synthesized and characterized for physical appearance, solubility, film forming properties and effect of colon microbial flora on the polymers. *In vitro* dissolution studies showed that flurbiprofen bearing hard gelatin capsules, coated with these polymers released drug only in simulated gastrointestinal fluid containing human fecal suspension, at pH 7.5. *In vivo* studies revealed that azo-aromatic and pH-sensitive polymer coating disintegrate only in colon following 10 h of oral administration. Hence these polymers can be successfully used to deliver drug at the colon.

Drug delivery to colon is desired not only for oral delivery of proteins but also to treat different diseases associated with the colon. The colonic region is recognized as having a less diversity and intensity of enzymatic activities than stomach and small intestine¹. The site specific drug delivery to the colon may be achieved in several ways¹. The use of prodrugs,³⁻⁴ microbially degradable polymers⁵ and redox sensitive polymers (azo polymer) are commonly described for this purpose⁶⁻⁷. Since it is known that azo function can be reduced in the colon⁸, a lot of novel polymers containing azo groups either in the polymeric backbone⁹ or in the crosslinks¹⁰⁻¹¹ have been synthesized. In order to promote further selective degradation in the vicinity of colonic environment, delivery systems have been designed that contain both pH sensitive acidic monomers and degradable azo aromatic crosslinks¹²⁻¹³. pH-sensitive polymers, which dissolves at or above pH 7 may also, be used for colonic delivery¹⁴⁻¹⁶. These pH sensitive polymers hinder the drug release in stomach and upper intestine. pH sensitive tertiary polymers of different azo compounds were reported to be more promising candidates for colon specific delivery¹⁷.

Colonic drug absorption is improved by increasing colonic residence time. Inflammatory bowel diseases and colitis have increased colonic permeability due to enterotoxins and cytotoxins¹⁸⁻¹⁹. Inflammation of colon is commonly treated by enema administration, which often fails to reach even the transverse colon. Hence, site-specific colon delivery through oral route is an effective alternative. Therefore, the present work is aimed at development of oral delayed release system for colon specific delivery of flurbiprofen.

MATERIALS AND METHODS

FDC Ltd. Mumbai, India generously supplied flurbiprofen as a gift sample. Methyl methacrylate (MMA), butyl methacrylate (BMA), polyacrylate and styrene procured from E. Merck, Mumbai. Sarabhai Chemicals, Vadodara, supplied hydroquinone and dibutyl phthalate. Hydroxy ethyl methacrylate (HEMA) and azo bis iso butyronitrile were purchased from Sigma, USA. Eudragit-[®]s was procured from Rhone Pharma, Germany. All other reagents were of analytical grade and water was double distilled.

Synthesis of azo polymers:

Azo-aromatic polymers were synthesized by bulk polymerization. Monomers in different ratios were taken in a round bottomed flask and 1% azo bis iso butyronitrile as an initiator and 2% DVAB as a cross-linker were added (Table 1). The

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TABLE 1: VARIOUS MONOMERS SELECTED FOR THE SYNTHESIS OF POLYMERS

Monomers selected for the study			Polymer code
Monomer (M 1)	Monomer (M 2)	Ratio (v/v)	
Styrene	Hydroxyethyl methacrylate	1:2	Poly-Styrene: HEMA 1:2
		1:3	Poly-Styrene: HEMA 1:3
		1:4	Poly-Styrene: HEMA 1:4
		1:5	Poly-Styrene: HEMA 1:5
Butyl methacrylate	Hydroxyethyl methacrylate	1:1	Poly-BMA : HEMA 1:1
		2:1	Poly-BMA : HEMA 2:1
		1:2	Poly-BMA : HEMA 1:2
Methyl methacrylate	Hydroxyethyl methacrylate	1:2	Poly-MMA : HEMA 1:2
		1:3	Poly-MMA : HEMA 1:3
		1:4	Poly-MMA : HEMA 1:4
		1:5	Poly-MMA : HEMA 1:5
Polyacrylate*			Polyacrylate
Eudragit®-S*			Eudragit-S

* Ready made polymer

flask was attached with a water condenser and stirred. Temperature was maintained at $70 \pm 2^\circ$. Polymerization was stopped by addition of 5 ml of 0.2% w/v solution of hydroquinone in ethanol, when the mixture attained a gel point. The polymers were isolated and purified by repeated dissolving and crystallizing in respective solvents. Finally, polymers were vacuum dried at 60° .

Characterization of polymers:

The polymers were visually evaluated for color, nature and state of polymer. Film forming property was evaluated by casting polymer solutions (2% w/w) containing 5% w/v (based on polymer weight) dibutyl phthalate (plasticizer) in respective solvents on a mercury substrate in a petridish. A glass ring of constant diameter was used to control the area of the film casting. The solvent evaporation was controlled by covering the petridish with an inverted glass funnel. The films were removed after complete evaporation of the solvent and dried to constant weight at 30° and stored in a dessicator.

Solubility was tested in a series of blend of solvents (20 ml each) in screw-capped test tubes by taking 500 mg

polymer and shaking the contents at constant speed and room temperature using a mechanical wrist action shaker. The time taken to dissolve the polymer was noted.

Effect of microbial flora of colon on polymer:

Glass cover slips were coated with the polymer, by dipping the cover slips in 2% w/v solution of polymer containing 5% w/v dibutyl phthalate as plasticizer and then dried aseptically. These polymer-coated glass cover slips were inoculated with 10 ml sterilized media (Schaefer broth) containing 2-3 ml freshly voided human fecal suspension and then 2 ml liquid paraffin was added to ensure anaerobic conditions. These test tubes were incubated at $37 \pm 1^\circ$ for 8 d.

Formulation of colon targeted drug delivery system:

About 0.1 g flurbiprofen was accurately weighed and filled in hard gelatin capsule (number 4) and sealed. Polymer solution (2% w/v) containing 2% v/v dibutyl phthalate, as plasticizer was prepared and the capsules were dipped six times in this polymer solution to form uniform coating and finally dried in a dessicator.

TABLE 2: CHARACTERIZATION OF SYNTHESIZED POLYMERS

Polymer code	Solubility					Appearance of polymer film	Polymer degradation
	S 1	S 2	S 3	S 4	S 5		
Poly-Styrene: HEMA 1:2	++	++	-	-	+++	Hard orange brownish colour	0
Poly-Styrene: HEMA 1:3	++	++	-	-	+++	Hard orange colour	0
Poly-Styrene: HEMA 1:4	+++	+++	+	-	+++	Tough brownish red colour	**
Poly-Styrene: HEMA 1:5	+++	+++	+	-	+++	Hard cream coloured transparent	***
Poly-BMA : HEMA 1:1	+	++	++	-	+	Hard crystalline reddish orange	0
Poly-BMA : HEMA 2:1	+	++	++	-	+	Tough solid, light yellowish	0
Poly-BMA : HEMA 1:2	+	++	++	-	+	Sticky mass pinkish white	0
Poly-MMA : HEMA 1:2	++	+++	++	-	++	Hard mass brown colour	0
Poly-MMA : HEMA 1:3	++	+++	+	-	++	Hard mass brown colour	0
Poly-MMA : HEMA 1:4	++	+++	++	-	++	Tough solid	**
Poly-MMA : HEMA 1:5	++	+++	++	-	++	Tough solid light brown colour	***
Polyacrylate	++	+++	++	-	++	Crystalline pink colour solid	0
Eudragit-S	+	+++	++	-	++	White solid powder	0

Solubility of the polymers was determined in various solvents i.e. toluene:ethanol, 80:20 (S 1); methylene chloride:ethanol, 50:50 (S2); methanol (S3); toluene (S4); methylene chloride (S5) and indicates by – as polymer insoluble, + as sparingly soluble, ++ as moderately soluble and +++ as highly soluble. Effect of microbial flora of colon on polymer was determined and shown by 0 as no pore formation, ** as partially porous film and *** as completely porous film.

In vitro dissolution study:

Dissolution study was performed using Souder and Ellenbogen extraction technique in simulated gastrointestinal fluids of different pH and using USP XIX dissolution test apparatus²⁰. Simulated gastrointestinal fluid of different pH was prepared by taking different volume ratios of simulated gastric fluid and simulated intestinal fluid (Table 3). *In vitro*

TABLE 3: PREPARATION OF SIMULATED GI FLUIDS OF DIFFERENT pH

pH of media	Simulated gastric fluid	Simulated intestinal fluid
1.2	100.0 parts	parts
4.5	39.0 parts	61.0 parts
6.8	22.5 parts	79.5 parts
7.5*	0.0 parts	100.0 parts

*-pH adjusted after addition of 10 ml human fecal suspension

dissolution study was carried out in simulated gastric fluid (pH 1.2) for 1st hour and mixture of simulated gastric and intestinal fluid (pH 4.5) for 2nd and 3rd hour. The dissolution media was then replaced with simulated intestinal fluid (pH 6.8) for 4th and 5th hour whereas simulated intestinal fluid (pH 7.5) containing 10% human fecal suspension was used in 6th hour.

Dissolution fluid was withdrawn at one-hour intervals for ten hours and samples were analyzed spectrophotometrically at 247 nm for drug content against respective blanks.

In vivo studies:

One hundred milligrams of barium sulphate was filled in hard gelatin capsule (number 4) instead of flurbiprofen and sealed. These capsules were then coated with the polymer by dipping into the selected azo-aromatic polymer, Poly-MMA:HEMA (1:5) and pH sensitive polymer Eudragit-S solution, separately using 5% v/v dibutyl phthalate as plasticizer. The capsule was administered orally to a human vol-

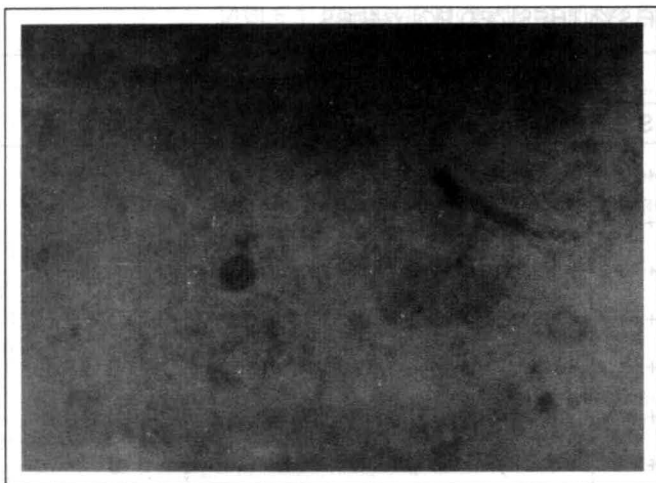


Fig. 1: Photomicrograph showing pH sensitive polymeric film after 8 days incubation in nutrient media (X 100)

unteeer with 250 ml water. After 5, 7 and 10 h intervals, the abdominal area was X-rayed to observe the capsule position in gastrointestinal tract.

RESULTS AND DISCUSSION

Various azo-aromatic co-polymers were synthesized using various combinations of monomers i.e., hydroxy ethyl methacrylate (HEMA), methyl methacrylate (MMA), butyl methacrylate (BMA) and styrene by bulk polymerization method (Table 1). Azo bis-iso-butyronitrile and DVAB were used as initiator and cross linker, respectively. Polyacrylate and Eudragit®-S were taken as pH sensitive polymers. The synthesized polymers were characterized for various physical attributes, i.e. film forming properties, solubility and effect of microbial flora of colon (Table 2).

Co-polymers of Poly-MMA- HEMA (1:4 and 1:5), Poly-Styrene-HEMA (1:4 and 1:5), Polyacrylate and Eudragit®-S showed good film forming property in presence of dibutylphthalate as plasticizer. The synthesized co-polymers were found to be freely soluble in various organic solvents (Table 2).

Isolated polymeric films were prepared with the different azo aromatic polymer and pH sensitive polymers. These polymeric films were incubated anaerobically for 8 d in sterilized media (Schaedler broth) containing freshly voided human fecal suspension. After stipulated period of incubation, the films were observed microscopically and compared with polymer films, which were incubated anaerobically with sterilized media but not inoculated with human feces (Table 2). The effect of colonic bacteria on films of co-polymers of

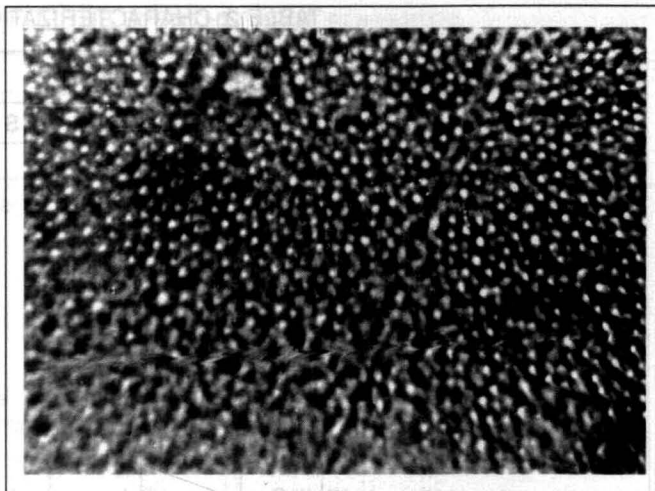


Fig. 2: Photomicrograph showing azo polymer coated film after 8 days incubation in nutrient media (X 100)

styrene-HEMA (1:4) and MMA-HEMA (1:4) was found to be less as compared to the effect on films of co-polymers of styrene-HEMA (1:5) and MMA-HEMA (1:5). The pores formed in the polymeric films were due to the cleavage effect of azoreductase enzyme released by the colonic bacteria on azo bonds of the polymers. The enzymes catalyses the reduction of the azo bonds leading to amines via amide intermediates (figs. 1 and 2). These results are in agreement with the results of Van den Mooter *et al.*¹⁷. In order to quantify the degradation of azo polymeric films by azoreductase, the intrinsic viscosity and permeability of isolated films before and after incubation in Schaedler broth, inoculated with human feces were determined. The control films were incubated in Schaedler broth only. All the polymeric films showed a significant increase in permeability after incubation compared to the control films due to the effect of colonic bacteria (azoreductase) on azo-aromatic polymeric films. No pore formation was seen in case of polymeric films prepared from polyacrylate and Eudragit®-S. It was due to the fact that they are pH sensitive polymers hence were not affected by azoreductase (Table 2; figs. 1 and 2).

The hard gelatin capsules bearing 100 mg flurbiprofen were coated with respective co-polymers using dibutyl phthalate as plasticizer and were used for further studies. *In vitro* dissolution study was performed using Souder and Ellenbogens extraction scheme in simulated gastrointestinal fluids of various pH using USP XIX dissolution test apparatus. The study revealed that the drug release from polymer coated capsule was independent of pH of dissolution media except with Polyacrylate and Eudragit®-S. The azo-

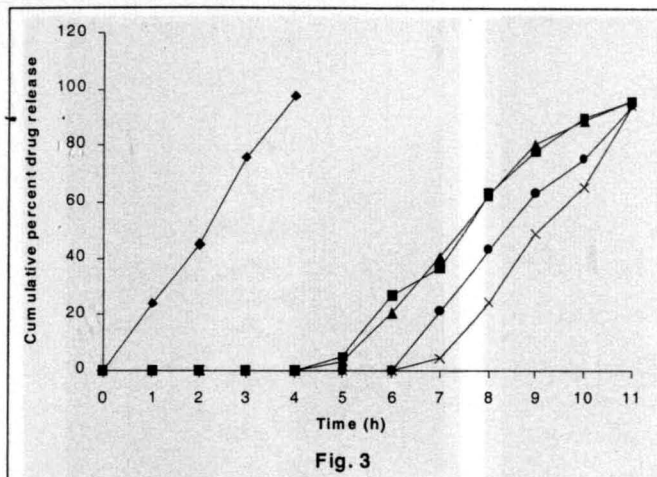


Fig. 3

Fig. 3: Cumulative percent drug release from styrene:HEMA-coated flurbiprofen bearing capsules
 Dissolution rate of uncoated capsules (◆), Styrene:HEMA 1:2 (■), Styrene:HEMA 1:3 (▲), Styrene:HEMA 1:4 (X) and Styrene:HEMA 1:5 (●) coated capsules was performed in presence of gastrointestinal fluid of different pH.

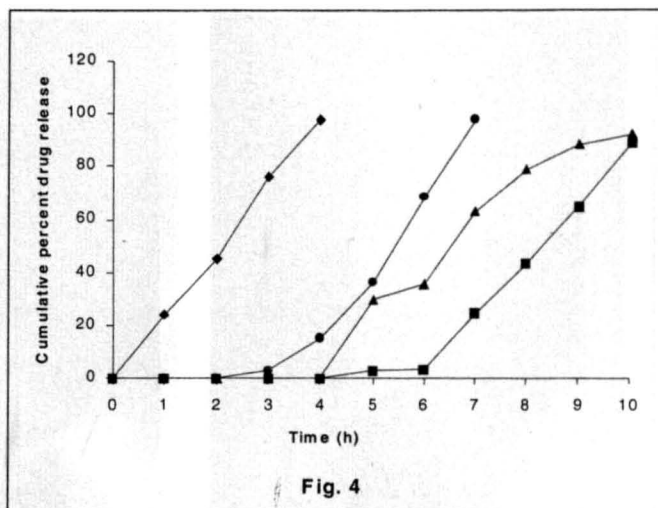


Fig. 4

Fig. 4: Cumulative percent drug release from BMA:HEMA-coated flurbiprofen bearing capsules
 Dissolution rate of uncoated capsules (◆), BMA:HEMA 1:1 (■), BMA:HEMA 1:2 (▲) and BMA:HEMA 2:1 (●) coated capsules was performed in presence of gastrointestinal fluid of different pH.

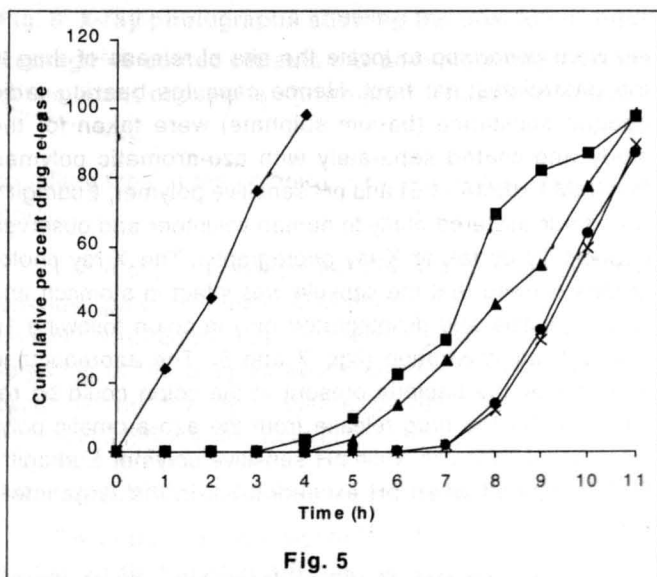


Fig. 5

Fig. 5: Cumulative percent drug release from MMA:HEMA-coated flurbiprofen bearing capsules
 Dissolution rate of uncoated capsules (◆), MMA:HEMA 1:2 (■), MMA:HEMA 1:3 (▲), MMA:HEMA 1:4 (X) and MMA:HEMA 1:5 (●) coated capsules was performed in presence of gastrointestinal fluid of different pH.

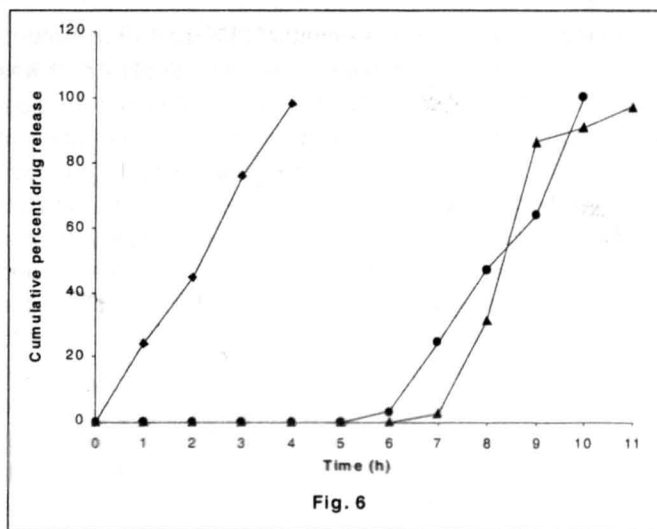


Fig. 6

Fig. 6: Cumulative percent drug release from polyacrylate- and Eudragit-S-coated flurbiprofen bearing capsules
 Dissolution rate of uncoated capsules (◆), Polyacrylate (●) and Eudragit®-S (▲) coated capsules was performed in presence of gastrointestinal fluid of different pH.

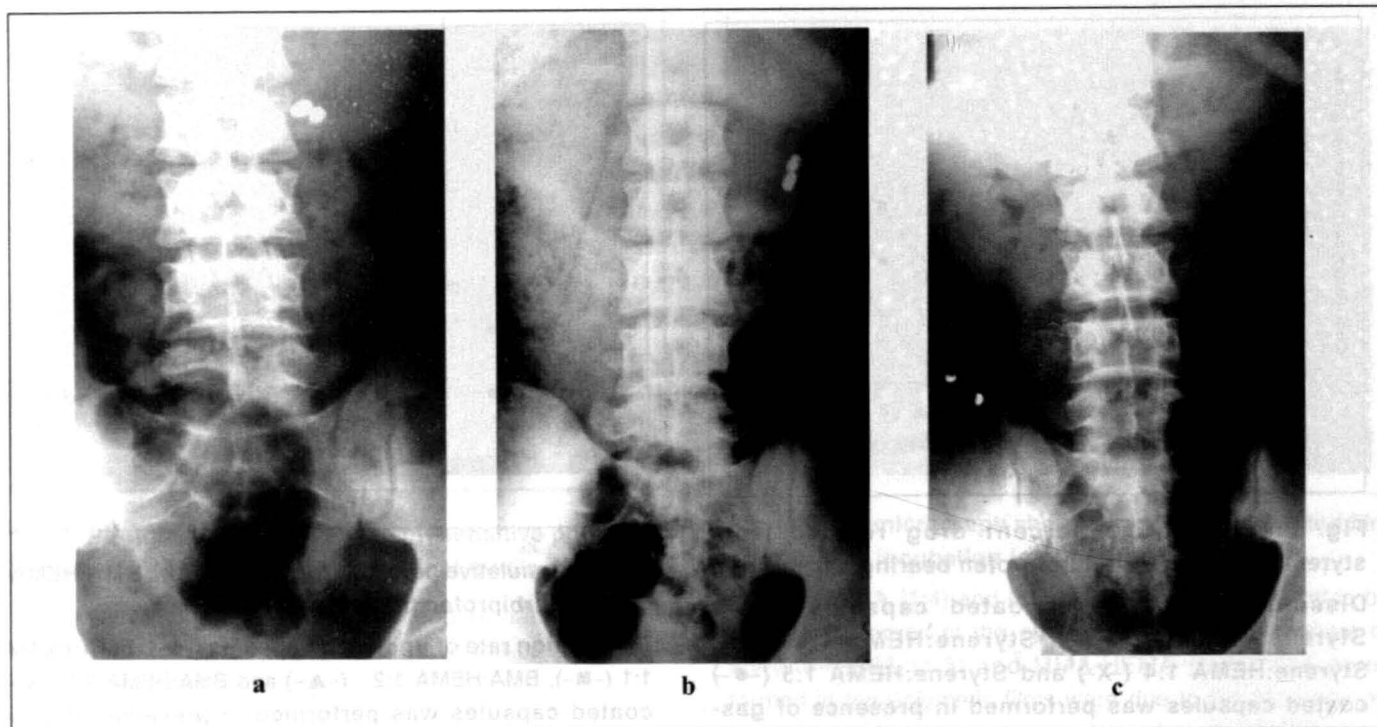


Fig. 7: X-ray photographs showing the position of azo polymer coated capsule bearing barium sulphate in the GIT
Azo polymer coated capsule was administered to human volunteer and X-ray photographs were taken after 5 h (a), 7 h (b) and 10 h (c).

aromatic polymer coated capsule released drug only in media containing human fecal suspension at pH 7.5. It was due to the effect of azoreductase, which degraded the azo-aromatic polymeric coat and facilitated the penetration of dissolution media and diffusion of drug outside the capsule. While, capsules coated with Polyacrylate and Eudragit®S also released drug in a media of pH 7.5 without human fecal suspension. As these are pH dependent polymers and contain ionisable carboxyl groups and when the pH of the media changes to alkaline range these polymers started to dissolve and released the drug in the media. No drug release was observed in a media having pH below 7.5 and devoid of human feces, except for capsules coated with Poly-MMA-HEMA (1:2 and 1:3), Poly-BMA-HEMA (1:1, 2:1, 1:2) and Poly-styrene-HEMA (1:2 and 1:3), which could be due to lack of cross linking of polymers (figs. 3 to 6). Khan *et al.*²¹ proposed a pH-dependent oral drug delivery system using combination of Eudragit® L100-55 and Eudragit® S100 and demonstrated that it is suitable for colon-targeting of drugs.

Poly-MMA-HEMA (1:5) and Eudragit®-S polymer coated capsules were selected for *in vivo* studies. The *in vivo* stud-

ies were performed to locate the site of release of drug in the gastrointestinal tract. Hence capsules bearing radio opaque substance (barium sulphate) were taken for the study and coated separately with azo-aromatic polymer, Poly-MMA-HEMA (1:5) and pH sensitive polymer, Eudragit®-S and administered orally to human volunteer and observed periodically by taking X-ray photographs. The X-ray photographs showed that the capsule was intact in stomach and small intestine and disintegrated only in colon following 10 h of oral administration (figs. 7 and 8). The azoreductase released by the bacteria present in the colon could be responsible for the drug release from the azo-aromatic polymer coated capsules while pH sensitive polymer Eudragit®-S disintegrated when pH exceeded 6.8 in the large intestine.

Van den Mooter *et al.*¹¹ performed *in vivo* studies of colon-specific drug delivery system on Wistar rats using hard gelatin capsule filled with fluorescein (as a tracer substance) and coated with the polymer. They observed that after 18 h the coating had become completely pale yellow and after 24 h it had a gelatinous appearance. The difference in the findings of Van den Mooter *et al.*¹¹ could be due

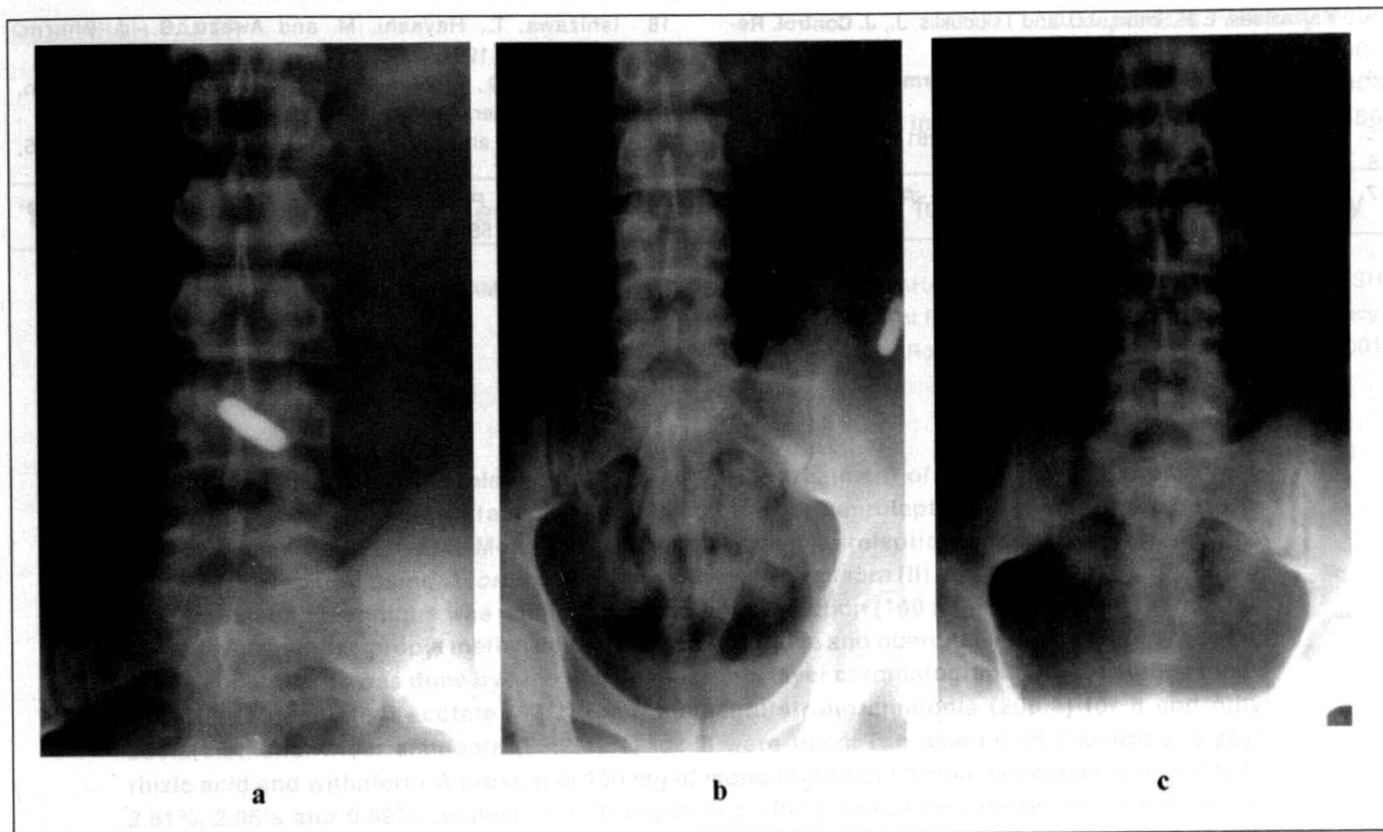


Fig. 8: X-ray photographs showing the position of Eudragit®-S coated capsule bearing barium sulphate in the GIT. Eudragit®-S coated capsule was administered to human volunteer and X-ray photographs were taken and the position of the intact capsule is shown after 5 h (a) and 7 h (b) while capsule was completely dissolved after 10 h (c).

to differences in the colonic microflora of rats and human being.

Therefore, it is concluded that the synthesized azo-aromatic polymer (Poly-MMA-HEMA:1:5) and pH sensitive polymer (Eudragit®-S) can successfully be used for colonic targeting of the drug. However, polymer structure elucidation and their toxicity studies to be explored clinically before launching into the market.

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