Design and Development of Hydrogel Beads for Targeted Drug Delivery to the Colon

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ABSTRACT

The purpose of this research was to develop and evaluate a multiparticulate system of chitosan hydrogel beads exploiting pH-sensitive property and specific biodegradability for colon-targeted delivery of satranidazole. Chitosan hydrogel beads were prepared by the cross-linking method followed by enteric coating with Eudragit S100. All formulations were evaluated for particle size, encapsulation efficiency, swellability, and in vitro drug release. The size of the beads was found to range from 1.04 ± 0.82 mm to 1.95 ± 0.05 mm. The amount of the drug released after 24 hours from the formulation was found to be 97.67% ± 1.25% in the presence of extracellular enzymes as compared with 64.71% ± 1.91% and 96.52% ± 1.81% release of drug after 3 and 6 days of enzyme induction, respectively, in the presence of 4% cecal content. Degradation of the chitosan hydrogel beads in the presence of extracellular enzymes as compared with rat cecal and colonic enzymes indicates the potential of this multiparticulate system to serve as a carrier to deliver macromolecules specifically to the colon and can be offered as a substitute in vitro system for performing degradation studies. Studies demonstrated that orally administered chitosan hydrogel beads can be used effectively for the delivery of drug to the colon.

KEYWORDS: Chitosan, pH-sensitive polymer, colon-specific drug delivery, hydrogel beads, multiparticulate system, satranidazole.

INTRODUCTION

Targeting of drugs specifically to the colon is advantageous in the treatment of diseases such as amoebiasis, Crohn's disease, ulcerative colitis, and colorectal cancer. In addition, it has shown great potential in the oral delivery of therapeutic peptides and proteins, which are unstable in the upper part of the gastrointestinal (GI) tract. The colonic region is recognized as having less diversity and intensity of enzymatic activities than the stomach and small intestine.[1] Various strategies are available for targeting drug release selectively to the colon.[2] The designing of prodrugs is based on the concept of preventing the release of drugs in the stomach and small intestine, and drug release is triggered by the use of some specific property at the target site such as altered pH or high activity of certain enzymes in comparison to nontarget tissues.[3,4] Since it is known that azo function can be reduced in the colon,[5] many novel polymers containing azo groups either in the polymeric backbone[6] or in the crosslinks[7,8] have been synthesized. Jain et al[9] exhaustively reviewed drug delivery using azo polymers. To promote further selective degradation in the vicinity of the colonic environment, delivery systems have been designed that contain both pH-sensitive acidic monomers and degradable azo aromatic crosslinks.[10,11] Polysaccharides such as chitosan, pectin, inulin, and guar gum have been explored for their potential in colon-specific drug delivery.[12-15] The polysaccharides remain intact in the hostile environment of the stomach and small intestine, and upon arrival in the colon they are degraded by polysaccharidases.[16] pH-sensitive polymers, which dissolve at or above pH 7.0, may also be used for colonic delivery. Ashford et al[17] showed that pH-sensitive polymers are not suitable for colon-targeted drug delivery systems because of poor site specificity. The long lag time at the ileocecal junction and fast transit indicate that a single unit may not be the best dosage form for a colon-targeted drug delivery system.

Chitosan is a functional linear polymer derived from chitin, the most abundant natural polysaccharide on the earth after cellulose, and it is not digested in the upper GI tract by human digestive enzymes.[18,19] Chitosan is a copolymer consisting of 2-amino-2-deoxy- D-glucose and 2-acetamido-2-deoxy-D-glucose units linked with β-(1–4) bonds. It should be susceptible to glycosidic hydrolysis by microbial enzymes in the colon because it possesses glycosidic linkages similar to those of other enzymatically depolymerized polysaccharides. The polysaccharide, on reaching the colon, undergoes assimilation by microorganisms or degradation by enzymes or break down of the polymer backbone leading to a subsequent reduction in molecular weight and thereby

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loss of mechanical strength and is unable to hold the drug any longer.[20] Chitosan has drawn attention for its potential to achieve site-specific delivery to the colon. Few reports have been published on the investigation of the application of chitosan in colon targeting.[21,22]

Because of the advantages of multiparticulate dosage forms over single unit preparations, such as more uniform dispersion in the GI tract, more uniform drug absorption, less inter- and intra- individual variability, and more flexible formulation process, interest in multiparticulates as oral drug delivery systems has been growing steadily. Because of their small particle size, multiparticulates can pass through the upper GI tract easily, can reach the colon quickly, and are retained longer in the ascending colon.[23] Therefore, a multiparticulate system of chitosan would be a desired dosage form for colon targeting.

The proposed multiparticulate system, hydrogel beads, combines the pH-sensitive property of enteric polymers as well as the biodegradability of chitosan in the colon for targeted delivery of satranidazole (SNZ) for the treatment of amoebiasis. The drug release is supposed to take place after dissolution of the enteric coating in the small intestine and biodegradation of the chitosan in the colon as a result of the presence of polysaccharidases in the colonic contents.

Materials and Methods

Materials

Chitosan (50 cps [purified viscosity grade], molecular weight: 150 KDa) was a kind gift sample from Panaecea Biotech, Chandigarh, India. Glutaraldehyde was from Spectrochem Pvt. Ltd (Mumbai, India). Toluene, Tris HCl buffer, and Triethyl citrate (TEC) was purchased from Central Drug House (New Delhi, India). Satranidazole was a gift sample from Alkem (Mumbai, India). Eudragit S-100 was procured as a gift sample from Degusssa (Darmstadt, Germany). All other solvents and reagents were of analytical grade.

Method

Preparation of chitosan hydrogel beads

Chitosan hydrogel beads were prepared using the method reported by Zhang et al,[24] with slight modifications. Chitosan was dissolved in different concentrations (1% to 4%) of acetic acid (1% wt/vol) and satranidazole (5 to 20 mg) was dissolved separately in dimethyl formamide (DMF). Then, the chitosan solution (2.5 mL) and the drug solution (7.5 mL) were mixed together to obtain 10 mL of chitosan drug solution. The chitosan drug solution was added dropwise (using a disposable syringe with a 22-gauge needle) into 40 mL of sodium chloride–saturated Tris HCl buffer solution containing glutaraldehyde-saturated toluene (GST) in different concentrations (1 to 3 mL). The beads were separated after 1 hour of curing time and subsequently decanted, washed twice with 3 mL of 0.05 M Tris-HCl buffer, and the beads were dried in a vacuum oven at 40°C.

Various formulation and process variables that could affect the preparation and properties of the beads were identified and optimized to get small, discrete, uniform, smooth-surfaced, and spherical beads. The various formulation and process variables are described in Table 1 (underlined values were used as constants).

Coating of chitosan hydrogel beads

The enteric coating layer of the chitosan hydrogel beads bearing satranidazole was performed by film coating in a conventional coating pan. The enteric coating solution was prepared as reported by Huyghebaert et al.[25] In brief, it was prepared by first making a milky latex of Eudragit S100 using 1M ammonia (1.5%). After 1 hour TEC (60%) was added and stirring was continued for 30 minutes. Talc was added to the milky latex as an antitacking agent. The enteric coating dispersion was passed through a 0.3-mm sieve before use. Throughout the coating process the coating dispersion was stirred using a magnetic stirrer. The parameters of the film-coating process were as follows: pan rotating speed, 20 rpm; atomizing air pressure, 2 bar; inlet air temperature, 60 to 70°C; outlet air temperature, 35 to 40°C; bead bed temperature, 38°C. The film-coated beads were not removed from the pan until complete weight gain was achieved. A series of coated products with different film thicknesses were produced, quantified by the % total weight gain (%TWG), by varying the amount of coating solution sprayed.

Characterization of the satranidazole-loaded chitosan hydrogel beads

Shape and surface morphology

Surface and shape characteristics of chitosan hydrogel beads were evaluated by means of a scanning electron microscope (FEI-Qunta-200 SEM, FEI Company, Hillsboro, OR). The samples for SEM were prepared by lightly sprinkling the beads on a double adhesive tape, which stuck to an aluminum stub. The stubs were than coated with gold to a thickness of ~300Å using a sputter coater and viewed under the scanning electron microscope (Figure 1a).

Particle size

After drying at 37°C for 48 hours, the mean diameter of the dried beads was determined by a sieving method using USP standard sieves. Observations are recorded in Table 1.

Table 1. Compositions and Characteristics of Different Chitosan Beads

Variables	Values	Formulation Code	Degree of Swelling	Average Particle Size (mm) (± SD*)	Encapsulation Efficiency (%)† (± SD*)
Satranidazole	0	D_0	1.22 ± 0.05	1.17 ± 0.08	—
(% wt/wt)	5	D_1	1.11 ± 0.18	1.60 ± 0.08	75.8 ± 4.19
	10	D_2	1.01 ± 0.01	1.71 ± 0.06	76.4 ± 2.39
	15	D_3	0.95 ± 0.09	1.92 ± 0.04	78.5 ± 1.72
	20	D_4	0.80 ± 0.12	1.60 ± 0.04	62.2 ± 0.33
Chitosan (% wt/vol)	1	C_1	0.83 ± 0.21	1.20 ± 0.10	80.0 ± 0.24
	2	C_2	1.11 ± 1.21	1.40 ± 0.11	80.8 ± 5.30
	3	C_3	1.26 ± 1.25	1.69 ± 0.09	81.4 ± 5.40
	4	C_4	1.38 ± 1.35	1.90 ± 0.05	84.0 ± 3.62
GST(mL)	1	G_1	1.50 ± 1.47	1.95 ± 0.05	74.3 ± 3.45
	2	G_2	1.15 ± 1.10	1.87 ± 0.02	76.2 ± 2.44
	3	G_3	0.69 ± 1.02	1.70 ± 0.02	79.5 ± 4.88
	4	G_4	0.34 ± 0.58	1.37 ± 0.01	82.1 ± 4.55
Cross-linking time (h)	10-minute	Ct_1	0.56 ± 0.52	1.95 ± 0.12	70.2 ± 0.22
	20-minute	Ct_2	0.57 ± 0.12	1.89 ± 0.22	85.7 ± 0.20
	40-minute	Ct_3	0.83 ± 0.05	1.68 ± 0.32	75.3 ± 1.01
Drying (°C)	Lyophilization	Dr_1	0.85 ± 0.52	1.36 ± 0.12	65.5 ± 0.12
	45	Dr_2	0.75 ± 0.72	1.04 ± 0.82	64.1 ± 0.12

* Data are expressed as mean ± SD of at least triplicate.

† Encapsulation efficiency was calculated based on the initial drug loading.

Underlined values indicate optimized parameter; D, Drug; C, Chitosan; GST, glutaraldehyde-saturated toluene; Ct, cross-linking time; Dr, drying temperature; —, not applicable.

Drug content and encapsulation efficiency

Encapsulation efficiency (EE) is the amount of added drug (in percent) that is encapsulated in the formulation of the beads. The EE of drug from hydrogel beads can be calculated in terms of the ratio of drug in the final formulation to the amount of added drug.

An accurately weighed amount (100 mg) of the formulation of beads was dispersed in 100 mL of Tris HCl buffer. The sample was ultrasonicated for 3 consecutive periods of 5 minutes each, with a resting period of 5 minutes each. It was left to equilibrate for 24 hours at room temperature, and then centrifuged at 3000 rpm for 15 minutes. The concentration of satranidazole in the decanted Tris HCl buffer and 2 washing solutions was determined by measuring the absorbance at 313.8 nm using a GBS Cintra 10-UV-Visible Spectrophotometer (Shimadzu, Japan). The determinations were made in triplicate, and results were averaged (Table 1).

Equilibrium swelling studies

Chitosan hydrogel beads (100 mg) were placed in phosphate-buffered saline (PBS) (pH 7.4) and allowed to swell up to a constant weight. The beads were removed, blotted with filter paper, and changes in weight were measured and recorded in Table 1. The degree of swelling (α) was then calculated from the formula:

$$\alpha = \frac{(w_g - w_o)}{w_o}, \qquad (1)$$

where, w_o is the initial weight of beads and w_g is the weight of beads at equilibrium swelling in the medium.

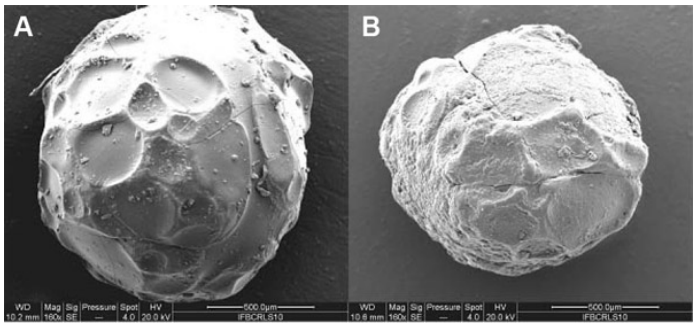

Figure 1. Scanning electron micrograph of chitosan hydrogel beads. A, Uncoated; B, Eudragit S100–coated.

Preparation of rat cecal and colonic medium (enzyme induction method)

The rat cecal and colonic medium was obtained by the induction method to assess the biodegradability of chitosan by colonic bacteria as proposed by Jain et al.[15] This medium is termed Type A rat cecal and colonic medium.

Two groups of 4 randomly chosen albino rats weighing between 150 and 200 g were selected for the present study and maintained on a normal diet. To induce the enzymes that specifically act on the chitosan during its passage through the colon, the rats were intubated with Teflon tubing and 1 mL of 1% wt/vol dispersion of chitosan in water was administered directly into the stomach. This treatment was continued for 3 days with the first group of 4 rats and 6 days with the second group. The rats were humanely killed and were dissected before release rate studies. The cecum was isolated, ligated at both ends, and cut loose and immediately transferred into simulated intestinal fluid of pH 7.4 previously bubbled with carbon dioxide. The cecal contents were individually weighed, pooled, and suspended in buffer to produce a final cecal concentration of 5% wt/vol. Drug release rate studies for the initial 5 hours were performed as described in the next section. From the sixth hour and onwards it was performed in simulated intestinal fluid containing rat cecal contents. The experiment was performed with a continuous supply of carbon dioxide into the dissolution media.

Preparation of rat cecal and colonic medium (extracellular and cell associated enzyme isolation method)

In the present study we observed drug release in the presence of extracellular and cell-associated enzymes and compared them with the enzyme induction method to assess the biodegradability of chitosan in colonic conditions.

The rat cecal and colonic contents were collected and then enzymes were isolated by a differential centrifugation technique.[26] Two randomly chosen albino rats weighing between 150 and 200 g were selected and maintained on normal diet (bengal grams soaked in water). These rats were anesthetized with anesthetic ether and then killed by decapitation. The cecum and colon contents were collected and diluted with cold 0.1 M isotonic acetate buffer at pH 5.0 to produce a final cecal concentration of 5% wt/vol. The suspended debris was removed by centrifugation at 500*g* for 15 minutes. Supernatants were then recentrifuged at 10 000*g* for another 30 minutes in a refrigerated centrifuge (Remi C-24, Mumbai, India) to obtain a clear supernatant containing extracellular enzymes and a ''bacterial pellet.'' It has been shown that this method is effective in separating bacteria from soluble elements.[27]

Because cell-associated enzyme systems from Bacteroides strains have been shown to degrade linear soluble polysaccharides,[28] it was necessary to prepare a second enzyme mixture from the isolated bacterial pellet. The resulting bacterial pellet was washed and resuspended in buffer solution. The cell walls were disrupted by sonication for 2 minutes in an ice bath. After ultrasonic disruption, the suspensions were then cleared of gross bacterial components by centrifugation at 10 000*g* to yield a clear second enzyme system containing the cell-associated enzymes. Such a rat cecal and colonic enzyme system was used as a release medium. This medium is termed Type B rat cecal and colonic medium.

In vitro drug release from beads

In vitro drug release studies were performed according to Chourasia and Jain's[29] extraction technique using USP dissolution test apparatus (apparatus 2). The dissolution studies were performed in 900 mL of dissolution medium, which was stirred at 100 rpm at 37 ± 0.1°C.

The scheme of using the simulated fluids at different pH was as follows:

- 1st hour: Simulated gastric fluid of pH 1.2
- 2nd to 3rd hour: Mixture of simulated gastric and intestinal fluid of pH 4.5
- 4th to 5th hour: Simulated intestinal fluid of pH 7.4
- 6th to 8th hour: Simulated colonic fluid of pH 7.0 (Type A/B)

In vitro drug release studies were performed as per the scheme in different simulated fluids. Simulation of GI transit conditions was achieved by using different dissolution media. Simulated gastric fluid (SGF) pH 1.2 consisted of NaCl (2.0 g), HCl (7 mL), and pepsin (3.2 g); pH was adjusted to 1.2 ± 0.5. simulated intestinal fluid (SIF) pH 7.4 consisted of KH_2PO_4 (6.8 g), 0.2N NaOH (190 mL), and pancreatin (10.0 g); pH was adjusted to 7.4 ± 0.1. SIF pH 4.5 was prepared by mixing SGF pH 1.2 and SIF pH 7.4 in a ratio of 39:61. The experiment was performed with a continuous supply of carbon dioxide into dissolution media. Aliquots of samples were withdrawn periodically and replaced with an equal amount of fresh dissolution media bubbled with carbon dioxide. The volume was made up to 10 mL and centrifuged. The supernatant was filtered through Whatman filter paper (Dawsonville, GA), and drug content was determined spectrophotometrically at 313.8 nm (UV 1601, Shimadzu, Japan). Each experiment was conducted at least in triplicate.

Statistical analysis

Experimental data have been represented as the mean with standard deviation (SD) of different independent determinations.

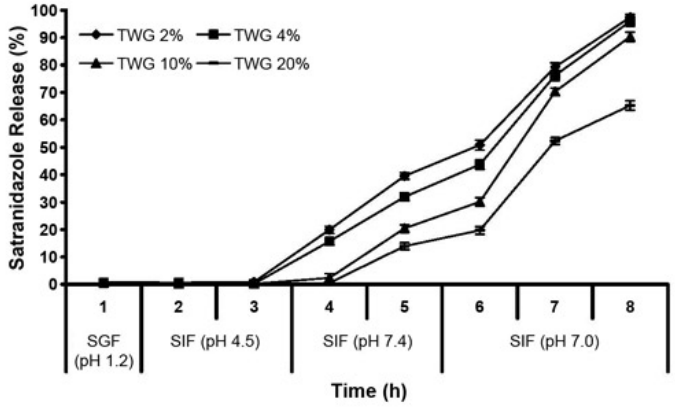

Figure 2. In vitro dissolution profiles for satranidazole from Eudragit S100–coated chitosan hydrogel beads as a function of coating thickness (TWG).

The significance of differences was evaluated by analysis of variance (ANOVA). Differences were considered statistically significant at $P < .005$.

Results and Discussion

Bead characteristics

These hydrogel beads had good spherical geometry. It is obvious that the surface of the beads shrank and a densely cross-linked gel structure was formed. This may explain the greater retardation of drug release from matrices of higher cross linker content. There was no significant variation in particle sizes among the different batches of beads; the mean diameter being 1.62 ± 0.13 mm. The average drug entrapment was found to be 76.85% ± 2.43% in the beads. The mean diameter of CHG beads ranged between 1.04 ± 0.82 mm and 1.95 ± 0.05 mm. The mean diameter of the drug-loaded CHG beads was increased on increasing the amount of drug, ie, as the drug content is increased from 5% to 15%, the beads size increased to 1.92 ± 0.04 mm but on further increasing the drug content, bead size decreased. Moreover, the longer the cross-linking time, the lower the average size of the CHG beads. ie, as cross-linking time is increased from 10 to 40 minutes, bead size decreased from 1.95 ± 0.12 to 1.68 ± 0.32 mm. The bead diameter decreased as the cross-linking time or concentration of cross-linker was increased since the cross-linker promotes the formation of cross-links between chitosan molecules and makes a more compact mass.

Optimization of coating thickness

The coating thickness over the drug-bearing beads was optimized in terms of total weight gain (TWG) of the beads after coating with enteric coating dispersion and their effect on in vitro drug release in simulated GIT fluids was studied. It was observed that in simulated intestinal fluid (pH 7.4) at the fifth hour, drug release was observed to be 39.5% and 31.9% with 2% and 4% TWG respectively, while 20.4% and 13.9% with 10% and 20% TWG. On changing the dissolution medium to simulated intestinal fluid (pH 7.0) at the end of 8 hours, drug release was observed to be 97.4%, 95.9%, 90.3%, 65.2% from coating thickness of 2%, 4%, 10%, 20% TWG, respectively. The formulation with the coating thickness of TWG 10% was deemed the most suitable coating parameter due to its optimum drug release by virtue of ionization, disruption, and dissolution of the coating, since beads with 20% TWG show hindered drug release (Figure 2). Ideally the coating should be thick enough to resist satranidazole release for a sufficient period of time equivalent to transit through the upper GI tract, yet not so thick that drug release under colonic conditions is hindered. Coating thicknesses of TWG = 10% coating appear to fulfill this criteria.

Under pH conditions resembling the upper GI tract, the cumulative amount of drug released in 8 hours in the dissolution test varied between 85.9% ± 1.09% and 87.4% ± 1.25% for Eudragit S100–coated chitosan hydrogel beads (Figure 3). The rate of release was inversely proportional to the thickness of the coat, thereby inferring that the film coat was controlling the release process. The mechanism of release was likely to be via diffusion followed by dissolution.

Drug content and encapsulation efficiency

Results of drug content and EE demonstrated that drug content increased from 9.18 ± 0.81 mg/100 mg to 25.62 ± 0.43 mg/100 mg of beads with increasing the amount of drug from 5% to 20% wt/wt. No significant increase ($P > .05$) in drug content was observed on further increasing the amount of drug, ie, above 15% wt/wt, which could be due to the

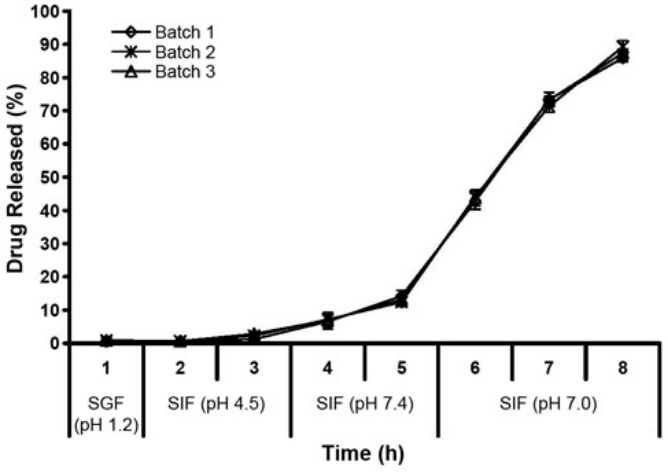

Figure 3. The in vitro release profiles of satranidazole from Eudragit S100–coated chitosan hydrogel beads in various simulated gastrointestinal fluids (n = 3).

limited solubility of the drug in DMF and that is endorsed from the presence of drug particles on the surface of the beads prepared with 20% of drug concentration. The percent EE was increased up to 85.7% ± 0.20% with increasing polymer concentrations to 4%. Concentration of the cross-linking agent exhibited no significant effect ($P > .05$) on percent EE.

Further, the EE increases from 75.8% ± 4.19% to 78.5% ± 1.72% until the drug concentration added was 15%. But on increasing the amount of drug to 20%, EE was decreased. This exhibited the maximum loading capacity of the polymer beads. The EE increased with the increased cross-linking time from 10 to 20 minutes; however, the EE decreased when the cross-linking time was increased beyond 20 minutes.

Drug release

In the case of uncoated chitosan beads, nearly 80% to 90% of the drug was released in the initial 4 to 5 hours. This situation is not acceptable for drugs that are required to be released locally in the colon. Chitosan hydrogel beads were coated with Eudragit S100 polymer to retard the release of the drug until the pH reaches above 7.0.

The effect of drug concentration, chitosan concentration, GST concentration, and cross-linking time on in vitro drug release was also observed. In vitro drug release after 5 hours was 84.2% ± 4.1% in the case of beads having 15% drug, while it was 89.2% ± 3.78% for beads with 20% drug. The effect of chitosan concentration on the release of drug was found to be meager. It is also observed that the amount of drug released from beads decreased on increasing cross-linking time. These properties are probably explained by the promotion of cross-links between chitosan chains and GST. GST concentration was also studied to determine its effect on satranidazole release. A decrease in drug release was also observed with an increase in GST concentration ($P < .05$). This may be because as the amount of glutaraldehyde was increased it produced microspheres with pronounced cross-linking between polymer chains that retarded the release of drug (Table 1). Sezer and Akbuga[30] also observed this release pattern in alginate beads. Freeze-drying of the samples resulted in larger and more porous beads and slightly faster disintegration times compared with air-dried beads. Freeze-drying had the advantage of avoiding drug extraction by immediately freezing and removing the water present within the beads by sublimation. When air-dried, the beads were exposed to water within for longer periods of time. Depending on drug solubility, this could result in drug extraction from the beads and a burst effect during the dissolution study.

In the second part of this investigation cross-linked chitosan beads were coated with Eudragit S-100. Figure 3 shows the drug release from various chitosan-TPP hydrogel beads in different simulated GIT fluids. The initial release of SNZ from the coated hydrogel beads was low. A small amount of SNZ could be measured in the pH 1.2 medium after 2 hours. Only 2.11% ± 0.6% was released after 3 hours and 6.87% ± 1.95% after 4 hours. After 8 hours, approximately 87.53% ± 1.38% drug had been released. Enteric coating has traditionally been used to prevent drug release in the upper GI tract. Ashford et al[17] used Eudragit S to protect tablets in the stomach and upper small bowel. The results of the present study prove that the amoebic drug was protected completely from acid and enzymes in gastric juice by a membrane coating of Eudragit S100, because Eudragit polymer contains carboxyl groups that ionize from neutral to alkaline media. As the ionization takes place, the integrity of the film is disturbed and releases the drug. At pH 7.4 (small intestine), the membrane coating dissolved, and the gel beads were exposed. Following the polymer matrix swelling and erosion, the drug was released.

The amount of SNZ released from enteric coated beads after the 5-hour study was found to be 13.27% ± 1.39%, which attests the ability of the chitosan to remain intact in the physiological environment of the stomach and small intestine. The relatively higher drug release after 5 hours reveals the presence of cracks and channels throughout the film coat. This increase in drug release suggests that most of the drug has dissolved and leached out through the cracks and channels in the coat, which is supposed to be most of the surface anchored drug. The drug released during 5-hour release rate studies, is because of the presence of unentrapped drug on the surface of the beads. These results are concordant with the results of Chourasia and Jain,[29] who have used enteric-coated chitosan microspheres for colon-targeted drug delivery where after 5 hours of testing in 0.1 M HCl and pH 7.4 Sorensen's phosphate buffer, 15.27% ± 0.58% of the drug was released from microspheres.

Conventional dissolution testing is less likely to accurately predict in vivo performance of colon delivery systems triggered by bacteria residing in the colon (because aspects of the colon's environment, ie, scarcity of fluid, reduced motility, and presence of microflora, cannot be simulated in conventional dissolution methods). Hence, release studies were performed in an alternate release medium (Sorensen's buffer containing rat cecal content at different concentrations) called rat cecal content release medium.

The in vitro drug release studies were also performed in SCF (pH 7.0) with and without using rat cecal contents. A significant difference ($P < .005$) was observed in the amount of SNZ released at the end of 24 hours from the dissolution medium having rat cecal content when compared with the study conducted without using rat cecal content. The amount of the drug released from the formulation was found to

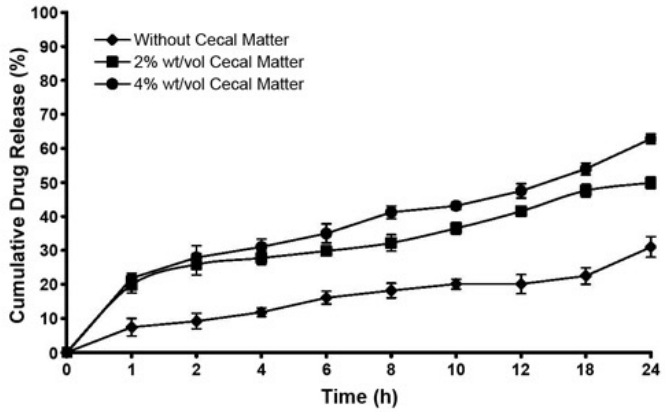

Figure 4. Comparative percent drug release in SCF (pH 7.0) from the chitosan hydrogel beads with and without rat cecal contents.

be 49.89% ± 2.39% with 2% wt/vol cecal matter after 24 hours. whereas in control study (without rat cecal contents in dissolution medium) only 31.02% ± 1.49% of drug was released (Figure 4). In case of dissolution medium having 4% cecal matter, 62.78% ± 1.42% of drug release was observed, which is considerably higher in comparison to the study involving no cecal matter. The study reveals that the release of the drug in the physiological environment of the colon is due to the degradation of chitosan by colonic bacteria present in rat cecal content.

The colonic bacterial action of rat cecal content medium (2% and 4% wt/vol) might not be sufficient to degrade such a gel barrier of the swollen beads and for this reason only 49.89% ± 1.71% and 62.78% ± 1.42% of drug was released after 24 hours with 2% and 4% wt/vol rat cecal content medium, respectively. Hence, rat cecal content was collected after the induction of enzymes responsible for the degradation of chitosan by administering 1 mL of 1% wt/vol aqueous solution of chitosan for 3 days. Studies were performed in the SCF containing 2% and 4% wt/vol rat cecal content collected after 3 days of enzyme induction and enhanced drug release was observed, ie, 57.04% ± 1.99% and 64.74% ± 1.91%, respectively after 24 hours (Figure 5). Induction of enzymes for 3 days resulted in improved activity of colonic enzymes that is reflected in the release of a higher amount of drug in the release rate study in comparison with those that involved rat cecal content without induction. In spite of the release of a higher percentage of drug after 3 days of induction compared with those without induction, there was a considerable amount of drug to be released and hence, enzyme induction was increased from 3 days to 6 days and then rat cecal content was collected and mixed with 2% and 4% wt/vol concentration in the SCF. The release of the drug was considerably improved with cecal content obtained after 6 days of enzyme induction in comparison with those without enzyme induction or 3 days induction. Drug released was observed to be 69.35% ± 1.09% and 96.52% ± 1.81% in 24 hours in SCF containing 6 days enzyme induced 2 and 4% cecal contents respectively, after 6 days of enzyme induction.

These results were also compared with drug release for 24 hours in the presence of Type B rat colonic medium just containing chitosanolytic extracellular enzymes isolated from rat cecal and colonic content. A significant difference ($P < .005$) was observed in the amount of SNZ released at the end of 24 hours from the dissolution medium having 2% rat cecal content after 3 and 6 days of enzyme induction as compared with 4% rat cecal content after 3 and 6 days of enzyme induction. The amount of the drug released after 24 hours from the formulation was found to be 97.67% ± 1.25% in presence of extracellular enzymes as compared with 64.71% ± 1.91% and 96.52% ± 1.81% release of drug after 3 and 6 days of enzyme induction in presence of 4% cecal content, respectively (Figure 5 and 6). This indicates that such a system might be useful for initial screening purposes without requiring rat induction studies or rat handling for 6 days.

CONCLUSION

Results of release studies indicate that Eudragit S100–coated chitosan beads offer a high degree of protection from premature drug release in simulated upper GIT conditions. Eudragit S100–coated chitosan beads deliver most of the drug load in the colon, an environment rich in bacterial enzymes that degrade the chitosan and allow drug release to occur at the desired site. Thus, spherical glutaraldehyde cross-linked chitosan hydrogel beads are a potential system for colon delivery of SNZ for chemotherapy of amoebic infection.

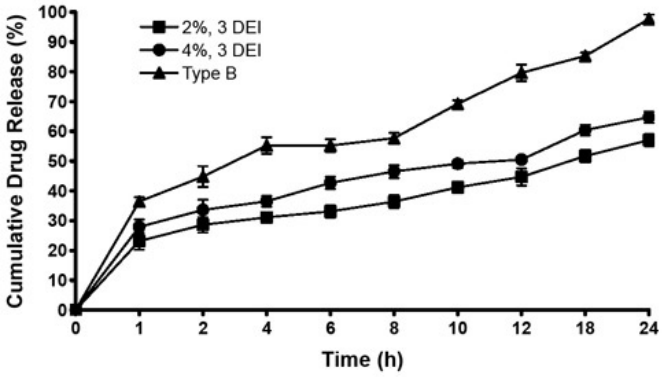

Figure 5. In vitro drug release from chitosan hydrogel beads ($CPGB_2$) in SCF pH 7.0 in the presence of Type A and B rat cecal contents (3 DEI, 3 days' enzyme induction).

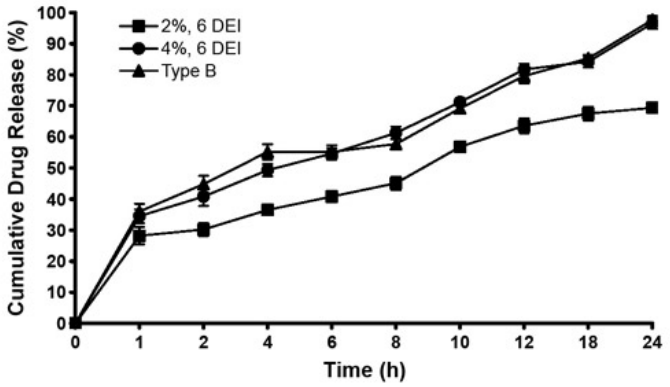

Figure 6. In vitro drug release from chitosan hydrogel beads ($CPGB_2$) in SCF (pH 7.0) in presence of Type A and B rat cecal contents (6 DEI, 6 days' enzyme induction).

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