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RESEARCH PAPER

Polysaccharide Matrices for Microbially Triggered Drug Delivery to the Colon

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ABSTRACT

Matrix tablets were prepared using xanthan gum (XG) and guar gum (GG) in varying proportions, and the suitability of the prepared tablets was evaluated for colon specific drug delivery. Indomethacin was used as a model drug. The ability of the prepared matrices to retard drug release in the upper gastrointestinal tract (GIT) and to undergo enzymatic hydrolysis by the colonic bacteria was evaluated. For this, drug release studies were carried out in the presence of rat cecal content. Further cecal content of rats with induced enzymatic activity were used. To ascertain the role of bacterial flora in carrying out the hydrolysis of the tablet, cecal content of rats treated with antibiotics were used in the dissolution media. Presence of XG in combination with GG in the tablets could retard drug release in the conditions of the upper GIT. However, the presence of GG and starch made these matrices microbially degradable. Guar gum alone as a drug release-retarding excipient in the matrices does not achieve the desired retardation. Presence of XG in the tablets not only retards the initial drug release from the tablets, but due to high swelling, makes them more vulnerable to digestion by the microbial enzymes in the colon.

Key Words: Xanthan gum; Guar gum; Colonic drug delivery; Colon targeting.

INTRODUCTION

There has been an increasing interest in the development of site-specific systems for release of drugs in the colon. Systems have been developed for topical treatment of inflammatory bowel disease, e.g., ulcerative colitis and Crohn's disease, and for systemic drug delivery. Studies are also on for the development of systems facilitating delivery of proteins and peptides to the colon, which is being visualized as a probable site for their administration.[1]

Several methods have been developed for confining drug release to the colon. One of the oldest and the most commonly employed method uses enteric

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polymers as coating materials over tablets, granules, or pellets. These rely upon the difference in pH values in the gastrointestinal tract (GIT).[2] Others include time-controlled release systems,[3] pressure controlled release systems,[4] prodrugs,[5] polysaccharide-based delivery systems[6,7] and osmotically controlled release systems.[8] Among the various systems developed for colon-specific drug delivery, prodrugs and polysaccharide-based delivery systems rely upon the enzymatic degradation of the carrier in the colon, thereby resulting in drug release. The inherent bacterial flora present in the colon carries out this enzymatic degradation. The enzyme-trigger mechanism in such delivery systems makes them highly site specific. Prodrugs, however, can only be formed with a limited number of drug moieties due to a chemical linkage required in their formation. Further, as new chemical entities, prodrugs require a detailed toxicological study to be performed before being used as drug carriers. Natural polysaccharides, however, fall under the category of ''GRAS'' (Generally Regarded As Safe) and with the use of these, the general problems associated with safety are resolved.

Natural polysaccharides, including chitosan, guar gum, pectin, dextran, cyclodextrin, inulin, etc.[6] remain undigested in the stomach and small intestine and are degraded by the vast anaerobic microflora of the colon, e.g., *bacteroides*, *bifidobacteria*, *eubacteria*, *clostridia*, to smaller monosaccharides, which are then used as energy source by the bacteria.

The present investigation is aimed at using the inexpensive, naturally occurring, and abundantly available polysaccharides for colon-targeted drug delivery. An attempt has been made to formulate a dosage that 1) retards drug release in the tracts of the upper GIT, 2) consists of biodegradable polysaccharides as the main constituent, 3) is degradable by a wider range of microbial species, 4) shows rapid drug release in the tracts of the colon due to the presence of a high concentration of degradable polysaccharides in the tablet, and additionally 5) could be formulated using the usual tableting techniques.

Working on this rationale, matrices were proposed for the above purpose. A drug release-retarding ingredient belonging to polysaccharides, i.e., xanthan gum, was selected for the study.[9,10] Guar gum, another polysaccharide being widely used for colon targeting, was selected as the other ingredient. Guar gum (GG) alone has earlier been used in colon-specific drug delivery as matrix forming material and as a compression coat.[11–13] Xanthan gum (XG) is known to have a greater drug release-retarding property and synergistically enhanced gel properties in presence of galactomannan gums such as guar.[14] So, a combination of these gums was proposed for achieving the above objective. A mixture of these gums was evaluated for its drug release-retarding properties under simulated gastrointestinal tract (GIT) conditions. This mixture was proposed to retard drug release more significantly in conditions of the upper GIT (as compared to guar alone) but still retained biodegradability due to the presence of guar gum.

Xanthan gum is a high molecular weight polysaccharide gum produced by pure-culture aerobic fermentation with gram negative bacterium, *Xanthomonas campestris*. It contains D-glucose and D-mannose as the dominant hexose units, along with D-glucuronic acid. It is used in oral and topical pharmaceutical formulations as a suspending, stabilizing, thickening, and emulsifying agent.[9]

Guar gum is a natural nonionic polysaccharide derived from the seeds of *Cyamopsis tetragonolobus* (Family: Leguminosae). It consists of linear chains of (1–4)-beta-D-mannopyranosyl units with alpha-D-galactopyranosyl units attached by[1–6] linkages. It is used in pharmaceutical preparations in the form of binders and disintegrating, suspending, thickening, and stabilizing agents. Starch, usually a filler in the tablet dosage form, was used in the formulation to take advantage of its biodegradation in the colon by the resident bacteria.[15]

MATERIAL AND METHODS

Materials

Guar gum (M.W. 220,000) was procured from Himedia Laboratories Limited, Mumbai, Maharashtra, India. Xanthan gum USNF was obtained as a gift sample from Dabur India Ltd., Ghaziabad, India. Indomethacin was obtained from Indian Drugs and Pharmaceuticals Ltd., Gurgaon, Haryana, India. Starch, talc, and magnesium stearate used for the preparation of tablets were of pharmacopoeial grade.

Preparation of Tables

Matrix tablets containing 25 mg of indomethacin, xanthan gum and guar gum (XG:GG) in varying ratios (as per Table 1) were prepared by wet granulation using 10% starch paste as the binder. The wet mass was passed through an 8 mesh sieve and the prepared granules were dried at 40° C for 6 h. The dried granules were passed through a 16-mesh sieve and were

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Table 1. Tablet code and the percentage of polysaccharide in the formulation.

Formulation code	Percentage of XG in the tablet	Percentage of GG in the tablet
XG:GG (0:30)	0	30
XG:GG (10:20)	10	20
XG:GG (15:15)	15	15
XG:GG (30:0)	30	0

lubricated with a mixture of talc and magnesium stearate (1.77% and 1.33%, respectively). The lubricated granules were compressed individually into tablets at a compression force of 4500–5500 kg. The tablets were tableted on a single-station tableting machine using 7.8 mm round punches. The compressed matrix tablets were tested for hardness, drug content, and drug release characteristics.

Swelling Studies

In order to understand the dissolution behavior of the drug from the matrices, swelling studies were conducted under conditions similar to those used for the dissolution studies. Tablets prepared with xanthan gum and guar gum in varying concentrations were subjected to swelling studies[8,9] at a temperature of 37±0.5° C. Initial studies were conducted in 75 mL of 0.1N HCl, pH 1.2 (2 h) followed by addition of 25 mL of 0.2 M trisodium phosphate and adjusting the pH to 6.8 (22 h). Swelling studies were conducted in triplicate for each formulation. Radial swelling of tablet width was noted manually from time to time (Figure 1).

Drug Release Studies

The ability of the prepared tablets to retard drug release in the physiological environment of the stomach and the small intestine was assessed by conducting drug release studies in simulated stomach and small intestinal pH, respectively. The changing pH media, Method 1, USP 23, for delayed release tablets was used. Dissolution test was conducted in USP 1 apparatus at 75 rpm and a temperature of 37±0.5° C. Initial drug release studies were conducted in 750 mL of 0.1N HCl for 2 h. Then, 250 mL of 0.2 M trisodium phosphate was added to the dissolution media and the pH adjusted to 6.8. Samples were withdrawn after regular intervals of time to evaluate drug release. These were analyzed spectrophotometrically at a wavelength of 318 nm.

Drug Release Studies in the Presence of Rat Cecal Content

To access the susceptibility of the prepared matrices to undergo degradation in the presence of the colonic bacteria, drug release studies were carried out in the presence of rat cecal content, since these are known to have similar contents to that of human intestinal microflora.[16] Wistar rats weighing 100–150 g were selected for the present study. These were maintained at a normal diet. To stimulate enzymes which specifically hydrolyze guar gum, enzyme

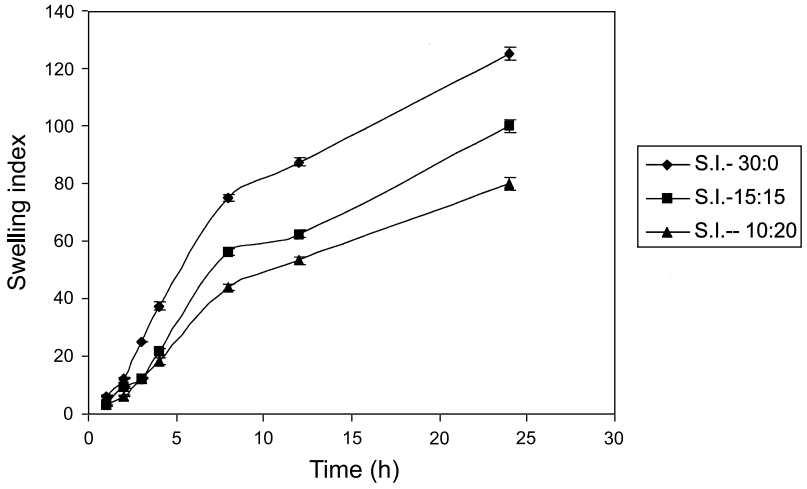

Figure 1. Swelling index vs. time graph for various XG:GG tablets.

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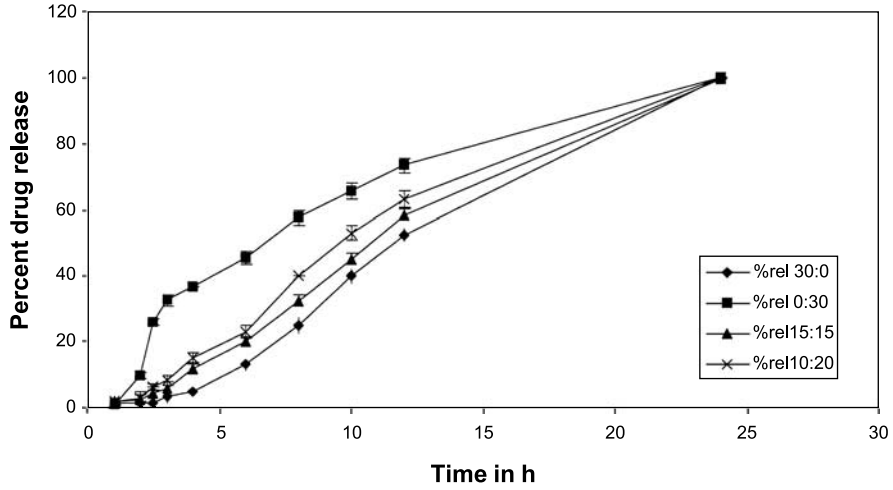

Figure 2. Percent drug release vs. time graph for various XG:GG combinations.

induction was done. For enzyme induction, 2 mL of a 1% dispersion of guar gum in water was administered to the rats daily for 7 days.

For the removal of cecal content, 45 minutes prior to its introduction into the dissolution media, five rats were killed by decapitation. The abdomens of the rats were cut open, and the ceca were isolated, then tied on both the ends and cut. The cecal content was individually transferred to a previously weighed beaker containing 10 mL of buffer pH 6.8 (previously bubbled with nitrogen). The weight of the pooled cecal content was taken. Nitrogen was continuously passed through the pooled content so as to keep the environment anaerobic.

Drug release studies were carried out using USP dissolution test apparatus. However, the procedure was slightly modified. The experiments were carried out in a 250-mL beaker immersed in water maintained in the jars of dissolution test apparatus. Initial studies were carried out in 150 mL of 0.1N HCl for 2 h. After this, 50 mL of 0.2 M trisodium phosphate was added and the pH was adjusted to 6.8. The study at a pH of 6.8 was continued for 3 h after which cecal content equivalent to 4 g and 8 g was added to 200 mL of buffer (pH 6.8) to give a final cecal dilution of 2% and 4%, respectively. Dissolution in the cecal content media was carried out until completion of 24 h. The experiments in cecal content media were carried out in the presence of a continuous supply of nitrogen. At different time intervals 1-mL samples were withdrawn from the dissolution medium and 1 mL of cecal content (2% or 4% as the case may be), maintained under anaerobic conditions, was replenished into the dissolution media. The volume of the sample was made up to 10 mL, filtered through G-5 filter, and the filtrate was analyzed for indomethacin content at 318 nm.[12]

Antibiotic Treatment

In order to deplete the microflora present in the cecum and the colon of rats, antibiotic treatment was given to the rats. This included administration of 50 mg/kg of metronidazole suspension (Aristo Pharma, India) once a day to rats for a period of 7 days.

RESULTS AND DISCUSSION

Swelling Studies

Swelling studies were carried out on all the prepared XG:GG tablet combinations in triplicate. The XG:GG (0:30) tablets started forming loose gel immediately after introduction of the tablet into the medium. This was probably indicative of disintegration or disentanglement of the outer layers of the gum immediately after introduction into the aqueous medium. The inner core of the tablet, which could be seen, however, showed progressive swelling. Formation of loose gel outside the tablet prevented any measurement of the swelling behavior of these tablets. The rest of the three formulations containing XG showed progressive swelling (Figure 1). These remained intact throughout the study, probably due to the presence of XG in the tablet. The swelling in XG:GG tablets in an increasing order were 10:20<15:15<30:0.

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Table 2. Percent drug release of the prepared tablets at different time intervals.

Tablet code	Cumulative percent drug release (5 h)	Cumulative percent drug release (24 h)
XG:GG (0:30)	42	100.01
XG:GG (10:20)	19	99.87
XG:GG (15:15)	16	99.56
XG:GG (30:0)	9	99.76

The Initial 2-h Study was Conducted in 0.1 N HCL (2 h) and further study at buffer pH 6.8.

Drug Release Studies

For the formulation of a delivery system for colon targeting, it is a prerequisite that the drug release should be minimal until the dosage form reaches the colon. The percent drug release vs. time profile (Figure 2 and Table 2) of the prepared matrices show that the presence of XG in the tablet retards the drug release. In XG:GG (30:0) tablets drug release is highly retarded and only 9% of the drug is released in the first 5 h of the dissolution (the usual upper GIT transit time) as against a 42% drug release observed in the case of XG:GG (0:30) tablets. In both cases, total drug release takes place in the next 19 h. Considering that the usual colonic transit time varies between 20 and 30 h,[17] complete drug release would take place in the colon. The XG:GG (30:0) tablets seem quite promising for colonic drug delivery, forming time-dependent delivery systems. A 42% drug release in XG:GG (0:30) observed in the first 5 h can be attributed to formation of a very loose gel upon introduction of the tablet in the dissolution media, leading to drug release from the outer layers of the tablet. This is in agreement with observations made in the swelling studies. The superficial gum layers disentangle and hydrate instantaneously in the dissolution media. Further, drug release from these tablets (0:30) takes place at a retarded rate due to swelling of inner layers of GG. However, a 30% guar gum concentration in the tablet alone could retard drug release for the present purpose. From the above results it can be inferred that presence of XG in the tablet retards drug release to a much higher extent as compared to GG.

In order to increase the degradable portion (making the formulation highly sensitive to arrival into the colon) of the tablet and thereby reduce the proportion of the nondegradable polysaccharide, i.e., xanthan gum in the formulation, various combinations of XG and GG were tried for formulation of an optimum dosage form, which would show minimal drug release in the upper GIT and still consist of a high percentage of microbially degradable polysaccharide. So, XG:GG (15:15) and XG:GG (10:20) were formulated.

Drug release studies in XG:GG (15:15) (Figures 3 and 4) showed a greater retardation in drug release as compared to XG:GG (10:20). This may be due to the presence of higher XG concentration in the former. During the initial 5 h of dissolution, 19% drug release was observed in XG:GG (10:20) tablets, which was further reduced to 16% in XG:GG (15:15) tablets. In

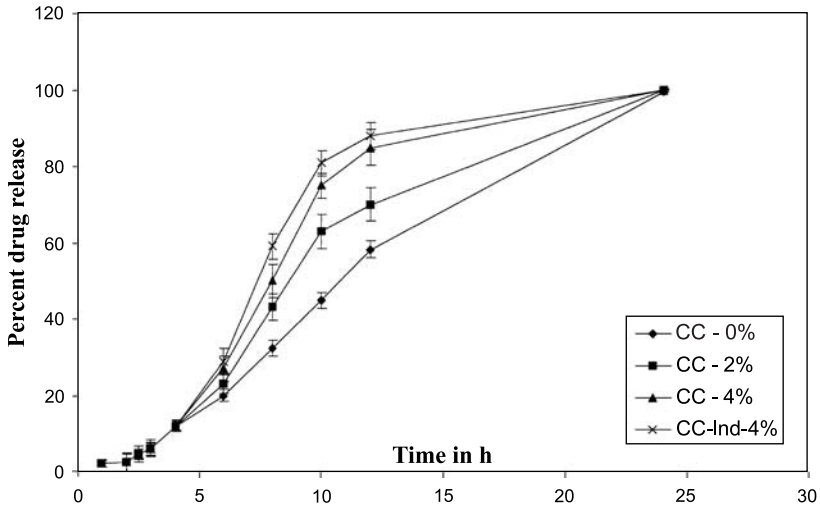

Figure 3. Percent drug release vs. time profile of XG:GG (15:15) tablets in 0%, 2%, 4%, cecal content (CC) and in presence of 4% cecal content of rats with enzyme induction (CC-Ind).

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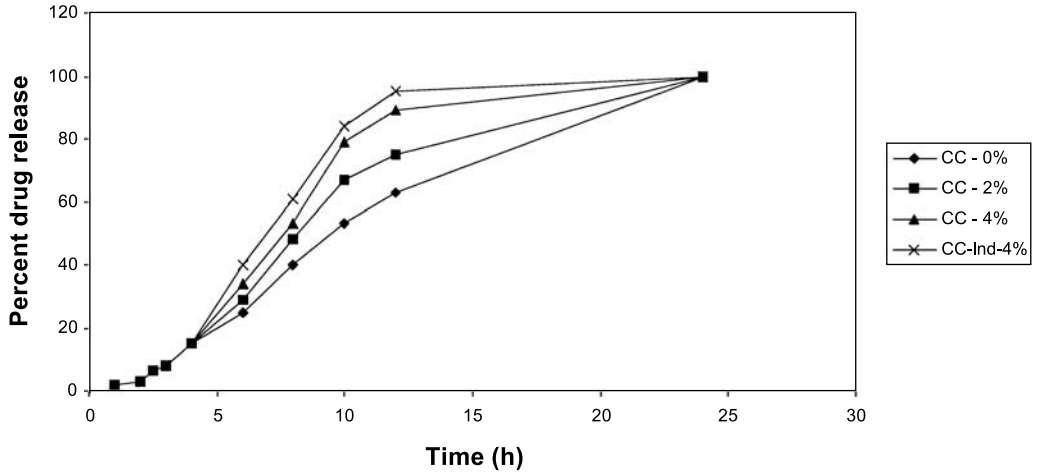

Figure 4. Percent drug release vs. time profile of XG:GG (10:20) tablets in 0%, 2%, 4% cecal content (CC) and in presence of 4% cecal content of rats with enzyme induction (CC-Ind).

both these tablets, as in the previous case, total drug was released from the tablet in the next 19 h. This can be explained on the basis that on exposing the tablets to the dissolution media, the gums with synergistic gelling properties form a viscous gel that slows down the further seepage of dissolution fluid into the tablet. The initial delay in drug release can also be attributed to the time taken for the glassy to rubbery transition by the gum combination.[18] The drug present on the surface of the tablet accounts for the initial release seen.

Drug release studies in the cases of XG:GG (10:20) and XG:GG (15:15) were carried out in the presence of cecal content in order to evaluate the susceptibility of the prepared matrices to be acted upon by the microflora of the colon. In the presence of 2% rat cecal content, drug release was enhanced in the cases of both the tablets and this was further increased upon increasing the percentage of the cecal content in the medium to 4%. Introduction of 4% cecal content of rats induced with guar gum also increased the drug release rate. In the case of XG:GG (15:15) tablets after 5 h (i.e., at the 10th h of dissolution) of incubation in 2% cecal content, a 64% drug release was observed (vs. 44% in the case of 0% cecal content), which increased to 75% in 4% cecal content and to 81% in the case of 4% cecal content of induced rats (Figure 3). A similar study of XG:GG (10:20) tablets showed an increase in drug release to 67% in the case of 2% cecal content as compared to 53% in the case of 0% cecal content and 79% in the case of 4%. Addition of 4% cecal content of induced rats increased the percent drug release to 84% (Figure 4).

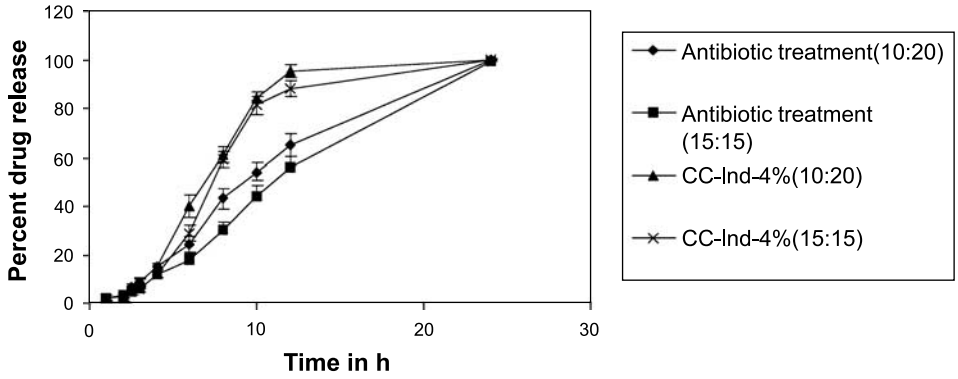

Figure 5. Percent drug release vs. time profile in XG:GG tablets after induction and antibiotic treatment.

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The increase in drug release upon increasing the concentration of cecal content from 2% to 4% may be attributed to the increase in the bacterial population available for utilizing these polysaccharides as substrates and carrying out their hydrolysis. The addition of cecal content of rats by giving guar gum increased the drug release. However, addition of cecal content of induced rats did not show a very marked increase as has been seen in earlier studies by Krishnaiah, Satyanaryana and Rama Prasad.[13] This may be because induction was carried out using guar gum, which only formed a small portion of the prepared tablet. Also, it was observed that drug release in the case of XG:GG (10:20) was faster as compared to XG:GG (15:15) tablets. The addition of 2%, 4%, and 4% cecal content of rat with induced enzymatic activity had greater effect on drug release in the case of XG:GG (15:15) tablets with respect to XG:GG (10:20) tablets, which had a higher content of GG in them. This may be explained on the basis that due to a higher content of XG in 15:15 tablets, they show higher swelling, which in turn increases the surface area available for the action of hydrolytic enzymes. So, although drug release is faster in 10:20 tablets, the presence of cecal content had greater effect on 15:15 tablets.

To evaluate the role of microflora in carrying out the hydrolysis of the tablet matrix, the cecal content of rats pretreated with antibiotic were used for dissolution studies (Figure 5). The presence of cecal content of these rats did not enhance the drug release. The effect of the presence of cecal content of rats with induced enzymatic activity in contrast to those given an antibiotic treatment is shown in Figure 5. This figure clearly shows the importance of these microflora in carrying out the hydrolysis of the matrices.

CONCLUSION

The prepared matrices (XG:GG, 30:0, 15:15, and 10:20) form enzyme-controlled delivery systems with nearly 55–75% of the tablet content being constituted by polysaccharides degradable by colonic microflora. These matrices form enzyme degradable delivery systems. However, in certain diseased conditions of the the GIT, with a disturbed microflora, these can provide drug delivery to the colon since these additionally form time-controlled release systems and shall provide a sustained drug delivery to the colon. Under normal GIT conditions, with a normal microflora they are designed to release the majority of the drug in the tracts of the ascending colon, e.g., in local colonic pathologies. The presence of XG in the tablets not only retards initial drug release in the upper GIT, but also increases the susceptibility of the matrices to microflora, as higher swelling increase surface area available for action of enzymes. So, the matrices prepared using the usual tableting techniques were highly site-specific and could prove ideal for delivery of such insoluble molecules to the colon. Guar gum used alone as a drug-release retarding constituent in the present concentrations is not able to provide drug delivery specifically to the colon.

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