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Development of Polysaccharide-Based Colon Targeted Drug Delivery Systems for the Treatment of Amoebiasis

Raghavendra C. Mundargi and Sangamesh A. Patil
Department of Chemistry, Center of Excellence in Polymer Science, Karnatak University, Dharwad, India

Sunil A. Agnihotri and Tejraj M. Aminabhavi
Drug Delivery Division, Center of Excellence in Polymer Science, Karnatak University, Dharwad, India

ABSTRACT The main focus of this study is to develop colon targeted drug delivery systems for metronidazole (MTZ). Tablets were prepared using various polysaccharides or indigenously developed graft copolymer of methacrylic acid with guar gum (GG) as a carrier. Various polysaccharides such as GG, xanthan gum, pectin, carrageenan, β-cyclodextrin (CD) or methacrylic acid-*g*-guar (MAA-*g*-GG) gum have been selected and evaluated. The prepared tablets were tested in vitro for their suitability as colon-specific drug delivery systems. To further improve the colon specificity, some selected tablet formulations were enteric coated with Eudragit-L 100 to give protection in an acidic environment. Drug release studies were performed in simulated gastric fluid (SGF) for 2 hr followed by simulated intestinal fluid (SIF) at pH 7.4. The dissolution data demonstrate that the rate of drug release is dependent upon the nature and concentration of polysaccharide/polymer used in the formulations. Uncoated tablets containing xanthan gum or mixture of xanthan gum with graft copolymer showed 30–40% drug release during the initial 4–5 hr, whereas for tablets containing GG with the graft copolymer, it was 70%. After enteric coating, the release was drastically reduced to 18–24%. The other polysaccharides were unable to protect drug release under similar conditions. Preparations with xanthan gum as a matrix showed the time-dependent release behavior. Further, in vitro release was performed in the dissolution media with rat caecal contents. Results indicated an enhanced release when compared to formulations studied in dissolution media without rat caecal contents, because of microbial degradation or polymer solubilization. The nature of drug transport was found to be non-Fickian in case of uncoated formulations, whereas for the coated formulations, it was found to be super-Case-II. Statistical analyses of release data indicated that MTZ release is significantly affected by the nature of the polysaccharide used and enteric coating of the tablet. Differential scanning calorimetry indicated the presence of crystalline nature of drug in the formulations.

KEYWORDS Colon targeted drug delivery, Polysaccharides, Metronidazole, In vitro release kinetics, Enteric coating

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Address correspondence to Tejraj M. Aminabhavi, Drug Delivery Division, Center of Excellence in Polymer Science, Karnatak University, Dharwad 580003, India; E-mail: aminabhavi@yahoo.com

INTRODUCTION

The delivery systems intended to release drugs in the colon require protection of the drug from the hostile environment of stomach and small intestine. This target-specific release is required for the topical treatment of diseases associated with colon such as amoebiasis, ulcerative colitis, Crohn's disease, and colon cancer. In addition, it has tremendous potentials for the oral delivery of therapeutic proteins and peptides because of the presence of favorable environment in colon in comparison to upper gastrointestinal tract (GIT) (Reddy et al., 1999). This is because the colon provides a less hostile environment for drugs caused by the low diversity and intensity of the digestive enzymatic activities as well as a near neutral pH. Moreover, colon transit time may last up to 78 hr, which is likely to increase the time available for drug absorption. Site-specific drug delivery to colon may therefore be achieved by different approaches. Among these, include coating with pH-sensitive polymers, coating with biodegradable polymers, fabrication of pro-drugs, timed-release systems, embedding in biodegradable matrices and hydrogels (Rubinstein, 1990; Vandamme et al., 2002; Chourasia & Jain., 2003). Coating of pH-sensitive polymers to tablets, capsules or pellets provide the delayed release and protect the active drug from the gastric fluid (Khan et al., 1999; Wilding et al., 1992). Microbially degradable polymers, especially azo-crosslinked polymers have been investigated in targeting drugs to colon (Jain et al., 2003; Vanden Mooter et al., 1993).

Amoebiasis is an infection of the large intestine caused by *Entamoeba histolytica*, a single-celled protozoan parasite. The trophozoite form of *E. histolytica* can invade the colonic epithelium, causing amoebic colitis. Tinidazole and metronidazole (MTZ) are the preferred drugs used in the treatment of intestinal amoebiasis, giardiasis, trichomoniasis, and anaerobic infections (Tracy & Webster, 1996). These drugs are to be delivered to the colon for their effective action against *E. histolytica*, wherein the trophozoites reside in the lumen of the caecum and large intestine and adhere to the colonic mucus and epithelial layers (McCoy et al., 1994). MTZ is completely absorbed after the oral administration (Lau et al., 1992), but in the conventional tablet form, it provides a minimal amount for local action in the colon and still results in the relief of amoebiasis, giardiasis, and other anaerobic infections, but with unwanted systemic effects. Hence, to overcome these side effects, colon targeted delivery system for MTZ is highly desirable.

Previous literature cites the use of polysaccharides, guar gum (GG) and xanthan gum (XG) as matrices for colon-specific delivery (Goldstein et al., 1973; Rama Prasad et al., 1998; Toti & Aminabhavi, 2004; Sinha & Kumria, 2002). GG is a natural nonionic polysaccharide derived from the seeds of *Cyamopsis tetragonolobus* (family: Leguminosae). It consists of linear chains of (1,4)-β-D-mannopyranosyl units with -α-D-galactopyranosyl units attached by (1,6) linkages. XG is a high molecular weight extracellular polysaccharide produced by the fermentation of gram-negative bacterium, *Xanthomonas campestris*. XG offers potential utility as a drug carrier because of its inertness and biocompatibility. It has been reported (Andreopoulos & Tarantili, 2001; Sinha & Kumria, 2002) by many researchers that XG can be used as an effective excipient for developing sustained release and colon targeted formulations.

Pectin remains intact in the stomach and the small intestine, but is degraded by the bacterial inhabitants of the human colon (Rubinstein et al., 1993; Kosaraju, 2005). However, β-cyclodextrin (CD) is degraded into small saccharides by the vast micro flora present in the colon, especially *Bacteroides* (Challa et al., 2005). Carrageenan is mainly used as the gelling and thickening agent; only a few studies have dealt with carrageenans for controlled release (CR) tablets (Bhardwaj et al., 2000; Takka et al., 1998). In our earlier study, methacrylic acid-*g*-guar gum (MAA-*g*-GG) was synthesized in the laboratory by free radical polymerization, which could be utilized for colon-specific delivery (Mundargi et al., 2006). Based on the above considerations and the previous literature, which had emphasis on a single polysaccharide, the present study is undertaken on five different polysaccharides and graft copolymer (MAA-*g*-GG) to identify the polysaccharide or a mixture of polysaccharides with graft copolymer for their suitability in colon-specific delivery of MTZ. In this sense, this work is of novel nature. Tablets were prepared using these polysaccharide/polymer combinations and in vitro drug release was performed with or without rat caecal contents. The release data have been fitted to an empirical equation proposed by Ritger and Peppas (Ritger & Peppas, 1987) to understand the mode of drug release; further, results were subjected to analysis of variance (ANOVA).

MATERIALS

MTZ (>99% purity), XG, and GG were supplied as gift samples from Vetcare and Himalaya Drug Co., Bangalore, India. Graft copolymer (MAA-*g*-GG) was synthesized in our laboratory as reported earlier (Mundargi et al., 2006). CD, carrageenan, pectin, and acetone were all purchased from SD Fine Chemicals, Mumbai, India. Eudragit-L 100 was obtained from Rohm GmbH Chemische Fabrik (Darmstadt, Germany).

METHODS

Preparation of MTZ Matrix Tablets

The required amount of polymer was mixed with MTZ (100 mg/tablet) to get a uniform distribution of drug in the polymer. The composition of different formulations along with the formulation codes is given in Table 1. Tablets were prepared by using an IR hydraulic pellet maker (Riken Seiki Co. Ltd., Japan) under a pressure of 300 kgf cm^{-2} for 15 s of dwell time uniaxially. Exactly weighed quantity of the powder mixture was filled into a die of 12.8 mm diameter using little pressure and then, hydraulic pressure was applied to form the tablet. Tablets were evaluated for hardness by using a Monsanto type hardness tester. Friability of the tablets was evaluated by a Roche Friabilator (Mumbai, India). Disintegration test was performed in distilled water maintained at 37°C using a USP disintegration tester (Electrolab, Mumbai, India). Thickness of the tablets was measured by using a vernier caliperse.

Determination of Drug Content

Five tablets were finely powdered; quantity equivalent to 100 mg of MTZ was accurately weighed and transferred to 100 mL volumetric flask containing 50 mL of methanol. This was allowed to stand for 6 hr with an intermittent sonication to ensure complete solubility of the drug. The solutions were made up to volume, filtered, suitably diluted, and estimated for MTZ content at λ_{max} value of 312 nm by using a UV-visible spectrophotometer (Model Anthelie, Secomam, France).

Coating of Tablets

Compressed tablets were coated by dip-coating technique. A 4% (w/v) Eudragit-L 100 in acetone without any plasticizer was used as the coating solution. Tablets were dipped in coating solution and then immediately dried at 50°C. The uncoated tablets with the formulation codes F5, F6, F7, and F8 were coated and assigned the formulation codes as C5, C6, C7, and C8, respectively.

Swelling Studies

The uncoated tablets in a wire basket were put into a 250 mL beaker containing 200 mL of pH 7.4 phosphate buffer and were allowed to swell at 37°C. Tablets were periodically removed and changes in weight were measured before and during swelling. The swelling ratio was then calculated using,

$$\text{Swelling ratio} = \left(\frac{A_t - A_0}{A_{tab}} \right) \quad (1)$$

where A_{tab} is weight of the dry tablet (mg), A_t and A_0 are the weights of tablet and basket at time t (h) and at the beginning, respectively.

Preparation of 4% Rat Caecal Content

Male Wistar rats weighing 125–150 g maintained on a normal diet were used for the study. Rats were asphyxiated using carbon dioxide (CO_2). The abdomens were

TABLE 1 Formulation of Tablets (Quantity in mg)

Formulation code	MTZ	Guar gum	MAA-*g*-GG	Pectin	Carrageenan	β-cyclodextrin	Xanthan gum
F1	100	–	–	400	–	–	–
F2	100	–	–	–	–	400	–
F3	100	–	–	–	400	–	–
F4	100	400	–	–	–	–	–
F5	100	–	–	–	–	–	400
F6	100	–	100	–	–	–	300
F7	100	–	200	–	–	–	200
F8	100	300	100	–	–	–	–

opened, the ceci were traced, ligated at both ends, dissected and immediately transferred into pH 7.4 phosphate buffer, previously bubbled with CO_2. The cecal bags were opened and the contents were individually weighed, pooled and then suspended in a phosphate buffer to provide 4% (w/v) dilution because the cecum is naturally anaerobic; all of these operations were performed under CO_2 atmosphere.

In Vitro Drug Release Studies

Dissolution experiments are carried in a USP 1 apparatus at 100 rpm at 37°C. Drug release studies were conducted in 500 mL of 0.1N HCl for the initial 2 hr, followed by 500 mL of pH 7.4 phosphate buffer. Samples were withdrawn at regular intervals of time and replaced with the same volume of fresh dissolution media. The samples were then analyzed spectrophotometrically at wavelengths of 277 nm and 321 nm in acidic and basic media, respectively.

Release behavior of the selected matrix tablets in the physiological environment of colon was assessed by performing the drug release studies in rat caecal content medium. Drug release studies were performed using the USP dissolution rate test apparatus (Apparatus 1, 100 rpm, 37°C) with slight modifications. A 250 mL beaker containing 200 mL of rat caecal content medium was immersed in water contained in 1000 mL vessel, which in turn, was kept in a water bath. The swollen formulations after completing the dissolution study in 0.1N HCl (2 hr) and pH 7.4 phosphate buffer (3 hr) were placed in baskets of the apparatus and immersed in the rat caecal content medium. As the caecum is naturally anaerobic, the experiment was performed with a continuous CO_2 supply into the beakers. At different time intervals, 1 mL of the sample was withdrawn and replaced with 1 mL of 4% rat caecal medium bubbled with CO_2. The experiment was continued for another 19 hr as the usual colonic transit time is 20–30 hr.

Differential Scanning Calorimetric (DSC) Study

The nature of drug present in the formulations was assessed by performing DSC on pristine MTZ, GG, XG, MAA-*g*-GG, and tablet formulations (F5, F6, and F8) using a DSC Rheometric Scientific, DSC SP, Surrey, UK. Accurately weighed samples (10 mg) were heated at the scanning rate of 10°C/min from the ambient temperature to 400°C under an inert nitrogen atmosphere at the flow rate of 10 mL/min.

Statistical Analyses

Statistical analyses were done by using the SPSS statistical package. Analysis of variance followed by the least significant difference (LSD) procedure was used for comparison of drug release rates from different formulations and $p < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

The present study is aimed at developing oral colon targeted formulations for MTZ using different polysaccharides and to identify the most suitable polysaccharide or a combination of XG with the (MAA-*g*-GG) graft copolymer and GG with graft copolymer. Various formulations were prepared and details along with the formulation codes are given in Table 1. Ideally, the drug delivery system targeted to colon should remain intact in the stomach and small intestine, thereby releasing the drug in the colon. Hence, attempts were made to formulate the matrix tablets using GG, XG, CD, carrageenan, pectin, MAA-*g*-GG, or a combination of polymers.

Physical Characteristics of Tablets

Results of drug content, hardness, thickness, friability, and disintegration time for formulations F5, F6, F7, and F8 are presented in Table 2. Hardness was found to be in the range from 4.0 to 6.0 kg cm^{-2}, which showed a dependence on the quantity and type of polysaccharide used in the tablet. Drug content, friability and thickness of the tablets were well within the acceptable limits. Drug content was analyzed on five tablets of each formulation individually, but only the mean values with standard deviations (±SD) are presented in Table 2. Drug content was found to vary between 99.9 and 101%, while the disintegration time for tablet formulations varied between 6 and 10 hr. This is attributed to the fact that tablets are composed of swelling matrix and contained no disintegration agents. This delay in disintegration time will allow drug to reach the colon.

TABLE 2 Physical Characteristics of the Tablets

Formulation code	Thickness average (mm)	Strength (kg/cm^2)	Friability (%)	Disintegration (h)	Drug content (%)
F5	3.18	5	0.85	8.00	100.4 ± 1.58
F6	3.20	5	0.8	7.00	101.0 ± 1.01
F7	3.26	6	0.4	6.00	99.90 ± 1.23
F8	3.24	4	1.2	10.00	100.19 ± 1.11

In Vitro Release Studies

Release data of tablets formulated with pectin, carrageenan, CD, GG, and XG are presented in Fig. 1. Formulation containing XG showed the slow release compared to other formulations. Among other formulations, GG containing tablets showed slower release rates. It is important to note that formulations containing pectin, carrageenan, and CD were disintegrated prematurely within 24 hr, releasing the majority of drug in the simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). This is attributed to the nature of polysaccharide used in the matrix tablet that is responsible for gel strength and resulted in the disintegration/erosion of the tablet. This also accounts for a fast release of the drug from the tablet matrices. However, tablets containing XG, mixture of XG with MAA-*g*-GG and GG with MAA-*g*-GG retained their physical integrity up to 24 hr of release.

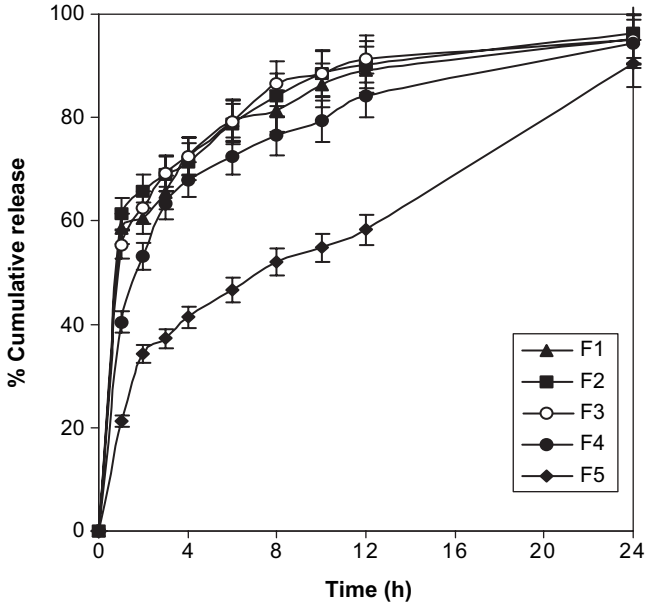

FIGURE 1 Percent drug release vs. time graph of F1, F2, F3, F4, and F5 formulations.

Comparison of drug release from tablets formulated using pectin, CD, carrageenan, GG, and XG was statistically evaluated by the ANOVA method. The F value was found to be 6.208 (df = 44, $p = 0.001$), which indicates a significant difference in the drug release rate from various formulations. Analysis of variance followed by LSD indicated a significant difference in the release rate from formulation containing XG. However, no significant difference was observed between other formulations (i.e., F1, F2, F3, and F4). Obviously, these matrices are not suitable for colon delivery because they would result in higher drug release before entering into the colon (see Table 3). It is evident from Fig. 1 that other than XG, GG was found to be marginally a better matrix material. Also, during the dissolution study, it was noticed that the physical integrity of GG containing tablets retained in tact. Hence, formulations F1, F2, and F3 were not subjected to any further studies.

Attempts were also made to prepare tablets using MAA-*g*-GG; unfortunately, because of its lower compressibility and lack of tablet strength failed in friability test and hence, the study was discontinued. Further, experiments were continued only with XG,

TABLE 3 Cumulative Percent Drug Release from the Tablets at Varying Time Intervals

Formulation code	% Drug release (4 hr)	% Drug release (12 hr)	% Drug release (24 hr)
F1	72.56	89.26	95.23
F2	71.42	90.24	96.42
F3	72.46	91.26	95.08
F4	67.99	84.26	94.29
F5	41.33	58.26	90.48
F6	30.50	56.48	85.02
F7	32.63	60.26	85.79
F8	71.15	87.21	98.86
C5	18.67	52.45	78.28
C6	19.37	53.95	79.73
C7	21.47	54.46	81.03
C8	23.23	64.3	86.6

mixture of XG with MAA-*g*-GG, and GG with MAA-*g*-GG matrices. These formulations were enteric coated with Eudragit-L 100 to give a protection in an acidic pH. Release data of these formulations with or without coating is shown in Fig. 2. Uncoated formulations containing XG or mixture of XG with MAA-*g*-GG released a lower amount of drug compared to GG with MAA-*g*-GG. The coated formulations (i.e., C5, C6, C7, and C8) released a negligible amount of MTZ in SGF. A comparison of drug release from the tablets before and after coating in SGF was statistically evaluated by ANOVA. The F value was found to be 5.699 (df = 12, $p = 0.021$), which indicated a significant difference in drug release before and after coating.

The coated formulations C5, C6, and C8 were further subjected to drug release study in 4% rat caecal content medium; release data are displayed in Fig. 3. It can be noticed that these formulations in rat caecal content showed enhanced drug release caused by the microbial degradation or polymer solubilization, suggesting a microbially triggered drug release. Comparison of drug release from tablets studied with or without rat caecal content was also statistically evaluated by ANOVA. The F value was found to be 1.495 (df = 53, $p = 0.583$), which indicated no significant difference in the drug release studied with or without the rat caecal content. This could be attributed to the fact that the colonic bacterial action of the rat caecal medium might not be sufficient to degrade such a gel barrier. However, the human caecal contents would be far better than what was used in the present study (Rama Prasad et al., 1998).

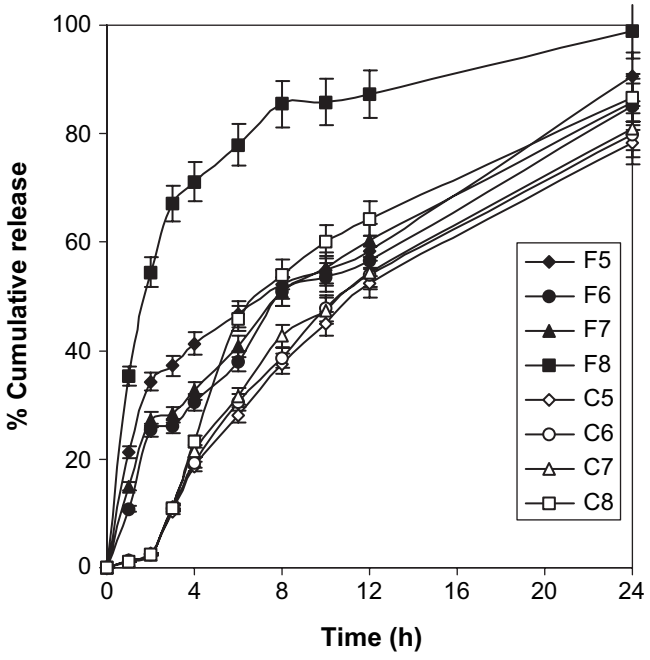

FIGURE 2 Percent drug release vs. time graph of F5, F6, F7, F8 and C5, C6, C7, C8 formulations.

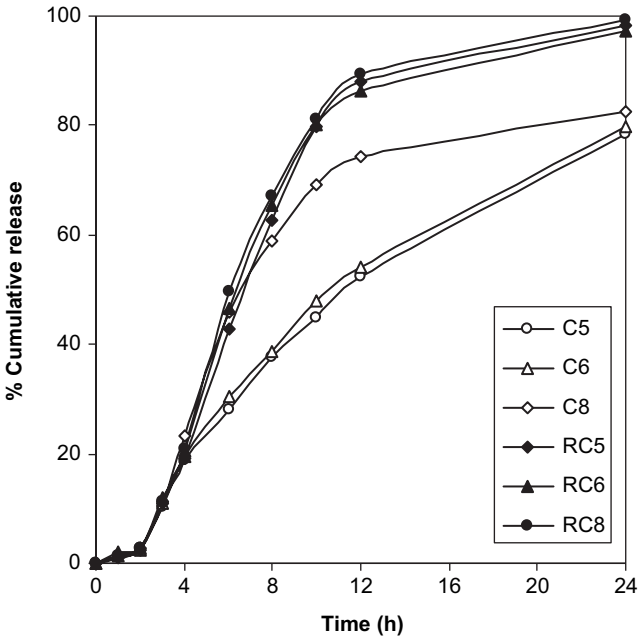

FIGURE 3 Percent drug release vs. time graph with rat caecal medium (RC5, RC6, RC8) and without rat caecal medium (C5, C6, C8).

The initial decrease in drug release rate of XG tablets can be explained on the basis that a higher concentration (as compared to other formulations) of XG might have led to an increase in hardness of the tablet, while porosity and capillary pore sizes have possibly reduced (Upadrashta et al., 1992). This in turn, could have reduced the wicking of water into the tablet and consequently, swelling and drug release rates are slowed down. The hydration of XG and GG seems not to be affected by the pH of the dissolution medium. The initial drug release may be attributed to the dissolution of the drug present on the surface of the tablet and the lag-time required for complete hydration of GG to form a viscous gel layer around the tablet.

Swelling study was performed on formulations containing XG, mixture of XG with MAA-*g*-GG, or GG with MAA-*g*-GG and these results are presented in Fig. 4. The formulations containing XG showed the highest swelling ratio up to 54 hr, whereas a least swelling ratio was noticed for formulation F7, which is MAA-*g*-GG plus XG. The formulation containing the mixture of

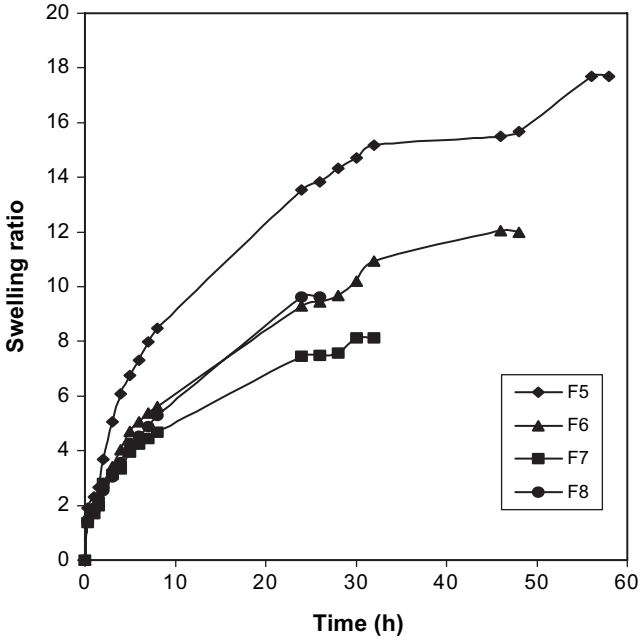

FIGURE 4 **Swelling ratio vs. time graph of various tablets at 7.4 pH (37°C).**

XG with MAA-*g*-GG exhibits a lesser swelling ratio when compared to formulations containing only XG, which is attributed a lesser amount of XG present in the matrix. A systematic trend was observed in case of formulations containing XG and mixture of XG with the graft copolymer; as the concentration of XG in tablets is reduced, swelling ratio was decreased and also, it was noticed that as the XG concentration decreased from 400 to 200 mg (F5 to F7), equilibrium swelling decreased from 54 to 32 hr. The swelling ratio of formulations containing the mixture of GG with MAA-*g*-GG is approximately equal to F6, but it reached equilibrium swelling in 24 hr only.

The present experiments on swelling study were performed on exact formulations to correlate with the release profiles (Tugcu-Demiroz et al., 2004). Drug release from the matrix system occurs by two simultaneous mechanisms: (i) erosion or attrition of the outermost, least consistent gel layer and (ii) dissolution of the active principle in the liquid medium and diffusion through the gel barrier when formed (Lapidus & Lordi, 1968). Studies (Talukdar & Kinget, 1995) performed on swellable matrices have shown that as the concentration of the swellable polymer is increased in the formulation, the gel thickness increases upon swelling. This increases the effect of diffusion path length, which decreases the drug release rates from the tablet.

In the present study, by varying the polysaccharide composition, swelling has increased, but after a certain lag phase, drug release has increased rather than being decreased, as is normally expected from a matrix tablet. This can be explained on the basis that these tablets upon swelling forms rather loose gel network which is attributed to very low concentration of the polysaccharide. Since the release of drug from these matrices takes place by polysaccharide erosion, which further depends upon the gel consistency (Marcos et al., 1991); the more susceptible the matrix is to erosion, the faster will be the drug release. This accounts for drug release behavior in case of formulations containing XG and mixture of XG with MAA-*g*-GG matrix. Hence, after an initial lag phase (time taken by the tablet to swell), swelling increases along with the drug release rates. Our study shows that the time-controlled release systems for colon targeting can be formulated using XG and a mixture of XG with MAA-*g*-GG as a matrix, which initially retards the drug release because of the lag-time required for swelling; after complete swelling, a rapid drug release was obtained. During the initial period, the gel strength of the barrier was too high to be broken, but after a lag-time the network was somewhat loosened to facilitate more release of the drug through the matrices.

In case of tablet formulations containing a mixture of GG with MAA-*g*-GG, an increase in swelling ratio (Fig. 4) was observed, but drug release was not much affected and no optimum lag-time was achieved as required to bypass the drug release in the upper parts of GIT. This may be attributed to lower swelling of GG tablets and probably because of the concentration of the gum, which was not sufficient to retard the release of the drug. Swelling was not affected by the pH of the medium. Drug release from the hydrophilic matrices such as those used in the present study is controlled by the rate of hydration of the matrix and the properties of the gel formed upon hydration, which influences drug diffusion and gel erosion. However, a rapid hydration is required to establish the gel layer and to prevent the drug release of an initial burst of the matrix. Thereafter, the thicker the gel layer, the longer is the diffusional path for the drug molecules to transport, but stronger will be the gel and hence, lesser will be its susceptibility to erosion (Wakerly et al., 1996).

Release Kinetics

To determine the mechanism of drug release, the initial portion (i.e., $M_t/M_\infty \leq 60\%$) of % drug release vs. time profiles were fitted to an empirical equation proposed by Ritger and Peppas. (1987):

$$\frac{M_t}{M_\infty} = Kt^n \qquad (2)$$

where $M_t/M_\infty$is the fraction of drug released at time t, K is a kinetic rate constant, and n is the diffusional exponent that characterizes the mechanism of drug release. For cylindrical systems, if $n = 0.45$ it suggests the Fickian diffusion; if $0.46 < n < 0.89$, it suggests the anomalous (non-Fickian) transport, for $n = 0.89$, the zero-order release is possible and if $n > 0.89$, a super-case-II transport is operative.

In order to predict and correlate the release behavior of drugs from the hydrophilic matrix of this study, it is necessary to fit them into release kinetic profiles. (Fickian, anomalous or super-case-II) (Ritger & Peppas, 1987). This will facilitate the understanding of mode of drug release such as whether the release is because of only diffusion or only erosion or caused by both diffusion and erosion. The n values calculated for selected formulations (i.e., F5, F6, F7, and F8) were found to vary between 0.40 and 0.62; for the coated formulations (i.e., C5, C6, C7, and C8), the n values are in the range of 1.55 to 1.93, indicating a super-case-II transport.

An analysis of release kinetics data on formulations F5, F6, F7, and F8 suggests that drug release occurred by the non-Fickian transport as indicated by the n values computed and possibly will drug release has occurred by the pore-diffusion mechanism. Non-Fickian release is described by two mechanisms: the coupling of drug diffusion and polymer relaxation. The release mechanism is known to be influenced by (i) nonhomogeneous gel microstructure as well as the existence of polymeric domains within the swollen gel, (ii) the rate of fluid ingress into the matrix, (iii) dissociation/erosion and total disentanglement at the dissolution front, and (iv) rate of matrix swelling, relaxation as well as molecular diffusion of drug through the swollen gel (Kim & Fassihi, 1997).

XG, an anionic natural derivative of cellulose, showed that drug release from this microbial exocellular polysaccharide follows almost a time-dependent release kinetics. Notice that XG is compatible with virtually all the salts and solution pH and temperature has very little effect on the viscosity of its gel. XG matrix swells in the presence of a solvent because of polymer relaxation, which is rather characterized by the formation of a gel-like network structure surrounding the system. For formulations containing only XG, the n value is found to be 0.4, indicating a non-Fickian trend, which is described by two types of mechanisms: the coupling of drug diffusion and polymer relaxation, when XG is used as the retarding hydrophilic polymer along with the graft copolymer; here, the drug release profiles suggest three mechanisms (i.e., swelling, erosion and diffusion fronts), which synchronize and contribute for the slow drug release. The formulation F6 has an n value of 0.62, which is slightly greater than observed for F5. This phenomenon is attributed to the physical changes induced in the polymer by the excipient. For formulation F7, which is a mixture of XG with the higher amount of MAA-*g*-GG, the n value was found to be 0.47. The formulation F7 also contributes in drug release by coupling of drug diffusion and polymer relaxation processes.

In general, solubility of drug itself crucially governs the rate and extent of diffusional release. For diffusion to occur, the first step is wetting of the drug by water, followed by its dissolution such that the drug molecule is available in its molecular form to diffuse out of the matrix. In the presently developed formulations, drug is in its crystalline state as evidenced by the DSC studies. Hence, the net release rate observed is a cumulative effect of drug's solubility (influenced by its structure, molecular weight and pka), polymer property (hydrophilicity/lipophilicity, molecular weight, tortuosity) as well as the relative ratio of drug and polymer in the tablet. In high concentrations of XG as in case of formulation F5, considering the high level of erodability of XG, it may be concluded that drug is released both by erosion and diffusion phenomena within the matrix. In this case the rate of polymer chain relaxation is much slower than the rate of diffusion of drug through the polymer matrix. Decreasing the XG concentration in formulations F5 to F7 has shifted the drug release kinetics, but no definite trend was observed. When GG along with the graft copolymer was used as the retarding polymer, the n value was found to be 0.58, suggesting the non-Fickian release kinetics.

Transport often occurs in rubbery polymers that possess a sufficient chain mobility to allow the water

penetration. It is an ideal case in which there is no interference of effects such as polymer chain rearrangement occurs. The overall rate of release of the drug from GG matrix is higher than that from the XG matrices; these results are a clear indication of the fact that XG has higher drug retarding ability than GG. In case of coated formulations (C5, C6, C7, and C8), the release is restricted by the coating layer in the SGF media, leading to a lesser cumulative release and following the super case-II kinetics, which is indicated by the increased swelling near the relaxing front for a longer time. In case of coated formulations, as a result of the coating, the release was restricted in SGF media when the release was continued in SIF media; here, the coating dissolves and tablet started to swell, leading to a lesser cumulative release with increased swelling at the relaxing front for a longer time.

DSC Studies

DSC studies were performed to understand the nature of the drug in the formulated tablets. Thermograms of the (a) pristine drug, powder samples of formulations (b) F5 and (c) F6 as well as (d) MAA-*g*-GG and (e) XG matrices are shown in Fig. 5. Since tablet formulation F7 contains the same ingredients as that of F6 with somewhat insignificant varying amount, it was excluded for the DSC study. Fig. 6 typically displays the data for (a) pristine drug, (b) formulation F8 (c) MAA-*g*-GG and (d) GG. A sharp endothermic peak corresponding to the melting point of MTZ was found at 164°C for the drug sample. An endothermic peak corresponding to the melting point of MTZ in the samples of matrix formulations F5, F6, and F8 are observed at 162, 163, and 160°C, respectively. A slight decrease in the value could be attributed to the bound water present in the formulations. On the other hand, thermograms of the powdered matrix formulations did not show any significant shift in the endothermic peak, indicating that drug in the matrix formulations is in a crystalline state; this could be advantageous in solid oral dosage forms. The degree of crystallinity of the pristine drug was calculated using the enthalpy of fusion. The results are found to be 90.22, 95.40, and 87.95% for formulations F5, F6, and F8, respectively, indicating the crystalline nature of the drug in the formulated products.

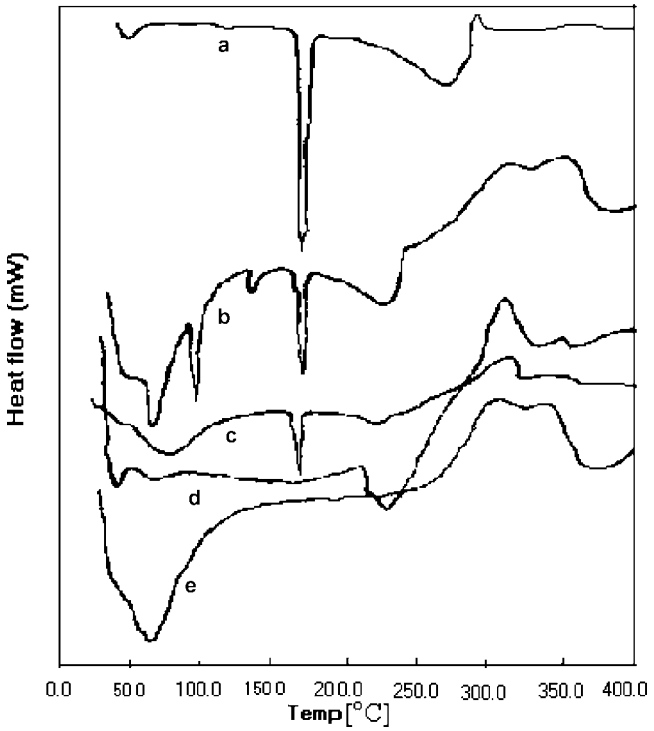

FIGURE 5 DSC thermograms of (a) MTZ, (b) F5, (c) F6, (d) MAA-*g*-GG and (e) XG.

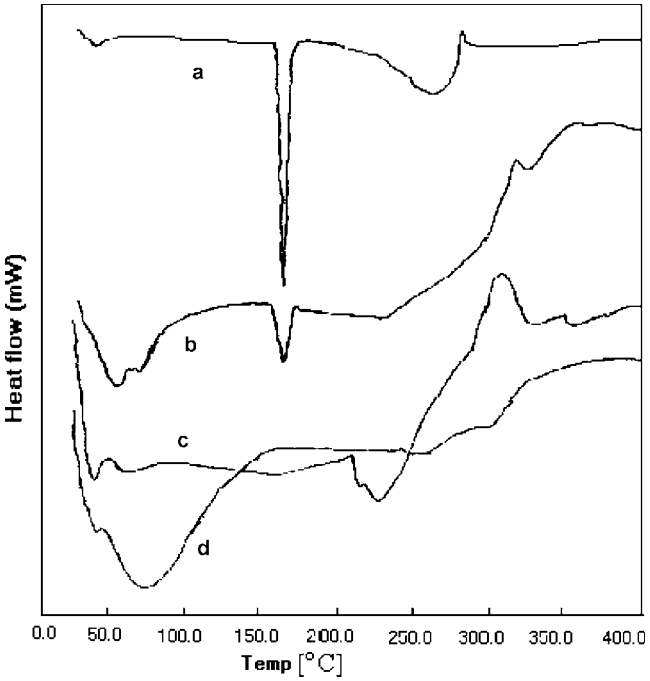

FIGURE 6 DSC thermograms of (a) MTZ, (b) F8, (c) MAA-*g*-GG and (d) GG.

CONCLUSION

The present investigation was performed to develop the colon targeted drug delivery systems for MTZ for an effective and safe therapy of amoebiasis. The dissolution

data obtained from the various formulations developed demonstrates that MTZ release rate is dependent upon the nature and concentration of polysaccharide used as a carrier. Among the various polysaccharides used in the formulation of tablets, XG and GG showed the acceptable in vitro release rates. Enteric coating of the tablets could help to control the release of drug during the initial time period. Preparations with XG as a matrix formed the time-dependent release formulations. The enteric coated matrix tablets containing XG, mixture of XG with MAA-*g*-GG, and GG with MAA-*g*-GG showed enhanced release rates in the simulated colonic fluids because of the microbial degradation or polymer solubilization phenomena. Swelling study indicated that XG has a maximum swelling ratio followed by the mixture of XG with MAA-*g*-GG, whereas a least swelling ratio was observed for formulation F7. The nature of drug transport was found to follow the non-Fickian trend in case of the uncoated formulations, whereas for the coated formulations, it was found to be super-case-II. Differential scanning calorimetry indicated the presence of crystalline nature of the drug in the formulated products.

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