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Studies on the development of oral colon targeted drug delivery systems for metronidazole in the treatment of amoebiasis

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Abstract

The aim of the present study is to develop colon targeted drug delivery sytems for metronidazole using guar gum as a carrier. Matrix, multilayer and compression coated tablets of metronidazole containing various proportions of guar gum were prepared. All the formulations were evaluated for the hardness, drug content uniformity, and were subjected to in vitro drug release studies. The amount of metronidazole released from tablets at different time intervals was estimated by high performance liquid chromatography method. Matrix tablets and multilayer tablets of metronidazole released 43–52% and 25–44% of the metronidazole, respectively, in the physiological environment of stomach and small intestine depending on the proportion of guar gum used in the formulation. Both the formulations failed to control the drug release within 5 h of the disolution study in the physiological environment of stomach and small intestine. The compression coated formulations released less than 1% of metronidazole in the physiological environment of stomach and small intestine. When the dissolution study was continued in simulated colonic fluids, the compression coated tablet with 275 mg of guar gum coat released another 61% of metronidazole after degradation by colonic bacteria at the end of 24 h of the dissolution study. The compression coated tablets with 350 and 435 mg of guar gum coat released about 45 and 20% of metronidazole, respectively, in simulated colonic fluids indicating the susceptibility of the guar gum formulations to the rat caecal contents. The results of the study show that compression coated metronidazole tablets with either 275 or 350 mg of guar gum coat is most likely to provide targeting of metronidazole for local action in the colon owing to its minimal release of the drug in the first 5 h. The metronidazole compression coated tablets showed no change either in physical appearance, drug content or in dissolution pattern after storage at 40 °C/75% RH for 6 months.

Keywords: Guar gum; Metronidazole; Colon targeting; In vitro dissolution; Compression coating

1. Introduction

Amoebiasis is an infection of the large intestine caused by *Entamoeba histolytica*, a single celled

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protozoan parasite. The current estimate is that *E. histolytica* causes between 34–50 million symptomatic infections each year (Walsh, 1986) and leading to the death of 40–100 thousands of people, which makes amoebiasis second only to malaria as a cause of death resulting from protozoan parasite (World Health Organization, 1997). The trophozoites of *E. histolytica* can invade the colonic epithelium, causing amoebic colitis.

The most preferred choice of drugs for intestinal amoebiasis is metronidazole and tinidazole (Tracy and 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). But the pharmacokinetic profile of metronidazole indicates that the drug is completely and promptly absorbed after oral administration reaching a concentration in plasma of about 10 μg/ml approximately 1 h after a single 500 mg dose (Lau et al., 1992). The administration of this drug in conventional tablet dosage form provides minimal amount of metronidazole for local action in the colon, still resulting in the relief of amoebiasis, but with unwanted systemic effects.

Various approaches (Van den Mooter and Kinget, 1995; Rama Prasad et al., 1996) available for colon specific drug delivery include (i) coating with pH dependent systems, (ii) design of timed release dosage forms and (iii) the use of carriers that are degraded exclusively by colonic bacteria. The pH dependent systems (Touitou and Rubinstein, 1986; Peters and Kinget, 1993) are designed to release the drug to above a particular pH of the GIT. The poor site specificity of pH dependent systems, because of large variation in the pH of the GIT, was very well established. The timed-release systems (Gazzaniga et al., 1994; Pozzi et al., 1994) release their load after a predetermined time period of administration. The time dependent formulations are designed to resist the release of the drug in the stomach with an additional non-disintegrating or lag phase included in the formulation (which equals to the small intestine transit time) and the release of the drug takes place in the colon. An example of such system is Pulsincap® (MacNeil and Stevens, 1990). This capsule consists of a non-disintegrating body having an enteric-coated cap. The enteric-coated cap dissolves in the small intestine and a hydrogel plug swells to create a lag phase. This plug ejects on swelling and releases the drug from the capsules. The large scale manufacturing of these systems, however, needs a lot of technological advancement and skills. Another limitation of the time dependent release systems are the variation in the gastric emptying time and small intestine transit time (Davis et al., 1984). But, due to the use of enteric coating over most of these systems, the large variation in gastric emptying is overcome by most of these systems (Watts and Illum, 1997). However, there is still likely to be a considerable variability in the in vivo performance of the timed-release systems by virtue of the variation in small intestinal transit time (Sinha and Kumria, 2001). The best alternative approach for colon specific drug delivery is the use of carriers that are degraded exclusively by colonic bacteria. The various carriers that are being evaluated for colon-specific drug delivery based on the colonic bacterial action are pectin and its salts (Ashford et al., 1993, 1994; Rubinstein et al., 1993; Wakerly et al., 1996a,b; Munjeri et al., 1997), chondroitin sulphate (Rubinstein et al., 1992a,b), amylose (Milojevic et al., 1995), inulin HP (Vervoot and Kinget, 1996) and guar gum (Rama Prasad et al., 1998; Krishnaiah et al., 1998a,b, 1999).

The present investigation is aimed at using the inexpensive, naturally and abundantly available guar gum for colon targeted delivery of metronidazole. Guar gum is a natural non-ionic polysaccharide derived from seeds of *Cyamopsis tetragonolobus* (Family: Leguminaciae). It consists of linear chains of (1→4)-β-D-mannopyranosyl units with α-D-galactopyranosyl units attached by (1→6) linkages (Goldstein et al., 1973). Guar gum was found to be a colon-specific drug carrier in the form of a matrix and compression coated tablets (Rama Prasad et al., 1998; Krishnaiah et al., 1998a,b, 1999). The present paper describes the development and in vitro evaluation of colon targeted drug delivery systems for metronidazole using guar gum as a carrier.

2. Materials and methods

2.1. Materials

Metronidazole (98–100.2% pure) and mebendazole were gift samples from M/s. Alkem Laboratories (India) Limited, Mumbai and M/s. ICI Pharmaceuticals, Chennai, India, respectively. Guar gum (viscosity of 1% aqueous dispersion is 125 cps at 25 °C) was obtained from Dabur Research Foundation, India, and was of USNF quality. The methanol used was of high performance liquid chromatography (HPLC) grade (Qualigens), and Triple distilled water (TD water) was used; other materials namely microcrystalline cellulose (Avicel, FMC Type pH-102), lactose, sodium starch glycollate, starch, magnesium stearate and talc were of US/NF quality.

2.2. Preparation of metronidazole matrix tablets

Matrix tablets of metronidazole were prepared by wet granulation technique using 10% starch paste as binder. Microcrystalline cellulose was used as diluent and mixture of talc and magnesium stearate at 2:1 ratio was used as lubricant. Metronidazole matrix tablets containing 30% of guar gum (M1) and 40% of guar gum (M2) were prepared. The composition of different formulations used in the study containing 200 mg of metronidazole in each case is shown in Table 1. Guar gum was sieved through a mesh (250 μm) and mixed with metronidazole (149 μm) and MCC (250 μm). The powders were blended and granulated with 10% starch paste. The wet mass was passed through a mesh (1190 μm) and the wet granules were dried at 50 °C for 2 h. The dried granules were passed through a mesh (1000 μm) and were lubricated with a mixture of talc and magnesium stearate (2:1). The lubricated granules were compressed with a maximum force of compression (4000–5000 kg) using 11 mm round, flat and plain punches on single station tabletting machine (Cadmach Machinery Co. Pvt Ltd, India).

Table 1
Composition of metronidazole matrix tablets containing 30% (M1) and 40% (M2) of guar gum

Ingredients	Quantity (mg) present per each matrix tablet	
	M1	M2
Metronidazole	200	200
Guar gum	135	180
MCC	56.5	11.5
Starch (added as paste)	45	45
Talc	9	9
Magnesium stearate	4.5	4.5
Total (mg)	450	450

Table 2
Composition of metronidazole multilayer tablets

Ingredients	Quantity (mg) present per each matrix tablet	
	G1M2	G2M2
Guar gum granules for bottom layer	50	100
Guar gum granules of matrix formulation M1	450	450
Guar gum granules for top layer	50	100
Total (mg)	550	650

2.3. Preparation of multilayer tablets

Multilayer tablets of metronidazole were prepared by using 50 and 100 mg of guar gum as release controlling layer on either side of the 40% (M2) guar gum matrix tablets of metronidazole. Granules of the matrix formulation (M2) prepared as described above containing 40% of guar gum were compressed with either 50 mg (G1M2) or 100 mg (G2M2) of guar gum granules as release controlling layer on both sides. The composition of multilayer tablets G1M2 and G2M2 is given in Table 2 and compressed with a maximum force of compression (4000–5000 kg) with 11 mm round, flat and plain punches on single station punching machine (Cadmach Machinery Co. Pvt Ltd) to obtain multilayer tablets.

2.4. Preparation of metronidazole compression coated tablets

The core tablets (average weight 200 mg) of metronidazole, for compression coating with guar gum, were prepared by direct compression technique. The composition of the core tablets is given in Table 3. Sodium starch glycollate was included in the formulation at 5% level to obtain metronidazole tablets with fast disintegration characteristics (disintegration time < 30 s). The drug, sodium starch glycollate, lactose, magnesium stearate and talc were thoroughly mixed and passed through mesh (149 μm). The uniformity of mixing was assessed by conducting content uniformity test on the samples of the powder mix. The mixture was compressed into tablets at an applied force of 4000 kg using 9 mm round, flat and plain punches on a single station tablet machine (Cadmach Machinery Co. Pvt Ltd). The core tablets were tested for hardness, content uniformity, friability and disintegration. After confirming the compliance with these tests, the core tablets were compression coated with different coat formulations LC1, LC2, and LC3 containing 275, 350, and 435 mg of guar gum (Table 4). About one-third quantity of the coat formulation was placed in the die cavity (diameter 11 mm), the metronidazole core tablet (diameter 6 mm) was carefully positioned in the center of the die cavity and was filled with the remainder of the coat formulation. It was then compressed around the core tablets at an applied force of 5000 kg using 11 mm round, flat and plain punches as described above.

Table 3
Composition of fast-disintegrating core tablets of metronidazole

Ingredients	Quantity (mg)
Metronidazole	200
Spray dried lactose	30
Sodium starch glycollate (dry)	12.5
Talc	5.0
Magnesium stearate	2.5
Average weight	250

Table 4
Composition of guar gum coat formulation

Ingredients	Quantity (mg) present in the coat formulation		
	LC1	LC2	LC3
Guar gum	275	350	435
MCC	160	85	0
Starch (added as paste)	50	50	50
Talc	10	10	10
Magnesium stearate	5	5	5
Total (mg)	500	500	500

All the formulations such as matrix, multilayer and compression coated tablets were tested for their hardness, drug content and in vitro drug release characteristics with a suitable number of tablets for each test. The hardness of the matrix tablets was determined by using Monsanto Hardness Tester.

2.5. HPLC analysis of metronidazole in tablet formulations and dissolution fluids

The quantitative determination of metronidazole was performed by HPLC. A gradient HPLC (Shimadzu HPLC Class VP series) with two LC-10AT VP pumps, variable wave length programmable UV–VIS Detector SPD-10A VP, CTO-10AS VP column oven (Shimadzu), SCL-10A VP system controller (Shimadzu) and RP C-18 column (250×4.6 mm^2 ID; particle size 5 μm) was used. The HPLC system was equipped with the software 'Class-VP series version 5.03 (Shimadzu)'.

The mobile phase used was acetonitrile and TD water (consisting of 0.4% triethylamine and pH adjusted to 3.6 with 5% orthophosphoric acid). The filtered mobile phase was pumped at a flow rate of 0.7 ml/min in the ratio of 44:56 (acetonitrile: water consisting of 0.4% triethylamine, and pH adjusted to 3.6 with 5% orthophosphoric acid). The column temperature was maintained at 40 °C. The eluent was detected by UV Detector at 254 nm and the data were acquired, stored and analysed with the software Class-VP series version

5.03 (Shimadzu). A standard curve was constructed for metronidazole in the range of 0.1–40 μg/ml using mebendazole (2 μg/ml) as an internal standard.

A good linear relationship was observed between the concentration of metronidazole and the ratio of the peak area of metronidazole to that of internal standard (mebendazole) with a high correlation coefficient ($r = 0.9999$). The required studies were carried out to estimate the precision and accuracy of this HPLC method of analysis of metronidazole. The standard curve constructed as described above was used for estimating metronidazole either in the matrix formulations, multilayer formulations, compression-coated tablets or in dissolution fluids.

2.6. Determination of drug content

The matrix, multilayer and the core tablets metronidazole used for compression coating were tested for their drug content. Ten tablets were finely powdered and quantities of the powder equivalent to 100 mg of metronidazole was accurately weighed and transferred to 100-ml volumetric flasks containing 50 ml of methanol and allowed to stand for 6 h with intermittent sonication to ensure complete solubility of the drug. The solutions were made up to volume and filtered. One milliliter of the filtrate added with internal standard after a suitable dilution was estimated for metronidazole content at 254 nm by reverse phase HPLC method as described above.

2.7. In vitro drug release studies

The ability of the matrix, multilayer and compression coated tablets of metronidazole to provide colon specific drug delivery was assessed by conducting in vitro drug release studies. These studies were carried out using a USP XXIII dissolution rate test apparatus (Apparatus 1, 100 rpm, 37 °C) for 2 h in 0.1 M HCl (900 ml) as the average gastric emptying time is about 2 h. Then the dissolution medium was replaced with pH 7.4 Sorensen's phosphate buffer (900 ml) and tested for drug release for 3 h as the average small intestinal transit time is about 3 h. At the end of the time periods 1 ml samples were taken, added with 20 μg of mebendazole (internal standard), the volume made up to 10 ml with TD water, filtered through 0.2 μm membrane filter and analysed for metronidazole by HPLC as described previously.

The ability of the matrix, multilayer and compression coated tablets of metronidazole to release in the physiological environment of colon was assessed by continuing the drug release studies in rat caecal content medium in view of our earlier report on the utility of guar gum as a carrier for colon-specific drug delivery (Rama Prasad et al., 1998). Male albino rats (supplied by M/s Ghosh Enterprises, Kolkata, India) weighing 105–115 g and maintained on a normal diet (Bengal gram purchased in local market and soaked in water, 25 g/rat) were used for the study. It was reported earlier from our laboratory that rat caecal content medium at 4% w/v level obtained after 7 days of enzyme induction with 1 ml of 2% w/v guar gum dispersion provide the best conditions for assessing the susceptibility of guar gum to colonic bacterial degradation (Rama Prasad et al., 1998). Hence, the rats were treated with guar gum dispersion for inducing the enzymes specifically acting on guar gum. The procedure involved oral treatment of rats with 1 ml of 2% w/v guar gum dispersion for 7 days. Thirty minutes before the commencement of drug release studies, six rats were euthanized, using carbon dioxide asphyxiation. The abdomen were opened, the caecai were traced, ligated at both ends, dissected and immediately transferred into pH 6.8 PBS, previously bubbled with CO_2. The caecal bags were opened, their contents were individually weighed, pooled and then suspended in PBS to give 4% w/v dilution. As the caecum is naturally anaerobic, all these operations were carried out under CO_2. The care of the rats was in accordance with institutional guidelines. The drug release studies were carried out using USP dissolution rate test apparatus (Apparatus 1, 100 rpm, 37 °C) with slight modifications. A beaker (capacity 150 ml) containing 100 ml of rat caecal content medium was immersed in the water maintained in the 1000-ml vessel, which in turn, was in the water bath of the apparatus. The swollen formulations after com-

pleting the dissolution study in 0.1 M HCl (2 h) and pH 7.4 Sorensen's phosphate buffer (3 h) were placed in the baskets of the apparatus and immersed in the rat caecal content medium. As the caecum is naturally anaerobic, the experiment was carried out with continuous CO_2 supply into the beakers.

At different time intervals, 1 ml of the sample was withdrawn without a pre-filter and replaced with 1 ml of fresh PBS bubbled with CO_2, and the experiment was continued for another 19 h as the usual colonic transit time is 20–30 h. One milliliter of methanol was added to the dissolution sample along with 20 μg of mebendazole (internal standard), the volume made up to 10 ml with TD water, centrifuged, the supernatant liquid was filtered through 0.2 μm membrane filter and analysed for metronidazole by HPLC as described previously. Methanol was added to the dissolution samples to ensure the complete dissolution of the slightly soluble metronidazole particles that may be eroded out of the guar gum tablets.

2.8. Stability studies

To assess the long-term stability (2 years), the compression coated formulations LC1 and LC2 were stored at 40 °C/75% RH for 6 months (Mathews, 1999) and were observed for physical change and drug content. At the end of storage period, the formulations LC1 and LC2 were also subjected to drug release studies in simulated gastric, intestinal and colonic fluids.

2.9. Statistical analysis

The cumulative percent of metronidazole released from the matrix, multilayer and compression coated tablets ($n = 3$) in the dissolution medium at 24 h with and without rat caecal contents was compared, and the statistical significance was tested by using Student's t-test. A value of $P < 0.05$ was considered statistically significant.

3. Results and discussion

The present study was aimed at developing oral colon targeted formulations for metronidazole using guar gum as carrier. It was earlier reported that guar gum could be used as a carrier for colon-specific drug delivery in the form of either a matrix tablet or as a compression coat over a drug core tablet (Krishnaiah et al., 1998a,b). Guar gum matrix tablets released about 21% of the drug (indomethacin) in the physiological environment of stomach and small intestine, but released majority of its drug content in the physiological environment of colon (Rama Prasad et al., 1998). However, the release of such a small percent of drug from the surface of the matrix tablets in the physiological environment of stomach and small intestine is a serious consideration for drugs showing deleterious effects on stomach and small intestine (for example, anticancer drugs in the treatment of colon cancer). In such a situation, it was suggested to apply guar gum as a compression coat over the drug core tablet (Krishnaiah et al., 1998b). In this direction, compression coated 5-aminosalicylic acid tablets were developed for colon targeting (Krishnaiah et al., 1999). The drug delivery system targeted to colon should remain intact in stomach and small intestine, but should release the drug in colon.

3.1. Matrix tablets of metronidazole

Since guar gum is found to have poor flow properties and is to be incorporated in the matrix tablets in a larger proportion, metronidazole tablets were prepared by wet granulation technique using starch paste as a binder. The uniformity of mixing of metronidazole, guar gum and MCC in the preparation of matrix tablets was assessed by assaying three samples of the powdered mix drawn randomly from the lot. The matrix tablets were prepared by applying maximum force of compression and the hardness of tablets was found to be in the range of 4.6–5.3 kg. Metronidazole tablets containing 30 and 40% of guar gum were prepared, and were subjected to drug content uniformity and in vitro drug release studies. The matrix tablets were found to contain 99.1–101.5% of the labelled amont of metronidazole indicating uniformity of drug content.

The matrix tablets were subjected to in vitro drug release studies in 0.1 M HCl (2 h), pH 7.4 Sorenson's phosphate buffer (3 h) and simulated colonic fluids (rat caecal content medium at 4% w/v level after 7 days of enzyme induction, 19 h). It was reported earlier from our laboratory that rat caecal content medium at 4% w/v level after 7 days of enzyme induction provide the best conditions for assessing the susceptibility of guar gum to colonic bacterial degradation (Rama Prasad et al., 1998). When the matrix tablets were subjected to in vitro drug release studies, metronidazole tablets containing 30 and 40% of guar gum (M1 and M2) remained intact and slightly swollen at the end of 5 h of dissolution study in the physiological environment of stomach and small intestine. The percent of metronidazole released from M1 (30% guar gum) at the end of 5 h (physiological environment of stomach and small intestine) was found to be 51.5 ± 3.5 where as in the next 19 h of dissolution study (pH 6.8 buffer without rat caecal medium; control study) it was 96.0 ± 2.8. When the guar gum content of the matrix tablets was increased to 40% (M2), the percent of drug release decreased to 43.8 ± 1.3 in the first 5 h and 79.2 ± 4.0 in the next 19 h of the dissolution study (Table 5). On exposure to the dissolution fluids, the gum gets hydrated and forms a viscous gel layer around the tablet that slows down further seeping-in of the dissolution fluids towards the core of the tablets. But it may be observed from the results that about 40% of the drug was released from the formulations (M1 and M2) in 0.1 M HCl. This may be because of the release of highly soluble (in 0.1 M HCl) metronidazole present on the surface of the matrix tablets and lag time required for the hydration of the gum.

The release of more percent of metronidazole from formulation M1 (96.02 ± 2.8), M2 (79.18 ± 4.0) in 0.1 M HCl, pH 7.4 Sorenson's phosphate buffer and PBS 6.8 buffer (control) after 24 h of dissolution study indicates that the drug is diffusing out of the formulations due to its high solubility in 0.1 M HCl, pH 7.4 phosphate buffer and pH 6.8 PBS. Hence, further studies on the in vitro dissolution of the formulations in simulated colonic fluids (rat caecal contents medium) were not carried out on formulations M1 and M2 as they also released almost 50% of its drug in physiological environment of stomach and small intestine. The results, thus, show that the matrix formulations of metronidazole containing either 30% (M1) or 40% (M2) of guar gum failed to control the drug release in the physiological environment of stomach and small intestine. Hence, it was planned to control the release of metronidazole by applying different amounts of guar gum as a release controlling layer over the metronidazole matrix formulations (M2).

Table 5
Percent of metronidazole released from matrix tablets containing 30% of guar gum (M1) and 40% of guar gum (M2) in 0.1 M HCl (2 h), pH 7.4 buffer (3 h) and pH 6.8 PBS (19 h)

Time (h)	Percent of ($\pm$s.d.) metronidazole released from M1	Percent of ($\pm$s.d.) metronidazole released from M2
2	43.24 ± 2.5	36.09 ± 1.2
5	51.45 ± 3.5	43.89 ± 1.3
10	65.21 ± 7.5	51.73 ± 1.7
15	83.52 ± 4.5	61.33 ± 3.7
20	90.61 ± 1.7	68.72 ± 3.9
24	96.02 ± 2.8	79.18 ± 4.0

Values shown in the Table indicate the mean $\pm$ s.d.

3.2. Multilayer tablets of metronidazole

Multilayer tablets of metronidazole were prepared to control the drug release in physiological environment of stomach and small intestine i.e. up to 5 h of dissolution study. Multilayer tablets were prepared by compressing a layer of granules containing either 50 mg (G1M2) or 100 mg (G2M2) of guar gum on either side of metronidazole guar gum matrix tablets (containing 40% of guar gum, M2). The hardness of the multilayer tablets was found to be in the range of 5–6 kg and metronidazole present in the multilayer tablets were found to be in the range of 98–102% of the labelled amount indicating uniformity of the drug content. Metronidazole multilayer tablets containing either 50 mg (G1M2) or 100 mg (G2M2) of guar gum as release controlling layer

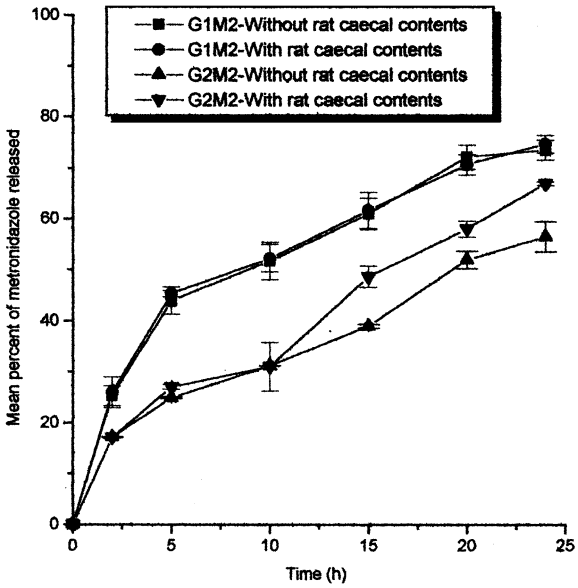

Fig. 1. Mean ($\pm$ s.d.) percent of metronidazole released from multilayer tablet formulation ($n = 3$) containing 50 mg (G1M2) or 100 mg (G2M2) of guar gum as a release controlling layer on either side of the matrix tablet containing 40% of guar gum in the dissolution study with and without rat caecal contents.

retained their physical integrity up to 24 h of the dissolution study conducted without rat caecal contents in the dissolution medium. In the first 5 h of dissolution study, the multilayer tablets G1M1 released about 45% of metronidazole whereas G1M2 formulations released only 26% of the drug indicating that G1M2 formulation could effectively control the drug release in the physiological environment of stomach and small intestine. The percent of metronidazole released from the multilayer tablets G1M2 with and without rat caecal contents is shown in Fig. 1. At the end of 24 h, the percent of metronidazole released from the multilayer tablets G1M2 in rat caecal content medium was found to be 78.1 ± 3.5 whereas in control study too, the formulation released $73.4 \pm 1.9\%$ of drug. However, the G2M2 formulation released $61.2 \pm 1.0\%$ of metronidazole in rat caecal content medium whereas in control study $56.5 \pm 2.9\%$ of drug at the end of 24 h of dissolution study (Fig. 1). No significant difference ($P > 0.05$) was observed in the amount of metronidazole released with rat caecal contents when compared to control study. The results clearly show that the colonic bacteria present in the rat caecal content medium did not act upon guar gum present in the multilayer formulations. Metronidazole is reported to be effective against the anaerobic microorganisms (Tracy and Webster, 1996). Mostly for this reason, metronidazole released from the formulations G1M2 and G2M2 in the presence of rat caecal contents might have inhibited the anaerobic microbial flora of the rat caecal contents. As a result, the guar gum multilayer tablets might have not degraded in the presence of rat caecal contents, and there was no significant difference in the percent of drug released from the G1M2 and GM2 formulations.

3.3. Compression coated metronidazole tablets

In view of the unsuccessful delivery of the guar gum multilayer tablets of metronidazole in the physiological environment of colon, it was essential either to prevent or minimize the release of metronidazole in the physiological environment of colon until the guar gum present in the formulation (G1M2 and G2M2) is acted upon by colonic bacteria. For this reason it was planned to apply guar gum as a compression coat over the fast disintegrating metronidazole core tablets. The fast disintegration of metronidazole core tablets is necessary to ensure fast release of the drug from the tablets soon after the degradation of the guar gum coat by the colonic bacteria.

Fast-disintegrating metronidazole core tablets were prepared by incorporating super disintegrant such as sodium starch glycollate. The hardness of the core tablets of metronidazole was found in the range of 2.5–3.0 kg. The core tablets of metronidazole were also found to comply with the friability test since the weight loss was found less than 0.6%. The core tablets were found to disintegrate within 30 s showing the required fast disintegration characteristics. The combined action of the superdisintegrant (sodium starch glycollate) and spray dried lactose (used as a diluent and direct compression vehicle) might have contributed to such a fast disintegration. Thus the core tablets of metronidazole formulated in the

study were found to have the required characteristics for colon targeting in the form of a guar gum compression coat over the drug core.

The core tablets of metronidazole prepared as above were compression coated with a coat formulation containing various quantities of guar gum. The coat formulations with various quantities of guar gum were prepared in the form of granules (Table 4) to impart both flowability and compressibility. The cumulative amount of metronidazole released from tablets coated with coat formulations containing 275 mg (LC1), 350 mg (LC2) and 435 mg (LC3) was found to be less than 1% after 5 h of the dissolution study in simulated gastric and intestinal fluids. Thus, guar gum in the form of a compression coat, is capable of protecting the drug from being released in the physiological environment of stomach and small intestine. To assess the integrity of the coats, drug release studies were carried out without the addition of rat caecal contents to pH 6.8 PBS. At the end of the 24 h of the dissolution study, all the tablets coated with coat formulations LC1, LC2 and LC3 were found intact and the mean percent drug released was 30.0 ± 0.8, 23.8 ± 0.6 and 19.0 ± 1.0, respectively. This indicates that until the coat is degraded, the gum will not permit the release of the bulk of the drug present in the core.

The drug delivery systems targeted to the colon should not only protect the drug from being released in the physiological environment of stomach and small intestine, but also have to release the drug in colon. Hence, in vitro drug release studies were carried out in pH 6.8 PBS containing 4% w/v of rat caecal contents (Rama Prasad et al., 1998). When the in vitro dissolution studies were carried out in the presence of rat caecal content medium, the percent drug released from metronidazole tablets coated with coat formulation LC3 was found to be only 20.4 ± 0.6 and the coat remained intact (Fig. 2). No significant difference ($P > 0.05$) was observed in the amount of metronidazole released at the end of 24 h of the dissolution study with rat caecal content medium when compared to the dissolution study without rat caecal contents. The presence of higher amount of guar gum (435 mg, LC3) might not have allowed complete degradation of the coat during the time period of testing. It may be noted that more or less the same amount of drug (19%) was released even without rat caecal contents. This clearly shows that the drug release is due to mechanical diffusion of the soluble metronidazole from the formulation, but not due to the action of colonic bacteria. Thus it is evident that unless the coat is degraded by colonic bacteria, drug release may not take place.

The percent drug released from metronidazole core tablets coated with coat formulation LC2 was found to increase from 20 h onwards indicating the commencement of disruption of the hydrated gum coats. The percent of drug released from LC2 formulation is shown in Fig. 3. The percent of drug released after 24 h of testing was 45.9 ± 2.3 and the tablet coat was found to be broken at one point making way for the release of the drug. A significant difference ($P < 0.001$) was observed in the amount of metronidazole released at the end of 24 h of the dissolution study with rat caecal content medium when compared to the dissolution study without rat caecal contents.

In case of tablets coated with coat formulations LC1, an increase in percent drug released was

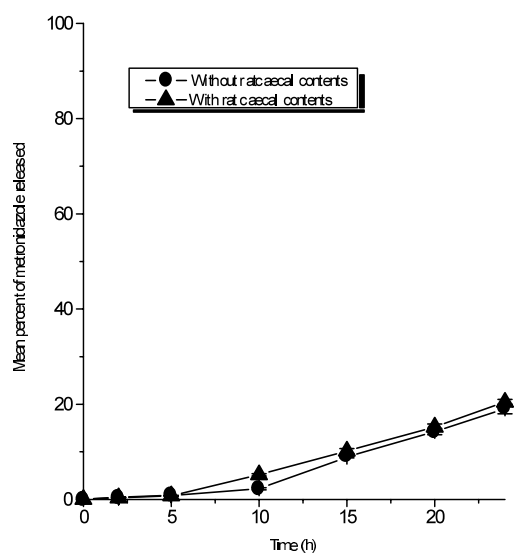

Fig. 2. Mean ($\pm$ s.d.) percent of metronidazole released from compression coated tablets ($n = 3$) containing 435 mg of guar gum coat in the formulation (LC3) in dissolution study with and without rat caecal contents.

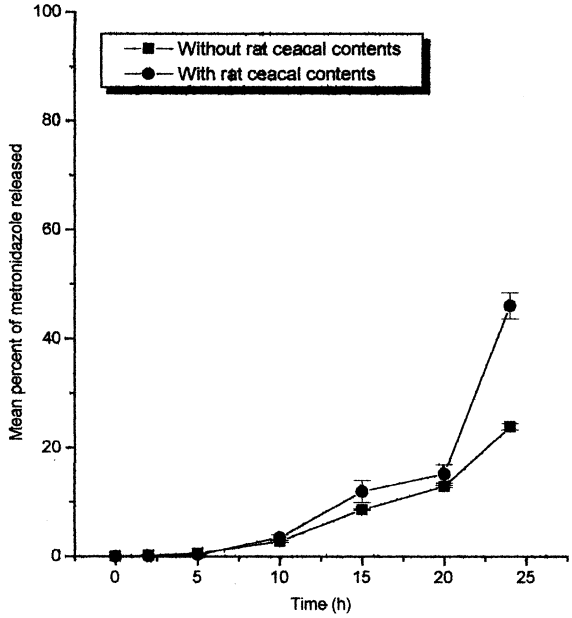

Fig. 3. Mean (± s.d.) percent of metronidazole released from compression coated tablets ($n = 3$) containing 350 mg of guar gum coat in the formulation (LC2) in dissolution study with and without rat caecal contents.

observed from 20 h onwards, and at the end of 24 h of dissolution study 62.2 ± 4.1% of metronidazole was released (Fig. 4). A significant difference ($P < 0.001$) was observed in the amount of metronidazole released at the end of 24 h of the dissolution study with rat caecal content medium when compared to the dissolution study without rat caecal content. The coat (LC1) was almost degraded in the presence of rat caecal contents thereby releasing the drug into the dissolution medium. Since the guar gum content of coat formulation LC1 (275 mg) was lesser compared to coat formulations and LC2 (350 mg) and LC3 (435 mg) the coat might have been completely hydrated and subsequently degraded by the caecal enzymes at a faster rate resulting in the release of about 62% of metronidazole. The results show that tight control of drug release from compression coated formulation LC1 and LC2 might have facilitated the colonic bacterial action on swollen guar gum and resulted in the degradation of the formulation thereby releasing the drug in the physiological environment of colon.

The compression coated formulation LC1 was completely degraded in simulated colonic fluids whereas LC2 formulation partially degraded in simulated colonic fluids. The results of the study indicate that metronidazole tablets compression coated with either 275 mg (LC1) or 350 mg (LC2) of guar gum would be potential formulations in delivering the drug to the colon.

The metronidazole compression coated formulation LC1 released almost 62% of its drug at the end of 24 h of the dissolution study. The formulation LC2 also released about 45% of its drug content in the physiological environment of colon. It is clear from these results that LC1 could target metronidazole to colon. The LC2 tablets are also considered as potential formulations for targeting of metronidazole to colon because of the fact that the human caecal contents would be far more than what was used in the present study. On increasing the proportion of guar gum coat just by an increment of 85 mg from 350 to 435 mg (LC3), only 20% of the drug were released at the end of 24 h of dissolution study. The gel strength

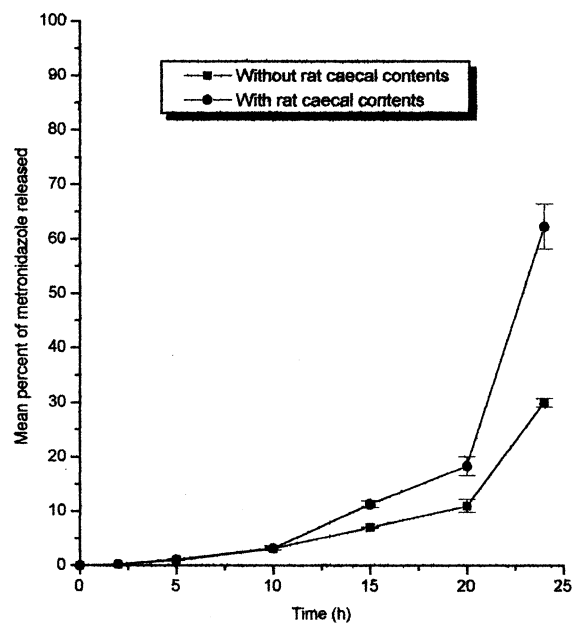

Fig. 4. Mean (± s.d.) percent of metronidazole released from compression coated tablets ($n = 3$) with 275 mg of guar gum coat in the formulation (LC1) in dissolution study with and without rat caecal contents.

Table 6
Percent of metronidazole released from LC1 and LC2 compression coated formulations ($n = 3$) before and after storage at 40 °C/75% RH for 6 months

Time (h)	Percent of metronidazole released from LC1		Percent of metronidazole released from LC2	
	Before storage	After storage	Before storage	After storage
2	0.24 ± 0.02	0.20 ± 0.01	0.08 ± 0.01	0.09 ± 0.02
5	1.08 ± 0.05	0.95 ± 0.02	0.29 ± 0.01	0.31 ± 0.03
10	3.22 ± 0.38	2.98 ± 0.01	2.42 ± 0.5	2.01 ± 0.1
15	11.2 ± 0.58	10.12 ± 1.04	9.94 ± 2.04	10.01 ± 1.23
20	18.2 ± 1.72	17.58 ± 1.01	15.1 ± 1.70	14.56 ± 1.02
24	62.2 ± 4.14	63.5 ± 3.56	45.9 ± 2.37	46.50 ± 2.01

Values shown in the table indicate the mean $\pm$ s.d.

of the swollen coat of guar gum might be too high and prevented the drug release from the formulation. The colonic bacterial action of the rat caecal medium might not be sufficient to degrade such a high strength gel barrier of the swollen LC3 compression coated formulation. Unless the coat of guar gum degrades, the drug release does not occur. Even in humans, in spite of higher caecal contents, the complete degradation of the LC3 may not be possible because of such a high quantity of guar gum in the coat formulation. However, the relative potential of the formulations LC2 and LC3 needs to be evaluated in human volunteers.

3.4. Stability studies

In view of the potential utility of LC1 and LC2 formulations for targeting of metronidazole to colon, stability studies were carried out at 40 °C/75% RH for 6 months (climatic zone IV conditions for accelerated testing) to assess their long term (2 years) stability. The protocol of the stability studies was in conformation with the recommendation in WHO document for stability testing of products intended for global market (Mathews, 1999). After storage, the formulations were observed for physical change and were subjected to assay of the drug and in vitro drug release studies. When the compression coated tablets LC1 and LC2 were stored at 40 °C/75% RH for 6 months there appeared no change either in physical appearance or in drug content. When the dissolution study was conducted in the simulated physiological environment of stomach, small intestine and colon as described above, no significant difference ($P > 0.05$) was observed in the cumulative percent of metronidazole released from both LC1 and LC2 stored at 40 °C/75% RH for 6 months when compared to that released from the same formulations before storage (Table 6). The insignificant change either in the physical appearance, drug content or in dissolution profile of LC1 and LC2 formulations after storage at 40 °C/75% RH for 6 months indicate that the formulations could provide a minimum shelf life of 2 years (Mathews, 1999).

4. Conclusions

The present investigation was carried out to develop colon targeted drug delivery systems for metronidazole for an effective and safe therapy of amoebiasis. Multilayer tablets of metronidazole with either 50 or 100 mg of guar gum as a release controlling layer on both sides of guar gum matrix formulation (containing 40% guar gum) failed to release the drug in the physiological environment of colon. Mostly, metronidazole released in simulated colonic fluids might have prevented anaerobic bacteria of rat caecal contents and as a result guar gum present in the formulation might not have been degraded. In view of this result, alternative colon targeted drug delivery systems were developed which could release minimal

quantity of metronidazole until the formulation is acted upon by colonic bacteria. Fast disintegrating metronidazole core tablets were compression coated with coat formulation containing various quantities of guar gum ranging from 275 to 435 mg. The compression coated metronidazole tablets coated with 435 mg of guar gum did not degrade in simulated colonic fluids where as the formulations coated with either 350 or 275 mg of guar gum were found degraded in dissolution medium containing rat caecal contents there by releasing about 45 and 62% of the drug, respectively. It appears that compression coated metronidazole tablets compression coated with either 350 or 275 mg of guar gum are most likely to provide targeted delivery of metronidazole to the colon.

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