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Development of Guar gum-Alginate Based Microcapsules of Metronidazole for Delivery to Colon

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Abstract

The present study was aimed to develop and evaluate suitable guar gum-alginate microcapsules for colon-specific delivery of Metronidazole for better treatment of amoebic colitis (important cause of death in the developing countries). Microcapsules were prepared by calcium chloride cross-linking method with different concentrations of sodium alginate and guar gum. Prepared microcapsules were treated for three different coatings with reduced molecular weight guar gum, chitosan and enteric coatings with cellulose acetate phthalate (CAP). Microcapsules were evaluated for size and morphology, thickness, strength and flexibility and drug content. *In vitro* drug release study was conducted by buffer change method to mimic GIT environment. In morphological study, the guar gum membrane was found to be transparent, porous and continuous in nature. Guar gum concentrations significantly affected the strength and flexibility of membrane. Drug loading was decreased with increase in the weight of either encapsulating polymer or guar gum and different coatings. *In vitro* drug release was found to be marginally decreased with increasing guar gum and alginate concentrations. The coated microcapsules exhibited significantly retarded and colon specific drug release as compared to uncoated microcapsules. Among the coatings, reduced molecular weight guar gum coating gave much lower drug release and also exhibited high colon specificity.

Keywords: Colon drug delivery, metronidazole, guar gum, sodium alginate, chitosan, microcapsules.

Introduction

Colon drug delivery has gained much interest for local as well as systemic delivery due to prolonged residence time of luminal contents (Davis,1990), reduced epithelial enzymatic activity (MacFarlane *et al.*, 1989), increased tissue responsiveness to absorption enhancers and natural absorptive characteristics (Taniguchi *et al.*,1980). The approaches known to achieve colon specific drug delivery includes coating with pH dependent polymers, design of timed release dosage forms and use of the carriers that are degraded exclusively by the colonic bacteria. The most promising of colon drug delivery systems are those based on the use of carriers, like polysaccharides, that are degraded exclusively by colonic bacteria. This is because of the poor site-specificity of both pH dependent systems and timed release dosage forms (Chavan Patil and Mishra,1999). Polysaccharides are biodegradable, abundantly available and also are cheap (Hovgaard and Bronsted,1996). They can be easily modified chemically and biochemically and are highly stable, safe, nontoxic, hydrophilic and gel forming. These include naturally occurring polysaccharides obtained from plant (guar gum, inulin),

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animal (chitosan, chondroitin sulphate), algae (alginates) or microbial (dextran) origin. These are broken down by the colonic microflora to simple saccharides. So these fall into the category of generally regarded as safe (GRAS) (Sinha and Kumaria, 2003). Many of the polysaccharides-based delivery systems shield the drug from the hostile environments of the upper GIT. When these delivery systems arrive into the colon the glycosidic linkages within the polysaccharides are hydrolysed releasing the drug candidate. The main saccharolytic species are *Bacterodies and Bifidobacterium* (Berthold *et al.*, 1996; Tozaki *et al.*, 1997a; Lorenzolamaosa *et al.*, 1998). Guar gum is a naturally occurring galactomannan polysaccharide consisting of a linear chain of β-D-mannospyranose joined by β-(1-4) linkage with α-D-galactopyranosyl units attached by 1,6-links in the ratio of 1:2. It is susceptible to microbial degradation in the large intestine (Balyiss and Houston, 1986; Tomolin *et al.*, 1989; Macfarlane *et al.* 1990) .Guar gum has also been investigated as a matrix tablet for delivery of water insoluble drugs to the colon. These tablets have shown promising results *in vitro* and *in vivo* (Kenyon *et al.* 1997; Rama Prasad *et al.*, 1998). Another study using compression coating also has shown suitability of this polymer for the above purpose (Krishnaiah *et al.*, 1999). Amoebiasis is an important cause of death by protozoal infections, next only to malaria in the developing world; even in advanced countries it has emerged as an important infection among immunosuppressed individuals. Metronidazole (Mz) has extremely broad spectrum of protozoal and antimicrobial activity. It is clinically effective in trichomoniasis, amoebic colitis and giardiasis (Webster, 1990). Its use in amoebic colitis is limited, because of its good absorption from upper GIT and very low amounts reaching the colon. Thus, a colon-specific delivery system of Mz is expected to be a promising formulation for the treatment of amoebic colitis as compared to conventional formulation of Mz. Hence, the aim of present investigation is to design a suitable guar gum-alginate microcapsular colon specific delivery system of Mz for the better treatment of amoebic colitis.

Materials and Methods

Materials: The gift samples of Metronidazole (Mz) from Alkem Labs Ltd, India; chitosan (medium viscosity grade) (Central Institute of Fisheries Technology, Kochi) and guar gum (DaburResearchFoundation,Sahibabad) were used. Cellulose acetate phthalate (CAP) (Nebulate Health Care Ltd,Chennai) and Sodium alginate (S.D. Fine Chem Ltd, Boisar) were purchased. All other chemicals used were analytical grade.

Preparation of guar gum-alginate microcapsules: The codes and contents of formulations prepared are shown in Table 1.

Guar gum dispersions of 0.1, 0.2 and 0.4% w/v were prepared by dispersing guar gum in distilled water with continuous stirring for 6 hrs followed by dissolving calcium chloride 1.5% w/v in the polymeric dispersion and adjusting the pH to 5.5 with 10% w/v sodium hydroxide solution and were then used in the encapsulating procedure. Alginate solutions of 1.5, 2.0 and 2.5% w/v were prepared by dissolving sodium alginate in distilled water containing dissolved Mz (1% w/v). Guar gum-alginate microcapsules (Mc) were prepared by following the reported method (Polk *et al.*, 1994; Mishra *et al.*, 2003) with slight modification. 20 ml of alginate solution was loaded into a syringe fitted with 23G needle. 100 ml of guar gum-calcium chloride solution was taken in a beaker and stirred at 100 rpm. Alginate-Mz solution was added at a constant rate of 30 ml / hr to guar gum-calcium chloride solution with constant stirring. A reaction time of 10 min was used. After forming Mc, it was filtered, washed with distilled water and hardened with acetone.

Table 1. Codes and contents of Mz microcapsules.

Batch Code	Microcapsule core	Encapsulating polymer	Outer coat
A	Mz + 1.5 % w/v SA	$CaCl_2$ + 0.4% w/v GG	-
B	Mz + 2.0 % w/v SA	$CaCl_2$ + 0.4% w/v GG	-
C	Mz + 2.5 % w/v SA	$CaCl_2$ + 0.4% w/v GG	-
D	Mz + 2.5 % w/v SA	$CaCl_2$ + 0.1% w/v GG	-
E	Mz + 2.5 % w/v SA	$CaCl_2$ + 0.2% w/v GG	-
F	Mz + 2.5 % w/v SA	$CaCl_2$ + 0.4% w/v GG	$CaCl_2$ + 0.4% w/v reduced mol.wt GG
G	Mz + 2.5 % w/v SA	$CaCl_2$ + 0.4% w/v GG	$CaCl_2$+0.4%w/v Chitosan solution*
H	Mz + 2.5 % w/v SA	$CaCl_2$ + 0.4% w/v GG	0.2 % w/v CAP in acetone

Mz – Metronidazole; $CaCl_2$ - Calcium chloride solution; SA – Sodium alginate solution; GG – Guar gum solution; CAP – Cellulose acetate phthalate; * 2 %w/v in acetic acid.

Three different types of outer-coated batches were also prepared as follows:

(i) ***Coating with reduced molecular weight guar gum solution:*** Guar gum solution (0.125% w/w) was treated with sodium nitrite solution (0.1% w/w) to reduce the molecular weight (Peninston and Johnson,1975; Mishra *et al.*,2003). Reduction in molecular weight was confirmed in term of the reduction in viscosity by the Mark-Houwink equation $[\eta]=k M^a$ where η is the intrinsic viscosity, M is the molecular weight, k and a are constants in relation to solute solvent system. Guar gum viscosity, before and after molecular weight reduction was 30 and 21 centipoise, respectively. Selected batches of Mc were added to reduced molecular weight guar gum (30% relative reduced viscosity)-calcium chloride solution in wet condition and stirred for 15 min and were allowed to settle. Excess coating solution was then aspirated off, washed with distilled water and dried as above .

(ii) ***Coating with chitosan:*** Batch C was treated with 0.4% w/v chitosan (in 2% w/v acetic acid) containing calcium chloride solution for 15 min and the rest of procedure was the same as in case of reduced molecular weight guar gum coating.

(iii) ***Enteric coating with CAP:*** Batch C Mc were added to 2% w/v solution of CAP in acetone and stirred for 15 min, filtered over a nylon mesh filter (aperture size 0.45μm) and air dried.

Morphological evaluation of Mc: Shape and surface characteristics of Mc were studied using Scanning Electron Microscope (SEM, JSM 840ASM,Jeol,Japan). Sizes of Mc were evaluated using optical microscope. Since Mc were irregular after drying the longest diameter was measured. Fifty Mc per formulation were evaluated. Average diameter was then calculated.

Strength and flexibility of Mc membrane: It was measured qualitatively by using a light microscope and forceps as reported method (Goosan *et al.*,1985;Mishra *et al.*,2003).To analyse, the strength and flexibility, the Mc were contacted with sodium citrate prior to test so as to liquefy the core and then were squeezed with forceps. The strength of the membrane was defined as the amount of deformation (i.e., change in diameter) a wet capsule could endure before rupturing. A rating of one (+) indicated that the Mc rupture with very small pressure

applied (~ 10% change in diameter),and a rating of four(++++) indicated that the Mc withstood significant deformation upto ~70% decrease in capsule diameter before rupturing. Similarly, flexibility was defined as the amount of deformation that could be applied and still have the membrane return to its original shape. A rating of one (+) indicated that the Mc permanently deformed with very little applied pressure (~ 10% change in diameter), and a rating of four (++++) indicated that the Mc withstood significant deformation (~70% change in capsule diameter) before being permanently deformed.

Drug loading: 50 mg of Mc were treated with 50 ml of phosphate buffer (pH 7.0) in a 100 ml amber coloured vial with stirring at 250 rpm. The temperature was maintained at 37 ± 0.2°C. At the end of 2 hrs, it was filtered, filtrate was analyzed spectrophotometrically at 320 nm (UV/VIS Spectrophotometer, Jasco, Model 7800, Japan) (Mishra *et al.*, 2003).

In vitro drug release study: *In vitro* drug release characteristics of Mc were evaluated following reported method (Polk *et al.*,1994; Tozaki *et al.*,1997b; Mishra *et al.*,2003) with little modification. Prepared Mc (50 mg) were placed in a 100 ml amber coloured vial and contacted with 20 ml of elution medium with maintained temperature (37 ± 0.2°C) and stirring (50 rpm) with small teflon coated magnetic bead, by placing the vial on energy regulated temperature controlled hot plate magnetic stirrer. A buffer change method was used to mimic GIT environment. Hydrochloric acid-potassium chloride buffer (pH 1.2) was used as an elution medium for first 2 hrs followed by phosphate buffer of pH 7.0 for next 4 hrs and phosphate buffered saline of pH 7.0 containing 33 % w/v freshly prepared rat cecal content suspension bubbling with CO_2 for last 4 hrs. Withdrawn samples were analysed by spectrophotometric analysis at 320 nm. Each Mc was evaluated *in vitro* in triplicate.

Results and Discussion

It was earlier reported (Polk *et al.*,1994) that pH and the concentration of calcium chloride in encapsulating solution do not affect the *in vitro* drug release but the strength and flexibility of Mc membrane. Hence, preliminary studies were carried out to see the effect of pH of guar gum dispersion and calcium chloride concentration in encapsulating solution on the flexibility and strength of Mc membrane. Mc strength and flexibility was very low at pH 4.0. As pH increase to 5.5, the durability of the membranes improves. Above pH 5.5, both the strength and flexibility decreased (data not shown).Thus, pH 5.5 was selected for use in all subsequent encapsulation procedures. As reports available (Polk *et al.*,1994; Goosen *et al.*,1985), the concentration of calcium chloride is generally used in the range of 1 - 2 % w/v for either encapsulation or bead formation. The strength and flexibility of Mc membrane remained constant when calcium chloride was used in the concentration range of 1.4 - 1.6% w/v (Data not shown). Thus, the optimum concentration appeared to be 1.5% w/v.

Shape and surface features of prepared Mc were studied by optical microscopy and Scanning electron microscopy (JSM-840 ASM, Jeol, Japan). It was observed that prepared Mc were spherical in wet conditions, irregular after drying (Fig.1) and also brown in colour probably due to alginate-drug core. The guar gum membrane was transparent, porous and continuous in nature (Fig.2) and the membrane was thick with irregular ridges (Fig.3).

The Mc ranged in size from 605 μm to 738μm, and an increase in size of Mc was observed with an increase in alginate concentration (Table 2) that is attributed to higher density of sodium alginate with its increasing concentration. Similarly when guar gum concentration was

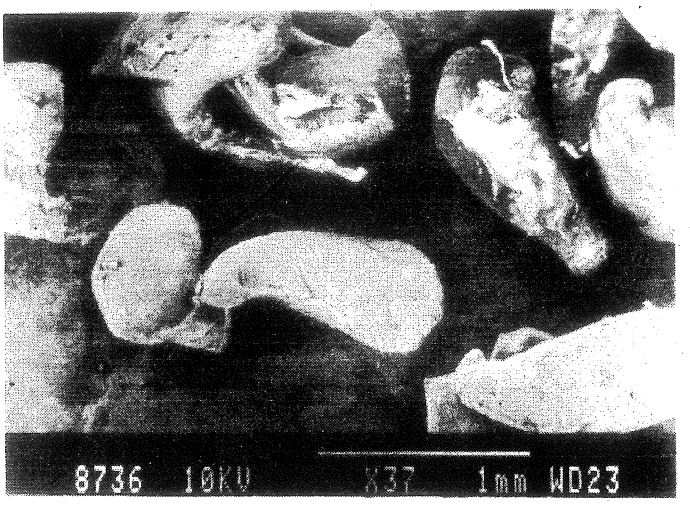

Fig.1. Scanning electron photomicrograph of guar gum-alginate Mc at 37 x magnification.

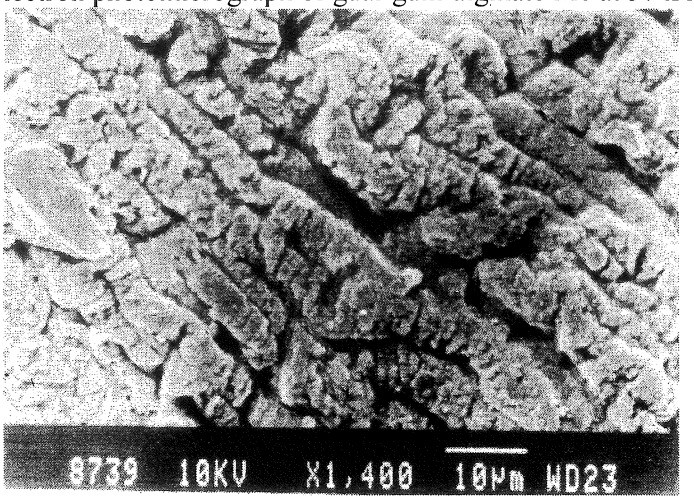

Fig.2. Scanning electron photomicrograph of guar gum-alginate Mc membrane with ridges at 1400x magnification.

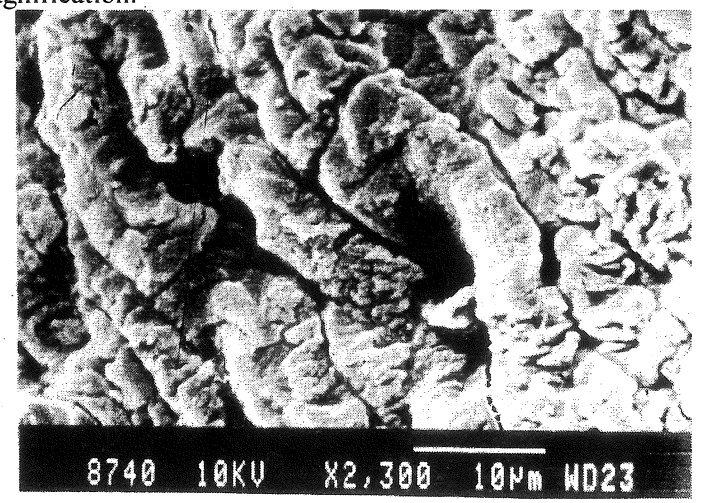

Fig.3. Scanning electron photomicrograph showing a close-up of a pore in guar gum-alginate Mc membrane at 2300x magnification.

increased, there was a increase in diameter of Mc. This might be due to high viscosity of guar gum dispersion.Different coatings also increased the thickness. Low concentration of guar gum produced weak and less flexible membrane while higher concentration produced strong and flexible membrane (Table 2). Guar gum is expected to cross-link with alginate core by precipitation. With increasing alginate concentration there was insignificant difference in strength and flexibility. Coating with CAP also did not exhibit any difference in strength and flexibility of membrane, while coating with reduced molecular weight of guar gum, increased the strength and flexibility (Table 2).

Table 2. Physical characteristics of prepared Mc {Data (Mean ± S.D.)}

Batch code	Size (μm)	Drug loading (μg/100mg)	Strength	Flexibility
A	684 ± 18	7857.81 ± 31.45	++	+++
B	705 ± 12	687.85 ± 21.53	++	+++
C	716 ± 12	628.04 ± 22.78	++	+++
D	634 ± 29	1005.61 ± 22.84	+	++
E	656 ± 28	927.10 ± 28.19	++	++
F	722 ± 10	542.06 ± 27.23	++++	++++
G	728 ± 10	504.73 ± 18.89	++++	++++
H	724 ± 12	501.58 ± 19.53	++	++

Strength: + very weak; ++ weak; +++ strong; ++++ very strong
Flexibility: + very fragile; ++ fragile; +++ flexible; ++++ very flexible

The strength and flexibility of guar gum membrane increased to a high level when coated with chitosan. This might be attributed to the strengthening of guar gum membrane by chitosan reacting with alginate core through ionic interaction or crosslinking by penetration.
Drug loading was decreased with increase in the weight of either encapsulating polymer or guar gum and different coatings (Table 2) but increased with the presence of drug in the capsule layer.
In vitro drug release study was conducted by buffer change method to mimic GIT environment (Mishra *et al.,* 2003). Three different alginate concentrations were used in order to study the effect of alginate on drug release from Mc. Accordingly, three batches containing 1.5% w/v, 2% w/v and 2.5% w/v of alginate based Mc were prepared (batches A, B and C). The results (Fig.4) of *in vitro* study indicated that amount of drug release decreased significantly ($P<0.01$), with an increase in alginate concentration, and is attributed to increase in the densities of the Mc core and also increase in the diffusional path length, which the drug molecules have to traverse.

Attempts to raise the alginate concentration above 2.5% were unsuccessful, as the solution became too viscous to extrude through a 23G needle during preparation of Mc.

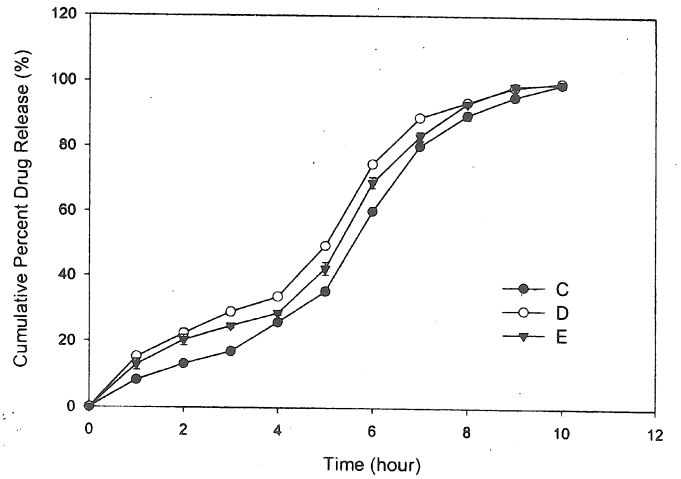

Fig. 4. *In vitro* release profiles of Mz from Mc prepared with different concentrations of alginate. Bars represent ± S.D. (n = 3).

In vitro drug release (Fig. 5) were also found to be marginally decreased with increasing the guar gum concentrations 0.1% w/v, 0.2% w/v and 0.4% w/v (batches D,E and C). Retarded drug release is due to 2.5%w/v alginate drug core and thicker guar gum membrane.

Coating with CAP (Batch H) gave comparatively lesser release in acidic medium pH 1.2, but with the increase in pH 7.0, higher drug release was obtained (Fig. 6). Thus, coating of Mc with CAP increased their enteric nature. Drug release (Fig.6) from Mc coated with CAP,chitosan and reduced molecular weight guar gum solution (batches H, G and F), were significantly retarded ($P<0.01$) till 6 hours, compared to uncoated Mc (batch C). *In vitro* drug release in anaerobic conditions (in the presence of rat cecal contents) showed rapid increase due to degradation of guar gum by the microbial flora of the colon (Bayliss and Houston, 1986;Kenyon *et al.*, 1996). When Mc coated with chitosan (batch G), no improvement in enteric nature was observed but a marginal one in the colon-specificity.

Among the three coatings, batch F gave much lower drug release as compared to batches G and H. However, in presence of rat cecal contents, batches G and H gave a significant increase in drug release, exhibiting thereby more colon specific drug release from batch F. Coated Mc (batches F, G and H) (Fig.6) exhibited more colon-specific drug release as compared to uncoated Mc (Fig.4).

Based on above observations, it was concluded that by adjusting Mc core compositions, coating thickness and further coatings; more colon specific drug delivery systems of Mz could be formulated.

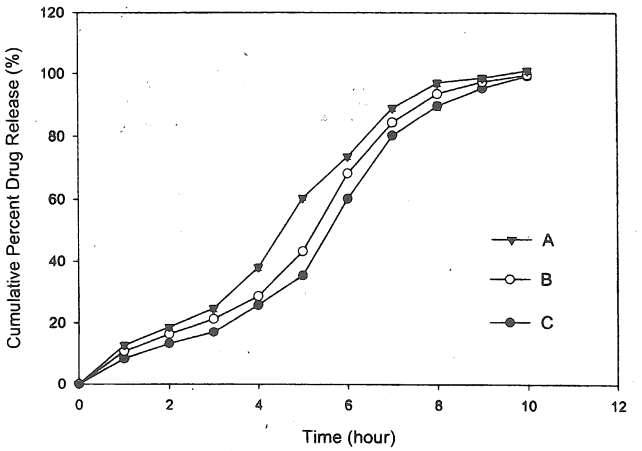

Fig. 5. *In vitro* release profiles of Mz from Mc prepared with different concentrations of guar gum. Bars represent ± S.D. (n= 3).

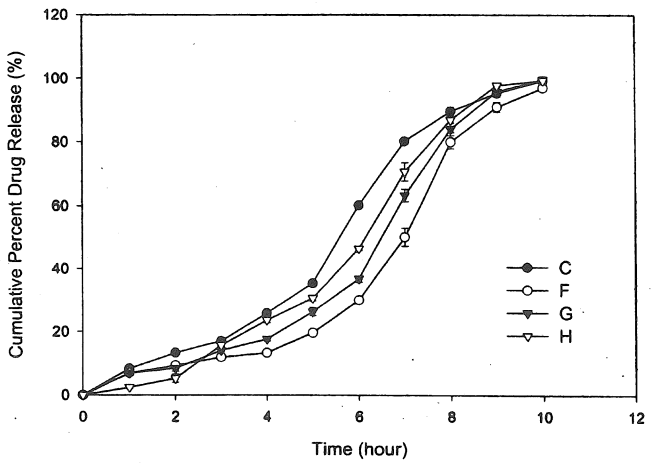

Fig. 6. *In vitro* release profiles of Mz from Mc prepared with different coatings. Bars represent ± S.D. (n = 3).

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