Expert Opinion

informa
healthcare

In vitro and *in vivo* evaluation of *Assam Bora* rice starch-based bioadhesive microsphere as a drug carrier for colon targeting

Mohammad Zaki Ahmad, Sohail Akhter, Iqbal Ahmad, Anjali Singh, Mohammad Anwar, Mohammad Shamim & Farhan Jalees Ahmad†
†*Department of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard, New Delhi, India*

Objective: The aim of this study is to develop, characterize and evaluate (*in vitro* and *in vivo*) a novel colon-targeted bioadhesive microsphere (BAM) containing metronidazole (MTZ).
Methods: BAMs are prepared using *Assam Bora* rice starch as a natural bioadhesive polymer by a double emulsion solvent evaporation method.
Results: The prepared microspheres showed a uniform spherical shape, with excellent retention time. The *in vitro* drug release study of the optimized formulations, in different physiological environments, confirmed the insignificant release of metronidazole in the physiological conditions of the stomach (10 – 12.5%) and small intestine (< 25%). Further, fast and major drug release in cecal content (> 90) indicated that the release of the drug was unaffected by the hostile environment of the gastrointestinal tract (GIT). *In vitro* bacterial inhibition studies illustrated that MTZ loaded BAMs, inhibiting metronidazole-sensitive *Bacteroides fragilis* and selected BAMs (F1 – F7), have an equivalent or higher zone of inhibition than the marketed formulation. An *in vivo* organ distribution study of MTZ revealed that *Assam Bora* rice starch-based microspheres were relatively intact in the upper part of GIT, and the drug was released only after reaching the colon, owing to the microbial degradation of *Assam Bora* rice starch by microflora residing in the colon.
Conclusion: MTZ release patterns exhibited slow and extended release over longer periods of time, which shows the potential of *Assam Bora* rice starch microspheres as a drug carrier for an effective colon-targeted delivery system.

Keywords: *Assam Bora* rice starch, bioadhesion, colon targeting, *in vitro* bacterial inhibition, metronidazole, organ distribution study

Expert Opin. Drug Deliv. (2012) 9(2):141-149

1. Introduction

The large intestine may be subjected to various disease conditions such as colorectal cancer, ulcerative colitis and Crohn's disease. Furthermore, life-threatening infectious diseases such as amebiasis, pseudomembranous colitis (also known as antibiotic-associated diarrhea), giardiasis and anaerobic infections are also associated with the large intestine, particularly amebiasis, which is caused by the protozoa *Entamoeba histolytica* and infects nearly 50 million people a year. After malaria, it is the second leading cause of death due to parasitic diseases in humans and estimated to be responsible for between 50,000 and 100,000 deaths worldwide every year [1-3]. Here in this study, metronidazole (MTZ) is taken as the model drug which is effective in anaerobic infections and represents the most common therapy in these pathogenic conditions [4]. MTZ in conventional tablet dosage forms gets rapidly and completely absorbed in the systemic

10.1517/17425247.2012.633507 © 2012 Informa UK, Ltd. ISSN 1742-5247

circulation after oral administration. As the drug does not reach the colon at therapeutic concentration, accordingly conventional therapy requires comparatively higher doses to compensate for the drug loss during passage through the upper gastrointestinal tract (GIT) leading to undesirable systemic side effects. Owing to these, colon-targeted drug delivery approaches have gained importance for the treatment of localized diseases conditions associated with the large intestine.

Among the different approaches to achieve colon-specific drug delivery, the use of polymers, specifically biodegraded by colonic bacterial enzymes, hold a special promise [5,6]. These polymers shield the drug from the environments of stomach and small intestine, and are able to deliver the drug to the colon. The important bacteria present in the colon such as *Bacteroides*, *Bifido bacterium*, *Eubacterium*, *Peptococcus*, *Lactobacillus* and *Clostridium* secrete a wide range of reductive and hydrolytic enzymes such as β-glucuronidase, β-xylosidase, β-galactosidase, α-arabinosidase, nitroreductase, azoreductase, deaminase and urea hydroxylase [7]. On reaching the colon, polymers undergo assimilation by microorganisms, degradation by enzymes or break down of the polymer backbone leading to a subsequent reduction in their molecular mass and thereby loss of mechanical strength. They are then unable to hold the drug entity to any further extent [8].

The present investigation is aimed towards the formulation and development of colon-targeted microspheres by using a natural, safe, economic and abundantly available *Assam Bora* rice starch. *Assam Bora* rice, locally known as *Bora Chaval*, was first introduced in Assam, India from Thailand or Myanmar by Thai-Ahoms [9-11], which is now widely cultivated throughout the Assam. The starch obtained from *Assam Bora* rice is characterized by its high amylopectin content (i.e., > 95%) with branched waxy polymer which shows physical stability and resistance towards enzymatic action [10-12]. *Assam Bora* rice starch hydrates and swells in cold water forming viscous colloidal dispersion or sols, which are responsible for its bioadhesive nature. Moreover, it is totally degraded by colonic bacteria but remains undigested in the upper GIT. So, on account of its bioadhesive nature, drug release retarding property and susceptibility to microbial degradation in the large intestine [10-12], *Assam Bora* rice starch is used in our study for targeting MTZ to colon in the form of bioadhesive microspheres (BAMs).

2. Materials and methods

Assam Bora rice was procured from local villagers of Dibrugarh district of upper Assam. All other chemicals used in the experiment were of analytical reagent grade and used without further purification. Starch was isolated by methods already established [11-14]. MTZ (> 99% purity) was obtained as a gift sample from Aarey Drugs & Pharmaceuticals Ltd, Mumbai, India. *Assam Bora* rice starch was isolated and further processed in our laboratory.

2.1 Preparation of microspheres

BAMs were prepared by a double emulsion solvent evaporation method described earlier by Sandra *et al.* 2005, with slight modifications [15]. Aqueous polymer dispersion was prepared by suspending the weighed amount of *Bora* rice starch (**Table 1**), in 75 ml of distilled water and further stirred at 800 r.p.m. for 6 h. The solution (A) was stored in a sealed container at 40°C for 24 h prior to use. Drug solution (B) was prepared by dissolving 500 mg of MTZ and 50 mg of magnesium stearate (anti-adherent) in 20 ml of methylene chloride and stirred at 1000 r.p.m. for 1 h. The first emulsion was prepared by emulsifying the solution B into 50 ml of solution A containing 0.2 ml of Tween 80 to enhance the process of emulsification. The compositions of different formulations along with their formulation codes are given in **Table 1**. Silverson homogenizer fitted with 6-blade butterfly propeller was used for the rapid mixing for 30 min. For the preparation of second emulsion, 30 ml of the first emulsion was added drop-wise (using 18 gauge hypodermic needle) to 300 ml of light liquid paraffin containing 1.5 % (v/v) span 80. The resulting double emulsion was stirred at 1000 r.p.m. for 1 h with heating at 40°C to promote the evaporation of water. Solid BAMs were subsequently separated from the oil by centrifugation at 10,000 r.p.m. and further washed in hexane and dried in hot air oven at 40°C for 6 h. For each polymer:drug ratio, five batches of BAMs were prepared for the purpose of assessing the reproducibility of drug loading.

2.2 Characterization

2.2.1 *Particle size, size distribution and morphology*

The prepared microspheres were mounted on the moving stage optical microscope (MOTIC, Xiamen, China) fitted with the calibrated ocular micrometer for analyzing the particle size range, size distribution and other morphological features. Morphology of the BAMs was studied by scanning electron micrographs (SEM) taken with a scanning electron microscope (Hitachi S-300N, Germany). The samples were gold coated (at about 100A°) on metal stabs with the aid of double-sided adhesive tape in KSE24M high vacuum evaporator. Selected regions that were scanned depicting distinct morphological feature were photographed.

2.2.2 *Entrapment efficiency*

Unentrapped drug was separated by centrifugation and the drug that remained entrapped in BAMs was determined after complete disruption by crushing 100 mg of BAMs in a glass mortar and dispersed in 100 ml of phosphate buffer (pH 7.4) and further analyzing the resultant solution for MTZ using HPLC (Shimazdu, Japan). Entrapment efficiency was calculated using the formula:

$$\textit{Entrapment Efficiency}\ (EF) = \frac{\textit{Actual}\ \text{drug content}}{\text{Initial amount of the drug added in the formulation}} \times 100 \quad (1)$$

Table 1. Composition of mucoadhesive microsphere formulation.

Formulation code	Metronidazole (mg)	*Assam Bora* rice starch (mg)	Magnesium stearate (mg)
F1	500	500	50
F2	500	1000	50
F3	500	1500	50
F4	500	2000	50
F5	500	2500	50
F6	500	3000	50
F7	500	3500	50

2.2.3 Flow properties

The flow properties of the microspheres were determined by measuring compressibility index and Hausner's ratio according to the following relationship:

$$compressibilityIndex = 100 \times \frac{\rho_{tapped} - \rho_{\text{bulk}}}{\rho_{\text{tapped}}} \quad (2)$$

$$HausnersRatio = \frac{\rho_{\text{tapped}}}{\rho_{\text{bulk}}} \quad (3)$$

where ρ_{tapped} and ρ_{bulk} are tapped density and bulk density, respectively.

2.2.4 Swelling index

The swelling studies on microspheres were performed with water, 0.1N HCl and phosphate buffer (pH 7) [16]. Accurately weighed 100 mg of BAMs were immersed in a little excess of double-distilled water, phosphate buffer (pH 7.4) and 0.1N HCl, respectively, and kept for 24 h. The swelling index (SI) was calculated by using the following formula:

$$\text{SI} = \left(\frac{W_i - W_s}{W_s} \times 100 \right) \quad (4)$$

where SI = percentage swelling of microsphere, W_i = initial weight of microsphere and W_s = weight of microsphere after swelling.

2.2.5 Bioadhesion test

The bioadhesive property of the BAMs was determined by the method already established [17] with a slight modification. This study was performed on freshly cut goat intestine (15 cm long piece of goat intestine). One gram microspheres were shifted on the mucosal surface, which was attached to 20×10 cm clean glass surface and the whole system was inclined at an angle of 45° relative to the horizontal plane. Phosphate buffer (pH 7.4) at 37 ± 0.5°C was passed over the mucosal surface at a rate of 2 ml/min. The time required for detaching all the microspheres from mucosal surface of goat intestine was recorded by visual inspection.

2.2.6 HPLC analysis of MTZ

MTZ was analyzed on reverse phase HPLC column (Supelco column 516, C18, 5 µm, 250 mm × 4.6 mm). The HPLC system (SCL-10 AVP, Shimadzu, Japan) consisted of a binary pump (LC-10 ATVP, Shimadzu) and a UV-VIS detector (SPD-10 AVP, Shimadzu). The mobile phase was composed of a mixture of methanol and sodium acetate buffer (pH 4.4) in a ratio of 15:85 v/v and passed through the column with a flow rate of 0.9 ml/min. The detection wavelength was set at 254 nm and the retention time of the MTZ was found to be 8 min. The assay was linear (r^2 = 0.9995) in the concentration range of 0.1 – 40 µg/ml with the lowest detection limit at 0.005 µg/ml. The percentage recoveries ranged from 99 to 100.1%. All the samples prior to the analysis were filtered through a 0.22 µm pore size membrane filter.

2.2.7 *In vitro* drug release study

To study the effect of different physiological environments in GIT on the release pattern from BAMs, dissolution study was carried out in 0.1N HCl (pH 1.2), phosphate buffer (pH 7.4) and goat cecal content (whole GIT of goat was commercially collected from the slaughter house immediately after sacrifice of goat and was stored in physiological solution (previously bubbled with CO_2) at 8°C before use). The test was performed in 900 ml of the above mentioned media, which was based on USP method (Dissolution apparatus I at 100 r.p.m. and 37 ± 0.5°C). Release of MTZ from the microspheres was studied for the initial 2 h in acidic medium, followed by phosphate buffer (pH 7.4) for 3 h and, further, the medium was replaced by goat cecal content and the dissolution was carried for next 19 h. Anaerobic environment of the cecal content was maintained by continuous CO_2 bubbling into the beaker containing cecal content and formulation. At regular intervals of time, 5 ml of the sample was withdrawn and replaced with 5 ml of fresh media to maintain sink conditions. Withdrawn samples were filtered through syringe filter (0.22 µm) and analyzed for MTZ by HPLC. Control study (without goat cecal content) was also performed with all the formulation. The drug release studies were performed in triplicate for every case.

2.2.8 Statistical analysis

The mean percentage of MTZ released from BAMs of various polymer ratios in the dissolution media (with and without goat cecal content) at 24 h was compared. The student *t* test was used to find the statistical significance. A value of $p < 0.05$ was considered statistically significant.

2.2.9 *In vitro* bacterial inhibition studies

Bacterial inhibition study was performed by using the agar diffusion method (Cup-plate technique). *Bacteroides fragilis* was chosen as bacterium strain sensitive to MTZ. *Bacteroides fragilis* MTCC 1045 was obtained from the Institute of Microbiology Technology, Chandigarh, India. The lyophilized strain was suspended in the sterile nutrient agar media and incubated at

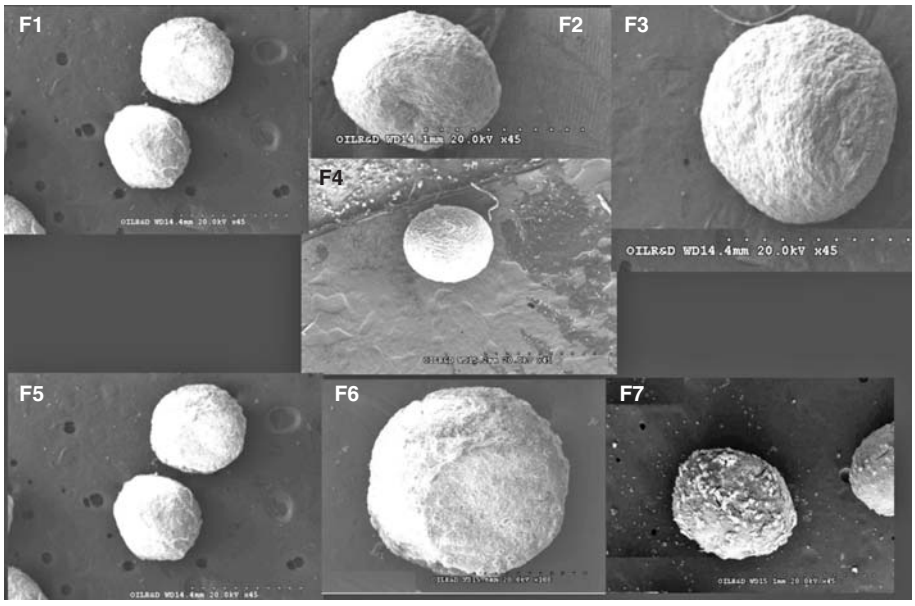

Figure 1. Scanning electron photomicrograph of different formulations.

37°C for 24 h in anaerobic conditions. One batch of this culture was harvested on nutrient agar slant and was incubated at 37°C for 36 h in anaerobic conditions. Culture broth of the following composition were used in the study: Water for injection (1500 ml), casein peptone (24 g), soya bean peptone (4.5 g), meat extract (7.5 g), glucose (4 g), sodium chloride (10 g), Agar (30 g) and dipotassium phosphate, K_2HPO_4 (4.5 g) at pH 7.4 ± 0.2 at 25°C. After testing the pH, broth was distributed in a series of 27 test tubes (50 ml in each). After filling, broth was sterilized by autoclave for 15 min at 121°C. Agar plate containing the bacterial strain was prepared as follows: the test tubes containing broth (50 ml) were inoculated with *B. fragilis* suspension. This suspension was mixed on vortex mixture and poured into Petri dishes (100 mm diameter) and left to solidify by placing the Petri dishes on a cool horizontal surface under the laminar air flow. After cooling and solidification, a 12 mm diameter well was bored in the center of each agar plate by using a sterilized cork borer. The agar plate was divided into nine groups (three in each group). In each of the seven groups, microspheres equivalent to 400 mg MTZ were taken from each batch (F1 – F7), respectively, and wetted with sterile physiological solution. In the eighth group, marketed MTZ tablet (Flagyl®, 400 mg) was placed. In the ninth group, sterilized water (0.25 ml) was used. All the plates were incubated at 37 ± 0.5°C for 36 h. For all incubation time (36 h), the microsphere's behavior was observed and diameter of the inhibition zone was measured and expressed in mm ± s.d. The results were subjected to statistical analysis.

2.2.10 *In vivo* organ distribution study

The animal protocol to carry out *in vivo* study was approved by the animal ethical committee and their guidelines were followed for the studies. Male albino rats, 7 – 9 weeks old and weighing 200 – 250 g, were used for the study. The animals were kept under standard laboratory conditions (temperature: 25 ± 2°C; relative humidity: 55 ± 5%). The animals were housed in polypropylene cages, with free access to standard laboratory diet (Bengal gram soaked in water). These animals were divided into three groups of six rats each. The first group served as control. The second group received 100 mg MTZ. The animals of the third group were given BAMs (F4) containing an equivalent amount of the drug. The formulations were orally administrated in suspension form following sufficient volume of drinking water. After 2, 5, 8 and 12 h, the rats were sacrificed following which the stomach, small intestine and colon were isolated.

These organs were homogenized by Micro Tissue Homogeniser (Remi Ltd, Mumbai, India) along with a small amount of phosphate buffer (pH 7.4); 1.5 ml of methanol was added to homogenate and kept for 30 min. After appropriate dilution of supernatants with the mobile phase, the drug content was determined by the HPLC method as described under Section 2.2.6, 'HPLC analysis of MTZ.'

3. Results

Microspheres formed were sufficiently rigid and SEM image showed spherical shape with slight rough surface (**Figure 1**). The mean particle size for the formulations (F1 – F7) ranged from 111 ± 0.012 to 485 ± 0.176 µm (**Table 2**). Entrapment efficiency (%) of selected formulations for MTZ is

Table 2. Mean particle size and encapsulation efficiency.

Serial no	Formulation code	Mean particle size (μm)	Entrapment efficiency (%)
1	F1	162 ± 0.087	85.49 ± 0.211
2	F2	251 ± 0.010	79.27 ± 0.279
3	F3	111 ± 0.012	75.78 ± 0.389
4	F4	305 ± 0.071	91.29 ± 0.031
5	F5	367 ± 0.098	89.67 ± 0.091
6	F6	425 ± 0.170	87.307 ± 0.371
7	F7	485 ± 0.176	78.29 ± 0.173

Each value represents the (mean ± s.d.) of three determinations.

Table 3. Swelling index bioadhesion time of BAMs.

Formulation code	Swelling index*			Adhesion time (h)		
	Water	0.1 N HCl	Phosphate buffer (pH 7.4)	Water	0.1 N HCl	Phosphate buffer (pH 7.4)
F1	44.68 ± 0.751	35.41 ± 0.661	79.92 ± 0.663	17	9	15
F2	49.98 ± 0.965	33.45 ± 0.168	83.36 ± 0.924	17	9	17
F3	54.60 ± 0.705	34.49 ± 0.861	83.92 ± 0.367	17	11	18
F4	54.36 ± 0.965	35.41 ± 0.421	84.82 ± 0.364	19	13	20
F5	56.43 ± 0.345	34.89 ± 0.172	85.92 ± 0.354	21	13	23
F6	57.08 ± 0.345	36.41 ± 0.192	87.89 ± 0.187	21	13	23
F7	58.92 ± 0.364	30.76 ± 0.195	89.87 ± 0.765	21	14	24

*Each value represents the (mean ± s.d.) of three determinations.
BAM: Bioadhesive microsphere.

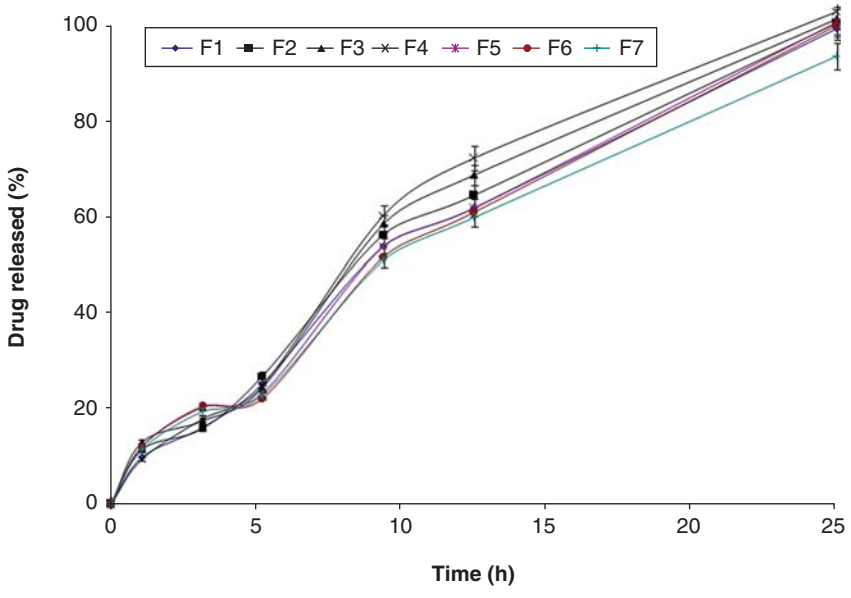

Figure 2. Percentage *in vitro* release from *Assam Bora* rice starch containing different drug:polymer ratios (F1 – F7) in simulated GIT fluid followed by cecal content (control).
GIT: Gastrointestinal tract.

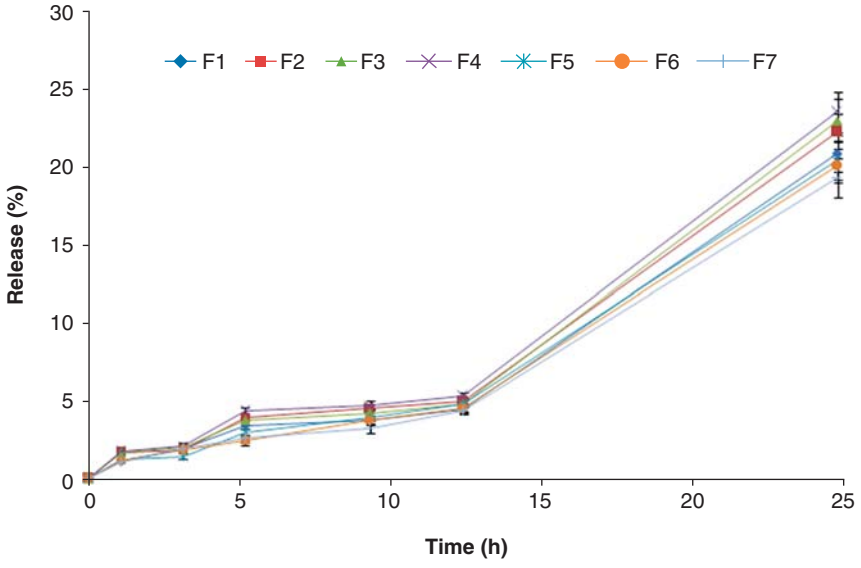

Figure 3. Percentage *in vitro* release from *Assam Bora* rice starch containing different drug:polymer ratios (F1 – F7) in simulated GIT fluid without cecal content (control).
GIT: Gastrointestinal tract.

Table 4. Zone of inhibition.

Formulation code	Diameter of zone of inhibition (mm)
F1	15.89 ± 0.15
F2	16.21 ± 0.89
F3	16.25 ± 0.25
F4	16.89 ± 0.09
F5	14.65 ± 0.45
F6	13.78 ± 0.87
F7	13.43 ± 0.25
Marketed formulation	12.34 ± 0.67
Sterile water	0

Each value represents the (mean ± s.d.) of three determinations.

summarized in the **Table 2** which varied from 75.78 ± 0.389 to 91.29 ± 0.031%.

Compressibility index and Hausner's ratio, the simple, faster and popular means of predicting flow properties, were chosen for flow characterization. They were proposed as an indirect method of determining bulk density, size and shape, surface area and cohesiveness. The compressibility index and Hausner's ratio of the formulated microspheres were in the range of 10 – 15 % and 1.10 – 1.16, respectively.

Results of swelling studies of microspheres in different media are summarized in **Table 3**, indicating variable SI of microspheres in water, acidic and alkaline medium with high swelling in phosphate buffer followed by water and 0.1N HCl.

The BAMs showed good adhesion/retention time (≥ 9 h) in selected biological media (**Table 3**). High percentages of microspheres were retained indicating the excellent mucoadhesion (which is important for the local drug targeting).

Drug release performance of selected BAMs in different media (0.1N HCl, phosphate buffer (pH 7.4) and goat cecal content) at regular intervals of time is depicted in **Figure 2**. There was no measurable drug release (10 – 12.5%) observed up to 2 h in simulated gastric fluid (pH 1.2), while at pH 7.4, the MTZ release was quite insignificant (< 25%) up to 5 h. The percentage release of MTZ from all the formulations at the end of 24 h with goat cecal content was found to be 89.61 ± 0.03 to 98.67 ± 0.04%, whereas in control study (without goat cecal contents in the dissolution media) only 19 ± 0.25 to 24 ± 0.56% of MTZ was released (**Figure 3**). This difference was found to be statistically significant ($p < 0.001$).

The optimized formulations were further tested for their bacterial inhibition performance by agar diffusion method. After completion of incubation period, they were observed and their zones of inhibition (millimeter) were measured. Clear zones of inhibition were obtained for formulations and marketed formulation (Flagyl). The diameter of zone of inhibition by Flagyl was 12.34 ± 0.67 mm, whereas for the prepared formulation (F1 – F7) it varies from 13.43 ± 0.25 to 16.89 ± 0.09 mm (**Table 4**).

The results of organ distribution indicated that maximum concentration (67 ± 5.4%) of MTZ was observed after 1 h in stomach following oral administration of plain MTZ and in the subsequent hour much less drug reached the small intestine and afterwards, no drug was found in the colon. *Assam Bora* rice starch-based BAMs were observed and found intact in the upper part of GIT. Approximately 2.5 – 4.5% of

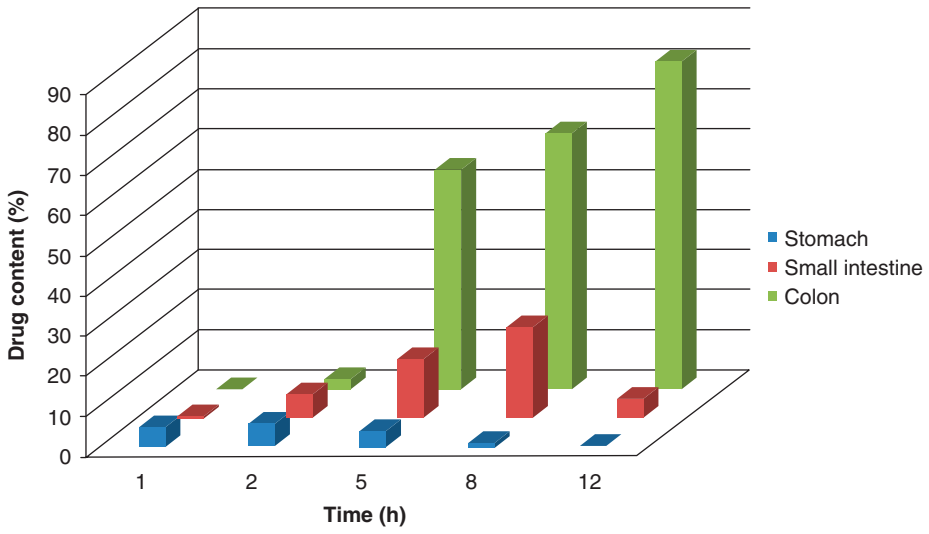

Figure 4. MTZ content in isolated organs of albino rat after oral administration of F4.
MTZ: Metronidazole.

total drug loaded was released during its transit through the upper GIT. After 12 h, the maximum percentage of drug was observed in the colon (81 ± 1.8%), and very insignificant amount of drug was found in the stomach and small intestine (**Figure** 4).

4. Discussion

During the optimization step of BAM synthesis, it was found that at the stirring speed of ≤ 800 r.p.m. resulted in the formation of undispersed, aggregated mass of BAMs. Furthermore, at the stirring speed above 1000 r.p.m., small sized and dispersed BAMs were formed due to the development of high shear and turbulence. However, the turbulence produced causes the adhesion of BAMs to the wall of container and the blade surface. So, the optimized stirring speed was kept at 800 – 1000 r.p.m. resulting in spherical and dispersed microsphere formation.

It was seen that with the increase of *Bora* rice starch concentration, the size of BAMs was increased. This may be due to the increase in viscosity of first emulsion, which in turn increased the droplet size during the second emulsification process, additionally accumulating starch polymer under the influence of chemical interactions such as H-bonding. Moreover, entrapment of drug affects the size but did not show linear relation with the particle size. MTZ, being slightly soluble in water, has a low tendency to diffuse out of BAM core and, therefore, high drug loading was achieved with *Bora* rice starch. Besides, high entrapment may also be due to inclusion of drug into hollow hilum of the *Assam Bora* rice starch.

Values for Carr's index and Hausner's ratio indicate good flow property (USP34-NF29) suggesting that BAMs can easily be encapsulated and handled during processing. Swelling property is directly related to bioadhesive capability of formulation, as reported earlier that adhesive nature and cohesiveness polymers are affected by swelling behavior [16,18]. BAMs are anticipated to take up water from the underlying mucosal tissues by absorption, swelling and capillary action leading to additive adhesion [19,20]. It may be due to greater gelling behavior of polymer in alkaline medium and followed by water and the least in acidic media (due to acidic hydrolysis of polymer backbone).

Results from the bioadhesion test clearly demonstrate the effect of physiological pH on bioadhesion of *Assam Bora* rice starch-based microspheres with mucus of GIT. Alkaline pH may be potentiating the binding of α-amylopectin–OH group with mucin of intestinal mucosa. While presence of acidic pH may lead to acidic hydrolysis of α-amylopectin–mucin complex as indicated in the result of low adhesion time in 0.1N HCl. Moderate adhesion time was seen in water due to the absence of alkaline and acidic pH. In addition, the results also revealed that as the bioadhesive content (*Bora* rice starch) increases, bioadhesive force also increases due to the involvement of increased number of α-amylopectin and mucin molecules.

The *in vitro* release studies indicate that minimal amount of drug was released from the *Assam Bora* rice starch-based BAMs in the physiological environment of stomach and intestine. Ideally, the oral drug delivery containing the MTZ should release no MTZ in the physiological environment of stomach and small intestine. Here, BAMs from *Assam Bora* rice starch appears to be promising as it releases the least amount of MTZ in the physiological environment of stomach and small intestine. This study shows that release of MTZ in the physiological environment of colon is due to microbial degradation of *Assam Bora* rice starch containing BAMs. The control study was performed to ensure that drug release was not due to mechanical erosion which is likely to occur

because of bowel movements. It is evident from **Figure 2** that formulation F4, having a drug polymer ratio 1:4, was found to show the better release behavior among the BAMs selected.

Bacterial inhibition studies indicate that the developed BAMs were therapeutically equivalent to Flagyl with the additional advantage of sustained/prolonged action and reduced systemic side effects. *In vivo* organ distribution study of optimized formulation was performed in order to establish its targeting potential in the colon. The release pattern of MTZ BAMs as seen in the results within the GIT can be understood by the protective nature of *Assam Bora* rice starch. The drug MTZ was released from BAMs only after reaching the colon owing to microbial degradation with microbial flora residing in the colon.

5. Conclusion

Studies reveal that *Assam Bora* rice starch-based BAM designed site-specific delivery of model drug (MTZ) may reduce the side effects of the drug caused by its systemic absorption when given in conventional forms. The developed formulation based on this polymer is safe, economical to use due to easy availability and easy to formulate. Experimental results demonstrate that *Assam Bora* rice starch microspheres exhibited good bioadhesive properties, and MTZ release from these BAMs was largely due to the microbial degradation followed by enzymatic, acid and alkali hydrolysis. Also, MTZ release pattern exhibits slow and extended release over longer periods of time that showed the potential of *Assam Bora* rice starch microspheres to be used as a drug carrier for an effective colon-targeted delivery system for MTZ-like drugs and those agents that are effective against colon/large intestine disease conditions.

Acknowledgement

The authors thank H Dubey, General Manager, R & D, Oil India Ltd, Duliajan, Assam, India for providing the instrument facility. They also thank HC Das, R Bordoloi and PK Borah of Oil India for cooperation in the laboratory and for providing the facilities to carry out parts of this investigation.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Walsh JA. Problems in recognition and diagnosis of amoebiasis: estimation of the global magnitude of morbidity and mortality. Rev Infect Dis 1986;8:228-38
2. WHOAmoebiasis report of a who expert committee. Technical Report Series 1969;421:1-52
3. WHO./PAHO/UNESCO ReportA consultation with experts on amoebiasis. Epidemiol Bull 1977;18(1):13-14
4. Philips MA, Stanley SL Jr. Chemotherapy of Protozoal infection: Amebiasis, Giardiasis, Trichomoniasis, Trypanosomiasis, Leishmaniasis, and other protozoal infection. In Goodman and Gilmanâ s: The Pharmacological basic of therapeutics 11th Edition, Laurence L, Brunton K, Parker L. editors. McGraw Hill Medical; New York: p. 1049-72, 2006
5. Krishnaiah YS, Bashkir Reddy PR, Satyanarayana V, et al. Studies on the development of oral colon targeted drug delivery systems for metronidazole in the treatment of amoebiasis. Int J Pharm 2002;236(1-2):43-55
   • **Deals with colon-targeted drug delivery.**
6. Sinha VR, Rachna K. Polysaccharides in colon-specific drug delivery. Int J Pharm 2001;224(1-2):19-38
   • **Describess the use of polysaccharides in colon-targeted drug delivery.**
7. Scheline RR. Metabolism of foreign compounds by gastrointestinal microorganisms. Pharmacol Rev 1973;25:451-23
8. Park K, Shalaby SWW, ParkH, Biodegradation. Biodegradable Hydrogels for Drug Delivery. Technomic Publishing Co.; USA: 1993. p. 13-34
9. Sharma SD, Vellanki JMR, Hakim KI, et al. Primitive and current cultivars of rice in Assam rich source of valuable genes. Curr Sci 1971;40:126-8
10. Ahmad MZ, Bhattacharya A. Isolation and Physicochemical characterization of Bora Rice Starch from Assam as Pharmaceutical Excipients. J Pharm Res 2009;2(8):1299-3
11. Ahmad MZ, Bhattacharya A. Isolation and physicochemical characterization of Assam Bora rice starch for use as a plasma volume expander. Curr Drug Deliv 2010;7:162-7
   • **Describes the unique property of *Assam Bora* rice starch.**
12. Bhattacharya A, Akhter S, Shahnawaz S, et al. Evaluation of Assam Bora rice starch as Plasma volume expander by polymer analysis. Curr Drug Deliv 2010;7(5):436-41
   •• **Deals with characterization of *Assam Bora* rice starch.**
13. Evans WE. Trease and Evans. Pharmacognosy. 15th edition. WB Saunders; London: 2006. p. 191-213
14. Song Y, Jane J. Characterization of barley starches of waxy normal and high amylase varieties. Carbohydr Polym 2000;41:365-77
   •• **Describes the isolation characterization of different varieties of starch.**
15. Sandra K, Gareth DR, John T, et al. Bioadhesive triclosan-loaded polymer microspheres for application to the oral cavity: preparation and controlled release characteristics. Eur J Pharm Biopharm 2005;59(1):207-16
16. Jain S, Chourasia M, Jain A, et al. Development and Characterization of bioadhesive microspheres bearing

salbutamol for nasal Delivery. Drug Deliv 2004;1:113-22

- **Describes the measurements of bioadhesive technique.**

17. Vyas SP, Talwar N, Karajgi JS, et al. An erythrocyte based bioadhesive system for nasal delivery of propranolol. J Control Release 1993;23:231-7

- **Describes the measurements of bioadhesive technique.**

18. Lehr CM, Bowstra JA, Tukker JJ, et al. Intestinal transit of bioadhesive microspheres in an in situ loop in the rat. J Control Release 1990;13:51-62

19. Mortazavi SA, Smart JD. An investigation into the role of water movement and mucus gel dehydration in mucoadhesion. J Control Release 1993;25:197-203

20. Duchene D, Ponchel G. Principle and investigation of bioadhesion mechanism of solid dosage forms. Biomaterials 1992;13:709-14

Affiliation

Mohammad Zaki Ahmad[1] PhD, Sohail Akhter[2] PhD, Iqbal Ahmad[3] PhD, Anjali Singh[3] PhD, Mohammad Anwar[3] PhD, Mohammad Shamim[4] PhD & Farhan Jalees Ahmad[†5]
[†]Author for correspondence
[1]Assistant Professor,
Dreamz College of pharmacy,
Khilra- Meramesit, Sundernagar-175036,
Mandi (Himachal Pradesh), India
[2]Research Scientist (CSIR),
Hamdard University,
Department of Pharmaceutics,
Faculty of Pharmacy,
New Delhi-110062, India
[3]Research Scholar,
Hamdard University,
Department of Pharmaceutics,
Faculty of Pharmacy,
New Delhi-110062, India
[4]Hamdard University,
Department of Chemistry,
Faculty of Science,
New Delhi-110062, India
[5]Professor,
Department of Pharmaceutics,
Faculty of Pharmacy,
Jamia Hamdard,
New Delhi-110062, India
E-mail: Email: farhanja_2000@yahoo.com