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Original Article

FORMULATION AND *IN VITRO* EVALUATION OF OIL ENTRAPPED BUOYANT BEADS FOR STOMACH SPECIFIC DELIVERY OF METRONIDAZOLE

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ABSTRACT

Objective: This work focused on the development of floating alginate beads for stomach specific delivery of Metronidazole towards the effective eradication of *Helicobacter pylori*, a major causative agent of peptic ulcers.

Methods: Different formulations of sunflower oil entrapped gel beads were prepared by emulsion gelation technique using sodium alginate as gelling agent. The prepared beads were evaluated for size, surface morphology, drug entrapment efficiency, *in vitro* floatuing and *in vitro* drug release properties.

Results: It was found that the percentage of oil plays an important role in controlling the floating of oil-entrapped alginate beads. All batches of beads floated for >24 hours with a very short lag time of 151–187 seconds. Scanning electron microscopy (SEM) revealed that the beads were spherical in shape with small pores on the surface. SEM of the cross section of the beads demonstrated minute oil globules throughout the inner portion of the beads confirming oil entrapment. The results clearly indicated that amount of polymer (% w/v) and amount of sunflower oil (% v/v) affected the bead size, floating, encapsulation efficiency and drug release of the beads. It was observed that the drug release kinetics was best fitted with the Higuchi model and drug release from the polymer matrix system was non fickian (anomalous transport) type.

Conclusion: The results provide evidence that formulated gel beads may be used to incorporate antimicrobials like Metronidazole and may be effective when administered locally in the stomach to cure *Helicobacter pylori* infection.

Keywords: Floating beads, Metronidazole, Sunflower oil, *In vitro* drug release, Scanning electron microscopy.

INTRODUCTION

Helicobactor pylori (*H. pylori*) is a small, spiral, microaerophilic, gram-negative bacteria with a 4-6 bulbous tipped sheathed flagella at one end, which helps it to penetrate the gastric mucosa and colonize on the gastric antrum.[1,2]

Although *H. pylori* is highly sensitive to most antibiotics, it's eradication from patients is very difficult, even with the current best therapies.[3,4] Conventional tablets or capsules are, in general, used for eradication therapy, but these preparations do not remain in the stomach for the long time. Therefore, it is difficult to reach minimum inhibitory concentrations in the gastric mucus where *H. pylori* colonize.

The bacteria penetrate the gastric mucus layer and fix itself to various phospholipids and glycolipids in the mucus gel. Therefore, access of antimicrobial drugs to the site is restricted from both the lumen of the stomach and the gastric blood supply. To overcome the problems in *H. pylori* treatment, a novel drug delivery system that will localize the antibiotic at the site of infection to achieve bactericidal concentration is highly desirable. Floating drug delivery systems have a bulk density lower than gastric fluids and thus remain buoyant in the stomach for a prolonged period of time, without affecting the gastric emptying rate.

While the system is floating on the gastric contents, the drug is released slowly at a desired rate from the system and remaining part of the residual system is slowly emptied from the stomach and enhanced GRT of the drug.[5-7] The extended retaintion of the drug can maintain a higher antibiotic concentration in the gastric region where *H. pylori* exists and thereby improve the therapeutic efficacy.

Based on this concept, in order to enhance the efficacy, we have made an attempt to develop oil entrapped floating alginate beads for stomach site specific delivery of Metronidazole for treatment of *H. pylori* infection. It is a nitroimidazole antibiotic used particularly for anaerobic bacteria and protozoa. It is indicated for the treatment of *Helicobacter pylori* infection. As part of a multi-drug regimen and is usually taken two or three times a day. The polymer used sodium alginate is an inexpensive, nontoxic product extracted from kelp. Literature reports indicate widespread use of sodium alginate for achieving sustained release of drugs,[8] targeting gastric mucosa,[9] and increasing the bioavailability of drugs[10]. Additionally it also reduces interfacial tension between an oil and water phase and is efficient for preparation of the emulsion.

MATERIALS AND METHODS

Materials

Sodium alginate (low viscosity grade; 250 cp of 2% solution at 25°C) was purchased from Loba Chemie Pvt. Ltd., Mumbai, India. Metronidazole was supplied by BS traders, Liluah, Howrah, India. Sunflower oil was purchased from Agro Tech Food Ltd., Secundrabad, India. Calcium chloride dihydrate (CCD) and Hydrochloric acid (35% GR) were purchased from E. Merck India Ltd., Mumbai, India. All materials were used with no further purification. Deionized water was used throughout the study.

Preparation of floating beads

Calcium alginate gel beads were prepared by emulsion gelation method. At first, the aqueous slurry of sodium alginate and Metronidazole in double distilled water was prepared. Sunflower oil was added drop wise to the aqueous slurry with stirring at 2500 rpm by a PMDC stirrer (RQ-121/D, Remi, India) fitted with stirring shaft (6×250 mm) with pitched-blade-type impeller.

The stirring was continued for 45 minutes to get a stable o/w type emulsion. Finally this emulsion was dropped through 18 G needle in to 10%w/v calcium chloride dihydrate (CCD) solution to get spherical beads. These beads were kept in contact with CCD solution for 15 minutes and then removed from CCD solution by straining, washed, dried in a hot air oven and stored in desicator for further study. The formulation variables are given in table 1.

Evaluation of the floating beads

Determination of bead size

The diameters of the dried beads were measured by Dial calipers [AEROSCAPE (150 X 0.2 mm)].

Morphology study

Morphological examination of the surface and internal structure of the beads was performed by using scanning electron microscope (SEM). The samples placed on the stubs using a carbon adhesive tape, coated with gold palladium alloy using a fine coat ion sputter (JEOL, fine coat ion sputter JFC-1100) and then surface topography was analyzed under scanning electron microscope (JSM 6100 JEOL, Tokyo, Japan) operated at an acceleration voltage of 15 kV.

Table 1: Formulation compositions for the preparation of drug loaded alginate beads

Formulations No.	Na-alginate (% w/v)	Drug(mg)	Sunflower oil (% v/v)	$CaCl_2.2H_2O$ (%w/v)
F1	3	400	5.00	10
F2	3	400	6.66	10
F3	5	400	5.00	10
F4	5	400	6.66	10

Percentage drug entrapment efficiency

A 100 mg portion of dried beads were placed into 100 ml of 0.1 N HCl. It was shaken for 6 hours and the resulting solution was filtered. The filtrate was suitably diluted and analyzed at 272.5 nm using a UV Spectrophotometer (UV-1700; SHIMADZU). The entrapment efficiency was calculated by the formula:

%Entrapment efficiency = (Calculated drug concentration/ Theoretical drug concentration) x 100

In vitro evaluation of floating ability

The in vitro buoyancy study for the beads was tested by visual observation in 0.1 N HCl. The experiment was carried out using a USP dissolution apparatus II, Veego. Twenty beads of each batch were placed into 500 ml 0.1 N HCl maintained at 37±0.5°C and 50 rpm. The time taken for the beads to float at the surface of the medium (known as floating lag time) and duration of floating were noted.

***In vitro* drug release study**

In vitro drug release studies were carried out for all products and for the pure drug in USP dissolution apparatus II, Veego using 500 ml 0.1 N HCl as a dissolution medium maintained at 37±0.5°C. Beads equivalent to 10mg of the pure drug were used for the study. A 2 ml of the aliquot was withdrawn at predetermined intervals and replaced with fresh dissolution medium. The aliquots were further diluted suitably and analyzed at 272.5 nm using UV-1700, SHIMADZU spectrophotometer.

RESULTS AND DISCUSSION

When emulsion of Na-alginate & Metronidazole with sunflower oil was dropped into calcium chloride solutions, spherical gel beads were then formed instantaneously due to intermolecular cross-linking between the divalent calcium ions and the negatively charged carboxyl group of alginic acid. Freshly prepared beads containing sunflower oil were almost spherical and white(Fig. 1) which after drying become brownish and dense(Fig. 2). Brown colour is due to the original color of sunflower oil.

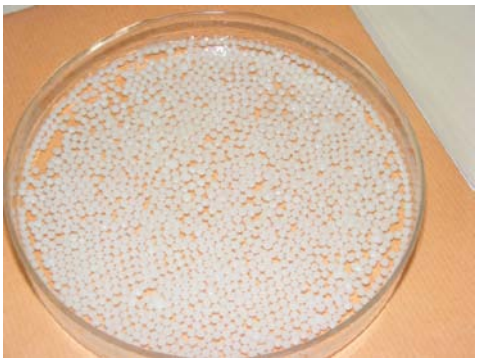

Fig. 1: Photograph of freshly prepared beads

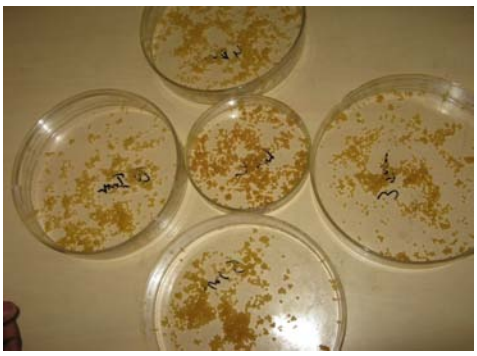

Fig. 2: Photograph of dried beads

Bead size

The average size of the dried spherical beads ranged from 1.954 mm to 2.064 mm(Table 2) depending on the concentration of the polymer and sunflower oil used. Size of the beads increases with the increase in the concentration of polymer at 10% fixed concentration of calcium chloride. This may be due to the increase in the viscosity of the polymer solution that in turn increases the droplet size during addition of the polymer solution to the cross-linking solution. It was also found that, if pressure is increased in the piston during ejection of slurry, the beads sizes are increased.

Entrapment Efficiency

Drug entrapment efficiency ranged from 76% to 84%(Table 2). It was observed that an increase in the amount of polymer increases drug entrapment efficiency due to increased space for drug molecules to be retained throughout a larger cross linked network of calcium alginate. It was also observed that increasing of the curing time decrease the drug entrapment efficiency which may be due to the solubility of Metronidazole in water.

Table 2: Physicochemical characterization of the prepared beads

Formulation No.	Average diameter(average of five values)(mm)	Drug entrapment efficiency (%)	Lag time(average of five values) (seconds)	Floating time (Hrs.)
F1	1.998	76.8712	187	>24
F2	1.954	79.0245	156	>24
F3	2.064	81.0231	179	>24
F4	2.032	84.2152	151	>24

Buoyancy study

All formulations floated within 187 seconds (Table 2) after being placed into the acidic medium (0.1 N HCl) and remained buoyant in the medium throughout the study irrespective of polymer and oil concentration. Primarily, sunflower oil entrapped in the bead is responsible for floating i. e. oil acts as floating aid. Secondarily during dissolution, drug released from the matrix of the beads and swelling reduces the bulk density, which also aid to float the beads.

Morphology

SEM study of alginate beads showed spherical morphology with small pores or minute channels (Fig.3). Small pocket structures (Fig.4) are found on the cross sectional area of the interior part of the beads i. e., matrix of the beads which prove that oil was entrapped in the alginate matrix.

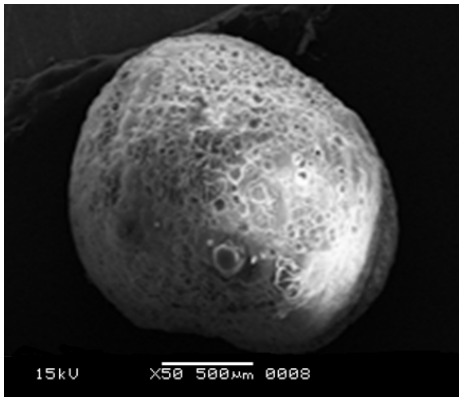

Fig. 3: SEM image of drug & oil incorporated alginate bead

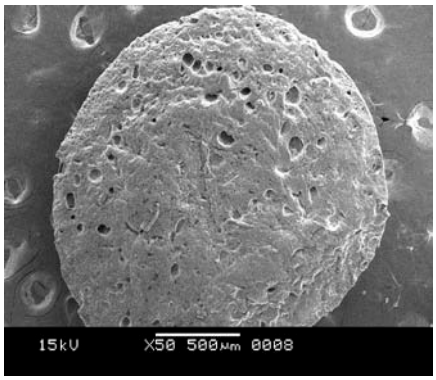

Fig. 4: SEM image of cross section of drug & oil incorporated alginate bead.

In vitro drug release

Different batches of the formulation were able to release up to about (92 to 98)% drug after 6 hours under study(Table 3). From the release profile, it was observed that the drug release kinetics was best fitted with the Higuchi model(Fig.5). From the results of correlation coefficients and release exponent data of different batches using the Korsemeyer–Peppas model, it was observed that the drug release from the polymer matrix system was non fickian (anomalous transport) type (1<n< 0.5) (Table 4). Anomalous transport or non-fickian diffusion refers to combinations of both diffusion and erosion controlled rate release. The initial fast release was attributed to the diffusion of the adsorbed drug particles on or near the outer surface of the beads, while subsequent release was due to slow diffusion of the entrapped drug from the interior core of the alginate matrix.

Table 3: Cumulative % drug release of different batches at different times(n=5).

Time (Hrs)	Cumulative % Release			
	F1	F2	F3	F4
0	0	0	0	0
0.5	60±2.49	59.375±0.61	67.5±1.85	61.25±2.55
1	68.6375±3.98	68.0075±1.68	71.1775±2.67	64.8775±2.13
2	72.0475±2.52	68.905±3.15	77.735±2.51	75.8025±2.51
3	77.98±2.65	78.59±2.44	80.5525±3.11	72.3375±2.47
4	79.5425±3.51	83.92±1.99	84.0075±2.74	77.6425±1.95
5	89.2675±2.50	88.015±2.36	86.22±2.19	80.4575±2.06
6	97.775±4.12	98.4±2.09	92.8325±1.57	94.5775±2.10

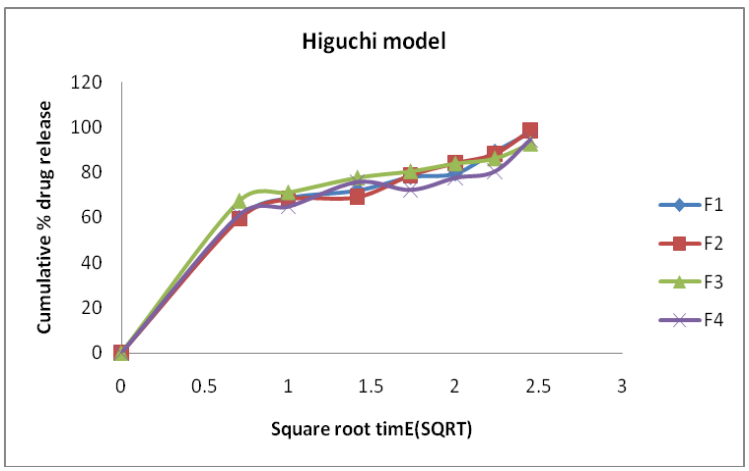

Fig. 5: Higuchi model of drug release for all formulations.

Table 4: Results of correlation coefficients and diffusion exponent for the drug release.

Formulation No.	Correlation Coefficients(R^2)			Model of best fit	Diffusion exponent (n)	Release mechanism
	Zero order	First order	Higuchi			
F1	0.625	0.834	0.837	Higuchi	0.727	Anomalous transport
F2	0.647	0.825	0.854	Higuchi	0.738	Anomalous transport
F3	0.508	0.823	0.752	Higuchi	0.685	Anomalous transport
F4	0.567	0.777	0.789	Higuchi	0.699	Anomalous transport

CONCLUSION

The designed formulations of Metronidazole combining an excellent buoyant ability and suitable drug release pattern could possibly be advantageous in terms of stomach targeting. We found that all of the formulations floated more than 24 hours with the minimum lag time. The longer residence time of dosage forms can maintain a higher metronidazole concentration in the gastric region where *H. pylori* exists, will allow more of the drug to penetrate through the gastric mucus layer to act on *H*. *pylori* and thereby improve the therapeutic efficacy of Metronidazole.

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CONFLICT OF INTERESTS

The authors show no conflict of interest.

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