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METRONIDAZOLE LOADED EUDRAGIT COATED ALGINATE BEADS FOR COLON TARGETING

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Abstract

The aim of present study was to investigate the possible application of Eudragit S®-100 coated alginate beads as a controlled release system of low molecular drugs with high solubility. Metronidazole has used as a model substance. The beads were prepared by the ionotropic gelation method and the effect of various factors (alginate, drug and calcium chloride concentrations) on bead properties was investigated. The addition of drug and alginate increased the drug loading capacity of the beads, thus larger beads were obtained. On the other hand, addition of calcium chloride retarded the drug release from beads. The erosion of the beads was suppressed by Eudragit coating. Coating of Eudragit on alginate beads exhibited no drug release at acidic pH, however continuous release of drug was observed from the formulation at colonic pH. It is concluded that Eudragit coated alginate beads may be used as a potential site specific release system of metronidazole.

Keywords: Alginate bead, Colon targeting, Eudragit, Metronidazole.

Introduction

Oral route is the most popular route of administration, and numbers of drugs have been administered effectively by this route. Problems such as acid- catalyzed degradation in the stomach, proteolytic breakdown in the gastrointestinal tract drug absorbed by upper GIT and first pass metabolism in the liver must be overcome for effective delivery of drugs to a specific site. Dosage form that delivers the drug into the colon rather than upper GIT has a numerous advantages. Oral delivery of drugs to the colon is advantageous in the treatment of disease of colon (Chron's disease, Ulcerative colitis), it also leads to higher drug concentration at a particular site and minimizes side effects due to release of drug in the upper GIT.[1,2]

Ideally, a targeted drug delivery system should release the drug in the right body compartment at the rate required for a specific treatment. Most available drug delivery systems use biodegradable, biocompatible, and natural biopolymers and are capable of rate and/or time controlled drug release. Considerable research efforts are being levied on oral sustained drug delivery systems which can distribute their drug load more uniformly in the

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gastrointestinal tract with the aim to reduce local irritation.[3-5]

Use of high molecular weight polysaccharides like alginate, pectin, guar gum, chitosan etc. have mostly been limited to pharmaceutical aids except for the colon targeting since last two decades. Various polysaccharides, viz., dextran, cyclodextrin, have been successfully utilized for the purpose of colonic drug delivery. Alginate has been investigated as a carrier material in different controlled release systems.[6,7] Alginate is a water soluble linear polysaccharide extracted from brown seaweed, composed of alternating blocks of 1-4 linked α-L-guluronic and β-D-mannuronic acid residue. The most important property of alginate is its ability to form complexes with divalent cations Ca^{2+}. In particular, alginate forms stable complexes with calcium ions that seems to assume the "egg box" model.[8] Alginate beads can be prepared by extruding the sodium alginate solution as droplet into a divalent cross linking solution such as Sr^{2+}, Ba^{2+} or Ca^{2+}.[9] Alginate has the following advantages: (a) Alginate is known to be nontoxic as taken orally. (b) It protects the mucosal membrane of the upper gastrointestinal tract from the irritation of the chemicals.[10] (c) Alginate can act as a controlled release-system due to its swelling property. (d) Alginate has the property of reswelling in susceptible to the environmental pH, incorporation of acid sensitive drugs into alginate beads protects from gastric juice.[11]

Alginate gel beads are used in many applications as matrices delivery system for controlled release or immobilization of drug. Calcium alginate gel beads, formed by calcium-induced inotropic gelation of alginate, have been extensively used for the oral delivery of a wide range of bioactive proteins and drugs.[12]

The beads could be utilised for colonic delivery of drug which do not reach colon or/and are absorbed from upper GIT, by enterically coating the alginate beads by specific enteric polymer viz. Eudragit. Hence disease like amoebiasis can be treated where drug concentration can be required in the colonic part where the parasite resides.[13]

Metronidazole was taken as the drug for the present study. Metronidazole is a broad spectrum antibacterial agent and is reported to be effective against oral, vaginal, topical and intracellular infection. Because of its rapid absorption through upper GIT it is probably less effective against parasite in the bowel lumen.[14]

The objective of this study was to explore the potential of calcium alginate and Eudragit-coated gel bead system as a device for efficient oral delivery of metronidazole. Two types of gel beads, calcium alginate and Eudragit-coated calcium alginate beads were prepared and characterized by examining and comparing their metronidazole entrapment efficiency, size distribution and *in vitro* release behaviors.

Materials and Methods

Materials

Alginate was procured from Himedia Laboratories Pvt.Ltd Mumbai. Metronidazole was generously supplied as a gift sample by M/s Broshell Remedies (Sagar, M.P., India) as a gift sample. All other chemicals used were of analytical reagent grade and were used as received.

Preparation of Calcium Alginate Gel Beads

The calcium alginate beads were prepared by method reported by Anal et al., (2005) with slight modification.[15] The drug-polymer solution in distilled water (9 ml) was dropped with a 10 ml syringe (5 gauge needle) in about 50 ml calcium chloride (2% w/v) solution with mild agitation and stirred slowly for 1 h to obtain the calcium alginate beads. Thereafter calcium alginate beads were collected, washed with distilled water and air-dried.

Coating of alginate beads

Eudragit coating of calcium alginate beads was performed by the method oil-in-oil solvent evaporation method reported by Simonoska et al., (2008) with slight modification.[16] Calcium alginate beads were dipped in 2% Eudragit coating solution prepared in acetone, propylene glycol solvent (20:1) and were stirred continuously at 100 rpm for 30 min, to get enteric coated beads.

Characterization of uncoated and coated alginate beads

Particle size, shape and surface morphology

Particle size analyzer (Cilas 1064L, Marcoussis, France) was used to determine the size of both uncoated and Eudragit coated calcium alginate beads. Average particle size was expressed as

volume mean diameter in mm (Table 1). The shape and surface morphology of beads (both uncoated and Eudragit coated) were studied using scanning electron microscopy. The sample was prepared by lightly sprinkling the beads on a double adhesive tape, which was stuck on aluminium stub. The stubs were then coated with gold to thickness of about 300 A ° using a sputter coater then viewed under scanning electron microscopy (Leo435 VP, Cambridge, UK) and shown in photomicrographs (Figs. 1 and 2).

Entrapment Efficiency

The drug entrapped in calcium alginate beads were determined by digesting them in 10 ml of 0.1 M phosphate-buffer saline (PBS, pH 7.4) for 12 h. The digested homogenate was centrifuged at 2000 rpm for 10 min, and the supernatant was filtered through 0.2 µm membrane filtered and injected into an HPLC (Schimadzu, LC-10, C-18 Column, Japan) column system. The detection wavelength was 318 nm and the mobile phase consist of acetonitrile-water (10:90) the flow rate was maintained at 1mL/min, and the analytical column used was reverse phase C-18 column (5µm, 250 mmX4.6 mm).[17]

***In vitro* Drug Release**

In vitro drug release studies of both uncoated and Eudragit coated calcium alginate beads were carried out according to Souder et al., (1985) using USP dissolution test apparatus type 2 (Paddle type) with minor modification.[18] The effect of drug release was studied in simulated gastrointestinal fluids of different pH in the following sequence, in order to mimic mouth-to-colon transit:

- 1st h: Simulated gastric fluid of pH 1.2.
- 2nd and 3rd h: Mixture of simulated gastric and intestinal fluid of pH 4.5.
- 4th and 5th h: Simulated intestinal fluid of pH 6.8.
- 6th h: Simulated intestinal fluid of pH 7.5.

Samples were withdrawn periodically and compensated with an equal amount of fresh dissolution media. The samples were analyzed for drug content by measuring absorbance at 318nm using HPLC analytical method.

Fractional release of metronidazole with respect to time was determined as follows:

$$\text{Fractional release (\%)} = \frac{M_o - M_t}{M_o} \times 100$$

Where, M_o is the amount of metronidazole initially entrapped in the beads and M_t is the amount of metronidazole remaining in the beads at a given time t.

Results and discussion

Eudragit coated calcium alginate beads were prepared with the aim of delivering metronidazole to colon. The matrices of polysaccharides are assumed to remain intact in the physiological environment of stomach and small intestine but once they reach in the colon, the Eudragit coating dissolves to release the drug. This multiparticulate system was based on and utilized pH-sensitive properties of polymer.

Preparation of Calcium alginate beads and its Eudragit coating

Calcium alginate beads of metronidazole were successfully prepared by gelation of alginic acid in the presence of calcium ion ($CaCl_2$) and their Eudragit coating were performed by oil-in-oil solvent evaporation method. Various formulation variables e.g. drug concentration, alginate concentration, and concentration of calcium chloride, which could affect the preparation and properties of beads were identified and studied. The formulation compositions of designed alginate beads are given in Table 1.

Particle Size and Entrapment Efficiency

Mean particle size of calcium alginate beads were determined by particle size analyzer (Cilas 1064L, Marcoussis, France)**.** Shape and surface morphology study was carried out using Scanning Electron Microscopy which showed smooth surface for Eudragit coated alginate beads in compared to uncoated beads. Mean particle size was found to increase on increasing the amount of the drug and alginate concentration. It is observed that as on increasing the concentration of drug from 10% to 40%, the size of calcium alginate beads is increased from 1.36±0.37 to 2.08±0.74 mm. The increase in entrapment of drug from 68.39±2.74% to 76.18±3.02% as on increasing the drug concentration from 10% to 40% could be due to availability of higher amount of drug for entrapment which also increases mean size of the alginate beads. A high concentration of polymer, produced beads of larger size, which varied from

1.21±0.57mm to 2.19±0.81mm for 1% to 4% alginate concentration respectively whereas drug entrapment efficiency increased from 68.24±2.85% to 77.85±3.17% respectively. The increase in entrapment efficiency of drug from 68.24±2.85% to 77.85±3.17% as on increasing the pectin concentration from 1% to 4% could be due to increase in size of beads but after 4% alginate concentration it remains unchanged which may be due to complete entrapment of available drug in the matrix of alginate beads.

Furthermore, on increasing concentration of calcium chloride (50 to 200 mmol/l) in the preparation of calcium alginate beads, the size of beads decreases from 2.36±0.32mm to 1.19±0.65 mm and percent drug entrapment efficiency increases from 67.38±1.78% to 78.62±2.77%. The effect can be attributed to the formation of more pronounced cross linking between polymer globules due to increase in calcium concentration which efficiently cross links the alginate beads due to rapid gelation of small polymer globules.

***In vitro* Drug Release**

In vitro drug release of calcium alginate beads were carried out in different pH of gastrointestinal fluids. The effect of drug concentration, alginate concentration and calcium ion concentration was observed on *in vitro* drug release. The amount of drug release was increased from 79.09±2.39% to 93.75±2.76% in 8h as drug concentration was increased from 10 to 40% respectively. But, the drug release was found to decrease from 94.36±2.48% to 78.37±2.48% in 8h as alginate concentration in beads was increased from 1 to 4% alginate concentration which could be due to the increase in beads size. Similar effect was observed for calcium alginate beads prepared by varying calcium ion concentration from increased from 50 to 200 mmol/l, the drug release was increased from 80.44±2.08% to 91.65±2.34% in 8 h which could be due to decrease in size of calcium alginate beads (Fig. 3). Calcium alginate beads which exhibited 50–60% *in vitro* drug release in 5 h were not selected for colon specific drug release.

In vitro release studies of Eudragit coated calcium alginate beads were performed in the similar fashion as that uncoated. Results clearly reveals that Eudragit coated beads show slow release, only 10-20 % drug release was observed in 5 h. 1-5% drug released was observed within 1 h of SGF (pH 1.2); only 10-20% drug was found to be release within 4 h of SGIF and SIF fluid (pH 4.5, 6.8). Sudden drug released was observed in colonic fluid and up to 80% of drug was found to be released within 3 h, this could be due to dissolution of the Eudragit coat at pH 6.8 and on exposure of the alginate beads to colonic fluid where alginate beads degrade due to colonic fluid and results higher percentage of drug release (Fig. 4).

Thus Eudragit coating of calcium alginate beads retard the release of metronidazole thereby prevents the release of drug in upper GIT until it reaches to desired site i.e. colon.

Thus highest release rate were observed with calcium alginate beads possibly due to the fact that they are not able to maintain their integrity in upper part of GIT and show maximum release rate within 5 h. While Eudragit coated calcium alginate beads maintain their integrity in upper GI tract and drug release was slow in comparison to uncoated calcium alginate beads. After an interval of 5 h when Eudragit coated calcium alginate beads reach ileo caecal region, the Eudragit coating dissolves and release of drug occur at colonic site. This similar fashion could be utilized *in-vivo* to treat amoebiasis.

Fig. No. 01: SEM photomicrograph of uncoated alginate beads.

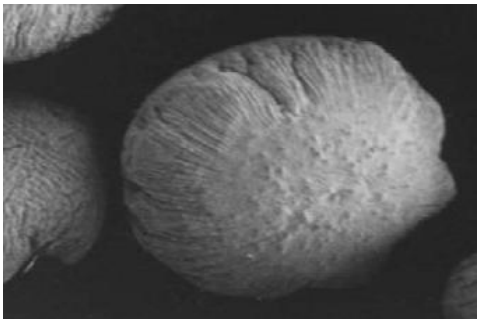

Fig. No. 02: SEM photomicrograph of Eudragit-coated alginate beads.

Table No. 01: Average particle size, entrapment efficiency and *in vitro* drug release from uncoated calcium alginate beads

Formulation Code	Variables	Values	Average bead size (mm)	Entrapment efficiency (%)	In vitro Drug Release after 8 hr (%)
ALD1	Concentration of Drug (%)	10%	1.36±0.37	68.39±2.74	79.09±2.39
ALD2		20%	1.55±0.52	72.56±2.91	83.56±2.75
ALD3		30%	1.87±0.63	75.77±2.79	87.86±2.25
ALD4		40%	2.08±0.74	76.18±3.02	93.75±2.76
ALP1	Concentration of Alginate (%)	1%	1.41±0.57	68.24±2.85	94.36±2.48
ALP2		2%	1.67±0.43	71.37±3.03	89.86±2.75
ALP3		3%	1.84±0.48	76.54±3.79	84.59±2.62
ALP4		4%	2.19±0.81	77.85±3.17	78.37±2.48
ALC1	Concentration of calcium chloride (mmol/ l)	50	2.36±0.32	67.38±1.78	80.44±2.08
ALC2		100	2.07±0.78	72.88±2.08	84.61±2.51
ALC3		150	1.75±0.53	76.92±2.73	87.53±2.83
ALC4		200	1.49±0.65	77.62±2.77	91.65±2.34

Each value is an average of three experiments (n = 3) ± SD.

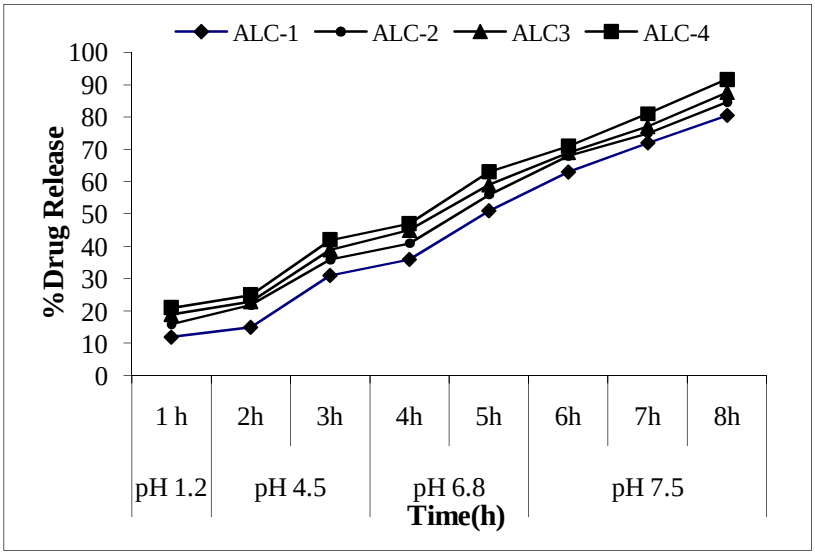

Fig. No. 03: *In vitro* release of metronidazole from uncoated calcium alginate beads containing different calcium ion concentration. (n=3, RSD≤5%)

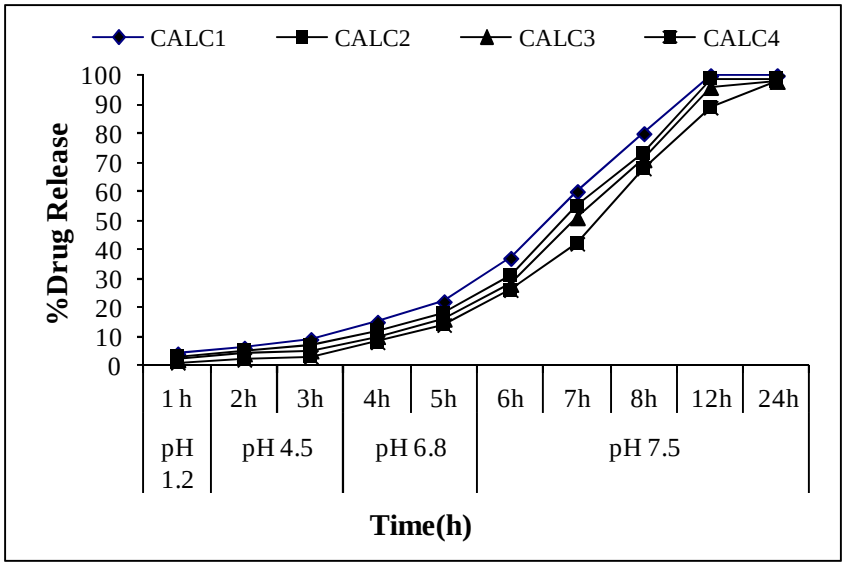

Fig. No. 04: *In vitro* release of metronidazole from Eudragit coated calcium alginate beads containing different calcium ion concentration. (n=3, RSD≤5%)

Conclusion

In the present study, ionotropic gelation method was utilized to produce metronidazole-loaded beads. Metronidazole-loaded Eudragit coated calcium alginate beads in particular demonstrated a satisfactory side specific delivery, suggesting that alginate is an effective natural polymer to control drug release from beads. Therefore, it can be concluded that Eudragit coated calcium alginate beads can be utilized and bear potential for the site specific delivery of the drug to the colon.

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