

Characterization of calcium alginate beads of 5-fluorouracil for colon delivery

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A multiparticulate system combining pH-sensitive property and specific biodegradability for colon targeted delivery of 5-fluorouracil (5-FU) was examined. The purpose of this study was to prepare and evaluate the colon-specific alginate beads of 5-FU for the treatment of colon cancer. Calcium alginate beads were prepared by extruding 5-FU loaded alginate solution to calcium chloride solution, and gelled spheres were formed instantaneously by ionotropic gelation reaction using different ratios of FU and alginate, alginate and calcium chloride, stirring speeds (500-1500 rpm), and reaction time. The core beads were coated with Eudragit S-100 to prevent drug release in the stomach and provide controlled dissolution of enteric coat in the small intestine and maximum drug release in the colon. Morphology and surface characteristics of the formulation were determined by scanning electron microscopy. *In vitro* drug release studies were performed in conditions simulating stomach to colon transit. No significant release was observed at acidic pH, however, when it reached the pH where Eudragit S-100 starts to dissolve, drug release was observed. Also, release of drug was found to be higher in presence of rat caecal content.

Key words: 5-FU, alginate, beads, colon-specific, Eudragit S-100, scanning electron microscopy

INTRODUCTION

The colon drug delivery has a number of important implications in the field of pharmacotherapy. Colon targeted drug delivery has the potential to deliver bioactive agents for the treatment of a variety of colonic diseases including inflammatory bowel disease (IBD) can be effectively treated by the local delivery of drugs to the large intestine. The treatment of colonic disease such as amoebas, Crohn's diseases, ulcerative colitis, and colorectal cancer is particularly improved by their local delivery to the bowel. By this technique, absorption of the drug from the stomach and small intestine can be minimized until the drug reaches the large intestine. Various drug delivery systems have been designed that delivers the drugs quantitatively to the large bowel and subsequently to trigger the release of active drug. The treatment of large intestine disorders, such as Crohn's disease, irritable bowel syndrome, colitis, colorectal cancer, and local infectious disease, where high concentration of drug is needed, that can be improved by colon specific drug delivery systems employing various mechanism of release. The site-specific delivery of the

drugs to the target receptor sites has the potential to reduce the side effects and improve the pharmacological response. However, for successful colonic drug delivery, many physiological barriers must be overcome, the major one being absorption or degradation of the active drug in the upper part of the gastrointestinal tract (GI) tract. For example, colon specific drug delivery systems protect peptide drugs from hydrolysis and enzymatic degradation in the duodenum and jejunum, and eventually release drugs in the ileum or colon, which leads to greater systemic bioavailability. The specific release in the colon also affects a time delay between administration and onset of action, which can be useful for diseases, such as asthma and arthritis. Various colon specific drug delivery systems are being developed, by taking advantage of the luminal pH in the ileum and microbial enzymes in the colon. Various strategies, currently available to target the release of drugs to colon, include formation of prodrug, coating of pH sensitive polymers, use of colon-specific biodegradable polymers, timed released systems, osmotic systems, and pressure controlled drug delivery systems. Among the different approaches to achieve targeted drug release to the colon, the use of polymers especially biodegradable by colonic bacteria holds great promise. Polysaccharidases are bacterial enzymes that are available in sufficient quantity to be exploited in colon targeting of drugs. Based on this approach, various polysaccharides have been investigated for colon specific

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drug release. These polysaccharides include pectin, alginate, guar gum, amylose, inulin, dextran, chitosan, and chondroitin sulphate. This family of natural polymers has an appeal to drug delivery, as it is comprised of polymers with a large number of derivatizable groups, a wide range of molecular weights, varying chemical compositions, and, for the most part, low toxicity and biodegradability yet high stability. The most favorable property of these materials is their approval as pharmaceutical excipients. Cancer is a term that is used to describe a wide variety of malignant diseases, the management of which requires several medical disciplines.^[1] Although relatively rare in the underdeveloped world, colon cancer is the second most common internal malignancy and the second leading cause of cancer deaths in western countries. Despite extensive experiments, investigation, and numerous theoretical considerations, the overall success in routine cancer therapy has been nominal. The main reason being the non-selectivity of the chemotherapeutic agents, which invariably leads to dose dependent systemic toxicities often warranting discontinuation of treatment.^[2] The development of techniques, which could selectively deliver drug molecules to the disease vasculature, is one of the most exhaustively pursued areas of research in experimental pharmacology and therapeutics. Hence, the present day cancer chemotherapy aims at selective toxicity that is, toxicity to neoplastic cells without adversely affecting to host cells. This is achieved by drug targeting or site-specific drug delivery, which involves preferential delivery of the drug to the desired site of action.^[3] Rationale for the calcium alginate beads is, it protects drug in the upper part of the GI tract due to its insolubility and is not degraded by gastric and intestinal enzymes. In comparison to single unit system, by reducing size of the delivery carrier (multiunit system) has marked advantages as it spreads over a large area and avoid the exposure of high concentration of drug to the mucosa and also will lead to longer residence in the colon.^[4,5]

MATERIALS AND METHODS

Materials

Materials used included 5-fluorouracil was kindly provided as a gift sample by Emerck (Bombay India) Limited. Sodium

alginate was purchased from Loba chemicals, Mumbai. Calcium chloride, hydrochloric acid, disodium hydrogen phosphate, potassium dihydrogen phosphate, methanol, dichloromethane was purchased from S.D. Chemicals, Boiser. Eudragit S-100 was provided as a gift sample from Alembic Limited, Vadodara and pepsin 1:10,000 was purchased from Loba Chemie, Bombay.

Methods

Preparation of core calcium alginate beads

Different formulations of calcium alginate beads were prepared as shown in Table 1, the two different solutions were prepared separately. First, amount of sodium alginate was dissolved in the distilled water with continuous stirring. The aqueous solution of calcium chloride was made simultaneously, it was prepared by the saturated solution of sodium chloride. Then drug (5-fluorouracil, having water-soluble properties) was dissolved in the previously prepared solution of sodium alginate. This polymeric solution containing the drug (5-fluorouracil) was added to the solution of calcium chloride, drop by drop using 26G1/2 syringe having needle of 0.45 mm inner diameter during agitation as shown in Figure 1. So the gelatinous precipitate is formed by chemical reaction between sodium alginate and calcium chloride. The prepared beads were left under stirring in the medium at 1000 rpm for 20 min and then removed by filtration and washed with distilled water and vacuum dried.^[6,7]

Coating of core calcium alginate spheroids

Optimized alginate beads were coated with Eudragit S-100 using solvent evaporation method.^[8] Beads were dispersed in

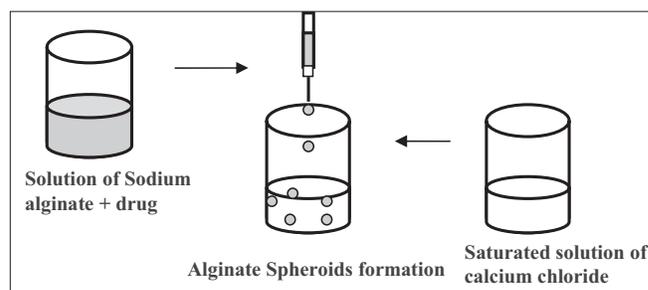


Figure 1: Schematic illustration of formation of alginate beads

Table 1: Various formulations of alginate beads

Formulation code	Polymer to calcium chloride ratio (wt/wt)	Drug to polymer ratio (wt/wt)	% entrapment efficiency	% release after 10 h
AL1	1: 1.0	1: 7	25.82 ± 2.1	92.55 ± 1.0
AL2	1: 1.5	1: 7	35.53 ± 1.4	90.93 ± 1.5
AL3	1: 2.0	1: 7	38.02 ± 1.8	88.66 ± 1.2
AL4	1: 2.5	1: 7	34.30 ± 2.3	94.46 ± 2.2
AL5	1.5: 1	1: 7	64.34 ± 0.9	80.64 ± 1.4
AL6	2.0: 1	1: 7	Less syringibility, big in size, drop shaped	
AL7	1.5: 1	1: 7	64.34 ± 1.0	90.44 ± 2.1
AL8	1.5: 1	1: 5	67.23 ± 1.3	79.57 ± 1.1
AL9	1.5: 1	1: 3	54.07 ± 1.5	88.55 ± 1.7
AL10	1.5: 1	1: 1	23.98 ± 1.2	90.62 ± 2.5

different strength of Eudragit S-100 in dichloromethane and methanol (4:1) ratio to obtain as 10% (E1), 13% (E2), and 15% (E3), weight gain. And the solvent was evaporated in a rotary evaporator by applying vacuum 300 mmHg and rotation rate was 50 rpm, then vacuum dried in desiccators. All the three batches were evaluated on the basis of release study.

Incorporation efficiency

Amount of drug-loaded alginate beads were dispersed in phosphate buffer pH 6.8 at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ and kept for digestion with continuous stirring up to 24 h. Then the sample was centrifuged at 1000 rpm for 10 min to remove any insoluble solids, the supernatant layer was removed and filtered. The drug content was determined using UV – visible spectrophotometry method. Incorporation efficiency was calculated using the following formula:

$$\text{Incorporation Efficiency} = \frac{b \times 100}{a}$$

where *a* is the theoretical drug content and *b* is the drug entrapped.

Surface morphology^[9]

The shape and surface characteristics of the beads were observed by scanning electron microscopy (SEM) (Philips, Eindhoven, Netharland). The samples were imaged using a 15-kV electron beam. The SEM study of core and coated alginate beads was performed.

In vitro drug release study from core alginate beads

Uncoated alginate beads were evaluated for the *in vitro* drug release in pH progression medium at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. Beads were weighed accurately and in 250 ml of dissolution medium. The content was rotated at 100 rpm at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. Perfect sink conditions prevailed during the drug dissolution study period. The simulation of GI transit condition was achieved by altering the pH of dissolution medium at different time intervals. The pH of the dissolution medium was kept 1.2 for 2 h using Simulated Gastric fluid (SGF). Then in Simulated Intestinal fluid (SIF) (KH_2PO_4 and $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$) were added to the dissolution medium, adjusting the pH to 5.0 with 1.0 N NaOH), and the release rate study was continued for an additional 2 h. Then, it was transferred in to the dissolution medium of PBS 7.4 (Phosphate Buffer Solutions) and maintained up to 24 h.^[10,11] The samples were withdrawn from the dissolution medium at various time intervals. The rate of 5-FU release was analyzed using UV – visible spectrophotometry method in 2nd order derivative spectrophotometry at λ_{max} 268.0 nm.

In vitro drug release study from coated alginate beads

Coated alginate beads (E1, E2, and E3), were performed for *in vitro* drug release study by the method similar to that of core beads, it was performed in pH progression medium at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. All dissolution studies were performed in

triplicate.

Preparation of rat cecal content (2%)^[12]

Rat cecal content was prepared by the method reported by Van den Mooter *et al.* Two wistar rats of uniform body weight (150-200 g) with no prior drug treatment, were weighed, maintained on normal diet, and administered 1 ml of 4% dispersion of alginate/ES in water, and this treatment was continued for 7 days for polymer induction to animals. Thirty minutes before starting the study, each rat was humanely killed and the abdomen was opened. The cecum were traced, dissected. The cecal bag was opened; the contents were weighed, homogenized, and then suspended in PBS (pH 7.4) to give the desired concentration (2%) of cecal content, which was used as simulated colonic fluid. The suspension was filtered and the mixture was centrifuged (Remi) at 1000 rpm for 20 min.

Stability study

Stability study was performed at 2-8°C and at ambient temperature for three months, and also at dark as well as light conditions.

RESULTS AND DISCUSSION

The effect of speed of stirring and reaction time were optimized on the basis of quality of beads and entrapment of the beads. It was evaluated by determining quality of beads at and entrapment efficiency at different speed and varying reaction time, it observes that at 1000 rpm for 20 min, proper shape and uniformity with optimized entrapment efficiency was formed.

Entrapment efficiency

Various batches were tried with different drug to polymer ratio and different polymer to CaCl_2 ratio. Prepared batches were optimized using 2nd derivative spectroscopy method for the estimation of drug (5-fluorouracil). As drug to polymer ratio and calcium chloride to polymer were increased, entrapment efficiency was also increased. Optimized ratio drug to polymer ratio and polymer to calcium chloride was 1: 5 and 1.5:1, the entrapment efficiency was 67.23%.

Surface morphology

By SEM study, the size of optimized alginate beads was 500 - 1000 μ . This shows that the Eudragit coated beads have smooth surface compared to core beads as shown in Figure 2.

***In vitro* drug release**

In vitro drug release of coated and uncoated beads was performed in pH progression medium at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$, in SGF, SIF, and in presence and in absence of colonic content. As compare to core alginate beads, coated beads shows about 5.13 % drug release after 4 h and rest of the drug releases up to 30 h as shown in Figures 3 and 4. So it protects the release

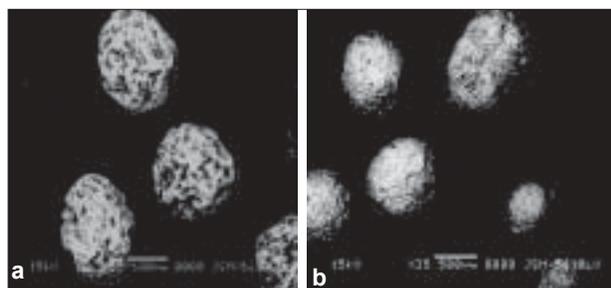


Figure 2: SEM study of, (a) uncoated alginate spheroids and (b) coated alginate spheroids

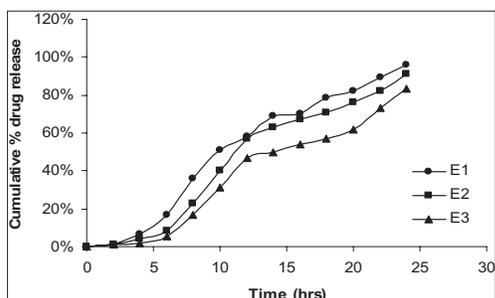


Figure 4: *In vitro* drug release study of coated alginate beads

of drug from the upper part of GI tract and minimizes the side effects. Above pH 7.0, Eudragit coating started to dissolve and exposed the alginate beads for drug release. Among these three trials, batch E3 showed good results in *in vitro* drug release study, almost only 2% drugs was released in 4 h. Therefore, we can conclude that if the Eudragit coated beads protect the drug from stomach and small intestine and start drug release upon arrival to colon and gives local action. It may provide site-specific release and reduce systemic side effects. It was further evaluated for drug release kinetics, data obtained from *in vitro* drug release studies were plotted in various kinetic models. Such as, First Order, Higuchi law, Korsmeyer-Peppas law, and Hixon-Crowell. All having R2 values greater than 0.9. In Korsmeyer-Peppas equation, the exponent $n=0.620$, indicating that the release mechanism is anomalous diffusion.

Stability study

Stability study was performed at different temperature and also at different conditions. But it showed that there is no significant reduction in the percentage drug retained in the formulation and also there was no significant difference in drug release profile for the sample storage at 2-8°C and at ambient temperature. Also, no significant difference was observed in sample stored in dark and light condition, but as 5-FU is a light sensitive drug, so it was suggested that, formulation should be protected from light.

CONCLUSION

The results of our study clearly indicate that there is great potential in designing site-specific delivery of 5-fluorouracil

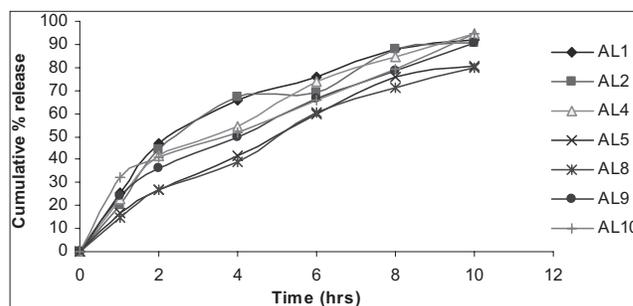


Figure 3: *In vitro* drug release study of core alginate beads

(5-FU) which may reduce the side effects of the drug caused by its absorption from the upper part of the GI tract when given in conventional dosage forms such as tablets, capsules and injections. In this study, the capability of alginate for the preparation of colon specific delivery was evaluated by investigation of different parameters on preparation and *in vitro* drug release from the alginate beads. The study provides an alternative for 5-FU delivery to the colonic region. Further pharmacokinetic studies are needed before establishing delivery of 5-FU as an alternative for colon cancer. Biocompatibility studies of the formulation additives must also be done. Sodium alginate is a biocompatible polymer and is expected to cause no harmful effects even if used for prolonged periods.

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