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RESEARCH PAPER

Gastroretentive Delivery Systems: Hollow Beads

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ABSTRACT

The objective of this study was to develop a floatable multiparticulate system with potential for intragastric sustained drug delivery. Cross-linked beads were made by using calcium and low methoxylated pectin (LMP), which is an anionic polysaccharide, and calcium, LMP, and sodium alginate. Beads were dried separately in an air convection type oven at 40°C for 6 hours and in a freeze dryer to evaluate the changes in bead characteristics due to process variability. Riboflavin (B-2), tetracycline (TCN), and Methotrexate (MTX) were used as model drugs for encapsulation. Ionic and nonionic excipients were added to study their effects on the release profiles of the beads. The presence of noncross linking agents in low amounts (less than 2%) did not significantly interfere with release kinetics. For an amphoteric drug like TCN, which has pH dependent solubility, three different pHs (1.5, 5.0, and 8.0) of cross-linking media were used to evaluate the effects of pH on the drug entrapment capacity of the beads. As anticipated, highest entrapment was possible when cross-linking media pH coincided with least drug solubility. Evaluation of the drying process demonstrated that the freeze-dried beads remained buoyant over 12 hours in United States Pharmacopeia (USP) hydrochloride buffer at pH 1.5, whereas the air-dried beads remained submerged throughout the release study. Confocal laser microscopy revealed the presence of air-filled hollow spaces inside the freeze dried beads, which was responsible for the flotation property of the beads. However, the release kinetics from freeze dried beads was independent of hydrodynamic conditions. Calcium-pectinate-alginate beads released their contents at much faster rates than did calcium-pectinate beads (100% in 10 hours vs. 50% in 10 hours). It appears that the nature of cross-linking, drying method, drug solubility, and production approach are all important and provide the opportunity and potential for development of a gastroretentive drug delivery system.

Key Words: Gastroretentive beads; Calcium-pectinate; Calcium-alginate-pectinate; Cross-linked beads; Proximal GI drug delivery.

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INTRODUCTION

Prolonged gastric retention of drug delivery systems in certain situations may be desirable to improve the bioavailability and the therapeutic efficacy of the drugs. Drugs that are absorbed in the proximal part of the gastrointestinal (GI) tract,[1] and drugs that are less soluble in or are degraded by the alkaline pH may benefit from prolonged gastric retention.[2,3] In addition, for local and sustained drug delivery to the stomach and proximal parts of the small intestine to treat certain conditions, prolonged gastric retention of the therapeutic moiety may offer numerous advantages, including improved bioavailability and therapeutic efficacy, and possible reduction of dose size.[4–6] It has been suggested that prolonged local availability of antibacterial agents may augment their effectiveness in treating *H. pylori* infection in peptic ulcer disease.[7] Moreover, it has been reported that the bactericidal effects of clarithromycin, garcinol, and reveratrol are time and concentration dependent.[8] Menon et al.[9] have compared the absolute bioavailability in dogs of furosemide in commercial products and a floating dosage form. The authors demonstrated higher bioavailability in floating dosage form than in nonfloating commercial products, which is attributed to the fact that the upper gastrointestinal tract is most probably the primary site of absorption for furosemide.

Various techniques and approaches including floating, bioadhesive, swelling, and high-density systems have been employed to prolong gastric residence of the delivery systems.[10–15] Due to unpredictable gastric emptying associated with migrating myoelectric complex (MMC) motility pattern, the above mentioned dosage forms have a high probability of leaving the stomach without delivering their contents. The objectives of this study were to develop a multiple-unit, oral floating dosage system to achieve an extended gastric residence time with potential for intragastric delivery of drug(s), and to investigate the in vitro behavior of the system compared to a nonfloating dosage form manufactured in the same manner from identical materials. The multiple-unit systems may be more advantageous than single-unit systems by avoiding ''all or none'' emptying from the stomach during MMC activity. With that in mind, in this work, a hollow bead system containing riboflavin and methotrexate as model drugs (both of which are primarily absorbed by active transporters located in the proximal part of the gastrointestinal tract)[16,17] was developed that remained buoyant in United States Pharmacopeia (USP) hydrochloride buffer media for over 12 hours.

MATERIALS AND METHODS

Low methoxylated pectin (LMP) with a degree of methoxylation of approximately 35% was provided by CP Kelco (Wilmington, DE). A medium viscosity sodium alginate (200 cP for 1% aqueous solution at 20°C) was obtained from TIC Gum (Belcamp, MD). Riboflavin HCl and tetracycline HCl were obtained from Sigma (St. Louis, MO). Methotrexate and Methotrexate tablets (Roxan, OH, lot# 158141B, Exp. 05/2003) were procured through Temple University Health Systems. All other reagents were of analytical grade and were used without further purification.

Preparation of Drug Encapsulated Calcium-Pectinate and Calcium-Pectinate-Alginate Beads

As part of ongoing research, in this present work 3% (w/v) pectin (LMP) solution was prepared in 100 mL deionized water using a magnetic stirring process. Before cross-linking with calcium chloride, 2% (w/v) riboflavin (B-2) and either a soluble (ionic/nonionic) or insoluble excipient were added in increments to a pectin solution for incorporation in the beads (Table 1). The additives, selected based on various ionization potentials and solubilities, were added to study their effects on encapsulation efficiency and release kinetics of the beads. The dispersions were then subjected to sonication (Solid-state Ultrasonic FS-9, Fisher Scientific, Pittsburgh, PA) for 30 minutes to remove any air bubbles that might have formed and trapped during stirring process. The dispersions were added dropwise via a 16-gauge needle into 500 mL of gently agitated 1 M calcium chloride aqueous solution by employing an eight channel peristaltic pump (Masterflex L/S Economy Drive, Cole Parmer, Vernon Hills, IL) at a rate of about 2 mL/min. The droplets instantaneously gelled into discrete calcium-pectinate spherical beads upon contact with calcium chloride solution. The beads were allowed to cure in a refrigerator at about 5°C for 24 hours. Thereafter, the beads were removed and washed with 3×500 mL volumes of deionized water. Half of the beads were dried in an air convection type oven at 40°C for 6 hours and the other half were dried in a freeze dryer (Labconco Stoppering Tray Dryer, Labconco Inc., Kansas City, MO) for 72 hours.

Table 1. Various formulations and their properties.

Pectin (% w/v)	Alginate (% w/v)	Drug (% w/v)	PH[b]	Additives (% w/v)	EC (%)[c]	$T_{50\%}$[d]
3	–	B-2 (2)[a]	6.1	–	55.5	8
3	–	B-2 (2)	6.1	–	55.5	10
3	–	B-2 (2)	6.1	Eudragit (1.5)	52.5	11
3	–	B-2 (2)	6.1	SLS (0.5)	57.5	7
3	–	B-2 (2)	6.1	NaCl (1.5)	52.5	9
3	–	B-2 (2)	6.1	Maltodextrin (1.5)	52.5	9
1.5	1.5	B-2 (2)	6.1	–	52.5	2
1.5	1.5	MTX (0.6)	6.1	–	48	1
1.5	1.5	TCN (2)	5.0	–	36	1
1.5	1.5	TCN (2)	1.5	–	22	1
1.5	1.5	TCN (2)	8.0	–	17.5	1

[a]Air-dried beads.
[b]pH of cross-linking media.
[c]EC = Entrapment capacity.
[d]$T_{50\%}$ = Time (hour) required to release 50% of the drug in HCL buffer at pH 1.5.

In preparing calcium-pectinate-alginate beads, separate solutions comprising 1.5% (w/v) pectin and 1.5% (w/v) of sodium alginate were prepared. For riboflavin inclusion, 2% (w/v) of the drug was added to the solutions, and beads were made as explained above. For methotrexate (MTX), 0.6% (w/v) drug was added to the polymer solutions and cross-linking was carried out following identical procedures except that the processes were done under minimum light. To study the influence of drug solubility on entrapment capacity, 2% (w/v) tetracycline (TCN) was added to the polymer solutions, and cross-linking was carried out in calcium chloride solution at different pH values adjusted to 1.5, 5.0, and 8.0.

Determination of Drug Entrapment Capacity

One hundred mg of each formulation was crushed in a glass mortar and pestle. Five hundred mL of hydrochloride buffer (pH 1.5) was added slowly and mixed well, which afforded liberation and dissolution of the drugs from the matrix. The solutions were vacuum filtered using a Millex 0.45 μm membrane filter. The filtrates were then made up to 900-mL volumes with hydrochloride buffer, pH 1.5. Aliquots of the solutions were subjected to ultraviolet (UV) spectroscopy in triplicate (HP 8453 diode array spectrophotometer, HP, Wilmington, DE) at 267 nm for riboflavin beads, at 271 nm for tetracycline beads, and at 307 nm for methotrexate beads. The entrapment capacity was determined by using the following relationship:[18]

$$\text{Drug entrapment capacity } (\%) = (\text{AQ}/\text{TQ}) \times 100$$

where AQ is the actual quantity of the drug present in the matrices and TQ is the 100% theoretical quantity of drug, which is supposed to be present in the matrices (loading quantity).

Internal Structures of the Beads

The images of the internal structures of the beads were captured by using a confocal laser scanning microscope (CLSM). For this purpose, an Olympus microscope equipped with Fluoview 2.1 for CLSM image processing (Olympus America, Inc., Melville, NY) was used. The samples were scanned with two interchangeable incident wavelengths (488 nm/568 nm) using argon-krypton laser light. The confocal fluorescence pictures were taken with a 40× objective lens, and the sequential images were stored as a 512 × 512 pixel box with 8-bit resolution. Riboflavin itself served the purpose of the marker.

In Vitro Release Studies

The release characteristics were evaluated in USP apparatus-I as well as in apparatus-II, and in a modified USP apparatus-II using USP hydrochloride buffer at pH

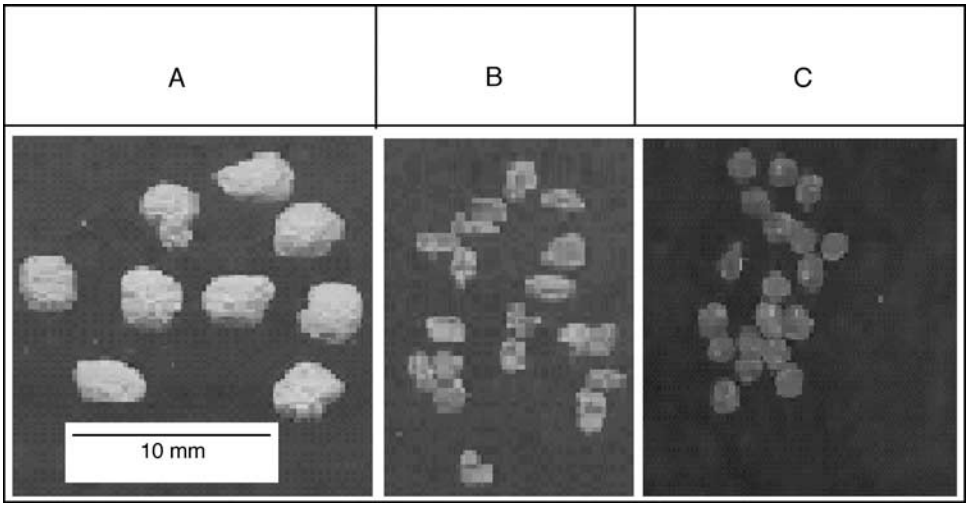

Figure 1. Appearances of freeze-dried and air dried beads. (A) Calcium-pectinate freeze-dried beads, (B) calcium-pectinate air-dried beads, and (C) calcium-alginate-pectinate air-dried beads. (*View this art in color at www.dekker.com.*)

1.5 (VK 7000, Vankel Industries, Cary, NC). The modified apparatus-II involved an added ring mesh device, which was used to prevent the dosage form from floating on the surface of the dissolution media. One hundred-mg beads were employed in each dissolution study. All studies were conducted in triplicate using an automated sampling procedure. Dissolutions of methotrexate tablets and beads were carried out in the absence of direct light.

Comparisons between dissolution profiles were made using f_2, similarity factor, as follows:[19]

$$f_2 = 50 \log \times \left[\left\{ 1 + \frac{1}{n} \sum_{t=1}^{n} W_t (R_t - T_t)^2 \right\}^{-0.5} \times 100 \right]$$

RESULTS AND DISCUSSIONS

The average size of the calcium-pectinate freeze-dried spherical beads was 2.7 (±0.1) mm, while the oven-dried beads had collapsed into nonuniform particulate shapes, and the calcium-alginate-pectinate oven-dried beads were spherical with an average diameter of 1.2 (± 0.1) mm (Fig. 1). Low methoxylated pectin (degree of methoxylation was about 35%) was used in this study, because high methoxylated pectin (degree of methoxylation was about 70%) disfavored interlink chain binding and gelation.

The drug entrapment capacity of the beads varied widely. The highest entrapment with riboflavin in calcium-pectinate beads was found to be 57.5% in the presence of 0.5% sodium lauryl sulfate (SLS), but without any additive it was about 55.5%. However, in the presence of SLS the beads exhibited tailing, which appears to be due to the reduction in surface tension of the drug-polymer solution mixture. The presence of other foreign materials, either insoluble or ionic or nonionic species up to the level of 1.5% (w/v), in the drug-polymer mix did not significantly affect the drug entrapment capacity or morphological character of the beads.

Drug entrapment is a function of various factors, including the physico-chemical properties of drug molecules, the amount of cross-linking agents, and the cross-linking environment. Basically, the drug and other foreign molecules remain entrapped within the spaces created by the cross-linking of the agents, as schematically presented in Fig. 2. The effect of drug solubility on entrapment capacity was observed from the tetracycline

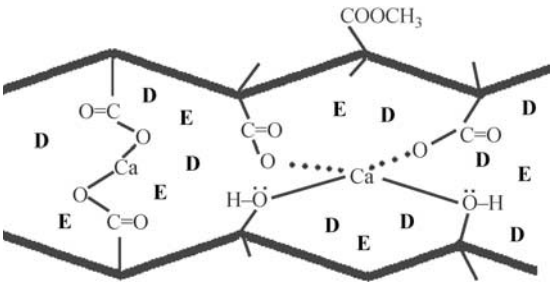

Figure 2. Schematic diagram of cross-linking of calcium and pectin. D is the drug molecule and E is the excipient. (*View this art in color at www.dekker.com.*)

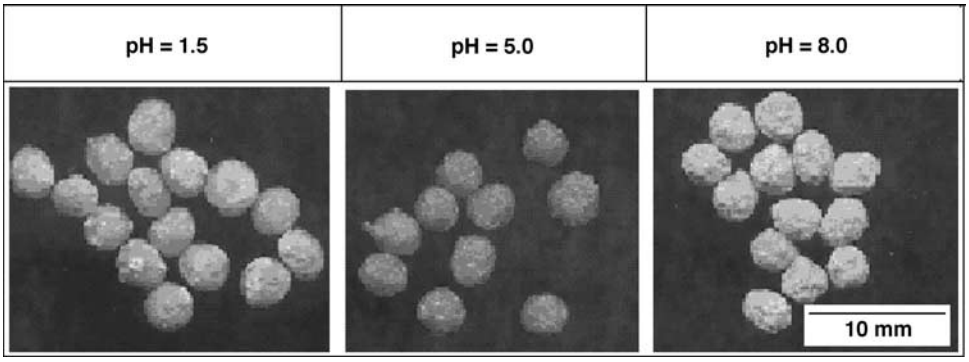

Figure 3. Appearances of freeze-dried calcium-alginate-pectinate beads of tetracycline made under different pH conditions. (*View this art in color at www.dekker.com.*)

beads made of calcium-alginate-pectinate. Tetracycline being an amphoteric drug, displaying both basic and acidic characteristics, is least soluble around pH 5 and highly soluble in low and high pHs (i.e., pH<2 and >8). This property influenced its entrapment efficacy in the beads. Hence, the intensity of the color of the beads made at pH 5.0 was higher than that of the other (Fig. 3). At pH 5.0, the highest entrapment (36%) observed was due to unfavorable solubility conditions, where the drug particles did not escape or leached out from the beads during the curing period.

The average cumulative release from riboflavin containing calcium-pectinate freeze-dried and air-dried beads approached 54% and 58% of the initial drug load, respectively, in USP apparatus-I in 12 hours. The air-dried beads exhibited almost twice the burst release of the freeze-dried beads (16.1% vs. 8.5% in the first half hour). This might be due to the migration of the drug molecules to the periphery along with water molecules during the air-drying and evaporation processes. The freeze-drying process, on the other hand, involves sublimation of water, which does not cause migration of drug molecules. The same freeze-dried beads released about 47% and 56% of their contents, respectively, when USP apparatus-II and modified USP apparatus-II were used for dissolution studies. However, the similarity factors, $f2$, among the profiles were greater than 50 (Using apparatus-I with freeze-dried beads as the reference for comparison. See Fig. 4). This signifies that the delivery system behaves almost independent of hydrodynamic conditions. In addition, the drug release was predominantly controlled by diffusion mechanisms. Thus, the square root of

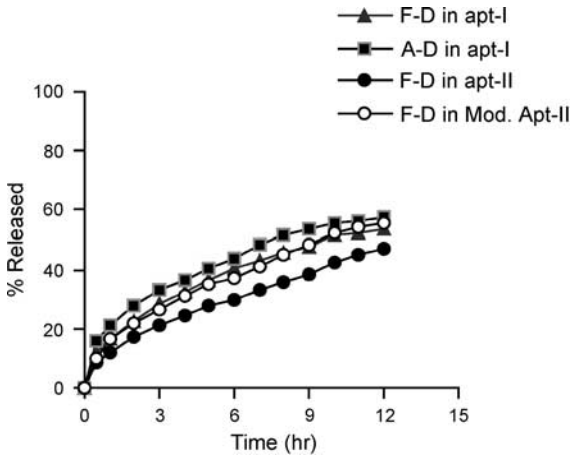

Figure 4. Dissolution profiles of calcium-pectinate air-dried (A-D) and freeze-dried (F-D) beads in HCl buffer at pH 1.5. (A) F-D in USP apparatus-I, (B) A-D in USP apparatus-I, (C) F-D in USP apparatus-II, and (D) F-D in modified USP apparatus–II. (*View this art in color at www.dekker.com.*)

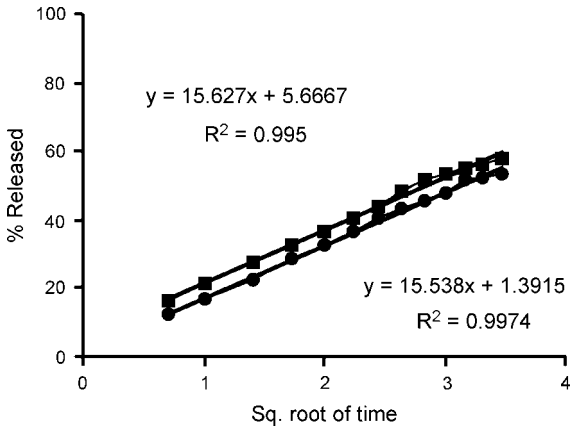

Figure 5. Kinetics of drug release from air-dried (left) and freeze-dried (right) beads.

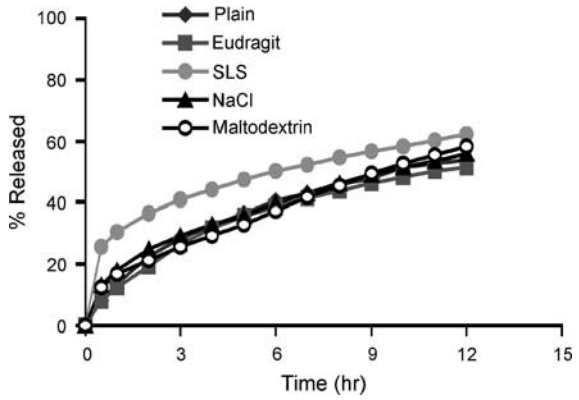

Figure 6. Release profiles of freeze-dried calcium-pectinate beads of B-2 containing added excipients. (*View this art in color at www.dekker.com.*)

release kinetics was observed,[20] as was expected from this kind of porous matrix (Fig. 5).

With added excipients, a small variation in cumulative % drug released in 12 hours was observed. In the presence of SLS, the extent of drug release was about 63% with a burst effect of almost five times higher than that of the plain beads. In contrast, the presence of an insoluble or soluble material within the stated level did not significantly change the extent of drug release for up to 12 hours (Fig. 6). Since the beads were hollow, the aqueous media could circulate inside the beads, and the drug release was controlled by the diffusion of drug through water-filled channels inside the beads.[19] Nevertheless, the rate of hydration of the matrix of the beads was primarily governed by the degree of cross-linking between anionic charged pectin sites and the divalent cationic calcium charges. Therefore, the presence of small amounts of foreign materials inside the beads did not seem to affect the hydration nor did it affect the release kinetics significantly.

The flotation aspects of freeze-dried and air-dried beads in the dissolution vessels differed significantly. Freeze-dried beads were found afloat immediately after placing them into the media and remained afloat until the end of the 12-hour dissolution run. On the contrary, the air-dried beads sank immediately after immersion into the media. During the freeze-drying process, water is removed by sublimation from the ice crystals inside the beads. Thus, upon drying, empty spaces filled with air inside the beads are created. From confocal laser microscopy work, the presence of these air pockets inside the beads and the distinct outer shell were discernable (Figs. 7 and 8).

Theoretically, for local drug delivery in the stomach from a gastroretentive system, it is desirable to have a burst followed by a slower and more sustained release.[21] In this case, the calcium-pectinate beads made of 3% w/v low methoxylated pectin and 2% w/v drug did not result in desirable release kinetics and bead formation. Reduction in concentration of pectin to 1.5% w/v also resulted in nonuniform beads. To achieve more uniform beads, another cross-linking agent, namely sodium alginate, was added to the mixture. The polymers, pectin and sodium alginate (1.5% w/v level of each), along with 2% w/v drug yielded well formed beads with high entrapment capacity. The release profiles of beads made with a mixture of two polymers were different from the one made with pectin alone. Within the first half hour of dissolution, the beads made with the binary mixture of polymers released about 25% of their initial contents; and in 10 hours almost 100% was released (Fig. 9). The freeze-dried beads, due to their hollow internal structure, allowed easy diffusion of the drug from the matrix structure, as the relaxation of the matrix was not a prerequisite for entry of the media into the beads with this particular formulation.

To evaluate the applicability of calcium-alginate-pectinate beads to other drugs, we used methotrexate (MTX) as another model drug and compared its in vitro release profiles with that of the commercially available methotrexate tablets. Methotrexate is an antineoplastic drug absorbed by active transport mechanism, and has low oral dose. In this case, beads were made using only 0.6% w/v drug in the 3% w/v polymer mix. It has been reported that at low doses MTX (< 30 mg/m^2) is well absorbed from the upper gastrointestinal tract, but at higher doses, lower relative

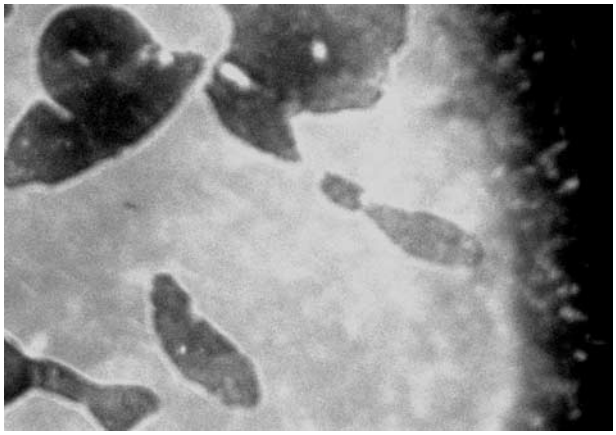

Figure 7. Confocal laser microscopy image of freeze-dried beads (Resolution X 40). The dark areas represent the air-filled spaces. The drug (B-2) itself was the marker. (*View this art in color at www.dekker.com.*)

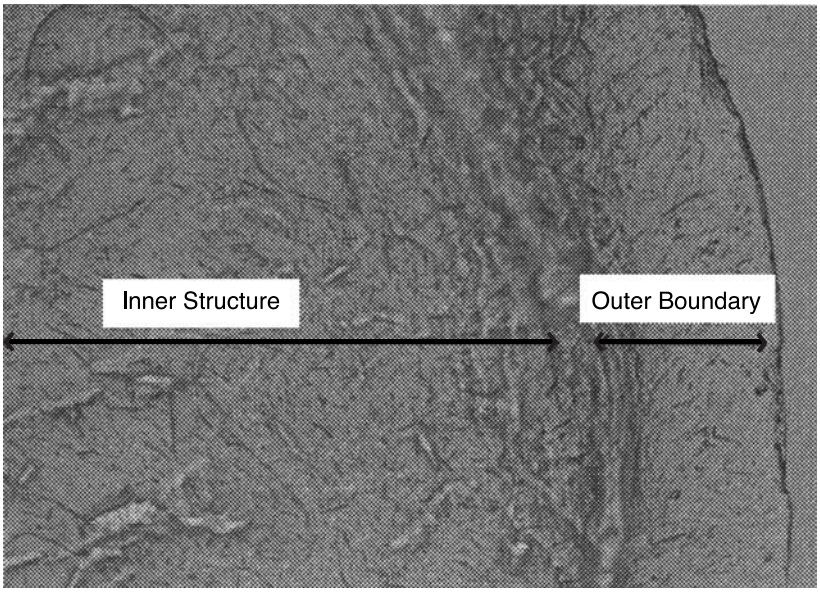

Figure 8. Confocal laser microscopy image of a nascent bead without the marker (Resolution X40). The distinct boundary is evident here.

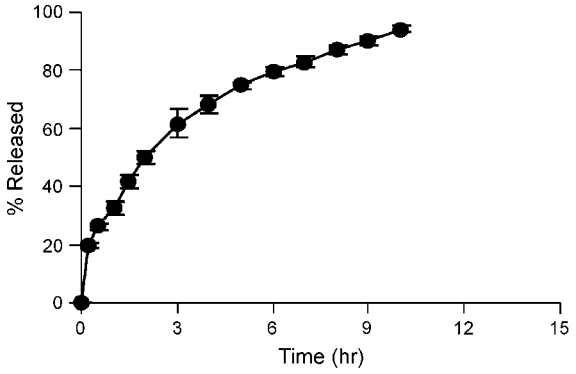

Figure 9. Release profiles of B-2 from beads made of binary mixture of polymers (n = 3).

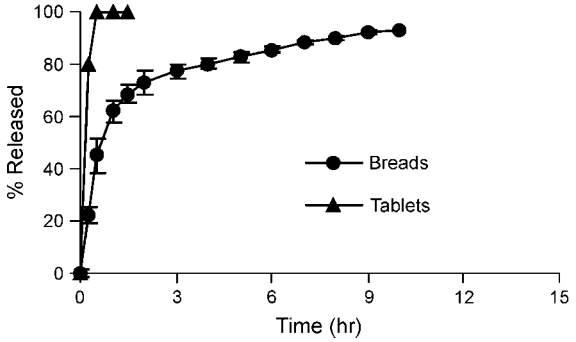

Figure 10. In vitro release profile of methotrexate (MTX) tablets and beads (n = 3).

bioavailability is observed due to possible saturation of the carriers.[17] In vitro evaluation of commercial MTX tablets showed 100% release of its contents within one half hour. However, the freeze-dried calcium-alginate-pectinate beads exhibited about 80% drug release in 5 hours (Fig. 10). At the end of dissolution, the beads were found to remain buoyant in the dissolution vessels with their structural integrity intact. To this end it is likely to utilize this type of gastroretentive delivery system to enhance the bioavailability potential of drugs that are either absorbed by active transport mechanism or in the proximal small intestine.

CONCLUSIONS

Via cross-linking of calcium, sodium alginate, and low methoxylated pectin, freeze-dried beads, which remain buoyant over 12 hours, were prepared. Depending on the physicochemical properties of the drugs, up to 100% drug release was achievable in about 10 hours, and release kinetics modulation was possible by controlling extent of cross-link formation and bead sizes. The presence of noncross-linking materials in low amounts, generally less than 2%, does not significantly interfere with release profiles. The release characteristics from the freeze-dried beads were independent of the hydrodynamic conditions. Therefore, this approach seems to

provide opportunity and potential for development of a gastroretentive drug delivery system for selectively targeting either the stomach, proximal intestine, or the entire GI tract for maximization of bioavailability and improvement of drug therapy.

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