Barium Chloride Crosslinked Carboxymethyl Guar Gum Beads for Gastrointestinal Drug Delivery

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ABSTRACT: A mild method for microencapsulation of sensitive drugs, such as proteins, employing a suitably derivatized carboxymethyl guar gum (CMGG) and multivalent metal ions like Ca^{++} and Ba^{++} is reported. Initially, guar gum is derivatized with carboxymethyl groups so that it forms durable, self-standing microbeads when its solution is dropped into $CaCl_2$ or $BaCl_2$ solutions. The swelling data of Ca^{++} and Ba^{++} crosslinked beads suggest that Ba^{++} crosslinks CMGG much more efficiently than Ca^{++}. The drug loading efficiency of these Ba^{++}/CMGG beads, as a function of concentration of both metal ion as well as drug, was then determined using Bovine Serum Albumin as a model drug. The ability of these beads to protect the drug from the acidic environment of the stomach was investigated. It was found that a very little amount of the drug is released from the beads when they are suspended in NaCl–HCl buffer of pH 1.2 for 6 h. The beads were also shown to release almost the entire encapsulated drug when exposed to TRIS–HCl buffer of pH 7.4. Thus, the results indicate that Ba^{++} crosslinked carboxymethyl guar gum beads can be used for gastrointestinal drug delivery. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 82: 3084–3090, 2001

Key words: carboxymethyl guar gum; microbeads; protein drugs; gastrointestinal delivery; microencapsulation; drug delivery systems; hydrophilic polymers; metal-polymer complexes

INTRODUCTION

Microencapsulation is increasingly being used to protect, target, and deliver sensitive drugs, thus increasing their therapeutic efficiency. A number of methods have been developed for this purpose, and more are being investigated.[1–3] However, most of them involve using materials and conditions that are not compatible with very sensitive drugs like proteins, peptides, and enzymes. Such sensitive drugs lose their activity when exposed to harsh conditions like elevated temperatures, organic solvents, and extremes of pH. One method, which is very mild and had been successfully used to encapsulate these drugs, is the crosslinking of sodium alginate with multivalent metal ions like Ca^{++} and Ba^{++}[4]. Sodium alginate is a polyanionic polysaccharide that gets ionically crosslinked by exchanging the univalent metal ions for the new multivalent ions that are supplied. The extensive amount of research work that has been done on this system is due to the exceptional ability of sodium alginate to form durable, acid-insensitive microparticles upon exposure to calcium chloride solution. This method is very mild, and is done at room temperature in aqueous medium by using only physiologically ac-

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ceptable chemicals. Because of the mildness of this procedure, several sensitive drugs, proteins, live cells, and organelles have been successfully encapsulated in sodium alginate microparticles.[5–10] Although theoretically it is possible to crosslink any anionic polymer to form such particles, attention has been riveted on alginate and to a lesser extent on pectinate.[11–13]

In the present study we have investigated the ability of carboxymethyl guar gum (CMGG) to form similar microparticles for the gastrointestinal delivery of sensitive drugs like proteins. Guar gum is a polygalactomannan derived from the seeds of a leguminacea plant, *Cyamopsis tetragonalobus*. Its anionic, carboxymethyl derivative is a high viscosity material and can be prepared in several degrees of derivatization.[14–18] Although this derivative finds its main application as an inexpensive thickener in textile and paper industries, it has also been investigated for food and drug delivery purposes. Crosslinking of carboxymethyl guar gum in to insoluble fibers by exposure to calcium chloride solution has been investigated to prepare ham-like vegetarian foods.[18] Beads of this material, containing Indomethacin, were made by dropping it in to either very cold aqueous solution or a nonsolvent.[19] In this study we have derivatized guar gum in such a manner that it gets crosslinked and gives nice beads when exposed to certain multivalent metal ions. Its ability to retain a protein model drug, bovine serum albumin (BSA) was also explored. BSA was chosen as model drug because it is a high molecular weight, globular protein that is well characterized, and is soluble in water. The drug loading efficiency of these beads and also *in vitro* release profiles are also presented.

MATERIALS AND METHODS

Guar gum with a viscosity of 3500 cps (1% in distilled water) was purchased from S.D.Fine-Chem Ltd., Mumbai, India. Before use, it was washed thoroughly by extracting with excess methanol for 4 h and dried. BSA was purchased from Sigma Chemicals, St. Louis, MO, and used as received. Sodium monochloroacetate, used for derivatizing guar gum, was bought from S.R.Drugs, Patancheru, Hyderabad, India, and was also used without further purification. Salts were also products of S.D.Fine-Chem Ltd., Mumbai.

Preparation of Carboxymethyl Guar Gum

CMGG was prepared using a slightly improved method over that reported by Montgomery et al.[17] In a 500-mL three-necked round-bottom flask equipped with a reflux condenser and N_2 supply, 20 g of guar gum was suspended in 200 mL distilled dioxane. While stirring, 35 g of sodium monochloroacetate, dissolved in 50 mL water, was added slowly over 15 min. The swollen guar dispersion was then gradually heated to 70°C. To this warm reaction mixture, 13 g of NaOH, dissolved in 25 mL water, was added drop wise over half an hour. The heating and stirring were continued for further 8 h, then it was allowed to cool to room temperature. Dioxane was decanted, and the solid settled was washed three times with 150 mL 30% aqueous methanol. During the last washing, pH of the suspension was adjusted to 7 using acetic acid. It was then washed finally with methanol and dried at 60°C for 2 h to obtain 24 g of carboxymethyl guar gum. It had a viscosity of 3500 cps (6% solution in distilled water, Brookfield viscometer, spindle #3). It had sodium content of 9%, which amounts to a degree of derivatization of 0.6.

Preparation of BSA Containing Carboxymethyl Guar Gum Beads

A homogeneous 6%(w/v) solution of the above-prepared CMGG was prepared in saline containing different amounts of BSA. About 100 mL of crosslinking solution of either $BaCl_2$ or $CaCl_2$ of known molarity was taken in a beaker and stirred, with the help of an overhead stirrer, gently. To this stirred solution 10 g of the CMGG solution containing BSA was added drop wise, using a disposable syringe with a needle of 0.8-mm internal diameter. The beads formed were stirred for 45 min more for hardening, filtered, washed, with distilled water and allowed to dry at room temperature until constant weight was attained.

Swelling Experiments

Test beads (0.15 g) were added to 50 mL distilled water and allowed to swell for 3 h. Swelling in g/g

was calculated from dry and swollen weights of the beads.

SEM

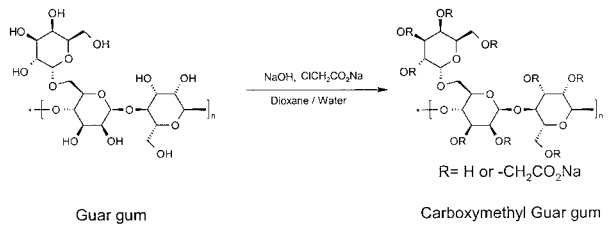
The particle size, shape, and morphology were studied using a Hitachi S 520 model scanning electron microscope. The beads were put on double sided tape, vacuum evaporated with gold, and examined.

In Vitro Release Studies

The *in vitro* release test of drug-loaded CMGG beads was carried out in simulated, enzyme-free gastric as well as intestinal fluids. Thus, 100 mg of dried beads were suspended in 100 mL of either pH 1.2 NaCl–HCl buffer (simulating gastric environment) or pH 7.4 TRIS–HCl buffer (simulating intestinal environment). Drug diffused into the extracting medium was estimated using Lowry's method of protein estimation.[20] The loading efficiency, or the amount of drug retained in the beads while preparing them, was estimated by digesting a known amount of beads in pH 7.4 PBS buffer. Each test was carried out in triplicate and plotted as a cumulative percentage of the total drug trapped in the beads against time of extraction.

RESULTS AND DISCUSSION

Since the advent of biotechnology many new drugs based on proteins and peptides have been discovered. Because of the sensitivity of these molecules towards several external conditions like solvents, heat, and extremes of pH, developing dosage forms for their oral delivery had become a challenge. One elegant method for protecting proteins from destruction in stomach and delivery to the intestine is coating or encapsulation in enteric materials. Carboxymethyl guar gum is



Scheme 1

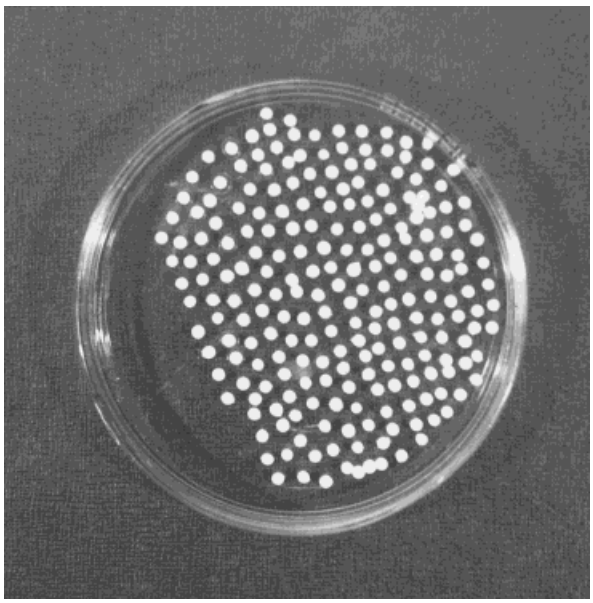


Figure 1 Photograph of Ba^{++}/CMGG beads.

an anionic polymer prepared from a plant polysaccharide called guar gum, by reacting with sodium monochloroacetate in the presence of NaOH (Scheme1). It is water soluble and gives very high viscosity solutions even at low concentrations (e.g.. 2% solution of commercial CMGG has a viscosity of 10,000 cps). Although it currently finds usage in nonfood applications like textile sizing, experiments have shown it to be biocompatible.[16] It is capable of forming insoluble complexes with calcium chloride, and it is this property that has been used in this study to form beads with enteric properties.

Preparation of CMGG Capable of Forming Durable, Self-Standing Beads with Multivalent Metal Ions

Dropping a neutralized solution of carboxymethyl guar gum into $CaCl_2$ crosslinks it into nascent beads. However, it was found that such beads, formed by using normal CMGG, used for textile and paper sizing get crosslinked only superficially to form a thick skin with a semiliquid interior. These beads lack strength, and once removed from water become flat, release contents, and never dry in to uniform beads. To improve the strength of these wet beads so that they can be dried in to spherical solid particles we have used different metal ion solutions like $CaCl_2$, $BaCl_2$, and $FeCl_3$ without success. Using

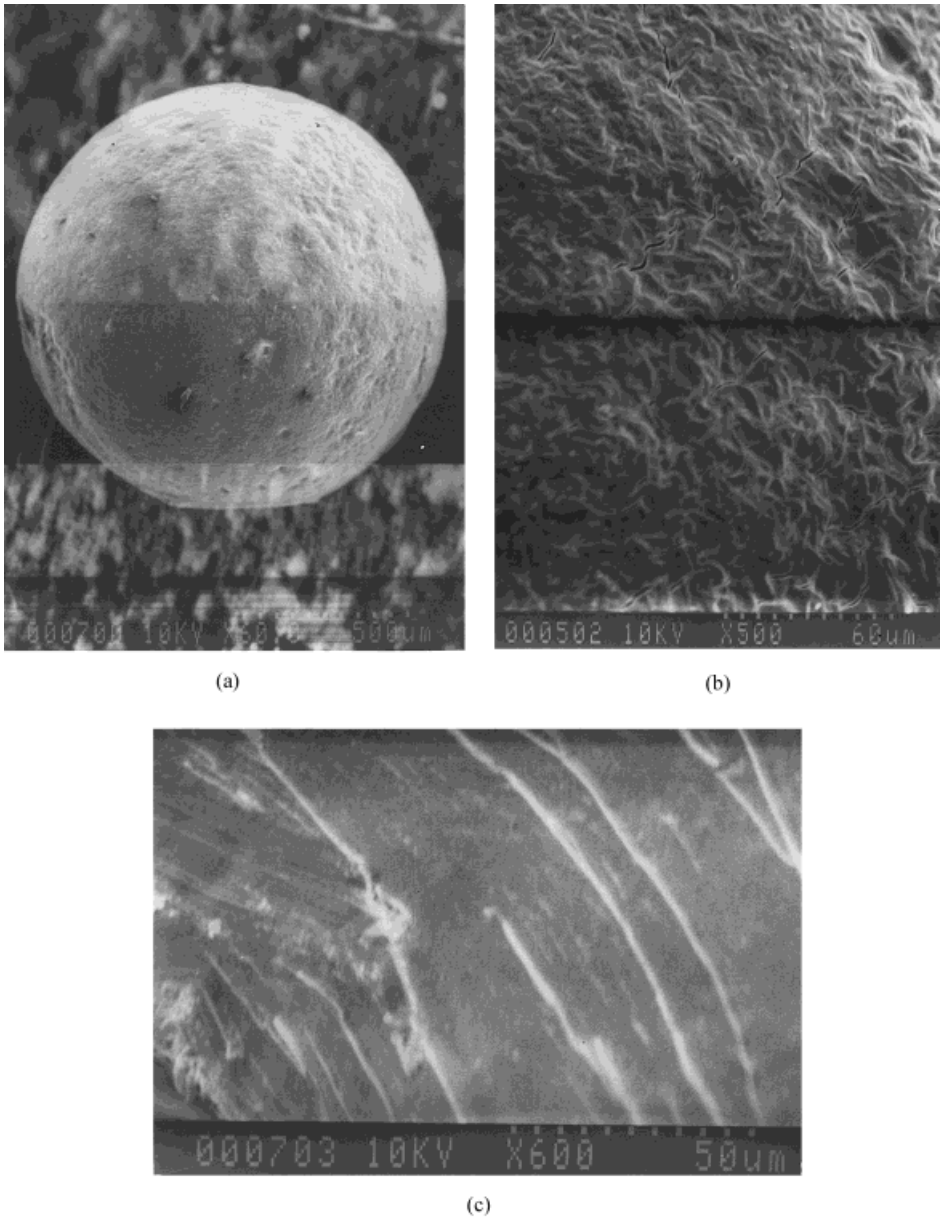


Figure 2 SEM micrographs of BSA containing CMGG beads. (A) Bead, (B) surface morphology, and (C) cross-section.

different concentrations of both CMGG and metal ions also did not improve the quality of the beads. Then, guar gum was derivatized using the above-mentioned method to obtain different extents of degree of derivatization (Scheme 1). CMGG, with a minimum degree of derivatization of 0.6 and far lower viscosity of 3500 cps (6% solution in distilled water), was found to give solely crosslinked spherical gel beads when exposed to either $CaCl_2$ or $BaCl_2$ (Fig. 1). These beads could be dried to form spherical particles without problem. As can be seen from the SEM pictures (Fig. 2), the dry beads are about 1200 μm in diameter. They appear to have fibrous surface and a solid core.

Ability of Metal Ions to Crosslink CMGG, Ca^{++} vs. Ba^{++}

To determine the extent of crosslinking that these metal ions are capable of forming with CMGG,

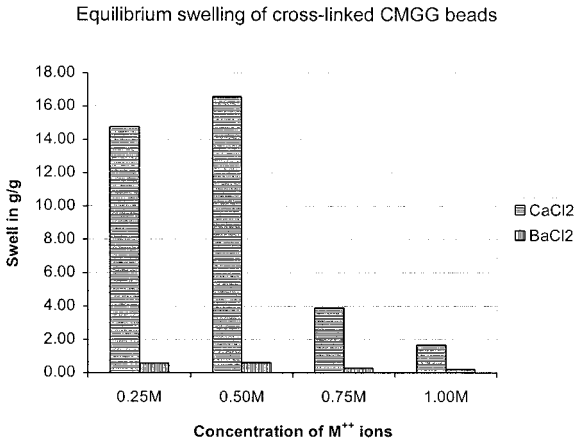


Figure 3 Equilibrium swelling of crosslinked CMGG beads in distilled water.

swelling studies were performed on beads made with these two ions at different concentrations. As is evident from Figure 3, $BaCl_2$ crosslinked CMGG much more efficiently than $CaCl_2$ at all concentrations tried.

This difference in the ability of one divalent metal ion to bind and crosslink an anionic polymer over another is not surprising. Unlike small molecular ions, polymeric molecules have conformational restrictions, which allow only certain size molecules to allow bond formation. Even in the case of sodium alginate, which is a linear polymer of mannuronic and guluronic acids, have such differences been noticed.[21] In the case of CMGG, the presence of a small branch of galactose on every alternate sugar residue adds to the conformational problem of bringing two different carboxylate ions close to effect crosslinking. Probably with its larger ionic size the Ba^{++} fits more appropriately in to the binding sites of this particular derivative than Ca^{++}.

Ability of Ba^{++}/CMGG Beads to Retain BSA

Successful crosslinking of CMGG into nonswelling uniform beads by $BaCl_2$ prompted us to investigate its utility as a possible gastrointestinal delivery vehicle for sensitive drugs. The efficiency of loading of BSA, as a model drug using different concentrations of Ba^{++} for crosslinking, was first studied. Beads were made by dropping a saline solution containing 6% CMGG, prepared as above, and 0.3% BSA into different molarity solutions of $BaCl_2$. Amounts of BSA retained as percent of total protein taken against concentration of $BaCl_2$ are presented in Figure 4. As can be seen, up to 53% of BSA taken up could be retained in the beads using a 1.25-*M* $BaCl_2$ solution.

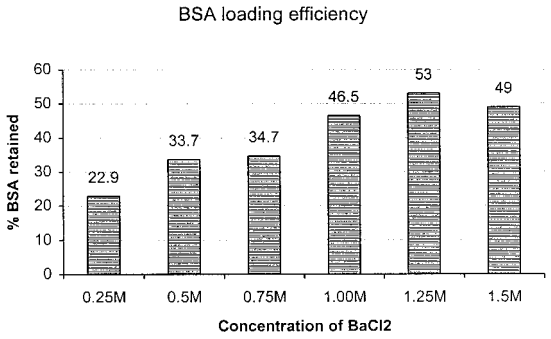


Figure 4 BSA loading efficiency as a function of $BaCl_2$ concentration.

Lower retention of BSA in the beads while using lower concentrations of $BaCl_2$ can be due to the lower rates of crosslinking, which allow more of the protein to diffuse into the crosslinking medium. However, retention of about 50% of the protein looks to be the maximum with the materials taken and under the conditions studied. Loading efficiency was also investigated as a function of the amount of BSA taken in the solution. Again, taking a solution containing 6% CMGG and different amounts of BSA, beads were made by using 1.0 *M* $BaCl_2$. As can be seen from Figure 5, maximum loading could be obtained using a solution containing 0.8% BSA. However, it should be noted that, even with lower loading efficiency, higher amounts of protein could be loaded if we take higher amounts of protein in the formulation.

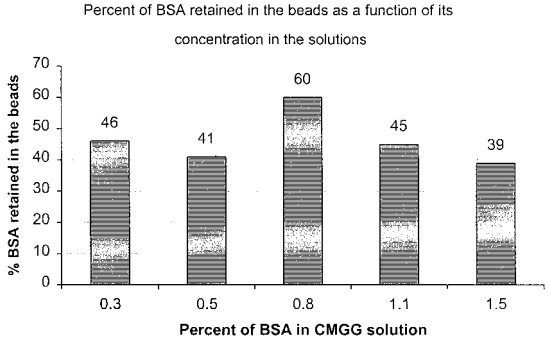


Figure 5 Percent of BSA retained in the beads as a function of its concentration.

Ability of Crosslinked CMGG Beads to Retain and Protect the Encapsulated Drug from a Gastric Environment

For these beads to be useful as gastrointestinal delivery vehicles for drugs it is essential that these beads should neither disintegrate nor release BSA in a gastric environment. Because the highly acidic pH of the stomach can denature protein drugs into useless fragments, it is imperative that these beads should safely take the drug past the stomach. To see this *in vitro*, beads prepared by dropping a solution containing 6% CMGG and 0.8% BSA in to 1.0 *M* $BaCl_2$ were suspended and stirred in pH 1.2 NaCl–HCl buffer, and BSA released was determined. As presented in Figure 6, hardly any BSA escaped from the beads up to 6 h. After 1 day stirring also, only about a third of the total drug encapsulated escaped. Thus, Ba^{++}/CMGG beeds seem to afford ample protection to the drug from the acidic environment of the stomach.

In Vitro Release of BSA from CMGG Beads in TRIS–HCl Buffer of pH 7.4

To estimate the behavior of the drug-loaded CMGG beads in an intestinal environment, the drug released from them was studied in pH 7.4 TRIS–HCl buffer. The cumulative release of BSA from the $BaCl_2$ crosslinked beads was studied over 24 h, and is presented in Figure 7. As can be seen, the drug release is very effective, and about 80% of the BSA encapsulated is released in 4 h. Because the transit time of food in the small intestine is not highly variable, and is usually 3–4 h, the rate of release appears to be optimal.

CONCLUSION

Carboxymethyl guar gum, capable of forming spherical crosslinked beads when exposed to Ca^{++} and Ba^{++}, has been prepared. As a model protein drug, BSA has been encapsulated in barium chloride crosslinked CMGG beads and its release *in vitro* in simulated gastric and intestinal buffers was investigated. It was found that $BaCl_2$ crosslinked beads protect the protein from low pH conditions and deliver it completely in simulated intestinal fluid.

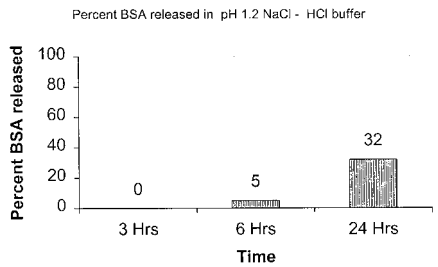


Figure 6 Release of BSA from Ba^{++} crosslinked beads in pH 1.2 NaCl–HCl buffer.

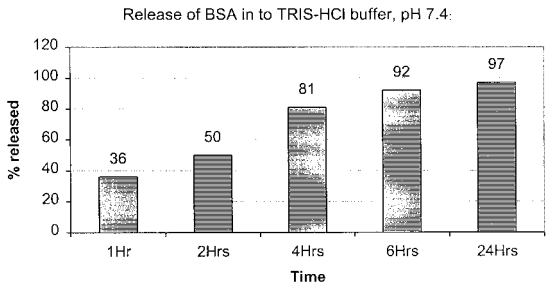


Figure 7 Release of BSA from Ba^{++}/CMGG beads in TRIS–HCl buffer of pH 7.4.

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