Chitosan/Calcium–Alginate Beads for Oral Delivery of Insulin

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SYNOPSIS

A mild chitosan/calcium alginate microencapsulation process, as applied to encapsulation of biological macromolecules such as albumin and insulin, was investigated. The microcapsules were derived by adding dropwise a protein-containing sodium alginate mixture into a chitosan–$CaCl_2$ system. The beads containing a high concentration of entrapped bovine serum albumin (BSA) as more than 70% of the initial concentration were achieved via varying chitosan coat. It was observed that approximately 70% of the content is being released into Tris-HCl buffer, pH 7.4 within 24 h and no significant release of BSA was observed during treatment with 0.1*M* HCl pH 1.2 for 4 h. But the acid-treated beads had released almost all the entrapped protein into Tris-HCl pH 7.4 media within 24 h. Instead of BSA, the insulin preload was found to be very low in the chitosan/calcium alginate system; the release characteristics were similar to that of BSA. From scanning electron microscopic studies, it appears that the chitosan modifies the alginate microspheres and subsequently the protein loading. The results indicate the possibility of modifying the formulation in order to obtain the desired controlled release of bioactive peptides (insulin), for a convenient gastrointestinal tract delivery system. © 1996 John Wiley & Sons, Inc.

INTRODUCTION

A wide range of bioactive molecules like proteins, peptides, and enzymes are commercially available as drugs. Many of these are stable only under physiological conditions and their therapeutic application is severely limited by their short half lives *in vivo*. Pulsed or self-regulated release from a polymer matrix may enhance the therapeutic effectiveness with a prolonged action. But, it is difficult to achieve due to their complex molecular properties as compared to small-molecular-weight conventional drugs. Several research attempts have been made to deliver the polypeptide molecules through polymer matrices, but these have limitations on appropriate delivery, biological activity etc.[1]

Alginates, a naturally occurring copolymer of guluronic and manuronic acid, are widely used in biomedical applications and are capable of being processed under mild conditions. Reports indicate that the biological activity is very much retained in the calcium alginate encapsulation process.[2] The simple, mild, aqueous-based gel formation of sodium alginate in the presence of divalent cations such as Ca^{2+} has been utilized to immobilize cells,[3] for hybrid artificial organs,[4] hemoglobin carriers,[5] macromolecular delivery,[2] drug delivery systems,[6–12] and, very recently, for oral delivery of transforming growth factor beta (TGF-β_1).[13] Alginate, being polyanionic, polycationic polymer coatings of polylysine,[2] polyvinylamine,[2] chitosan,[5,12,14] etc., are employed to increase the stability of alginate capsules or to minimize the loss of encapsulated material.

Chitosan, a natural polysaccharide, having structural characteristics similar to glycosaminoglycans, seems to be nontoxic and bioabsorbable,[15] and has been explored for the release of several drugs.[16,17] The use of chitosan in the development of oral sustained release preparations was indicated based on the intragastric "floating" tablets of chitosan.[12] Chitosan has gel-forming properties in the low pH range, and also, with its antacid and antiulcer characteristics, it has the potential for use in oral sustained delivery systems.

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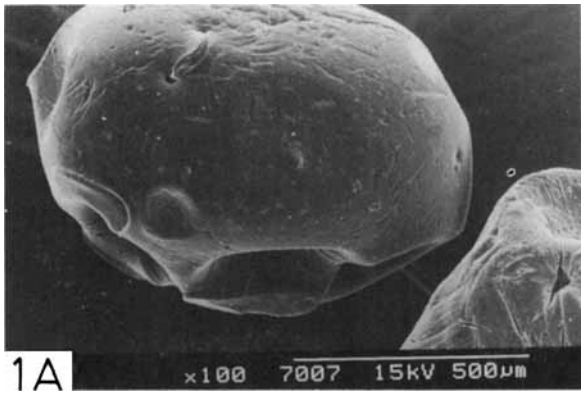
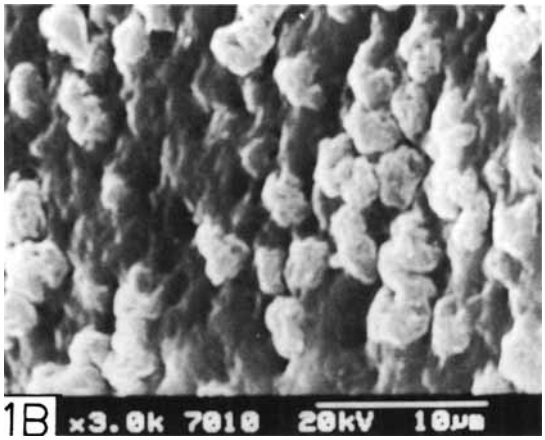




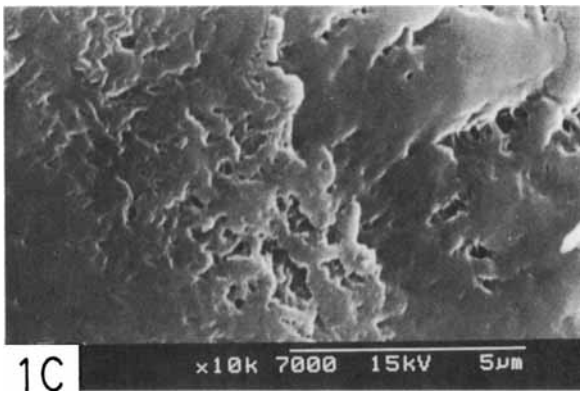
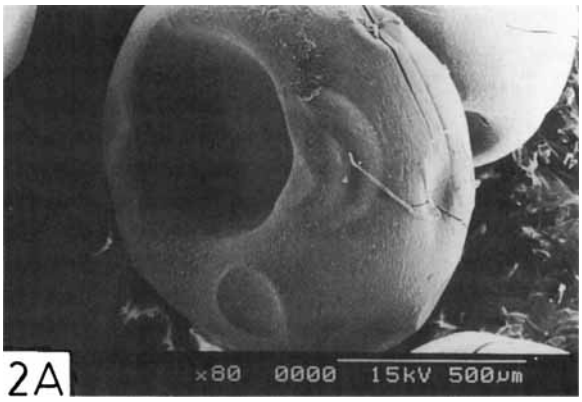
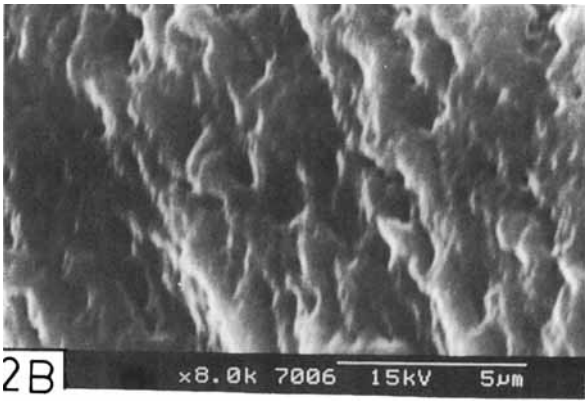


Figure 1 SEM Micrographs. (A) Dry alginate beads, (B) surface morphology, and (C) its cross section.

Presently, our aim is to utilize calcium alginate as a matrix to deliver insulin orally in the gastrointestinal tract. A chitosan coating is employed to increase the payload by forming a membrane at the bead surface during preparation. BSA, a well-characterised protein, which is large in size and readily soluble in water, is chosen as a model for encapsulation. The *in vitro* release profile of BSA has been





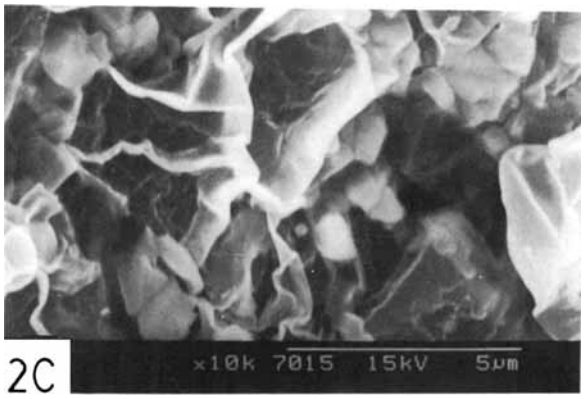
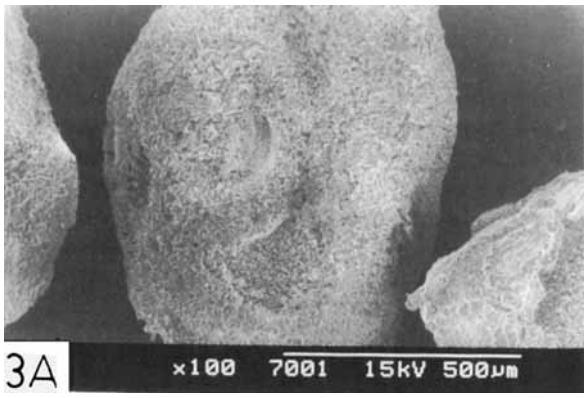
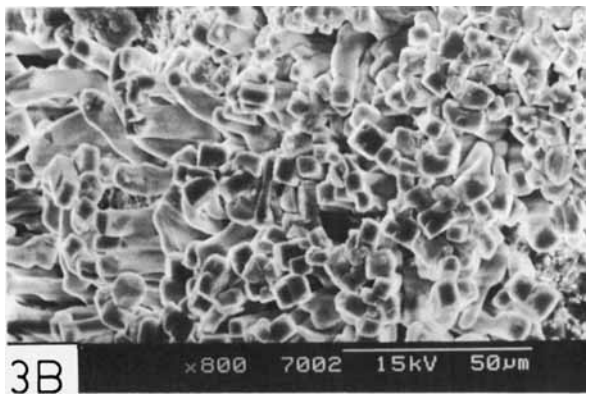


Figure 2 SEM of (A) alginate–chitosan (0.3%) beads, (B) surface morphology, and (C) its cross section.





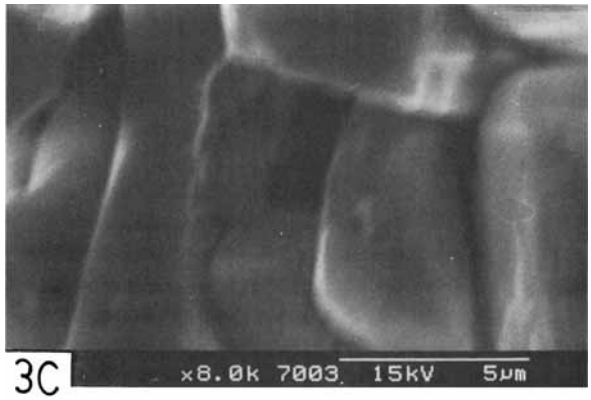


Figure 3 SEM of (A) albumin-loaded alginate chitosan (0.3%) beads, (B, C) surface morphology, and (D, E) its cross section.

evaluated. The optimized system is further selected for encapsulation of insulin and for release studies.

MATERIALS AND METHODS

Sodium alginate of medium viscosity ($\simeq$ 3500 cps for a 2% solution at 25°C) is obtained from Sigma Co. (St. Louis, MO; A-2023). Chistosan, a gift from Central Institute of Fisheries Technology (Cochin, India), is derived from prawn shell chitin by greater than 85% deacetylation, of particle size 1–3 mm and inherent viscosity 5.13 dL/g, measured at a concentration of 0.5 g/dL in 2% acetic acid at 30°C, and is used as received. BSA, fraction V, is a product of Spectrochem, India. $CaCl_2$ and NaCl are from Sarabhai chemicals and Glaxo Lab., India, respectively. The insulin is from Boots insulin injection preparation (IP, 40U/mL, the Boots Co., Bombay, India). Phosphate-buffered saline (PBS) and Tris-HCl

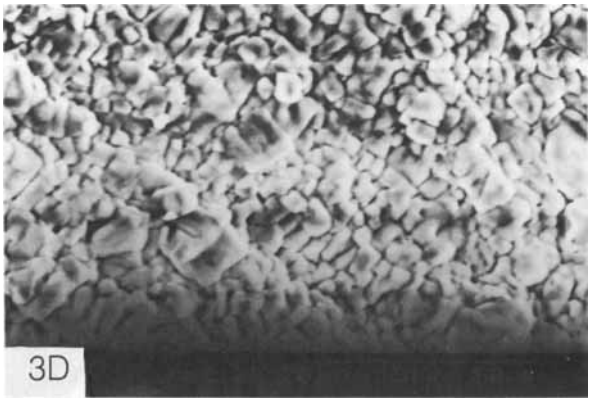


Figure 3 (*Continued*)

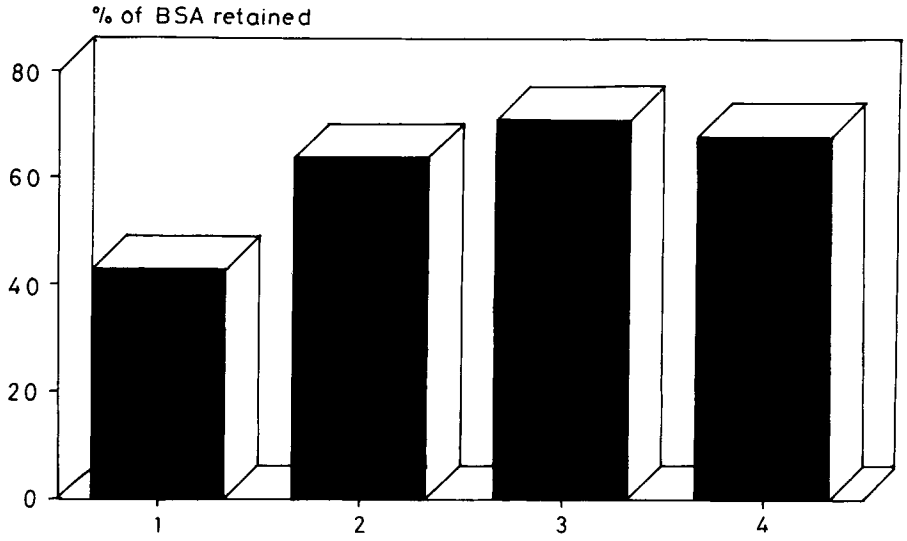


Figure 4 Percentage BSA retained in the bead. (1) Alginate, (2) alginate–chitosan (0.1%), (3) alginate–chitosan (0.3%), (4) alginate–chitosan (0.5%).

buffer solutions are freshly prepared as needed using distilled, deionized water.

Bead Formation

Sodium alginate (2% w/v) is allowed to dissolve in distilled, deionized water containing BSA (0.3g%) or insulin (400 IU%) and 0.9 g% NaCl. Approximately 20 mL of this solution is dropped through a needle (0.15 mm diameter), from a plastic syringe into a beaker containing 100 mL of $CaCl_2$ solution (1.5 g%) under gentle stirring. The capsule formed is allowed to harden for 15 min in $CaCl_2$ solution, and then is filtered and rinsed with distilled water. In another method, a chitosan solution of various concentrations (100 mL) containing 1.5 g% $CaCl_2$ is used for gellation of alginate. A series of chitosan solution in 0.01*M* HCl (0.1, 0.3, and 0.5%) is prepared and the pH is adjusted to 5.7 using 0.1*N* NaOH solution, and filtered to get a clear solution. The capsules are prepared from these chitosan solutions and the alginate, as mentioned above. The rinsed capsules are allowed to dry in air at room temperature until constant weight is achieved.

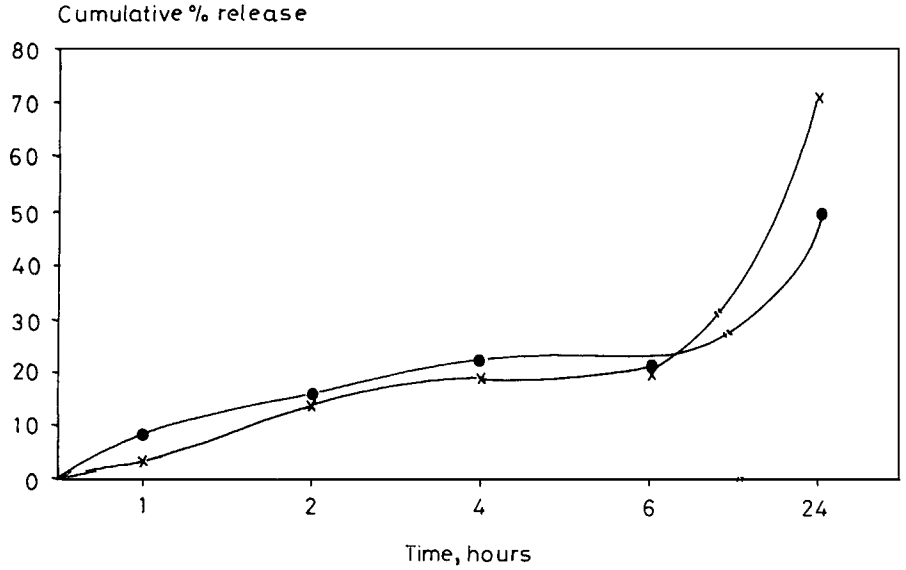


Figure 5 BSA release profile into Tris-HCl buffer pH 7.4. (●) Alginate, (x) Alginate–chitosan (0.3%).

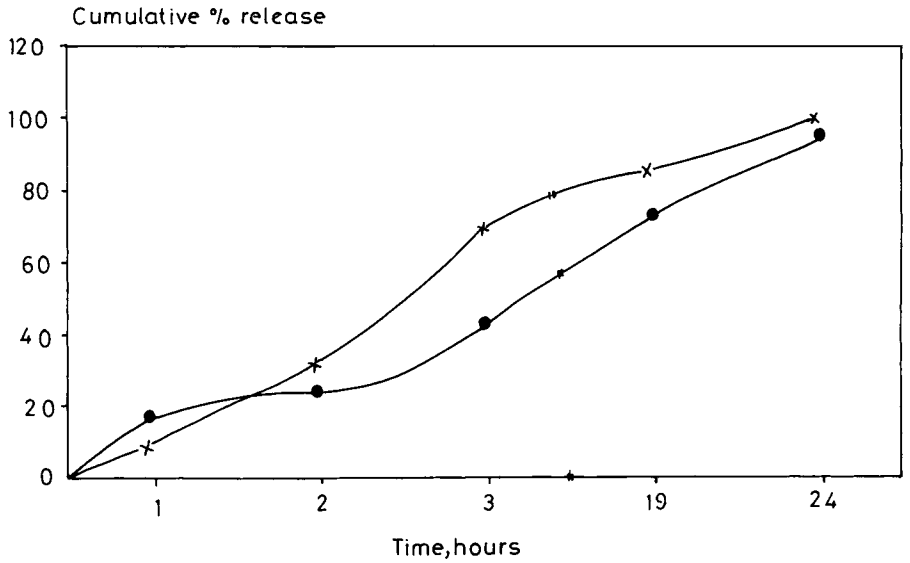


Figure 6 BSA release profile into Tris-HCl, pH 7.4 after acid treatment (0.1*M* HCl) for 4 h. (●) Alginate, (x) alginate–chitosan (0.3%).

Scanning Electron Microscopy (SEM)

The surface morphology and internal structure of the beads are examined using a scanning electron microscope (Hitachi, Model S-2400). Samples are mounted on metal stubs, using double-sided adhesive tape, gold coated under vacuum, and then examined.

In Vitro BSA/Insulin Release

The release of encapsulated proteins is carried into Tris-HCl buffer, pH 7.4 at room temperature ($\simeq$ 30°C) and samples at appropriate intervals are withdrawn and assayed using Lowry's method[19] for protein estimation. An equal volume of same dissolution medium is added to maintain a constant volume. The release profile into Tris-HCl/PBS pH 7.4 after 4 h acid treatment (in 0.1*M* HCl) is also evaluated. The amount loaded is estimated by dissolving a known amount of beads in PBS, pH 7.4. Each determination is carried out in triplicate and the release results are plotted as the cumulative percentage of the content into dissolution medium versus time.

RESULTS AND DISCUSSION

SEM micrographs of dried alginate beads, their surface morphology, and cross section are shown in Figure 1. The beads are about 900–1000 μm in size, not spherical in shape, and have a relatively smooth surface with few wrinkles [Fig. 1(A)]. The surface morphology [Fig. 1(B)] and the internal structures [Fig. 1(C)] appear to have micropores (<0.5 μm), as is evident from the figures.

Figure 2 shows the SEM micrographs of alginate–chitosan (0.3%) beads, their surface, and internal morphology. The incorporation of chitosan does not cause any change in the overall shape or size [Fig. 2(A)], however, the surface and internal morphology have been modified with this membranous structure with intermitent micropores (0.5 μm), as indicated in Figure 2(B) and (C). In other words, the presence of chitosan modifies the surface and the internal structure of the alginate beads.

Scanning electron micrographs of albumin-loaded alginate–chitosan (0.3%) beads, their surface, and their internal morphology are depicted in Figure 3(A)–(E). It appears that the presence of albumin in the alginate–chitosan beads has greatly altered the surface and internal structures [Fig. 3(A)–(C)], when compared with the unloaded beads [Fig. 2(A)–(C)]. Also, relatively poor bead formation can be noticed. From the Figure 3(A)–(E), microscopically one can say that several rectangular rod-like structures are stacked loosely to form a bead. Possibly, these are due to the alginate–BSA interaction[2] and also due to the loss of entrapped BSA during the process.

A minimum concentration of 2.0% solid content in solution of this particular alginate is required to get a dry solid bead form, under these process conditions. The wet capsules just after preparation are found to be spherical in shape, but upon drying in air at room temperature the sphericity is very much lost, as is evident from SEM figures. Freeze drying

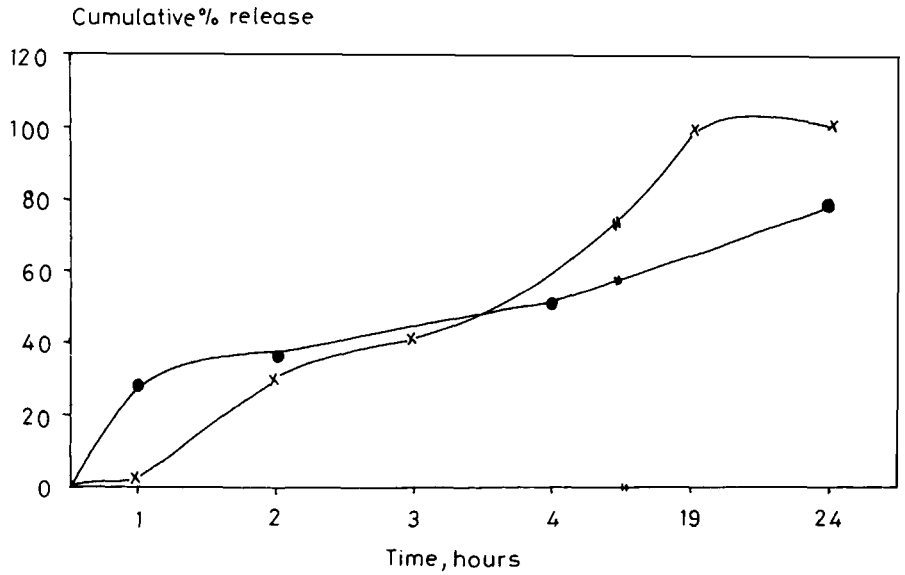


Figure 7 Insulin release profile from chitosan-coated (0.3%) alginate beads. (●) Tris-HCl, pH 7.4 before acid treatment, (×) PBS, pH 7.4 after acid treatment.

or lyophilization may result in retention of the sphericity of the beads. The degree of swelling in deionized, distilled water remains almost the same in both alginates, bare and chitosan skin-coated ones. The equilibrium water contents are attained within 1 h and are found to be 52.3 and 54.5%, respectively.

BSA-Loaded Alginates—Dissolution Studies

The amount of protein retained in various alginate capsules is shown in the Figure 4. It is observed that approximately 43.0% of BSA added is retained in the bare alginate capsules at the end of the microencapsulation procedure. However, it is evident from Figure 4 that the payload is raised to 70% by providing a skin coating of chitosan (0.3% solution) *in situ*. But further increases in payload are not found for higher concentrations of chitosan solution. The use of the calcium alginate/chitosan system for BSA encapsulation appears to increase the protein within the matrix compared to the classical system of protein entrapment by calcium alginate gellation. The enhanced loading of BSA may be due to the reduction in the release rate of the protein by ionic interaction between the alginate and chitosan, resulting in a chitosan skin coating. Huguet et al.[5] also loaded more hemoglobin to calcium alginate beads in the presence of chitosan.

The release of encapsulated BSA is noted in Tris-HCl buffer pH 7.4 before and after treatment with 0.1*M* HCl for 4 h, and the cumulative percentage release is depicted in Figures 5 and 6, respectively. Albumin releases very slowly in Tris-HCl buffer, pH 7.4 during 6 h of incubation, which reaches a plateau of 50 and 70% release after 24 h (Fig. 5) from the alginate and alginate–chitosan systems, respectively. Even after 48 h, a fraction of protein (10–15%) remained within the matrix (not shown in the figure). It is evident that the chitosan coating has increased the protein delivery in this system, which may be due to the increased payload of BSA and reduced internal gellation in the matrix. Probably the enhanced BSA release is partially due to the poor bead formation of the albumin-loaded alginate–chitosan system as evident from Figures 3(A) and 5. However, more detailed studies are needed to confirm these observations. During the low pH treatment (0.1*M* HCl), no significant release of protein from the beads is observed. But the release rate of BSA from the acid-treated beads is very much enhanced by Tris-HCl buffer, pH 7.4 (Fig. 6). Almost 100% of the entrapped BSA is found to be released within 24 h.

Insulin Loaded Alginates

The optimum concentration of chitosan, i.e., 0.3% in 0.01*M* HCl, for BSA loading is chosen for the insulin-encapsulated alginate capsule formation. During the dissolution of beads in PBS pH 7.4, it is found that the percentage loading is 11.22 ± 2.35, from 4 separate observations of 3 batches. Approximately, 78.8% of loaded insulin has released during exposure to 0.01*M* Tris-HCl buffer pH 7.4 within 24 h (Fig. 7). As in the case of BSA, no significant

release of insulin is observed during acid treatment (0.1 *M* HCl, 4 h). But the acid-treated ones released 100% of the content into PBS pH 7.4 medium within 24 h (Fig. 7) where the beads are swollen rapidly and disintegrated.

At low pH alginates do not swell, but a reversal of shrinkage takes place and the contents are not released. However, an increase in the dissolution rate of beads is observed in PBS pH 7.4 (Figs. 6 and 7) after acid treatment. This phenomenon is in agreement with the reports of TGF-β_1 release from alginate by Mumper et al.[19] According to them, upon acid treatment, the hydrolysis of alginate takes place, which simultaneously reduces the Ca^{2+} concentration within the beads resulting in increased dissolution at neutral pH.

The development of calcium–alginate–chitosan beads, containing insulin, can provide an excellent gastrointestinal delivery system for insulin. It has been reported that purified alginate is nontoxic and biodegradable when taken orally.[13,20] In addition, alginate has been found to have bioadhesive properties and can also be effective in protecting mucous membranes of the gastrointestinal tract.[13,21] Chitosan matrix has also been shown to be acceptable orally[22] and has proven to be a promising vehicle for sustained release preparations.[16,17] Hence, the present alginate–chitosan matrix has its own unique functions for delivering bioactive molecules in the intestine. More detailed investigations are needed and are being planned to increase the insulin payload within the alginate–chitosan system. However, further *in vivo* studies are needed to confirm these observations, and the biocompatibility of the encapsulated insulin.

CONCLUSION

The shrinkage of calcium alginate capsules at low pH enables the encapsulated drug to be released into the gastrointestinal tract. Besides, the acid treatment of alginate enhances the dissolution rate at neutral pH. The payload of insulin can be increased by encapsulating lipoinsulin,[23] glycosylated insulin[24] etc. In addition, the bioactivity biocompatibility, and immunoreactivity of the encapsulated insulin is to be evaluated by *in vivo* tests and such results are awaited in the future.

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