CHAPTER II
LITERATURE SURVEY

2.1 Alginate Based Material for Drug Delivery Studies

Alginate is produced from brown seaweeds (*Phaeophyceae,* mainly *Laminaria*). It is a linear polymer containing β-(1→4)-linked D-mannuronic acid (M) and α-(1→4)-linked L-guluronic acid (G) residues. The structure of alginate is shown in Fig. 2.1. Although these residues are epimers (D-mannuronic acid residues being enzymatically converted to L-guluronic after polymerization) and only differ at C_5, they possess very different conformations; D-mannuronic acid being $^4C_1$ with diequatorial links between them and L-guluronic acid being $^1C_4$ with diaxial links between them. It is not random copolymers but, according to the source algae, consist of blocks of similar and strictly alternating residues (i.e. MMMMMM, GGGGGG and GMGMGMGM), each of which has different conformational preferences and behavior. They may be prepared with a wide range of average molecular weights (50-100000 residues) to suit the application. The main uses of alginates are gelling and thickening agents and emulsion stabilizers in the food industry, thickening agents for use in the textile and paper industries, wound healing, microencapsulating and controlled release systems. The use of alginate (particularly high-G) gelled encapsulation and release systems has a large literature and includes the immobilization and controlled release of pesticides, biocatalysts and drugs. In the presence of divalent cations, such as calcium, alginic acid/alginate is able to form gels because of association between the calcium ion and two guluronic acid residues. The cross-linking in alginate gels is therefore ionic, based on calcium bridges, with the degree of cross-linking depending upon the concentration of calcium ions and the number of guluronic acid sequences in the polysaccharide (Lloyd *et al.*, 1998).

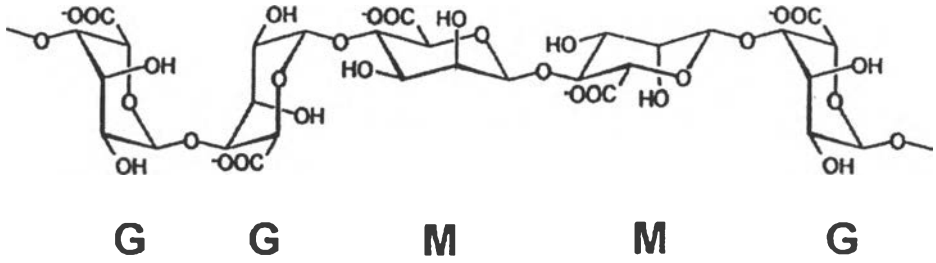

Figure 2.1 Chemical structure of alginate.

Homopolymeric sequences of β-D-mannopyranosyl uronate and α-L-gulopyranosyl uronate in seaweed alginate, although to widely differing extents, and heterotypic mixed sequences are usually presented. From their structures one might predict that the homopolymeric regions of poly(β-D-mannuronate) would behave similarly to cellulose chains. However, the increased chain flexibility due to the axial, rather than equatorial hydroxyl at the C-2 position and cation binding by the charged polyanion would influence any ordered conformation.

The axial linkage of homopolymeric poly(α-L-guluronate) leads to a buckled ribbon configuration with limited flexibility. In both the free acid and salt forms it packs as twofold chains. Large interstices exist between the chains packed in this fashion. Cooperative interactions are strengthened by these spaces being filled with either water molecules or cations. Evidence has accumulated that this is indeed the case, cations particularly calcium fill these interstices, strengthening the interaction between residues. Circular dichroism studies and mathematical modeling indicate that the buckled ribbons of poly(α-L-guluronate) pack together, with the cations strongly coordinated in cavities between the chains, like eggs in a box. For each chain the likely coordination site involves a carboxylate oxygen and an O-5 from one residue, with the glycosidic oxygen and O-2 and O-3 of the next residue toward the nonreducing terminus (Figure 2.2).

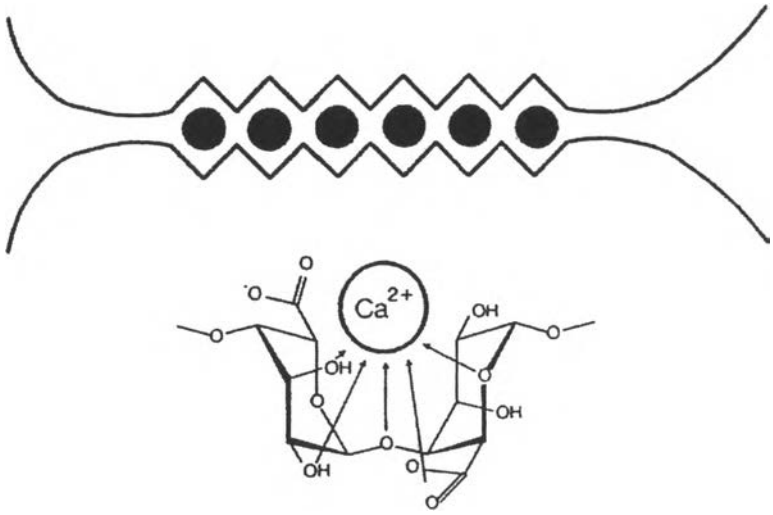

Figure 2.2 The egg-box model for binding of divalent cation to alginates.

In the presence of calcium, alginate chains associate to form hydrated networks (gels). In alginate, the structurally regular homopolymeric sequences are interrupted by the occurrence of other residues. These interruptions act to terminate intermolecular associations through structural junction zones, with the consequent exchange of partners building three-dimensional networks. The primary mechanism of interchain association on calcium-induced gelation of alginate is dimerization of polyguluronate chain sequences with interchain chelation of Ca^{2+}. Calcium polyguluronate junctions are terminated by D-mannuronate residues. In the presence of excess Ca^{2+} uninterrupted polyguluronate chains form a solid precipitate.

The effect of pH and drug solubility on the release kinetics of sodium alginate matrices has been studied by Hodsdon *et al.* (1995). It was found that the release of highly soluble model drug, chlorpheniramine meleate, was significantly faster in simulated gastric fluid than in simulated intestinal fluid, whereas the opposite effect was observed for hydrochlorothiazide, a drug of poor solubility. This can be explained in terms of the internal microscopic structure of the hydrated surface layer formed on matrix hydration and by the different hydration kinetics of the polymer in these two media. Drug release mechanisms seemed to depend on the solubility of drug and the structure and properties of the surface layers formed by alginate matrices when hydrated in different pH media.

Lee *et al.* (1997) prepared polyelectrolyte complex of sodium alginate and chitosan or its derivatives for biomedical application in the form of microcapsule. Chitosan used was reacetylated with acetic anhydrides to control the N-acetyl content of the chitosan. The drug release rate was also investigated. It was found that the release rate was controlled by the pH. The minimum release occurred at pH 4.8. The release rate varied with pH due to loop formation of backbone chains of polyelectrolyte. The long N-acyl groups introduced to the chitosan enhanced the release rate remarkably.

Yan *et al.* (2001) prepared chitosan-alginate polyelectrolyte films with chitosan of different molecular weights. It was found that chitosan of low molecular weight appears to react more completely with sodium alginate than chitosans of higher molecular weights. Films prepared from the low molecular weight chitosans have lower water vapor permeability and water uptake in aqueous acid. The polyelectrolyte complex films have potential for biomedical applications, because they exhibited well *in vitro* biocompatibility with mouse and human fibroblasts.

Mi *et al.* (2002) prepared a novel crosslinking reinforced chitosan-alginate beads using a naturally occurring crosslinker, genipin, for drug delivery. It was found that the release rate of the drug coming out of the chitosan-alginate beads could easily be modified by regulating the production process, such as pH or concentration of alginate in the gelling solution.

2.2 Chitosan Based Material for Drug Delivery Studies

Chitin is a naturally occurring polysaccharide found in the supporting material of crustaceans, insects, etc. It is a (1→4)-β-linked glycan composed of 2-acetamido-2-deoxy-D-glucose (N-acetylglucosamine) (Figure 2.3). It is a highly insoluble material resembling cellulose in its solubility and low chemical reactivity. It may be regarded as cellulose with hydroxyl at position C-2 replaced by an acetamido group. Like cellulose, it functions naturally as a structural polysaccharide. Chitin is a white, hard, non-elastic, nitrogenous polysaccharide and the major source of surface pollution in coastal areas. In nature, chitin serves as a 'glue' for chemical components making up the delicate wings of insects and the crunchy integuments of

crustaceans such as crabs and shrimps. To obtain chitin from crustacean shell waste, it is usually ground and mixed with a dilute aqueous sodium hydroxide solution to dissolve protein. The residual material is then treated with a dilute aqueous hydrochloric acid solution to dissolve calcium carbonate, leaving behind chitin as a white powder.

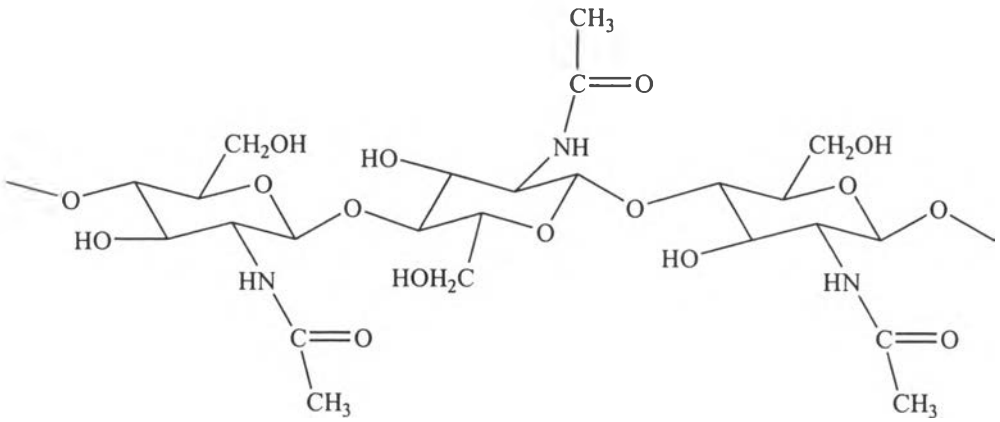

Figure 2.3 Chemical structure of chitin.

Chitosan, a polyaminosaccharide, [β(1→4)-2-amino-2-deoxy,β-D-glucan], is the N-deacetylated derivative of chitin, although this N-deacetylation is almost never complete. The structure of chitosan is shown in Fig. 2.4.

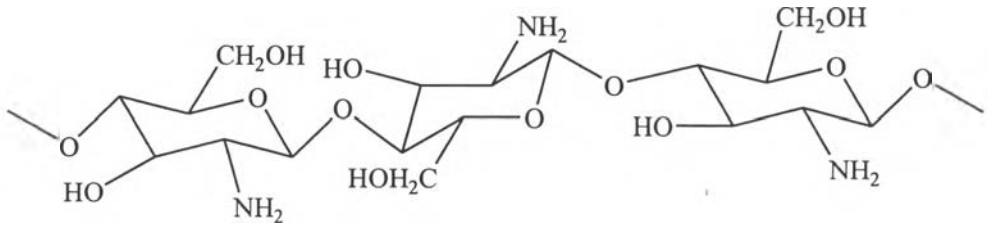

Figure 2.4 Chemical structure of chitosan.

Chitosan is a natural polycationic polymer which possesses valuable properties as a metal recovering and water-purifying agent. The other applications are wastewater treatment for heavy metal and radioisotope removal and valuable metal recovery, potable water purification for reduction of unwanted metals, complex binding of iron in precooked food to reduce 'warmed –over flavor'.

Chitosan is biocompatible with its degradation products being known natural metabolites and can be produced in powder, film, bead, fiber and fabric formats. Many of chitosans properties depend upon its cationic nature. At acidic pH's it is linear polyelectrolyte with a high charge density, one positive charge per glucosamine residue and so will interact with negatively charged molecules including proteins, anionic polysaccharides and nucleic acids, many of which are located in skin. It was shown that in the area of wound healing chitosan can reduce the scar tissue (fibroplasia) by inhibiting the formation of fibrin in the wounds, it is hemostatic and can form a protective film/coating. One reason postulated for the ability of chitosan to enhance its biodegradability. It is a substrate for lysozyme with the degradation products being adsorbed and possibly even having some nutrient value. Also chitin, chitosan and chitosan derivatives affect macrophage activity, which will influence the wound healing process (Lloyd *et al.*, 1998).

Chitosan is non-toxic and easily bioabsorbable with gel-forming ability at low pH. Moreover, chitosan has antacid and antiucler activities, which prevents or weakens drug irritation in the stomach. Also, chitosan matrix formulations appears to float and gradually swells in an acid medium. All these interesting properties of chitosan make this natural polymer suitable for controlled drug release formulations (Kumar, 2000). Chitosan has many useful physical properties in the delivery of drugs. One of the first considerations leading to the development of biologically active polymers was the hope of achieving controlled release of active substances. A controlled release system is one which regulates or controls the release of some type of biologically active agent. A number of chitosan-controlled drug delivery systems have been successfully developed to control the rate of drug administration and to prolong the duration of therapeutic action as well as to target the delivery of drugs.

Risbud *et al.* (2000) investigated a pH-sensitive freeze-dried and air-dried hydrogels of chitosan and poly(vinyl pyrolidone) for antibiotic delivery. Amoxicillin was used as model drug. It was found that freeze-dried hydrogels exhibited superior pH-dependent swelling properties over non-porous air-dried hydrogels. Therefore, the amounts of amoxicillin released from freeze-dried hydrogels was higher than those from air-dried hydrogels, which may suggest that freeze-dried hydrogels could serve as potent candidates for antibiotic delivery in an acidic environment.

Gupta and Kumar (2000) studied drug release behavior of chitosan beads and microgranules by using diclofenac sodium as model drug. The release rate of diclofenac sodium from the beads was found to be slower in comparison to the microgranule. The percent and the amount of diclofenac sodium release were much higher in acidic solution than in basic solution due to the swelling property of the matrix at acidic pH.

Puttipipatkhachorn *et al.* (2001) studied the drug-polymer interaction and drug release behavior from chitosan films. Four different grades of chitosan varying in molecular weight and degree of deacetylation were used. The model drugs used were salicylic acid and theophylline. The results of Fourier Transform Infrared Spectrum and solid-state ^{13}C NMR spectroscopy demonstrated the drug polymer interaction between salicylic acid and chitosan, whereas no drug-polymer interaction was observed in theophylline-loaded chitosan films. Most chitosan film loaded with either salicylic acid or theophylline exhibited a fast release pattern in distilled waters. The sustained release action of salicylic acid from the high viscosity chitosan films was due to the drug-polymer interaction.

Zhang *et al.* (2001) prepared microcapsules of chitosan/sodium carboxymethylcellulose (NaCMC). The swelling behavior, encapsulation efficiency, and release behavior of the microcapsules with different chitosan contents and pH conditions were investigated by using bovine serum albumin (BSA) as a model compound. It was found that the microcapsules have a high encapsulation efficiency (75%). The BSA in the microcapsules was speedily released at pH 7.2 and hardly released at pH 1.0. The BSA release was reduced with increase of the chitosan content from 17 to 38%.

Citrate cross-linked chitosan films for drug controlled release were prepared by Shu *et al.* (2001). The swelling ratio of citrate/chitosan films was sensitive to pH, ionic strength. It was found that the lower concentration and the higher pH of citrate solution resulted in a larger swelling ratio and quicker riboflavin release. Heparin, pectin, and alginate were used to improve the drug controlled release properties of citrate/chitosan film by further coating on the film surface. It was found that among them only the coating of alginate prolonged riboflavin release noticeably.

2.3 Controlled Drug Delivery Systems

Controlled drug delivery technology represents one of the most rapidly advancing areas of science in which the chemists and chemical engineers are contributing to human health care. Controlled drug delivery occurs when a polymer, whether natural or synthetic, is judiciously combined with a drug or other active agent in such a way that the active agent is released from material in a predesigned manner. The release of the active agent may be constant over a long period, or it may be triggered by the environment or other external events.

The benefit behind controlling the drug delivery is to achieve more effective therapies while eliminating the potential for both under- and overdosing. Other advantages of using controlled delivery systems can include the maintenance of drug level within a desired range, the need for fewer administrations, optimal use of the drug in question, and the increase in the patient compliance.

The goal of many of the original controlled-release release systems was to achieve a delivery profile that would yield a high drug level over a long period of time. With traditional tablets or injections, the drug level in the blood follows the profiles shown in Figure 2.5 (a), in which the level rises after each administration and then decreases until the next administration. The key point with traditional drug administration is that the level of the agent should remain between a maximum value, which may represent a toxic level, and a minimum level, below which the drug is no longer effective. In controlled release system designed for long term administration, the drug level in the blood follows the profile shown in Figure 2.5 (b), remaining constant, between the desired maximum and minimum, for an extended period of time.

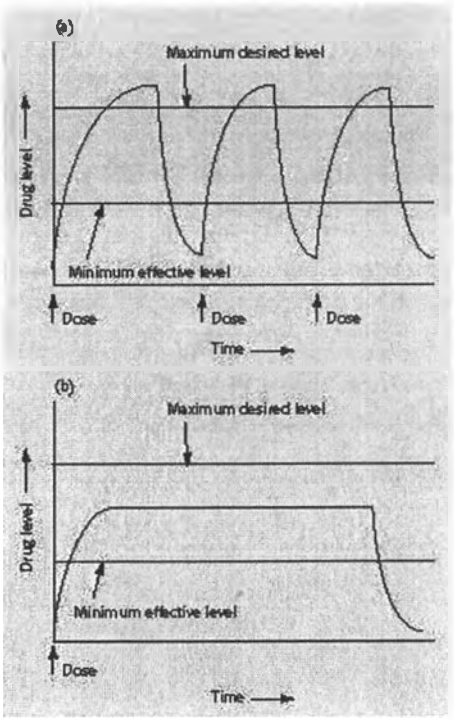

Figure 2.5 Drug level in the blood from (a) traditional drug administration and (b) controlled delivery dosing.

There are three primary mechanisms by which active agents can be released from a delivery system described as follows.

2.3.1 Diffusion Controlled Release

Diffusion occurs when a drug or other active agent passes through the polymer that forms the controlled-release device. The diffusion can occur on a macroscopic scale—as through pores in the polymer matrix—or on a molecular level, by passing between polymer chains. Examples of diffusion-release systems are shown in Figures 2.6. In this Figure, a polymer and active agent have been mixed to form a homogeneous system, also referred to as a matrix system. Diffusion occurs when the drug passes from the polymer matrix into the external environment. As the release continues, its rate normally decreases with this type of system, since the active agent has a progressively longer distance to travel and therefore requires a longer diffusion time to release.

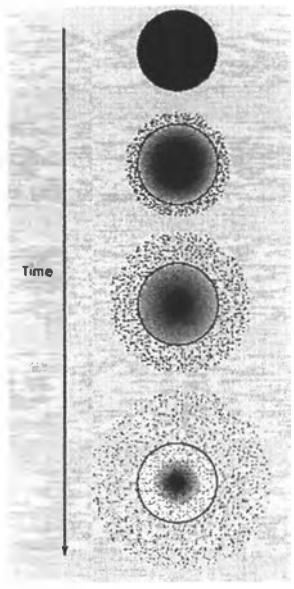

Figure 2.6 Drug delivery from a typical matrix drug delivery system.

For the diffusion-controlled systems described thus far, the drug delivery device is fundamentally stable in the biological environment and does not change its size either through swelling or degradation. In these systems, the combinations of polymer matrices and bioactive agents chosen must allow for the drug to diffuse through the pores or macromolecular structure of the polymer upon introduction of the delivery system into the biological environment without inducing any change in the polymer itself.

2.3.2 Swelling Controlled Release

It is also possible for a drug delivery system to be designed so that it is incapable of releasing its agent or agents until it is placed in an appropriate biological environment. Swelling-controlled release systems are initially dry and, when placed in the body, will absorb water or other body fluids and swell. The swelling increases the aqueous solvent content within the formulation as well as the polymer mesh size, enabling the drug to diffuse through the swollen network into the external environment. Most of the materials used in swelling-controlled release systems are based on hydrogels, which are polymers that will swell without dissolving when placed in water or other biological fluids. These hydrogels can absorb a great deal of

fluid and, at equilibrium, typically comprise 60–90% fluid and only 10–30% polymer. One of the most remarkable, and useful, features of a polymer's swelling ability manifests itself when that swelling can be triggered by a change in the environment surrounding the delivery system. Depending upon the polymer, the environmental change can involve pH, temperature, or ionic strength, and the system can either shrink or swell upon a change in any of these environmental factors. A number of these environmentally sensitive or "intelligent" hydrogel materials are listed in Table 2.1. For most of these polymers, the structural changes are reversible and repeatable upon additional changes in the external environment. The diagrams in Figure 2.7 illustrate the basic changes in structure of these sensitive systems. Once again, for this type of system, the drug release is accomplished only when the polymer swells. Because many of the potentially most useful pH-sensitive polymers swell at high pH values and collapse at low pH values, the triggered drug delivery occurs upon an increase in the pH of the environment. Such materials are ideal for systems such as oral delivery, in which the drug is not released at low pH values in the stomach but rather at high pH values in the upper small intestine.

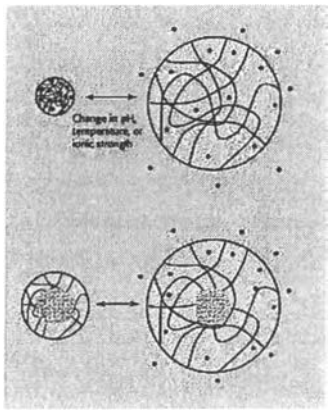

Figure 2.7 Drug delivery from environmentally sensitive release systems.

Table 2.1 Environmentally sensitive polymers for drug delivery

Stimulus	Hydrogel	Mechanism
pH	Acidic or basic hydrogel	Change in pH ------swelling-------- release of drug
Ionic strength	Ionic hydrogel	Change in ionic strength, change in concentration of ions inside gel ----change in swelling--- release of drug
Chemical species	Hydrogel containing electron accepting groups	Electron donating compounds, formation of change/ transfer complex ----change in swelling--- release of drug
Enzyme-substrate	Hydrogel containing immobilized enzymes	Substrate present, enzymatic conversion product changes ----change in swelling--- release of drug

2.3.3 Erosion Controlled Release

All of the previously described systems are based on polymers that do not change their chemical structure beyond what occurs during swelling. However, a great deal of attention and research effort are being concentrated on biodegradable polymers. These materials degrade within the body as a result of natural biological processes, eliminating the need to remove a drug delivery system after release of the active agent has been completed. Most biodegradable polymers are designed to degrade as a result of hydrolysis of the polymer chains into biologically acceptable and progressively smaller compounds. Degradation may take place through bulk

hydrolysis, in which the polymer degrades in a fairly uniform manner throughout the matrix, as shown schematically in Figure 2.8a. For some degradable polymers, the degradation occurs only at the surface of the polymer, resulting in a release rate that is proportional to the surface area of the drug delivery system (see Figure 2.8b).

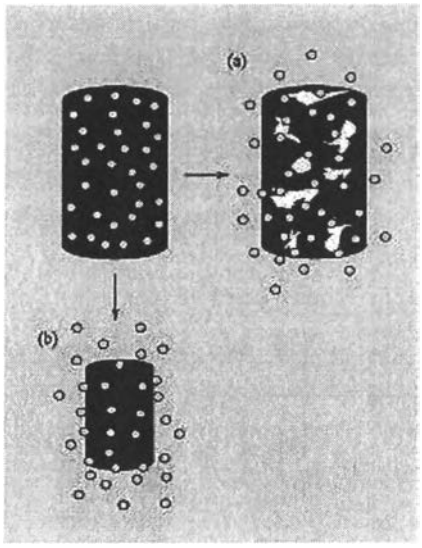

Figure 2.8 Drug delivery from (a) bulk erosion and (b) surface erosion systems.

2.4 Chitosan-Coated Calcium Alginate Based Material for Drug Delivery Studies

Murata *et al.* (1993) prepared chitosan-reinforced alginate gel beads and studied the release patterns of coloring matter (Brilliant Blue G) held within them. It was found that the release rates of Brilliant Blue G from the gel beads were much slower after an initial lag time when they were incubated with chitosan compared with the original intact gels prepared without chitosan. The initial release rates were reduced gradually in proportion to the increases in the chitosan concentration and incubation times used when preparing the gel beads. Furthermore, erosion of the gel beads was suppressed by chitosan treatment.

Huguet *et al.* (1994) prepared chitosan/calcium alginate beads for encapsulation of hemoglobin. It was found that the high concentration of hemoglobin was retained inside the beads. The effects of molecular weights of

chitosan (M_v 245,000 or 390,000) and pHs of its solution (2,4, or 5.4) on the encapsulation of hemoglobin were also studied. It was found that the best retention being obtained with beads prepared at pH 5.4. The hemoglobin released during the bead storage in water was found to depend on the conditions of their formation and especially on the chitosan molecular weight. The best retention during storage in water was obtained with beads prepared with the high viscosity-average molecular weight chitosan solution at pH 2.

Hari *et al.* (1996) prepared chitosan/calcium alginate beads for oral delivery of insulin and bovine serum albumin (BSA). It was found that the beads containing a high concentration of entrapped BSA as more than 70% of the initial concentration were achieved via varying chitosan coated. It was observed that approximately 70% of the content is being released into Tris-HCl buffer pH 7.4 within 24 h. Instead of BSA, the insulin preload was found to be very low in the chitosan/calcium alginate system. However, the release characteristics were similar to that of BSA.

Gonzalez-Rodriguez *et al.* (2002) prepared alginate/chitosan particles by ionic gelation (Ca^{2+} and Al^{3+}) for the sodium diclofenac release. The ability to release the active substance was examined as a function of pH of dissolution medium. It was found that the release of sodium diclofenac was prevented at acidic pH, while it was complete in a few minutes when pH was raised up to 6.4 and 7.2. The alginate/chitosan ratio and the nature of the gelifying cation allowed a control of the release rate of the drug.