

## REVIEW: CHITOSAN BASED HYDROGEL POLYMERIC BEADS – AS DRUG DELIVERY SYSTEM

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Chitosan obtained by alkaline deacetylation of chitin is a non-toxic, biocompatible, and biodegradable natural polymer. Chitosan-based hydrogel polymeric beads have been extensively studied as micro- or nano-particulate carriers in the pharmaceutical and medical fields, where they have shown promise for drug delivery as a result of their controlled and sustained release properties, as well as biocompatibility with tissue and cells. To introduce desired properties and enlarge the scope of the potential applications of chitosan, graft copolymerization with natural or synthetic polymers on it has been carried out, and also, various chitosan derivatives have been utilized to form beads. The desired kinetics, duration, and rate of drug release up to therapeutic level from polymeric beads are limited by specific conditions such as beads material and their composition, bead preparation method, amount of drug loading, drug solubility, and drug polymer interaction. The present review summarizes most of the available reports about compositional and structural effects of chitosan-based hydrogel polymeric beads on swelling, drug loading, and releasing properties. From the studies reviewed it is concluded that chitosan-based hydrogel polymeric beads are promising drug delivery systems.

*Keywords:* Chitosan; Hydrogel; Polymeric beads; Drug delivery system; Drug release

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### INTRODUCTION

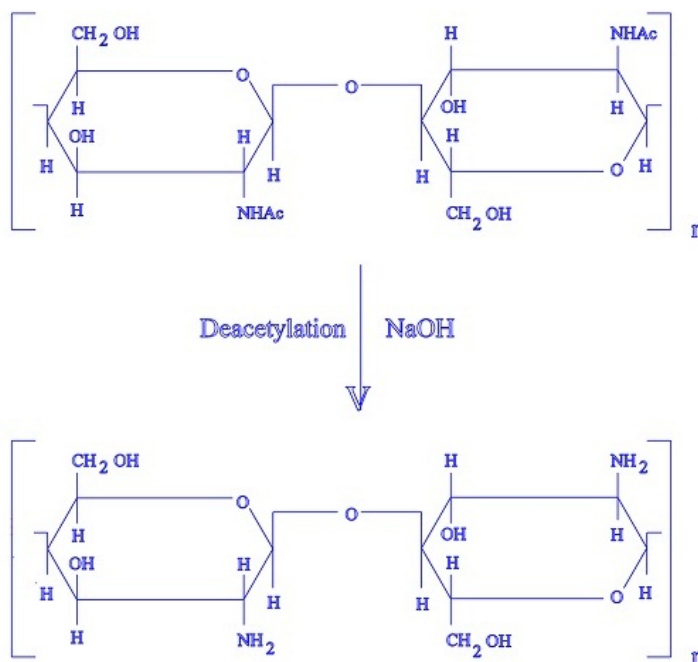
Recently efforts have been made to design novel drug dosage formulations so that more and more effectiveness can be achieved relative to the conventional dosage forms. To achieve this goal, controlled release technology was originated in 1980 that developed the commercial methodology by which predecided and reproducible release of a drug up to therapeutic level into a specific environment over a prolonged time period could be maintained. Such drug delivery systems function according to the changes in physiological signals within the body and target the drug for the site of action to minimize any side effects. Controlled release medications thus reduce toxicity and enhance the efficacy, safety, and reliability of chemotherapy, which results in improvements in patient compliance and convenience (Langer 1998; Brouwers 1996). By now the concept of a drug delivery system has become well known and is utilized in the treatment of a variety of diseases (Labhasetwar et al. 1997). Nano- and micro-beads of polymers have been formulated using either synthetic or natural polymeric material (Jagur-Grodzinski 1999; Uhrich et al. 1999; Kumar and Kothari 1999). Drug release from such

types of formulated polymeric beads in which a drug is entrapped by polymeric material involves its diffusion from or through polymeric material slowly and in a controlled manner. Dispersing a drug in a polymeric matrix or covalently attaching drugs to biodegradable polymer may also be able to release them by erosion/degradation of such macromolecules. Therapeutic molecules complexed by polymers capable of forming a gel may also be released by diffusion. Hence, drug delivery systems require a polymeric matrix that is non-toxic, biocompatible, and biodegradable.

## **CHITOSAN**

Biodegradable polymers derived from renewable sources have recently generated much interest for pharmaceutical applications either in drug transport or in controlled release in vivo (Zhu et al. 2006; Atyabi et al. 2005; Lin et al. 2005; Ruel-Gariepy et al. 2004; Tapia et al. 2004; Thierry et al. 2005; Van der Merwe et al. 2004). Chitosan is regarded as such a valuable natural biocompatible polymer because it is nontoxic, biodegradable (George and Abraham 2006; Gan et al. 2005), mucoadhesive (He et al. 1998; Schnurch et al. 1998), easily bioabsorbable (Yalpani et al. 1992), and also possesses gel-forming ability at low pH (Tseng et al. 1995). Moreover, it has antacid and antiulcer activities (Ito et al. 2000), which prevent or weaken drug irritation in the stomach. All of these interesting properties of chitosan make this natural polymer an ideal element for formulating drug delivery devices (Yalpani et al. 1992; Illum 1998; Chandy and Sharma 1990, 1991; Graham 1990; Gupta and Ravi Kumar 2000a; Mi et al. 2002a). This material has also been used for several other biomedical purposes, e.g. in gene therapy (Danielsen et al. 2004; Mansouri et al. 2004), gene delivery (Wong et al. 2006), for sutures and wound healing materials (Okamoto et al. 1992), skin culture (Damour et al. 1994; Stone et al. 2000; Mao et al. 2003), vascular grafting (Zhu et al. 2005), nerve cell generation (Zielinski and Aebischer 1994), cartilage regeneration (Iwasaki et al. 2004), vaccine delivery (Lubben et al. 2002; Zaharoff et al. 2007; Amidi et al. 2007), and making contact lenses (Markey et al. 1989). Besides pharmaceutical applications it has been used in pollution control to remove toxic metal (Kumar et al. 1998; Nair and Madhavan 1984) and dyes (Ravi Kumar et al. 1998; Harry 1989; Weber 1992; Sosulski 1962; Dutta and Ravi Kumar 1998; Knorr 1982, 1983), in photography (Muzzarelli 1997) to improve quality of film, in cosmetics (Mark et al. 1985) due to its fungicidal and fungistatic properties, as a food and nutrition supplement (Knorr 1991; Nicol 1991; Spreen et al. 1984; Zikakis et al. 1982), and for paper finishing and sizing (Ashori et al. 2005).

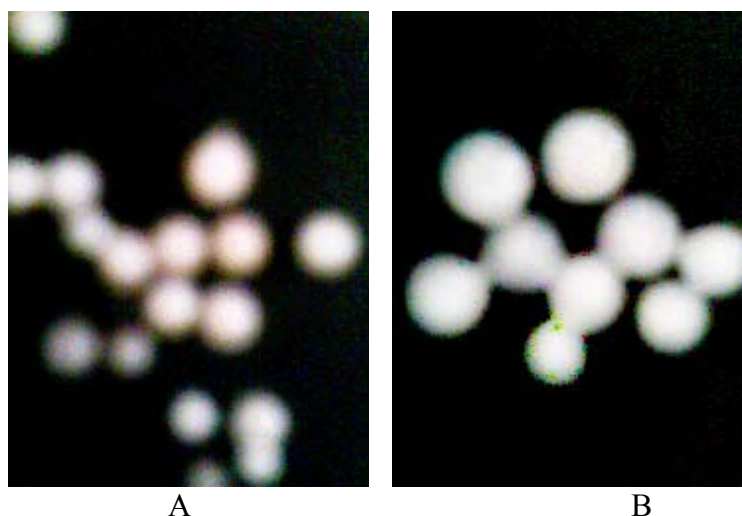
Chitosan is obtained by N-deacetylation of chitin, which is a naturally abundant muco polysaccharide that forms the exoskeleton of crustaceans, insects, etc. It is well known to consist of 2-acetamido 2-deoxy- $\beta$ -D-glucose through a  $\beta$  (1 $\rightarrow$ 4) linkage (Ravi Kumar 2000). N-deacetylation of chitin is carried out by alkaline hydrolysis with NaOH at 120°C for 1-3 hr according to the chemical reaction shown in Scheme 1. The alkali treatment produces 40-80% deacetylated chitosan. Thus, chitosan is a hetero-polymer having (1 $\rightarrow$ 4) 2-amino 2-deoxy  $\beta$ -D-glucose units together with (1 $\rightarrow$ 4) 2-acetamido-2-deoxy- $\beta$ -D-glucose units of the original chitin in polymeric chain.



**Scheme 1.** Deacetylation of chitin into chitosan

The ratio of 2-amino-2-deoxy- $\beta$ -D-glucose units to 2-acetamido-2-deoxy- $\beta$ -D-glucopyranose is an important parameter called the degree of deacetylation, which determines the polymer's solubility and solution properties. A number of analytical methods for determining the degree of deacetylation (Baxter et al. 1992; Maghami and Roberts 1988; Domard 1986; Domard 1987; Wei and Hudson 1993; Sashiwa et al. 1991; Sashiwa et al. 1993; Raymond et al. 1993; Niola et al. 1983; Pangburn et al. 1984; Raathke and Hudson 1993) have been reported. When chitin is converted into chitosan, the molecular weight is lowered due to deacetylation, and the charge distribution in acidic solvent is changed due to amino group formation. The average molecular weight of chitosan has been determined using high performance liquid chromatography (Wu 1998), light scattering (Muzzarelli et al. 1987), and viscometry methods (Chen and Hwa 1996).

Chitosan is a highly basic polysaccharide, so it can form poly-oxysalts, films, chelate metal ions, and optical structures (Hench 1998). It is soluble in dilute acids such as acetic acid, formic acid, etc. It has the characteristic of forming hydrogels that are highly swollen hydrophilic polymer networks, capable of absorbing large amounts of water, such that they have become widely used in controlled release systems (Kost et al. 1987). Figure 1 shows dried chitosan beads and swollen beads after absorption of water. Recently, pH-sensitive hydrogels (Yao et al. 1994) have exhibited potential for use in site-specific delivery of drugs. Some of the most appealing characteristics of chitosan are its bioadhesive properties and its ability to promote cell proliferation and consequently, tissue regeneration (Berger et al. 2004a,b). These properties of chitosan are enhanced upon decreasing the polymer's degree of acetylation (Amaral et al. 2005a,b) and are of utmost importance for biomedical engineering. Further, owing to its particular structure and self-assembly properties, chitosan has proved to be a useful excipient in the pharmaceutical industry (Bernkop-Schnurch 2000; Bernkop-Schnurch and Kast 2001; Janes et al. 2001a; Krauland et al. 2006; Takeuchi et al. 2005).



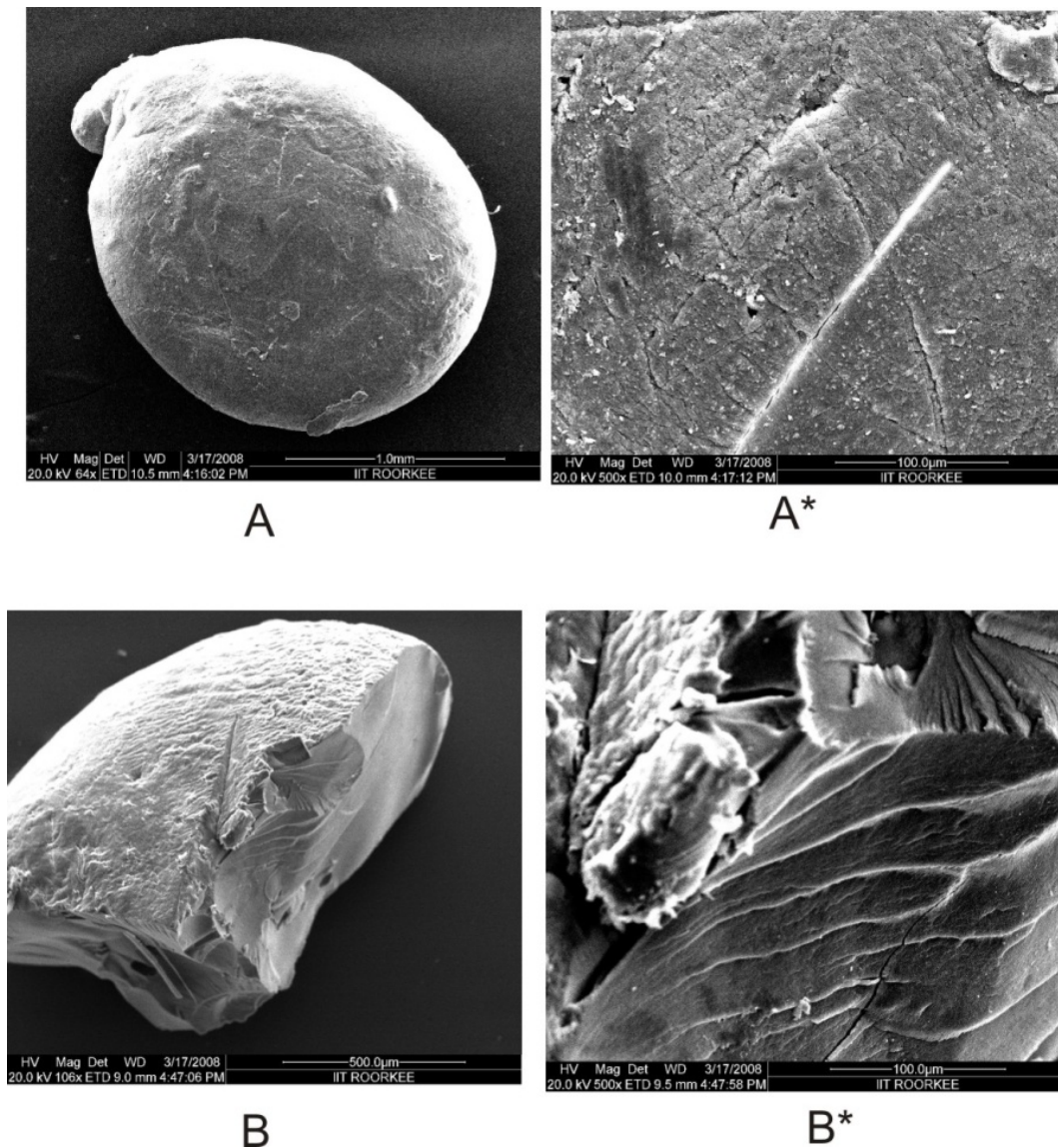
**Figure 1.** Photographs for chitosan hydrogel beads (A) and swollen beads (B) after absorbing water

The poor solubility of unmodified chitosan in both water and organic solvents make its utilization limited in the pharmaceutical field. One of the reasons for the intractability of chitosan lies in its rigid crystalline structure that is related to the acetamido and primary amino groups that induce relevant conformational features through intra- and /or inter-molecular hydrogen bonding (Nishimura et al. 1991). Replacement of one or both of the primary amino group's hydrogens by other substituents, through suitable chemical modification, is a possible means for partial destruction of its rigid crystalline structure, increasing the solubility of the polymer in aqueous or organic media (Liu et al. 2005). Numerous efforts have been made to prepare functional and soluble derivatives of chitosan (Delben et al. 1994; Kurita et al. 2002; Muzzarelli 1988; Muzzarelli et al. 1984; Skorik et al. 2003). Thus, chemical modifications have been carried out on the chitin and chitosan glucopyranoside ring, conferring to the new materials surprising properties and additional possibilities of engaging in a wide range of chemical reactions (Holappa et al. 2004, 2005, 2006).

## CHITOSAN-BASED HYDROGEL POLYMERIC BEADS

Chitosan-based hydrogel beads are solid, spherical, micron- or nano-sized drug carrier particles constituting a matrix type of structure. SEM micrographs of external and internal structures of pure chitosan beads crosslinked with glutaraldehyde are shown in Fig. 2. The drug may be either absorbed in the spherical beads or entrapped within them. In other words, these are just like a vesicular system, where the drug exists within a cavity surrounded by the polymeric solid. These polymeric beads are advantageous over pellets due to their relatively higher intercellular uptake. Their charge properties influence the uptake by intestinal epithelia. The beads obtained from hydrophobic polymers have been found to exhibit higher uptake as compared to the beads prepared from more hydrophilic surfaces (Jung et al. 2000). So nano-/ micro-beads surface charges

and increased hydrophobicity of the polymeric matrix have been found to be effective for the gastrointestinal uptake in a positive sense. A number of methods for preparing beads from various polymers (Reis et al. 2006; Kreuter et al. 1983; Soppimath et al. 2001) have been reported in the literature. Various methods for the preparation of chitosan beads are described (Agnihotri et al. 2004; Aktas et al. 2005). Sinha et al. (2004) has also described various methods for loading different categories of drugs in chitosan-based beads, which may release the drugs to specific regions of living beings in a controlled manner. They also discussed different parameters affecting the entrapment efficiency of the drug in chitosan beads and also the release characteristics of drugs from beads. It is well known that drug release from hydrogels beads depends on their chemical properties in response to pH (Mitra et al. 1984; Kost 1990).



**Figure 2.** SEM micrographs showing external morphology (A) with its magnification (A\*) and internal structure (B) with its magnification (B\*) of pure chitosan bead crosslinked with glutaraldehyde

Chitosan-based beads have been formulated for controlled release of drugs, as beads formulated from chitosan matrix appear to float and gradually swell in an acidic medium. Recently, nano- or micro-beads have acquired great importance, because of having a tendency to accumulate in an inflamed area of body. Beads, due to their attractive properties and applicability, have secured a unique position in drug delivery technology. Several chitosan-based hydrogel polymeric beads useful as drug delivery system have been discussed in the literature and may be now classified on the basis of material used to prepare them as follows:

### **Pure Chitosan Beads**

Very simple coacervation methods for the preparation of chitosan beads have been adopted by scientists (Nishimura et al. 1986; Genta et al. 1998). In this method, the solution of chitosan is prepared in aqueous acetic acid or formic acid. This solution is then blown into NaOH or NaOH-methanol using a compressed air nozzle. In this way, tiny droplets or coacervates are obtained, which are finally filtered and washed with hot and cold water successively. The diameter of the tiny droplets can be controlled by varying the extrusion rate of chitosan solution or the nozzle diameter. Chitosan beads formed in this way possess porous surfaces and are applicable in various fields, e.g. immobilization of protease (Hayashi and Ikada 1991), carriers for the cancer drug adriamycin (Nishimura et al. 1986), oral sustained delivery of nefedipine (Chandy and Sharma 1992), ampicillin (Chandy and Sharma 1993), and various steroids (Chandy et al. 1987). Xu and Du (2003) studied the effect of molecular structure of chitosan on release rate of protein from chitosan nanoparticles.

The hardening of the above-described tiny chitosan beads can be carried out by crosslinking with glutaraldehyde, glyoxal, or glycol. Chitosan-crosslinked beads with glutaraldehyde have been studied by Jameela and Jayakrishnan (1995) and Kulkarni et al. (2007); containing mitoxantrone and diclofenac sodium drugs, respectively. Yao et al. (1995) have studied the release profiles of rotundine drug from chitosan beads crosslinked with glutaraldehyde and found higher release in acidic medium (pH 1-2) than in alkaline medium (pH 7.2 - 7.4). They have also prepared crosslinked chitosan beads coated with polysaccharide (carboxy methyl chitin etc) and lipids (e.g dipalmitoyl phosphatidyl choline) separately for intelligent drug delivery of 5-fluorouracil (5-Fu) and concluded that polysaccharide-coated beads were more promising. Agnihotri and Aminabhavi (2004) have also carried out studies for controlled release of clozapine from chitosan beads crosslinked with glutaraldehyde and found that the release of clozapine was dependent on the extent of matrix crosslinking and the amount of drug loading. Glutaraldehyde and glyoxal crosslinked beads have been prepared using chitosan with different molecular weights and degree of deacetylation for sustained release of centchroman by Gupta and Jabrail (2006), who found that the glyoxal-crosslinked chitosan beads were more compact and hydrophobic and showed better sustained release as compared to glutaraldehyde-crosslinked beads. They concluded that chitosan with medium molecular weight ( $1134 \text{ kg mol}^{-1}$ ) and 62% (w/w) degree of deacetylation showed improved controlled release characteristics for centchroman. Chitosan gel beads have also been prepared by ionotropic crosslinking or interpolymer linkage with tripolyphosphate (TPP) (Alsarra et al. 2004; Mi et al. 1999a; Shiraishi et al. 1993). The



influence of the pH, TPP concentration, and ionic strength of the gelling medium in the entrapment, release, and activity of the drug in chitosan hydrogels have been monitored.

### **Chitosan /Natural Polymer Beads**

Several reports are found in the literature in which beads have been prepared using chitosan with natural polymers, e.g. alginate, gelatin, cellulose derivatives, etc. Some of them are described here.

#### *Chitosan alginate beads*

Haemoglobin encapsulation in chitosan-calcium alginate beads was reported by Huguet et al. (1994). The loading and release of haemoglobin was found to be dependent on the molecular weight of chitosan and the pH of the releasing medium. Chitosan-alginate beads have also been evaluated for proteins by Polk et al. (1994). Aral and Akbuga (1998) have prepared alginate-reinforced chitosan gel beads using bovine serum albumin for protein release as a model protein and studied the effects of different factors such as initial BSA amount, sodium alginate concentration, the addition of pectin, and glutaraldehyde on the properties of the formulated beads.

Chitosan / calcium alginate beads for entrapment of biological macromolecules such as albumin and insulin were investigated by Hari et al. (1996). The microcapsules were obtained by adding dropwise a protein-containing sodium alginate mixture into a chitosan and calcium chloride system. The beads contained a high concentration of entrapped bovine serum albumin (BSA), as more than 70% of the initial concentration was achieved by varying the chitosan coat. It was observed that approximately 70% of the content was being released into Tris-HCl buffer of pH 7.4 within 24 hours. But the acid-treated beads had released almost 100% entrapped protein into tris-HCl pH 7.4 media within 24 hours. The insulin preload was found to be very low in the chitosan/calcium alginate system. Coppi et al. (2001) have also prepared chitosan alginate microparticles as a protein carrier.

Mi et al. (2002a) have prepared crosslink-reinforced chitosan alginate complex beads by dropping chitosan droplets into alginate–crosslinker co-existing gelling solution. A novel naturally occurring material genipin extracted from gardenia fruit was used as a crosslinking agent. The swelling ratio of the crosslinked reinforced chitosan alginate complex bead decreased as the pH or concentration of alginate in the gelling solution was decreased. Contrary to the swelling properties, the rate of indomethacin drug released out of the chitosan–alginate complex beads increased with the decrease in pH or concentration of alginate in the gelling solution, due to the decreased crosslinking of the beads.

Murata et al. (2000) obtained chitosan-alginate dried beads constituting metronidazole. They investigated the release profile of metronidazole and also its delivery to the stomach mucosa by oral administration to guinea pigs. The chitosan alginate beads floated on the gastric juice and released the drug into the stomach.

#### *Chitosan/gelatin beads*

A chitosan/gelatin network of polymeric beads for controlled release of cimetidine has also been investigated by Yin et al. (1996). To prepare drug-loaded beads, chitosan and gelatin (1:1 by weight) in addition to cimetidine were dissolved in 5% acetic acid, Tween 80. Liquid paraffin at a water:oil ratio of 1:10 was added to this mixture with

agitation at 30°C. Aqueous glutaraldehyde was then added to the inverse emulsion and kept for 2 hours. Finally, liquid paraffin was evaporated under vacuum to obtain beads. Drug-release experiments were performed in acidic (pH 1.0) and alkaline (pH 7.8) solution. The release rates were found to be dependent on pH of release medium, composition of beads, and degree of deacetylation of chitosan. Yao et al. (1995) also investigated a novel hydrogel of chitosan/gelatin crosslinked with glutaraldehyde. Levamisole, cimetidine and chloramphenicol were used as model drugs. A pH-dependent release of these drugs from gel was reported.

Scientists (Shu and Zhu 2000) obtained drug (fluorescein, isothiocyanate dextran and brilliant blue) loaded chitosan-gelatin beads under coagulation conditions at 4°C, crosslinked with tripolyphosphate. The loading efficiency of the model drug was varied by more than 90%. Effects of crosslinking time, tripolyphosphate solution, and pH were investigated. The release period of brilliant blue was more than 2 months at a fairly constant rate in 0.9% NaCl at pH 7.4, but for fluorescein isothiocyanate dextran it was only 1-2 days under the same conditions.

#### *Chitosan/methyl cellulose beads*

Rokhade et al. (2006) prepared novel interpenetrating polymer network (IPN) beads of chitosan and methyl cellulose by emulsion crosslinking in the presence of the crosslinker glutaraldehyde. Theophylline, an antiasthmatic drug, was incorporated into IPN beads under varying composition of methyl cellulose and chitosan, percent drug loading, and amount of glutaraldehyde added. The IPN had better mechanical properties than pure chitosan beads, indicating the suitability of the beads. Beads with spherical shape having smooth surfaces were produced with the size range of 119 - 318 µm. The release of the drug was found to be dependent on the extent of matrix crosslinking, the amount of drug loading, and the methyl cellulose content of the matrix.

#### *Chitosan / carboxy methyl konjac glucomannan beads*

Carboxymethyl konjac glucomannan (CKGM) is a water-soluble hetero-polysaccharide extracted from the konjac plant that grows mainly in Asia. It has 1,4 linked β-D-mannopyranose and β-D-glucopyranose units in a molar ratio of 1.6:1 with a low degree of acetyl groups as side substituents (6 position) (Li et al. 2004). Due to its high viscosity, stability, and emulsifiability in aqueous solution, it shows promise in biomedical applications, e.g. drug delivery (Wang et al. 2002; Pathak and Barman 2003; Slepian and Massia 2001) and cellular therapy. Du Jian et al. (2006) obtained pH-sensitive polyelectrolyte carboxymethyl konjac glucomannan-chitosan beads as a drug carrier for bovine blood protein (BSA). The formation and properties of polymer complexes depend on the charge ratio of anionic to cationic polymer, the degree of neutralization, the ionic strength, and the valency of simple ions in the electrolyte solution. When chitosan was dropped into carboxymethyl konjac glucomannan solution, inter- and intra-molecular electrostatic attractions occurred between the anionic carboxyl groups from carboxy methyl konjac glucomannan and cationic amino groups of chitosan, which are responsible for making the macromolecular curled chains of chitosan and carboxymethyl konjac glucomannan, and which ultimately give rise to an insoluble carboxymethyl konjac glucomannan and chitosan complex. It was found that the swelling rate of beads was larger in alkaline medium (pH 7.4) than in acidic medium (pH 5.3) and was the



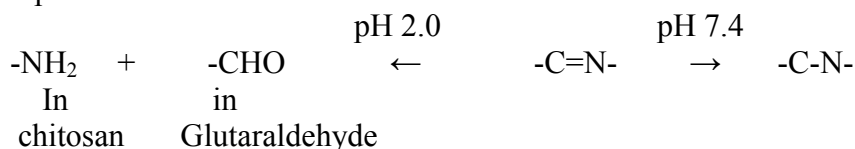
smallest at pH 3.5. Drug release profiles for BSA were studied at pH 1.2, 5.0, and 7.4.

### Chitosan – Synthetic Polymer Beads

Stimuli-sensitive materials that are able to modify their conformation and properties in response of changes in different physiological variables are receiving increasing attention as components of biosensors and therapeutic devices. In particular, polymers that have a low critical solubility temperature or with ionisable groups, which provide a network that undergoes reversible phase transitions, show an enormous potential to develop drug delivery systems useful to control both the site and release rate of drugs (Yuk and Bae 1999; Peppas et al. 2000; Soppimath et al. 2002). Hence, the incorporation of synthetic co-monomer is utilized to obtain pH sensitivity and also to provide a sufficient loading capacity (Alvarez-Lorenzo and Concheiro 2002; Moritani and Alvarez-Lorenzo 2001). An interpenetrating network is an assembly of two crosslinked polymers, at least one of which is synthesized and crosslinked in the presence of the other. If only one component of the assembly is crosslinked, leaving the other in linear form, the system is termed a semi-IPN (Sperling 1981). If both of the components of the assembly are crosslinked, the system is termed a full IPN. Chitosan beads obtained in combination with various synthetic polymers reported in literature are represented here:

#### *Chitosan – poly (ethylene glycol) beads*

Semi-interpenetrating polymer network beads of chitosan and poly-(ethylene glycol) for oral sustained drug delivery were synthesized by Gupta and Ravi Kumar (2001a,b). A viscous homogeneous solution of chitosan and poly-(ethylene glycol) was extruded in the form of droplets into NaOH-methanol solution using a syringe and then the particles were crosslinked using glutaraldehyde. They suggested that crosslinking of biodegradable polymer was potentially important to control the degradation rates and rate of swelling of the matrix and thus, the release of drug is dependent on the degree of crosslinking and solution pH. The crosslinking also helped in optimizing drug entrapping capacity and its sustained release for an extended period. The structural changes in the beads were investigated using infra-red spectroscopy. It was concluded that during crosslinking, formation of the C=N group of the imine involved a reaction between the amino group of chitosan with aldehyde group of glutaraldehyde in chitosan-poly-(ethylene glycol). A complexation through cooperative hydrogen bonding also took place at pH 2.0. The imine group in the network was found to be protonated as a result of the hydrogen bonding dissociation, promoting swelling of the beads. The formation of the  $\text{NH}_3^+$  group within the beads when swollen in pH 2.0 solution was supported by IR data. The rate of swelling was slower in pH 7.4 solution than in the case of pH 2.0. It was found that imine groups within beads were not protonated at pH 7.4, leading to a lower swelling of the beads, and at pH 7.4 the C=N bond did not break on swelling but was transformed from C=N to C-N. The changes of the imine bond within the network at pH 2.0 and 7.4 can expressed as follows:



#### *Chitosan - poly (ethylene oxide) beads*

These hydrophilic, spherical micro-beads were found to have potential application for the administration of drug molecules (Jagur-Grodzinski et al. 1999; Kreuter 1998). The preparation of hydrophobic-hydrophilic polymeric beads as drug carriers required the use of an organic solvent. They have a limited protein loading capacity (Vandorpe et al. 1997; Amiji and Park 1994; Lehr et al. 1992; Pornsak 1996). To overcome these limitations, Calvo et al. (1997a) reported the preparation of beads made completely from hydrophilic polymer. They reported a new approach of preparation technique based on an ionic gelation process. The method constituted of mixing of two aqueous phases, one containing chitosan and other a di-block copolymer of ethylene oxide and the poly-anion sodium triphosphate at room temperature. These beads had a greater protein loading capacity (80% of bovine serum albumin) and provided a continuous release for up to 1 week.

#### *Chitosan – $\beta$ - glycerol phosphate beads*

A novel approach to obtain a pulsatile delivery system for insulin was developed by Kashyap et al. (2007). Pulsatile release is defined as the rapid and transient release of a drug within a short time period of release immediately after a predetermined pre-release period. Pulsatile release is observed during and after intake of food when blood glucose level rises steeply. Pulsatile delivery of insulin has a greater hypoglycemic effect and is more efficient in controlling diabetes in comparison to continuous delivery (Koopmans et al. 1996). In such an intelligent insulin delivery self-regulated system, the drug release rates are adjusted by the system in response to the feedback information (Hisamitsu et al. 1997; Podual et al. 2000a). These systems are capable of adjusting drug release to the physiological needs of body without external intervention. These are enzymatically controlled self-regulated systems that have high specificity of interaction with their substrate. They react with a desired substrate by containing a specific enzyme and then releasing the drug. Kashyap et al. (2007) used glucose oxidase as a glucose sensor in chitosan- $\beta$ -glycerol phosphate beads. Glucose oxidase (Shiino et al. 1995; Podual et al. 2000b) is one of the most extensively studied sensors, which has to be incorporated into a hydrogel for sensing glucose level and modulating insulin release accordingly. The pH-sensitive hydrogel containing immobilized glucose oxidase oxidizes glucose to gluconic acid in the presence of oxygen. Formation of gluconic acid results in a decrease of pH, which triggers swelling in the pH-sensitive hydrogel, producing a change in pore size and consequently a change in the release of insulin by a diffusion process.

#### *Chitosan –poly acrylic acid beads*

Peniche et al. (1999) and Borzacchiolo et al. (2001) reported the preparation and characterization of chitosan/polyacrylic acid networks by the free-radical polymerization of acrylic acid in the presence of chitosan. The reaction proceeds at 37°C, indicating that chitosan acts as an activator for the decomposition of the initiator at low temperature. They also reported that during polymerization under mild conditions some grafting of polyacrylic acid chains onto chitosan molecules produced super-absorbent hydrogels, which showed water uptake rates as high as 560 g of water per gram of drug sample. The

mechanical properties of these gels were enhanced by copolymerization of acrylic acid with methyl acrylate without loss of the swelling property (Borzacchiolo et al. 2001). Chitosan-polyacrylic acid beads were prepared by a one-step method using inverse suspension free-radical polymerization of acrylic acid in the presence of chitosan, involving sunflower oil as the continuous phase (Peniche et al. 2003). These beads have been evaluated as a drug delivery system for meclofenamic acid drug. The swelling was found to be pH-dependent; however, the release of meclofenamic acid from beads with the drug was modulated by the water solubility of the drug.

#### *Chitosan –poly (ethylene oxide-g-acryl amide) beads*

Agnihotri and Aminabhavi (2006) synthesized capecitabine-loaded semi-interpenetrating network hydrogel beads of chitosan-poly-(ethylene oxide g-acrylamide) by emulsion crosslinking using glutaraldehyde. Poly-(ethylene oxide) was grafted with poly-acrylamide by free-radical polymerization using ceric ammonium nitrate as a redox initiator. Capecitabine is an anticancer drug and is converted to fluorouracil in the body tissues following the administration. It is widely used in the treatment of metastatic colorectal cancer and breast cancer, since it is readily absorbed from the gastrointestinal tract. The recommended daily dose is large, i.e. 2.5 g/m<sup>2</sup>, with a short elimination half life of 0.5-1h (Judson et al. 1999). Formulation of controlled released dosage for capecitabine is necessary to minimize its adverse effects, such as bone marrow depression, cardiotoxicity, diarrhoea, nausea and vomiting, stomatitis, dermatitis, etc., and to remain at the therapeutic limits for a longer time. Agnihotri and Aminabhavi (2006) successfully loaded capecitabine into beads by changing experimental variables such as grafting ratio of the graft copolymer to chitosan, the amount of crosslinking agent, and the percentage of drug loading in order to optimize process variables on drug encapsulation efficiency, release rate, size, and morphology of the beads. Encapsulation efficiency was found to be 70 to 87 %. The release of capecitabine was found to continue up to 10 h.

#### *Chitosan –acrylamide –poly (vinyl alcohol) beads*

The hydrophilicity of chitosan, due to the presence of amino and hydroxyl functional groups in its repeating unit, makes it soluble in dilute acidic solutions and gives rise to a rubbery hydrogel in neutral water. Poly-(vinyl alcohol), on the other hand, has some preferable physical properties, such as elasticity and high hydrophilicity (Schellekens and Bastiansen 1991), making it appropriate to blend with other polymers like chitosan. Logically, blending of chitosan with acrylamide-grafted poly-(vinyl alcohol) produces a biodegradable blend system, which was studied by Rao et al. (2006) for control release of cefadroxil, an antibiotic drug. The rate of drug release was found to be pH-sensitive and to continue for more than 10 hours.

#### *Chitosan–poly(vinyl pyrrolidone) beads*

To improve the hydrophilicity of chitosan, a suitable amount of hydrosoluble polymer is added to obtain a semi-interpenetrating network (Liu et al. 2001; Li et al. 2002; Patel et al. 1996; Risbud et al. 2000) with improved swelling behaviour throughout the complete pH range. Risbud et al. (2000) studied semi-interpenetrating networks of chitosan and poly-(vinylpyrrolidone). Marsano et al. (2005) obtained crosslinked chitosan-poly-(vinylpyrrolidone) beads using ethylene glycol diglycidyl ether as

crosslinker. Different ratios of crosslinker/chitosan were used to modulate the extent of crosslinking in the final beads. The beads showed the capability of reversible swelling in water at room temperature at pH 7.0.

### **Chitosan Derivatives Beads**

Owing to its preferable characteristics, chitosan is very important for medical and pharmaceutical purposes. However, its activities are limited to acidic conditions because of its poor solubility above pH 6.5, where chitosan starts to lose its cationic nature (Marsano et al. 2005; Jae et al. 2003; Tsai and Su 1999; Helander et al. 2001). Chitosan is generally insoluble under physiological conditions because of a strongly hydrogen bonding network structure; thus further investigations for the biological activities and development have been restricted. To overcome these shortcomings, various derivatives have been synthesized by chemical modification, and such derivatives have proved to be valuable as drug carriers. In the drug delivery system, chitosan and its derivatives have been proposed as matrices in numerous pharmaceutical formulations in the form of gels, films, emulsions, transmucosal devices, microspheres, and so on for the prolonged release of drugs (Sezer and Akbuga 1999; He et al. 1999), delivery of vaccines (Alpar et al. 2001; Mi et al. 1999b), DNA (Alaxakis et al. 1995), insulin (Hari et al. 1996), and anticancer agents such as 5 fluoracil (Ohya et al. 1993), among others. Mani Prabaharan (2008) reviewed the various methods of preparation of chitosan derivatives intended for controlled drug delivery and concluded that chitosan derivatives are promising materials for controlled drug and nonviral gene delivery. Some of the chitosan derivatives reported in the literature as drug delivery beads are given below:

#### *Chitosan O - derivatives*

Beads containing methotrexate were prepared from o-carboxy methylate chitosan and an isoelectric critical technique under ambient conditions (Jeong et al. 1998). Controlled drug release studies were carried out in numerous media including stimulated gastric fluid, intestinal fluid, and 1% fresh mice serum. It was concluded that a faster release of the drug took place in acidic media as compared to neutral media. The effect of methotrexate and o- carboxymethylate chitosan ratio and amount of crosslinking agent on drug release in different media was determined. The changes in bead size and effective radius of formulated beads were detected by Scanning electron microscopy and laser light scattering system before and after the drug release. Chang and Zhongguo (1996) concluded that the o- carboxymethylate chitosan beads constitute a promising alternative to other anticancer drugs and enzyme carriers.

BSA-loaded beads were formed by incorporation of tripolyphosphate and chitosan o-(2-hydroxyl)propyl-3-trimethyl ammonium chitosan chloride (O-HTCC), which was synthesized by coupling of glycidyl trimethyl ammonium chloride to chitosan, whose functional  $-NH_2$  groups were protected (Sun and Wan 2007). They were found to have high BSA loading efficiency (87.5%) and loading capacity (99.5%), and release profiles of BSA from beads had an obvious burst effect, and a slowly continuous release phase followed.

### *Chitosan N - derivatives*

Dunn et al. (1993) attempted to develop better membrane forming properties of poly-L-lysine in chitosan by extending the length of the spacer arm, i.e the amino group. In a chemical modification, chitosan was first reacted with  $\alpha$ -bromo acyl bromide and finally with an amine, but no significant difference was obtained in membrane properties between modified and unmodified chitosan. Ultimately, a two-step synthetic method was developed to attach a long alkyl amine side chain to chitosan's amino group. In the first step chitosan was reacted with n-N (bromo alkyl) phthalimide, followed by hydrazine (i.e.  $N_2H_4$ ) to produce flexible alkylamine side chains to the chitosan polysaccharide backbone, which had two amino groups in the newly formed side chain and enhanced the membrane forming properties. All the amino alkylated products were characterized by  $^1H$  and  $^{13}C$  NMR. This synthetic chitosan derivative was also used for entrapments of drugs in the form of beads and capsules. Onishi et al. (2005) developed a chitosan-prednisolone conjugate by amide coupling between chitosan and succinyl prednisolone. These microspheres were prepared and utilized as oral dosage forms for the treatment of inflammatory bowel disease. Chitosan prednisolone microspheres and enteric-coated chitosan prednisolone microspheres were described for their useful features (Onishi et al. 2008), which were realized by transition properties in the gastrointestinal tract based on the particle characteristics and their drug release profiles (Oosegi et al. 2008).

Thanou et al. (2000) obtained chemically modified N-trimethyl chitosan chloride, a quaternized chitosan derivative and concluded that the N-trimethyl chitosan derivative was effective to alter the permeation of hydrophilic drug molecules across the mucosal epithelia by opening the tight junctions. Recently Chen et al. (2007) made modifications in N-trimethyl chitosan chloride nanoparticles and evaluated them as a protein delivery system. Chitosan succinate and chitosan phthalate were synthesized and studied as potential matrices for colon-specific orally administered drug delivery systems (Aiedeh and Taha 1999). N- triethylated and N-trimethylated oligosaccharides have also been synthesized to overcome the low solubility of chitosan in neutral pH media.

### *Other derivatives of chitosan*

Kotze et al. (1999) prepared and studied the properties of a number of chitosan derivatives to enhance intestinal permeability, with emphasis on solubility in neutral and basic solutions. Lee et al. (1998) synthesized hydrophobically modified chitosan containing the deoxycholic group. This modified chitosan derivative formed self-assemblies in aqueous media. A charged complex was developed between the positively charged self-aggregated entities and negatively charged plasmid DNA. Hence, these self-aggregates were studied as an in vitro vehicle for the transfection of genetic material in mammalian cells. Park et al. (2006) formulated adriamycin-loaded glycol-chitosan, which was capable of forming self-aggregates in aqueous medium. When the adriamycin content was in the range of 2.0 to 5.0 weight percent, the self-aggregates were spherical in shape and had mean diameters of 238 - 304 nm, depending on the adriamycin content. The critical aggregation concentrations were as low as  $1.0-2.5 \times 10^{-2}$  mg /ml. The size of self-aggregates was not affected by the polymer concentrations in the range from 50-2000  $\mu$ g/ml and was maintained up to 8 days in phosphate buffered saline (pH 7.4), indicating high colloidal solubility. The release of adriamycin was dependent on the pH of the medium and higher in acidic (pH 4) medium than in alkaline (pH 7) medium.

Giunchedi et al. (1998) obtained a new derivative of chitosan, methyl pyrrolidone chitosan, in which the pyrrolidone chitosan group was covalently linked to the polysaccharide backbone. This chitosan derivative combines the hydrophilic properties of the pyrrolidone moiety (Muzzarelli 1992) and the biocompatibility of chitosan (Muzzarelli et al. 1998). The beads were loaded with a model drug ampicillin, and release rate were found to be dependent upon the nature of the chitosan used. Chen et al. (2003) studied the derivative of chitosan by coupling it with linoleic acid through 1-ethyl 3-(3-dimethyl amino propyl) carbodiimide-mediated reaction to increase its amphipathicity for improved emulsifications. Linoleic acid-chitosan self-aggregated beads were used to encapsulate the lipid soluble model compounds, retinal acetate, with 50% efficiency.

A chitosan derivative with galactose groups was prepared by inducing galactose groups into the amine group of chitosan (Zhang et al. 2004). The results indicated that a 20% degree of galactosylation took place, which changed physical properties such as crystallinity, solubility, stability, etc., relative to the original chitosan. It was concluded that galactosylated chitosan beads could be used for passive and active hepatic targeting. Jayakumar et al. (2007) developed chitosan-based beads possessing covalent attachment of thiol moieties, which were synthesized by graft co-polymerization technique. Thiol-containing chitosan beads were prepared by using tripolyphosphate, at pH 4.0. The in-vitro drug release behaviour was studied in phosphate buffer solution at various pH, using indomethacin as a model drug. The release rate of indomethacin at pH 7.4 was higher than the release rate at pH 1.4 due to ionization of thiol groups and high solubility of indomethacin in an alkaline medium. Various water-soluble compounds of chitosan were synthesized by chemical modifications (Lim and Hudson 2003).

### **Crosslinked Chitosan Network Beads with Spacer Groups**

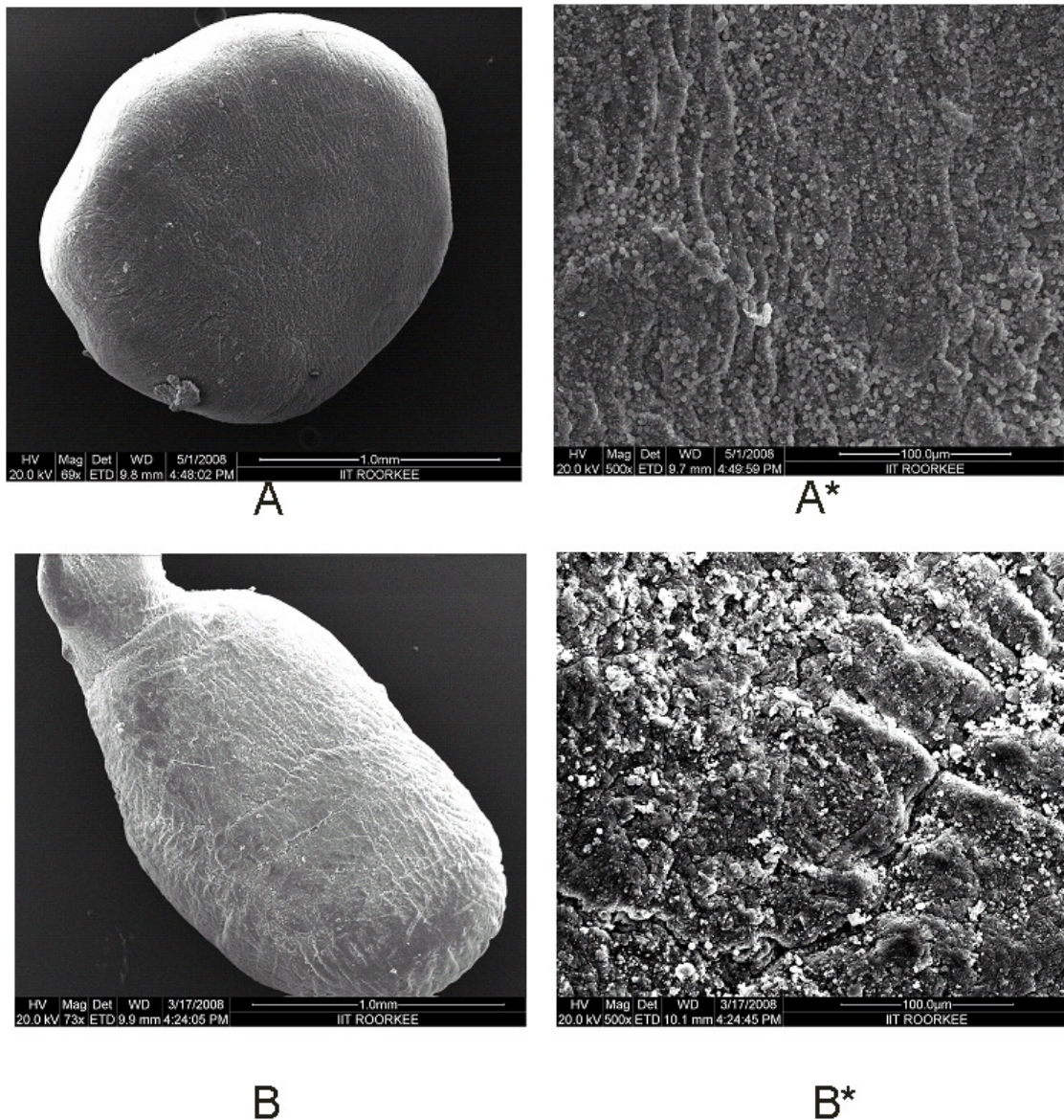
A novel technique for the preparation of the pH-sensitive beads of chitosan is reported in which amino acids like glycine (Gupta and Ravi Kumar 2000a-c), glutamic acid (Kumari and Kundu 2008), or alanine (Kumari and Kundu 2007) were employed as spacer groups to enhance the flexibility of polymer networks and influence the swelling behaviour through macromolecular interactions. Rani et al. (2010) have reported a comparative study for pH responding beads of chitosan and chitosan-aminoacids crosslinked with glutaraldehyde. The swelling behaviour, solubility, hydrolytic degradation, and drug loading capacity of beads were investigated. Effects of the crosslinker were studied by varying the amount of glutaraldehyde. The beads showed increased pH sensitivity. The pH-sensitive swelling was due to the transition of the bead network between collapsed and expanded states, which were related to the ionization degree of amino groups on chitosan in different pH solutions.

Li et al. (2000) synthesized carbohydrate-branched chitosan derivatives to get water solubility and novel biological activities. Curti et al. (2003) reported an alternative method, exploring functionalized compounds such as choline dichloride, carrying the preformed trimethyl ammonium group that can react with chitosan to yield highly cationic chitosan. N-[(2-hydroxy-3-trimethyl-ammonium)-propyl] chitosan chloride (HTCC) was prepared by reacting chitosan with glycidyl trimethyl ammonium chloride (Lim and Hudson 2004).



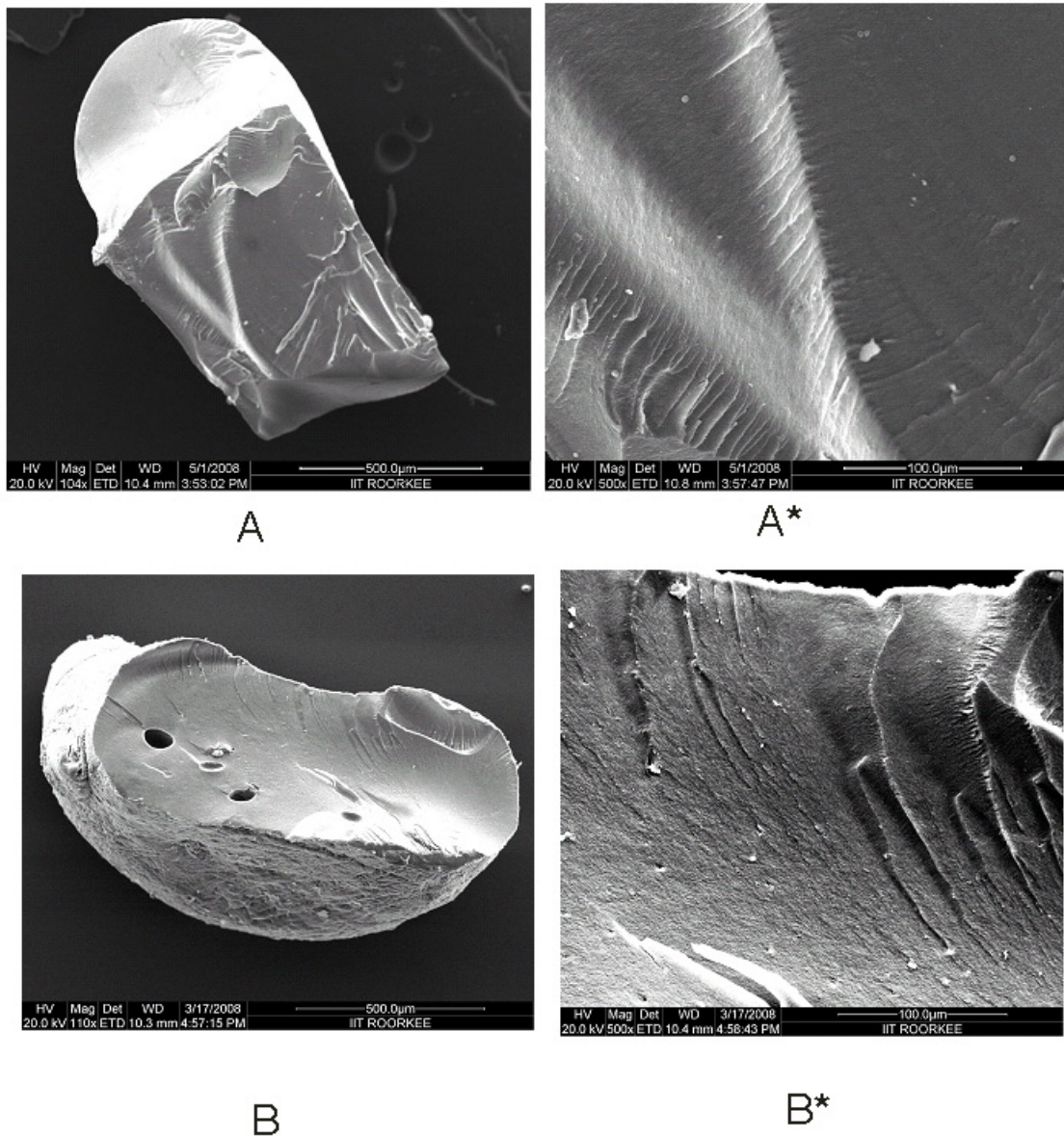
## DRUG LOADING

A successful polymeric-based hydrogel bead may have drug-loading capacity to reduce the quantity of the carrier required for administration. The polymeric composition can be manipulated to influence the permeation and diffusion characteristics of chitosan-based hydrogel polymeric beads, and through this manipulation, beads can be prepared to accommodate a variety of drugs loaded into the matrix. These include hydrophilic and hydrophobic substances, charged or neutral small molecules, and macromolecules. External morphology and internal structure differentiating drug loaded and unloaded chitosan-glutamic acid beads crosslinked with glutaraldehyde are presented in Figs. 3 and 4.



**Figure 3.** SEM micrographs showing external morphology for drug loaded (A) with its magnification (A\*) and drug unloaded (B) with its magnification (B\*) for chitosan-glutamic acid bead crosslinked with glutaraldehyde





**Figure 4.** SEM micrographs showing internal structure for drug loaded (A) with its magnification (A\*) and drug unloaded (B) with its magnification (B\*) for chitosan-glutamic acid bead crosslinked with glutaraldehyde

Drug loading into the chitosan-based hydrogel polymer beads can be achieved by two general methods. In one method, the polymeric hydrogel is mixed with the drug and an initiator, with or without a crosslinker, and the material is allowed to polymerize, trapping the drug within the matrix i.e. the incorporation method. In the second approach, after the formation of beads, they are allowed to swell to equilibrium in a suitable drug solution, i.e. the incubation method. In the incorporation method the drug is physically embedded into the polymeric matrix, whereas in the incubation method drug is adsorbed onto the surfaces. The latter method has some advantages over the first method, because

polymerization conditions may have deleterious effects on drug properties, and there may be difficulties in purification after loading, since polymerization ingredients often remain. To improve the efficiency of loading, several methods of loading have been developed, depending upon the method of preparation as well as physicochemical properties of the drug. Incorporation of the drug during bead formation can provide maximum drug loading, but it may be affected by the process parameters such as the method of preparation, the presence of additives, etc. Both water-soluble or water-insoluble drugs can be loaded into chitosan-based hydrogel polymeric beads. The incorporation method for preparing chitosan-based polymeric beads is well adapted for water-soluble drugs, and then beads preparation is carried out by any suitable method. A drug that gets precipitated in acidic pH can be loaded by the incubation method into preformed beads. In this method loading depends upon the swelling of beads in water (Kawashima et al. 1985). The percentage loading of drug has been found to decrease with increasing crosslinking due to decreased swelling.

A drug that is insoluble in water can also be loaded using the multiple emulsion technique. In this method the drug is dissolved in a suitable solvent and then emulsified in chitosan solution to form an oil-in-water (o/w) type emulsion by using a surfactant to stabilize the suspension. An o/w emulsion or suspension prepared in this way can be further emulsified into liquid paraffin to obtain an oil-water-oil (o/w/o) multiple emulsion. The resulting tiny drops can be hardened by using a suitable crosslinking agent.

Rokhade et al. (2006, 2007) have prepared chitosan-methyl cellulose hydrogel beads using an emulsion crosslinking method. In this method theophylline drug was mixed in homogenous polymeric chitosan methyl cellulose hydrogel. This solution was added slowly to light liquid paraffin containing 1% (w/w) Span-80. To this w/o emulsion, glutaraldehyde was added, and spherical beads with 50% drug loading capacity were formulated. A similar method was used by Agnihotri and Aminabhavi (2006) and Rao et al. (2006) for loading capecitabine and cefadroxil, respectively, into chitosan-based hydrogel beads. Agnihotri and Aminabhavi (2004) loaded clozapine drug into the pre-formed chitosan beads crosslinked with glutaraldehyde by soaking them in drug solution for 30 minutes and sonications for 5 minutes, yielding a mean particle size of 550-700  $\mu\text{m}$ . By this method, the percent entrapment efficiency was found to be in the range between 91.14 and 98.97%.

Gupta and Jabrail (2006) loaded two types of chitosan beads crosslinked with glutaraldehyde and glycol using the incubation method by keeping beads in phosphate-buffered solution containing a known amount of drug for 48 h for glutaraldehyde-crosslinked beads at pH 5 and for glyoxal-crosslinked beads at pH 4. The effects of chitosan molecular weight, degree of deacetylation, and degree of crosslinking were studied on drug loading capacity of beads. Medium molecular weight chitosan beads crosslinked with glyoxal showed a maximum loading of 40.5 % w/w, and beads crosslinked with glutaraldehyde showed 37.5 % w/w. However, beads prepared with chitosan having low and high molecular weights and crosslinked with glutaraldehyde showed higher loading capacity than the beads crosslinked with glyoxal. It was noted that the drug loading of centchroman increased with increasing molecular weight, degree of deacetylation, and also degree of crosslinking.

Hejazi and Amiji (2002) prepared chitosan hydrogel polymeric beads using different methods. In one method, tetracycline was mixed with chitosan solution and then simultaneously crosslinked and precipitated. Spherical beads having an average diameter of only 2.3  $\mu\text{m}$  with 8% (w/w) drug loading capacity were formulated. In another method, the drug was incubated into previously formed beads for 48 h. It could be loaded with 69% of drug. Jameela et al. (1994) obtained bovine serum albumin and diphtheria toxoid loaded chitosan beads crosslinked with glutaraldehyde by preparing the beads first and then loading them by passive absorption of drug from aqueous solution.

## DRUG RELEASE

Drug release from chitosan-based hydrogel polymeric beads depends upon the extent of crosslinking within the polymer, the polymeric composition and morphology, the size and density of the beads, as well as the physicochemical properties of the incorporated and incubated drug. In-vitro release also depends upon pH, polarity, and the presence of enzymes in the dissolution media. The release of drug from these beads involves the

- (a) desorption of the surface bound / adsorbed drug
- (b) diffusion through the bead polymeric matrix
- (c) bead polymeric matrix erosion, and
- (d) a combined erosion /diffusion process.

Thus, diffusion and biodegradation govern the process of drug release (Sujjaareevath et al. 1998). In case of drug release from the surface of the bead, adsorbed drug may instantaneously dissolve into the releasing medium when it comes in contact with it. If the drug is entrapped in the surface layer of the bead, the release mechanism is the same, and the released drug leads to a bursting effect by which most of the drug is released within a few minutes. This type of burst release of drug can be prevented either by increasing the crosslinking density or by washing the beads with a proper solvent, but washing leads to reduced encapsulation efficiency. Drug release by diffusion involves three steps. First, water penetrates into the polymeric system of beads, which causes swelling of the matrix. Secondly, the conversion of glassy polymer into rubbery matrix takes place. The third step is the diffusion of drug from the swollen rubbery matrix. Hence, the release is slow initially, and later it becomes fast (Chien 1982; Peppas and Sahlin 1989).

Although a number of methods have been developed to study in-vitro release, there are still some technical difficulties in the study of in-vitro drug release from beads (Washington 1990; Magenheim and Benita 1991). A most promising and simple technique was adopted by Calvo et al. (1996), in which the beads were added directly into the dissolution medium. However, the method is not very sensitive for studying rapid-release formulations, but can only be used for formulations having release times longer than 1 hour (Calvo et al. 1997a,b).

Release profiles of drugs from beads depend upon the nature of the delivery system. In the case of a matrix device, the drug is uniformly distributed or dissolved in the matrix and the release occurs by diffusion or erosion of the matrix. If the distribution of the drug is faster than matrix degradation, then drug release occurs mainly by a

diffusion mechanism. Otherwise, it depends upon degradation. Rapid initial release is attributed to the fraction of the drug that is adsorbed or weakly bounded to the large surface area of the beads, compared to the fraction of the drug incorporated within the beads. Calvo et al. (1996) obtained almost similar release profiles for indomethacin from both beads and nano capsules (coated beads), which indicated that the polymer coating did not show any barrier properties for the drug release.

### Kinetics of Drug Release

To describe the kinetics of the drug release from the drug formulations, mathematical models such as zero order (Wagner 1969; Gibaldi and Feldman 1967), first order, Higuchi's model (Higuchi 1961; Higuchi 1963; Cobby et al. 1974), Hixson Crowell's model (Hixson and Crowell 1931), Korsmeyer and Peppas model (Korsmeyer et al. 1983; Peppas 1985; Harland et al. 1988), and Weibull's model (Langenbucher 1988; Goldsmith et al. 1978; Romero et al. 1991; Vudathala and Rogers 1992) have been used. The criterion for selecting the most appropriate model is based on a goodness of fit test (Bamba et al. 1979). Hence the release data needs to be analysed with the following mathematical models:

#### *Zero order model*

$$Q_t = k_o t \quad (1)$$

In Equation 1,  $Q_t$  is the percentage of drug released at time  $t$ , and  $k_o$  is the zero order constant. In this model, the plots of percent of drug released versus time are linear.

#### *First order model*

$$\ln Q_t = \ln Q_o - k_f t \quad (2)$$

In Equation 2,  $Q_t$  and  $t$  are the same as above,  $Q_o$  is the initial amount of drug present in the bead, and  $k_f$  is the first order constant. In this model the plots of  $\ln(Q_o - Q_t)$  versus time  $t$  are linear. This approach assumes that the drug molecules diffuse out through a gel-like layer formed around the drug during the dissolution process.

#### *Higuchi's model*

A large number of modified release dosage forms contain some sort of matrix system. In such instances, the drug dissolves from this matrix. The dissolution pattern of the drugs is dictated by the water penetration rate (diffusion method), and thus the following relationship applies:

$$Q_t = k_n t^{1/2} \quad (3)$$

where  $k_n$  is the Higuchi constant. In the Higuchi model, a plot of the percentage of drug released versus square root of time is linear. A good release data fit obtained suggests that the release rate depends upon the rate of diffusion through the crosslinked polymeric system of beads. It has been demonstrated that the rate of release depends upon the size

of microspheres. Release from smaller-size beads is faster than from large-sized beads due to a smaller diffusional path length for the drug and the large surface area of contact of small particles with the dissolution medium.

#### *Hixon Crowell model*

Some specialized formulations contain many drug particles of the same size and shape of their agglomerates that dissolve evenly. In such instances, the cube root law can express the dissolution process. If the dissolution pattern of the drug is dictated by the actual dissolution of drug molecule, then the following relationship applies,

$$Q_t = [100(1/3 - (k \cdot t))]^3 \quad (4)$$

Where  $k$  is the Hixon Crowell constant. In this model, the percentage of drug released versus cube root of time is linear.

#### *Weibull Distribution model*

When applied to the released data the Weibull equation expresses the accumulated fraction of material in solution at time  $t$ , by

$$Q_t = 1 - \exp [-(t - t_i) b/a] \quad (5)$$

where  $a$  is a scale parameter that defines the time scale of the process,  $t_i$  is the location parameter, which represents the lag period before the actual onset of dissolution (in most cases  $t_i = 0$ ), and  $b$  is the shape parameter.

#### *Korsmeyer and Peppas model*

The most commonly used equation for diffusion controlled matrix system is the following empirical equation used by Ritger and Peppas (1987), in which the early time release data can be fitted to obtain the diffusion parameters:

$$Q_t = k t^n \quad (6)$$

where  $n$  is an empirical parameter characterizing the release mechanism based on the diffusional exponent (Peppas and Korsmeyer 1987). Drug transport is classified as Fickian ( $n=0.5$ ), case II transport ( $n=1$ ), non Fickian or anomalous ( $0.5 < n < 1$ ), or super case II ( $n > 1$ ). In this model, a plot of percentage drug released versus  $\log t$  is linear.

In the swelling-controlled release system, the drug is dispersed within a glassy polymer. Upon contact with biological fluid, the polymer swells, but no drug diffusion occurs initially through the polymer phase. As the penetrant enters the glassy polymer, the glass transition temperature of the polymer is lowered due to relaxations of the polymer chains so that drug could diffuse out of the swollen rubbery polymer. This type of system is characterized by two moving boundaries, the front separating the swollen rubbery portion and the glassy region, which moves with a front velocity, and the polymer fluid interface. The rate of drug release is controlled by the velocity and position of the front dividing the glassy and rubbery portions of the polymer.

## PHARMACEUTICAL APPLICATIONS

A new dimension for the use of chitosan-based hydrogel polymeric beads as drug delivery device in pharmaceutical and biomedical science has been explored. Some important applications are as follows:

### Drug Delivery to Colon

Chitosan for the oral administration to human has been recognized as safe by scientists (Lee et al. 2002), hence can be used as an absorption enhancer in solid oral dosage forms delivery of drugs that are either poorly absorbed or destroyed in gastrointestinal tract. These may be introduced to the systemic circulation through the colonic absorption mode (Hejazi and Amiji 2003). As chitosan is biodegradable by the colonic bacterial flora (Pantaleone et al. 1991; Zhang et al. 2002), it is a promising polymer for colon drug delivery. The effect of chitosan tripolyphosphate beads on the absorption of insulin was studied by measuring the decrease of the plasma glucose concentration and the relative pharmacological availability (Tozaki et al. 2002). Chitosan tripolyphosphate showed excellent association with insulin and improved the intestinal absorption of insulin to a great extent. Chitosan succinate and chitosan phthalate loaded with sodium diclofenac (Aiedeh and Taha 1999) have been found to resist dissolution under acidic conditions and showed improved dissolution under basic conditions, suggesting their suitability for colon-specific drug delivery systems. Chitosan-pectin complexes (Feranandez-Hervas and Fell 1998) were also found to be suitable for colon-specific delivery of indomethacin and paracetamol. Chitosan-alginate beads loaded with a model protein, bovine serum albumin, were investigated to explore the temporary protection of protein against acidic and enzymatic degradation during gastric passage. During incubation in gastric fluid (pH 1.2), the beads showed swelling and started to float but did not show any sign of erosion. Release studies were also carried out in gastric fluid (pH 1.2) and subsequently in intestinal fluid (pH 7.5), where the beads were found to erode, burst, and release the protein (Anal et al. 2003).

### Mucosal Delivery

In recent times mucosal surfaces such as nasal, peroral, and pulmonary surfaces are receiving a great deal of attention as alternative routes of systemic administration. Chitosan has mucoadhesive properties, and therefore it seems particularly useful to formulate the bioadhesive dosage forms for mucosal administration (Genta et al. 1998). Chitosan has been found to enhance drug absorption through mucosa without damaging the biological system. Here, the mechanism of action of chitosan was suggested to be a combination of bioadhesion and a transient widening of the tight junctions between the epithelial cells (Artursson et al. 1994).

### Nasal Delivery

Nasal mucosa has high permeability and can provide easy access of the drug to the absorption site. The particulate delivery to peroral mucosa is easily taken up by the Peyer's patches of the gut-associated lymphoid tissue. The influence of glutaraldehyde on drug release and mucoadhesive properties of chitosan beads has been studied by (Genta et al. 1998). The ability of insulin-loaded chitosan beads to enhance the nasal absorption

of insulin was investigated in a conscious rabbit model. Studies (Fernandez- Urrusuno et al. 1999) have revealed that chitosan beads enhanced the nasal absorption of insulin to a greater extent than an aqueous solution of chitosan. Vander Lubben et al. (2001a,b, 2003) incorporated the model protein ovalbumin into chitosan beads, and the uptake of ovalbumin associated with chitosan beads in murine peyer's patches was demonstrated using confocal laser scanning microscopy. The researchers investigated the ability of chitosan beads to enhance both systemic and local immune responses against diphtheria toxoid vaccine after the oral and nasal administration in mice. They found that systemic and local IgG and IgA immune responses against diphtheria toxoid vaccine associated to chitosan beads were strongly enhanced after the oral delivery in mice. Chitosan / hyaluronan beads (Lim et al. 2002) for the nasal delivery of gentamicin have been developed. Chitosan glutamate, hyaluronic acid, and a combination of both polysaccharides were used in this study, and authors concluded that the bioavailability of drug was maximum from chitosan / hyaluronan beads and minimum from hyaluronan beads, while intermediate from chitosan beads. Extensive work on development of nasal vaccines based on chitosan have been carried out, and the findings have been summarized (Jabbal-Gill et al. 1998; Iqbal et al. 2003; McNeela et al. 2000,2004; Illum 2003; Illum et al. 2001; Westerink et al. 2002).

### **Gastro Enteric Delivery**

Schipper et al. (1999) claim that chitosan has effects on the intestinal epithelium, and they studied the effects of chitosans with varying molecular weights and degree of acetylation on the absorption of a poorly absorbed model drug atenolol in intestinal epithelia cell layers with or without a mucous layer. The observations suggest that chitosan had pronounced effect on the permeability of mucous-free epithelial layer and enhanced the permeation of atenolol. Chitosan formulations have also been used for the relief of break-through pain in cancer patients (Pavis et al. 2002).

### **Ocular Delivery**

The primary requirement for an ocular delivery system is bioadhesiveness that increases the contact time with the cornea, leading to improved drug absorption at the site. In consideration of the chitosan bioadhesiveness, attempts were made to take advantage of cationic properties of chitosan in ocular delivery. The ability of chitosan hydrochloride to enhance the transcorneal permeability of the drug has been demonstrated (Di colo et al. 2002). Poly-(ethyleneoxide) was used as a base material to which ofloxacin-containing chitosan beads were added and powder compressed, resulting in circular inserts (6mm). The addition of increasing concentration of chitosan exhibited accelerated erosion and release of the entrapped drug. Experiments in the rabbit's eye formed a superficial gel that adhered to the applications site, gradually spread over the cornea, and eroded with time. The irritation in the eye, in chitosan based systems was found to be lower as compared to chitosan-free inserts. The release and bioavailability rates were much higher for the poly-(ethyleneglycol)/chitosan hydrochloride when compared with the plain inserts of either. De Campos et al. (2001) investigated the potential of chitosan beads as a new vehicle to improve the delivery of drugs to ocular mucosa. Cyclosporin-A was chosen as a model drug. A modified ionic gelation method was used to prepare Cyclosporin-A loaded chitosan nano-beads. The in-vitro release



studies, performed under sink conditions, revealed fast release during the first hours, followed by a more gradual drug release during the 24 hours period. The in-vivo experiments showed that after topical instillation of cyclosporine-A loaded chitosan nanobeads to rabbits, therapeutics concentrations were achieved in the external ocular tissues within 48 hours, while maintaining negligible or undetectable cyclosporin-A levels in the inner ocular structures blood and plasma. These levels were significantly higher than those obtained following the installation of chitosan solution containing drug and an aqueous drug suspension. The study indicated that chitosan nano-beads could be used as a vehicle to enhance the therapeutic index of the clinically challenging drugs with potential applications at the extraocular level.

### **Topical Delivery**

Having an excellent bioadhesive property and ability of sustain the release of active molecules, chitosan has been used to obtain topical delivery systems. Chitosan beads for topical sustained release of cetyl pyridinium chloride have been investigated (Conti et al. 1998). These chitosan beads loaded with cetyl pyridinium chloride improved microbiological activity. Conti et al. (2000) obtained beads composed of chitosan defined as powders for topical wound healing properties. They prepared blank and ampicillin drug loaded beads of chitosan by a spray drying technique. In-vivo evaluation in albino rats showed that both drug-loaded and blank beads exhibited good wound healing properties. Kojuji et al. (2004) have worked on chitosan beads incorporated with the model inflammatory drug prednisolone. Here, sustained release of prednisolone from chitosan gel beads was observed. In order to investigate the therapeutic efficacy of sustained prednisolone release, against local inflammation, the formulation was induced on the dorsal surface of mice. The results indicated that the chitosan beads show sustained release of the minimum effective dose of prednisolone locally and furthermore, this approach prolongs the duration of drug activity. Furthermore, with minimum dose of drug release, the possibility of side effects is also reduced.

### **Implants**

Khor and Lim (2003) discussed various applications of chitosan implants in a recent review. Chitosan was also tested as a local implant in the form of micro-beads and fibres as sustained release depots for endothelial growth factors (Elcin et al. 1996). Endothelial cell growth factors have a relatively short half-life. This antigenic factor should therefore be administered frequently in order to reach an effective concentration. Drug loaded matrixes implanted subcutaneously in rats showed a burst effect; however, the release continued for more than three weeks. N-succinyl-chitosan mitomycin-C as an implant for controlled drug release and its antitumor activity has been evaluated (Song et al. 1996). Sustained release implants of herbs extracts using chitosan-gelatin matrix were developed (Zhao et al. 2002). In-vitro and in-vivo degradation studies using lysozymes and female Wister rats were performed, respectively. No side effects during the use of in-vivo implants were observed, suggesting their use as medical devices. The biodegradability of these implants has a further advantage, as no surgical removal of the exhausted implants is needed (Zhao et al. 2002).

Chitosan-based beads used as implants having small particles size, low crystallinity, and good sphericity were prepared by spray-drying method followed by

crosslinking with genipin and glutaraldehyde (Mi et al. 2002b). Histological study of both kinds of the crosslinked chitosan beads has been made by injecting them into the skeletal muscle of a rat model, which showed less inflammatory reaction with beads crosslinked with genipin than its glutaraldehyde crosslinked beads. On the other hand, the degradation studies suggested that glutaraldehyde-crosslinked beads started to degrade by 12 weeks, whereas the genipin-crosslinked beads were intact until 20 weeks, this suggesting that these implants are suitable for long term applications. More recently, chitosan and sodium hyaluronate implants for controlled release of insulin were studied (Surini et al. 2003).

Intramuscular injections constituting progesterone-loaded chitosan beads exhibited a constant plasma concentration of approximately 1-2 mg/ml for up to 5 months in rabbits (Jameela et al. 1998). Immunogenicity studies on Wistar rats using diphtheria toxoid-loaded chitosan beads exhibited a constant antibody titer for more than 5 months (Jameela et al. 1994).

### **Cancer Therapy**

Chitosan nano-beads loaded with gadolinium diethylene triamine pentaacetic acid (GdDTPA) were prepared for gadolinium neutron capture therapy (Gd-NCT), a cancer therapy using  $\gamma$ - rays, which were emitted during the reaction Gd-157 to kill tumor cells (Sashiwa et al. 2000,2003). Strong retention of GdDTPA and releasing properties in tumor tissue of mice indicated that these beads are successfully useful to suppress the growth of tumor (Shikata et al. 2002; Tokumitsu et al. 2000). Gd-content within the beads was increased by using more percentage of deacetylated chitosan. It was found that 100% deacetylated chitosan showed the highest Gd concentration (15% Gd-DTPA) and fine size of beads (452 nm), while lower deacetylated chitosan produced large sized beads constituting low Gd-DTPA content.

Jameela et al. (1996) obtained antitumor-active chitosan beads crosslinked with glutaraldehyde loaded with mitoxantrone. Their antitumor activity was evaluated against Ehrlich ascites carcinoma in mice by intra peritoneal injections. Drug-loaded micro-beads increased the mean survival time of mice up to 50 days. This is in contrast to 2.1 days when the mice received free mitoxantrone drug. In other studies (Jameela and Jayakrishnan 1995), the authors evaluated the in-vitro release of mitoxantrone, which was controlled for 4 weeks in phosphate buffer at 37 °C. The antitumor effect of doxorubicin-dextran loaded chitosan nanobeads was evaluated by Mitra et al. (2001) in macrophage tumor cells implanted in mice. The result of in-vivo studies suggests that the beads reduced the side effects and also improved therapeutic efficacy in treating tumors. Janes et al. (2001b) also obtained chitosan nanoparticles using the ionic gelation method as a carrier for doxorubicin by complexing it with dextran sulfate and studied their antitumor activity.

### **Gene Delivery**

Maclaughlin et al. (1998) and Mumper et al. (1995) were the scientists who first described the potential of chitosan as a gene carrier. The interaction between polycationic chitosan and negatively charged DNA is electrostatic (Mao et al. 2001). These interactions are so strong that the chitosan-DNA complex does not dissociate until it has entered the target cell (Roy et al. 1999). Chitosan-DNA nano beads have been prepared

by Mao et al. (2001) to examine their transfections efficiency, which was cell type dependent. The presence of 10% fetal bovine serum did not interfere with their transfection ability. Self-aggregated nano-particles were prepared (Lee et al. 1998) by hydrophobic modifications of chitosan with deoxycholic acid in aqueous media. These were able to form complexes on mixing with plasmid DNA.

Trimethyl chitosan oligomers were examined for their potency as DNA carriers (Florea et al. 2000). Chitosan and lactosylated chitosan carriers were investigated for their transfection efficiencies in-vitro (Erbacher et al. 1998). Recently, galactosylated chitosan-dextran- DNA complexes have also been prepared (Park et al. 2000). Galactose groups were chemically bound to chitosan for liver specificity, and dextran was grafted to increase the stability of the complex in water. It was found that the chitosan-dextran-DNA complex system could efficiently transfect the liver cells.

Chew et al. (2003) explored the use of chitosan-DNA nano-beads for oral immunization to induce the immune responses. Recently, Cui et al. (2001) investigated the topical application of chitosan plasmid DNA nanobeads as a potential approach to genetic immunization. Borchard (2001) has also published a review on the efficient non viral gene delivery using cationic polymers as DNA condensing agents. The review examined the advances made in the applications of chitosan and chitosan derivatives to non-viral gene delivery. It gave an overview of the transfections studies performed by using chitosan as a transfection agent.

## CONCLUSIONS

Chitosan-based polymeric hydrogel beads have a wide range of applications and may be used to solve numerous biomedical problems. Chitosan and its derivatives can be easily obtained as beads and also digested by lysozymal enzymes in the digestive tract. Therefore, chitosan and its derivatives can be utilized as a delivery system for a number of drugs, vaccines, hormones, and anti cancer agents to release them in a controlled manner. This concept of chitosan-based polymeric hydrogel bead systems and macromolecular drug formulations is expected to be useful for enhancement of efficacy and minimization of toxic side effects. From the studies reviewed, it is concluded that chitosan and its derivatives as polymeric hydrogel bead systems are promising materials for controlled drug delivery. Some researchers have also been exploring applications of chitosan and its derivatives by obtaining a system for non-viral gene delivery, but such approaches will need more attention. However, most of the studies concerning drug release from chitosan-based drug-carrying beads carried out, so far, are only in in-vitro release. There is a need to carry out more and more studies in-vivo. Gene therapy and the interaction with living tissues seem to be major topics in the current research on chitosan based beads.

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