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Review

Chitosan microspheres as a potential carrier for drugs

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Abstract

Chitosan is a biodegradable natural polymer with great potential for pharmaceutical applications due to its biocompatibility, high charge density, non-toxicity and mucoadhesion. It has been shown that it not only improves the dissolution of poorly soluble drugs but also exerts a significant effect on fat metabolism in the body. Gel formation can be obtained by interactions of chitosans with low molecular counterions such as polyphosphates, sulphates and crosslinking with glutaraldehyde. This gelling property of chitosan allows a wide range of applications such as coating of pharmaceuticals and food products, gel entrapment of biochemicals, plant embryo, whole cells, microorganism and algae. This review is an insight into the exploitation of the various properties of chitosan to microencapsulate drugs. Various techniques used for preparing chitosan microspheres and evaluation of these microspheres have also been reviewed. This review also includes the factors that affect the entrapment efficiency and release kinetics of drugs from chitosan microspheres.

Keywords: Chitosan; Microspheres

1. Introduction

Chitosan, a natural linear biopolyaminosaccharide is obtained by alkaline deacetylation of chitin, which is the second abundant polysaccharide next to cellulose (Muzzarelli, 1977; Roberts, 1992). Chitin is the principal component of protective cuticles of crustaceans such as crabs, shrimps, prawns, lobsters and cell walls of some fungi such as *aspergillus* and *mucor*. Chitin is a straight homopolymer composed of β-(1,4)-linked *N*-acetyl-glucosamine units while chitosan comprises of copolymers of glucosamine and *N*-acetyl-glucosamine (Kas, 1997; Singla and Chawla, 2001; Kato et al., 2003). Chitosan has one primary amino and two free hydroxyl groups for each C_6 building unit (Fig. 1). Due to the easy availability of free amino groups in chitosan, it carries a positive charge and thus in turn reacts with many negatively charged surfaces/polymers and also undergoes chelation with metal ions (Fukuda, 1980) like cobalt (Onsoyen and Skaugrud, 1990). Thus, it is utilized for separation of metals.

Chitosan is a weak base and is insoluble in water and organic solvents, however, it is soluble in dilute aqueous acidic solution ($\text{pH} < 6.5$), which can convert the glucosamine units into a soluble form $R–NH_3^+$ (Chandy and Sharma, 1990). It gets precipitated in alkaline solution or with polyanions and forms gel at lower pH. It also acts as flocculant for the treatment of waste water (Demarger-Andre and Domard, 1994).

Commercially, chitosan is available in the form of dry flakes, solution and fine powder. It has an

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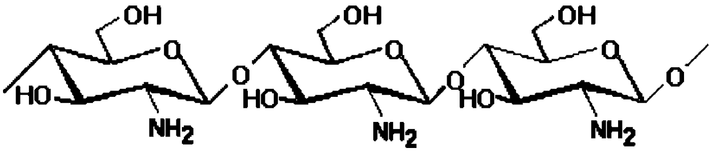


Fig. 1. Structure of chitosan.

average molecular weight ranging between 3800 and 2,000,000 and is from 66 to 95% deacetylated (Kas, 1997). Particle size, density, viscosity, degree of deacetylation, and molecular weight are important characteristics of chitosan which influence the properties of pharmaceutical formulations based on chitosan.

Properties such as biodegradability, low toxicity and good biocompatibility make it suitable for use in biomedical and pharmaceutical formulations (Chandy and Sharma, 1990; Illum et al., 2001), e.g. it is used for hypobilirubinaemic and hypocholesterolemic effects (Furda, 1980; Nagyuvary, 1982), antacid and antiulcer activities (Ito et al., 2000), wound and burn healing properties (Tachihara et al., 1997), immobilization of enzymes and living cell and in ophthalmology (Felt et al., 1999). Since chitosan has a capacity of forming film it has been suggested as a biopolymer of choice for the development of contact lens (soft and hard contact lenses). Chitosan has been used for the manufacturing of ocular bandage lenses used as protective devices for acutely or chronically traumatized eyes (Markey et al., 1989). Chitosan membranes have also been found useful as artificial kidney membranes because of their suitable permeability and high tensile strength (Amiji, 1995).

Among pharmaceutical applications it has been used as a vehicle for directly compressed tablets (Kristmundsdottir et al., 1995; Sabnis et al., 1997; Illum, 1998), as a disintegrant (Nigalaye et al., 1990), as a binder (Upadrashta et al., 1992), as a granulating agent (Ilango et al., 1997), in ground mixtures (Hou et al., 1985), as a drug carrier for sustained release preparations (Kawashima et al., 1985a; Miyazaki et al., 1990; Akbuga, 1993; Kristl et al., 1993) as well as a co-grinding diluent for the enhancement of dissolution rate and bioavailability of water insoluble drugs (Miyazaki et al., 1981; Sawayanagi et al., 1982a; Shiraishi et al., 1990).

Chitosan has been shown to possess mucoadhesive properties (Lehr et al., 1992; Needleman and Smales, 1995; Rillosi and Buckton, 1995; He et al., 1998; Shimoda et al., 2001; Kockisch et al., 2003) due to molecular attractive forces formed by electrostatic interaction between positively charged chitosan and negatively charged mucosal surfaces. These properties may be attributed to:

(a) strong hydrogen bonding groups like –OH, –COOH (Schipper et al., 1997);
(b) strong charges (Dodane et al., 1999);
(c) high molecular weight (Schipper et al., 1996; Kotze et al., 1998);
(d) sufficient chain flexibility (He et al., 1998); and
(e) surface energy properties favoring spreading into mucus (Lueβen et al., 1994).

Chitosan possesses no toxicity and can be applied onto the nasal epithelium. It swells and forms a gel like layer in aqueous environment (by absorbing water from the mucous layer in the nasal cavity), which is favorable for interpenetration of polymer and glycoprotein chains into mucous (Felt et al., 1998). The positive charge on chitosan polymer gives rise to strong electrostatic interaction with mucus or negatively charged sialic acid residues on the mucosal surface (Illum et al., 1994). Chitosan also shows good bioadhesive characteristics and can reduce the rate of clearance of drug from the nasal cavity thereby increasing the bioavailability of drugs incorporated in it (Soane et al., 1999). Membranes prepared from chitosan have shown greater permeability for acidic drugs than basic drugs (Sawayanagi et al., 1982b). In this review, chitosan was studied as a carrier for microsphere drug delivery. Chitosan microspheres are the most widely studied drug delivery systems for the controlled release of drugs viz., antibiotics, antihypertensive agents, anticancer agents, proteins, peptide drugs and vaccines.

2. Chitosan microspheres

The use of microsphere-based therapy allows drug release to be carefully tailored to the specific treatment site through the choice and formulation of various drug–polymer combinations. The total dose of medication and the kinetics of release are the variables, which can be manipulated to achieve the desired result. Using innovative microencapsulation technologies, and by varying the copolymer ratio, molecular weight of the polymer, etc., microspheres can be developed into an optimal drug delivery system which will provide the desired release profile. Microsphere-based systems may increase the life span of active constituents and control the release of bioactive agents. Being small in size, microspheres have large surface to volume ratios and can be used for controlled release of insoluble drugs. Extensive research is being carried out to exploit chitosan as a drug carrier to attain the desirable drug release profile.

Chitosan microspheres are used to provide controlled release of many drugs and to improve the bioavailability of degradable substances such as protein or enhance the uptake of hydrophilic substances across the epithelial layers. These microspheres are being investigated both for parenteral and oral drug delivery. Studies have shown that polyethylene oxide containing triblock copolymer (pluronics) modified chitosan microspheres were used as potential blood compatible hemoadsorbants (Queen et al., 2000). Chitosan has also been used as a potential carrier for prolonged delivery of drugs, macromolecules and targeted drug delivery. Magnetic chitosan microspheres used in targeted drug delivery are expected to be retained at the target site capillaries under the influence of an external magnetic field (Gallo and Hassan, 1988). Also, strong interaction between cationic microspheres and anionic glycosaminoglycan receptors can retain the microspheres in the capillary region (Gallo and Hassan, 1988; Hassan et al., 1992).

3. Preparation of chitosan microparticles

Reacting chitosan with controlled amounts of multivalent anion results in crosslinking between chitosan molecules. The crosslinking may be achieved in acidic, neutral or basic environments depending on the method applied. This crosslinking has been extensively used for the preparation of chitosan microspheres. The processes that have been used for the preparation of the microspheres are given below. Fig. 2 outlines various methods which have been used for the preparation of chitosan microspheres.

3.1. Interaction with anions

3.1.1. Ionotropic gelation

The counterions used for ionotropic gelation can be divided into three categories viz., low molecular weight counterions (e.g. pyrophosphate, tripolyphosphate, tetrapolyphosphate, octapolyphosphate, hex-

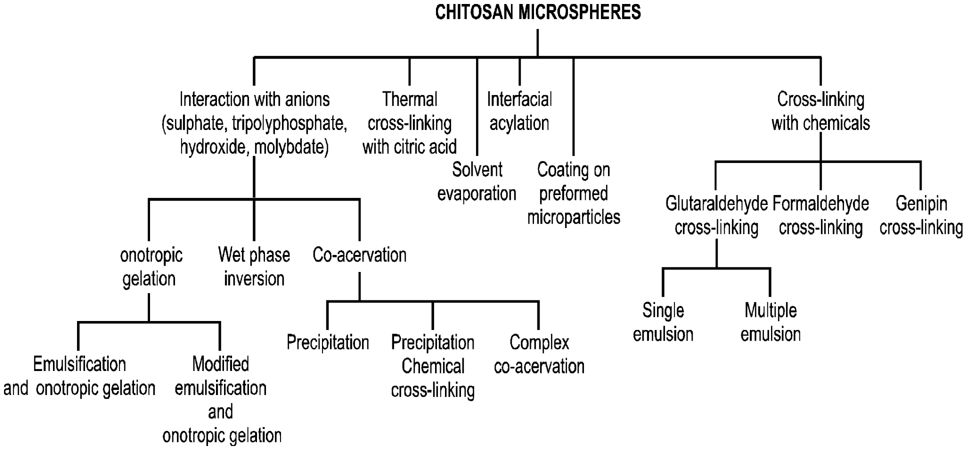


Fig. 2. Methods for preparation of chitosan microspheres.

ametaphosphate and $(Fe(CN)_6)^{-4}/(Fe(CN)_6)^{-3}$), hydrophobic counterions (e.g. alginate, κ-carragenan, poly-1-hydroxy-1-sulphonate-propene-2, polyaldehydro-carbonic acid), high molecular weight ions (e.g. octyl sulphate, lauryl sulphate, hexadecyl sulphate, cetylstearyl sulphate) (Skaugrud, 1991). The chitosan solution in acetic acid was extruded dropwise through a needle into different concentrations of aqueous solutions of magnetically stirred tripolyphosphate or some other anion. The beads were removed from the counter ion solution by filtration, washed with distilled water and dried (Sawayanagi et al., 1983; Kawashima et al., 1985a,b; Nishimura et al., 1986; Bodmeier et al., 1989a,b; Bodmeier and Paeratakul, 1989; Kim and Rha, 1989a,b; Shiraishi et al., 1993; Chandy and Sharma, 1991, 1993; Wan et al., 1994; Acikgoz et al., 1995, 1996; Sezer and Akbuga, 1995; Aydin and Akbuga, 1996).

Dambies et al. (2001) prepared chitosan gel beads using molybdate as the gelling agent. It was observed that this new gelation technique led to a structure different from one produced during alkaline coagulation of a chitosan solution. Instead of a morphology characterized by large open pores, gel beads produced in a molybdate solution, under optimum conditions (pH 6; molybdate concentration, 7 g/l), were found to have a double layer structure corresponding to a very compact 100 μm thick external layer and an internal structure of small pores.

3.1.1.1. Wet phase inversion. In this method of preparation, chitosan solution in acetic acid was dropped into an aqueous solution of a counterion sodium tripolyphosphate through a nozzle. Microspheres formed were allowed to stand for 1 h, washed and crosslinked with 5% ethylene glycol diglycidyl ether. Finally, the microspheres were washed and freeze-dried to form porous chitosan microshperes (Mi et al., 1999). Changing the pH of the coagulation medium could modify the pore structure of the chitosan microsphere.

3.1.2. *Emulsification and ionotropic gelation*

In this method the dispersed phase, which consists of an aqueous solution of chitosan, is added to a non-aqueous continous phase (*iso*-octane and emulsifer) to form w/o emulsion. Sodium hydroxide (1 N) solution is then added at different intervals leading to ionotropic gelation. The microspheres thus formed are removed by filtration, washed and dried (Lim et al., 1997).

3.1.3. *Modified emulsification and ionotropic gelation*

Shu and Zhu (2001) investigated the possibility of three kinds of anions (tripolyphosphate, citrate and sulphate) to interact with chitosan by turbidimetric titration. The results indicated that there are electrostatic interactions between the above anions and chitosan in a certain region of solution pH (1.0–7.5 for sulphate/chitosan, 4.5–7.5 for citrate/chitosan and 1.9–7.5 for tripolyphosphate/chitosan), that is related to the natural characteristics of the anions. Out of the pH region where anions interacted with chitosan, no microspheres were formed. However, even in the pH region where anions interacted with chitosan, only irregular microparticles were obtained in the case of the conventional emulsification and ionotropic gelation method, while spherical microspheres with diameters in the range of tens of microns were obtained when a modified process was employed. The key point of the modified process was the introduction of gelatin and allowing the ionic crosslinking process of chitosan/gelatin w/o emulsions to take place under coagulation conditions at a low temperature. The surface of sodium sulphate crosslinked chitosan/gelatin and sodium citrate crosslinked chitosan/gelatin microspheres was very smooth, but large gaps were observed on the surface of tripolyphosphate/chitosan microspheres. The increase of stirring speed led to a decrease in diameter and a narrowing in size distribution.

Kim et al. (2003) attempted to design a novel type of porous chitosan scaffold containing transforming growth factor-beta1 (TGF-beta1). The microspheres loaded with BSA, a model protein, were also prepared (Kim et al., 2003) using tripolyphosphate. From the release experiments, it was found that both proteins were slowly released from the microspheres over 5 days in a phosphate buffer solution (pH 7.4) in which the release rate of TGF-beta1 was much lower than that of BSA. It was observed that the scaffold containing microspheres of TGF significantly augmented the cell proliferation and production of extracellular matrix. These results suggested that the chitosan scaffold containing microspheres of TGF possessed a promising potential as an implant to treat cartilage defects.

3.1.4. Floating hollow chitosan microspheres by ionic interaction with sodium dioctyl sulfosuccinate

Floating microcapsules containing melatonin were prepared by the ionic interaction of chitosan and a negatively charged surfactant, sodium dioctyl sulfosuccinate (El-Gibaly, 2002). The characteristics of the floating microcapsules generated were compared with the conventional non-floating microspheres prepared from chitosan and sodium tripolyphosphate. The use of dioctyl sulfosuccinate solution in coagulation of chitosan produced well-formed microcapsules with round hollow core and 31.2–59.74% drug incorporation efficiencies. Chitosan concentration and drug–polymer ratio had a remarkable effect on drug entrapment in dioctyl sulfosuccinate/chitosan microcapsules. The dissolution profiles of most of microcapsules showed near zero order kinetics in simulated gastric fluid (SGF, pH 1.2). Moreover, release of the drug from these microcapsules was greatly retarded lasting for several hours (t_{50} in SGF was 1.75–6.7 h, depending on processing factors). Drug release from non-floating microspheres was almost instant. Most of the hollow microcapsules tended to float over simulated biofluids for more than 12 h. Swelling studies conducted on various drug-free formulations indicated that dioctyl sulfosuccinate/chitosan microcapsules showed less swelling than tripolyphosphate/chitosan microspheres. Therefore, it was concluded that the floating hollow microcapsules could form an interesting gastroretentive controlled drug delivery system.

Paclitaxel (taxol)-containing chitin and chitin-Pluronic F-108 floating microparticles were formulated as biodegradable systems for localized administration in solid tumors by Nsereko and Amiji (2002). Lysozyme-induced degradation and in vitro release of paclitaxel was examined in PBS at 37 °C. Due to higher porosity, chitin-Pluronic microparticles were able to imbibe higher swelling medium and degraded much faster in the presence of lysozyme than chitin microparticles. After 48 h 51% of incorporated paclitaxel was released from chitin-Pluronic microparticles as compared to 28% from chitin microparticles. The results of this study showed that chitin and chitin-Pluronic F-108 microparticles are biodegradable drug delivery systems that can be useful for localized delivery of paclitaxel in solid tumors.

3.1.5. Coacervation

In this process, the polymer is solubilized to form a solution. This is followed by addition of a solute, which forms insoluble polymer derivative and precipitates the polymer. This process avoids the use of toxic organic solvents and glutaraldehyde used in the other methods of preparation of chitosan microspheres. Berthold et al. (1996a) prepared prednisolone sodium phosphate loaded chitosan microspheres using sodium sulphate as a precipitant. Addition of sodium sulphate to the solution of chitosan in acetic acid resulted in decreased solubility of chitosan, leading to precipitation of chitosan as a poorly soluble derivative.

3.1.6. Complex-coacervation

Chitosan microparticles can also be prepared by complex coacervation. Sodium alginate, sodium carboxymethylcellulose, κ-carregeenan and sodium polyacrylic acid can be used for complex coacervation with chitosan to form microspheres. These microparticles are formed by interionic interaction between oppositely charged polymers. Formulation of coacervate capsules of chitosan–alginate and chitosan–κ-carrageenan was carried out by interaction of the above-mentioned solutions with potassium chloride and calcium chloride, respectively. The obtained capsules were hardened in the counterion solution before washing and drying (Daly and Knorr, 1988; Ohtakara et al., 1989a,b; Nishioka et al., 1990; Li et al., 1991; Pandya and Knorr, 1991; Ohya et al., 1993).

Chitosan–alginate microparticles were prepared to control the release characteristics and physicochemical properties of drugs (Kim and Rha, 1989a,b). Chitosan–CMC complex microparticles have also been used to immobilize cell culture (Shioya and Rha, 1989). Chellat et al. (2000) prepared microspheres of a polyelectrolyte complex hydrogel from chitosan and xanthan after interaction between the two-polyionic polymers. The results suggested a better control of the degradation kinetics when chitosan was complexed to xanthan.

The preparation of chitosan–alginate nanospheres was also described by De and Robinson (2003) and their properties were compared to the poly-L-lysine-alginate system. The mass ratio range of sodium alginate:$CaCl_2$:cationic polymer (poly-L-lysine or chitosan) to prepare nanospheres was 100:17:10. This mass ratio ensured that the calcium alginate was maintained in the pre-gel phase and sufficient cationic polymer was present to form nanospheres. At low cationic polymer concentrations, nanospheres were not formed, whereas microspheres were formed at higher concentrations.

3.2. *Crosslinking with other chemicals*

Crosslinking agents such as glutaraldehyde, formaldehyde and genipin have been used for preparation of chitosan microspheres. Detailed procedure of the methods are given in the following sections.

3.2.1. *Emulsion crosslinking method*

In this process chitosan solution (in acetic acid) is added to liquid paraffin containing a surfactant resulting in formation of w/o emulsion. A crosslinking agent of varying amount is added depending upon the crosslinking density required (Thanoo et al., 1992; Jameela and Jayakrishnan, 1995; Akbuga and Bergisadi, 1996, 1999; Al-Helw et al., 1998; Jameela et al., 1998; Denkbas et al., 1999). The microspheres formed are filtered, washed with suitable solvents and dried. The literature abounds in reports wherein chitosan microspheres have been prepared by glutaraldehyde crosslinking. It has also been reported that glutaraldehyde crosslinking leads to the formation of microspheres with rough surface which can be overcome by using toluene saturated glutaraldehyde (Gohel et al., 1994).

3.2.2. *Multiple emulsion method*

Water insoluble drugs are simply dispersed in chitosan solution and entrapped by emulsion crosslinking process. In case, where the drug gets partitioned more into the oily phase, multiple emulsion is the way to increase the entrapment efficiency. This method involves formation of (o/w) primary emulsion (non-aqueous drug solution in chitosan solution) and then addition of primary emulsion to external oily phase to form o/w/o emulsion followed by either addition of glutaraldehyde (crosslinking agent) and evaporation of organic solvent (Pavanetto et al., 1996). Chitosan microspheres prepared by multiple emulsion method, loaded with hydrophobic drug (ketoprofen) were found to have good morphological character and satisfactory production yield when prepared using this method.

Recently, genistein chitosan microspheres were prepared by the o/w/o multiple emulsion method by Wu and Li (2002). The authors carried out optimization studies using Central Composite Design and reported that the theoretical drug content was 13–15%, the concentration of organic phase was 30–40% and the concentration of oil phase was 68–72%.

3.2.3. *Precipitation–chemical crosslinking*

This process involves the precipitation of the polymer followed by chemical crosslinking. Precipitation can be done by sodium sulphate followed by chemical crosslinking using glutaraldehyde (Berthold et al., 1996b) or formaldehyde.

In a study performed by Aggarwal et al. (2001), an aqueous solution of chitosan (3% (w/v) in 4% (v/v) glacial acetic acid) was added into agitating medium and stirring continued to obtain wet microsphere, which were then filtered, washed and finally dried at room temperature.

A study conducted by El-Shafy et al. (2000) reported that solvent emulsification technique (w/o) can also be used to prepare microspheres using heat as a crosslinking agent, thereby, avoiding the use of a chemical as a crosslinking agent.

3.2.4. *Crosslinking with a naturally occurring agent*

Mi et al. (2002) prepared an injectable-chitosan-based delivery system with low cytotoxicity. The chitosan microspheres with small particle size, low crystallinity and good sphericity were prepared by a spray-drying method followed by crosslinking with a naturally occurring crosslinking agent (genipin). The results of the study demonstrated that the genipin-crosslinked chitosan microspheres had a superior biocompatibility and a slower degradation rate than the glutaraldehyde-crosslinked chitosan microspheres. It was concluded that the genipin-crosslinked chitosan microspheres may be a suitable polymeric carrier for long-acting injectable drug delivery.

3.3. *Miscellaneous methods*

In addition to the use of above counterions, a number of microencapsulation processes have been reported for the preparation of chitosan microspheres using gelling properties of chitosan. These processes are described in the following sections.

3.3.1. *Thermal crosslinking*

Orienti et al. (1996) prepared indomethacin loaded chitosan microspheres by thermal crosslinking using citric acid. Chitosan solutions of varying concentrations were prepared maintaining a constant molar ratio between chitosan and citric acid. The above chitosan-crosslinker solution was then cooled at 0 °C and added to corn oil followed by thermal crosslinking at 120 °C.

3.3.2. *Solvent evaporation method*

This method involves the formation of an emulsion between polymer solution and an immiscible continuous phase whether aqueous (o/w) or non-aqueous (w/o). Lim et al. (2000) investigated the comparison of mucoadhesive microspheres of hyaluronic acid, chitosan glutamate and a combination of the two prepared by solvent evaporation with microcapsules of hyaluronic acid and gelatin prepared by complex coacervation.

Bogataj et al. (2000) prepared microsphere by solvent evaporation method using the solvents liquid paraffin/acetone. The drug solution (in acetone) was dispersed in chitosan solution and this mixture was emulsified in liquid paraffin and stirred. The suspension of microspheres was filtered, washed and dried. Agglomeration preventing agent magnesium stearate was also added. The results showed that average particle size decreased with increasing amount of magnesium stearate used for microsphere preparation.

In a modified method called “Dry-in-oil”, chitosan solution in acetic acid was dropped into oil and the temperature of the system was raised and pressure reduced, resulting in evaporation of the solvent and formation of microspheres (Li et al., 1991).

3.3.3. *Spray drying*

Chitosan microspheres can also be prepared by spray drying. Chitosan solution is sprayed, air-dried followed by the addition of a crosslinking agent. He et al. (1999a) used formaldehyde as a crosslinking agent. He et al. (1999b) further reported a novel method in which cimetidine and famotidine were entrapped in microspheres prepared by spray drying of multiple emulsion (o/w/o or w/o/w). They found that the release of the drugs from microspheres by this novel method was significantly sustained as compared to those prepared by conventional spray drying or o/w emulsion method.

In another study low, medium and high-molecular-weight chitosan and hydroxypropyl methylcellulose (HPMC), in different drug–polymer ratios was used for the preparation of spray-dried chitosan microspheres (Filipovic-Grcic et al., 2003). The results indicated that the entrapment efficiency and carbamazepine release profile depended on polymeric composition and drug–polymer ratios of the microspheres prepared. The best entrapment efficiencies were obtained when chitosan of low-molecular-weight were used for the microencapsulation. The microspheres with low carbamazepine loading (6.3%, w/w) showed better control of drug release when compared to higher drug loadings.

3.3.4. *Interfacial acylation*

Chitosan microspheres containing oxytetracycline were prepared by spray hardening and interfacial acylation methods (Mi et al., 1997). The objective of this study was to prepare oxytetracycline-containing microspheres for oral administration and injection using different molecular weight chitosan (70,000–2,000,000). By the spray hardening method, microspheres with particle sizes between 5 and 30 μm could be obtained. On the other hand, chitosan microspheres with the ability to extend the dissolution period of oxytetracycline in low pH medium were prepared by the interfacial acylation method. The result indicated that the release of oxytetracycline from various acylated chitosan microspheres was decreased with increasing the molecular weight of chitosan.

3.3.5. *Coating by chitosan solution*

In this method, previously formed microparticles are coated with chitosan (Nishioka et al., 1989; Takahashi et al., 1990; Meshali and Gabr, 1993; Murata et al., 1993, 1996; Vural et al., 1994; Hari et al., 1996). Human serum albumin (HSA) microspheres were prepared and added to various concentrations of chitosan–acetic acid solutions and mixed. The coated microparticles were filtered and dried.

Lin and Kang (2003) coated poly(epsilon-caprolactone) (PCL) microparticles with chitosan and gelatin. The release of indomethacin from uncoated microparticles followed a two-exponential release profile, where indomethacin was rapidly released within 4 h during the first release phase, after that approximately 20% of the drug was released continuously and slowly up to 24 h in the second phase. The similar release profile was observed from coated microparticles irrespective of the times of coating and the types of coating material. Both the natural coating materials, chitosan and gelatin, efficiently reduced the initial burst release in the first phase of drug release, but did not alter the second phase of drug release.

Takishima et al. (2002) prepared ethylcellulose microparticles and coated them with chitosan solution. When fluorescein isothiocyanate (FITC)-labeled chitosan-coated ethylcellulose microparticles without drug were administered intraduodenally, they moved slowly in the intestine, that is, most of them were retained at the upper and middle parts of the small intestine for more than 8 h, which is considered appropriate due to mucoadhesive properties of coated chitosan.

3.3.6. Reacetylated chitosan microspheres

Chitosan microspheres containing 5-fluorouracil (5-FU), tegafur (FT), and doxifluridine (DFUR) were prepared by the "Dry-in-oil" method using silicone oil with no surfactant as a dispersion medium (Yoshino et al., 2003). The microspheres with fairly large size (300–900 μm) and good drug content (4–22%, w/w) could be obtained. For DFUR-containing chitosan microspheres (DFUR-M), reacetylation with acetic anhydride DFUR-M and coating using chitosan and glutaraldehyde was performed. The former two microspheres were examined for in vivo degradation after subcutaneous implantation in mice, and in vivo plasma concentration–time profiles after implantation in rats. DFUR-M showed high initial rapid release, which was suppressed to some extent by reacetylation or chitosan coating, but remained to be improved for drug release properties.

Portero et al. (2002) also described the preparation of reacetylated chitosan microspheres for the controlled release of active anti-microbial agents viz., amoxycillin and metronidazole. The reacetylated chitosan microspheres exhibited a controlled water swelling capacity and gelified at acidic pH, resulting in prolonged release of the encapsulated antibiotics. The reacetylation time was found to be the key factor that affected not only drug release, but also encapsulation efficiency and anti-microbial activity of the encapsulated compound.

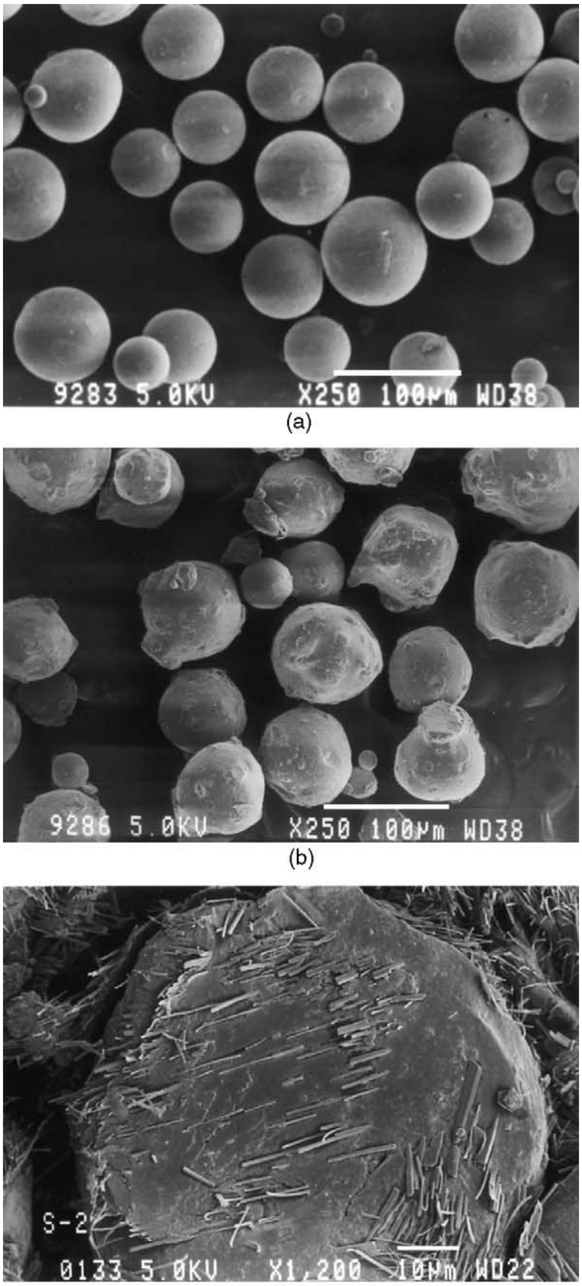
4. Factors affecting the entrapment efficiency of the drugs in chitosan microspheres

Many factors affect the entrapment efficiency of the drugs in the chitosan microspheres, e.g. nature of the drug, chitosan concentration, drug polymer ratio, stirring speed, etc. Generally a low concentration of chitosan shows low encapsulation efficiency (Orienti et al., 1996). However, at higher concentrations, chitosan forms highly viscous solutions, which are difficult to process.

A number of reports have shown that entrapment efficiency increases with an increase in chitosan concentration. This may be explained on the basis that an increase in viscosity of the chitosan solution with increase in concentration prevents drug crystals from leaving the droplet. A study carried out by Nishioka et al. (1990) also revealed that the cisplatin content increased with increasing chitosan concentration. Further Nishioka et al. (1990) also proved that the incorporation of chitin in the carrier matrix produced a more pronounced increase in drug content.

Genta et al. (1998) obtained satisfactory ketoprofen contents in all batches of chitosan microspheres with a theoretical polymer/drug ratio 1:2 w/w. Microspheres made with a mixture of high molecular weight/low molecular weight chitosan (1:2 w/w) showed good drug content and encapsulation efficiency and these were independent of polymer/drug ratio.

Pavanetto et al. (1996) revealed that the acetic acid concentration in the polymeric solution influenced the ketoprofen content of the microspheres. Maximum drug encapsulation efficiency was obtained for the lowest theoretical drug chitosan ratio. Singla et al. (2001) reported that when nifedipine was dispersed in the chitosan solution with stirring during preparation of mirospheres, the entrapment efficiency increased. Further, Dhawan and Singla (2003) reported that with increase in loading, the entrapment efficiency decreased. Scanning electron microscopy indicated that the roughness on the surface of the microspheres increased with increase in loading (Miglani, 2002). Fig. 3a depicts the surface topography of unloaded



(a)

(b)

(c)

Fig. 3. Scanning electron micrograph of (a) unloaded chitosan microspheres (bar: 100 μm; Dhawan and Singla, 2003); (b) nifedipine loaded (27.1%) chitosan microspheres (bar: 100 μm; Dhawan and Singla, 2003); (c) internal structure of nifedipine loaded chitosan microspheres (bar: 10 μm; Miglani, 2002).

chitosan microspheres while Fig. 3b shows the surface of nifedipine loaded chitosan microspheres. Nifedipine was found to be in crystalline state in the microspheres (Fig. 3c). The decrease in entrapment effiency was attributed to the removal of crystals present on the surface before washing.

5. Drug loading by adsorption onto chitosan microspheres

In an attempt to incorporate the drug onto previously formed chitosan microsphes, prednisolone sodium phosphate was adsorbed to previously manufactured chitosan microspheres (Berthold et al., 1996a,b). The drug adsorption was found to be dependent upon the initial drug concentration. A higher initial concentration led to a higher loading efficiency. It was also observed that lipophilic steroids were adsorbed in lower amounts as compared to their hydrophilic derivatives.

Since the encapsulation of isoniazid tends to be limited by its hydrophilic characteristics, therefore, the drug was adsorbed onto pre-formed chitosan alginate microspheres prepared by complex coacervation (Lucinda-Silva and Evangelista, 2003). The adsorption was probably of chemical nature, i.e. there was an ionic interaction between the drug and the surface of the particles.

Interleukin-2 was also incorporated into the preformed microspheres by diffusion from an external aqueous solution (Liu et al., 1997). Fluoresceinisothiocyanate labeled bovine serum albumin (FITC-BSA) was incorporated into the microspheres by mixing the protein with the polysaccharide solution of chitosan and microspheres were prepared by gelation of chitosan and sodium alginate followed by lyophilization. The microspheres thus formed were porous in structure.

Hejazi and Amiji (2002) prepared chitosan microspheres by ionic crosslinking and precipitation with sodium sulfate. Two different methods were used for drug loading. In first method, tetracycline was mixed with chitosan solution before the simultaneous crosslinking and precipitation. In second method, the drug was incubated with pre-formed microspheres for 48 h. When the drug was added to the polymer solution before crosslinking and precipitation, only 8% (w/w) was optimally incorporated in the final microsphere formulation. On the other hand, when the drug was incubated with the pre-formed microspheres, a maximum of 69% (w/w) could be loaded. This signifies that the drug can be adsorbed on to the chitosan microspheres to a greater extent using the latter method.

An investigation by Jameela et al. (1994) revealed the possibility of incorporating biological macromolecules (bovine serum albumin and toxoid), which are very sensitive to organic solvents, pH and temperature, by a passive adsorption technique into degradable biopolymer matrices, thereby, preserving their biological integrity. It was also observed that drugs were adsorbed onto such matrices due to swelling behavior. Moreover, the drug was not released completely in the initial burst.

Nascimento et al. (2001) prepared chitosan microspheres by the coacervation-phase separation method (induced by the addition of a non-solvent, NaOH, 2.0 M solution). The microspheres were crosslinked with glutaraldehyde, reduced with sodium cianoborohydride and grafted with poly(acrylic acid). The impregnation of aspirin into chitosan/poly(acrylic acid) copolymer microspheres was achieved by the dissolution of the drug in water:ethanol (2:1), which was adsorbed by the microspheres for 24 h at 25 °C. The efficiency of aspirin impregnation was approximately 94%.

6. Parameters affecting the release characteristics of drugs from chitosan microspheres

Many parameters determine the drug release behavior from chitosan microspheres. These include concentration and molecular weight of the chitosan, the type and concentration of crosslinking agent, variables like stirring speed, type of oil, additives, crosslinking process used, drug chitosan ratio, etc. Various researchers have proposed different models for the release of drugs from chitosan microspheres. Acikgoz et al. (1996) reported that the best fit for release of diclofenac sodium from chitosan microspheres was obtained by Higuchi equation. Sezer and Akbuga (1995) observed that when the release data of piroxicam from chitosan microspheres was subjected to simple power law equation, the mode of release was found to be non-fickian and super case II type.

6.1. *Effect of molecular weight of chitosan*

Drug release studies from chitosan microspheres have generally shown that the release of the drug decreases with an increase in molecular weight of chitosan. Shiraishi et al. (1993) investigated the effect of molecular weight of chitosan hydrolysate on the release and absorption rate of indomethacin from gel beads. The release rate of indomethacin was found to decrease with increasing molecular weight of chitosan. Similar results were obtained when oxytetracycline release from acylated chitosan microspheres was studied. It was observed that the release decreased with increasing molecular weight of chitosan (Mi et al., 1997). Similarly, Al-Helw et al. (1998) reported that release of phenobarbitone from crosslinked chitosan microspheres was slower from high molecular weight chitosan when compared to medium and low molecular weight chitosan.

In another study, Polk et al. (1994) reported that chitosan molecular weight was a key variable in the release of albumin from chitosan microspheres. The molecular weight of chitosan was varied from 1.25×10^6 to 0.25×10^6 through a nitrite oxidation reaction with sodium nitrite. Decreasing the molecular weight increased the release of albumin (from 37% release at 4 h with high molecular weight chitosan to 77% release with low molecular weight chitosan). Capsules produced with high molecular weight chitosan and a combination of high and low molecular weight chitosan gave the best results for reducing elution of albumin in the first 4 h and increasing elution in the following 20 h.

However, Genta et al. (1998) reported that the fastest ketoprofen dissolution profile from chitosan microspheres was obtained from medium molecular weight chitosan. This may be attributed to swelling behavior of chitosan microspheres. An increase in molecular weight of chitosan leads to increase in viscosity of the gel layer, which influences the diffusion of the drug as well as erosion of the microspheres.

6.2. *Effect of concentration of chitosan*

Nishioka et al. (1990) reported that the rate of cisplatin release reduced with the increasing concentration of chitosan. Aiedeh et al. (1997) observed that the method of chitosan interfacial crosslinkage by ascorbyl palmitate in water/oil dispersion was suitable to produce biodegradable system for insulin. The microcapsules obtained had release kinetics approaching zero order and a release rate, which could be increased by decreasing the chitosan content in the preparative solution.

6.3. *Effect of drug content in the microspheres*

A number of reports studying the effect of drug release have shown that the release of the drug from the microspheres increases with increase in drug content in the microspheres (Bayomi et al., 1998). However, contrary results have also been reported. Akbuga and Durmaz (1994) reported that furosemide release from chitosan microspheres followed the Higuchi matrix model. As the amount of furosamide incorporated increased, furosemide release was also increased. While Bodmeier et al. (1989a) reported that the release of sulfadiazine (a water insoluble drug) decreased with increase in drug content in the microspheres.

6.4. *Physical state of the drug in the microspheres*

The physical state of a drug is also an important parameter while investigating the drug release kinetics from a dosage form. The physical state of the drug may vary from molecular dispersion to well defined crystalline structures. He et al. (1999a) observed that cimetidine and famotidine were molecularly dispersed inside the microspheres, in the form of a solid solution. Therefore, the drug release was fast accompanied by a burst effect.

6.5. *Effect of density of crosslinking*

The crosslinking density has a remarkable effect on the release of drugs from the microspheres. Jameela et al. (1998) revealed that highly crosslinked microspheres released only 35% of the progesterone in 40 days compared to 70% release from microspheres crosslinked lightly.

Mitoxantrone release from chitosan microspheres was also controlled by the extent of crosslinking. The crosslinking was carried out using glutaraldehyde-saturated toluene (Jameela and Jayakrishnan, 1995). Only about 25% of the drug was released over 36 days from highly crosslinked microspheres.

Shu and Zhu (2002a) adopted a modified chitosan crosslinking method, i.e. chitosan/gelatin droplet coagulated at low temperature were crosslinked by anions (sulfate, citrate and tripolyphosphate) and prepared chitosan beads. Sulfate and citrate crosslinked chitosan beads swelled and even dissociated in simulated gastric fluid and hence, model drug (riboflavin) released completely in 5 h. In simulated intestinal fluid, beads remained in a shrinkage state and drug released slowly (release percentage less than 70% in 24 h). However, swelling and drug release of tripolyphosphate/chitosan beads was usually insensitive to media pH. Chitosan beads, crosslinked by a combination of tripolyphosphate and citrate (or sulfate) together, not only had a good shape, but also improved pH-responsive drug release properties. These results indicated that ionically crosslinked chitosan beads might be useful in stomach specific drug delivery.

Ko et al. (2002) prepared felodipine loaded chitosan microparticles with tripolyphosphate (TPP) by ionic crosslinking. On examining the morphologies of TPP–chitosan microparticles with scanning electron microscopy, it was observed as pH of TPP solution decreased and molecular weight of chitosan increased, microparticles had more spherical shape and smooth surface. Chitosan microparticles prepared with lower pH or higher concentration of TPP solution resulted in slower felodipine release from microparticles. With decreasing molecular weight and concentration of chitosan solution, release of the drug was increased. The release of drug from TPP–chitosan microparticles decreased when crosslinking time increased.

Kumar et al. (2002) encapsulated curcumin (upto an extent of 79.49 and 39.66%) in bovine serum albumin and chitosan to form a depot forming drug delivery system. Microspheres were prepared by emulsion–solvent evaporation method coupled with chemical crosslinking of the natural polymers. The concentration of the crosslinking agent had remarkable influence on the drug release. In vitro release studies indicated a biphasic drug release pattern, characterized by a typical burst-effect followed by a slow release which continued for several days.

Dini et al. (2003) studied the synthesis and characterization of glutaraldehyde crosslinked chitosan microspheres containing hydrophilic drug, hydroquinone. It was found that slower drug release rates were obtained from microspheres prepared by using a higher initial concentration of chitosan, a higher molecular weight of chitosan or/and a lower drug concentration. It was established that the release rate of hydroquinone was mainly controlled by the polymer crosslinking density and the degree of swelling of the hydrogel matrix.

6.6. *Effect of additives*

Lim and Wan (1998) prepared chitosan microspheres by emulsification–coacervation technique using pentasodium tripolyphosphate as a counterion. This led to a high degree of aggregation. The aggregation was markedly reduced by the incorporation of magnesium stearate in the dispersed phase. However, this addition did not affect the drug release. Additionally, with an increasing magnesium stearate content, larger sized microspheres were produced.

Bogataj et al. (2000) reported that higher pipemidic acid content in the chitosan microspheres was observed when higher amounts of magnesium stearate were used. The study also showed that the average particle size decreased with an increasing amount of magnesium stearate. The particle size and concentration of the magnesium stearate significantly influenced the dissolution rate of the drug. The physical state of pipemidic acid changed from crystalline to amorphous after its incorporation in microspheres.

Lim et al. (1997) reported that when chitosan microspheres were prepared by emulsification ionotropic gelation, the Span 85 concentration had a marked effect on the surface of the microspheres. At high Span 85 concentration, the microspheres had collapsed surfaces. The same effect was also reported by Singla et al. (2001) (Fig. 4).

Addition of chitin during preparation of microspheres was found to suppress the burst effect of cisplatin loaded chitosan microspheres prepared by emulsion crosslinking technique. The release kinetics was observed to be of first order. The average diameter was found to be 74.8 μm and the drug content was about 20.83 ± 0.36% (Wang et al., 1996a).

7. Stability of chitosan microspheres

A few studies have reported the instability of chitosan microspheres (prepared by precipitation) in acidic medium. Berthold et al. (1996a) prepared chitosan microspheres by sodium sulfate precipitation but the acid stability of the microspheres was found to be poor. Further, Berthold et al. (1996b) made an extensive investigation into the acid stability of the microspheres and observed that crosslinking with glutaraldehyde can increase the same. The acid instability of the microspherses can be explained by the manufacturing process of the microspheres. The addition of sodium sulfate to acetic acid solution of chitosan leads to poorly soluble chitosan derivatives by ionic neutralization of the positively charged amino groups.

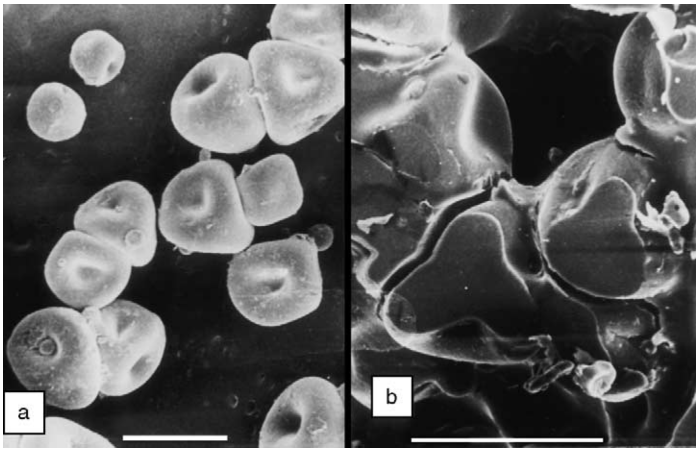


Fig. 4. Scanning electron micrograph of (a) external surface and (b) internal structure of unloaded chitosan microspheres at 1.5% (w/v) Span 85 concentration. Bar: 100 μm (Singla et al., 2001).

On addition of the acid (increased proton concentration) the equilibrium is shifted towards solubilization of chitosan and microspheres dissolve (Illum, 1998).

Bodmeier et al. (1989a) prepared sulfadiazine loaded chitosan beads using tripolyphosphate and observed that the beads had poor mechanical strength.

8. Different categories of drugs microencapsulated

Microparticles prepared using chitosan are being extensively investigated for various classes of drugs. The findings of these research studies are summarized in the following sections.

8.1. Anticancer drugs

8.1.1. Fluorouracil (5-FU)

Li et al. (1991) prepared 5-FU loaded chitosan microspheres by "Dry-in-oil" method. Results of the study suggested that chitosan was able to slow down the release rate of the drug. In another study, anionic polysaccharides, 6-*O*-carboxymethyl-*N*-acetyl-α-1,4-polygalactosamine, 6-*O*-carboxymethyl-chitin, alginate and heparin have also been used to coat chitosan gel microspheres of 1-[*N*-(5-aminopentyl)-carbamoyl]-5-fluorouracil by polyelectrolyte complex membrane formation (Ohya et al., 1993). These microspheres released the drug in a controlled manner and were found to have targetability to specific organs/cells due to the presence of polysaccharide chains on the surface of microspheres which are further recognized by the saccharides specific receptor cell surface. In another study, glutaraldehyde crosslinked chitosan microspheres of 5-FU were prepared and the effect of variables viz., drug and chitosan concentration, crosslinking process, the type of oil and stirring rate were examined (Akbuga and Bergisadi, 1996). 5-FU release from crosslinked chitosan microspheres was characterized by an initial rapid release of drug. Release properties of 5-FU were also influenced by the addition of substances such as chitin, alginic acid, agar, stearic acid and sodium caprylate. 5-FU loaded chitosan microspheres for chemoembolization have also been prepared by a suspension crosslinking technique using glutaraldehyde (Denkbas et al., 1999). The particle size range of the microspheres varied between 100 and 200 μm depending upon the emulsifier concentration, stirring rate, chitosan/solvent ratio and drug/chitosan ratio. About 60% of the loaded drug was released within first 24 h.

Chandy et al. (2000) investigated the potential of chitosan-coated PLA (polylactic acid)/PLGA (polylactic-*co*-glycolic acid) microspheres for the targeted delivery of 5-FU to treat cerebral tumors. Scanning electron microscopy revealed that the chitosan-coated microspheres had less surface micropores compared to polyvinyl alcohol-coated microspheres. The amount of drug release was much higher initially (approximately 25%), followed by a constant slow release profile for a 30-day period of study, thus exhibiting biphasic pattern.

8.1.2. Cisplatin

Coated albumin microspheres of cisplatin with chitin and chitosan have shown to possess sustained release characteristics and have also been found useful in hepatic artery embolization (Nishioka et al., 1989, 1990). The rate of cisplatin release reduced with increasing concentration of chitosan. The time taken for 50% cisplatin release from microspheres prepared with 1.0% of chitosan was less (0.5 h) compared to microspheres prepared using 5.0% of chitosan (4.5 h), indicating about nine-fold prolongation. The addition of chitin further resulted in retardation of the rate of release of cisplatin. Chitosan microspheres were shown to undergo enzymatic degradation by lysozymes.

Kyotani et al. (1992) investigated the antitumor activity of cisplatin containing albumin microspheres, treated with chitin or chitosan, on rabit hepatic tumors. Tumor growth rates of chitin containing and chitosan-treated albumin microspheres, showed lower growth rates of 160 and 120%, respectively, as compared to non-treated groups with a rate of 580%.

Wang et al. (1996b) found chitosan microspheres of cisplatin, to be good chemo-embolization agent. Cisplatin loaded chitosan microspheres were also prepared using a w/o emulsion system (Akbuga and Bergisadi, 1999). Variables such as chitosan concentration, cisplatin, glutaraldehyde concentration, type of chitosan and oil were studied and found to have a significant effect on cisplatin entrapment in chitosan microspheres. Incorporation efficiency was found to be between 28 and 29%. The type of oil used was found to affect release properties of cisplatin, which showed an initial burst effect. Pharmacokinetics, targeting, embolization effects and alteration of liver function using cisplatin chitosan microspheres were studied after hepatic arterial embolization in dogs. Results showed a remarkable decrease in the number of arterioles in liver, necrosis of nodules and hepatic cell degeneration in the embolized region.

Chandy et al. (2002) demonstrated the possibility of entrapping an antiproliferative agent, cisplatin, in a series of surface-coated biodegradable microspheres composed of poly(lactic acid) poly(caprolactone) blends, with a mean diameter of 2–10 pm. The microspheres were surface coated with polyethylene glycol (PEG), chitosan, or alginate. A solution of cisplatin and a 50:50 blend of polylactic acid (PLA)–polycaprolactone (PCL) dissolved in acetone–dichloromethane mixture was poured into an aqueous solution of PEG (or polyvinyl alcohol or chitosan or alginate) with stirring using a high speed homogenizer, for the formation of microspheres. Cisplatin recovery in microspheres ranged from 25 and 45% depending on the emulsification system used for the preparations. The amount of drug release was much higher initially (20–30%), this was followed by a constant slow-release profile for a 30-day period of study. It was observed that drug release was dependent on the amount of entrapped drug, on the presence of extra cisplatin in the dispensing phase, and on the polymer coatings. This microsphere formulation might have potential for the targeted delivery of antiproliferative agents to treat restenosis.

8.1.3. *Mitoxantrone*

Microspheres of mitoxantrone were prepared by crosslinking technique using glutaraldehyde-saturated toluene. Implantation of placebo chitosan microspheres in skeletal muscle of rats showed biocompatability and biodegradability of the microspheres for long duration (Jameela and Jayakrishnan, 1995). The antitumor activity after intraperitoneal injection of mitoxantrone loaded chitosan mirospheres against Ehrlich ascites carcinoma in mice was also studied (Jameela et al., 1996). The activity was found to be much higher demonstrating the potential of these microspheres for sustained drug delivery to minimize drug toxicity and maximize therapeutic efficacy.

8.1.4. *Oxantrazole*

Magnetic chitosan microspheres containing oxantrazole were prepared using a combined emulsion/polymer crosslinking/solvent evaporation technique (Hassan et al., 1992). In addition to the central composite experimental design, statistical optimization procedures were used to evaluate the parameters required for optimized formulation. Entrapment of anticancer agents into biodegradable microspheres is difficult because of low aqueous drug solubility and porosity of the microspheres. The latter effect was circumvented by a chitosan crosslinking step that resulted in approximately 3% (w/w) oxantrazole entrapment in the microspheres. The combined formulation and statistical optimization strategy provided a basis to develop other microparticulate systems and led to a dosage form that can be used for future in vivo investigation.

In another study, magnetic cationic microspheres containing oxantrozole were found to have an ability to enhance the brain delivery of the drug 100 times more as compared to oxantrazole solution, due to the cationic anionic interaction of magnetic microspheres with the blood–brain barrier (Hassan and Gallo, 1993).

8.1.5. *Cytarabine*

Chitosan microspheres loaded with cytarabine were embedded in a poly(lactide-*co*-glycolide) film to constitute a co-matrix system (Blanco et al., 2000). Co-matrices were subcutaneously implanted in the back of rats. After 6 months of implantation, most of the microspheres of the matrix seemed to be intact; the co-matrix appeared surrounded by conjunctive tissue and small blood vessels. Nerve packets were detected in the periphery of the implant. Maximum plasma cytarabine concentration after 48 h of the device implantation was 18.5 ± 1.5 μg/ml and the drug was detected in plasma for 13 days.

8.1.6. *Methotrexate*

Sustained plasma levels of methotrexate were obtained when drug loaded chitosan and chitin microspheres were administered to mice bearing Ehrlich ascites tumors (Singh and Udupa, 1998). The physicochemical characteristics were affected by various parameters viz., stirring speed, concentration of chitosan and chitin. The $t_{1/2}$ for in vitro release varied depending on the concentration of chitosan and chitin and the amount of glutaraldehyde used.

8.1.7. Taxol

The possibility of encapsulating taxol-loaded polylactic acid (PLA) microspheres within heparin–chitosan spheres to develop a prolonged release co-matrix form was investigated by Chandy et al. (2001). The amount of taxol/heparin release was initially much higher, followed by a constant slow release profile for a prolonged period.

8.2. Antiinflammatory drugs

8.2.1. Indomethacin

Shiraishi et al. (1993) prepared indomethacin loaded chitosan microspheres by polyelectrolyte complexation of sodium tripolyphosphate and chitosan. A pH-dependent disintegration of the beads was observed in the in vitro study. The plasma concentrations of indomethacin after oral administration of chitosan gel beads to beagle dogs exhibited a sustained-release pattern. Good correlation was observed between the molecular weight of chitosan and dissolution rate constant or the mean absorption time or area under the plasma concentration–time curve. It was concluded that the chitosan gel beads composed of chitosan hydrolysate (molecular weight: 25,000) might be suitable for the sustained-release preparation of indomethacin. Effect of molecular weight of the chitosan on the release and absorption of indomethacin was also investigated by Bodmeier and Paeratakul (1989). Beads were prepared by dispersing the drug in solutions of the ionic polysaccharides chitosan or sodium alginate. These dispersions were then dropped into solutions of the respective counterions tripolyphosphate or calcium chloride ($CaCl_2$). Strong spherical beads with a narrow particle size distribution and low friability could be prepared with high yield and a drug content approaching 98%. The flow properties of micronized or needle-like drug crystals were significantly improved by this agglomeration technique when compared to nonagglomerated drug crystals. The ionic character of the polymers resulted in pH-dependent disintegration of the beads.

Another study reported the preparation of inomethacin loaded chitosan microspheres using only aqueous solvents (Aggarwal et al., 2001). The influence of formulation variables on indomethacin content in the microspheres and time for release of indomethacin from the microspheres was also investigated.

In another study, indomethacin loaded chitosan microspheres were coated with human serum albumin, in order to slow their clearance from the synovial fluid. While evaluating the effect of type of coating material and chitosan concentration on the release of the drug it was found that decrease in the release rate was due to the viscosity of chitosan (Vural et al., 1991).

Citric acid crosslinked microspheres of indomethacin were developed by Orienti et al. (1996). Increase in drug loading was observed with increased concentration of chitosan. Drug release was higher at higher pH. The release kinetics of the drug was seen to be Fickian-anomalous (or approaching zero order) but it shifted to zero order at higher pH values (7.4) and at higher chitosan concentration.

8.2.2. Diclofenac sodium

Murata et al. (1996) studied the effects of a complex of chondroitin sulphate and chitosan on the release of diclofenac sodium and flurbiprofen from alginate gel beads. It was observed that the chitosan chondroitin sulfate complex suppressed the disintegration of the gel beads and release of diclofenac sodium from the beads.

Chitosan microspheres of diclofenac were prepared by the coacervation phase separation method and the in vivo bioavailability study was carried out in rabbits (Gohel et al., 1994). The microspheres exhibited good spherical geometry and release kinetics of the drug was according to the Higuchi model.

Tapia et al. (1993) prepared the pellets of diclofenac sodium by extrusion and spheronization and observed that chitosan had a retardant effect on the release of the drug while Gonzalez-Rodriguez et al. (2002) reported the preparation of alginate/chitosan particles ionic gelation (Ca^{2+} and Al^{3+}) and investigated the release of sodium diclofenac.

A biodegradable diclofenac sodium microsphere system using chitosan has been characterized and evaluated in vitro and in vivo by Acikgoz et al. (1995). The release rate data were investigated by using zero-order, first-order, Hixson-Crowel and Higuchi kinetics. The optimum diclofenac sodium chitosan microsphere formulation, unloaded chitosan microspheres, sustained release commercial product and plain drug were evaluated for pharmacological activity and the ulcerogenic index in rabbits. A slow release of diclofenac was observed and a good fit to the Higuchi model was demonstrated. Although improvement in anti-inflammatory activity was exhibited, no improvement in ulcerogenicity was observed.

In another study, the effect of varying experimental conditions on release kinetics of diclofenac sodium from chitosan microspheres was studied using factorial design experiment (Acikgoz et al., 1996). The independent variables in the 3^3 factorial design were chitosan, tripolyphosphate concentration and stabilization time. The dependent variables studied were $t50\%$ and total drug content. The release was found to be of the order of Higuchi equation.

In order to exploit the colon specific biodegradation of chitosan, diclofenac sodium microcores with chitosan were prepared (Lorenzo-Lamosa et al., 1998). These were further entrappsed in acrylic polymeric coatings using different enteric coating polymers. So, this method combined colon specific biodegradability and pH-dependent release of the drug. The method involved entrapment of the drug within chitosan microspheres by spray-drying method, which was then microencapsulated into Eudragit L-100 and Eudragit S-100 using an o/o solvent evaporation method. A continuous release for a variable time (8–12 h) was achieved by this system due to the dissolution of the Eudragit coating alongwith swelling of the chitosan microcore.

Though chitosan is a very interesting biomaterial for drug delivery, its use in oral administration is restricted by its fast dissolution in the stomach and limited capacity for controlling the release of drugs. To overcome this limitation, a microparticulate chitosan controlled release system, consisting of hydrophilic chitosan microcores entrapped in a hydrophobic cellulosic polymer, such as cellulose acetate butyrate or ethylcellulose was proposed by Remunan-Lopez et al. (1998). These microparticles were obtained with different types of chitosan and various core/coat ratios, with the particle size in all cases being smaller than 70 μm. Two drugs sodium diclofenac and fluorescein-isothiocyanate labeled bovine serum albumin (FITC-BSA) were used. Results showed that the entrapment efficiency of sodium diclofenac was very high. Furthermore it was possible to modulate the in vitro release of the encapsulated molecules by changing the core properties viz., chitosan salt, molecular weight, core to coat ratio or the coating polymer. The microparticles were stable at low pH and thus, suitable for oral delivery without requiring any harmful crosslinking treatment.

Kumbar et al. (2002) prepared chitosan microspheres of diclofenac sodium with three different crosslinking methods, i.e. glutaraldehyde, sulphuric acid and heat treatment. Chitosan microspheres were produced in a w/o emulsion followed by crosslinking using one of the above methods. Among all the systems studied, the 32% glutaraldehyde crosslinked microspheres showed the slowest release, i.e. 41% at 420 min, and a fastest release of 81% at 500 min was shown by heat crosslinking for 3 h.

8.2.3. Prednisolone

A precipitation process using sodium sulphate as a precipitant was used to prepare chitosan microspheres loaded with prednisolone sodium phosphate (Berthold et al., 1996a). The drug was adsorbed on to the surface of the chitosan microspheres and a loading upto 30.5% was achieved. Drug release from microspheres was found to be dependent on the drug–polymer ratio.

Mooren et al. (1998) studied the transport of prednisolone using microspheres or a solution of the drug (control) across HT-29B6 cell monolayers, a polarized human colonic cell line. The transport of prednisolone across HT-29B6 cell monolayers was drastically enhanced by the use of chitosan microspheres compared to a solution of the drug.

Chitosan forms a gel in solutions with a pH above 12, and the gelation occurs at pH of about 9 in 10% amino acid solutions. In a study carried out by Kofuji et al. (2000), enzymatic degradation and the drug release profile of chitosan gel beads was investigated. The degradability of the chitosan gel beads was affected by the degree of deacetylation of chitosan. The release of prednisolone from the chitosan gel beads was sustained significantly compared with the gel prepared with sodium hydroxide only. However, the release was not sustained by the increment of sodium hydroxide concentration in the solution employed for the preparation of chitosan gel beads.

8.2.4. Ketoprofen

Effect of molecular weight of chitosan on drug loading and drug release was studied using ketoprofen as a model drug (Genta et al., 1998). Chitosans with molecular weight between 70,000 and 2,000,000 were found to be suitable carriers for ketoprofen that could modulate drug release within 48 h.

Chitosan microspheres of ketoprofen have also been prepared by a multiple emulsion (o/w/o), which produced satisfactory yield of microspheres (Pavanetto et al., 1996). Yamada et al. (2001) prepared ketoprofen microspheres by the "Dry-in-oil" method using ethylcellulose as a matrix polymer. Chitosan-coated ketoprofen microparticles were prepared by the precipitation of droplets of chitosan solution containing microspheres. The adhesion of chitosan-coated ketoprofen microparticles was tested using rat small intestinal mucosa. It was observed that chitosan-coated ketoprofen microparticles showed a good mucoadhesion. The maximum plasma concentration of ketoprofen for chitosan-coated ketoprofen microparticles was less than one-third of that for ketoprofen powder suspension. Chitosan-coated ketoprofen microparticles tended to show higher and steadier plasma level than microspheres.

8.2.5. *Ibuprofen*

Spherical pellets of poorly soluble drugs (micronized griseofulvin, ibuprofen, indomethacin, sulfadiazine, or tolbutamide) were prepared by dispersing the drug in solutions of the ionic polysaccharides chitosan or sodium alginate, and then dropping these dispersions into solutions of the respective counterions tripolyphosphate or $CaCl_2$ (Bodmeier and Paeratakul, 1989). The droplets instantaneously formed gelled spheres by inotropic gelation. Strong spherical beads with a narrow particle size distribution and low friability could be prepared with high yield and a drug content approaching 98%.

8.2.6. *Acetaminophen*

Bead formulations containing acetaminophen were prepared using the combination of carboxymethylcellulose sodium, cellulose microcrystalline (Avicel RC-591) and chitosan (Sea Cure) (Goskonda and Upadrashta, 1993). The study indicated that the higher viscosity grades of chitosan yielded beads with rough surfaces and slower drug release properties. The release of acetaminophen from beads varied with the dissolution method used.

8.2.7. *Piroxicam*

Piroxicam-loaded chitosan gel beads were prepared by dropping the drug containing chitosan solution into varying concentrations of triphosphate (tripolyphosphate) solutions of pH 5–9 (Sezer and Akbuga, 1995). The drug was successfully encapsulated at pH 5. The method of drying and chitosan concentration were found to be the key factor controlling the release of the drug.

8.2.8. *Sulfasalazine*

Vural et al. (1994) prepared sulfasalazine microspheres of albumin (human serum albumin) and treated them with 0.5–3% chitosan. These microspheres were evaluated for release. Incorporation of chitosan into the microspheres retarded the rate of dissolution. In addition, the release rate was influenced by the chitosan concentration.

8.2.9. *Dexamethasone*

Spray-dried microspheres of chitosan with a molecular weight of 70,000 or 750,000 were loaded with dexamethasone and studied with respect to morphology, drug content, particle size distribution, thermal behavior, powder X-ray diffraction properties, and drug release profiles (Genta et al., 1995). Dexamethasone was entrapped with good encapsulation efficiency. Drug entrapment in the chitosan polymeric network significantly improved the drug dissolution rate. This improvement did not occur with a physical mixture of drug and polymer.

8.3. *Cardiac agents*

8.3.1. *Calcium channel blockers*

8.3.1.1. Diltiazem hydrochloride. Casein–chitosan microspheres containing diltiazem hydrochloride were prepared by colloidal coacervation technique (Bayomi et al., 1998). The interaction between chitosan solution in acetic acid (5%, v/v) and casein solution in 0.5 M sodium hydroxide formed the basis of formation of microspheres. Formaldehyde was used as the crosslinking agent. The concentration of casein, chitosan, drug and stirring speed affected the properties and performance of the microspheres. Drug release was retarded by increasing the concentration of casein and the stirring time while fast drug release was obtained upon increasing the concentration of chitosan and a high initial drug loading. The entrapment efficiency of the microspheres varied between 14.5 and 53.7%.

Casein–chitosan microspheres containing diltiazem hydrochloride were also prepared using aqueous coacervation technique (al-Suwayeh et al., 2003). The formed microspheres were not suitable for tableting by direct compression due to their poor binding properties. Therefore, effect of different concentrations on the dry binders viz., hydroxypropylmethylcellulose, ethylcellulose, carbopol 940 and egg albumin on the properties of the tablets was studied. Each blend of microspheres with dry binder and 2% (w/w) magnesium stearate as glidant was tableted. It was concluded that the formulated tableted microspheres provided an acceptable delivery for diltiazem over an extended period of time.

8.3.1.2. Nifedipine. Nifedipine and nifedipine–cyclodextrin complexes were encapsulated in chitosan microspheres. Varying the crosslinking density, particle size and initial drug loading in the microspheres modified drug release profile. More than 70% of drug entrapment efficiency was achieved. The drug release from cyclodextrin complex was reduced significantly though the solubility of the drug was enhanced by the complexation (Filipovic-Grcic et al., 1996).

8.3.1.3. Propranolol hydrochloride. Lim and Wan (1998) reported that propranolol loaded chitosan microspheres prepared by emulsification coacervation technique had convoluted surface and ill-defined shape. Magnesium stearate was incorporated in the disperse phase to prevent aggregation of the microspheres. The size of the drug-loaded microspheres decreased with increasing magnesium stearate content. The release of propranolol hydrochloride from the microspheres was fast, irrespective of the content of magnesium stearate. Drug encapsulation efficiency was enhanced when a greater amount of magnesium stearate was used.

8.3.1.4. Isosorbide-5-mononitrate. The formulation and in vitro release profile of the isosorbide-5-mononitrate-loaded chitosan microspheres was investigated by Farivar et al. (1993). The release of the drug from chitosan microspheres was compared with marketed formulations.

8.4. Antibiotics

8.4.1. Amoxycillin

Amoxycillin and metronidazole loaded chitosan microspheres for stomach specific delivery were prepared for the treatment of *Helicobacter pylori* infection (Shah et al., 1999). The microspheres were prepared by crosslinking in addition to precipitation with sodium tripolyphosphate. In vitro studies in simulated gastric fluid showed that the total amount of drug was released in 2 h due to the high porosity of the drug-loaded microspheres. However, amoxycillin showed 40% degradation in 10 h in simulated gastric fluid while metronidazole was stable for 24 h. This study showed the usefulness of porous metronidazole containing chitosan microspheres for eradication of the above infection.

8.4.2. Ampicillin

A new derivative of chitosan, methyl-pyrrolidinone chitosan was used to prepare ampicillin microparticles by spray-drying technique. Scanning electron microscopy, particle size analysis, differential scanning calorimetery and in vitro drug release studies were carried out to characterize the microparticles (Giunchedi et al., 1998). Microbiological assays were also performed using different bacterial strains. Results of the assay showed that ampicillin microspheres were able to maintain the antibacterial activity of the drug.

Chandy and Sharma (1993) monitored the in vitro release profile of ampicillin from chitosan beads and microgranules. It was observed that the amount and percentage of drug release was much higher in HCl solution compared to the phosphate solution, probably due to the gelation properties of the matrix at acidic pH. The release rate of ampicillin from the chitosan matrix was slower for the beads as compared to the granules. Scanning electron microscopic studies revealed that the drug formed a crystal structure within the chitosan beads, which dissolved out slowly to the dissolution medium through the micropores of the chitosan matrix.

8.4.3. Tetracycline

Hejazi and Amiji (2003) examined the gastric residence time of tetracycline loaded chitosan microspheres (prepared by ionic crosslinking and precipitation method) following their oral administration in gerbils. Gastric retention studies were performed by administering radioiodinated [^{125}I] chitosan microsphere suspension in the nonacid-suppressed and acid-suppressed states. At different time points, animals were sacrificed and the radioactivity in tissues and fluids was measured with a gamma counter. The results indicated that chitosan microspheres did not provide a longer residence time in the fasted gerbil stomach. The tetracycline concentration profile in the stomach following administration of microsphere formulation was similar to that of aqueous solution.

8.4.4. Sulfadiazine

Bodmeier et al. (1989a) prepared sulfadiazine loaded chitosan microspheres by dropping chitosan solution into tripolyphosphate solution. Maximum drug content was achieved using high payloads, short gelation times, low tripolyphosphate concentration and a low internal to external phase ratio. The beads showed pH-dependent swelling and dissolution behavior. In 0.1 N HCl the release was found to decrease with increasing concentration of tripolyphosphate, but the release was independent of tripolyphosphate concentration in intestinal fluids.

8.4.5. Griseofulvin

Spherical pellets of micronized griseofulvin were prepared by dispersing the drug in solutions of the ionic polysaccharides chitosan or sodium alginate. These dispersions were then dropped into solutions of the respective counterions tripolyphosphate or calcium chloride (Bodmeier and Paeratakul, 1989). Strong spherical beads with a narrow particle size distribution and high drug content (approaching 98%) could be prepared. The flow properties of micronized or needle-like drug crystals were significantly improved by this agglomeration technique when compared with nonagglomerated drug crystals. Chitosan beads disintegrated in 0.1 M HCl, while calcium alginate beads stayed intact in 0.1 M HCl but rapidly disintegrated in simulated intestinal fluids.

8.4.6. Sulfathiazole

Wong et al. (2002) studied the influence of microwave irradiation on the drug release properties of alginate–chitosan and chitosan beads prepared by extrusion. Though the drug was chemically stable in microspheres, it underwent polymorphic changes on exposure to microwave radiations. Polymorphic changes were prevented by means of drug–alginate interaction in alginate and alginate–chitosan beads. Changes in the polymorphic state of drug were found to have insignificant effect on the drug release profiles of chitosan beads. The release-retarding property of alginate and alginate–chitosan beads was significantly enhanced by subjecting the beads to microwave irradiation. The authors interpreted that the reduction in rate and extent of drug released from the microwave-treated beads was primarily due to additional formation of non-ionic bonds, involving alginate crosslinkage and alginate–chitosan complexation. The results showed that microwave technology can be employed in the design of solid dosage forms for controlled-release application without the use of abnoxious chemical agents.

8.5. Antithrombotic agent

Gallo and Hassan (1988) formulated a new magnetic microsphere carrier that could localize drugs by both biochemical and physical means. The microspheres were formulated to have a controlled cationic character and with mean diameter of 0.70 μm and a magnetite content of 16% (w/w). Formation of complexes between chitosan and heparin and between the microspheres and heparin was investigated. Heparin served as a model glycosaminoglycan. The chitosan:heparin complex ratio was found to be 1:1 based on charge and was formed between ammonium ions on the chitosan and SO^{3-} groups on heparin. Neutralization of the charge on the microspheres prevented their complexation with heparin.

8.6. Steroidal drugs

Chitosan microspheres in size range 45–300 μm containing progesterone were prepared by glutaraldehyde crosslinking technique (Jameela et al., 1998). In vivo bioavailability study after intramuscular injection in rabbits showed the maintenance of plasma concentration of 1–2 ng/ml for upto a period of 5 months without a high burst effect. Chitosan microspheres, therefore, seem to be very promising agents for prolonged drug delivery of steroids.

8.7. Anticalcification agents

Implantation or injection of microspheres containing pamidronate and suberoylbisphosphate for site-specific therapy in treating several pathological conditions associated with bone destruction was investigated (Patashnik et al., 1997). Microspheres of antiresorption and anticalcification agents, pamidronate and suberoylbisphosphate, respectively were prepared from a w/o emulsion. These microspheres were coated with polymer and were found to be biocompatible. The coated microspheres had a considerably retarded drug release rate. In vitro and in vivo studies showed that the in vitro release of bisphosphates was faster than in vivo release. Implantation results showed relatively increased disposition in the tibialis muscle. Bisphosphonate released from microspheres effectively inhibited biprosthetic tissue calcification in the rat subdermal model.

8.8. Proteins

8.8.1. Antigens

Porous chitosan microspheres were prepared for the controlled delivery of the antigen of new castle disease, which was immobilized into the pores of microspheres (Mi et al., 1999). High porosity of microcrospheres with an open structure could be obtained by changing the pH value of the coagulation medium, i.e. aqueous tripolyphosphate solution to 8.9. Higher adsorption efficiency and slower release rate of the antigen were obtained by chemical modification of the microspheres with 3-chloro-2-hydoxypropyltrimethyl ammonium chloride.

8.8.2. Bovine serum albumin (BSA)

BSA was adsorbed from aqueous solutions into pre-formed glutaraldehyde crosslinked chitosan microspheres (Jameela et al., 1994). A sustained release of the macromolecules was obtained from the microspheres. Polk et al. (1994) investigated a polymeric delayed-release protein delivery system with albumin as a model drug. Chitosan was reacted with sodium alginate in the presence of calcium chloride to form microcapsules with a polyelectrolyte complex membrane. The pH of the extracapsular environment was found to affect the release of albumin significantly (15% release over 24 h at pH 3.0 and 73% release at pH 8.0).

8.8.3. Salmon calcitonin

The salmon calcitonin-loaded chitosan beads were prepared by dropping a drug containing solution of chitosan into tripolyphosphate solution (Aydin and Akbuga, 1996). The mean diameter of beads was about 0.9 mm and encapsulation efficiency of drug was 54–59%. The drug was successfully encapsulated at pH 6. The amount of salmon calcitonin did not affect the drug release from beads. The release of salmon calcitonin from the beads could be sustained for a period of 27 days.

8.9. Amino acids

Several layers of phenylalanine or tryptophan were coated covalently on chitosan beads using N_2-plasma, carbodimide or glutaraldehyde treatments (Beena et al., 1994). The surface modified chitosan beads exhibited high binding affinity for gamma globulin compared to bare beads. This study revealed that modified chitosan surfaces may be an excellent sorbent system for haemoperfusion due to their high binding affinity for immunoproteins and blood compatibility.

8.10. Antidiabetic agents

8.10.1. Insulin

A study performed on insulin loaded chitosan mirospheres showed that oral chitosan-microcapsulated insulin had the antihyperglycemic effect on the blood glucose level of streptozotocin-diabetic rats (Huang et al., 2001). The potential of chitosan nanoparticles as a system for improving the systemic absorption of insulin following nasal instillation was also investigated by Fernandez-Urrusuno et al. (1999). Chitosan nanoparticles were found to be efficient vehicles for the transport of insulin through the nasal mucosa.

8.11. Growth factors

Brain derived neurotrophic factor (BDNF) was microencapsulated using biodegradable chitosan microspheres (Mittal et al., 1994). The biological assays confirmed that the released BDNF remains biologically active in vitro. Microencapsulation of BDNF and other trophic factors may represent a viable alternative to currently employed delivery systems in vivo and holds potential for eventual therapy of neurodegenerative disorders, particularly for peripheral neuropathies and central nervous system disorders where supplementation of neurotrophins retards or prevents neural degeneration.

For the controlled and localized release of endothelial cell growth factor (ECGF), chitosan–albumin microspheres in the size range 400–600 μm were formed in 0.5 M sodium hydroxide–methanol solution and incubated with ECGF (Elcin et al., 1996) to facilitate adsorption. In vivo experiments were carried out in rat groin fascia and showed an initial burst release of 22–27% of ECGF within first 2 h followed by 2–7% release during week and 1–4% release during the second week. Further during the third week ECGF release of 0.5% per day was maintained.

8.12. DNA encapsulation

Calf thymus DNA was immobilized within chitosan-coated alginate microspheres (Vitrexakis et al., 1995). Microcapsules were prepared by emulsification/internal gelation using interfacial polymerization of chitosan and alginate. The microspheres of size range 20–500 μm and encapsulation efficiency of about 96% were obtained. No leakage of DNA was observed upon core dissolution.

Chitosan is considered to be a good candidate for gene delivery since cationically charged chitosan can be complexed with negatively charged plasmid DNA. Hence, the effect of different factors such as plasmid size, chitosan concentration and plasmid addition techniques on characterization and in vivo transfection of DNA–chitosan microspheres was investigated (Aral et al., 2000). Two types of plasmids, large pMk3 and pUC18, were used in the study. In vitro release studies carried in phosphate buffer showed that in pMk3 chitosan microspheres prepared using low chitosan concentration (kM2) released a higher level of DNA in comparison to other formulation (kM5). In case of plasmid pUC18, no significant difference between microspheres prepared using low and high concentration was observed. In vivo experiments were performed in rats by injecting naked and encapsulated plasmid into the muscle and monitoring β-galactosidase production. The highest level of β-galactosidase expression was obtained with low dose DNA chitosan microspheres and injection of naked plasmid DNA to rats resulted in negligible expression. These results provide preliminary data for development of systems for gene delivery.

Alexakis et al. (1995) also encapsulated calf thymus DNA within crosslinked chitosan membranes, or immobilized within chitosan-coated alginate microspheres. Microcapsules were prepared by interfacial polymerization of chitosan.

Ozbas-Turan et al. (2003) encapsulated two different plasmid DNAs (pGL2 and pMK3) in the same microsphere structure investigated the in vivo transfection characteristics of chitosan microspheres. They reported that two plasmids could be encapsulated in chitosan microspheres without affecting their structural and functional integrity.

8.13. Diuretics

8.13.1. Furosemide

Akbuga and Durmaz (1994) prepared furosemide loaded crosslinked chitosan microspheres and investigated the effect of several factors on microsphere properties. It was concluded that the properties of chitosan microspheres containing furosemide were affected by various preparation variables, including type and concentration of chitosan, drug concentration and stirring rate.

8.14. Central nervous system (CNS) acting agents

8.14.1. Theophylline

Theophylline granules coated with polyelectrolyte complex of tripolyphosphate and chitosan were prepared by Kawashima et al. (1985a). Effect of thickness and hardness of coating film on the drug release rate of theophylline granules coated with chitosan–tripolyphosphate complex was also investigated (Kawashima et al., 1985b). Lin and Lin (1992) studied the effect of acid type, acetic acid and sodium carboxy methylcellulose (NaCMC) concentration on the dissolution and floating properties of theophylline chitosan microspheres was observed. It was observed that the greater the amount of NaCMC used, the slower the release and the larger the particle size of the microcapsules obtained. The interaction between chitosan and NaCMC in microcapsules caused the formation of a water insoluble complex, and this complex significantly affected the formation, micromeritic property and release behavior of chitosan microspheres. The higher the concentration of acetic acid used for the preparation of microspheres faster was the release rate of the drug from the microcapsules.

Thanoo et al. (1992) prepared theophylline, aspirin and griseofulvin loaded chitosan microspheres by glutaraldehyde crosslinking. Dioctyl sulphosuccinate was used as a stabilizing agent. Good drug loading efficiency (exceeding 80%) could be achieved for all the drugs. Release profile of all the drugs was observed in gastric as well as intestinal fluids. Theophylline and griseofulvin showed similar release profile in both the fluids while aspirin showed a slightly faster rate of release in intestinal fluid as compared with gastric fluid probably due to ionization and higher solubility of aspirin in intestinal fluid.

8.14.2. Phenobarbitone

Glutaraldehyde crosslinking of an aqueous acetic acid dispersion of chitosan in light-liquid paraffin containing sorbitan mono-oleate (as a stabilizing agent) was used to prepare chitosan microspheres containing phenobarbitone (Al-Helw et al., 1998). Uniform and spherical microspheres with a loading efficiency upto 57.2% were obtained. Release rate of the drug from the microspheres prepared from high molecular weight chitosan was slow in comparison with that prepared from medium and low molecular weight chitosan. Rapid initial drug release (20–30%) was seen in all the microspheres followed by slow release of the remaining amount of the drug.

8.14.3. Phenytoin

To determine the effects of chitosan on gel matrix erosion, chitosan reinforced calcium alginate gel beads were prepared and release characteristics of phenytoin was investigated (Murata et al., 1993). The release rates of phenytoin 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 increase in the chitosan concentration and/or incubation times used while preparing the gel beads. Also, erosion of the gel beads was suppressed by chitosan treatment.

8.14.4. Pentazocine

Sankar et al. (2001) investigated the potential of bioadhesive chitosan microspheres of pentazocine for intranasal systemic delivery thus avoiding the first pass effect and improving the bioavailability and achieving sustained and controlled blood level profiles. This might be used as an alternative therapy to injection with an improved therapeutic efficacy in the treatment of chronic pain such as cancer, trauma and post-operative pain, etc. The formulation variables were drug loading, polymer concentration and stirring rate during crosslinking. Application of in vitro data to various kinetic equations indicated matrix diffusion controlled drug delivery from chitosan microspheres. Drug loading, polymer concentration and stirring speed influenced the drug release profiles significantly while oils had negligible effect. In vivo studies indicated significantly improved bioavailability of pentazocine from microspheres with sustained and controlled blood level profiles as compared to i.v., oral and nasal administration of drug solution. Good correlation was observed between in vitro and in vivo data.

8.14.5. Lidocaine hydrochloride

Chitosan gel beads containing lidocaine hydrochloride were prepared in amino acid solutions of pH 9 (Kofuji et al., 1999). The above phenomenon was observed not only in amino acid solutions but also in solutions of compounds having amino groups. A solute concentration of more than 10% was required for preparation of gel beads at a pH of 9. The release of lidocaine hydrochloride from the chitosan gel beads was prolonged. Chiou et al. (2001) investigated the effect of post-coating PLLA (poly-L-lactic acid) microspheres with different chitosans on the initial burst and controlling the drug release of the microspheres. Coating by chitosan retarded the burst release of lidocaine.

8.14.6. Opioid analgesics

Morphine administered nasally to humans as a simple solution is only absorbed to a limited degree with a bioavailability of the order of 10% compared with intravenous administration. Illum et al. (2002) investigated the development of nasal morphine formulations based on chitosan microspheres, which, in the sheep model, provided a highly increased absorption with a five- to six-fold increase in bioavailability over simple morphine solution. The chitosan–morphine nasal formulations were also tested in healthy volunteers in comparison with a slow intravenous infusion (over 30 min) of morphine. The results showed that the nasal formulation was rapidly absorbed with a t_{max} of 15 min or less and a bioavailability of nearly 60% was obtained. The shape of the plasma profile for nasal delivery of the chitosan–morphine formulation was similar to the one obtained for the slow intravenous administration of morphine. It was concluded that a properly designed nasal morphine formulation can result in a non-injectable opioid product capable of offering patients rapid and efficient pain relief.

8.15. Corticosteroids

Huang et al. (2002) prepared chitosan microspheres by a spray drying method using type-A gelatin and ethylene oxide–propylene oxide block copolymer (Pluronic F-68) as modifiers. Betamethasone disodium phosphate loaded microspheres demonstrated good drug stability (<1% hydrolysis product), high entrapment efficiency (95%) and positive surface charge (37.5 mV). The in vitro drug release from the microspheres was related to gelatin content. Further, Huang et al. (2003a) observed that betamethasone disodium phosphate-loaded microspheres demonstrated good drug stability (<1% hydrolysis product), high entrapment efficiency (95%) and positive surface charge (37.5 mV). The results also indicated that yield and size of particle was increased with increasing betamethasone amount but both zeta potential and tap density of the particles decreased with increasing betamethasone loaded amount. The in vitro release of betamethasone showed a dose-dependent burst followed by a slower release phase that was proportional to the drug concentration in the concentration range between 5 and 30% (w/w) (Huang et al., 2003b).

8.16. Lipid regulating agent

Martinac et al. (2002) evaluated the ability of the macromolecular conjugates and microspheres to modify the release rate of gemfibrozil. Gemfibrozil was covalently linked to two similar polymers: poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)] (PHEA) and poly[α,β-(*N*-3-hydroxypropyl-DL-aspartamide)] (PHPA) by an ester linkage. Microspheres, composed of chitosans of different molecular weight alone or as a mixture with (2-hydroxypropyl)methylcellulose (HPMC), PHPA or PHEA and with different theoretical polymer/drug ratio (2:1 and 3:1 w/w) were prepared by spray drying. The best conditions were achieved by microspheres composed of the low molecular weight chitosan combined with PHPA or HPMC with either 2:1 or 3:1 (w/w) polymer/drug ratio. The PHEA–gemfibrozil conjugates exhibited rapid gemfibrozil release within less than 2 h, while the PHPA–gemfibrozil conjugate showed sustained gemfibrozil release profiles over a 10-h period.

8.17. Anti-infective agents

8.17.1. Chlorhexidine diacetate

Giunchedi et al. (2002) investigated the development of buccal tablets based on chitosan microspheres containing chlorhexidine diacetate. The microparticles were prepared by a spray-drying technique. The loading of chlorhexidine into chitosan was able to improve the anti-microbial activity of the drug, particularly against Candida albicans. Buccal tablets were prepared by direct compression of the microparticles with mannitol alone or sodium alginate. After their in vivo administration, the determination of chlorhexidine in saliva showed the capacity of these formulations to give a prolonged release of the drug in the buccal cavity.

8.17.2. Pipemedic acid

Microspheres containing the mucoadhesive polymer chitosan hydrochloride, with matrix polymer Eudragit RS were prepared using pipemidic acid as a model drug (Bogataj et al., 2000). The release of pipemedic acid from mucoadhesive microspheres adhered on pig vesical mucosa was investigated by Burjak et al. (2001). The results obtained on pathologically changed mucosa model supported the indication of the role of glycosaminoglycans and polymer charge in the mucoadhesion process on vesical mucosa. Analysis of release data showed that the drug dissolution profiles follow the Higuchi kinetics better than the release profiles from adhered microspheres and different kinetics might be a consequence of different release mechanisms.

8.17.3. Nitrofurantoin

Hari et al. (1996) prepared microcapsules by adding dropwise a solution of sodium alginate containing nitrofurantoin into a chitosan–$CaCl_2$ system. About

70–80% of the drug was released into phosphate buffer, pH 7.4 within 6 h. Drug release into the gastric medium was found to be relatively slow compared to that into the intestinal medium. From scanning electron microscopic studies, it appeared that the chitosan modifies the nitrofurantoin–alginate microspheres.

8.18. Gastrointestinal agents

8.18.1. Metoclopramide hydrochloride

Usefulness of metoclopramide microspheres of chitosan and chondroitin sulphate for controlled release of the drug for oral administration was investigated by Ganza-Gonzalez et al. (1999). Microspheres were prepared by spray drying technique-using formaldehyde as a crosslinking agent. The in vitro release kinetics was measured at different pH. Chondroitin sulphate microspheres did not retard drug release whereas chitosan microspheres prepared with more than 15% formaldehyde showed good control release and further the release rate was independent of the pH of the dissolution medium. Release of drug from chitosan microspheres was best fitted by models in which release rate was highly governed by rate of diffusion through the matrix.

8.18.2. H_2-antagonist drugs

Non-crosslinked and crosslinked chitosan microspheres of cimetidine and famotidine were prepared by spray-drying method. It was observed that decreasing the amount of crosslinking agent increased both the particle size and zeta potential of the microspheres. Both cimetidine and famotidine were found to be molecularly dispersed within the microsphere matrix. The release of the drugs from the microspheres was fast and was accompanied by a burst effect (He et al., 1999a).

8.18.3. Enzymes

The effect of glutaraldehyde concentration on the binding of the dextranase to chitosan was investigated (Struszczyk et al., 1994). α-Galactosidase and glucoamylase have also been immobilized in porous chitosan beads (Ohtakara et al., 1989a,b).

Urease loaded capsules of a blended chitosan polyvinyl alcohol mixture were prepared by the coacervation salting-out method using sodium sulphate as a coagulation solution followed by further treatment with a solution containing formaldehyde, sulphuric acid and sodium sulphate (Miguez et al., 1997). The enzyme extract was fully immobilized and the enzymatic assay showed the presence of the enzyme in an active form with a shift in the pH maximum activity to a lower pH.

8.19. Others

8.19.1. Chlorpheniramine maleate

Chlorpheniramine maleate/ion exchange particles embedded in glutaraldehyde crosslinked chitosan particles were studied in vitro (Huang et al., 1999). The microspheres displayed controlled drug release in simulated gastric and intestinal fluids with release further delayed by crosslinking.

8.19.2. Acyclovir

In order to prolong the release of acyclovir and to increase its ocular bioavailability, drug loaded chitosan microspheres were prepared by an emulsification technique (Genta et al., 1997). This microparticulate drug carrier showed a promising effect in topical administration of acyclovir to the eye. Results showed that 90% of the particles were about 25 μm in size and the in vitro dissolution profile of the drug was slow. In vivo occular studies on rabbit showed a prolonged high concentration of acyclovir and increased area under the curve values.

8.19.3. Bilirubin

Chandy and Sharma (1992) reported that polylysine-modified chitosan may be an excellent sorbent system for hemoperfusion due to its high binding affinity, capacity, and blood compatibility. The binding capacity was proportional to the amount of polylysine bonded to the chitosan beads. The hemolytic potential of all modified beads was compatible with polystyrene control tubes. Studies were also performed against albumin as proof of specificity toward bilirubin binding. The albumin-coated beads showed the highest blood compatibility and selectivity over the other modified beads.

8.19.4. Benzoic acid

In a study carried out by Mitani et al. (1995), benzoic acid was adsorbed onto swollen chitosan beads. The presence of glucose and mannitol stimulated the adsorption of benzoic acid.

8.19.5. *Saccharomyces cerevisiae*

Chitosan has also been used to increase the stability of calcium alginate beads with entrapped yeast cells (Li, 1996). Polyelectrolyte complexes were used as surface-coating materials by dropping alginate into chitosan. The coacervation agent sodium tripolyphosphate was needed for the formation of the polyelectrolyte complexes. Gel beads obtained by coacervation method maintained their rigidity and were chemically stable in phosphate buffer. No differences in ethanol production were found between *Saccharomyces cerevisae* immobilized in calcium alginate and in calcium alginate coated with polyelectrolyte complexes.

8.19.6. *Lipoprotein lipase*

Lipoprotein lipase was covalently immobilized onto the surface of porous chitosan beads with or without spacers of different lengths (Itoyama et al., 1994). Lipoprotein lipase immobilized directly to the surface of chitosan beads without any spacer gave a higher stability than that immobilized with spacer, in spite of the lower relative activity. The spacer effect on relative activity could be explained in terms of the mobility of the immobilized lipoprotein lipase molecule.

8.19.7. *Haemoglobin*

Huguet et al. (1994) investigated the retention capacity of alginate beads coated by chitosan. When beads were stored in water or in a 0.9% NaCl solution, the chitosan coating was stable for more than 5 months or less than 2 months, respectively. The greater the concentration of chitosan in the formation solution of the beads, the lower the haemoglobin release.

8.19.8. *Rosemary oil*

Magdassi et al. (1997) prepared microcapsules, which contained rosemary oil. The first step involved the formation of oil-in-water emulsions, by using lecithin as emulsifier, thus imparting negative charges on the oil droplets. This was followed by the addition of a cationic biopolymer, chitosan, in conditions that favored the formation of an insoluble chitosan–lecithin complex.

8.19.9. *Prodrug*

Filipovic-Grcic et al. (1995) encapsulated a polymeric prodrug, α,β-poly(*N*-hydroxyethyl)-DL-aspartamide-levodopa adduct, in sodium alginate–chitosan microspheres. The gel/matrix material of alginate–chitosan complex protected the adduct from hydrolysis by the surrounding medium.

8.19.10. *Tetrasulphonated copper phthalocyanine*

Tetrasulphonated copper phthalocyanine, in the salt form, was incorporated into a blend of chitosan/poly(vinylalcohol) and microspheres were produced using the method of salt coacervation with sodium sulphate (Stolberg et al., 1999). Spectroscopic analysis, differential scanning calorimetry and thermogravimetry analysis were carried out to characterize the form in which the macro-complex was immobilized in the blend. Alkaline treatment of the coagulating medium produced species which were more stable, but with a different morphology observed by scanning electronic microscopy. The kinetic data revealed a decrease in the capacity of sorption of the microspheres that had received the alkaline treatment.

8.19.11. *Marker*

Chitosan-coated alginate microspheres containing a lipophilic marker dissolved in edible oil, were prepared by emulsification/internal gelation. The potential use of these microspheres as an oral controlled release system was investigated by Ribeiro et al. (1999). Higher overall levels of release were obtained with uncoated microspheres, possibly due to binding of marker to the chitosan membrane coat.

El-Shafy et al. (2000) reported the improved nasal bioavailability of fluorescein isothiocyanate–dextran (FITC–dextran) encapsulated in non-mucoadhesive and mucoadhesive microspheres in New Zealand white rabbits.

8.19.12. *Gadopentetic acid*

Biodegradable gadopentetic acid (Gd-DTPA)-loaded chitosan microparticles (Gd-microCPs) were prepared as a device for gadolinium neutron-capture therapy (Gd-NCT) by an emulsion-droplet coalescence technique (Tokumitsu et al., 1999). The results suggested that Gd-microCPs could be a useful device for intratumoral injection into solid tumor on Gd-NCT.

8.19.13. *Bromothymol blue*

Ramdas et al. (1999) attempted to develop a formulation that could bypass the acidity of the stomach and release the loaded drug for long periods into the intestine by using the bioadhesiveness of polyacrylic acid, alginate, and chitosan. Bromothymol blue was taken as a model drug. The formulation exhibited bioadhesive property and released the drug for an 8-day period in vitro.

8.19.14. Brilliant blue

Shu and Zhu (2002b) studied the release behavior of brilliant blue from chitosan-coated calcium-alginate gel beads (CCAGB). The CCAGB were prepared by dropping alginate solution into $CaCl_2$/chitosan solution (method 1(a)), or into chitosan solution then gelled by $CaCl_2$ (method 1(b)), or into $CaCl_2$ solution then coated by chitosan (method 2). Compared to calcium alginate gel beads, the dried CCAGB had poorer shape and rougher surface morphology especially in methods 1(a) and (b); moreover, CCAGB was found to be more instable in 0.9% NaCl and serious burst of beads occurred when high concentration of alginate (3.0 and 5.0%, w/v) was used. Under un-dried bead state in method 1(a), the increase of chitosan content prolonged brilliant blue release in 0.9% (w/v) NaCl; while in method 2, the increase of chitosan concentration over 0.1% (w/v) (3.0% (w/v) alginate concentration was used) resulted in more serious burst of beads and hence facilitated brilliant blue release.

8.19.15. Ecaltonin

Kawashima et al. (2000) prepared PLGA (poly-lactic-*co*-glycolic acid) nanospheres with elcatonin by the emulsion solvent diffusion method and coated the surface of the resultant nanospheres with a mucoadhesive polymer such as chitosan, poly(acrylic acid), and sodium alginate. Even under nonfasting conditions, the mucoadhesion of chitosan-coated nanospheres was unaltered and the reduction in blood elcatonin levels was maintained satisfactorily.

8.19.16. Toxoid

Diphtheria toxoid was loaded by passive absorption into crosslinked chitosan microspheres (Jameela et al., 1994). Immunogenicity studies on wistar rats showed sustained action of the toxoid for a period of 5 months. In addition, the microspheres also showed histocompatibility.

8.19.17. Hormones

Melatonin, a pineal hormone, valuable for those suffering from a disordered circardian rhythm was loaded into chitosan microspheres by the emulsion melting/cooling method using stearyl alcohol. Release profile of the drug from the microspheres was found to be independent of the pH. The above method provided an alternative method for the preparation of an oral sustained release dosage form of melatonin without the use of harmful organic solvents (Lee et al., 1998).

8.19.18. Fibroblast growth factor

Methylpyrrolidinone chitosan (MPC), a water-soluble derivative of chitosan, was investigated as a carrier material for basic fibroblast growth factor (bFGF), a combination intended for the treatment of wound healing deficiencies (Berscht et al., 1994). Soft and flexible fleeces of methylpyrrolidinone chitosan were prepared by freeze-drying. The growth factor was incorporated either before drying of the fleeces by mixing bFGF solution with MPC solution or by soaking bFGF solution into a previously prepared fleece and subsequent freeze-drying. Release studies using an immunological assay, radioactivity measurements and cell culture techniques revealed a sustained release of biologically active bFGF from the fleeces.

8.19.19. Mammalian cell

The cells were encapsulated in a chitosan–alginate bi-polymer membrane by Kim and Rha (1989a). The encapsulation process was based on the electrostatic interaction between chitosan and crystalline methyl cellulose (Shioya and Rha, 1989). Another study carried upon mammalian cell encapsulation using chitosan reported that latter prevented excessive cell clumping and necrosis (Muzzarelli, 1994).

8.19.20. Interleukin-2 (IL-2)

Alginate/chitosan porous microsphere complex was used for the entrapment of IL-2. The immune activity study of IL-2 released from the microspheres suggested that an improved tumor immunotherapy might be obtained due to controlled release of interleukin (Liu et al., 1997).

Ozbas-Turan et al. (2002) prepared chitosan microspheres by using the precipitation technique and evaluated them for sustained-release of recombinant human interleukin-2 (rIL-2). The average diameter of microspheres was between 1.11 and 1.59 μm. The rIL-2 encapsulation efficiency in these microspheres was high (75–98%). The encapsulated rIL-2 remained biologically active and rIL-2 was released from chitosan microspheres in a sustained manner. The efficacy of rIL-2 loaded chitosan microspheres was studied using two model cells, HeLa and L-strain cell lines. Chitosan microspheres were added to the cells at different concentrations, and the amount of rIL-2 was assayed using the ELISA (enzyme-linked immunosorbent assay) kit. Cell culture studies indicated that microspheres were uptaken by cells, and rIL-2 was released from the microspheres. Cellular uptake of rIL-2-loaded microspheres was dose-dependent. It can be said that chitosan microsphere is a suitable carrier for rIL-2 delivery.

9. Conclusion

Chitosan is a versatile polymer whose applications range from weight supplement in the market to a drug carrier in formulation research. Chitosan has been shown to improve the dissolution rate of poorly soluble drugs and thus can be exploited for bioavailability enhancement of such drugs. Reacting chitosan with controlled amounts of multivalent anion results in crosslinking between chitosan molecules. This crosslinking has been used extensively for the preparation of chitosan microspheres. Other crosslinking agents such as glutaraldehyde, formaldehyde and naturally occurring crosslinking agent genipin have also been used for preparation of microspheres. Apart from crosslinking, chitosan microspheres have also been prepared by a number of other processes viz., coacervation, multiple emulsion method, solvent evaporation, etc. The particle size of chitosan microspheres can be modified approximately for the oral, nasal and parentral delivery of drugs. Following oral administration, drug loaded chitosan microspheres dissolve in the gastric medium in the stomach, thus liberating drug initially. The microspheres can be stabilized by coating chitosan with hydrophobic polymer such as ethylcelluolose. The entrapment efficiency of drugs in the chitosan microspheres is dependent upon the chitosan concentration. The entrapment efficiency increases with increase in chitosan concentration. The drugs can also be loaded via a passive absorption method by adding microspheres to a drug solution. Drugs are loaded by using the swelling properties of the microspheres in the drug solution. Release of drug from chitosan microspheres is dependent upon the molecular weight of chitosan, concentration of chitosan, drug content and density of crosslinking. Various therapeutic agents such as anticancer, anti-inflammatory, antibiotics, antithrombotic, steroids, proteins, amino acids, antidiabetic and diuretics have been incorporated in chitosan microspheres to achieve controlled release.

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