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An overview of modified release chitosan, alginate and eudragit RS microparticles

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ABSTRACT

In this paper, different formulation types of microparticulate systems such as beads, microbeads, microspheres and microsponges using to special attention chitosan, alginate and eudragit RS 100 are reviewed. Chitosan and alginates are natural, anionic or cationic, biocompatible, biodegradable and non-toxic polymers. They have excellent potential for pharmaceutical and biopharmaceutical applications. Eudragit RS is an acrylic copolymer and it has well-established mucoadhesive characteristics. It is still being used as a sustained release coating materials in pharmaceutical field. Various techniques used for preparing chitosan, alginate and eudragit RS microparticles have also been reviewed. This review also includes non steroidal anti-inflammatory drug (NSAID) microparticle formulations which have been prepared with these polymers to minimize side effects and to obtain controlled release drug delivery systems. Moreover, literatures and patents underline a widespread use of alginate, chitosan and eudragit RS are covered in this paper.

Keywords: Chitosan, Alginates, Eudragit RS, Microparticles, Polymer.

INTRODUCTION

There has been considerable interest in recent years in developing controlled or sustained drug

delivery systems by using biopolymers. Modified-release oral delivery systems provide many advantages in comparison with conventional forms: reduced side-effects, drug concentration kept at effective levels in plasma, and improved utilization of drug and decreased dosing frequencies [1].

Non-steroidal antiinflammatory drugs (NSAIDs) are used in humans and animals for the treatment of inflammatory and rheumatic disorders such as dysplasia of the hip, chronic arthritis, spondylitis and the like. As mentioned before, one of the most common side effects of NSAIDs is irritation of gastrointestinal tract [2]. This side effect can be attributed to local irritation due to the penetration of the drug in the gastric mucosal cells, but it can also be caused by enterohepatic recirculation or by the inhibition of prostaglandin synthesis due to the inhibition of the cyclooxygenase enzyme [3].

NSAIDs are widely used therapeutic agents that have antiinflammatory, analgesic, and antipyretic activities. NSAIDs are involved in the suppression of prostaglandin synthesis by inhibiting cyclooxygenases, enzymes that catalyze the formation of prostaglandin precursors from arachidonic acid. It has been reported that inflammatory processes are associated with the pathophysiology of Alzheimer's disease and that treatment with NSAIDs reduces the risk of Alzheimer's disease [4]. Our previous study reported biodegradable alginate and chitosan microparticles, prepared to compare with modified release Eudragit microsponges containing mefenamic acid (MA). We demonstrated the potential effects of MA microparticles and the polymers used their formulations on DNA damage induced by kainic acid as well as in vitro release of MA microparticles prepared by three different polymers (alginate, chitosan and Eudragit RS 100). Our results showed that DNA damage was higher in MA-loaded Eudragit microsponges than MA-loaded biodegradable chitosan or alginate microparticles [5].

A new mefenamic acid-alginate bead formulation prepared by ionotropic gelation method using 3×2^2 factorial design has shown adequate controlled release properties in vitro [6]. In addition, the irritation effects of mefenamic acid (MA), a prominent non-steroidal anti-inflammatory drug, were evaluated on rat gastric and duodenal mucosa when suspended in 0.5% (w/v) sodiumcarboxymethylcellulose (NaCMC) solution and loaded in alginate beads [7].

POLYMERS

The use of natural polymers and polymethacrylates as drug carriers is one of the main objectives of researchers dealing with long acting dosage forms.

Natural polymers

Over the past few years, the use of natural polymers in the design of drug delivery formulation has received much attention due to their excellent biocompatibility and biodegradability [8].

Alginates

Alginates, which are naturally occurring substances found in brown seaweed and algae, have received much attention for use in pharmaceutical dosage forms, particularly as a vehicle for controlled drug delivery. The formation of a matrix upon hydration causes a gelatinous layer which can act as a drug diffusion barrier. Alginate is a family of polysaccharides composed of α -

L-guluronic acid and β -D-mannuronic acid residues, arranged in homopolymeric blocks of each type and in heteropolymeric blocks. The structural formula of alginate is shown in figure 1. [9].

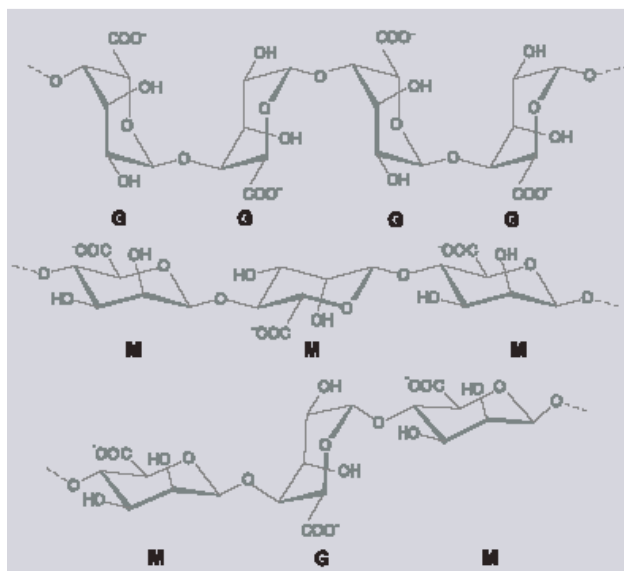


Figure 1. The structural formula of alginate

Alginates form hydrogels in the presence of divalent cations like Ca^{2+} . It can be ionically crosslinked by the addition of divalent cations in aqueous solution. The native alginate is mainly present as an insoluble Ca^{2+} crosslinked gel. The viscosity of alginate solutions depends primarily on the molecular weight of the material. The divalent cations bind to the α -L-guluronic acid blocks in a highly cooperative manner and the size of the cooperative unit is more than 20 monomers. Complex coacervation of oppositely charged poly electrolytes has been commonly used as a method for preparing microbeads. In the alginate–chitosan system, the complex is formed by spraying a sodium alginate solution into the chitosan solution. The resultant alginate–chitosan microbeads are mechanically strong and stable over a wide pH range [10-12].

Alginate is an anionic, biodegradable and biocompatible natural polymer and alginate gels have been used to encapsulate other delivery systems including microspheres and liposomes. They could potentially be useful as an oral delivery system for micro- or nanoparticles. Also alginate is a bioadhesive polymer which can be advantageous for the site specific delivery to mucosal tissues. When it is taken orally and it protects the mucous membrane of the upper gastrointestinal tract from the irritation of chemicals. The alginate monomer composition is reported to have a major impact on the drug release properties of the different formulation systems [13]. When exposed to low pH can therefore undergo a reduction in alginate molecular weight which results in faster degradation and release of a molecule when the gel is reequilibrated in a neutral pH solution. The release of the drug is dependent on both dissolution of the gel and diffusion of the drug into the GI fluid. The release of macromolecules from alginate beads in low pH solutions is also significantly reduced which could be advantageous in the development of an oral delivery system. A crosslinked alginate matrix delivery system. Alginate gels have been used to encapsulate other delivery systems including microspheres. As research and development continues with alginate polymeric delivery systems, we expect to see many innovative and exciting applications in the future [14].

Lee and Kim explored the possible applicability of alginate gel beads as an oral controlled release system of macromolecular drugs. The drug release from alginate beads at pH 6.8 showed nearly zero-order release rate, which was more rapid than that at pH 1.2. Since the release of blue dextran as the model of macromolecular drugs could be controlled by the regulation of the preparation conditions of alginate beads, the alginate beads may be used for a potential oral controlled release system of such macromolecular drugs as vaccines and polypeptide drugs [15]. Sankalia *et al.* reported that reversed chitosan–alginate polyelectrolyte complex (PEC) beads were prepared by dropping chitosan containing alpha-amylase into a sodium alginate solution without any salt. A higher concentration of the polymers and pH values corresponding increased charge density of both the polymers and resulted intense cross-linking with small micropores. The increased viscosity at higher concentrations of chitosan resulted in larger particles. At pH 2.0, the ionic interaction between chitosan and alginate is very reduced because of rapid swelling and lack of significant ionic interaction between the chitosan and alginate. When the pH increases up to neutral, the swelling tends to decrease due to increasing number of carboxylate groups, which interact with the ammonium group of chitosan. Moreover, the solubility of chitosan decreases at higher pH values, and it acts as a sustained-release matrix. Korsmeyer–Peppas' power law is the best-fit model in describing the dissolution behavior of α -amylase from the chitosan–alginate PEC. Stability testing was carried out according to the International Conference on Harmonization (ICH) guidelines for zones III and IV. The shelf-life of the enzyme-loaded beads was found to increase to 3.68 years, compared with 0.99 years for the conventional formulation [16].

Alginates have been widely exploited in pharmaceutical industry for controlled drug release. Anal *et al.* prepared multilayer chitosan beads containing ampicillin by ionotropic gelation. Chitosan and Ca^{2+} as cationic components and alginate and tripolyphosphate as anions were used. When ionic interactions between alginate–chitosan complex increase, entrapment efficiency of the drug in beads higher but chitosan beads are more efficient in the entrapment of the drug as chitosan drops loose less of their drug content during ionotropic gelation. Drug release was studied in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). The release studies were also carried out in SIF only, without prior incubation in SGF. The beads swelled and started to float without erosion in SGF. Single layer chitosan–alginate beads released 70% of the drug within 4 h. Multilayer beads released only 20–30% in the same period of time. Both single and multilayer beads continued to release drug in SIF. Approximately 70% of the entrapped ampicillin was released about in 12 h from chitosan–TPP beads but TPP multilayer beads release only 25–30%. The multilayer beads, cross-linked with TPP are suitable for the oral sustained release of low molecular and highly hydrophilic compounds [17].

Matrices incorporating either a single alginate salt or a combination of salts have been employed to successfully sustain release of many drugs *in vitro* and *in vivo*. They concluded that optimum formulation released the drug slower than the other formulations and the developed formulations could be proposed as a promising system for the treatment of breast cancer [18,19]. Some researchers prepared alginate beads containing 5-fluorouracil (5-FU) by the gelation of alginate with calcium cations. Alginate beads loaded with 5-FU were prepared at 1 and 2% (w/v) polymers. They found that when the drug load increased, larger beads were obtained in which the resultant beads contained higher 5-FU content and the amount of 5-FU released from the alginate beads increased with decreasing alginate concentrations [19].

Alginate is used as an excipient due to thickening, gel-forming, and stabilizing properties in drug dosage forms. The ability of alginate to form two types of gel dependent on pH, i.e., an acid gel and an ionotropic gel. At low pH hydration of alginic acid leads to the formation of a high-viscosity "acid gel." Since the property of reswelling is susceptible to the environmental pH, the incorporation of acid-sensitive drugs into the beads protects them from the gastric juice [20].

In pharmaceutical technology, alginates have been used as tablet binders, disintegrants, viscosity, enhancing agents and sustained release matrices [21].

Alginate is a suitable negatively charged agent which may interact with positively charged chitosan. Recently, alginate beads containing several substances have been prepared by the gelation of alginate with calcium cations [22].

In a study, the effects of the alginate composition, the calcium addition, and the dissolution medium on drug release were investigated. The capsules containing sodium/calcium salts of alginate showed the slowest drug release at neutral pH but the fastest in acidic medium. It was similar that higher alginate viscosity slowed down the drug release rate at neutral pH but enhanced the release rate in the acidic medium. The drug release in acidic medium showed a non-Fickian diffusion-controlled release but in water at neutral pH exhibited a Super Case II transport mechanism. To examine the effects of the various grades of alginate, the calcium added to the formulation, and the dissolution medium on drug release, the dissolution studies were performed using US Pharmacopeia (USP) dissolution apparatus II. [23].

Thanou et al. described that trimethyl chitosan chloride (TMC) and Mono-carboxymethylated chitosan (MCC) as chitosan derivatives have been evaluated to overcome chitosan's limited solubility and effectiveness as absorption enhancer at neutral pH values such as intestinal pH. TMC has been shown to increase the permeation and/or absorption of neutral and cationic peptide analogs across intestinal epithelia. MCC polymers are promising absorption enhancers, especially for anionic macromolecules such as heparins across intestinal epithelia [24].

In a recent invention, oligosaccharides are oxidated in presence of the oxidant, and produce the oxidated products. It was reported that the algin oligosaccharides and their derivatives can be used in the manufacture of medicaments for preventing Alzheimer's dementia [25].

Another invention relates to sustained-release formulations using alginate gel beads or particles. The formation of the sustained-release gels consist of the co-precipitation of alginate gel beads with a biologically active agent. This approach provides an efficient and high loading of high loading of active agent within the alginate gel for sustained-release delivery. Moreover protein protection, decreased degradation and increased stability can be achieved [26].

A process for preparing the gel beads of calcium alginate includes dispersing the aqueous solution of sodium alginate, which contains insoluble calcium salt, in the atoleine containing surfactant via inorganic membrane, reducing pH value to release Ca^{2+} ions and gelatinizing reaction. Its advantages are controllable granularity (1-100 microns), and low energy consumption [27]. A basic drug (e.g. pindolol, procatechol or nifedipine) is enclosed in alginic acid gel beads. Although commercially available sodium alginate can be used to prepare the

alginate gel, sodium alginate having about 10000-100000 molecular weight is especially preferably used for sustained release of the drug. A 1% aqueous solution of the above-mentioned sodium alginate has ≤ 100 cps viscosity and a good sustained release system can be designed by using the solution according to the release pattern of the drug [28]. In a recent study, sulindac loaded alginate beads were prepared by ionotropic gelation method for a mucoprotective and controlled drug release. Sulindac beads were investigated *in vitro* for a possible sustained drug release and their use *in vivo* as a gastroprotective system. Sulindac loaded alginate beads led to a significant reduction of macroscopic histological damage in the stomach and duodenum in mice. Also microscopic analyses of the mucosal damage demonstrated a significant mucoprotective effect of all bead formulation compared to the free drug [29].

Chitosan

Chitosan, or B (1,4) 2-amino-2-deoxy- D- glucose a hydrophilic biopolymer obtained industrially by hydrolysing the aminoacetyl groups of chitin, After deacetylation of chitin, the chitosan obtained is dissolved in acid, filtered and the precipitate is washed and dried to get amine free chitosan. The structural formula of chitosan is shown in figure 2. [30].

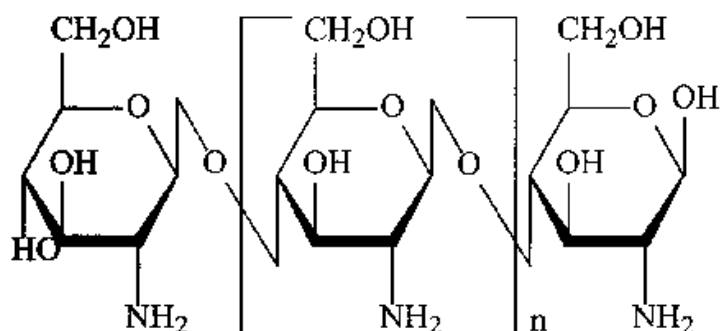


Figure 2. The structural formula of chitosan

Chitosan is a cationic polymer, which is the second most abundant polymer in nature after cellulose. Chitin is the primary structural component of the outer skeletons of crustaceans, and is also found in many other species such as molluscs, insects and fungi. The most commonly obtained form of chitosan is the α -chitosan from crustacean chitin obtained from crab- and shrimp shell wastes [31].

Also Chitosan is a biodegradable, hydrophilic, biocompatible and natural linear biopolyaminosaccharide with good potential for pharmaceutical applications due to its high charge density, non-toxicity and mucoadhesion. In addition, chitosan was studied as a carrier for microsphere drug delivery. 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. They are the most widely studied drug delivery systems for the controlled release of drugs such as antibiotics, antihypertensive agents, anticancer agents, proteins, peptide drugs and vaccines [32,33].

Chitosan, a linear polyelectrolyte at acidic pHs, is soluble in variety of acids and interacts with polyanionic counterions. It forms gels with a number of multivalent anions and also with glutaraldehyde. It has a high charge density, adheres to negatively charged surfaces and chelates metal ions. Since many minerals carry negative charges, the positive charge of chitosan interacts strongly with negative surfaces. Molecular weight, viscosity and degree of deacetylation are the intrinsic properties. Also it has some biological properties such as biocompatibility, bioactivity, wound healing acceleration, and immune system stimulant effect [34].

Chitosan in the form of a salt is a preferred choice for use as the cationic bioadhesive material. Chitosan is non-toxic and is present in the diet. It is a positively charged biopolymer at gastric pH. Chitosan is prepared by the deacetylation of chitin. The chitosan should have a molecular weight of greater than 5,000 D, Chitosan can be employed as a chitosan salt (eg the glutamate, lactate, chloride or acetate salt) or as a chitosan derivative such as N-trimethyl chitosan chloride [35,36].

The degree of deacetylation (DD) is one of the more important property for chitosan. The arbitrary DD of ≥ 40 defining chitosan, plays an important role in defining the use of chitosan as DD influences the physical, chemical and biological properties of chitosan such as the tensile strength of films, ability to chelate metal ions and immunoadjuvant activity [37].

Chitosan is soluble in most organic acidic solutions at pH less than 6.5 including formic, acetic, tartaric, and citric acid. It is insoluble in phosphoric and sulfuric acid. Chitosan is available in a wide range of molecular weight and degree of deacetylation. Molecular weight and degree of deacetylation are the main factors affecting the particle size, particles formation and aggregation [38].

Chitosan is available in the form of dry flakes, solution and fine powder. It has been shown to possess mucoadhesive properties. Chitosan microspheres are used to provide controlled release of many drugs and to improve the bioavailability of degradable substances These microspheres are being investigated both for parenteral and oral drug delivery. The use of microsphere-based therapy allows drug release to the specific treatment site through the choice and formulation of various drug-polymer combinations. Using innovative preparation of 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. Chitosan has also been used as a potential carrier for prolonged delivery of drugs, macromolecules and targeted drug delivery [39].

Chitosan microspheres have been described for use in oral delivery to provide local treatment of diseases of the stomach, or to improve the intestinal absorption of drugs which have a limited absorption capacity in the small intestine. Lisbeth *et al.* found that the compositions of the microspheres have a low density and initially float on the contents of the stomach following administration with a suitable dosing liquid. When the stomach is emptied of its contents, the particles adhere to, and coat, the stomach wall [35]. 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 [40]. One of invention was made for administration to mucosa. The drug delivery composition includes a pharmacologically active compound and particles, powder or microspheres, chitosan or a chitosan derivative or salt where in the particles are either solidified or partially cross-linked. They have a zeta potential of +0.5 to +50 mV. Solidified particles were made by treating particles made from a water soluble chitosan salt with an alkaline agent such as sodium hydroxide in a non-acid containing water to make them insoluble [41].

In a recent invention, the novel systems for the sustained release of drugs for ophthalmic use were developed. The invention relates to a novel solid complex which is insoluble in an aqueous environment and in dry form. The complex is formulated based on a viscoelastic solution derived from HA (Biovisc(R)), drugs such as a steroid, quinolone, a non-steroid anti-inflammatory, an immunosuppressor or compatible drug and chitosan, all of which are conjugated to produce biodegradable polymer matrices for the treatment of ocular ailments [42].

A method for inhibition of brain cell damage using a high molecular weight water-soluble chitosan was studied. In particular, the use of chitosan in a food composition inhibits brain cell damage by aging or can prevent general aging symptoms and a brain disease such as Alzheimer's disease [43].

Chitosan is a good immobilizing agent for biological cells and other biologically-active materials such as monoclonal antibodies, vaccines and pharmaceuticals. The aqueous solution of chitosan generally has a concentration of from 0.15 to about 3 wt.%, The procedure is effected at a pH of about 5.6 to 6.0. Polyanionic polymer, an alkali metal alginate, or sodium alginate can be used as they are reversibly gelled [44].

Chitosan microspheres can be prepared by reacting chitosan with controlled amounts of multivalent anion resulting in cross-linking between chitosan molecules. The cross-linking may be achieved in acidic, neutral, or basic environments depending on the method applied. Chitosan microspheres can be prepared by various methods such as cross-linking with anions, precipitation, complex coacervation, modified emulsification and ionotropic gelation, precipitation- chemical cross-linking, glutaraldehyde cross-linking, thermal cross-linking. The cross-linking of polymers affects the mucoadhesive strength of the microspheres. The *in vitro* evaluation of the mucoadhesive properties of polymeric microspheres is a basic step in the development of a mucoadhesive microparticle drug delivery system. As the process of mucoadhesion is a consequence of interaction between the mucus layer on mucosa and mucoadhesive polymer, it is greatly dependent upon mucus and polymer structure including their charges [45].

Preparation of chitosan microparticles

Ionotropic gelation

Ionotropic gelation method was used to entrap MA into calcium alginate beads as a potential drug carrier for the oral delivery of this anti-inflammatory drug. The microbeads were investigated *in vitro* for a possible sustained drug release and their use *in vivo* as a gastroprotective system for MA [6,7]. Process parameters such as the polymer concentration, polymer/drug ratio, and the amount of hardening agent were analysed for their influences on the

microbead properties.

Propranolol–HCl-loaded calcium alginate (ALG) beads, propranolol–resin complex (resinate)-loaded calcium alginate (RALG) beads and polyethyleneimine (PEI)-treated RALG (RALG–PEI) beads were prepared by ionotropic gelation/ polyelectrolyte complexation method and investigated *in vitro* release profiles in simulated gastric fluid (SGF, 0.1(N) HCl, pH 1.2) and simulated intestinal fluid (SIF, phosphate buffer, pH 6.8) as dissolution media. The release of drug from all the beads was slow and incomplete in SGF owing to considerably less swelling of the beads. The release of drug from the ALG beads in SIF was rapid and complete in 1.5 h. When the larger the amount of Ca^{2+} ions diffused inwardly into the resinate-loaded alginate beads and consequently the larger the amount of the drug displaced from the resinate by the Ca^{2+} ions, resulting in a decrease in drug entrapment efficiency. [46].

Another invention relates to a preparation method of floatation-bioadhesion synergistic type microgranules. Preparation method includes the following steps: firstly, utilizing drying system in the water to prepare ethyl cellulose floatation microspheres, then using sodium alginate to make coating in the emulsification system, finally the sodium alginate and ethyl cellulose microparticles are dispersed in chitosan solution to produce ion gelatification and form chitosan film to obtain the invented floatation-bioadhesion synergistic type microgranule [47]. In another study, controlled release beads were prepared using alginate, konjac glucomannan, and chitosan by ionotropic gelation method. Bovine serum albumin and insulin were used as model proteins for *in vitro* assessments [48].

Alginate/chitosan particles were prepared by ionotropic gelation (Ca^{2+} and Al^{3+}) for the sodium diclofenac release. The drug encapsulation yield was more than 98%, and the efficacy was neither affected by the alginate amount nor the crosslinking ion used. Thus, this method is useful to encapsulate ionic drugs with a high water solubility. The use of Ca^{2+} resulted acceptable sphericity and a notable surface porosity. The morphology of the particulates prepared with Al^{3+} ions didn't exhibit spherical morphology the particles were flattened, disk-shaped with a collapsed center. The trivalent ions cause more points of aggregation between two contiguous alginate chains, binding them strictly and quickly that they can't be in spherical forms during their formation. The neutralization between oppositely charged alginate and chitosan decreases the solubility of the alginate/chitosan particles. This mechanism gives rise to the high efficiency of the microsphere formation in depleting diclofenac from the solution. At pH 6.4 a rapid increase of the release rate was observed because the deprotonation of the alginic acid causes the disintegration of the microsphere systems and the increasing deprotonation of chitosan weakens the extent of the interactions inside the microspheres [49].

Also ionotropic gelation method was used to entrap sulindac into calcium alginate beads as a potential drug carrier for the oral delivery of this anti-inflammatory drug. Beads were investigated *in vitro* for a possible sustained drug release and their use *in vivo* as a gastroprotective system for sulindac. Process parameters such as the polymer concentration, polymer/drug ratio, and different needle diameter were analysed for their influences on the bead properties [29].

Extrusion-spheronization

Chitosan pellets were prepared using the extrusion/spheronization technology. Increasing the acetic acid concentration to > 0.2 N acetic acid leads to a sticky extrudate or pellets with a rod-like appearance. The particle size decreases with increasing chitosan quantity when the powder is extruded with the same acid concentration and with constant power (180 W). Chitosan particles have a flake-like character. Therefore, the chitosan particles are partly dissolved in the solution and stuck to pellets. A low abrasion is achieved when the acid concentration and the quantity of granulation liquid is adjusted to the higher amount of chitosan [50].

Solvent evaporation technique

The solvent evaporation technique method is the most popular and very common technique. In the general microencapsulation technique using an o/w emulsion system (e.g. solvent evaporation methods) the drug is dissolved, dispersed or emulsified in an organic polymer solution which is then emulsified in an external aqueous or oil phase. As the organic solvent is removed by evaporation, the drug and polymer are precipitated in the droplets, thus forming the microspheres. In the phase separation method, the polymer is precipitated around a dispersed drug phase through the addition of a non solvent, an incompatible polymer or temperature change. This method results in the formation of microcapsules (core-shell structure) vs. microspheres (matrix structure) formed by the solvent evaporation method [51].

Triclosan-loaded microspheres prepared from chitosan by double-emulsion solvent evaporation technique has been shown to exhibit controlled-release behaviour over extended time periods. The kinetics of the release have been shown to obey the Baker and Lonsdale model appropriate for diffusion-controlled spherical matrices [52].

Spray drying method

Spray drying was a suitable technique for preparing spherical microparticles of zolmitriptan and chitosan with a narrow particle size range and high drug loading. The dissolution profiles for amorphous and crystalline zolmitriptan were similar: 100% dissolved in approximately 35 min. Thorough mixing of the drug with the chitosans (glassy solid solution) and the development of interactions between them could have played a role in controlling the release of drug from the formulations. Chitosans have previously been shown to control the release of highly soluble drugs [53].

Precipitation coacervation method

Protein-loaded microparticles is developed by Precipitation coacervation method for mucosal delivery system. Chitosan anionic precipitation/coacervation is accomplished by the addition of sodium deoxycholate (DCA). Bovine serum albumin (BSA) and DCA were simply dipped into a chitosan solution under stirring. Platelet-like and/or spherical microparticles were obtained with high protein loading efficiency. BSA release profiles for chitosan–DCA formulations have an initial burst release in PBS pH 7.4 and HCl 0.1N but more than 55% of the BSA remains protected inside the microparticles [54].

Amoxicillin and metronidazole loaded chitosan microspheres for stomach specific delivery were prepared for the treatment of *Helicobacter pylori* infection. Drug-loaded porous chitosan microspheres were prepared by simultaneous crosslinking and precipitation with sodium

tripolyphosphate. Due to the high porosity of drug-loaded chitosan microspheres, all the amoxicillin and metronidazole were released in 2 h in simulated gastric fluid (SGF, pH 1.2). Metronidazole and amoxicillin were highly permeable through the gastric mucin gel layer. Therefore, acid-stable antibacterial agents could be useful for the eradication of *H. pylori* infection [55]. Hejazi and Amiji examined the gastric residence time of tetracycline loaded chitosan microspheres following their oral administration in gerbils. Gastric retention studies were performed by administering radioiodinated (¹²⁵I) chitosan microsphere suspension in the nonacid-suppressed and acid-suppressed states. Chitosan microspheres were prepared by ionic cross-linking and precipitation with sodium sulfate. Radioactivity in gerbils' tissues and fluids was measured with a gamma counter. The results indicated that chitosan microspheres did not provide a longer residence time in gerbil stomach. The tetracycline concentration profile in the stomach following administration of microsphere formulation was similar to that of aqueous solution [56].

Coating by chitosan

Alginate microspheres prepared by emulsification/internal gelation were chosen as carriers for a model protein and the microspheres coated by chitosan by an uninterrupted method, in order to simplify the coating process and minimize protein losses during production. High encapsulation efficiency have been established. Optical microscopic observation of microspheres confirms aggregation phenomena during chitosan-coating which can be explained by strong electrostatic interaction between alginate and chitosan, two polyelectrolytes of opposite charge. chitosan membrane showed a high porosity. Microspheres swelling was ion-sensitive and the model protein fast release from coated alginate matrix in phosphate buffer suggested a weak chitosan diffusion barrier [57].

Calcium-induced alginate gel beads containing chitosan salt (Alg-CS) was prepared using nicotinic acid (NA), a drug for hyperlipidemia, and investigated its two functions which are NA release from Alg-CS, and uptake of bile acids into Alg-CS in gastrointestinal tract NA was rapidly released from Alg-CS in diluted HCl solution (pH 1.2) or physiological saline without disintegration of the beads. when increasing of bile acid concentration, the uptake amount increased according to this result, linear relationship existed among them [58].

Polycaprolactone and chitosan coated epichlorohydrin-crosslinked alginate Polycaprolactone coated and chitosan coated epichlorohydrin crosslinked alginate (PACE-A) microspheres were prepared by a reproducible polymer dispersion technique that produced recombinant protein-containing particles averaging 8.2 micron in size. The PACE-A microspheres of the invention were coated with chitosan and polycaprolactone to increase the mechanical strength and stabilization and to modify the time of antigen release [59].

Emulsion crosslinking method

A novel double emulsion procedure (water-in-oil-in water; w/o/w) could be used for polar drugs. In the presence of both polar and non-polar drugs, the bioadhesive cationic polymer is provided in an aqueous phase, being either the aqueous phase of the oil-in-water emulsion, or the external aqueous phase of the water-in-oil-in-water emulsion. Controlled release gastroretentive microspheres were prepared using a novel emulsion-spray drying method (water in oil in water (w/o/w) and oil in water (o/w) emulsions) and the microspheres displayed enhanced retention in

the stomach of human subjects [35].

Glycolic acid loaded chitosan microspheres were prepared by the dry-in-oil emulsion method. 2% Span 20 was used as light mineral oil. Glutaraldehyde aqueous solution was used as crosslinking agent. A dialysis method was used to perform the 'in vitro' release test of all batches of glycolic acid loaded microparticulate systems. Amounts of microsphere suspensions were placed in the dialysis sacks. Chitosan microspheres do not control glycolic acid release even after crosslinking[60].

A porous matrix of chitosan was described. It was stated that the chitosan may be crosslinked by various agents to include glutaraldehyde, glyoxal, epichlorohydrin and succinaldehyde. The use of microspheres for bioadhesion or gastroretention was not suggested [61].

In patent US5840341 (A), cross-linked chitosan particles were prepared to improve the absorption of drugs across mucosal tissue for example the vagina, rectum, lungs, eye, colon or nasal cavity. The chitosan microspheres were prepared by spray drying a solution of chitosan (0.05% w/v-0.5% at pH 3-7) containing an appropriate amount of glutaraldehyde or formaldehyde, the size of the cross-linked or solidified microspheres were 1-200 μm [41].

In another study, an aqueous process involving precipitation chemical crosslinking was used to prepare the microspheres. Sodium sulphite was used as a precipitant and glutaraldehyde as a crosslinking agent [62]. In patent ZL 96115714.3, the W/O emulsification cross-linking reaction was employed to prepare microspheres. Chitosan was dissolved in acetic acid aqueous solution (1%, v/v) and dropped into toluene (oil phases) containing 1% (v/v) Tween-80 and 1% span-80. The mixture was stirred vigorously for half an hour and formaldehyde was added into the reaction system for 1 h, then chitosan microspheres were separated, washed with deionized water. After that, chitosan microspheres were treated with H_2O_2 , washed repeatedly and dehydrated successively with ethanol (30, 50, 80, 95, and 100%) and finally vacuum dried over night [63].

Another invention provides water soluble chitosan microspheres which can be used for loading drug and a preparation method. The structure of the microsphere is that the outside of crosslinking state water soluble chitosan core which can load the drug is orderly enwrapped with a crosslinking state water soluble chitosan coating layer and a crosslinking solidified outer surface layer, which can be used for loading and controlling releasing the drug [64].

An another invention relates to preparing chitosan microspheres by phase separation, firstly, adding the chitosan solution into a nontoxic oil phase, then adding an emulsifying agent and a dispersing agent, adding sulfuric acid solution for cross-linking, after reaction, washing the resultant product, drying by vacuum and getting chitosan microsphere cross-linked with sulfuric acid. The microspheres of the invention are smooth, have a good sphere configuration, could be used for a medicinal controllable carrier [65]. A process for preparing the porous microspheres of high-amino cross-linked chitosan includes such steps as using formaldehyde to react with the amino radical of chitosan to generate schiff base for protecting amino radicals, adding epoxy chloropropane, cross-linking, and adding diluted acid to remove schiff base. Its advantages are uniform granularity, high amino content and high sphericizing performance [66].

Thermal crosslinking

Crosslinked chitosan microparticles were prepared by crosslinking the chitosan gel to get a non-sticky glassy gel. Crosslinking of chitosan was done by using glutaraldehyde (GA) along with clozapine. Microparticles have shown more than 50% swelling instantly within the first minute and later, swelling becomes slow. In vitro drug release was performed in pH 7.4 phosphate buffer solution for 12 h. Higher release rates are observed due to the dissolution of surface-adhered drug. At longer time, drug release is due to the diffusion process, which is much slower when compared to the initial release. None of the formulations have shown 100% release and complete release of the drug from the matrix occurs only after complete erosion or degradation of the chitosan matrix. In vivo studies indicated that drug release from microparticles were in a controlled way [67].

Chitosan has been shown to improve the dissolution rate of poorly soluble 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. Apart from crosslinking, chitosan microspheres have also been prepared by a number of other processes. The entrapment efficiency of drugs in the chitosan microspheres is dependent upon the chitosan concentration. The entrapment efficiency increases with increase in chitosan concentration. Release of drug from chitosan microspheres is dependent upon the molecular weight of chitosan, concentration of chitosan, drug content and density of crosslinking [39].

Acrylic resin, Eudragit

Eudragit RS 100 is commonly used for the enteric coating of tablets and the preparation of controlled-release drug forms, and represents a good material for the dispersion of drugs. Eudragit RS is composed of poly (ethylacrylate-methylmethacrylate-trimethylammonioethyl methacrylate chloride) copolymers with ratios of 1:2:0.1. containing a low level of quaternary ammonium groups in 4.5–6.8%. RS has a lower content of charged groups (4.5– 6.8%), it contains only 5% w/w of hydrophilic units and it exhibits a very low permeability, enabling sustained release formulation manufacture and its well-established mucoadhesive characteristics. Eudragit RS is insoluble at aqueous media but swells in water. It is permeable and has pH-independent release profiles [68].

In a recent study microspheres containing acetazolamide were prepared by solvent evaporation method using acetone/ liquid paraffin system. Magnesium stearate was added to the formulations as droplet stabilizer to overcome the problem of droplet coalescence during solvent evaporation. The drug release rate from the microspheres were studied at pH 1.2 and pH 7.4 using the USP XXIII paddle method. Acetazolamide release rates from Eudragit RS microspheres were very slow and incomplete for all formulations at both pH values. When polymer: drug ratio was too low (1:1, w/w) no spherical particles were obtained independent of stirring speed of the system (750 or 500 rpm). The release rate data were found to fit first-order kinetics [69].

Kilicarslan *et al.* reported that verapamil hydrochloride (VRP) microspheres were prepared with Eudragit RS 100 by solvent evaporation method. In vitro dissolution tests were done by using dissolution media with three different pH in sequence as half-change method. As dissolution media, simulated gastric fluid (pH 1.2), phosphate buffer (pH 6.8), and simulated intestinal fluid

(pH 7.5) were used and these dissolution media with different pH were used in sequence for 2 h (pH 1.2), 3 h (pH 6.8) and 4 h (pH 7.5) for half-change method. Drug/polymer ratio, particle size and viscosity were evaluated. The particle size, drug release and the viscosities decrease with the increasing polymer amount. In pH 1.2 dissolution medium drug dissolution is faster than in the other dissolution media with different pH and the lowest dissolution data were determined with pH 7.5 as expected from a weak basic drug [70].

Modified or controlled release NSAID microparticles

Non-steroidal anti-inflammatory drugs (NSAIDs) are usually good candidates for the development of controlled release preparations, particularly through the oral route. However, adverse effects on the gastric mucosa have been observed by several authors, furthermore short biological half-lives can require different daily administrations. Therefore, microencapsulation has been used for the preparation of oral formulations [71-73]. Ketoprofen microsponges were prepared by quasi-emulsion solvent diffusion method. Eudragit RS 100 used to obtain modified release of microsponges. The drug loading capacity was not so much affected by drug to polymer ratio but production yield was changed from minimum ratio to maximum. When the amount of solvent increases, polymer and drug concentration decrease. As a result of the decrease in the polymer concentration, microsphere particle size increases. Also effect of mixing speed was studied and observed that mixing speed of 350 rpm for the preparation of the ketoprofen microsponges was suitable. Drug release of microsponges' kinetics was Higuchi matrix model [72].

A recent patent is based on an antiinflammatory drug (piroxicam) incorporated in a pellet composition further comprising a selected combination of a microcrystalline cellulose and at least one suitable adjuvant, i.e. more precisely comprising suitable respective amounts of a microcrystalline cellulose and a swellable polymer and/or a drug dissolution enhancing amount of a cyclodextrin derivative [3].

A number of recent reports could be assumed that biodegradable NSAID-loaded alginate or chitosan beads were promising pharmaceutical forms by providing controlled-release drug delivery systems and by covering the gastric lesions that NSAIDs may cause [74,75].

In a study carried out by Kofuji *et al.*, enzymatic degradation and the drug release profile of chitosan gel beads was investigated. Prednisolone was used as a model drug. 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 [76].

Yamada *et al.*, 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 [77].

In a recent study, the microsphere formulations containing flurbiprofen were prepared by quasi-emulsion solvent diffusion method with Eudragit RS 100 [78].

Kawashima et al. prepared ketoprofen microspheres for oral sustained release systems, to protect the gastric mucous membrane from drug irritation. ketoprofen microspheres with an Eudragit RS was developed by utilizing sugar esters as solvent diffusion modifiers. The microspheres were produced via transient o/w emulsion droplets of the polymer, which was formed by the interaction of drug and ethanol. The main factor determining the formation of microspheres in this technics was the diffusion rate of solvent from the emulsion droplets (oil phase) to the water phase at the initial stage. When the solvent diffusion rate was very rapid the stable o/w emulsion droplets were not formed, whereas the slow diffusion of solvent led to aggregation of microspheres during processing. In the evaluation of additives to modify the solvent diffusion, sugar esters codissolved in organic phase successfully enhanced the rate of solvent diffusion. The presence of sugar ester in the oil phase improved microsphere formation [79].

Taken together, controlled-release formulations have many advantages over immediate-release formulations. With these formulations, less frequent drug administration is possible, lower plasma peak concentrations can be obtained to avoid adverse effects, and patient compliance can correspondingly be improved.

REFERENCES

- [1] Khan, M.S.Y., Akhter, M., *Pharmazie*, **2005**, 60(2), 110-114.
- [2] Jelen, M.G., Jamnig, D., Schabus, H., Pipam, W., Likar, R., *Acute Pain*, **2008**, 10, 131-136.
- [3] Remon, J.P., Debonne, A., Controlled release pharmaceutical pellet compositions, EP1252886A1, (**2002**).
- [4] Asanuma, M., Nishibayashi-Asanuma, S., Miyazaki, I., Kohno, M., Ogawa, N., *J Neurochem*. **2001**, 76, 1895–1904.
- [5] Sevgi, F., Yurdasiper, A., Kaynarsoy, B., Turunc, E., Guneri, T., Yalcin, A., *AAPS PharmSciTech*, **2009**, 10(1), 104-112.
- [6] Sevgi, F., Kaynarsoy, B., Ertan, G., *Pharm Dev Technol.*, **2008**, 13(1), 5-13.
- [7] Sevgi, F., Kaynarsoy, B., Ozyazici, M., Pekcetin, C., Ozyurt, D.A., *Pharm Dev Technol.*, **2008**, 13(5), 387-392.
- [8] Kaplan, D. L., Wiley, B. J., Mayer, J. M., Arcidiacono, S., Keith, J., Lombardi, S. J., Ball, D., Allen. A. L., Shalaby S. W., *Biomedical polymers: designed-to-degrade systems*. Hanser Publishers, New York, N.Y. **1994**, *Biosynthetic polysaccharides*, 189-212.
- [9] <http://www.fmcbiopolymer.com>
- [10] Seiyaku, F., Sustained release dilazep hydrochloride tablets containing sodium alginate, Japanese Patent 01025721, (**1989**).
- [11] Viernstein, H., Retarded-release drug tablet with alginic acid-sodium alginate matrix, Austrian Patent 385200, (**1988**).
- [12] Connick, W.J. Jr., Controlled release of bioactive materials using alginate gel beads, US Patent 4401456, (**1983**).
- [13] Takka, S., Acarturk, F., *J Microencapsul.*, **1999**, 16, 275–290.
- [14] Gombotz, W.R., Wee, S. F., *Adv Drug Deliver Rev.*, **1998**, 31, 267–285.
- [15] Kim, C.K., Lee, E.J., *Int J Pharm.*, **1992**, 79, 11-19.
- [16] Sankalia, M. G., Mashru, R. C., Sankalia, J. M., Sutariy, V. B., *Eur J Pharm Biopharm.*, **2007**, 65, 215–232.
- [17] Anal, K.A., Stevens, F.W., *Int J Pharm.*, **2005**, 290, 45–54.

- [18] Turkoglu, M., Gursoy, A., Eroglu, L., Okar, I., *STP Pharma Sci.*, **1997**, 7, 135-140.
- [19] Calis, S., Arica, B., Kas, H.S., Hincal, A.A., 5-Fluorourasil-loaded alginate microspheres in chitosan gel for local therapy of breast cancer, *Chitosan in pharmacy and chemistry*, Italy, Atec., **2002**, 65-69.
- [20] Segi, N., Yotsuyanagi, T., Ikeda, K., *Chem Pharm Bull.*, **1989**, 37, 3092-3095.
- [21] Bodmeier, R., Paeratakul, O., *J Pharm Sci.*, **1989**, 78(11), 964-967.
- [22] Tonnesen, H.H., Karlsen, J., *Drug Dev Ind Pharm.*, **2002**, 28, 621-630.
- [23] Sriamornsak, P., Sungthongjeen, S., *AAPS PharmSciTech.*, **2007**, 8(3) Article 51.
- [24] Thanou, M., Verhoef, J.C., Junginger, H.E., *Adv Drug Deliver Rev.*, **2001**, 52, 117-126.
- [25] Meiyu, G., Xianliang, X., Guangqiang, S., Algin oligosaccharides and the derivatives thereof as well as the manufacture and the use of the same, EP1736160 (a1), (**2006**).
- [26] Goldenberg, M.S., Beekman, A.C., Sustained-release alginate gels, US Patent 6656508, (**2003**).
- [27] Xiudong, L., Xiaoju, M., Qun, L., Membrane emulsifying/internal gelatinizing coupled process for preparing gel beads of calcium alginate, CN1373000 (A), (**2002**).
- [28] Masaki, O., Teruko, I., Sustained release pharmaceutical using natural polymer, JP2167220 (A), (**1990**).
- [29] Arica, Y., B., Brice, M., Durlu-Kandilci, N. T., Korkusuz, P., Pellequer, Y., Lamprecht, A., *J microencapsul.*, **2007**, 24(4), 371-82.
- [30] <http://www.nutrimart.com>
- [31] George, M., Abraham, T. E., *J. Control Release*, **2006**, 114, 1-14.
- [32] Cho, C.S., Park, I.K., Jiang, H.L., Yoo, H.S., Shin, N.R., Chitosan microspheres for vaccine delivery to enhance immune activity against atrophic rhinitis of swine, 10-2003-0088872, (**2003**).
- [33] Ho, C.S., Park, I.K., Jiang, H.L., Yoo, S.G., Kang, Pegylated chitosan microspheres for vaccine delivery to enhance immune activity against atrophic rhinitis of swine, 10-2004-0080359, (**2004**).
- [34] Kas, H.S., *J Microencap*, **1997**, 14(6), 689-711.
- [35] Lisbeth, I., He, P., Gastroretentive controlled release microspheres for improved drug delivery, JP 5339149, (**2001**).
- [36] Capan Y, Jiang G, Giovagnoli S, Na K-H, DeLuca PP. *AAPS PharmSciTech.*, 2003;4:E28.
- [37] Tan, S. C., Khor, E., Tan, T. K., Wong, S. M., *Talanta* **1998**, 45, 713-719.
- [38] Peniston, Q. P., Johnson, E., Process for the manufacturer of chitosan, US Patent 4,195,175, (**1980**).
- [39] Sinha, V.R., Singla, A.K., Wadhawan, S., Kaushik, R., Kumria, R., Bansal, K., Dhawan, S., *Int J Pharm.*, **2004**, 274, 1-33.
- [40] Aral, C., Ozbas-Turan, S., Kabasakal, L., Keyer-Uysal, M., Akbuga, J., *STP Pharm Sci.*, **2000**, 10, 83-88.
- [41] Watts, P.J., Illum, L., Drug delivery composition containing chitosan or derivative thereof having a defined z. Potential, US5840341 (A), (**1998**).
- [42] Jimenez, B.A., Quintana-Hau, J.D., Tornero-Montano, J.R., Lopez-Sanchez, M.I., Figueroa-Ponce, H., Implants and Microspheres for the Sustained Release of Drugs for Ophthalmic Use and Preparation Methods Thereof, US2008102099(A1), (**2008**).
- [43] Jang, T.S., Kim, H.M., Method for inhibiting brain cell damage using chitosan and food composition containing chitosan to inhibit the same, KR20020073696 (A), (**2002**).
- [44] Moo-Young, M., Bols, N.C., Overgaard, S.E., Scharer, J.M., Immobilization of biologically

active material in capsules prepared from a water-soluble polymer and chitosan acetate, US5116747, (1992).

- [45] Dhawan, S., Singla, A.K., Sinha, V. R., *AAPS PharmSciTech*, 2004; 5(4): article 67.
- [46] Halder, A., Maiti, S., Sa, B., *Int J Pharm.*, **2005**, 302, 84-94.
- [47] Zheng, M.X., Method for preparing floating-biological adhesion synergistic microparticle, CN1891208 (A), (2007).
- [48] Wang, K., He, Z., *Int J Pharm.*, **2002**, 244, 117-126.
- [49] Gonza'lez-Rodriguez, M.L., Holgado, M.A., Sanchez-Lafuente, C., Rabasco, A.M., Fini, A., *Int J Pharm.*, **2002**, 232, 225-234.
- [50] Steckel, H., Mindermann-Nogly, F., *Eur J Pharm Biopharm*, **2004**, 57, 107-114.
- [51] Re, M., Biscans, B., *Powder Technol.*, **1999**, 101, 120-133.
- [52] Kockischa, S., Reesb, G. D., Tsbouklisc, J., Smart, J. D., *Eur J Pharm Biopharm.*, **2005**, 59, 207-216.
- [53] Alhalaweh, A., Andersson, S., Velaga, S. P., *Eur J Pharm Sci.*, **2009**, 38, 206-214.
- [54] Lameiro, M. H., Lopes, A., Martins, L. O., Alves, P. M., Melo, E., *Int J Pharm.*, **2006**, 312, 119-130.
- [55] Shah, S., Qaqish, R., Patel, V., Amiji, M., *J Pharm Pharmacol.*, **1999**, 51, 667-672.
- [56] Hejazi, R., Amiji, M., *Pharm Dev Technol.*, **2003**, 8, 253-262.
- [57] Ribeiro, A. J., Silva, C., Ferreira, D., Veiga, F., *Eur J Pharm Sci.*, **2005**, 25, 31-40.
- [58] Murata, Y., Toniwa, S., Miyamoto, E., Kawashima, S., *Eur J Pharm Biopharm.*, **1999**, 48, 49-52.
- [59] Lillard, J.W., Koritala, P.R., Palaniappan, R., *Pace-a microspheres for delivery of antigens*, WO03062199 (A2), (2003).
- [60] Perugini, P., Genta, I., Pavanetto, F., Conti, B., Scalia, S., Baruffini, A., *Int J Pharm.*, **2000**, 196, 51-61.
- [61] Cardinal, J.R., Curatolo, W.J., Ebert, C. D., *Chitosan compositions for controlled and prolonged release of macromolecules*, US4895724, (1990).
- [62] Agrawal, A., Kaur, S.T., iwary, A.K., Gupta, S., *J Microencap*, **2001**, 18 (6), 819-823.
- [63] Chen, X.G., Liu, W.S., Lang, G.H., Liu C.S., Cong, R.S., *Manufacture of chitin microspheres*, Chinese Patent ZL 96115714.3, (2002).
- [64] Yubao, L., Junfeng, L., Zhang, L., Zuo, Y., Sun A., Sichuan U., *Water-solubility chitosan microsphere for carrying medicament and preparation thereof*, CN101214228 (A), (2008).
- [65] Zhenqiu, Y., Baozhen, S., *Ion cross-linked chitosan microballoon, its preparing process and use*, CN1485362, (2004).
- [66] Ling, X., D., Yumin, S., Xiaowen, *Process for preparing porous microspheres of high-amino cross-linked chitosan*, CN1328083 (A), (2001).
- [67] Agnihotri, S. A., Aminabhavi, T.M., *J Control Release*, **2004**, 96, 245-259.
- [68] Kibbe, A.H., **2000**, *Handbook of Pharmaceutical Excipients*, Washington, DC: American Pharmaceutical Association and Pharmaceutical Press.
- [69] Haznedar, S., Dortunc, B., *Int J Pharm.*, **2004**, 269, 131-140.
- [70] Kilicarlan, M., Baykara, T., *Int J Pharm.*, **2003**, 252, 99-109.
- [71] Perumal, D., *Int J Pharm.*, **2001**, 218-211.
- [72] Comoglu, T., Gonul, N., Baykara, T., **2003**. *Il Farmaco.*, 58, 101-106.
- [73] Mateovic-Rojnik, T., Frlan, R., Bogataj, M., Bukovec, P., M, A., *Chem Pharm Bull.*, **2005**, 53(1), 143-146.

- [74] Arica, B., Calis, S., Atilla, P., Durlu, N.T., Cakar, N., Kas, H.S., Hincal, A.A., *J Microencapsul.*, **2005**, 22(2), 153-165.
- [75] Acikgoz, M., Kas, H.S., Hascelik, Z., Milli, U., Hincal, A.A., *Pharmazie*, **1995**, 50, 274-277.
- [76] Kofuji, K., Ito, T., Murata, Y., Kawashima, S., *Chem Pharm Bull.*, **2000**, 48, 579–581.
- [77] Yamada, T., Onishi, H., Machida, Y., *Yakugaku Zasshi*, **2001**, 121, 239–245.
- [78] Orlu, M., Cevher, E., Araman, A., *Int J Pharm.*, **2006**, 318, 103-117.
- [79] Kawashima, Y., Iwamoto, T., Niwa, T., Takeuchi, H., Hino, T., *J Microencapsul.*, **1993**, 10, 329-340.