AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Drug delivery systems using chitosan nanoparticles

Yateendra Shanmukha Puvvada*[1], Saikishore.V[1], Srikanth.K[1], Satyanarayana. J[2]

1. Bapatla college of pharmacy, Bapatla, Andhra Pradesh, India-522101.

2.Hetero labs, Jeedimetla, Hyderabad

ABSTRACT

In the recent years considerable research efforts have been directed towards developing safe and efficient chitosan-based particulate drug delivery systems. Chitosan nanoparticles are good drug carriers because of their good biocompatibility and biodegradability, and can be readily modified. The primary hydroxyl and amine groups located on the backbone of chitosan allow for chemical modification to control its physical properties. When the hydrophobic moiety is conjugated to a chitosan molecule, the resulting amphiphile may form self-assembled nanoparticles that can encapsulate a quantity of drugs and deliver them to a specific site of action. Chemical attachment of the drug to the chitosan throughout the functional linker may produce useful prodrugs, exhibiting the appropriate biological activity at the target site. Mucoadhesion and absorption enhancement properties of chitosan increase the *in vivo* residence time in the gastrointestinal tract and improve the bioavailability of various insoluble drugs. The present review outlines the major new findings on the pharmaceutical applications of chitosan-based nanoparticulate drug delivery systems. The first part of the review is concerned with the organ-specific delivery system using chitosan and its derivatives. The subsequent section covers methods of their preparation, drug loading, release characteristics, and applications. Chemically modified chitosan have increased attention for their wide applications and their research discussed critically to evaluate usefulness of these systems in delivering the bioactive molecules. From literature survey, it is realized that research activities on chitosan nanoparticulate systems containing various drugs for different therapeutic applications have increased at the rapid rate. Hence, the present review is timely.

Key Words: Chitosan, nanoparticles

*Corresponding Author Email: chowdharypuvvada@yahoo.com

Received 23 December 2011, Accepted 16 January 2012

Please cite this article in press as: Puvvada YS *et al.*, Drug Delivery Systems Using Chitosan Nanoparticles . American Journal of PharmTech Research 2012.

INTRODUCTION

In the very recent past, there has been considerable interest in developing potential site specific delivery systems especially, biodegradable nanoparticles as effective drug delivery devices in view of their applications in controlled release of drugs, their ability to target particular organs/tissues[1]. Nanoparticles generally include liposomes, polymeric nanospheres, Nanoemulsions, polymer micelles, hydro gels, and solid nanoparticles out of which Liposomes have been used as potential drug carriers instead of conventional dosage forms because of their unique advantages which include ability to protect drugs from degradation, target the drug to the site of action and reduce the toxicity or side effects. However, developmental work on liposomes has been limited due to inherent problems such as low encapsulation efficiency, rapid leakage of water soluble drug in the stability. On the other hand, polymeric NP's offer some specific advantages over liposomes. For instance, Nanoparticles help to increase of drug/proteins and possess useful controlled release properties[2]. Additionally Polymeric nanoparticles possess better reproducibility and stability profiles than liposomes; have been proposed as alternative drug carriers that overcome many of these problems.

Most of nanoparticles prepared from water-insoluble polymers are involved heat, organic solvent or high shear force which doubts the stability of the drug. Moreover, some preparation methods such as emulsion polymerization and solvent evaporation are complex and require a number of preparation steps that are more time and energy consuming. In contrast, water-soluble polymers offer mild and simple preparation methods without the use of organic solvent and high shear force. Among water-soluble polymers available, chitosan is one of the most extensively studied. This is because chitosan possesses some ideal properties of polymeric carriers for nanoparticles such as biocompatible, biodegradable, nontoxic, and inexpensive. Furthermore, it possesses positively charge and exhibits absorption enhancing effect. These properties render chitosan a very attractive material as a drug delivery carrier. In the last two decades, chitosan nanoparticles (chitosan NP) have been extensively developed and explored for pharmaceutical applications.

Chitosan, a linear aminopolysaccharide composed of randomly distributed (1→4) linked D-glucosamine and N-acetyl-D-glucosamine units, is obtained by the deacetylation of chitin, a widespread natural polysaccharide found in the exoskeleton of crustaceans such as crab and shrimp[5]. The extraction of polymer involves preparation of the samples from collected crude skeleton followed by deproteinization with 4M NaOH and crushed into pieces. The ground exoskeleton is then demineralised using 1% Hcl and followed by deacetylation with 50% NaOH

to obtain chitosan and finally exacted by dissolving chitosan in acetic acid and reprecipitated with P^H change. This cationic polysaccharide has drawn increasing attention within pharmaceutical and biomedical applications, owing to its abundant availability, unique mucoadhesivity, inherent pharmacological properties, and other beneficial biological properties such as biocompatibility, biodegradability, non toxicity and low-immunogenicity[6-8]. The physicochemical and biological properties of chitosan are greatly influenced by its molecular weight and degree of deacetylation. Detailed characteristics of chitosan for biomedical applications are well described in several comprehensive reviews[6-8]. The presence of reactive functional groups in chitosan offers great opportunity for chemical modification, which affords a wide range of derivatives such as quaternized chitosan (N, N, N-trimethyl chitosan;TMC), carboxyalkyl chitosan, thiolated chitosan, sugar-bearing chitosan, bile acid-modified chitosan and cyclodextrin-linked chitosan[8-14]. Various synthetic strategies for the modification of the chitosan have been extensively reviewed elsewhere[6, 15, 16]. These chitosan derivatives have been designed to improve specific properties of native chitosan. For example, thiolation of chitosan remarkably improves its mucoadhesive properties because of the formation of disulfide bonds with cysteine-rich subdomains of mucus glycoproteins[23]. The chemical modification of chitosan imparts amphiphilicity, which is an important characteristic for the formation of self-assembled nanoparticles, potentially suited for drug delivery applications. The hydrophobic cores of the nanoparticles could act as reservoirs or micro containers for various bioactive substances. Because of their small size, nanoparticles can be administrated via intravenous injection for targeted drug delivery. Conjugation of the targeting moieties to the surface of drug-loaded nanoparticles may improve therapeutic efficiency of the drug[24]. However, the nanoparticulate drug delivery systems offer numerous advantages over the conventional dosage forms. These include improved efficacy, reduced toxicity and improved patient compliance[1, 3, 18, 19].

Different methods have been used to prepare chitosan particulate systems. Selection of any of the methods depends upon factors such as particle size requirement, thermal and chemical stability of the active agent, reproducibility of the release kinetic profiles, stability of the final product and residual toxicity associated with the final product. In this review the methods of preparation, optimization of methods, and characterization is discussed. The present review details the latest developments on the chitosan polymers as well as surface modification aspects for cell entry[3, 4]. However, selection of any of these methods depends upon the nature of the active molecule as well as the type of the delivery device. Since we are concerned only with the nanoparticulate systems of chitosan and its derivatives, we will restrict our discussions only on these aspects.

Ionic Cross-Linking

The use of complexation between oppositely charged macromolecules to prepare chitosan nanospheres has attracted much attention because the process is very simple and mild. As a widely used method for preparing chitosan nanoparticles, ionic cross-linking is generated by auto-aggregation between chitosan or its derivatives and macromolecules of opposite charge, or when ionic cross-linking agent exists. The most commonly used cross-linking agent is sodium tripolyphosphate. When it is added continuously to a water solution (as shown in the figure) of chitosan with constant stirring under a moderate temperature, two components with opposite charges will combine to form nanoparticles[20]. As ionic cross-linking can be performed at room temperature to avoid the use of organic solvent, and uniform nanoparticles with an adjustable size can be obtained easily, cross-linking has been widely used in encapsulation of protein and gene drugs. Using ionic cross-linking, chitosan nanoparticles were prepared by Du et al[21]and then loaded with Ag^{+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, andFe^{2+}. The zeta potential of chitosan nanoparticles is intensified significantly by positive charge of ions thus improving the stability of nanoparticles and greatly enhancing the antibacterial potential of nanoparticles [21].

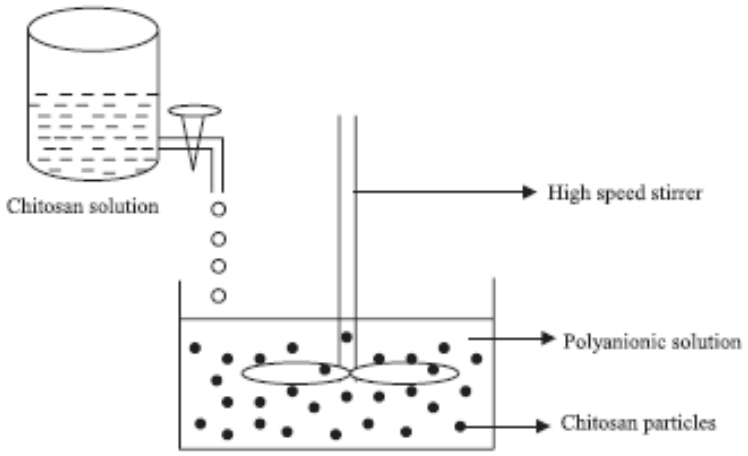


Figure 1 Ionic cross-linking technique.

Covalent Cross-Linking

Chitosan and its derivatives can be prepared as nanoparticle drug carriers by using covalent cross-linking, [21, 22] which involves mainly the formation of covalent bonds between the chitosan chain and a functional cross-linking agent. This method was first used to prepare chitosan nanoparticles by encapsulating neoplastic drugs by cross-linking glutaraldehyde with amino groups in the molecular chain of chitosan.[22, 23]. Commonly applied agents also include polyethylene glycol (PEG) dicarboxylic acid, glutaraldehyde, or mono functional agents such as

epichlorohydrin[24, 25]. Fe_3O_4 chitosannanoparticles were prepared by Qu et al [24] by covalent crosslinking. Oleic acid-coated Fe_3O_4 nanoparticles are absorbed by chitosan and cross-linked with glutaraldehyde, resulting in Fe_3O_4chitosan nanoparticles of average size of 10.5 nm and a narrow size distribution. These nanoparticles have been shown to have a highly saturated magnetization effect, super paramagnetic properties, and a sufficiently high temperature to induce hyperthermia[26].

Coacervation / Preparation

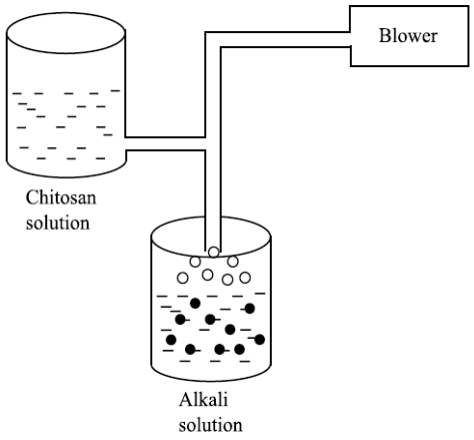


Figure 2: Preparation of nanoparticles with Coacervation phase separation technique.

This method utilizes the physicochemical property of chitosan since it is insoluble in alkaline pH medium, but precipitates/coacervate when it comes in contact with alkaline solution. Particles are produced by blowing chitosan solution into an alkali solution like sodium hydroxide, NaOH-methanol or ethanediamine using a compressed air nozzle to form coacervate droplets[28]. Separation and purification of particles was done by filtration/centrifugation followed by successive washing with hot and cold water. Varying compressed air pressure or spray-nozzle diameter controlled the size of the particles and then using a cross linking agent to harden particles can control the drug release (as shown in the figure). In another technique[29], sodium sulphate solution was added drop wise to an aqueous acidic solution of chitosan containing a surfactant under stirring and ultrasonication for 30 min. nanospheres were purified by centrifugation and re-suspended in demineralised water. Particles were cross-linked with glutaraldehyde. Particles produced by this method have better acid stability than observed by other methods.

Polymerization

Radical polymerization was initially developed by Chauvierre et al.[30,31] It was later used to prepare chitosan and thiolated chitosan-poly (isobutyl cyanoacrylate) core-shell nanoparticles by

Bravo-Osuna et al.[32] Diluted nitric acid solution of chitosan was stirred continuously together with a mixed solution of ceric ammonium nitrate, nitric acid, and isobutylcyanoacrylate at 40^0C for 40 minutes in an argon environment. After cooling to room temperature, sodium hydroxide was added to adjust pH to 4.5 and a nanoparticle suspension was then obtained.[33] Radical polymerization, by combining different monomers, can regulate both nanoparticle shell and core properties.

Emulsion Cross-Linking

This method utilizes the reactive functional amine group of chitosan to cross-link with aldehyde groups of the cross-linking agent. In this method, water-in-oil (w/o) emulsion is prepared by emulsifying the chitosan aqueous solution in the oil phase. Aqueous droplets are stabilized using a suitable surfactant. The stable emulsion is cross-linked by using an appropriate cross-linking agent such as glutaraldehyde to harden the droplets. nanospheres are filtered and washed repeatedly with n-hexane followed by alcohol and then dried[34]. By this method, size of the particles can be controlled by controlling the size of aqueous droplets. However, the particle size of final product depends upon the extent of cross-linking agent used while hardening in addition to speed of stirring during the formation of emulsion. However, this emulsion cross-linking method has few drawbacks since it involves tedious procedures as well as use of harsh cross-linking agents, which might possibly induce chemical reactions with the active agent. Simultaneously difficulty in complete removal of the un-reacted crosslinking agent involved in the process.

Spray-drying

Spray-drying is a well-known technique to produce powders, granules or agglomerates from the mixture of drug and excipient solutions as well as suspensions. The method is based on drying of atomized droplets in a stream of hot air. In this method, chitosan is first dissolved in aqueous acetic acid solution, drug is then dissolved or dispersed in the solution and then, a suitable cross-linking agent is added. This solution or dispersion is then atomized in a stream of hot air. Atomization leads to the formation of small droplets, from which solvent evaporates instantaneously leading to the formation of free flowing particles[35]. Various process parameters are to be controlled to get the desired size of particles. Particle size depends upon the size of nozzle, spray flow rate, atomization pressure, , inlet air temperature and extent of crosslinking and also high negative voltage to instantly evaporate the particles sprayed from the nozzle as elctrospray disposition technique (new technique in which instead of the pressure high negative voltage is used, which atomizes the solid particles) as shown in the figure 3.

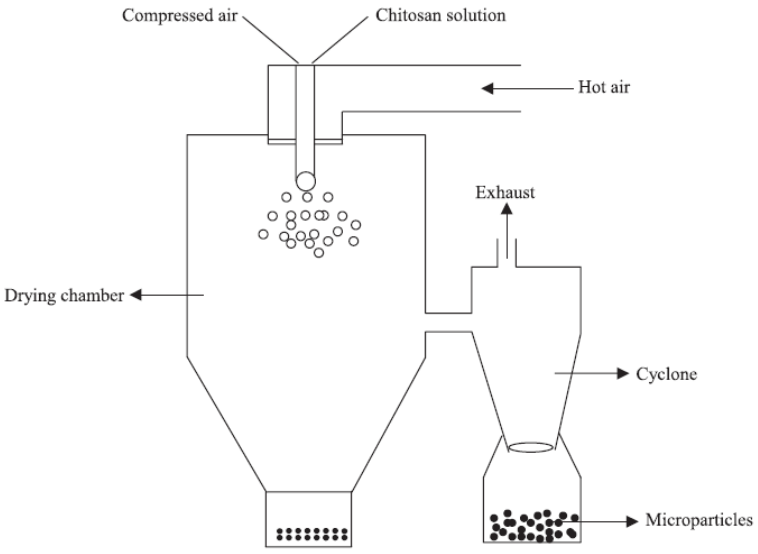


Figure 3: Preparation of nanopartilcels using spray drying

Emulsion-droplet coalescence method

The novel emulsion-droplet coalescence method was developed by Tokumitsu et al.[36], which utilizes the principles of both emulsion cross-linking and precipitation. However, in this method, instead of cross-linking the stable droplets, precipitation is induced by allowing coalescence of Chitosan droplets with NaOH droplets. First, a stable emulsion containing aqueous solution of chitosan along with drug is produced in liquid paraffin oil and then, another stable emulsion containing chitosan aqueous solution of NaOH is produced in the same manner. When both emulsions are mixed under high-speed stirring, droplets of each emulsion would collide at random and coalesce (as shown in the figure 4), thereby precipitating chitosan droplets to give small size particles.

Reverse Micellar Method:

Reverse micelles are thermodynamically stable liquid mixtures of water, oil and surfactant. Macroscopically, they are homogeneous and isotropic, structured on a microscopic scale into aqueous and oil microdomains separated by surfactant-rich films. One of the most important aspects of reverse micelle hosted systems is their dynamic behaviour. Nanoparticles prepared by conventional emulsion polymerization methods are not only large (N200 nm),but also have a broad size range. Preparation of ultrafine polymeric nanoparticles with narrow size distribution could be achieved by using reverse micellar medium[37]. Aqueous core of the reverse micellar droplets can be used as a nanoreactor to prepare such particles. Since the size of the reverse micellar droplets usually lies between 1 and 10 nm [38], and these droplets are highly

monodispersed, preparation of drug-loaded nanoparticles in reverse micelles will produce extremely fine particles with a narrow size distribution. Since micellar droplets are

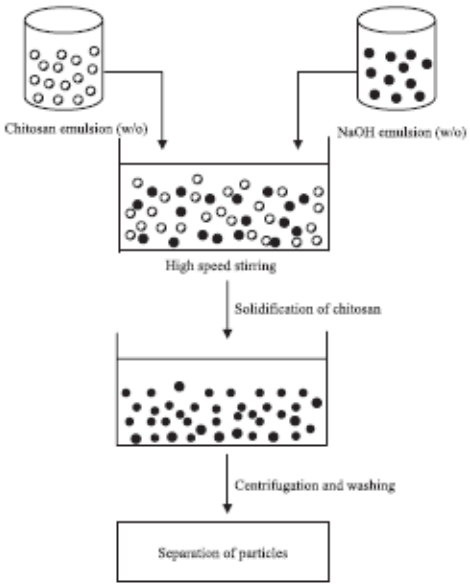


Figure 4: Preparation of nanoparticles using emulsification droplet technique.

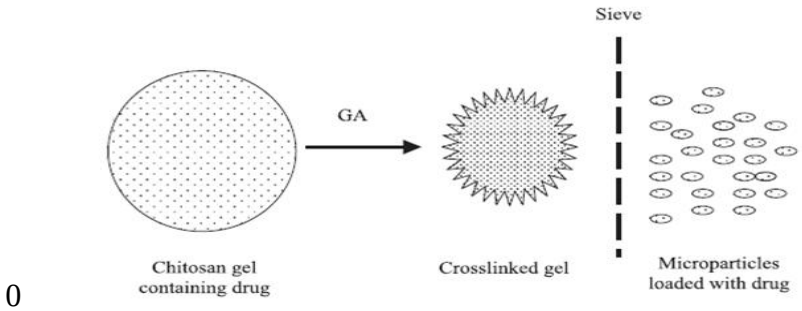
in Brownian motion, they undergo continuous coalescence followed by re-separation on a timescale that varies between millisecond and microsecond[39]. The size, polydispersity and thermodynamic stability of these droplets are maintained in the system by a rapid dynamic equilibrium. In this method, the surfactant is dissolved in a organic solvent to prepare reverse micelles. To this, aqueous solutions of chitosan and drug are added with constant vortexing to avoid any turbidity. The aqueous phase is regulated in such a way as to keep the entire mixture in an optically transparent nanoemulsion phase. Additional amount of water may be added to obtain nanoparticles of larger size. To this transparent solution, a cross-linking agent is added with constant stirring, and cross-linking is achieved by stirring overnight. The maximum amount of drug that can be dissolved in reverse micelles varies from drug to drug and has to be determined by gradually increasing the amount of drug until the clear nanoemulsion is transformed into a translucent solution. The organic solvent is then evaporated to obtain the transparent dry mass. The material is dispersed in water and then adding a suitable salt precipitates the surfactant out. The mixture is then subjected to centrifugation. The supernatant solution is decanted, which contains the drug-loaded nanoparticles. The aqueous dispersion is immediately dialyzed through dialysis membrane or about 1 hr and the liquid is lyophilized to dry powder.

Embedded Quantum Dots Technology Lumiscent semiconductor quatum dots have ideal optical properties and have been used to tag bio-molecules for bio-detection and bio imaging. It

can be prepared by following method. Dissolve chitosan 0.1M acetic acid solution which serves as stock solution. The stock solution was then filtered with a 0.2µm filter for subsequent use. Add quantum dots in aqueous solution chitosan stock solution while stirring. 1-ethyl 1-3-(3dimethylaminopropyl) carbodiimide hydrochloride (EDC) aqueous solution was next added and this was left to react overnight at room temperature. Chitosan nanoparticles were centrifuged down at a rotation speed of 4000 rpm at which free quantum dots remain in the solution and then washed with Distilled water

Sieving Method

Recently, Agnihotri and Aminabhavi[17] have developed a simple, yet novel method to produce chitosan nanoparticles. In this method, nanoparticles were prepared by cross-linking chitosan to obtain a non-sticky glassy hydrogel followed by passing through a sieve. A suitable quantity of CS was dissolved in 4% acetic acid solution to form a thick jelly mass that was cross-linked by adding glutaraldehyde. The non-sticky cross-linked mass was passed through a sieve with a suitable mesh size to get nanoparticles (as shown in the figure 5). The nanoparticles were washed with 0.1 N NaOH solution to remove the un-reacted excess glutaraldehyde and dried overnight in an oven at 40^{o}C.



0

Figure 5: Preparation of nanoparticles using sieving method

Drug Loading into Nanoparticles of Chitosan

Drug loading in nanoparticulate systems can be done by two methods, i.e., active loading and passive loading during the preparation of particles (incorporation) and after the formation of particles (incubation) respectively. In the former system, the drug is physically embedded into the matrix or adsorbed onto the surface. Various methods of loading have been developed to improve the efficiency of loading, which largely depends upon the method of preparation as well as physicochemical properties of the drug. Maximum drug loading can be achieved by incorporating (active loading) the drug during the formation of particles, but it may get affected by the process parameters such as method of preparation, presence of additives, etc. Both water-

soluble and water-insoluble drugs can be loaded into chitosan -based particulate systems. Water soluble drugs are mixed with chitosan solution to form a homogeneous mixture, and then, particles can be produced by any of the methods discussed before. Whereas in the later ones drug was incubated with the pre-formed nanospheres for 48 h for the sufficient entry of the drug (as shown in the figure 6).

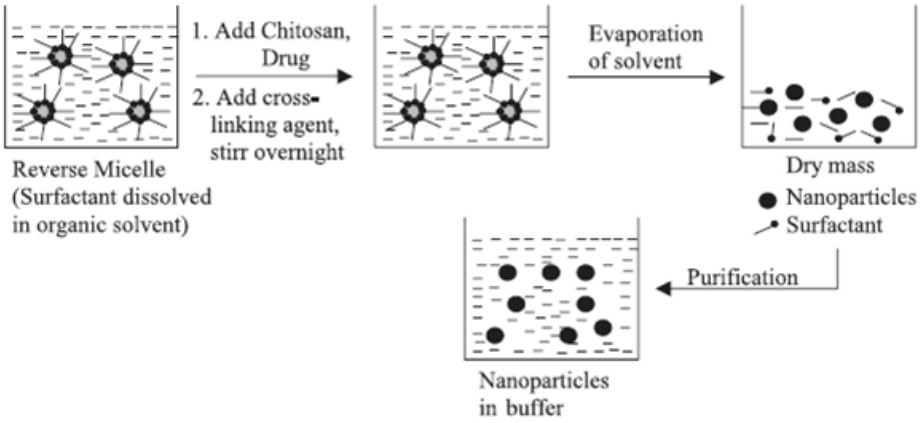


Figure 6: Preparation of nanoparticles by Drug loading method

MODIFICATION OF CHITOSAN NANOPARTICLES FOR TARGETED DELIVERY:

In order to improve targeting and bioavailability of chitosan nanoparticles, an increasing number of studies are focusing on modification of chitosan. Modified chitosan nanoparticles are characterized by pH sensitivity, thermo sensitivity, and targeting accuracy.

Modification for pH Sensitivity

A pH-sensitive nanocarrier is a drug delivery system that increases drug release by changing carrier properties under a certain acid-base environment in vivo, and targets the lesion tissue. Poly (propyl acrylic acid) (PPAA) is a polymer that is highly sensitive to pH. At a pH lower than 6.0, its high membrane fragmentation ability was shown to cause rupture of endosomal membrane and release vesicular materials into cytochylema.[40] Therefore, Kiang et al[41] added PPAA to chitosan–DNA complex to improve gene transfection efficiency. The results showed that adding PPAA to chitosan– DNA complexes enhanced gene expression in both HEK293 and HeLa cells compared with chitosan nanoparticles alone. Chitosan nanoparticles prepared with sodium tripolyphosphate and glycidoxypropyltrimethoxysilane cross-linking were pH sensitive.[42] with antihuman IgG antibody as a model protein drug, the release of antibody was increased from 5.6% to 50% when solution pH was adjusted from 7.4 to 6.0. Therefore, chitosan nanoparticles prepared by two-step cross-linking are a potential drug carrier sensitive to pH.[42]

Modification for Thermo sensitivity

Drug release is regulated by structural change of thermo sensitive drug carriers at different temperatures. Poly(N-isopropylacrylamide) is a well-known thermo sensitive polymer widely used in drug carriers.[43,44] Chitosan polyvinylcaprolactan graft copolymer nanoparticles were sensitive to temperature, with a critical solution temperature at 38^0C.45 With 5-fluorouracil as a model drug, drug release mainly occurred above 38^0C with high toxicity to tumor cells but low toxicity to normal cells.

Modification for Targeting:

Active targeting can be obtained in chitosan nanoparticles through chemical modification, so as to make the drug identify the target accurately. With resveratrol as a model drug, Yao[45] prepared chitosan nanoparticles using ligands of both avidin and biotin to modify the nanoparticles. The resulting delivery system passively targeted the liver and positively targeted hepatoma cells.[46] Two kinds of targeting mechanisms were thus combined in the new drug delivery system to achieve targeting to specific cells in specific tissues, further improving therapeutic effects and reducing toxic and side effects Chitosan nanoparticles modified by glycyrrhizic acid, strengthened the active liver-targeting delivery of drug-loaded carriers through the mediation of glycyrrhizic acid because there were binding sites of glycyrrhizic acid on the surface of liver parenchyma.[47] Kim et al[14] used hydrophobic cholanic acid to modify glycol chitosan and prepare nanoparticles through self-assembly. The antitumor drug cisplatin could be encapsulated easily in a hydrophobic core of nanoparticles. It was proven that due to prolonged circulating time in vivo and strengthened cell permeability and drug effect, drug-loaded nanoparticles were concentrated in tumor tissues of mice successfully with better antitumor effect and lower toxicity.

APPLICATION OF CHITOSAN NANOPARTICLES AS DELIVERY CARRIERS:

Oral Route

The oral route is considered the most convenient and comfortable means of drug administration, because of its non-invasive nature. It reduces the risk of infection, and do not require trained personnel. However, the bioavailability of orally administered proteins is usually poor, because of the hostile gastric and intestinal environments, and also the limited gastrointestinal mucosal permeability. It has been reported that chitosan nanoparticles could delivery the drug into the specific target, thereby decreasing drug doses[48]. Lin *et al.*[48] reported the preparation of

nanoparticles composed of chitosan and poly-γ-glutamic acid (γ-PGA) for insulin delivery. The stability and functionality of nanoparticles *in vitro*, using Caco-2 cell monolayers, and *in vivo*, in a rat model were studied. The authors observed that the chitosan nanoparticles could transiently and reversibly open the tight junctions between Caco-2 cells, thus enhancing the paracellular permeability. Sonaje *et al.*[49] prepared self-assembled nanoparticls, by mixing γ-PGA with chitosan in the presence of MgSO4 and TPP. The efficacy of nanoparticles for oral delivery and intestinal absorption of insulin was investigated in a diabetic rat model. The *in vitro* results showed that the mucoadhesive properties of nanoparticles are affected by the pH and additionally, the transport of insulin across Caco-2 cell monolayers is pH-dependent. In addition, oral administration of insulin-loaded nanoparticles demonstrated a significant hypoglycemic action for at least 10 hours, in diabetic rats.

Nasal Delivery

The nasal mucosa is an attractive route for the delivery of vaccines because it has a relatively large absorptive surface and low proteolytic activity[51-56]. Importantly, nasally administered vaccines can induce both local and systemic immune responses. However, most proteins are not well absorbed from the nasal cavity when administered as simple solutions. The major factors limiting the absorption of nasally administered proteins are the poor ability to cross the nasal epithelia, and the mucociliary clearance, which rapidly removes protein solutions from the absorption site[51,52,57]. Mucoadhesive, hydrophilic chitosan nanoparticles have received much attention to overcome these obstacles and deliver protein antigens via the nasal route, because they strongly attach the mucosa increasing mucin viscosity. Amidi and colleagues[58] prepared and characterized protein loaded Trimethyl chitosan nanoparticles as a nasal delivery system. It was observed that trimethyl chitosan nanoparticles have a high loading efficiency and capacity up to 50%. The release studies showed that more than 70% of the protein remained associated with the nanoparticles for at least 3h of incubation in PBS (pH 7.4), at 37ºC. *In vivo* uptake studies indicated the transport of the protein across the nasal mucosa. Zhang *et al.*[59] used polyethylene glycol-grafted chitosan nanoparticles to improve the systemic absorption of insulin, following nasal administration. *In vitro* release studies showed an initial burst, followed by a slow release of insulin. The nasal delivery of insulin using chitosan-acetyl-L-cysteine nanoparticles was proposed by Wang *et al.*[60]. These authors observed that intranasal administration of modified chitosan-based nanoparticles in rats enhanced the absorption of insulin by the nasal mucosa, as compared with unmodified chitosan nanoparticles and control free insulin solution.

Colon Delivery

Colon targeted drug delivery is useful in improving the absorption of peptide drugs via the GI tract. Site specific drug delivery to the colon is of special interest for drugs instable in the upper part of the GI tract, because of the peptidase activity in the small intestine. The colon is thought to have lower enzymatic activity than other regions of the GI, hence a greater absorption efficiency in this region would be expected, as long as the proteins/peptides are released locally[62]. Due to negligible activity of brush-border membrane and much less activity of peptidases and pancreatic enzymes, the colon has been considered suitable for the delivery of peptides and proteins. Bayat *et al.*[61] developed a nanoparticulate system using two new quarternized derivatives of chitosan, triethylchitosan (TEC) and dimethylethylchitosan (DMEC) nanoparticles, for insulin colon delivery. The three kinds of nanoparticles showed a positive charge that could facilitate insulin uptake, allowing a low bursting effect and a steady release of insulin *in vitro*. DMEC nanoparticles and TEC nanoparticles had smaller particle size, higher insulin loading capacity and improved transport and absorption of insulin, as compared with chitosan nanoparticles. The blood glucose lowering effect of TEC nanoparticles and DMEC nanoparticles, after injection into ascending colon, was superior to that obtained with free insulin or chitosan nanoparticles. This study indicated that nanoparticles prepared from quaternized derivatives of chitosan might be a promising vehicle of administration of proteins and peptides via colon absorption.

Brain Drug Delivery:

The blood brain barrier (BBB) represents an insurmountable obstacle for a large number of drugs including antibiotics, antineoplastic agents and a variety of central nervous system (CNS) active drugs .Currently, chitosan nanoparticles are used as drug delivery vehicles to deliver such drugs to brain by infiltrating BBB and these may provide a significant strategy to break this impasse[63-65],.These drug delivery systems offer numerous advantages over conventional dosage forms, including improved efficacy, reduced toxicity and improved patient compliance. Nanoparticles can also be utilized in the form of carriers in drug delivery [66]. The colloidal systems allow access across the BBB of non- transportable drugs by masking their physicochemical characteristics through their encapsulation in these systems. Among the particulate carriers, nanospheres are being increasingly investigated for targeted delivery of antiretroviral drugs to HIV infected cells and to achieve sustained release kinetics. Their encapsulation into such systems may provide improved efficacy, decreased drug resistance, the reduction in dosage, a decrease in systemic Simar preet Kaur et al Prepared Rivastigmine loaded chitosan nanoparticles by ionic gelation

Method. Three batches of chitosan used nanoparticles formulated shown to have appreciable entrapment efficiency with small size distribution. In-vitro release pattern showed sustained and controlled release for the short half-life drugs such as rivastigmine[67].

FUTURE PROSPECTS

From this review, it is concluded that chitosan nanoparticles have been used as drug delivery carriers. As a drug delivery system, chitosan nanoparticles have attracted increasing attention because of their good biocompatibility, degradability, and nontoxicity. Absorption and bioavailability of drug encapsulated into chitosan nanoparticles can be improved, recently, chitosan is also extensively explored in gene delivery, so they can be used to deliver protein drugs, gene drugs, and other drugs and can protect them effectively from enzyme degradation in vivo. Chitosan nanoparticles are now being modified for sustained/ controlled release and targeting. . These systems have great utility in controlled release and targeting studies of almost all class of bioactive molecules as discussed in this review. While great progress has been achieved in the application of chitosan nanoparticles as drug carriers, some problems remain to be resolved urgently. Majority of studies carried out so far are only in in vitro conditions. More in vivo studies need to be carried out. Chemical modifications of chitosan are important to get the desired physicochemical properties such as solubility, hydrophilicity, etc. For example, chitosan has poor solubility and unmodified chitosan nanoparticles can encapsulate only some hydrophilic drugs. However, studies toward optimization of process parameters and scale up from the laboratory to pilot plant and then, to production level are yet to be undertaken Although chitosan can be modified easily to encapsulate hydrophobic drugs, further investigation is required on the biocompatibility of modified chitosan and its derivatives. In conclusion, chitosan and its derivatives as drug carriers have potential for a wider application. The published literature indicates that in the near future, chitosan-based particulate systems will have more commercial status in the market than in the past.

REFERENCES:

1. Kumar MN, Muzzarelli RA, Muzzarelli C. Chitosan Chemistry and Pharmaceutical Perspectives. Chem Rev 2004; 104:6017–6084.
2. Kumaresh S, Soppimath, Tejraj M, Aminabhavi, Anandrao R, Kulkarnia, Walter E. Rudzinski, Biodegradable Polymeric Nanoparticles as Drug Delivery Device. J Controlled Release 2001;70 (1) :1–20.

3. Waree Tiyaboonchai, Chitosan Nanoparticles A Promising System for Drug Delivery Naresuan University Journal 2003; 11(3): 51-66.
4. Kreuter J. Nanoparticles International Journal. Kreuter (Ed.), Colloidal Drug Delivery Systems, Marcel Dekker, New York, 1994; 219–342.
5. Knight CG (Ed.), Liposomes From Physical Structure To Villa-Jato, M.J. Alonso, Enhancement of Nasal Absorption of Therapeutic Applications, Elsevier, Amsterdam, 1981.
6. Patel MP, Patel RR, Patel JK. Chitosan Mediated Targeted Drug Delivery System: A Review J Pharm Pharma Sci (www.cspsCanada.org) 2010;13(3) :536 - 557,
7. Kumar MN, Muzzarelli RA, Muzzarelli C. Chitosan Chemistry and Pharmaceutical Perspectives. Chem Rev 2004; 104:6017–6084.
8. Illum L, Chitosan and its use as a Pharmaceutical Excipient, Pharm. Res. 1998; 15 (5) 1326–1331.
9. Felt O, Buri P, Gurny R, Chitosan: a Uniquepolysaccharide for Drug Delivery, Drug. Dev. Ind.Pharm. 1998; 24 (3): 979–993.
10. Schnurch A, Guggi D, Pinter Y, Thiolated chitosans: Development and InVitro Evaluation of a Mucoadhesive, Permeation Enhancing Oral Drug Delivery System. J Control Release 2004; 94 (1):177–186.
11. Amidi M, Romeijn S.G, Borchard G, Junginger H.E, Hennink W.E, Jiskoot W, Preparation and Characterization of Protein-Loaded N-trimethyl Chitosan Nanoparticles as Nasal Delivery system, J Control Release 2006;111 (6) :107–116.
12. Wang Y, Jiang Q, Li R, Liu L, Zhang Q, Wang Y, Zhao J, Self-Assembled Nanoparticles of Cholesterol-Modified O Carboxymethyl Chitosan as a Novel Carrier for Paclitaxel, Nanotechnology 2008;19 (5) :145101.
13. Park JH, Cho YW, Chung HK, Won IC, Jeong SY. Synthesis and Characterization of Sugar Bearing Chitosan Derivatives: Aqueous Solubility and Biodegradability, Biomacromolecules 2003; 4 (2):1087–1091.
14. Trapani A, Fuentes M.G, Alonso M.J, Novel Drug Nanocarriers Combining Hydrophilic Cyclodextrins and Chitosan, Nanotechnology 2008;19 (7): 185101.
15. Kim K, Kwon S, Park J.H, Chung H, Jeong S.Y, Kwon I.C, Kim I.S, Physicochemical Characterizations of Self-assembled Nanoparticles of Glycol Chitosan–Deoxycholic acid conjugates, Biomacromolecules 2005;6 (4) :1154–1158.

16. Kurita K, Chitin and Chitosan: Functional Biopolymers from Marine Crustaceans, Mar. Biotechnol. (NY) 2006; 8 (3): 203–226.
17. Mourya V.K, Inamdar N.N, Chitosan- medications and Applications: Opportunities Galore. React Funct Polym 2008; 68: 1013–1051.
18. Agnihotri SA, Mallikarjuna NN, Tejraj M, Aminabhavi, Recent advances on Chitosan-Based micro- and Nanoparticles in drug delivery. J Controlled Release 2004;100 : 5 –28
19. Peppas BL. Recent advances on the use of Biodegradable Microparticles and Nanoparticles in the controlleddrug delivery. Int J Pharm 1995; 116 : 1– 9.
20. Couvreur P, Grislain L, Lenaerts V, Brasseur F, Guiot P, Polymeric Nanoparticles and Microspheres, CRC Press, Boca Raton, FL, 1986. 28-94.
21. Amidi M, Mastrobattista E, Jiskoot W, Hennink WE, Chitosan-based Delivery Systems for Protein Therapeutics and Antigens. Adv Drug DelivRev 2010; 62(1):59–82.
22. Du W, Niu S, Xu Y, Xu Z, Fan C. Antibacterial Activity of Chitosan Tripolyphosphate Nanoparticles Loaded with various metal ions. Carbohydr Polym 2009;75(3):385–389.
23. Jun Jie, WangZhao Wu, ZengRenZhong, XiaoTianXieGuang, Lin ZhouShu, Ling Wang. Recent Advances of Chitosan Nanoparticles. Int J Nanomedicine 2011:6 765–774
24. Prabaharan M, Mano J. Chitosan-based particles as controlled drug delivery systems. Drug Deliv 2004;12(1):41–57.
25. Ohya Y, Shiratani M, Kobayashi H, Ouchi T. Release behavior of 5-fluorouracil from Chitosan-gel Nanospheres Immobilizing5-fluorouracil coated with polysaccharides and their cell specific cytotoxicity. J MacromolSci Part A. 1994; 31(5):629–642.
26. Goldberg M, Langer R, Jia X. Nanostructured Materials for Applicationsin Drug Delivery and Tissue Engineering. J BiomaterSciPolym Ed 2007; 18(3):241–268.
27. Bodnar M, Hartmann J, Borbely J. Preparation and Characterization of Chitosan-based Nanoparticles. Biomacromolecules 2005;6 (5):2521–2527.
28. Qu J, Liu G, Wang Y, Hong R. Preparation of Fe3O4- Chitosan Nanoparticles used for Hyperthermia. Adv Powder Technol 2010; 21(4):461–467.
29. Nishimura K, Nishimura S, Seo H, Nishi N, Tokura S, Azuma I, Macrophage activation with multiporous beads prepared from partially deacetylated chitin. J Biomed Mater Res 1986;20 :1359– 1372.
30. Berthod A, Kreuter J, Chitosan microspheres– Improved Acid Stability and Change in Physicochemical Properties by cross-linking, Proc. Int. Symp. Control. Release Bioact Mater 1996; 23:369– 370.

31. Chauvierre C, Labarre D, Couvreur P, Vauthier C. Radical Emulsion Polymerization of Alkylcyano Acrylates Initiated by the Redox system dextran-cerium (IV) under acidic aqueous conditions. Macromolecules 2003; 36(16):6018–6027.
32. Chauvierre C, Labarre D, Couvreur P, Vauthier C. Plug-in Spectrometry With Optical Fibers as a novel Analytical tool for Nanoparticles Technology: Application to the Investigation of the Emulsion Polymerization of the Alkylcyanoacrylate. J Nanopart Res 2003; 5(3):365–371.
33. Chauvierre C, Labarre D, Couvreur P, Vauthier C. Novel Polysaccharide Decorated Poly (isobutyl cyanoacrylate) Nanoparticles. Pharm Res 2003;20(11):1786–1793.
34. Osuna BI, Vauthier C, Farabollini A, Palmieri GF, Ponchel G. Mucoadhesion Mechanism of Chitosan and Thiolated chitosan-poly(isobutyl cyanoacrylate) core-shell nanoparticles. Biomaterials 2007; 28(13): 2233–2243.
35. Akbuga J, Durmaz S, Preparation and Evaluation of Crosslinked Chitosan Microspheres Containing Furosemide. Int J Pharm 11 (1994) 217– 222.
36. He P, Davis SS, Illum L. Chitosan Microspheres Prepared by Spray Drying. Int J Pharm 1999;187: 53– 65.
37. Tokumitsu H, Ichikawa H, Fukumori Y, Chitosan– Gadopentetic acid Complex Nanoparticles for Gadolinium Neutron Capture Therapy of Cancer: Preparation by Novel Emulsion Droplet Coalescence Technique and Characterization. Pharm Res 1999; 16:1830– 1835.
38. Leong YS, Candau F. Inverse Microemulsion Polymerization. J Phys Chem 1982; 86 :2269– 2271.
39. Maitra A, Determination of size Parameters of Water– Aerosol OT– oil reverse micelles from their nuclear magnetic resonance data, J Phys Chem 1984;88 : 5122– 5125.
40. Luisi PL, Giomini M, Pileni MP, Robinson BH. Reverse Micelles as Hosts for Proteins and small molecules. Biochim Biophys Acta 1988; 947:209–246.
41. Jones R, Cheung C, Black F, et al. Poly (2-alkylacrylic acid) Polymers Deliver Molecules to the Cytosol by pH-Sensitive Disruption of Endosomal vesicles. Biochem J 2003; 372:65–75.
42. Kiang T, Bright C, Cheung C, Stayton P, Hoffman A, Leong K. Formulation of Chitosan-DNA Nanoparticles with poly (propyl acrylic acid) Enhances Gene Expression. J Biomater Sci Polym Ed. 2004; 15(11): 1405–1421.

43. Pan A, Wu B, Wu J. Chitosan Nanoparticles Cross linked by Lycidoxypropyl Trimethoxysilane for pH Triggered Release of Protein. Chin Chem Lett. 2009;20(1):79–83.
44. Chung J, Yokoyama M, Aoyagi T, Sakurai Y, Okano T. Effect of Molecular Architecture of Hydrophobically Modified Poly (N-isopropylacrylamide) On the Formation of Thermoresponsive Core-shell Micellar Drug Carriers. J Control Release 1998; 53(1–3):119–130. 43.
45. Chung J, Yokoyama M, Yamato M, Aoyagi T, Sakurai Y, Okano T. Thermo-responsive drug Delivery from Polymeric Micelles Constructed using block Copolymers of Poly (N-isopropylacrylamide) and Poly (butylmethacrylate). J Control Release. 1999;62(1–2):115–127.
46. Rejinold N S, Chennazhi KP, Nair SV, Tamura H, Jayakumar R. Biodegradable and Thermo-Sensitive Chitosan-g-poly(N-vinylcaprolactam) nanoparticles as a 5-fluorouracil carrier. Carbohydr Polym 2011; 83(2): 776–786.
47. Yao Q. Study on the two-ligand modified chitosan nanoparticles actively targeting to malignant liver cells. Doctoral Paper of Sichuan University. 2006. 6: 765–774.
48. Huang Y, Lin AH, Zhang X. Targeting Binding of chitosan Nanoparticles with Glycyrrhizin Surface Modification to Hepatic Parenchymal Cells Invitro. Tradit Chin Drug Res Pharmacol 2008; 19(6):495–498.
49. Murthy N, et al., A Novel Strategy for Encapsulation and Release of Proteins: Hydrogels and Microgels with Acid-Labile Acetal Cross-Linkers. J Am Chem Soc 2002. 124(42): 12398-12399.
50. Murthy N, et al., A macromolecular Delivery Vehicle for Protein-Based Vaccines: Acid-Degradable Protein-Loaded Microgels. Proc. Natl. Acad. Sci. USA. 2003. 100(9): 4995-5000.
51. Leonard M, et al., Hydrophobically Modified Alginate Hydrogels as Protein Carriers with Specific Controlled Release Properties. J Control Release 2004. 98(3): 395-405.
52. Soane R J, et al., Evaluation of the Clearance Characteristics of Bioadhesive Systems in Humans. Int J Pharm 1999. 178(1): 55-65.
53. Soane RJ, et al., Clearance Characteristics of Chitosan Based Formulations in the Sheep Nasal Cavity. Int J Pharm 2001. 217(1-2): 183-191.
54. Davis SS, Nasal vaccines. Chitosan as a novel *nasal delivery system for vaccines.* Adv Drug Deliv Rev 2001. 51(1-3): 21-42.

55. Illum L, Farraj N F and Davis SS. Chitosan as a Novel Nasal Delivery System for Peptide Drugs. Pharm Res 1994. 11(8):1186-1189.
56. Illum L, et al., Chitosan as a Novel Nasal Delivery System for Vaccines. Adv. Drug Deliv Rev 2001. 51(1-3):81-96.
57. Almeida AJ, Alpar HO. Nasal delivery of Vaccines. J Drug Target 1996. 3(6):455-467.
58. Illum, L., Nasal Drug Delivery--Possibilities, Problems and Solutions. J Control Release 2003. 87(1-3): 187-198.
59. Amidi, M., et al., Preparation and Characterization of Protein-Loaded N-Trimethyl Chitosan Nanoparticles As Nasal Delivery System. J Control Release 2006; 111(1-2): 107-116.
60. Zhang X, et al., Nasal Absorption Enhancement of Insulin using PEG-grafted Chitosan Nanoparticles. Eur J Pharm Biopharm 2008. 68(3): 526-534.
61. Wang X, et al., Chitosan-NAC Nanoparticles as a Vehicle for Nasal Absorption Enhancement of Insulin. J Biomed Mater Res Part B: Appl. Biomater. B, 2009. 88: 150-161.
62. Bayat A, et al., Nanoparticles of Quaternized Chitosan Derivatives as a Carrier for Colon Delivery of Insulin: *Ex-vivo* and *In-vivo* studies. Int J Pharm 2008. 356(1-2): 259-266.
63. Asghar LF, Chandran S. Multi particulate Formulation Approach to Colon Specific Drug Delivery: Current Perspectives. J Pharm Sci 2006. 9(3):327-338.
64. Chakraborty C, Sarkar, B, Hsu CH, Wen ZH, Lin CS, Shieh, PC. Future prospects of nanoparticles on brain targeted drug delivery. J Neurooncol 2009, 93, 285–286.
65. Chen Y, Dalwadi, G, Benson H AE. Drug Delivery across the Blood-Brain Barrier Curr Drug Deliv 1994, 1(4), 361-376.
66. Lockman PR, Mumper RJ, Khan MA, Allen DD. Nanoparticle technology for drug delivery across the blood-brain barrier. Drug Dev Ind Pharm 2002; 28(1):1-13.
67. Kaur SP, Rekha Rao, Afzal Hussain, Sarita Khatkar. Preparation and Characterization of Rivastigmine Loaded Chitosan Nanoparticles. J Pharm Sci Res 2011;3(5):1227-1232
68. Saha P, Goyal, AK, Rath G. Formulation and Evaluation of Chitosan-Based Ampicillin Trihydrate Nanoparticles Trop J Pharm Res 2010, 9 (5), 483-488.