An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

CHITOSAN NANOPARTICLE FOR DRUG DELIVERY – A MINI REVIEW

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ARTICLE INFO

Key Words

Chitosan,
Nanoparticles,
Cancer,
Drug delivery,
Biocompatible

ABSTRACT

Chitosan, a natural based-polymer obtained by alkaline deacetylation of chitin, is nontoxic, biocompatible, and biodegradable. These properties make chitosan a good candidate for the development of conventional and novel drug delivery systems. Their size allows them to be used orally, through mucosal routes and parenteral administration. Clinical investigations suggest that therapeutic nanoparticle can enhance efficacy and reduce side effects compared with conventional cancer therapeutic drugs. Their use as unique drug delivery systems will be a significant value to current cancer therapeutics. The present review paper includes published papers on chitosan Nanoparticle, methods of their preparation, drug loading, release characteristics, and applications. From literature survey, it is realized that research activities on chitosan nanoparticulate systems containing various drugs for different therapeutic applications have increased at a rapid rate.

INTRODUCTION:

The word 'Nano' which is a Greek word simply means dwarf. Nano size refers to one thousand millionth of a meter (10^{-9}m)[1,2]. Nanoparticles generally include liposomes, polymeric nanospheres, Nanoemulsions, polymer micelles, hydro gels, and solid nanoparticles and they 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 [3]. Polymeric nanoparticles that are used for preparation of nanoparticles can be natural polymers or synthetic polymers. A number of natural polymers such as heparin, dextran, albumin, gelatin, alginate, chitosan have been intensively investigated. Synthetic polymers includes polyethylene glycol,polyglutamic acid,polylactic acid etc.[4]. Liposomal nanoparticles are very good candidates for drug delivery for last 50 years. Drug delivery systems based on unmodified liposomes are limited by their short circulation time since they are cleared by macrophages of reticuloendothelial system[5]. Apart from the polymeric and liposomal nanoparticles gold and iron nanoparticles are also developed and have been applied to the development of new a new generation of anti-cancer drug delivery systems.Several gold nanoparticle anticancer drug delivery systems have been reported and showed good invitro results [6]. Magnetic drug targeting is a drug delivery system that can be used in loco-regional cancer treatment. Coated magnetic particles, called carriers, are very useful for delivering chemotherapeutic drugs [7].

CHITOSAN

Chitosan is a linear polysaccharide composed of randomly distributed β-(1-4) linked D-glucosamine and N-acetyl-D-glucosamine units, and is obtained by the deacetylation of chitin which is found in the exoskeleton of crustaceans. Together with chitin, chitosan is considered the second most abundant polysaccharide after cellulose. Compared to chitin in chitosan, majority of the N-acetyl groups are hydrolyzed. Most of the nanoparticles are water insoluble, Chitosan is one of the most extensively studied water soluble polymer. This is because of the ideal properties of chitosan such as biocompatible, biodegradable, nontoxic and inexpensive [3].

Preparation methods for Chitosan Nanoparticles

There are many different methods reported for the preparation of chitosan nanoparticles. The methods are ionotropic gelation, micro emulsion, emulsification solvent diffusion and polyelectrolyte complex formation. 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 [8].

Ionotropic gelation

Chitosan Nanoparticle prepared by ionotropic gelation technique was first reported by Calvo *et.al* [9].The mechanism of chitosan nanoparticle formation is based on electrostatic interaction between amine group of chitosan and negatively charge group of polyanion such as tripolyphosphate (TPP). Different mutations of chitosan nanoparticles produced by the ionic gelation of chitosan by TPP has also been studied by Xu and Du [10].This technique offers simple and mild preparation method in aqueous environment. Dissolve chitosan in acetic acid in the presence or absence of stabilizing agent which can be added in the chitosan solution before or after the addition of polyanion. Polyanion was then added and nanoparticles were spontaneously formed under mechanical stirring at room temperature.

Microemulsion method

Chitosan NP prepared by microemulsion technique was first developedby Maitra *et al.*,[11]. This technique is based on formation of chitosan NP in the aqueous core of reverse micellar droplets and subsequently cross-linked through glutaraldehyde. In this method, a surfactant was dissolved in N-hexane. Then, chitoan in acetic solution and glutaraldehyde were added to surfactant/hexane mixture under continuous stirring at room temperature. Nanoparticles were formed in the presence of surfactant. The system was stirred overnight to complete the cross-linking process, which the free amine group of chitosan conjugates with glutaraldehyde. The organic solvent is then removed by evaporation under low pressure. The yields obtained were the cross-linked chitosan NP and excess surfactant. The excess surfactant was then removed by precipitate with $CaCl_2$ and then the precipitant was removed by centrifugation. The final nanoparticles suspension was dialyzed before lyophilyzation. This technique offers a narrow size distribution of less than 100 nm and the particle size can be controlled by varying the amount of glutaraldehyde that alter the degree of cross-linking. Nevertheless, some disadvantages exist such as the use of organic solvent , time-consuming preparation process, and complexity in the washing step [12].

Emulsification solvent diffusion method

El-Shabouri [13] reported chitosan NP prepared by emulsion solvent diffusion method, (which originally developed by Niwa *et al.*,[14] employing PLGA. This method is based on the partial miscibility of an organic solvent with water. An o/w emulsion is obtained upon injection an organic phase into chitosan solution containing a stabilizing agent (i.e. poloxamer) under mechanical stirring,

followed by high pressure homogenization. The emulsion is then diluted with a large amount of water to overcome organic solvent miscibility in water. Polymer precipitation occurs as a result of the diffusion of organic solvent into water, leading to the formation of nanoparticles. This method is suitable for hydrophobic drug and showed high percentage of drug entrapment. The major drawbacks of this method include harsh processing conditions (e.g., the use of organic solvents) and the high shear forces used during nanoparticle preparation. Mucoadhesive polymer coated chitosan nanoparticles were prepared by emulsion polymerization technique by Sailaja *et al.*, [16].

Polyelectrolyte complex (PEC)

Polyelectrolyte complex or self assemble polyelectrolyte is a term to describe complexes formed by self-assembly of the cationic charged polymer and plasmid DNA. Mechanism of PEC formation involves charge neutralization between cationic polymer and DNA leading to a fall in hydrophilicity as the polyelectrolyte component self assembly. Several cationic polymers (i.e. gelatin, polyethylenimine) also possess this property. Generally, this technique offers simple and mild preparation method without harsh conditions involved. The nanoparticles spontaneously formed after addition of DNA solution into chitosan dissolved in acetic acid solution, under mechanical stirring at or under room temperature [15]. The complexes size can be varied from 50 nm to 700 nm.

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. The nanoparticles were washed with 0.1 N NaOH solutions to remove the un-reacted excess glutaraldehyde and dried overnight in an oven at 40^0 C.

Modification of Chitosan Nanoparticles

To improve the targeting, sustained or controlled release and bioavailability of Chitosan Nanoparticles, an increasing number of studies are focusing on modification of chitosan. Modified chitosan nanoparticles are characterized by pH sensitivity, thermosensitivity and targeting accuracy.

Modification of 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. Fan *et al.*,[18] investigated the effect of the pH of chitosan solution on the formation of chitosan/TPP particles by adjusting the pH from 3.6-5.5.The results indicated that the critical mass ratio of chitosan to TPP for the formation of an opalescent solution decreased with the pH decreasing.

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 [19,20]. Chitosan polyvinylcaprolactan graft copolymer nanoparticles were sensitive to temperature, with a critical solution temperature at 38^0 C [21]. With 5-fluorouracil as a model drug, drug release mainly occurred above 38^0 C with high toxicity to

tumor cells but low toxicity to normal cells. The effect of the temperature of chitosan solution on the particle size was studied by Fan et al., [18]. The particle size displayed a clear tendency to diminish when the temperature was increased from 10^0C to 60^0C, while when the temperature was above 60^0C, the particle size was slightly decreased.

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 [22] prepared chitosan nanoparticles using ligands of both avidin and biotin to modify the nanoparticles. The resulting delivery system passively targeted the live and positively targeted hepatoma cells. 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 [23] Kim *et al.,* [24] 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. Narayanan *et al.,* [25] synthesized, characterized and performed a preliminary *in vitro* evaluation of PTH 1-34 loaded chitosan nanoparticles for controlled delivery of the peptide for osteoporosis treatment.

Modification of sustained release

Nanoparticles made with chitosan or lactic acid –grafted chitosan were developed for high drug loading and prolonged drug release, further increase drug encapsulation, and increase chitosan solubility in solution of neutral pH , chitosan modified with lactic acid onto amino groups in chitosan without using a catalyst. Chitosan nanoparticles for cytarabine were developed to sustain the drug release for I.V administration, which may help to improve the patient compliance[26]. A Dustgani *et al.,*[27] synthesized and characterized novel biodegradable nanoparticles based on chitosan for encapsulation of dexamethasone sodium phosphate.

Applications of chitosan nanoparticles

Route of administration

Routes of administration of chitosan nanoparticles have been developed. Oral nanoparticles can protect drugs from degradation in the gastrointestinal tract and improve drug absorption [28]. Yin *et al.,* [29] developed a promising vehicle for oral delivery. Trimethyl chitosan–cysteine conjugate (TMC–Cys) was synthesized in an attempt to combine the mucoadhesion- and the permeation-enhancing effects of TMC and thiolated polymers related to different mechanisms for oral absorption. The TMC–Cys nanoparticles, obtained via self-assembly, possessed spherical morphology, uniform size, positive zeta potentials, and high insulin encapsulation efficiency. Mucoadhesion and permeation enhancing effects of TMC–Cys nanoparticles were significantly higher than those of TMC nanoparticles. Biocompatibility assessment revealed lack of toxicity of TMC–Cys nanoparticles [29]. Because of poor stability and intestinal absorption of catechins, Dube *et al.,* [30] encapsulated (+)-catechin (C) and (−)-epigallocatechin gallate (EGCg) in chitosan nanoparticles. The encapsulation significantly enhanced intestinal absorption and the cumulative amounts transported after encapsulation were significantly higher [30] An insulin-loaded, pH-sensitive chitosan

nanoparticle was formulated by ionic cross-linking with hydroxypropyl methylcellulose phthalate (HPMCP) as a pH-sensitive polymer [31]. *In vitro* results revealed a superior acid stability of CS–HPMCP nanoparticles with a significant control over insulin release and degradation in simulated acidic conditions with or without pepsin. Moreover, fluorescently labeled CS–HPMCP nanoparticles showed a 2- to 4-fold improvement in the intestinal mucoadhesion and penetration compared with CS–TPP nanoparticles [31]. Amidi *et al.,* [32] investigated the potential of N-trimethyl chitosan (TMC) nanoparticles as a carrier system for the nasal delivery of proteins. TMC nanoparticles have an excellent loading capacity for proteins, and a positive surface charge, suitable for attaching to nasal mucosa. In vivo experiments showed that TMC nanoparticles loaded with fluorescein isothiocyanate–albumin, when administered in the nasal cavity, were able to cross the mucosal layer, be taken up by rat nasal epithelia and NALT cells, and transported to submucosal layers. TMC nanoparticles are a potential new delivery system for protein transport through the nasal mucosa [32]. Wang *et al.,* [33] who prepared estradiol-loaded chitosan nanoparticles and investigated the levels of estradiol in blood and cerebrospinal fluid in rats after intranasal administration showed that estradiol levels in the cerebrospinal fluid after intranasal administration were significantly higher than after intravenous administration. The drug targeting index (DTI) of the nasal route was 3.2 and drug targeting percent (DTP%) was 68.4% [33]. The combination of bioadhesion and paracellular transport effects has led to chitosan to be considered for the delivery of estradiol via the nasal cavity. Huo *et al.,* [34] who used N-octyl-O-glycol chitosan (OGC) as the carrier of paclitaxel for intravenous administration, found that OGC for intravenous administration had good biocompatibility and no toxicity. Moreover, paclitaxel-loaded OGC micelles had low toxicity and a higher tolerated dose. *In vivo* studies of chitosan-fluorescent nanoparticles (CS-fl) prepared for ocular administration showed that the amounts of CS-fl in cornea and conjunctiva were significantly higher for CS-fl nanoparticles than for a control CS-fl solution, these amounts being fairly constant for up to 24 hours [35].

Carrier for various drugs

Carrier of gene drugs

As a gene carrier, conventional virus has the disadvantages of low transfection rate and cell toxicity [36] and even causes serious immune response. As a nonvirus carrier, chitosan has excellent biocompatibility and biodegradation, which has led to increasing application of chitosan nanoparticles in gene drug delivery [37,38]. Gene silencing mediated by double-stranded small interfering RNA (siRNA) has been widely investigated as a potential therapeutic approach for diseases caused by genetic defects [37]. However, its application is restricted by rapid degradation and poor cell absorption. Drug loading of chitosan nanoparticles prepared by ionic gelation by Katas and Alpar [37] reached 100%, protecting well siRNA from nuclease degradation. With natural positive ion chitosan as a carrier material and using electrostatic interaction of polyelectrolyte, siRNA of silencing green fluorescent protein was compounded directly by Liu [38] to prepare stable siRNA nanoparticles with a complex rate of 83% to 94%. It was also found that more stable nanoparticles with positive surface charges could be generated by siRNA and chitosan with high molecular weight and degree of deacetylation. The product was not only easily adsorbed onto the cell surface to increase chance of cellular endocytosis, but also could protect siRNA activity effectively during the transfection in cells to improve gene silencing efficiency. However, gene transfection efficiency of chitosan nanoparticles is low generally and also influenced by molecular weight, degree of deacetylation, the chitosan:DNA ratio, environmental pH, and nanoparticle preparation method [39]. Improving transfection efficiency is a challenge for using chitosan nanoparticles as a gene carrier. Transfection efficiency of chitosan

with different degrees of deacetylation and molecular weights was studied by Lavertu *et al.,* [40] who found that maximum transgene expression occurred when the ratio of the degree of deacetylation (DDA) to the molecular weight (MW) moves from high DDA/low MW to low DDA/high MW. Moreover, several chitosan–pDNA (plasmid DNA) complex formulations achieved levels of transgene expression approaching those of the positive controls (Lipofectamine™, Life Technologies, Carlsbad, CA; and FuGENE® 6, Roche Diagnostics, Basel, Switzerland), while two optimal conditions (92-10-5:1 and 80-10-10:1 [DDA-MW-N:P ratio] both at pH 6.5) were particularly effective, showing equivalent transfection efficiency compared with the best positive control [40]. This provided a good example for the application of chitosan nanoparticles in gene transfection. Moreover, chitosan could also be modified by folic acid to improve transfection efficiency. Folic acid could be easily absorbed by cells, promoting the targeting and internalization of drug. Mansouri *et al.,* [41] used folic acid to modify chitosan for improving gene transfection efficiency. They studied systematically the characteristics of folic acid for gene treatment, finding that folic acid-modified chitosan nanoparticles had low cell toxicity and could condense DNA effectively with ideal size and zeta potential. The results showed that folic acid-modified chitosan nanoparticles were a nonvirus gene carrier with a good application potential.

Carrier of protein drugs

Protein drugs can be degraded easily by enzymes in vivo and have poor permeability and stability as well as a short half-life. However, chitosan can protect protein well and promote the contact between drug and biomembrane, thereby improving bioavailability. Gan and Wang [42] showed that changing the size and surface charge of chitosan–bovine serum albumin nanoparticles could regulate the encapsulation efficiency and release kinetics of bovine serum albumin, but it was difficult to control the burst release of protein of high molecular weight. Zhang *et al.,* [43] used insulin and cationic β-cyclodextrin to form a complex encapsulated into alginate–chitosan nanoparticles. Binding rate and drug-loading amount were 87% and 9.5%, respectively, and cumulative release of insulin in simulated intestinal fluid reached 40%. Insulin was protected well in the nanoparticle core, avoiding the degradation in simulated gastric fluid, as well as the structure of insulin during release. Glycol chitosan nanoparticles modified by 5β-cholanic acid (HGC) and RGD (Arg-Gly-Asp) polypeptide were easily encapsulated into nanoparticles with a drug-loading amount greater than 85% [44]. RGD–HGC nanoparticles showed a one-week sustained-release effect. RGD–HGC displayed antiangiogenic efficacy by inhibiting human umbilical venous cord endothelial cell adhesion to a βig-h3 protein-coated surface, markedly suppressing bFGF-induced angiogenesis as well as decreasing hemoglobin content in Matrigel plugs in vivo. Therefore, RGD-HGC nanoparticles can inhibit tumor cell growth and reduce microvessel density significantly [44].

Carrier of anticancer chemical drugs

Chitosan itself has a certain antitumor activity and its positive charge can neutralize the negative charge on the tumor cell surface, resulting in selective absorption. Thus, chitosan nanoparticles can increase drug concentration in the tumor site and improve therapeutic effects. Doxorubicin/methoxy PEG grafted carboxymethyl chitosan nanoparticles with higher cell toxicity could enter cell and inhibit tumor-cell proliferation effectively [45].Paclitaxel chitosan nanoparticles had a high encapsulation rate of 94.0% ± 16.73% with sustained-release effect [46]. Cell toxicity testing showed that paclitaxel–chitosan nanoparticles had a higher toxicity than that of paclitaxel alone, and with a higher cell uptake rate. Using an evaporation method of composite microemulsion solvent, Trickler *et al.,* [47] combined glyceryl monooleate (GMO) and chitosan to prepare nanoparticles with a hydrophobic core and a hydrophilic shell. When encapsulated in the nanoparticle, paclitaxel had clear sustained-

release characteristics; cell uptake increased four-fold and median lethal dose (IC_{50}) of paclitaxel decreased 1000-fold, leading to a maximum reduction of the side effects of paclitaxel. Kim *et al.*, [48] linked 5β-cholanic acid to the main chain of glycol chitosan for the preparation of amphiphilic HGC nanoparticles. Encapsulated into nanoparticles by the dialysis method, paclitaxel had a drug-loading amount greater than 80%. With significant sustained-release effect, paclitaxel–HGC nanoparticles had low toxicity to B16F10 melanoma cells but a clear anticancer cell effect.

Carrier of other drugs

Chitosan nanoparticles also can load other drugs including antivirus drugs, antiallergic drugs, and hormone drug. Hao and Deng [49] prepared acyclovir-loaded chitosan nanoparticles with a drug loading of 17.8% and an encapsulation rate of 87.5% by an ionic cross-linking method. Li and Luan [50] prepared tranilast-loaded chitosan nanoparticles for allergic diseases with a particle size of 285.5 nm and an encapsulation rate of 82.4%.

CONCLUSION

From this review, it can be clearly understood that chitosan nanoparticles have been used extensively as drug delivery systems because of their good biocompatibility, degradability and non toxicity. Chitosan nanoparticles are now being modified for sustained/controlled release and targeting. Their nanosize facilitates the drug uptake through the cell membrane. While great progress has been achieved in the application of chitosan nanoparticles as drug carriers, some problems remain to be resolved urgently. For example, chitosan has poor solubility and unmodified chitosan nanoparticles can encapsulate only some hydrophilic drugs. Chitosan nanoparticles offer versatile routes of administration. In conclusion, chitosan and its derivatives as drug carriers have potential for a wider application in the market than in the past.

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