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REVIEW ARTICLE

Chitosan Nanoparticle - A Drug Delivery System

Ashish Kumar Kosta*, T.Mohit Solakhia, Dr. Shikha Agrawal

Swami Vivekanand College of Pharmacy, Khandwa Road, Near Tolnaka, Indore,(M.P), India

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ABSTRACT

Chitosan Nanoparticles have gained more attention as drug delivery carriers because of their better stability, low toxicity, simple and mild preparation method, and providing versatile routes of administration. Their sub-micron size not only suitable for oral application, but also applicable for mucosal routes of administration as well as parental administration, Chitosan Nanoparticles are good drug carriers because of their good biocompatibility and biodegradability, and can be readily modified. As a new drug delivery system, they have attracted increasing attention for their wide applications. This paper reviews published on Chitosan Nanoparticles, including its preparation methods, modification, and applications.

Keywords: Chitosan Nanoparticle, drug delivery carriers, sub-micron size.

INTRODUCTION

Nanotechnology is being increasingly explored in science and industry for widely different applications. Nanotechnology and polymers have captivated a tremendous interest in many areas such as the pharmaceutical industry and therapeutic innovation among others. Natural and synthetic polymers have been used as a promising tool for nanoscale drug carrier systems, especially in oral administration of poorly absorbed therapeutic drugs. In recent years, great developments have been made in the field of mucoadhesive polymer systems in formulations that increase the residence time of drugs on mucosal membranes and subsequently, enhance the bioavailability of drugs with poor oral absorption. Chitosan polymers are extensively used for the delivery of an active pharmaceutical ingredient. They can form a matrix or membrane that can control the release of a drug over a prolonged period, thus avoiding repetitive dosing. They can also be used to form (nano) carriers to deliver drugs, in particular poorly soluble drugs or biotechnology-based drugs. Both systems can protect the drug from degradation. Moreover, when the carrier is functionalized by a targeting agent, the encapsulated drug may be selectively released inside or near a specific tissue or organ. Polymeric delivery systems can modify the pharmacokinetics of a drug, leading to a higher therapeutic index by decreasing the side effects and/or increasing efficacy.

NANOPARTICLE

The word 'Nano' is derived from Latin word, which means dwarf. Nano size refers to one thousand millionth of a particular unit thus nanometer is one thousand millionth of a meter (i.e. $1n=10^{9}m$).

Nanoparticles have received much attention for the delivery of macromolecular drugs, such as peptides, proteins, and genes, due to their ability to protect protein and peptides from degradation in the gastrointestinal tract by proteolytic enzymes. Nanoparticles possess marked mucoadhesion properties that have been related to the combination of the particle size and the particle superficial charge. Furthermore Nanoparticles have been referred to as capable of being absorbed into mucosal tissue. Chitosan received attention as a material for Nanoparticles for the decade. Chitosan Nanoparticles are potential delivery system for hydrophilic drugs due to its outstanding physicochemical and biological properties.

The advantages of Nanoparticles as a drug delivery system include the following:

- Particle size and surface characteristics of Nanoparticles can be easily manipulated to

***Corresponding Author:** Ashish Kumar Kosta**, Email:** ashishkosta046@gmail.com

achieve both passive and active drug targeting after parenteral administration.

- They control and sustain release of the drug during the transportation and at the site of localization, altering organ distribution of the drug and subsequent clearance of the drug so as to achieve increase in drug therapeutic efficacy and reduction in side effects.
- Controlled release and particle degradation characteristics can be readily modulated by the choice of matrix constituents. Drug loading is relatively high and drugs can be incorporated into the systems without any chemical reaction; this is an important factor for preserving the drug activity.
- Site-specific targeting can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance.

The system can be used for various routes of administration including oral, nasal, parenteral, intra-ocular etc.

Advantages of polymeric Nanoparticles over liposome- liposome has Low encapsulation efficiency, rapid leakage of water-soluble drug in the presence of blood components and poor storage stability while they help to increase the stability of drugs/proteins and possess useful controlled release properties.

CHITOSAN

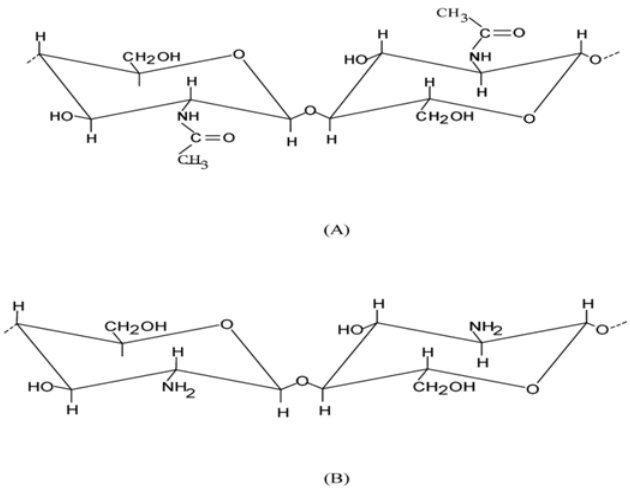
Chitosan is a natural polymer obtained by the hydrolysis of chitin, a native polymer present in shellfish. Together with chitin, chitosan is considered the second most abundant polysaccharide after cellulose. The use of chitosan as an excipient in pharmaceutical formulations is a relatively new development. The polymer differs from chitin in that a majority of the N-acetyl groups in chitosan are hydrolyzed. The degree of hydrolysis (deacetylation) has a significant effect on the solubility and rheological properties of the polymer. The amine group on the polymer has a pKa in the range of 5.5 to 6.5, depending on the source of the polymer. At low pH, the polymer is soluble, with the sol-gel transition occurring at approximate pH 7. The pH sensitivity, coupled with the reactivity of the primary amine groups, make chitosan a unique polymer for oral drug delivery applications. Chitosan obtained from partial deacetylation of chitin, is a polysaccharide comprising copolymers of glucosamine and N-acetylglucosamine.

In recent decades there has also been considerable interest in the pharmaceutical field in using chitosan as an excipient, in various applications. In addition to the good biocompatibility of chitosan and the abundance of natural sources of the material, chitosan has a number of desirable properties that make study of it interesting. Because it is a poly cationic polymer, chitosan forms gels most readily in acidic environments, such as that in the stomach. This makes chitosan interesting in relation to the development of slow release dosage forms for oral administration.

Chemical structure of chitosan

Chitosan [a (1→4) 2-amino-2-deoxy-b-D-glucan] is obtained by the alkaline deacetylation of chitin. Chitosan molecule is a copolymer of N-acetyl-D-glucosamine and D-glucosamine (**Fig 1**). The sugar backbone consists of b-1, 4-linked D-glucosamine with a high degree of N acetylation, a structure very similar to that of cellulose, except that the acetylamino group replaces the hydroxyl group on the C-2 position. Thus, chitosan is poly (N-acetyl-2- amino-2 deoxy-D-glucopyranose), where the Nacetyl- 2-amino-2-deoxy-D-glucopyranose (or Glu-NH) units are linked by (1→4)-b-glycosidic bonds.

Fig 1: Structures of chitin (A) and chitosan (B).



Properties of chitosan

Chitosan consist of repeating units of glucosamine and Nacetyl-glucosamine, the proportions of which determine the degree of deacetylation of the polymer. With a pKa of approximately 6.5 on the amine groups, chitosan is insoluble at neutral pH but is soluble and positively charged at acidic pH. By affecting the number of protonatable amine groups, the degree of deacetylation fundamentally determines the polymer properties including solubility, hydrophobicity, and the ability to interact electrostatically with polyanions. The solubility of chitosan in neutral and basic pH can be improved by quaternization to form trimethyl chitosan derivatives. The molecular weight of chitosan, which is available over a wide range, is also of fundamental importance. Generally, chitosan having lower molecular weights and

lower degrees of deacetylation exhibit greater solubility and faster degradation than their high-molecular-weight counterparts.

Properties of chitosan making it suitable for oral delivery

- Biocompatibility and biodegradability
- Permeation enhancing effect
- Mucoadhesiveness
- pH sensitiveness
- Mild gelation conditions

CHITOSAN NANOPARTICLE

Chitosan based Nanoparticles have advantages particularly for the design and engineering of novel Nanoparticulate drug delivery systems, due to their desirable properties such as:

- Biocompatibility,
- Biodegradability,
- Bio– and mucoadhesivity, and
- Hydrophilic character that facilitate the administration of poorly absorbable drugs across various epithelial barriers, such as corneal, nasal and intestinal mucosa.

Chitosan Nanoparticles have been shown to provide sustained release of both hydrophilic and hydrophobic drugs and are prepared by three distinct methods including ionic gelation, precipitation using tripolyphosphate and crosslinking methods using gluteraldehyde. The method used for preparation determines the entrapment efficiency, loading efficiency, and particlesize. Particle size of the Chitosan Nanoparticles generally depends on molecular weight of chitosan used, concentration of chitosan solution and amount of cross linker. Increasing the concentration of chitosan increases the viscosity of chitosan solution thus making smaller sized particle formation difficult.

An additional advantage of this type of system is that they can be produced under aqueous and fairly mild conditions, thus effectively, being especially suitable to preserve the bioactive conformation of delicate macromolecules (e.g. hormones, antigens, DNA, RNA, growth factors), that otherwise would be prone to enzymatic degradation and hydrolysis. Most frequently Chitosan Nanoparticles are formed according to a bottom–up approach as a result of a self–assembling or crosslinking processes in which the molecules arrange themselves into ordered nanoscale structures either by physical or covalent inter– or intramolecular interactions. In these nanostructures, the drug can be entrapped or attached to the Nanoparticles matrix. Chitosan Nanoparticles have been prepared by several methodologies, including physical crosslinking by ionic gelation by specific ions such as pentasodium tripolyphosphate (TPP) or EDTA. In particular, chitosan –TPP Nanoparticles have been utilized as a drug delivery platform for a wide range of active molecules.

Preparation of Chitosan Nanoparticles

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.

1. Emulsion Cross linking

In this process, chitosan solution is emulsified in oil (w/o emulsion). The aqueous droplets are stabilized using a suitable surfactant. The emulsion is then reacted with an appropriate crosslinking agent such as glutaraldehyde, to stabilize the polysaccharide droplets. The Nanoparticles are then washed and dried. Ohya *et al* reported for the first time the preparation of Chitosan Nanoparticles containing 5-fluorouracil using w/o emulsion method followed by glutaraldehyde cross linking. These pioneering studies demonstrated the feasibility of preparing Chitosan Nanoparticles that could bind and delivery drugs. Major drawbacks of this method are associated with the use of organic solvents and cross linking agents that may adversely affect the stability of proteins. Moreover, glutaraldehydes cross linked Nanoparticles present negative effects on cell viability.

2. 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. 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, and inlet. Huang et al. prepared chitosan–iron oxide Nanoparticles with various chitosan: iron oxide ratios by spray-drying. Atomic absorption

spectrometry results implied that chitosan had strong chelation with iron. Meanwhile, Fe3O4 was crystallized and distributed in the chitosan matrix. These chitosan–iron oxide Nanoparticles were stable in water with strong superparamagnetism.

3. Reverse Micellar Method

In this method surfactant is first dissolved in an organic solvent to produce reverse micelles. To this, an aqueous solution of chitosan and drug are added with constant vortexing to avoid any turbidity. The aqueous solution is kept in such a way as to keep the entire mixture in an optically transparent microemulsion phase. Additional amount of water may be added to obtain Nanoparticles of larger size. To this solution, a crosslinking agent is added and the mixture kept overnight under constant stirring. The organic solvent is then evaporated to obtain the transparent dry mass. The material is dispersed in water, followed by the addition of a suitable salt, which helps to precipitates the surfactant out. It is then centrifuged and the supernatant decanted, which contains the drug-loaded Nanoparticles. The aqueous dispersion is immediately dialysed through dialysis membrane for about 1 hr. and the liquid is lyophilized to dry powder.

4. Template Polymerization

In this technique, chitosan is firstly dissolved in an acrylic monomer solution under magnetic stirring. Due to the electrostatic interaction, the negatively charged acrylic monomers align along the chitosan molecules. After complete dissolution of chitosan, the polymerization is started by adding the initiator (K2S2O8) under stirring at 70ºC. The complete polymerization leads to the appearance of an opalescent solution, indicating the Nanoparticles formation. The Nanoparticles solution are then filtered and dialysed to remove the residual monomers and initiator. The obtained Nanoparticles are positively charged and present a size in the range of 50 to 400 nm.

5. 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. The complexes size can be varied from 50 nm to 700 nm.

6. Precipitation

There are two kinds of approaches for Nanoparticle precipitation. One is desolvation, in which flocculant (commonly sodium sulfate) is added to a water solution of chitosan and solubility of chitosan is decreased by the combination of water and sodium sulfate, leading to the precipitation of Nanoparticles due to hydrogen bonding between molecules. This method was first applied by Berthold et al to prepare chitosan microspheres. Technical improvements then enabled Tian and Groves prepare 600- to 800-nm chitosan Nanoparticles. The other type is based on diffusion of emulsified solvent. Under the action of emulsified solvent, the water phase containing chitosan is dispersed in the organic phase encapsulating the drug, where turbulence appears between the interfaces of the two phases and chitosan is precipitated, resulting in the generation of Nanoparticles. In this method, organic solvent is used and the large Nanoparticles obtained restricting their application.

7. Coacervation or ionic gelation method

Much research has been focused on the preparation of Nanoparticles using biodegradable hydrophilic polymers such as chitosan, gelatine and sodium alginate. Calvo and co-workers developed a method for preparing hydrophilic Chitosan Nanoparticles by ionic gelation. The method involves a mixture of two aqueous phases, of which one is the polymer chitosan, a di-block co-polymer ethylene oxide or propylene oxide (PEO-PPO) and the other is a polyanionn sodium tri polyphosphate. In this method, positively charged amino group of chitosan interacts with negative charged TPP to form coacervates with a size in the range of nanometer. Coacervates are formed as a result of electrostatic interaction between two aqueous phases, whereas, ionic gelation involves the material undergoing transition from liquid to gel due to ionic interaction conditions at room temperature.

Modification of Chitosan Nanoparticles

In order to improve targeting, sustained or controlled relase 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.

- **Modification of thermosensitivity**

Drug release is regulated by structural change of thermo sensitive drug carriers at different temperatures. Poly (N-isopropyl acrylamide) is a well-known thermo sensitive polymer widely used in drug carriers.

Chitosan –polyvinylcaprolactan graft copolymer Nanoparticles were sensitive to temperature, with a critical solution temperature at 38°C. (Rejinold NS,et al) With 5-fluorouracil as a model drug, drug release mainly occurred above 38°C with high toxicity to tumor cells but low toxicity to normal cells.

- **Modification of 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 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. 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.

- **Modification of sustained release**

Nanoparticles of made with chitosan or lactic acid-grafted chitosan were developed for high drug loading and prolonged drug release further increase drug encapsulation, prolong drug release, and increase chitosan solubility in solution of neutral pH, chitosan was modified with lactic acid by grafting D,L-lactic acid onto amino groups in chitosan without using a catalyst. With increased protein concentration, the drug encapsulation efficiency decreased and drug release rate increased. Unlike chitosan, which is generally soluble only in acid solution, the chitosan modified with lactic acid can be prepared from solutions of neutral pH, offering an additional advantage of allowing proteins or drugs to be uniformly incorporated in the matrix structure with minimal or no denaturization.

Pharmaceutical Applications of Chitosan Nanoparticles

- **Ocular administration**

Among mucoadhesive polymers explored now, chitosan has attracted a great deal of attention as an ophthalmic drug delivery carrier because of its absorption promoting effect. Chitosan not only enhance cornea contact time through its mucoadhesion mediated by electrostatic interaction between its positively charged and mucin negatively charged, its ability to transient opening tight junction is believed to improve drug bioavailability. Felt et al. found that chitosan solutions prolonged the cornea resident time of antibiotic in rabbits. The same effects were also observed employing Chitosan Nanoparticles as demonstrated by De Campos et al. that Chitosan Nanoparticles remained attached to the rabbits' cornea and conjunctiva for at least 24 hr. In addition, De Campos et al. found that after ocular administration of Chitosan Nanoparticles in rabbits, most of drug was found in extraocular tissue, cornea and conjunctiva, while negligible drug were found in intraocular tissues, iris/ciliary body and aqueous humor. Together, these results suggested that Chitosan Nanoparticles showed to be attractive material.

- **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. 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 solution from the absorption site. 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 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 3 hr of incubation in PBS (pH 7.4), at 37ºC. *In vivo* uptake studies indicated the transport of the protein across the nasal mucosa.

- **Delivery of vaccines**

Nanoparticles often exhibit significant adjuvant effects in parenteral vaccine delivery since they may be readily taken up by antigen presenting cells. Moreover, oral and nasal delivery of Nanoparticles are thought to have the potential to provide mucosal protective immune responses, one of the most desired goals of modern vaccinology. The submicron size of Nanoparticles allows them to be taken up by M-cells, in mucosa associated lymphoid tissue(MALT) i.e. gut-associated, nasal-associated and bronchus-associated lymphoid tissue, Illum et al initiating sites of vigorous immunological responses. Immunoglobulin A (IgA), a major immunoglobulin at mucosal surface, and the generation of B-cell expressing IgA occur primarily in MALT. The B-cell then leave the MALT and reach systemic circulation wherethey clonally expand and mature into IgA plasma cells. Therefore, providing not only protective IgA at the pathogen entered sites, but also systemic immunity.

- **Chitosan nanoparticles for oral gene delivery**

Increasingly, nucleic acids are being applied as drugs, both for vaccination and therapeutic gene expression. Chitosan–DNA Nanoparticles may be readily formed by coacervation between the positively charged amine groups on the Chitosan and negatively charged phosphate groups on the DNA. Mao et al explored the conditions under which chitosan–DNA Nanoparticles formed and found that discrete particles formed at chitosan concentrations of 50–400 μg/mL and DNA concentrations of 40–80 μg/mL.

CONCLUSION

Chitosan Nanoparticles showed to be prospective drug delivery carriers as they offer many advantages. Chitosan is considered as a safe material as it is natural polymer that possesses biocompatible and biodegradable properties and also water-soluble polymers which is an ideal property for drug delivery carriers, therefore, simple and mild preparation methods can be applied. This renders Chitosan Nanoparticles as promising drug delivery carriers that are suitable for a broad category of drugs including macromolecules and labile drugs. Chitosan is available in a wide range of molecular weights and is easily chemically modified by coupling with ligands providing flexibility in formulation development. Their nano-sized facilitates the drug uptake through the cell membrane. Together, the absorption enhancing effect and nano-sized particles exhibited ability to improve drug bioavailability. Chitosan Nanoparticles offer versatile routes of administration, especially non-invasive routes, i.e. peroral, nasal, and ocular mucosa, which are preferable routes administration. Furthermore, Chitosan Nanoparticles demonstrated to be good adjuvant for vaccine delivery.

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