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Chitosan Nanoparticles: A Boon for drug delivery

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The ability of nanoparticles to manipulate the molecules and their structures has revolutionized the conventional drug delivery system. The chitosan nanoparticles, because of their biodegradability, biocompatibility, better stability, low toxicity, simple and mild preparation methods, offer a valuable tool to novel drug delivery systems in the present scenario. Besides ionotropic gelation method, other methods such as microemulsion method, emulsification solvent diffusion method, polyelectrolyte complex method, emulsification cross-linking method, complex coacervation method and solvent evaporation method are also in use. The chitosan nanoparticles have also been reported to have key applications in parentral drug delivery, per-oral administration of drugs, in nonviral gene delivery, in vaccine delivery, in ocular drug delivery, in electrodeposition, in brain targeting drug delivery, in stability improvement, in mucosal drug delivery in controlled drug delivery of drugs, in tissue engineering and in the effective delivery of insulin. The present review describes origin and properties of chitosan and its nanoparticles along with the different methods of its preparation and the various areas of novel drug deliverywhere it has got its application.

Key words chitosan; nanoparticle; ionotropic gelation; solvent evaporation; complex coacervation

I. INTRODUCTION

Recent years have witnessed unprecedented growth of research and applications in the area of nanoscience and nanotechnology. There is increasing optimism that nanotechnology, as applied to medicinal science, will bring significant advances in in the diagnosis and treatment of disease (Jong and Borm, 2008).The physical approach to alter the pharmacokinetic and pharmacodynamics properties of active pharmaceutical ingredient (API) is the particulate drug delivery system (nano and microparticles) approach. Nanoparticles have attracted a lot of attention of the pharmaceutical scientist in the drug delivery system due to versatility in targeting tissues, accessing deep molecular targets and controlling drug release. Nanoparticles are solid colloidal drug carriers ranging from 10—1000 nm in diameter and are composed of synthetic, natural or semi-synthetic polymers encapsulating the drug molecule. Due to its biodegradability, biocompatibility, easier formulation techniques and versatility in application aided with low toxicity chitosan offers certain advantages over others amongst the polymeric carriers for nanoparticulate drug delivery. The reason why these nanoparticles (NPs) are attractive for medicinal purposes is based on:

- Larger surface to mass ratio than other particles
- Quantum properties
- Ability to adsorb and carry other compounds

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1.1. Chitosan

Chitosan is a mucopolysacharide closely related to cellulose. Chitosan is obtained by deacetylation of chitin, the major compound of exoskeletons in crustaceans. It was first described by Rouget in 1859 and in 1894; and was formally named by Hoppe-Seyler. Deacetylation of chitin is established by boiling chitin from crab and shrimp shells in sodium hydroxide after decolourization with potassium permanganate.). It is insoluble in phosphoric and sulphuric acid. Chitosan is available in a wide range of molecular weight and degree of deacetylation. Molecular weight and degree of deacetylation are the main factors affecting the particle size, particles formation and aggregation.

1.2. Structural Features

When the number of *N* acetylglucosamine units exceeds 50%, the biopolymer is termed as chitin, whereas the term "chitosan" is used to describe an *N*-acetyl-glucosamine unit content less than 50%.1) The unique structural feature of chitosans is the presence of the primary amine at the C-2 position of the glucosamine residues. Few biological polymers have such a high content of primary amines. These amines confer important functional properties to chitosan that can be exploited for biofabrication.10)

1.3. Properties

The properties of chitosan are dependent on the molecular weight, degree of deacetylation and viscosity.1) The degree of deacetylation affects the solubility, hydrophobicity and its ability to interact electrostatically with polyanions by affecting the number of protonatable amine groups of chitosan.11—13) It has also been reported that chitosan having a low degree of deacetylation (DA), which are active as absorption enhancer at both low and high molecular weights, shows a clear dose-dependent toxicity.14) However, chitosan having a higher DA is active enhancer at high molecular weight, but show low toxicity at low molecular weight. As far as toxicity is concerned, it depends on the structural features of the chitosan polymer and not alwaysrelated to its absorption enhancing effect. The molecular weight of chitosan also displays fundamental importance. Generally, chitosan with a lower molecular weights and lower DA, exhibit greater solubility and faster degradation than its high-molecular-weight counterparts.13,15—19)

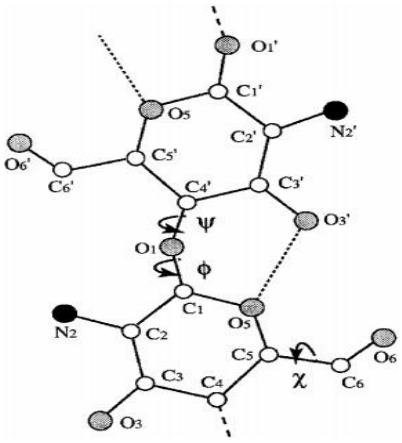

Figure 1.1: Structure of chitosan

(Suheyla Kas, 1997)

1.4. Chitosan Nanoparticles

Chitosan has been reported to be very suitable for preparation of nano- and microparticles for controlled drug release. Chitosan, particularly, chitosan nanoparticles offer many advantages due to their better stability, low toxicity, simple and mild preparation methods, providing versatile routes of administration and has gained more attention as a drug delivery carrier.[22] They have ability to control the release of active agents. They avoid the use of hazardous organic solvents while fabricating particles since they are soluble in aqueous acidic solution. Moreover, chitosan is a linear polyamine containing a number of free amine groups that are readily available for cross linking whereas its cationic nature allows for ionic cross
linking with multivalent anions.[29]

II. PREPARATION OF CHITOSAN NANOPARTICLES

The methods of preparation of chitosan nanoparticles have been described in Table 1.

Name	Procedure of preparation	Merits	Demerits	Ref.
Ionotropic gelation method	The chitosan was dissolved in acetic acid (presence/ absence of stabiliser) followed by the addition of polyanion or anionic polymer under mechanical stirring at room temperature.	1. Simple and mild method gelation 2.Uses aqueous enviornment		30) 31) 42) 115) 116) 117)
Microemulsion method	To surfactant dissolved in *n*-hexane, chitosan solution (dissolved in acetic acid) and glutraldehyde were added under continuous stirring at room temperature. The resulting nanoparticles were stirred overnight. The organic solvent was removed by evaporation under low pressure and the excess surfactant was removed by precipitation by CaCl2 followed by centrifugation,	1. Offer a narrow size distribution of less than 100 nm	1. Time consuming process 2. Quite complex washing steps 3. Use of organic solvents	22) 118)

	dialysis and lyophillization			
Emulsification solvent diffusion method	Firstly, o/w emulsion was prepared by injecting an organic phase into chitosan solution containing a stabilizing agent (*i.e.* poloxamer). Then, under mechanical stirring and high pressure homogenization, the emulsion was diluted with a large amount of water to overcome organic solvent miscibility in water. Polymer precipitation then leads to the formation of nanoparticles.	Suitable only for hydrophobic drugs	1.Harsh processing conditions 2.High shear forces 3.Use of organic solvents	2) 44) 119) 120)
Polyelectrolyte complex (PEC) method	To the cationic polymer (chitosan solution dissolved in acetic acid, gelatin, polyethylenimine), anionic (Alg, dextran sulfate DNA solution) solution was added under mechanical stirring under room temprature		1. Simple and mild preparation 2. Absence of harsh conditions 3. Formation of nanoparticles is spontaneous in nature	22) 44) 121) 122)
	To lginate (such as sodium alginate) dilute solution, Ca2_ solution (such as CaCl2) (at a certain ion concentration) was added. A pregel state forms a continuous system to which aqueous polycationic solution (like chitosan) is added. Leading to the formation of a polyelectrolyte complex, stabilizing the Alg pre-gel nucleus into individual sponge-like nanoparticles.			51) 60) 122) 38)

	To sodium Alg solution in water (1.0% w/v;1 ml), AOT solution in methylene chloride (5% w/v; 1 ml) was added, vortexed and emulsified for 1 min over ice bath leading to the formation of primary emulsion. To this emulsion, 15 ml of aqueous poly vinyl alcohol (PVA) solution (2% w/v) was added and again emulsified by sonication which leads to the formation of secondary w/o/w emulsion. To this, 5 ml of aqueous calcium chloride solution (60% w/v) was added gradually and stirred at room temperature for 18 h to evaporate methylene chloride followed by ultracentrifugation, washing and lyophillization			51) 122) 60) 38)
Complex coacervation method	To positively charged polyelectrolyte (*e.g.* chitosan solution in acetic acid (1%), pH 5.5), negatively charged polyelectrolyte (*e.g.* pDNA solution in sodium sulphate/dextran sulphate) was added. The solution was preheated to 50—55 °C and then vortexed for 45 s leading to the formation of chitosan nanoparticles.	1. Process can be performed entirely in an aqueous solution and at low temperature 2. Offers a better chance to preserve activity of the encapsulated substances	1. Low drug loading efficiency 2. Poor stability 3. Crosslinking of the complex by chemical reagents such as toxic glutaraldehyde is necessary	12) 13) 56) 83)
Solvent	To chitosan solution (in			

evaporation method	ethanol), poly-L-lisin (PLL) solution (in ethanol) was added and mixed by inversion. To this, pDNA-Tris buffer was added with rapid pouring of ethanol under magnetic stirring, The solvent was removed under reduced pressure to yield nanoparticles.			
	To sodium Alg solution (9.5 ml, 0.06% w/v) containing antituberculosis drugs (ATD), calcium chloride (0.5 ml, 18mM) was added. To this, chitosan solution (2 ml, 0.05% w/v) was added and stirred for 30 min and the mixture was kept overnight at room temperature, centrifuged at 19000 rpm for 30—45 min and washed.			
Coprecipitation method	The lactic acid-grafted chitosan (LA-g-chitosan) was prepared by dehydrating the solvent cast thin film of chitosan containing lactic acids. The LA-g-chitosan nanoparticles were fabricated *via* a co-precipitation process by LA-g-chitosan in ammonium hydroxide to form coacervate drops. Spherical and uniformly dispersed chitosan and lactic acid-modified chitosan (LA-g-chitosan)	1.High degree of size uniformity 2.High encapsulation efficiency		62)

	nanoparticles were prepared.			

III. APPLICATIONS OF CHITOSAN NANOPARTICLES

3.1. In Parentral Drug Delivery The biodistribution of nanoparticles can vary depending on their size, surface charge and hydrophobicity.[63)] The particles with diameter greater than 100 nm are rapidly taken up by the reticuloendothelial system (RES), while smaller ones tend to have a prolonged circulation time. Hydrophilic coating (such aspolyethylene glycol (PEG) or a nonionic surfactant) on hydrophobic carriers significantly improves the circulation time.[64,65)] Following intravenous injection, chitosan NP exhibited a marked tendency to accumulate in a number of tumors.[65,66)] One of the possible reasons for this phenomenon may be the leakiness of tumor vasculature.[67,68)] Nano-sized particles can be administered intravenously because the diameter of the smallest blood capillary is approximately 4m m.[63)]

3.2. In Per-oral Administration Being verified by both *in vitro* and *in vivo* study, the absorption promoting effect of chitosan has been found to be due to a combination of mucoadhesion and transient opening of tight junctions in the mucosal cell membrane.[69,70)] Further, an interaction between positively charged chitosan and negatively charge of mucin provides a prolonged contact time between the drug and the absorptive surface, and thereby promoting the absorption.[71)] The report chitosan increases the half time of its clearance, also supports its mucoadhesion.[71)] Besides this, *in vitro* studies in Caco-2 cells have shown that chitosan is able to induce a transient opening of tight junctions thus increasing membrane permeability particularly to polar drugs, including peptides and proteins.[72,73)] Further, Pan *et al.* reported that hypoglycemic effect was observed in induced diabetic rats after orally administration of chitosan nanoparticles.[31)] Moreover, oral allergen-gene immunization with chitosan–DNA nanoparticles has been found to be effective in modulating murine anaphylactic responses, indicating its prophylactic utility in treating food allergy.[56)] Therefore, chitosan can be employed as a coating material for liposomes, micro/nanocapsules to enhance their residence time, thereby improving drug bioavailability.[74,75)]

3.4. In Vaccine Delivery Chitosan is one of the most extensively studied vaccine carriers.[84,85)] Its absorption promoting effect is believed to improve mucosal immune response. Chitosan acts as an adjuvant for systemic vaccine delivery. Activation of macrophages has found to be initiated after the uptake of chitosan.[85—87)] Chitosan has widely been explored for the application for DNA mucosal vaccines. A chitosan-based DNA flu vaccine has been developed by Illum *et al.* that showed high antibody level in mice after intranasal administration.[84)] Plasmid pCMVArah2 encoding peanut allergen gene was successfully incorporated into chitosan NP with good antigen expression and good protection after oral administration in mice.[22,56,88)] The association of vaccines to some of the particulate systems as nanoparticles has shown to enhance the antigen uptake by mucosal lymphoid tissues, thereby inducing strong systemic and mucosal immune responses against the antigens.[1)]

3.5. In Ocular Drug Delivery

Since chitosan is a low toxic material, ophthalmic formulation based on chitosan has exhibited an excellent tolerance after applied chitosan onto the rabbit's corneal surface.[89,90)] Besides employing chitosan NP to improve drug transport *via* ocular, chitosan-coated nanoparticles are also utilized as it exhibit ability to enhance

the corneal penetration.[89,90] De Campos *et al.* have shown that chitosan NP remained attached to the rabbits' cornea and conjunctiva for at least 24 h.[43] The mucoadhesive chitosan (CS)-sodium Alg nanoparticles have been investigated as a new vehicle for the prolonged topical ophthalmic delivery of antibiotic, gatifloxacin.[56,91]

3.6. In Electrodeposition

Chitosan suspended in its solution can mediate the selective assembly of nanoparticles in space. The 100 nm particles *i.e.*, fluorescent latex spheres got assembled onto the cathode surface with high lateral resolution in the *x–y* direction. The control experiments demonstrated that chitosan is required for nanoparticle assembly. A further analysis indicated the nanoparticles entrapment throughout the chitosan matrix in the *z* direction. Thus this chitosan-mediated electrodeposition provides a mean to assemble nanoscale particles into higher-order structures, a requirement that is necessary to exploit one of the unique properties of nanoparticles.[92]

3.8. In Stability Improvement

The chitosan–TPP nanogels containing drugs, genes, or proteins have been utilized as drug delivery systems successfully in human fluids. When the particles are loaded with macromolecules or drugs, the gel network effectively make particles much more stable due to the interaction between them.[37] The chitosan–caseinate complexes have also been reported to have better stability. The different properties with different conditions may modify foods to novel textures, novel optical properties, or increased stabilities. The nanoparticles formed as a result of interactions between these biomacromolecules have been used in the encapsulation and controlled release of drugs, nutraceuticals and other bioactive compounds.[97]

IV. CONCLUSION

Chitosan nanoparticles are most suitable for controlled drug delivery of a drug, effectiveness for mucosal drug delivery, ability to improve the stability of drugs, genes or proteins when formulated as chitosan nanocarriers and better option for tissue engineering applications. The chitosan nanoparticles act as a good adjuvant for vaccine delivery also. These have a tendency to accumulate in a number of tumors to carry anti-tumours thus proving a promising nonviral gene delivery vector. These also have excellent tolerance to the corneal surface and act as better insulin and other therapeutic polypeptides' carrier. Chitosan nanoparticles, coated with Polysorbate 80, have a great potential for brain targeting. The various applications of chitosan are mainly due to its physiochemical properties.

- Being a natural polymer, it is considered as a safe material that has biocompatibility and biodegradability.
- Its water solubility is an ideal property as a drug carrier. That is why; it is suitable for wide variety of drug as a carrier. In the present review, various drug molecules, including proteins, plasmid DNA, and oligonucleotides formulations have been demonstrated.
- It improves the drug bioavailability due to its absorption enhancing effect and facilitates the drug uptake through the cell membrane due to its nanosize.
- These offer a versatile route of administration, especially non-invasive routes like per oral, nasal, ocular and transdermal which are the most preferable.

- Chitosan has a readily modifiable pH responsive solubility which allows it to respond by assembling as a thin film.
- Chitosan shows mucoadhesion as it is able to open tight junctions.
- Chitosan reactivity allows it to be readily functionalized. Proteins can be assembled onto its stimuli responsive backbone by the action of enzymes.
- Chitosan provides a greater flexibility in the development of a formulation as it is available in a wide range of molecular weight. By coupling with a suitable ligand it can be chemically modified easily.

All these versatile capabilities of chitosan and its nanoparticles suggest that this biopolymer has a very bright future in the field of pharmaceutical nanotechnology.

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