Review

Chitosan Nanoparticles: A Promising System in Novel 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 non-viral 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 delivery where it has got its application.

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

1. Introduction

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.

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.[1—4] Deacetylation of chitin is established by boiling chitin from crab and shrimp shells in sodium hydroxide after decolourization with potassium permanganate.[1,4]

Chitosan is digested by chitinases after oral administration. Toxicity tests revealed the LD_{50} of chitosan in mice exceeding 16 g/kg.[4] Chitinases are secreted by intestinal microorganisms and also present in plant ingredients of food, or by lysozymes.[5,6] The amount of derivatized groups and the physical form of chitosans have also shown to contribute to the biodegradability of chitosans.[7] Because of its low production costs, biodegradability, biocompatibility and recent FDA approval, the pharmaceutical and food applications of chitosan have increased remarkably over recent years.[8,9]

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 always

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related 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)]

Chitosan has a pK_a of approximately 6.5 on the amine groups. At pH less than about 6, chitosan's amines are protonated reflecting polycationic behavior of chitosan. Therefore, at pH less than 6.5, chitosan is soluble in most organic acidic solutions including formic, acetic, tartaric, and citric acid.[20—22)] It is insoluble in phosphoric and sulfuric acid.[13,22,23)] The solubility of chitosan in neutral and basic pH can be improved by quaternization to form trimethyl chitosan derivatives.[13,24)] Trimethyl chitosan derivatives are soluble at higher pH than unmodified chitosan.[25)] At pH above about 6.5, chitosan's amines are deprontonated and are reactive and thus can undergo interpolymer associations leading to fiber and network (*i.e.*, film and gel) formation.[10)]

Despite tremendous efforts in investigating the suitability of chitosan in drug delivery and the large number of chitosan manufacturers, it is still very difficult to obtain chitosan which is fully standardized with respect to molecular weight and degree of deacetylation for pharmaceutical research.[1)]

1.4. Nanotechnology According to a working group of the European Science Foundation in 2004, "Nanomedicine" is built on complex systems of nanometer-scale size consisting of at least two components, one of which is an active pharmacological ingredient and the whole system leading to a special function related to the diagnosis, treatment, or prevention of disease.[26)] In this context, nano-scale is taken to include active components or objects in the size range from 1 nm to 100 s of nanometers. Nanomedicines in the form of drug carriers (*e.g.*, particles, liposomes, dendrimers, *etc.*) play an important role to warrant safe and efficient delivery of active compounds to their intended site of action.

The added advantages of nanoparticles over microparticles include the ability to improve drug encapsulation, pharmacokinetics, bioavailability as well as therapeutic efficacy.[27)] Nanotechnology has opened up a new era in the field of drug delivery.[28)] For nanoparticulate drug delivery, the polymeric carriers should be easy to synthesize and characterize inexpensive, biocompatible, biodegradable, non-immunogenic, non-toxic and water soluble.[27)]

1.5. 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)]

2. Preparation of Chitosan Nanoparticles

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

2.1. Ionotropic Gelation Method The method is based on electrostatic interaction between amine group of chitosan and negatively charged group of polyanion such as tripolyphosphate.[30,31)] The size and surface charge of nanoparticles can be changed by changing the ratio of chitosan and stabilizer.[32,33)] For a high yield of nanoparticles, the critical processing parameter chitosan : tripolyphosphate (TPP) weight ratio should be controlled and was found to be within the range of 3 : 1—6 : 1.[34,35)] The mean particle size of nanoparticle decrease with increasing solution temperature in ultrasonic radiation samples.[36)] With the change in physicochemical conditions, like pH of the medium, a volume phase transition takes place.[37)] Structural changes can also be introduced by ionic strength variations like presence of KCl at low and moderate concentrations emphasize swelling and weakness of chitosan–TPP ionic interactions, in turn particle disintegration.[37)] Using chitosan–TPP ionotropic gelation, the estradiol (E2)-loaded chitosan nanoparticles with an average size of 269.3±31.6 nm, a zeta potential of +25.4 mV has been reported with 64.7% entrapment efficiency as 1.9 $mg \cdot ml^{-1}$.[29)]

Besides this, the insulin containing dilute alginate (Alg) solution have been used by inducing an ionotropic pre-gel with calcium counter ions, followed by polyelectrolyte complex coating with chitin.[38,39)] Using this mehod, proteins and peptides,[40)] including insulin,[41)] Z-DEVD-FMK, a caspase inhibitor peptide,[42)] cyclosporine,[43)] small interfering RNA (siRNA) and a basic fibroblast growth factor (bFGF) have been loaded.

2.2. Microemulsion Method Free amino group of chitosan conjugates with gluaraldehyde. Particle size can be controlled by varying the amount of glutraldehyde which changes the degree of cross-linking.

2.3. Polyelectrolyte Complex (PEC) Method The

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Table 1. Desrciption of Methods of Preparation of Chitosan Nanoparticles

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 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 $CaCl_2$ followed by centrifugation, dialysis and lyophillization.	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)
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 method 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, Ca^{2+} solution (such as $CaCl_2$) (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) 83) 56) 55) 58)
Solvent evaporation method	To chitosan solution (in 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.	—		83)

Table 1. Continued

Name	Procedure of preparation	Merits	Demerits	Ref.
	To sodium Alg solution (9.5 ml, 0.06% w/v) containing antituberculosis drugs (ATD), calcium chloride (0.5 ml, 18 mM) 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.	—	1. Process good at small scale but not quite suitable for large scale pilot production	123)
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) nanoparticles were prepared.	1. High degree of size uniformity 2. High encapsulation efficiency	—	62)

mechanism of formulation of chitosan nanoparticles by polyelectrolyte complex method is the electrostatic interactions between the negative group of anion like carboxylic groups of Alg or sulfate groups of dextran sulfate and the positive group of cation like amine groups of chitosan and thus charge neutralization between cationic polymers and anionic group, which in turn lead to a fall in hydrophilicity due to the self assembly of the polyelectrolyte components.[22,44—46] The size of the complexes can be varied from 50 to 700 nm.[22,44] Chitosan and Alg are polycation and polyanion polyelectrolyte respectively, that can be used to form a polyelectrolyte complex for the delivery of proteins,[47] peptide drugs[48] and DNA.[49] Fourier transform (FT)-IR spectra of insulin-loaded nanoparticles revealed amide absorption bands characteristic of protein (loaded in nanoparticles) spectra and confirms the formation of new chemical entities.[50]

2.4. Emulsification-Cross Linking Method This method involves the preparation of novel surfactant-polymer nanoparticles for efficient encapsulation and sustained release of water-soluble drugs.[51] The nanoparticles are formulated using dioctyl sodium sulfosuccinate (Aerosol OTi; AOT) and sodium Alg AOT is an anionic surfactant that is approved as oral, topical and intramuscular excipient (US Food and Drug Administration's Inactive Ingredients Database). Sodium Alg is a naturally occurring polysaccharide polymer, extensively investigated for drug delivery and tissue engineering applications.[52,53] AOT–Alg nanoparticles can sustain the release of water-soluble drugs such as doxorubicin and verapamil over a period of 4 weeks.[51]

2.5. Complex Coacervation Method By coacervation between the positively charged amine groups on the chitosan and negatively charged phosphate groups on the DNA, chitosan–DNA nanoparticles have been reported to form readily.[13,54,55] The weight ratio of the two polymers plays a major role in controlling particle size, surface charge, entrapment efficiency and release characteristics of the nanoparticles to be formulated.[56,57] The physicochemical and release characteristics of the chitosan (CS)–dextran sulfate (DS) nanoparticles can be varied by varying the ratios of two ionic polymers.[56,57]

The CS–DS nanoparticles were developed for the delivery of water-soluble small and large molecules, including proteins, Plasmid DNA, Rhodamine 6G (R6G) and bovine serum albumin (BSA) under mild conditions.[57]

2.6. Solvent Evaporation Method In this method, the emulsification of the polymer solution into an aqueous phase followed by the evaporation of the polymer solvent which induces the precipitation of polymer as nanospheres is required. Alg (a biodegradable and biocompatible co-polymer of guluronic acid and mannuronic acid) has already been acclaimed permission from US FDA for human use and is commonly administered orally for the treatment of reflux esophagitis and has been utilized as nanoparticulate delivery system for frontline antitubercular drugs (ATDs) (rifampicin, isoniazid, pyrazinamide and ethambutol).[58,59] The dosing frequency of ATDs by applying this methodology has been reduced thus improved the patient compliance. Alg nanoparticles were prepared by the cation induced controlled gelification of Alg[59,60] and have been employed for encapsulating other antibiotics.[60,61]

2.7. Coprecipitation Method In coprecipitation method, chitosan-based nanoparticles with a high degree of size uniformity were prepared by grafting D,L-lactic acid on chitosan to serve as a drug carrier for prolonged drug release. 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) nanoparticles with a mean diameter of *ca.* 10 nm were prepared. Albumin encapsulation efficiency as high as 92% and 96% was attained for chitosan and LA-g-chitosan nanoparticles, respectively.[62]

3. 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 as

polyethylene 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 4 μm.[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]

3.3. In Non-viral Gene Delivery Chitosan was first proposed by Mumper as a promising gene delivery vector.[76] Chitosan polymer posses a significantly lower toxicity than poly-L-lysine and PEI, is less immunogenic and lack mutational potential.[22,45,76] Additionally, it enhances the transport of drug across cell membrane. To increase the transfection efficiency, cell targeting ligands were attached to the chitosan particles. Park *et al.* developed liver targeted delivery system by preparing galactosylated-chitosan–graft-dextran DNA complexes, as galactose is well known for liver targeted delivery.[77] Similarly, Mao *et al.* prepared transferrin–chitosan–DNA nanoparticles for a targeted drug delivery.[78] Transferrin can be taken up by receptor-mediated endocytosis mechanism as transferrin receptors are found on many mammalian cells. Unfortunately, they showed transfection efficiency less than the expected. However, when KNOB (C-terminal globular domain of fiber protein) conjugated to the chitosan, the transfection efficiency in Hela cells was improved by 130 fold.[22] Alg–chitosan nanoparticles were able to mediate transfection of 293 T cells four times that achieved by chitosan nanoparticles in 48 h, The transfection efficiency was as high as with Lipofectamine™, with significantly reduced cytotoxicity. Overall, as the presence of Alg results in the reduction of the strength of interaction between chitosan and DNA, the Alg inclusion improved the vector properties of chitosan-based nanoparticles contributing to improved transfection.[78] Due to their high binding capacity and loading efficiency, chitosan–TPP nanoparticles with entrapped siRNA have been shown to be better vectors as siRNA delivery vehicles compared to chitosan–siRNA complexes. Chitosan–TPP nanoparticles show much potential as viable vector candidates for safer and cost-effective siRNA delivery.[79,80] The chitosan nanoparticles have also been used as non-viral vectors for gene delivery or as delivery carriers for protein molecules.[81—83]

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 dem onstrated 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]

3.7. In Brain Targeting Chitosan nanoparticles have been utilised for brain targeting of the drugs after coating them with polysorbate 80.[93] A cholinesterase inhibitor have been utilized for targeting it to brain *via* nasal route.[94] Chitosan has been utilised to improve the brain targeting efficiency by the direct nose to brain pathway especially for drugs for treatment of central nervous system disorders. Further it has been used to combine the active drug for targeting to the olfactory region with controlled release bioadhesive characteristics for maintaining the drug on the absorption site.[95]

Besides this, the estradiol chitosan nanoparticles have higher estradiol concentration in CSF at each sampling time following intranasal delivery. This proves a better utility of

chitosan nanoparticles as a suitable formulation for estradiol delivery to the central nervous system (CNS).[29] It has also been reported that both chitosan structural features and molecular weight play a key role on promoting the intranasal absorption of 2,3,5,6-tetramethylpyrazine phosphate (TMPP).[96]

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]

3.9. In Mucosal Drug Delivery Numerous studies have demonstrated that chitosan and their derivatives (*N*-trimethyl chitosan, mono-*N*-carboxymethyl chitosan) are effective and safe absorption enhancers for the improvement of mucosal (nasal, peroral) delivery of hydrophylic macromolecules such as peptide and protein drugs and heparins.[1,98,99] Mucus composed of molecules like salts, lysozyme and mucins, which are highly hydrated glycoproteins primarily responsible for the viscoelastic properties of mucus. Sialic acid residues on mucin have a pK_a of 2.6 that makes it negatively charged at physiological pH.[98,100,101] It has also been reported that the absorption enhancing effect of chitosans is caused by opening of the intercellular tight junctions, thereby favouring the paracellular transport of macromolecular drugs.[1] Also the presence of mucus affects free drug permeability as well as the uptake of particulates by forming both a physical barrier to diffusion as well as by interacting electrostatically with cationic molecules, such as chitosan. Derivatives of chitosan such as trimethyl chitosan retain their mucoadhesive properties, but to a lesser extent than unmodified chitosan.[102] In addition, formation of chitosan into micro- and nano-particles also preserves their mucoadhesion.[103—105]

Chitosan nanocapsules have been reported to have the ability to enhance and prolong the intestinal absorption of salmon calcitonin (sCT), mainly ascribed to their mucoadhesive character and intimate interaction with the intestinal barrier.[99] The positively charged chitosan will bind to cell membranes and is reported to decrease the *trans*-epithelial electrical resistance (TEER) of cell monolayers as well as increase the paracellular permeability.[106,107] Chitosan solutions have shown to increase trans and *para*-cellular permeability in a reversible, dose-dependent manner depending on the molecular weight and degree of deacetylation of the chitosan.[108] The mechanism of action, which have been reported to be mediated by the positive charges on the chitosan, includes interactions with the tight junction proteins occludin and ZO-1, redistribution of F-actin, and slight destabilization of the plasma membrane.[107,109,110] This ability of chitosan to enhance permeation is also influenced by the pH of the environment. It has also been reported that chitosan trimethyl derivative with 61.2% quaternization was able to decrease TEER of Caco-2 cells and increase mannitol permeability at pH 7.4, unlike unmodified chitosan hydrochloride or 12.3% quaternized trimethyl chitosan.[25]

3.10. In Controlled Drug Delivery Chitosan nanoparticles are also suitable for controlled drug delivery system. Chitosan forms colloidal particles and entraps bioactive molecules. The mechanisms, like chemical crosslinking, ionic crosslinking and ionic complexation are reported to be involved. A possible alternative of chitosan by chemical modification is useful for the association of bioactive molecules to polymer for controlling the drug release profile. Due to the high affinity of chitosan for cell membranes, it has been used as a coating agent for liposome formulations also.[34]

3.11. In Tissue Engineering Chitosan is a better option for tissue engineering application. Being a natural polymer, chitosan is linkages and is susceptible to the lysozymes present in human body.[111]

3.12. In Insulin Delivery Chitosan–dextran sulphate and chitosan Alg nanoparticles have been utilized as a promising insulin and other polypeptides' carriers.[46] Chitosan nanoparticles improve the systemic absorption of insulin following nasal instillation.[41] Also, the insulin-loaded NPs have been reported to effectively reduce the blood glucose level in a diabetic rat model.[112] The chitosan nanoparticles did not improve the absorption enhancing effect of chitosan in solution or powder form. The chitosan powder is the most effective formulation for nasal delivery of insulin in the sheep model.[113] The chitosan nanoparticles enhanced the nasal absorption of insulin to a greater extent than an aqueous solution of chitosan. The amount and molecular weight of chitosan did not have a significant effect on the insulin response.[41,114]

4. 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 non-viral 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.

1. Being a natural polymer, it is considered as a safe material that has biocompatibility and biodegradability.
2. 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.
3. It improves the drug bioavailability due to its absorption enhancing effect and facilitates the drug uptake through the cell membrane due to its nanosize.
4. These offer a versatile route of administration, especially non-invasive routes like per oral, nasal, ocular and transdermal which are the most preferable.
5. Chitosan has a readily modifiable pH responsive solubil-

ity which allows it to respond by assembling as a thin film.
6. Chitosan shows mucoadhesion as it is able to open tight junctions.
7. Chitosan reactivity allows it to be readily functionalized. Proteins can be assembled onto its stimuli responsive backbone by the action of enzymes.
8. Chitosan provides a greater flexibility in the development of a formulation as it is avilable 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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