

Chitosan: A Promising Biopolymer in Drug Delivery Applications

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Received date: August 19, 2015, Accepted date: October 06, 2015, Published date: October 13, 2015

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Abstract

Drug delivery system with controlled release technology has emerged as a powerful tool for the treatment of various diseases. The therapeutic index of the active agent can be enhanced by increasing its stability, solubility and bioavailability, along with specific site delivery. Polymers have been playing an integral role as carrier in formulating an efficient drug delivery system by their stability, drug loading capacity and tunable properties. 'Chitosan' the natural cationic polymer derived from chitin has received growing attention mainly due to their biodegradable, biocompatible, non-toxic, mucoadhesive and ability to target specific delivery properties. Chitosan has itself many medicinal properties like antimicrobial, antioxidant, low immunogenicity etc. which enhance its potential in different biomedical applications. The various techniques for preparation of chitosan micro/nano particles are discussed in this review. Various types of chitosan based drug delivery systems are surveyed to elucidate its role in different biomedical applications.

Keywords: Drug delivery; Controlled release; Micro/nano particles; Biomedical applications

Introduction

Origin, history and chemical structure of chitosan

Chitosan is a cationic linear copolymer polysaccharide made up of random distribution of β (1 \rightarrow 4) linked 2- amino- 2- deoxy- D- glucose (D-glucosamine) and 2- acetamido- 2- deoxy- D- glucose (N- acetyl- D- glucosamine) units. The structure is shown in Figure 1 and it is very similar to cellulose, in which the C-2 hydroxyl groups are replaced by acetamido residue. However owing to the presence of large percentage of nitrogen (6.89%), chitosan shows much commercial interest than synthetically substituted cellulose (1.2%). This provides chitosan chelating properties. Chitosan is typically obtained by extensive deacetylation of chitin, an abundant polysaccharide found in crustacean shells.

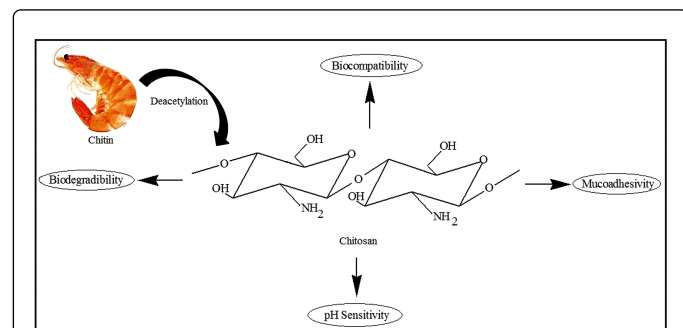


Figure 1: Structure of Chitosan along with its characteristics properties.

Chitin was first identified by Henri Braconnot, Director of botanical garden in Nancy, France in 1811 [1]. He used the name 'fungine' as a component of mushroom cell. Odier introduced the name 'chitin' in 1823. It was derived from the Greek word 'chiton' which means 'covering', 'tunic' or 'envelop'. The structure was solved by Albert Hofmann in 1929 [2]. It is present in many natural resources viz. in cell walls of fungi, in exoskeletons of arthropods mainly in crustaceans (e.g. crabs, lobster and shrimps), insects, radulae of molluscs and in beaks and internal shells of cephalopods (e.g. octopus and squid). Depending upon the source, chitin occurs in three different crystalline forms i. e. α , β and γ - form [3]. However γ - chitin is nothing but a variant of α - chitin [4].

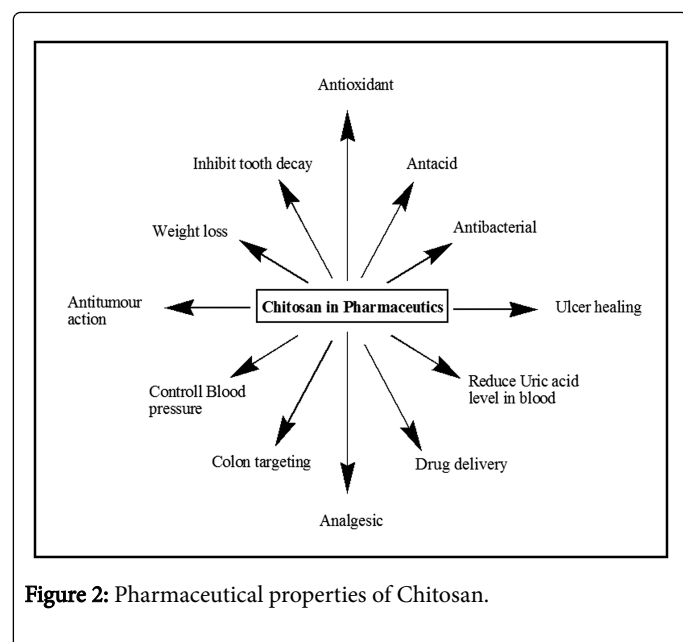
For industrial preparation, the major source for chitin is still the crustacean shells. The source is cheap as crab and shrimp shells can be obtained from the sea food industries as waste or by products. These shells contain 20- 40% of chitin along with proteins, calcium and small amount of pigments. But this type of supplies are limited to fishing industry site and available only in particular season. This problem can be solved by the waste mycelium obtained from industries of fungal products like antibiotics, enzyme or citric acid. It has been reported that annual world production of citric acid estimated at around 400,000 tons resulting into approx. 80,000 tons of waste mycelium of *Aspergillus niger* [5]. Moreover, this type of source contains lower percentage of inorganic materials in comparison to crustacean shells, due to which demineralization is not needed during processing.

Deacetylation process of synthesis of chitosan from chitin is generally done by hydrolysis under alkali condition at high temperature. There are four steps in the preparation method i. e. deproteinization, demineralization, decoloration and deacetylation [6]. Deproteinization involves overnight alkaline treatment with 3- 5% aqueous NaOH (w/v) solution at room temperature. The inorganic ingredients are then removed by treating with 3- 5% aqueous HCl (w/v) solution at room temperature for 5 h. After that, crude chitosan is obtained, which is then purified by precipitating the chitosan from its acetic acid solution by NaOH and washing with distilled water till

neutralization. Commercially chitosan is obtained with different molecular weight (MW) and degree of deacetylation (DD) depending on the percentage of primary amino groups present in the polymer backbone [7].

Properties and applications in different fields

Chitosan offers outstanding biological properties due to which it has gained enormous importance in various applications in pharmaceutical and biomedical areas e.g. in drug delivery, tissue engineering, gene delivery etc. the biological properties include biocompatibility, biodegradability, safe and non-toxicities. Besides this, it is haemostatic, fungistatic, bacteriostatic, spermicidal, anticholesteremic and anticarcinogenic. Some of its applications are summarized in Figure 2. The primary amino groups in the polymer backbone of chitosan provide positive charge on its surface. Due to its unique structure with polycationic surface, along with capability of forming inter and intramolecular H- bonding, chitosan has been regarded as a good candidate for the development of novel pharmaceutical products



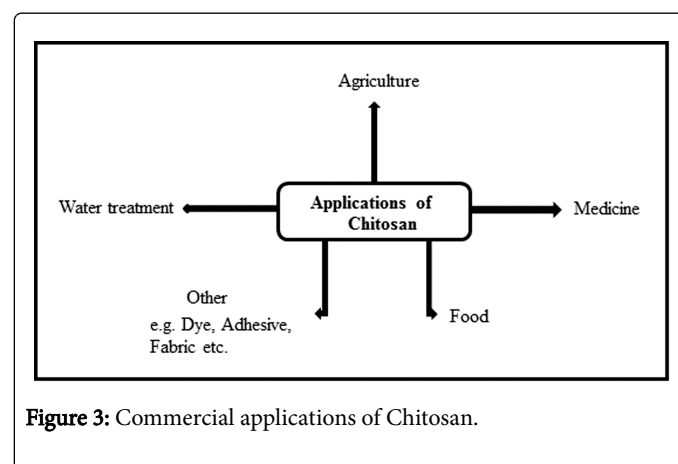
The bioadhesiveness of chitosan provides the polymer to adhere to hard/ soft tissues, the character which has numerous applications in dentistry, orthopedics, ophthalmology and in surgical measures [8]. Jiang et al. studied the optical and wave guiding properties of chitosan based films. They prepared film with uniform, dense and homogeneous structure with smooth surface having good thermal stability, thereby proving it's prospective in ophthalmology [9].

The antibacterial properties of chitosan facilitate its use in the pharmaceutical products as it limits the threat of infections. Chitosan based materials have been therefore widely used in treatment of wounds and burns [10]. Dai et al. has reported that not only the antimicrobial property but also their ability to convey the extrinsic antimicrobial agents to the wounds and burns have made them perfect material for these applications [11]. Chitosan can form compatible, tough, and oxygen permeable water absorbent films. These films can be readily degraded by enzymes. In burn accidents, many patients generally suffered from severe loss of skin due to burning. They then

suffered from danger of severe infections and fluid loss. In this regard, chitosan contains similar structural characteristics as glycosamine glycans, therefore, they are considered as suitable material for designing substratum for skin replacement [12]. Malette et al. studied the effect of chitosan and saline solution on healing and fibroplasia of wounds in skin of abdominal surface of dogs [13].

Tissue engineering involves the development of substituents which would replace damaged/ diseased/ injured part of the body through complex construction of cells, bioactive molecules which support cell attachment, proliferation and differentiation. The artificial implantation must be biocompatible and having functional as well as mechanical stability. This is an interdisciplinary area which utilizes the principles and methods of both biological science and engineering for the designing of artificial substituents in order to maintain or improvement of bio-function of the effected site of the body. The various properties like gel formation, porous structure, high affinity towards biological molecules, ease of modification etc. has led the researchers to focus on chitosan for tissue engineering applications [14]. Especially the chitosan based hydrogels have been developed for this purpose. However pure chitosan does not recommended due to its fragile nature and low mechanical strength. Therefore chitosan has been combined with other materials, e.g. collagen, other polymers, hydroxyapatite etc. in order to improve mechanical strength as well as to mimic the nanostructure of the tissue [15].

Due to the bioadhesion property, chitosan based compounds has been developed for preparing biological adhesives used in surgeries. They are suitable for tissue adhesion, sealing of leakage of air or body fluid, during surgical procedures, hemostasis etc. Photochemically crosslinked chitosan hydrogel has been tried as bioadhesive on rabbit model and found to be effective in sealing the puncture in the carotid arteries/lung made artificially by a needle [16].



Apart from biomedical applications, chitosan has many other commercial applications as shown in Figure 3. It has been found that chitosan can increase photosynthesis process in plant, promotes plant growth, nutrient uptake, germinations and sprouting [17]. These properties have made the polymer suitable for agricultural applications, especially in seed treatment, as enhancer and also as biopesticide substance to work against fungal infections due to its antifungal property [18]. The antifungal property of chitosan has made it suitable in horticulture applications also. This property enables chitosan to control pre and post-harvest plant diseases [19]. Again chitosan has been applied to prolong the self-life of treated fruit and vegetables through minimizing the rate of respiration and water loss.

Therefore it can be widely used as plant protector. As a biopesticide, chitosan has been used in crop protection and food preservations also.

Chitosan based materials have been investigated in the treatment of waste water due to their ability to adsorb heavy metal ion. Weltrowski et al. synthesized chitosan N- benzylsulfonates derivative for use as a sorbent for removal of metal ion in acidic medium [20]. Kang et al. studied the metal adsorption (Cu^{2+} , Mn^{2+} , and Pb^{2+}) capacity of amidoximated chitosan bead-g-PAN copolymer [21].

In cosmetics products, chitosan based materials have been widely used owing to their fungicidal, fungistatic nature, antioxidant property, and most importantly cationic nature [22]. Such materials are applied generally in cosmetics of hair care, skin care and oral care. Dutta et al. reported that chitosan based material could reduce skin irritation in case of skin care products [23].

Chitosan in drug delivery

To deliver the pharmaceutically active agent at the right place, at the right concentration for the right period of time is still a challenge for the researchers. To achieve this objective 'drug delivery systems' has introduced which are based on association of the active agent with a suitable carrier [24]. The drug delivery systems offer many advantages in therapy (Figure 4), which include: (a) reduce toxicity, (b) increase therapeutic index of drug and (c) prevent frequent, expensive and unpleasant dosing.

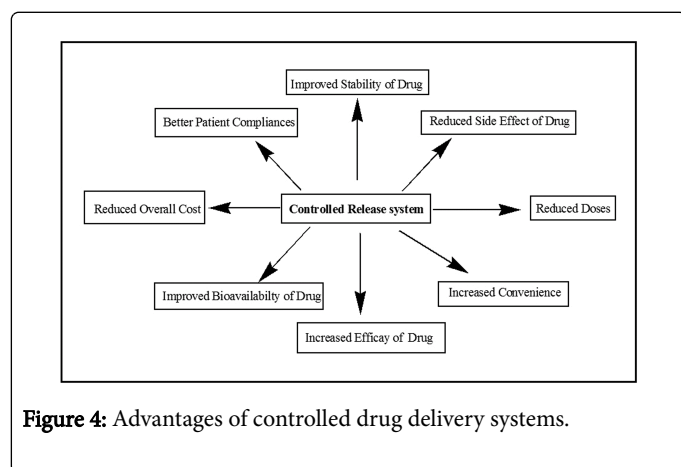


Figure 4: Advantages of controlled drug delivery systems.

Such delivery system with 'controlled drug release' characteristics has emerged as a promising tool in treatment of various diseases. These systems allow the delivery of the active agent at a predetermined rate locally or specifically for a specified period of time [25]. In case of conventional drug delivery systems, after each dose the drug level increases, perhaps exceeds the maximum level, which then followed by continuous declination upto below minimum effective level until the next administration. As a result conventional system requires several doses of drug to maintain the average drug level, which is both uneconomical and health hazardous. But in case of controlled drug release system the possibility of under and over dosing has not taken place. They also suppress complications like degradation of the active agent, low drug solubility, low bioavailability, fast clearance rate, inability of the drug molecules to cross the biological barrier etc.

After achieving the aim of releasing the drug in controlled manner, the next challenge has become the delivery of the drug specifically to the site of action. This idea leads to the development of advanced form of controlled release systems named as 'targeted drug delivery systems'.

Here the active agent is allowed to encapsulate within such a specially designed system which can carry and deliver it specifically to the target tissue, cell or organ. It requires appropriate choice of carrier, route and target [26]. The main aim of designing such delivery system is to reduce the side effect of the drug (especially in cancer therapeutics).

Polymer based drug delivery systems can improve the pharmacokinetic of a drug, improve their therapeutic index, decrease their side effects and hence increase the efficiency of the whole system. Different types of polymeric drug delivery systems have been developed in these years including microsphere, micelles, hydrogels, nanoparticles etc. [27]. The drug molecules release from the polymer matrix through different mechanisms such as diffusion, erosion, degradation of the polymer etc. Biodegradable and biocompatible polymers are best suggested for this application because of the need of appropriate release of the drug as well as easy removal of the carrier after drug administration.

The remarkable physical, chemical and biological properties like easily modifiable and nontoxicity make chitosan a potential material in designing formulations for drug delivery in the gastrointestinal tract. The polycationic nature of chitosan makes it able to interact with the negatively charged mucous membrane and thereby increases the adhesion to the mucosa and as a result enhances the time of contact for penetration of drug molecules through it. It can act as permeation enhancer for hydrophilic drugs which have poor oral bioavailability due to interaction with cell membrane which may open the tight junctions in the membrane. Thanou investigated the role of chitosan as permeation enhancer for peptide drugs across mucosal epithelia [28].

Another important feature of using chitosan as drug carrier is its metabolic degradation in the body. Chitosan provides easy elimination process after drug administration, generally by renal clearance; however, this applies for chitosan with suitable molecular weight. For very large molecular weight chitosan, enzyme degradation is required. This rate of degradation depends on the molecular weight and degree of acetylation of the polymer. The possible site of degradation may be liver and kidney. Funkhouser et al. reported the presence of three chitinases enzymes which showed activity of chitosan degradation [29].

Chitosan can be used as a diluent/ filler in the drug delivery systems. It act as inactive ingredient added to tablet or capsule in order to control the release of drug [30].

Demerits and remedies

Despite of having all the above advantages chitosan suffers mainly from drawbacks of low solubility. As it is a weak base ($\text{pK}_a = 6.2 - 7$), chitosan has low solubility at physiological pH of 7.4. It is soluble only in acidic aqueous solution in which the glucosamine units having $-\text{NH}_2$ groups gets converted into the soluble protonated polycation form $-\text{NH}_3^+$. Another disadvantage of which chitosan suffers is its high swelling tendency in aqueous environment resulting fast release of drug in case of drug delivery applications. This suggests the modification of chitosan to tailor mode for various application.

Numerous studies have been performed till today in order to overcome these demerits of chitosan. 'Derivatisation' is the most popular way to solve these complications. The chemical modifications are performed either on $-\text{NH}_2$ groups of glucosamine units or on $-\text{OH}$ groups of the polymer. Varieties of chitosan derivatives have been developed throughout these years depending upon the requirements for various applications in biomedical areas. These modifications have

been done with a view to bring improvements in the properties of the polymer.

Preparation technique of micro/ nano particles of chitosan

The following are some of the synthesis techniques of micro/ nano particles of chitosan:

Coacervation/ ionic gelation: This method is generally used for the preparation of nanoparticles of biodegradable hydrophilic polymers. It is the separation of two liquid phases in a colloidal system. The more concentrated phase in the system is the coecervate while the other phase is the equilibrium solution. For preparing chitosan nanoparticle, this technique is widely used based on the ability of cationic chitosan to interact with polyanions like TPP, sodium sulphate etc. to form the coacervate. Coacervates are formed from the electrostatic interaction between two aqueous phases and ionic gelation involves the transition from liquid to gel due to the ionic interactions. A number of factors e.g. concentration, pH, ratio of components, method of mixing etc. influence the preparation of particles [31].

Emulsion: The most common emulsion used for preparing chitosan micro/nano particle is the water-in- oil emulsion, where an aqueous chitosan solution is emulsified in an oil phase. Surfactants are applied for stability of the formed particles. They are then crosslinked by crosslinking agents. This method is generally used in preparation of particles with larger size (in micro range). The demerits of this method lie in the use of toxic organic solvent, initiators, surfactants and crosslinker etc. Wang et al. reported the preparation of uniform sized chitosan microparticles by using paraffin and petroleum ether as the oil phase and glutaraldehyde saturated toluene (GST) as the crosslinking agent [32]. Pavenetto et al. used an o/w/o multiple emulsion technique for synthesis of ketoprofen loaded chitosan microsphere [33].

Emulsion droplet coalescence: This is first introduced by Tokumitsu et al. using both emulsion crosslinking and precipitation technique [34]. They synthesized chitosan nanoparticles of 100 % deacetylation with particle size 452 nm. In this method, they first prepared two emulsions: one containing chitosan in water and liquid paraffin along with drug and the other contains alkaline chitosan solution having NaOH. Both the emulsions were then mixed under high speed stirring, resulting into random collisions of droplets of each emulsion. Coalescence of chitosan droplets with NaOH droplets took place and thereby precipitated chitosan in small solid particles. Stavudine loaded chitosan and Eudragit nanoparticles synthesized via this method were found to have good anti- viral effect and cumulative drug release [35].

Reverse micelle: This method is used to prepare Ultrafine polymer nanoparticles with a narrow size range. At first surfactant was added to produce reverse micelle in an organic solvent. An aqueous solution of chitosan and drug were then added to that solution under constant vortexing. The solution was kept in such a way to keep the entire mixture in an optically transparent microemulsion phase. Larger particle sized nanoparticles could be obtained by adding additional amount of water in the solution. The particles were then crosslinked and the solution was kept under stirring for overnight. The organic solvent was evaporated, the transparent mass obtained was then dissolved in water and the surfactant was precipitated out by adding salt to that solution. The solution was then centrifuged and the aqueous supernatant was dialysed for about 1 h and the lyophilized to dry powder. Mansouri et al. prepared Bovine Serum Albumin loaded

chitosan nanoparticles with particles in the range of 143 to 428 nm using the reverse micelle method [36].

Sieving: Particles within micro range size can be obtained by this technique. Agnihotri et al. used this technique to prepare chitosan microsphere [37]. At first an acidic solution of chitosan was crosslinked by glutaraldehyde. The crosslinked chitosan was then allowed to pass through a sieve having suitable mesh size to obtain microparticles of chitosan. The particles were then washed with NaOH solution and dried. This method was simple but prepared particles were of irregular surface. Gopinath et al. reported a simple and reproducible sieving method for preparation of Metronidazole loaded chitosan alginate microcapsules [38].

Spray drying: In spray drying technique, the solution of polymer with crosslinker is atomized in a stream of hot air. This results into the formation of small droplets from which the solvent evaporates and form free flowing particles. Different factors such as size of nozzle, spray flow rate, inlet air temperature, atomization pressure, extent of crosslinking etc. influence the particle size. Huang et al. optimized the parameters for preparing betamethasone loaded chitosan microparticles having good, spherical and smooth surface and dose dependent sustained release with the help of spray drying technique [39].

Different types of chitosan formulations in drug delivery applications

Chitosan biopolymers fabricated via different preparation protocols have been widely studied as carriers and control release for drugs, therapeutic proteins and genes in recent years with varying degree of effectiveness and drawbacks [40]. Chitosan has a cationic character because of its primary amino groups which impart it lots of properties such as controlled drug release, mucoadhesion, in situ gelation, transfection, permeation enhancement, and efflux pump inhibitory properties use in drug delivery systems [41]. Over the last few decades, considerable amount of work has been published on chitosan and its fabricated products in drug delivery. Felt and his co-workers have focused on many potential applications of chitosan as a pharmaceutical drug carrier [42]. Park et al. have provided an insight into various target-specific carriers, based on chitosan and its derivatives, towards low molecular weight drug delivery [43]. They illustrated the organ specific delivery of low molecular drugs and also the recent developments of drug delivery carriers for cancer therapy with special focus on various targeting strategies using chitosan and its derivatives. Marcos et al. has summarized the work covering the development of chitosan as drug carrier, potentiality of chitosan nanocarriers for applications in vaccination, transmucosal protein delivery and gene therapy [44]. They have also put forwarded the perspective on the plausible advances in this area in the near future.

Sonia and Sharma have mentioned the recent applications of chitosan nano/microparticles in oral and/or buccal delivery, stomach-specific drug delivery, intestinal delivery, colon-specific drug delivery, and gene delivery, giving special emphasis to oral drug delivery [45]. Werle et al. has highlighted the synthesis and characterization procedures of thiolated chitosans, their special features important for oral drug delivery, different formulation approaches and the applicability of thiolated chitosans for the oral delivery of various substance classes including peptides and efflux pump substrates etc. [46]. Agnihotri et al. has critically reviewed the different chemical modifications of chitosan or its derivatives, and their micro- and

nanoparticles used in drug delivery [47]. Some of the practical applications of chitosan and its fabricated product are briefly discussed below-

Grafted chitosan: Yu et al. has prepared a copolymer of poly(L-lysine) with chitosan and compared its efficacy towards plasmid DNA binding ability as well as gene transfection effect in HEK 293T cells with pristine chitosan polymer [48]. In both the cases, the copolymer (PLL-g-Chi) showed good results and the gene delivery was followed to be dependent on the composition of the copolymer. In another study the transfection of gene in HeLa cells was evaluated using self-branched chitosan. Simple modification was done for self-branching of chitosan without any change in its chemical composition. This new material showed profound improvement in gene transfer compared to its linear counterpart. The nanoparticles formed with self-branched chitosan showed higher colloidal stabilities and enhanced cellular uptake levels than that of the linear polymer [49]. A galactosylated grafted chitosan copolymer (GC) was used in coating of polystyrene which was in turn used for hepatocyte adhesion. In the experiment it was found that this new material had excellent adhesion and spheroid formation of hepatocytes due to the galactose-specific recognition between GC molecules and that of hepatocytes which was much lower in case of the chitosan coated polystyrene only [50]. A PEG grafted chitosan entity has been prepared. PEG was chemoselectively inserted at the C6 position of the repeating unit of chitosan polymer. The potential of the nanoparticles prepared from the former towards gene delivery was monitored with the help of a fluorescent labeled control siRNA. These nanoparticles provide adequate gene transfer efficacy with no toxicity when tested in neuronal cells [51]. Another copolymer of quaternized chitosan (Chitosan-N-trimethyl aminoethylmethacrylate chloride) with PEG was successfully synthesized. This new material showed better solubility in physiological environment along with enhanced biocompatibility. Compared to the quaternized chitosan alone. The hemolysis assay indicated that PEG modification could significantly reduce the cytotoxicity of quaternized chitosan [52]. Zhang et al. prepared nanoparticles of polyethylene glycol-grafted chitosan (G-g-chitosan) by ionic gelation method [53]. These nanoparticles had excellent insulin binding capacity. The insulin release was affected by the molecular mass of chitosan as well as PEG polymer and also the degree of substitution. The nasal absorption of insulin was improved prominently compared to G-g-chitosan suspension and control insulin solution.

Nanoparticles: Nanoparticles of chitosan with tri-polyphosphate have excellent capacity for association of insulin. Insulin loading on the nanoparticles displayed high positive charge and rapid insulin release kinetics. These make the nanoparticles an interesting system for nasal drug delivery [54]. Loading of insulin on nanoparticles of polyelectrolyte complex of chitosan and alginate showed significant enhancement in intestinal absorption of insulin following oral administration. Many fold improvement in hypoglycemic effect and insulinemia levels were observed in this new system than those obtained from oral insulin solution and physical mixture of oral insulin and empty nanoparticles [55]. The efficacy of the nanoparticles prepared from a chitosan derivative conjugated with multiple galactose residues in an antennary fashion (Gal-m-CS) was studied for targeting hepatoma cells. It was found that the nanoparticles suspended in an aqueous environment were quite stable during storage and had a high affinity to HepG2 cells. Thus this new novel material has many significant scopes in specific liver-targeting drug/gene delivery [56]. Ko et al. has studied the effect of different parameters on the release

property of felodipine loaded into chitosan-tripolyphosphate nanoparticles and [57]. Their experiments revealed that pH and concentration of the crosslinking agent, MW and concentration of wall material and curing time played a vital role in the release behavior. Shu & Zhu have introduced a novel method for preparing chitosan tripolyphosphate beads for effective drug delivery [58]. They prepared the nanomaterial under coagulation condition at 4°C in the presence of gelatin. The drug loading capacity obtained for brilliant blue and FITC-dextran was more than 90% and was accompanied by many fold increase in mechanical strength.

Banik et al. prepared a nanoparticle of chitosan-montmorillonite loaded with isoniazid drug and studied the effect of particle size on the release properties [59]. Swelling and release of isoniazid from the nanoparticles were found to increase with decrease in the pH of the medium and the size of the nanoparticles. But, cytotoxicity was found to increase with decrease in size of the nanoparticles. Incorporation of clay into the system decreases the cytotoxicity. They found that mucoadhesivity of the nanoparticles was better in gastric pH and increased with the decrease in particle size.

Quaternization: In an *in vivo* experiment Hamman et al. has studied the effect of degree of quaternization of N-trimethyl chitosan chloride on absorption [¹⁴C]-mannitol in the nasal route of rats [60]. It was observed that at pH 7.4 the optimum degree of quaternization for TMC was 48% for maximum absorption and no further significant increase was observed on further increasing the degree of quaternization. Initial increase in charge density with degree of quaternization followed by steric effect on further quaternization was the probable reason for such discrepancy. Another study of the effect of degree of quaternization on enhancing the permeability of ofloxacin across rabbit corneal epithelium, reconstituted *in vitro* was reported [61]. Chitosan of low and high molecular weight were quaternized with varying degrees and they found that the polymer with intermediate degree of quaternization had significant enhancement in permeability irrespective of polymer molecular weight. Transcorneal permeability-enhancing property was further confirmed by the *in vivo* tests on rabbit eyes. N-Trimethylated Chitosan Chloride (TMC) also improved the intestinal permeation of the peptide Drug Buserelin by opening the paracellular pathway in a reversible way [62]. The efficiency of TMC was studied *in vitro* (Caco-2 cell monolayers) and *in vivo* (rats). The influence of the degree of quaternization of N-trimethyl chitosans (TMCs) on the mucoadhesive and penetration enhancement properties towards buccal mucosa (model molecule-Fluorescein isothiocyanate dextran) was studied [63]. Mucoadhesive property was dependent on the quaternization degree and the influence of trimethylation on enhancement of penetration of the polymer was found to be effective when pH 6.4 buffer was used.

Nanoparticles prepared from N-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride (HTCC) with tripolyphosphate (TPP) was used in binding of protein drug Bovine serum albumin (BSA). Encapsulation efficiency was up to 90% and *in vitro* release studies showed a burst effect followed by slow and continuous release of the drug. Encapsulation efficiency was dependent on BSA and TPP concentrations. The burst effect of BSA was decreased when modified with PEG and sodium alginate and at the same time encapsulation rate was also affected [64].

Thiolation: Sakloetsakun and his co-workers have explored the possibility of chitosan-thioglycolic(chitosan-TGA) acid conjugate system in the presence of oxidizing agents as a promising *in situ* gelling system in controlled drug release activities [65]. Chitosan-TGA rapidly

shifted to gel in a couple of minutes on addition of oxidizing agents. Within a short time the viscoelastic properties of thiolated chitosan were increased to many thousand folds in the presence of H₂O₂. Four different oxidizing agents were tested and in each case decrease in time of gel formation and increase in viscoelasticity were obtained.

Martien et al. in their experiment concluded that thiolated chitosan produced by introducing thioglycolic acid (TGA) to chitosan via amide bond formation mediated by a carbodiimide had promising scopes in oral delivery of therapeutic gene [66]. They synthesized nanoparticles of pDNA with thiolated chitosan. They applied it efficiently in gene transfer in Caco-2 cells with improved stability toward nuclease. Another advantage of this material is its low cytotoxicity.

In a study, Dünnhaupt et al. used a sulphur protected (protecting molecule is 6-mercaptopyridine) thiolated chitosan in mucosal drug delivery [67]. In the *in vitro* mucoadhesion studies, promising results were obtained and the water uptake capacity as well as cytotoxicity were reduced to many fold compared to corresponding thiolated and unmodified chitosans. On the other hand, cohesive properties were strongly improved.

Thiolated chitosan capable of inhibiting P-glycoprotein (P-gp) enhanced the transport of acyclovir (ACY) across rat intestinal mucosa and Caco-2 cell monolayers. The inhibitory effect was described to be dependent on the average molecular mass of the applied chitosan [68].

Lactosaminated N-succinyl-chitosan (Lac-Suc) was prepared and employed as a liver-specific drug carrier in mice and the activity was monitored using fluorescein thiocarbonyl in combination. When injected intravenously Lac-Suc is cleared much faster from the systemic circulation than Suc itself, and simultaneously a considerable amount of Lac-Suc was quickly taken up by the liver via the asialoglycoprotein receptor. The liver accumulation of Lac-Suc was dependent on the dose used and the later was retained in liver for a long period [69].

Lorenzo-Lamosa et al. in a study described a new colonic drug delivery system, consisting of chitosan core-coated microspheres. These microspheres could release the encapsulated drug continuously over a prolonged and adjustable period of time at pH 7.4. They also concluded that the crosslinking of Chitosan using pH-sensitive polymers made it more useful for oral drug delivery [70].

Carboxymethylation: Chitosan has been modified to hydrophilic by inserting carboxymethyl group onto the polymer backbone. Carboxymethyl chitosan has been widely used in drug delivery applications. Chen et al. used this chitosan derivative for preparation of pH dependent hydrogels for protein delivery. Genipin, a natural crosslinker was applied to crosslink the BSA loaded carboxymethyl chitosan- alginate based hydrogel system [71]. Tan et al. further modified carboxymethyl chitosan with linoleic acid to evaluate *in vitro* studies [72]. The formulation showed sustained release profile of adriamycin and the degree of substitution of linoleic acid was found to influence the rate of drug release. The anti-tumor activity of the nanoparticles against HeLa cells was also satisfactory. Sodium acrylate was grafted onto carboxymethyl chitosan in order to increase the carboxylic groups in the polymer by developing Ca²⁺ crosslinked hydrogel microspheres. This derivative has been successfully evaluated for delivery of hydrophobic drug such as chitosan [73]. Jeong et al. evaluated the *in vitro* anti-tumor activity of hydrophobic drug, doxorubicin loaded into polyethylene grafted carboxymethyl chitosan derivative in glioma cells [74]. Numerous benefits of using carboxymethyl chitosan in drug delivery areas lie in its unique

biological and physicochemical properties like outstanding biocompatibility, biodegradability, mucoadhesiveness, pH sensitive controlled drug release capacity, site specificity etc. These properties are attributed to the active functional groups i.e. -NH₂ and -COOH present in it [75].

Phosphorylation: Phosphorylated chitosan has been used successfully as potential carrier in drug delivery purposes. Phosphorylation increases the solubility of chitosan due to the incorporation of methylene phosphonic group onto the polymer backbone. Heras et al. prepared phosphorylated chitosan using chitosan, phosphorous acid and formaldehyde [76]. Win et al. reported the use of phosphorylated chitosan for synthesis of pH sensitive gel beads as controlled release system in gastrointestinal fluid [77]. They also synthesized polyelectrolyte complex microsphere using phosphorylated chitosan and studied the effect of proteolytic enzymes on the drug release in gastrointestinal fluids [78]. Pramanik et al. synthesized a novel bioanalogue hydroxyapatite chitosan phosphate nanocomposite [79]. Due to the incorporation phosphorus containing chitosan the new material showed good cytocompatibility, osteocompatibility and was highly osteogenic *in vitro*. Moreover, improvement in mechanical properties of the composite is a good sign for bioactivity. Chitosan beads containing phosphorus were synthesized and the *in vitro* drug release behavior in various pH solutions was studied using indomethacin as a model drug at two different concentrations. The release percent of the drug was found to increase with the increase in pH. Ionization of phosphorous groups in different pH was one of the factors governing the release rate [80].

Modification with nanomaterials: Metal nanoparticles such as zinc oxide, titanium dioxide, montmorillonite, iron oxide etc. have recently been widely investigated in drug delivery applications (specially in cancer therapeutics) due to their ability to improve drug loading, control the drug release rate and targeting to specific location in the body. ZnO nanoparticles stabilized with cationic chitosan coating has applied for preparation of doxorubicin loaded ZnO quantum dots which showed significant drug loading capacity along with controlled drug release pattern [81]. Abdelhady reported the antimicrobial and UV protection ability of chitosan/ ZnO nanoparticles [82]. The cotton fabrics decorated with those nanoparticles showed considerable antibacterial activities against both gram positive and gram negative bacteria and also good UV protection ability which in turn increased with increase in the concentrations of the nanoparticles. Ti metal based Titania nanotubes array incorporated with hydrophilic chitosan matrix were studied as carrier for indomethacin drug [83]. The system showed significant improvement in drug release with extended release pattern.

Clay nanoparticles particularly montmorillonite (MMT) has been investigated as a safe and useful material in drug delivery area. It improves the drug delivery properties and is nontoxic. With increase in the concentration of MMT in the system Betaxolol hydrochloride (BH) loaded MMT/ chitosan nanoparticle showed improvement of drug encapsulation efficiency. The *in vivo* studies showed that the system did not cause any irritation or damage to the cornea, conjunctiva or iris of rabbit eyeball [84]. Banik et al. evaluated the cell viability of isoniazid loaded carboxymethyl chitosan/ MMT nanoparticles via MTT assay and found that MMT incorporation improved the cell viability of the system indicating its good cytocompatibility with human lymphocytes [85].

Chitosan in organ targeting drug delivery

Since the functional groups of chitosan provide site for modifications, desirable properties can be achieved through chemical

modifications. Due to this property, researchers have been attempting to use chitosan based systems as carrier in targeted drug delivery.

Target site	Formulation	Drug	Significant results
Colon	1. Chitosan microcores entrapped within acrylic microspheres (Eudragit) [71]	Sodium diclofenac	Drug release rate was found to depend on chitosan type and its molecular weight. The coating of Eudragit made the system pH dependent. Drug release was proposed to be achieved through the following steps: a) dissolution of Eudragit coating, b) swelling of chitosan core, c) dissolution of the drug and its diffusion through chitosan gel core.
	2. Citrate crosslinked chitosan film [86]	Riboflavin	Drug release was pH sensitive. In acidic condition, swelling was more and consequently drug was released within 2 h took place while in neutral pH only 40% release in 24 h. To control the release chitosan film was further coated with alginate.
	3. Chitosan succinate and chitosan phthalate [87]	Sodium diclofenac	pH dependent release: reduced drug release was in acidic conditions while improved dissolutions under alkaline conditions.
	4. Chitosan capsule [88]	5-Aminosalicylic acid (5-ASA)	A significant increase in the release of drug from chitosan capsule was observed in the presence of the rat cecal content. Chitosan based capsule was suggested to accelerate healing of 2,4,6-trinitrobenzene sulfonic acid sodium salt (TNBS)-induced colitis in rats.
	5. Chitosan coated liposome [89]	Alendronate	The chitosan coating of liposomes significantly increased cellular uptake of alendronate in Caco-2 cells and also enhanced the oral bioavailability of alendronate in rats.
Ocular	1. Chitosan hydrogel with disodium α -D-Glucose 1-phosphate (DGP) [90]	Levocetirizine dihydrochloride (LD)	The result showed an initial rapid drug release followed by a sustained release and remarkable cornea penetration of LD. Ocular irritation studies revealed an excellent ocular tolerance of the hydrogel. The ocular residence time was also significantly prolonged.
	2. Low molecular weight chitosan coated liposome [91]	Diclofenac sodium	Chitosan coating was found to bring remarkable modification to liposome on its ocular drug delivery behaviors. Chitosan coating also improved transcorneal drug penetration rate, which was attributed to the penetration enhancing effect of low molecular weight chitosan.
	3. Chitosan coated poly-caprolactone nanocapsules [92]	Indomethacin	Chitosan coating of poly-caprolactone capsule significantly increased the ocular bioavailability of indomethacin.
Liver	1. Glycyrrhetic acid-modified chitosan/poly(ethyleneglycol) nanoparticles [93]	Doxorubicin	The nanoparticles were remarkably targeted to the liver and maintained high concentration of drug for prolonged period of time. The nanoparticles could effectively inhibit tumor growth in H22 cell-bearing mice.
	2. Methoxy poly (ethylene glycol) (PEG)-graft chitosan and lactose conjugated PEG-graft-chitosan micelle [94]	Diammonium glycyrrhizinate (DG)	Pharmacokinetic experiments carried out using rats showed that the area under the curve (AUC) values of DG for PIC micelles were higher than that for DG injection. The lactose-conjugated PIC (Lac- PIC) micelles delivered more DG to the liver than conventional PIC micelles, indicating that Lac-PIC micelles were promising liver targeted nanocarriers for DG
	3. Galactosylated chitosan nanoparticles [95]	5-Fluorouracil	In vitro and in vivo studies demonstrated that sustained releases of GC/5-FU nanoparticles were more effective at targeting hepatic cancer cells than 5-FU monotherapy in the mouse orthotopic liver cancer mouse model.
	4. Glycyrrhizin (GL) conjugated chitosan nanoparticles [96]	Adriamycin	High drug loading efficiency was found (91.7%). Flow cytometry and confocal laser microscopy studies exhibited preferential accumulation of the nanoparticles in hepatocytes. Cellular uptake was time and dose dependent.
Kidney	1. Low molecular weight chitosan [97]	prednisolone	The prednisolone loaded chitosan system in the kidney was found to be 13 fold higher than that of prednisolone alone.
Lung	1. Chitosan- poly(lactic coglycolic acid) nanoparticles [98]	paclitaxel	The in vitro uptake study revealed that the uptake of the nanoparticles on a lung cancer cell line (A549) was significantly increased by chitosan modification.
	2. Chitosan- poly(lactic coglycolic acid) nanoparticles [98]	paclitaxel	The nanoparticles become highly positive under acidic condition and thereby strongly interact with the negatively charged tumor cells.

Table 1: Chitosan based targeted drug delivery systems.

Chitosan has been either derivatised or conjugated with other polymer/ targeting ligand/ magnetic moiety to target an active agent to

specific site. Some of such chitosan based systems are summarized in Table 1.

Conclusion

Chitosan has the desired properties for safe use in biomedicine, pharmacology and waste water treatment. Chitosan and chitosan derivatives based systems has considered as promising material for the development of safe and effective drug delivery systems owing to their unique physicochemical characteristics. Being mucoadhesive polymer, chitosan enhances the residence time of the system and consequently the bioavailability of the drug. This bioadhesive property of the polymer leads to the development of target specific carriers. Owing to its unique properties, along with the pharmaceutical applications chitosan has commercial importance also. The inclusion of fillers such as ZnO, TiO₂ etc. into the chitosan is one of the effective ways to widen the horizon of applications of chitosan. This review presents the use of different formulations of chitosan for different biomedical applications. Product of chitosan and its derivatives containing different nanomaterials like clay, TiO₂, ZnO etc. have not yet gained much importance as pharmaceutical excipient due to lack of sufficient information regarding their mode of action and toxicity. A thorough understanding of the mechanism of their action and toxicity is needed in order to optimize the chitosan based formulations. A methodical study is necessary which may shower some light on the interaction of the remaining polymer, filler, if any, after drug delivery with the biological system. It is envisaged that chitosan and its derivatives either alone or in combination with the other polymers along with fillers may further widen and strengthen their existence in the pharmaceutical area in near future.

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