e-ISSN : 2455-5258

Research in Pharmacy and Health Sciences

Review Article

Chitosan: A Multifunctional Polymer

Rupinder Kaur, Ujjwal Nautiyal, Alok Semwal*

Department of Pharmacy, Himachal Institute of Pharmacy, Paonta Sahib-173025, HP, India

ABSTRACT

Chitosan, one of the natural multifunctional polymer, due to its unique and versatile biological property is regarded as a useful compound in medical and pharmaceutical technology. In recent times chitosan has been far and wide used as a popular formulation excipient due to its inimitable characteristics in the field of pharmaceutical sciences as binding, disintegrating, stabilizing, suspending, tablet coating, and film forming material. Recently, considerable research effort has been made in order to develop safe and efficient chitosan products. This brief editorial epitomizes the potential applications of chitosan in novel drug delivery systems.

Received: 21-11- 2016

Revised: 17-12-2016

Accepted: 20-12-2016

***Correspondence to:**
Mr. Alok Semwal
Email:
alokm.pharm01@gmail.com

Funding: Nil
Competing Interests: Nil

Keywords: Chitin, Chitosan, Biodegradability, Drug Delivery, Pharmaceutical Applications.

INTRODUCTION:

Chitosan, the principle derivative of chitin, is one of the most abundant natural amino polysaccharides extracted from the exoskeleton of shellfish such as shrimp, lobster or crabs and cell wall of fungi etc [1]. Chitosan (Figure 1) is the simplest linear polysaccharide composed of α, 1-4 linked D-glucosamine (GlcN) and N-acetylD-glucosamine (GlcNAc), with various compositions of these two monomers [2].

Chitin is formed by a linear chain of acetylglucosamine groups while chitosan is recovered by removing enough acetyl groups (CH_3-CO) from chitin, therefore the chitin molecule and the resultant product is found to be soluble in most diluted acids. The actual variation between chitin and chitosan is the acetyl content of the polymer [3].

Chitosan is a non-toxic biodegradable polymer of high molecular weight. Chitosan is one of the promising renewable polymeric materials for its broad application in the pharmaceutical and biomedical industries for enzyme immobilization [4]. Chitosan is used in the chemical waste water treatment and food industries for food formulation as binding, gelling, thickening and stabilizing agent [5]. Research into the chemical properties of chitosan has demonstrated its suitability for the preparation of enzymatic biosensors for the analysis of metallic elements, proteins and lipids [6 (a)]. Chitosan also exhibits a number of interesting biological activities, including antimicrobial activity, induced disease resistance in plants, and diverse stimulating or inhibiting activities toward a number of human cell types[6(b), 6(c)]. Moreover, chitosan can be used to prevent or treat wound and burn infections due to its intrinsic antimicrobial properties and its ability to deliver extrinsic antimicrobial agents to wounds and burns. Due to its easy bio-degradability and biocompatibility, chitosan is currently in use for varieties of pharmaceutical and commercial purposes, specially during water purification for detoxification from hazardous metal wastes [7].

Chitosan A N-deacetylated derivative of chitin is primarily characterized by its molecular weight (MW) and the degree of acetylation (DA). Commercially chitosan is available with >85% deacetylated units (DA < 15%), and molecular weights (MW) between 100 and 1000 kDa. There is no a specific standard to define MW, but it is accepted that Low MW < 50 kDa, Medium MW 50 – 150 kDa, and High MW > 150 kDa [8]. A common method for the synthesis of chitosan is the deacetylation of chitin using sodium hydroxide in excess as a reagent and water as a solvent. The process of deacetylation involves the removal of acetyl groups from the molecular chain of chitin, leaving behind a compound (chitosan) with a high degree of chemically reactive amino group (–NH_2). This reaction pathway, when allowed to go to completion (complete deacetylation) yields up to 98% product [9].

The following four steps in chronological order of the process are needed to produce chitosan from crustacean shells (Figure 2, Figure 3): (i). Deproteinization, (ii). Demineralization, (iii). Decolouration, and (iv). Deacetylation [10].

Chitosan obtained by deacetylation of chitin (a naturally occurring polymer) has been shown to possess mucoadhesive properties owing to the molecular attractive forces formed by electrostatic interaction between positively charged chitosan and negatively charged mucosal surfaces [11]. Chitosan and their derivatives (*N*-trimethyl chitosan, mono-*N*-carboxymethyl chitosan) are effective and safe absorption enhancers that improve mucosal (nasal, peroral) delivery of hydrophylic macromolecules such as peptide and protein drugs and heparins, by favouring the paracellular transport of macromolecular drugs [12].

Due to its mucoadhesiveness and ability to cross epithelial barriers, chitosan has been widely studied as a vaccine adjuvant or co-adjuvant as it was shown to enhance bioavailability and immunogenicity of antigens after oral, nasal, or subcutaneous administration [13-15]. Superior haemostatic efficacy of chitosan through platelets activation and thrombin generation is also known, enabling its application in wound dressings [16, 17]. The polymer is also considered as a promising candidate in obesity and hypercholesterolemia treatment as it is able to combine bile acids in the digestive tract and in consequence increase their excretion [18]. Numerous data have drawn attention to the use of chitosan as an antifungal and antibacterial agent [19-21]. Furthermore, chitosan has been recently employed as an adjunctive for an antimicrobial drug in order to increase its pharmacological activity [22-23].

Chitosan film is regarded as biofunctional material, well tolerated by living tissues, particularly applicable as edible coatings to prolong shelf-life and preserve quality of fresh foods [24]. In medical field, chitosan films have been tested as curative wound dressing and as scaffolds for tissue and bone engineering [25]. Additionally, the reactive functional groups present in chitosan (amino group at the C2 position of each deacetylated unit and hydroxyl groups at the C6 and C3 positions) can be readily subjected to chemical derivatization allowing the manipulation of mechanical and solubility properties, enlarging its biocompatibility [26].

2. CHEMISTRY OF CHITOSAN

2.1 Structure

The primary unit in the chitin polymer is 2-deoxy-2-(acetylamino)glucose. These units are combined by 1-4 glycosidic linkages, forming a long chain linear polymer. Removal of most of the acetyl groups of chitin by treatment with strong alkalis yields chitosan (Figure 4) [27].

2.2 Solubility

Although chitin is insoluble in most solvents, the properties of chitosan relate to its polyelectrolyte and polymeric carbohydrate character. It is insoluble in water or in alkaline solutions at pH levels above about 6.5 [27]. It dissolves readily in dilute solutions of most organic acids, including formic, acetic, tartaric, and citric acids. Chitosan is soluble to a limited extent in dilute inorganic acids except phosphoric and sulphuric acids [29]. The solubility of chitosan depends on its biological origin, molecular weight and degree of acetylation. Since chitosan is soluble in diluted acid solutions, films can be readily prepared by casting or dipping, resulting in dense and porous structure [30, 31].

The water-solubility of chitosan at neutral pH increases with increasing DA, and a chitosan with DA 0.6 is fully water-soluble at all pH values. The dependence of solubility upon DA may be explained by a decrease in the apparent pK_0-value with DA, or by a decreased possibility of aligning polymer chains when increasing the amount of randomly distributed GlcNAc units.

The selective introduction of saccharide residues at the chitosan amine function facilitates the conversion of the water-insoluble chitosan into various soluble, branched derivatives. The branched chitosan derivatives are soluble in either neutral or slightly acidic (pH 5–6) aqueous medium, with solubility being attained for some products at DA as low as 0.14. Chitosan can be reacetylated with acetic anhydride to obtain water soluble partially reacetylated chitin.

The formation of derivatives suitable for industrial applications with good solubility in various organic solvents can be effected through the introduction of hydrophobic substituents by acylation with long chain fatty acyl halides or anhydrides [32].

2.3 Technical products and impurities

Chitosan could be defined as chitin sufficiently deacetylated to form soluble amine salts. The degree of deacetylation necessary to obtain a soluble product must be 80 to 85 percent or higher; *i.e*., the acetyl content of the chitosan product must be less than 4-4.5 percent. Chitosan products are highly viscous, resembling natural gums [27].

Chitosan is available in several forms. Practical grade chitosan from crab shells has a minimum of 85 percent deacetylation and a viscosity >200 cps (Brookfield, 1% solution in 1% acetic acid); it may contain foreign matter. High molecular weight (600,000 D) chitosan is a coarsely ground polymer prepared from crab or shrimp shells with a viscosity of 800-2000 cps. Low molecular weight (150,000 D) chitosan is 75-85 percent deacetylated and has a viscosity of 20-200 cps [33].

2.4 Structural modification

Chemical modifications of chitosan are increasingly studied as it has the potential of providing new applications. Chitosan can be modified by physical or chemical processes in order to improve the mechanical and chemical properties. Various chemical properties of chitosan that may affect structural modification of chitosan are mentioned in Figure 5.

One of the important strategies to increase both the solubility and positive charge density of chitosan is based on the introduction of quaternary ammonium groups into chitosan. This modification has got the commonly accepted term "quaternization of chitosan" (Figure 6). Thus, derivatives soluble in water and in both acidic and basic physiologic circumstances may be good candidates for the polycationic biocides [35]. Studies with quaternary salts of chitosan revealed that the antimicrobial activity against bacteria is higher than that of chitosan [36]. Jia *et al*. [37], reported that the activity of N-propyl-N,N-dimethyl chitosan against *E. coli* is 20 times higher than that of chitosan, indicating that the derivatives with cationic charge exhibit particularly high activity.

2.5 Elemental analysis

A known amount of chitosan is heated for 1 h at 600ºC, and the residue is weighed to find the quantity of inorganic material. The percentages of nitrogen in fully deacetylated chitosan (8.695), in fully acetylated chitin (6.896), and in the organic fraction of the analyzed material (%N) are related to the DA by following formula.

$$\%\mathrm{DA} = \frac{(8.695 - \%\mathrm{N})100}{8.695 - 6.896}$$

Samples with varying DA (degree of acetylation) present relatively small variations in nitrogen content, thus results obtained by elemental analysis (EA) are not precise, especially if contaminants are present. This technique was used to define chitosan as having a nitrogen content of more than 7% and chitin with less than 7% nitrogen[39, 40].

2.6 Degree of deacetylation of chitosan

The process of deacetylation involves the removal of acetyl groups from the molecular chain of chitin, leaving behind a compound (chitosan) with a high degree of chemically reactive amino group ($-NH_2$). The solubility of chitosan depends on the degree of deacetylation, pH and on the protonation of free amino groups [41].

2.7 Molecular weight of chitosan

The physicochemical, biological, and rheological properties of chitosan vary significantly as a function of its molecular weight and molecular weight distribution [42, 43]. It is therefore important, and in some cases critical, to know precise and accurate values of the molecular weight of chitosan. It is well known that the determination of the molecular weight of polyelectrolytes is complex [44]. In the case of chitosan, this situation is exacerbated due to the marked tendency of this polymer to form resilient aggregates in solution [45].

The most direct method for molecular weight characterization is aqueous size exclusion chromatography, SEC (or gel filtration chromatography). SEC provides the number-average molecular weight, Mn, and the weight-average molecular weight, Mw, and therefore the polydispersity index Mw/Mn in a single measurement. It has been used extensively in studies of chitosans, with the first SEC characterization of chitosan being reported by Wu and coworkers in 1976, who devised a protocol involving injection of chitosan solutions (5–10 mg/mL) into a column combination of 18 ft total length packed with glycerol-coated glass particles eluted with a 2% acetic acid solution. Refractive index, RI, and ultra-violet absorption, UV detection were employed and molecular weights were determined relative to dextran standards [46].

Chitosan molecular weight also affects the binding or agglutination of red blood cells [47]. Several chitosan samples with Mw from 2000 to 4000 kDa and DD from 90 to 70% were tested. It was found that solid-state chitosan with a high DD bound more platelets and was more haemostatic [48].

2.8 Persistence length of chitosan

The dimensions of chitosan chains and their related hydrodynamic volume and viscometric contribution depend on the semi-rigid character of the polysaccharide chains. Since chitosan in an acid medium is a polyelectrolyte, these properties are influenced by the ion concentration. A conformational analysis of chitins with different degrees of deacetylation concluded that chitin and chitosan are semi-rigid polymers characterized by a persistence length (asymptotic value obtained at high degree of polymerization) that depends moderately on the DA(different average) of the molecule [49] (Figure 7).

The local stiffness is related to the conformation of the molecule, and especially to the intra-chain H bond network formed as shown in Figure 8. The decrease of the stiffness of chitosan as temperature increases is shown by 1H NMR and follows the prediction from molecular modeling. A critical temperature around 40 ^{0}C is found where Lp (Persistence length) starts to decrease more rapidly, behavior that is certainly related to the destabilization of H bonds as temperature increases [51].

2.9. Complex formation by chitosan

2.9.1 Complex formation with metals

Chitosan is known to have good complexing ability; the $–NH_2$ groups on the chain are involved in specific interactions with metals [52]. A mechanism for complex formation with copper at pH 4.5, was proposed in agreement with X-ray data on chitosan–copper stretched films [53]. Recently, the mechanism of complex formation with copper in dilute solution was re-examined and two different complexes were proposed, depending on the pH and copper content [54]. This chelation depends on the physical state of chitosan (powder, gel, fiber, film). Better chelation is obtained for greater degrees of deacetylation of chitin. Thus chelation is related to the $–NH_2$ content as well as to the $–NH_2$ distribution [55]. It is also related to the depolymerization(DP) of oligo-chitosans; the complex starts to form when DP>6 [56]. The two forms proposed are:

$$[Cu(-NH_2)]^{2+}, 2OH^-, H_2O \text{ and}$$

$$[Cu(-NH_2)_2]^{2+}, 2OH^-$$

The first complex is formed at pH between 5 and 5.8, while the second forms above pH 5.8; the maximum amount of copper fixed is $[Cu]/[NH_2] = 0.5$ mol/mol.

The nature of the cation is very important in the mechanism of interaction; the affinity of chitosan for cations absorbed on film shows selectivity following the order(for divalent and trivalent cations) [56]:

$$Cu^{+2} >> Hg^{+2} > Zn^{+2} > Cd^{+2} > Ni^{+2} > Co^{+2} > Ca^{+2}$$

$$Eur^{+3} > Nd^{+3} > Cr^{+3} > Pr^{+3}$$

2.9.2 Electrostatic complexes

Chitosan, as a polyelectrolyte, is able to form electrostatic complexes under acidic conditions. Two different types of complexes are considered here: electrostatic complexes with an oppositely charged surfactant (SPEC) and polyelectrolyte complexes (PEC) [57].

2.9.3 Complexes with surfactants

A general behavior of polyelectrolytes is demonstrated with chitosan and sodium dodecyl sulfate (SDS). An electrostatic complex is formed in the presence of a low DA chitosan involving cooperative stacking of surfactant alkyl chains. Apparently the association forms a micellar system that precipitates out, but for very small amounts of added surfactant, interesting interfacial properties are observed. A critical aggregation concentration (c.a.c.) around 100-fold smaller than the c.m.c. of the surfactant alone is detected by surface tension measurements [58, 59].

The cooperativity of the observed interaction depends directly on the charge density of the chitosan (in fact, it depends on the distance between two adjacent ionic sites), as is shown for carboxymethylchitin in the presence of tetradecyltrimethylammonium bromide (TTAB) [60].

In addition, a capsule is formed when a chitosan solution is dropped into a SDS surfactant solution; a chitosan gel layer (characterized by an ordered nanostructure) crosslinked by charged surfactant micelles is formed in the interfacial film [61]. Babak et al. [62] showed that this structure can encapsulate enzymes.

This electrostatic interaction has been compared with covalent analogs obtained by grafting alkyl chains on a chitosan backbone. The interfacial properties of the chitosan-derived polymer surfactant has relatively low surface tension acitivity but interesting bulk properties. The role of sulfated N-acyl chitosan (S–Cn–Chitosan) in a lipid membrane was compared with that of SDS to show that SDS dissociates the membrane, whereas the polymer stabilizes the membrane, and even increases its rigidity, suggesting low toxicity in bioorganisms. In solution, when the alkyl chain in S–Cn–chitosan is longer than 10 units, the polymers form more stable micelles than those formed by the same alkyl chain surfactant alone [63].

2.9.4 Complexes with oppositely charged polymers (proteins, polyanions, DNA)

There are no good examples of polymer/polymer complex formation based on chitosan and neutral polymers, although many electrostatic PEC between chitosan and synthetic or natural polymers are cited in the literature: e.g. polyacrylic acid, sodium salt (PAA), carboxymethylcellulose (CMC), xanthan, carrageenan, alginate (extracted from brown algae), pectin, heparin, hyaluronan (HA), sulfated cellulose, dextran sulfate, N-acylated chitosan/ chondroitin sulfate [64, 65]. The main applications of these electrostatic complexes are as antithrombogenic materials, controlled release systems, encapsulation of drugs, immobilization of enzymes and cells, and gene carriers [66].

3. DERIVATIVES OF CHITOSAN

3.1 O-and N-Carboxymethylchitosans

Carboxymethylchitosan (CM-chitosan) is the most fully explored derivative of chitosan; it is an amphoteric polymer, whose solubility depends on pH. Under controlled reaction conditions (with sodium monochloracetate in the presence of NaOH), one gets O and N-carboxymethylation. The yield of substituents on the three positions was determined by NMR[67(a,b)]. This reaction extends the range of pH (pH>7) in which chitosan is water-soluble, but a phase separation due to the balance between positive and negative charges on the polymer was observed at $2.5 < pH < 6.5$. Most interesting

is the preparation of N-carboxymethylchitosan by reaction with glyoxylic acid in the presence of a reducing agent. The distribution of monosubstituted (–NH–CH2COOH) and disubstituted (–N ($–CH_2COOH)_2$) groups was established by 1H and 13C NMR[67(c)]. Disubstitution is easily obtained, giving an interesting derivative for ion complexation. A specific oxidation of the C-6 position hydroxyl group was realized using the TEMPO reactant on chitin to produce a chitin based hyaluronic acid analog. This derivative is water soluble in a wide range of pH, but only if it is prepared from a fully acetylated chitin [68].

3.2 Chitosan 6-O-sulfate

This derivative is an anticoagulant; it was first prepared as an O- sulfated derivative[69,70] and more recently as N-sulfated chitosan [71].

3.3 N-methylene phosphonic chitosans

The anionic derivatives, with some amphoteric character synthesized under various conditions are proved to have good complexing efficiency for cations such as Ca^{2+}, and those of transition metals (Cu (II), Cd (II), Zn (II) etc.) [72, 73]. The complexation provides corrosion protection for metal surfaces [74]. These derivatives were also modified and grafted with alkyl chains to obtain amphiphilic properties that have potential applications in cosmetics [75].

3.4 Trimethylchitosan ammonium

This cationic derivative, water soluble over all the practical pH range, is obtained by quaternization of chitosan with methyl iodide in sodium hydroxide under controlled conditions, and can be fully characterized by NMR [76,77]. A large decrease of molecular weight during this reaction is observed under all conditions. These polymers show good flocculating properties with kaolin dispersions, suggesting applications to paper making [78]. Other quaternized derivatives have been prepared, are claimed to have antistatic properties [79].

3.5 Carbohydrate branched chitosans

Carbohydrates can be grafted on the chitosan backbone at the C-2 position by reductive alkylation. For this purpose, disaccharides (cellobiose, lactose, etc.) having a reducing end group, are introduced, in the presence of a reductant, on chitosan in the open chain form [80]. These derivatives are water soluble. Carbohydrates can also be introduced without ring opening on the C-6 position. These derivatives are important as they are recognized by the corresponding specific lectins and thus could be used for drug targeting. A special case of carbohydrate branched chitosan is the grafting of a cyclic oligosaccharide, cyclodextrin [81].

3.6 Chitosan-grafted copolymers

One of the most explored derivatives is poly(ethylene glycol)- grafted chitosan, which has the advantage of being water soluble, depending on the degree of grafting: higher molecular weight PEG at low DS gives higher solubility than low molecular weight PEG. PEG can be also be introduced by reductive amination of chitosan using PEG-aldehyde [82]. Polypeptides have been grafted by reaction with N-carboxyanhydrides of amino acids with the purpose of developing new biomaterials, but the degree of polymerization of the grafted chains cited in this work remains low (DP = 5.9–6.6)[83].

3.7 Alkylated chitosans

These are very important as amphiphilic polymers based on polysaccharides. The first derivative having these characteristics was a C-10-alkyl glycoside branched chitosan with a high degree of substitution (DS =1.5), which gelled when heated over 50^0C [84]. Another approach was used for selective N- and O-palmitoylation giving a derivative with two or three long alkyl chains per monomeric unit. This reaction involved protection and deprotection of the C-6 position [85]. By using carboxylic anhydrides with different chain lengths on CM-chitosan, highly substituted derivatives with low regularity were obtained. They were insoluble in water and their biodegradability was decreased [86]. Using the reductive amination, a series of amphiphilic derivatives were produced with different chain lengths (Cn from 3 to 14) and controlled DS (usually lower than 10% to maintain water solubility in acidic conditions) [87]. This technique was also used to introduce n-lauryl chains [88]. Alkylated chitosans with good solubility in acidic conditions (pH<6) have a number of very interesting properties. First, they exhibit surface activity and they were compared with corresponding low molecular weight surfactants [89, 90]; for the same amount of alkyl chains with the same length, they have a relatively low effect on the decrease of the surface tension but they improve much the stability of the interfacial film. It is interesting to mention that alkyl chitosans are compatible with neutral and cationic surfactants; it was demonstrated that cationic surfactant adsorbed on the alkyl chain grafted on chitosan, promotes its solubilization [91].

3.8 Cyclodextrin-linked chitosans

The cyclic oligosaccharides, namely α-, β-, γ-cyclodextrins (CD), are important because of their ability to encapsulate hydrophobic molecules in their toroidal hydrophobic cavity, whose selectivity depends on the number of glucose units (respectively 6, 7, 8 D-glucose units) [92-94]. For various applications, it is interesting to graft the cyclodextrin on a polymeric backbone such as a biocompatible polysaccharide. A synthesis of α- and β-cyclodextrin-chitosans with relatively high degree of substitution has been described [95].

A β-cyclodextrin with a specific modification on one of the –OH groups on its small side was grafted to chitosan by reductive amination. At a DS lower than 10%, these derivatives are water soluble in acidic conditions with loose inter-chain interactions [96]. The authors found that these new derivatives had the ability to differentially recognize and retain certain guest compounds based on their molecular shapes and structures. They proposed to use these polymers as supports for reverse-phase adsorption or as adsorbents in controlled release systems [97].

4. APPLICATIONS OF CHITOSAN

4.1 Chitosan applications on crops

Chitosan derivatives have a wide scope of application and regulate the immune system of plants and induce the excretion of resistant enzymes. Moreover, chitosan not only activates the cells, but also improves its disease and insect resistant ability [98]. Application of chitin and chitosan to soybean leaf tissues increased the activities of Phenylalanine Ammonia Lyase (PAL) and Tyrosine Ammonia-Lyase (TAL). The elevation of PAL and TAL activity was dependent on the chain length of the oligomers and time after treatment [99]. Lee *et al.* reported that chitosan, a component of fungal cell walls, reduced the size of stomatal aperture and inhibited light induced stomatal opening in tomato epidermis by inducing Reactive oxygen Species (ROS) such as superoxide and H_2O_2 , which inhibit stomatal opening and promote stomatal closing [100].

Guan *et al.* [101] examined the use of chitosan to prime maize seeds. Although chitosan had no significant effect on germination under low temperatures, it enhanced germination index, reduced the mean germination time, and increased shoot height, root length, and shoot and root dry weights in two tested maize lines. In other studies, it has been reported that seed priming with chitosan improved the vigor of maize and wheat seedlings. It was also reported that such treatment led to an increase of seed resistance to certain diseases and improve their quality and/or their ability to germinate.

4.2 Topical delivery

Jaleh Varhosaz *et al.*[102] prepared the gel containing lidocaine (LC) as a local anesthetic agent with three different molecular weights (MW) and concentrations of chitosan for prolonging anaesthetic effect of this drug for transdermal delivery. Lecithin was used as permeation enhancer. Viscosity, bio-adhesion, drug release from synthetic membranes, drug permeation through the biological barrier (rat skin) was studied. It was found that by increasing the concentration and MW of chitosan, there was increase in both the rate and extent of drug release, and was probably because of the increase in repulsive forces between LC and chitosan cations.

4.3 Water filteration

Chitosan can also be used in water processing engineering as a part of a filtration process. Chitosan causes the fine sediment particles to bind together, and is subsequently removed with the sediment during sand filtration. It also removes phosphorus, heavy minerals, and oils from the water. Chitosan is an important additive in the filtration process. Sand filtration apparently can remove up to 50% of the turbidity alone, while the chitosan with sand filtration removes up to 99% turbidity[103]. Chitosan has been used to precipitate caseins from bovine milk and cheese making [104]. In combination with bentonite, gelatin, silica gel or other fining agents, it is used to clarify wine, mead, and beer [105].

4.4 Manufacturing of Bioinspired materials

A manufacturing concept inspired by natural nacre, shrimp carapace or insect cuticles, [106] has led to development of methods to manufacture large scale consumer objects using chitosan [107] (Figure 9). This method is based on replicating the molecular arrangement of chitosan from natural materials into fabrication methods, such as injection molding or mold casting [108]. Once discarded, chitosan-constructed objects are biodegradableand non-toxic [109]. The method is being explored to engineer human organs or tissues using three-dimensional bioprinting[110, 111]. Pigmented chitosan objects can be recycled[112] with the option of reintroducing or discarding the dye at each recycling step, enabling reuse of the polymer independently of colorant[113, 114]. Unlike other plant-based bioplastics (e.g. cellulose, starch), the main natural sources of chitosan are from marine environments and do not compete for land or other human resources[115].

4.5 Antimicrobial activity

The bactericidal activity of chitosan-derivatives is reported to be stronger than that of unmodified chitosan. The extent of the antimicrobial action of chitosan is influenced by intrinsic and extrinsic factors such as MW, DDA, pH, temperature, solubility, derivatization, type of organism, etc. Jia *et al.* [37] showed the N-propyl-N, N-dimethyl chitosan presents bactericidal activity against *Escherichia coli* 20 times higher than that of chitosan with 96% deacetylation . The antimicrobial activity of N, N, N-trimethyl chitosan (TMC) is 500 times higher than that of unmodified chitosan. It has been shown that other chitosan-derivatives such as hydroxypropyl chitosan, O-hydroxyethylchitosan, and carboxymethyl chitosan, among others, also exhibit significant antimicrobial activity [116-119]. It has been reported that the quaternary salts of alkyl chitosan derivatives exhibit antifungal property against plant fungi *Pythium debaryanum*, *B. cinerea a*nd *Fusarium oxysporum*[120].

4.5.1 Mechanisms proposed for antimicrobial activity of chitosan

- Electrostatic interaction between positively charged chitin/chitosan molecules and negatively charged microbial cell membranes. This electrostatic interaction results in two fold interference: i) by promoting changes in the properties of membrane wall permeability, thus provoke internal osmotic imbalances and consequently inhibit the growth of microorganisms[121, 122] and ii) by the hydrolysis of the peptidoglycans in the microorganism wall, leading to the leakage of intracellular electrolytes such as potassium ions and other low molecular weight proteinaceous constituents (e.g. proteins, nucleic acids, glucose, and lactate dehydrogenase) [123-126]. Since such mechanism is based on electrostatic interaction, it suggests that the greater the number of cationized amines, the higher will be the antimicrobial activity [127, 128]. This suggests that chitosan has higher activity than that found for chitin and this has been confirmed experimentally [127, 129]. It is worth observing that the amount of polycationic chitosan available to bind to a charged bacterial surface is apparently reduced as the concentration of chitosan increases [130, 131].

- Another proposed mechanism is the binding of chitosan with microbial DNA, which leads to the inhibition of the mRNA and protein synthesis via the penetration of chitosan into the nuclei of the microorganisms [121, 132, 133]. In this the chitosan molecules is assumed to be able to pass through the bacterial cell wall, composed of multilayers of cross-linked murein, and reach the plasma membrane. Observation by confocal laser scanning microscopy [134] confirmed the presence of chitosan oligomers (a chain with few number of monomer units) inside *E. coli* exposed to chitosan under different conditions. Raafat *et al*. [135] stated that the prevailing contention is that chitosan acts essentially as an outer membrane disruptor rather than as a penetrating material [135, 136].

- The third mechanism is the chelation of metals, suppression of spore elements and binding to essential nutrients to microbial growth [137, 138]. It is well known that chitosan has excellent metal-binding capacities where the amine groups in the chitosan molecules are responsible for the uptake of metal cations by chelation [139]. In general, such mechanism is more efficient at high pH, where positive ions are bounded to chitosan, since the amine groups are unprotonated and the electron pair on the amine nitrogen is available for donation to metal ions. In a recent model proposed by Wang *et al*. [140], the metal is arranged as an electron acceptor connected to one or more chitosan chains via $–NH_2$ and by forming bridges to hydroxyl groups, as illustrated in Figure 10.

4.6 Gene delivery

Chitosan's cationic qualities have the aptitude to interact with negative molecules such as DNA and form complexes, facilitate transfection and inhibit degradation of the same. Chitosan as (safer to other non-viral vectors) non-viral vector for gene delivery put forward quite a few advantages that are not producing endogenous recombination, oncogenic and immunological effects. The molecular mass and deacetylation extent of the chitosan, chitosan to DNA/siRNA charge proportion (N/P ratio) and its strength, the chitosan salt form utilized, pH, serum, additives, chitosan/nucleic acid particles preparation process and routes of administration and diverse formulation allied factors influence the transfection efficiency [141].

4.7 Gastro retentive drug delivery systems (GRDDS)

Suitability of utilizing chitosan in making these particular floating drug delivery systems has been successfully achieved by ionic interaction of chitosan and negatively charged surfactant sodium dioctyl sulfosuccinate [142]. In an additional endeavor chitosan granules prepared with prednisolone demonstrated good buoyancy and controlled release when it was added to acidic and neutral media provide evidence of its appropriateness in making such category of formulations [143]. Effervescent floating drug delivery systems [144] and Sustained Release floating tablets using a mixture of sodium bicarbonate, citric acid and chitosan were prepared and evaluated effectively. Bioadhesiveness and floating capabilities of chitosan microspheres shown to have a high potential in developing GRDDS especially for the drugs which are all poorly soluble in intestinal medium and readily soluble in acidic medium. Chitosan microspheres are having capability to stay longer in stomach and facilitate the stomach-specific drug delivery. Chitosan capsules have been used in the specific delivery of insulin to the colon [145].

4.8 Cosmetics

Chitosan can be tailored to produce different forms for use in different cosmetic fields such as skin care, hair-care and deodorants. It is an essential component in skin-care creams, shampoos and hairsprays due to its antibacterial properties. It forms a protective, moisturizing, elastic film on the surface of the skin that has the ability to bind other ingredients that act on the skin. In this way, it can be used in formulating moisturizing agents such as sunscreens, organic acids, etc. to enhance their bioactivity and

effectiveness. Its applications includes: maintenance of skin moisture, minimize acne problem, protect the epidermis, reduces the static electricity in hair, reduces dandruff, improve suppleness of hair, makes hair softer[146].

4.9 Chitosan microspheres as drug delivery carriers

These can be prepared by the water-in-oil emulsion solvent diffusion method, being ethyl acetate the oil phase. Figure 11 displays Scanning Electron Microscopy (SEM) image of the chitosan microspheres with drug entrapment[147].

4.10 As permeation enhancer

It has been reported that chitosan, due to its cationic nature is capable of opening tight junctions in a cell membrane. This property has led to a number of studies to investigate the use of chitosan as a permeation enhancer for hydrophilic drugs that may otherwise have poor oral bioavailability, such as peptides. Because the absorption enhancement is caused by interactions between the cell membrane and positive charges on the polymer, the phenomenon is pH and concentration dependant.

Furthermore increasing the charge density on the polymer would lead to higher permeability. This has been studied by quaternizing the amine functionality on chitosan. Chitosan was able to increase the paracellular permeability of peptide drugs across mucosal epithelia [148]. Chitosan derivatives have been evaluated to overcome chitosan's limited solubility and effectiveness as absorption enhancer at neutral pH values such as those found in the intestinal tract. Trimethyl chitosan chloride (TMC) has been synthesized at different degrees of quaternization. This quaternized polymer forms complexes with anionic macromolecules and gels or solutions with cationic or neutral compounds in aqueous environments and neutral pH values. TMC has been shown to considerably increase the permeation of neutral and cationic peptide analogs across $CaCO_2$ intestinal epithelia.

4.11 Paper industry

Biodegradable chitin and chitosan can strengthen recycled paper and increase the environmental friendliness of packaging and other products. Chitosan is already involved in the manufacture of paper because chitosan molecules greatly resemble those of cellulose, the constituent of plant walls [149].

5. CONCLUSION

In this review we aim to present an overview of the state of art in the knowledge and technical applications of chitin and chitosan. Nevertheless, this is an ambitious project; and the very large number of papers published on a wide range of properties and applications forces us to make a selection from the most significant results obtained by the many groups working around the world.

Chitin is a natural polymer for which we try to point out some unique features and its potential for useful development. Chitin can be transformed and used as fiber, film, sponge or powder. The preparation of derivatives that are soluble, especially in aqueous media, makes it possible to take advantage of the special properties of chitin: this polysaccharide is a film-forming polymer, biodegradable and renewable; it also has antibacterial and fungistatic properties; the semi-rigid character of chitin is valuable for thickening properties but also promotes the inter-chain interactions that cause difficulties in characterization. Unlike chitin, chitosan is water soluble in acidic media, or under precisely specified conditions at neutral pH, allowing much development in the domains of solutions and hydrogels.

The advantage of chitosan over other polysaccharides (cellulose, starch, galactomannans, etc.) is that its chemical structure allows specific modifications without too many difficulties at the C-2 position, as described in this review. Specific groups can be introduced to design polymers for selected applications.

Finally, the natural biological properties of chitin and chitosan are valuable for both plant and animal applications, and such developments can be considered as valuable extensions of the use of chitosan and its derivatives.

ACKNOWLEDGMENTS

The author expresses the deep sense of gratitude to the Director, Himachal Institute of Pharmacy, Paonta Sahib (H.P) for providing Library facilities for this review article and encouragement. We are thankful to all researchers in chitin and chitosan field whose works have been a great source to prepare the manuscript.

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LIST OF FIGURES

Figure 1: Chitosan [2]

Figure 2: Crustacean shells [10]

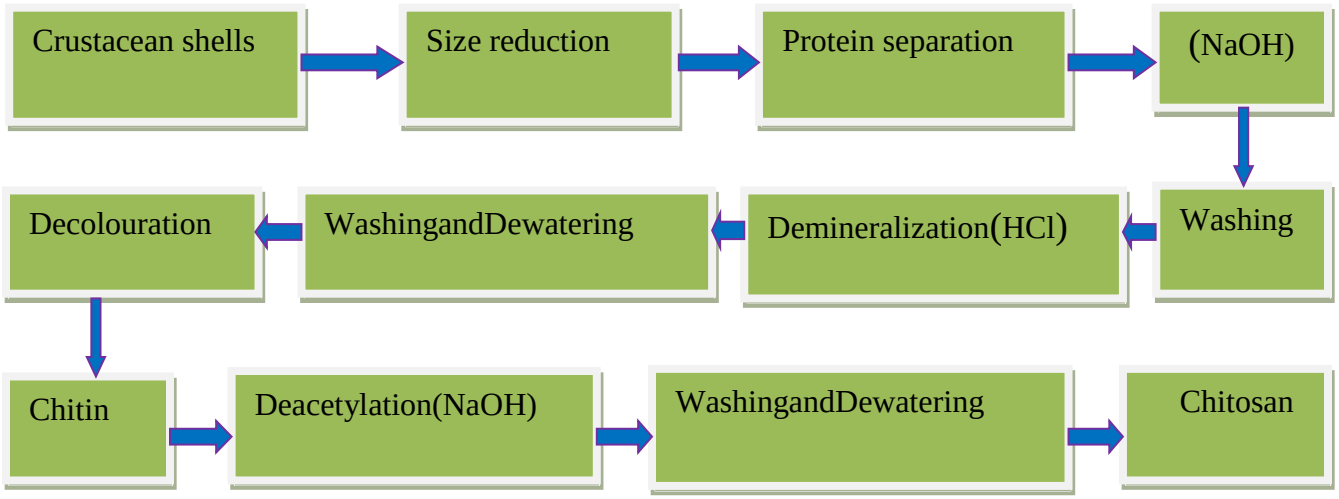

Figure 3: Isolation of chitosan from crustacean shells [9,10]

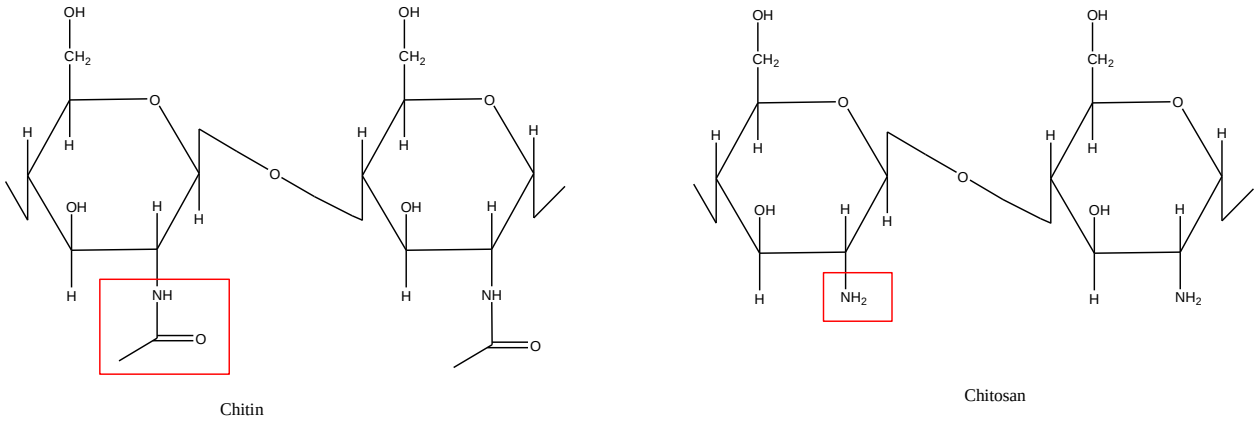

Figure 4: Chemical structure of chitin and chitosan[28]

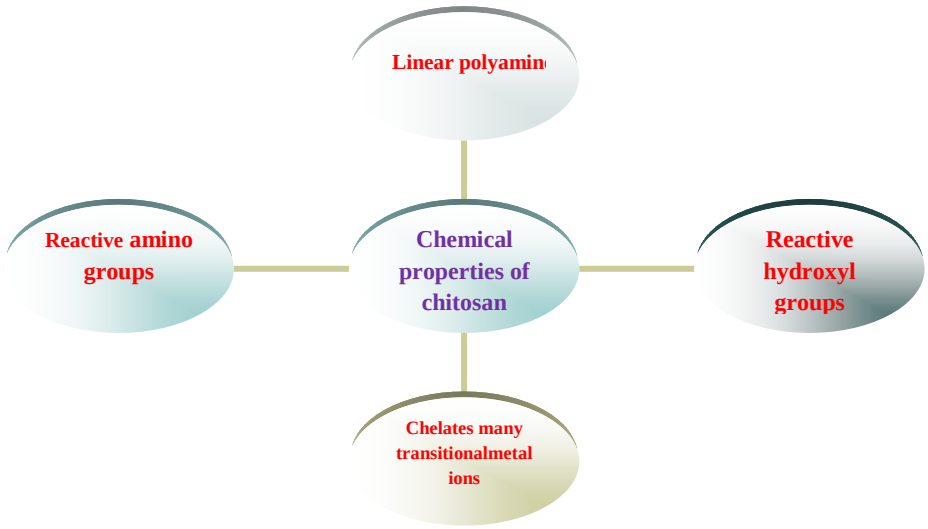

Figure 5: Chemical properties of chitosan[34]

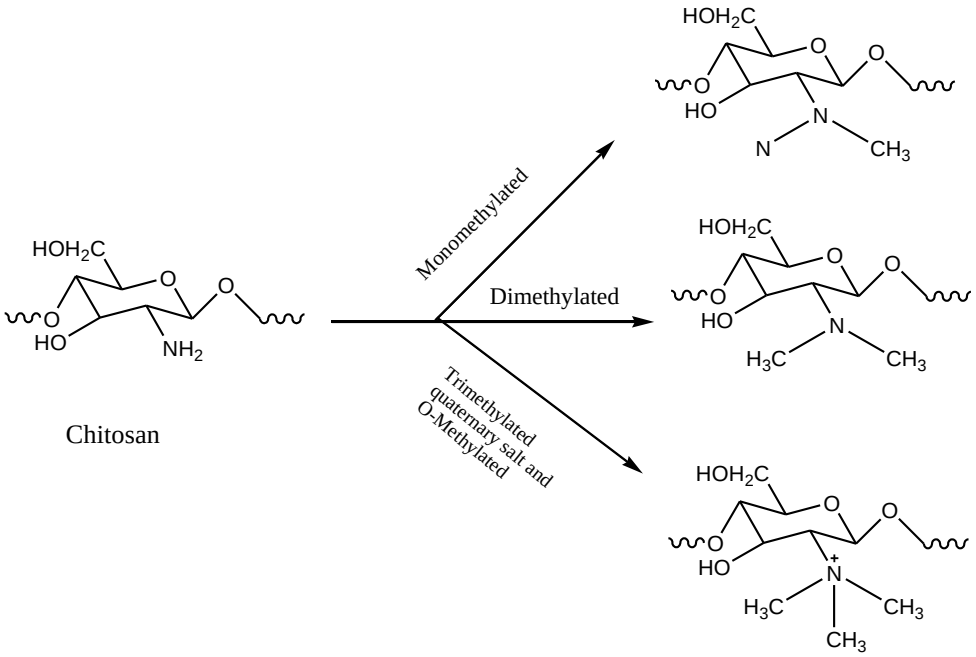

Figure 6: Schematic representation of the reaction leading to the quaternization of the amino groups of chitosan and resulting in N, N, N-trimethylchitosan[38]

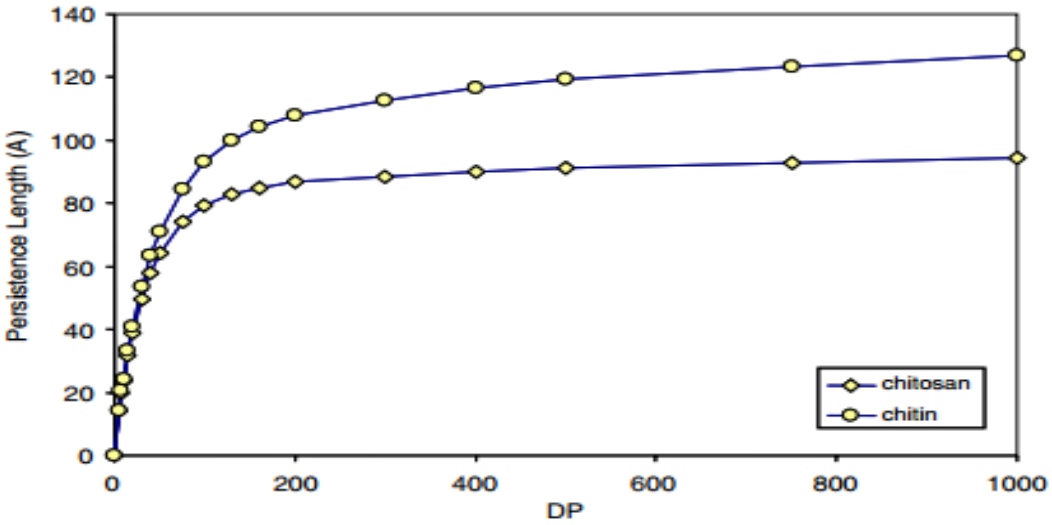

Figure 7: Persistence length as a function of the degree of polymerization(DP) for chitin and chitosan obtained from molecular modelling at 25 ºC with a dielectric constant D=80[50]

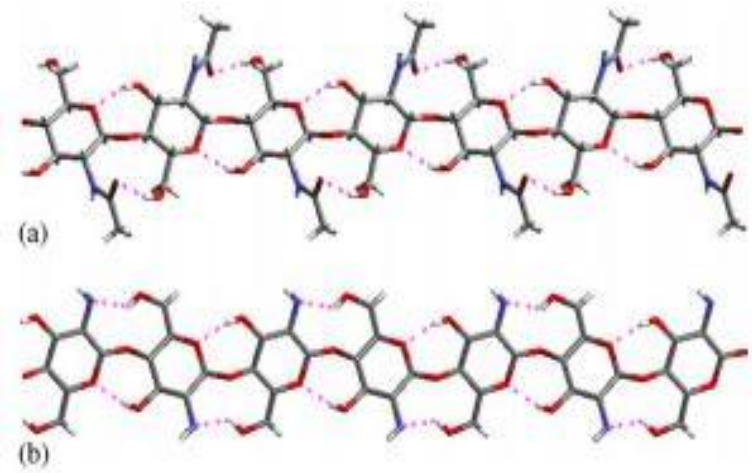

Figure 8: Molecular modelling: (a) of a chitin chain with two H bonds (1). between—OH 3 and O 5, (2). between—OH 6 and O of CQO; and (b). of a chitosan chain with two H bonds (1). between—OH 3 and O 5, and (2), between—OH 6 and N [51]

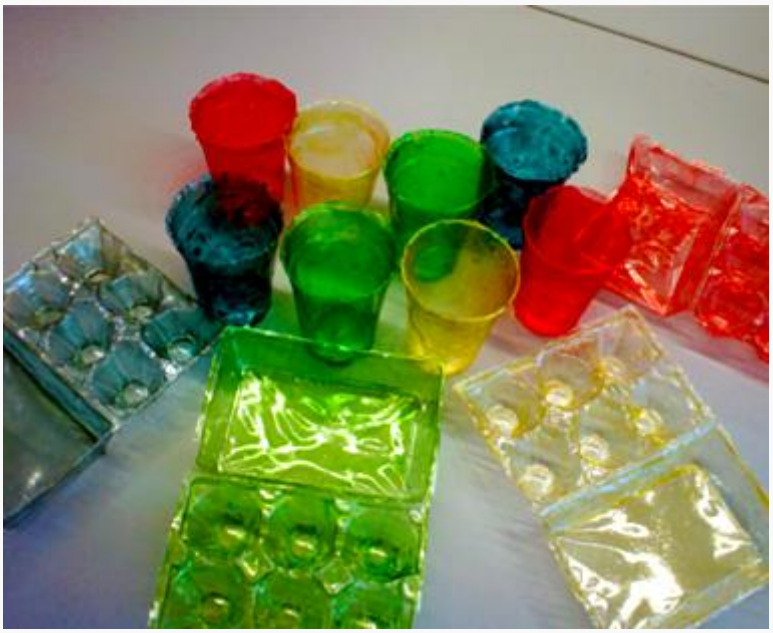

Figure 9: Large three-dimensional functional objects made from chitosan [112]

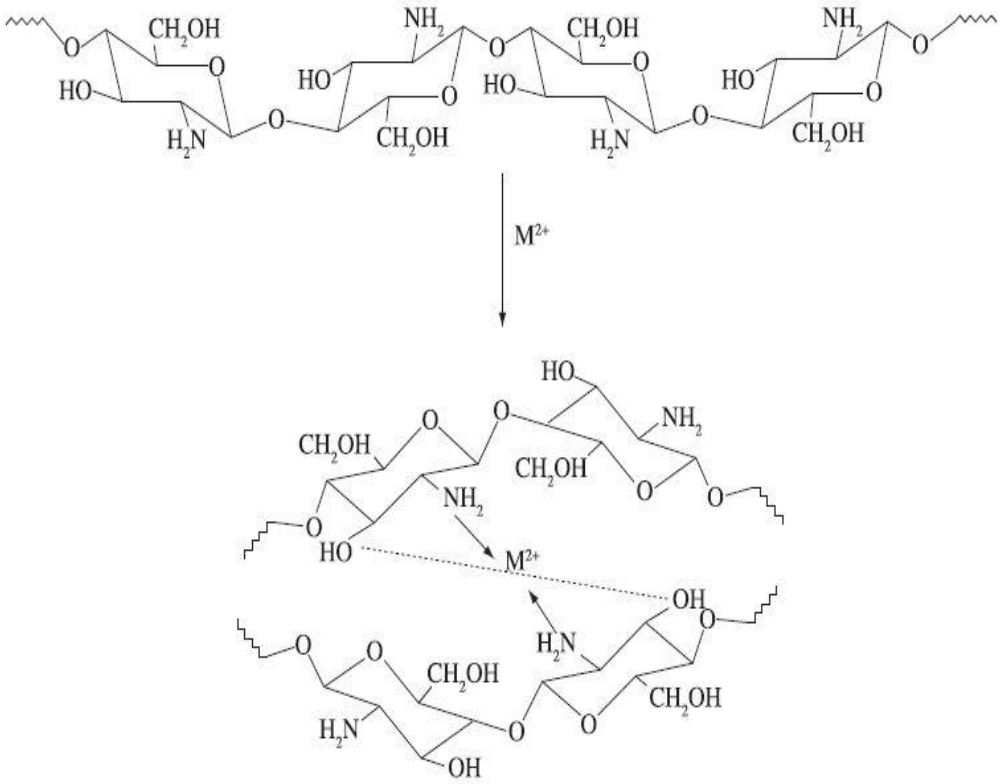

Figure 10: Metal-chitosan complexation model according to Wang *et al*.[140]

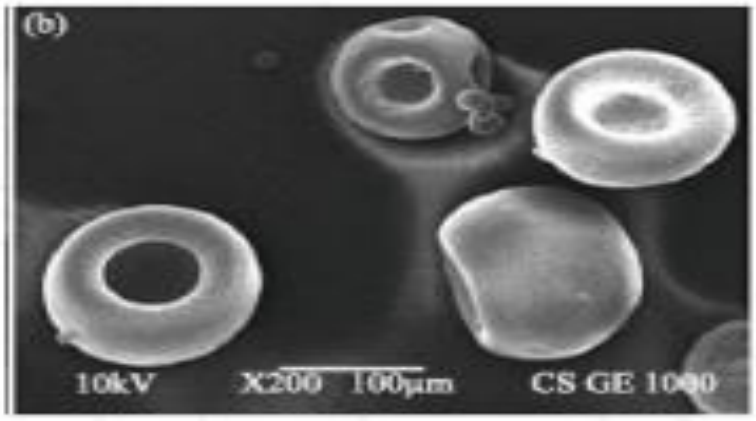

Figure 11: Scanning Electron Microscopy (SEM) of drug-loaded chitosan microparticles [147]

Cite this article as: Kaur R, Nautiyal U, Semwal A. Chitosan: A Multifunctional Polymer. Res Pharm Healt Sci. 2017;3(1):256-276.