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Chitosan: A Natural Antimicrobial Agent- A Review

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ABSTRACT

Chitosan is the second most abundant natural polymer after cellulose in the world; primarily composed of glucosamine and N-acetyl glucosamine residues with a 1, 4-β-linkage. Chitosan is considered as most promising materials for future applications on account of its excellent biodegradability, biocompatibility, antimicrobial activity, non-toxicity, and its economic advantages. This review presents an overview of sources, solubility, chemical properties, biological properties and mode of action of antimicrobial activities of chitosan.

Keywords: Chitosan, chitin, antimicrobial, degree of deacetylation (DD), biocompatibility.

INTRODUCTION

Chitosan receives a lot of attention because it has numerous desirable qualities and it is produced from chitin, which is the second most abundant biopolymer after cellulose in the world[1]. Naturally, chitosan occurs in the cell walls of some fungi, but today's commercial production is by partial chemical de-acetylation of chitin which is extracted from crustaceans (insects, crabs, shrimp shell or squid pen wastes) by deproteination, decalcification and decolorization and from fungi by enzymatic extraction as shown in figure 1.When the fraction of acetylated amine group is reduced to 40-50%, the resultant 2-amino-2-deoxy-β-D glucan is then referred to as chitosan. The structure of chitosan is primarily characterized by its molecular weight and degree of deacetylation (DD). The only difference between structure of chitin and chitosan lies in the DD. Chitin is an inexhaustible renewable resource with high crystallinity and in consequence with low solubility in aqueous solvents with an estimated production $10^{10\text{-}11}$ at annually. A lot of highly promising applications have been proposed for chitosan, the partially deacetylated counterpart of chitin. Due to deacetylation there is formation of a free amino group which fetches a positive charge to the polymer at somewhat acidic pH values, making it more hydrophilic. Due to its polycationic nature, chitosan has a number of interesting physico-chemical properties in aqueous solution, for example spontaneous formation of nanoparticles and an ability to form physical hydrogels. In addition, the cultivation of fungi can provide an alternative source of chitosan with the recent advances in fermentation technology. The cationic nature is also thought to be slightly responsible for the broad range of biological functionalities reported, such as antimicrobial activities and disease resistance inducing activities in plants, the mechanism of which are discussed in the pending of the review[2-10].

Chitosan is insoluble in most of the solvents but is soluble in dilute organic acids such as acetic acid, formic acid, succinic acid, lactic acid, and malic acid. The use of chitosan is limited because of its insolubility in water, high viscosity, and tendency to coagulate with proteins at higher pH. Many efforts have been reported to prepare functional derivatives by chemical modifications to increase the solubility in water [10-19]. Chitosan is inexpensive, biodegradable, and nontoxic for mammals which make it suitable for use as an additive in the food industry, as a hydrating agent in cosmetics, and more recently as a pharmaceutical agent in biomedicine [20,21]. Chitosan, however, shows its antibacterial activity only in an acidic medium, which is usually assigned to the poor solubility of chitosan at high pH. The solubility of chitosan depends on its origin, molecular weight and degree of acetylation. The reported antimicrobial activities of chitosan might be the effect of dissolved chitosan in acidic media such as acetic acid, lactic acid, glutamic acid and hydrochloric acid. The antimicrobial activity of chitosan was reported to be dependent on its molecular weight and degree of deacetylation (DD). The antibacterial effect of chitosan oligomers was also investigated, but the media was still acidic [22-29].

Sources: Chitosan was first discovered by Rouget in 1859 during boiling of chitin in a concentrated potassium hydroxide solution, which resulted in deacetylation of chitin. It was formely named by hoppe Seyler in 1894[124].

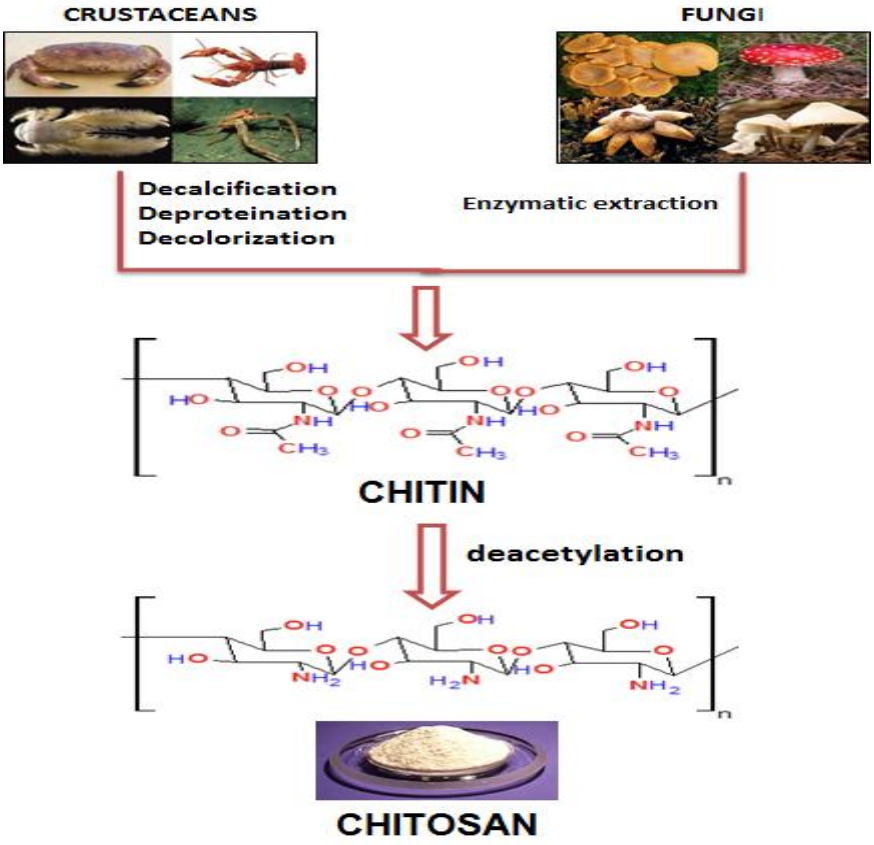


Figure 1. Showing extraction of chitin and chitosan from different sources

Chitin is widely distributed both in the animal and the plant kingdom. The lists of organisms that contain chitin are Fungi, Algae, Echiruda, Annelida (Segmented worms), Mollusca, Cnidaria (jellyfish), Aschelminthes (roundworm), Entoprocta, Bryozoa (Moss or lace animals), Phoronida (Horseshoe worms), Brachiopoda (Lamp shells), Arthropoda and Ponogophora[30]. Chitin being the main source of chitosan is the major component of arthropod exoskeletons, tendons, and the linings of their excretory, respiratory and

digestive systems, as well as insect's external structure and some fungi. It is also found in the iridophores (reflective material) of both eyes and epidermis of cephalopods and arthropods of phylum Mollusca and the epidermal cuticle of the vertebrates. Paralipophrys trigloides's epidermal cuticle is also chitinous in nature [31, 32]. Shell wastes of shrimp, lobsters, crabs and krill are the main commercial sources of chitin. Therefore, we can say that chitin is not only present in invertebrates but is also present in vertebrates.

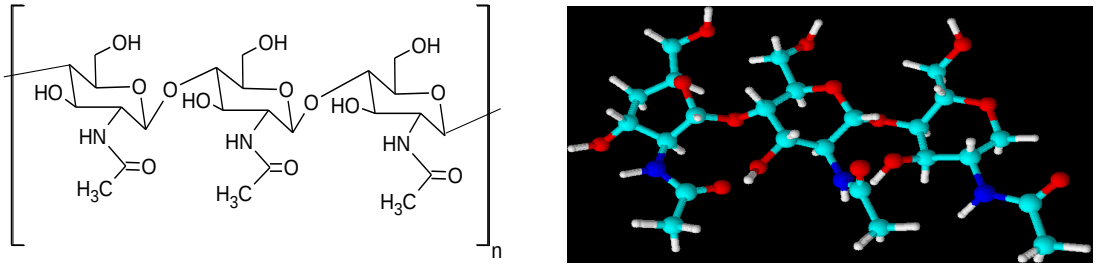
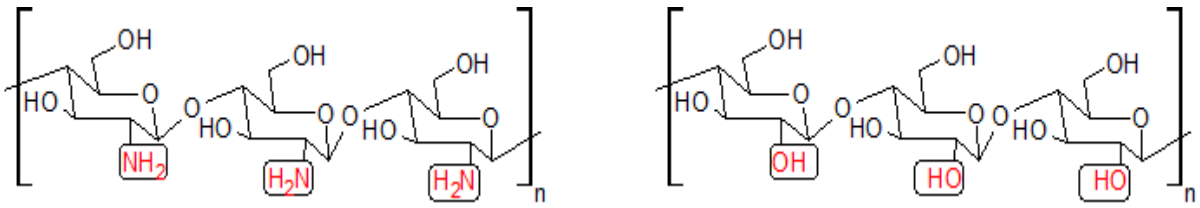


Figure 2. Structure of chitin

Types: There are three forms of chitin viz α, β, and γ chitin. The α-form is composed of polysaccharide strands aligned in alternating antiparallel fashion which is mainly obtained from crab and shrimp. α–Chitin is the most abundant one; the anti-parallel arrangement in α-chitin gives rise to strong hydrogen bonding and consequently makes it more stable**.** The rarer β-chitin is composed of parallel strands of polysaccharides, it is found in association with proteins in squid pens [33, 34] and in the tubes synthesized by pogonophoran and vestimetiferan worms [35, 36]. Both α and β chitin are commercially available. β-form can be converted into α-form form, but reverse is not possible[119-121]. The γ-form of chitin contains two parallel and one anti-parallel strands of chitin [37]. Use of lithium thiocyanate leads to conversion of γ-chitin into α-chitin [122]. The chains are associated with one another by very strong hydrogen bonding between the amide groups and carbonyl groups of the adjuncted chain. Hydrogen linkages are responsible for the great insolubility of chains in water and for the formation of fibrils.

Physico-chemical characteristics: Chitin is a structural biopolymer, which has a role similar to that of collagen in the higher animals and cellulose in terrestrial plants [38-40]. Plants produce cellulose in their cell walls and insects and crustaceans produce chitin in their shells [41, 42]**.**



a b

Figure 4. showing structures of a.chitosan, b.cellulose

Cellulose and Chitosan provide structural integrity and protection to plants and animals, respectively and share very similar structure.[43, 44] The only difference between structure of chitosan and cellulose is the amine (NH_2) group at position C-2 of chitosan instead of the hydroxyl (-OH) group found in cellulose(Figure. 4). Thus, chitosan is a biopolymer which consists of N-acetyl-2-amino-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose, where the repeating units are linked by β-(1→4)-glycosidic bonds [45]. After purification, chitosan has a rigid crystalline structure through inter-molecular and intra-molecular hydrogen bonding and show polymorphism. However, unlike plant fiber, chitosan possesses net ionic positive charges, which give it the ability to chemically bind with negatively charged fats, lipids, cholesterol, metal ions, proteins, and macromolecules, etc. [46]. In this respect, chitin and chitosan have attained increasing commercial interest as suitable resource materials due to their excellent

properties including biocompatibility, biodegradability, non-toxicity, adsorption, and ability to form films, and to chelate metal ions [47].

Solubility: Chitosan is insoluble in most of the solvents but is soluble in dilute organic acids such as acetic acid, formic acid, succinic acid, lactic acid, and malic acid below pH 6.0. This is because chitosan can be considered a strong base as it possesses primary amino groups with a pKa value of 6.3. The presence of the amino groups (Figure.4) indicates that pH substantially alters the charged state and properties of chitosan [48]. At low pH, these amines get protonated and become positively charged and that makes chitosan a water-soluble cationic polyelectrolyte. On the other hand, as the pH increases above 6, chitosan's amine group becomes deprotonated and the polymer loses its charge and become insoluble. The transition between solubility and insolubility occurs at its pKa value around pH between 6 and 6.5. As the pKa value is highly dependent on the degree of N-acetylation. The solubility of chitosan is dependent on the degree of deacetylation, distribution of the acetyl groups along the main chain, molecular weight and the method of deacetylation used [123**,** 49]. The degree of ionization depends on the pH and the pKa with respect to studies based on the role of the protonation of chitosan. The presence of hydrochloric acid and organic acids such as acetic, formic, and lactic acids can dissolve chitosan [50-52]. The best solvent for chitosan was found to be formic acid, where solutions are obtained in aqueous systems containing 0.2–100% of formic acid (FA) [53]. The most commonly used solvent is 1% acetic acid (as a reference) at about pH 4.0. Chitosan is also soluble in 1% hydrochloric acid and dilute nitric acid but insoluble in sulphuric and phosphoric acids. But concentrated acetic acid solutions at high temperature can cause depolymerization of chitosan [50, 51]. The concentration of the acid plays a great importance to impart desired functionality [54]. Chitosan as stated above is soluble at pH below 6. It is known that the amount of acid needed depends on the quantity of chitosan to be dissolved [49].

Bio-chemical properties of chitosan: The various chemical and biological properties of chitosan are as follows:

- Natural polymer
- Linear polyamine
- Reactive amino groups
- Reactive hydroxyl groups available [55].
- Chelates many transitional metal ions [56].
- Biocompatible [57].
- Biodegradable to normal body constituents [58].
- Safe and non-toxic [59, 60].
- Binds to mammalian and microbial cells aggressively [61].
- Accelerate the formation of osteoblast responsible for bone formation [62].
- Haemostatic [63].
- Fungistatic [64].
- Spermicidal [65].
- Antitumor [66, 67].
- Anticholesteremic [68].
- Accelerate bone formation [69].
- Immunoadjuvant [70].
- Drug delivery agent [71].

Antimicrobial Properties of Chitosan: The antimicrobial properties of chitosan are more often discussed in the recent article which depends on its physical properties. This biopolymer can be used against a broad spectrum of target organisms like bacteria, fungi, viruses or algae [72-76]. Microbial growth on the surface of food is a major cause of food spoilage and food-borne illness [77, 78]. Chitosan possesses unique properties that make it an ideal ingredient for development of antimicrobial edible film. Chitosan is an antimicrobial non-toxic biopolymer that has been proven to serve as a matrix to obtain edible films

containing essential oils [79, 80]. The interaction (binding or chelation) of chitosan with endotoxins of gram-negative bacteria decreased their acute toxicity. Because of the strong chelating ability of chitosan, external chelating agents such as EDTA may not be required, when antimicrobial agents such as nisin are added to chitosan to control gram negative bacteria. Chitosan's ability to inhibit a wide variety of bacteria, fungi, yeasts, and viruses make its application in a broad range or variety of antimicrobial agent in experiments involving in vivo and in vitro interactions in different forms (solutions, films and composites) [81,82]. Again like physico-chemical properties; the antimicrobial activity of chitosan is influenced by its molecular weight, degree of deacetylation, concentration in solution, and pH of the medium [83, 84]. In general, acetic acid, lactic acid, and formic acids were more effective in inhibiting bacterial growth than propionic and ascorbic acids. Chitosan shows stronger antimicrobial activity for gram-positive than gram-negative bacteria [85, 86]. Chitosan has been observed to act more quickly on fungi and algae than on bacteria [87] Because of the positive charge on the C-2 of the glucosamine monomer below pH 6, chitosan is more soluble and has a better antimicrobial activity than chitin [88].

Mechanism of antimicrobial activity of chitosan: The exact mechanism of the antimicrobial action of chitin, chitosan, and their derivatives is still imperfectly known, but different mechanisms have been proposed. One of the reasons for the antimicrobial character of chitosan as discussed in previous sections is its positively charged amino group which interacts with negatively charged microbial cell membranes, leading to the leakage of proteinaceous and other intracellular constituents of the microorganisms. It has been reported that quaternized chitosan with a higher degree of substitution of the quaternary ammonium exhibited a strong interaction with negative charges on the bacterial cell surface and showed better antibacterial activity than chitosan[89-92]. This interaction results (a) by promoting changes in the properties of membrane wall permeability, thus provoke internal osmotic imbalances and consequently inhibit the growth of microorganisms [93, 94] and (b) 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 (example: proteins, nucleic acids, glucose, and lactate dehydrogenase) [95-99]. The mechanisms of the antimicrobial activity of chitosan were different for Gram-positive and Gram-negative bacteria has been reported in the comparative study of the effect of chitosan on S. aureus (Gram-positive) and on Escherichia coli (Gram-negative). For Gram-positive S. aureus, the antimicrobial activity increased on increasing the molecular weight of chitosan. Besides, for Gram-negative E. coli, the antimicrobial activity increased on decreasing molecular weight [100]. Chitosan generally showed stronger effects for gram-positive bacteria such as *B. cereus, Staphylococcus aureus, Lactobacillus plantarum, Listeria monocytogenes, Bacillus megaterium, L. brevis, L. bulgaris, etc.* than for gram-negative bacteria such as *Salmonella typhymurium, E. coli, Pseudomonas fluorescens, Vibrio parahaemolyticus*, etc.[101-103]. Chitosan show interaction with the membrane of the cell which leads to alteration in permeability of cell. For example, fermentation with baker's yeast is inhibited by certain cations, which act at the yeast cell surface to prevent the entry of glucose. UV-absorption studies indicated that chitosan caused considerable leakage of proteinaceous material from *Pythium oaroecandrum* at pH 5.8[104]. The charge density on the surface of cell is a determinant factor to establish the amount of adsorbed chitosan. Highly adsorbed chitosan would obviously result in higher changes in the structure and in the permeability of the cell membrane. This would indicate that the antibacterial mode of action is dependent upon the host microorganism [105]. 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 [106-108]. 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 [109] 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. stated that in spite of been accepted as a possible mechanism, the probability of it occurring is rather low [110].

The third proposed mechanism is the chelation of metals, suppression of spore elements and binding to essential nutrients to microbial growth [111-113]. 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 [114]. Similarly to bacteria, the chitosan activity against fungus is assumed to be fungistatic rather than fungicidal with a potential to communicate regulatory changes in both the host and fungus [115, 116]. The antifungic mechanism of chitosan involves cell wall morphogenesis with chitosan molecules interfering directly with fungal growth, similarly to the effects observed in bacteria cells [117]. Microscopic observation reported that chitosan oligomers diffuse inside hyphae interfering on the enzymes activity responsible for the fungus growth [118].

CONCLUSIONS

Chitosan has received considerable attention as a possible excipient in pharmaceutical and other biomedical applications due to 1. its good biocompatibility and low toxicity 2. its possible formulation in nanoparticles or in gels, and 3. its cationic nature. During the last decade, there is growing demand of food without chemical preservatives. It has driven the research on new products with natural antimicrobial properties. Since chitosan is recognised as a safe biopolymer suitable for oral administration and its antifungal, antibacterial and antiviral properties; it is very interesting for food industry. In agriculture, it is used in various ways such as soil enrichment, foliar spraying, coating seed, supplement in hydroponic, and supplement in plant tissue culture media in which coating seed and foliar spraying are useful. The applications of chitosan in various disciplines are shown in table1.

Table 1: Application of chitosan and its derivatives in different areas [94,125]

S.No.	Areas	Applications
01	Agriculture	Defensive mechanism in plants Stimulation of plant growth Seed coating, Frost protection Time release of fertilizers and nutrients into the soil
02	Water & waste Treatment	Flocculant to clarify water (drinking water, pools) Removal of metal ions Ecological polymer (eliminate synthetic polymers) Reduce odors
03	Bio pharmaceutics	Immunologic, antitumoral Hemostatic and anticoagulant Healing, bacteriostatic
04	Food & beverages	Not digestible by human (dietary fiber) Bind lipids (reduce cholesterol) Preservative Thickener and stabilizer for sauces Protective, fungistatic, antibacterial coating for fruit
05	Cosmetics & toiletries	Maintain skin moisture Treat acne Improve suppleness of hair Reduce static electricity in hair Tone skin Oral care (toothpaste, chewing gum)

06	Potential Biomedical Applications	Surgical sutures Dental implants Artificial skin Rebuilding of bone Corneal contact lenses Time release drugs for animals and humans Encapsulating material
07	Antimicrobial agent	Bactericidal Fungicidal Measure of mold contamination in agricultural commodities
08	Edible film industry	Controlled moisture transfer between food and surrounding environment Controlled release of antimicrobial substances Controlled release of antioxidants Controlled release of nutrients, flavours and drugs Reduction of oxygen partial pressure Controlled rate of respiration Temperature control Controlled enzymatic browning in fruits Reverse osmosis membranes
09	Additives	Clarification and deacidification of fruits and beverages Natural flavour extender Texture controlling agent Emulsifying agent Food mimetic Thickening and stabilizing agent Colour stabilization
10	Nutritional quality	Dietary fibre Hypocholesterolemic effect Livestock and fish feed additive Reduction of lipid absorption Production of single cell protein Antigastritis agent Infant feed ingredient
11	Purification of water	Recovery of metal ions, pesticides, phenols and PCB's Removal of dyes
12	Recovery of solid materials from food processing wastes	Affinity flocculation Fractionation of agar
13	Material science	Electrochemistry (biosensors) Packaging films/composite coating formulations Textile finishing (dye binding) Polymeric membrances
14	Other applications	Hydrocolloid Enzyme immobilization Encapsulation of nutraceuticals Chromatography

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