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Application of Chitosan as an Antimicrobial Agent

Muhammad Shoaib[1] and Wang Zhouping[2]*

[1]School of Food Science and Technology, Jiangnan University, Wuxi, China
[2]Synergetic Innovation Center of Food Safety and Quality Control of Jiangsu Province, Jiangnan University, China

Abstract

Chitosan is a naturally occurring polysaccharide from chitin. The low solubility of neutral chitosan and alkaline limits its use. Nevertheless, chemical modification into composites or hydrogels brings to its new functional properties for different forms. Chitosan is recognized as a versatile biomaterial because of its non-toxicity, low allergenicity, biocompatibility, and biodegradability. Chitosan and chitosan based-materials show higher antimicrobial with their wide applications in medicine, pharmacy, food, and textile industries. The major antibacterial mechanism of Chitosan reported as:

• Ionic interaction of positive charges of the chitosan based-materials with different molecules located on the surface of bacterial cells.

• Penetration of chitosan chains into the cells and their interaction with negatively charged particles like mRNA, inhibiting protein synthesis, and so on.

• Realization of an external coating that chelates essential metals involved in microbial growth.

All these actions are subjected to the bacterial strain, the growth stage of bacterial cells and all these events can take place individually or collectively with different strengths. The researchers with the updated knowledge summarize the data related to the production, classification, modification, and the application of chitosan mainly as a natural antimicrobial agent.

Keywords: Chitosan; Antimicrobial Agent; Mechanism of Action; Chitosan Based-Materials; Application of Chitosan

Correspondence to: Wang Zhouping, Synergetic Innovation Center of Food Safety and Quality Control of Jiangsu Province, Jiangnan University, Wuxi, 214122, China; E-mail: wangzp@jiangnan.edu.cn

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Introduction

Chitin, a biopolymer synthetized by crustacean [1], fungi, mushrooms and insects. After cellulose, it's the second most abundant substance on biosphere. Although, chitin is synthetized by many categories of organisms, the main sources for chitin extraction are crab and shrimp shells. The difference between cellulose, that is a biopolymer made from D-glucose units linked via β (1→4) bonds, and chitin is that the units in chitin have N-acetyl-glucosamine (more exactly 2-acetamide-2 deoxy-D-glucopyronose) linked by β (1→4) bonds as shown in figure 1. In fact, not all the units in natural chitins are N-acetylglucosamine, some of these units are deacetylated as well. Chitosan is obtained by deacetylation of chitin, either in alkaline conditions (chemical deacetylation in concentrated NaOH) or using chitin deacetylase (enzymatic deacetylation). When the ratio of acetylated units (1→4)-2-acetamide-2-deoxy-β-D-glucan versus (1→4)-2-amine-2-deoxy-β-D-glucan) is higher than 40% the product is considered to be chitin, but when the acetylated units decrease under 40%, the polymer is named chitosan. Chitin can be extracted from producing organisms by chemical methods (demineralization with strong acids and deproteinization with strong bases) and biological (enzymatic) methods.

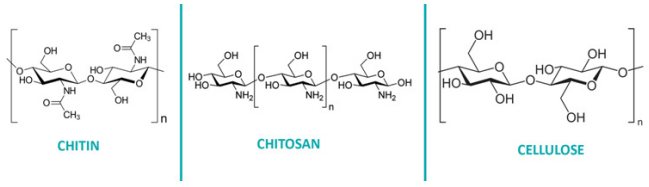

Figure 1: Chemical structures of chitin, chitosan and cellulose.

Chitosan and its derivatives are primarily used as antibacterial agents [2]. The degree of acetylation and the molecular weight have a significant role in the antibacterial activity of chitosan-based products. To increase its low solubility, the raw chitosan is chemically modified either at its primary amino or the primary alcohol groups [3]. Due to the incomplete characterization of chitosan-based materials, it is rather complicated to compare them and to control the influence of various types of factors that affect the antibacterial activity and mode of action of chitosan. Even the activity of chitosan was investigated as an antimicrobial agent against a broad range of organisms, like bacteria, yeasts, fungi or algae, in experiments involving *in-vitro* or *in-vivo* interactions, it is not yet clear if chitosan has a bactericidal or bacteriostatic activity. The problem associated with synthetic polymers such as toxicity and non biodegradability is suppressed by the use of biopolymers. The important characteristic of biopolymer is its ability to degrade by natural enzymes with their immunogenic behavior. The development of biomaterials using natural polymers is an important and promising channel for research. Chitin is the second largest biopolymer found in nature. It is mainly derived from the exoskeletons

of crustaceans, insects, mollusks and the cell wall of microorganisms. Chitosan is the deacetylated result of chitin that is expressed as a copolymer of á-(1, 4) glucosamine (C6H11O4N), consisting diverse amount of N-acetyl groups. It ranges from white to light red coloured concrete fine particles, unable to mix in water but can be mixed in organic acids. Chitin and chitosan are present in large amount in nature and are biodegradable polymers having outstanding features such as biocompatibility, non-poisonous and adsorption [4]. The reaction of chitosan is more versatile than cellulose due to the presence of -NH2 groups.

Recently, some attempts were made to introduce the rules and limits in classification of chitosan samples according to the molecular weight (MW) and the degree of deacetylation (DA) [2]. Apart from the influence of MW and DA of chitosan samples, which has to be characterized with precision to compare the antibacterial effect (like minimal inhibitory concentration MIC), other factors like pH, temperature, and salinity can also play a significant role in the antibacterial activity. Due to chitosan solubility at lower pH values, chitosan-based products have higher antibacterial activity in acidic environments [5]. The experiments have proven that, in most of the cases, the antibacterial activity of chitosan is increased at higher temperature (until 40°C) and lower pH values (between 4 and 6). Probably due to solubility issues, it seems that the molecular weight (MW) of chitosan has a more significant influence on antibacterial activity than the degree of acetylation (DA). Studies on *Bacillus cereus, E. coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella enterica, B. subtilis, Listeria monocytogenes,* and *Klebsiella pneumoniae* have shown that the chitosan with smaller MW have a higher antibacterial activity, as short polymers have higher mobility and stronger interactions with the bacterial walls, than the chitosan with high molecular weights. Studies on some Gram-positive and Gram-negative bacteria have revealed that the antibacterial activity is higher at lower DA [1].

Production of Chitosan

The raw material for the production of chitosan is chitin. The primary sources are the shells of crustaceans, mainly crabs and shrimps. The purification process is easier for shrimp shells, which are thinner. Usually, shells of the same size and species are grouped, cleaned, dried, and ground into small shell pieces. There is no standard purification method as different chitin sources require different treatments due to the diversity in their structures. Conventionally, the protocol is divided into demineralization, deproteinization, and decolorization, which can be carried out using chemical or biological (enzymatic treatment or fermentation) treatments. The end-products need to be highly purified if they are to be used for biomedical or pharmaceutical purposes, as residual proteins, minerals or pigments can cause serious side effects. Conversion of chitin to chitosan can be achieved by enzymatic or chemical deacetylation. Chemical deacetylation is more commonly used for commercial preparation because of economic issues and feasibility for mass production. No matter which method is used, depolymerization is inevitable. The processes involved in the chemical and biological development of chitosan from crustacean shells are illustrated in figure 2.

Properties

The applications of the chitosan depend on the characteristics, such as the appearance of polymer, turbidity of the polymer solution, degree of deacetylation, and molecular weight. The degree of deacetylation can be determined by different techniques, such as infrared spectroscopy, potentiometric titration, and more advanced methods like 1H liquid

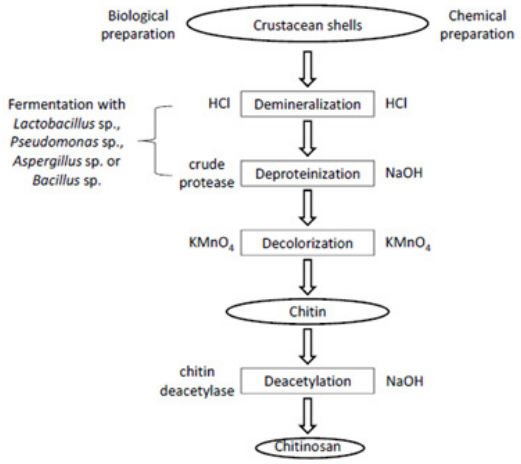

Figure 2: A schematic presentation of chitosan preparation from raw materials (Cheung *et al.,* 2015).

state and solid-state 13C NMR. The average molecular weight of chitosan is usually obtained from steric exclusion chromatography equipped with a viscometer and light scattering detector or matrix-assisted laser desorption/ionization-mass spectrometer. Different characterization techniques for determining molecular weight, degree of de-acetylation and crystallinity are summarized in table 1. Chitosan obtained from deacetylation of chitin becomes soluble in aqueous acidic solutions when the average degree of deacetylation is above 0.5, but not at an alkaline or physiological pH. The physical properties of chitosan in aqueous solution depending on the degree of deacetylation and the acetyl group distribution in the polymer chains. Uneven acetyl group distribution will lower its solubility and make it form aggregates quickly [6]. The solubility problem hinders its applicability.

Modification of chitosan at the molecular level increases its solubility and stability and thus makes it more versatile biopolymer. The presence of free amino groups on the chitosan chains allows

Table 1: Physicochemical characteristics of chitosan and their methods of determination.

Physiochemical Characteristics	Method of Determination
Molecular weight	Viscometry; gel permeation chromatography; light scattering; high performance liquid chromatography; matrix-assisted laser desorption/ionization-mass spectrometer
Degree of deacetylation	Infrared spectroscopy; ultraviolet spectrophotometry; nuclear magnetic resonance spectroscopy (H-NMR and C-NMR); conductometric titration; potentiometric titration; differential scanning calorimetry
Crystallinity	X-ray diffraction

modifications under mild conditions. Chitosan usually reacts with other small molecules or polymers and is transformed into derivatives or composites. Chitosan hydrogel is one of the various forms of its composites. It is composed of a cross-linked network of polymer chains with a high content of hydrophilic groups. Thus, it is a super absorbent of water, but is water-insoluble because of the chemical or physical bonds formed between the polymer chains hydrogel [7], chitosan-alginate composite [8], chitosan-collagen composite chitosan-hydroxyapatite composite [9] and chitosan-tricalcium phosphate composite [10]. They can be molded into different shapes and forms (films, fibers, sponges, beads, and solutions). These materials are mainly applied in bone tissue engineering scaffold [11], drug delivery system, wound healing materials and metal and dye absorbent for polluted water [12].

Antimicrobial Activity

Mechanism of Action

Chitin and Chitosan have been examined as an antimicrobial substantial contrary to an extensive variety of target organisms such as algae, bacteria, yeasts, and fungi in experimentations including *in vivo* and *in-vitro* interfaces with chitosan in diverse procedures (solutions, films, and composites). Initial investigation expressing the antimicrobial possibility of chitin, chitosan, and their modifications dated from the 1980-1990s. Generally, in these studies the chitosan is considered to be bactericidal (kills the live bacteria or some fraction therein) or bacteriostatic (hinders the growth of bacteria but does not imply whether or not bacteria are killed), often with no distinction between activities. Recent data in literature tends to characterize chitosan as bacteriostatic rather than bactericidal. Although the exact mechanism is not fully understood therefore several other factors may contribute to the antibacterial action. Three models have been planned and the widely accepted is the model of interaction amidst positively charged Chitin/Chitosan molecules and the negatively charged microbial cell membranes. In this model, the interaction is mediated by the electrostatic forces between the protonated NH_3^+ groups and the negative residues presumably by competing with Ca_2^+ for electronegative sites on the membrane surface.

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 hampers the growth of microbes and ii) through the hydrolysis of the peptidoglycans in the wall of microorganism, which leads to the leak of intracellular electrolytes such as potassium ions and other small molecular mass proteinaceous constituents (e.g. proteins, nucleic acids, glucose, and lactate dehydrogenase) [13]. This model was investigated in a recent work, who observed under transmission electron microscope the ultrastructural changes of *S. simulans* cells upon exposure to positively charged chitosan. It was possible to observe and identify chitosan molecules attached to bacterial cell surfaces. In the interacting sites, it was observed that the cell membrane became locally detached from the cell wall, giving rise to "vacuole-like" structures underneath the wall. The detachment generates ions and water efflux; provoking decreases in the internal bacteria pressure. Visual confirmation of effective membrane lysis has also been reported for gram-negative and gram-positive bacteria. 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. This suggests that chitosan has higher activity than that found for chitin and this has been confirmed experimentally. It is worth observing that the amount of polycationic chitosan available to bind to a charged bacterial surface is reduced as the concentration of chitosan increased. A possible explanation is that in the presence of a larger number of charged sites, the chains tend to form clusters by molecules aggregation while they are still in solution [15]. Observations have confirmed that at higher concentrations, the chitosan tends to form a coating over the bacteria, not necessarily attached to the surface and independent of the bacteria type. In such condition, modifications on pH could be conclusive for a decent solubility and to retain the chains separately.

Regarding the bacteria surface polarity, the external membrane of gram-negative bacteria constitutes vital part of lipopolysaccharides having phosphate and pyrophosphate groups which provides to the surface a bulk of negative charges greater to that observed for gram-positive ones (membrane composed by peptidoglycan associated to polysaccharides and teichoic acids [15]. This maintains the evidence that the leakage of intracellular material detected by chitosan in gram-negative is higher to that stated in gram-positive bacteria. The bacterial effectiveness of gram-positive or gram-negative bacteria is, however, somewhat controversial. Several authors have detailed that chitosan usually displayed stronger special effects for gram-positive bacteria (e.g. *Listeria monocytogenes*, *Bacillus megaterium*, *B. cereus*, *Staphylococcus aureus*, *Lactobacillus plantarum*, *L. brevis*, *L. bulgaris*, etc.) than for gram-negative bacteria (e.g. *E. coli*, *Pseudomonas fluorescens*, *Salmonella typhymurium*, *Vibrio parahaemolyticus*, etc. [16]. Conversely, the hydrophilicity that gram-negative bacteria possess is suggestively advanced than in gram-positive bacteria, constructing them as most delicate to chitosan. These findings are confirmed by several in vitro experiments in which gram-negative bacteria appear to be very sensitive to chitosan, exhibiting increased morphological changes on treatment when compared to gram-positives. The charge density on the cell surface is a determinant factor in establishing the amount of adsorbed chitosan. More adsorbed chitosan would result in greater changes in the structure and the permeability of the cell membrane. This would suggest that the antibacterial mode of action is dependent upon the host microorganism. Another anticipated method is the attachment of chitosan with microbial DNA, which progresses to the inhibition of the mRNA and protein production through the infiltration of chitosan into the nuclei of the microorganisms. In this the chitosan molecules are assumed to be able to pass through the bacterial cell wall, composed of multilayers of cross-linked murrain, and reach the plasma membrane. The current issue is that chitosan acts essentially as an outer membrane disruptor rather than as a penetrating material. The third mechanism is the chelation of metals, suppression of spore elements and binding essential nutrients to microbial growth. It is widely known that chitosan has outstanding metal-attachment capabilities where the amine groups in the chitosan molecules are accountable for the acceptance of metal cations by process of chelation [17].

In universal, such procedure is more effective at a higher pH, where positive ions are restricted to chitosan as the amine groups remain unprotonated and the electron pair on the amine nitrogen is accessible for giving away to metal ions. An ideal projected grounded on the scheme chitosan-Cu, relate the pH dependency on the amount of accessible positions for interacting in polysaccharide backbone. At pH < 6, the complexation involves only one NH_2 group and three hydroxyls or H_2O molecules, while at pH > 6.7 is likely to have two NH_2 involved in the complex formation. For higher pHs, i.e., 7-9, the deprotonation of hydroxyl groups are considered to occur and the predominant complexation is ruled by two -NH_2 and two hydroxyl groups are dissociated. 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. It is unquestionable that chitosan molecules in bacteria surrounds might complex metals and blockage some essential nutrients to flow, contributing to cell death. Nevertheless, this is, evidently, not a determinant antimicrobial action since the sites available for interaction are limited, and the complexation reaches saturation in the function of metal concentration.

Gram-Positive Bacteria

Antibacterial effects of chitosan on gram-positive bacteria is the non-covalent binding of chitosan to teichoic acid incorporated in the peptidoglycan layer. The surface localized teichoic acid molecules are essential for cell division, and interaction with chitosan can impair this process, and possibly other means equally crucial for bacterial growth.

The roles of teichoic acids are to protect the cells against environmental stress, to control the enzymatic activity, and to assure a cationic concentration of the cell surface to facilitate the binding of the cell to receptors. The significance of teichoic acid towards chitosan action was shown on *S. aureus* mutants in genes involved in its biosynthesis. The mutant species of *S. aureus* were more resilient compared to the wild type. This proves that poly-anionic teichoic acids are the target sites of chitosan antibacterial activity towards gram-positive bacteria. At least in the case of small molecules of chitosan (shorter than 5 kDa) it was advocated that the polymer can enter in the bacterial cell and block the synthesis of DNA emphasizing the fact that the molecular weight of chitosan is an essential factor that can affect the mode of action of this polymer. There are articles describing the antibacterial activity of chitosan in the form of nanoparticles. At least for *S. aureus*, the nanoparticles of chitosan proved to have a lower bactericidal concentration (4 μg/mL) compared to soluble chitosan (32 μg/mL). The antimicrobial activity is improved when chitosan nanoparticles are loaded with cupper (2 μg/mL). There are reported cases when chitosan films have not shown any antimicrobial activity, at least against *Staphylococcus aureus* and *Staphylococcus epidermidis*, although the chitosan**.**

Gram-Negative Bacteria

One mechanism that is believed to be involved in the interaction of chitosan with gram-negative bacteria is correlated to the chelation effect of chitosan with cations when the pH is above pKa. Another mechanism of chitosan is its electrostatic interaction with anionic parts of lipopolysaccharides from the outer membrane of gram-negative bacteria. It is also possible that chitosan (at least polymers with low molecular weight) pass through the membrane and interferes with DNA/RNA synthesis. Taking into account the difference in MW of the chitosan-based products, it appears that oligo-chitosan has a lower antibacterial activity than small, medium and high MW chitosan. The differences in antibacterial activities of unlike chitosan with different MW are rather small and seem to be mostly dependent on the type of bacteria. Considering the fact that it was observed to have a higher antimicrobial activity with increasing the degree of deacetylation, electrostatic interactions could be the major factor determining the antibacterial activity of chitosan. Chitosan (pKa, 6.3-6.5) has the highest antibacterial activity at low pH due to the protonated amino groups. The reaction of chitosan is more versatile than cellulose due to the presence of -NH2 groups. Like cellulose, chitin and chitosan can undergo many reactions such as etherification, esterification and crosslinking. The main parameter that affects the characteristics of chitosan is its molecular weight and degree of deacetylation (representing the proportion of deacetylated units. Unlike other naturally occurring polysaccharides such as cellulose, dextran, pectin, alginic acid which is either acidic or neutral, chitosan is highly basic in nature. Due to this unique property, chitosan has several functional properties such as polyoxy salt formation, ability to form films, chelate metal ion and optical structural characteristics. Chitosan is non-toxic therefore it is used in food industry. Microcrystalline chitin (MCC) is used as flavoring and coloring agent, shelf life as well as dietary fiber in baked food. Chitin and chitosan are inexpensive and non-hazardous. They can be used for the preparation of dosage forms in commercial drugs such as spread dry chitosan acetate and ethyl cellulose can be used as new compression coats for 5-aminosalicylic acid (ASA) tablets. Chitin/chitosan controlled delivery systems are in developing stage. It is being used for a wide variety of re-agents in several environments. That's why chitosan is more effective than chitin. Quaternized chitosan derivatives have a better solubility than chitin and raw chitosan, and an improved antibacterial activity, due to permanent positive charges (Viegas de Souza et al., 2013). Other non-covalent interactions between chitosan and molecules from the bacterial surface can be considered to explain the mechanism of chitosan antibacterial activity. For example, chitosan can interact with cholesterol molecules and destabilize the bacterial membrane [18].

It was confirmed that the antimicrobial activity increases with the chain length of the alkyl substituent. Hydroxypropyl and carboxymethyl chitosan derivatives also have antibacterial activities. Hydroxypropyl chitosans, grafted with maleic acid are soluble derivatives of chitosan and at neutral pH present an antibacterial activity higher than that of raw chitosan. Although carboxymethyl chitosan derivatives can have both negative and positive substituent groups, it seems that the influence of carboxymethyl part is less significant than the presence of positive charges on the polymer chain, or its molecular weight. Other types of chitosan derivatives have also shown improved antibacterial activities. For example, acylthiourea chitosan derivatives have higher antimicrobial activity against *S. aureus* and *Sarcina sp*. Similarly, thymine-chitosan, sulfonated chitosan [20], and alkyl sulfonated chitosan [20] showed a superior antimicrobial activity against *S. aureus*. Chitosan and its derivatives have been shown to have antimicrobial activities and the results are summarized in table 2.

Applications of Chitosan

Areas of particular CS or CNSP applications in the past 16 years are the food and pharmaceutical industry, including tissue engineering and drug transport, which correspond to 20 and 21%, respectively. However, the versatility of CS applications can be demonstrated by the variety of uses in many areas, ranging from metal-contaminated water purification to the formation of nanotubes and use as an antimicrobial agent. This versatility directly contributes to the growth of studies conducted with this macromolecule, which is intrinsically related to new applications of this biopolymer. The Food and Drug Administration (FDA) agency in the U.S. approved CS as a food additive in 1983. Since it is considered a Generally Recognized as Safe (GRAS) compound, CS has, thus, been widely applied as a functional food, in environmental protection and as a safe biotechnology product to be used to promote health in human beings and animals. Many biological activities have been reported for CS, such as antimicrobial, anticancer, antioxidant, and immune stimulatory effects, that are dependent on its physicochemical properties (Table 1). Food applications have already been approved by the Regulatory Agencies regarding food consumption and drug administration in Japan, Italy and Finland, as well as the U.S.58 (Figure 3).

Food Industry

Another industry that can benefit from the use of antimicrobial polymers is the food industry. Here, the main application of polymers with antibacterial properties is the realization of packages that prevent the development of microbial cells. Because of its favorable properties of negligible human toxicity and antibacterial effectiveness, nisin was approved to be used as a food preservative. Nisin was impregnated in films created from chitosan-poly-lactic acid, from where it was slowly released during the period of validity of food products. These films have shown to have high antimicrobial activity against *Staphylococcus aureus* [21]. Chitosan based products were used to enhance fish preservation during storage to improve the quality of fresh-cut broccoli or to control bacterial contamination during brewing. Another advantage of using chitosan based-product in food packages is the fact that it was found

Table 2: Antimicrobial activities of Chitosan and its derivatives (Yong et al., 2015).

Gram-negative bacteria	*E. Coli ATCC 25922*	Chitosan 8 μg/mL chitosan nanoparticles 0.0313 μg/mL; Cu loaded chitosan nanoparticles 0.313 μg/mL
	E.Coli 0157	a-Chitosan 9 μg/mL a-Chitosan 9 μg/mL
	P. aenginosa	Chitosan 0.0125%; Chitosan-Zn complex 0.00625%; a-chitosan 9 μg/mL, a-chitosan 9 μg/mL, N-N diethyl-Nmethylchitosan 32 μg/mL
	P. mirabilis	Chitosan 0.025%, Chitosan-Zn complex 0.00625%,
	S. enteritidis	Chitosan 0.05%, Chitosan-Zn complex 0.00625%
Gram-positive bacteria	*S. aureus*	Chitosan 0.05%, Chitosan-Zn complex 0.00625%, a-Chitosan 9 μg/mL, a-chitosan μg/mL, N-ethyl-NN-dimethyl Chitosan 4 μg/mL
	S. aureus ATCC 25923	Chitosan 8 μg/mL, Chitosan nanoparticles 0.125 μg/mL, Chitosan nanoparticles 0.0625 μg/mL
	Corynebacterium	Chitosan 0.025%, Chitosan-Zn complex 0.0313%
	S. epidermidis	Chitosan 0.025%, Chitosan-Zn complex 0.0125%, a-Chitosan 5 μg/mL, a-Chitosan 5 μg/mL
Fungi	*C. krusei*	Chitosan 5 μg/mL
	C. globrate	Chitosan 20 μg/mL
	P. digitatum	Chitosan 65 μg/mL
	P. italicum	Chitosan 57.5 μg/mL
	F. proliferatum	Chitosan 2.5 μg/mL
	H. avellanea	Chitosan 2.5 μg/mL
Virus	IC_{50} of cytopathic effect by $HIV\text{-}1_{mb}$	WMQ-Chitosan oligomers 48.01 μg/mL
	IC_{50} of p24 production by $HIV\text{-}1_{mb}$	QMW-Chitosan oligomers 6.35 μg/mL
	IC_{50} of p24 production by $HIV\text{-}1_{bl}$	WMQ-Chitosan oligomers 98.37 μg/mL
	IC_{50} of production by $HIV\text{-}1_{KTMDR}$	QMW-Chitosan oligomers 81.03 μg/mL WMQ- Chitosan oligomers 144.02 μg/mL
	IC_{50} of luciferase expression by $HIV\text{-}1_{RF}$	QMW-Chitosan oligomers 68.13 μg/mL WMQ-Chitosan oligomers 163.94 μg/mL
	IC_{50} of interaction between gp41 and CD4 By HIV-1	QMW-Chitosan oligomers 38.13 μg/mL WMQ-Chitosan oligomers 51.48 μg/mL

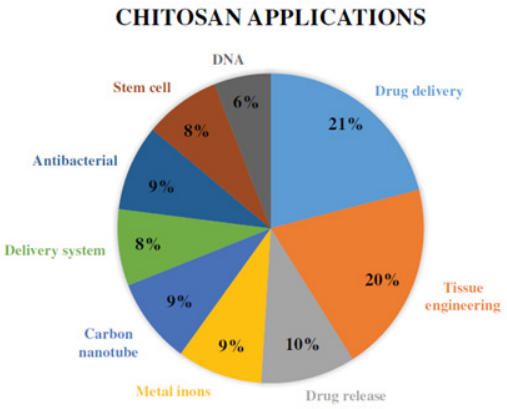

Figure 3: Chitosan applications in various fields.

to have noticeable improvement in the sensory quality during storage. This was observed in the boxes of chicken meat [22], cherry tomato fruits, and the fruits or red table grapes.

Spoilage and Microbial Growth Control

Among the different chitosan bioactivities, perhaps the most applicable in the food chain production is its antimicrobial activity, enhancing food safety and preservation, while also impacting biosecurity, the food business and community health, since it can be effective in controlling foodborne pathogens without antibiotics. The antimicrobial activity of chitosan is influenced by several intrinsic and extrinsic factors, which can be classified into 4 categories (a) microbial factors related to the species of the target organism and cell age; (b) chemical properties of the chitosan molecule (density of positive charges, MW, concentration, hydrophilic/hydrophobic characteristics and chelating potential); chitosan physical state and water solubility and (d) environmental factors (ionic strength of the medium, pH, temperature and pathogen exposure time pathogen).

Chitosan presents antimicrobial activity against a broad spectrum of microorganisms, including Gram-positive and Gram-negative bacteria, filamentous fungi and yeast. Chitosan exerts antifungal effects on different fungi developmental stages by suppressing sporulation, spore germination, mycelia growth, spore viability and the production of virulence factors. The observed antibacterial activity is a complicated process that differs between Gram-positive and Gram-negative bacteria, due to their distinct cell surface characteristics. Discrepancies exist however, since in several studies. Chitosan displays stronger antibacterial activity against Gram-negative when compared to Gram-positive bacteria, while in other studies Gram-positive bacteria were more susceptible. Some studies described the mechanism of action as a result of the interaction between the chitosan macromolecules, that are positively charged, and the membrane of the microbial cell, that is negatively charged, with subsequent breakage and, consequently, leakage of intracellular components, including proteins and nucleic acids causing alterations in cell membrane permeability, chitosan antimicrobial activity is enhanced by its ability to act as a chelating agent, selectively binding to trace metals, thus inhibiting the toxin production and microbial growth.

Chitosan also activates various defense processes in the host tissue, acting as a binding agent to water and as an inhibitor to several enzymes. The alternative mechanism is microbial growth inhibition by the interaction between the positively charged chitosan, intracellular fungi and bacteria DNA. It consequently inhibits the RNA and protein synthesis, is considered effective only for low molecular weight chitosan (LMW-CS), which can penetrate microorganism cells. Although this mechanism of action based on ionic interactions with DNA is still controversial. It could explain the inhibition of both Gram-positive and Gram-negative bacteria and fungi by chitosan, establishing a similar mechanism of action for all microorganisms, regardless of their cell membrane structure.

Chitosan definitely demonstrates a strong inhibitory effect on

microorganisms growing in low pH media, confirmed by the fact that its antimicrobial activity is weakened with increasing pH values, causing low protonation of amino groups, which in turn also influences the solubility of the biopolymer. Due to apparent discrepancies, the interactions of chitosan and its derivatives with the outer membrane barrier of bacteria should be better understood, but they certainly depend on the binomial combination of MW and DD. LMW-CS of less than 10 kDa have greater antimicrobial activity compared to high molecular weight chitosan (HMW-CS), ranging between 10-500 kDa. However, studies conducted with hydrochloride D-glucosamine, demonstrated that the chitosan monomer was not effective in inhibiting bacterial growth, suggesting that the antimicrobial activity of chitosan is related not only to the cationic nature of the de-acetylated glucosamine, but also to the chain length of the biopolymer. Although some results regarding the bactericidal activity of LMW-chitosan are comparable, it has been reported that, depending on the bacteria strain, the conditions of the biological assays and chitosan physico-chemical characteristics (MW and DD), the results are not always in agreement with each other.

Studies testing the 9.3 kDa chitosan have shown inhibition of *E. coli* growth, while the 2.2. kDa chitosan promotes the growth of the same bacteria. On the other hand, the 4.6 kDa chitosan was most active against Gram positive bacteria, yeast and fungi. Thus, results with LMW-chitosan are still somewhat controversial and unclear, indicating that additional experimental data are required to understand the antimicrobial mechanisms that take place. Evaluating these studies, it seems that chitosan antimicrobial action depends on MW, but also on the different physical states of its derivatives, that may, thus, provide distinct mechanisms of growth inhibition. Similar to the LMW-chitosan, water-soluble ultrafine nanoparticles can penetrate bacterial cell walls, interfering in the microorganism nuclei by binding to DNA and RNA, as well as inhibiting both the mRNA and protein synthesis. Despite the apparent discrepancies regarding chitosan effects, the natural antimicrobial properties of chitosan and its derivatives have resulted in their extensive use as commercial disinfectants, since some chitosan have an advantage over other disinfectants due to their high antimicrobial and broad spectrum of activity but low toxicity to mammalian cells, allowing them to be discarded with less damage to the environment [23]. Recently, it has been demonstrated that bioplastic films composed by chitosan and its derivatives also display antimicrobial activity. The potential use of CS-based films and their derivatives may be directly dependent on particle size, film thickness and the structure of the matrix-forming fibers. In a previous study, two chitosan films with distinct structures and particle sizes were tested, where particles ranged between 37-40 μm. The films exhibited superior antimicrobial activity against *S. aureus* when smaller-sized, spherical shaped particles were used, which provides greater specific surface contact. It is accepted that chitosan nanoparticle-based films can be effectively used in the food industry, as they provide various benefits, including good edibility, biocompatibility with human tissues, an aesthetically pleasing appearance, displaying barrier properties against pathogenic microorganisms, toxicity, and are non-polluting and made from low cost material.

Preservation of Fresh and Processed

Food Quality

Conventional food packaging systems are supposed to passively protect food, acting as a barrier between the packaged food and the surrounding environment. Antimicrobial food packages have decreased the growth of pathogenic and a spoilage microorganism on food surface, where microbe dominates. Antimicrobial nanocomposite methods are mainly interesting, since resources in the nanoscale variety have a greater surface-to-volume ratio when paralleled to their microscale counterparts. Nano-materials are thus more efficient, since they are able to produce more copies of microbial molecules and cells. Chitosan films have shown potential to be used as a packaging material for the quality preservation of a variety of foods. Chitosan has also been widely used in antimicrobial films to provide edible protective coating, and in the dipping and spraying of food products, due to its antimicrobial properties. Coatings based on chitosan have been used as an antifungal agent, which resulted in the enhancing of germination and quality of artichoke seeds. The effect of the formulation and thickness on seed germination (G%), fungi activity and vegetative growth were evaluated, and results indicated that significant differences between treatments regarding seed germination were observed, where all chitosan coatings reduced the number of fungi strains and increased plant growth. In another study, apples were heat-treated at 38°C for 4 days (Heat treatment) before or after being coated by 1% chitosan. The combination of the heat treatment plus chitosan fruit coating showed the lowest respiration rate, malondialdehyde levels, membrane leakage, ethylene evolution and the highest firmness and consumer acceptance among the treatments [24]. When applied on wounded wheat leaves, chitosan induced lignification and, consequently, restricted the growth of nonpathogenic fungi in wheat. Chitosan also inhibited the growth of *A. flavus* and aflatoxin production in liquid cultures, pre-harvest maize and groundnut, and enhanced phytoalexin production in germinating peanut plants. In addition, chitosan also improved the microbiological quality of fresh cut broccoli. Edible coatings consisting solely of chitosan or a combination of chitosan with other biopolymers, such as sodium caseinate, were applied to carrots, cheese and salami. The sodium caseinate/chitosan films inhibited bacteria and yeast growth and can be potentially applied to several food matrices. In other studies, acetic or propionic acid were incorporated into a chitosan matrix in Bologna ham, baked ham and fresh salmon, with positive effects. The application of high concentrations of chitosan is considered effective in the control of fruit coloring, decreasing fruit darkening and maintaining anthocyanin content, a pigment directly related to food freshness. Enzyme activity is influenced by the presence of O2 concentrations inside the fruit. Chitosan forms a physical barrier around the fruit, and, consequently, darkening is reduced. Furthermore, the positive charges present in the coating can stabilize anthocyanin pigments, aiding in maintaining fruit color, sensory attributes and antioxidant features [25]. Other studies were performed using chitosan included biodegradable packaging and edible coatings for the preservation of fresh-cut fruits and vegetables [26]. The use of chitosan-based edible films was also tested to preserve the quality of pork meat hamburgers. Their importance in the modulation of the oxygen permeability of films in order to avoid the undesirable effects of metmyoglobin (MtMb) formation was promoted by lower partial oxygen pressure in the surface of the coated hamburgers. The interest in edible coatings is on the rise, due to their ability to reduce fruit respiration and transpiration rates, and consequently increases storage time and consistency retention. The use of chitosan also decreased the respiration rate and production of ethylene in raspberries and has a high selective permeability to respiratory gases, acting as a passing barrier for O_2. This gas control between the fruit and the environment reduces respiration rates, as well as the enzymatic action of 1-carboxylic-1- aminocyclepropane oxidase and synthases, which are highly influenced by the presence of O_2. The decrease of mass loss with chitosan applications has also been related to the formation of a selective barrier around the surface

of the fruit, improving moisture loss and reducing respiration and the main metabolic processes that lead to loss of water [27]. Chitosan has also been used for juice clarification with good results for apple, carrot, grape, lemon, orange and pineapple juices. Chitosan anti-oxidative properties, especially in food products that contain high amounts of unsaturated fatty acids, which are sensitive to oxidation during storage, have also been reported. Chitosan scavenges free radicals or chelates metal ions from the donation of hydrogen or lone pairs of electrons, increasing its antioxidant ability and free radical scavenging activity [28].

Food Nanotechnology

The United States Department of Agriculture implemented a project to develop green nanotechnology aimed at eliminating foodborne pathogens [29]. In this study, the USDA envisaged the development of a nanoparticle wash treatment with the capability of significantly reducing or eliminating pathogenic bacteria associated with fresh or fresh-cut fruits and vegetables, to be used with minimal processing. The specific tasks involve the design, synthesis and characterization of ultra-potent chitosan nanoparticles coated by antimicrobial peptides, the evaluation of peptide-enhanced nanoparticles as a lysis agent in realistic food processing environments and the development of a postharvest nanoparticle electric field treatment for decreasing the bacterial loads of fresh fruits and vegetables [30]. Food-grade nanoparticles and micro particles can be fabricated from a range of different ingredients, including biopolymers, lipids, surfactants, and minerals. Biopolymer particles are often classified according to their structures, such as (filled) hydrogel particles, inclusion complexes, and polyelectrolyte complexes. However, the dimensions of the biopolymer particles alter their functional performance in foods [31]. Nanoparticles composed of different materials (including silicates, silver, magnesium, and zinc oxide) have been incorporated into packaging materials, where they afford greater protection to foods due to several effects that include reduced gas and odor permeation, blocking of ultraviolet radiation, enhanced mechanical properties and thermal stability. Studies of the health effects of these particles are especially important, because the packaging may have direct contact with the food. Functional bioactive ingredients have received much attention in recent years from the scientific community, consumers and food manufacturers. Potential functional bioactive ingredients include vitamins, probiotics, bioactive peptides, antioxidants, among others. Micro/nanostructured chitosan can be used as bioactive ingredient carrier and have the potential for the development of novel encapsulation or immobilization carriers [32]. They also display muco-adhesive properties, which may prolong the contact, time between bioactive and absorption sites, thereby increasing absorption. Chitosan particles are especially useful for the encapsulation of hydrophilic macromolecules, which are associated through electrostatic interactions or hydrogen bonding [33]. Encapsulation of bioactive compounds is a relatively old concept and was initially focused on protecting vitamins from oxidation. Since then, many other types of active ingredients have been the focus of encapsulation technologies, and encapsulation is currently one of the most intensively studied application areas of micro particle and nanoparticle biopolymers. Generally, two types of active ingredients can be distinguished, bioactive molecules (nutraceuticals) and bioactive living cells (probiotics). Chitosan was successfully used in applications regarding the encapsulation of different bioactive compounds [34] chitosan produces biopolymer particles to encapsulate proteins in combination with gellan gum [35] and colon specific delivery systems for peptides and proteins demonstrated that a chitosan/vitamin C nanoparticle system successfully increased the shelf life and delivery of vitamin C in rainbow trout during 20 days of storage. Demonstrated that chitosan nanoparticles could be used to encapsulate DNA, which was then beneficially incorporated into shrimp feed to protect them from white spot syndrome virus. Other additives encapsulated by chitosan described in the literature are shark liver oil in combination with calcium alginate beads and tuna oil droplets. The use of chitosan nanoparticle-based edible films as food coating has been reported with respect to a variety of foodstuffs, including cheese and meat products, such as fermented sausages. In another study, the possibility of producing food-grade stable nanoparticles with simple processing techniques was demonstrated, using lecithin and sodium caseinate, which could be further used as base systems for the production of Nano capsules [36].

Water Treatment

Chitosan is observed as one of the largely effectual materials for adsorption of contaminants in water treatment systems. The presence of amino and hydroxyl groups in chitosan allows its adsorption, interactions with contaminants such as dyes [37], metals [38] and organic compounds, etc. Besides, these functional groups are subjected to modifications (cross-linking and grafting), which enhance the absorption efficiency and specificity [39]. For example, crosslinking the functional groups of chitosan improves the adsorption efficiency of chitosan at low pH. Grafting with sulfur or nitrogen improves specificity and capacity for some metal ions [40]. The dye adsorption performance by unmodified chitosan is excellent; however, its low stability has prompted many researchers to consider modifying them. Dissimilar alterations (attached amino group, carboxyl group, sulfur group and alkyl group; cross-linked epichlorohydrin, ethylene glycol diglycidyl ether, glutaraldehyde and tripolyphosphate) were considered and engaged to develop the adsorption efficiency as well as the mechanical and physical properties. The original properties of chitosan have been altered and are more suitable for the adsorption of different dyes. Chitosan can perform as a chelating polymer for attaching poisonous heavy metal ions. Metal cations could be chelated by the chitosan amine groups in near-neutral circumstances. For metal anions, the adsorption depends on electrostatic attraction on protonated amine groups in acidic conditions. Chitosan modified with different derivatives offers a wide range of properties for specific adsorption of metal ions. Biological contaminants, comprising phenolic compounds, polycyclic aromatic hydrocarbons, organic pesticides, and herbicides, produce health and environmental harms due to their lethal effects together with meager biodegradability. Chitosan adsorption for organic pollutants offers high adsorption capacities, insensitivity to poisonous substances, good modifiability as well as recoverability. The method relies on the features together with the nature of the toxins and is complex. There is no easy theory or single procedure to clarify adsorption features. Some interactions related to the adsorption mechanism include partition, diffusion, cation exchange, hydrogen bond, Van der Waals forces, dipole-dipole interactions, and electrostatic interaction. Quaternary tetra-alkyl-ammonium chitosan derivatives can be utilized in the form of a cheap perchlorate-specific solid-phase extraction anion exchange cartridge in conjunction with colorimetric analysis for perchlorate removal or analysis [41].

Magnetic hydroxypropyl chitosan/oxidized multi-walled carbon nanotubes composites were good for the elimination of lead ions from aqueous solutions with pseudo-second-order kinetics. The optimum interaction limit and pH were 120 min and pH 5.0, respectively. Sips model is more appropriate than Langmuir, Freundlich, and Dubinin-

Radushkevich models for describing the adsorption process, which was endothermic and spontaneous (Wang et al., 2015a). Protonated poly-amidoamine attached chitosan beads loaded with Zr (IV) ions, created by amination of chitosan beads by ethylenediamine via Michael addition and trailed by protonation, eradicated fluoride ions from aqueous solutions with higher selectivity than other metal ions. The adsorption was spontaneous and endothermic (Prabhu & Meenakshi, 2015). Chitosan nanofibers formed by electrospinning with 5% chitosan in acetic acid as the spinning solution was cross-linked with glutaraldehyde to eliminate chromium from water by adsorption with a pseudo-second-order kinetic stereotype, following a diverse isotherm of Freundlich and Langmuir. The maximum nanofiber adsorption capacity was more than double of chitosan powders. Sodium, calcium magnesium, nitrate, and chloride, but not sulfate ions, had nil or negligible effect on adsorption, which involved hydroxyl as well as amino groups of chitosan [42]. Chitosan along with polyphenol oxidase, was used in conjunction with elimination of bisphenol derivatives from aqueous solutions based on adsorption of enzymatically generated quinine derivatives on chitosan beads or chitosan powders. The maximum temperature and pH settings were 40 °C and pH 7 for bisphenols B, E, O, and Z; 30 °C and pH 7 for bisphenols C and F; and 40 °C and pH 8 for bisphenol T. The removal time could be reduced by using more chitosan beads or chitosan powders of a smaller size [43]. Sorption of Cd(II) ions onto cross-linked small-molecular-mass chitosan pyruvic acid derivative followed Langmuir isotherm prototype and showed pseudo-second-order kinetics. Two levels of Cd(II) concentration (1 or 3 mg/L, temperature (45 or 70 °C) and solution pH (6.0 or 10.0) were measured. The features and their interface influence on the efficiency of cadmium elimination followed the order: Cd (II) concentration > solution pH > interaction between solution pH and Cd(II) concentration > interaction between solution pH, temperature and Cd(II) concentration.

A procedure for concurrent elimination of cyanobacterial harmful algal blooms and public health threat was devised by employing chitosan-modified local soil flocculation and microcystins, which may pose a microbe-modified soil capping. Breaking of toxin was a result of the joint actions of flocculation plus microcystin-degrading bacteria in the covering material, which prevents dilution of bacterial biomass, enriches the algal cells, sequesters the liberated toxins, and promotes toxin biodegradation. Chitosan nano-rod with a minimum particle size smaller than 100 nm was produced by crosslinking chitosan of low molecular weight with polyanion sodium tripolyphosphate and was then physicochemical characterized (using AFM, DSC, FT-IR, SEM, TGA, and XRD) for wastewater treatment. Its sorption capability and sorption isotherms for chromium were considered. By what means the initial concentration of chromium ions, sorbent amount, and extent of shaking and solution pH influenced sorption ability were also inspected. The findings disclosed that in the solid-state nano chitosan was rod-shaped, and it effectively sorbed chromium (VI) to chromium (III) ions. The sorption capability of chitosan nanoparticles is extraordinarily high; furthermore, the adsorbent supports multilayer adsorption grounded on the Langmuir, the Freundlich, and the Temkin sorption isotherms. The adsorption follows pseudo-second-order kinetics, which infers the transformation of chromium (VI) to chromium (III). Hence nano chitosan is a good biosorbent for chromium removal from water (Sivakami et al., 2013).

Dry Mouth Syndrome Treatment

Chitosan-thioglycolic-mercaptonicotinamide conjugates, which were non-toxic against Caco-2 cells and synthesized by the oxidative S-S coupling of chitosan-thioglycolic acid with 6 mercaptonicotinamide, manifested improved swelling and cohesive characteristics compared with unmodified chitosan and were promising for therapy of dry mouth syndrome in which lubrication and mucoadhesiveness of the mucosa are wanting [44]. Gene Silencing in Disease Vector Mosquito Larvae Chitosan/interfering RNA nanoparticles mixed with food and consumed by larvae of *Aedes aegypti* (vector of the dengue and yellow fever) and *Anopheles gambiae* (vector of the primary African malaria vector) represented a methodology that can be applied to many insects and pests.

Cardiovascular Diseases Treatment

Administration of chitosan-oligosaccharides by gastric gavage to apolipoprotein E deficient mice (apoE-/-) fed a high fat diet for 12 weeks lowered triglyceride and cholesterol levels in non-high density lipoprotein fractions, weakened atherosclerosis, amplified atherosclerotic plaque strength, upregulated hepatic expression of small bulk lipoprotein receptor, scavenger receptor BI and also the appearance of macrophage scavenger receptor BI and ATP attaching cassette transporter A1. There was no effect on the plasma lipid level in LDL-R mice with a deficiency of low-density lipoprotein receptors and cholesterol absorption in wild-type mice [45].

Treatment of Age-Related Diseases

The potential utility of chitosan, chitooligosaccharides, their derivatives, and chitosan-based functional food in forestalling and therapy of aging-associated diseases has been discussed in this paper. The pathophysiological roles played by oxidative stress, oxidation of low-density lipoprotein, an increase of tissue stiffness, conformational changes of protein, aging-associated, and chronic inflammation have been reviewed.

Drug Delivery System

Chitosan has been widely used in the pharmaceutical industry for drug delivery systems in different forms, like tablets, microspheres, micelles, vaccines, nucleic acids, hydrogels, nanoparticles, and conjugates. Chitosan and its derivatives can be used in drug delivery systems in both implantable as well as injectable forms through oral, nasal, or ocular routes. Besides, they facilitate trans mucosal absorption, which is essential in nasal and oral delivery of some polar drugs like peptides along with protein vaccines for their administration [46]. It is commonly used as an excipient in tablet formulation for oral medication. High-molecular-weight chitosan is more viscid and postpones the discharge of the active element, extends the period of drug action, recovers healing efficacy as well as decreasing the harmful effects of oral tablets. Chitosan microspheres have been broadly explored for measured discharge of drugs and vaccines via oral and nasal distribution. They were created by complexation among the cationic chitosan in accumulation to the anionic complexes such as tripolyphosphate or alginates. Different drugs or vaccines were loaded in the microspheres, they were protected, especially drugs which are protein in nature, in the digestive tract and absorbed through the paracellular route on the epithelial layer.

Chitosan surface activity is low because it does not retain any hydrophobic parts. It can be developed by chemical adjustments at its glucosidic group with a hydrophobic substituent. The chitosan create micelles with an exterior hydrophilic protection and an interior hydrophobic center. The hydrophobic drugs were secured in the center with improved solvability and bio-accessibility. The chitosan micelles

were formed by the electrostatic repulsions between oppositely charged polymers. The chitosan hydrogels are three-dimensionally structured hydrophilic polymers that can absorb and hold up to thousands of times more fluids than their dry weights used in drug delivery. The drugs are loaded in the hydrogels by diffusion, entrapment, and tethering. Usually, the loaded hydrogel is injected into the body, and the drug is diffused into the neighboring tissues. The recent development of *in situ* forming depots using chitosan-based hydrogel has attracted much attention as a new method for controlled drug release. The original thermos sensitive chitosan-based polymer is in solution form at room temperature. When it is inoculated into the body, it produces semi-solid hydrogels at the physiological temperature. It showed protection for the drugs from physiological degradation, combined with prolonged and steady release of drugs. Chitosan nanoparticles demonstrate exceptional biodegradability plus biocompatibility, which have been considered largely as drug carriers. They can be administered through non-invasive means such as ocular, nasal, oral, and pulmonary routes. The drugs are secured from chemical and enzymatic dilapidation in the digestive system. Besides, they bind strongly to mucus, which enhances the adsorption through intestinal epithelial cells [47].

They can be prepared by several methods such as ionotropic gelation, emulsion cross-linking, emulsion-solvent extraction, emulsification solvent diffusion, emulsion-droplet coalescence, complex coacervation, reverse micro emulsion technique in addition to self-assembly. The most common method is the ionotropic gelation method, in which the preparation conditions are mild and less time-consuming. It is centered on natural accumulation of positively-charged chitosan with a negatively-charged sodium polymer such as tripolyphosphate. The drugs are dissolved in either component. Then a nanoparticle suspension is formed upon addition of the other element under vigorous agitation. The chitosan derivatives conjugated with antitumor agents to create a right partner for targeted drug delivery in cancer treatment. They manifest reduced side effects compared with the original drug because of a predominant distribution into the cancer cells and a gradual release of the free drug from the conjugates.

An intermolecular compound formed from a 1:1 ratio by weight of 30-kDa chitosan and sulfobutyl ether â-cyclodextrin was less soluble than either component. At pH 1.2, the drug famotidine was gradually discharged from the less-solvable chitosan/sulfobutyl ether â-cyclodextrin complex produced rapidly on the tablet upon disclosure to water, followed by disbanding of the inter-polymer complex and, finally, breakdown of the tablet. At pH 6.8, gel creation by chitosan was accountable for the slow discharge. The slow release of the tablet was seen in the drug absorption *in vivo* following treatment of rats via the oral route [48]. Glycol chitosan Nano gel uptake took place essentially by means of endocytosis mediated by flotillin-1 and Cdc42 and macropinocytosis with the participation of the actin cytoskeleton, and internalization mechanisms through the folate receptor. About half of the nano gel population was found in endolysosomal compartments, while the rest was located in undefined cytoplasmic compartments at the end of seven hours of incubation with HeLa cells. Glycol chitosan nano gels may be useful as drug delivery vectors for targeting different intracellular compartments [49].

Controlled-release, floating and mucoadhesive beads of glipizide were developed by polyionic complexation technique using chitosan as cationic and xanthan gum as anionic polymers. The beads displayed pH-dependent swelling kinetics, good bio adhesive strength and comparable floating capacity in the gastric fluids. Altering the chitosan to xanthan gum ratio did not affect the drug release [50]. Insulin was physically and chemically stable in a polyelectrolyte complex composed of insulin and 13-kDa low-molecular-weight chitosan in Tris-buffer (pH 6.5). Solubilization of the insulin-low-molecular-weight chitosan polyelectrolyte complex in a reverse micelle system, given to hyperglycemic rats, constituted an oral bioactive insulin delivery system [51]. The colon is a drug delivery target because of the long transit time and thus a prolonged drug absorption time. Progesterone has an abbreviated half-life, much first-pass metabolism, and low oral bioavailability oral Zn-pectinate/chitosan multi particulate system prepared by ionotropic gelation, allowing increased oral bioavailability of progesterone as well as progesterone residence time in plasma for colonic-specific progesterone delivery, was developed. Negligible progesterone release in simulated gastric fluids was observed, but there was a burst release at pH 6.8 and sustained release at pH 7.4 [52]. Incorporation of glutaraldehyde augmented the drug entrapment efficacy of the Boswellia resin-chitosan polymer composites in phosphate buffer (pH 6.8). The drug release rate surged to 92% as the gum resin concentration in the composites was elevated to 80%. The composites released 60%–68% drug load in seven hours [53]. Water in oil forming a nano sized systems containing low-molecular-weight chitosan-insulin polyelectrolyte complexes was constructed and their hypoglycemic activity was assayed in diabetic rats. The 1.3-kDa chitosan with 55% (among 55%, 80% and 100%) deacetylation possessed the most potent hypoglycemic activity among three molecular weights (namely, 1.3, 13 and 18 kDa) and different extents of deacetylation [54]. Higher retention of conjugate formed between chitosan and catechol derived from mussel adhesive proteins, in the gastrointestinal tract *versus* unmodified chitosan, owing to production of irreversible catechol mediated-crosslinking with mucin, may be advantageous for production of new mucoadhesive polymers to be employed for mucosal drug delivery [55]. Microcapsules loaded with fish oil were produced from oil-in-water emulsions by both membrane emulsification and ultrasonic emulsification employing *N*-stearoyl *O*-butylglyceryl chitosan as shell material. The microcapsules produced by membrane emulsification displayed a larger diameter and more desirable loading capacity and encapsulation efficiency. Microcapsules from both membrane emulsification and ultrasonic emulsification demonstrated sustained release of fish oil which had higher thermosability [56].

Medical Industry

The medical industry is one of the primary beneficiaries of any materials that present antibacterial activity. The surface of any medical instrument is susceptible to microbial infection. Although there is notable progress in materials and procedures, most hospital-acquired diseases derive from medical devices. To diminish biofilm development and to increase the long-term use of medical devices a coating copolymer of 4-vinyl-nhexylpyridinium bromide (VP) and dimethyl (2-methacryloyloxyethyl) phosphonate. (DMMEP) active against several pathogenic bacteria (*Staphylococcus ureus, Staphylococcus epidermidis, Streptococcus sanguinis, and Escherichia coli*) was proposed. Antimicrobial peptides, that are also involved in modulation of the immune response, antimicrobial wound-dressing containing antimicrobial peptides grafted to chitosan (Polycationic polymer) or to alginate (Polyanionic polymer) presented a high antimicrobial effect (in the range of 4-6 log reduction) for *Staphylococcus aureus* and *Klebsiella pneumonia* with no toxic effect against human dermal fibroblasts [57]. Composite gels based on chitosan and ZnO, containing gentamicin that was slowly released under planktonic and surface-attached conditions have shown highly effective antimicrobial activities against *Pseudomonas aeruginosa* and *Staphylococcus aureus*

[58-67]. The system has the advantages to be transferred to any other soluble antibiotic or any other type of drugs as the active molecules remain trapped in the Chitosan-ZnO composite gel, and, most important, when used in a wound dressing device, it maintained a moist environment to the wound.

Conclusion

Chitosan is chitin derived biopolymer with many interesting applications. Many of its applications in medicine, pharmacy, textile, or food industries are derived from antibacterial activities of chitosan based-materials. The effectiveness of antimicrobial properties can be modulated by selecting the range of molecular weight of the polymeric chains and the degree of acetylation of amino groups. Further improvement of antibacterial efficiency of chitosan based-materials can be realized by derivatization of amino or hydroxyl groups of monomeric units of the polymeric chain. Depending on the application, chitosan-based materials can be presented in the soluble form, films, or nanoparticles dispersed in a suitable environment. Thus, one can utilize the adjustable parameters of Chitosan to produce chitosan based-materials with antibacterial activities against gram-positive and gram-negative bacteria.

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