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Is Chitosan a New Panacea? Areas of Application

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1. Introduction

Polysaccharides are extremely common in nature and cellulose is the most common organic compound on the planet. It is said that the second most common polysaccharide in the world after cellulose is chitin. "Chitin is to shellfish what cellulose is to trees".

It's been more than two centuries since chitin was discovered formally and considered very important from the scientific and industrial point of view, as it has many applications in many different areas.

The development of commercial applications for chitin and chitosan has progressed. The first known use of chitosan was a durable, flexible film used as a component in the varnish applied to Stradivarius violins, however new efforts are changing its vision in the market. The emphasis on environmentally friendly technology has stimulated interest in biopolymers, which are more versatile and far more biodegradable than their synthetic counterpart.

The purpose of this chapter is to highlight the basic concepts of chemistry and the application of this polysaccharide that is gaining much interest due to the properties it presents and the many applications in various fields. Thousands of scientific articles have been reported in the last 20 years where companies appeared engaging and exploiting this material worldwide. Through investigation many questions have arisen but have not yet answered, however, this polysaccharide has been very successful in many applications.

Furthermore, this chapter aims to convince young readers to further research on possible technology that tend to care for the environment and health.

2. The origin and discovery of chitin

The universe began about 15 million years ago. Materials with high temperature and density were expanded, released energy, then cooled and gave birth to stars, planets and all living beings. The sun was born 5 billion years and 0.4 million years later gave birth to Earth.

Why talk about the birth of the earth? This is because the chitin could be a constituent of the first living cell. It actually came into existence long before the dinosaurs. In the late Precambrian period, two billion years ago, living cells appeared with nuclei containing chitin around it. In the Silurian period 440 million years, land plants appeared containing cellulose. Fish appeared in the Carboniferous period and later, the arthropods in the Devonian period. The first dinosaurs lived two hundred million years ago and during the second half of the Jurassic period the crab rich in chitin appeared.

After dinosaurs occupied the Earth for 100 million years, from the Jurassic to Cretaceous, they were extinguished by a comet that crashed into the Yucatan Peninsula 65 million years ago, but crabs and small animals escaped this catastrophe.

Since living beings appeared, cellulose and chitin have been beneficial in general and both maintained an ecological balance. Chitin is the animal version of the cellulose and it is the second most abundant in nature, but Professor M Peter has challenged that assumption by saying that Chitin is certainly a very abundant material even if much of it is not readily accessible for industrial use and suggested that hemicelluloses, which occur in conjunction with cellulose in trees and other plants, are actually more abundant than chitin. The hemicellulose component averages about half of the cellulose component, whereas the normal estimate of chitin production is that it is one whole order of magnitude less than that of cellulose. Another possible contender is lignin, which again occurs in conjunction with cellulose in most plants and, like hemicelluloses, averages about half of the cellulose component. A fourth possible contender is starch which like cellulose it is a major component of vegetable matter where it acts as a reserve material rather than a structural component [1].

The English word “chitin” comes from the French word *chitine*, which first appeared in 1836. These words were derived from the Greek word *chitōn*, meaning mollusk that is influenced by the Greek word *khitōn*, meaning “tunic” or “frock”. That word may come from the Central Semitic word **kittan*, the Akkadian words *kitû* or *kita’um*, meaning flax or linen, and the Sumerian word *gada* or *gida*. A similar word, “chiton”, refers to a marine animal with a protective shell (also known as a “sea cradle” [2].

It is normally accepted as a fact that chitin was first isolated from mushrooms and called “fungine” by the French chemist Henri Braconnot in 1811. Charles Jeuniaux suggested in a paper presented at the 1st International Conference of the European Chitin Society held in Brest in 1995, that chitin had previously been isolated from arthropod cuticle by the English scientist A Hachett in 1795. However, as pointed out by Professor Jeuniaux, Hachett only reported the presence in the cuticle of an organic material particularly resistant to the usual chemical reagents but did not investigate it further. Braconnot on the other hand carried out chemical analysis on his fungal culture, and reported the formation of acetic acid from it on treatment with hot acid, and concluded it was a new material. Braconnot may be considered the discoverer of chitin even though his name for the new material, ‘fungine’, was soon replaced by its current name “chitin” which was first proposed by Odier [3,4].

Chitin is a big molecule composed of β -1,4-N-acetylglucosamine (GlcNAc) monomers. There are three forms of chitin: α , β , and γ chitin. The α -form, is mainly obtained from crab and shrimp. Both α and β chitin/chitosan are commercially available [5].

3. Sources of chitin

In the book "Chitin" published by Muzarelli in 1977, we can find a complete list of organisms that contain chitin: Fungi, Algae, Cnidaria (jellyfish), Aschelminthes (round worm), Entoprocta, Bryozoa (Moss or lace animals), Phoronida (Horseshoe worms), Brachiopoda (Lamp shells), Echinurda, Annelida (Segmented worms), Mollusca, Arthropoda and Pogonophora [6]. Herring, P.J in 1979 wrote that chitin is the main component of arthropod exoskeletons, tendons, and the linings of their respiratory, excretory, and digestive systems, as well as insects external structure and some fungi. It is also found in the reflective material (iridophores) of both epidermis and eyes of arthropods and cephalopods (phylum: Mollusca) and the epidermal cuticle of the vertebrate Paralipophrys trigloides (fish) is also chitinous [7,8]. The main commercial sources of chitin are the shell wastes of shrimp, lobsters, crabs and krill. There are three forms of chitin: α , β , and γ chitin. The α -form, is composed of alternating antiparallel polysaccharide strands and is mainly obtained from crab and shrimp. α -Chitin is by far the most abundant; it occurs in fungal and yeast cell walls, krill, lobster and crab tendons and shells, shrimp shells, and insect cuticle. The rarer β -chitin is composed of parallel strands of polysaccharides, is found in association with proteins in squid pens [9,10] and in the tubes synthesized by pogonophoran and vestimentiferan worms [11,12]. It also occurs in aphrodite chaetae [13] as well as in the lorica, built by some seaweeds or protozoa [14,15,16]. And 2 parallel chains alternating with an antiparallel strand constitute gamma chitin and are found in fungi [15].



Figure 1. Chitinous structure of worm and insects

4. Chitin from crustacean

Currently most commercial production of chitin is based on extracting it from the exoskeleton of shrimp, prawn, crab and other crustaceans. This source contains a high percentage of inorganic material, primarily CaCO_3 and a rough calculation indicates that for every tonne of chitin produced, 0.8 tonne of CO_2 is released into the environment. In view of current concerns about global warming this cannot be considered to be a truly environmentally friendly process [3].

Another source of chitin that is more environmentally friendly, although much more limited in volume, is squid pen. This waste contains very little in the way of inorganic material and very little, if any CO₂ would be released in the extraction and purification process. Another and perhaps more sustainable source in the long run is vegetable chitin from fungal sources such as waste mycelia. There is extensive literature on the topic, but it is only recently that it has become commercially available [3].



Figure 2. Exoskeleton of crustacea, this is the source of commercial chitin

5. Chitin from fungi

Chitin is widely distributed in fungi, occurring in *Basidiomycetes*, *Ascomycetes*, and *Phycomycetes*, where it is a component of the cell walls and structural membranes of mycelia, stalks, and spores. The amounts vary between traces and up to 45% of the organic fraction, the rest being mostly proteins, glucans and mannans. However, not all fungi contain chitin, and the polymer may be absent in one species that is closely related to another. Variations in the amounts of chitin may depend on physiological parameters in natural environments as well as on the fermentation conditions in biotechnological processing or in cultures of fungi [4].

The chitin in fungi possesses principally the same structure as the chitin occurring in other organisms. However, a major difference results from the fact that fungal chitin is associated with other polysaccharides which do not occur in the exoskeleton of arthropods. The molecular mass of chitin in fungi is not known. However, it was estimated that bakers' yeast synthesizes rather uniform chains containing 120 ± 170 GlcNAc monomer units which corresponds to $24,000 \pm 34,500$ Daltons [4].

6. Chemical methods to prepare chitin

Several procedures have been developed through the years to prepare chitin; they are at the basis of the chemical processes for industrial production of chitin and chitosan. Various methods are reported in Muzzarelli's book such as: Method of Rigby (1936 and 1937); Hackman (1954); Foster and Hackman (1957); Horowitz, Roseman and Blumenthal (1957);

Whistler and Be Miller (1962); Takeda and Abe (1962); Takeda and Katsuura (1964); Broussignac (1968); Lovell, Lafleur and Hoskins (1968); Madhavan and Ramachandran (1974) [6]. There is also a review that summarizes methods of preparation of various chitin and its conversion to chitosan [17].

7. Enzymatic methods to prepare chitin

A new process for deproteinization of chitin from shrimp head was studied [18]. Recovery of the protein fraction of the shrimp waste has been widely studied by enzymatic hydrolysis method [19,20]. The enzymatic deproteinization process has limited value due to residual small peptides directly attached to chitin molecules ranging from 4.4% to 7.9% of total weight [21]. As these processes are costly because of the use of commercial enzymes, there is now a need to develop an efficient and economical method for extracting proteins from shellfish waste. One interesting new technology for extraction of chitin that offers an alternative to the more harsh chemical methods is fermentation by using microorganisms. Fermentation has been envisaged as one of the most ecofriendly, safe, technologically flexible, and economically viable alternative methods [22-28]. Fermentation of shrimp waste with lactic acid bacteria results in production of a solid portion of chitin and a liquor containing shrimp proteins, minerals, pigments, and nutrients [26,29]. Deproteinization of the biowaste occurs mainly by proteolytic enzyme produced by *Lactobacillus* [30]. Lactic acid produced by the process of breakdown of glucose, creating the low pH condition of ensilation; suppress the growth of microorganisms involved in spoilage of shrimp waste [31]. The lactic acid reacts with calcium carbonate component in the chitin fraction leading to the fermentation of calcium lactate, which gets precipitated and can be removed by washing. There is now a need to develop an efficient, simpler, eco- friendly, economical, and commercially viable method.

8. Chitosan

Despite the wide spread occurrence of chitin, up to now the main commercial sources of chitin have been crab and shrimp shells. In industrial processing, chitin is extracted from crustaceans by acid treatment to dissolve calcium carbonate followed by alkaline extraction to solubilized proteins. In addition a decolorization step is often added to remove leftover pigments and obtain a colorless product. These treatments must be adapted to each chitin source but by partial deacetylation under alkaline conditions, one obtains "chitosan" [16]. Chitosan is the most important derivate of this naturally occurring polymer being one of the most abundant polysaccharides after cellulose. Chitosan is a copolymer composed of N-acetyl-D-glucosamine and D-glucosamine units. It is obtained in three different ways, thermochemical deacetylation of chitin in the presence of alkali, by enzymatic hydrolysis in the presence of a chitin deacetylase, or naturally found in certain fungi as part of their structure. In chitosan part of the amino groups remain acetylated. It is generally accepted that N-acetylglucosamine residues are randomly distributed along the whole polymer chain. In an acid medium, amino groups are protonated and thus determine the positive charge of

the chitosan molecule. Thus, chitosan behaves like a polycation in solution [32]. Properties of chitosan, such as the mean polymerization degree, the degree of N-deacetylation, the positive charge, and the nature of chemical modifications of its molecule, strongly influence its biological activity.

Chitin contains 6–7% nitrogen and in its deacetylated form, chitosan contains 7–9.5% nitrogen. In chitosan, between 60 to 80% of the acetyl groups available in chitin are removed [33]. The chain distribution is dependant on the processing method used to obtain biopolymer [34–36]. It is the N-deacetylated derivative of chitin, but the N-deacetylation is almost never complete [35]. Chitin and chitosan are names that do not strictly refer to a fixed stoichiometry. Chemically, chitin is known as poly-N-acetylglucosamine, and in accordance to this proposed name, the difference between chitin and chitosan is that the degree of deacetylation in chitin is very little, while deacetylation in chitosan occurs to an extent but still not enough to be called polyglucosamine [37].

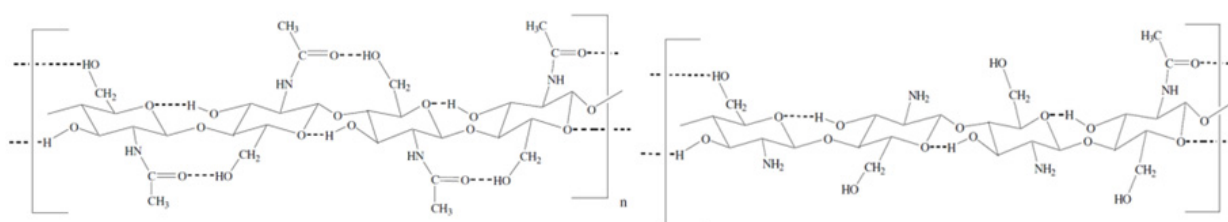


Figure 3. Chitin and chitosan chemical structure

9. Sources of chitosan

Chitosan is commercially produced from deacetylated chitin found in shrimp and crab shell. However, supplies of raw materials are variable and seasonal and the process is laborious and costly [38]. Furthermore, the chitosan derived from such process is heterogeneous with respect to its physiochemical properties [38]. Recent advances in fermentation technology provide an alternative source of chitosan. Fungal cell walls and septa of *Ascomycetes*, *Zygomycetes*, *Basidiomycetes* and *Deuteromycetes* contain mainly chitin, which is responsible for maintaining their shape, strength and integrity of cell structure [38]. The production of chitosan from fungal mycelia has a lot of advantages over crustacean chitosans such as the degree of acetylation, molecular weight, viscosity and charge distribution of the fungal chitosan. They are more stable than crustacean chitosans. The production of chitosan by fungus in a bioreactor at a technical scale offers also additional opportunities to obtain identical material throughout the year. The fungal chitosan is free of heavy metal contents such as nickel, copper [39–41]. Moreover the production of chitosan from fungal mycelia gives medium-low molecular weight chitosans ($1\text{--}12 \times 10^4$ Da), whereas the molecular weight of chitosans obtained from crustacean sources is high (about 1.5×10^6 Da) [41]. Chitosan with a medium-low molecular weight has been used as a powder in cholesterol absorption [42] and as thread or membrane in many medical-technical applications. For these reasons, there is an increasing interest in the production of fungal chitosan.

There are some examples of chitosan extracted from fungi. Chitosans isolated from *Mucorales* typically show Mw in the range 4×10^5 to 1.2×10^6 Daltons and FA values between 0.2 and 0.09. Amino acid analysis of chitosan prepared from *Aspergillus niger* reveals covalently bound arginine, serine, and proline. Nadarajah et. al., 2001, studied chitosan production from mycelia of *Rhizopus* sp KNO1 and KNO2, *Mucor* sp KNO3 and *Asperigullus niger* with the highest amount of extractable chitosan obtained at the late exponential phase. *Mucor* sp KNO3, produced the highest amount of 557mg per 2.26 g of dry cell weight /250 ml of culture. Kishore et. al.(1993), examined the production of chitosan from mycelia of *Absidia coerulea*, *Mucor rouxii*, *Gongronella butieri*, *Phvcomyces blakesleeanus* and *Absidia blakesleeanana*. Chitosan yields of *A. coerulea*, *M. rouxii*, *G. butieri*, *P. blakesleeanus* and *A. blakesleeanana* were 47–50, 29–32, 21–25, 6 and 7 mg/100 mL of medium, respectively. The degree of acetylation of chitosan ranged from 6 to 15%; the lowest was from strains of *A. coerulea*. Viscosity average molecular weights of fungal chitosans were equivalent, approximately 4.5×10^5 Daltons. Wei-Ping Wang et.al., (2008) evaluated the physical properties of fungal chitosan from *Absidia coerulea* (AF 93105), *Mucor rouxii* (Ag 92033), and *Rhizopus oryzae* (Ag 92033). Their glucosamine contents and degrees of deacetylation (DD) were over 80%, differences had been observed in their molecular weight (Mw), ranging from 6.6 to 560 kDa. Chitosan was isolated and purified from the mycelia of *Rhizomucor miehei* and *Mucor racemosus* with a degree of deacetylation of 97 y 98 respectively [43-45].

Considerable research has been carried out on using mycelium waste from fermentation processes as a source of fungal chitin and chitosan. It is argued that this would offer a stable non-seasonal source of raw material that would be more consistent in character than shellfish waste, but so far this route does not appear to have been taken up by chitosan producing companies. Currently there is only one commercial source of fungal chitosan and is produced by the company Kitozyme. However their raw material is not mycelium waste from a fermentation process, which is what is normally envisaged when fungal chitosan is referred to, but actually conventional edible mushrooms grown under contract in France and shipped to Belgium for processing. So mycelium waste still remains a vast and as yet untapped potential source of chitosan.

10. Genetic engineering approach to produce chitin

It is difficult to obtain pure carbohydrates, especially chitin, through conventional techniques. Bacterial cells have been engineered in an effort to overcome this problem [46]. *E. coli* has been engineered to produce chitobiose. This method took advantage of NodC, which is a chito-oligosaccharide synthase, and genetically engineered chitinase to make a cell factory with the ability to produce chito-oligosaccharides [47]. Recombinant chito-oligosaccharides have also been obtained using *E. coli* cells which expressed nodC or nodBC genes [48]. By expressing different combinations of nod genes in *E. coli*, O-acetylated and sulfated chito-oligosaccharide have been produced [49].

11. Parameters influencing the behavior of the biopolymer

The main parameters influencing the characteristics of chitosan are its degree of deacetylation (DD) and molecular weight (Mw), which affect the solubility, rheological and physical properties. Various grades of chitosan are available commercially, which differ primarily in the degree of deacetylation and molecular weight. Different conditions such as type and concentration of reagents, time and temperature employed throughout the processing can affect the physical characteristics and performance of the final chitosan product [50]. However, both DD and molecular weight can be further modified. For example, DD can be lowered by reacetylation [51-55] and molecular weight can be lowered by acidic or enzymatic depolymerisation [56-58].

12. Degree of Deacetylation (DD)

Deacetylation describes a reaction that removes an acetyl functional group. When the degree of deacetylation of chitin reaches about 50% (depending on the origin of the polymer), it becomes soluble in aqueous acidic media and is called chitosan. The solubilization occurs by protonation of the $-NH_2$ function on the C-2 position of the D-glucosamine repeat unit, whereby the polysaccharide is converted to a polyelectrolyte in acidic media. Chitosan is the only pseudonatural cationic polymer and thus, it finds many applications that follow from its unique character (flocculants for protein recovery, depollution, etc.). Being soluble in aqueous solutions, it is largely used in different applications as solutions, gels, or films and fibers.

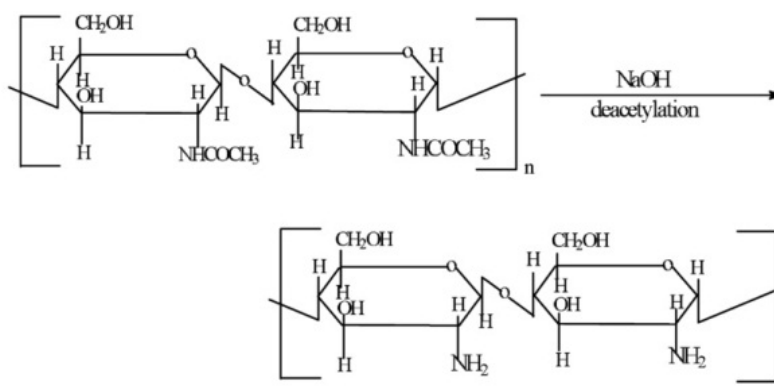


Figure 4. Chitin deacetylation

A highly deacetylated polymer has been used to explore methods of characterization [59]. The solution properties of a chitosan depend not only on its average DA but also on the distribution of the acetyl groups along the main chain in addition of the molecular weight [60-62]. Several methods have been proposed for alkaline deacetylation to obtain chitosan [6,17]. The conditions used in the deacetylation determines the polymer molecular weight and degree of deacetylation (DD).

Chitosan has been largely employed in many areas, such as photography, biotechnology, cosmetics, food processing, biomedical products (artificial skin, wound dressing, contact

lens, etc.), system of controlled liberation of medicines (capsules and microcapsules), treatment of industrial effluents for removal of metallic and coloring ions. The amino groups are responsible for the distinct characteristics attributed to this basic polymer (compared to an acidic biopolymer). Therefore, the characterization of the polymer in either chitin or chitosan is extremely important according to the structure-properties relationship, defining a possible industrial application. Thus many techniques are available to determine the degree of deacetylation. Elson Santiago de Alvarenga (2011) published online describing the most important parameters to be evaluated in chitosan as “deacetylation degree” (DD) [63].

The methods for carrying out the analysis of the degree of deacetylation are: Elemental analysis; Titration; HPLC; Infrared; ^1H nuclear magnetic resonance; CP-MAS ^{13}C NMR; CP-MAS ^{15}N NMR; steric exclusion; nitrous acid deamination; thermal analysis.

13. Molecular weight

Another important characteristic to consider for these polymers is the molecular weight and its distribution. The first difficulty encountered in this respect concerns the solubility of the samples and dissociation of aggregates often present in polysaccharide solutions [16, 57, 64, 65, 66]. As to choice of a solvent for chitosan characterization, various systems have been proposed, including an acid at a given concentration for protonation together with a salt to screen the electrostatic interaction. The solvent is important also when molecular weight has to be calculated from intrinsic viscosity using the Mark–Houwink relation.

14. Biological properties of chitosan

14.1. Biocompatibility

Biocompatibility of a biomaterial refers to the extent to which the material does not have toxic or injurious effects on biological systems [67, 68]. One of the present trends in biomedical research requires materials that are derived from nature as natural materials have been shown to exhibit better biocompatibility with humans and because chitosan's monomeric unit, N-acetylglucosamine, occurs in hyaluronic acid, an extracellular macromolecule that is important in wound repair. Additionally, the N-acetylglucosamine moiety in chitosan is structurally similar to glycosaminoglycans (GAGs), heparin, chondroitin sulphate and hyaluronic acid in which they are biocompatible, and hold the specific interactions with various growth factors, receptors and adhesion proteins besides being the biologically important mucopolysaccharides and in all mammals. Therefore, the analogous structure in chitosan may also exert similar bioactivity and biocompatibility [69, 70].

The potential of chitosan stems from its cationic nature and high charge density in solution. An effective approach for developing a clinically applicable chitosan is to modify the surface of the material that already has excellent biofunctionality and bulk properties [71]. Altering

the physical and chemical properties of the chitosan in order to improve its medicinal quality will also influence its biocompatibility [69,70].

The excellent biological properties of chitosan can be potentially improved with a variety of additional chemicals such as polyethylene glycol and carboxymethyl N-acyl groups in order to produce biocompatible chitosan derivatives for use as wound dressings [72]. Chitosan's positive surface charge enables it to effectively support cell growth [73]. Chitosan-gelatin sponge wound dressing had demonstrated a superior antibacterial effect. Additionally, chitosan gelatin sponge allowed the wound site to contract markedly and shortened the wound healing time, as compared with sterile Vaseline gauze [74]. Widely used surface modification techniques include coating, oxidation by low temperature plasma for better printing and adhesion and surfactant addition for antistatic. Blends are often used to improve tensile properties and to provide a stronger structural component for separation media that supports the active polymer. The physical properties of a polymer can also be altered by introducing a second polymer that improves the properties of the original polymer in certain aspects, such as hydrophobicity, lowered melt temperature, raised glass transition temperature, etc [75]. A thorough understanding of cell and proteins interactions with artificial surfaces is of importance to design suitable implant surfaces and substrates. The surface properties of newly synthesized biomedical grade chitosan derivatives, including surface composition, wettability, domain composition, surface oxidation, surface charge and morphology, may influence protein adsorption and subsequently, the cellular responses to biomaterial implants [76-81].

15. Biodegradability

The claim "biodegradable" is often associated with environmentally friendly products. It is defined as being able to be broken down by natural processes, into more basic components. Products are usually broken down by bacteria, fungi or other simple organisms [82].

An important aspect in the use of polymers as drug delivery systems is their metabolic fate in the body or biodegradation. In the case of the systemic absorption of hydrophilic polymers such as chitosan, they should have a suitable Mw for renal clearance. If the administered polymer's size is larger than this, then the polymer should undergo degradation. Biodegradation (chemical or enzymatic) provides fragments suitable for renal clearance. Chemical degradation in this case refers to acid catalysed degradation i.e. in the stomach. Enzymatically, chitosan can be degraded by enzymes able to hydrolyse glucosamine-glucosamine, glucosamine-N- acetyl-glucosamine and N-acetyl-glucosamine-N-acetyl- glucosamine linkages [83]. Even though depolymerisation through oxidation-reduction reaction [84] and free radical degradation [85] of chitosan have been reported these are unlikely to play a significant role in the in vivo degradation.

Chitosan is thought to be degraded in vertebrates predominantly by lysozyme and by bacterial enzymes in the colon [83, 86]. However, eight human chitinases (in the glycoside hydrolase 18 family) have been identified, three of which have shown enzymatic activity

[87]. A variety of microorganisms synthesises and/or degrades chitin, the biological precursor of chitosan. In general, chitinases in microorganisms hydrolyze N-acetyl- β -1,4-glucosaminide linkages randomly i.e. they are endo-chitinases (EC 3.2.1.14). Chitinases are also present in higher plants, even though they do not have chitin structural components.

Chemical characterisation assays determining the degradation of chitosan commonly use viscometry and/or gel permeation chromatography to evaluate a decrease in Mw [88]. Lysozyme has been found to efficiently degrade chitosan; 50% acetylated chitosan had 66% loss in viscosity after a 4 h incubation in vitro at pH 5.5 (0.1 M phosphate buffer, 0.2 M NaCl, 37 °C) [88]. This degradation appears to be dependent on the degree of acetylation with degradation of acetylated chitosan (more chitin like) showing the faster [89,90].

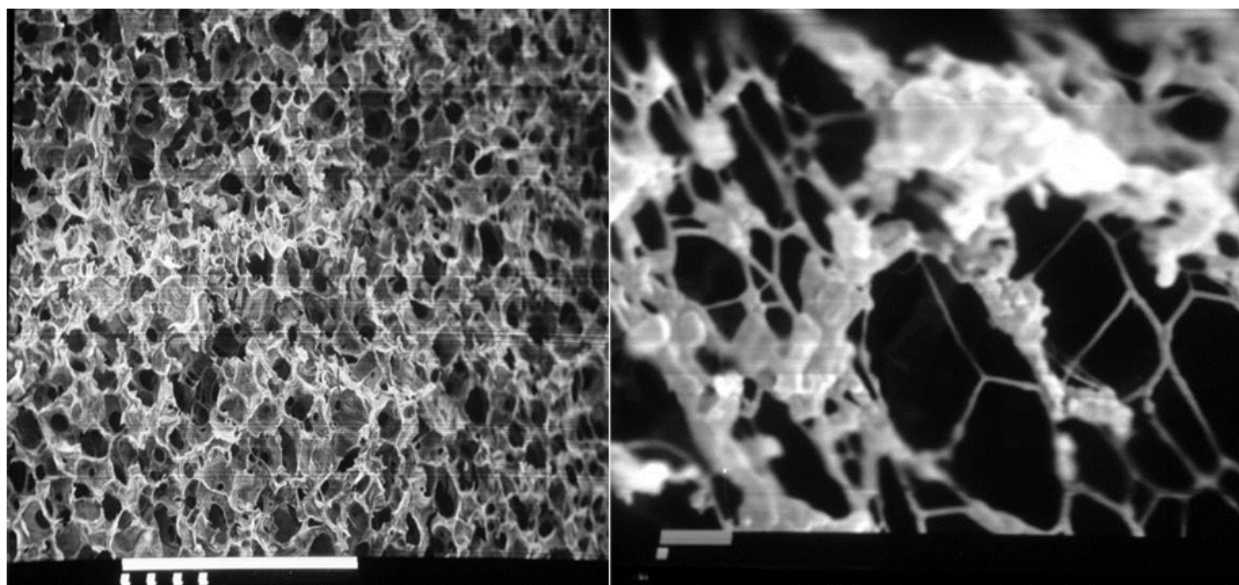


Figure 5. Biodegradation of chitosan thermosensible gel inside the rat's body after 5 days.

16. Safe biomaterial

Chitosan is a potentially biologically compatible material that is chemically versatile ($-NH_2$ groups and various Mw). These two basic properties have been used by drug delivery and tissue engineering to create a great amount of formulations and scaffolds that show promise in healthcare. It is approved for dietary applications in Japan, Italy and Finland [91] and it has been approved by the FDA for use in wound dressings [92] but is not approved for any product in drug delivery. The term "Chitosan" represents a large group of structurally different chemical entities that may show different biodistribution, biodegradation and toxicological profiles.

The formulation of chitosan with a drug may alter the pharmacokinetic and biodistribution profile. The balancing, or reduction, of the positive charges on the chitosan molecule has effects on its interaction with cells and the microenvironment, often leading to decreased uptake and a decrease in toxicity. The modifications made to chitosan could make it more or less toxic and any residual reactants should be carefully removed. In addition, the route of administration determines the uptake, concentration, contact time and cell types affected.

Chitosan details (DD, MW)	Modification	Assessment	IC50
95% DD, 18.7 kDa	Steric acid conjugation micelle	In vitro ,A549 cells	369±27 µg/ml
95% DD, 18.7 kDa	teric acid conjugation and entrapment in micelle	In vitro ,A549 cells	234±9 µg/ml
97% DD, 65 kDa	N-octyl-O-sulphate	Invitro, primary rat hepatocytes	>200 mg/ml
87% DD, 20, 45, 200, 460 kDa	None, aspartic acid salt	In vitro, Caco-2 cells, pH 6.2	0.67±0.24, 0.61±0.10, 0.65±0.20, 0.72±0.16 mg/ml
87% DD, 20, 45, 200, 460 kDa	None, glutamic acid salt		0.56±0.10, 0.48±0.07, 0.35±0.06, 0.46±0.06 mg/ml
87% DD, 20, 45, 200, 460 kDa	None, Lactic acid salt		0.38±0.13, 0.31±0.06, 0.34±0.04, 0.37±0.08 mg/ml
87% DD, 20, 45, 200, 460 kDa	None, hydrochloride salt		0.23±0.13, 0.22±0.06, 0.27±0.08, 0.23±0.08 mg/ml
78% DD, <50 kDa	None, lactic acid salt	In vitro B16F10 cells	2.50 mg/ml
82% DD, 150–170 kDa	None, lactic acid salt	In vitro B16F10 cells	2.00±0.18 mg/ml
>80% DD, 60–90 kDa	None, glutamic acid salt	In vitro B16F10 cells	2.47±0.14 mg/ml
77% DD, 180–230 kDa	None, lactic acid salt	In vitro B16F10 cells	1.73±1.39 mg/ml
85% DD, 60–90 kDa	None, hydrochloric acid salt	In vitro B16F10 cells	2.24±0.16 mg/ml
81% DD, 100–130 kDa	None, hydrochloric acid salt	In vitro B16F10 cells	0.21±0.04 mg/ml
100% DD, 152 kDa	Glycol chitosan	In vitro B16F10 cells	2.47±0.15 mg/ml
100% DD, 3–6 kDa	20, 44, 55% Trimethyl chitosan, chloride salt	In vitro, MCF7 and COS7 cells, 6 h & 24 h	>10 mg/ml
100% DD, 3–6 kDa	94% Trimethyl chitosan, chloride salt	In vitro, MCF7, 6 h	1.402±0.210 mg/ml
100% DD, 3–6 kDa	94% Trimethyl chitosan, chloride salt	In vitro, COS7, 6 h	2.207±0.381 mg/ml
100% DD, 100 kDa	36% Trimethyl chitosan, chloride sal	In vitro, MCF7, 6 h	0.823±0.324 mg/ml
100% DD, 100 kDa	36% Trimethyl chitosan, chloride sal	In vitro, COS7, 6 h	>10 mg/ml

Chitosan details (DD, MW)	Modification	Assessment	IC ₅₀
84.7% DD, 400, 100, 50, 25, 5 kDa	40% Trimethyl chitosan	In vitro, L929 cells, 3 h	30, 70, 90, 270, >1000 µg/ml
84.7% DD, 1.89 MDa	12% PEG modified 40% trimethyl chitosan	In vitro, L929 cells, 3 h	220 µg/ml
84.7% DD, 3.6 MDa	25.7% PEG modified 40% trimethyl chitosan	In vitro, L929 cells, 3 h	370 µg/ml
84.7% DD, 300 kDa	6.44% PEG modified 40% trimethyl chitosan (and all PEG modified TMC with lower Mw)	In vitro, L929 cells, 3 h	>500 µg/ml
97% DD, 65 kDa	N-octyl-O-sulphate	In vivo, IV, mice	102.59 mg/kg
97% DD, 65 kDa	N-octyl-O-sulphate	n vivo, IP, mice	130.53 mg/kg

Table 1. Toxicity of chitosan and chitosan derivatives

Table taken from [94]

In a series of articles are described the effects of chitosans with differing molecular weights and degree of deacetylation in vitro and in vivo. Toxicity was found to be degree of deacetylation and molecular weight dependent. At high DD the toxicity is related to the molecular weight and the concentration, at lower DD toxicity is less pronounced and less related to the molecular weight [93].

A summary of toxicities of chitosan and derivatives assessed through IC₅₀ values is presented in the next table [94].

From this table it can be gathered that most chitosans (and derivatives) are not significantly toxic compared to a toxic polymer such as polyethylenimine [94].

It appears that the toxicity of chitosan is related to the charge density of the molecule, toxicity increases with increasing density. It appears that there is a threshold level below which there are too few contact points between a molecule and the cell components to produce a significantly toxic effect. This balance is between 40 and 60% DD, or degree of trimethylation, although any sufficiently small chitosan (<10 kDa) is not appreciably toxic. Modifications that do not increase the charge on the molecule seem to have little effect on the toxicity beyond that of the native molecule [94].

17. Antimicrobial activity

The exact mechanism by which chitosan exerts its antimicrobial activity is currently unknown, it has been suggested that the polycationic nature of this biopolymer that forms from acidic solutions below pH 6.5 is a crucial factor. Thus, it has been proposed that the positively charged amino groups of the glucosamine units interact with negatively charged components in microbial cell membranes, altering their barrier properties, and thereby

preventing the entry of nutrients or causing the leakage of intracellular contents [95-100]. Another reported mechanism involves the penetration of low- molecular weight chitosan into the cell, binding to DNA and the subsequent inhibition of RNA and protein synthesis [101]. Chitosan has also been shown to activate several defence processes in plant tissues and it inhibits the production of toxins and microbial growth because of its ability to chelate metal ions [102,103].

El-Ghaouth et. al.(1992) have proposed possible antibacterial actions of chitosan and its derivatives [104]. They asserted that chitosan reacted with the cell surface, altered cell permeability, and further prevented the entry of material or caused the leakage of material. However, no evidence has been provided to demonstrate the relationship between the antibacterial activity of chitosan and the surface characteristics of the bacterial cell wall. Antimicrobial activity of chitosan has been demonstrated against many bacteria, filamentous fungi and yeasts [105-108]. Chitosan has wide spectrum of activity and high killing rate against Gram-positive and Gram-negative bacteria, but lower toxicity toward mammalian cells [109,110]. Variations in chitosan's antimicrobial efficacy arise from various factors. These factors can be classified into four categories as follows: (1) microbial factors, related to microorganism species and cell age; (2) intrinsic factors of chitosan, including positive charge density, Mw, concentration, hydrophilic/hydrophobic characteristic and chelating capacity; (3) physical state, namely water-soluble and solid state of chitosan; (4) environmental factors, involving ionic strength in medium, pH, temperature and reactive time [111].

Although owning a broad spectrum of antimicrobial activity, chitosan exhibits different inhibitory efficiency against different fungi, Gram-positive and Gram-negative bacteria. Chitosan exerts an antifungal effect by suppressing sporulation and spore germination [112]. In contrast, the mode of antibacterial activity is a complicating process that differs between Gram positive and Gram-negative bacteria due to different cell surface characteristics.

Based on the available evidences, bacteria appear to be generally less sensitive to the antimicrobial action of chitosan than fungi. The antifungal activity of chitosan is greater at lower pH values [113]. For a given microbial species, age of the cell can influence antimicrobial efficiency.

Chitosan has a broad spectrum of unique biological activities, including its ability to induce resistance to viral infections in plants, inhibit viral infection in animal cells, and prevent the development of phage infection in infected microbial cultures. High polymeric chitosan, when added to a nutrient medium, prevents the accumulation of the infectious phage progeny in infected cultures of Gram-positive and Gram-negative organisms. The yield of infectious DNA containing phage can decrease by several orders of magnitude in the presence of chitosan [114, 115]. The observed effect also depends on the concentration, degree of polymerization, and molecular structure of chitosan. Thus, chitosans with a polymerization degree of 250 and higher were much more effective in inhibiting coliphage infection than their fragments with a polymerization degree of 15–19. On the other hand, chitosan oligomers were more effective than their polymeric precursor in inhibiting the

replication of 1-97A phage in *Bacillus thuringiensis* cultures. Factors determining such strong differences are currently unclear. Anionic derivatives of chitosan, such as 6-O-sulfate and N-succinate-6-O-sulfate, caused no effect on phage infection [114]. This finding showed that the positive charge of a chitosan molecule is important for inhibition of phage infection.

It has been suggested that chitosan can inhibit the replication of bacteriophages by several mechanisms: it can (a) decrease the viability of cultured bacterial cells, (b) neutralize the infectivity of mature phage particles in the inoculum and/or daughter phage particles, and (c) block the replication of the virulent phage [114].

The condition of the phage culture is known to be of paramount importance for the development of phage infection because phages can reproduce only in viable cells. However, there is evidence that chitosan displays a bactericidal activity toward many microbial species including *Escherichia coli* [116, 117] and species of the genus *Bacillus* [118]. Chitosan, because of its polycationic nature, binds to the external membrane of Gram negative microorganisms by electrostatic forces, which is demonstrated in experiments with core phosphate groups of lipid A, thereby decreasing the potency of endotoxin. It was suggested that the antibacterial effects of chitosan and many other cationic agents are based on their ability to increase the permeability of the outer membrane of Gram negative microorganisms to an extent incompatible with their viability [116-119].

Great amount of literature support the essential importance of polycationic structure in antimicrobial activity. A higher positive charge density leads to strong electrostatic interaction. Therein, the positive charge is associated with DD or degree of substitution (DS) of chitosan or its derivatives, which affect positive charge density. Concerning chitosan derivatives, antimicrobial activity mostly depends on DS of the grafting groups.

There are several works regarding the influence of the molecular weight of chitosan on its antimicrobial properties [120-126]. Some of them have demonstrated that COS (chitoooligosaccharides), which are soluble in water, were the least effective in terms of biocide properties [124-126]. Recent work carried out by Qin et al. 2006, on the evaluation of chitosan solutions against the growth of *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus*, it has shown that only water insoluble chitosan in organic acidic solutions, i.e. chitosonium salts, exhibit efficient biocide properties. On the other hand, a research performed by Fernandes et al, 2008 showed that the growth of *E. coli* was markedly inhibited by COS, and this inhibition decreased slightly as molecular weight increased. In another work performed by Fernandez-Saiz et al. 2009, changes in molecular weight of the chitosan materials tested, i.e. 310–375 and 50–190 kDa, did not lead to significant variations in biocide properties [127, 128,129].

Concerning DD, there are several works that consider this feature, and there is no doubt that the antimicrobial properties of chitosan increased with this variable. The antimicrobial performance tends to increase upon an increase in the DD of chitosan, which is related to an increase in the positive charge of the polymer [130-133].

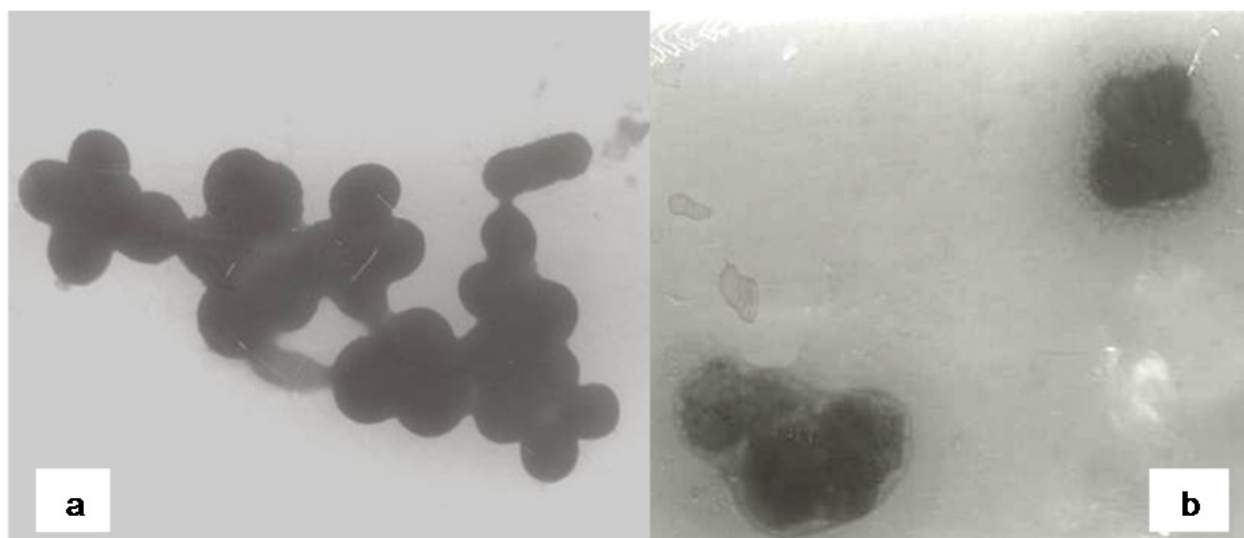


Figure 6. Staphylococcus bacteria (a), covered with chitosan (b)

18. Analgesic activity

Some investigators reported that chitin and chitosan induce analgesia. Allan in 1984 found that chitosan provide a cool and pleasant soothing effect when applied to open wounds. Ohshima et al in 1987, reported that chitin proved excellent pain relief in 83 out of 91 cases who received the agent topically over open wounds such as burns, skin abrasion, skin ulcers, skin graft areas and so on [134-135]. Minami in 1993 and Okamoto in 1993, reported that animals did not feel pain when their wounds were covered with chitin and chitosan [136-137].

Chitin and chitosan have been found to reduce the inflammatory pain due to intraperitoneal administration of acetic acid in rats [138]. When the chitosan suspension was mixed with acetic acid, the amino groups in C2 the position were protonated to NH_3^+ subsequently the particles resolved in the solution. Bradykinin is one of the main substances related to pain and the levels of this substance decrease in the presence of chitin and chitosan. Chitin absorbs Bradykinin more extensively than chitosan and this could be one of the main analgesic effect [138].

19. Antitumor activity

In some medical applications of chitin/chitosan, as antitumor compounds, for example, their degradation products are preferred, as they have a lower viscosity and a better solubility in water. The antitumor activity of chitin/chitosan is manifested by the stimulation of the immune system (production of lymphokines, including interleukins 1 and 2, stimulation of NK, etc.) [139-141]. Jeon and Kim in 2002, tested the antitumor activity of three kinds of COSs (high molecular weight ranging from 6.5 to 12 kDa – HMWCOS, medium molecular weight ranging from 1.5 to 5.5 kDa -MMWCOS, and low molecular weight ranging from 0.5 to 1.4 kDa – LMWCOS) against Sarcoma 180 solid (S180) and Uterine cervix carcinoma No.

14 (U14) [140]. The efficiency of tumor growth inhibition for both types of tumor cells in mice was best in the case of MMWCOS. There are many reports of S180 tumor cells being used for testing the antitumor activity of chitosan [141-142].

Maeda and Kimura 2004, investigated the antitumour effect of three water-soluble low molecular weight chitosans (21 kDa, 46 kDa, 130 kDa) and various doses of 650 kDa chitosan in sarcoma 180-bearing mice. They found that LMWC (21 and 46 kDa) and also smaller oligosaccharides could activate the intestinal immune system of animals, thus preventing tumor growth. But no antitumor effect was observed after the oral administration of chitosan samples, even of low molecular weight (46 kDa). The same authors confirmed that high molecular weight chitosan (650 kDa) prevents the adverse reactions of some cancer chemotherapeutic drugs [141]. Qin et al. in 2002 also tested the antitumor activity of LMWC against sarcoma 180, but they came to the opposite conclusions [143]. They noted that oral administration of LMW chitosan decreases the weight of the tumour [139, 142], although administration by intraperitoneal injection led to a higher inhibitory rate [142]. It was reported the higher the MW of LMWC, the better the tumor inhibitory effect [139]. The introduction of acidic groups as a result of chitosan oxidation has the opposite effect, and an increase in MW decreases antitumor activity. Qin Cai-qin et al in 2002, prepared low molecular weight chitosans by oxidative degradation with H_2O_2 . They found that carboxylic contents increased with decrease in molecular weight (M_n). They also found that the introduction of carboxylic group is advantage to water-solubility of chitosan, but more acidic groups decrease the function of amino groups of chitosan against sarcoma 180 tumor. There is a correlation between the activity and the molecular weights of the oxidized chitosans, and a maximum of inhibition was found around 4 100 [143]. The influence of LMWC and COS (including the pentamer, hexamer, and higher oligomers) on the growth inhibition of Ehrlich ascites tumor (EAT) cells and tumor induced neovascularization was investigated [144]. Based on experimental results concerning the inhibition of angiogenesis and the induction of apoptosis, it was confirmed that COSs seem to be more potent angiogenic inhibitory and antitumor compounds. Wang et al. reported that chitin oligosaccharides (DP 1-6) also reduced the number of K562 cells (human erythromyeloblastoid leukemia cell line) [145].

20. Chitosan applications

20.1. Biomedical

Potential applications of chitosan can only be exploited if its usable forms are properly developed and prepared. In solution and gel, it can be used as a bacteriostatic, fungistatic and coating agent. Gels and suspensions may play the role of carriers for slow release or controlled action of drugs, as an immobilising medium and an encapsulation material. Film and membranes are used in dialysis, contact lenses, dressings and the encapsulation of mammal cells, including cell cultures. Chitosan sponges are used in dressings, and to stop bleeding in mucous membranes. Chitosan fibres are used as resorbable sutures, non-wovens for dressings, and as drug carriers in the form of hollow fibres.



Figure 7. These are some ways you can shape the chitosan: films, gels, microspheres, tubes, sponges, powder

21. Artificial skin

The preparation of artificial skin by natural materials such as gelatin, pectin, starch, cellulose, alginate, chitin, collagen, polyamino acids, and dextran has been established to enhance the healing process. The structures of these natural materials are analogs of protein and growth factor structures in the human body that may be more relevant for stimulating the appropriate physiological responses required for cellular regeneration and tissue reconstructing in wounds [146].

Dressing materials based on chitin, chitosan and derivatives are well-known on the market, and are produced mainly in Japan and the US. JEX KK Co produces composite dressings made of synthetic resins, chitosan and materials of collagen and acetylchitosan. Eisai Co is manufacture of chitin dressings in sponge form (Chitopack C®) or a PET non-woven modified with chitin (Chitopack C®). The Japanese Unitika Co offers a dressing non-woven of chitosan fibres. The American 3M proposes a chitosan gel preparation (Tegasorb®) and a hydrocolloid (Tegaderm®) designed for the healing of extensive internal wounds. ChorioChit sponge is a biological dressing obtained by lyophilisation of human placenta blended with MCCh.

22. Scaffold for the regeneration of tissue

Chitin and its derivatives have been used as scaffolds for bone and other natural tissue regeneration [147] as well as structures by which three-dimensional formation of tissues are supported [148]. While looking for a good material for a good scaffold, there are at least four important factors that should be taken into account: (1) ability to form temporary matrix, (2) ability to form porous structure for tissue to grow, (3) biodegradability, and finally (4) non-toxic byproducts from the digestion [149,150]. Thus, neither the physical nor biological properties of such biomaterials should be ignored [147]. Chitin and its derivatives have been shown to possess these criteria.

23. Haemostasis and wound healing

Hemostasis through blood coagulation is an important step for wound healing. The main cellular components in blood coagulation are platelets. It has been shown that chitosan has a hemostatic effect [151]. Okamoto et al 2003, reported that chitin is an effective agent for hemostasis maintenance through aggregating platelets, and suggested that the effect of chitin and chitosan is due to both physical and chemical properties of these biopolymers, especially their amino groups [152].

Haemostatic dressings containing chitin and chitosan as bioactive agents are also well known, notably the Syvek patch, RDH (Marine Polymer Technologies), Clo-Sur PAD (Medtronic-Scion), Chito-Seal (Abbot), the M-Patch and Trauma DEX (Medafor).

24. Peripheral nerve prosthesis

Prosthesis is made from various forms of utility polysaccharides. The main objective of research is to develop replacement implants that will not be rejected by the body of the recipient and offering the ability to regenerate damaged nerve. Because chitin has high mechanical strength under physiological conditions (low for chitosan) chitin has the potential to be a good nerve guidance channel. Ferrier et al, 2005, used this fact and made chitin tubes that could support nerve cell adhesion and neurite outgrowth [153]. In a research related to nerve regeneration, it was shown that rabbits with the crushed common peroneal nerve exhibit better improvement in peripheral nerve regeneration in the presence of chitooligosaccharide. As a result, chito-oligosaccharide can be used as neuroprotective material with an ability to improve injured peripheral nerve regeneration [154].

25. Immunology

The key property of chitin-derived products for application in various biomedical applications is the immuno-modulating effect [155,156]. Some mechanisms of immuno-enhancement activity of chitin and its derivatives have been reported, for example, chitosan exhibited the ability to boost NO production from macrophages in the presence of interferon- γ (IFN- γ) through the NF- κ B signaling pathway [157]. Minami et al. in 1998 found that chitin and chitosan affected C3 and C5 components of complement system and concluded that complement system is activated by chitin and chitosan through the alternative pathway. After activating the complement, C5 is produced followed by an increase in migration of polymorphonuclear cells (PMN) to the injured tissue. This is a normal inflammatory reaction but in the presence of chitin and chitosan, there are no inflammatory symptoms, such as erythema, temperature elevation and abscess formation [158]. The intensity of complement [158] and macrophage [159] activation of chitin is less than chitosan; therefore, chitin is more immunomodulatory.

26. Blood cholesterol control

Chitin and chitosan are among the candidates to battle obesity and hypercholesterolemia. It has been reported that they can reduce the amount of cholesterol in rats [160]. Several mechanisms have been proposed to explain this phenomenon. One is through electrostatic interaction between lipids and aminopolysaccharides [161]. Chitin binds to lipid (cholesterol) micelles and inhibits their absorption. Another proposed mechanism is increasing the excretion of bile acid by which the amount of fecal fat increases [162]. The hypocholesterolemic effect of chitosan has also been found in humans. The proposed cholesterol lowering mechanism of chitosan was that it combines bile acids in the digestive tract, and excretes them into the feces, thus decreasing the resorption of bile acids, so that the cholesterol pool in the body was decreased and the level of serum cholesterol consequently decreased [163].

27. Drug delivery carriers

It is important for a drug delivery carrier to be efficiently removed after delivering drugs. In other words, it must not accumulate in the body nor must it be toxic [164].

Chitosan offers several advantages, and these include its ability to control the release of active agents and avoid the use of hazardous organic solvents while fabricating particles since it is soluble in aqueous acidic solution. Chitosan in the form of colloidal structures can entrap macromolecules by various mechanisms. These associated macromolecules have been shown to transport through mucosa and epithelia more efficiently [165]. Cationic chitosan in combination with other natural polymers has been shown to enhance the drug encapsulation efficiency of liposomes via the layer-by-layer (L-b-L) self-assembly technique [166]. Nanoparticles made of chitosan in association with polyethylene oxide have been used as protein carrier [167]. Moreover, an oral delivery system has been developed by using chitosan and tripolyphosphate. In this system, micro- and nano-particles were entrapped in beads made from chitosan in solution of tripolyphosphate [168].

28. Food

28.1. Chitosan films

Edible films and coatings have received considerable attention in recent years because of their advantages including use as edible packaging materials over synthetic films. This could contribute to the reduction of environmental pollution.

By functioning as barriers, such edible films and coatings can feasibly reduce the complexity and thus improve the recyclability of packaging materials, compared to the more traditional non-environmental friendly packaging materials, and may be able to substitute such synthetic polymer films [169].

Edible films are defined as a thin layer of material which can be consumed and provides a barrier to moisture, oxygen and solute movement for the food. The material can be a complete food coating or can be disposed as a continuous layer between food components [170]. Edible films can be formed as food coatings and free-standing films, and have the potential to be used with food as gas aroma barrier [171].

Chitosans are described in terms of the degree of deacetylation and average molecular weight and their importance resides in their antimicrobial properties in conjunction with their cationicity and their film forming properties [172]. Chitosan can form semi-permeable coatings, which can modify the internal atmosphere, thereby delaying ripening and decreasing transpiration rates in fruits and vegetables [173-176]. Films from aqueous chitosan are clear, tough, flexible and good oxygen barriers [177,178].

29. Bread

Applications of chitosan for extension of shelf life of bread by retarding starch retrogradation and/or by inhibiting microbial growth have been documented. Park and others in 2002 investigated the effect of chitosan (493 kDa) coating on shelf life of baguette [179].

Chitosan coating may offer a protective barrier for moisture transfer through the bread surface, thus reducing weight loss, retarding hardness, retrogradation, inhibiting microbial growth, retarding oxidation [179- 181].

30. Eggs

Several problems are encountered during storage of eggs, such as weight loss, interior quality deterioration, and microbial contamination [182-183]. The movement of carbon dioxide and moisture from the albumen through the shell governs quality changes in albumen and yolk, and weight loss of [184,185].

Chitosan coating may offer a protective barrier for moisture and gas transfer from the albumen through the egg shell, thus extending the shelf life of eggs [182, 186].

31. Fruits and vegetables

The major postharvest losses of fruits are due to fungal infection, physiological disorders, and physical injuries [102, 104, 187]. One of the potential approaches to extend the storability of these perishable commodities is to apply edible coatings on the surface, followed by a cold storage [188]. Edible coatings can be used as a protective barrier to reduce respiration and transpiration rates through fruit surfaces, retard microbial growth and color changes, and improve texture quality of fruits [171]. Coating fruits with semipermeable film has generally been shown to retard ripening by modifying the endogenous CO₂, O₂, and ethylene levels of fruits [102]. Chitosan coating is likely to modify

the internal atmosphere without causing anaerobic respiration, since chitosan films are more selectively permeable to O₂ than to CO₂ [189]. Therefore, chitosan coating with its ability to modify internal atmosphere in the tissue and fungistatic property has a potential to prolong storage life and control decay of fruits.

32. Juice and beverages

Processing of clarified fruit juices commonly involves the use of clarifying aids, including gelatin, bentonite, silica sol, tannins, polyvinylpyrrolidone, or combinations of these compounds [190]. Chitosan with a partial positive charge has been shown to possess acid-binding properties [191] and to be effective in aiding the separation of colloidal and dispersed particles from food processing wastes [192,193]. These properties make chitosan an attractive processing aid in fruit juice production.

33. Mayonaise

Few studies have been conducted on the use of chitosan to enhance emulsification in mayonnaise preparation. Lee (1996) reported that addition of chitosan (1500 kDa, 0.1% based on egg yolk weight) increased emulsifying capacity of egg yolk by about 10% and enhanced emulsion stability of mayonnaise by 9.4% compared with those of the control [186]. Kim and Hur (2002) also suggested the use of chitosan as an emulsion stabilizer in commercial mayonnaise preparation [194].

Chitosan possesses a positive ionic charge and has both reactive amino and hydroxyl groups, which give it the ability to chemically bond with negatively charged protein. When pH is less than 6.5, chitosan solution carries a positive charge along its backbone. Because of its polar groups, chitosan also provides additional stabilization due to hydration forces [195]. According to Filar and Wirick in 1978, chitosan functions only in acid systems to show possible utility as a thickener and stabilizer [196].

34. Meat

Meat or meat products are highly susceptible to lipid oxidation, which leads to rapid development of rancid or warmed-over flavor. Chitosan possesses antioxidant and antibacterial capacity [126, 197], and may retard the lipid oxidation and inhibit the growth of spoilage bacteria in meat during storage.

Darmadji and Izumimoto in 1994 observed that addition of 1.0% chitosan to beef decreased the TBA value by about 70% compared to that of the control sample after 3 days of storage at 4 °C. Chitosan has a desirable effect on the development of the red color of beef during storage [198]. Sagoo et al in 2002, demonstrated that chitosan was an effective inhibitor of microbial growth in chilled comminuted pork products and that the effect of chitosan was concentration dependent [199].

35. Milk

A few attempts have been made to evaluate the possibility of using chitosan to improve the quality and shelf life of milk. Ha and Lee in 2000, investigated the effectiveness of water-soluble chitosan (0.03%) to minimize the microbial (bacterial and yeast) spoilage of processed milk [200]. Complete inhibition of microbial growth was observed in the banana-flavored milk containing chitosan, in contrast to that observed in control milk (without chitosan), during storage for 15 days at 4 and 10 °C. The banana-flavored milk containing chitosan also maintained relatively higher pH than that of control milk during storage for 15 d at both temperatures [200].

36. Sausages

Sodium nitrite is generally used as a curing agent for color and flavor development as well as preservative effect in sausages [201]. However, nitrite reacts with amine in meat and may produce nitrosoamine, a strong toxicant detrimental to human health. Several workers [202] have investigated the possible role of chitosan, in lieu of sodium nitrite, as curing agent in sausage, and found that addition of chitosan could reduce or replace the use of nitrite without affecting preservative effect and color development.

37. Seafoods and seafood products

Seafood products are highly susceptible to quality deterioration due to lipid oxidation of unsaturated fatty acids, catalyzed by the presence of high concentrations of hematin compounds and metal ions in the fish muscle [203]. Furthermore, seafood quality is highly influenced by autolysis, contamination by and growth of microorganisms, and loss of protein functionality [204].

The oxidative stability of fish flesh with added chitosans was compared with those added with conventional antioxidants, butylated hydroxyanisole + butylated hydroxytoluene (BHA + BHT, 200 ppm) and tert butylhydroquinone (TBHQ, 200ppm), during storage at 4 °C. Chitosan was most effective in preventing lipid oxidation than the others. The antioxidant capacity of chitosan added to the fish muscle depended on the molecular weight and concentration of chitosan [204]. Similarly, Kim and Thomas in 2007 also observed that the antioxidative effects of chitosan in salmon depended on its molecular weight [205].

38. Chitosan in agriculture

Due to the antifungal, antibacterial and antiviral properties of chitosan, it has been used successfully in agriculture in recent years: in plant protection, like growth promoter, in soil correction, enhancer of secondary metabolites production, and activator of defense mechanisms to mention a few.

39. Seed coating

Chitosan application can be done by different ways: in the seed, in the soil or by foliar way. In seeds, it has been used as a coating material for cereals, nuts, fruits and vegetables [206-208]. It has been shown that this way of application alters permeability of the seed plasma membrane, increasing the concentrations of sugars and proline, and enhancing peroxidase (POD), catalase (CAT), phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL) activities [207,209]. By this way, germination rates increases significantly [210] and seedlings germinate quicker, better, and vigorously [211-214]. Chitosan is used not only in seed coatings, but also in fruits and vegetables, because it gives more firmness and it promotes diminution of the normal microbiological charge [215] increasing the product life.

40. Leaf coating

Chitosan foliar application increases stomatal conductance and reduces transpiration, without affecting plant height, root length, leaf area or plant biomass [216]. When chitosan is sprayed in leaves, abscisic acid (ABA) content increases [217]. It promotes the activation of defense mechanisms which allow plants to deal with stress and to defend against diseases due to the antiviral, antifungal and antibacterial nature of chitosan [218,219].

41. Fertilizer

By applying chitosan in soil, it has been demonstrated that it stops plant wilting because it acts as a potent fertilizer due to the high concentration of nitrogen content in its molecular structure [220,221]. Also, it has been used as a soil amendment, controlling diseases caused by fungal species like *Fusarium acuminatum*, *Fusarium sp*, *Cylindrocladium floridanum* and *Aspergillus flavus* [208, 218, 222].

42. Plants growth promoter

Chitosan acts as plant growth promoter in some crops like Faba bean plant, radish, passion fruit, potato, gerbera, cabbage, soybean and other crops when it is incorporated in solution, increasing plant production and protecting plants against pathogens too. Chitosan has a significant effect on growth rates of roots, shoots, flowering, and number of flowers [219, 223].

43. Plant self defense

Plants react naturally against most of biological and environmental adverse conditions, but sometimes defense has to be induced in order to fight against harder threats. It has been reported that chitosan is a great biopolymer used for this purpose, because it induces defense reactions in some plants, sensitizing them in order to increase their responses against pathogens attack. Some substances that get favored due to the presence of chitin and

chitosan are phytoalexins, pathogenesis related proteins (PR), protein inhibitors, chitinases and glucanases, as well as Reactive Oxygen Species (ROS) and hydrogen peroxide generation [224]. This is because chitosan interacts with cellular DNA generating multiple biochemical reactions in the plant, generating a rapid response in the plant against pathogens attack. For this reason, chitosan has been considered as an elicitor, namely a defense mechanism activator in plants, generating a process at cellular level in which plant cells get and transduce biological signals in order to activate defense responses [225]. There are some specific elicitor-binding proteins which act like physiological receptors in signal transduction cascades, varying their specificity depending on the studied system, which allows researchers to find the molecular bases that origin the signal interchanges between host plants and microbial pathogens [225-227].

Not only at biochemical level but also at microbiological level, chitosan is effective on plant protection. It has been found that application of chitosan in plants by the ways mentioned in sections above reduces visibly the damages caused in the plants by pathogenic fungi because of the antibiotic nature of chitosan [215, 218]. Because of being a polysaccharide, chitosan acts as a bioremediator molecule that stimulates the activity of beneficial microorganisms in the soil such as *Bacillus*, fluorescent, *Pseudomonas*, *Actinomycetes*, *Mycorrhiza* and *Rhizobacteria* [228-233], which alter the microbial equilibrium in the rhizosphere disadvantaging plant pathogens, making them able to compete through mechanisms such as parasitism, antibiosis, and induced resistance [234,235].

44. Bioinsecticide

Chitosan research has been focused principally in controlling bacterial and fungal burden; nevertheless there are some investigations about the use of chitosan as bioinsecticide. One of the first findings was that chitosan is active against some insects like lepidopterous and homopterous, with a mortality of 80%, and this percentage increases when increasing oligo-chitosan concentration too [236].

Not only chitosan, but also its derivatives (as N-acetyl (NAC) and N-benzyl (NBC) chitosan derivatives) had shown significant insecticidal activities superior to those of chitosan itself, particularly against species like *Spodoptera littoralis*, an important destructive pest of subtropical and tropical agriculture in northern Europe, affecting cotton, vegetable and ornamental crops [237]. Some other insects have been successfully attacked by chitosan derivatives, like *Helicoverpa armigera* (Hübner), *Plutella xylostella* (L.), *Aphis gossypii* (Glover), *Metopolophium dirhodum* (Walker), *Hyalopterus pruni* (Geoffroy), *Rhopalosiphum padi* L., *Sitobium avenae* (Fabricius) and *Myzus persicae* (Sulzer) [238].

Active chitinases from chitosan are relevant enzymes for biopesticide control mechanisms, being the hydrolysis of chitin-containing media a common practice to evaluate the efficiency of bioinsecticide organisms. It has been considered to add chitin derivatives to formulations

containing these microorganisms to increase biopesticide effectiveness, to provide a favorable developmental environment and resistance against adverse conditions [239]. New chitosan derivatives with insecticidal or fungicidal properties may thus serve as good alternatives for broad-spectrum and highly persistent pesticides because they are non-toxic to vertebrates and humans, and have a biodegradable matrix.

45. Biopesticide

Trichoderma sp. and *Bacillus* sp. are microorganisms which often increase chitin and chitosan production, enhancing its efficiency to control pathogenic microorganisms and pests [238]. Native populations of biocontrol microorganisms became increased by adding chitin in soils infected with pathogenic agents. Thereafter, these endogenous control strains can be isolated, cultured and potentially used as biological controls. It has also been demonstrated a significant increase in chitinolytic microorganisms even in very infertile soils like in dunes, improving soil microbiota and its properties [239, 241].

46. Bionematicide

Nematodes proliferation can be controlled when chitosan is applied in soil, because chitinolytic microorganisms proliferate destroying nematode eggs and degrading the chitin-containing cuticle of young nematodes [240]. Because of the high content of nitrogen in chitosan and chitin molecules, concentrations of ammonia emissions increase turning toxic to nematodes which principally affect plant roots and shoots [239, 243].

Further research is still required to find more applications of chitosan in agriculture, but nowadays this polymer means to be a cheap and easy material to deal with crop problems pre-harvest, harvest and post-harvest level.

47. Conclusions

Scientific databases reveal thousands of articles and patents related to chitin, chitosan and its derivatives and increasingly opens up new possibilities to produce new derivatives as well as new applications.

The answer to the question if the chitosan is a “new panacea”, is given by the multiple applications for this new biopolymer and its predecessor, the chitin. Two hundred years have passed since its discovery and this biopolymer has shown unique qualities that many other polymers do not have, as it can be applied in different areas like in the agricultural and medical field or in related areas such as pharmacy and biomedical.

As seen in this chapter, chitosan's behavior in different applications within diverse areas, is governed by its molecular weight, degree of deacetylation, degree of polymerization and source of obtention. Twenty years ago the articles published did not provide data on the characterization of material but today most papers focus on the properties of the polymer before the application.

Also, through the study of this biopolymer and due to the great demand of chitin and chitosan, it is very important to direct all efforts to seek methods of production through environment-friendly processes and on the other hand, through genetic engineering methods, finding the way to produce a more uniform material with characteristics previously designed, especially for medical or pharmaceutical items.

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