CHITOSAN: A CRITICAL REVIEW OF STRUCTURAL CHARACTERISTICS – PROPERTIES RELATIONSHIP

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Dedicated to the memory of
Acad. Bogdan C. Simionescu (1948 – 2024)

Recognized for its potential for over 50 years, chitosan continues to captivate researchers aiming to develop innovative biomaterials across a wide spectrum of applications including biomedicine, agriculture, environmental protection, cosmetics, and food technology. Following extensive investigations into its safety, properties, and possible uses, chitosan has now entered a phase of maturity, where the focus shifts toward translating research into market-ready products. To achieve this goal, a comprehensive understanding of the influence of the structural parameters of chitosan on its functional properties is necessary. These parameters, which are highly dependent on the source and method of preparation, significantly affect not only physicochemical characteristics, such as crystallinity, solubility, and viscosity, but also key bioactivities including biodegradability, antimicrobial efficacy, hemostatic potential, and anti-inflammatory effects. In this context, the present review aims to provide an analysis of the relationship between structural parameters of chitosan and its properties, offering a valuable insight into the rational design of chitosan-based products with real-world applicability.

Keywords: chitosans, overview, polycationic, structure, properties

INTRODUCTION

Chitosan is a linear polysaccharide distinguished among others by its cationic nature, conferred by the presence of amine units. It is naturally occurring in some species of fungi, and usually, it is obtained through the chemical modification of chitin, a structural component abundantly found in the exoskeletons of crustaceans, insects, and in fungal cell walls.[1] This origin from a renewable natural resource makes chitosan a sustainable biopolymer and a promising candidate for replacing petroleum-derived plastics.

The high value of chitosan is prompted by its good biocompatibility, biodegradability, non-toxicity, and a wide spectrum of bioactivities, which make it of extensive interest in various fields, including biomedical and environmental ones. It is important to highlight that many of chitosan's bioactivities are closely related to the presence of its primary amine groups, which confer distinctive biological interactions.

Due to its natural origin, the physicochemical characteristics of chitosan, such as molecular weight, polydispersity degree and pattern of deacetylation, vary significantly depending on the source of the raw material and the specific conditions used during the deacetylation process. This is a serious challenge for researchers, taking into consideration that chitosan's properties are directly correlated to these characteristics, making the reproducibility a challenging task.

Taking into consideration these facts, the present review paper is focused on the main characteristics of chitosan, the structure–properties relationship, and routes for improving the intrinsic activities, either by chemical modification or by formulation.

CHITOSAN OBTAINING, PURITY AND SAFETY

Chitosan is a naturally derived biopolymer obtained through the deacetylation of chitin, a structural polysaccharide found in the

exoskeletons of crustaceans and insects, as well as in the cell walls of fungi.[2–5] Based on its origin, chitosan can be classified as either animal-derived or vegan. This is relevant, as animal-derived chitosan may face restrictions in certain consumer segments and religious contexts. Furthermore, in pharmaceuticals, cosmetics and medical devices, source transparency is often required for regulatory approval and labelling. This is because marine-derived chitosan may contain traces of heavy metals depending on the harvesting location and allergen risks from residual shellfish proteins,[6] which can be problematic in biomedical, cosmetic and food-contact applications. On the other hand, fungal-derived chitosan is allergen-free, making it safer for sensitive populations.

Industrial chitosan production predominantly relies on chemical extraction due to its high efficiency and rapid processing. This method typically involves four sequential steps (Fig. 1):

- *Demineralization* – to remove calcium carbonate and other minerals using strong acids (*e.g.*, hydrochloric acid);
- *Deproteinization* – to remove proteins with strong alkalis (*e.g.* sodium hydroxide);
- *Discoloration* – to remove different pigments (*e.g.* melanin, astaxanthin, carotenoids) in order to yield an off-white product;
- *Deacetylation* – to hydrolyse acetyl groups into amines through alkaline treatment, converting chitin into chitosan.

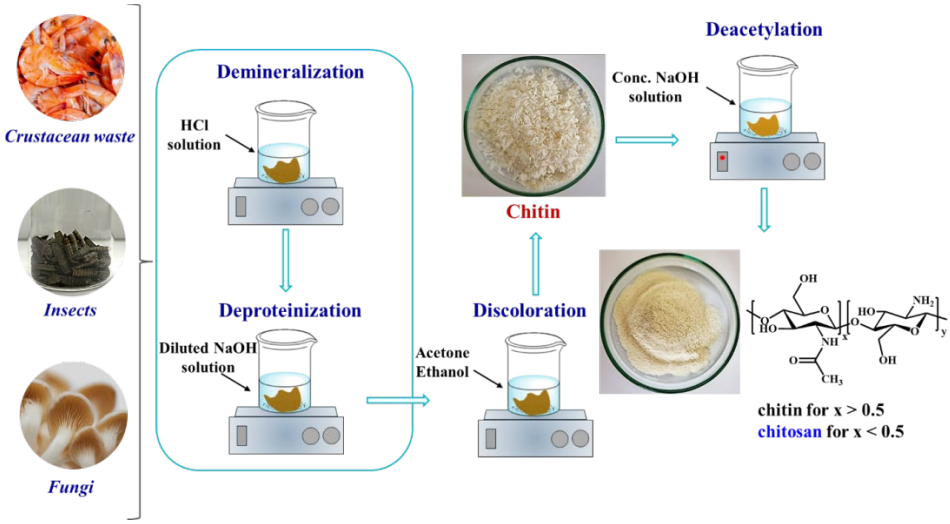

Figure 1: Representation of the sources of chitosan and the obtaining steps

Although this process is cost-effective and well-established for large-scale applications, it requires harsh chemicals, which can contribute to environmental pollution and cause the degradation of valuable co-products, such as proteins and minerals.[2] To address these environmental drawbacks, green extraction techniques were explored, including enzymatic and microbial methods, as well as physical and solvent-assisted approaches, such as microwave irradiation, ultrasound treatment, ionic liquids, deep eutectic solvents, electrochemical methods, and pulsed electric fields.[7] These strategies offer advantages, such as higher purity and yield, along with milder reaction conditions that minimize the ecological impact. However, their relatively lower efficiency, compared to that of chemical extraction, limits implementation in industrial-scale production.

A more ecological approach appears to be the obtaining of vegan chitosan from fungi *via* fermentation under controlled conditions, avoiding seasonal fluctuations and overfishing concerns, and reducing the need for harsh demineralization steps, since fungal chitin contains little calcium carbonate.[5]

Another pathway, less explored, is obtaining of chitosan from insects, *via* a procedure similar with that of marine chitosan, but which offers the advantage of a better control of its characteristics.[8]

STRUCTURE AND CHARACTERISTICS OF CHITOSAN

Chitosan is defined through a series of structural parameters, including: molecular weight (Mw), polydispersity index (PDI), degree of deacetylation (DD) and pattern of acetylation (PA) (Fig. 2). These characteristics vary very much as a function of the source of chitosan and its extraction methods, leading to a wide variability in structural parameters, which results in distinct physicochemical and biological properties. Therefore, rather than referring to chitosan as a single biopolymer, it is more accurate to consider it a class of biopolymers, collectively referred to as "chitosans".

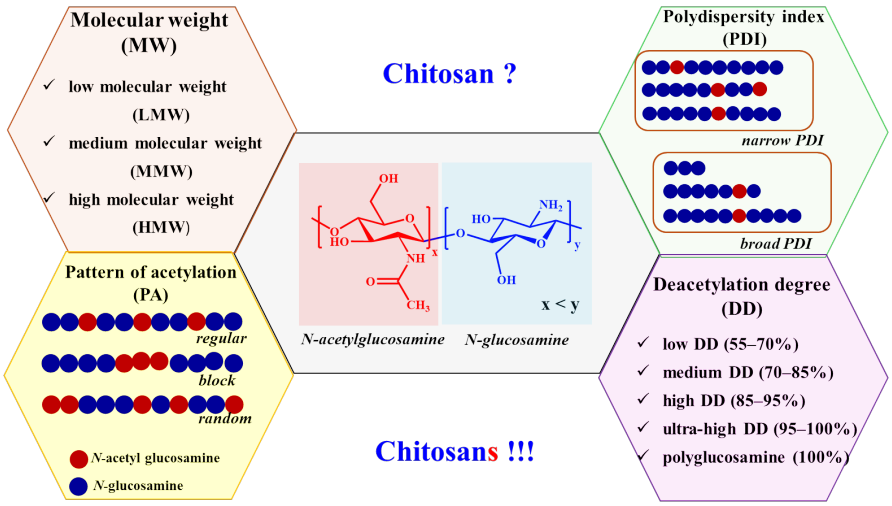

Figure 2: Representation of the structural parameters affecting the properties of chitosan

Degree of deacetylation

Chitosan is obtained from chitin through partial deacetylation, a process in which part of the acetyl groups are removed. Consequently, chitosan is a linear random copolymer consisting of glucosamine (deacetylated units) and *N*-acetylglucosamine (acetylated units) linked by glycosidic bonds (Figs. 1, 2). The DD of chitosan is the percentage of N-acetylglucosamine units in the polymer chain that have been converted to D-glucosamine units during the deacetylation process. There is no clear DD to define the boundary between chitosan and chitin. Some authors consider chitosan having a DD greater than 70%, while others propose a threshold above 50%. From a structural and functional perspective, a 50% DD can be considered a logical cut-off, as it represents an equal proportion of the two monomeric units, leading to significant changes in the polymer's physicochemical and biological properties.

Based on the DD, chitosan can be classified in: low DD (55–70%), medium DD (70–85%), high DD (85–95%), and ultra-high DD (95–100%).[9] Chitosan consisting entirely of glucosamine units (100% DD) is referred to as "fully deacetylated chitosan", "full chitosan" or "polyglucosamine". Due to the simultaneous occurrence of deacetylation and degradation in alkaline media, obtaining fully deacetylated chitosan under these conditions is challenging. However, it can be found as a commercial product, used by many researchers in order to prepare chitosan with controlled DD through acetylation reactions, for better reproducibility of properties when used for specific applications.[10,11]

Since amine groups are mainly responsible for chitosan's bioactive properties, the DD plays a key role in determining its physicochemical and biological performance.[9] An increase in DD strengthens the hydrogen-bonding network, which enhances crystallinity, but reduces solubility. Furthermore, a higher proportion of amine groups improves bioactivities directly dependent on them, such as antimicrobial activity, hemostatic performance, and mucoadhesion. However, high DD also reduces biodegradability and may lower biocompatibility, as highly deacetylated chitosan is more resistant to enzymatic degradation and may trigger stronger inflammatory responses.[12]

Molecular weight

Mw is the main characteristic of chitosan that determines its solubility, viscosity, degradation

rate, mechanical strength, bioactivity, and application suitability. Based on Mw, chitosan can be classified into three categories:

- low molecular weight (LMW), 20–150 kDa;
- medium molecular weight (MMW), 150–700 kDa;
- high molecular weight (HMW), >700 kDa.

These ranges may vary slightly among authors due to the absence of a standardized IUPAC definition.[13] In addition, chitosan oligomers, or chitooligosaccharides, are defined as chitosan molecules with Mw below 20 kDa.[14,15]

LMW chitosan, with its higher solubility and more rapid degradation, is suitable for fast-release drug delivery or applications where rapid clearance is desired, such as antimicrobial treatments and wound healing. Conversely, HMW chitosan, characterized by higher mechanical strength and slower degradation, is ideal for long-term biomedical and industrial uses, such as film and coating applications as antimicrobial food packaging or protective biomedical coatings, water purification and heavy metal adsorption, where higher molecular weight enhances adsorption capacity and material stability in aqueous systems.

The molecular weight of chitosan can be tailored through various approaches, including enzymatic degradation,[16] controlled depolymerization[17] and fractionation by ultrafiltration.[18] Such control enables the customization of chitosan's properties for specific biomedical, pharmaceutical, and industrial applications.

Polydispersity index

Usually, obtaining chitosan *via* alkaline hydrolysis yields chitosan with a broad PDI. The influence of the PDI on the properties of chitosan-based biomaterials is mainly related to its influence on the crystallinity degree, which is reflected in solubility and mechanical performance. It is expected that a narrow PDI, corresponding to a more uniform molecular weight distribution, promotes better packing of chitosan chains into crystalline domains, leading to a decline in solubility because of stronger intermolecular interactions.[9]

Conversely, a bimodal molecular weight distribution, containing both high- and low-molecular-weight fractions, generally conducts to a reduction of the crystalline degree, which improves the solubility and ultimately can compromise the mechanical strength of biomaterials.[19] To achieve better control of chitosan's properties, it is recommended that commercial chitosan be purified prior to use, including a stage to narrow its PDI.

Pattern of acetylation

The PA, defined by the spatial distribution of acetylated and deacetylated units along chitosan's backbone, influences key attributes such as solubility, bioactivity, biodegradability, and interactions with biological systems. Chitosan can exhibit various deacetylation patterns, including random, block-like, or alternating distributions (Fig. 2).[20] These patterns are influenced by the source of chitin and the method of deacetylation, whether homogeneous or heterogeneous. Recent studies have revealed that chitosan obtained *via* heterogeneous deacetylation exhibits a non-random, regular PA, with acetylated units preferentially located at every third position along the polymer chain.[21] In contrast, chitosan obtained by controlled acetylation of polyglucosamine with acetic anhydride led to a random PA.[22,23]

A random deacetylation pattern typically enhances water solubility due to the irregular distribution of hydrophilic and hydrophobic regions, facilitating better interaction with aqueous environments. In contrast, a block-like arrangement of acetyl groups promotes stronger intermolecular interactions, contributing to increased crystallinity and reduced solubility. The PA plays an important role in enzymatic degradation of chitosan in presence of lysozyme or chitinase, as recognition and cleavage are highly dependent on the sequence and spacing of acetylated units.[24] Furthermore, it was demonstrated that PA is essential for signalling pathways in human cells and plants.[25]

However, despite its crucial impact on the properties, PA is often neglected in chitosan studies, because of the difficulty to analyse it.[26]

PROPERTIES OF CHITOSAN

Chitosan is the only naturally occurring polycationic polysaccharide, due to its amine groups, which protonate in acidic environments, transforming in transient positive charges. This cationic nature facilitates strong electrostatic interactions with negatively charged biological sites, including cell membranes, DNA, and proteins.[27–29] Such interactions enhance the capacity of chitosan to bind and stabilize

negatively charged drugs or bioactive agents, making it highly suitable for drug delivery systems, especially those requiring controlled release or gene transfer *via* complexation with nucleic acids.[30,31] Furthermore, the protonated amines improve the antimicrobial activity, muchoadhesion and cellular uptake (Fig. 3). On the other hand, the cationic nature of chitosan is "a double-edged sword", also presenting disadvantages. First, it should be taken into consideration that chitosan's cationic nature is only active under acidic conditions (pH < 6.3), restricting its functionality in neutral or alkaline environments characteristic of biological fluids. While improving cellular uptake by opening the tight junctions of cell membranes is an advantage for improving the efficiency of drug delivery, the excessive cationic charge density can disrupt mammalian cell membranes, leading to haemolysis, inflammation or cell death. The strong electrostatic interactions promoted by cationic charges are of course an advantage for binding drugs or bioactive compounds, but in complex biological environments, this can result in off-target effects or reduced selectivity.

In this view, the properties of chitosan will be critically exposed, highlighting the advantages and disadvantages.

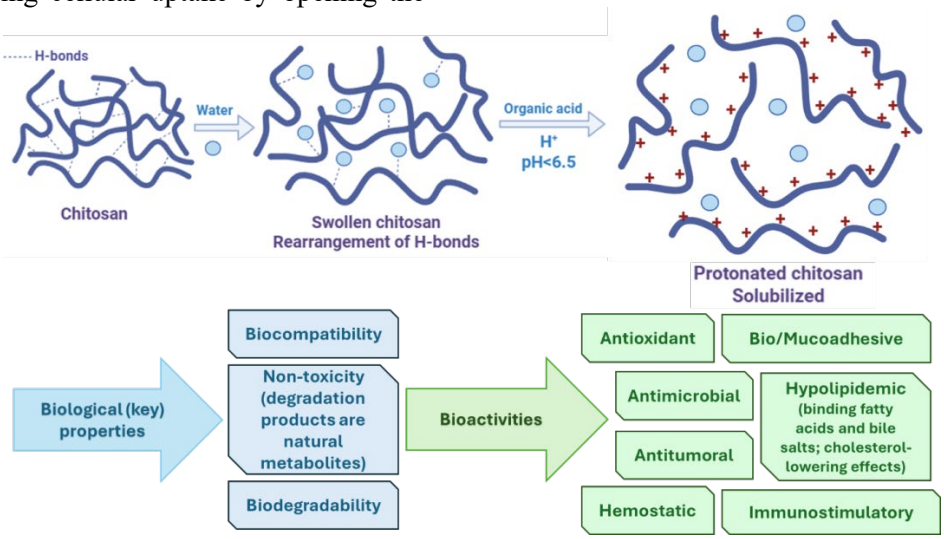

Figure 3: Solubilisation of chitosan in acidic environment and most relevant properties induced by the presence of positive charges (created in Biorender – https://BioRender.com)

Physical properties

Crystallinity

Chitosan is recognized in literature as having a semicrystalline state, consisting of both crystalline and amorphous regions, reaching a crystallinity index up to 0.9.[32] The organization of chitosan chains into crystalline clusters is an outcome of its linear structure and strong intermolecular forces, which can develop between its chains. The crystallinity of chitosan is a very important parameter, as it influences the solubility, biodegradation and mechanical properties of its biomaterials. This is because the well-packed chains in the crystalline domains hinder the access of solvent molecules. Furthermore, higher crystallinity generally leads to increased strength and stiffness, while reduced crystallinity can result in greater flexibility and ductility. The crystallinity degree is correlated with the Mw, DD, PDI and PA. Shorter chitosan chains (lower Mw) will have the tendency to crystallize easier than longer chains, while a higher DD will facilitate the crystallization.[33] A higher PDI will also hinder the crystallization. However, the crystallization conditions play a decisive role in the crystallinity degree.[34]

Solubility and viscosity

The high number of hydroxyl groups of chitosan contributes to a dense hydrogen-bonding network, rendering it insoluble in water and most organic solvents under neutral or basic conditions. However, in acidic environments (typically pH < 6.5), the protonation of the amine groups disrupts this network, significantly enhancing solubility (Fig. 3). Organic acids, such as acetic, formic, lactic, tartaric, malic and citric acids, at pH less than 6.5 were successfully used to solubilize

chitosan. Nevertheless, some of them appear to impact the degradation of chitosan.[35] As a general rule, monoprotic acids dissolve chitosan better than multiprotic ones. For example, phosphoric acid or sulfuric acid are not good solvents for chitosan. Other systems, such as pressurized CO_2-water[36] or urea-alkali[37] have also been reported as being efficient in chitosan dissolution. On the other hand, in neutral or basic media, characteristic of biological fluids, the protonated groups are transformed into amine units, rendering chitosan insoluble. This is an important aspect that should be taken into consideration when designing chitosan biomaterials for *in vivo* applications.

The solubility of chitosan is influenced by Mw, DD, and PDI. As rationally expected, lower Mw will induce better solubility due to better mobility of the shorter chains, forming less viscous solutions, with lower chain entanglements and intermolecular hydrogen bonding. On the other hand, higher Mw will conduct to solutions with higher viscosity, which can be advantageous in preparation of materials such as hydrogels. In literature, there are reported even water-soluble chitosan oligomers, when Mw was around 2000–6500 g/mol.[38] A higher DD will improve the solubility by increasing the protonated sites and consequently increasing the repulsive forces amongst the macromolecular chains.[39] However, the DD does not impact the solubility and viscosity of chitosan to the same extent as Mw.[39] It is expected higher polydispersity will lead to an inhomogeneous solubility because of the longer macromolecular chains.

The temperature is also helpful in improving the solubility of chitosan by destabilization of H-bonds network by increasing chain mobility. It was shown that, in acidic solutions, at around 40 °C, the H-bonds are hindered and consequently the fluidity of chitosan solution is improved.[40]

pH sensitivity

The presence of the protonable amine groups endows chitosan with pH-responsive behavior. In acidic environments, the amine groups become protonated, leading to electrostatic repulsion between polymer chains and increased chain mobility (Fig. 3). This results in swelling and enhanced solubility, which are beneficial for applications requiring material expansion or drug release. Conversely, in alkaline conditions, the amine groups are deprotonated, allowing the formation of hydrogen bonds between polymer chains. This promotes chain aggregation, increased structural integrity, and reduced solubility. This pH-sensitive behavior is particularly valuable in the design of chitosan-based hydrogels, nanoparticles, and drug delivery systems, where controlled swelling, gelation, and release kinetics can be finely tuned in response to the environmental pH.[41,42]

Mechanical properties

It is often pointed out that chitosan has weak mechanical properties, which, of course, affects the mechanical properties of the chitosan biomaterials. Generally, chitosan films are known for their brittle nature, but their properties can be enhanced using plasticizers, crosslinking agents, or by incorporating other materials like nanoparticles.[43,44] The mechanical properties of chitosan biomaterials, like tensile strength and flexibility, can be significantly impacted by DD, Mw and the presence of additives or chemical modifications. Chitosan of higher Mw can lead to stronger films, but the Mw–mechanical properties relationship can be complex.[45,46]

Key biological properties

Toxicity

Chitosan has gained recognition among researchers as a biocompatible, biodegradable, and non-toxic biopolymer. The reputation of chitosan as a non-toxic biopolymer stems from its natural origin, structural similarity to glycosaminoglycans (key components of the extracellular matrix), and favorable interactions with biological systems. Chitosan is well tolerated in humans, with no adverse effects following oral administration of up to 6.75 g per day for 8 weeks.[47] It is approved by GRAS and currently applied for dietary use, cosmetics, wound dressings and cartilage repairing formulations.[48] However, while chitosan is undeniably less toxic than the majority of synthetic polymers and holds immense promise for biomedical use, the claim of non-toxicity should not be accepted uncritically. Numerous studies have shown that the toxicity profile of chitosan is highly context-dependent, influenced by factors such as Mw, DD, concentration, dosage, formulation and delivery route. Chitosan proved cytotoxicity for concentrations higher than 2.0 mg/mL in most cell lines.[49] It was also shown that the cytotoxicity increases for higher DD, an effect attributed to the increase of cationic charge density, which may disrupt cell membranes.[50] It was also

demonstrated that chitosan's toxicity varies significantly between oral, topical, and injectable forms. For instance, intranasal or intravenous formulations may elicit immune responses or mucosal irritation, which were not observed in topical use.[49,51]

Biocompatibility

Chitosan has exceptional biocompatibility due to its structural similarities with glycosaminoglycans and the presence of functional groups that develop interactions with cellular environments. Unlike many synthetic polymers, chitosan is typically non-allergenic, and exhibits low immunogenicity, minimizing the risk of adverse reactions and supporting its safe use in clinical settings.[52] Because in biological fluids chitosan undergoes biodegradation, it is important to note that the biodegradation products of chitosan, namely glucosamine and chitooligosaccharides, are themselves biocompatible and have demonstrated beneficial effects on tissue regeneration and immunomodulation.[53] This makes chitosan particularly suitable for long-term applications, as it does not accumulate in the body and contributes positively to tissue health. However, it should be taken into consideration that Mw, DD and chemical modification of chitosan can influence chitosan's biocompatibility. A high DD can lead to an excessive cationic charge, which may cause haemolysis or cytotoxicity at elevated concentrations.[54] Conversely, LMW chitosan tends to be better tolerated by cells, offering improved metabolic processing and cellular uptake.

Biodegradability

Chitosan is biodegradable, its breakdown occurring predominantly through enzymatic hydrolysis. Key enzymes involved in this process include lysozymes, chitinase, chitosanases, and nonspecific proteases, which cleave the polymer into chitooligosaccharides and eventually into glucosamine monomers.[51,55] The rate of chitosan's degradation is influenced by Mw, DD, PA and crystallinity, as well as external conditions, such as pH, temperature, and the presence and activity of specific enzymes.[24,56,57] Generally, chitosan with lower Mw and DD exhibits faster degradation, owing to its enhanced solubility and greater accessibility to enzymatic attack. Lysozyme, an enzyme that is naturally present in the human body, is active at acetylated sites, thus chitosan with low DD is more susceptible to degradation, while regular or alternating patterns enhance enzymatic recognition and cleavage. On the contrary, chitosanase is more efficient at degrading highly deacetylated chitosan. This is an important aspect for the design of drug delivery systems or scaffolds for tissue engineering: for optimal degradation, the Mw and DD of chitosan should be chosen as a function of the predominant enzyme in the target tissue, *e.g.* for oral or nasal delivery, which are lysozyme-rich environments, chitosan with moderate DD should be used. Different techniques were developed to control the degradation rate, including the incorporation of lysozyme in the architecture of chitosan biomaterials.[58]

Bioactivities

Antimicrobial activity

Chitosan exhibits notable antimicrobial activity against a broad spectrum of bacteria, fungi, and viruses, primarily due to its polycationic nature.[59] Even if an exact mechanism of action is not still elucidated, it was hypothesised that the protonated amine groups play a key role, by their ability to interact electrostatically with the negatively charged microbial cell walls (Fig. 4). This interaction disrupts membrane integrity, leading to increased permeability, leakage of intracellular contents, and ultimately cell lysis.[60–62] Additionally, chitosan can chelate essential metal ions (*e.g.*, Mg^{2+}, Ca^{2+}) from microbial surfaces, interfering with metabolic processes and enzyme function. Another proposed mechanism suggests that low molecular weight chitosan can penetrate microbial cells, where it may bind to DNA, thereby inhibiting replication and transcription.

The antimicrobial efficacy of chitosan appears to be influenced by its Mw and DD, even though the literature provides often contradictory findings. A systematic review came to the conclusion that the strongest antimicrobial activity belongs to chitosan with a low to intermediate Mw, while those with higher Mw had lower activities and chitosan oligomers were almost inactive.[63] Generally speaking, lower Mw and higher DD should enhance solubility and increase the likelihood of interaction with microbial membranes, thereby improving antimicrobial performance.[64] The acetylation pattern appears to also play a role in antimicrobial activity. Even if not so many studies on this topic were reported, there are a few suggesting that

block-like PA provide improved antibacterial activity compared to randomly PA with similar DD, attributed to denser areas of positive charges along the chain.[65]

The antimicrobial activity of chitosan is also pH-dependent. Under acidic conditions, the protonation of amine groups intensifies its positive charge, strengthening its interaction with microbial surfaces and enhancing antimicrobial potency. Further, the antimicrobial activity of chitosan can be modulated through chemical modifications, such as: quaternization to introduce permanent positive charges,[66,67] imination with antimicrobial aldehydes,[64,68] or incorporation of active agents, including metal nanoparticles, essential oils, or polyphenols, which synergistically boost its antimicrobial spectrum and effectiveness.[69–71]

Antioxidant activity

Chitosan exhibits intrinsic antioxidant activity, primarily due to the presence of amine (-NH_2) and hydroxyl (-OH) functional groups capable of neutralizing various free radicals, superoxide radicals, and hydroxyl radicals, by donating electrons or hydrogen atoms. Additionally, chitosan can chelate transition metals like Fe^{2+} and Cu^{2+}, thereby inhibiting their participation as catalysts in the formation of reactive oxygen species (ROS), thus reducing their pro-oxidant activity.[72,73]

The antioxidant capacity of chitosan is influenced by its molecular weight (Mw) and degree of deacetylation (DD). Lower Mw and higher DD enhance radical scavenging efficiency by increasing the availability of free amine groups and improving solubility.[73,74] Similarly to its antimicrobial behavior, chitosan's antioxidant activity is pH-dependent, with optimal performance observed under slightly acidic conditions, where protonation enhances its reactivity. Furthermore, chitosan's antioxidant potential can be enhanced through chemical modifications or incorporation of antioxidant agents, such as polyphenols, vitamins, or metal nanoparticles, which synergistically improve its ability to combat oxidative stress.[73] The antioxidant activity of chitosan is a valuable property that can reduce oxidative stress at injury sites, thereby promoting wound healing, reducing inflammation, and supporting tissue regeneration.[75] It can also modulate redox activity in cancer therapy,[72] regulate oxidant activity in skin with anti-aging effects[76] and extend shelf life while preserving nutritional quality in food industry.[77]

Muco- and bio-adhesivity

Chitosan exhibits strong mucoadhesive and bioadhesive behavior, primarily due to its cationic nature, which facilitates electrostatic interactions with negatively charged biological surfaces, particularly mucosal tissues rich in mucins (Fig. 4). In addition to ionic bonding, hydrogen bonds formed between the amine and hydroxyl groups of chitosan and glycoproteins in the mucus layer further enhance adhesion.[78] The mucoadhesive strength of chitosan is influenced by its Mw and DD. Higher Mw provides longer polymer chains that promote entanglement with mucosal surfaces, while increased DD offers a greater number of protonable amine groups, intensifying electrostatic interactions. Moreover, chemical modifications with units able to bind to mucin, such as thiol, carboxyl, or quaternary amine groups, can significantly improve mucoadhesive performance.[79] This mucoadhesion ability of chitosan is particularly advantageous in drug delivery systems, by prolonging residence time on mucosal surfaces (oral, ocular, nasal, pulmonary, vaginal), thereby enhancing bioavailability. It is also of interest in wound dressings, improving the adherence to the wound site and forming a protective barrier against infection, and in scaffolds for tissue regeneration, by supporting cell attachment, proliferation, and matrix integration.[80]

Hemostatic activity

The polycationic character of chitosan endows it with potent hemostatic activity, making it highly effective in wound management and bleeding control. Its ability to promote blood clotting is attributed to multiple synergistic mechanisms: (i) electrostatic interactions between positively charged amine groups and negatively charged red blood cells (RBCs) and platelets lead to their aggregation, initiating the coagulation cascade and accelerating fibrin formation,[81] and (ii) the hydrophilic nature of chitosan allows it to absorb wound exudates, concentrating clotting factors at the injury site and promoting coagulation,[82] forming a strong physical barrier that adheres to wet tissues and seals the wound (Fig. 4). Remarkably, chitosan was shown to induce clot formation even in anticoagulated blood, making it particularly valuable for patients with coagulation disorders.[83,84]

It was reported that the hemostatic efficacy of chitosan is modulated by its Mw and DD. A higher DD increases cationic charge density, enhancing interactions with platelets and improving clot formation. Additionally, chemical functionalization, such as quaternization or oxidation, can further boost hemostatic performance.[82,85,86] However, such modifications must be carefully evaluated, as they may introduce cytotoxic effects that compromise the biocompatibility. A medium-high molecular weight and medium DD appeared to be recommended for hemostatic properties.[86] It is rationally expected that the PA also influences the hemostatic activity. A regular or block-like deacetylation pattern may create localized regions of high charge density, favourable for interactions and promoting cell aggregation and clot formation. Conversely, a random pattern may distribute charges more diffusely, potentially reducing the intensity of interaction at any given site.

Chitosan's hemostatic properties are especially useful in traumatic injuries, surgical incisions, and burns, where rapid bleeding control is critical, hemostatic dressings for both internal and external bleeding sites, offering a safe and effective alternative to conventional agents. As a function of the targeted site, various chitosan-based formulations in various shapes (*e.g.* sponges, hydrogels, nanofiber membranes or foams) were investigated and some of them are already on the market.[82]

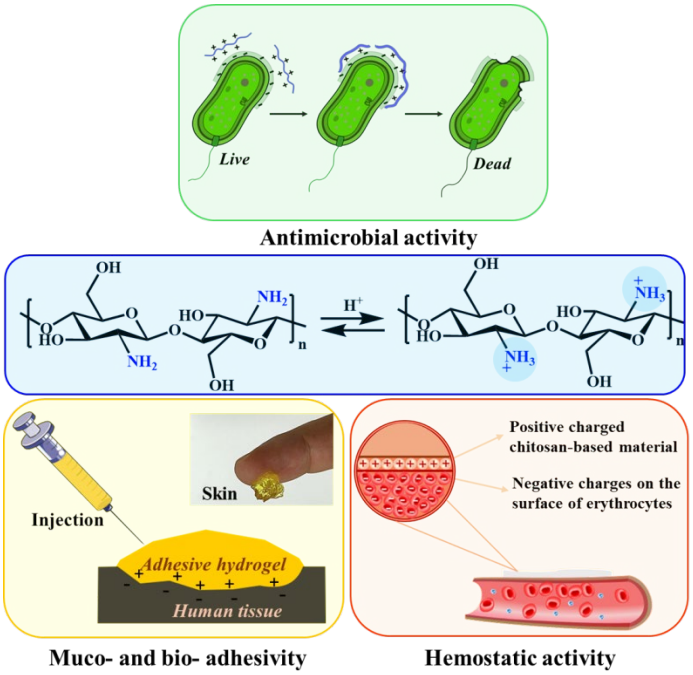

Figure 4: Representation of the bioactivities induced by the protonation of chitosan's amine groups

Cell adhesion and proliferation

Given that biocompatibility is a fundamental requirement for biomedical applications, chitosan has been extensively investigated for its interaction with human tissues and cells. Numerous studies have demonstrated that chitosan provides a good substrate for cell adhesion and proliferation, particularly supporting the growth of epithelial cells, fibroblasts, osteoblasts or neurons.[87,88] Its surface chemistry, combined with the presence of functional groups capable of forming hydrogen bonds and electrostatic interactions, facilitates cell attachment, spreading, and matrix integration, making it highly suitable for applications in tissue engineering, wound healing, and bio-scaffold development. However, the effectiveness of chitosan in promoting cell growth is not uniform and is significantly influenced by its Mw and DD. *In vitro* studies have shown that the cell adhesion is better for chitosan with higher DD, attributed to higher crystallinity.[89]

Anti-inflammatory activity

Many studies reported that chitosan possesses anti-inflammatory activity through various mechanisms, implying the modulation of cytokine expression, scavenging free radicals, inhibiting

inflammatory enzymes and regulating immune responses.[90] The anti-inflammatory activity of chitosan was observed in wound healing, where it promotes tissue granulation and inhibits pro-inflammatory cytokines.[91]

Immunostimulatory activity

It is often affirmed that chitosan has immunostimulatory activity. Indeed, *in vitro* and *in vivo* investigations indicated that chitosan boosts the innate and adaptive immunities, in a dose dependent manner, stimulating the secretion of TNF-alpha and IL-beta cytokines from macrophages.[92–95] It has been also reported that chitosan stimulates the host immune system against viral and bacterial infection.[96] It appears that Mw, DD and PA all play an important role in the mechanism of immunomodulation. As an example, it was shown that the randomly acetylated chitosan is less prone to inducing the production of inflammatory markers/cytokines.[97] Also, it was shown that the immunostimulatory effect is increased by formulation with antioxidant agents.[98] Nevertheless, this property remained less explored than others.

Anticancer activity

Chitosan has predominantly been explored as a carrier, matrix, or adjuvant in antitumor drug formulations, while its intrinsic antitumor potential has received less attention.[72,99] Some studies suggested that chitosan may exert synergistic antitumor effects due to its antioxidant and antimicrobial activities, or due to the ability to enhance the biodistribution *via* increased mucoadhesion and membrane permeability. It was also reported that chitosan can contribute to tumour angiogenesis inhibition, supporting its potential as a multifunctional component in cancer therapy.[99]

Regarding the influence of Mw and DD on the anticancer activity of chitosan, literature offers quite contradictory results. Thus, chitosan oligomers with high DD showed *in vitro* antitumor effects against prostate, lung, hepatoma, and oral squamous cell carcinoma cell lines.[100–102] On the other hand, LMW and HMW chitosan showed similar selective cytotoxicity against osteosarcoma, breast, and cervical cancer cells.[103] *In vivo*, chitosan oligomers reduced tumour growth in sarcoma-bearing mice, whereas higher MW chitosan did not.[104] Chitosan nanoparticles demonstrated dose- and size-dependent antitumor activity in hepatoma models.[105] These findings suggest that solubility, rather than MW alone, plays a critical role in chitosan's antitumor efficacy, reinforcing its potential as biocompatible adjuvant in cancer therapy.

Hypolipidemic effect

Chitosan is well recognized for its hypolipidemic effects, contributing to a reduced risk of cardiovascular diseases. Its lipid-binding capacity arises from a combination of electrostatic and hydrophobic interactions, which vary depending on the lipid type and environmental conditions. In acidic media, protonated amine groups on chitosan interact with negatively charged lipids, such as fatty acids and bile salts, forming insoluble complexes that are eliminated from the body.[106] Chitosan also binds neutral lipids, like cholesterol and triglycerides, *via* hydrophobic interactions, particularly when these lipids are incorporated into micelles or emulsions, facilitating coprecipitation under optimal pH and ionic strength.[107] Studies evaluating chitosans with varying structural parameters found no significant differences in bile acid or fat-binding capacity, which ranged from 1077 to 1239 g oil/g.[108] Additionally, both low and high molecular weight chitosan demonstrated comparable cholesterol-lowering effects,[109] suggesting that solubility, rather than molecular weight alone, may be the key factor influencing its lipid-binding efficiency.

CONCLUDING REMARKS

Chitosan is a remarkable biopolymer that is already present in numerous commercial products. Its continuous expansion into new innovative products is promising, but a deeper and more systematic understanding of the relationship between structural parameters and functional properties is necessary. The considerable variability in molecular weight, polydispersity, deacetylation degree, and acetylation pattern lead to pronounced differences in chitosan's physicochemical and biological performance. Given this complexity, it is more accurate to refer to chitosan not as a single, uniform material, but rather as a family of structurally diverse biopolymers: *chitosans*. This diversity requires precise characterization and standardized reporting of structural parameters prior to any formulation or application.

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