REVIEW

PROPERTIES AND EMERGING APPLICATIONS OF CHITOSAN NANOPARTICLES: A BRIEF REVIEW

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Abstract

The review article presents current research on obtaining chitosan nanoparticles (NPs). It highlights their key properties and potential applications in agriculture. Various methods of chitosan nanoparticle formation were considered: emulsion method in the presence of special agents, drying method, crosslinking of molecules through covalent bonds, self-organisation processes and obtaining interpolyelectrolyte complexes, and ionotropic gelation. Some examples of the chitosan NPs application have also been reviewed.

Keywords: *chitosan, nanoparticles, ionic gelation technique, nanocapsules, bioactivities, eco-friendly*

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1. Nanomaterials and Methods for Obtaining Chitosan Nanoparticles

Regardless of the extraction source, chitin, chitosan, and their derivatives attract the attention of researchers from year to year. In particular, the unique properties of chitosan (CS), such as biocompatibility, biodegradability, and non-toxicity, as they are extracted from a renewable source, expand the areas of their application. High reactivity of chitosan, due to the presence of electron-donor groups in its molecule, contributes to an increase in research on the modification and functionalisation of macromolecules of natural polysaccharide.

Currently, research is being conducted worldwide to develop innovative solutions based on green chemistry. We can contribute to this field through research focused on the extraction of CS and the synthesis of its derivatives, which fully adhere to the principles of green chemistry. These principles include minimising the quantity of reagents used, reducing hazards associated with synthesis processes and products, employing safer chemicals, conserving energy, utilising renewable raw materials, streamlining technological stages, and ensuring biodegradability, biocompatibility, and non-toxicity. These valuable properties of chitin and chitosan broaden the potential application areas of chitosan-based preparations across various sectors of the national economy. It should be emphasised that with the development of nanotechnology, new opportunities are opening up for obtaining nanostructured modifications of chitin and chitosan that regulate their structure, morphology, and, accordingly, reactivity [1, 2].

Chitosan-based nanomaterials are promising materials that have received special attention in the last decade. Nanomaterials have distinctive properties from massive materials and atoms due to their smaller size and high surface tension, which determines their increased chemical and biological activity. They can be conditionally divided into the following groups: nanogels, nanodots, nanocarrier systems, nanofibers, and nanodispersions.

Nanoparticles (NPs) are divided into natural or synthetic, which are sometimes referred to as non-engineered and engineered NPs, respectively. Non-engineered NPs, naturally present in the environment, originate from events such as terrestrial dust storms, erosion, volcanic eruptions, and forest fires. Engineered NPs (ENPs), on the other hand, are intentionally produced by man using many different materials, such as carbon (graphene and fullerene), metals/metal oxides (including Ag, Zn, Cu, SiO_2, Al_2O_3), polymers (alginate, chitosan, hydroxyethylcellulose, etc.). These ENPs are able to enter into and exit plant cells and can transport DNA and chemicals into plant cells. The unique physical and chemical properties of NPs could boost plant metabolism [3].

The uniqueness of compounds containing NPs is that their size, shape, total charge of the surface layer, morphological and hydrodynamic parameters differ radically from each other depending on the method of their production. Currently, there are a huge number of methods and combined methods for the production of NPs, such as chemical, mechanochemical, physical, thermolysis, electrolysis, biochemical, radiation, microbial, enzymatic, etc. Accordingly, all the methods for the formation of NPs can be divided into two unique techniques [4]:

- ‘top-down’, that is, bulk materials are decomposed to nanoscale sizes;
- ‘bottom-up’, in which the source material is dissolved and then it undergoes shape changes, for example, by cross-linking or the electrospinning technique.

The ‘top-down’ approach involves producing nanochitosan by deacetylating nanochitin. While this method is effective for the fabrication of NPs from chitin, it does not allow for

the formation of chitin-based nanofibers, as the deacetylation process leads to the almost complete amorphization of the polymer's crystalline structure.

The 'bottom-up' approach is one of the most common and accessible methods for obtaining chitosan NPs and nanofibers. It can be conditionally divided into the following techniques [5]:

1. emulsion method in the presence of special agents;
2. drying method;
3. crosslinking of molecules through covalent bonds;
4. self-organisation processes and obtaining interpolyelectrolyte complexes;
5. ionotropic gelation.

1.1. Emulsion Method in the Presence of Special Agents

Emulsion methods are widely used for the production of nanoparticles, providing high encapsulation efficiency, high stability, and low toxicity. Transparent nanoemulsions typically range in size from 20 to 500 nm, and their stability depends on droplet size and the hydrodynamic parameters of the NP. These systems are widely applied in the food industry, for example, to improve the quality and extend the shelf life of bakery products, dairy products, meat, fruits, and vegetables [6, 7].

1.2. Drying Method

A new technique known as the freeze-dissolve method has been developed for the isolation of NPs or ultrafine powder and is considered a more efficient and sustainable approach. This technique allows for the production of monodisperse nanoparticles with a narrow size distribution [8].

1.3. Crosslinking of Molecules Through Covalent Bonds

One of the effective methods for obtaining polymer nanostructures is the introduction of metal ions into their macromolecules as a cross-linking agent. Chitosan NPs containing Cu^{2+} ions were obtained by regulating the conformation of macromolecules [9, 10]. It was found that during the complexation of chitosan with Cu^{2+} and Co^{2+}, the pH of the medium is of fundamental importance; by varying it, it is possible to obtain polymer-metal complexes (PMCs) of various compositions and types. For the first time, it was shown that at pH 4 - 5, chitosan - acting as a macroligand - forms nanostructured PMCs through cross-linking with Cu^{2+} and Co^{2+} ions [11].

This technique is widely used in the formation of nanostructures in solutions of carboxymethyl chitosan (5 - 116 nm) [12], pectin (300 - 700 nm) [13], and other polymers.

1.4. Self-organisation Processes

This is an important approach to nanoparticle synthesis, based on molecular rearrangement that leads to the formation of nanostructures with improved functional properties [5].

1.5. Obtaining Interpolyelectrolyte Complexes

Recently, there has been an increase in demand for new materials based on polyelectrolyte complexes (PEC), which represent a special class of polymers formed as a result of cooperative reversible interactions of oppositely charged macroions [14, 15].

The formation of PEC occurs in the presence of two or more oppositely charged polycations, polyanions or polyampholytes, in which the internal and external charges compensate each other. Complexation is one of the most effective methods for modifying

the properties of polymers, where the interaction of lyophilising and blocking agents forms binary, layered colloidal dispersions and/or films [16].

The uniqueness of the interpolyelectrolyte complex (IPEC) formation method lies in its simplicity, low cost, low energy consumption, and high efficiency. Non-stoichiometric complexes containing an excess of one polyion have increased sorption activity with respect to medicinal substances, dyes, and proteins due to the presence of a net charge [17].

In excess of polyanion, complexation leads to the formation of non-stoichiometric IPEC (NIPEC), which is practically a block copolymer, the hydrophilic regions of which are represented by free (unbound) anionic units and hydrophobic fragments of mutually neutralised anionic and cationic units. The negative charge imparts colloidal stability to NIPEC particles in aqueous solutions, facilitating their binding with d-metal ions or positively dispersed particles [18].

Water-soluble NPECs can be obtained from oppositely charged synthetic and natural polyelectrolytes under varying conditions. A common method for preparing NPECs involves directly mixing aqueous solutions of positively and negatively charged polyelectrolytes in nonequivalent ratios, at a pH where both are charged, and in the presence of small amounts of a low-molecular weight electrolyte [19].

In this context, the preparation of various chitosan complexes and their modifications, particularly binary or three-component IPEC, as well as the study of their physicochemical properties and potential applications, are of great scientific and practical importance. The fundamental and theoretical principles underlying the formation of thermodynamically stable interpolyelectrolyte complexes involving chitosan and sodium carboxymethylcellulose (Na-CMC), collagen, and pectin have been investigated [18, 20].

When obtaining IPEC, the complexes tend to aggregate due to charge neutralisation; therefore, in order to avoid aggregation and regulate the size of nanoparticles, at least two conditions are mandatory:

- polyelectrolyte solutions must be diluted;
- one of the polyions must be in excess.

Compliance with such rules leads to the formation of nanostructures of polyelectrolyte complexes [21, 22]. The results of microscopic studies indicate that in the CS-collagen complexes, regardless of the ratio of the initial macromolecules, under the selected synthesis conditions, non-spherical nanoparticles of 50 ± 20 nm are formed and have a unimodal character.

It was also found that the interaction of CS with Na-CMC resulted in relatively large nanoparticles, which are due to the structural features of rigid-chain macro ions [23]. The hydrodynamic sizes of CS/Na-CMC NPs varied from 200 nm (at pH = 3.5) to 1000 nm (at pH = 6.3) with high bioactive properties and thermodynamic stability for 3 months. It was found that stoichiometric interpolyelectrolyte complexes can be effective structure formers against soil erosion.

1.6. Ionotropic Gelation

Ionotropic gelation is the most effective and widespread method among all methods of obtaining NP CS (Table 1). Formaldehyde, polyethylene glycol, phosphates, and other low-molecular compounds are used as crosslinkers [5].

NPs of CS have enormous potential as nanocarriers. The empty pores within their cross-linked structures can be filled with active substances, medicinal preparations, and bioactive compounds, enabling targeted delivery to specific cells and sustained release. Chitosan micro- and nanoparticles were obtained using the ion gelation technique.

Table 1. Methods of CS NPs formation by ionotropic gelation.

CS NPs synthesis conditions	Subject of research	CS NPs characteristics	[Ref]
NPs formed at 25°C C(CS) = 0.1 - 0.5 - 2.75 - 5 mg/ml C[η] < ~4, 85 mM NaCl DA (CS) = 20% - 35% - 50% Mw = 125 - 450 kDa magnetic stirrer 750 rpm NH_2/PO_4^{-3} = 0.5 – 1 - 1.5	influence of molecular mass (MM) and degree of deacetylation (DD) of CS, C(CS), the NH_2/PO_4^{-3} molar ratio, and ionic strength of the solution (NaCl)	microparticles 2500 nm nanoparticles 200 nm suspension	[24]
C(CS) 0.05 - 0.5% w/v C(Na-TPP) = 0.25 - 2 mg/ml Tween 80 0.5% (v/v) pH = 4.6 - 4.8 Ag	concentration of components stability of NPs	168.4 ± 15 nm, PDI = 0.266, ZP = 50.3 mV at C(CS) = 0.5 mg/ml suspension	[25]
MM (CS) = 15 and 50 kDa DD (CS) = 55, 80, and 90% C(CS) = 0.5 mg/ml in 0.2 mg/ml CH_3COOH pH = 3.6, 60°C, τ = 10 min, centrifugation: 30 min at 10000 rpm drying 48 h at –80°C flow rate of TPP(s) 0.25 - 2.5 ml/min	C(TPP) MM and DD of CS mixing speed and method TPP addition rates freeze-drying method	145.73 nm; ZP = +4.32 mV 724.23 nm; ZP = +43.67 mV nanopowders	[26]
DD (CS) = 98.65% η (CS) = 210 cPa C(X3)=0.2%, C(TPP) = 0.1% CS:TPP = 5:1 volume ratio Tween-80	magnetic stirrer stirring time 1 h	228.74 nm suspension with a yield of 80.67%	[27]
DD (CS) = 85% m(CS) = 60 mg in 20 ml 2% CH_3COOH C(Na-TPP) =0.1% in 0.1N HCl	the effect of the pH of the TPP solution	opalescent suspension	[28]

Table 1. (continued) Methods of CS NPs formation by ionotropic gelation.

CS NPs synthesis conditions	Subject of research	CS NPs characteristics	[Ref]
C(CS) = 0.1% (w/v) in 0.05% CH_3COOH C(TPP) = 0.1% (w/v) centrifugation at 16,000 rpm for 30 min freeze-drying	CS:TPP = 10 mg:1 mg CS:TPP = 10 mg:2 mg CS:TPP = 10 mg:3 mg	$D_{average}$ 100 and 272 nm ZP 32 and 40 mV nanopowders	[29]
CS/TPP layer capsule: DD (CS) = 90%; 0.1% Na-TPP pentabasic. 1%, 2% CS(s) in 0.1M CH_3COOH C(TPP) = 0.1% glutaraldehyde and a curcumin:CS:TPP=5:2:2 stirring at 1000 rpm for 30 min centrifugation at 12,000 rpm for 15 min and freeze-drying	CS:TPP = 1:1	hollow capsule size 17.33 nm Cur:CS:TPP 72 ± 3.12 nm and 135.2 nm ZP = +12.9 mV nanopowders	[30]
low molecular weight CS (LWCS) (pH = 5) 30% H_2O_2 water soluble CS (WSCS) (pH = 7) centrifugation at 6000 rpm for 30 min	Mv(LWCS) 17 kDa Mv(WSCS) 15 kDa 'flowerlike' cluster	for WSCS sample: d = 29.4 and 62.0 nm PDI = 0.158 ZP 30 mV For LWCS sample: d = 45.7 nm PDI = 0.149 ZP 36 mV	[31]
Mw (CS) =22,000 DD(CS) = 97% C(CS) = 0.3% (w/v) in 1% CH_3COOH V(CS) = 20 ml C(TPP) = 0.075% (w/v) in H_2O pH = 4.6 - 4.8 with 1M NaOH	C(CS) = 1 - 100 mg/ml effect and mechanism of germination and seedling growth	A_{av} = 200 nm stable for 6 months	[32]
MM (CS) = 130; 152; 165 kDa DD (CS) = 85.8%; 96.5%; 94.4% DC (CS)= 25%; 32%; 30% C(CS) = 0.5 and 10 mg/ml in 2% CH_3COOH C(TPP) = 1 mg/ml; pH=3.8; 4.7; τ = 4 - 24 h stirring at 300 rpm for 20 min centrifugation at 7,000 rpm freeze-drying for 4 h at 50 - -55°C	pH C(CS) MM DD degree of crystallinity (DC)	pH = 3.8: 363 nm pH = 4.7: 642 nm interaction energy of 50.4 - 69.4 kcal/mol	[33]

The main factors influencing particle formation were analysed. It was found that the average hydrodynamic diameter of the particles formed strongly depended on the initial concentration of chitosan solutions and its molecular weight characteristics. Additionally, the degree of acetylation (DA) of chitosan and the presence of salts in the reaction medium also significantly affected particle size. Specifically, controlling the initial chitosan concentration, pH, and ionic strength of the solution was crucial. Particles were not formed at chitosan solution concentrations above 5 mg/ml or below 0.1 mg/ml in the presence of NaCl. Particle formation occurred only under dilute and semi-dilute mode ($C[\eta] < \sim 4$), and the presence of 85 mM NaCl facilitated the production of particles up to 500 nm in size [34]. Viscometric measurements indicated that the presence of NaCl in CS solutions led to a decrease in particle size (compactification of macromolecules), suppressing the polyelectrolyte effect of the polycation.

Agarwal *et al.* [25] conducted fundamental studies to establish the optimal conditions for obtaining suspensions containing relatively small and stable CS nanoparticles for 8 weeks. The interaction of the polycation with low-molecular counter ions was carried out by varying the concentrations of chitosan and sodium tripolyphosphate (Na-TPP) in the ranges of 0.05 - 0.5% w/v and 0.25 - 2 mg/ml, respectively. To prevent the agglomeration of chitosan nanoparticles, the surfactant Tween 80 (0.5% (v/v)) was added to the reaction mixture, and the pH was adjusted to 4.6 - 4.8 using 1N NaOH. At a concentration of 0.5 mg/ml for both chitosan and Na-TPP solutions, NPs with an average size of 168.4 ± 15 nm, a polydispersity index of 0.266, and a zeta potential of 50.3 mV were formed. Silver ions were subsequently added to the chitosan NP solution to enhance bioactivity.

Obtaining CS nanopowders via ionotropic gelation is a multifactorial process. Al-Nemrawi *et al.* [26] studied the influence of the concentration of TPP, molecular mass (MM), and degree of deacetylation (DD) of CS; the rate and method of stirring; the rate of adding TPP; and the method of sublimation drying on the size, polydispersity, and zeta potential of solutions containing nanoparticles, which directly affect their biological properties and stability. To obtain nanopowder, CS was dissolved in CH_3COOH (0.2 mg/ml) to obtain 0.5 mg/ml of CS solution at pH = 3.6. Then it was preheated in a water bath at 60°C for 10 minutes. In another beaker, TPP was dissolved in water in various concentrations and cooled to a temperature of 2 - 4°C. Then, 5 ml of TPP solution was added to 10 ml of CS solution and stirred for 10 minutes. After centrifuging the suspension for 30 min at a speed of 10,000 rpm, the nanopowders were dried for 48 h at 80°C. It has been found that CS NP parameters such as size, polydispersity, and zeta potential affect their biological properties and stability. It has been found that the size of the nanoparticles decreases with increasing mixing speed and MM of CS and becomes more monodisperse at higher TPP concentrations.

The magnetic stirrer operation speed was also found as a crucial factor in obtaining homogeneous and stable nanoparticles from chitosan (CS from *Penaeus monodon*). To produce spherical chitosan nanoparticles with an average diameter of 228.74 nm, a 0.2% chitosan solution (50 ml; $\eta = 210$ cPa; DD = 98.65%) was intensively stirred using a magnetic stirrer. A 0.1% (w/v) TPP solution was then slowly added at a chitosan-to-TPP volume ratio of 5:1. After suspension formation, the reaction mixture was stirred for 1 h. To prevent agglomeration of nanoparticles, an emulsifier, Tween-80, was added to the solution, which creates steric hindrance and protects particles from each other [27].

A method for obtaining spherical nanoparticles of CS by ionotropic gelation in the size range from 100 nm to 272 nm, with a zeta potential of 32 mV and 40 mV, was developed by interacting a 0.1% solution of CS in 0.05% acetic acid with a 0.1% solution of TPP

during fractional titration. The nanopowders were isolated from the solution by centrifugation at a speed of 16,000 rpm for 30 min [29].

By modifying the existing method of chitosan nanoparticle synthesis, Vishwakarma *et al.* [30] successfully obtained chitosan nanocapsules with an average size of 17.33 nm. It is possible that an insignificant amount of the cross-linking agent - glutaraldehyde - led to a sharp narrowing of the macromolecules and a decrease in the particle size. It was shown that decreasing the mass ratio of chitosan to TPP leads to smaller particle sizes. Conversely, when the CS:TPP mass ratio is increased to 4:1, the particle size rises to approximately 300 nm.

A method for obtaining NPs of chitosan aliphatic is proposed based on low-molecular fractions of LWCS (15 kDa), as well as on water-soluble forms of WSCS (17 kDa). A combination of three methods, such as peroxide destruction, ultrasonic treatment (only for LWCS samples), and ionotropic gelation, made it possible to obtain monodisperse 29.4, 62.0 (LWCS), and 45.7 nm (WSCS) NPs with a narrow distribution of PDI 0.158 and 0.149, respectively [31]. Certainly, a relatively high positive value of the zeta potential contributes to excellent adhesion of the nanopolymer on the surface of negatively charged cells. Moreover, the small size ensures its penetration through the membrane and increases the efficiency of nanocompounds.

At pH 4.6 - 4.8, CS nanoparticles (Mw = 22,000; DD = 97%) with an average size of 200 nm were obtained by ionotropic gelation. For this purpose, 20 ml of a 0.3% CS solution (prepared in 1% CH_3COOH) was titrated dropwise with 0.075% Na-TPP solution. As a result, NPs of CS with an average size of 200 nm were obtained, which had a narrow polydispersity index and were stable for 6 months. These polysaccharide-based nanoparticles demonstrated potential as nanostimulators for wheat seed growth [32].

By varying the molecular weight characteristics and concentration of the chitosan solution, as well as adjusting the pH of the reaction mixture from 3.8 to 4.7, CS NPs ranging in size from 363 nm to 642 nm were obtained. Theoretical calculations of the interaction energy were carried out, which were equal to 50.4 - 69.4 kcal/mol [33].

Luthfiyana *et al.* [35] described a method for obtaining nanochitosan from shell waste, which poses an environmental hazard. The authors proposed a method for producing chitosan nanopowders with a size of 468.1 ± 21.63 nm, using Tween-80 as an emulsifier and sodium tripolyphosphate as a surfactant. Optimisation of the stirring speed effectively prevented nanoparticle agglomeration.

Over the past two decades, there has been a growing trend toward the use of plant extracts for chitin-chitosan extraction and enhancement of its bioactivity. In a recent study [36], the antibacterial and antioxidant properties of nanochitosan-based films were investigated. The films were obtained by adding Tween-80 as an emulsifier, glycerin as a plasticiser, and up to 2% of prickly *Mentha spicata* (mint) essential oil (EO), pomegranate peel, and grape seed extracts to a nanochitosan solution. The resulting nanochitosan films exhibited significant antibacterial and antioxidant activities, with effectiveness changing in the following order: *M. spicata* EO > grape seed extract > pomegranate peel extract. These findings suggest the potential application of such films in mitigating microbiological and chemical contamination in the food industry.

Chitosan NPs obtained by ionotropic gelation, due to their structural features and the valuable properties of chitosan and their technological effectiveness, are excellent carriers of active substances. Also, the increased cationic nature of chitosan NPs determines their mucoadhesive ability on the negatively charged surface of pathogen cells. Unlike chitosan, chitosan NPs have a high zeta potential value and can be used as a nanocontainer for both water-soluble and water-insoluble bioactive compounds. Active substances, in particular,

metal/metal oxide nanoparticles, can be added during the formation of NPs or after the formation of chitosan nanoparticles.

The polycationic nature of chitosan enhances its bioadhesive properties and solubility, allowing it to bind readily to negatively charged pathogen membranes via electrostatic interactions. Thus, it improved the adhesion to the membrane and extended the contact time for active substances to penetrate it [5, 13]. At present, there are quite a lot of published works on methods for obtaining chitosan nanoparticles, the advantages and disadvantages of which are summarised in Table 2.

Table 2. Comparative analysis of methods for obtaining low molecular weight chitosan derivatives: advantages and disadvantages [5].

Type of method	**Advantages**	**Disadvantages**
covalent cross-linking	possible to obtain small-sized NPs with a narrow size distribution high mechanical properties of NPs	cross-linking agents are toxic to living organisms absence of pH-dependent drug release and lack of swelling
self-organisation processes and PEC	simple and mild method without harsh conditions and any harmful cross-linkers and solvents prolongation effect adjusting surface charge limiting the number of molecules that self-organise	pH-depending reaction difficulty of forming small-sized NPs limiting the content of natural polycations complex solvent composition polymer incompatibility
ionic cross-linking (ionic gelation)	reduces undesirable effects and improves biocompatibility easy size control of NPs	poor stability of complexes lower encapsulation efficiency compared to other techniques
emulsion technique	effective size control and appropriate for both hydrophobic and hydrophilic drugs	require strong cross-linking agents and high mechanical energy multistep process and a limited number of special cross-linkers
drying methods	simple and fast method high quality of NPs strongly size and distribution adjusting	multifactor process require high temperature, which led to the destruction of some compounds

Chitosan NPs are mainly used in medicine and the agro-industrial field as antimicrobial, mucoadhesive, and biostimulant agents [37]. Therefore, selecting an appropriate method for chitosan NPs obtaining is crucial to ensure their biological efficiency, resistance to aggregation, and oxidative stability, as well as technological applicability and extended shelf life.

1.7. Preliminary Mechanism of Chitosan and Na-TPP Interaction

Research into the kinetics of CS/Na-TPP complex formation began in the 90s of the 20th century. Since then, scientists have actively investigated the influence of various factors on the behaviour and reactivity of these components. It has been established that chitosan exhibits both regio- and chemoselectivity [38].

CS is a rigid-chain polymer and a compositionally heterogeneous polysaccharide. It is characterised by molecular polydispersity, pH-dependent solubility, and a tendency to form H-H bonds. It is these properties of chitosan that determine its uniqueness and unpredictability.

The CS macromolecule exhibits chemoselectivity, specifically due to the deprotonated amino groups and the –OH groups located at the C-3 and C-6 carbon positions (Figure 1), which allow CS to undergo reactions of nucleophilic substitution S_N and addition A_N. It should be noted that by varying the synthesis conditions, it is possible to obtain *–N* and *–O* derivatives of CS due to $–NH_2$ and –OH groups in the macromolecule. That is, varying the pH of the reaction system promotes chitosan transition from the salt to neutral form, which affects the conformation of the polymer. It should be noted that the dissociation of the Na-TPP molecule also changes accordingly with the change in pH. Studying the interaction of chitosan with sodium tripolyphosphate, it was found that the hydroxyl and phosphate groups of the low-molecular counterions compete in the interaction with the counterions of the amino groups of the polymer. As a result, at pH > 6, the deprotonation mechanism dominates, where chitosan amino groups interact via –OH groups of sodium tripolyphosphate. At pH < 6, crosslinking occurs in the presence of Na-TPP phosphate groups and protonated amino groups of polycation [39].

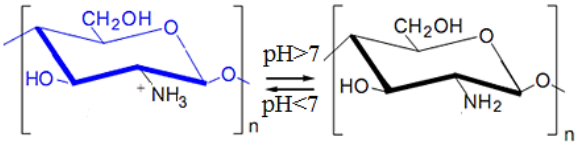

Figure 1. Protonation and deprotonation of chitosan unit [5].

It should be noted that, in addition to other influencing factors, the pH of the reaction medium is one of the important factors in regulating the interaction mode and stability of the resulting complexes, which plays a decisive role in the use of nanochitosan as a nanocontainer. In acidic environments, the abundance of positively charged amino groups of chitosan contributes to their tight binding with counterions [39].

However, in alkaline media, the polyelectrolyte effect of CS is suppressed, and the local concentration of protonated amino groups of chitosan decreases, which leads to a reduction in the degree of cross-linking (effective bonds) between CS and TPP, and, accordingly, the consumption of Na-TPP solution decreases.

Quantum-chemical modelling has proposed a mechanism for the interaction of chitosan macromolecules with deprotonated and protonated TPP molecules. According to the authors, when CS interacts with deprotonated TPP, low-molecular counterions $P_3O_{10}^{5-}$ strongly bind to the –OH group (C-6) and amino group (C-2) of CS. In the case of protonated TPP ($H_4P_3O_{10}^-$), an ionic bond arises between one amino group of CS and the phosphate group of TPP [33].

Bhumkar *et al.* [28] also studied the mechanism of crosslinking of chitosan macromolecules Na-TPP by examining the effect of reaction pH on the binding, since both the cross-linking agent and the polymer matrix are pH-selective. Sodium tripolyphosphate

($Na_5P_3O_{10}$), when dissolved in water, dissociates and hydrolyses, forming both hydroxyl and phosphorylated compounds. At pH = 9, both OH^- and phosphate anions are present in the interaction with $–NH_3^+$ of chitosan. According to the results of the titration of the CS solution (pH = 3.05) with Na-TPP solutions of different acidity (pH = 3 and pH = 9), it was found that the conductivity of the solution increases after 13 and 26 millilitres of TPP with pH = 3 and pH = 9, respectively. This evidence indicates that at pH = 3, cross-linking occurs via amino groups and phosphate groups. Whereas at pH=9, TPP interacts with CS via phosphate and hydroxyl groups. It should be emphasised that in both cases, the pH of the reaction system did not vary significantly. This variation may be due to the buffer effect of TPP [28].

Thus, the analysis of scientific literature shows that the listed methods for obtaining nanochitosan allow obtaining nanopowders and suspensions containing nanoparticles with an average particle diameter of up to 200 nm. To increase the bioactivity of the polymer, it is desirable to synthesise particles less than 100 nm. Obtaining polymer-metal complexes of chitosan, as well as using nanochitosan as a nanocontainer of metal nanoparticles, can be a unique technique to solve this problem.

2. Chitosan as a Macroligand

The development of the chemistry of macromolecular coordination compounds, formed at the junction of the chemistry of high-molecular compounds and coordination chemistry, began in the late 50s - early 60s of the twentieth century. The study of metal complexes with macroligands reached its peak in the 80s of the last century. In the chemistry of polymer-metal complexes (PMC), significant progress has been made in the study of natural and synthetic PMCs, their application in medicine and agriculture, and the creation of new polymer materials with special properties [40–42]. The biopolymer chitosan binds well to molecules such as pesticides [43, 44], proteins [45], and dyes [46], unlike some natural polymers, such as cellulose derivatives [47, 48]. The free amino group C-2 enables the formation of complexes with transition metal ions [49–51], which results in higher bioactive properties compared to chitosan itself. It should be noted that for these purposes, the use of biogenic metals creates new opportunities in the development of chitosan preparations with improved properties, due to the synergistic effect of the properties of the polymer itself and the ions of biogenic metals.

3. Nanochitosan as an Effective Drug in Agriculture

Currently, problems in obtaining nanoparticles and nanomaterials can be associated with high surface tension, i.e., high surface energy, which, accordingly, makes it difficult to regulate the size, composition, morphology, and structure of NPs, as well as to obtain thermodynamically and aggregatively stable nanoparticles [52–57]. It is obvious that nanochemistry is developing at a rapid pace; in the last 5 years (Table 3), the following can be noted [58–63]:

1. reduction of the sizes of particles to 1 - 3 nm and synthesis of subnanoparticles less than 1 nm,
2. obtaining not only spherical particles but also particles of other shapes: cubic, ring, tube, nesting doll, and needle; expansion of work on core-shell-type structures,
3. controlling the self-organisation process of nanoparticles by adjusting the temperature and pH,
4. obtaining hybrid particles, including inorganic and organic compounds.

Table 3. Application of nanochitosan and its derivatives in plant growing.

Preparations	Purpose	Bioactivity against phytopathogens	[Ref]
chitosan nanoparticles were prepared via polymerisation of methacrylic acid for the incorporation into NPK fertilisers: (CS-PMAA)	nanofertilisers: urea-loaded chitosan: (CS-PMAA-(NH_2CONH_2)), calcium phosphate-loaded chitosan (CS-PMAA-($Ca(H_2PO_4)_2$ H_2O)), potassium chloride-loaded chitosan (CS-PMAA(KCl)	The potential of chitosan NPs as controlled-release NPK fertilisers was investigated. The stability and interaction of chitosan NP suspensions containing NPK were evaluated by FTIR spectroscopy, and the results showed the existence of electrostatic interactions between chitosan nanoparticles and the nutrient elements, like N, P, and K	[3] [64]
chitosan NPs	efficacy of CS NPs on fungal growth and chilli seed quality	CS NPs at a concentration of 0.6% (w/v) significantly delayed mycelia growth of *Rhizopus* spp., *Colletotrichum* spp. (*C. capsici, C. gloesporoides*), and *Aspergillus niger* compared to the control	[65]
ZnO -25 nm 1.5 and 10 ppm activities of SOD and GPX increased more than CATT (wheat)	high stress	The review describes the role of a number of nanoparticles: Ag, SiO_2, TiO_2, SeO_2, which can affect the physiological and biochemical response of plants to counteract stress caused by high or low temperatures and the influence of UV-B. It shows how nanoparticles are absorbed in different parts of plants and how they move, as well as the factors affecting their absorption and movement	[66]
CS NPs < 100 nm 0, 100, 200, 400 mg/l	against cold stress banana	Decreased levels of reactive oxygen species (ROS) and malondialdehyde (MDA), as well as an accumulation of soluble carbohydrates, proline, and amino acids	[66]
CS-TPP NPs as nanocontainer	CS NPs + herbicide	The review systematises data on the role of nanotechnology in plant and animal husbandry. Results on nanopesticides, nanofungicides, nanobacteriocides, nanosensors, nanobarcodes, and nanocarriers, which are obtained based on polymers and metal/metal oxide nanoparticles, are analysed. For instance, paraquat herbicide encapsulated in chitosan/tripolyphosphate nanoparticles was found to be an effective herbicide. Even after encapsulation, the herbicidal efficacy of paraquat was not reduced	[67]

Table 3. (continued) Application of nanochitosan and its derivatives in plant growing.

Preparations	Purpose	Bioactivity against phytopathogens	[Ref]
CS as Plant Disease Suppressor, antimicrobial mechanisms of CS	CS, CS NPs, CS-Cu	This review emphasises the role and mechanisms of CS and CS NPs as plant growth promoters and disease suppressors, as well as their future implications in agriculture	[68]
CS NPs Me NPs	transport and activity of NPs	The authors summarised the results of studies on the influence of size, surface charge, and chemical composition of nanoparticles on absorption and transport in leaves and roots using various methods. It was established that at d (Me) = 50 nm, NP can penetrate into plant leaves through the stomatal pathway, but with an increase in particle size, their penetration via leaves decreased. For example, some authors applied fluorescein isothiocyanate-labelled ZnO NPs (30 nm) to wheat leaves, and microscopy results indicated that the NPs mainly passed through the epidermis of the leaves via the stomatal route and then accumulated in chloroplasts	[69]
natural polymers agrochemicals	CS, nano-CS, NPs of polysaccharide/metal/metaloxide	The review summarises the results of studies on replacing harmful pesticides with non-toxic nanopreparations containing chitosan, nanochitosan, and polymer-stabilised metal nanoparticles to solve problems such as soil treatment with fertilisers, fungicides, insecticides, drought problems, etc	[70]
Ag NPs and Ag, etc.	nanocomposites for insect control and fungal plant diseases	This chapter highlights the applications of silver nanomaterials for controlling agricultural pests and pathogens. For instance, fungicide is used to treat mildew and root rot caused by *Pythium* and *Phytophthora* species	[71]
intelligent $CuSO_4$-containing CS microparticles on the cellulose substrates	moisture-sensitive smart packaging materials based on chitosan-Cu^{2+}-TPP were obtained $CuSO_4$-loaded microcapsules, at different Na-TPP-to-chitosan ratios, were not cytotoxi	The best conditions were achieved at 180°C, liquid flow rate of 290 ml/h, an aspiration rate of 90%, and nebulising gas flow rate of 667 nl/h. These conditions successfully produced 4 μm chitosan capsules containing $CuSO_4$. The samples were evaluated under different humidity conditions, from 0% to 100% relative humidity, which resulted in a colour change from dark brown to blue	[72]

Especially for the creation of nanomaterials for the agro-industrial complex, the production of nanoparticles by chemical reduction in the presence of biopolymers such as ‘nanosphere’ or ‘nanocapsule’ has its advantages, such as the ability to regulate the hydrodynamic and dimensional characteristics of metal nanoparticles, non-toxicity, biocompatibility, and biodegradability of the preparation.

4. Proposed Mechanisms of Nanoparticle Bioactivity and Toxicity

According to both theoretical and experimental findings, the mechanism of action of chitosan and its derivatives may be explained by the following hypotheses [73–75]:

1. Interaction of the chitosan with the host plant and its induction of its (defensive responses), including the production of H_2O_2, soluble phenols, salicylic acid, and avenalumins, as well as increasing the activity of defence-related enzymes such as chitinase, beta-1,3-glucanase, phenylalanine ammonia-lyase, etc.
2. Direct action of chitosan on microorganisms: this effect depends on MM and DD of CS; high MM and DD values provide effective electrostatic interaction with the negatively charged cell surface, resulting in the formation of a dense polymer layer on the pathogen’s surface; in contrast, low MM and DD values promote the penetration of chitosan molecules into microbial cells, leading to the degeneration and flocculation of intracellular components. This procedure disrupts normal physiological and metabolic processes or may involve direct interactions with the genetic material.

Furthermore, chitosan can form coordination compounds with biogenic metal ions, which are important for the growth of bacteria and fungi. The bioactivity of chitosan and its derivatives is overlooked by entomopathogenic fungi [68]. Chitosan nanomaterials have important potential uses in the areas of nanomedicine, pharmaceuticals, nutraceuticals, food, and the environment. In different size ranges, nanochitosan has potential applications in both medicine (200 - 500 nm) and microbiology (70 - 400 nm).

Systematisation and comparison of the results indicate that the mechanism of action of chitosan preparations is a multifactorial process. It depends not only on the molecular weight and size characteristics of chitosan and its derivatives but also on factors such as the biological structure of the target organism, the pH of the surrounding environment, and other conditions, all of which play a key role.

The high value of the total positive charge of CS and its modifications ensures its electrostatic interaction with negatively charged cell membranes of microorganisms, therefore leading to agglutination, destruction, change of the cell membrane, and death of the organism [76, 77]. This phenomenon is characteristic of both gram-positive bacteria (the cell wall contains teichoic acid from peptide glycans) and gram-negative microbes (the surface of the wall has a negative charge due to the phosphate groups of lipopolysaccharides). The higher the zeta potential of the solution, the more pronounced the adhesive property of the preparations is [68]. Currently, there is a worldwide trend to replace various chemical fertilisers, pesticides, and other agricultural products with polymer nanopreparations. This is due to their negative impact on biodiversity and the ecosystem.

Therefore, nanoparticles are a preferred choice for consumers because of their compact size and high efficiency. Although the positive effects of nanopreparations are obvious, research on their mechanism of action and potential risks that may arise after entering living organisms through plants is still insufficient, and further research is needed [67].

5. Conclusions

To conclude, the biopolymer CS could be used as an effective macroligand and stabiliser of metal nanoparticles. According to literature data, chitosan is used in more than 80 sectors of the national economy. Certainly, this is due to the valuable properties of chitosan, such as non-toxicity, biocompatibility, biodegradability, solubility, etc. Chitosan is the only natural cationic polysaccharide, and its molecular mass characteristics and degree of deacetylation can be regulated during the extraction processes, allowing it to be tailored for use in various applications. Despite enormous studies on the biochemical significance of chitosan and its derivatives, the preliminary mechanism of their action has not been identified yet, although there are various fundamentally and theoretically substantiated assumptions. In the future, scientists will have to study the role of chitosan preparations in more detail and deeper at the molecular level [69, 77].

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7. References

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