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Oligochitosan and Oligochitosan Ascorbat: Preparation and Properties

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Abstract: The use of chitosan and its derivatives with antimicrobial, immunomodulating and phytoactive properties is of great interest in the world, especially in the medicine and agriculture. This review discusses the current understanding of chitosan derivatives, isolated from crustaceans and pupae of the silkworm *Bombyx mori*, as oligochitosan and oligochitosan ascorbate, about their physicochemical and biologically active properties.

Keywords: *review, chitosan, oligochitosan, oligochitosan ascorbate, Bombyx mori, ascorbic acid, acid hydrolysis, oxidative depolymerization, biologically active properties.*

Introduction

The water-soluble chitosan derivatives production from a promising new source of silkworm pupae, Bombyx mori, expands the applications range of this natural amino polysaccharide in agriculture and biomedical fields. The chitosan solubility in water depends on a number factors, including the molecular weight and deacetylation degree (DAD) of the polymer, the amino groups protonation degree in the macromolecule (pH solution) [1, 2, 3]. Chitosan is characterized by molecular weight, deacetylation degree, acetylated and deacetylated residues location in the polymer chain, and polydispersity degree. The first three characteristics affect the chitosan solubility in water. In turn, the biologically active properties manifestation by chitosan largely depends on the solubility [4, 5].

So in [6, 7], the antibacterial activity of narrowly dispersed samples of oligochitosans obtained from crustacean chitosan and differing in molecular weight was investigated at different pH solution values. It was shown that under acidic conditions, a stronger inhibitory effect is samples characteristic with a higher molecular weight, and under slightly alkaline conditions, chitosan forms, which are close to oligomeric ones, are more active. It is assumed that the chitosan antibacterial activity is determined by its amino groups protonation degree, which is variable and depends both on the molecular weight of the substance and on the medium pH [8].

Numerous works analysis shows that the water solubility and biological activity of low molecular weight chitosan (oligochitosan), especially antiviral and antibacterial, as well as biocompatibility, improve with a decrease in molecular weight compared to high molecular weight chitosan [7-10].

Currently, there are several main methods for depolymerizing chitosan: acid and enzymatic hydrolysis, oxidative depolymerization with hydrogen peroxide, periodic acid (sodium periodate) or nitrous acid (sodium nitrite), as well as their various modifications. All of these methods have their own advantages and disadvantages. Enzymatic hydrolysis requires the use of a large amount of expensive pure enzymes or enzyme complexes, as well as the subsequent purification of the hydrolysis product from the used enzymes and impurity proteins. Oxidative depolymerization of chitosan proceeds much faster than acidic and, moreover, enzymatic hydrolysis; however, it leads to a change in the chitosan chemical structure (partial deamination and oxidation) the more the deeper the depolymerization degree. Unfortunately, in the chemical depolymerization process, chitosan molecules often undergo chemical modification, leading to the carboxyl and aldehyde groups' appearance, deamination, and the schiff bases formation, the amounts of which are determined and depend on the methods used for the chitosan isolation and depolymerization [11]. On the other hand, the acid hydrolysis method in the hydrochloric acid presence is effective and technologically acceptable, and also does not lead to the reaction formation by-products, except for lower chitooligosaccharides.

As is known, there are various methods for obtaining oligochitosan and the ability to control the polymerization degree of the obtained derivatives, since the chitosan biological activity and its derivatives depends on the molecular weight. At the same time, the chitosan depolymerization control process and the oligochitosan production and its derivatives remains an urgent task. In order to obtain oligosaccharides, the oxidative depolymerization method is used in the hydrogen peroxide, sodium nitrite presence, etc., also during hydrolysis with sodium nitrite, in comparison with the acid hydrolysis method, the most dramatic decrease in the molecular weight characteristics of the samples occurs. Despite this, as is known from the literature, a functional groups redistribution is observed in the oligochitosan structure, and aldehyde groups are also formed [12, 13].

In the work of *Allan G.G.* and etc. showed that in order to suppress the side effects of oxidative depolymerization, the reaction must be carried out using a potassium nitride and hydrochloric acid mixture at 25-65°C temperature [13]. In the work, an alcoholic solution of ammonium hydroxide is used as a precipitant. The work also noted that in the oxidative depolymerization process of chitosan in the sodium borohydride presence, the side effects level is significantly reduced. To study the chitosan depolymerization kinetics by the nitride method, we determined the change in the KNO_2 concentration during the reaction, the chitosan depolymerization rate, and the rate constant of the process was estimated from the time dependence of the concentration logarithm. The results obtained show that with increasing temperature, there is an increase in the rate constant [13]. It is also noted in the work that in the sodium nitride presence, a selective attack on the chitosan amino groups occurs and, as a result, hydroxyl groups are formed due to the deamination reaction. In the oxidative depolymerization process, not only amino groups' deamination occurs, but also glycosidic bonds cleavage with the aldehyde groups' formation is observed. As indicated in the work, imine bonds are formed between the aldehyde and chitosan amino groups. Therefore, this method is less applicable in the oligochitosan synthesis.

As is known from the literature, in $NaNO_2$ presence, aldehyde groups are formed in chitosan macromolecules and this leads to the intermolecular bonds formation. To suppress such side reactions, it is necessary to add reducing agents such as sodium borohydride, etc. In the work of *Kristoffer T. and* etc. [14] showed that not only deacetylated oligochitosans but also acetylated oligo derivatives are synthesized based on the oxidative depolymerization method. In order to obtain N-acetylated chitosan derivatives, ammonium acetate salts are used as an acetylating agent and the synthesis is carried out with $NaNO_2$ supplement at 4°C temperature for 12 hours. The resulting product is isolated by centrifugation, since the final acetylated oligomers do not dissolve in dilute hydrochloric acid.

It is known that with an increase in $NaNO_2$ concentration, the chitosan molecular weight sharply decreases. However, in high molecular weight chitosan, the glycosidic bonds cleavage occurs at a higher rate than in low molecular weight. Also, the oligochitosans solubility increases with decreasing molecular weights. The authors of [15] indicate that no change in the samples cytotoxicity was found with an increase in the chitosan molecular weight. Chitosan is widely used as a gene delivery vehicle and these properties depend on the molecular weight of the polysaccharide. The molecular weight of depolymerized chitosan is influenced by the solution concentration and the original chitosan source. In the oxidative hydrolyse chitosan process under constant conditions, the molecular weight decreases linearly. Thus, chitosan low molecular weight fractions can potentially be used to develop drug delivery systems due to improved solubility properties.

In [12], it is confirmed that an increase in temperature leads to an increase in the chitosan depolymerization degree. In the oxidative depolymerization process in an open reactor, with an increase in temperature, a slight decrease in the depolymerization degree is observed in comparison with a closed system. This can be attributed to the excess acid nitride evaporation.

As is known, the chitosan toxicity depends on its molecular weight and the deacetylation degree. Most of the commercially available chitosans are high molecular weight. In the pharmaceutical field, it is important to develop a reproducible and simple method for producing chitosan with low molecular weight. Typically, low molecular weight chitosan can be obtained from high molecular weight chitosan by depolymerization using enzymatic degradation, oxidative degradation, acid hydrolysis, and ultrasonic degradation.

During ultrasonic treatment of a chitosan solution, an unstable degradation rate and a decrease in molecular weight are observed, and therefore the chitosan macromolecules degradation process is unpredictable [16].

The authors [15, 16] studied the chitosan depolymerization using $NaNO_2$, H_2O_2, and HCl. As a result, it was found that $NaNO_2$ showed the best characteristics in the oxidative depolymerization reaction. For example, with an increase in the duration up to 60 minutes, a sharp decrease in the molecular weight of the initial chitosan was found; after that, with an increase in the reaction duration, there was no significant change in the molecular weight of oligochitosan. Also, the chitosan depolymerization, to a large extent, depends on the $NaNO_2$– concentration with an increase in the salt concentration, the chitosan depolymerization degree increases.

The chitosan properties change dramatically depending on its molecular weight. Therefore, a large number of scientific studies are devoted to the production of low molecular weight chitosan and its nano derivatives by various methods [17-19]. The main method for obtaining oligochitosan is homogeneous acid catalytic hydrolysis. It is carried out in solutions of mineral and organic acids - hydrochloric, acetic, lactic, and formic. The hydrolysis rate depends on many synthesis factors, such as acid concentration, temperature, and reaction duration [17, 20]. To obtain low-molecular-weight chitosan, oxidative destruction in an acetic acid medium by means of hydrogen peroxide is also used. However, in this case, not only hydrolysis occurs, but also the hydroxyl and amino groups oxidation [18, 21].

To isolate the chitosan hydrolysis products from solution, alkaline reagents, acetone or ethyl alcohol are usually used, bringing the pH to a neutral value. Along with this traditional method, ionotropic gelation is also used in a sodium tripolyphosphate solution presence [22–25], in which the sodium salt of tripolyphosphoric acid can act as oppositely charged stabilizers.

The physical methods include sonication [26]. In [27-30], the main regularities of the heterogeneous hydrolysis of polysaccharides were formulated, which revealed an insignificant effect of the

macromolecules structure (configuration of glycosidic bonds, spatial arrangement of hydroxyl groups, the elementary unit composition) on the formation of low-molecular-weight chitosan. Since there are amorphous and crystalline regions in the supramolecular structures of chitosan, and the reagents access to the crystalline regions is limited, the reaction proceeds mainly in amorphous regions, which leads to the production of hydrolysis products with a limiting degree of polymerization [30].

IR and H^1-NMR spectroscopic studies confirm that during oxidative depolymerization in the presence of hydrogen peroxide, the 1,4-ß-D- glucosidic bonds of chitosan are broken. Also, the X-ray structural analysis results show that the chitosan crystallinity degree during depolymerization gradually decreases due to a decrease in intermolecular hydrogen bonds. In addition, the authors of [31] concluded that the chitosan depolymerization rate depends on the deacetylation degree of the initial chitosan and the synthesis conditions (hydrogen peroxide concentration and reaction temperature). The authors note that with reaction duration of more than 4 h, the molecular weight of the obtained oligochitosans changes insignificantly, and when using a 1.0 M H_2O_2 solution, water-soluble fractions of oligochitosans were obtained.

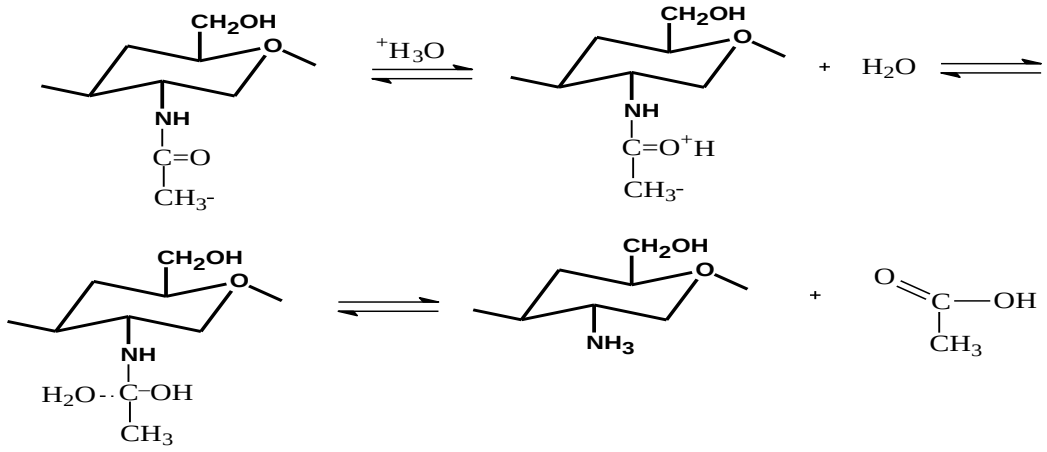
Also, the authors, studying the biological activity, found that oligochitosan nanoparticles obtained by chemical modification by ionotropic gelation can be used as antigen carriers for nasal administration. In this case, oligochitosan was synthesized by oxidative depolymerization using $NaNO_2$, and sodium tripolyphosphate was used to obtain oligochitosan nanoparticles. As a synthesis result, nano-oligochitosans with a particle size of 200-300 nm was obtained [31]. It was confirmed that oligochitosan nanoparticles containing bioactive macromolecules included in the nanostructured oligochitosan structure are the most effective for nasal use. These nanoparticles can form chemical bonds with epithelial cells. It is even more interesting that oligochitosan nanosystems have shown a greater ability to associate proteins [32]. These characteristics are significantly more attractive than those of hydrophobic polymers.

It is possible to conditionally divide chitosans depending on their molecular weight. Low molecular weight chitosan and oligochitosan are deep depolymerization products of high molecular weight chitosan. It is confirmed in the literature that, depending on the “Kuhn” segment and the macromolecules rigidity, for chitosan containing less than 100 units of monomeric units (MM <∼16 kDa), the name “oligochitosan” is used [33-35].

The water-soluble oligochitosans production by acid hydrolysis is the most widespread and cost-effective method, since the inaccessible reagents and modern devices participation is not required. Acid hydrolysis of chitosan proceeds in a dilute solution of hydrochloric acid (sulfuric acid is also sometimes used) at 30-100°C temperature; recrystallization and dialysis of the obtained oligochitosans are not required. The obtained products properties depend on the acid concentration and the optimal concentration of hydrochloric acid in the range of 1-12 M. The chitosan depolymerization rate in a 12M HCl solution is approximately a magnitude order higher than the N-deacetylation rate of chitosan. In dilute acid (1-6M), the rate of depolymerization is equal to the deacetylation reaction rate [35, 36].

Also, the chitosan depolymerization rate in a hydrochloric acid solution depends on the amino groups’ distribution and acetyl groups located in the polymer chains of chitosan. It is known from the literature that in the acid hydrolysis process with an increase in the deacetylation degree of the initial chitosan, the depolymerization rate of the final products decreases [35]. In the course of acid hydrolysis, partial deacetylation occurs; therefore, the acetylation degree of the resulting oligochitosan decreases in comparison with the initial chitosan. Thus, the depolymerization rate and the deacetylation degree depend on the acid and the reaction temperature concentration. From a practical point of view, the chitosan depolymerization method in the presence of hydrochloric acid is more effective, since this method does not affect the chemical structure of oligochitosan, but the process takes place when the system is heated for a long time (in the range

of 1-8 hours). The oligochitosan reaction formation by acid hydrolysis proceeds according to the following scheme:



Scheme 1: Reaction of oligochitosan formation by acid hydrolysis

Several other acids (phosphoric, sulfuric, and hydrofluoric acids) have also been used to depolymerize chitosan and have shown many disadvantages [36]. When sulfuric acid is used, a redox reaction occurs during acid hydrolysis, which makes it difficult to vary the depolymerization of chitosan.

As a result of the acid hydrolysis method, a significant swelling of chitosan occurs and the ends of the chain form mobile groups when the glycosidic bonds are broken. This can cause "recrystallization" and an increase in the degree of ordering in these areas compared to the ordering of the initial chitosan, which leads to an increase in the oligochitosan crystallinity degree in comparison with the initial chitosan [37].

Oligochitosan is more soluble in an acidic aqueous medium in comparison with high molecular weight chitosan, but its antimicrobial activity decreases with an increase in the acetylation degree (AD) and an increase in pH above the critical pH point for a chitosan solution. In [33], N-acetylated oligochitosans were additionally investigated in a wide pH range depending on molecular weight and AD for antibacterial activity against. *Staphylococcus aureus* and *Escherichia coli*. The results show that reacetylated oligochitosan with MW ≤ 11 kDa is completely soluble in an alkaline medium (up to pH 12.5) if its AD is at least 16%. Reacetylated chitosans with AD ~ 30% are soluble in the entire pH range up to 12.5, but they aggregate and precipitate from solution at pH ≥ 8. Taking into account the influence of AD and MM, the antibacterial activity of reacetylated oligochitosans is observed in the AD range of 16-28% at pH 7,4. These results demonstrate a wide range of practical applications of oligochitosan in pharmaceutical, cosmetic and other industries.

The literature describes the preparation of reacetylated oligochitosan derivatives by acid hydrolysis of high molecular weight chitosan with MM 334 kDa, AD 4% in 1 M hydrochloric acid solution at 70 °C for 4–24 hours according to the indicated methods [38].

In the work of the authors *Einbu A. and Vårum K.M.* [39] it was found that the rate of de-N-acetylation increases with an increase in the acid concentration from 2 to ~ 6 M. A further increase in the acid concentration leads to a decrease in the rate of de-N-acetylation. On the basis of NMR spectroscopic studies, the authors determined the activation energy for the hydrolysis of the N-acetyl bond. The results obtained indicate that the activation energy of acid hydrolysis is 102-116 kJ / mol at a hydrochloric acid concentration of 3, 6, and 12 M, respectively. It should be noted that the maximum values of the activation energy were found when using a 6 M hydrochloric acid solution, indicating that the concentration of water plays an important role in the deacetylation process of acetamide groups; the degree of depolymerization of

polysaccharides not only depends on the acid concentration, but also, possibly, depends on the amount of water.

Oligochitosans are used as antimicrobial substances in various industries, in particular in perfumery, medicine and pharmaceuticals. They show high fungistatic activity against *Candida* species and clinical *Candida albicans*, which, in turn, are resistant to a number of classical antibiotics. Also, oligochitosan inhibits the formation of hyphal structures in *C. Albicans*. The antimicrobial properties of oligochitosans mainly depend on the molecular weight and concentration of the solution. Oligochitosan with a molecular weight of 10 to 20 kDa showed the maximum activity in suppressing pathogenic fungi. [40].

Oligochitosan is used not only in medicine, but also in agriculture as a biostimulant. For example, when treating corn seeds, a 0.001% aqueous solution of oligochitosans (OCH) with a molecular weight (MW) was used: 6.9 kDa; 18.4 kDa; 28.6 kDai found that OCH stimulates root growth and an increase in seedling mass. Oligochitosan with a molecular weight of 28.6 kDa had the highest elicitor activity compared to the control variant. Seed treatment with 0.001% aqueous solution of OCH with varying degrees of acetylation (AD): 7.0%; 9.7%; 16.4%; 34.7% led to an increase in the total mass of seedlings regardless of AD [41]. This result indicates that the molecular weight of oligochitosan (OCH) significantly affects the germination of corn seeds.

One of the most promising and urgent areas of modern chemistry and technology is the production of water-soluble natural polymers, the study of the structure, properties, physical and chemical modifications, the identification of promising areas of their application in various fields.

Chitosan derivatives are of great interest in the world, including chitosan ascorbate (CHA) and oligochitosan ascorbate (OCHA), which have bactericidal, growth-stimulating and antifungal properties. In the form of a gel, chitosan ascorbate is used against dental periodontitis. In this case, an 8% chitosan ascorbate gel has the best effect [42]. Also, chitosan derivatives with ascorbic acid are used in the fight against hypercholesterol, a hypolepidemic effect is observed [43]. It should be noted that water-soluble forms of oligochitosan with organic acid have the most pronounced antimicrobial properties against *Streptococcus mutans, Lactobacilli brevis in vitro* and in vivo compared to the corresponding forms of high molecular weight chitosan[44].

Oligochitosan ascorbate also has antioxidant properties against a number of bacteria, such as *E. coli, P. aeruginosa, S. typhimurium, L. Monocytogenes, and S. aureus*, and at low concentrations [45, 46].

Chitosan ascorbates and its oligomers are prepared in the form of effervescent tablets. They include: citric acid, sodium bicarbonate, lemongrass and green tea extracts. The dosage form is characterized by an expansion of the therapeutic effect and a decrease in ulcerogenic activity [47, 48]. Also oligochitosan is used as a carrier of tocopherol, ascorbic, lipoic acids and amino acids in pharmacy and cosmetology [49].

The mechanism of the antimicrobial activity of chitosan derivatives and chitosan itself is not fully understood to date, since the biological activity of chitosan derivatives depends on many factors, such as molecular weight (MM), deacetylation degree (DAD), acetylation degree (AD), concentration and pH of the solution. The MM of chitosan is one of the main characteristics that affect the activity of the polymer towards plant pathogens. *Badawy etc.* [50] studied the antifungal effect of chitosan samples and found that the antimicrobial activity, in addition to MM, depends on the concentration of the solution. Using samples for inhibiting fungi *Fusarium gramine arum, F. oxysporum and Rhizoctonia solani*, their effective inhibitory concentration was compared. The authors confirmed that chitosan at a concentration of 1000 mg/L inhibited the growth of some fungi. It was shown that the inhibitory concentration of chitosan oligomers and macromolecules decreased with decreasing MM in *in vitro* experiments. The minimum effective concentration for oligochitosan with a molecular weight of 5 kDa was 1392 mg/l, and with a molecular weight of 290 kDa it was 2407 mg/l [51]. From the results obtained in [50], it follows that the effectiveness

of the oligochitosans action depends not only on MM, but also on the composition of the cell wall of the tested pathogen. The authors suggested that the cationic form of the primary amino groups of glucosamine units in the polymer chain as a result of electrostatic interaction with anionic groups on the surface of the cell wall leads to its destabilization and the formation of pores, which ultimately leads to the death of the pathogen. The authors of a study on the effect of chitooligosaccharides on the cell membranes of microbes came to similar conclusions [52]. It was shown that chitosans with low AD and lower pH values were more effective. At low pH values, the activity of chitosan increased with an increase in MW, regardless of the bacteria tested. However, at neutral pH, the activity of chitosan increased with decreasing MM. The authors explain this by a decrease in the solubility and total positive charge of chitosan in solution at pH 7.0 [53]. From the publications discussed above, it follows that chitosan with a low MM and high DAD has a higher antibacterial activity, which is probably due to the higher solubility of the biopolymer.

The authors of the study [51] suggested that if the activity of chitosan is due to its polycationic properties, then the transformation of derivatives due to amino groups (cationic groups) in the structure of the molecule should enhance the antimicrobial activity by the introduction of additional charged groups into the polymer molecule by a chemical method, for example, oligochitosan ascorbate, carboxymethyl chitosan etc., will lead to an increase in the biologically active properties of the obtained derivatives [54]. It should be noted that of the whole variety of water-soluble chitosan derivatives, the highest antibacterial activity is possessed by quaternized chitosan derivatives containing quaternary ammonium groups in their structure with a large positive charge, which interacts with negatively charged bacterial cell walls. Based on the above, it can be assumed that the antibacterial activity against gram-negative bacteria was further increased by a decrease in MM, while the opposite effect was observed for gram-positive bacteria [55-60]. The results of confocal microscopy also provide evidence that low molecular weight fluorescently labeled chitosan, when interacting with *F.oxysporum* cells, was localized in the cytoplasmic membrane, and the results of scanning electron microscopy showed that the pathogen caused significant morphological changes on the cell surface [56].

In general, based on the results obtained by the researchers, the antimicrobial effect of oligochitosan and its derivatives is associated with different forms of the nitrogen atoms of the polymer molecule. Many studies have shown that certain types of bonds at the nitrogen atom, such as quaternary donor-acceptor bonds of the amino group [51, 58], can significantly increase the antimicrobial activity of this derivative. An increase in the density of a positive charge in oligochitosan ascorbate macromolecules causes a greater interaction with the anionic components of the cell wall of a microorganism; therefore, acidic derivatives of chitosan with stronger electronegative groups may exhibit a more pronounced antimicrobial effect. It was already mentioned above that the molecular weight affects the activity of the polymer, but high-molecular-weight samples cannot pass through the cell membranes and only interact with the cell surface, changing its permeability or forming a film that prevents the transfer of nutrients through the membranes of the microbial cell [59-61]. And low-molecular water-soluble chitosan derivatives or their nanoparticles can penetrate the cell walls of bacteria and interact with DNA, blocking its transcription and mRNA synthesis [62]. Chitosan derivatives (including metal ions, minerals, and nutrients) interact with cytoplasmic components, thereby preventing the growth of pathogens [60–62].

The efficiency of the operating mechanism can be increased due to the smallest size of chitosan nano-derivatives due to the large surface area that is in contact with pathogens. Their small size can also increase the absorption and penetrating properties of nano-derivatives of chitosan through the seed coat, plant tissues, and cell membranes of pathogens, which leads to an improvement in plant immunity and defense responses [63].

In recent years, the demand for agricultural products has been increasing on the world market and amounted to about 2 million tons. At the same time, China is the main consumer and uses 1.8 million tons of drugs per year, followed by the USA and Argentina with 500 thousand and 236 thousand tons per year, respectively. Malaysia uses 49.2 tons/year in agriculture [64-66]. According to world estimates, in 2020 the global volume of agricultural products has almost doubled and is 3.5 million tons [64]. Chitosan derivatives are registered as active ingredients of several safe products for crop production, including *Chito Plant* (ChiPro GmbH, Bremen, Germany), *Biochit* (Agritalia, Italy), *Stemicol* (Plant Response Biotech, Spain). In China, a disease resistance inducer based on chitosan U-lemei (Qingdao Jingling Ocean Technology Co., Ltd) is produced and exported to many countries, which protects vegetable, fruit and tea plants from fungal, bacterial and viral diseases. In South Korea, Kunpoong Bio Co. Ltd. «produces the Biofarm preparation based on chitosan, which effectively protects a number of crops from a complex of diseases. In the USA, the first preparation based on chitosan was registered in 1989 by “Bentech Laboratory Inc.” as a biopesticide recommended for use in agriculture for grain crops. Currently in the United States, chitosan as an active ingredient is approved by the Environmental Protection Agency (EPA) for most crops, including cereals, vegetables, potatoes, citrus fruits, fruits, and berries, ornamental and floral, cotton and grapes, for processing seeds and spraying plants. On its basis, preparations registered as biochemical fungicides have been created, including *Elexa*, *Greenland* and PDB (Plant Defense Booster) (Safescience Products, Inc.), (Yield Enhancing Agent) (DCV Inc.) for seed treatment and spraying plants. These formulations have undergone extensive field tests showing that they increase plant resistance to fungal, bacterial and viral diseases, improve plant growth and increase yields. Also, a new generation of preparations based on nanosized particles of chitosan was created in 2008 by the American company “Agri House Inc, Denver”, trade mark YEA – “Yield Enhancing Agent”. This drug was registered as a new generation of natural elicitors based on chitosan for use in plant protection [66]. In Russia, commercial forms of chitosan with the commercial names "Narcissus", "Ecogel" and "Agrokhit" are also proposed for use in crop production. Their growth-stimulating ability was tested on cucumbers grown in open ground and in greenhouses [67]. The results obtained indicate that chitin-containing preparations are effective and popular growth stimulants, as well as antimicrobial, antiviral and antifungal agents for protecting agricultural plants in open ground and greenhouse cultivation.

In the Republic of Uzbekistan, at the Institute of Chemistry and Physics of Polymers of the Academy of Sciences of the Republic of Uzbekistan, on the basis of chitosan *Bombyx mori*, the drug "UZKHITAN" has been developed as a seed dressing agent against root rot, as well as a growth stimulator, development and increase in yield, registered by the State Commission for Chemicalization and Plant Protection. This drug has been tested as a wheat seed dresser with a stimulating effect [68].

Treatment of wheat leaves with preparations based on chitosan led to an increase in the concentration of phenolic acids, especially ferulic acid, in the composition of plant organs. In the composition of plant tissues, chitosan and its derivatives have a stimulating effect on the formation of lignin precursors (p-coumaric, ferulic, sinapic acids), as well as on the synthesis of phenolic acids with antimicrobial activity - benzoic, p-coumaric, caffeic, protocatechuic, chlorogenic, ferulic and gallic acids [69, 70]. In wheat germ treated with preparations based on chitosan, in comparison with the control, there is a significant increase in the growth index, germination rate, and moisture content in the grain, root length and activity, physiological parameters changed: the activity of superoxide dismutase, peroxidase, catalase, the content of malondialdehyde and chlorophyll [71].

Treatment of rice and wheat with nano-derivatives of chitosan reduced the incidence of plants [72] and stimulated the secretion of the enzyme polyphenol oxidase, which has a catalytic effect in the biosynthesis of lignin. Consequently, increasing the amount of these enzymes can interpret the process of enhancing the

formation of lignin, which creates a barrier to the penetration of pathogens [73]. Also, nanopreparations stimulate the synthesis of catalase enzymes, which protects the plant cell from oxidative damage by reactive oxygen species. After seed treatment with nanopreparations, an increased activity of catalase is observed, and at the same time, germination and an increase in resistance to temperature stress accelerated [74]. Consequently, catalase protects plant cells from the active form of oxygen, as a result of which oxygen is released in the form of a radical, which contributes to the lethal effect on plant pathogens.

It is now recognized that salicylic acid (SA) is one of the key molecules of the signaling pathway for the formation of induced plant resistance; however, determining its role is complicated by the fact that each plant has its own specificity of the participation of salicylic acid in the induction of defense mechanisms [75]. At the same time, the use of preparations based on oligochitosan and salicylic acid leads to an increase in the amount of AD in plant tissues and an increase in resistance against root gall nematode. It has been shown that various methods of processing tomatoes with solutions of complexes of chitosan with salicylic acid (immersion of roots in solution, spraying of plants, root application) reduce root contamination by root gall nematode. Thus, the addition of salicylic acid to chitosan enhanced its ability to stimulate plant defense against the pathogens studied [76].

It should be noted that the use of chitosan-based nanopreparations for growing agricultural crops is preferable, since the preparations have a synergistic effect, at low concentration they contribute to high yields, and minimize agrochemical pollution of soils and water bodies.

When using drugs based on nanochitosan and oligochitosan (oligochitosans form nanostructures) as growth stimulators of agricultural crops, an increase in biomass accumulation occurs due to an increase in the absorption of nutrients, chlorophyll content and the rate of photosynthesis. For example, when processing the leaves of *Robusta coffee* seedlings by spraying with a nanochitosan solution with particle sizes of 420 nm, 750 nm, 970 nm, the best absorption of nutrients N, K, P, Ca and Mg was observed compared to untreated plants. Plant hormones are widely used to increase efficiency. At the same time, nanochitosan with optimal release and high bioavailability is used as a nanocarrier of the hormones gibberellin, auxin, abscisic acid and cytokinin. The resulting nanostructural complexes stimulate the development of bean roots and leaves to a greater extent than the initial hormones [77].

A number of agricultural preparations for processing agricultural crops are produced on the basis of chitosan nano-derivatives in the world. For example, in the USA (Agro Nanotechnology Corp.) developed nanopreparations, trade mark Nano-GroTM plant growth stimulator. In India (JU Agri Sciences Pvt. Ltd, Janakpuri, New Delhi) a plant growth regulator is produced on the basis of nanochitosan and organic acids (including amino acids), trade mark Nanomax NPK fertilizer. In Thailand (Pannaraj Intertrade, Thailand) water-soluble analogs of nanochitosan with salicylic acid are produced, the trade mark "Master nanochitosan organic fertilizer" [77]. The results of literature data show that nanopreparations are of great interest not only in medicine, but in recent years this direction has been developing on a large scale in agriculture as well.

The synthesized complexes of chitosan with ascorbic, α-lipoic acid using the stabilizer Tween-80 and sodium tripolyphosphate (TPPNa) increase the antioxidant properties of plants. The results of studies using 2,2-diphenyl-1-picrylhydrozil have determined that chitosan nanoascorbate with TPPNa has high antioxidant properties[78]. The antioxidant properties of chitosan ascorbate are enhanced with an increase in the content of ascorbic acid. The obtained films, in comparison with the films of chitosan acetate, have the most pronounced antioxidant activity. The authors of [79] suggest that the obtained films of chitosan ascorbate can be used as packaging materials for storing food products.

It was noted in [80] that chitosan ascorbate at a component ratio of 1:1 contains about 23% ascorbic acid and the reaction product is water-soluble. The research results confirm that, in comparison with the initial chitosan, chitosan ascorbate has a minimum inhibitory concentration to *Escherichia coli* of 5 mg/ml.

These results are the basis for the use of chitosan ascorbate complex as a cosmetic ingredient due to its antioxidant, moisturizing, and antibacterial properties.

The penetrating properties of chitosan ascorbate through the mucous membrane of the mouth and intestines into the cells of the bacterium Caco-2 were also investigated. The study compared similar complexes of hydrochloride, chitosan lactate, and the results of studies confirm that chitosan ascorbate has the best penetrating properties and showed cytotoxicity to intestinal Caco-2 [81].

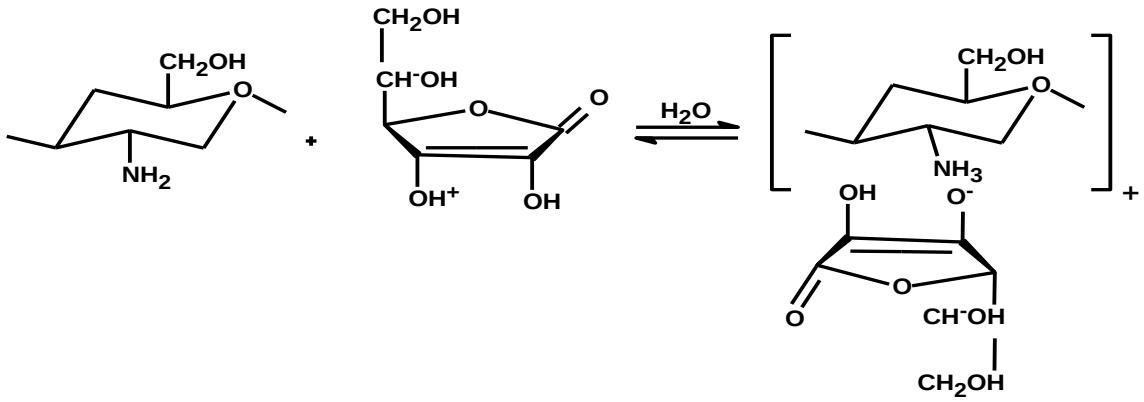
It is known that waste waters and water bodies pollution of fish farms with heavy metals, such as Cd^{2+} ions, causes a change in the level of alanine aminotransferase, aspartate aminotransferase in liver tissues, which leads to a decrease in catalase activity and, as a result, the viability of fish decreases. As indicated in the work, the use of chitosan ascorbate in the composition of feed protects fish from the toxic effect of Cd^{2+} ions due to antioxidant properties and promotes an increase in viability [82].

Despite the interest shown by scientists in the formation and biological activity of chitosan and oligochitosan ascorbate, the kinetic parameters and synthesis conditions are insufficiently studied and their synthesis raises many questions.

In [83, 84, 85], the kinetic parameters of the chitosan ascorbate formation were studied for the first time and the rate constant and activation energy were determined, the value of which is confirmed by the formation of donor-acceptor bonds between the amino groups of chitosan and the yenol groups of ascorbic acid. It has been shown that in the chitosan and oligochitosan ascorbate formation, the chitosan ratio (CH) and ascorbic acid (AA) CH:AA, and the degree of deacetylation of the initial chitosan, play the most important role [86]. When chitosan derivatives are formed with ascorbic acid, it is necessary to control the reaction temperature, because with an increase in the synthesis temperature to more than 65 ° C, the rate of the reaction of formation of chitosan ascorbate decreases; under the influence of high temperatures, the rate constant of the reverse process increases [87]. The conductometric and potentiometric titration results confirm that the chitosan ascorbate reaction formation lasts up to 60 minutes, and a further increase in the synthesis time insignificantly affects the formation of final products [88]. Also, the results of quantum-chemical calculations indicate that during the formation of chitosan derivatives with ascorbic acid, donor-acceptor bonds between the amino groups of chitosan and the enol group of ascorbic acid are energetically efficiently established [89-93]. Therefore, the authors suggest that the formation of the ascorbate anion is due to the most acidic hydrogen atom at C-3 [94-96].

Despite the above, there are different points of view in the literature on the mechanisms of interaction of chitosan with ascorbic acid, but the study of the structural characteristics of chitosan ascorbate remains relevant due to the fact that in the process of formation of chitosan ascorbate, the interaction of components occurs on the basis of three approaches [94-101]:

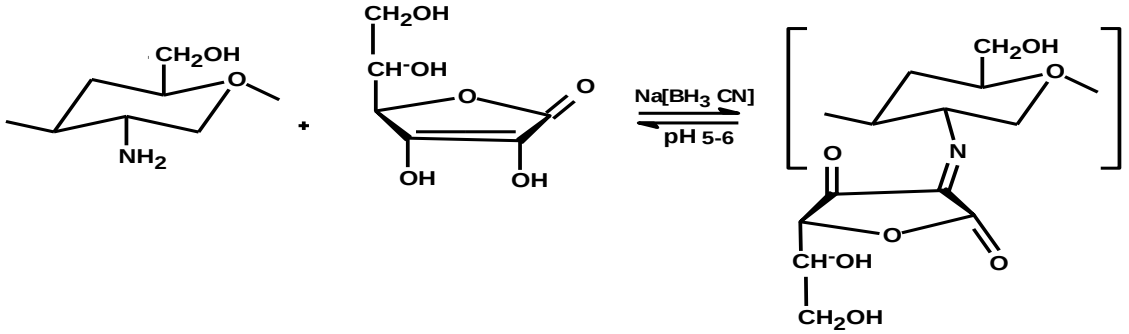
- The first approach is based on the interaction of the third (C_3-OH) enol hydroxyl group of ascorbic acid and the amino group of chitosan with the formation of a donor-acceptor bond [89-96];



Scheme 1. The mechanism of chitosan ascorbate formation using a donor-acceptor bond

A number of authors have shown that due to the more reactive third (C_3-OH) enol hydroxyl group of ascorbic acid with the amino group of chitosan, donor-acceptor bonds are formed. The synthesis was carried out in an aqueous solution with varying the pH of the solution in the range of 4.5-6.5. In the reaction of the lone electron pair, the amino groups of chitosan have donor properties. The authors confirm the structure of chitosan ascorbate using NMR and IR spectroscopy [94-96].

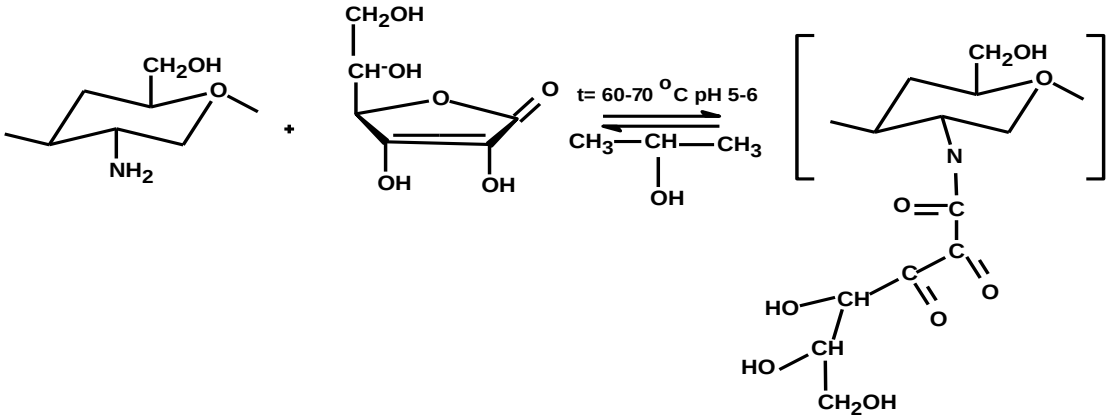
-The second approach is to interconnect the amino group of chitosan with the second (C_2-OH) enol hydroxyl group of ascorbic acid by means of reducing agents - the Schiff reaction [97, 98, 99]



Scheme 2: Reaction of chitosan ascorbate formation with imine bonds.

As can be seen from the presented scheme, due to the second (C_2-OH) enol hydroxyl group of ascorbic acid with the amino group of chitosan, the Schiff reaction occurs and covalent imine bonds are formed, since in the presence of a reducing agent no donor-acceptor bond is formed in reaction systems. In the synthesis of chitosan ascorbate by this method, the final products are obtained by recrystallization or dialysis. Therefore, this method requires a long time and expensive reagents. The authors also confirm the structure using IR spectroscopy methods. [97-99].

- The third approach is based on a reaction leading to the opening of the lactone ring of ascorbic acid at high temperatures. The lactone ring of ascorbic acid is opened by exposure to 60-70 °C temperature of the water-isopropyl mixture. As a result, the interaction of the carboxyl groups of ascorbic acid and the amino groups of chitosan with the formation of an amide bond occurs [100].



Scheme 3. Reaction of chitosan ascorbate formation with amide bond formation

As a result of the interaction of chitosan with ascorbic acid in an aqueous solution, chitosan ascorbate is formed due to the donor-acceptor interaction. When reducing agents are present in the reaction system, in particular sodium cyanoborohydride ($Na[BH_3CN]$), covalent imine bonds are formed.

There are literary sources that confirm that reaction systems heating leads to the formation of a covalent bond in the presence of chitosan amino groups and carboxyl groups of ascorbic acid, the reaction of which is accompanied by opening of the lactone ring of this acid [100]. In these works, ^{13}C-ЯМР spectroscopy showed that in the presence of isopropyl alcohol in an inert atmosphere with varying temperature and pH, covalent amide bonds are formed.

The detected signals at 162 and 165 ppm fully characterize the amide bonds between chitosan and ascorbic acid. The reaction occurs due to the opening of the lactone ring of ascorbic acid. In [101-102], a comparative analysis of the biological activity of nano-derivatives of chitosan with organic acids, including ascorbic acid, was carried out. The increased interest in the nano-derivative of chitosan with ascorbic acid is explained, since the product obtained as a result of the reaction is considered non-toxic to the environment and has biological activity. The work also shows a pronounced biological activity of chitosan ascorbate and chitosan nanoascorbate to diseases of humans and plants.

The synthesis of oligochitosan ascorbate by various methods based on local raw materials opens up a wide possibility of creating new oligomeric preparations that are interesting both from a theoretical and applied point of view. Despite the interest of scientists in the formation and biological activity of oligochitosan ascorbate, there is no information in the literature confirming the synthesis based on chitosan *Bombyx mori*. Until now, chitosan ascorbate is obtained on the basis of chitosan from crab sources, for the first time we were able to obtain oligochitosan ascorbate based on *Bombyx mori* chitosan. It was found that oligochitosan ascorbate and oligochitosan effectively suppress the growth of *Fusarium oxysporum* in comparison with the control and the reference [103-105]. As a result, it was found that the optimal duration of chitosan acid hydrolysis in a solution of 1 M hydrochloric acid at 75^0C, leading to the oligochitosan production with a molecular weight of less than 16 kDa, should be considered 4-5 hours. Also, for comparison, chitosan depolymerization was carried out using sodium nitrite in solution, resulting in oligochitosan with a molecular weight of 6 kDa. On the basis of oligochitosan samples obtained by two methods, their ascorbates were obtained. The composition, structure, and molecular weight characteristics of oligochitosan ascorbate samples were confirmed by physicochemical methods.

Thus, we studied modern scientific sources covering the production and physicochemical properties of oligochitosan, oligochitosan ascorbate obtained from a different source of chitosan. This literature review provides an unambiguous direction for determining the optimal conditions for the synthesis and field of

obtained oligochitosan and oligochitosan ascorbate application. From a practical point of view, the chitosan depolymerization method in the hydrochloric acid presence is more effective, since this method does not affect the chemical structure of oligochitosan, the process proceeds when the system is heated in a time interval of 1-8 hours at a 50-80 °C temperature. The depolymerization rate and the deacetylation degree of the initial chitosan depend on the acid concentration and reaction temperature, as well as the synthesis duration, which plays an important role in the oligochitosan synthesis. The chitosan interaction with ascorbic acid is analyzed on the basis of literature sources. Literature data indicate that the chitosan and oligochitosan interaction with ascorbic acid in an aqueous solution leads to the chitosan and oligochitosan ascorbate formation, which differs in molecular weight from chitosan ascorbate, through the donor-acceptor mechanism.

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