

Chemical Modification of Chitosan for Improving Its Effective Industrial Application

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The work is devoted to the chemical modification of the natural chitosan polymer to improve its quality and suitability for further use in chemical processes. Chitosan samples with an increased degree of deacetylation and a reduced molecular weight were synthesized by the purification with sodium hydroxide with subsequent re-precipitation. Additionally, the chitosan succinate, chitosan hydrochloride, and thyoled chitosan were obtained. These compounds are characterized by solubility in a wider pH range compared to the commercially available samples. For each of the synthesized samples, infrared diffusion reflection spectra were obtained. The modified samples of chitosan were used for obtaining nanoparticles and finely-dispersed films.

1. Introduction

Chitosan is one of the promising products of biomass processing (waste from fisheries and seafood processing). It is a product of the deacetylation of chitin, which is the main component of the exoskeleton of arthropods and other invertebrates, as well as the cell walls of fungi, bacteria, and blue-green algae (Cogollo-Herrera et al., 2018). Currently, a large amount of seafood and fishery waste containing thousands of tons of chitin is generated annually (Yadav et al., 2019). These wastes reduce useful production areas and make an inappropriate sanitary and hygienic situation for the fishing industry and seafood processing. Therefore, the processing of chitin-containing waste into useful products is of great interest. Due to the complexity of the process of chitosan obtained from chitin, the properties of the resulting products (molecular weight, degree of deacetylation, content of amino groups) differ significantly. The reaction of alkaline deacetylation of chitin is typically carried out with concentrated aqueous solutions of NaOH at temperatures of 100 - 180 °C for 0.5 to 10 days (Di Nardo et al., 2019). Deacetylation is accompanied by simultaneous cleavage of the glycoside bonds of the polymer. This leads to the production of a polydisperse biopolymer in terms of composition and molecular weight. The molecular weight of chitosan varies widely depending on the sources of feedstock and the method of production and can be from 100 to 1,200 kDa (Yui et al., 1994).

Chitosan contains primary amines as well as hydrophilic hydroxyl groups. Due to the unique structure of the natural polymer, chitosan has a wide range of applications and is used in many fields of science, industry (Yeul and Shrivastava, 2012), and laboratory research (Razmi et al., 2017). In addition, the prospects for the use of this biopolymer are expanding because of its prevalence in nature, environmental friendliness, and complete biodegradability (Frigaard et al. 2022). However, in the chemical engineering processes, as well as in the production of nanoparticles and films from chitosan, it is very important to use a biopolymer homogeneous in composition and properties. Chitosan characterized by an increased degree of deacetylation, low molecular weight, and good solubility is considered to be the most valuable for practical use (Aranaz et al., 2021). For nanoengineering purposes, the commercial chitosan samples have an insufficient degree of purity (they contain a large amount of non-deacetylated chitin residues, protein impurities, pigments, etc.) and should be additionally purified (Berth and Dautzenberg., 2002). A treatment with heated alkali solutions is the most common method of chitosan modification (Boominathan and Sivaramakrishna, 2021). It is applied to remove impurities of proteins and pigments for partial destruction of the glycoside bonds to form a short-length

polymer chain and reduce the molecular weight. To obtain water-soluble forms of chitosan, its treatment with functional reagents can also be used (Kaczmarek et al., 2019).

The main purpose of this work is the chitosan modification using different methods while studying the main physics-chemical characteristics of the resulting samples. The modification was carried out to improve the quality of chitosan for its further application in industrial processes. For example, the modified chitosan can be used as a support for enzyme immobilization, as a component for biodegradable packing materials, etc.

2. Materials and methods

In this work, the commercial samples of low-viscous, middle-viscous, and high-viscous chitosan («Biochimika», Japan) were used. Besides, the chitosan sample was obtained from chitin («Biochimika», Japan) according to the procedure described below. 5 g of chitin fraction with a particle size below 0.2 mm and 75 mL of 50 wt. % aqueous solution of NaOH was subsequently placed into a three-necked flask with a volume of 100 mL equipped with a paddle stirrer, reflux condenser, and thermometer. The resulting suspension was thermostatted at 96-98 °C for 30 min. Then, the reaction mixture was cooled to 25 °C using a water bath. The separation of the suspension was carried out by filtration through a glass fiber using a Büchner funnel. The solid residue was washed with water several times and dried in the air until the constant weight.

To modify chitosan samples, the following methods were used:

- Method 1: 5 g of chitosan was mixed with 400 mL of 2 wt. % solution of acetic acid in bidistilled water for 16 h at a constant stirring with a rate of 300 min⁻¹. The resulting solution was boiled for 15 min for denaturation and sedimentation of the proteins. Then, the mixture was centrifuged at 5,000 rpm for 10 min, and the supernatant was filtered through the filter with 1 µm size pores. 1 N solution of NaOH was added dropwise to the filtrate until the pH was 9.0 to precipitate chitosan from an aqueous phase. The precipitate was redispersed in distilled water with pH 9.0 and centrifuged twice. The resulting chitosan sample was dried using lyophilization and stored at 4 °C.
- Method 2: 5 g of chitosan was mixed with 50 mL of 1 M NaOH aqueous solution. The mixture was heated up to 70 °C and maintained for 2 h at a constant stirring. The resulting suspension was filtered using a Büchner funnel. An insoluble NaOH chitosan sample was washed with distilled water and dried at 40 °C for 12 h. The purified chitosan flakes were resolved in a 0.1 M solution of acetic acid and filtered through a filter paper to remove insoluble particles. 1 M solution of NaOH was added dropwise to pH 8.0 to precipitate the purified chitosan. The precipitate was filtered, washed with deionized water, and dried under a vacuum for 24 h.
- Method 3: To obtain the chitosan succinate, 0.17 g of succinic acid was put into the round-bottom three-necked flask with a total volume of 250 mL. The flask was equipped with a mechanical stirrer, reflux, and thermometer. In the flask, 100 mL of distilled water was added at a temperature of 20 °C and a constant stirring. After the dissolution of the succinic acid, the mixture was filtered to remove the mechanical impurities. The filtrate was put into the flask, and 0.17 g of chitosan (mean molecular weight of 30 kDa) was added at a stirring rate of 200 min⁻¹. The reaction mixture was stirred for 2 h. After the reaction, 75 mL of ethanol was added to the mixture to precipitate the chitosan succinate. The mixture was centrifuged, and the solid was dried at 30 °C overnight.
- Method 4: To obtain a water-soluble chitosan chloride, the preliminarily swelled chitosan sample was treated with hydrochloric acid. The method allows the chitosan to be converted into salt and other derivatives in mild conditions using a minimum solvent without specific equipment. A partial decomposition leading to a decrease in molecular weight also takes place. The reaction product remains solid and can be easily removed, washed, and dried. For these purposes, 10 g of chitosan was added to 65 mL of 2M hydrochloric acid and stirred constantly for 1 h. Then, 15 mL of 2M solution of sodium nitrite was added to the reaction mixture and stirred for 15 min. After the reaction, 50 mL of ethanol was added to the mixture to precipitate the chitosan salt. The solid product was filtered, washed with ethanol, and dried at 30 °C overnight.
- Method 5: Thioled form of chitosan was obtained by the covalent binding with N-acetylcysteine. 500 mg of chitosan was dissolved in 1 wt. % solution of hydrochloric acid. The solution pH was increased up to 5 by 1M solution of NaOH. 4 g of N-acetylcysteine was dissolved in the demineralized water. The carboxylic groups of N-acetylcysteine were activated by carbodiimide for 20 min. pH value was maintained in the range of 4-5 during the experiment. The mixtures obtained were joined and stirred at room temperature for 6 h. The conjugate was isolated by the dialysis using 1mM HCl containing 2 mM of EDTA (once), the same solution containing 2 wt. % of NaCl (twice), and 0.5 mM of HCl. The conjugate pH was kept by 1 M NaOH at a value of 4.5. The isolated product was frozen and lyophilic dried.

To analyze the chitosan samples, FTIR spectra of diffuse reflection were obtained using the IR Prestige-21 spectrophotometer («Shimadzu», Japan). The estimation of the molar weight of the modified samples was carried out using a glass viscometer («Soyuznauchpribor», Russia). The molecular weight of chitosan samples was calculated using a viscosity index and the Marc-Houwink-Sakurada equation (Kasaai et al., 2000). To evaluate the deacetylation degree of chitosan, a potentiometric titration of 1 wt. % chitosan solution in 0.1 N HCl by 0.03 N solution of NaOH was carried out using a pH meter (Pérez-Álvarez et al. 2018).

To estimate the number of insoluble impurities, the chitosan samples were resolved in an acetate buffer with pH 4.3 for 10 min. The solutions were filtered through a paper filter, and the residue weights were obtained using an analytical scale. The number of insoluble impurities was calculated as a percentage.

Chitosan nanoparticles were synthesized by mixing of 10 mL of chitosan solution (1.5 mg/mL in acetate buffer with pH 4.3) with 10 mL of the solution of sodium tripolyphosphate (0.25 mg/mL in the distilled water). The mean diameter of the nanoparticles was measured by the dynamic light scattering method using 90Plus spectrometer (Brookhaven, USA).

Chitosan films were obtained by the deposition of 1 wt. % solution of chitosan in 0.2 M acetic acid in the Petri dish (0.2 mL/cm²) with the following drying in the air for 24 h. The film thickness was measured using IRPrestige-21 spectrophotometer («Shimadzu», Japan).

3. Results and discussion

Figure 1 presents FTIR spectra of the initial middle-viscous chitosan and the samples obtained according to methods 1 and 2.

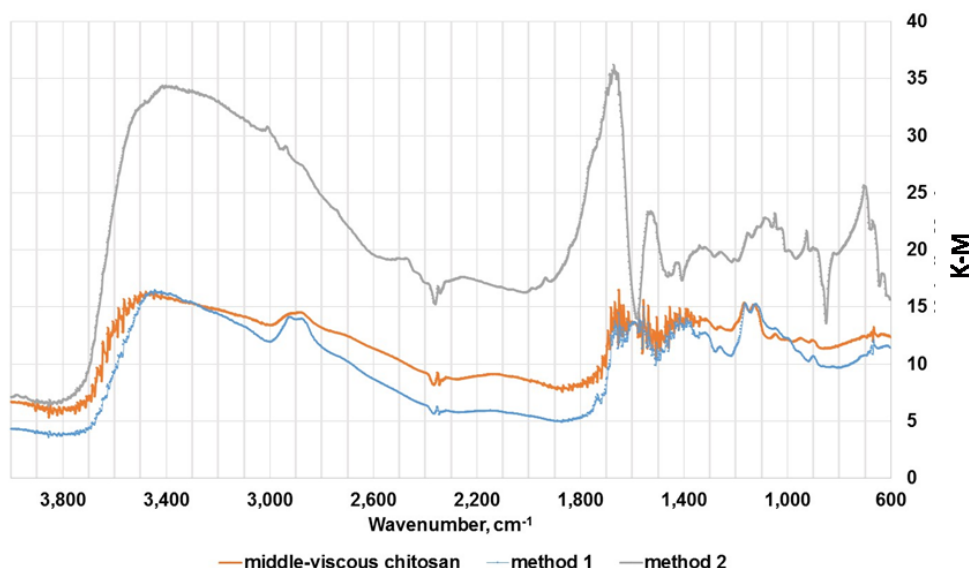


Figure 1: FTIR-spectra of the chitosan samples purified by methods 1 and 2 compared to the original chitosan

From Figure 1, it is seen that all spectra obtained have absorption bands at 3,440 cm⁻¹ and 1,655 cm⁻¹. These bands can be correlated to amino-group vibrations. The band at 3,440 cm⁻¹ is partially surpassed by the band corresponding to the vibrations of intermolecular and intramolecular bonded OH-groups. The strength of these bands is significantly higher in the sample purified according to method 2. This can be explained by the drastic increase in the free amino-group concentration in the sample. All spectra contain distinct absorption bands at 3,000–2,800 cm⁻¹, which correspond to the vibrations of the methylene group of the pyranose ring. Besides, the spectra of the initial chitosan sample contain pronounced absorption bands at 1,653–1,620 cm⁻¹ (amide band I) and 1,553 cm⁻¹ (amide band II). These bands can be correlated to the non-deacetylated residues of chitin as well as to the protein impurities. A decrease in the intensity of the bands in the spectra for purified samples confirms an increase in the deacetylation degree of chitosan and the removal of impurities during the purification. In the spectra of chitosan purified by method 1, a significant decrease of peak intensity at 1,100–900 cm⁻¹ corresponded to the glycoside bonds between the ether bonds and pyranose rings can be observed. It can indicate a decrease in the length of the polymeric chain and the reduction of the molecular weight of the sample as well as an increase in its deacetylation degree. The spectra of the samples have a significant

difference in the range of 1,700-800 cm^{-1} . This correlates to a drastic decrease in the number of impurities in the purified samples and an increase in the deacetylation degree of chitosan.

Figure 2 presents the FTIR spectra of the synthesized chitosan succinate and an initial chitosan sample in the wave number of 1,800-1,600 cm^{-1} .

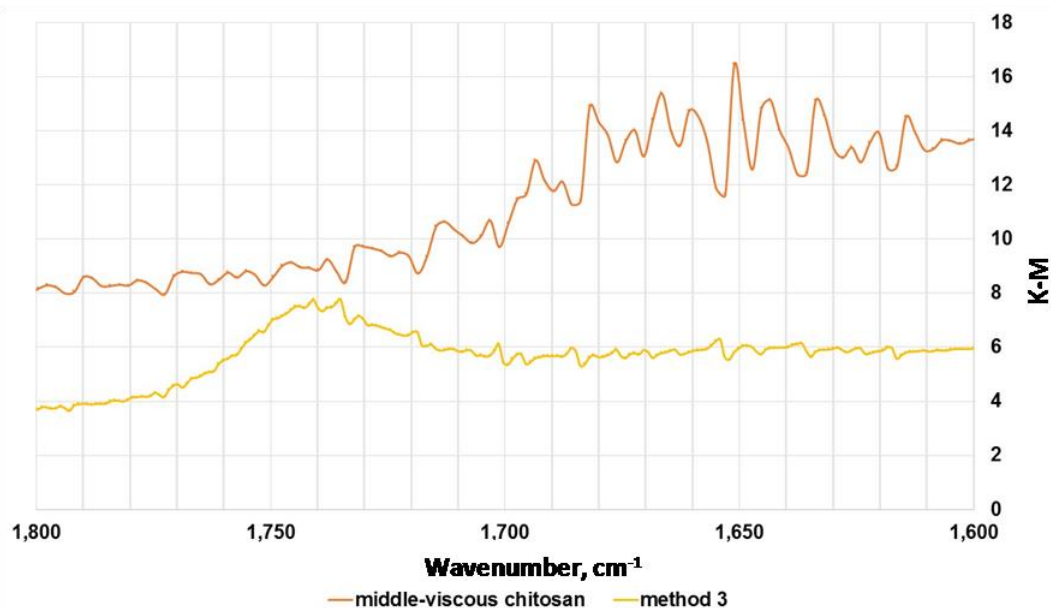


Figure 2: FTIR-spectra of the chitosan sample purified by method 3 compared to the original chitosan

In the spectra of succinate, the intensity of the adsorption bands corresponding to the free amino groups (at 1,655 cm^{-1}) is lower than for the initial chitosan. Also, the adsorption band at 1,740-1,730 cm^{-1} related to the succinate-ions can be seen. This confirms the successful synthesis of the chitosan derivative.

Figure 3 presents the FTIR spectra of the thyoled form of chitosan in comparison with the initial one. Besides the adsorption bands corresponding to the functional groups of chitosan, in the spectra of “chitosan-cysteine” conjugate, the additional bands at 970-960 cm^{-1} related to the thiol group of cysteine can be seen.

The calculated molecular weight of the samples, deacetylation degree, and the number of insoluble impurities are presented in Table 1.

The data in Table 1 show that the chitosan purification methods using NaOH solution lead to a significant decrease in the molecular weight as well as to an increase in the deacetylation degree. This facilitates the synthesis of the films from chitosan characterized by the smaller size and polydispersity. Thiolysation and treatment with succinic acid did not affect the molecular weight and deacetylation degree significantly. However, these modification methods allowed the chitosan solubility range to be widened. The initial chitosan can be dissolved at pH values 1÷5, while the modified ones can be dissolved at pH1÷8. This allows the biopolymer to be used for purposes where excessive acidity is prohibited, i.e., for the immobilization of the cells and enzymes, as well as in pharmaceutical and cosmetics.

The results obtained significantly expand the prospects for the use of modified chitosan in chemical engineering processes, including for the production of nanoparticles, films, and supports for metals, cells, enzymes, and drugs. Because of the reduction the molecular weight, the synthesis of nanoparticles with smaller diameters and films with smaller thicknesses and uniform structure becomes possible. An increase in the degree of chitosan deacetylation makes it possible to increase the content of free amino groups on the surface, as well as to increase the capacity of the biopolymer matrix for adsorption and covalent cross-linking. Reducing the content of insoluble impurities significantly increases the uniformity of synthesized nanoparticles, films, and supports.

The target modification of chitosan makes it possible to achieve a significant improvement in its properties and expand the range of its applicability in chemical engineering.

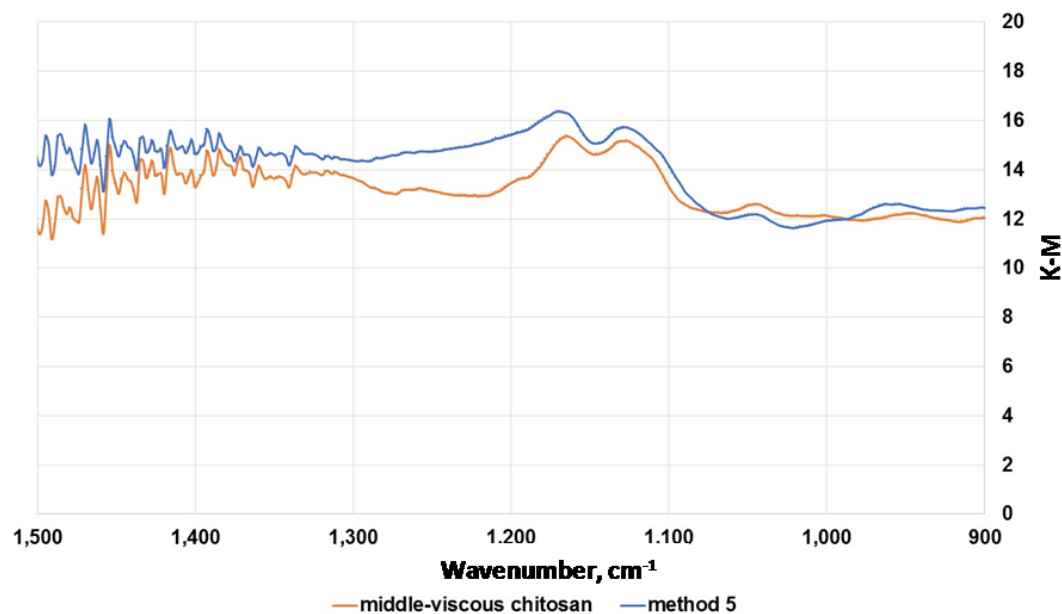


Figure 3: FTIR-spectra of the chitosan sample purified by method 5 compared to the original chitosan

Table 1: Properties of the studied chitosan samples

No	Sample	Modification method	Molecular weight, kDa	Deacetylation degree, %	Amount of insoluble impurities, wt. %
1	Low-viscous chitosan	-	223	86.4	0.35
		1	196	92.6	0.33
		2	187	93.7	0.31
		3	212	87.9	0.32
		4	210	88.8	0.34
2	Middle-viscous chitosan	5	209	85.3	0.32
		-	387	83.7	0.87
		1	232	91.7	0.32
		2	198	90.3	0.30
		3	380	83.3	0.35
3	High-viscous chitosan	4	386	82.9	0.41
		5	378	84.6	0.33
		-	651	76.5	1.25
		1	455	84.3	0.99
		2	422	85.1	0.97
4	Chitosan synthesized from chitin	3	587	75.9	0.99
		4	591	74.3	0.79
		5	588	73.1	0.86
		-	588	64.3	5.43
		1	466	75.4	2.21
		2	455	76.8	2.65
		3	571	72.5	3.11
		4	575	73.1	2.95
		5	589	72.0	2.88

The characteristics of nanoparticles and films obtained from the modified chitosans are presented in Table 2. Experiments showed that all modification methods resulted in a decrease in the mean size of nanoparticles as well as a decrease in the thickness of the films. The results obtained confirm the effectiveness of the modification methods used for chitosan.

Table 2: Characteristics of nanoparticles and films obtained from the modified chitosans

Nº	Modification method	Mean particle diameter, nm	Film thickness, μm
1	without modification	171.2	101.4
2	1	156.3	90.3
3	2	148.8	89.0
4	3	155.4	88.9
5	4	159.1	87.4
6	5	160.9	90.3

4. Conclusions

In this work, the properties of commercial and synthesized chitosan samples before and after modification were investigated. For each of the synthesized samples, infrared diffusion reflection spectra were obtained. The degree of deacetylation, molecular weight, and the number of insoluble impurities were determined. Modification of chitosan makes it possible to significantly increase the deacetylation degree of the samples and reduce the molecular weight of the biopolymer and the content of insoluble impurities. All the investigated modification methods resulted in a decrease in the mean diameter and thickness for nanoparticles and films prepared from the obtained chitosan samples. Nanoparticles and fine-dispersed films are the prospective supports for the immobilization of live cells and enzymes and can be effectively used in the pharmaceutical, cosmetics, and food industries.

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