Conference Paper

Effect of Heterogeneous Deacetylation on the Properties of Northern Shrimp Chitin and Chitosan

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Abstract

The effect of alkaline treatment of shrimp chitin on the molecular weight, the degree of deacetylation and degree of crystallinity of the resulting chitosan is studied. The viscosity of chitosan solutions from repeatedly deacetylated chitin is studied. It is shown that repeated treatment of chitin/chitosan with alkali causes the destruction of polysaccharide macromolecules. After four-time deacetylation and one-time deacetylation of chitin/chitosan for four hours, the molecular weight of the polysaccharide decreases by ten times. The maximum degree of chitosan deacetylation under experimental conditions was 92.0 -92.5%. The diffractograms of chitin and chitosan from the Northern shrimp are of the form typical for samples containing an amorphous phase in addition to a crystalline phase. The degree of crystallinity of chitin from Northern shrimp was 40.8%, of chitosan samples after one-, two-, and three-time deacetylation was 62-65%. For a sample of chitosan obtained after four-time deacetylation, recrystallization, and drying in a freeze dryer, the degree of crystallinity is close to the degree of crystallinity of shrimp chitin. The investigated acetic acid chitosan solutions with a concentration of 5% (wt.) and the chitosan molecular weight of 250, 160 and 130 kDa in their rheological properties are liquid-like non-Newtonian systems, their viscosity decreasing with increasing shear stress. After four-time deacetylation of chitin, the viscosity of chitosan solutions practically does not change with increasing shear stress, which apparently can be due to a significant decrease in the molecular weight of chitosan under these conditions.

Keywords: chitin, chitosan, alkaline deacetylation, degree of deacetylation, crystallinity.

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1. Introduction

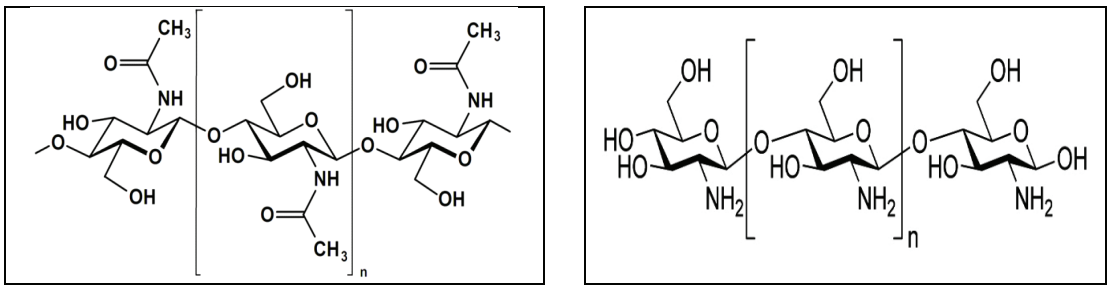
Chitin and chitosan are used in many industries, medicine, cosmetics, agriculture. Chitosan, obtained by the chitin deacetylation, is a unique object of research due to its complex chemical, physico-chemical and biological properties, among which biocompatibility, non-toxicity, biodegradability should be emphasized. Development and improvement of the technology of chitosan production from chitin is of interest for fishing industry, as an area of waste-free processing of marine crustaceans.

Conducting the deacetylation of chitin in a heterophase system leads to heterogeneity of properties both between different particles and molecules, and in the depth of one particle. Due to the presence of crystalline and amorphous structures in chitin and chitosan particles, the action of chemical reagents leads to the formation of macromolecules of these polysaccharides with heterogeneous properties along the length of the polymer chain of a single molecule. As a result, polymers with the same chemical composition will exhibit different physicochemical and chemical properties due to the different distribution of substituents [1].

Chitin is a linear aminopolysaccharide consisting of N-acetyl-2-amino-2-deoxy-D-glucopyranose links.

The structural formula of chitin is shown in Figure 1a, where there is a monomeric unit-2-acetamido-2-deoxy-β-D - glucopyranose in square brackets, n is the number of pyranose links.

Chitosan is a 2-amino-2-deoxy-β-D-glucan aminopolysaccharide. The structural formula of chitosan is shown in Figure 1b. The monomeric unit of 2-amino-2-deoxy-β-D-glucopyranose is square bracketed; n is the number of monomer units, the degree of chitosan polymerization.



a) b)

Figure 1: Structural formula of chitin (a) and chitosan (b).

The widespread use of chitin and chitosan in various industries and human life requires a detailed study of their properties. Therefore, it is important to study these polysaccharides by various methods of analysis.

This paper presents the results of studying the effect of the method of alkali treatment of shrimp chitin on the molecular weight, the degree of deacetylation and degree of crystallinity of chitosan, the viscosity of solutions of repeatedly deacetylated chitin / chitosan is also studied.

2. Methods and Equipment

Chitin from the shell of the Northern shrimp *Pandalus borealis* obtained according to the technology described in the monograph [2] was used in the work.

The molecular weight (MW) of chitin was determined by the method of high-performance liquid chromatography using the technique described in [2]. Chromatographing was performed on LC-10AVP HPLC system ("Shimadzu Corp.", Japan) using a column TSK-gel Alpha-2500 (30 x 0.78 cm) and TSK-gel Alpha-4000 (30 x 0.78 cm) with guard column TSK-guardcolumn Alpha (6 x 0.4 cm) ("TOSOH", Japan). The molecular weight of chitosan samples was determined by viscometric method, using Ubbelode viscometer (capillary diameter of 0.54 mm). The method is based on determining the intrinsic viscosity of the chitosan solution and calculating the average molecular weight by Mark-Houwink-Kun equation [3].

The degree of chitin deacetylation (DD) was determined by infrared spectroscopy method and calculated by the method described in [4]. IR absorption spectra were recorded on IR spectrophotometer IR-420 (Shimadzu, Japan) in the frequency range from 4000 to 400 cm^{-1}. [5--7]. The degree of chitosan deacetylation was determined by potentiometric titration method using the Anion 4151 ionomer (`Infraspak -- Analyte'', Russia) with an eluent addition step equal to 0.1 cm^3 [2].

Chitosan was obtained by chitin deacetylation with 50 % sodium hydroxide solution in the mass ratio chitosan: NaOH = 1: 30 and at a temperature t=95±3°C for 60 minutes, followed by water washing to a neutral medium and air drying at room temperature. The samples obtained thereby were subjected to two-, three-, and four-time deacetylation. After three-time deacetylation, the chitosan sample was re-precipitated from the solution in acetic acid, washed with distilled water, dried in a freeze dryer, and a fourth deacetylation was performed.

Chitosan samples were also obtained by one-time deacetylation of chitin at a temperature t=95±3°C for 1 to 4 hours and deacetylation of chitin for four hours while maintaining the constant alkali concentration (50% wt.) every hour during deacetylation.

X-ray phase analysis of chitin and chitosan samples was performed in the Institute of Chemistry and Technology of Rare Elements and Mineral Raw Materials of the Russian Academy of Sciences Kola Science Center on the diffractometer Shimadzu (Japan) LabX XRD-6000 in the laboratory of physicochemical methods of analysis. X-ray phase analysis was performed using x-ray tube radiation-Cu-Ka, a graphite monochromator with a monochromatic x-ray wavelength λ =1.54178 Å. The qualitative x-ray phase analysis was performed, consisting in the identification of crystalline phases based

on their inherent values of interplanar distances *d(hkl)* and the lines of corresponding intensities *I (hkl)* of the x-ray spectrum. The interplanar distances were calculated using the Wulf-Bragg equation [8]. To calculate the degree of crystallinity the diffractograms were processed according to the methods given in [9--11].

To measure the rheological characteristics of chitosan solutions, the maximum possible under experimental conditions concentration of chitosan in 0.33% acetic acid was selected, at which a homogeneous chitosan solution without inclusions dissolved particles is formed. This concentration was 5% by weight. The viscosity of chitosan solutions was measured in the mode of shear deformations atthe modular compact rheometer PhysicaMCR302 (AntonPaar, Austria) using the measurement cell "cone-plane".

3. Results

The molecular weight and degree of deacetylation of chitosan obtained by repeated deacetylation of chitin are shown in Figure 2

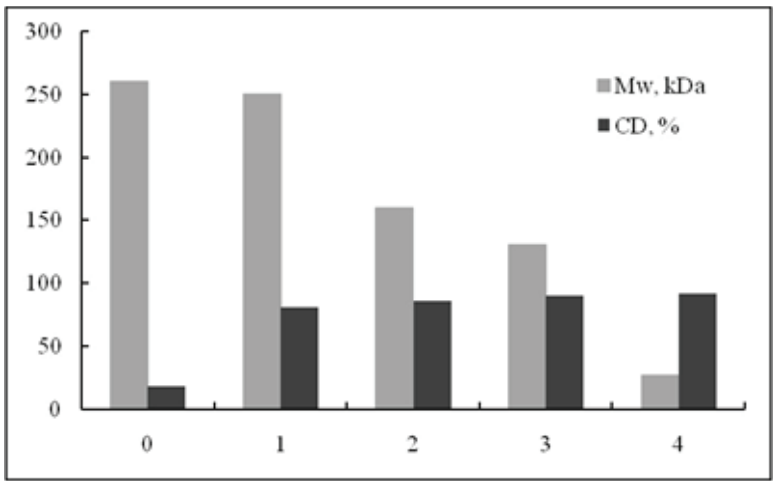


Figure 2: Molecular weight and degree of deacetylation of chitin and chitosan samples obtained by repeated chitin deacetylation, where sample 0 is the original chitin; samples 1-4 are chitosan obtained by one-time (1), two-time (2), three-time (3) and four-time (4) deacetylation.

The molecular weight and degree of deacetylation of chitosan obtained by one-time deacetylation of chitin for four hours and deacetylation of chitin while maintaining the alkali concentration constant every hour during the deacetylation process are shown in Figure 3.

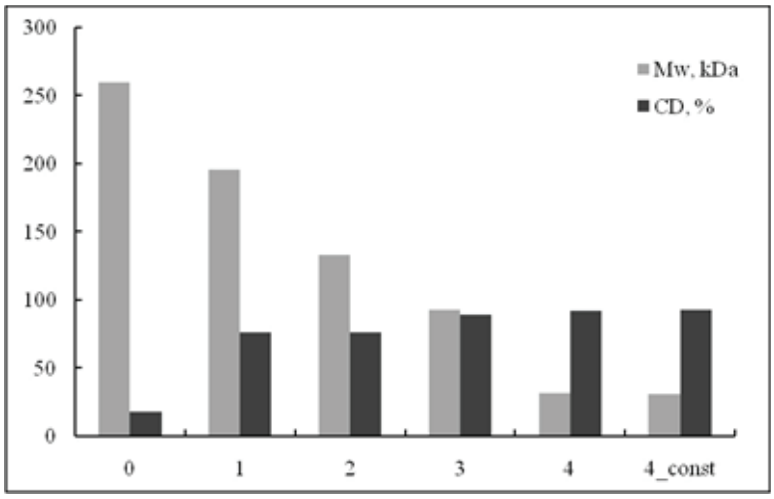


Figure 3: Molecular weight and degree of deacetylation of chitin and chitosan obtained by one-time deacetylation of chitin, where sample 0 is the original chitin; samples 1-4 are chitosan obtained by deacetylation for 1, 2, 3 and 4 hours; sample 4 const is chitosan obtained during 4 hours while maintaining a constant NaOH concentration.

4. Discussion

From the data given in figures 2 and 3, it follows that under experimental conditions, the degree of chitosan deacetylation increases after three-time deacetylation of samples and after three hours of deacetylation. The maximum degree of deacetylation of the samples obtained by the four-time deacetylation or deacetylation of chitin while maintaining the alkali concentration constant every hour during the deacetylation for four hours was 92.0-92.5%. Under the experimental conditions, the degree of deacetylation close to 100% is not achieved.

The treatment of chitin/chitosan with 50% solution of sodium hydroxide in the mass ratio chitosan: NaOH = 1: 30 at t=95±3°C for more than one hour causes the destruction of polysaccharide macromolecules which leads to a decrease in molecular weight. Thus, after the first deacetylation, the molecular weight of the samples practically does not change in comparison with the molecular weight of the initial chitin. After the second and third deacetylation, the molecular weight decreases by 1.5-2 times (Figure 2). After four-time deacetylation (Figure 2) and deacetylation for four hours while keeping the alkali concentration constant every hour during the deacetylation process (Figure 3) the molecular weight of the polysaccharide is reduced by about ten times compared to the molecular weight of the original chitin.

It is known from the literature data that natural and synthetic polymers contain both crystalline and amorphous phases. The ordered (crystalline) and disordered (amorphous) areas of the polymer are strongly interconnected and form a single whole. To characterize such polymers, the concept of the crystallinity degree is used, which

characterizes the ratio of the volumes of the crystalline and amorphous phases. The degree of crystallinity for most polymers is in the range of 20-80% [12].

All the shrimp chitin and chitosan diffractograms obtained by us were in the form typical for the samples containing an amorphous phase in addition to a crystalline one. The chitin and chitosan diffractograms contain the peaks having a broad line (Gallo) at the base with an angular width of 2Θ =10-20˚. Figure 4 shows the diffractogram of a shrimp chitin sample as an example.

For shrimp chitin, the position of the peak with maximum intensity I_0 is located in the region 2Θ=20˚. In the field of interplanar spacings d=8.7Å there is a peak corresponding to 2Θ=10.1˚. (Figure 4). On the chitosan diffractograms, the positions of the peaks with maximum intensity I_0 are in the region 2Θ=20.2-20.7˚. They correspond to the interplanar distances d =4.3-4.4 Å. In the field of interplanar spacings d=8.0-8.6 Å the diffractograms exhibit peaks corresponding to 2Θ=10.3-11.1˚. The results obtained by us are consistent with the literature data on studying the crab chitin diffraction [9, 13--15].

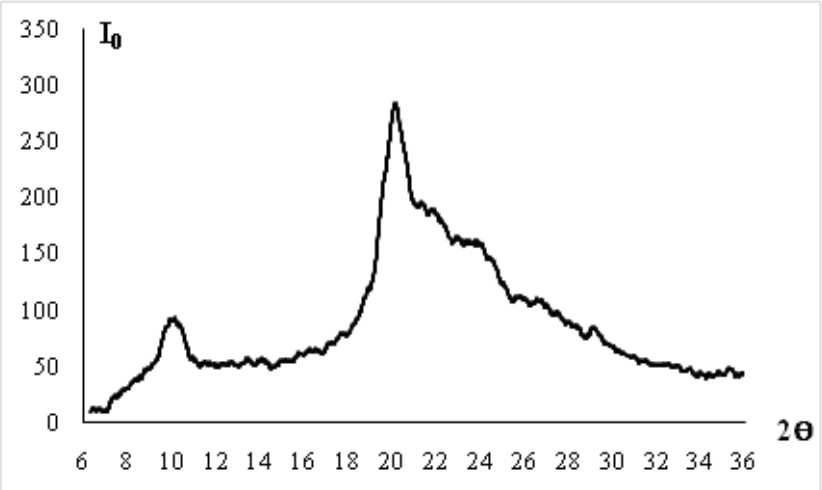


Figure 4: Diffractogram of chitin obtained from the shell of Northern shrimp.

The results of processing the diffractograms showed that there is an increase in the degree of crystallinity of chitosan samples compared with the degree of chitin crystallinity with increasing the degree of chitosan deacetylation by repeated deacetylation to 81-92%. Thus, the degree of crystallinity of shrimp chitin was 43.9%, the degree of crystallinity of chitosan samples obtained by the repeated deacetylation was 62-65%. It is known from the literature data that a new crystal structure of chitosan is formed at the deacetylation degree above 90% [9, 16]. Chitosan, like chitin, is an amorphous crystalline polymer for which the phenomenon of polymorphism is observed. Thus, it is noted in [17] that chitosan obtained by the deacetylation of crab chitin with MW 600-750 kDa is of six polymorphic crystalline modifications. Apparently, under the conditions of

multiple alkaline deacetylation of shrimp chitin, a new chitosan structure begins to form at DD above 80%. It should be noted that multiple deacetylation leads to a decrease in the molecular weight of the investigated samples. Probably, the decrease in the molecular weight contributes to the formation of the crystal structure of the chitosan samples.

For a sample of chitosan obtained after four-time deacetylation, recrystallization and drying in a freeze dryer, the degree of crystallinity was close to that one of the original shrimp chitin and was 43.9%. Apparently, under these conditions, the crystal structure of chitosan is destroyed.

The results of rheological measurements for chitosan solutions are presented in Figure 5 as flow curves (dependence of effective viscosity (η, Pas) on shear stress (σ, Pa).

As it can be seen from Figure 5, chitosan samples exhibit non-Newtonian behavior after one-time, two-time, and three-time shear-flow deacetylation (viscosity was measured one day after the solution preparation). With increasing the shear stress σ in the studied range the viscosity η significantly reduces. It was not possible to achieve the minimum Newtonian viscosity η_∞ at high voltages (speeds) of the shift (Figure 5) under experimental conditions due to the transition to an unstable flow at high shear rates.

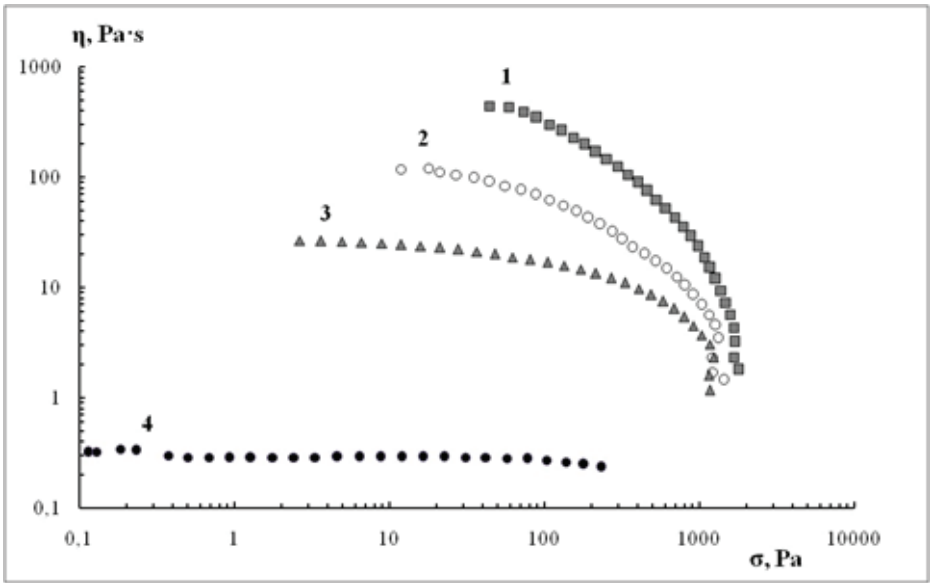


Figure 5: Dependence of chitosan solution viscosity on shear stress. T=20°C, $C_{Chitosan}$ = 5%, with $C_{CH3COOH}$ = 0.33 N. Chitosan obtained by one-time (1), two-time (2), three-time (3) and four-time (4) deacetylation.

The rheological behavior of chitosan solutions with concentration of 0.1-1.0% and MW of some 200-300 kDa was studied in [18]. It was found that according to their rheological properties, such solutions are systems close to Newtonian fluids. The authors of this

work suggested the possibility of compaction of chitosan macromolecules due to the formation of intramolecular hydrogen bonds.

The acetic acid chitosan solutions with a concentration of 5% and MW=130,160 and 250 kDa investigated by us are liquid-like non-Newtonian systems according to their rheological properties. Probably, in the volume of the solution at the concentration of chitosan $C_{Chitosan}$ = 5%, polysaccharide macromolecules form fragile reversible structures that are easily destroyed when the shear rate increases. The experimental data obtained by us indicate that chitosan does not form any gel in the acetic acid solution, even though the concentration is high.

After four-time chitin deacetylation, the viscosity of the chitosan solution decreases slightly with increasing the shear stress (Figure 5, curve 4). Apparently, the difference in rheological behavior of chitosan samples after four-time deacetylation and one-, two -, three-time deacetylation is mainly due to a significant difference in the molecular weight of chitosan (26.6 kDa and 250, 160 and 130 kDa, respectively).

5. Conclusion

The treatment of chitin/chitosan with alkali at t=95±3°C for more than one hour causes destruction of the polysaccharide macromolecules, leading to a decrease in molecular weight. After four-time deacetylation and one-time deacetylation for four hours, the molecular weight of the polysaccharide decreases by about 10 times compared to the MW of the original shrimp chitin. The results obtained allow choosing the conditions for heterogeneous alkaline deacetylation of chitin for producing the high - or low-molecular chitosan.

The diffractograms of shrimp chitin and chitosan are of the form typical for the samples containing crystalline and amorphous phases. The positions of the peaks with the maximum intensity (I_0) are in the region of 2Θ= 20.0-20.7°. With an increase in the degree of deacetylation of chitosan obtained by repeated chitin deacetylation from 81% to 92%, an increase in the degree of crystallinity of the samples is observed.

Rheological behavior of chitosan solutions with 5% mass concentration in 0.33% acetic acid depends on the molecular weight of chitosan

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Conflict of Interest

The authors have no conflict of interest to declare.

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