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STUDYING THE KINETICS OF THE ACID HYDROLYSIS REACTION AND THE STRUCTURAL CHARACTERISTICS OF LOW-MOLECULAR CHITOSAN BOMBYX MORI

Abstract: *This article investigates oligochitosan and low-molecular-weight chitosan, mainly chitosan derivatives that contains bactericidal, growth-stimulating and antifungal properties and are non-toxic to the environment. As these points were considered under world interest, samples of low molecular weight chitosan were obtained by acid hydrolysis of high molecular weight chitosan isolated from Bombyx mori. Research pinpoints and makes scrutinize analyzes on the kinetic regularities of the synthesis of oligosaccharides. It also mentions the rate constant for the formation of low molecular weight (oligo) chitosan Bombyx mori, which is* $K = 4.6 \times 10^{-6}$ *g mol / min, was determined depending on the degree of depolymerization of the process on time and from the slope. In conclusion, Structural and physicochemical properties were studied using AFM and IR spectroscopy.*

Key words: *Bombyx mori chitosan, Bombyx mori oligochitosan, acid hydrolysis, rate constant.*
Language: *English*
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Introduction

UDC 678.5, 547.64

Chitosan macromolecules are characterized by different molecular weights (polymerization degree), deacetylation degree, protonation degree (pKa value), solutions viscosity, acetylated and deacetylated residues arrangement in the polymer chain and molecular weight distribution. Most of the aforecited characteristics affect the chitosan solubility in water, in its turn, the manifestation of biologically active properties by chitosan largely depends on the solubility [1-4]. The work [4-6] investigated the antibacterial activity of narrowly dispersed oligochitosans samples, differing in molecular weight, at different pH values. It was shown that under acidic conditions a stronger inhibitory effect is characteristic of samples with a higher molecular weight, and under slightly alkaline conditions, chitosan forms close to oligomeric are more active. It is assumed that the antibacterial chitosan activity is determined by its amino groups protonation degree, which is a variable and depends both on the molecular weight of the substance and on the pH environment.

Numerous works analysis shows that water solubility and biological activity of low molecular weight chitosan (oligochitosan), especially antiviral and antibacterial activity, as well as biocompatibility

improves with decreasing molecular weight compared to high molecular weight chitosan [7-11].

As it's known, until now oligochitosan is obtained on the basis of chitosan from crab sources. For the first time we have obtained oligochitosan on the basis of *Bombyx mori* chitosan.

It determined that oligochitosan effectively inhibits the *Fusarium oxysporum* growth compared to control and standard [12-15]. In order to determine the kinetic laws acid hydrolysis was carried out with varying synthesis conditions.

Research methods.

In this work it was used high molecular weight chitosan samples derived from silkworm pupae *Bombyx mori* with 190 kDa molecular weight, determined by viscometry data. Molecular weight of low molecular weight chitosan determined by viscometry method using an Ubelode viscometer based on the Mark-Kuhn-Houwink equation.

Deacetylation samples determined by conductometric titration method in the Mettler Toledo device. To study the change in particle size of the obtained samples, AFM studies were performed using an *Agilent 5500* atomic force microscope (AFM).

It was also studied the structural characteristics of the obtained low-molecular-weight chitosan samples, X-ray structural studies were carried out on a DRON-3M diffractometer using monochromatic CuK_α-radiation with a wavelength λ = 1,54 Å.

The chitosan samples crystallinity degree is determined based on the formula below:

$$CD = \frac{S_k}{S_k + S_a} \times 100 \qquad (1),$$

where CD–crystallinity degree;

S_k – crystal area;

S_a – amorphous area.

In order to study the acid hydrolysis reaction kinetics of chitosan, the reaction was carried out in 1 M hydrochloric acid solution at 60 °C for 6 hours.

Results and discussion.

Based on the obtained viscometric data, α depolymerization degree and its dependence on the synthesis time were calculated. The depolymerization constant of the initial chitosan was determined and the results are presented in Table 1.

Table 1. Physicochemical properties dependence of low molecular weight chitosan on the synthesis time

Samples	Synthesis time, min.	Nitrogen content *, %	DAD, %	MM	Particle size, AFM	Degree de.p., α ×10^{-3}
CH-init.	-	8,18	76,0	190,0	600 nm to 3 micron	-
CH -1	60	8,25	79,0	103,0	400-500 nm	1,65
CH -2	120	8,34	84,0	96,0	80-200 nm	1,75
CH -3	180	8,49	91,0	82,0	80-150 nm	1,99
CH -4	240	8,49	91,0	71,7	70-100 nm	2,27
CH -5	300	8,49	91,0	63,2	38-100 nm	2,58
CH **-6**	**360**	**8,49**	**91,0**	**51,0**	**30-100 nm**	**3,20**

* *The nitrogen content is determined based on conductometric titration.*

The results obtained show that with increasing duration is a significant decrease in the molecular weight of the original chitosan from 190 to 51 kDa. At the same time, there is an increase in the nitrogen content from 8.18% to 8.49%, which is evidence of acetamide groups' deacetylation by means of hydrochloric acid. It was shown that in the acid hydrolysis process, not only the glycosidic bonds cleavage occurs, but at the same time acetamide groups' deacetylation of the initial chitosan is observed [13-15]. It was found that with an increase in duration to 180 minutes, the deacetylation degree (DAD) increases from 76% to 91%, then, with an increase in the synthesis time, the nitrogen and DAD content of the obtained low-molecular-weight chitosan changes insignificantly.

Also, the AFM results show, that with an increase in the depolymerization degree, the particle size decreases from 3 μm to 30 nm, respectively[1] (Fig. 1.).

[1] ***The authors are grateful to the junior researcher Kulumbetov A.S. for obtaining data from AFM studies.***

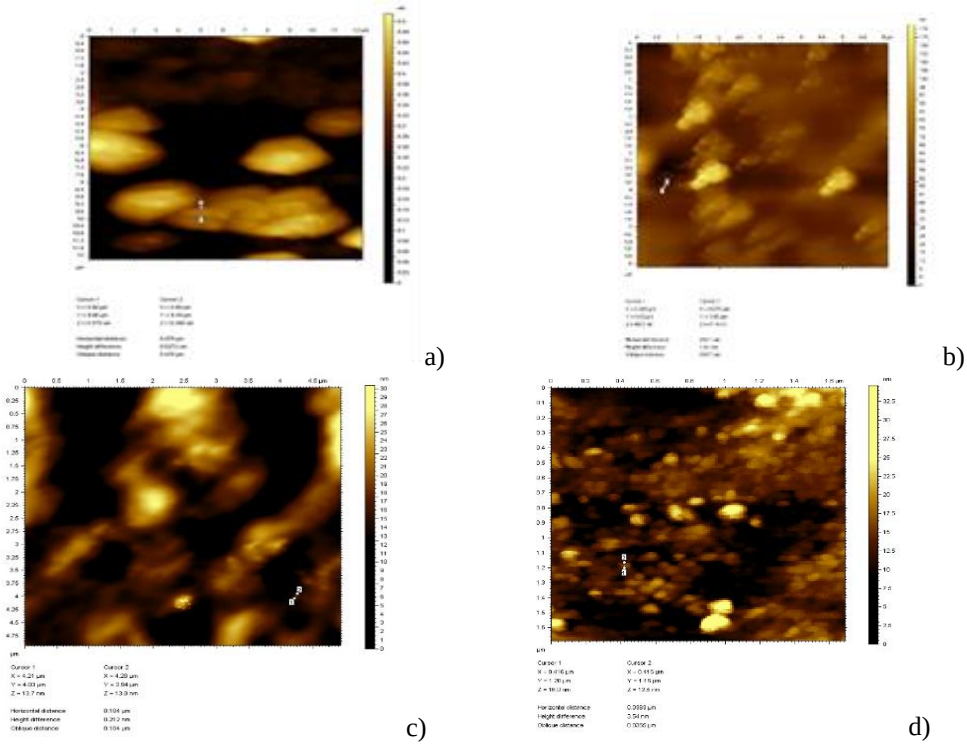

Fig. 1. AFM studies results of oligochitosan samples a, b, c, d.

To estimate the rate constant of the acid hydrolysis reaction, the depolymerization degree dependence of the process on time was plotted and the slope tangent was used to determine the reaction rate constant value for the low molecular weight formation (oligo) chitosan *Bombyx mori*, which is equal to K=4,6 × 10^{-6} g.mol / min.

The obtained X-ray diffraction chitosan patterns revealed characteristic reflexes in the ranges 2Θ=9,1-10,3; 19,9-20,0, inherent in the functional acetamide, chitosan amine groups, respectively, and indicating a decrease in reflections for the crystal structure of the chitosan crystalline regions[2]. The data obtained are shown in Figure 2.

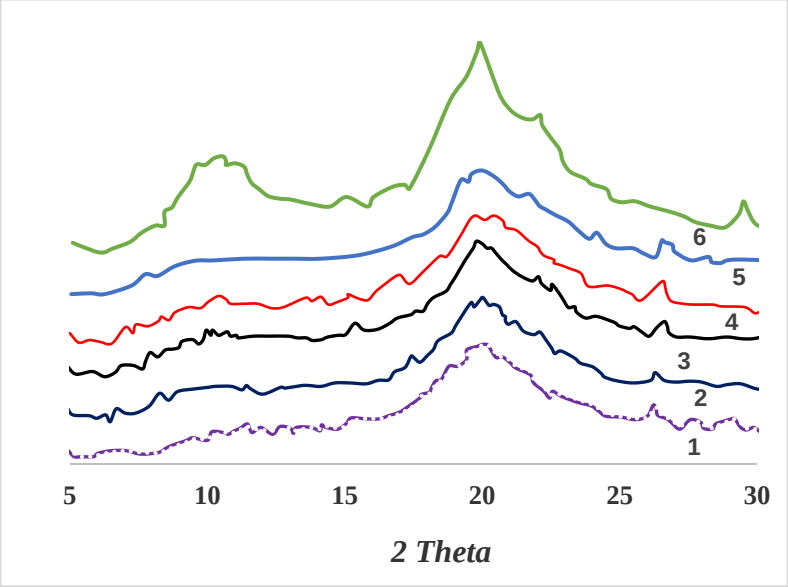

Fig. 2. RSA results of oligochitosan samples 1, 2, 3, 4, 5 and initial chitosan-6.

[2] ***The authors are grateful to Ph.D. Ashurov N.Sh. for receiving RSA data***

X-ray structural data confirm weakening of the intermolecular hydrogen bonds and the crystallinity degree, since the acidic hydrolysis of the initial chitosan leads to these bonds cleavage.

RSA results confirm that with the acid hydrolysis, a significant decrease in the crystallinity degree first occurs, which indicates a sharp decrease in the molecular weight of the initial chitosan. Then, with increasing duration, the degree of crystallinity of the samples increases slightly due to partial recrystallization of the mobile parts of chitosan macromolecules. Changes in the intensity of the identified characteristic reflections in the RSA-spectrum of low-molecular-weight chitosan in comparison with RSA of the initial chitosan indicate the formation of low molecular weight chitosan. The change in the crystallinity degree is presented in table 2.

Table 2. The crystallinity degree of low-molecular-weight chitosan samples

Sample	2θ	D, A	L, A	Degre krist. %
CH init.	10,3	8,58	39,9	53.4
	19,9	4,46	80,7	
CH -1	10,3	8,59	79,8	32.7
	19,9	4,46	80,7	
CH -2	10,5	8,59	36,3	43.7
	19,9	4,46	80,7	
CH -3	10,5	8,42	19,96	48,0
	20,0	4,43	44,85	
CH -4	10,3	8,59	26,6	41,0
	20,0	4,43	40,4	
CH -5	9,1	9,12	79,8	30.2
	20,0	4,44	80,7	

The table shows that for all samples obtained by acid hydrolysis of the initial chitosan at different durations, characteristic reflections of the crystal structure CH 2θ — from 10,3° to 20° are observed, associated with interplanar distances (100) - (111). However, there are differences in diffraction angles, crystallinity intensity and degree.

The structure of the samples was confirmed using IR spectroscopy. In order to compare the structural characteristics the IR spectra of the initial chitosans and oligochitosans have been obtained. The results are shown in Figure 3. In the IR spectrum of chitosan (Fig. 3 a), characteristic absorption bands at 1633 and 1577 cm^{-1} were found corresponding to the functional groups of acetamide (amide I) and amine. In the range of 1310–1420 $cm^{-1}$and at 2900 cm^{-1}, absorption bands of methylene groups were revealed. In the range 3200–3350 cm^{-1}, absorption bands of the hydroxyl group were found.

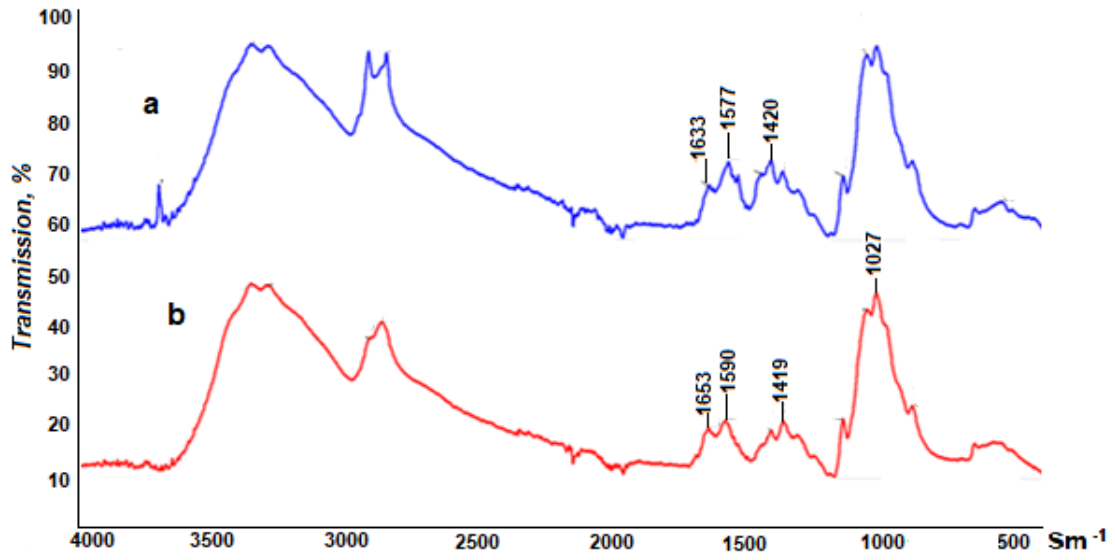

Figure 3. IR-spectra of chitosan-1 (a) and oligochitosan *Bombyx mori* (b)

In the IR - spectra of chitosan and oligochitosan (Fig. 3, a. and b), characteristic absorption bands corresponding to the amino group and acetamide groups at 1650 and 1590 cm^{-1} were found. In the range of 2900-3000 cm^{-1}, a decrease in the intensity of the absorption band of hydroxyl and methylene groups was revealed, which indicates a decrease in the intermolecular hydrogen bond, taking into account the acidic hydrolysis of the initial chitosan.

Conclusion.

Thus, the acid hydrolysis process of chitosan has been studied in 1M hydrochloric acid solution at 60 ^{0}C for 6 hours. It was shown that with an increase in the hydrolysis duration molecular weight of chitosan decreases from 190 kDa to 51 kDa, in this case, a decrease in the particle size of the obtained samples from 3 microns to 30 nanometers is observed, there is also an increase in the resulting products deacetylation degree by deacetylation of acetamide groups with hydrochloric acid. The rate constant of the acid hydrolysis reaction of chitosan *Bombyx mori*, which value is equal to $K=4{,}6 \times 10^{-6}$ g.mol/min.

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