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MACROMOLECULAR CHEMISTRY AND POLYMERIC MATERIALS

Kinetics of Base Deacetylation of Chitin and Chitosan as Influenced by Their Crystallinity

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Abstract—Structural properties of the initial and reprecipitated chitin and chitosan samples in dry and wet states were studied.

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Base hydrolysis of chitin (CTN) and chitosan (CSN) is one of the main procedures for deacetylation of these natural polysaccharides [1].

The kinetics of deacetylation of chitin and chitosan was analyzed in [2–4]. It was found that complete deacetylation of chitin and chitosan cannot be attained in a single-stage treatment of these polysaccharides with concentrated alkali. Several explanations were suggested for the decrease in the rate of chitin deacetylation in the alkaline solutions.

This paper is devoted to elucidation of the mechanism of chitin deacetylation on the basis of published data and our experimental results.

EXPERIMENTAL

Chitin and chitosan were prepared in laboratory by the known procedure [5] from shell-containing wastes from king crab processing.

Chitin and chitosan were deacetylated in a 50% NaOH solution at 100°C. Samples with various degrees of deacetylation were prepared from chitin and chitosan by repeated treatments with 50% NaOH solution at 100°C.

Prior to reprecipitation from NaOH, chitin was dissolved in alkali by the procedure described elsewhere [6]. A suspension of chitin (3 g) in a 40% aqueous solution of sodium hydroxide (75 g) was stored for 3 h at 25°C under reduced pressure. The resulting alkaline chitin was mixed with crushed ice (225 g) at approximately 0°C. The resulting chitin solution with a 10% alkali concentration was filtered through a Schott filter in a vacuum. Then, chitin was precipitated by gradual neutralization of this solution with HCl to pH 6.5. The precipitate was separated by centrifugation, and a part of the wet reprecipitated chitin was dried at 60°C.

Reprecipitation of chitin from acid solutions was performed as follows. Chitin (10 g) was mixed with cold concentrated HCl (200 ml), and the resulting mixture was stored for a day with intermittent stirring. Then, the chitin solution was filtered through a Schott filter in a vacuum. The resulting solution was diluted with cold distilled water (3 l) under stirring. The resulting suspension was kept until the precipitation was complete, and then the chitin precipitate was separated by centrifugatoin. The precipitate was washed with distilled water to pH 6.5, and a part of the resulting reprecipitated wet chitin was dried at 60°C.

Chitosan was precipitated from an acid solution. For this purpose, chitosan (2 g) was mixed with a 0.1 M HCl solution (200 ml), and the resulting mixture was kept for 18 h until the chitosan completely dissolved. The resulting solution was filtered through a Schott filter under reduced pressure and then was neutralized with stirring to pH 7.5 with a 0.5 M NaOH solution. The chitosan precipitate was separated by centrifugation and washed with distilled water. A part of the wet chitosan was dried at 60°C.

The degree of chitosan deacetylation was determined by potentiometric titration of the chitosan so-

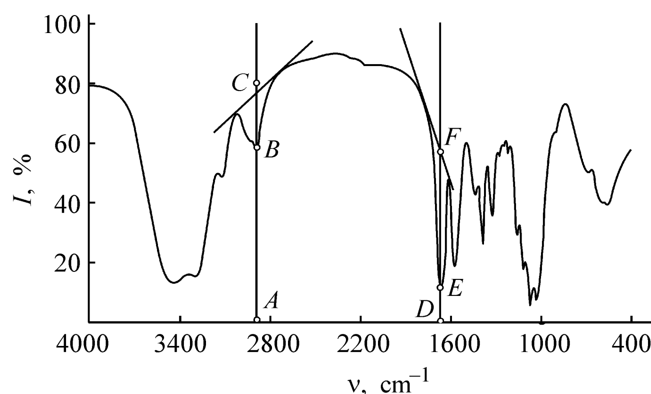

Fig. 1. IR absorption spectrum of a chitin sample. (I) Transmission and (ν) wavenumber. *Lines and points* illustrate the graphical processing of the data for DD calculation.

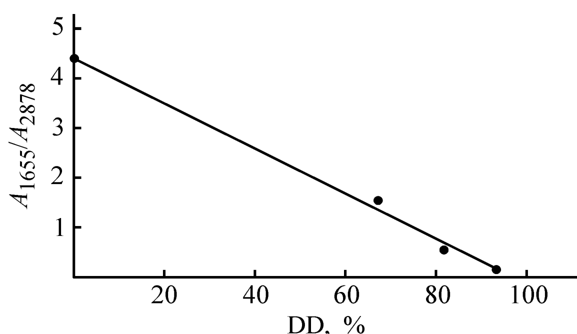

Fig. 2. Absorption of chitin in the IR spectra vs. the degree of deacetylation DD. (A_{1655}/A_{2878}) Ratio of the optical densities of the bands at 1655 and 2878 cm^{-1}.

lution with a 0.1 M HCl solution. The solution pH was measured on an Anion 4151 pH-meter (Infraspak-Analit Research and Production Enterprise, Russia). The absolute error in determining the degree of deacetylation was ±0.5%. The chitosan sample (0.2 g) weighed with an accuracy of ±0.0001g was dissolved in a 0.1 M HCl solution (20 ml), and the resulting solution was titrated with a 0.1 M NaOH solution with the resulting potentiometric titration curve plotted, pH = $f(V_{NaOH})$. Then, the points of the maxima of the first derivative, which correspond to the equivalence points in titration equivalence of excess HCl (V_1) and amino groups (V_2), were determined.

The degree of deacetylation DD (%) was calculated using the following expression:

$$DD = \frac{203.2}{42.0 + \dfrac{1000m}{c_{NaOH}(V_2 - V_1)}} \times 100,$$

where m is the content of chitosan in the sample, g; c_{NaOH}, concentration of the NaOH solution, M; $V_2 - V_1$, volume of the NaOH solution consumed in titration of the amino groups, cm^3; 203.2, molecular weight of the acetylated monomeric unit of the polysaccharide; 42.0, difference between the molecular weights of the acetylated and deacetylated monomeric units; 1000, factor of conversion of milliliters to liters; and 100, factor of conversion of DD to percents.

The degree of deacetylation of chitin and chitosan was also determined by IR spectroscopy [7, 8].

The IR spectra were recorded on an IR-420 Shimadzu spectrophotometer (Japan) in the 4000–400 cm^{-1} range using KBr pellets. The pellets were prepared using samples ground in a TI-100 mill (C.M.T. Co, Japan) for 10 min and dried to constant weight at 100°C. KBr was recrystallized from an aqueous solution and calcined at 600°C. The purity of KBr was checked by IR spectroscopy. The weight ratio of the sample and KBr in the pellet was 1 : 49. To prepare a pellet with diameter and thickness of 13 and 0.42 mm, respectively, the resulting mixture (120 mg) was compacted at a 650-kg cm^{-2} pressure for 1 min at room temperature on an SSP-1 hand press (Shimadzu, Japan).

The procedure for DD determination was improved for its use in a wide DD range. Using the samples with DD varying from 0 to 98%, we found that the IR absorption band at 2878 cm^{-1} is the most appropriate for measurements. This absorption band corresponds to the stretching vibrations of the C–H bond [8], and its optical density obeys Beer's law in the entire DD range studied. The bending vibrations of the amino group (amide I) are observed at 1655 cm^{-1}. The graphical processing of the IR spectrum is illustrated in Fig. 1.

The optical density was calculated as follows:

$$A_{2878} = \log(AC/AB),$$
$$A_{1655} = \log(DF/DE).$$

The degree of deacetylation was calculated from the calibration dependence obtained for samples with the known DD (Fig. 2). The resulting regression equation can be represented as

$$A_{1655}/A_{2878} = -0.04529\text{DD} + 4.4214,\ r^2 = 0.9946,$$

where r is the linear-regression coefficient.

The degree of crystallinity of the samples was determined by the known procedures [10, 11].

The structural properties of chitin and chitosan samples were studied at the Tananaev Institute of Chemistry and Technology of Rare Elements and Mineral Raw Materials (Kola Scientific Center, Russian Academy of Sciences) on a DRON-2 diffractom-

eter (Burevestnik Research and Production Association; Cu_{K_α}, Ni filter, 24 kV, 10 mA) in the range $2\theta = 6°–36°$. The X-ray diffraction (XRD) patterns were recorded from the initial wet samples and samples suspended in mineral oil deposited on sapphire supports. The reflections were identified using JCPDS tables [12] and published data [13].

The XRD patterns were mathematically processed using the Graph Digitizer Ver. 1.9 (Nick's Production), PeakFit Ver. 4.12 (SeaSolve Software Inc.), and Microsoft Excel 2002 program packages.

Kurita et al. [2] attributed the features of deacetylation to the crystal structure of natural chitin.

It is known that chitin is formed in nature in various crystal modifications. In most cases, it has a highly ordered structure, named α-chitin, which is typical of the majority of crustacea and insects [14].

Other modifications, such as β- and γ-chitin, are also known [15]; they differ in the mutual orientation of adjacent chitin macromolecules. Chitosan is also characterized by high crystallinity, but its structure differs from that of α-chitin [11].

As shown in [1, 4, 11], strong intermolecular hydrogen bonds should affect the rate of chemical reactions of the functional groups of macromolecules involved in formation of the crystal structure.

The degree of crystallinity, χ_{cr}, is frequently determined from the ratio of the integral intensity of the peak of the crystalline regions in the diffraction pattern to the total integral peak [10, 11].

To calculate the intensity of the peak originating from the crystalline regions in the samples, the total area under the diffraction curve S_{tot} and the area of the amorphous component S_{am} were determined graphically.

The degree of crystallinity, χ_{cr}, was calculated by the following expression:

$$\chi_{cr} = \frac{S_{tot} - S_{am}}{S_{tot}} \times 100,$$

where S_{tot} is the total area under the diffraction curve in the angle range $2\theta = 6°–36°$, and S_{am}, the area of the amorphous component in the same range.

The degrees of crystallinity evaluated by the above procedure are listed in the table.

It should be noted that the XRD patterns exhibit a series of peaks typical of the crystal structure of α-chitin, described in [12, 13].

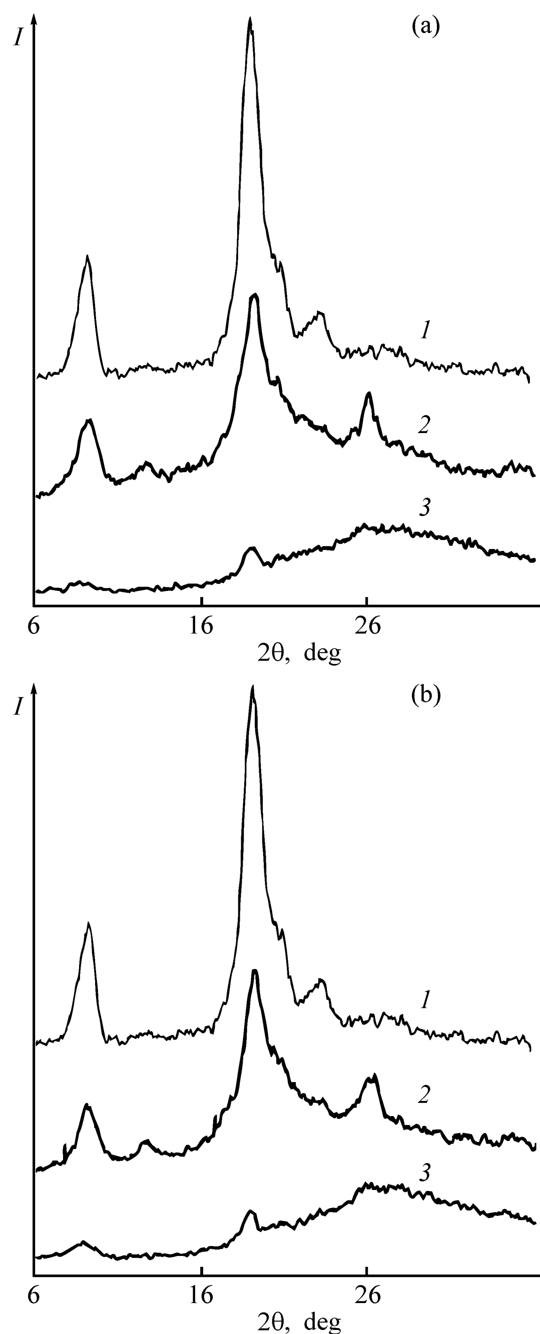

Fig. 3. XRD patterns of (*1*) initial chitin and (*2*) dry and (*3*) wet chitin reprecipitated from solutions of (a) NaOH and (b) HCl. (*I*) Peak intensity and (2θ) diffraction angle; the same for Fig. 4.

The results listed in the table show that reprecipitation virtually completely restores the crystal structures of chitin and chitosan. The diffraction peaks of the restored structures of the chitin samples recrystallized from solutions of NaOH (Fig. 3a) and HCl (Fig. 3b) are broader as compared to the initial chitin, but their positions in the XRD patterns are similar.

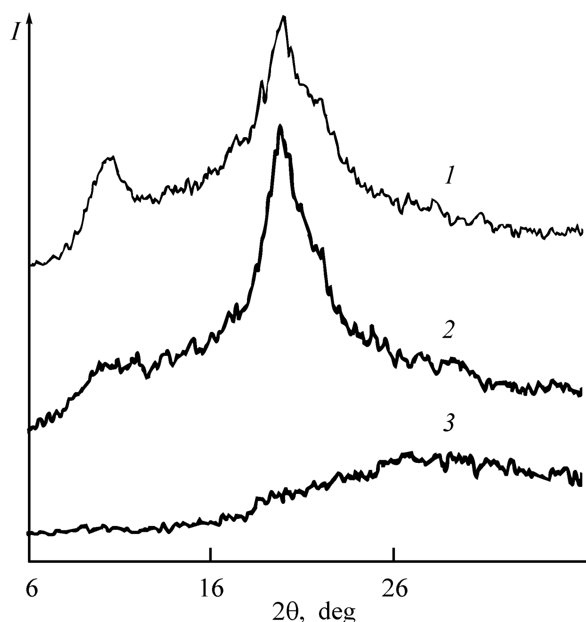

Fig. 4. XRD patterns of (*1*) initial chitosan and (*2*) dry and (*3*) wet chitosan reprecipitated from HCl solutions.

To compare the crystal structures of the chitin samples reprecipitated from acid and alkali, their XRD patterns were normalized as in [16]. It was found that dry chitin samples reprecipitated from NaOH and HCl exhibit similar XRD patterns, which suggests similarity of their structures.

Our results agree with experimental data reported in [2, 16].

It is assumed [2] that, in the course of recrystallization, the α-chitin structure is restored only partly, simultaneously with the formation of other crystal forms and amorphization.

Degree of crystallinity, χ_{cr}, of chitin and chitosan samples

Sample	χ_{cr}, %
Initial dry chitin	61.1
Chitin reprecipitated from NaOH, dry:	
from NaOH, dry	55.5
from HCl, dry	60.7
from NaOH, wet	8.8
from HCl, wet	8.5
Initial dry chitosan, DD 82%	35.8
Chitosan reprecipitated:	
from HCl, DD 82%, dry	37.5
from HCl, DD 82%, wet	3.8

As for chitosan samples, their crystal structure was also restored during reprecipitation (Fig. 4), with certain differences from the initial chitin structure. Two main peaks in the XRD pattern of chitosan are shifted as compared to α-chitin. In the XRD patterns of chitin with DD less than 71%, two peaks at 2θ 9.2° and 19.1° are observed. In the case of chitosan with DD 95%, these two peaks are shifted to 2θ 11.2° and 20.1°–20.4°, respectively. These experimental data do not contradict those reported in [2, 11, 16–18].

Partial restoration of the α-chitin structure after recrystallization and drying of the samples of α- and β-chitin was also confirmed by NMR, IR, and Raman spectroscopy [19].

On the whole, the above data suggest that the crystal structure of chitin and chitosan is restored after recrystallization of the initial sample from alkali and acid and their drying (see table).

The XRD patters of the wet chitin and chitosan samples show that they are virtually amorphous (Figs. 3 and 4, curves *3*).

It should be noted that the weak peaks observed in the XRD patterns of the wet recrystallized chitin (Figs. 3a, 3b) are absent in the XRD patterns of the wet recrystallized chitosan (Fig. 4).

It has been suggested previously [2, 20] that deacetylation of crystalline chitin proceeds more slowly than that of the amorphous compound.

Ottoy et al. [21] studied the DD distribution of various fractions of partly deacetylated chitosan exhibiting different solubilities in acid. Their experimental results suggest that deacetylation proceeds by the diffusion mechanism in which the deacetylation rate is controlled by the rate of alkali diffusion into the solid particles, rather than by the ratio of the crystalline and amorphous regions in the polysaccharide structure.

Chang et al. [22] showed that the deacetylation rate depends on the alkali concentration: as the NaOH concentration increases, the reaction rate constant becomes higher. Chang et al. suggested that the rate of heterogeneous deacetylation can be controlled by both the chemical reaction and the diffusion of the reactant and reaction products in the polysaccharide particle.

Thus, our experimental results and published data suggest that the kinetics of deacetylation of dry reprecipitated chitin and chitosan should be similar to that of the initial samples, whereas the rate of deacetylation of the wet recrystallized samples should be higher. However, our experimental kinetic de-

pendences of deacetylation do not fit in the model analyzing the effect of the degree of crystallinity of chitin on the degree of its deacetylation.

It is known that, as DD increases, the crystallinity of chitin decreases and, when DD reaches 90–95%, a new crystal structure typical of chitosan starts to form [2, 17]. In this case, the rate and degree of chitin deacetylation should increase with the initial DD owing to the rise in the content of amorphous regions. Previously, Novikov et al. have shown [23] that the rate of deacetylation of chitin and chitosan are independent of the initial degree of deacetylation of the corresponding polysaccharide. The shapes of the kinetic curves for chitin and chitosan with various initial DD (up to 95%) were similar [23, 24].

Yaghobi and Mirzadeh [25] explained the increase in the degree of deacetylation in the course of repeated alkaline treatments as follows. They suggested that the initial structure of chitin hinders penetration of NaOH molecules to all the polysaccharide molecules. When the alkaline treatment is performed continuously, the rate of deacetylation decreases at DD 75%. The authors assume that water-washing of chitin or partly deacetylated chitin after the first stage of deacetylation is of particular importance. Washing changes the chitin morphology, causing its swelling, and makes chitin macromolecules more accessible to NaOH molecules. At the same time, the authors attribute the decrease in the rate of continuous deacetylation to a decrease in the concentration of NaOH molecules from 50 to 43%, i.e., to a concentration at which, as they believe, deacetylation is terminated.

However, this explanation contradicts the experimental data. Novikov et al. have shown [26] that the NaOH concentration decreases insignificantly (from 50 to 49.4%). If the decrease in the rate of deacetylation were due to a decrease in the alkali concentration below some limit, then its increase would accelerate deacetylation. However, the rate of deacetylation does not increase in the presence of excess NaOH [26]. Probably, the deacetylation rate is determined by formation of solvated structures owing to interaction of polysaccharide macromolecules and low-molecular-weight electrolyte with the solvent.

For example, Percot et al. [27] suggested formation of NaOH hydrates, which are less active in chitin deacetylation than nonhydrated alkali molecules.

Previously, we suggested in [23, 24, 28] that OH^- complexes with chitin are formed and showed that deacetylation can be described by two parallel reactions of pseudo-first order: (1) deacetylation and (2) formation of chitin complex with hydroxide ions.

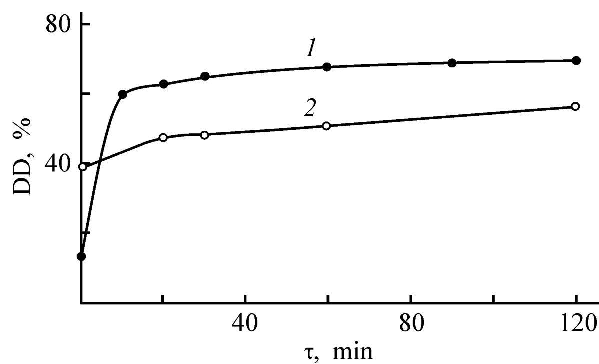

Fig. 5. Kinetic curves of deacetylation of (*1*) dry chitin and (*2*) wet chitin reprecipitated from 50% NaOH solution at 100°C. (DD) Degree of deacetylation and (τ) time.

Figure 5 shows the kinetic dependences of deacetylation of dry chitin (curve *1*) and wet chitin reprecipitated from NaOH solution (curve *2*). As can be seen, the rate of deacetylation of wet reprecipitated chitin (χ_{cr} 4.0%) is lower than that of the dry sample (χ_{cr} 59.0%). This fact suggests solvation (hydration) of polysaccharide molecules in NaOH solution. The water dipoles are located in a certain order near the acetamide and glycoside bonds of the chitin macromolecules. In accordance with the mechanism of deacetylation in the alkaline medium [29], hydrolysis of the acetamide bonds starts with a nucleophilic attack of the carbonyl carbon atom of the acetamide group by OH^- ions. The hydrate shell probably hinders this nucleophilic attack, causing a decrease in the deacetylation rate.

The above results suggest that the influence exerted by the degree of crystallinity of the samples studied on the deacetylation rate is significantly weaker than that exerted by the hydrate shell probably formed around the polysaccharide macromolecules, which hinders nucleophilic attack of the hydroxide ions and decreases the deacetylation rate.

CONCLUSIONS

(1) The IR spectrophotometric procedure for evaluating the degree of deacetylation of chitin and chitosan samples was improved.

(2) The possibility of formation of a hydrate shell around the macromolecules and its effect on the deacetylation rate were suggested.

(3) The influence exerted by the degree of crystallinity of the samples on the deacetylation rate is

weaker thanthat exerted by the hydrate shell formed around the polysaccharide macromolecules. This shell hinders nucleophilic attack of the hydroxide ions and decreases the deacetylation rate.

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