

Mechanical Activation as a Method to Obtain Water-Soluble Forms of Chitin and Chitosan in the Solid Phase

GALINA L. IVASHCHENKO^{1,3}, TATIANA P. SHAKHTSHNEIDER^{2,3}, VLADIMIR V. BOLDYREV^{2,3}, NATALIA G. BAZARNOVA¹, ANATOLY V. IVANOV⁴ and OL'GA R. GARTMAN⁴

¹Altay State University, Pr. Lenina 61, Barnaul 656099 (Russia)

E-mail: galina@solid.nsk.su

²Institute of Solid State Chemistry and Mechanochemistry, Siberian Branch of the Russian Academy of Sciences, Ul. Kutateladze 18, Novosibirsk 630128 (Russia)

³Centre for Research and Advanced Education "Molecular Design and Ecologically Safe Technologies", Ul. Pirogova 2, Novosibirsk 630090 (Russia)

⁴Altay State Medical University, Pr. Lenina 42, Barnaul 656099 (Russia)

Abstract

The paper deals with the effect of mechanical treatment on chitin and chitosan isolated from *Gammarus* (Amphipoda) of Altay. Water-soluble carboxymethyl ethers of chitin and chitosan in the solid phase were obtained using mechanochemical activation in planetary centrifugal mill SPEX 8000. The obtained samples were characterized by means of IR spectroscopy, carboxymethyl group content was determined, as well as molecular masses, solubility in water, relative viscosity of alkaline solutions.

INTRODUCTION

Mechanochemistry of organic compounds has been intensively developed during the recent years. This is especially true for pharmacy since the application of mechanochemistry allows one to modify medicines increasing their biological availability, to perform solvent-free syntheses of biologically active substances under ecologically safe conditions [1–3].

Interest to investigations of biopolymers, such as chitin and chitosan, is caused by their unique properties allowing them to be used in diverse areas of human activities, including pharmacy, cosmetics, agriculture, food industry. Chitin is a polysaccharide composed of unbranched chain of β -(1,4)-bonded 2-acetamido-2-desoxy-*D*-glucose (N-acetyl-*D*-glucosamine) groups. Three types of chitin exist in nature. They are: α -, β -, and γ -chitin, differing by the spatial positions of molecule chains and by the presence of bonded water molecules [4]. A promising derivative of chitin is chi-

tosan, which is deacetylated biopolymer (*D*-glucosamine). The presence of the amine group renders polycationic character to this polymer and broadens the possibilities to modify it, in particular, to obtain N-derivatives [5].

Poor solubility of biopolymers in water limits their application range. At present, various water-soluble forms of chitin (C) and chitosan (CT) have been obtained [5, 6]. One of the promising directions is to obtain carboxymethyl ethers of chitin (CMC) and chitosan (CMCT). The solubility of these derivatives in water, as well as conservation of biological activity characteristic of the initial compounds, broaden the possibilities of their application [7]. The majority of the existing methods of obtaining CMCT implies the use of organic solvents and is expensive. The development of a solid-phase synthesis method is interesting from the viewpoint of the intensification of processes and the creation of ecologically safe technologies for the production of these polymers.

TABLE 1

Chemical composition of dried *Gammarus*

Raw	Moisture, %	Lipids, %	Total nitrogen, %	Mineral substances, %	Chitin, %
<i>Gammarus</i> [10]	10.8	7.7	7.8	26.0	7.0
<i>Gammarus</i> of Altay	9.7	–	–	27.3	8.7

Earlier, the effect of grinding in various comminuting devices on the structure and properties of chitosan obtained from crab and shrimps shells was investigated [8, 9]. It was demonstrated that more profound changes in the structure and properties of chitosan occur during grinding in extruder than in ball mill [8]. The synthesis of the carboxymethyl ethers of chitin and chitosan was performed with the help of extruder [7]. The process was carried out in two stages; at the first stage, alkaline chitosan was obtained from chitin and sodium hydroxide; at the second stage, a mixture of alkaline chitosan with sodium monochloroacetate in solution or in the solid phase was supplied into extruder. Thus the samples with carboxymethyl group substitution extent of 0.4–1.4 were obtained; however, their solubility in water was lower (70 %) than the samples obtained by suspension method.

Among raw material sources of chitin, *Gammarus* Amphipoda crayfish is becoming the most important one. It is common in the lakes of West Siberia. Comparative evaluation of the chemical composition of *Gammarus* and such kinds of chitin-containing raw material as crabs, shrimps demonstrated reasonableness of the use of *Gammarus* to isolate chitin [10].

The goal of the present study was to perform solid-phase synthesis of the carboxymethyl ethers of chitin and chitosan isolated from *Gammarus*, using mechanical activation in high energy strain mills.

ed flakes soluble in concentrated inorganic acids; chitosan was white hard flakes soluble in diluted acids, in particular in 2 % acetic acid.

X-ray diffraction patterns were performed with a DRON-4 diffractometer with CuK_α radiation within the range $2\theta = 4\text{--}40^\circ$. To avoid the formation of texture in samples, X-ray phase analysis of chitin samples was carried out by adding X-ray amorphous substances (Vaseline, paraffine; 1 : 1 by mass). Specific surface of the samples was measured according to BET procedure (thermal desorption of argon).

Carboxymethyl ethers of chitin and chitosan were synthesized in two types of mills: AGO-2 planetary centrifugal mill (ISSC&M SB RAS) – steel drums cooled with water, drum volume: 40 ml; number of balls: 50; ball diameter: 6 mm, and vibratory-ball SPEX 8000 mill (USA) – steel drum, drum volume: 50 ml; number of balls: 15; ball diameter: 6 mm. Sodium salt of monochloroacetic acid (Na-MCAA) and sodium hydroxide, both of chemically pure reagent grade, were used as carboxymethylating agents. A mixture of chitin (chitosan), solid sodium hydroxide and alkylating agent (Na-MCAA), at a molar ratio of 1 : 1 : 1 as calculated per one OH group, was loaded into the drum. The reaction mixture was mechanically treated and sampled after definite time intervals. The products were thoroughly washed from unreacted initial compounds sequentially with ethanol acidified with acetic acid (pH 5), and pure ethanol.

EXPERIMENTAL

The composition of *Gammarus* was studied by means of the chemical analysis. Chitin was obtained from the *Gammarus* using a conventional acid-base method. Chitosan was obtained by high-temperature alkaline treatment of chitin [11]. The resulting chitin was cream-color-

TABLE 2

Chitosan characteristics

Characteristic	Mean-weight
Deacetylation extent, %	97.0
Characteristic viscosity, dl/g	26.3
M_w	1.6×10^6

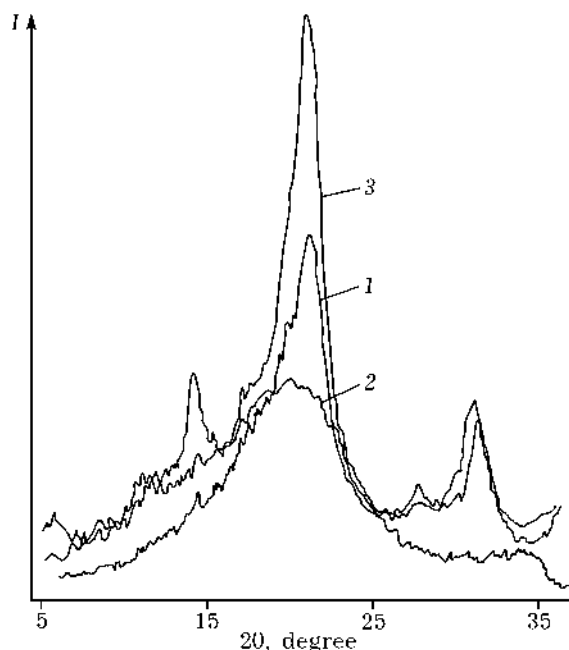


Fig. 1. X-ray diffraction patterns of chitin samples: 1 – initial, 2, 3 – mechanically activated in AGO-2 for 8 and 25 min.

Viscometric measurements of chitosan were performed in Ubbelode viscosimeter with the 0.54 mm diameter of capillary at 25 °C in 2 % acetic acid solution; 0.5 M NaOH solution was used in viscometry of carboxymethyl products. Molecular masses (M_w) of samples were calculated using the characteristic viscosity of polymer solutions according to the equation $[\eta] = 1.38 \cdot 10^{-4} M_w^{0.74}$ proposed in [12]. Chitosan deacetylation extent was determined by titration [13].

Carboxymethyl group (CMG) content was determined with the help of ANION-410 conductometer according to procedure described in [14]. The solubility of CMC and CMCT was determined by dissolving the product in water

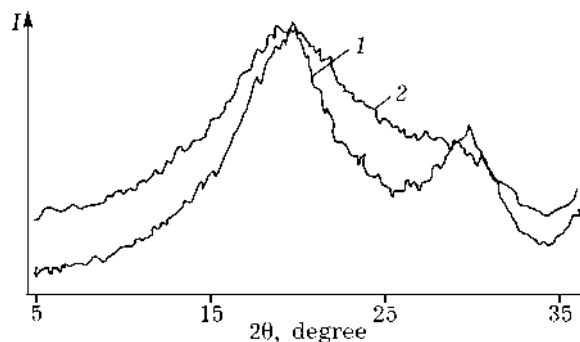


Fig. 2. X-ray diffraction patterns of chitosan samples: 1 – initial, 2 – mechanically activated in AGO-2 for 10 min.

and filtering the solution through a glass porous filter POR/60 [13].

The IR spectra of initial samples and carboxymethylated products were recorded with VECTOR 22 spectrophotometer (Bruker).

RESULTS AND DISCUSSION

It is known that chitin content of a raw material depends not only on its kind but also on the environment characteristics and the age of organisms [4]. Table 1 shows the composition of the *Gammarus* involved in the present study in comparison with literature data for the raw material of another origin.

It follows from these data that the Altay *Gammarus* is not worse than the *Gammarus* from other regions. At the same time, higher polymer content makes it a promising source for obtaining chitin and chitosan.

The parameters of the chitosan obtained in this study are listed in Table 2.

Comparison with the results available from literature [8] suggests that the chitosan obtained in the present study is high-molecular with low acetylation degree. Chitosan samples obtained under similar conditions from shrimps and crab shells had lower mean-weight molecular mass and higher acetylation extent (11–18 %) [8].

X-ray diffraction patterns of initial compounds and samples subjected to mechanical treatment are shown in Figs. 1 and 2. The presence of few broad reflections points to low-ordered type of structures with low crystallinity degree. For both chitin and chitosan, the initial material and mechanically activated one

TABLE 3

Specific surface (S_{sp}) of initial and mechanically activated samples

Sample	S_{sp} , m ² /g
Chitosan:	
initial	0.83
MA	2.63
Chitin:	
initial	7.23
MA	11.48

Note. MA – mechanical activation in AGO-2 (20 g), 15 min.

TABLE 4

Properties of carboxymethylated chitin at different synthesis duration
(C : Na-MCAA : NaOH = 1 : 1 : 1 mol)

t , min	Carboxy- methyl groups, %	Substitution degree, %	Solubility in water, %	Relative viscosity	$M_w \times 10^{-4}$
<i>AGO-2 (20 g)</i>					
2	12.2	33.5	—	—	—
5	17.1	47.0	74.3	1.55	2.43
8	19.6	53.8	81.7	1.47	0.73
10	21.7	59.6	89.5	1.42	0.56
15	24.7	63.2	91.2	1.34	0.48
<i>SPEX 8000</i>					
3	10.8	29.7	—	—	—
6	11.5	31.6	—	—	—
9	14.3	39.3	40.3	—	—
15	20.2	55.5	77.6	1.46	1.78
20	21.2	58.2	80.4	1.43	1.44

have identical sets of reflections differing only in intensities.

It is known that intense mechanical action on polymers can cause mechanical cracking of macromolecular chains and shifts of separate structural fragments [15, 16]. The result is distortion of initial packing of chains and the loss of ordering. In the case of chitosan, the X-ray diffraction patterns of mechanically activated sample exhibited decrease of intensity and increase of peak width which is charac-

teristic of the amorphous product. After short mechanical treatment for about 10–15 min, the similar phenomenon was observed with chitin sample. However, the presence of broader and more intensive maximum at $2\theta = 19.5^\circ$ for mechanically activated chitin, compared to the initial polymer, is the evidence of partial recrystallization of the amorphous phase. This effect is not a consequence of texture formation in sample, since it was also observed when recording the substance with the addition of

TABLE 5

Properties of carboxymethylated chitosan at different synthesis duration
(CT : Na-MCAA : NaOH = 1 : 1 : 1 mol)

t , min	Carboxy- methyl groups, %	Substitution degree, %	Solubility in water, %	Relative viscosity	$M_w \times 10^{-4}$
<i>AGO-2 (20 g)</i>					
5	13.1	25.2	22.3	—	—
10	24.4	47.8	90.5	1.36	0.45
15	26.4	50.9	91.4	1.30	0.24
<i>SPEX 8000</i>					
5	12.3	23.7	—	—	—
10	17.6	33.9	41.8	—	—
15	19.3	37.2	67.6	1.38	4.88
20	21.6	41.6	77.4	1.35	3.56

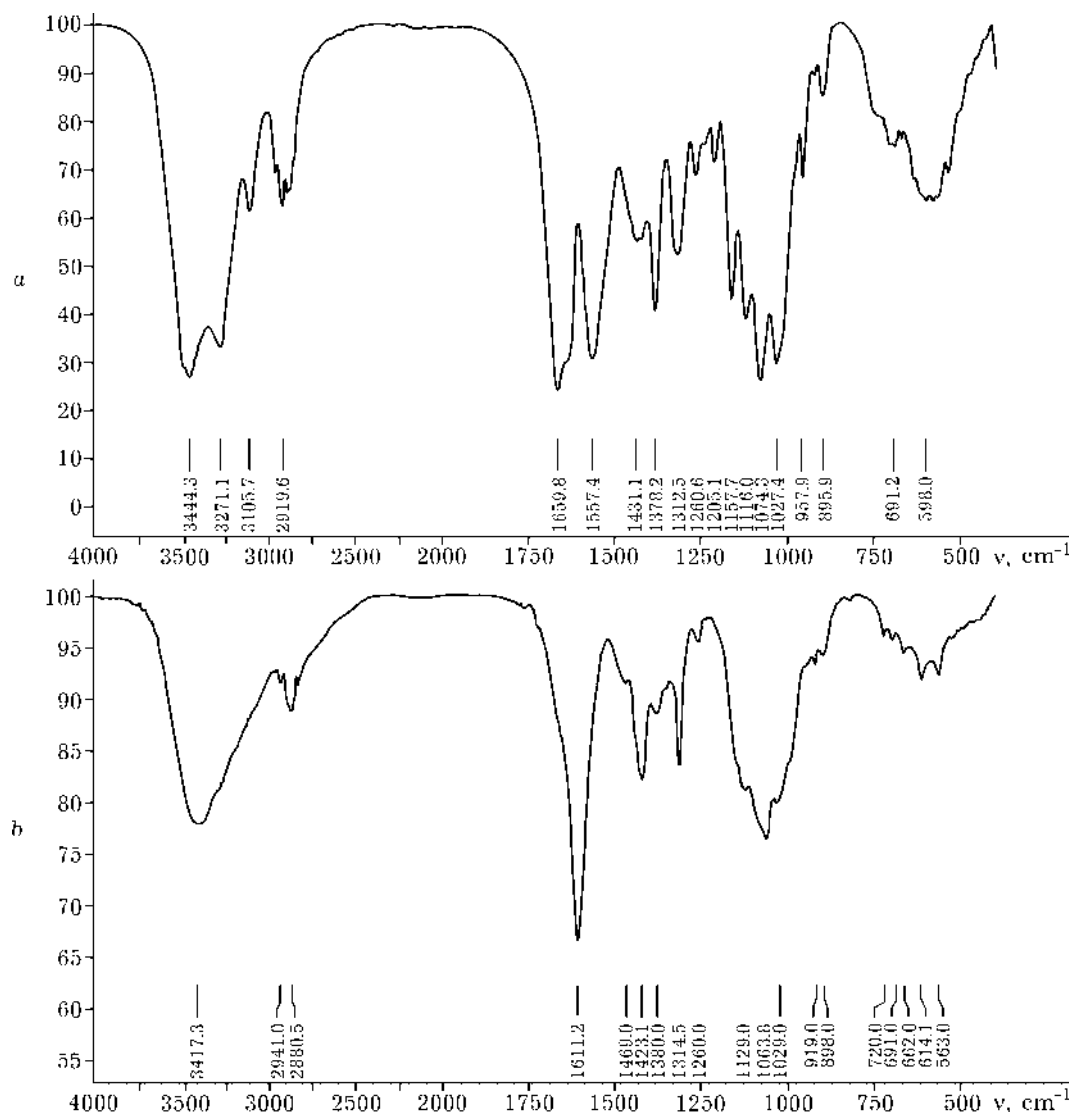


Fig. 3. IR spectra of chitin samples: *a* – initial, *b* – carboxymethylated by treatment in AGO-2 for 15 min.

X-ray amorphous Vaseline or paraffin. This allows one to assume that in course of long mechanical treatment, because of high mobility of destruction fragments with low molecular masses, the rearrangement of intra- and intermolecular hydrogen bonds occurs resulting in the formation of new packing of macromolecular chains. Recrystallization of chitin samples was observed after the treatment both in AGO-2 and in SPEX 8000. However, in planetary centrifugal mill the effect was observed after treatment for shorter time, which is likely to be explained by more intensive action of mechanical pulses. Unlike chitin, no increase of the crystallinity degree of product was observed after prolonged mechanical treatment

of chitosan. This may be explained by higher packing density of the macromolecules in the sample.

The results of measurements of specific surface of samples are shown in Table 3. Mechanical treatment of the polymers leads to the increase of the specific surface of chitin by a factor of 1.7 and chitosan by a factor of 3. Specific surface of chitosan is much smaller than that of chitin.

Tables 4, 5 show the properties of carboxymethyl ethers of chitin and chitosan obtained by mechanical activation for different time intervals. It follows from the analysis of the data that depending on the time of mechanical treatment the samples are partially

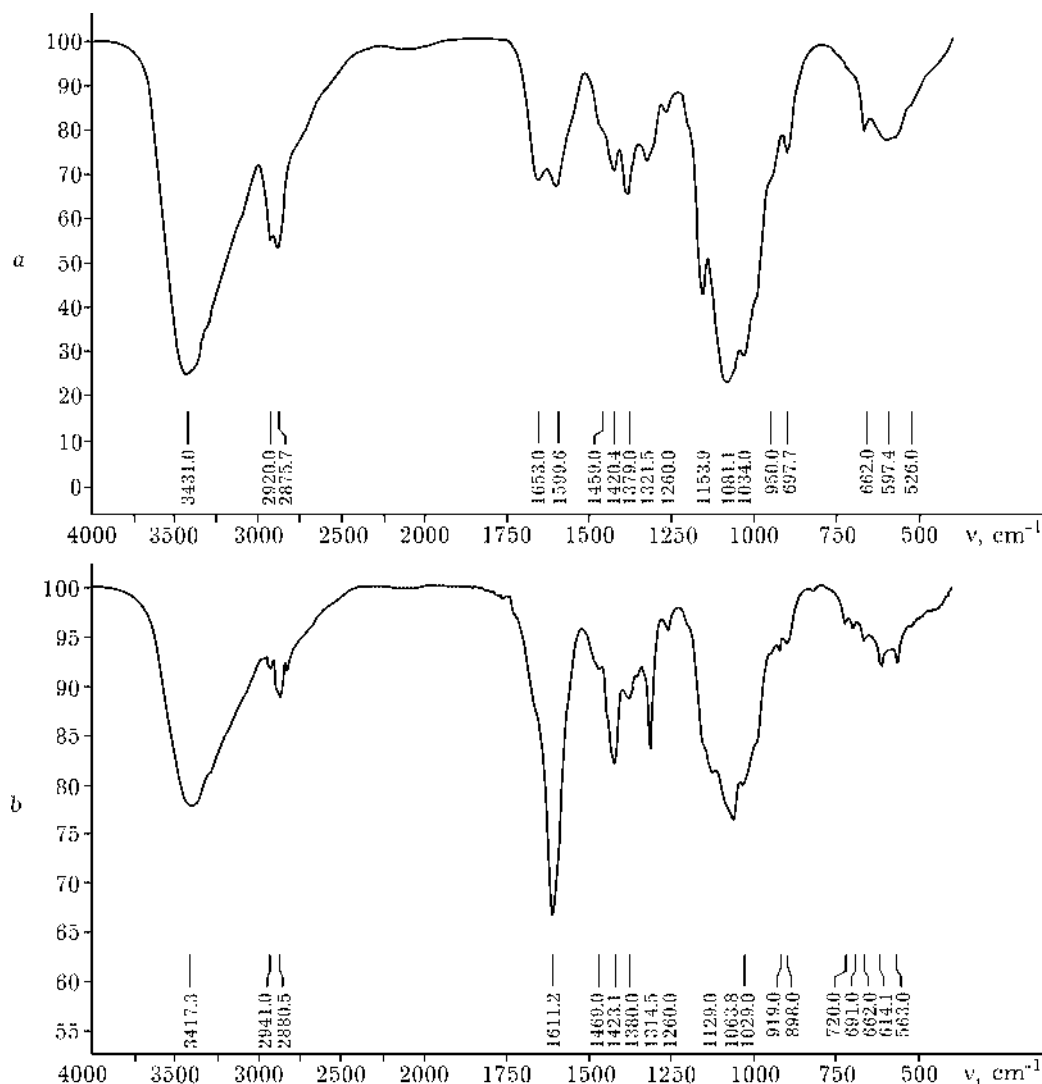


Fig. 4. IR spectra of chitosan samples: *a* – initial, *b* – carboxymethylated by treatment in AGO-2 for 15 min.

or completely soluble in water and form solutions with rather low relative viscosity. With increasing degree of mechanical activation, the degree of substitution of functional groups in chitin and chitosan increase, which causes the increase of the solubility of these derivatives in water.

The efficiencies of the substitution of functional groups in carboxymethylated samples in AGO-2 and SPEX 8000 are different from each other. Higher carboxymethyl group content in products obtained by mechanical activation in AGO-2 is likely to be connected with more intensive action of shear deformations.

It is known that nonselective carboxymethylation of chitosan results in the formation

of both O-substituted and N-substituted, or O, N-substituted derivative, depending on synthesis conditions. Correspondingly, carboxymethylation of chitin is accompanied by the formation of mono- or di-O-substituted derivative. Judging from total hydroxyl group content of chitin, which is 16.8 %, carboxymethyl ether may contain 36.4 % CMG as a maximum. The 24.7 % CMG introduced in CMC evidence that the reaction results in the product which is close to the equilibrium bi-O-substituted derivative.

Chitosan contains OH- (21.1 %) and NH₂- (9.96 %) groups; so, maximally this sample can contain 51.9 CMG (among them, 20.9 % corresponding to the substitution of only OH groups). The introduction of 24.8 % CMG points to non-

uniform substitution of functional groups in the product.

The results of the determination of molecular masses of samples point that destruction goes most intensively within the first minutes of the synthesis. Close molecular masses of the carboxymethyl ethers of chitin and chitosan obtained in AGO-2 after the synthesis for 10 and 15 min are likely to be explained by the phenomenon which is characteristic of polymer grinding, namely, destruction limit which cannot be overcome, regardless of how the treatment is carried out. The mobility of macromolecules is considered to be very high within the limiting destruction region; so, mechanical energy which is supplied to the solid is consumed for intermolecular shifts of macromolecular chains [15, 16].

IR spectroscopic studies revealed that the intensity of the band at $3445\text{--}3400\text{ cm}^{-1}$ corresponding to the stretching vibrations of OH groups is noticeably decreased while the carboxymethylation degree increases (Figs. 3, 4). The appearance of a new band at 1611 cm^{-1} in the spectra of carboxymethylated samples is connected with the vibrations of the carbonyl group in the carboxymethyl fragment. The decrease of the band intensity in the region of $3270\text{--}3300\text{ cm}^{-1}$ in CMC spectrum, corresponding to stretching vibrations of NH groups, points to partial deacetylation of chitin during mechanical activation in alkaline medium.

CONCLUSIONS

1. The possibility to use *Gammarus* of Al-tay as chitin-containing raw material is demonstrated. Chitosan obtained from *Gammarus* is more high-molecular, with the structure similar to that of the samples of biopolymer obtained for other types of raw material.

2. Solid-phase synthesis of water-soluble carboxymethyl ethers of chitin and chitosan was performed with the help of mechanical treatment in planetary-centrifugal and vibratory mills. It was demonstrated that the process performance in planetary mill is accompanied by the formation of products with the highest carboxymethyl group content, giving solutions with lower relative viscosity.

Acknowledgements

The investigation was supported by the Civilian Research and Development Foundation (CRDF) REC-008, Grant of the RFBR No. 99-03-32482 and grants from the Ministry of Education of RF.

REFERENCES

- 1 A. M. Dubinskaya, *Khim.-farm. zhurn.*, 23 (1989) 755.
- 2 A. M. Dubinskaya, *Uspekhi khimii*, 68 (1999) 708.
- 3 T. P. Shakhtshneider and V. V. Boldyrev, in E. Boldyrev and V. Boldyrev (Eds.), *Reactivity of Molecular Solids*, Wiley, Chichester, 1999, p. 271.
- 4 E. P. Feofilova, *Biol. nauki*, 6 (1981) 5.
- 5 E. A. Plisko, L. A. Nud'ga, S. N. Danilov, *Zurn. obshch. khimii*, 43 (1972) 1243.
- 6 E. A. Plisko, L. A. Nud'ga, S. N. Danilov, *Uspekhi khimii*, 46 (1977) 1470.
- 7 T. A. Akopova, C. Z. Rogovina, G. A. Vikhoreva, S. N. Zelenetsky, *Vysokomolekulyar. soyed.*, 37 (1995) 1797.
- 8 T. A. Akopova, C. Z. Rogovina, I. N. Gorbachyeva *et al.*, *Ibid.*, 38 (1996) 263.
- 9 A. I. Gamzazade, N. A. Golikov, V. P. Savchenko *et al.*, *Materialy V Mezhdunar. konf. "Novyye perspektivy v issledovanii khitina i khitozana"*, Moscow, 1997, p. 29.
- 10 R. A. Muzzarelli, *Chitin*, Pergamon Press, Oxford, 1977.
- 11 Pat. 2117673 RF, 1996.
- 12 W. Brown and D. Henly, *Makromol. Chem.*, 79 (1964) 68.
- 13 A. V. Obolenskaya, V. P. Shchegolev, E. P. Akim *et al.*, *Prakticheskiye raboty po khimii drevesiny i tsellyulozy*, Lesn. prom-st', Moscow, 1970.
- 14 Natriy-karboksimetiltsetyuloza, TU 6-55-40-90.
- 15 C. Simionescu, C. Oprea, *Mecanochimia compusilor macromoleculari*, Bucuresti, 1967.
- 16 N. K. Baramboy, *Mechanokhimiya vysokomolekulyarnykh soyedineniy*, Khimiya, Moscow, 1978.