Available online at www.sciencedirect.com
SCIENCE DIRECT
Carbohydrate Polymers 64 (2006) 184–189
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Synthesis and properties of acylated chitin and chitosan derivatives

V.A. Vasnev *, A.I. Tarasov, G.D. Markova, S.V. Vinogradova, O.G. Garkusha

Institute of Organoelement Compounds, Russian Academy of Sciences, 28, Ul. Vavilova, GSP-1, V-334, 119991 Moscow, Russia

Received 16 June 2005; received in revised form 7 November 2005; accepted 8 November 2005
Available online 30 January 2006

Abstract

Acylating of chitin and chitosan by *p*-nitrobenzoyl- and myristic chlorides was carried out by the interfacial and homofacial esterification. Chitosan salts with monocarboxylic and hydrochloric acids were obtained and the influence of the method of their isolation from aqueous solutions on their repeated water solubility was studied. The amidation of chitosan salts of carboxylic acids was carried out through the pyrolysis of films. It was found that crosslinked acryloyl- and methacryloyl derivatives of chitosan can be prepared directly from the corresponding salts.

Keywords: Chitin; Chitosan; Acylating of chitin and chitosan; Chitosan salts

1. Introduction

After cellulose chitin, highmolecular linear polymer building from *N*-acetyl-glucoseamine units connected by β(1–4)glucoseimide bonds—the most widespred naturally polysaccaride (Muzzarelli, 1977). Chitin is a component of the outward cover of arthropoda. He is found in mushrooms and lichens. Particularly plenty of chitin is contained as a horny substance in crab and lobster testae. Its yearly production constitutes milliard tons in nature. However, in contrast to cellulose, the systematic investigation of chitin and its derivatives and their practical applications have begun relatively recently.

Chitin molecules possess the high hardness and the tendency to the intermolecular association with the formation of high-orientated supermolecular structures. Difficulties related to extraction, purification and transfer of chitin to a form suitable for processing have restricted the scientific interest to this polymer. This, in turn, hampered the search for practical applications of this potentially unlimited and reproducible raw material. The discovery that chitin and a water-soluble product of chitin acetylation, chitosan, possess unique properties, such as, antibacterial, antiviral, intoxic and inallergic, high radiation resistance, the capability for the immunological protection against pathogenes, biocompatibility, biodegradability and etc. gave impetus to their study.

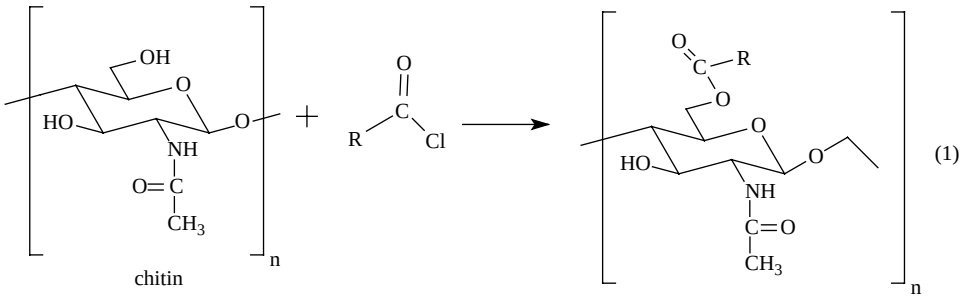
The existance in the molecules of these polysaccharides reactive hydroxyl and amino groups offer wide possibility for obtaining of new perspective polymer systemes. However, since chitin is highly crystalline and contains strong hydrogen bonds its heterophase modification presents a problem.

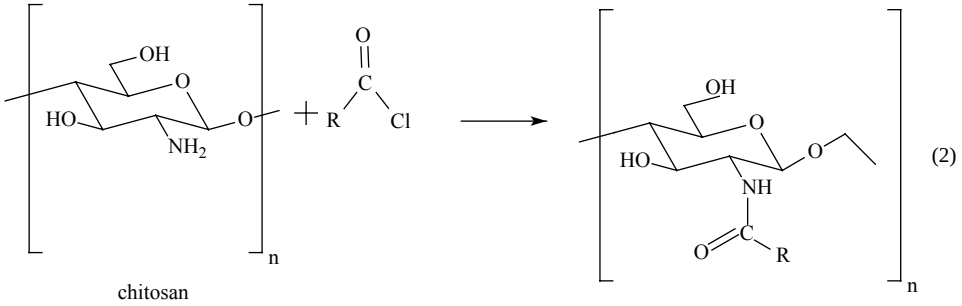
Chitosan prepared from chitin through alkaline hydrolysis is the most preferable research subject. The existence of two hydroxyl groups and one amino group in the monomer unit of chitosan and its solubility in aqueous acidic solutions offer wide possibilities for various transformations of chitosan aimed at obtaining more easily processible products or for the synthesis of new compounds with unusual properties. This approach also provides a more suitable route to preparing chitin derivatives, reconverting a modified (e.g. tosylated via OH groups at a C6 position (Kurita, Yoshino, Yokota, Ando, Inoue and Ishii, 1992)) chitosan into chitin through *N*-acetylation. Therefore, this chemical transformation of chitosan has received much attention in recent years (Xu, McCarthy, & Gross, 1996; Senso, Franco, & Oliveros, Minguillon, 2000; Zong, Kimura, Takahashi, & Yamane, 2000).

The goal of this work was to design efficient methods for acylation of chitin (1) and chitosan (2) using acid chlorides of *p*-nitrobenzoic acid (NBAC) and myristic acid (MAC)

* Corresponding author. Tel.: +7 8095 135 9212; fax: +7 8095 135 50 85.
E-mail address: vasnev@ineos.ac.ru (V.A. Vasnev).

0144-8617/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2005.11.019





where R, –p–O_2N–C_6H_4–; $CH_3(CH_2)_{12}$– as well the study of chitosan salts with monocarboxylic and hydrochloric acids.

2. Materials and methods

2.1. Materials

Chitin (the Akvatoriya Company, Russia) was vacuum dried at 130 °C; the water content was 0.97%. A dry chitosan (the Sonat Company, Russia) had $M = 79 \times 10^3$ and the acetylation degree 15% (according to the ^{1}H NMR data); the water content was 0.29%.

Chitosan on the basis of chitin from crabs ($M = 2.1 \times 10^5$ and the deacylation degree 90%) was used for obtaining chitosan salts. Aqueous solutions of salts were obtained at room temperature by adding an equimolar amount of acid (relative to amine groups) to an aqueous 3% chitosan suspension. Chitosan salts were isolated from aqueous solutions by evaporating water or precipitating by an organic precipitant and dried in vacuum at room temperature to a constant weight.

p-Nitrobenzoyl chloride (Merck) with $T_m = 74$–75 °C and hexafluoroisopropanol (HFIP) (PIM Company, Russia) with $T_b = 58$ °C were used as received.

Myristoyl chloride was obtained by treating myristic acid with excessive thionyl chloride and distilled two times in vacuum (117 °C/133 Pa); the purity of the final product was checked by elemental analysis data and ^{1}H NMR spectra.

Acrylic and methacrylic acids, pyridine, trifluoroacetic acid, acetic acid, methylene chloride, and dimethylacetamide (DMA) were purified according to conventional techniques; their parameters corresponded to the literature data.

2.2. Acylation of chitin at the solid–liquid interface

After a three-necked flask equipped with a stirrer and a capillary for feeding inert gas was purged with argon, chitin (0.5 g, 0.0025 mol) and HFIP (10 ml) containing dissolved NBAC (0.97 g, 0.0053 mol) were added. Chitin was dissolved under stirring to form a high-viscosity solution, the feeding of the inert gas was ceased, and the flask was connected to a vacuum pump. The flask was heated to 40 °C in a water bath, and HFIP was very rapidly distilled off in vacuum 2.66 kPa to yield a loose chitin mass. The vacuum pump was disconnected, the inert gas was fed again, and a dry pyridine (12 ml, 0.152 mol) was added. The as-obtained reaction mixture was allowed to stir for 2 h at room temperature. The precipitate was filtered off, washed successively with distilled water and methanol and vacuum dried at 60 °C over a period of 6 h.

2.3. Acylation of chitin in a DMA–LiCl solution

The three-necked flask equipped with the stirrer, the capillary for feeding inert gas and a reflux condenser was charged with chitin (1 g, 0.005 mol) and a 5% solution of LiCl in DMA (50 ml) was added at room temperature for 4 h. After dissolution of chitin, NBAC (2.18 g, 0.012 mol) was loaded and upon its dissolution, pyridine (6 ml, 0.076 mol) was added. The reaction was carried out for 4 h. The product was precipitated into water, filtered off, washed with distilled water and methanol and dried in vacuum at 60 °C for 6 h.

2.4. Acylation of chitosan at the liquid–liquid interface

Chitosan (1 g, 0.0062 mol) was dissolved in 2% aqueous solution of acetic acid (50 ml) in the tissiue microgrinder of the

RT-2 type. After a solution NBAC (1.4 g, 0.0077 mol, 20 ml) in methylene chloride was loaded, an aqueous solution of KOH (8 g, 0.03 mol, 20 ml) was added dropwise to the intensely stirred solution (5000 rpm) over a period of 20 min and the reaction mixture was stirred for another 40 min, KOH was added in an amount necessary for neutralization of the whole amount of acetic acid and the assumed amount of hydrochloric acid, which should release upon NBAC interaction with chitosan. The emulsion formed was destroyed by heating to 50 °C to remove methylene chloride. The precipitated *p*-nitrobenzoic acid was filtered off, and the pH of the solution adjusted to 8.5–9. The gel-like precipitate was centriifuged from solution, washed with distilled water and methanol and dried in vacuum at 60 °C for 6 h.

2.5. Homogeneous acylation of chitosan in the acetone–water system

Chitosan (0.5 g, 0.0031 mol) was dissolved in 15 ml of the aqueous solution of trifluoroacetic acid (0.2 ml, 0.0026 mol). When calculating the amount of acid, the degree of chitosan acetylation was taken into account. Acetone (40 ml) was added under intense stirring. Tertiary amine (pyridine or triethylamine (TEA) (0.034 mol) dissolved in acetone (20 ml) was added. Separately, a solution of MAC (8.3 ml, 0.031 mol) in acetone (20 ml) was prepared. In the case of TEA, acid chloride and base were simultaneously added in the reaction solution, while in the case of pyridine, pyridine was first added dropwise and a solution of acid chloride was then loaded. The reaction time was 2 h, of which 1 h was spent adding the base dissolved in acetone. The product was precipitated into water, washed with methanol and dried in vacuum at 60 °C.

2.6. Acylation of chitosan in a DMA solution

Chitosan (5 g, 0.0031 mol) was dissolved in 20 ml of aqueous solution of trifluoroacetic acid (0.2 ml, 0.0026 mol), and DMA (40 ml) was added to the intensively stirred solution. Water was distilled off in vacuum (2.26 kPa) from a mixture of solvents. Pyridine or TEA (0.0182 mol) was added to activate NH_2 groups. When pyridine was used, it was directly added to the salt form of chitosan and a solution of MAC (4.2 ml, 0.0156 mol) in DMA (25 ml) was loaded. In the case of TEA, acid chloride and base were simultaneously added dropwise. The reaction time was 2 h, of which 1 h was spent gradually adding the corresponding components. The product was dried in vacuum at 60 °C for 6 h.

2.7. Preparation of chitosan carboxylates

Carboxylic acid (0.0158 mol) was dissolved in H_2O (40 ml), and chitosan (3 g, 0.0186 mol) was added. When the complete dissolution of chitosan was achieved, the resulting solution was poured into a Petri dish and dried at room temperature for 96 h until the film was obtained. Films were dried in vacuum at 60 °C for 6 h. Pyrolysis of films cast from chitosan salts was carried out at 120–170 °C over a period of 1–6 h.

2.8. Calculation of acetylation and substitution degrees

The acetylation degree (AD) was estimated from the ^{1}H NMR (Kubota, Tatsumoto, Sano, & Toya, 2000) and IR data (Brugnerotto, Lizardi, Goycoolea, Arguelles-Monal, Desbrieres and Rinaudo, 2001). The substitution degree (SD) was determined based on the elemental analysis data (from ratios C: N, C: H and N: H) and ^{1}H NMR spectra.

2.9. Investigation techniques

IR spectra were taken on a Nicolet 750 Magna spectrometer using samples prepared as KBr pellets, and ^{1}H NMR spectra were measured on a Bruker WP-200SY spectrometer operated at 200.13 MHz. Thermomechanical studies were conducted on a UIP-70 testing machine at a constantly applied load of 100 g. The diameter was 4 mm and the heating rate was 300 °C/h. The dynamic TGA testing was performed on a MOM Q-1500D derivatograph (Hungary) at a heating rate of 5 °C/min.

3. Results and discussion

Since, chitin is highly crystalline and contains strong hydrogen bonds (Gardner & Blackwell, 1971), its heterophase modification presents a problem. We made an attempt to reduce the crystallinity degree of chitin and to make its functional groups more sterically accessible. To this end, chitin and NBAC (two hydroxyl groups of chitin per mol of NBAC) were dissolved in HFIP. The solvent was rapidly distilled off in vacuum to yield a foamed chitin in the form of thin scales containing a uniformly distributed NBAC. Pyridine was added to the as-prepared mixture. It served as both the solvent of NBAC and an acceptor-catalyst. The reaction was carried out at room temperature for 2 h.

Fig. 1 shows the IR spectrum of the acylated chitin. As is seen, the IR spectrum exhibits intense absorption bands at 1730 and 1538 cm^{-1}, assigned to ester and nitro groups, respectively; these bands are absent in the spectrum of chitin. The SD of chitin calculated from the elemental analysis data was 0.22. If a crystalline nonactivated chitin was acylated, an appreciable SD (0.10) was achieved only after the reaction mixture was heated for 6 h at 80 °C. Thus, loosening of the chitin structure markedly improves its capability for interaction with acid chloride under heterophase conditions even at room temperature.

The acylation of chitin with NBAC was accomplished in the DMA–LiCl solution in the presence of pyridine. According to IR spectroscopy, no reaction takes place if the equivalent amounts of pyridine and acid chloride are added. It appears that an appreciable amount of LiCl occurring in solution (pyridine: LiCl = 0.2 mol/mol) binds nucleophilic sites of pyridine, thereby hampering the occurrence of interaction. Indirect evidence for this assumption is provided by the fact that the hydrolysis of acetic anhydride effected in the presence

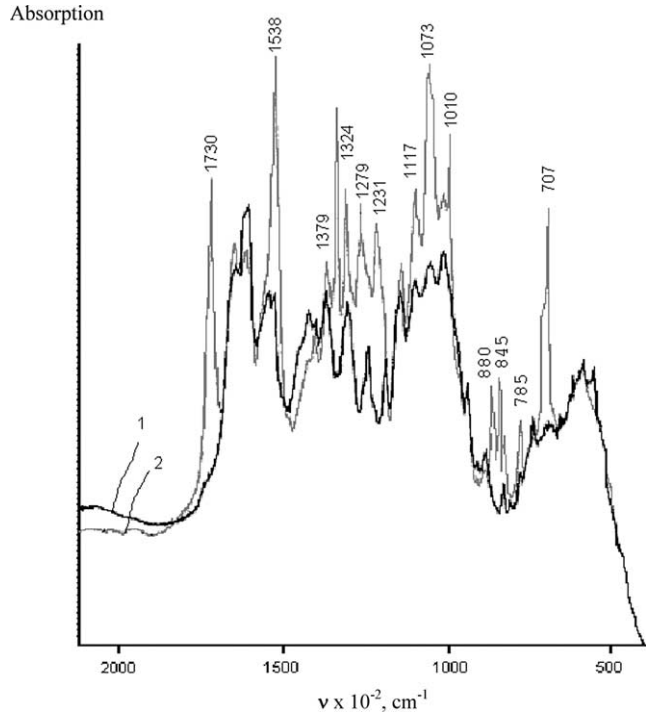


Fig. 1. IR spectra of (1) the initial chitin and (2) its *O*-(*p*-nitrobenzoyl) derivative.

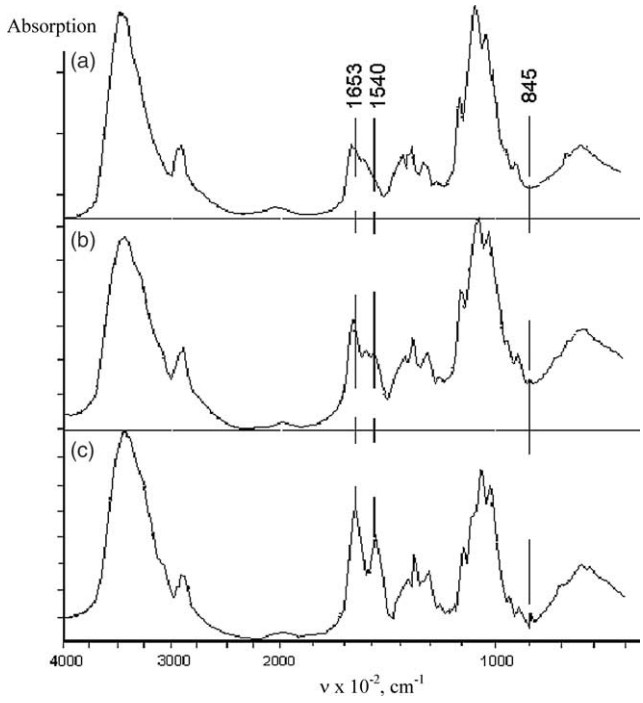


Fig. 2. IR spectra of (a) the initial chitosan and its *N*-*p*-nitrobenzoyl derivative obtained at a stirring rate of (b) 200 and (c) 5000 rpm.

of pyridine is decelerated by sodium perchlorate. (Fersht & Jencks, 1970). Therefore, we conducted acylation in the presence of an excessive pyridine, which enabled us not only to bind the evolving HC1 but also to neutralize the detrimental effect of LiCl. The IR spectrum of this product corresponded to the spectrum of the product obtained by solid-phase acylation (Fig. 1). It was found that when the reaction was accomplished at room temperature for 4 h and at the pyridine-to-LiCl molar ratio equal to 1.1: 1.0, SD can be as high as 0.4.

Chitosan is well soluble in acidic aqueous solutions. In principle, this makes it possible to acylate chitosan under homogeneous conditions; however, the occurrence of many reactions involves difficulties because of the poor solubility of acylating agents in water. In this work, *N*-acylation of chitosan with NBAC and MAC was implemented at the liquid–liquid (water–CH_2Cl_2) interface in the presence of KOH. The reaction was conducted at various stirring rates at room temperature for a period of 1 h.

Fig. 2 demonstrates the IR spectra of the starting chitosan (a) and its *p*-nitrobenzoyl derivative obtained at a stirring rate of 200 (b) and 5000 (c) rpm. As is seen, no absorption band at 1730 cm^{-1}, characteristic of an ester bond, is observed in the IR spectra. Moreover, the intensity of the Amide-1 absorption band (at 1650 cm^{-1}) increases and the absorption band at 1540 cm^{-1} due to NO_2 groups appears. With an increase in the rate of stirring, these changes become more pronounced. For stirring rates of 200 and 5000 rpm, the SD estimates based on the NMR spectra are 0.24 and 0.33–035, respectively.

The acylation of chitosan with MAC was studied in more detail. In this case, not only the rate of stirring but also the duration of reaction and the ratio of reagents were varied. As can be seen from Table 1, SD can be widely varied from 9 to 85%. If the reaction is effected in the presence of pyridine (sample 7) at a stirring rate of 200 rpm, *O*-acylation (SD = 0.26) proceeds concurrently with *N*-acylation (SD = 0.85)

One of the methods of obtaining acylated chitosan derivatives includes the thermolysis of its acylammonium salts in the solid state (Toffey & Glasser, 2001). We used this method to prepare chitosan amides derived from acids, such as acrylic, methacrylic, trifluoroacetic, acetic and myristic. Thermogravimetric studies of chitosan salts based on these acids revealed that a dramatic weight loss, which is apparently related to the degradation of chitosan, is observed even at 180 °C. Therefore, the thermolysis of salts was performed at temperatures below 180 °C. Films of chitosan salts with

Table 1
Effect of the molar ratio of MAC: chitosan and the stirring rate on the substitution degree of NH_2 groups of chitosan

Sample	MAC:chitosan molar ratio	Condition of the stirring		Substitution degree of NH_2 groups
		The rate, rpm	The reaction time, h	
	0.1	200	2	0.09
2	0.5	200	2	0.26
3	1.0	200	2	0.40
4	10	200	2	0.85
5	0.5	200	4	0.50
6	1.0	5000	2	0.85
7[a]	5	200	2	0.85

The acetylation degree of the initinal chitosan is 0.15.
[a] The process was carried out in the presence of pyridine (pyridine: MAC = 1:1); SD with respect to OH groups was 0.26.

unsaturated acids lose solubility in water even after heating for 2 h at 120 °C, which is possibly due to formation of a spatial network via double bond opening. The formation of amide bonds in the heated films of chitosan salts was monitored from their IR spectra. In the course of heating, the intensity of the Amide 1 absorption band at 1650 cm^{-1} markedly increased.

Thermomechanical curves plotted for films of chitosan salts are shown in Fig. 3 In the case of unsaturated acid derivatives, small strains (up to 10%) are observed even at 50 °C. Upon further heating to 200 °C and above, film strains remain unchanged. This observation can apparently be rationalized as being due to formation of the spatial network through polymerization via double bonds. Films of chitosan acrylates that were preliminarily heated at 160 °C for 6 h begin to buckle only at 170 °C, and even at 300 °C their deformation is as low as −10%. This finding confirms the presence of the three-dimensional network structure. Upon thermolysis, films based on chitosan salts with saturated acids show more pronounced deformations than the starting films. Thus, in the case of chitin acetate, appreciable deformations of the film begin only at 240 °C. After thermolysis, a 20% strain manifests itself even at 50 °C and achieves 50% at 200 °C.

When studying the behavior of solutions of chitosan salts in mixed solvents, we found that, upon the addition of acetone or DMA to the aqueous solution of chitosan and trifluoroacetic acid, no precipitation of salts was observed. The removal of water from a DMA–water (1:2 v/v) mixture does not cause any salt segregation. This enabled us to implement the acylation of chitosan in DMA after removal of water and in the acetone–water mixture. Table 2 illustrates the homophase acylation of the chitosan salt with MAC in the presence of various bases. As is seen, pyridine is a more efficient acceptor catalyst than TEA in both the mixed solvent(samples 1, 2) and DMA (samples 3, 4).

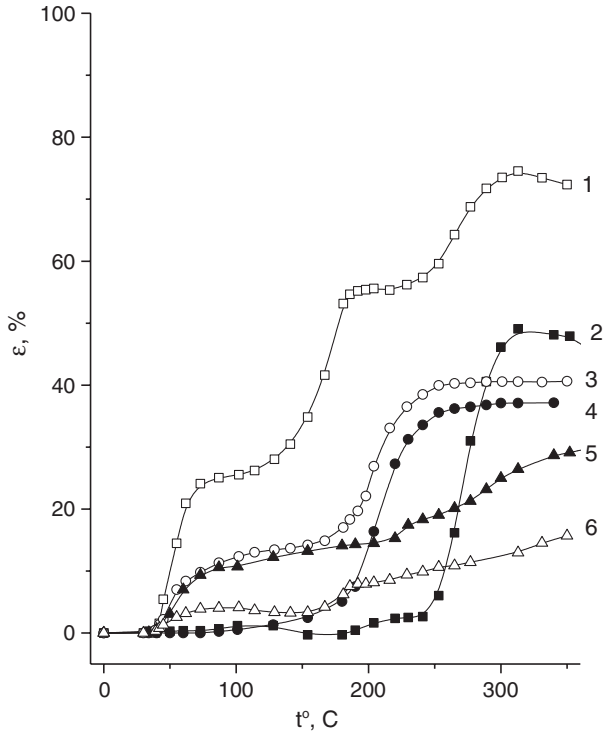


Fig. 3. Thermomechanical curves of chitosan salts with (1,2) acetic, (3,4) trifluoroacetic, and (5,6) acrylic acids (2,4,6) before and (1,3,5) after pyrolysis of film at 160 °C for 6 h.

Table 2
Conditions of acylation and SD of chitosan in reaction of chitosan trifluoroacetate with MAC

Sample	MAC: chitosan molar ratio	Solvent	Base[a]	Degree of substitution with respect to groups[b]	
				OH	NH_2
1	10	Acetone–water	Pyridine	1.0	1.70
2	5	Acetone–water	Triethylamine	0.85	0.35
3	5	DMA	Pyridine	1.0	1.40
4	5	DMA	Triethylamine	0.85	0.80

The acetylation degree of starting chitosan is 0.15.
[a] Base-to-chitosan molar ratio is 11 (sample 1) or 6 (samples 2–4).
[b] Calculated from the ^{1}H NMR spectra. If the substitution with respect to NH_2 groups is complete, SD is two.

Thus, to accompish the acceptor-catalytic esterification of chitin with carboxylic acid chlorides under mild temperature conditions, one can either activate chitin by dissolving it in hexafluoroisopropanol, followed by rapid removal of the solvent, or by conducting the reaction in the DMA–LiCl mixture in the presence of a great excess of pyridine, thereby diminishing the detrimental effect of lithium chloride.

Chitosan can be acylated to advantage under the conditions of interphase condensation in a system comprising an acidic aqueous solution of chitosan and a solution of acid chloride in organic solvent in the presence of KOH. Varying the ratios of reagents, one can widely change the substitution degree of chitosan. Using DMA or the acetone–water mixture as a solvent of chitosan trifluoroacetate, one can achieve acylation simultaneously via NH_2 and OH groups due to addition of tertiary amine. The reaction proceeds with a higher efficiency in the presence of pyridine (see Table 2). As it has been noted below, we studied also the repeated solubilities of chitosan salts in water depending on a method of their isolation from the aqueous solution. It was shown that all the studied chitosan salts with carboxylic acids and hydrochloric acid retain solubility in water after isolation from the aqueous solution through water evaporation. A more complex situation is observed when water-soluble chitosan salts are precipitated from aqueous solutions with organic solvents. In this case, either water-soluble or water-insoluble chitosan salts can be obtained depending on various factors (Table 3). Thus, after precipitation from the aqueous solution, muriatic chitosan preserves its solubility in water, whereas acetic chitosan does not dissolve further in water.

The analysis of the Table 3 shows, that the repeated solubilities of chitosan salts depend essentially on the structure and pK_a of the salt-building acid and the permittivity of the precipitant.

Table 3
The appearance of chitosan salts after their precipitation from aqueous solutions and their solubility in water Acid (pK_a) characteristics of salts after precipitation from aqueous solution

Acid (pKa)	Methanol ($\varepsilon=32.6$)	Isopropanol ($\varepsilon=17.7$)	Acetone ($\varepsilon=20.7$)	Dioxane ($\varepsilon=2.2$)
	The kind of precipitate			
	The solubility			
Hydrochloric	Gel	Gel	Fibrous	Fibrous
	Soluble	Soluble	Soluble	Soluble
Trifluoro acetic (0.23)	Does not precipitate	Gel	Gel	Gel
	–	Soluble	Soluble	Soluble[a]
2,4-Dichloro-benzoic (2.68)	Does not precipitate	Gel	Gel	Gel
	–	Soluble	Soluble	Soluble[a]
Formic (3.75)	Gel	Fibrous	Fibrous	Fibrous
	Soluble	Soluble	Soluble	Insoluble (gel)
Lactic (3.86)	Gel	Fibrous	Fibrous	Fibrous
	Soluble	Soluble	Soluble	Insoluble[b]
Benzoic (4.18)	Gel	Fibrous	Fibrous	Fibrous
	Soluble	Insoluble (gel)	Insoluble	Insoluble
Acrylic (4.26)	Gel	Fibrous	Fibrous	Fibrous
	Insoluble	Insoluble	Insoluble	Insoluble
Acetic (4.76)	Gel	Fibrous	Fibrous	Fibrous
	Insoluble	Insoluble (gel)	Insoluble	Insoluble

[a] Dissolves during 8 days.
[b] Swells insignificantly.

Undoubtedly these circumstances must be taken into account in the usage of chitosan salts as starting compounds for the synthesis of various chitosan derivatives by polymer-analogical substitution as well in the production of films, fibers etc directly from water-organic mixtures. These results are promising for using chitosan derivatives for obtaining both the water-resistant and the water-soluble articles, which are especially important from the ecological point of view. Taking into account their destruction in nature.

According to X-ray analysis, all chitosan salts obtained from aqueous solutions have an amorphous structure, irrespective of the structure of the acid and the method of salt isolation. We suppose that the observed discrepancy in the solubility of salts in water is apparently due to formation of supermolecular structures, which develop when chitosan salts are precipitated from aqueous solutions by an organic precipitant. The development of these structures depends on the degree of charge separation within chitosan salt.

4. Conclusion

The peculiarities of chemical modification of chitin and chitosan have been studied and shown. Efficient methods for acylation of chitin and chitosan by acid chlorides of *p*-nitrobenzoic and myristic acids have been developed. Chitosan salts with monocarboxylic and hydrochloric acids have been obtained and studied. The possibility of its obtaining both as water-resistant and water-soluble samples has been established.

Acknowledgements

This work was supported by the Russian Foundation for Basic Research (project N 01-03-32215).

References

Brugnerotto, J., Lizardi, J., Goycoolea, F. M., Arguelles-Monal, W., Desbrieres, J., & Rinaudo, M. (2001). An infrared investigation in relation with chitin and chitosan characterization. *Polymer*, *42*, 3569–3580.

Gardner, K. H., & Blackwell, J. (1971). The substructure of crystalline cellulose and chitin microfibrils. *Journal of Polymer Science Part B*, *36*, 327–340.

Fersht, A. R., & Jencks, W. P. (1970). The acetylpyridinium ion intermediate in pyridine-catalyzed hydrolysis and acyl transfer reactions of acetic anhydride. Observation, kinetics, structure-reactivity correlation and effects of concentrated salt solutions. *Journal of the American Chemical Society*, *92*, 5432–5442.

Kubota, N., Tatsumoto, N., Sano, T., & Toya, K. (2000). A simple preparation of half N-acetylated chitosan highly soluble in water and aqueous organic solvents. *Carbohydrate Research*, *324*, 268–274.

Kurita, K., Yoshino, H., Yokota, K., Ando, M., Inoue, S., Ishii, Sh., et al. (1992). Preparation of tosylchitins as precursors for facile chemical modifications of chitin. *Macromolecules*, *25*, 3786–3790.

Muzzarelli, R. A. A. (1977). *Chitin* (339 pp). London: Pergamon Press.

Senso, A., Franco, P., Oliveros, L., & Minguillon, C. (2000a). Characterization of doubly substitutes polysaccharide derivatives. *Carbohydrate Research*, *329*, 367–376.

Senso, A., Oliveros, L., & Minguillon, C. (2000b). Preparation of mixed 10-undecenoyl/phenylaminocarbonyl derivatives of chitosan. *Carbohydrate Research*, *328*, 617–622.

Toffey, A., & Glasser, W. G. (2001). Chitin derivatives. III. Formation of amidized homologs of chitosan. *Cellulose*, *8*, 35–47.

Xu, J., McCarthy, S. P., & Gross, R. A. (1996). Chitosan film acylation and effects on biodegradability. *Macromolecules*, *29*, 3436–3440.

Zong, Z., Kimura, Y., Takahashi, M., & Yamane, H. (2000). Characterization of chemical and solid state structures of acylated chitosans. *Polymer*, *41*, 899–906.