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Selective mono-N-2-carboxyethylation of chitosan in the presence of magnesium halides

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ABSTRACT

The solubility of chitosan in aqueous solutions of lithium and magnesium halides varies in the order: LiCl < LiBr < LiI; $MgCl_2$ < $MgBr_2$ < MgI_2. A method for selective production of mono-N-(2-carboxyethyl)chitosan (NCE-chitosan) was developed by synthesis in gel (concentration of chitosan 4–20%) of the magnesium halides solution (1.1–3.5 M) using acrylic acid. Using for reaction of MgI_2 or $MgBr_2$ provides relative greater amount of the monosubstituted amino groups (73–87%) in comparison with their absence (50–70%) at the high degree of substitution (DS) more than 0.7. DS of the prepared NCE-chitosan and the ratios mono-:disubstitution of amino groups was determined by ^{1}H NMR spectroscopy. The chemical structure of the obtained NCE-chitosan was characterized by FT-IR and ^{13}C NMR spectroscopies.

1. Introduction

Chitosan is structural analogue of cellulose and has many biochemical properties (Chirkov, 2002; Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004; Rabea, Badawy, Stevens, Smagghe, & Steurbaut, 2003). Among the carboxyalkylated derivatives of chitosan, N-(2-carboxyethyl)chitosan (NCE-chitosan) is known as the polymer also having high biological activity. It was demonstrated that NCE-chitosan has antioxidant and antimutagenic properties (Sashiwa, Yamamori, Ichinose, Sunamoto, & Aiba, 2003), antimicrobic activity (Kogan et al., 2004), biodegradability (Mincheva, Manolova, Paneva, & Rashkov, 2005), haemocompatibility and weak anticoagulant activity (Mincheva, Manolova, Paneva, & Rashkov, 2006) and can be used as drugs delivery system (Orienti, Luppi, & Zecchi, 1999). The examples of composites of NCE-chitosan with other polymers which form hydrogel with multifunctional bioactive application are known (Jiang et al., 2005; Mincheva et al., 2006; Weng, Romanov, Rooney, & Chen, 2008). Different methods of NCE-chitosan preparation are described in the literature: standard procedure in the diluted solutions by addition of acrylic acids (Sashiwa et al., 2003) or using of 3-halopropionic acids (Skorik, Gomes, Vasconcelos, & Yatluk, 2003), and more productive gel technology of chitosan transformations with the same reagents (Pestov, Skorik, Kogan, & Yatluk, 2008; Pestov, Zhuravlev, & Yatluk, 2007). Experimental simplicity of transformation proceeding, considerably smaller reaction time and greater yield of product per unit of reaction volume are the main advantages of last method. Moreover the important distinctive feature of gel technology of polymers transformations as against modifying of chitosan in the diluted solution was revealed during researches. It was shown, that proceeding of transformation in gel-state synthesis conditions allows not only functionalization of chitosan, but also simultaneous cross-linking (Pestov et al., 2007).

Due to specific reaction ability of the mentioned above reagents all methods make with exclusive selectivity the NCE-chitosan, that is a general characteristic of transformation methods both in solution and in gel. But owing to statistical features proceeding of reactions by all methods allow to prepare products with the mono-:disubstituted amino groups ratio not less 1 at DS higher 1, i.e. the statistical irregular copolymers. Any biologically active natural polymer containing the D-glucosamine (hyaluronic acid, heparin, chondroitin-sulfates) have the functional groups in strictly defined points. With this point of view, as well as from the point of view of organic chemistry, advantage of each reaction is regio- and chemical selectivity. In this paper, we present a development method for selective mono-N-2-carboxyethylation of chitosan.

2. Materials and methods

2.1. Materials

Chitosan was purchased from JSC "Sonat" (Moscow, Russia). Degree of acetylation (DA) was determined by ^{1}H NMR spectroscopy to be 0.16; while the average molecular mass of 2.5×10^5

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was established using viscometry according to Gamzazade et al. (1985). Acrylic acid, lithium and magnesium halides were purchased from Sigma–Aldrich and were used without further purification.

2.2. Instrumentation

Degree of substitution (DS) of the prepared NCE-chitosan was determined on the basis of their ^{1}H NMR spectra as previously described (Pestov et al., 2007; Skorik et al., 2003). NMR spectra were recorded in D_2O/DCl using a Bruker DRX 400 spectrometer. FT-IR spectra were recorded on a «Nicolet 6700» FT-IR spectrometer using the Smart Orbit Accessory for Single-Reflection Attenuated Total Reflectance (ATR). The solubility of the product in magnesium halides solution was evaluated by acid–base titration.

2.3. Preparation of N-(2-carboxyethyl)chitosan

The typical reaction procedure in solution was as follows: chitosan (0.66 g, corresponding to 4 mmol NH_2) was dissolved in 60 ml water containing 0.54 ml of acrylic acid (4 mmol). The solution was kept at 60 °C for 24 h, cooled, and 0.45 g of potassium hydroxide in 20 ml of water was added. The product was precipitated with acetone and dried in a vacuum at 25 °C to constant weight. DS = 0.3.

The typical reaction procedure in aqueous gel was as follows: a mixture of 3.3 g (0.02 mol) of chitosan and 2.7 g (0.04 mol) of acrylic acid in 11 ml of water was kept at room temperature until a gel formed, heated at 60 °C for 24 h, and cooled; then, 80 ml of 2.8% aqueous potassium hydroxide was added. The product was precipitated with acetone and dried in a vacuum at 25 °C to constant weight. DS = 0.96.

The typical reaction procedures in Mg^{2+}-solution gel was as follows:

(1) Chitosan 1 g was dissolved in 40 ml solution of MgI_2 preparated from 18.4 ml water and 84.4 g $MgI_2{\cdot}8H_2O$. The insoluble fraction was separated by filtration and 0.27 ml (4 mmol) acrylic acid was added to 15 ml of filtrate. The mixture was kept at 60 °C during 48 h, the product was precipitated in acetone to remove it from the excess of low molecular contaminants. Reprecipitation from solution of HCl was repeated twice then the product was filtered and dried at 25 °C in vacuum up to constant weight. DS = 0.07.
(2) Chitosan 0.6 g was dissolved in 60 ml solution of MgI_2 preparated from 27.6 ml water and 126.6 g $MgI_2{\cdot}8H_2O$. Acrylic acid 0.27 ml (4 mmol) was added to 15 ml of this mixture and it was kept at 60 °C during 48 h. The product was isolated as in previous experiment. DS = 0.12.
(3) The mixture 0.33 g (2 mmol) of chitosan, 0.27 ml (4 mmol) of acrylic acid and 1.1 ml of water containing 0.58 g (2 mmol) of $MgBr_2{\cdot}6H_2O$ was kept at room temperature until a gel formed and heated at 60 °C during 24 h. The product was isolated similarly. DS = 0.80.
(4) The mixture 0.33 g (2 mmol) of chitosan and 8.25 ml 3.5 M solution of MgI_2 was kept at room temperature during 24 h, then 0.27 ml (4 mmol) of acrylic acid was added to this mixture and heated at 60 °C during 24 h. The product was isolated similarly. DS = 0.91.
(5) The mixture 0.33 g (2 mmol) of chitosan and 8.25 ml 3.5 M solution of MgI_2 was kept at room temperature during 24 h, then 0.27 ml (4 mmol) of acrylic acid was added to this mixture and heated at 90 °C during 200 h. The product was isolated similarly. DS = 1.25.

3. Results and discussion

3.1. Solubility of chitosan in solutions of lithium and magnesium halides

It is known, that cellulose and chitin are capable to pass in a plastic state or to form a colloid solutions in concentrated aqueous solutions of some alkaline and alkaline earth metals halides. Chitosan with a high degree of acetylation (DA = 0.8) is capable to colloid dissolution, for example, in methanol solutions of calcium or magnesium chloride (Tokura & Seo, 1994), solution of lithium chloride in *N*,*N*-dimethylacetamide (Shigemasa et al., 1999). But the regular data for chitosan on the given phenomenon in the literature are not present.

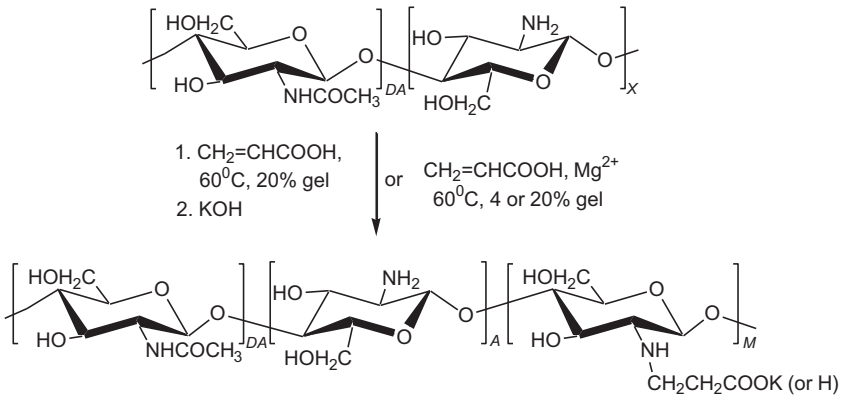
We studied experimentally an ability of chitosan with a low DA to dissolve under the same conditions, which were used for cellulose and chitin. Comparison amount of chitosan (Table 1), which was dissolved in the solution of lithium halides, shows that ability of chitosan salt to dissolve increases in the order LiCl < LiBr < LiI, as was established for cellulose and chitin. The solubility data of chitosan in solutions of magnesium halides are shown in Table 2. The solubility increases in the order $MgCl_2$ < $MgBr_2$ < MgI_2. It should be noted that only an insignificant part of chitosan is dissolved homogeneously, other weight of polymer is in the swollen state (gel). The structural analysis by ^{1}H NMR spectroscopy of the dissolved and not dissolved parts of chitosan showed that DA was constant. Thus, the essentially influencing factor on ability of chitosan to be dissolved is a molecular weight and molecular-mass distribution. It should be noted the tendency to form chitosan gels at high polymer concentrations. In contrast gels formed from solutions of chitosan in acid in which degradation of a polymers chain proceeds, the systems

Table 1
Solubility of chitosan in water solutions of lithium halides.

Salt	Concentration of salts solution, mol/l	Concentration of chitosan, %	
		Calculated	Actual
LiI	6.0	1	0
		2.5	0.7
		5	Gel
	4.8	5	0.72
	3.7	5	0.55
	2.7	5	0.19
LiBr	17.3	2.5	0.29
		5	Gel
LiCl	13.7	5	0.1

Table 2
Solubility of chitosan in water solutions of magnesium halides.

Salt	Concentration of salts solution, mol/l	Concentration of chitosan, %	
		Calculated	Actual
MgI_2	2.0	1	0.18
		2.5	0.35
		5	0.90
	3.5	1	0.74
		2.5	1.00
		5	1.26
$MgBr_2$	3.3	1	0.10
		2.5	0.21
		5	0.42
	4.2	1	0.15
		2.5	0.25
		5	0.68
$MgCl_2$	4.8	1	0.07
		2.5	0.20
		5	0.34



Scheme 1. 2-Carboxyethylation of chitosan by using acrylic acid under various condition (Table 1); *DA*, *X*, *A* and *M* are the molar fractions of appropriate glucosamine units.

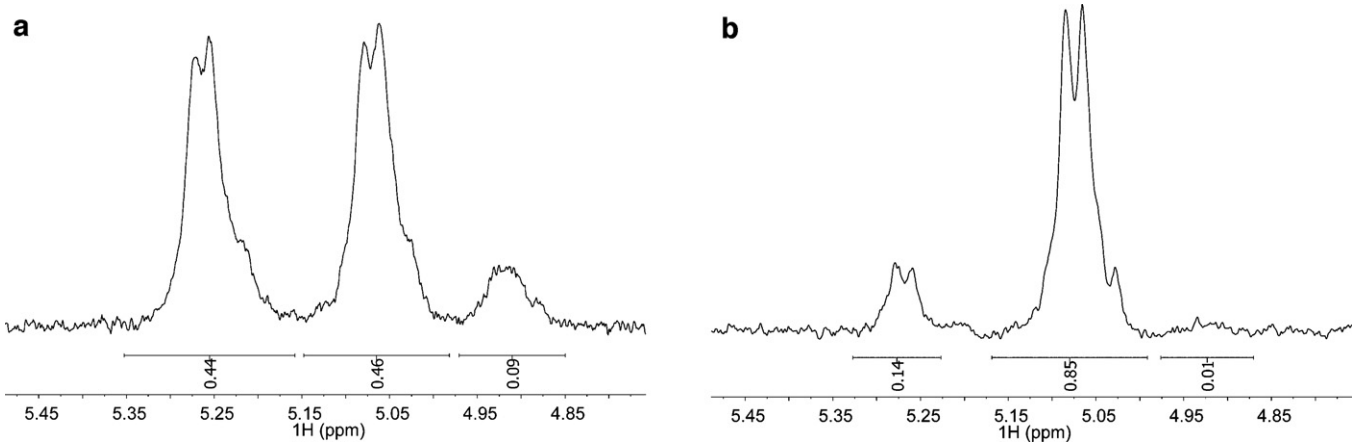


Fig. 1. The ^{1}H NMR spectrum (400 MHz, δ, ppm) fragment of *H*-1 atoms of NCE-chitosan obtained (a) in gel on the basis of acrylic acid solution (Table 3, N 12) and (b) on the basis of MgI_2 solution (Table 3, N 8) in D_2O/DCl solution at 70 °C: 4.92 (d, *H*-1 of $GlcNH_2$), 5.07 (d, *H*-1 of GlcNHR), 5.26 (d, *H*-1 of $GlcNR_2$).

on a basis of lithium or magnesium halides solutions are not acidic (pH ≥ 7). This allows them to be successfully used, for example, for a further transformations without any degradation of polymer.

3.2. Mono-N-2-carboxyethylation of chitosan

The problem of monoaddition acrylic acids to monomeric compounds is solved by using of amine excess and subsequent separation its from a reaction product (Salov, Zil'berman, & Krasnov, 1985). For the polymers such operation is impossible, since lead to the addition of only part of a reagent to a polymeric chain, i.e. to a low DS. Existence of an accessible way of preparation of monoaddition products does not stimulate the search for reagents providing monoaddition at equivalent quantity of reaction components. As the greatest solubility was established for MgI_2 in present research, the addition of acrylic acid to chitosan was carried out in solutions of $MgBr_2$ and MgI_2 according to Scheme 1.

The obtained products were soluble in water and solutions of acid and alkali. The chemical structure of the obtained NCE-chitosan was determined by elemental analysis, FT-IR and ^{1}H NMR

Table 3
N-2-carboxyethylation of chitosan, 60 °C, 48 h.

N	Conditions of reaction			Characteristic of product	
	Solvent	Concentration of chitosan, %	Molar ratio acrylic acid:chitosan	DS	Molar ratio mono-:disubstituted amino groups
1	3.5 M MgI_2	1	1	0.07	0.07:0
2[a]	3.5 M MgI_2	1	1	0.12	0.12:0
3	3.5 M MgI_2	1	5	0.16	0.16:0
4[a]	3.5 M MgI_2	1	5	0.13	0.13:0
5[b]	H_2O	1	2	0.31	0.31:0
6	8 M LiI	5.5–gel	2	0.21	0.21:0
7	3.5 M MgI_2	4–gel	2	0.19	0.19:0
8[c]	3.5 M MgI_2	4–gel	2	1.25	3:1
9[b]	3.5 M MgI_2	20–gel	2	0.91	3.1:1
10[b]	1.1 M $MgBr_2$	20–gel	2	0.80	4.2:1
11[b]	2.2 M $MgBr_2$	20–gel	2	0.69	3.3:1
12	H_2O	20–gel	5	1.32	1:1
13[b]	H_2O	20–gel	2	0.96	2.4:1

[a] It was used dispersed chitosan.
[b] Time of reaction 24 h.
[c] 90 °C, 200 h.

spectroscopies. Using ^{1}H NMR spectroscopy data, we have concluded that addition of acrylic acid to chitosan yields NCE-chitosan only as in the methods of preparation NCE-chitosan described earlier (Pestov et al., 2008, 2007; Sashiwa et al., 2003; Skorik et al., 2003). Various chemical shift of *H*-1 atom of the D-glucosamine residue depending from character of amino group substitution allows calculating exactly the mono-:disubstituted amino groups ratio (Fig. 1a and b). The reaction conditions and the characteristic of the obtained products are shown in Table 3.

The mechanism of dissolution and swelling of chitosan in solution of MgI_2 is most probably due to the complex formation between magnesium ion and functional groups of chitosan (Scheme 2) (Varma, Deshpande, & Kennedy, 2004).

Indeed, the addition of acrylic acid to chitosan in the presence of MgI_2 gave products with a low DS (Table 3, N 1–4 and 5). On the other hand, proceeding of the same reaction for a dispersed chitosan in solution of MgI_2 (Table 3, N 2 and 4) resulted in higher DS. Consequently, the magnesium ions forming complexes with amino groups of chitosan hamper of addition reaction due to significantly decreasing of nucleophility of amino groups. Using the reaction mixture as gel (Table 3, N 6–14) in comparison with the mixture as solution (Table 3, N 1–5) allows considerably to increase DS that correspond to the gel-effect, which was investigated by us recently for gels of chitosan in solutions of acrylic and halopropionic acids (Pestov et al., 2008, 2007). Decreasing a rate of addition because of complex formation is observed in gels as well as in solutions (Table 3, N 10 and 11 – consecutive increasing the amount of $MgBr_2$ decreases a degree of addition). Presence of LiI influences the value of DS to a smaller extent than presence of MgI_2 (Table 3, N 6 and 7), that is caused a smaller complex formation ability of lithium ion compared with magnesium ion. Replacement MgI_2 on $MgBr_2$ decreases a DS (Table 3, N 9, 10 and 11) because of the decreasing of dissolving ability of appropriate salt. The increasing of temperature, reaction time and gel concentration promotes increasing of DS (Table 3, N 8, 7 and 9), that is provided by the known factors influencing on rate of reaction.

The ratio between mono- and disubstituted amino groups of glucosamine units at low degree of substitution ($DS < 0.5$) is comparable with the same ratio in the presence of the used salts. However, at the high degree of substitution ($0.5 < DS \sim 1$) using of MgI_2 or $MgBr_2$ provides relative greater amount of the monosubstituted amino groups (Table 3, 73–87% by using of salts – N 8–11 and 50–70% in their absence – N 12–13). Thus, it is necessary to conclude, that using of MgI_2 or $MgBr_2$ hamper of addition process of acrylic acids to amino groups of chitosan. However, addition of the first molecule of acid is slowed less than the second molecule, that provides increasing of monoaddition selectivity. Using the FT-IR spectroscopy for characteristic of preparated products was shown, that distinctions between selectively monosubstituted product and polymer with equal amount of mono- and disubstituted amino groups of glucosamine units are shown obviously enough on reflection spectra in region of carbonyl group absorption (Fig. 2). Distinctions consist not only of different ratio absorption band of carboxyl (at 1706, 1710, 1723 cm^{-1}) and amide (at 1631, 1620 cm^{-1}) groups, but also of absorption bands value of carboxyl groups.

The analysis of structure of obtained derivatives using ^{13}C NMR spectroscopy has shown feature of reaction proceeding depending on composition of reaction gel. The ^{13}C NMR spectrum of product (Fig. 3) obtained by modifying of chitosan in gel on basis of acrylic acids solution showed as expected chemical shifts of carbonyl atoms of carboxyl (183 ppm) and acetamide groups (175 ppm) as the signal at 166 ppm corresponding to carbonyl atoms of propionamide group. This group appears parallel during reaction of addition of acrylic acids, forming cross-linked structure of polymer. The subsequent alkaline hydrolysis of thus obtained NCE-chitosan

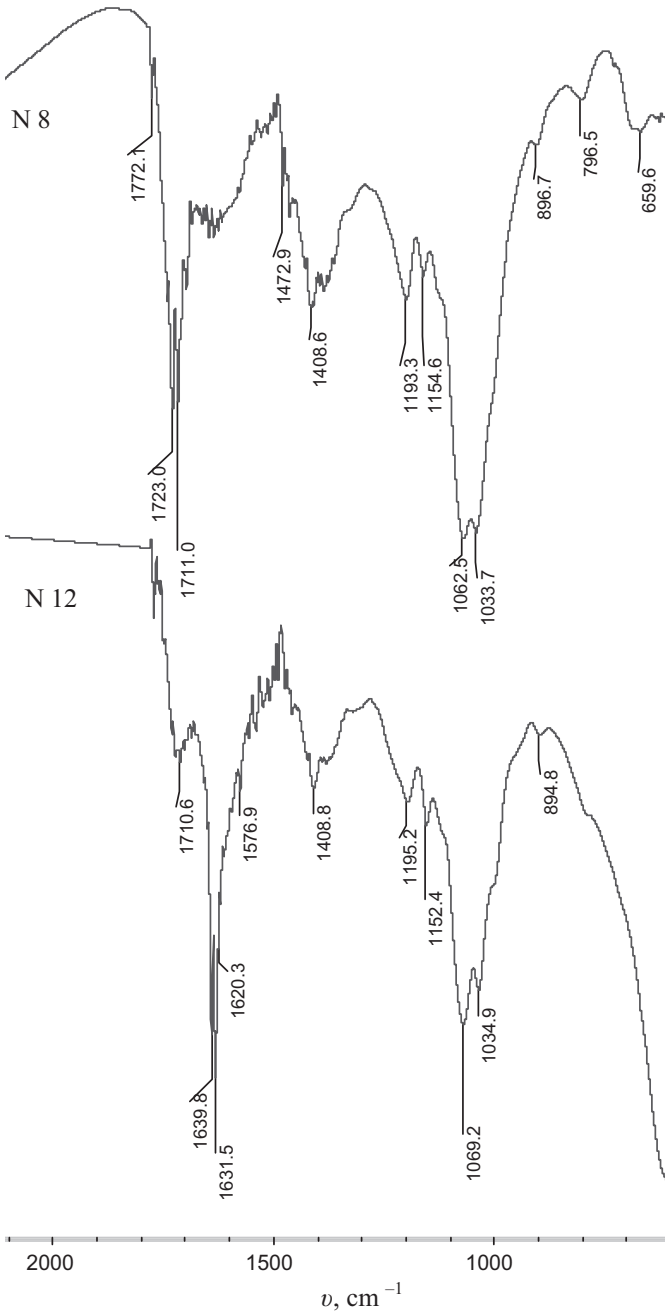
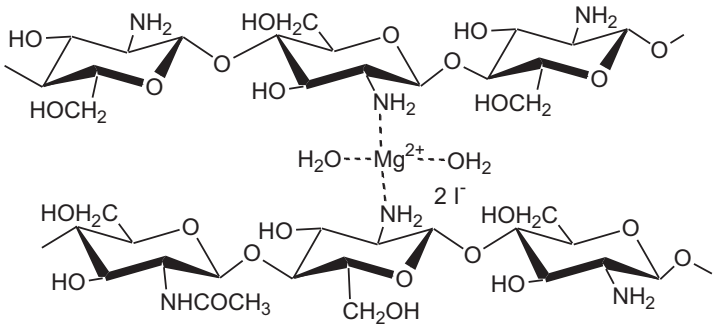


Fig. 2. FT-IR spectra of NCE-chitosan: selective monosubstituted product (N 8) and polymer with mono-:disubstituted amino groups ratio 1:1 (N 12).

results in disappearance of given signal (Pestov, Kogan, Skorik, Yatluk, & Pajtinka, 2006). In the ^{13}C NMR spectrum of product modified in gel on the basis of MgI_2 solution such signal (166 ppm) is not observed, that testifies to other character of addition, namely, about significant decrease of reaction ability of amino group in reactions of Michael addition and addition–elimination with formation of amide due to its coordination with ions of magnesium.

Thus, it is necessary to assume the mechanism of addition reaction, which includes a stage of coordination the nucleophilic and electrophilic centres by magnesium ion, as to a matrix (Scheme 3).

The chelate ring formed as result of reaction impedes sterically of addition the second molecule of acrylic acids. However, as the magnesium metallocentre has weak ability to complex formation and MgI_2 or $MgBr_2$ are dissolved in acetone distinct from NCE-



Scheme 2. Magnesium ion coordination mode of amine groups of chitosan.

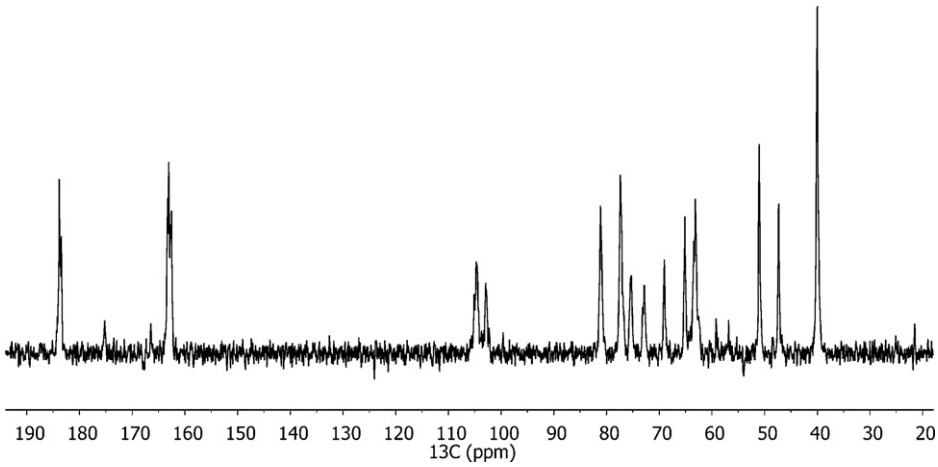
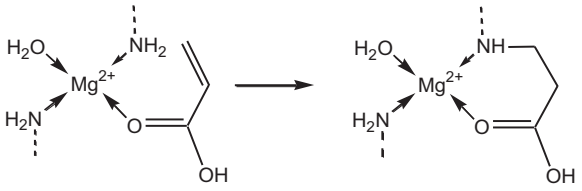


Fig. 3. The 100 MHz ^{13}C NMR spectrum of NCE-chitosan obtained in gel on the basis of acrylic acid solution (Table 3, N 8) in D_2O/DCl solution at 70 °C: (δ, ppm) 22 ($NHCOCH_3$), 40 (CH_2COOH of $GlcNR_2$ and GlcNHR), 47 (NCH_2 of GlcNHR), 51 (NCH_2 of $GlcNR_2$), 63–69 (*C*-6, *C*-2), 72–82 (*C*-3, *C*-4, *C*-5), 102–105 (*C*-1), 166 ($NHCOCH_2$), 175 ($NHCOCH_3$), 183 (COOH).



Scheme 3. Probable mechanism of 2-carboxyethylation of chitosan by acrylic acid in the presence Mg^{2+}, including coordination stage of carboxyl and amine groups by magnesium ion.

chitosan, problems with isolation and purification of polymeric product do not arise.

4. Conclusion

It has been demonstrated that a novel process modification of chitosan – gel-state synthesis – can provide higher DS and increase yield in a shorter reaction time than similar reactions carried out in true solutions. Some results show that the process of transformation is accompanied by the cross-linking reaction with formation of the amide bonds. Using the established facts of gel formation of chitosan in the aqueous solutions of MgI_2 or $MgBr_2$ it has been shown that such systems also can serve as the reaction mixture for carboxyethylation of chitosan by acrylic acid. Therefore it can be concluded that chitosan modification using the gels on the basis of salts (lithium and magnesium halides) solutions allow to prepare products with other structure than by using acidic gels or solutions of chitosan. Owing to statistical features proceeding reaction of acrylic acid addition by all methods (in solution and in gel) allow to prepare products with the mono-:disubstituted amino groups ratio not less 1 at DS higher then 1, i.e. the statistical irregular copolymers. We have established that realization of reaction in the gel of chitosan on the basis of solutions of MgI_2 or $MgBr_2$ allows to prepare selective monosubstituted product at high DS. Thus, parallel cross-linking reaction does not proceed.

It is known that bioactivity of carboxymethylchitosan against fungi strongly depends both on a ratio mono-:disubstitution of amino groups (Liu, Guan, Yang, Li, & Yao, 2001). We believe that the present researches should be taken into account at comparison of biological activity of NCE-chitosan with equal DS, but a different ratio mono-:disubstitution of amino groups of glucosamine units. Generally, the results of the present work show the first example of selective monocarboxyethylation of chitosan by acrylic acid in the presence of magnesium halides.

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