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Carboxyalkylation of chitosan in the gel state

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ABSTRACT

This study presents a new approach for direct carboxyalkylation of chitosan in the gel state by using aza-Michael addition and substitution reactions. Various reagents were applied including acrylic and crotonic acids, and α-, β-, γ-, δ-, and ε-halocarboxylic acids. The reaction of chitosan with γ- and δ-halocarboxylic acids showed no target product formation either in solution or in the gel state. In the case of acrylic, crotonic, α- and β-halocarboxylic acids, the reaction performed in the gel state (concentration of chitosan 20–40%) shows higher degree of substitution at lower reaction time and temperature than in diluted solutions (concentration of chitosan 0.5–2%). The results were discussed in terms of kinetics of the target and side reactions. ^{1}H and ^{13}C NMR confirmed that in all cases the carboxyalkylation of chitosan proceeds exclusively at the amino groups.

1. Introduction

Among naturally occurring polysaccharides, considerable attention has recently been focused on the polyaminosaccharide chitosan, the deacetylated derivative of chitin that is easily obtained at relatively low cost (Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004; Kurita, 2001; Rinaudo, 2006). A number of studies of chitin and chitosan have been aimed at chemical modification of the polymer with hydrophilic groups to improve its solubility across the whole pH range. Carboxyalkyl groups represent useful hydrophilic substituents, as their ionization in the alkaline range improves the polymer solubility and also impart useful new chemical properties. Carboxyalkylation is traditionally performed under heterogeneous or homogeneous conditions by using the reactions of nucleophilic substitution, addition, and addition–elimination with subsequent reduction, in which chitosan acts as a polymer nucleophile (Jayakumar et al., 2010; Pestov & Yatluk, 2007).

The only carboxyalkylated chitosan that have been prepared by nucleophilic addition is (2-carboxyethyl)chitosan. A great variety of acrylic acid derivatives have been used for its preparation (Aoi et al., 2000; Dong, Yuan, Wu, & Wang, 2000; Lee, Shin, & Noh, 1991; Loubaki, Sicsic, & Legoffic, 1989; Nudga, Plisko, & Danilov, 1975; Sashiwa, Kawasaki, et al., 2003; Sashiwa, Shigemasa, & Roy, 2000; Sashiwa, Yamamori, Ichinose, Sunamoto, & Aiba, 2003b; Tokura, Nishi, Nishimura, & Ikeuchi, 1983). The lowest DS was evident if the acrylic acid was used (Sashiwa, Yamamori, Ichinose, Sunamoto, & Aiba, 2003a). Acrylic acid is believed to have a very low reactivity with amines because the formed ammonium salt considerably impairs the nucleophilic properties of the amine. Thus, acrylic acid, which is a weak electrophile compared to its esters, cannot participate fully in the Michael addition reaction. However, this opinion is erroneous, since many investigations have demonstrated that a direct addition of this kind is possible even with weakly nucleophilic aromatic amines (Farahani, Antonucci, & Karam, 1998; Skorik, 2012; Skorik, Romanenko, Gomes, Neudachina, & Vshivkov, 2002) because of the equilibrium between the amine and the ammonium salt. In the case of chitosan, the reaction with acrylic acid leads to the formation of N-carboxyethylated derivative (Sashiwa, Yamamori, Ichinose, Sunamoto, & Aiba, 2003a).

Another direct method for the preparation of carboxyalkylated chitosan derivatives is the nucleophilic substitution reaction, in which chitosan serves as a polymer substrate and the corresponding halocarboxylic acid acts as reagent. This method allows for the preparation of products of different structures as a function of the pH of the reaction medium. An alkaline medium is used in a heterogeneous reaction, ensuring synthesis of an N,O-substituted product (Chen, Tian, & Du, 2004; Gladyshev et al., 1990; Liu, Guan, Yang, Li, & De Yao, 2001; Rinaudo, Ledung, Gey, & Milas, 1992; Shigemasa et al., 1995). A selectively substituted product can be prepared only if special protection of functional groups is provided (Kurita, 2001). The reaction will result in formation of only an N-substituted product if conducted in a low-alkaline medium in homogeneous conditions (An, Thien, Dong, & Le Dung, 2009; Skorik, Gomes, Vasconcelos, & Yatluk, 2003). The chitosan modification reactions are conducted in

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dilute solutions (1.5%), and therefore high DS can be achieved only after a prolonged period of time and two- or three-fold treatment (Kogan et al., 2004; Skorik et al., 2005, 2003).

A common characteristic of the known methods for preparation of carboxyalkylated chitosans is the use of dilute solutions or suspensions (0.5–2%). The use of dilute solutions is impractical considering the inefficient consumption of solvent in both the reaction and isolation of the product, the low yield of product per reaction volume, and the desirable use of chitosan with a low molecular weight for formation of slightly viscous solutions. In addition, considerable degrees of substitution (DS) result from the use of extremely large excess of the reagent or from over-prolonged reaction times. All of these factors make production of carboxyalkylated chitosans on a large scale quite inconvenient. We have recently taken these drawbacks into account by proposing a method of using gel technique for carboxyethylation of chitosan with acrylic acid (Pestov, Kodess, Matochkina, & Yatluk, 2011; Pestov, Zhuravlev, & Yatluk, 2007) and 3-halopropionic acids (Pestov, Skorik, Kogan, & Yatluk, 2008). The proposed method conserves considerable amounts of solvents and reagents, increases the DS of the product, and omits stirring from the synthesis process.

The aim of this study is to compare the effectiveness and usefulness of direct carboxyalkylation of chitosan using aza-Michael addition and substitution reactions performed in dilute solutions and in the gel state.

2. Experimental

2.1. Materials and methods

Chitosan was purchased from JSC "Sonat" (Moscow, Russia). Degree of acetylation (DA) was determined as 0.16 by ^{1}H NMR spectroscopy; while the average molecular mass of 2.5×10^5 was established using viscometry according to Gamzazade et al. (1985). Acrylic acid (99.5%), crotonic acid (98%), 3-iodopropionic acid (99%), chloroacetic acid (99%), bromoacetic acid (98%), 3-bromopropionic acid (97%), 2-bromopropionic acid (98%), 4-bromobutyric acid (97%), 5-bromovaleric acid (97%) were purchased from AlfaAesar; and 5-chlorovaleric acid (98%), 6-bromocaproic acid (97%) were obtained from Sigma–Aldrich and were used without further purification. 6-Chlorocaproic acid was prepared starting from 6-caprolactone by using the procedure described in (Seligsohn Gabrieli & Co., 1978).

Degree of substitution (DS) of the prepared *N*-carboxyalkylated derivatives of chitosan was determined on the basis of their ^{1}H NMR spectra as previously described (Skorik et al., 2003). Spectra were recorded in D_2O/DCl using a Bruker DRX 400 spectrometer at 70 °C. The differences in DS between similar treatments were found to be less than 10% (relative).

2.2. Carboxyalkylation of chitosan using addition reactions

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, and then heated at 70 °C for 12 h, cooled, and 80 ml of 2.8% aqueous potassium hydroxide was added. The product was precipitated with acetone and dried *in vacuo* at 25 °C to constant weight. ^{1}H NMR (D_2O/DCl): δ, ppm = 2.07 (NHCOCH_3), 2.93 (CH_2COOH), 3.24–3.32 (*H*-2 of GlcNH$_2$, GlcNHR and GlcNR$_2$), 3.50–4.20 (NCH_2, *H*-2 of GlcNAc, *H*-3,4,5,6), 4.63 (*H*-1 of GlcNAc), 4.92 (*H*-1 of GlcNH$_2$), 5.07 (*H*-1 of GlcNHR), 5.26 (*H*-1 of GlcNR$_2$). DS = 1.12. ^{13}C NMR (D_2O/DCl): δ, ppm = 22 (NHCOCH_3), 40 (CH_2COOH of GlcNHR and GlcNR$_2$), 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), 165 (NHCOCH$_2$), 175 (NHCOCH$_3$), 183 (COOH).

The reaction with crotonic acid was performed similarly: a mixture of 0.33 g (0.002 mol) of chitosan and 0.68 g (0.008 mol) of crotonic acid in 1 ml of water was kept at 70 °C until a gel formed, and then heated at 75 °C for 96 h, cooled, and 5.5 ml of 6% aqueous sodium hydroxide was added. The product was precipitated with acetone, reprecipitated from HCl solution, and dried *in vacuo* at 25 °C to constant weight. ^{1}H NMR (D_2O/DCl): δ, ppm = 1.15 (CH_3), 2.07 (NHCOCH_3), 2.88 (CH_2COOH), 3.20–3.38 (*H*-2 of GlcNH$_2$, GlcNHR and GlcNR$_2$), 3.62–4.10 (NCH_2, *H*-2 of GlcNAc, *H*-3,4,5,6), 4.65 (*H*-1 of GlcNAc), 4.90 (*H*-1 of GlcNH$_2$), 5.03 (*H*-1 of GlcNHR). DS = 0.68. The reaction in the presence of Se was performed as above but included 48 mg Se.

2.3. Carboxyalkylation of chitosan using substitution reactions

The typical reaction procedure in gel was as follows: a mixture of 0.66 g (0.004 mol) of chitosan and 0.8 g (0.004 mol) of 3-iodopropionic acid in 2 ml of water was kept at 60 °C until a gel formed and then a mixture of 3.2 g (0.016 mol) of 3-iodopropionic acid and 7.26 g (0.098 mol) of Li_2CO_3 in 2 ml of water was added. The reaction mixture was heated at 60 °C for 24 h, cooled, and then 5 ml of water was added. The product was precipitated with acetone, reprecipitated from water, and dried *in vacuo* at 25 °C to constant weight. ^{1}H NMR (D_2O/DCl): δ, ppm = 2.07 (NHCOCH_3), 2.93 (CH_2COOH), 3.24–3.32 (*H*-2 of GlcNH$_2$, GlcNHR, and GlcNR$_2$), 3.50–4.20 (NCH_2, *H*-2 of GlcNAc, *H*-3,4,5,6), 4.63 (*H*-1 of GlcNAc), 4.92 (*H*-1 of GlcNH$_2$), 5.07 (*H*-1 of GlcNHR), 5.26 (*H*-1 of GlcNR$_2$). DS = 1.02. ^{13}C NMR (D_2O/DCl): δ, ppm = 23 (NHCOCH_3), 30–36 (CH_2COOH of GlcNHR and GlcNR$_2$), 41 (NCH_2 of GlcNHR), 43 (NCH_2 of GlcNR$_2$), 57–64 (*C*-6, *C*-2), 69–77 (*C*-3, *C*-4, *C*-5), 96 (*C*-1), 174 (NHCOCH$_3$), 176–178 (COOH).

The reactions with chloroacetic, 2-bromopropionic, and 5-bromovaleric acids were performed similarly. The reactions with 5-chlorovaleric and 6-chlorocaproic acids used DMSO and a water–ethanol mixture instead of water.

Kinetic studies of bromide ion release in the reaction mixtures were carried out as follows: chitosan (0.33 g; 0.002 mol) was dissolved in 20 ml of aqueous methanol (50 vol%) containing 0.01 mol of the respective bromocarboxylic acid. The solution was stirred and maintained at the required temperature for different time periods (from 1 to 120 h). Bromide ion content was determined as described previously (Pestov et al., 2008); all analytical experiments were performed in triplicate.

3. Results and discussion

3.1. Addition reactions

The results on the reaction of chitosan with acrylic acid are summarized in Table 1. A comparison of the data obtained with different chitosan concentrations clearly shows that a tenfold increase in the chitosan concentration leads to an increase in the DS by a factor of 2–7. Note that the reaction time is also considerably reduced, the temperature of the process is lower, and the yield of the product per reaction volume grows. This actually makes the process advantageous for the production of large quantities of the product. In all cases, the formation of an N-substituted product was confirmed by ^{1}H NMR spectroscopy. Thus, acrylic acid is an excellent selective electrophilic reagent for N-carboxyethylation in concentrated aqueous systems. The observed increase in the addition reaction rate upon transition from solution to gel reaction systems is most likely due predominantly to the increased concentration of the reagents, which leads to a greater reaction rate.

Table 1
Carboxyethylation of chitosan by acrylic acid.

0.5–2% chitosan solutions				20% chitosan gel			
Acrylic acid:chitosan mole ratio	T (°C)	Reaction time (h)	DS	Acrylic acid:chitosan mole ratio	T (°C)	Reaction time (h)	DS
2[a]	50	24	0.14	2[b]	50	24	0.96
2[a]	50	48	0.27	5[b]	50	36	1.24
2[a]	50	96	0.46	5[b]	50	48	1.16
3[a]	50	24	0.34	3[b]	50	24	1.06
4[a]	50	24	0.40	4[b]	50	24	1.11
2[a]	75	24	0.49	2[b]	70	12	1.12
2[a]	100	24	0.64	2[b]	90	12	1.34
5[c]	25	96	0.48	2[b]	60	8.5	0.47

[a] Sashiwa et al. (2003a).
[b] Pestov et al. (2007).
[c] Present work. Ultrasonic treatment (45 kHz) was applied for the initial 40 min.

As shown in Fig. 1, an optimal range of the polymer concentration exists around 20–40%, which provides the highest DS. In contrast, a further increase in the polymer concentration in the reaction system leads to a decrease in the total DS. This phenomenon can be related to a manifestation of the gel effect in polymer transformations. The initial increase in the DS with the concentration of chitosan obeys the law of mass action. When the polymer concentration grows, the viscosity of the reaction medium increases, which decreases the diffusion rate of the reagent to the chitosan amino groups, thereby limiting the rate of the target reaction. Thus, the gel effect of polymer transformations results in a concentration of the polymer in the gel that ensures the same type of diffusion of the reagent to the functional groups of the polymer as would occur in a dilute solution.

The products synthesized in solutions of different concentrations differ not only in their DS, but also in their supramolecular structures. If solutions with a low chitosan concentration are used, the side reactions are addition of water to the acrylic acid and the formation of 3-hydroxypropionic acid (Pestov et al., 2008). The gel reaction is accompanied by a side reaction that involves a cross-linking of the macromolecules (Pestov et al., 2007). This is beneficial, for example, if the product is to be used later as a chelating polymer. If a water-soluble product is required, then, when the reaction is complete, the gel can be treated with an alkali to break the intermolecular cross-linking. The product can then be isolated as a salt, essentially as is done for reactions in dilute solutions.

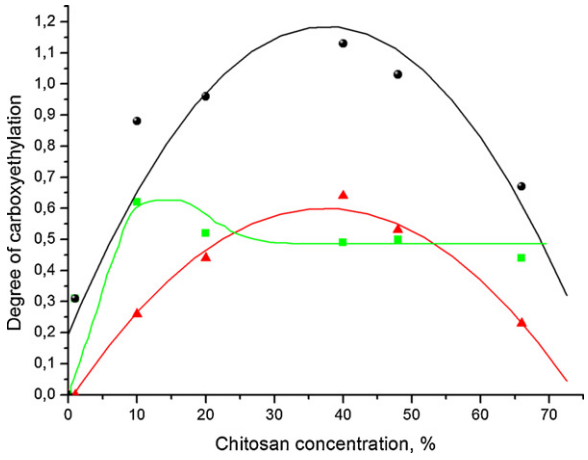


Fig. 1. The dependence of the total degree of substitution (●), the degree of mono-substitution (■) and disubstitution (▲) on the concentration of chitosan (expressed on a dry basis) in the reaction mixture with acrylic acid, mole ratio acrylic acid:chitosan = 2:1, 60 °C, 24 h.

The amide character of the intermolecular cross-linking has been confirmed by FTIR (Pestov et al., 2007) and ^{13}C NMR spectroscopy (Pestov et al., 2011). The signal at 183 ppm corresponds to C=O in the carboxylic group; at 175 ppm, to C=O in the acetamide group; and at 165 ppm, to C=O in the amide group for intermolecular cross-linking. When this product is boiled with an alkali, the ratio between the signals at 183 and 165 ppm increases: this is indicative of additional amide cross-linkages, which are then decomposed during boiling with alkali.

A comparison of the conditions used for the addition reaction in dilute and concentrated systems and the structures of the formed products also revealed different molecular architectures of the polymers. Use of gel technique makes it possible to form a polymer material with grafted functional groups and a cross-linked molecular structure in a single stage. This cannot be achieved by approaches that use dilute solutions.

The use of crotonic acid in an aza-Michael reaction with chitosan in the gel state is also effective, in spite of its lower reactivity when compared to acrylic acid (Table 2). To speed up the reaction with the amino group of chitosan, crotonic acid (or *trans*-2-butenoic acid) should be converted to its *cis*-isomer, isocrotonic acid; the reaction can be considerably enhanced by the addition of elemental selenium or by increasing the temperature (Dear and Pattison, 1963). Indeed, as shown in Table 2, the DS value is doubled in the presence of selenium. Increasing the concentration of the gel allows one to use a slight excess of crotonic acid to obtain a reasonably high DS. As in the case of acrylic acid, the optimum concentration of chitosan in gel is 20–33% (Table 2). Thus, the proposed gel technique can be used in an aza-Michael reaction with a wide range of reagents to synthesize a variety of chitosan derivatives.

3.2. Substitution reactions

Synthesis of 2-carboxyethylated chitosan requires the use of 3-halopropionic acids. The chitosan modification reactions were conducted in dilute solutions (1.5%), and therefore high DS can be

Table 2
Methylcarboxyethylation of chitosan by crotonic acid in gel at 75 °C.

Crotonic acid:chitosan mole ratio	Chitosan concentration (%)	Time (h)	DS
4	11	48	0.24
4[a]	11	48	0.47
4	11	96	0.59
4	20	96	0.68
2	20	96	0.49
2	33	96	0.59
2	50	96	0.21

[a] Reaction was performed in the presence of Se.

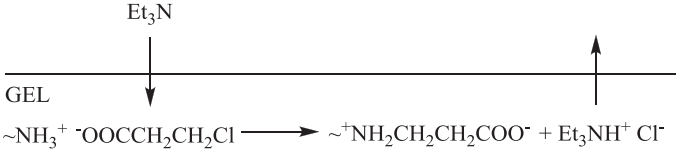
Table 3
Carboxyethylation of chitosan by 3-halopropionic acids at 60 °C, mole ratio 3-halopropionic acid:chitosan = 5:1.

1.1–1.5% chitosan solutions				15–20% chitosan gels			
Halogen	Base	Time (h)	DS	Halogen	Base	Time (h)	DS
Cl[a]	–	48	0.03	Cl[a]	–	24	0.26
Br[a]	–	48	0.02	Br[a]	–	24	0.35
I[a]	–	48	0.03	I[a]	–	24	0.37
Cl[a]	Li_2CO_3	48	0.20	Cl[a]	Li_2CO_3	24	0.83
Br[a]	Li_2CO_3	48	0.22	Br[a]	Li_2CO_3	24	1.00
I[a]	Li_2CO_3	48	0.24	I[a]	Li_2CO_3	24	1.02
Cl[b]	$NaHCO_3$	24	0.48	Br[c]	Li_2CO_3	24	0.40
Br[b]	$NaHCO_3$	72	0.92	Br[c]	Li_2CO_3	48	0.50
Br[c]	Et_3N	48	0.23	Cl	Et_3N	24	0.96

[a] Pestov et al. (2008).
[b] Reaction was performed using the procedure described in Skorik et al. (2003).
[c] The gel is formed gradually in the course of the reaction.

achieved only after a prolonged period of time and two- or three-fold treatment (Kogan et al., 2004; Skorik et al., 2005, 2003). In this case, we also propose to use a gel technique for chitosan modification. In contrast to the reaction with acrylic acid that takes place in acidic conditions, modification by 3-halopropionic acids is effected first in a mild acidic and then in a mild alkaline medium. Here the sequence of gel formation of the reaction mixture becomes important. As shown in Table 3, the duration of the process can be considerably reduced by increasing the concentration of the chitosan solution when a nucleophilic substitution reaction is used for chitosan modification.

In this approach, the reaction mixture is also a homogeneous gel, which requires no stirring during the reaction. Note again that the main difficulty in carboxyethylation by 3-halopropionic acids with an inorganic base consists in proper formation of the gel, which determines the final DS. This drawback is eliminated if an organic base, *e.g.*, triethylamine, is also used. In this case, the gel is made up of chitosan and 3-chloropropionic acid and is placed in triethylamine. As a result, the reaction takes place at the interface:



Thus, triethylamine acts as a base and a catalyst for the phase transfer, since the amine itself dissolves poorly in water and forms immiscible layers, while its hydrochloride dissolves well. The gel does not increase the volume during the reaction, pointing to a reversible penetration of triethylamine into the gel phase.

^{1}H NMR spectroscopic analysis of the chemical structure of the products revealed a selective N-substitution. An examination of the ^{13}C NMR spectra showed the absence of amide cross-links (Fig. 2), unlike the product of the reaction with acrylic acid. Since the pH of the reaction gel is nearly 6.5, all of the carboxyl groups are in the carboxylate form, which is not favorable for the formation of amide bonds. Thus, the nucleophilic substitution reaction in the gel has an advantage over the same process in a solution in that it provides the same or higher DS within a shorter time (Table 3). Note again that the carboxyethylation system (such as 3-chloropropionic acid-triethylamine) can be very easily used in practice.

When chitosan is modified using gel technique, other halogen derivatives of carboxylic acids can be used, in addition to 3-halopropionic acids. We used both chloroacetic and 2-bromopropionic acids, which are α-halocarboxylic acids, because these are more reactive substrates for nucleophilic substitutions (Table 4).

The data given in Tables 3 and 4 show that β-halocarboxylic acids provided higher DS at the same reaction conditions. In all likelihood, this is how the "reverse side" of a higher concentration of the reaction mixture appears. A more reactive reagent will react more quickly not only with chitosan, but also with the base and the solvent. The literature shows that chitosan has been carboxymethylated by chloroacetic acid in the presence of 28-fold excess acid due to a simultaneous and rapid hydrolysis reaction (Xie, Xu, & Liu, 2001). For this reason, gel carboxymethylation is followed by a considerable increase in side reactions, thereby reducing the rate of the desired reaction, and hence decreasing the DS. The use of 1-bromopropionic acid, a more sterically hindered substrate, decreases the rate of the desired reaction to the greatest extent, and hence the DS is the lowest among all other α-halocarboxylic acids (Table 4). The 1-carboxyethylation of chitosan has been reported previously; it is accomplished either by the nucleophilic substitution in an alkaline medium (Shigemasa et al., 1995) or by reduction of the corresponding Schiff base in an acid medium (Chiessi, Palleschi, Paradossi, Venanzi, & Pispisa, 1991). The same is true of chloroacetic acid, where the carboxymethylation of chitosan takes place in an alkaline (Rinaudo et al., 1992) and low-alkaline medium (An et al., 2009), as well as when glyoxylic acid is used with subsequent borane reduction (Ledung, Milas, Rinaudo, & Desbrieres, 1994; Muzzarelli, Tanfani, Emanuelli, & Mariotti, 1982). We have been the first to use a nearly neutral medium (pH 6.5) for carboxymethylation and 1-carboxyethylation of chitosan to perform these reactions in the gel state.

Table 4
Carboxyalkylation of chitosan by halocarboxylic acids in gel, 60 °C, 24 h, mole ratio halocarboxylic acid:chitosan = 5:1.

Reagent	Solvent	Base	DS
$ClCH_2COOH$	H_2O	Li_2CO_3	0.44
	H_2O	Et_3N	0.33
$CH_3CHBrCOOH$	H_2O	Li_2CO_3	0.17
$Br(CH_2)_4COOH$	H_2O	–	0
	H_2O	Li_2CO_3	0
$Cl(CH_2)_4COOH$	DMSO	–	0.04
	H_2O/EtOH	–	0.03
	H_2O/EtOH	Et_3N	0.05
$Cl(CH_2)_5COOH$	DMSO	–	0.04
	H_2O/EtOH	–	0.04
	H_2O/EtOH	Et_3N	0.05

The use of less reactive 5-chlorovaleric, 5-bromovaleric, or 6-bromocaproic acids showed that performing the reaction in gel conditions using different solvents did not favor the target reaction either under acidic conditions or with the addition of a base (Table 4).

To investigate the impact of the target and side reactions, kinetic studies of the rate of bromide ion release were carried out for ω-bromocarboxylic acids in a water/methanol solution in the absence of a base (Fig. 3). These curves show the total outcome from the target and side reactions. As can be seen from Fig. 3, the observed rate of bromide ion release is in the order 4-bromobutyric > 5-bromovaleric ≫ 3-bromopropionic > bromoacetic > 6-bromocaproic acid. However, the degree of carboxyalkylation of the isolated products (corresponding to the last points on the kinetic curves) decreases in order bromoacetic (0.2) > 6-bromocaproic (0.11) > 3-bromopropionic (0.02) > 4-bromobutyric (0) = 5-bromovaleric acids (0). These data clearly show that for all ω-bromocarboxylic acids the rate of the side reactions exceeds that of the target reaction. In most cases, the carboxyalkylation of chitosan occurs to a very small extent, which could be due to suppression of the nucleophilicity of nitrogen as a result of the formation of ammonium salts at mild acidic pH. Production of strong hydrobromic acid in the outcome of the reactions contributes further to the inhibition of the target reaction. The reagents are consumed mostly for side processes, such as hydrolysis characteristic of α- and β-halocarboxylic acids,

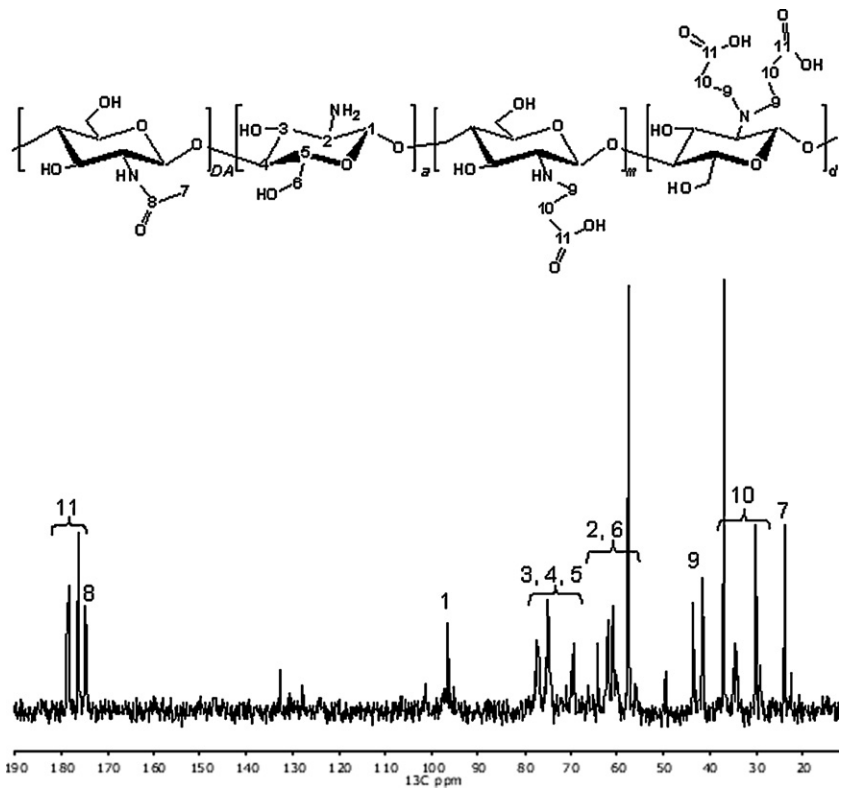


Fig. 2. ^{13}C NMR spectrum (100.6 MHz, D_2O/DCl, 70 °C) of N-(2-carboxyethyl)chitosan obtained in 15% chitosan gel by reaction with 3-iodopropionic acid in the presence of Li_2CO_3 at 60 °C; mole ratio 3-halopropionic acid:chitosan = 5:1.

or dehydrohalogenation that leads to the formation of acrylic acid or correspondent γ-, δ-, or ε-lactones.

In the transition from solution to gel state, the relative rates of the target and side reactions change ambiguously for different reagents. Generally, α- and especially β-halocarboxylic acids provide higher degrees of substitution in the gel state as compared with those achieved in a dilute chitosan solution. In contrast, ε-halocaproic acid shows a slightly higher yield of the desired reaction when performed in solution. The use of γ- and δ-halocarboxylic acids for chitosan carboxyalkylation did not favor the target reaction either in solution or in the gel state.

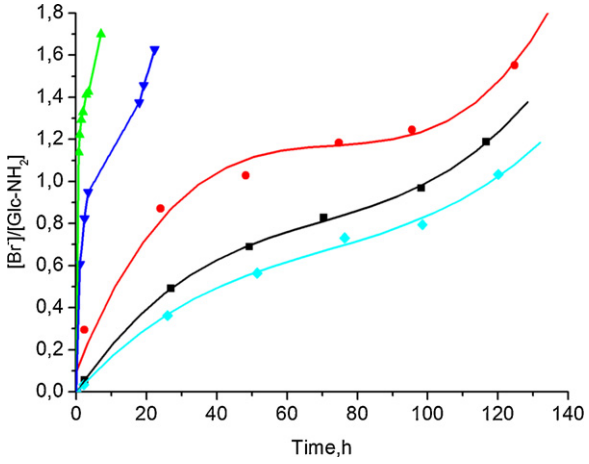


Fig. 3. Kinetic curves of bromide release at the reaction of chitosan with bromoacetic (■), 3-bromopropionic (●), 4-bromobutyric (▲), 5-bromovaleric (▼), and 6-bromocaproic (♦) acids. [Br^-]/[Glc-NH_2]: mole ratio of bromide to the chitosan free amino group; 50 vol% aqueous methanol, initial mole ratio ω-bromocarboxylic acid:chitosan = 5:1, 40 °C. Data represent mean values from the experiments performed in triplicate.

4. Conclusion

As mentioned in Section 1, carboxyalkylation is traditionally accomplished under heterogeneous or homogeneous conditions. The new method proposed in the present study for chitosan carboxyalkylation is based on gel systems, which are neither homogeneous nor heterogeneous. Various reagents for carboxyalkylation of chitosan were applied using aza-Michael type addition and substitution reactions. In the case of acrylic, crotonic, α- and β-halocarboxylic acids, the reaction performed in the gel state offers considerable advantages over the same reaction in solution, including a higher degree of substitution, higher yield of the product per reaction volume, lower reaction time and temperature, lower consumption of solvents and reagents, and no need for stirring of the reaction mixture. All of these factors make the gel technique advantageous for large-scale production. Reactions performed with less reactive γ- or δ-halocarboxylic acids showed no target product formation either in solution or in the gel state.

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