N-Alkylation of Chitosan by β-Halopropionic Acids in the Presence of Various Acceptors

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ABSTRACT: *N*-carboxyethylation of chitosan by β-halopropionic acids in the presence of various proton and halogen ion acceptors was investigated. It has been observed that carboxyethylation of chitosan in aqueous medium is accompanied by the by-processes of hydrolysis and dehydrohalogenation of the β-halopropionic acids yielding β-hydroxypropionic acid, bis(2-carboxyethyl) ether, and acrylic acid. Degree of carboxyethyl substitution (DS) of chitosan and the relative rates of the by-processes varied significantly depending on the conditions used and nature of the proton or halogen ion acceptor. At carboxyethylation of chitosan with the alkaline β-bromopropionates, the DS increased in the order $Cs^+ < Rb^+ < K^+ \sim Na^+ < Li^+$. For alkaline earth salts $BrCH_2CH_2COOM_{0.5}$ (M = Be^{2+}, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}), the highest DS was obtained with strontium and barium salts, which could be subsequently removed from the reaction mixture by precipitation as sulfates. Among the organic bases applied (tetrabutylammonium hydroxide, triethylamine, trimethylamine, pyridine, 4-*N*,*N*-dimethylaminopyridine, 2,6-lutidine, and 1,5-diazabicyclo[4.3.0] non-5-ene), the highest DS was obtained using a moderately strong base triethylamine. For the halogen acceptors (Pb^{2+}, Ag^+, Tl^+), the stoichiometrically highest DS was achieved in a system comprising iodopropionic acid *plus* Tl^+ and a comparable conversion rate was obtained using also a combination of chloropropionic acid and Ag^+. A novel alternative preparative approach—gel-state synthesis—was suggested that provides for the highest DS at the optimum reaction conditions. J Appl Polym Sci 108: 119–127, 2008

Key words: chitosan; carboxyethylation; β-halopropionic acids; halogen and proton acceptors; gel-state synthesis

INTRODUCTION

Chitin and its deacetylated derivative chitosan possess a series of unique properties, including biocompatibility, biodegradability, negligible toxicity, high adsorption capacity toward heavy metal ions and radionuclides, and antimicrobial activity.[1–3] Unfortunately, the insolubility of chitin in water solutions and the limited solubility of chitosan in solutions having pH below 6.0 poses certain obstacles for broad practical application of these two polymers. For this reason, much effort has been applied to the development of suitable procedures for preparation of water-soluble derivatives of chitin and chitosan that would be feasible for use in a broad scale of applications from plant protection to clinical administration in human medicine.[3–5]

Carboxymethylation of chitosan using chloroacetic acid is a well-known process that has been described in detail,[6–8] while there is only limited data available on alkylation by halopropionic acids. Use of α-chloropropionic acid was described by Shigemasa et al.[9] Orienti et al.[10] synthesized *N*-(2-carboxyethyl)-chitosan (*N*-CE-chitosan) by treating chitosan with β-bromopropionic acid in aqueous pyridine. We have previously reported a synthesis of *N*-CE-chitosan by means of alkylation with chloro-, bromo-, and iodopropionic acids in the presence of sodium hydrogen carbonate.[11]

Lee et al.[12] described synthesis of *O*-CE-chitosan by means of hydrolysis of the beforehand prepared cyanoethylated derivative. Aoi et al.,[13] Sashiwa et al.,[14] and Skorik et al.[15] applied hydrolysis of acrylamide, methylacrylate, or ethylacrylate derivatives of chitosan, whereas Sashiwa et al.[16] and Skorik et al.[15] carried out direct addition of acrylic acid.

The novel water-soluble *N*-CE-chitosan revealed pronounced antioxidant[17] and antimutagenic[15,17] properties when tested on a model microorganism *Euglena gracilis*, showed high chelating efficiency toward

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transition metal ions,[18] and had better biodegradability (compostability[16] and enzymatic degradability[19]) than chitosan itself. *N*-CE-chitosan also appears suitable for mediating transport of hydrophilic drugs such as vitamin B6 through the skin[10] or as a nitric oxide carrier for use in a variety of medical applications, in which an effective dosage of nitric oxide is indicated as a preferred method of treatment.[20]

In the present study, a systematic investigation of chitosan carboxyethylation by applying chloro-, bromo-, and iodopropionic acids in the presence of various bases or metal cations serving as halogen acceptors is described and the influence of the by-processes both promoting and hampering the formation of the final product is investigated. Such thorough exploration of the process under study will allow for suggestion of a convenient (in terms of reaction time and high degree of substitution) procedure of the preparation of the desired product.

EXPERIMENTAL

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 was established using viscometry according to Gamzazade et al.[21] Halopropionic acids were purchased from Merck-Schuchardt (Darmstadt, Germany). All other chemicals were of analytical grade and were used without further purification.

Carboxyethylation of chitosan

Chitosan carboxyethylation with halopropionic acids

Chitosan (0.33 g; 0.002 mol) was dissolved in 20 mL of aqueous methanol (50 vol %) containing 0.01 mol of the respective halopropionic acid. The solution was stirred and kept at required temperature for different time periods (from 1 to 120 h). The product was precipitated with 250 mL of acetone and separated by centrifugation. Then, it was reprecipitated twice at the same conditions and dried *in vacuo*. The filtrate was dried on a rotary evaporator.

Chitosan carboxyethylation with halopropionic acids in the presence of sodium hydrogen carbonate

Chitosan (0.33 g; 0.002 mol) was dissolved in 30 mL of aqueous methanol (50 vol %) containing an equimolar amount of the respective halopropionic acid. In another 10 mL of the similar aqueous methanol solvent, 0.78 g (0.0093 mol) $NaHCO_3$ was dissolved and 0.008 mol of the same halopropionic acid was added. The solutions were mixed together and left at 40°C for different time periods (from 1 to 60 h). The reaction mixture was treated as described above.

Chitosan carboxyethylation with acrylic acid

Chitosan (0.33 g; 0.002 mol) was dissolved in 30 mL of aqueous methanol (50 vol %) containing 0.68 mL (0.01 mol) of acrylic acid. The solution was stirred and kept at required temperature for different time periods (from 24 to 190 h). The reaction mixture was treated as described above.

Chitosan carboxyethylation with halopropionates of alkaline and alkaline-earth metals

Chitosan (0.33 g; 0.002 mol) was dissolved in 20 mL of H_2O containing 0.002 mol of halopropionic acid. Alkaline or alkaline-earth metal carbonate [0.0046 mol; M_2CO_3 (M = Li, Na, K, Rb, Cs) or MCO_3 (M = Be, Mg, Ca, Sr, Ba), respectively] was dissolved or suspended (in case of the water-insoluble carbonates) in 10 mL of H_2O and 0.008 mol halopropionic acid was added. Upon neutralization, the solution was mixed with the prepared solution of chitosan and left at 60°C for 48 h. The reaction mixture was treated as described above.

Chitosan carboxyethylation with bromopropionic acid in the presence of organic bases

Chitosan (0.33 g; 0.002 mol) was dissolved in 20 mL of H_2O containing 0.31 g (0.002 mol) of β-bromopropionic acid. Then, 1.12 g (0.008 mol) of β-bromopropionic acid was admixed to the solution of 0.0046 mol of the organic base (triethylamine, trimethylamine, tetrabutylammonium hydroxide, 2,6-lutidine, pyridine, 4-*N*,*N*-dimethylaminopyridine or 1,5-diazabicyclo[4.3.0]non-5-ene) in 10 mL of water and the mixture was added to the chitosan solution. The resulting reaction mixture was stirred at 60°C for 48 h. The reaction mixture was treated as described above.

Chitosan carboxyethylation with halopropionic acids in the presence of metal cations–acceptors of halogens

Chitosan (0.33 g; 0.002 mol) was dissolved in 20 mL of H_2O containing 0.006 mol of a respective β-halopropionic acid. Metal nitrate solution (0.004 mol) in 5 mL of H_2O and 0.224 g (0.004 mol) of KOH in 5 mL of H_2O were then added and the mixture was left at ambient temperature for 24 h. The reaction mixture was treated as described above.

Reaction in gel state was performed under similar conditions, however only 2.5 mL of water was used

instead of 20 mL, which resulted in formation of chitosan gel rather than a solution.

Characterization of the products

DS of the prepared *N*-alkyl derivatives of chitosan was determined on the basis of their ^{1}H NMR spectra as previously described.[11] Spectra were recorded in D_2O/DCl using a Bruker DRX 400 spectrometer. Signal assignments were carried out based on comparison with the spectra of the model compounds (β-halopropionic acids, β-hydroxypropionic acid, bis(2-carboxyethyl)ether, acrylic acid).

Analytical determination

Determination of halogen ions

The determination was carried out as described by Cheronis and Ma.[22] Briefly, about 5 g of analyzed solution (aliquot) was placed in a titration flask and the following reagents were added: 5 mL of 6*N* HNO_3, 5 mL of 0.1*N* $AgNO_3$, 1–3 drops of saturated ferric nitrate solution, and 2–3 drops of nitrobenzene. The mixture obtained was titrated with 0.1*N* NH_4SCN until a dark red coloration appeared. The control titration was carried out without the analyzed sample. The concentration of the assayed halogen ion was determined from the difference of ammonium thiocyanate volumes used in the control and analytical titrations.

Determination of acrylic acid

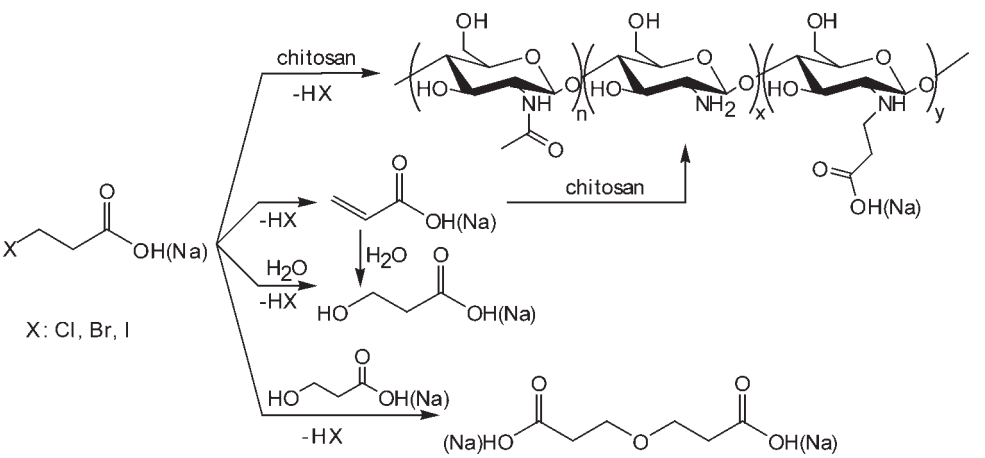
The determination was carried out as described by Cheronis and Ma.[22] Briefly, 0.3 g of the analyzed solution (aliquot) was placed in a titration flask and 5 mL of water and three drops of phenolphthalein were added. NaOH (0.05*N*) was added dropwise until a pink coloration appeared. Ten milliliters of H_2O was added and the solution was thoroughly stirred. Five milliliters of a saturated solution of sodium bromide in methanol and 200 mg of solid NaBr were added. The flask contents were stirred and placed in an ice bath. Five milliliters of 0.075*N* solution of bromine complex with NaBr was added and the flask was stopped and left for 1 h. Then, the flask was again cooled in an ice bath, 10 mL of methanol and 5 mL of 3% potassium iodide solution were added, the flask was left in the dark for 10 min, and its contents were subsequently titrated with 0.05*N* sodium thiosulfate until a straw-yellow coloration appeared. Five drops of 1% starch solution were added and titration was resumed until the coloration disappeared. The control titration was carried out without the analyzed solution and the concentration of acrylic acid was calculated from the difference of the volumes of thiosulfate used for analytical and control titrations.

RESULTS AND DISCUSSION

Chitosan carboxyethylation with halopropionic acids

First, we investigated the interaction between chitosan and halopropionic acids in aqueous methanol and established the composition of products, as well as the kinetics of the release of halogen ions.

The composition of the reaction mixture and relative content of the low-molecular-weight products were determined based on the ^{1}H NMR spectra of the residue isolated from the supernatant solution upon separation of the polymeric substances. It was found that besides the main reaction products, all supernatant solutions contained the starting β-halopropionic acid, β-hydroxypropionic acid, bis(2-carboxyethyl) ether, and acrylic acid (as the reaction took place in aqueous methanol, small amounts of methyl esters of the corresponding acids produced due to esterification were also detected). Summarizing, the process occurs according to Scheme 1.



Scheme 1

TABLE I
Degree of Carboxyethylation of the Obtained CE-Chitosans and Molar Fraction (%) of the Reaction Products in the Mother Solution of Chitosan Alkylation with XCH_2CH_2COOH (50 vol % Aqueous Methanol, 40°C)

Reaction conditions	Chitosan:XCH_2CH_2COOH = 1 : 5 (5 days)			Chitosan:XCH_2CH_2COOH:$NaHCO_3$ = 1 : 5 : 4.9 (2.5 days)		
Halogen (X)	Cl	Br	I	Cl	Br	I
DS	0.03	0.02	0.03	0.33	0.28	0.40
XCH_2CH_2COOH	95	76	81	13	20	1
$HOCH_2CH_2COOH$	2	5	6	43	42	50
$O(CH_2CH_2COOH)_2$	1	18	10	19	27	17
CH_2=CHCOOH	1	1	3	22	7	27
CE-chitosan	1	0	0	3	4	5

The obtained chitosan derivatives were characterized by their degrees of substitution. The established DS values of the formed CE-chitosans enable determination of the ratio of the products of the alkylation, hydrolysis, and dehalogenation reactions that concurrently take place in the reaction mixture. The data obtained are presented in Table I.

As can be seen, carboxyethylation of chitosan occurs to a very low extent, which could be due to suppression of the nucleophilicity of nitrogen as a result of the formation of ammonium salt at mild acidic pH:

$$\text{Glc-NH}_2 + \text{HOOCCH}_2\text{CH}_2\text{X} \rightarrow \text{Glc-NH}_3^+ + {}^-\text{OOCCH}_2\text{CH}_2\text{X}$$

Production of strong hydrohalic acids HX in the outcome of all reactions additionally contributes to the inhibition of *N*-carboxyethylation.

The consumption (conversion) of halopropionic acids due to their participation in the reactions of nucleophilic substitution and elimination corresponds to the order Br > I > Cl (the conversion values are 24, 19, and 5%, respectively).

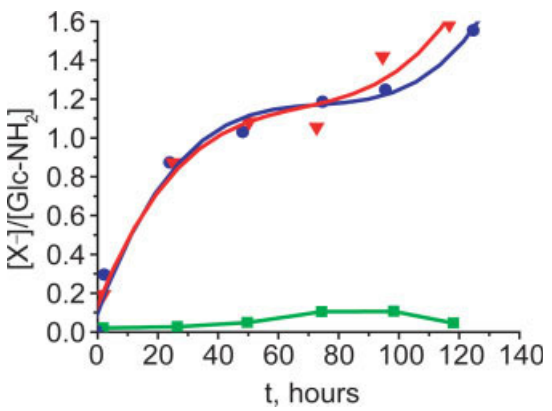


Figure 1 Kinetic curves of halogen ion release at reaction of chitosan with halopropionic acids. (■) 3-Chloropropionic acid, (●) 3-bromopropionic acid, (▼) 3-iodopropionic acid. [X^-]/[Glc-NH_2]: molar ratio of halide to the chitosan free amino group (50 vol % aqueous methanol, 40°C, chitosan:XCH_2CH_2COOH = 1 : 5). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

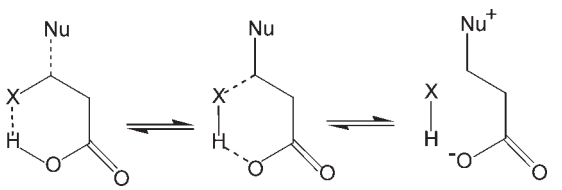
The data shown in Table I illustrate the result of the carboxyethylation process after 120 h. To investigate the reaction course in time, the kinetic studies of the rate of halogen ion release were carried out, i.e. the total outcome from the main and side reactions. The kinetics of the halogen ion release is presented in Figure 1. As can be seen from the figure, the observed rate of halogen release is in the order Br ~ I ≫ Cl.

A regular order of reactivity of the leaving groups in the nucleophilic substitution reactions is I > Br > Cl. However, in this case, this order is violated probably because of participation of the adjacent carboxyl group (Scheme 2).

Thus, the reaction rate is not only affected by the reactivity of the leaving groups, but also depends on the stability of the cyclic intermediate formed. Regarding the bond length values, the intermediate corresponding to the bromopropionic acid is probably the most stable.

Chitosan carboxyethylation with halopropionic acids in the presence of $NaHCO_3$

A similar investigation was carried out in a system comprising chitosan–halopropionic acid-$NaHCO_3$ in a water:methanol mixture (50 vol %). The reaction products ratio is presented in Table I. As can be seen from the obtained DS values, addition of sodium hydrogen carbonate resulted in at least a tenfold decrease in the order I > Cl > Br, and the reactivity of halopropionic acids was also in the same order I > Cl > Br with the corresponding conversion values of 99% > 87% > 80%.



Scheme 2

This order of reactivities of the halopropionic acids does not contradict the order of reactivity of the leaving groups mentioned above at the reactions of nucleophilic substitution and elimination, because in this case the total result includes contributions from both reactions. In fact, a relative increase in the amounts of acrylic acid produced is observed with chloro- and iodopropionic acids. These observations indicate the increased rate of the by-processes of hydrolysis and dehydrohalogenation that occur under alkaline conditions. In the case of bromopropionic acid, under both acidic and mild alkaline conditions, the prevailing process was hydrolysis as can be judged by the predominant content of β-hydroxypropionic acid and bis(2-carboxyethyl) ether.

The kinetics of the halogen ion release in the presence of $NaHCO_3$ is demonstrated in Figure 2. As can be seen from this figure, during the initial phase, the rate of transformation of halopropionic acids was in the order Br > I > Cl. These data imply that with bromopropionic acid the rate of the by-processes (hydrolysis and dehydrohalogenation) is so high that the consumption of the acid limits the possibility of efficient alkylation of chitosan. When compared to the similar kinetic data obtained in the absence of $NaHCO_3$ (Fig. 1), it is clear that in a less alkaline medium, the by-processes for bromopropionic acid are markedly slowed down.

To prove the assumption that carboxyethylation of chitosan could be, to a certain extent, affected by the addition of the acrylic acid that is produced as a result of the dehydrohalogenation of halopropionic acids, the kinetics of the addition of acrylic acid to chitosan, as well as the kinetics of its accumulation in the reaction mixture during the reaction of chitosan with halopropionic acids were investigated. The data obtained are shown in Figure 3.

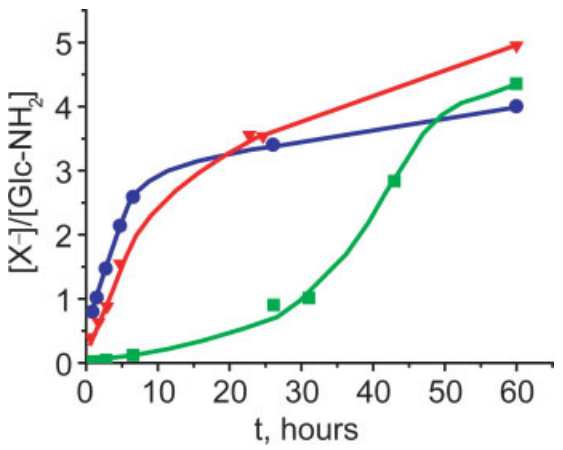


Figure 2 Kinetics of halogen ion release at reaction of chitosan with halopropionic acids in the presence of $NaHCO_3$. (■) 3-Chloropropionic acid, (●) 3-bromopropionic acid, (▼) 3-iodopropionic acid. $[X^-]/[Glc\text{-}NH_2]$: molar ratio of halide to the chitosan free amino group (50 vol % aqueous methanol, 40°C, chitosan:XCH_2CH_2COOH:$NaHCO_3$ =1 : 5 : 4.9). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

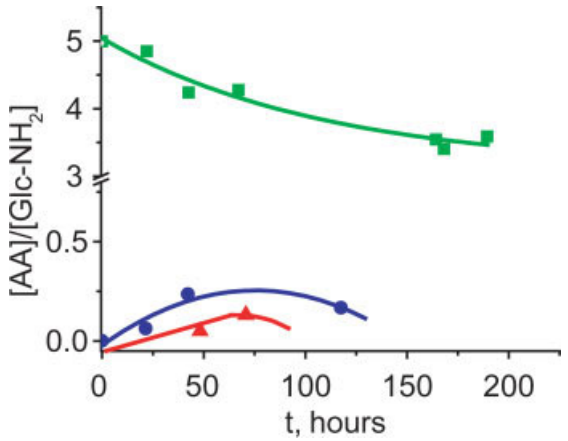


Figure 3 Kinetics of addition of acrylic acid to chitosan (■) and of its formation during the reaction of chitosan with halopropionic acids in the presence of $NaHCO_3$. (●) 3-Iodopropionic acid, (▲) 3-chloropropionic acid. [AA]/$[Glc\text{-}NH_2]$: molar ratio of acrylic acid to the chitosan free amino group (50 vol % aqueous methanol, 40°C, chitosan:acrylic acid = 1 : 5 or chitosan : XCH_2CH_2COOH : $NaHCO_3$ =1 : 5 : 4.9). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

In case of iodo- and chloropropionic acids, a noticeable contribution to overall alkylation due to the addition of acrylic acid was observed. The formation of acrylic acid from the bromopropionic acid was insignificant and undetectable by the analytical procedure used. Higher pH values led to lower substitution, as can be seen from the data presented in Table II, which indicates that use of sodium acrylate in the alkylating mixture resulted in decreased DS as well as in a diminished yield of CE-chitosan.

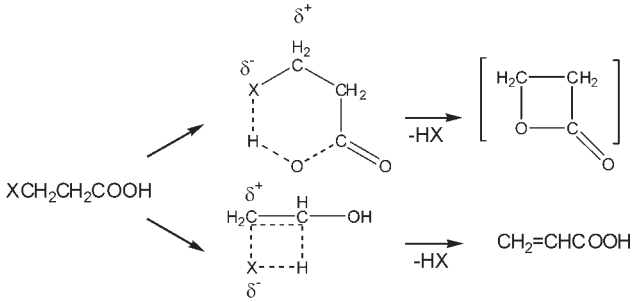
The results obtained imply that acrylic acid is produced from the halopropionic acids via the regular mechanism of elimination reactions at high pH values, whereas its addition to nucleophiles takes place at low pH values. The formation of acrylic acid is most significant in the case of iodopropionic acid.

Thus, the combination of the above mentioned factors, i.e., the highest inclination of bromopropionic

TABLE II
Degree of Carboxyethylation and Molar Fraction (%) of the Reaction Products at Chitosan Alkylation with Acrylic Acid (I) and a Mixture of Acrylic Acid–Sodium Acrylate (II) in 50% Aqueous Methanol

Alkylating reagent	I	II
DS	0.44	0.20
$HOCH_2CH_2COOH$	2	0.5
$O(CH_2CH_2COOH)_2$	0	0.5
CH_2=CHCOOH	89	95
CE-chitosan	9	4

Temperature = 40°C; molar ratio of chitosan:acrylic acid = 1 : 5; reaction duration = 120 h.



Scheme 3

acid to hydrolysis; the dependence of the rates of substitution and elimination reactions on the pH value, which varies in the course of the reaction; and the participation of acrylic acid, the formation and reactivity of which also depend on pH, in the overall process of carboxyethylation, leads to an unusual picture of the dependence of the degree of carboxyethylation on the nature of the halogen in halopropionic acid. Because of these circumstances, bromopropionic acid appears to be the less suitable reagent.

Several mechanisms of the nucleophilic halogen substitution are feasible. Chuchani et al.[23,24] dealing with the investigation of the gas phase elimination of hydrogen halides from halocarboxylic acid suggested a mechanism of anchimeric interaction of the leaving chlorine atom with the acidic hydrogen of the carboxyl group with the subsequent formation of propiolactone, or an alternative mechanism involving a four-membered cyclic transition state (Scheme 3).

In solution, a six-membered intermediate can further react with the nucleophiles or to form propiolactone at higher pH values. Products of interaction of this propiolactone with nucleophiles crucially depends on the pH value[25,26] (Scheme 4).

In this way, several mechanisms of formation of the detected products can be realized concurrently.

Chitosan carboxyethylation with the alkaline and alkaline-earth metal salts of halopropionic acids

The possibility of application of the salts of halopropionic acids for the carboxyethylation of chitosan has also been studied. Because the addition of sodium hydrogen carbonate was accompanied by the formation of persistent foam, it was more convenient to use a solution of salt prepared beforehand from the corresponding metal carbonate and halopropionic acid. Ten different alkaline and alkaline-earth metal salts were used in the reaction involving bromopropionic acid. As can be seen from the results shown in Table III, the highest degree of alkylation was obtained using lithium salts. For this reason, only lithium salt was used in the experiments involving the other two halopropionic acids (Table III).

The influence of the type of metal ion on the degree of chitosan alkylation was apparent. The largest amount of acrylic acid that subsequently underwent addition to chitosan was formed using the lithium salt. In other words, in this case, a by-process contributed positively to the desired result.

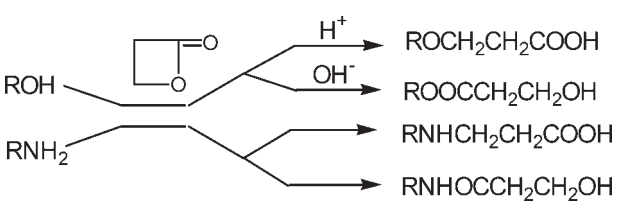
Among alkaline earth metals, the highest DS values were obtained with barium and strontium salts. Comparing the absolute values, it can be seen that using these salts resulted in somewhat lower DS values than in case of lithium salts, however, a simple procedure of removal of Ba^{2+} and Sr^{2+} cations, in the form of insoluble sulfates, makes their application rather advantageous.

Chitosan carboxyethylation with bromopropionic acid in the presence of organic bases

Seven nitrogen compounds were used as organic bases of different strength. The results presented in Table IV indicate that the basicity of the compound did not have a straightforward effect on the outcome of the reaction. Generally speaking, increased basicity resulted in a higher degree of alkylation. However, in the case of a very strong base, such as tetrabutylammonium hydroxide, the established DS value was significantly lower, most probably due to the prevalence of the by-process of hydrolysis. On the other hand, in the case of pyridine, the reaction was hampered by a process of quaternization, which was prevented in the case of the sterically hindered 2,6-lutidine. However, rather low basicity of 2,6-lutidine did not permit to obtain higher DS values in contrast to stronger bases such as trimethylamine or 1,5-diazabicyclo[4.3.0]non-5-ene (Table IV). Thus, the highest degree of substitution is rendered by the moderately strong organic bases, of which triethylamine provided for the best results.

Chitosan carboxyethylation with halopropionic acids in the presence of acceptors of halogen ions

To date, there have been no reference in the literature on the application of the salts of metals that form insoluble halides in the chemical transformation under investigation. It could be expected that the highest degree of carboxyethylation would be achieved using silver salts—traditional halogen acceptors used in organic syntheses.



Scheme 4

TABLE III
Degree of Carboxyethylation and Molar Fraction (%) of the Reaction Products at Chitosan Alkylation with Alkaline and Alkaline Earth Metal Halopropionates

X	Cl	Br									I
M^{n+}	Li^+	Li^+	Na^+	K^+	Rb^+	Cs^+	Be^{2+}	Mg^{2+}	Ca^{2+}	Sr^{2+}	Ba^{2+}	Li^+
DS	0.20	0.22	0.15	0.15	0.10	0.05	0.08	0.08	0.05	0.2	0.18	0.24
XCH_2CH_2COOH	19	2	5	4	5	9	3	3	5	11	3	16
$HOCH_2CH_2COOH$	32	42	45	63	66	58	57	52	56	38	47	36
$O(CH_2CH_2COOH)_2$	40	19	45	28	22	36	31	33	29	30	30	36
CH_2=CHCOOH	4	38	5	5	5	3	4	5	6	10	9	8
CE-chitosan	5	4	3	3	2	1	5	7	4	11	11	4

Solution = water; temperature = 60°C; molar ratio of chitosan:$XCH_2CH_2COOM(H)$ = 1 : 5; reaction duration = 48 h.

The results on chitosan alkylation with halopropionic acids in the presence of halogen acceptors are presented in Table V. The data demonstrate that the lowest degree of alkylation is observed when using lead, while the highest degree of alkylation occurred with thallium. In each case, the content of nonreacted halopropionic acid was the highest for lead, i.e., all described reactions of its transformation occurred in the presence of this metal to the lowest extent. In the case of silver, the smallest consumption was observed with bromopropionic acid. With iodopropionic acid, the major reaction was the hydrolytic replacement of halogen, and with chloropropionic acid, the formation of a significant amount of a bis-product, bis(2-carboxyethyl) ether, was also observed. Using thallium salts, by-processes occurred to the highest extent. It should be noted that in the presence of all metal salts, only a negligible amount of acrylic acid was formed.

The attempt to expand the range of metals used by introducing a salt of Hg^+ was unsuccessful: when mercury salt was added to the reaction mixture, chitosan precipitated from the solution. In general, the results presented in this section are characterized by poorer reproducibility than those described previously, due to the difficulties associated with the separation of the precipitating sediments of the metal halides.

Generally speaking, the best results were provided by a system comprising thallium and iodopropionic acid, however due to the significant toxicity of thallium salts, it is more appropriate to use a combination of silver + chloropropionic acid. Still, none of the mentioned systems yielded such high degrees of substitution as were obtained by the application of bases. Because all metals used as halogen acceptors form weak bases, the low solubility of the resulting halides is probably a critical factor affecting reaction rate. As demonstrated, the use of halogen acceptors led to an increased degree of carboxyethylation; however, since acrylic acid is not produced in this case, the total degree of carboxyethylation cannot be increased by the addition of the produced acrylic acid. Hence, the use of halogen acceptors is not optimal.

Chitosan carboxyethylation in gel state

To find optimal reaction conditions, we carried out the carboxyethylation reaction in solution. However, this procedure required use of rather dilute solutions. It seems feasible to overcome this drawback by using physical hydrogels of chitosan recently described by Montembault at al.[27] The authors have shown that the gelation may occur in aqueous solutions of chitosan at

TABLE IV
Degree of Carboxyethylation and Molar Fraction (%) of the Reaction Products at Chitosan Alkylation with Bromopropionic Acid in the Presence of Organic Bases

Base	Py[a]	2,6-Lu	DmaPy	Me_3N	Et_3N	Dabcn	Bu_4NOH
pKa (the most basic)	5.23	6.62	9.5	9.8	11.01	>13	Strong
DS	0.02	0.16	0.14	0.17	0.23	0.21	0.15
$BrCH_2CH_2COOH$	0	4	10	0	0	0	11
$HOCH_2CH_2COOH$	15	39	45	50	40	49	33
$O(CH_2CH_2COOH)_2$	17	48	34	44	40	34	41
CH_2=CHCOOH	0	8	8	4	17	13	7
CE-chitosan	0.4	3.2	2.8	3.4	4.6	4.2	3

Solution = water; temperature = 60°C; molar ratio of chitosan:$BrCH_2CH_2COOH$ = 1 : 5; reaction duration = 48 h.
Py = pyridine; 2,6-Lu = 2,6-lutidine; DmaPy = 4-*N*,*N*-dimethylaminopyridine; Dabcn = 1,5-diazabicyclo[4.3.0]non-5-ene.
[a] In the case of pyridine, the formation of carboxyethylated (quaternized) pyridine was also observed; molar fraction 64%.

TABLE V
Degree of Carboxyethylation and Molar Fraction (%) of the Reaction Products at Chitosan Alkylation in the Presence of Metal Cations–Halogen Acceptors

X	Cl			Br			I		
M^{n+}	Pb^{2+}	Ag^{+}	Tl^{+}	Pb^{2+}	Ag^{+}	Tl^{+}	Pb^{2+}	Ag^{+}	Tl^{+}
DS(NMR)	0	0.12	0.10	0	0	0.01	0.01	0.03	0.18
$HalCH_2CH_2COOH$	87	30	94	87	43	74	78	10	9
$HOCH_2CH_2COOH$	3	32	2	9	31	6	4	71	47
$O(CH_2CH_2COOH)_2$	9	34	1	4	25	19	16	16	34
CH_2=CHCOOH	1	0	0	0	0	1	1	3	~0
CE-chitosan	0	4	1	0	0	1	1	0	7

Solution = water; temperature = 20°C; molar ratio of chitosan:XCH_2CH_2COOH:$M(NO_3)_x$:KOH = 1 : 3 : 2 : 2; reaction duration = 24 h.

any degree of acetylation and in the absence of organic solvent or crosslinking agent. However, no polymer transformations have been carried out in such gel systems to date. In this work, we found that under conditions shown in Table VI, chitosan formed a physical hydrogel. The results provided in Table VI testify that even in the absence of a base, high degrees of alkylation can be obtained and formation of foam is eliminated. At the addition of the base (lithium carbonate was shown to be the most efficient among alkaline carbonates), the degree of alkylation still increased in the same order of chloro-, bromo-, and iodopropionic acid. The obtained values of DS could be attained in true solutions only in 2 or more days.[11] Under gel-state conditions, the reactivity of halopropionic acids is approximately equal, hence it can be suitable to use the least expensive chloropropionic acid.

Thus, the suggested procedure for chitosan carboxyethylation in a gel state using halopropionic acid and lithium carbonate seems to be the most convenient, because it requires the shortest time and provides for the highest values of DS.

CONCLUSIONS

Investigation of the composition of the reaction solution indicated that carboxyethylation of chitosan was accompanied by the reactions of hydrolysis and dehydrohalogenation of halopropionic acids. The product of dehydrohalogenation—acrylic acid—was subsequently added to chitosan yielding the desired end-product, and thus the ratio of the main product and the by-products has been permanently changing in the course of the reaction.

The results obtained indicate that the highest degrees of chitosan alkylation using alkaline metal halopropionates can be attained under slightly alkaline conditions in the presence of excess $NaHCO_3$.[11] However, this reaction has the drawback of being accompanied by the formation of a stable foam. As an alternative, a mixture of halopropionic acid with its salt can be used; the acid is applied in a stoichiometric amount required for dissolution of chitosan and its salt is added in an excess amount. This reaction scheme prevents the formation of carbon dioxide and subsequent foaming; however, the resulting degree of alkylation is somewhat lower.

During carboxyethylation of chitosan with the alkaline salts of bromopropionic acid, the DS increased in the order $Cs^{+} < Rb^{+} < K^{+} \sim Na^{+} < Li^{+}$. Use of lithium salts resulted in the predominant occurrence of dehydrohalogenation that led to the formation of acrylic acid, which in the end resulted in a higher degree of carboxyethylation of the end-product. Of all halopropionic acids, using bromopropionic acid yielded the lowest DS.

When using the salts of alkaline earth metals $XCH_2CH_2COOM_{0.5}$ (M = Be^{2+}, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}), the highest degrees of alkylation were obtained by using strontium and barium salts, which is very advantageous since these cations can be subsequently easily removed from the reaction mixture by precipitation as sulfates.

Among the organic bases applied, the highest degree of alkylation was achieved using the moderately strong base triethylamine. In the case of pyridine, a significant amount of quaternized *N*-(2-carboxyethyl)pyridinium was formed.

When using the addition of halogen acceptors—salts of lead, silver, and thallium—the highest yields of the alkylated product were obtained using a system comprising thallium and iodopropionic acid; however, binding of the released halogen did not

TABLE VI
Degree of Carboxyethylation of Chitosan in Gel State

X	Conditions	DS
Cl	20% gel	0.26
Br	20% gel	0.35
I	20% gel	0.37
Cl	15% gel, 4.6 equiv. Li_2CO_3	0.83
Br	15% gel, 4.6 equiv. Li_2CO_3	1.00
I	15% gel, 4.6 equiv. Li_2CO_3	1.02

Solution = 5 equiv. halopropionic acid; temperature = 60°C; reaction duration = 24 h.

contribute to a marked increase in the degree of alkylation and the yield was not higher than that achieved by using bases. This result can be explained by the fact that only an insignificant amount of acrylic acid was produced in this process.

It can therefore be concluded that chitosan alkylation using the alkaline metal salts of halopropionic acids gives comparable or even better results than the addition of acrylic acid.[15,16]

The drawback of both processes, i.e., the necessity of using rather diluted solutions, can be circumvented by using an in-gel procedure for chitosan carboxyethylation.

Thus, the following results have been obtained in this work dealing with elaboration of a fast and straightforward method of chitosan carboxyethylation:

- Application of moderately strong bases, such as lithium carbonate or triethylamine, is most suitable for attaining the highest degree of carboxyethylation
- It has been demonstrated for the first time that a novel process modification—gel-state synthesis—can provide higher DS and increased yield in a shorter reaction time than similar reactions carried out in true solutions

Therefore, it can be recommended that, to achieve the best results, carboxyethylation of chitosan should be carried out using gel technology and applying moderately strong bases. The suggested approach can find application at the preparation of various derivatives of chitosan.

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