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Polyampholytes from natural building blocks: synthesis and properties of chitosan-*O*-alginate copolymers

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Chitosan-*O*-alginate comb copolymers with a different degree of chitosan substitution were synthesized using chitosan protected by phthaloylation and "activated" by tosylation, and reduced low-molecular-weight sodium alginate. The obtained chitosan derivatives were characterized by FT-IR and ^{1}H NMR spectroscopy, functional group analysis, viscometry and potentiometric titration. The novel nature-friendly polyampholytes contained a 15–25-fold excess of carboxyl groups and were soluble in neutral and alkaline water. The intrinsic viscosity and molecular weight of these chitosan derivatives were relatively low, suggesting a significant breakdown of chitosan backbone under derivatization. The solution viscosity of the polyampholytes had a tendency to decrease by lowering the pH toward a supposed isoelectric point at pH 3.5–4.0. Comb-shaped copolymers in aqueous solutions are expected to form tight coils which are stabilized by intra- and intermolecular complexes between carboxyl and amine groups.

Key words: chitosan, sodium alginate, comb copolymer, polyampholyte, nature-friendly

INTRODUCTION

Polyampholytes are charged macromolecules carrying both acidic and basic groups [1, 2]. Under appropriate conditions, e. g. in aqueous solutions, these groups dissociate, leaving ions on chains and counterions in solutions. After ionization, there are positively and negatively charged groups on the polymer chain. Examples of polyampholytes include denatured proteins (e. g. gelatin), proteins in their native state (such as bovine serum albumin), and synthetic copolymers made of monomers containing acidic and basic groups.

Chitosan is a cationic copolymer of glucosamine and *N*-acetylglucosamine, prepared by partial deacetylation of the natural polymer chitin which is one of the most abundant polysaccharides in nature and is mostly derived from the exoskeleton of crustaceans [3–5]. Chitosan and its derivatives are used in various fields: water treatment [6, 7], biomedicine [8–10], cosmetics [11, 12], agriculture [13], food industry [14–17]. However, applications of chitosan suffer from severe limitations since chitosan is normally insoluble at a neutral or alkaline pH because of its very strong hydrogen bonds and stable crystalline structure. Chitosan has reactive amino and hydroxyl groups, both of which can be used to chemically alter chitosan properties under mild conditions.

The number of publications concerning chitosan *O*-derivatives containing polymeric or oligomeric grafts is not large. Liu et al. reported a synthesis of chitosan-*graft*-polycaprolactone copolymer which as an amphoteric natural / synthetic hybrid material was expected to be of considerable importance in many fields [18, 19]. Coupling of chitosan and PEG afforded water-soluble copolymers that were of great interest because of their biodegradability and wide potential applications as biomedical materials [20–23]. Derivatization of chitosan with mannose [24] or maltose [25] resulted in water-soluble products that were interesting from the viewpoint of their distinctive bioactivities. No publications were found on the modification of chitosan with negatively charged natural oligomers, e. g. alginate.

Alginate is a salt form of alginic acid. It is a linear binary copolymer consisting of (1–4)-linked β-D-mannuronic and α-L-guluronic acid residues. The relative amount of the two uronic acid monomers and their sequential arrangement

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along the polymer chain vary widely depending on the origin of the alginate. Commercial alginates are produced mainly from *Laminaria hyperbolia, Macrocystis pyrifera, Laminaria digitata, Ascophyllum nadosum, Laminaria japonica, Eclonia maxima, Lessonia nigrescens, Durvillea antarctica* and *Sargassum* spp. Alginic acid has already established its applications in food, pharmaceutical and medical industries. However, not much has been reported on the chemical modification of alginic acid or its salts.

The present work is focused on a detailed study of the synthesis of chitosan-*O*-alginate comb copolymers by attaching oligomeric alginate to the hydroxyl functionalities of chitosan. Novel polyampholytes carrying a positively charged backbone and negatively charged side chains are expected to be interesting as materials made from naturally occurring building blocks and thus being nature-friendly and biodegradable, which is very important in laundry applications.

EXPERIMENTAL

Materials

Chitosan (M_w 400 kDa, deacetylation degree 72%) was purchased from Aldrich. The degree of deacetylation (DA) of chitosan was calculated according to the content of primary amino groups and the content of nitrogen in chitosan determined experimentally by potentiometric titration and elemental analysis, respectively [26]. Sodium alginate (viscosity of 2% aqueous solution at 25 °C ~250 cps) was purchased from Aldrich. Phthalic anhydride was from Aldrich, and toluene-4-sulfonyl chloride (tosyl chloride) was from Fluka. All other reagents and solvents were of analytical grade.

Preparation of low molecular weight sodium alginate

A 3.4% aqueous solution of sodium alginate (18 g) was acidified to pH 5.6 by addition of aqueous 0.1 M HCl and heated for 3.5 h at 95 °C under nitrogen in an oven. The solution was cooled to room temperature, adjusted to pH 3.6 by adding 0.1 M HCl and heated for additional 4 h at 95 °C under nitrogen. The resulting solution was cooled and neutralized using aqueous 0.1 M NaOH. The product was precipitated by pouring the solution into acetone, filtrated, washed with acetone and dried in an oven to give 15.7 g of the product (yield 87%).

Reduction of the hydrolyzed sodium alginate

7 g of hydrolyzed sodium alginate was dissolved in 230 ml of aqueous 0.1 M sodium phosphate (pH 8), and 11.3 g of $NaBH_4$ was added. The reaction was carried out for 2 h under stirring at room temperature. The reaction was terminated by adding 10 ml of concentrated HCl in order to decompose the reducing agent $NaBH_4$. The resulting solution was dialyzed for a week against distilled water using a Visking (SERVA) dialysis membrane (MWCO 12–14 kDa) or subjected to ultrafiltration through an Omega (FILTRON) membrane (MWCO 1 kDa). The product was precipitated by pouring the solution into acetone, filtrated, washed with acetone and dried in an oven to give 6 g of the product (yield 86%).

Preparation of *N*-phthaloyl chitosan and 6-*O*-tosyl-*N*-phthaloylchitosan

The synthesis of *N*-phthaloyl chitosan was done according to the procedure described elsewhere [27, 28]. Anal. Calcd for $[C_{14}H_{13}O_6N]_{72}[C_8H_{13}O_5N]_{28}$: C 55.50%, H 6.10%, N 5.27%. Found: C 54.08%, H 5.5%, N 5.20%. ^{1}H NMR spectrum (DMSO-d_6, ppm): δ = 1.8 (CH_3 in acetamide), δ = 2.7–4.4 (pyranose ring), δ = 7.5–7.8 (aromatic ring).

The synthesis of 6-*O*-tosyl-*N*-phthaloyl chitosan was done by the method described before using *N*-phthaloyl chitosan as a raw material [29]. Anal. Calcd for $[C_{21}H_{19}O_8SN]_{72}[C_{15}H_{19}O_7SN]_{28}$: C 55.15%, H 4.52%, N 3.33%. Found: C 54.97%, H 4.02%, N 3.52%.

Synthesis of chitosan-*O*-alginate copolymers

6-*O*-tosyl-*N*-phthaloyl chitosan (0.1 g, 0.24 mmol) and NaOH (0.23 g, 5.75 mmol) were dissolved in DMF (100 ml). The reduced sodium alginate (3 g, 0.33 mmol) was dissolved in distilled water (300 ml), and the resulting solution was added slowly to the solution of 6-*O*-tosyl-*N*-phthaloyl chitosan. The reaction between 6-*O*-tosyl-*N*-phthaloyl chitosan and sodium alginate was carried out for 48 h at 50 °C under stirring and nitrogen atmosphere. The solution containing the product was dialyzed for a few days against distilled water using a Visking (SERVA) dialysis membrane (MWCO 12–14 kDa), ultrafiltrated using 30 kDa Omega (FILTRON) membrane and lyophilized at –40 °C using Christ ALPHA 2–4 LSC freeze-dryer to give 1.55 g of the product (yield 70%).

In order to remove the protecting phthaloyl groups, 1.3 g of *N*-phthaloyl chitosan-*O*-alginate was spread in 130 ml of DMF, and 13 ml of hydrazine monohydrate was added dropwise. The reaction mixture was stirred for 2 h at 80 °C under nitrogen atmosphere, and the resulting solution containing a target copolymer was dialyzed and lyophilized to give 1.03 g of the product (yield 83%).

Analytical procedures

FT-IR spectra were recorded with a Perkin–Elmer Spectrum BX spectrometer under dry air at 20 °C by the KBr pellet method. ^{1}H NMR spectra of the samples dissolved in D_2O were recorded on a UNITY INOVA VARIAN spectrometer at 300 MHz and 29 °C.

The intrinsic viscosity of polyampholyte solutions in distilled water and in acetate buffer (aqueous 0.5 M CH_3COOH / 0.5 M CH_3COONa) at 25 °C was measured using a dilution type Ubbelohde viscometer.

pH-Potentiometric titrations were carried out in aqueous solutions using a CyberScan pH6000 pH-meter with a glass electrode. 0.05 g of a polyampholyte was dissolved in 10 ml of water or 0.1 M NaCl, and the solutions were titrated with 0.01 M NaOH.

For determination of the isoelectric point of the polyampholytes, samples containing 0.05 g of a chitosan-*O*-alginate copolymer in 10 ml of water were titrated with aqueous 0.01 M NaOH measuring the flow time of the solution through a capillary of a viscometer at fixed values of pH differing by about 0.5.

The content of primary amino groups was determined by a spectrophotometric assay procedure [30]. Solutions of chitosan-*O*-alginate copolymers containing $2 \cdot 10^{-5}$–$6 \cdot 10^{-4}$ mol/l of amino groups were prepared by dissolving polyampholytes in 4 M acetate buffer (pH 5.2). The ninhydrin reagent was prepared as follows: 1 g of ninhydrin was dissolved in 38 ml of DMSO while flushing with nitrogen, 13 ml of 4 M acetate buffer (pH 5.2) was added, and the resulting solution was further bubbled with nitrogen; 1 ml of a sample solution was poured into a test-tube, mixed with 1 ml of acetate buffer and 2 ml of ninhydrin reagent, and the test-tube was kept in a boiling water bath for 30 min. The solution was cooled down to room temperature, and its UV absorbance was measured at 570 nm wavelength. A standard absorbance curve was generated using aqueous solutions of D-glucosamine.

The degree of chitosan substitution (DS, %) indicates the average number of alginate chains per 100 monosaccharide residues of chitosan. The DS of chitosan was calculated referring to the content of primary amino groups in the products by the following equation:

$$\mathrm{DS} = \frac{m - 173 \cdot c}{9000 \cdot c} \cdot 100,$$

where m is the concentration of a copolymer in a test sample, g/l; c is the concentration of amino groups, mol/l; 173 is the average molecular weight of a monosaccharide residue of chitosan (DD 72%); 9000 is the viscosity average molecular weight of sodium alginate.

RESULTS AND DISCUSSION

Preparation of low-molecular-weight reduced sodium alginate

Alginate is a salt form of alginic acid and consists of linear chains composed of two monosaccharides, D-mannuronic acid (mannuronate) (M) and L-guluronic acid (guluronate) (G). The content and distribution of M and G along the chains vary considerably, leading to a large span in rheological and gel-forming properties.

Degradation of polysaccharides occurs via cleavage of glycosidic linkages. Compared to other sugars, glycosidic linkages between uronic acids, such as M and G, are relatively resistant to hydrolysis in very strong acids, e. g. under conditions used to convert polysaccharides to monosaccharides [31]. At pH values close to the value of pKa of alginate (at pH 1–4), the degradation rate is less dependent on pH. In this range, the protonated (-COOH) form of M and G contributes to the hydrolysis by intramolecular catalysis in addition to the free H^+ ions.

The degradation of sodium alginate is presented in Scheme 1. Due to the viscous solution of the polysaccharide, the reaction proceeded fairly slowly. When the hydrolyzed sodium alginate was precipitated, the material was white, but it became slightly coloured under drying. The intrinsic viscosity of the raw sodium alginate was 4.46 dL/g (in 0.1 M NaCl) but decreased to 0.31 dL/g after hydrolysis, confirming drastic changes in the molecular weight of the polysaccharide.

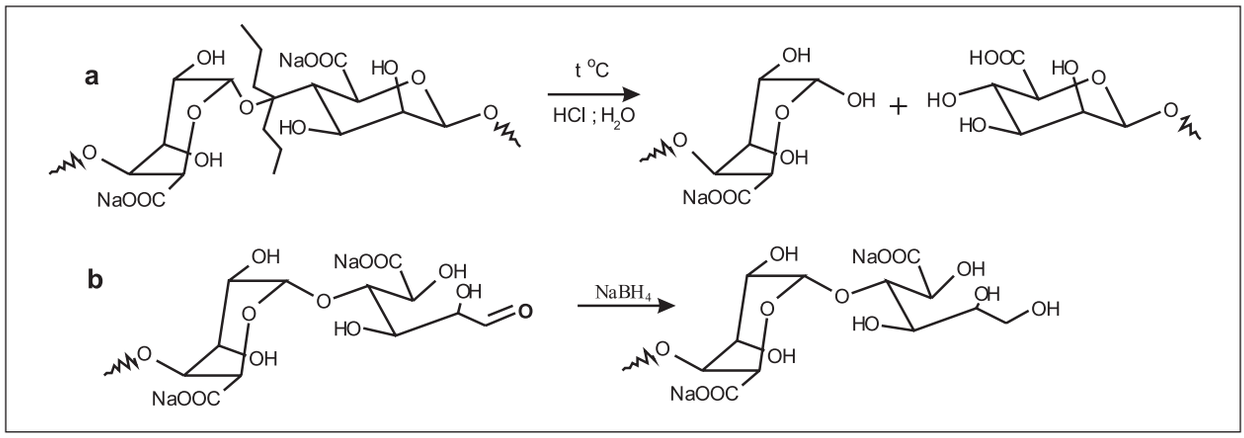
The FT-IR spectra of degraded alginates did not reveal new absorption bands; hence, no considerable alterations in the structure of the alginates had occurred. The bands corresponding to the stretching vibrations of carbonyl in a protonated and unprotonated carboxylic group are at 1730 and 1631 cm^{-1}, respectively. When the proton is displaced by Na ions, the absorption bands are observed at 1614 cm^{-1} and 1431 cm^{-1}, and they are assigned to the symmetric and antisymmetric stretching vibrations of COO^-, respectively [32]. The FT-IR spectrum of the degraded sodium alginate had absorption bands at 1032 cm^{-1} (OH), 1418 cm^{-1} (COO^-) and 1619 cm^{-1} (COO^-).

The M and G ratio in the chains of degraded alginate was estimated analysing the ^1H NMR spectra of a compound [33]. The signal at δ 4.95 ppm was assigned to H-1 protons of guluronic acid residue while the signal at δ 4.75 ppm to the sum of H-1 protons of mannuronic acid residue and H-5 protons of mannuronic acid residue having a neighboring G residue. The signal at δ 4.55 ppm was attributed to H-5 protons of the guluronic acid residue having a neighboring G residue. The calculated amount of guluronic acid residues was ca 52%, i. e. the content of both M and G along the chains was almost equal.

Alginate, like the majority of polysaccharides, has a reducing end. Reduction of the hydrolyzed alginate using $NaBH_4$ resulted in an alginate containing a primary hydroxyl group at the end of its macromolecule (Scheme 1). The presence of just one primary hydroxyl group in the reduced alginate was a keystone for coupling the reduced alginate with 6-*O*-tosyl-*N*-phthaloyl chitosan and getting comb-like derivatives of chitosan. Since sodium borohydride is more stable in alkaline solutions [34], the reaction of alginate reduction was carried out at pH 8. The amount of aldehyde groups was evaluated by a spectrophotometric assay using alkaline 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole reagent (Purpald) [35]. The initial alginate solution gave red colour, while reduced alginate had no colour.

Purification of the reduced sodium alginate from low-molecular-weight substances was complicated. Membrane dialysis was very slow, the volume of the dialysate rose 10–20 times, and the yield was low, hardly reaching 50%. Ultrafiltration was a more efficient method of purification lasting only one day and giving a yield of ca 90%.

The molecular weight of the hydrolyzed reduced alginate was estimated by the viscometric method. Viscosity-average molecular weight (M_v) was calculated using the Mark–Houvink equation for alginates described elsewhere [36]: $[\eta] = 7.30 \times 10^{-5} \times M^{0.92}$. Calculations based on intrinsic



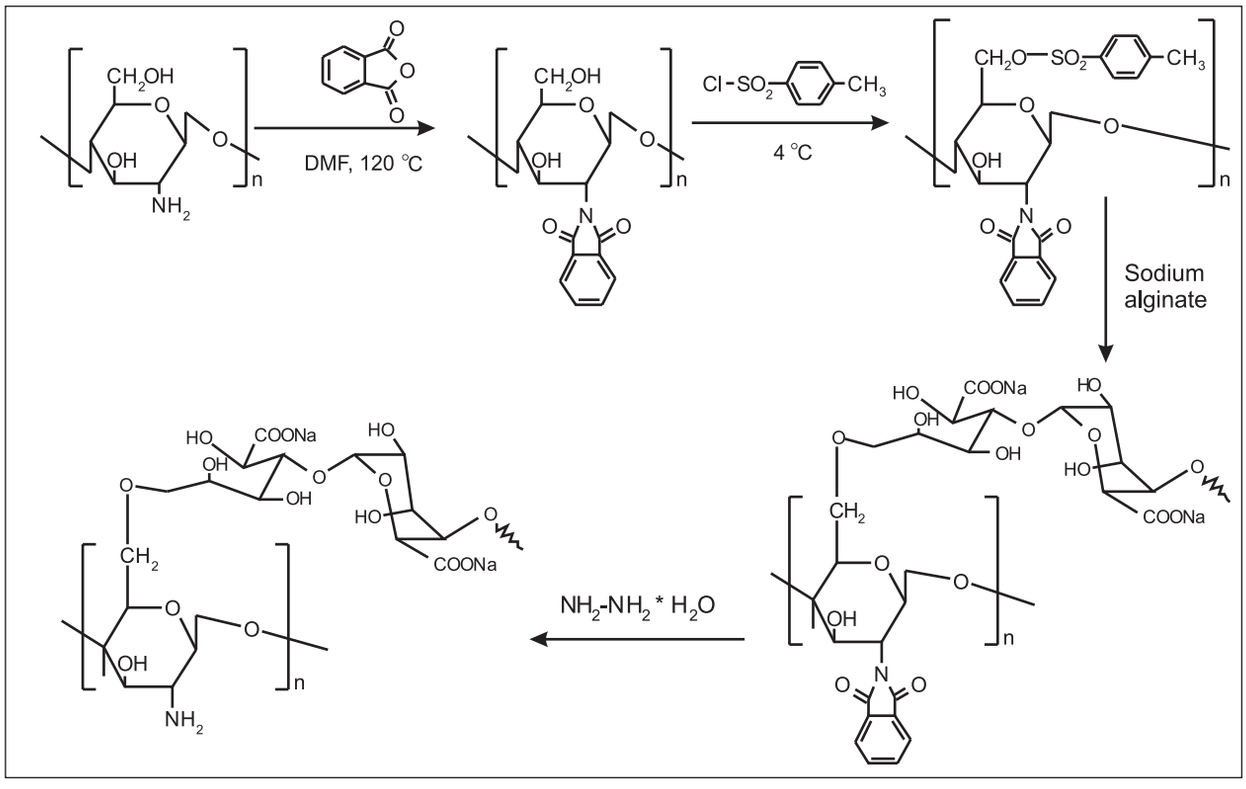
Scheme 1. Acidic hydrolysis (a) and sodium alginate reduction (b)

viscosity [η] = 0.31 dL/g resulted in M_v = 9000. The value of the viscosity average molecular weight of alginate, M = 9000, was used for calculating the DS of chitosan. Because of uncertainty about the absolute molecular weight and polydispersity of alginate, the DS of chitosan in its derivatives should be considered as apparent.

Synthesis and structure of chitosan-*O*-alginate comb copolymers

The higher reactivity of amino groups present in the backbone of chitosan requires protection to limit their participation in reactions under modification of chitosan. This was done by the use of phthalic anhydride in DMF (Scheme 2) according to the procedure reported earlier [37]. *N*-Phthaloylation was complete under certain conditions proved by a negative ninhidrin test [29].

The (*p*-tolylsulfonyl)oxy group is one of the most effective leaving groups widely used in carbohydrate chemistry [38]. Since primary hydroxyl groups are more reactive than secondary ones, the regioselective reaction is expected and proved in many cases. Treatment of *N*-phthaloyl chitosan with a 10-fold excess of tosyl chloride and triethylamine in *N,N*-dimethylacetamide afforded 6-*O*-tosyl-*N*-phthaloyl chitosan with a very high degree of tosylation. It was proved



Scheme 2. Synthesis of chitosan-*O*-alginate comb copolymers

that primary hydroxyl groups of both glucosamine and *N*-acetyl glucosamine units could be completely changed to tosyl groups [29].

O-Tosyl-*N*-phthaloyl chitosan is an "activated" form of chitosan, which can be used as intermediate ideally suited for production of a wide variety of chitosan *O*-derivatives. Alginate through its primary hydroxyl group present in the terminal mannuronic or guluronic acid unit was grafted onto "activated chitosan" (Scheme 2). The reaction was carried out in a DMF-alkaline water mixture under homogeneous conditions. The reaction time was long (48 h) in order to achieve the maximal DS at a given ratio of the main reactants. The molar ratio between chitosan and alginate was changed from 1 : 0.21 to 1 : 1.5, and the obtained DS varied from 25 to 44% (Table). The final products – chitosan-*O*-alginate copolymers – were received by deprotection of amino functionalities using hydrazine monohydrate. New absorption bands at 1615 cm^{-1} and 1417 cm^{-1} in the FT-IR spectra of *N*-phthaloyl chitosan-*O*-alginate copolymers were attributed to carboxylate groups of alginate (Fig. 1). The absorption band of the tosyl group at 1176 cm^{-1}, present in the spectrum of 6-*O*-tosyl-2-*N*-phthaloyl chitosan disappeared. These results demonstrated that the grafting of alginate to chitosan "activated" by tosyl groups was successful. The removal of protective phthaloyl groups was also efficient, whereas the absorption band at 1716 cm^{-1}, attributed to phthaloyl groups, disappeared.

The obtained *N*-phthaloyl chitosan-*O*-alginate copolymers were water-soluble over the entire pH interval. Formation of polyelectrolyte complexes between carboxyl and amine groups of the constituents was excluded because of the protection of amine functionality. Thus, the water solubility of *N*-protected derivatives of chitosan manifested formation of a new compound containing covalent bonds between chitosan and alginate.

^{1}H NMR spectra confirmed the presence of the structural units of both chitosan and alginate and, in particular cases, allowed evaluating the DS of chitosan (Fig. 2). Protons that belong to alginate moieties (H-2'–H-5') have signals inbetween 3.6 and 3.9 ppm; these signals overlap with the signals of the protons H-3–H-6 of the chitosan backbone. The signal

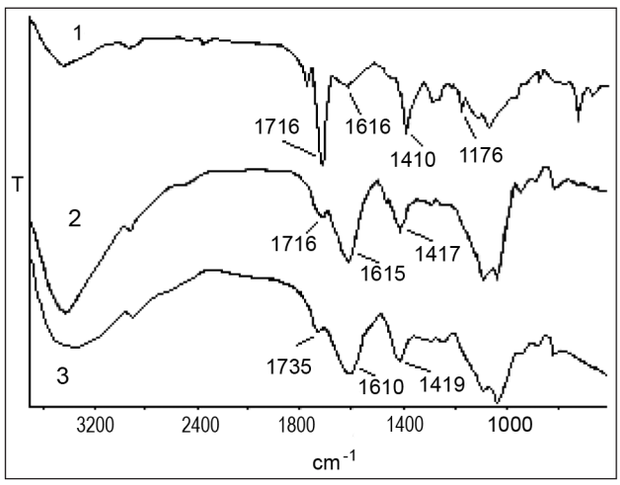


Fig. 1. FT-IR spectra of 6-*O*-tosyl-2-*N*-phthaloyl chitosan (1), *N*-phthaloyl chitosan-*O*-alginate (2) and chitosan-*O*-alginate (Chital-2) (3)

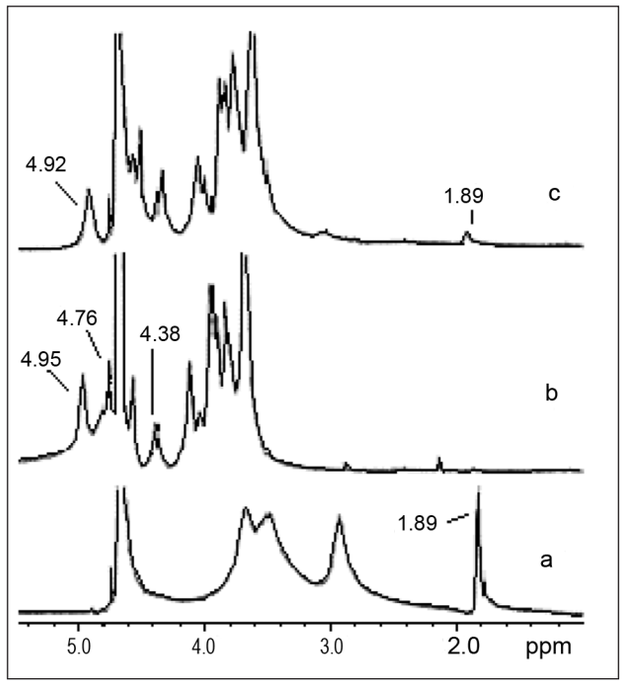


Fig. 2. ^{1}H NMR spectra of chitosan (a) in D_2O / acetic acid-d_4, hydrolyzed reduced sodium alginate in D_2O (b) and chitosan-*O*-alginate (Chital-1) in D_2O (c)

Table. **Results of the analysis of chitosan-*O*-alginate copolymers**

Compound	Molar ratio[a]		NH_2[b], %	Alg[c], %	DS[d], %	DS[e], %	Yield, %	[η][f], dL/g
	Chitosan	Alginate						
Chital-1	1	0.21	0.67	92.8	25	16	52	0.177
Chital-2	1	0.37	0.49	94.6	34	–	61	0.191
Chital-3	1	1.5	0.39	95.8	44	–	43	0.221

[a] Molar ratio of tosylated chitosan glucosamine units and alginate in the reaction mixture;

[b] Content of NH_2 groups in the copolymers determined spectrophotometrically;

[c] Content of alginate in the copolymers calculated from the content of NH_2 groups;

[d] Calculated according to the content of NH_2 groups;

[e] Calculated from ^{1}H NMR spectra of the copolymers;

[f] In acetate buffer.

at 4.9 ppm was attributed to the H-1' proton of guluronic acid residue, and that at 1.89 ppm was assigned to the protons in the acetyl group of *N*-acetyl glucosamine units of chitosan. Calculation of the DS of chitosan is based on the ratio of the signal at 1.89 ppm to the signal at 4.9 ppm.

The intrinsic viscosity [η] of chitosan-*O*-alginate copolymers in acetate buffer was relatively low, confirming the low molecular weight of the polyampholytes (Table). It had already been reported [24, 29, 39] that a certain breakdown of chitosan backbone took place under its derivatisation if *N*-phthaloylation had been used to protect the amino functionalities of chitosan. A large breakdown of chitosan backbone, resulting in graft oligomers, was determined during the synthesis of chitosan-*O*-PEG copolymers [22], which was thought to depend on the amino group protection–deprotection procedure. The extent of the breakdown of chitosan main chain was suggested to be consistent with the molecular weight of the attached chains [29]. Since the molecular weight of the alginate used for grafting to chitosan was relatively high (M_v = 9000), the low molecular weight of the novel polyampholytes was comprehensible and even expected.

Solution properties of novel polyampholytes

Chitosan-*O*-alginate copolymers were soluble in alkaline and neutral media. Dissolution of the copolymers in distilled water gave the pH of the solutions 4.0–4.5. The insolubility of polyampholytes in acidic media was explained by a high content of alginate in the copolymers, resulting in a 15–25-fold excess of carboxylic groups over amine groups. Solutions of chitosan-*O*-alginate copolymers showed colour reactions with 2,4,6-trinitrobenzoic acid and nynhidrin, specific of amine groups [30, 40]. Mixing solutions of chitosan-*O*-alginate copolymers with solutions of $CaCl_2$, $CuCl_2$ and $FeCl_3$ gave jelly-like dregs which indicated the chelating properties of the novel polyampholytes, confirming the presence of carboxyl groups.

Determination of the intrinsic viscosity of chitosan-*O*-alginate copolymers in water failed since in water the chitosan derivatives showed a strongly expressed polyelectrolyte effect which was evidenced by an increase in viscosity at lower polymer concentrations. The viscosity of aqueous solutions of chitosan-*O*-alginate copolymers was affected by ionic strength: addition of the low-molecular electrolyte NaCl (0.1–0.5 mol/l) gave a lower reduced viscosity irrespective of pH (Fig. 3). Such behaviour is usually characteristic of polyelectrolytes containing one type of charged groups [41]. The obvious effect of ionic strength on the viscosity of chitosan-*O*-alginate copolymers is explained by a considerable excess of carboxylic groups over amine groups in these polyampholytes. In the polyelectrolyte regime with no added salt, the polyampholyte chains were stretched by the repulsion among carboxylate anions. When a salt had been added, the Debye length decreased, and the repulsion between excess charges began to be screened once the Debye length became smaller than the chain size.

As is usual for polyelectrolytes, the viscosity of chitosan-*O*-alginate solutions depends on pH (Fig. 3). In neutral and slightly alkaline media, carboxyl groups of alginate are ionized, and repulsion among negatively charged carboxylate anions takes place, resulting in a relatively higher viscosity of solutions. In slightly acidic media (at pH 4–6), amine groups become protonated and part of carboxylate anions become neutralized. The contribution of partial neutralization of carboxylate anions to repulsion between the neighbouring groups is more significant since carboxyl groups are in large excess, and this results in a decrease of solution viscosity. The lowest viscosity should be at a pH value when chitosan-*O*-alginate has an equal number of positive and negative charges, i. e. at the isoelectric point. Since alginate chains are composed of mannuronic and guluronic acid units whose pK_a values are reported as 3.38 and 3.65, respectively [42], and the pK_a value of chitosan is at 6.3–6.7 [43, 44], the isoelectric point of polyampholyte chitosan-*O*-alginate with a 15–25-fold excess of carboxylic groups is expected at pH 3.5–4.0. A decrease of solution viscosity at lower pH confirms these expectations (Fig. 3). Unfortunately, at pH 3.5–4.0 chitosan-*O*-alginate copolymers becomes insoluble, disabling the determination of the isoelectric point.

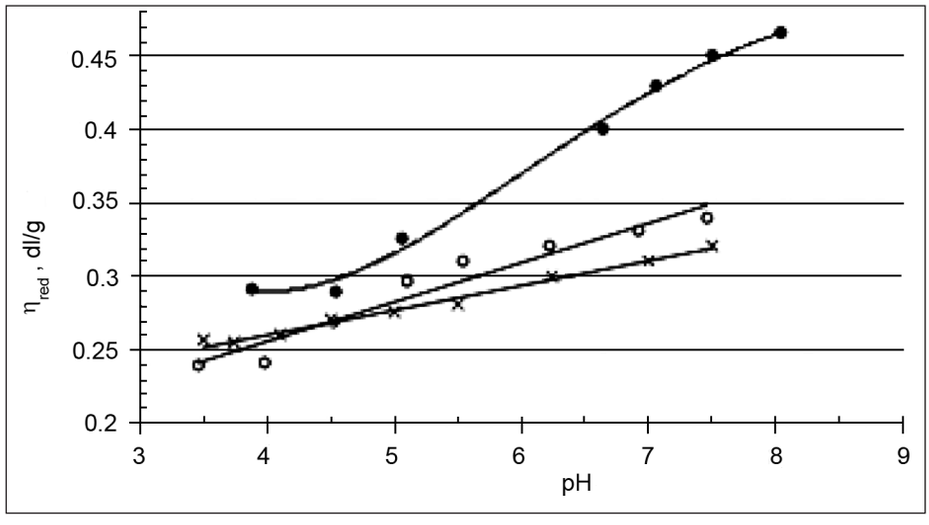


Fig. 3. Reduced viscosity of chitosan-*O*-alginate (Chital-3) in water (-•-), 0.1 M NaCl (-○-) and 0.5 M NaCl (-×-) as a function of pH

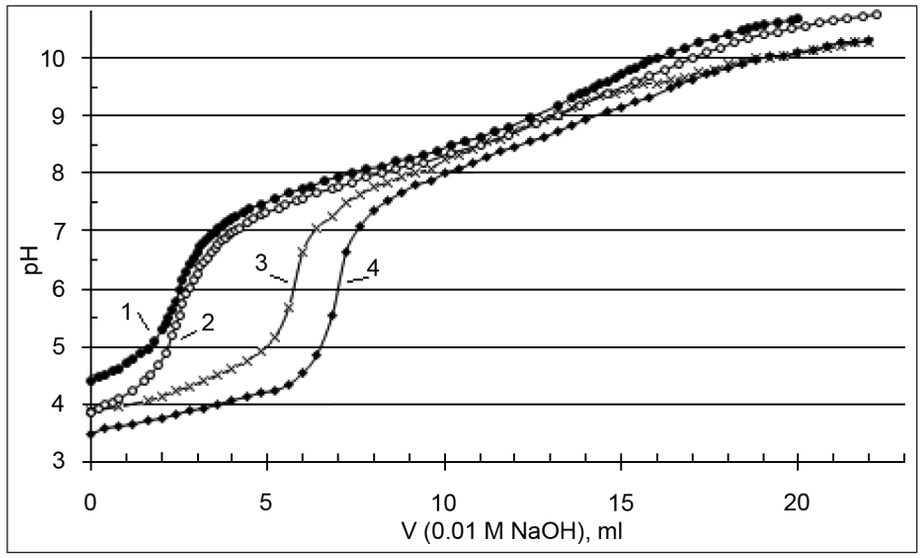


Fig. 4. Potentiometric titration curves of chitosan-*O*-alginate copolymers Chital-2 (1, 2) and Chital-3 (3, 4) in water (1, 3) and 0.1 M NaCl (2, 4)

The alteration in the viscosity of the novel polyampholytes with pH is small compared to typical polyelectrolytes [45, 46]. This is possibly related to the comb-like structure of the copolymers forming relatively compact coils, even in ionized state. An increase in the ionic strength of the solution makes the alteration marginal (Fig. 3).

The potentiometric titration of chitosan-*O*-alginate copolymer solutions in water and 0.1 M NaCl with 0.01 M NaOH gave titration curves with two inflections (Fig. 4). It should be noted that these titration curves could not serve for determination of carboxyl groups since the copolymers were not titrated at low pH where they were insoluble. The first inflection (at pH about 5–6.5) corresponds to the neutralization of carboxyl groups, while the second is related to the neutralization of chitosan amino groups. The second inflection is badly resolved, apparently broadened and almost hidden for copolymers with a higher DS of chitosan. One can assume that at least part of amine groups of chitosan were protonated by carboxyl groups since only these groups being attached to grafted alginate chains formed complexes of different stability. The stability of intra- and intermolecular complexes between carboxylic groups from the alginate and amine groups of chitosan (or, the same, pH of neutralization of bound carboxylic groups) depends on several factors including accessibility of functional groups, conformation of chitosan-*O*-alginate copolymers, "neighbour" effect, etc. A tight packing of comb-shaped copolymers makes the accessibility of the functional groups of novel polyampholytes more difficult compared to complexes of linear polyelectrolytes. Addition of the external electrolyte NaCl shielded charges, enhancing the ionization of both carboxyl and amino groups and partially destroying complexes between alginate and chitosan chains. These changes were reflected in potentiometric titration curves (Fig. 4, curves 2 and 4) which were shifted down and right, showing a higher acidity of the polyampholytes in the presence of an external electrolyte.

CONCLUSIONS

Novel chitosan-*O*-alginate comb copolymers were synthesized using chitosan protected by phthaloylation and "activated" by tosylation, and reduced sodium alginate. The obtained nature-friendly polyampholytes contained a 15–25-fold excess of carboxyl groups and were soluble in neutral and alkaline water. The intrinsic viscosity and molecular weight of these chitosan derivatives were relatively low, suggesting a significant breakdown of the chitosan backbone under derivatization. The solution viscosity of the novel polyampholytes had a tendency to decrease by lowering the pH toward a supposed isoelectric point at pH 3.5–4.0. Comb-shaped copolymers in aqueous solutions are expected to form tight coils which are stabilized by intra- and intermolecular interactions between carboxyl and amine groups.

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POLIAMFOLITAI IŠ GAMTINIŲ POLIMERŲ FRAGMENTŲ: CHITOZANO-*O*-ALGINATO KOPOLIMERŲ SINTEZĖ IR SAVYBĖS

S a n t r a u k a

Pirmą kartą susintetinti įvairaus pakeitimo laipsnio „šepečio“ struktūros chitozano-*O*-alginato kopolimerai, pasižymintys poliamfolitų savybėmis. Mažos molekulinės masės redukuotas natrio alginatas priskiepytas prie chitozano, pastarojo amino grupę blokuojant ftalinimu ir pirmines hidroksigrupes „aktyvuojant“ tozilinimu. Chitozano darinių struktūra įrodyta pasitelkus FT-IR ir ^{1}H BMR spektrus, savybės ištirtos naudojant viskozimetriją ir potenciometrinį titravimą. Karboksigrupių šiuose „gamtai draugiškuose“ poliamfolituose buvo 15–25 kartus daugiau nei aminogrupių, jie buvo tirpūs neutraliame ir šarminiame vandenyje. Poliamfolitų molekulinė masė ir ribinis klampos skaičius santykinai mažas, o tai leidžia teigti, kad skiepijimo reakcijų metu iš dalies skilo chitozano grandinė. Vandeniniuose tirpaluose „šepečio“ struktūros poliamfolitų makromolekulės yra kompaktizuotos dėl chitozano aminogrupių ir alginato karboksigrupių vidumolekulinių kompleksų.