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Polyelectrolytes from natural building blocks: synthesis and properties of chitosan-*O*-dextran graft copolymers

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Chitosan-*O*-dextran graft copolymers with the degree of substitution (DS) of chitosan varying from 25 to 90% were synthesized using dextran with the molecular weight 1500 and 6000. Grafting of dextran on the C-6 position of glucosamine residues of chitosan was achieved by protecting amino functionality by phthaloylation and "activating" primary hydroxyl groups of chitosan by tosylation. The chemical structure of the chitosan derivatives was confirmed by FT-IR and ^{1}H NMR spectroscopy, the DS of chitosan in its derivatives was determined from the content of nitrogen in the products. Chitosan-*O*-dextran graft copolymers were hydrophilic products soluble in aqueous solutions in a wide pH range and exhibited an apparent polyelectrolytic effect. The intrinsic viscosity of these chitosan derivatives was relatively low, especially of those containing dextran-6000 grafts. Attachment of dextran at the C-6 position of glucosamine residues significantly enhanced the breakdown of the chitosan backbone during the amino group protection–deprotection procedure using *N*-phthaloylation.

Key words: chitosan, dextran, graft copolymer, phthaloylation, tosylation, polyelectrolyte

INTRODUCTION

Chitin is one of the most important natural polysaccharides, and it is widely found in the shells of arthropods. Chitosan is its principal derivative. These substances have many applications in flocculation and coagulation, in food processing, heavy-metal ion recovery from wastewaters, cosmetics, drug delivery systems, artificial skin and wound repairing materials because of their good biocompatibility and biodegradation, as well as nontoxicity [1–5].

Modification of chitosan is difficult because of its lacking solubility. The reactions under heterogeneous conditions are accompanied by various problems, including poor extents of reaction, the structural ambiguity of the products, and partial degradation due to harsh reaction conditions. Chitosan contains two different types of functionalities – amino and hydroxyl groups in every monosaccharide residue, which are able to undergo many reactions of amines and alcohols. There are a lot of publications concerning modification of chitosan by amino groups. Nevertheless, modification of chitosan by hydroxyl groups may have an advantage because of free amino groups in the derivatised product and its retained cationicity.

The number of publications concerning the *O*-derivatives of chitosan containing polymeric or oligomeric grafts is not big. Liu et al. reported a synthesis of a chitosan-*graft*-polycaprolactone copolymer that as an amphoteric natural / synthetic hybrid material was expected to be of considerable importance in many fields [6, 7]. Coupling of chitosan and PEG afforded the water-soluble copolymers that were of great interest because of their wide potential applications as biodegradable and biomedical materials [8–11]. Derivatisation of chitosan with mannose [12] or maltose [13] resulted in water-soluble products that were interesting from the viewpoint of their distinctive bioactivities. Surprisingly, chitosan was not yet derivatised with oligomeric dextran.

Dextran is a water-soluble polysaccharide that consists mainly α-1,6 linked D-glucopyranose residues with a low percentage of α-1,2, α-1,3 and α-1,4 linked side chains. Usually dextrans are considered as linear polysaccharides or oligosaccharides. Generally, they are obtained by a species of *L. mesenteroides*, NRRL B-512F, which produces water-soluble dextran containing 95% linear α-(1→6) linkage and 5% α-(1→3) linkage [14]. Dextrans and their derivatives are of considerable biomedical and industrial importance and have a broad spectrum of applications ranging from drilling fluid additives and chromatographic support media, to drug carriers and blood plasma extenders.

In this paper, we report the synthesis of novel water-soluble chitosan-*O*-dextran derivatives. Derivatisation of chitosan through its hydroxyl groups is more complicated as compared with the preparation of chitosan-*N*-dextran copolymers [15] since it requires protection of more active amino groups. The novel materials were characterized by chemical, spectroscopic and viscometric methods.

EXPERIMENTAL

Materials

Chitosan (average M_v 400 kDa, degree of deacetylation 72%) and phthalic anhydride were purchased from *Aldrich*. Two

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oligomeric dextrans with molecular weight 1500 and 6000 (dextran-1500 and dextran-6000) and toluene-4-sulfonyl chloride (tosyl chloride) were purchased from Fluka. All other reagents and solvents were of analytical grade.

Activation of chitin and chitosan (syntheses that were done by the foregone methods)

a) Preparation of *N*-phthaloyl chitosan [16, 17]

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).

b) Preparation of *N*-salicyliden chitosan [18]

Anal. calcd for $[C_{13}H_{15}O_5N]_{72}$ $[C_8H_{13}O_5N]_{28}$: C 55.60%, H 5.73%, N 5.86%. Found: C 55.0%, H 5.9%, N 6.0%.

c) Tosylation of chitin [19]

Anal. calcd for $[C_{15}H_{19}O_7SN]$: C 50.3%, H 5.6%, N 3.89%, S 8.9%. Found: C 50.0%, H 5.9%, N 3.91%, S 8.7%.

d) Iodination of 6-*O*-tosylsalicyliden chitosan and chitin [20]

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

Triethylamine (9.5 ml, 68 mmol) and toluene-4-sulfonyl chloride (3.6 g, 18.8 mmol) dissolved in 20 ml *N,N*-dimethylacetamide (DMA) were gradually added to a cooled to 4–8 °C solution of *N*-phthaloylchitosan (0.5 g, 1.88 mmol) in DMA (50 ml), and the mixture was stirred at 8 °C overnight. The precipitate obtained by pouring the solution into ice water was collected by filtration, washed with chloroform, and dried to give 0.63 g of the product (yield 79%).

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%.

Preparation of 6-*O*-tosyl-salicyliden chitosan

A mixture of salicyliden chitosan (1.13 g, 4.5 mmol) in 45 ml of 42% aqueous sodium hydroxide was left standing at a reduced pressure for 3 h, and then 113 g of crushed ice made from deionised water was added to this mixture [20]. The mixture was cooled in an ice bath, and 33.9 g (0.18 mol) of toluene-4-sulfonyl chloride dissolved in 90 ml of chloroform was added under vigorous stirring. After 2 h the ice bath was replaced by a water bath (a temperature around 18 °C), and the mixture was stirred for additional 2 h. The mixture was poured into a large amount of deionased water under stirring, and the precipitant was washed until neutral pH. The resulting white fibrous product was washed with methanol and ether and dried to give 2 g of the product (yield 106%).

Synthesis of chitosan-*O*-dextran graft copolymers

Synthesis using 6-O-tosyl-N-phthaloyl chitosan intermediate

6-*O*-tosyl-*N*-phthaloyl chitosan (0.1 g, 0.24 mmol) and NaOH (0.24 g, 0.6 mmol) were spread in DMF (10 ml) and stirred for 1 h under nitrogen atmosphere at 40 °C. Dextran (0.36 g, 0.24 mmol) dissolved in 10 ml of H_2O was slowly added to the reaction mixture. The reaction was carried out for 48 h at 40 °C under stirring. The solution containing the resulting copolymer was dialyzed for 4 days using the Visking (SERVA) dialysis membrane (MWCO 12000–14000) against distilled water and lyophilized at –40 °C using the Christ ALPHA 2–4 LSC freeze-dryer to give 0.22 g of *N*-phthaloyl chitosan-*O*-dextran (yield 52%).

In order to remove protecting phthaloyl groups, 0.2 g of *N*-phthaloyl chitosan-*O*-dextran was dissolved in 30 ml of formamide, and then 4 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 the target copolymer was dialyzed and lyophilized according to the procedure described above to give 0.12 g of the product (yield 63%).

Anal. Calcd for $[C_{60}H_{101}O_{49}N]_{62}$ $[C_{62}H_{103}O_{50}N]_{28}$ $[C_6H_{11}O_4N]_{10}$: C 44.00%, H 6.2%, N 1.63%. Found: C 44.5%, H 6.0%, N 1.54% (DS 90%).

^{1}H NMR spectrum (D_2O, ppm): δ = 1.9 (CH_3 in acetamide), δ = 3.4–3.8 (pyranose ring), δ = 4.9 (H-1 in dextran).

Synthesis using iodosalicyliden chitosan intermediate

Dextran (1.56 g, 1 mmol) and NaOH (0.17 g, 4.25 mmol) were added to a dispersion of iodosalicyliden chitosan (0.1 g, 0.17 mmol) in 20 ml of DMF. The reaction was carried out at room temperature for 24 h under stirring. Then 15 ml of methanol was added ant the mixture was stirred for additional 30 min. The resulting precipitate was filtrated, washed with DMF, methanol and ether, and dried.

In order to remove protecting salicyliden groups, 0.6 g of salicyliden chitosan-*O*-dextran was dispersed in a mixture of 12 ml hydrazine monohydrate and 24 ml ethanol. The reaction was carried out at 70 °C for 24 h under stirring. After cooling, the reaction mixture was diluted with water and concentrated until solid residue using a rotating evaporator; this procedure was repeated several times. The residue was dissolved in deionised water and purified using gel-filtration chromatography (Sephadex-G25). 0.3 g of the graft copolymer chitosan-*O*-dextran was obtained (yield 83%).

Synthesis using iodochitin intermediate

Grafting of dextran on iodochitin was done by the method described above except the separation of the product. The reaction mixture was purified using a Visking (SERVA) dialysis membrane (MWCO 12000–14000) and concentrated until a solid residue using a rotating evaporator. In order to remove *N*-acetyl groups, the solid residue was dispersed in 20 M NaOH solution and kept overnight at 70 °C under nitrogen atmosphere. Then the reaction mixture was neutralized with HCl and purified successively by membrane dialysis and gel-filtration chromatography (Sephadex G-25)

Determination of the yield of chitosan derivatives and of the degree of *O*-substitution of chitosan

The yield of chitosan derivatives was evaluated by the ratio of the amount of the product obtained experimentally (in grams) to the amount calculated according to the chemical equation.

The degree of substitution of dextran to the monosaccharide residue of chitosan (DS %) was calculated referring to the content of nitrogen in the product by the following equation:

$$DS = \frac{N - 8.22}{\frac{1008}{143 + M} + \frac{392}{185 + M} - 8.22} \cdot 100,$$

which was derived from the equation used for the theoretical calculation of the content of nitrogen in copolymers:

$$N = \left[\frac{14}{161+M-18} \cdot 0.72 \cdot DS + \frac{14}{203+M-18} \cdot 0.28 \cdot DS + \right.$$
$$\left. + \frac{14}{161} \cdot 0.72 \cdot (1-DS) + \frac{14}{203} \cdot 0.28 \cdot (1-DS) \right] \cdot 100,$$

where 14 – the molecular weight of nitrogen, 161 – the molecular weight of glucosamine monosaccharide residue, *M* – the molecular weight of dextran, 18 – the molecular weight of excluded water, 203 – the molecular weight of *N*-acetylated monosaccharide residue, 0.72 – the degree of deacetylation of chitosan.

Determination of the content of primary amino groups

The content of primary amino groups was determined by potentiometric titration [21]. 0.1 g of a copolymer was accurately weighed, dissolved in 20 ml of aqueous 0.1 M HCl and titrated with 0.1 M NaOH. A blank experiment was carried out in the same conditions. The content of amino groups (NH_2, %) in a copolymer was calculated as follows:

$$NH_2 = \frac{(V_1 - V_2) \cdot C \cdot E}{m \cdot 1000} \cdot 100,$$

where V_1 and V_2 – the volume (ml) of NaOH solution used for titration of the bland and sample solutions, respectively; *C* – the concentration (mol/l) of NaOH solution; *E* – the molecular weight of the amino group (16); *m* – the sample weight (g).

Spectroscopic measurements and viscometry

The infrared absorption 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 300 MHz spectrometer at 29 °C.

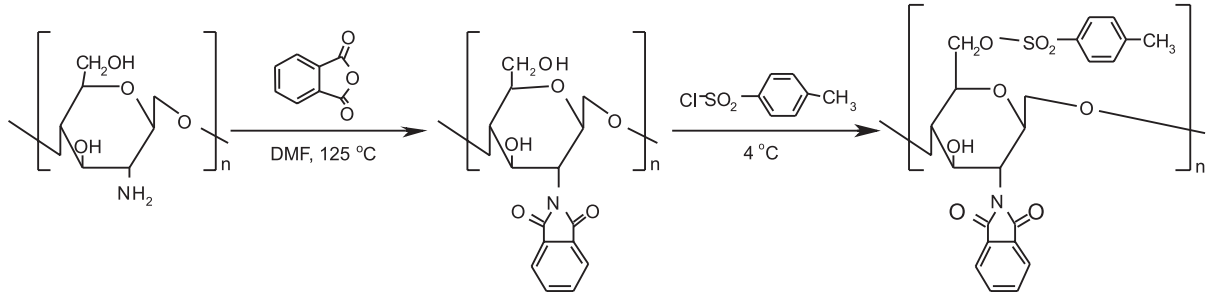
The intrinsic viscosity of copolymer solutions in distilled water and in the acetate buffer (aqueous 0.5 M CH_3COOH/0.5 M CH_3COONa) at 25 °C was measured using a dilution type Ubbelohde viscometer.

RESULTS AND DISCUSSION

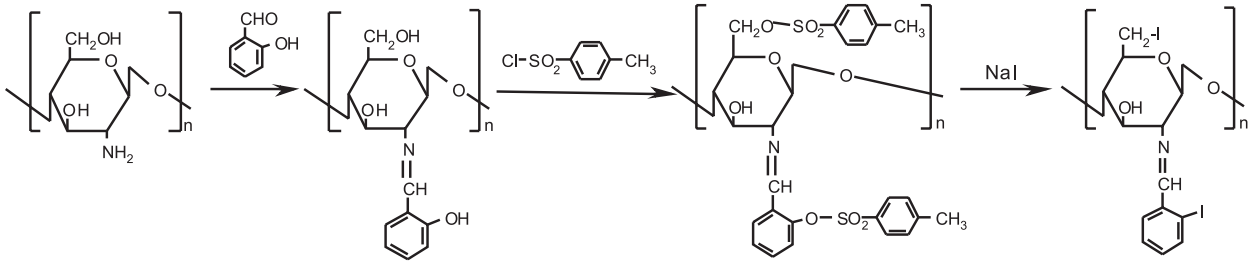
"Activation" of chitosan directed to *O*-derivatives

The higher reactivity of amino groups present in the main 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 dry DMF (Scheme 1) according to the procedure reported elsewhere [16]. Introduction of bulky phthaloyl groups prevents formation of intra- and intermolecular hydrogen bonds and consequently improves the solubility of chitosan in organic solvents such as DMF and DMSO [16, 17]. Solubilization of *N*-phthaloyl chitosan in several organic solvents is of the last importance for the further derivatisation of chitosan in homogeneous conditions. Besides, phthaloyl groups could be removed easily regenerating free amino groups in the derivatised chitosan. Though *N*-phthaloylation of chitosan requires experience of working with dry reactants, under certain conditions it was complete, which was proved by a negative ninhidrin test.

An alternative possibility to protect the amino functionality of chitosan is the use of salicyl aldehyde [22] resulting in a Schiff base compound containing an imine group (-RC=N-) (Scheme 2). Treatment of chitosan with salicyl aldehyde resulted in a yellow hydrogel within 15–20 min. In order to achieve a complete protection of amino groups, the reaction was carried



Scheme 1. Successive phthaloylation and tosylation of chitosan



Scheme 2. Successive salicylidenation, tosylation and iodination of chitosan

out for one hour. *N*-Salicyliden chitosan was insoluble, however, in common organic solvents, thus governing the further derivatisation of chitosan in heterogeneous conditions. It was expected that a strong acid should remove the salicyl group from a chitosan derivative, but practically neither hydrochloric acid nor formic acid worked. The deprotection of amino functionality was done by the use of hydrazine monohydrate.

Formation of *N*-phthaloyl chitosan and *N*-salicyliden chitosan was proved by FT-IR spectra. In the spectrum of *N*-phthaloyl chitosan (Fig. 1b), absorption at 1711 and 1777 cm^{-1} are characteristic of carbonyl in phthalimide and absorption at 721 cm^{-1} of aromatics. The bands at 1390 cm^{-1} and at about 1287 cm^{-1} can be attributed to -CH_2 bending or twisting modes. The spectrum of *N*-salicyliden chitosan (Fig. 2a) showed a characteristic absorption at 1631 cm^{-1} (-C=N-) and 1578 cm^{-1} (aromatics) [18].

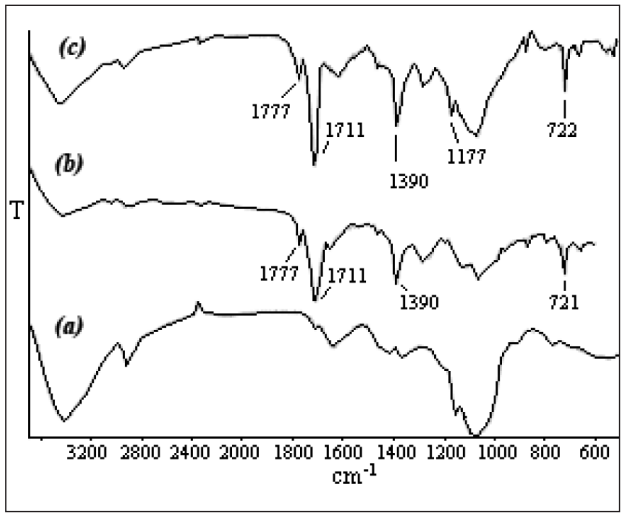


Fig. 1. FT-IR spectra of chitosan (*a*), *N*-phthaloyl chitosan (*b*) and 6-*O*-tosyl-*N*-phthaloyl chitosan (*c*)

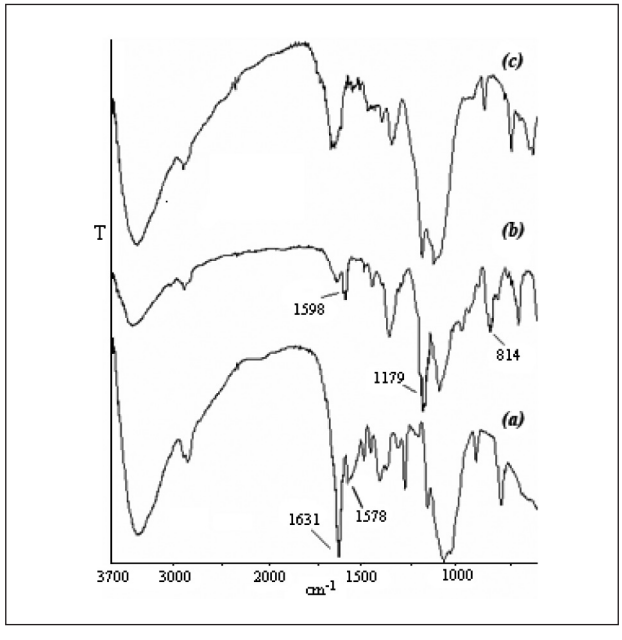


Fig. 2. FT-IR spectra of salicyliden chitosan (*a*), 6-*O*-tosyl-salicyliden chitosan (*b*) and iodosalicyliden chitosan (*c*)

The (*p*-tolylsulfonyl)oxy group is one of the most effective leaving groups widely used in carbohydrate chemistry [20]. 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 DMA afforded 6-*O*-tosyl-*N*-phthaloyl chitosan with a very high degree of tosylation (Scheme 1, Table 1). It was proved that primary hydroxyl groups of both glucosamine and *N*-acetyl glucosamine units could be completely changed to tosyl groups. The degree of chitosan tosylation depended on the ratio of the initial components and reached ca 100% (Table 1). The use of triethylamine in the tosylation of chitosan was essential for the neutralisation of hydrogen chloride generated during tosylation. The IR spectrum of 6-*O*-tosyl-*N*-phthaloyl chitosan showed a characteristic absorption at 1177 cm^{-1} due to tosyl groups (Fig. 1).

Table 1. **Results of the analysis of 6-*O*-tosyl-*N*-phthaloyl chitosan**

Run No	Molar ratio at tosylation			Product	
	N-Phthaloyl chitosan	Triethylamine	Tosyl chloride	N, %	Degree of tosylation, %
1	1	35	10	3.30	100
2	1	10	8	4.0	80
3	1	10	5	4.33	65
4	1	10	3	4.60	49

Tosylation of *N*-salicyliden chitosan proceeded under heterogeneous conditions. The reaction was severely dependent on the stirring efficiency and temperature control because of its rapid rate at the interface. Vigorous stirring at 0 °C in the initial stage of the reaction allowed reaching the degree of tosylation ca 100%. The FT-IR spectrum of 6-*O*-tosyl salicyliden chitosan showed characteristic absorption bands at 1598 cm^{-1} and 814 cm^{-1} (*p*-phenilen) and at 1179 cm^{-1} (SO_2 of tosyl groups) (Fig. 2). The absence of the band at 1631 cm^{-1} (-C=N-) evidenced that the tosylation of the salicyliden group took place also (Scheme 2).

The tosylation of chitin was done under homogeneous conditions [19]. Tosyl chitin was obtained as a brown powder highly swelling in solvents as DMSO, DMF, pyridine and water. The FT-IR spectrum of tosyl chitin showed typical absorption bands at 1598 cm^{-1} (aromatics), 1176 cm^{-1} (SO_2) and 816 cm^{-1} (C–O–S) attributed to the tosyl group.

Iodination reactions of both 6-*O*-tosyl-*N*-salicyliden chitosan and tosyl chitin proceeded under heterogeneous conditions. Iodosugars were obtained as pale-yellow powdery substances in moderate yields. The FT-IR spectra of these substances showed the absence of tosyl groups since the bands at 1598 cm^{-1}, 1176 cm^{-1} and 814 cm^{-1} characteristic of these groups disappeared completely. Iododerivatives were insoluble in organic solvents, thus governing the grafting of dextrans under heterogeneous conditions.

Synthesis, structure and properties of chitosan-*O*-dextran derivatives

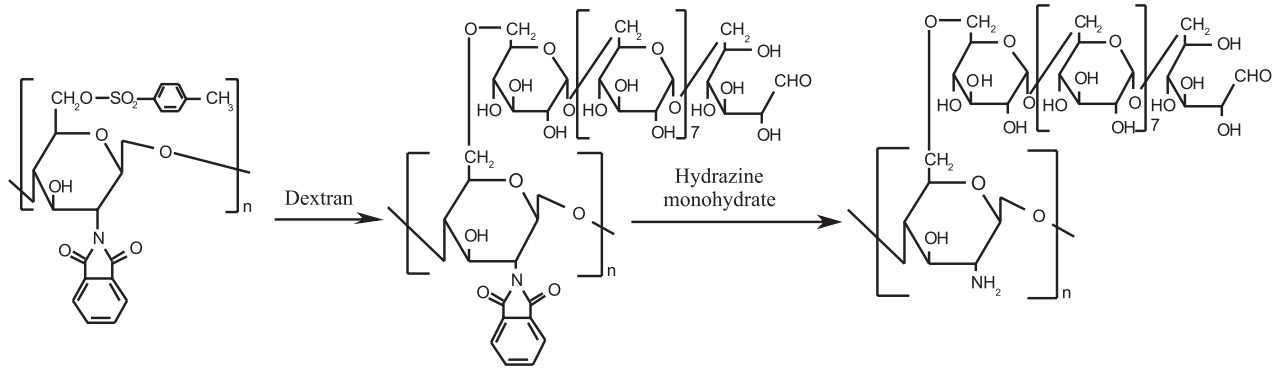
O-Tosyl-*N*-phthaloyl chitosan is an activated form of chitosan that can be used as an intermediate ideally suited for production of a wide variety of chitosan *O*-derivatives. Dextran through its primary hydroxyl group present in the terminal glucopyranose unit only was grafted onto "activated chitosan" (Scheme 3). The reaction was carried out in a DMF – alkaline water mixture under heterogeneous conditions for a few hours until the mixture became homogeneous. The reaction time was extended to 24 h or even to 48 h in order to achieve the maximal DS at a given ratio of the main reactants. A high degree of chitosan substitution was attained during the reaction between chitosan and dextran-1500, however, only 46 % was reached using dextran-6000 (Table 2). The obtained *N*-phthaloyl chitosan-*O*-dextran copolymers were easily soluble in water, but insoluble in organic solvents. Deprotection of amino groups of the graft copolymers was carried out using hydrazine monohydrate according to Kurita et al. [12]

Separation and purification of *N*-phthaloyl chitosan-*O*-dextran copolymers as well as successive deprotected derivatives chitosan-*O*-dextran is a serious problem. Precipitation of these copolymers from aqueous solutions was found impossible because of a highly hydrophilic nature of both chitosan and dextran chains. Salting out with an aqueous saturated ammonium sulphate solution did not work, either. The use of membrane dialysis was more efficient, although the completeness of the removal of unreacted dextran was hardly evaluable. The dialysate was concentrated until a solid residue using a rotating evaporator, and the obtained product was tried to dry in a vacuum oven at room temperature. An insoluble product was received, however, irrespective of the ratio of chitosan and dextran used for modification. Besides, the dialysate turned cloudy during concentration. Freeze-drying was the only suitable method to receive soluble chitosan-*O*-dextran copolymers.

Iodosalicyliden chitosan is insoluble in a common organic solvent and water, therefore, the reaction between the latter and dextran proceeded under heterogeneous conditions. The obtained *N*-salicyliden chitosan-*O*-dextran was insoluble in water, but it became soluble after deprotection of the amino functionalities of chitosan. The DS value of chitosan in the graft copolymers prepared through salicyliden intermediates was 3–4% only as calculated from the content of primary amino groups and elemental analysis of the products.

The reaction between iodochitin and dextran was much more successful, giving a reasonable amount of chitin derivative. Deacetylation of this compound in a highly alkaline aqueous solution at an elevated temperature resulted in a chitosan-*O*-dextran graft copolymer with DS 20%, but the intrinsic viscosity of the copolymer was only about 0.1 dL/g.

When salicyl aldehyde was used for the protection of amino groups of chitosan, additional hydroxyl groups that can participate in the modification reactions of chitosan were introduced. Moreover, it was difficult to deprotect amino groups. Reactions between chitin and dextran proceeded under heterogeneous conditions and did not give products with a high DS. In order to obtain water-soluble block copolymers with a high DS, *N*-phthaloyl chitosan as an intermediate compound was used.



Scheme 3. Synthesis of chitosan-*O*-dextran graft copolymers

Table 2. **Results of the analysis of chitosan-*O*-dextran graft copolymers**

Compound	Molar ratio [a]		N, %	NH_2 [b], %	DS, %	[η] [c], dL/g
	Chitosan	Dextran				
Chitosan	–	–	8.10	6.7	–	8.40
Chitosan-*O*-dextran-1500	1	1.6	1.50	0.75	90	0.277
Chitosan-*O*-dextran-1500	1	1	4.20	1.27	50	0.290
Chitosan-*O*-dextran-6000	1	1	4.30	0.40	46	0.151
Chitosan-*O*-dextran-6000	1	0.5	6.10	0.64	25	0.176

a) Molar ratio of chitosan glucosamine units and dextran in the reaction mixture.
b) Experimentally determined content of primary amino groups in the copolymer.
c) Acetate buffer, 25 °C.

Both chitosan and dextran are polysaccharides, and their FT-IR spectra are similar. Grafting of dextran on *O*-tosyl-*N*-phthaloyl chitosan could not be evaluated qualitatively by analysis of their FT-IR spectra because of the absence of a well-resolved band attributed either to chitosan or to dextran. In the spectrum of chitosan-*O*-dextran derivative as compared to the spectrum of the graft copolymer before removal of protective phthaloyl group, the absorption band at 1707 cm^{-1} (carbonyl) had disappeared, while the band at 1046–1152 cm^{-1} (pyranose) remained (Fig. 3). Moreover, an amino stretching band at 1643 cm^{-1}, attributed to chitosan, was observed, indicating an active dephthaloylation.

^{1}H-NMR spectra of *N*-phthaloyl chitosan-*O*-dextran and chitosan-*O*-dextran derivatives confirm formation of new structural units under grafting (Fig. 4). Protons that belong to dextran moieties (H-2'–H-6') have signals in the region between 3.3 and 3.9 ppm; these signals overlap with the signals of the protons H-3–H-6 of the chitosan backbone; the signal at 4.9 ppm is attributed to H-1' protons of dextran residues, and the signals at 2.7 and 4.5 ppm belong to the protons H-2 and H-1 of chitosan. The signal at 7.5 ppm, which belongs to the phthaloyl group, fully disappeared after deprotection of amino functionality of the chitosan derivative.

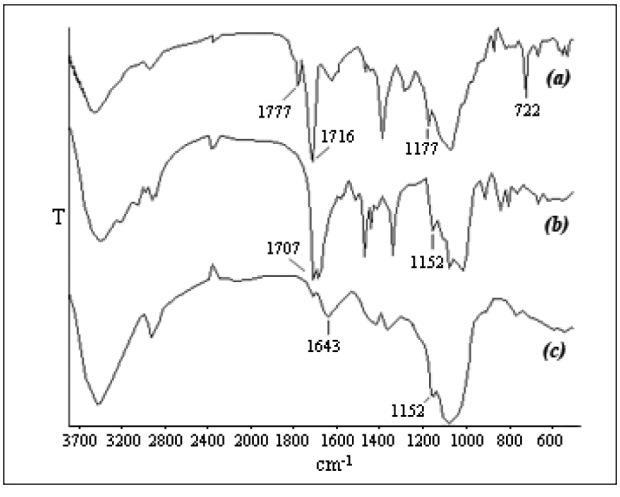


Fig. 3. FT-IR spectra of 6-*O*-tosyl-*N*-phthaloyl chitosan (*a*), *N*-phthaloyl chitosan-*O*-dextran (*b*) and chitosan-*O*-dextran with DS 90% (*c*)

An attempt to calculate the DS of chitosan in its derivatives by comparing the signal at 1.96 ppm, attributed to the protons in the acetyl group of *N*-acetyl glucosamine units of chitosan, to the signal at 4.9 ppm attributed to H-1' protons of dextran residues was done. Because of a partial overlapping of the signal of the protons H-1' of dextran with the signal of the solvent (water) and a very weak signal of acetyl groups, the calculation of the copolymer composition was, however, inaccurate (in the case of dextran-1500) or even almost impossible (in the case of dextran-6000).

Chitosan-*O*-dextran derivatives were soluble in water over the entire pH range, but they were insoluble in common organic solvents such as acetone, methanol and DMF. The viscosity of chitosan-*O*-dextran graft copolymers in dilute aqueous solutions was increasing with a decrease in polymer concentration (Fig. 5). This effect is due to long-range interactions between macromolecules of the polyelectrolyte, causing an increase in their volume. The polyelectrolyte effect was screened by adding an acetate buffer (Fig. 5, B). In the presence of added electrolytes, the polyelectrolytes behaved as non-ionic polymers, and no chain expansion was observed. The intrinsic viscosity of the initial chitosan in the same acetate buffer is 8.4 dL/g. Significantly lower values of the intrinsic viscosity of chitosan derivatives are related to the segregation of chitosan backbones, collapse of the ordered structure and a decrease of hydrogen bonding due to the attachment of the bulky dextran graft as well as breakdown of the chitosan backbone during the deprotection procedure.

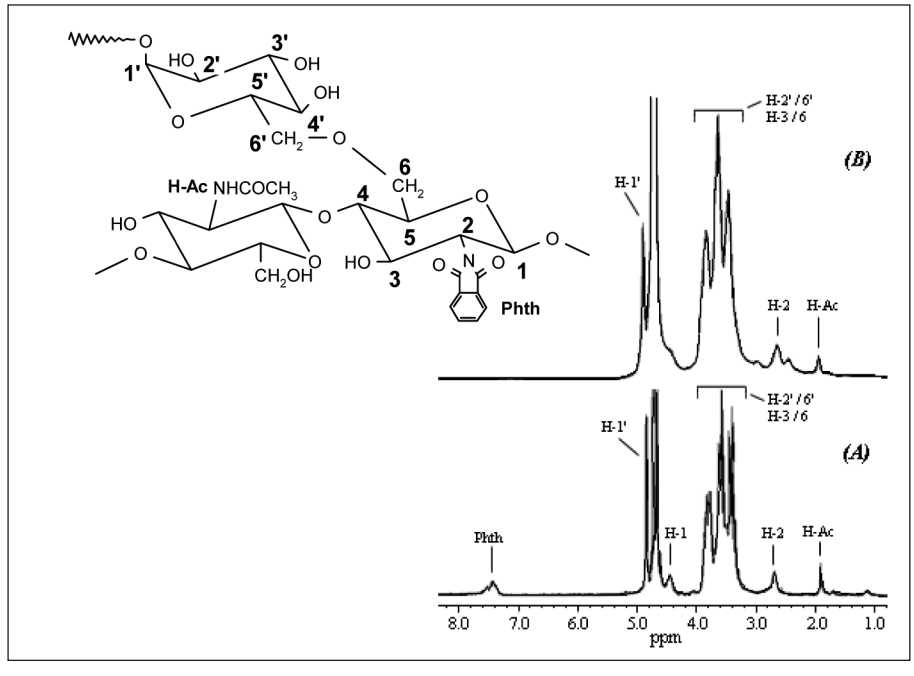


Fig. 4. ^{1}H NMR spectra of *N*-phthaloyl chitosan-*O*-dextran-1500 (*A*) and chitosan-*O*-dextran-1500 (*B*) with DS 90% in D_2O. Inserted fragment of the copolymer structure illustrates assignment of the protons

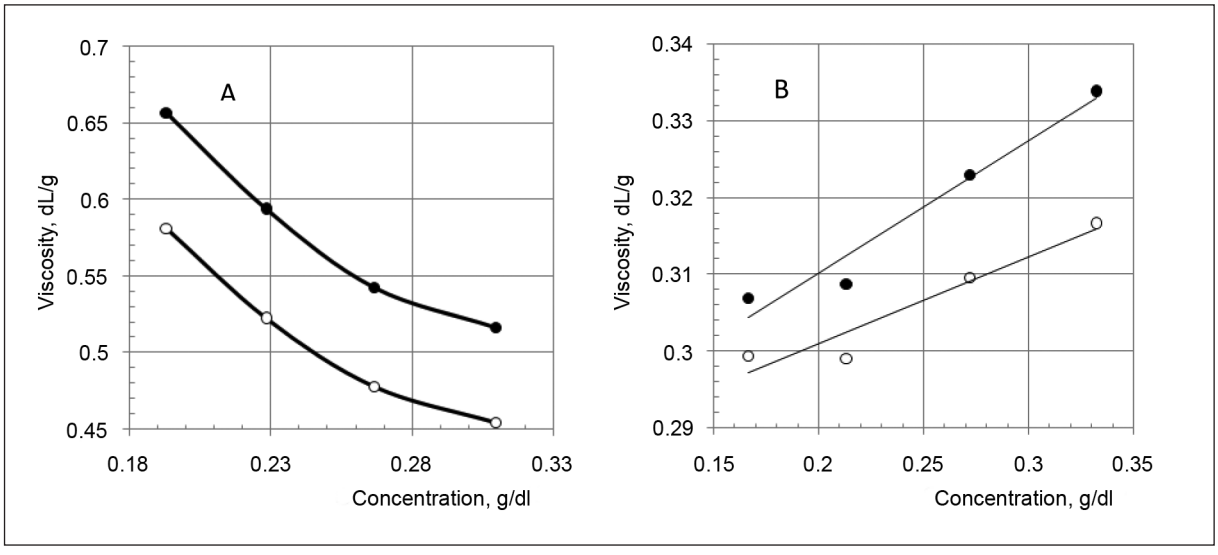


Fig. 5. Concentration dependence of reduced (●) and inherent (○) viscosity of chitosan-*O*-dextran-1500 (DS 90%) in water (A) and in acetate buffer (B)

Breakdown of chitosan backbone in its derivatives under amino group phthaloylation – dephthaloylation procedure

The greatest problem facing the synthesis of chitosan-*O*-dextran graft copolymers was the deprotection of primary amino groups in derivatised chitosan. It has already been reported [12, 23] that a certain breakdown of the chitosan backbone took place under its derivatisation if *N*-phthaloylation had been used to protect the amino functionalities of chitosan. An especially large breakdown of the chitosan backbone, resulting in graft oligomers, was determined during the synthesis of chitosan-*O*-PEG copolymers [10], which was thought to depend on the amino group protection–deprotection procedure. Referring to the above publications, one can speculate that the extent of breakdown of the chitosan main chain is consistent with the molecular weight of the attached chains. The breakdown is moderate, if low molecular compounds are attached, but it becomes substantial in the case of attached oligomers or polymers. In order to prove this hypothesis, the effect of amino group protection–deprotection procedure on the breakdown of the chitosan backbone in both derivatised and initial chitosan was studied.

The deprotection of amino functionality both in *N*-phthaloyl chitosan and its copolymers were studied using pure hydrazine monohydrate and its solutions in DMF, DMSO, formamide (FA), water and water–organic mixtures. It was determined that chitosans obtained by removing the protective phthaloyl groups had a several times lower intrinsic viscosity as compared to the initial chitosan, irrespective of the deprotection conditions (Table 3). Conditions of deprotection practically had no influence on the values of intrinsic viscosity. A reduction in the intrinsic viscosity of chitosan solutions could be caused both by disarrangement of abundant H-bonds characteristic of polyelectrolyte solutions and a certain breakdown of the main chain.

The dephthaloylation of *N*-phthaloyl chitosan-*O*-dextran copolymers resulted in chitosan derivatives a possessing relatively low intrinsic viscosity (Table 4). Moreover, the intrinsic viscosity of the copolymer solutions depended substantially on the conditions of dephthaloylation. The lowest [η] value, hardly exceeding 0.01 dL/g, was characteristic of the chitosan derivative deprotected from phthaloyl groups in hydrazine. It is obvious that the

Table 3. **Results of deprotection of *N*-phthaloyl chitosan**

Medium	Time, h	Yield, %	[η], dL/g
Hydrazine:DMF = 1 : 2.5	2	68	1.04
Hydrazine	2	61	1.01
Hydrazine	15	78	1.18
Hydrazine : water = 1 : 2.5	15	76	1.08

Table 4. **Results of dephthaloylation of chitosan-*O*-dextran derivatives**

Compound	Reaction medium	Yield, %	[η], dL/g
N-phthaloyl chitosan-*O*-dextran-1500	–	–	0.314
Chitosan-*O*-dextran-1500	Hydrazine	11	0.012
Chitosan-*O*-dextran-1500	FA*: hydrazine = 7.5 : 1	90	0.277
N-phthaloyl chitosan-*O*-dextran-6000	–	–	0.137
Chitosan-*O*-dextran-6000	DMSO : hydrazine = 7.5 : 1	66	0.140
Chitosan-*O*-dextran-6000	Water : hydrazine = 6 : 1	20	0.081
Chitosan-*O*-dextran-6000	FA*: hydrazine = 7.5 : 1	53	0.120

* Formamide.

destruction of the polysaccharide chains is tremendous in this case. Diluted solutions of hydrazine affected the intrinsic viscosity of the copolymers to a lesser extent. Hydrazine solutions in formamide were ascertained being optimal for the deprotection of chitosan graft derivatives. The deprotection reaction is recommended to be carried out for 2 h at 80 °C under nitrogen atmosphere. Nevertheless, the intrinsic viscosity of chitosan-*O*-dextran copolymers dephthaloylated under optimal conditions never exceeded 0.3 dL/g (dextran-1500 grafts) or 0.14 dL/g (dextran-6000 grafts), i. e. were several times lower as compared to analogous chitosan-*N*-dextran derivatives [15]. Thus, the present study proves that oligomeric attachments significantly enhance the breakdown of chitosan backbone under amino group protection–deprotection procedure, which could be related to impeded relaxation processes of the chains containing bulky substituents.

CONCLUSIONS

Novel chitosan-*O*-dextran copolymers with the degree of chitosan substitution varying from 25 to 90% were synthesized using dextran-1500 and dextran-6000. Chitosan-*O*-dextran graft copolymers were hydrophilic products soluble in aqueous solutions in a wide pH range and exhibited a polyelectrolyte effect, indicating that these copolymers retained the cationic properties of chitosan. The intrinsic viscosity of these chitosan derivatives was relatively low, especially of those containing dextran-6000 grafts. It has been proven that dextran attachment at the C-6 position of glucosamine residues significantly enhances the breakdown of the chitosan backbone under the amino group protection–deprotection procedure using *N*-phthaloylation.

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POLIELEKTROLITAI IŠ GAMTINIŲ POLIMERŲ FRAGMENTŲ: CHITOZANO-*O*-DEKSTRANO KOPOLIMERŲ SINTEZĖ IR SAVYBĖS

S a n t r a u k a

Pirmą kartą susintetinti chitozano-*O*-dekstrano skiepytieji kopolimerai, kuriuose chitozano pakeitimo laipsnis kinta nuo 25 iki 90%. Oligomerinio dekstrano, kurio molekulinė masė 1500 arba 6000, priskiepijimas prie chitozano C-6 padėties gliukozamino grandyse pasiektas, blokuojant amino grupę ftalinimu ir „aktyvuojant“ pirmines chitozano hidroksigrupes tozilinimu. Chitozano darinių struktūra įrodyta FT-IR ir ^{1}H BMR spektroskopijos pagalba, chitozano pakeitimo laipsnis apskaičiuotas pagal azoto kiekį produktuose. Chitozano-*O*-dekstrano skiepytieji kopolimerai yra hidrofilinės medžiagos, lengvai tirpstančios vandenyje esant įvairioms pH vertėms, joms būdingas stipriai išreikštas polielektrolitinis efektas. Šių chitozano darinių, ypač turinčių dekstrano-6000 skiepus, ribinis klampos skaičius yra santykinai mažas. Įrodyta, kad prie C-6 padėties gliukozamino grandyse prijungus dekstraną, gerokai sustiprėja chitozano pagrindinės grandinės destrukcija amino grupės blokavimo ftalio anhidridu–deblokavimo procedūrų metu.