CHEMIJA. 2013. Vol. 24. No. 4. P. 325–334


# Quaternization of chitosan and partial destruction of the quaternized derivatives making them suitable for electrospinning

**Rūta Gruškienė[1,2],**

**Rasa Deveikytė[2],**

**Ričardas Makuška[2]***

[1] *Sector of Applied Biocatalysis, Institute of Biotechnology, Vilnius University, V. A. Graičiūno St. 8, LT-02241 Vilnius, Lithuania*

[2] *Department of Polymer Chemistry, Vilnius University, Naugarduko St. 24, LT-03225 Vilnius, Lithuania*

Quaternization of chitosan by the use of (2,3-epoxypropyl) trimethylammonium chloride (EPTMAC) was studied. In acidic or neutral media, *N*-quaternized derivatives of chitosan (*N*-HTCC) with a degree of quaternization (DQ) varying from 30 to 68% were synthesized while additional quaternization of *N*-HTCC in alkaline media through its hydroxyl groups enabled to increase DQ of chitosan up to 95%. Quaternization of chitosan present in chitosan-dodecyl sulfate complexes was less successful resulting in chitosan derivatives with DQ about 20% which were insoluble in water. In order to decrease dynamic viscosity (DV) of the solutions of the quaternized chitosans and make them suitable for production of nanofibers by electrospinning, partial degradation of the polymers by UV irradiation or enzymatic hydrolysis was studied. UV irradiation helped to decrease DV of the solutions of the quaternized chitosans with high DQ (>50%). Enzymatic hydrolysis of the quaternized chitosans by pectinase or cellulase in their solutions was very fast resulting in the products with thirtyfold lower DV within 2–3 hours. Enzymatic hydrolysis of the quaternized chitosans stopped at a certain stage producing oligomeric cationic chitosan derivatives instead of the quaternized glucosamine.

**Key words:** chitosan, quaternized derivatives, UV irradiation, enzymatic hydrolysis

## INTRODUCTION

Polymers containing quaternary ammonium groups are cationic polyelectrolytes with permanent charges, differing from polymeric amines which become charged in acidic media only. Chitosan belongs to polymeric amines but can be modified to quaternized derivatives by several methods. Quaternized chitosans have high moisture-retention capacity, superior bioadhesive properties, permeation enhancing effects and antimicrobial properties even at neutral conditions [1–4]. It was determined that antifungal activity was enhanced by the presence of positive charges, and a quaternized chitosan had better antifungal activity than initial chitosan, Schiff bases of chitosan or *N*-substituted chitosan [2, 5]. Quaternized derivatives of chitosan can be prepared into several different formulations, such as micro- and nanofibres, hydrogels, microspheres and nanoparticles. J. Wu et al. [1] prepared uniform-sized pH-sensitive quaternized chitosan microspheres which could be located accurately on the target site and increase

* Corresponding author. E-mail: ricardas.makuska@chf.vu.lt

bioavailability of the used drug with low side-effects. These microspheres can also be utilized as drug carriers targeted to organs with different pH values. X. Wang et al. [6] synthesized a series of biocompatible quaternized chitosan / rectorite nanocomposites as novel non-viral carriers for gene therapy by oral administration and injection, and investigated their biophysical and biochemical properties.

Nanofibers webs are excellent candidates for many applications: in medical textile (wound dressing, medical prothesis, drug delivery), filters, composites, protective clothes, etc. Nanofibers containing ionogenic polymers have been successfully electrospun from mixed solutions of an ionogenic polymer and non-ionic polymer. Electrospun nanofibre mats with antibacterial properties have been prepared recently from the quaternized chitosan as an ionogenic polymer and poly(vinyl alcohol) [7] or poly(vinyl pyrrolidone) [8] as a non-ionic polymer. Morphology and diameters of electrospun fibers depend on a number of parameters, that include properties and composition of the spinning solution such as polymer type, conformation of polymer chain, viscosity (concentration) of the solution, conductivity, polarity and surface tension of the solvent, and electrospinning conditions – applied field strength, distance between the capillary and collector, and feeding rate [8]. It was determined that continuous and uniform fibers can be produced using chitosan solutions with viscosity ranging from 480 to 590 cP which corresponds to 7–7.5% solution of chitosan with molecular weight 106 000 [9].

Although several different reagents were used to prepare quaternized chitosans, the main were methyl iodide [10, 11] and glycidyltrimethyl ammonium chloride (GTMAC) [1, 12–16]. GTMAC reacts mainly with amino groups of chitosan which are nucleophilic enough to open the epoxy ring of GTMAC under acidic or neutral conditions [17]. It is known that under alkaline conditions GTMAC can react with hydroxyl groups and is used for quaternization of starch [18, 19]. Preparation of *O*-quaternized chitosan derivatives is complicated because of insolubility of chitosan in alkaline media. Moreover, the synthesis of *O*-quaternized chitosans requires protection of more active amino groups. No publications were found on quaternization of chitosan directly through hydroxyl groups. *O*-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride (*O*-HTCC) was synthesized by the use of *N*-benzylidene chitosan as a precursor [20].

The present work focuses on quaternization of chitosan by the use of GTMAC with the aim to prepare *N*- and (or) *O*-quaternized chitosans. Quaternization of chitosan through hydroxyl groups should allow obtaining quaternized chitosan derivatives containing primary amino groups or chitosan derivatives with a very high degree of quaternization. Another aim of the present study was partial destruction of the quaternized chitosans making them suitable for the preparation of nanofibers by electrospinning.

## EXPERIMENTAL

### Materials

Chitosan (medium molecular weight, degree of deacetylation (DD) 72%), sodium dodecyl sulfate (SDS), poly(ethylene glycol) monomethyl ether (MPEG) ($M_r$ 2000) were obtained from FLUKA. 2,3-Epoxypropyl trimethyl ammonium chloride (glycidyl trimethyl ammonium chloride) (GTMAC) (technical, ≥90%) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were purchased from ALDRICH. Tris-(hydroxymethyl)-aminomethane (TRIS) was obtained from APPLICHEM. Low molecular weight (LMW) chitosan was prepared according to the procedure described before [21]. All other reagents and solvents were of analytical grade and used without further purification.

### Quaternization of chitosan

*A: In acidic solution*

*N*-quaternized derivative of chitosan (*N*-HTCC) was synthesized according to the procedure presented before [13]. Briefly, chitosan (1 g, 5.78 mmol) was dissolved in 1% acetic acid (50 ml), and then GTMAC (2.52 g, 16.6 mmol) was added. The reaction was carried out at 70 °C for 24 h. The reaction mixture was cooled down and poured into cold acetone. The white product was collected by filtration, washed by acetone several times, and vacuum dried at room temperature to give 1.49 g of the product (yield 91%).

*B: In neutral solution*

Synthesis of *N*-HTCC in neutral media was done according to the procedure described elsewhere [1]. Briefly, GTMAC (2.52 g, 16.6 mmol) was dissolved in 7 ml deionized water, and the solution was mixed with 10 ml aqueous chitosan suspension containing 1 g (5.78 mmol) of chitosan. The suspension was stirred for 4 h at 80 °C. Then the reaction mixture was cooled down and poured into cold acetone. The resultant suspension was stirred in a refrigerator overnight. After being washed by acetone several times, the white product was collected by filtration and vacuum dried at room temperature to give 1.53 g of the product (yield 94%).

*C: In alkaline solution*

Chitosan (1 g, 5.78 mmol) was dispersed in 20 ml alkaline aqueous solution (1% NaOH). GTMAC (1.75 g, 11.6 mmol) was dissolved in 5 ml deionized water, and the GTMAC solution was added into the chitosan suspension. The suspension was stirred for 24 h at 70 °C. Then the reaction mixture was cooled down to room temperature, diluted with 20 ml of methanol and left under stirring for 3 h. Finally, the reaction mixture was poured into cold acetone, and the resultant suspension was stirred in a refrigerator overnight. After being washed by acetone several times, the white product was collected by filtration, extracted with methanol in a Soxhlet's apparatus and vacuum dried at 40 °C to give 1.08 g of the product (yield 58%).

$^1$H NMR spectrum of the quaternized chitosan in $D_2O$ containing one drop of DCl: δ = 1.9 ($CH_3$ in acetamide), δ = 2.99 (H-2), δ = 3.1 (-$^+N(CH_3)_3$), δ = 3.3–3.8 (pyranose ring and (-O-$CH_2$-)).

FT-IR (KBr): ν ($cm^{-1}$) = 3 430 (OH), 1 640 (imide I), 1 560 (imide II), 1 482 ($CH_3$ in quaternary ammonium), 1 150–950 (C–O, pyranose).

**Preparation of SDS/chitosan complexes**

SDS/chitosan complexes (SCC) were prepared by mixing acidic solutions of chitosan and SDS according to the procedure described elsewhere [22]. Chitosan (3 g, 17 mmol) and SDS (5 g, 17 mmol) were dissolved in 200 ml and 70 ml of 2% acetic acid, respectively. The SDS solution was poured into the chitosan solution under vigorous stirring, and the mixture was gently stirred for 2 hours at room temperature. The resulting precipitates were filtered off, washed three times with distilled water, and finally freeze-dried to give 6.2 g of the white product (yield 98%).

$^1$H NMR spectrum (in DMSO d-6): δ = 0.86 ($CH_3$ in SDS), δ = 1.25 (-$(CH_2)_9$- in SDS), δ = 1.49 (-$\underline{CH_2}CH_2$-O in SDS), δ = 1.87 ($CH_3$ in acetamide of chitosan), δ = 2.88 (H-2 of chitosan), δ = 3.2–3.5 (H-3 – H-6 of chitosan (pyranose ring)), δ = 3.69 (-$CH_2\underline{CH_2}$-O in SDS).

FT-IR (KBr): ν ($cm^{-1}$) = 3 600–3 100 (O–H, N–H), 2 930–2 830 ($CH_2$, SDS), 1 640 (imide I), 1 530 (imide II), 1 240 (S = O, SDS), 1 150–950 (C–O, pyranose), 815 (C–O–S, SDS).

**Synthesis of O-quaternized chitosan using SDS/chitosan complexes**

GTMAC (2.1 g, 13.5 mmol) dissolved in 20 ml deionized water was added to 70 ml DMSO solution containing 1 g (2.7 mmol) SCC. The resulting mixture was stirred at 70 °C for 24 h. The reaction mixture was dialyzed against 15% TRIS solution (pH 8) for 48 h and then against water for 3 days, concentrated by rotary evaporation and vacuum dried at room temperature to give 0.74 g of the product (yield 84%).

$^1$H NMR spectrum of the quaternized chitosan in $D_2O$ containing one drop of DCl: δ = 1.9 ($CH_3$ in acetamide), δ = 2.99 (H-2), δ = 3.1 (-$^+N(CH_3)_3$), δ = 3.3–3.8 (pyranose ring and (-O-$CH_2$-)).

FT-IR (KBr): ν ($cm^{-1}$) = 3 430 (OH), 1 640 (imide I), 1 560 (imide II), 1 478 ($CH_3$ in quaternary ammonium), 1 150–950 (C–O, pyranose).

**Enzymatic hydrolysis of the quaternized chitosan**

0.24 g Pectinase from *Aspergillus niger* or cellulase from *Trichoderma reesi* were dissolved in 5 ml of deionized water, and the solution was added to 100 ml HTCC solution (4%) in acetate buffer (pH 4.6) under stirring. The solution was kept at 37 °C for a certain time and then poured into acetone. The resulting precipitates were filtered off, washed several times with acetone, and finally vacuum dried at room temperature to give 2.7 g of the product (yield 67%).

**UV-Irradiation of the quaternized chitosan**

25–100 ml solution of the quaternized chitosan (7%) in 0.5% $CH_3COOH$ were placed in a 150 ml quartz ampoule equipped with a mechanical stirring bar. The homogeneous solution was irradiated with a 250 W UV lamp at a distance of 24 or 30 cm at room temperature under stirring for a fixed duration. Destruction of the quaternized chitosan was evaluated according to dynamic viscosity of the solution.

**Analytical procedures**

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 were recorded on a UNITY INOVA VARIAN spectrometer (300 MHz, Varian). The samples of chitosan and its derivatives were prepared in $D_2O$ containing one drop of DCl. The samples of SDS/chitosan complexes and its derivatives were prepared in dimethyl sulfoxide-$d_6$.

The content of primary amino groups in chitosan derivatives was determined by potentiometric titration [23]. The degree of quaternization of chitosan by GTMAC (DQ, %) was calculated according to the content of residual amino groups by the equation disclosed before [24] with slight modification:

$$DQ = \frac{1152 - 173 \cdot X}{M \cdot X + 1600} \cdot 100,$$

where $X$ is the content of amino groups in copolymers (%), and $M$ is the molecular weight of GTMAC (151.63).

DQ of chitosan was calculated also according to the content of $Cl^-$ ions in the quaternized derivatives of chitosan determined by argentometric titration according to the procedure described elsewhere [12].

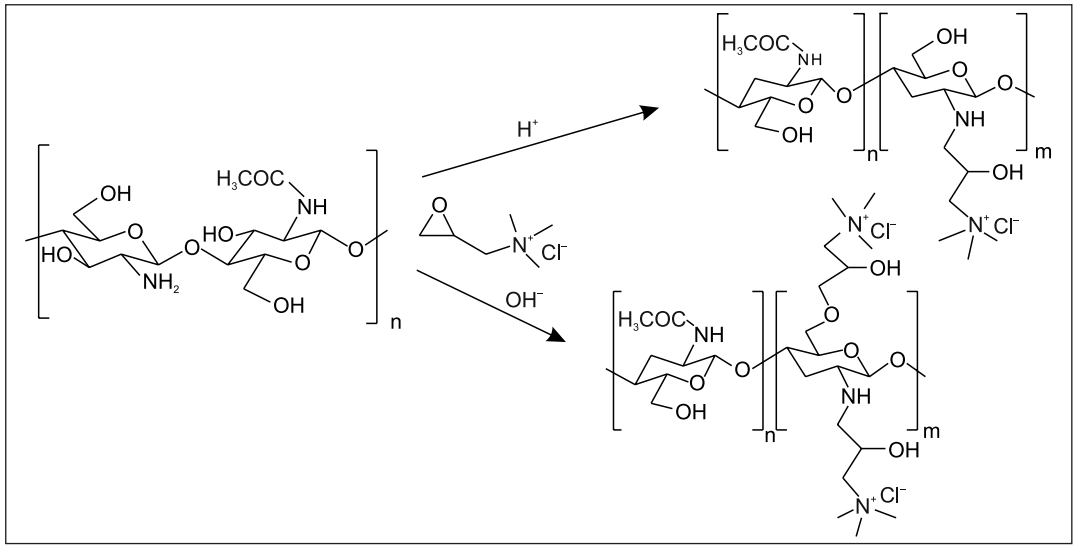
Calculation of DQ of chitosan from $^1$H NMR spectra was based on the ratio between the peak integration of the protons from the quaternary ammonium group at 3.1 ppm and from the methyl group in residual acetyl groups of chitosan at 1.8–1.9 ppm.

Intrinsic viscosity of chitosan derivatives in aqueous 0.1 M $CH_3COONa$/0.2 M $CH_3COOH$ at 25 °C was measured using a dilution type Ubbelohde viscometer. Reduced viscosity of the solutions at c = 0.3 g/100 ml was measured using an Ostwald viscometer. Dynamic viscosity of the solutions of the quaternized chitosans was measured using a Brookfield viscometer type DV-II at 18 °C.

## RESULTS AND DISCUSSION

**Quaternization of chitosan by the use of GTMAC**

Subject to pH of the medium, the reaction between chitosan and glycidyltrimethyl ammonium chloride (GTMAC) is expected to give different products. In acidic and neutral aqueous solutions, hydroxyl groups of chitosan are not sufficiently nucleophilic to induce ring opening of GTMAC, whereas the amino groups of chitosan are nucleophilic



**Scheme 1.** Quaternization of chitosan by GTMAC

enough to do that [12]. In alkaline solutions both amino and hydroxyl groups can react with GTMAC resulting in *N*-,*O*-substituted chitosan.

In the present study, quaternization of chitosan by the use GTMAC in acidic, neutral and alkaline aqueous solutions was done in parallel (Scheme 1). Although in acidic and neutral solutions preparation of the same product *N*-[(2-hydroxyl-3-trimethylammonium)propyl] chitosan chloride (*N*-HTCC) is expected, quaternization of chitosan under these conditions should proceed in a different way since chitosan is soluble in acidic solutions but insoluble at neutral pH.

The degree of quaternization of chitosan (DQ) depends on the reaction conditions such as reaction time, reaction temperature, and molar ratio of GTMAC to amino groups of chitosan [17]. It was shown by Seong et al. [17] that DQ increased significantly with increasing reaction time suggesting 18 h as an optimal time for the reaction at 50 °C and the molar ratio of GTMAC to $NH_2$ of chitosan equal to 4. It was determined also [17] that DQ depended directly on the reaction temperature up to 50 °C, while above 50 °C an increase in DQ was minimal. The reaction of quaternization of chitosan carried out in our laboratory in acidic media at various ratios of GTMAC to chitosan resulted in *N*-HTCC with DQ 35 to 70% (Table 1). An agreement between DQ estimated according to the residual amino group content and chloride anion content was rather good (Table 1). DQ was further confirmed by $^1$H NMR analysis for selected samples of the quaternized chitosan. Evidently, an optimal ratio of GTMAC to chitosan was approx. 4 since no significant increase in DQ was observed at higher concentration of the quaternizing reagent. Maximal DQ of chitosan reached in acidic conditions (about 70%) was consistent with the data published before [25]. Successful quaternization of chitosan was confirmed by elemental analysis and FT-IR and $^1$H NMR spectra of the products which were consistent with the spectra reported before [13].

Quaternization of chitosan in aqueous dispersions at neutral pH was successful as well, and sufficiently high DQ of chitosan was achieved during a relatively short time (Table 1). The results of quaternization of low-molecular-

Table 1. **The results of quaternization of chitosan by GTMAC in acidic and neutral media**

| No. | Chitosan, reaction medium | GTMAC: chitosan, mol | $NH_2$, % | $DQ_{NH2}$, % | Cl, % | $DQ_{Cl}$, % | $DQ_{NMR}$, % | $\eta_{red}$, dL/g (c = 0.3%) |
|---|---|---|---|---|---|---|---|---|
| 1. | Commercial chitosan, acidic medium | – | 6.00 | – | – | – | – | 14.6 |
| 2. | | 1 | 2.65 | 35 | 5.61 | 36 | – | 10.4 |
| 3. | | 2 | 1.34 | 51 | 7.52 | 54 | 37 | 8.95 |
| 4. | | 4 | 0.32 | 67 | 8.98 | 71 | 55 | 5.87 |
| 5. | | 6 | 0.24 | 68 | 9.06 | 72 | 69 | 3.69 |
| 6. | Commercial chitosan, neutral medium | 1 | 3.04 | 30 | 4.86 | 29 | 57 | 11.4 |
| 7. | | 2 | 1.48 | 49 | 6.86 | 47 | – | 9.36 |
| 8. | | 4 | 0.61 | 62 | 8.16 | 61 | 81 | 6.32 |
| 9. | | 6 | 0.47 | 64 | 8.58 | 66 | 84 | 4.61 |
| 10. | Low molecular weight chitosan, neutral medium | – | 5.66 | – | – | – | – | 3.01 |
| 11. | | 1 | 3.11 | 30 | 5.12 | 32 | 28 | 2.46 |
| 12. | | 4 | 0.55 | 63 | 8.50 | 65 | – | 1.38 |
| 13. | | 6 | 0.22 | 68 | 8.82 | 69 | 76 | 1.27 |

Table 2. **The results of quaternization of chitosan by GTMAC in alkaline media**

| No. | GTMAC: chitosan, mol | Reaction medium | $NH_2$, % | $DQ_{NH2}$, % | Cl, % | $DQ_{Cl}$, % | $DQ_{NMR}$, % | [η], dL/g |
|---|---|---|---|---|---|---|---|---|
| 1. | 2 | 0.3% NaOH | 5.24 | 10 | 1.79 | 10 | 25 | 8.14 |
| 2. | 2 | $NH_3$ in methanol, pH~9 | 5.37 | 9 | 0.52 | 2.6 | – | 5.53 |
| 3. | 2 | 0.8% NaOH | 5.61 | 7 | 0.22 | 5.1 | 19 | 7.20 |
| 4. | 4 | 0.8% NaOH | 5.08 | 11 | 0.94 | 4.8 | – | 4.22 |
| 5*. | 4 | 0.5% NaOH | – | – | 10.5 | 95 | 105 | 3.41 |
| 6*. | 4 | NaOH/2-propanol (v/v 1/1) | – | – | 9.9 | 84 | 94 | 4.68 |

5* – Additional quaternization of *N*-HTCC (No. 5 in Table 1); 6* – Additional quaternization of *N*-HTCC (No. 8 in Table 1).

weight chitosan were almost identical to those of commercial chitosan. High DQ of chitosan under the reaction in neutral solutions under heterogeneous conditions was somewhat unexpected the rather that acetic acid was declared being a catalyst activating the epoxy ring of GTMAC towards the reaction with amino groups [17]. DQ of chitosan quaternized under neutral conditions was about 64% (at molar ratio of GTMAC to amino groups of chitosan 4), whereas DQ about 50 was reported earlier using chitosan with DD 89% [1, 12].

Reduced viscosity $\eta_{red}$ of *N*-HTCC prepared from commercial medium molecular weight chitosan varied from 11.4 to 3.69 dL/g declining at higher DQ (Table 1). The quaternized derivatives showed lower intrinsic viscosity compared to the initial chitosan. This can be related to more compact structure of the quaternized chitosan [26].

Under alkaline conditions, GTMAC can react with both amino and hydroxyl groups of chitosan [17]. Quaternization of starch by GTMAC at high values of pH was found to be very efficient [18, 19, 27]. Unfortunately, quaternization of chitosan in alkaline media did not prove itself since DQ of chitosan hardly exceeded 10% (Table 2). Low DQ is possibly related to insolubility of chitosan in alkaline media restricting the reaction between epoxy groups of GTMAC and hydroxyl groups of chitosan to surface layers of chitosan particles. Neither higher concentration of sodium hydroxide nor the use of ammonia solution in methanol (1/3, v/v) in which chitosan was finely dispersed did not help increasing DQ of chitosan.

An attempt was made to increase DQ of chitosan by additional quaternization of *N*-HTCC synthesized in acidic or neutral conditions. *N*-HTCC with DQ 70 and 62% (samples No. 5 and No. 8 from Table 1) were subjected to further reaction with GTMAC changing the reaction media to alkaline (Table 2, Nos. 5, 6). Additional quaternization of *N*-HTCC enabled to increase significantly DQ of chitosan (Table 2). Since degree of deacetylation of chitosan was 72%, one could expect that in both cases partial quaternization of chitosan through hydroxyl groups took place resulting in *N,O*-[(2-hydroxyl-3-trimethyl-ammonium)propyl] chitosan chloride (*N,O*-HTCC). Thus, additional quaternization of *N*-HTCC through its hydroxyl groups is an efficient method to be used for the preparation of chitosan derivatives with very high DQ.

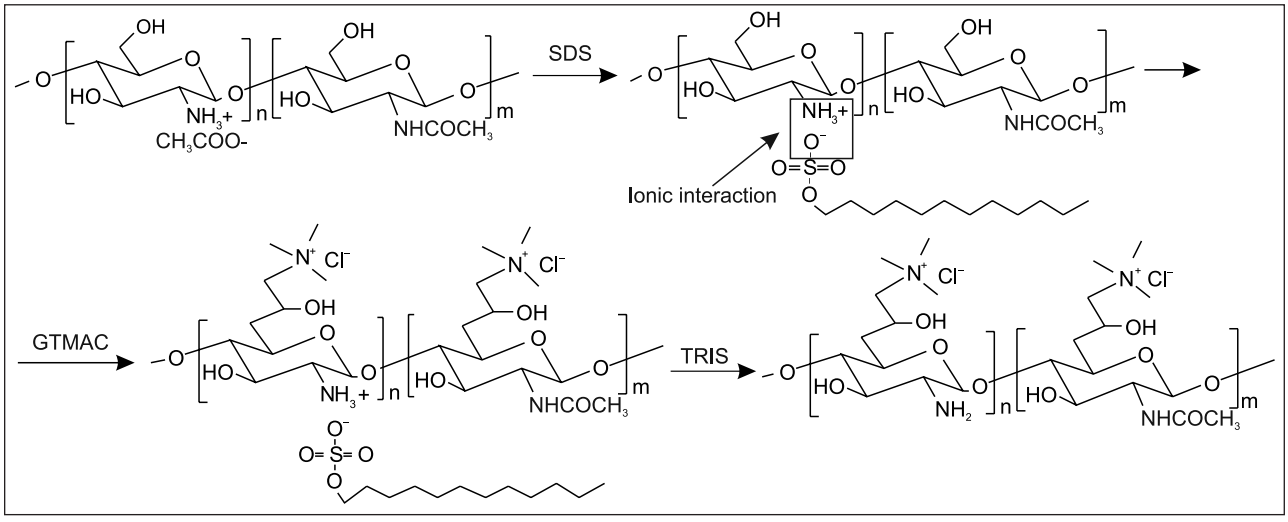
**Quaternization of chitosan present in its complexes with sodium dodecyl sulphate**

Quaternization of chitosan exclusively through its hydroxyl groups is studied faintly [28] and deserves more attention. Possible advantages of the chitosan derivatives prepared by chitosan quaternization through its hydroxyl groups are (a) very high charge density and (b) the presence of tertiary amino groups alongside with quaternary ammonium groups.

Quaternization of chitosan exclusively through its hydroxyl groups requires protection of more active amino groups of chitosan. Using *N*-benzylidene chitosan as a precursor, *O*-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride (*O*-HTCC) with DQ 33% was synthesized recently [28]. *N*-Benzylidene chitosan and *N*-phtaloyl chitosan as precursors for the synthesis of *O*-derivatives of chitosan have some drawbacks, however, associated with insolubility or partial destruction of the products under protection-deprotection procedures [29, 30]. For protection of amino functionality of chitosan, a different approach was used in the present study recently proposed for regioselective modification of chitosan [22]. The method is based on the use of sodium dodecyl sulfate (SDS)-chitosan complexes (SCC) which are soluble in DMSO. SCC were prepared by simply mixing acidic aqueous chitosan and SDS solutions at equimolar amounts of the components (Scheme 2).

The complex was separated, solubilized in DMSO and reacted with excess amount of GTMAC. SDS was removed from the quaternized chitosan by the use of strong base of *TRIS* which acted as a decomplexing agent. The results of *O*-quaternization of chitosan are summarized in Table 3.

Formation of SCC and *O*-HTCC was confirmed by FT-IR and $^1$H NMR spectroscopy (Figs. 1, 2). In the FT-IR spectrum of chitosan, the absorption bands at 1 656 $cm^{-1}$ and ~1 560 $cm^{-1}$ were assigned to the amide-I and amide-II stretching, respectively. Absorption band at 1 560 $cm^{-1}$ apparently decreased during quaternization. Characteristic absorption bands of SCC were at 2 930–2 830 $cm^{-1}$ ($CH_2$-), 1 242 $cm^{-1}$ (S=O) and 815 $cm^{-1}$ (C-O-S). In the FT-IR spectrum of *O*-HTCC, a band at 1 479 $cm^{-1}$ attributed to the methyl groups of ammonium arose, and the bands at 1 242 $cm^{-1}$ and 815 $cm^{-1}$ associated with sulfate groups disappeared. The FT-IR spectrum of *O*-HTCC is almost identical with the FT-IR spectrum of *N*-HTCC, and the both contain the signals characteristic for quaternized chitosan.




**Scheme 2.** *O*-Quaternization of chitosan through sodium dodecyl sulfate–chitosan complex intermediate

Table 3. **Quaternization of chitosan by the use of SCC as an intermediate**

| No. | Reaction medium | GTMAC : SCC, mol | Cl, % | $DQ_{Cl}$, % | $DQ_{NMR}$, % | [η], dL/g |
|---|---|---|---|---|---|---|
| 1. | DMSO/$H_2O$* | 2 | 0.18 | 0.9 | 12 | 2.45 |
| 2. | (1 : 0.8 v/v) | 4 | 0.21 | 1.1 | 21 | 1.39 |
| 3. | DMSO/$H_2O$ | 2 | 2.21 | 11 | 18 | 4.22 |
| 4. | (1 : 0.8 v/v) | 4 | 3.33 | 19 | 37 | 4.01 |
| 5. | DMSO/2-propanol | 2 | 2.30 | 12 | 16 | 4.20 |
| 6. | (1 : 0.8 v/v) | 4 | 3.34 | 19 | 32 | 4.03 |
| 7. | | 6 | 4.59 | 28 | 42 | 3.88 |

* – NaOH : GTMAC = 1 : 1.25 mol.

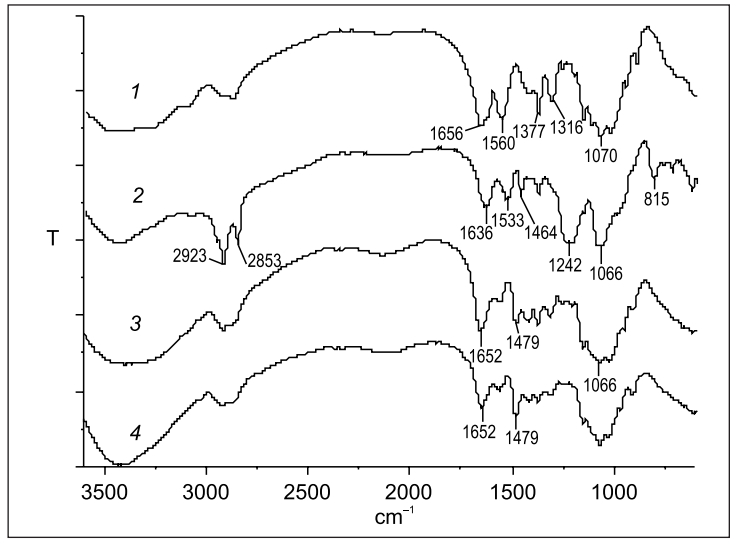



**Fig. 1.** FT-IR spectra of chitosan (*1*), SCC (*2*), *O*-HTCC (DQ 28%) (*3*) and *N*-HTCC (DQ 68%) (*4*)

In the $^1$H NMR spectrum of *O*-HTCC, the characteristic signals at 0.86, 1.25 and 3.69 ppm assigned to ($CH_3$), (-($CH_2$)$_9$-) and (-$CH_2\underline{CH_2}$-O) protons of SDS, respectively, disappeared confirming that SDS was removed completely. The appearance of a strong signal at 3.0–3.2 ppm attributed to the protons in methyl groups of the quaternary ammonium confirmed successful *O*-quaternization of chitosan. Calculation of DQ of chitosan was possible from the ratio of the signal at around 3.1 ppm attributed to the protons from the quaternary ammonium groups to the signal at 1.8–1.9 ppm attributed to the protons in the residual acetyl group of chitosan. Unfortunately, a strong signal of the protons from the quaternary ammonium was partially overlapping with the signal of H-2 of chitosan making evaluation of DQ less reliable than from elemental analysis.

*O*-Quaternization of chitosan was carried out in a mixture of DMSO and water (1 : 0.8 v/v), both in neutral and alkaline medium, or in a mixture of DMSO and

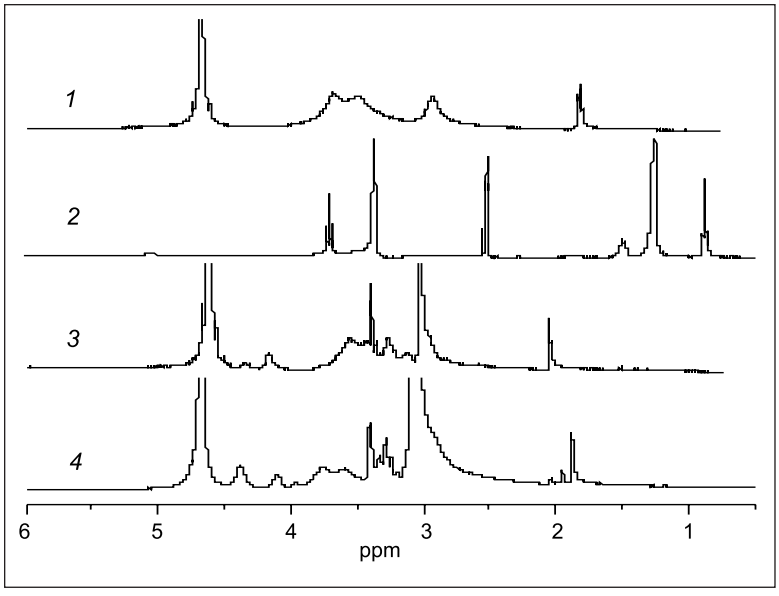



**Fig. 2.** $^1$H NMR spectra of chitosan (*1*), SCC (*2*), *O*-HTCC (DQ 28%) (*3*) and *N*-HTCC (DQ 68%) (*4*). The spectra of chitosan, *N*-HTCC and *O*-HTCC were recorded in $D_2O$ while that of SCC in DMSO-$d_6$

2-propanol (1 : 0.8 v/v). *O*-(2-Hydroxyl) propyl-3-trimethyl ammonium chitosan chloride (*O*-HTCC) with DQ 19 and 28% was synthesized by using DMSO/$H_2O$ and DMSO/2-propanol mixtures, respectively. Quaternization in alkaline medium was unsuccessful resulting in the products with very low DQ. Taking into account high viscosity of the *O*-quaternized chitosans, one can expect that the complexation / decomplexation processes did not invoke destruction of the chitosan skeleton. Unfortunately, *O*-quaternized chitosans were insoluble in water and not suitable for electrospinning.

**Partial destruction of the quaternized chitosans**

Viscosity of polymer solutions plays a major role in the production of nanofibers and is one of the most studied parameters in electrospinning [31]. It is well known [32] that as the concentration (and viscosity) of the polymer solutions or melts increases, the fiber diameter increases exponentially. Uniform fibers can be produced at relatively low viscosity ranging from 1 to 215 P [32]. What concerns spinning of chitosan, for the production of continuous and uniform fibers solution concentrations of 7–7.5% are optimal with viscosities ranging from 4.8 to 5.9 P [9].

It was determined that dynamic viscosity (DV) of 7% solution of *N*-HTCC with DQ 60% in 0.5% acetic acid was 338 P while that of *N*-HTCC with DQ 28% was even 2 930 P. The required viscosity at about 5 P can be achieved by dilution of the above solutions to 2.0–2.8% which is apparently low concentration for the production of nanofibers. It is obvious that the molecular weight of the quaternized chitosans is too high for getting solutions with the required viscosity.

Partial destruction of quaternized chitosans in their solutions could be carried out by chemical or physical techniques including gamma irradiation, a combination of ozone and UV irradiation, and sonication. For example, molecular weight of the quaternized carboxymethyl chitosan was reduced by the use of hydroperoxide [33]. An alternative possibility is enzymatic hydrolysis of chitosan and its derivatives [34]. It was determined that enzymatic hydrolysis of trimethylated chitosans by the use of the enzyme lysozyme depended on the degree of deacetylation (DA): polymers with a DA ≤ 17% were less susceptible to lysozyme-catalyzed degradation than the re-acetylated polymers with a DA ≥ 49%.

In the present study, partial destruction of the quaternized derivatives of chitosan was carried out by UV irradiation or enzymatic hydrolysis. Homogeneous solutions of the quaternized chitosans were irradiated by 250 W UV lamp varying concentration of HTCC, volume of the sample and a distance from the lamp to the quartz tube. It was determined that UV irradiation helped to decrease DV of the solutions of the quaternized chitosans with high DQ (Fig. 3, curves 1, 4). DV of *N*-HTCC (DQ 50%) dropped within 3 hours by 70 times reaching the values suitable for electrospinning. Behavior of more viscous solutions of the quaternized chitosans with lower DQ under irradiation was different (Fig. 3, curves 2, 3, 5). During the initial period of irradiation (30–40 min) DV decreased (Fig. 3, curve 2) or remained almost constant (Fig. 3, curves 3, 5). Under further irradiation DV turned to increase reaching very high values of 16 000 P for the quaternized chitosan with DQ 30%. Thus UV irradiation cannot be applied for partial destruction of the quaternized chitosans in their solutions if degree of quaternization is relatively low (<50%). On the other hand, this method is suitable for highly quaternized chitosans (DQ > 50%).

An increase in viscosity of the solutions of partially quaternized chitosan under UV-irradiation could be related to coupling reactions taking place in viscous solutions. Degradation of polymers under UV irradiation proceeds by free-radical mechanism, i. e. a cleavage of macromolecules goes through formation of polymeric or oligomeric free-radicals [35, 36]. Macroradicals are terminated by various reactions including chain transfer to other macromolecules

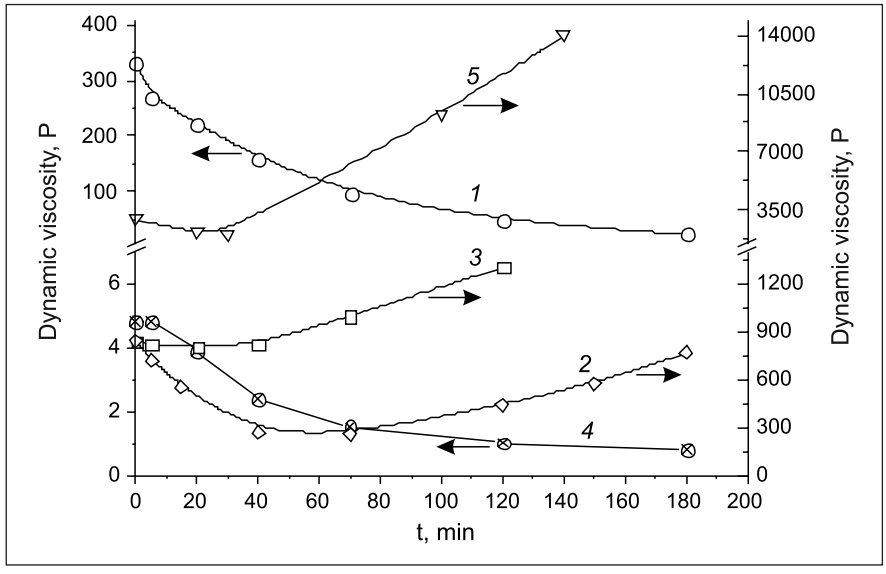



**Fig. 3.** UV irradiation of the 7% solution of *N*-HTCC with DQ 49% (*1*), 40% (*2, 3*), 68% (*4*) and 30% (*5*). The distance from the lamp to the quartz tube was 24 cm (*1, 2, 4, 5*) and 35 cm (*3*)

or low molecular compounds, and coupling with other macroradicals [37]. Quaternized chitosans with high DQ (low and moderate viscosity) can be attributed to quaternary ammonium salts which are relatively stable against cross-linking and gelation under ageing, including accelerated ageing under UV irradiation. Contrarily, quaternized chitosans with lower DQ (high viscosity) contain more residual amino groups and can be attributed to polymeric amines which are not stable against ageing and tend to cross-linking. Of course, an increase in molecular weight under UV irradiation could be related also to high viscosity of the solutions of quaternized chitosans with a relatively low DQ in which lifetime of the radicals is much longer and possibility for bimolecular coupling is much higher.

Enzymatic hydrolysis of the quaternized chitosans by the use of pectinase from *Aspergillus niger* or cellulase from *Trichoderma reesi* was carried out in various media including citrate and acetate buffers, and acetic and formic acids. The hydrolysis was successful in all the cases (Table 4). Since citrate anions were difficult to separate and the hydrolyzed product was contaminated by citrate residues, acetic acid was used for the detailed study of enzymatic destruction of the quaternized chitosans.

Enzymatic hydrolysis of 4% solutions of the quaternized chitosans was very fast resulting in the products with 10–30 times lower DV within 2–3 hours (Fig. 4). Concentration of an enzyme had little effect. Although both enzymes were efficient, a few better results were achieved using cellulase. Enzymatic hydrolysis during 5 h enabled to reduce DV of 4% solutions of the quaternized chitosans with DQ 30–40% to 1–2 P (Fig. 4). Similar results were obtained under hydrolysis of 7% solutions of the quaternized chitosans (Fig. 5).

In order to evaluate the scope and limitations of the method, further enzymatic hydrolysis was carried out up to 10 days. Degradation of the quaternized chitosan was evaluated by a decrease in intrinsic viscosity which neared to 0.7 dL/g after 10 days of the hydrolysis and did not change later (Fig. 6). It means that enzymatic hydrolysis of the quaternized chitosans stops at a certain stage.

Although enzymatic hydrolysis of the quaternized chitosan by pectinase from *Aspergillus niger* or cellulase from *Trichoderma reesi* does not lead to the quaternized glucosamine or other low molecular fragments, it helps a lot in getting polymer solutions with the viscosity prerequisite for electrospinning. For example, degradation of 7% solution of *N*-HTCC (DQ 30%, DV 2930 P) in the presence of pectinase from *Aspergillus niger* for 22 h at 37 °C gave DV of 5 P.

Solutions of enzymatically degraded *N*-HTCC were tested for production of nanofibers by electrospinning [38]. The electrospinning solutions were prepared by mixing 8 wt.%

Table 4. **Dynamic viscosity (DV) of the 2% solution of N-HTCC (DQ 49%, DV = 290 cP) after enzymatic hydrolysis in various media at 18 °C**

| Reaction medium | Enzyme | Time of enzymatic hydrolysis, h | DV, cP |
|---|---|---|---|
| Citrate buffer, pH 3.0 | Pectinase (0.04 g/1 g HTCC) | 24 | 41 |
| Acetate buffer, pH 4.6 | Pectinase (0.1 g/1 g HTCC) | 18 | 30 |
| | Cellulase (0.1 g/1 g HTCC) | 18 | 21 |
| Acetic acid, pH 3.25 | Pectinase (0.04 g/1 g HTCC) | 24 | 41.6 |
| | Cellulase (0.04 g/1 g HTCC) | 6.5 | 58 |
| Formic acid, pH 2.7 | Pectinase (0.04 g/1 g HTCC) | 24 | 38.8 |

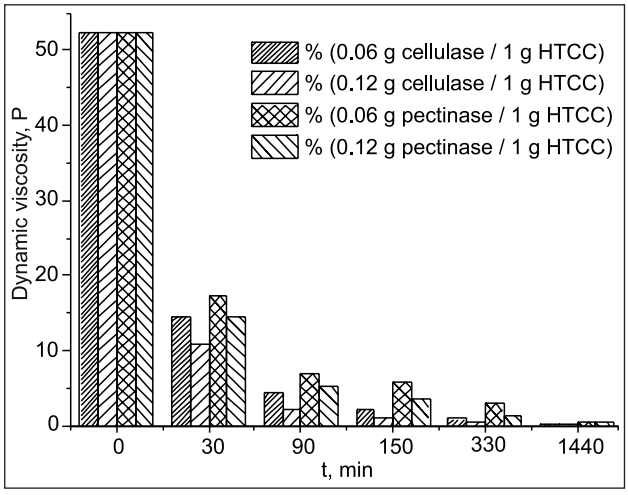



**Fig. 4.** Dynamic viscosity of the 4% solution of *N*-HTCC (DQ 40%) in acetate buffer versus the amount of pectinase from *Aspergillus niger* or cellulase from *Trichoderma reesi* and the time of enzymatic hydrolysis at 18 °C

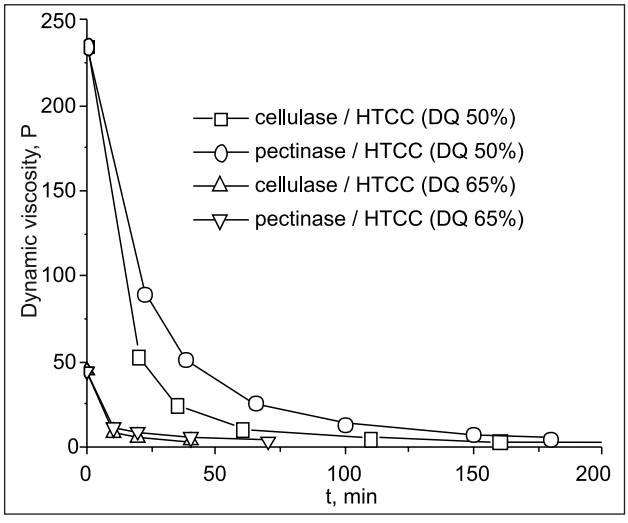



**Fig. 5.** Dynamic viscosity of the 7% solution of *N*-HTCC in acetate buffer versus the enzyme type and the time of enzymatic hydrolysis at 18 °C (0.12 g of enzyme to 1 g of HTCC)

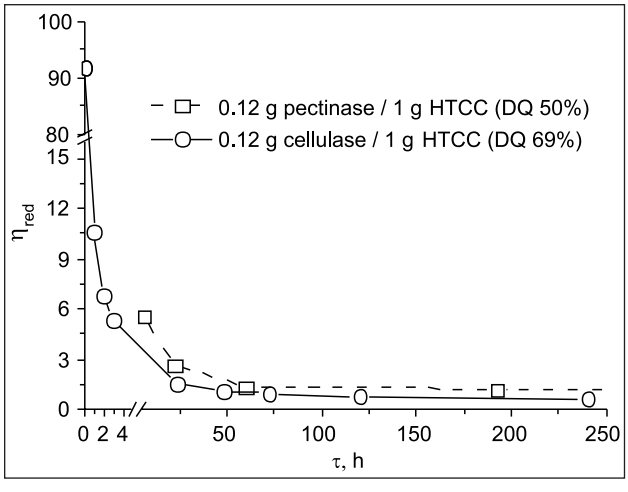



**Fig. 6.** Reduced viscosity of the 4% solution of *N*-HTCC versus the time of enzymatic hydrolysis at 18 °C

solution of poly(vinyl alchohol) (PVA) with 8 wt.% solution of the quarternized chitosan (DQ 30%) at weight ratios HTCC : PVA = 15 : 85 to 25 : 75. Electrospinning from HTCC/PVA solutions resulted in nanofiber webs possessing cationic properties [38]. More uniform nanofibers were formed from the compositions containing lower content of *N*-HTCC.

## CONCLUSIONS

1. *N*-Quaternized derivatives of chitosan (*N*-HTCC) with a degree of quaternization (DQ) varying from 30 to 68% were synthesized by the reaction of chitosan with (2,3-epoxypropyl) trimethylammonium chloride (EPTMAC) in acidic or neutral media. Additional quaternization of *N*-HTCC in alkaline media through its hydroxyl groups enabled to increase DQ of chitosan up to 95% resulting in chitosan derivatives *N,O*-HTCC with a very high charge density.

2. For protection of the amino functionality of chitosan and quaternization of chitosan exclusively through its hydroxyl groups (synthesis of *O*-HTCC), chitosan-dodecyl sulfate complexes were prepared and reacted with EPTMAC. Quaternization of chitosan by this pathway was unsuccessful resulting in *O*-HTCC with DQ about 20% which were insoluble in water.

3. Partial destruction of the quaternized chitosans with high DQ (>50%) by UV irradiation helped to decrease dynamic viscosity (DV) of the solutions by tenfold and more making them suitable for electrospinning. This method cannot be applied to the quaternized chitosans with lower DQ since secondary reactions of coupling prevail in this case significantly increasing viscosity of the solutions.

4. Enzymatic hydrolysis of the quaternized chitosans by pectinase or cellulase in their solutions was very fast resulting in the products with tenfold or even thirtyfold lower DV within 2–3 hours. This method is suitable for the reduction of DV of the solutions of the quaternized chitosans irrespective of DQ. Enzymatic hydrolysis of the quaternized chitosans stops at a certain stage producing oligomeric cationic chitosan derivatives instead of the quaternized glucosamine.

Received 4 July 2013
Accepted 5 September 2013

### References

1. J. Wu, W. Wei, L. Y. Wang, Z. G. Su, G. H. Ma, *Coll. Surf. B: Biointerfaces*, **63(2)**, 164 (2008).
2. Z. Y. Guo, R. E. Xing, S. Liu, et al., *Carbohydr. Res.*, **342**, 1329 (2007).
3. D. D. Britto, R. C. Goy, S. P. C. Filho, A. B. G. Assis, *Int. J. Carbohydr. Chem.*, **2011**, Article ID 312539, doi: 10.1155/2011/312539 (2011).
4. H. Tan, R. Ma, C. Lin, Z. Liu, T. Tang, *Int. J. Mol. Sci.*, **14**, 1854 (2013).
5. R. Li, Z. Guo, P. Jiang, *Carbohydr. Res.*, **345**, 1896 (2010).

6. X. Wang, X. Pei, Y. Du, Y. Li, *Nanotechnology*, **19(37)**, 375102.1 (2008).
7. M. Ignatova, K. Starbova, N. Markova, N. Manolova, I. Rashkovet, *Carbohydr. Res.*, **341(12)**, 2098 (2006).
8. M. Ignatova, N. Manolova, I. Rashkov, *Eur. Polym. J.*, **43**, 1112 (2007).
9. X. Geng, O. H. Kwon, J. Jang, *Biomaterials*, **26**, 5427 (2005).
10. R. Belalia, S. Grelier, M. Benaissa, V. Coma, *J. Agric. Food Chem*., **56**, 1582 (2008).
11. A. Dormard, M. Rinaudo, C. Terrassin, *Int. J. Biol. Macromol.*, **8**, 105 (1986).
12. J. Wu, W. Wei, L. Y. Wang, Z. G. Su, G. H. Ma, *Biomaterials*, **28**, 2220 (2007).
13. S. Channasanon, W. Graisuwan, S. Kiatkamjornwang, V. P. Hoven, *J. Colloid Interface Sci.*, **316**, 331 (2007).
14. C. Qin, Q. Xiao, H. Li, et al., *Int. J. Biol. Macromol.*, **34**, 121 (2004).
15. Y. Lapitsky, T. Zahir, M. S. Shoichet, *Biomacromolecules*, **9**, 166 (2008).
16. E. Faizuloev, A. Marova, A. Nikonova, I. Volkova, M. Gorshkova, V. Izumrudov, *Carbohydr. Polym*., **89**, 1088 (2012).
17. H. S. Seong, H. S. Whang, S. W. Ko, *J. Appl. Polym. Sci*., **76**, 2009 (2000).
18. R. Klimaviciute, A. Riauka, A. Zemaitaitis, *J. Polym. Res.*, **14**, 67 (2007).
19. D. Sidlauskiene, R. Klimaviciute, A. Zemaitaitis, *J. Appl. Polym. Sci.*, **107**, 3469 (2008).
20. Y. Sun, A. Wan, *J. Appl. Polym. Sci*., **105**, 552 (2007).
21. F. S. Kittur, A. B. Vishu Kumar, R. N. Tharanathan, *Carbohydr. Res.*, **338(12)**, 1283 (2003).
22. G. Cai, H. Jiang, K. Tu, L. Wang, K. Zhu, *Macromol. Biosci*., **9(3)**, 256 (2009).
23. N. Gorochovceva, R. Makuska, *Eur. Polym. J.*, **40**, 685 (2004).
24. U. Janciauskaite, V. Rakutyte, J. Miskinis, R. Makuska, *React. Funct. Polym.*, **68**, 787 (2008).
25. J. Cho, J. Grant, M. Piquette-Miller, C. Allen, *Biomacromolecules,* **7**, 2845 (2006).
26. A. M. M. Sadeghi, F. A. Dorkoosh, M. R. Avadi, et al., *Eur. J. Pharm. Biopharm.*, **70(1)**, 270 (2008).
27. J. Bendoraitiene, R. Kavaliauskaite, R. Klimaviciute, A. Zemaitaitis, *Starch / Stärke*, **58**, 623 (2006).
28. K. G. Skryabin, G. A. Vihoreva, V. P. Varlamov, *Chitin and Chitosan. Production, Properties and Use*, Nauka, Moscow (2002).
29. R. Makuska, N. Gorochovceva, *Carbohydr. Polym*., **64(2)**, 319 (2006).
30. J. Holappa, T. Nevalainen, J. Savolainen, et al., *Macromolecules,* **37(8)**, 2784 (2004).
31. S. Sukigara, M. Ghandi, J. Ayutsede, M. Micklus, F. Ko, *Polymer*, **44(19)**, 5721 (2003).
32. J. Lyons, F. Ko, *Polymer News*, **30**, 1 (2005).
33. L. Sun, Y. Du, L. Fan, X. Chen, J. Yang, *Polymer*, **47**, 1796 (2006).
34. R. J. Verheul, M. Amidi, M. J. van Steenbergen, E. van Riet, W. Jiskoot, W. E. Hennink, *Biomaterials*, **30**, 3129 (2009).
35. A. A. Andrady, A. Torikai, T. Kobatake, *J. Appl. Polym. Sci.*, **62**, 1465 (1996).
36. V. A. Sharpatyi, *High Energy Chem.*, **40**, 18 (2006).
37. W. Kleeberg, W. Rogler, W. V. Gentzkow, R. Rubner, US Patent 4283505.
38. R. Makuška, R. Kulbokaitė, J. Bendoraitienė, E. Adomavičiūtė, R. Milašius, *International Conference "Polyelectrolytes 2008". Book of Abstracts,* Coimbra, Portugal, 199 (2008).

**Rūta Gruškienė, Rasa Deveikytė, Ričardas Makuška**

## CHITOZANO KVATERNIZAVIMAS IR KVATERNIZUOTŲ DARINIŲ DALINĖ DESTRUKCIJA SIEKIANT PADARYTI JUOS TINKAMUS ELEKTROVERPIMUI

*S a n t r a u k a*

Ištirtas chitozano modifikavimas veikiant jį *N*-2,3-epoksipropil-*N,N,N*-trimetilamonio chloridu (EPTMAC). Rūgštinėje ir neutralioje vandeninėje terpėje susintetinti kvaternizuoto chitozano (*N*-HCTT) dariniai, kuriuose chitozano kvaternizavimo laipsnis (KL) siekia iki 68 %. *N*-HCTT papildomai kvaternizuojant šarminėje terpėje, kur EPTMAC prijungiamas prie chitozano ir per hidroksigrupę, KL padidintas iki 95 %. Chitozano-dodecilsulfato kompleksuose esančio chitozano kvaternizavimas buvo neefektyvus, kadangi KL siekė tik apie 20 %, produktas buvo netirpus vandenyje.

Siekiant sumažinti kvaternizuoto chitozano tirpalų klampą ir padaryti juos tinkamus nanopluoštų formavimui elektroverpimo būdu, ištirta dalinė šių polimerų destrukcija veikiant UV spinduliuote ir fermentais. UV spinduliuotė mažino kvaternizuoto chitozano su dideliu KL (>50 %) tirpalų klampą, tačiau netiko chitozano dariniams su mažu KL. Kvaternizuotų chitozano darinių hidrolizė jų tirpaluose, veikiama fermentų pektinazės ir celulazės, yra labai greita, tirpalų klampa per 2–3 valandas sumažėja 10–30 kartų. Kvaternizuotų chitozano darinių fermentinė hidrolizė vyksta ne iki galo, todėl gaunamas ne kvaternizuotas gliukozaminas, o oligomeriniai kvaternizuoto chitozano dariniai.