Quaternized and Unmodified Chitosans: Hydrodynamic Properties

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Abstract: Molecular properties of N-(2-hydroxy-3-trimethylammonium)propyl]chitosan (modified chitosan) series with the averaged quaternization degree 90% have been studied in comparison with the unmodified chitosan series by the method of translation isothermal diffusion, viscometry and static light scattering in dilute solutions in 0.33M CH₃COOH-0.2M CH₃COONa at pH 3.54. Molecular mass, translation diffusion coefficient, and hydrodynamic size of the homologues samples in the modified/unmodified series have been determined as well as their chain rigidity and Mark-Kuhn-Houwink equations at acidic pH. It was established that the size of modified chitosan molecules might be smaller than the initial polysaccharide of an equal polymerization degree in the same solvent, which was explained by the change of thermodynamic conditions and the change of the ratio of thermodynamic/electrostatic contributions to the total chain rigidity. Quaternized chitosan molecules displayed the different hydrodynamic behavior in 0.33M CH₃COOH-0.2M CH₃COONa and in 0.2M NaCl (neutral pH). Solution properties of quaternized chitosan at neutral pH had been identified as the concentration dependent. The threshold influence of the secondary amino group protonation on the hydrodynamic properties of modified chitosan molecules was detected in 0.2M NaCl at the solute concentration range 0.001-0.004 g/cm³.

Keywords: Polysaccharides, chitosan, quaternization, hydrodynamic behavior.

1. INTRODUCTION

Insolubility of chitosan in water at neutral pH, favorable for biomedical applications, limits a direct utilization of this polysaccharide and compels to improve its solubility by means of chemical modification. It is well known that chitosan (deacetylated chitin) dissolves at pH less than 6.5 owing to protonation of its amino groups i.e. at acidic conditions only. Insertion of the charged quaternary ammonium moieties onto the chitosan chain is one of the possibilities to improve its solubility in wide pH range [1].

Quaternary ammonium containing chitosans are attractive derivatives for the expansion of application of this practically important polysaccharide [1-10]. During the last decade it was shown that chitosan quaternizes in different ways and exhibits the enhanced bacteriostatic and microbiostatic activity being compared to the initial analogue [2-5]. It was also shown that quaternized chitosan can be used as the effective drug delivery carrier and gene-material carrier [1, 6-8]. Predominantly, quaternized chitosans are the objects of interest in the field of biomedical applications due to their low toxicity, biodegradability, and biocompatibility. But the food science [9] and textile manufacturing [2-5, 10] industries also continue to display a significant attention to chitosan derivatives containing quaternary ammonium.

Quaternization of chitosan by means of the two most commonly known ways leads to increase of a total positive charge and appearance of water-solubility of the modified polysaccharide [1, 10, 11]. The simplest way of quaternization is related to formation of quaternary ammonium moieties on the backbone of chitosan using its own primary amino groups [11]. The second way is based on the reaction of amino groups of chitosan with an epoxy-group of quaternary ammonium containing compounds, which is implemented by the inclusion of quaternary ammonium moieties to the side-substituents of polysaccharide chain. Lim and Hudson were the first who had shown that glycidyl trimethylammonium chloride is a favorable compound for quaternization of chitosan by the second way [10].

In contrast to chitosan, whose solution properties are relatively well known [12-16], the molecular behavior of quaternized chitosans are not sufficiently studied in dilute solutions at the variable conditions. One of possible reasons for this is a modern tendency to accelerate the study of biomacromolecules by means of maximally fast transition to the practical plane. Another reason is related to a sustainable opinion that quaternization just increases a total charge, but weakly influences molecular properties of chitosan. In the majority of publications devoted to quaternized chitosans, a characterization of the samples just concerns the confirmation of changes in chemical structure of initial polysaccharide and determination of its degree of quaternization (DQ)
without accounting for possible changes in molecular behavior of the modified polysaccharide [2-5, 10, 11, 17, 18]. Quaternized chitosanes, whose biological activity is doubtless [10, 17-20], continue to be the object of investigations. Data on molecular properties, including a hydrodynamic mobility, hydrodynamic dimension, chain rigidity and conformation of quaternized chitosan derivatives at different pH, ionic strength and in a different countertions media are not yet systematized, that may lead to ambiguous conclusions on their properties.

In the present work a comparative study of hydrodynamic properties of \( N \)-[(2-hydroxy-3-trimethylammonium)propyl]chitosan (HTPC) in acidic solvent \( 0.33 \text{M CH}_3\text{COOH}+0.2 \text{M CH}_3\text{COONa} \) and in the solvent with neutral pH (0.2M NaCl) have been performed. A comparison of hydrodynamic properties of HTPC and initial chitosan samples in the same buffer (0.33M CH\(_3\)COOH+0.2M CH\(_3\)COONa) has also been made.

HTPC is active as an antimicrobial/antibacterial agent [10, 17, 18] and can be used as an agent of the delivery systems [19]. Properties of HTPC continue to be under discussion. For instance, the opposite tendencies of antibacterial activity of HTPC against \( E. \) coli at weak acidic and at alkaline conditions were detected in [17, 18].

2. EXPERIMENTAL

2.1. Materials

Two chitosan products of Bioprogress Ltd. (Russia) (degree of deacetylation of chitin 82% and 90%, respectively, weight fraction of sol less than 5%) were used as the parent polysaccharide samples for further depolymerization and chemical modification.

Original chitosan products were preliminarily reprecipitated and lyophilized. Series of chitosan samples with various molecular mass were produced by depolymerization of the high-molecular chitosan products using the procedure described in [20]. A high degree of deacetylation of the received chitosan samples was confirmed by \(^1\text{H NMR} \) data and was similar to the parent products within a standard accuracy.

Series of HTPC samples in the salt form were synthesized according to a methodology described in detail earlier [19, 21], and using the received chitosan series as the initial polymers for modification. Synthetic methodology is based on the adapted methodology suggested by Lim and Hudson [10, 22]. It gives a possibility to receive HTPC of high degree of quaternization (DQ). The HTPC samples with the mean DQ 90±5% were selected for the present study.

Chitosan sample and HTPC sample have the same number in Table 1, if a corresponding chitosan was used as the initial sample for modification.

2.2. Solvents

The solvent, \( 0.33 \text{M CH}_3\text{COOH}+0.2 \text{M CH}_3\text{COONa} \), was prepared using a bidistilled water, glacial acetic acid, and trihydrate sodium acetate (reagent grade, Vekton, St. Petersburg, Russia). The HTPC and chitosan samples were dissolved directly in \( 0.33 \text{M CH}_3\text{COOH}+0.2 \text{M CH}_3\text{COONa} \) during one to two days at room temperature under stirring. In the same way, the solutions of HTPC in 0.2M NaCl were prepared. Sodium chloride (Vekton, St. Petersburg, Russia) and trihydrate sodium acetate were used after drying under vacuum.

At the temperature 298 K, the solvents had the following physical properties: density of 1.02 g/cm\(^3\), viscosity of 1.165 cP, refractive index of 1.3395 — 0.33M CH\(_3\)COOH+0.2M CH\(_3\)COONa, and density of 0.99 g/cm\(^3\), viscosity of 0.90 cP, refractive index of 1.3398 — 0.2M NaCl.

2.3. Methods

The following molecular properties of chitosan and HTPC molecules have been determined experimentally: translational diffusion coefficient \( D \), intrinsic viscosity \([\eta]\), hydrodynamic radius \( R_h \), molecular mass \( M \), and chain rigidity \( A \).

The translational diffusion coefficients \( D \) of polymers were determined by the isothermal translational diffusion method using a Tsvetkov's diffusometer-interferometer [23]. This method is attributed to so
called “transport methods” in polymers’ hydrodynamics, that are based on mass transfer through the solvent-solution boundary under the treatment of any external force. The method used permits to observe the diffusion process in the natural gravity field and to operate in the region of highly dilute solution due to sensitivity of the interferometric scheme of registration. A quartz cell of 3 cm in length was applied, where the diffusion interface was formed by laying the solvent beneath the solution. The position of the diffusion boundary was recorded on a digital array at fixed time intervals. The diffusion interferograms were processed on a PC by the method of “maximum ordinate and area” [23], using an automatic algorithm, which is based on fitting the image contrast and the use of Lame’s procedure for exact description of the interference curve in the coordinate grid [24]. Coefficient $D$ was calculated from the slope of the experimental dependence of the dispersion of the diffusion curve $\sigma^2$ on the duration of the experiment run $t$ (Figure 1) according to the equation

$$\sigma^2 = 2Dt$$ (1).

Coefficients $D$ were determined at the several solute concentrations $c$ within a range $(0.03-0.08)\times10^{-2}$ g/cm$^3$ and then extrapolated to the zero concentration (according to relationship (2)), so that to determine the translational diffusion coefficient $D_0$ at infinite dilution.

$$D_0 = \lim_{c \to 0} D,$$ (2)

We do not observe any concentration dependence of $D$ for chitosan and HTPC in 0.33M CH$_3$COOH+0.2M CH$_3$COONa. $D_0$ was determined as the concentration averaged value.

The hydrodynamic radius $R_h$ of macromolecules was calculated using Stokes-Einstein equation for

<p>| Table 1: Molecular Properties of Chitosan and HTPC in 0.33M CH$_3$COOH + 0.2M CH$_3$COONa at 298 K |</p>
<table>
<thead>
<tr>
<th>Sample</th>
<th>$M_w\times10^3$, g/mol</th>
<th>$P$</th>
<th>$[\eta]\times10^{-1}$, cm$^2$/g</th>
<th>$k'$</th>
<th>$D_0\times10^4$, cm$^2$/s</th>
<th>$A_0\times10^{10}$</th>
<th>$^6M_0\times10^3$, g/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitosan</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1</td>
<td>100±10</td>
<td>600</td>
<td>4.0±0.1</td>
<td>0.33</td>
<td>1.3±0.1</td>
<td>3.73±0.06</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>60±5</td>
<td>360</td>
<td>3.2</td>
<td>0.35</td>
<td>1.7</td>
<td>3.61</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>300</td>
<td>2.5</td>
<td>0.38</td>
<td>2.0</td>
<td>3.69</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>240</td>
<td>2.0</td>
<td>0.35</td>
<td>2.3</td>
<td>3.65</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>120</td>
<td>0.78±0.05</td>
<td>0.33</td>
<td>3.9</td>
<td>3.60</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>200±20</td>
<td>1100</td>
<td>9.7±0.1</td>
<td>0.30</td>
<td>0.7</td>
<td>3.40</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>45±5</td>
<td>280</td>
<td>2.7</td>
<td>0.34</td>
<td>2.0</td>
<td>3.86</td>
<td>-</td>
</tr>
<tr>
<td>HTPC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HTPC-1</td>
<td>200±20</td>
<td>$^6$660/500</td>
<td>1.6</td>
<td>0.42</td>
<td>1.5±0.1</td>
<td>-</td>
<td>152</td>
</tr>
<tr>
<td>HTPC-4</td>
<td>80±10</td>
<td>260/280</td>
<td>1.2</td>
<td>0.40</td>
<td>2.0</td>
<td>-</td>
<td>86</td>
</tr>
<tr>
<td>HTPC-5</td>
<td>40±5</td>
<td>130/120</td>
<td>0.56±0.05</td>
<td>0.34</td>
<td>3.5</td>
<td>-</td>
<td>35</td>
</tr>
<tr>
<td>HTPC-5'</td>
<td>20</td>
<td>70/70</td>
<td>0.33</td>
<td>0.35</td>
<td>5.0</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>HTPC-6</td>
<td>500±20</td>
<td>1600/1300</td>
<td>4.0±0.1</td>
<td>0.40</td>
<td>0.8</td>
<td>-</td>
<td>400</td>
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<tr>
<td>HTPC-7</td>
<td>80±5</td>
<td>270/250</td>
<td>1.2</td>
<td>0.39</td>
<td>2.1</td>
<td>-</td>
<td>75</td>
</tr>
</tbody>
</table>

$^6M_0$, was calculated according eq. (6) with the mean value of hydrodynamic invariant $A_0=\langle 3.66±0.06\rangle\times10^{-10}$ g cm$^2$/(s$^2$ deg mol$^{1/3}$) determined for chitosan series with deacetylation degree 90%.

Figure 1: The dependence of the dispersion of diffusion curve $\sigma^2$ on the time $t$ of diffusion process in solutions of chitosan samples 1-5 (curve number correspond to sample’s number in Table 1), HTPC-1 (a), HTPC-4 (b), and HTPC-5' (c) at solute concentration $0.05\times10^{-2}$ g/cm$^3$ in 0.33M CH$_3$COOH + 0.2M CH$_3$COONa.
spherical particles (Eq. (3)) and the experimental $D_0$ values.

$$R_h = kT / 6\pi\eta_0D_0$$  \hspace{1cm} (3)

Here, $k$ is the Boltzmann constant, $T$ is the absolute temperature, and $\eta_0$ is the solvent viscosity.

From the translational diffusion data, we were also able to calculate the refractive index increment $dn/dc$ of the polymer-solvent system using the area encompassed by the curved interference fringe [23]. Sample-averaged refractive index increments $dn/dc = 0.153 \pm 0.007$ (chitosan – 0.33M CH$_3$COOH – 0.2M CH$_3$COONa system) and $dn/dc = 0.144 \pm 0.006$ (HTPC–0.33M CH$_3$COOH–0.2M CH$_3$COONa system) have been utilized for processing of the static light scattering data.

The viscosity was measured with an Ostwald capillary viscometer with the outflow time (84.2±0.1) s of 0.33M CH$_3$COOH+0.2M CH$_3$COONa, and (64.0±0.1) s of 0.2M NaCl. The intrinsic viscosity value [$\eta$] of the polymer solution was determined according to Huggins’ graphical extrapolation of $\eta_{ip}/c$ value to zero solute concentration (Eq.(4)), (Figure 2).

$$\eta_{ip}/c = [\eta] + k'[\eta]^2c + \cdots,$$  \hspace{1cm} (4)

where $\eta_{ip}/c = (\eta - \eta_0)/\eta_0$, $c = (t - t_o)/t_o$, $\eta$ and $\eta_0$ are the viscosities of the solution and solvent, respectively; $t$ and $t_o$ are the outflow times of the solution and solvent in the viscometer; $k'$ is the Huggins constant corresponding to the slope of the linear function $\eta_{ip}/c = f(c)$.

The intensity of the light scattering from the chitosan solutions was measured within the scattering angle range $\theta = 30^\circ$–$130^\circ$ on a PhotoCor Complex setup (Russia). The temperature of solutions during measurements was controlled at 298.00±0.05 K. A diode laser operating at wavelength $\lambda_o=654$ nm was used as a light source.

The molecular mass $M_w$ and the second virial coefficient $A_2$ were determined from the static light scattering data using equation (5) and corresponding plotting (Figure 3) [25].

$$Hc / I(0,c) = 1 / M_w + 2A_2c$$  \hspace{1cm} (5)

where $H = 4\pi^2 n_0^2 (dn/dc)^2 / N_0 A_0^4$ is the scattering constant, $n_0$ is the refractive index of the solvent, $I(0, c)$ is the Rayleigh factor that was determined for each solute concentration $c$ under the condition $\theta \to 0$.

Finally, the molecular mass $M_w$ was calculated from the intercept of the function $Hc/I(0, c) = f(c)$, and the second virial coefficient $A_2$ was determined from the slope of the same function.

Dynamic light scattering was also applied to control the hydrodynamic sizes and distribution of particles in solutions. Autocorrelation functions of the scattered intensity of light have been analyzed using a DynaLS Software product. The translation diffusion coefficients
D have been determined from the dynamic light scattering data using the relationship $D = (1/\eta q^2)_{q=0}$, where $q = 4\pi n_0 \sin(\theta/2)/\lambda_0$ is the scattering vector [25].

Molecular mass $M_{Dq}$ of HTPC samples was determined from the diffusion data and viscometric measurements, using equation (6).

$$M_{Dq} = (A_0 T / \eta_n) D_0^{100} / [\eta],$$

(6)

Where, $A_0$ is the hydrodynamic invariant, which usually has a constant value for the same class of polymers [23].

The hydrodynamic invariant $A_0$ was calculated for chitosan samples, using eq. (6) and molecular mass $M_w$, determined for them by means of the independent method. The $A_0$ values are presented in Table 1. The sample-averaged value $A_0=3.66\pm0.05\times10^{-10}$ g cm$^2$ /c$^2$ deg mole$^{1/3}$ obtained for chitosan series, corresponds well to the typical hydrodynamic invariant value for a rigid-chain polymers $A_0=3.6\times10^{-10}$ g cm$^2$ /c$^2$ deg mole$^{1/3}$ [23]. Experimental sample-averaged value of $A_0$ was used for calculation of $M_{Dq}$ of HTPC samples, which also are shown in Table 1.

The Spectrophotometer SF-2000 (UV/Vis, resolution 1 nm), managed automatically by original program of OKB “Spectr” (St.Petersburg, Russia), has been used for the study of absorbance of HTPC samples in 0.2M NaCl. Quartz cells of 1 cm in length have been used for measurements. Spectra were recorded at solute concentrations (0.4-0.03)x10$^{-2}$ g/cm$^3$ in a spectral region of 190-400 nm.

The measurements of pH in solutions of modified chitosan have been performed with pH meter (FEP20, Mettler Toledo) calibrated using three standard buffers NIST, DIN19266, and DIN19268. The accuracy of pH determination was ±0.01.

3. RESULTS AND DISCUSSION

3.1. Hydrodynamic Properties Chitosan and HTPC in Acidic Solvent

From the structural point of view, both objects under investigation are polymers having the same backbone and charged side-substituents of different structure. A position of charged groups relating to backbone is different for chitosan and HTPC. Amino groups of chitosan, protonated and charged in acidic solvent, are situated very close to the backbone, while the charged quaternized amino groups of HTPC are removed from the polysaccharide main chain at a distance of ~ 0.5-0.6 nm. It was shown earlier, that the secondary amino groups of HTPC do not protonate at acidic conditions [21]. In spite of HTPC being in the salt form, the polyelectrolyte nature of both polymers under investigation justifies the use of buffer for comparative study of hydrodynamic properties of their molecules. The buffer 0.33M CH$_3$COOH+0.2M CH$_3$COONa was selected for comparison of chitosan and HTPC molecular properties taking into account the following additional reasons: a) this acetic buffer is a traditional solvent for chitosan [13-16, 26, 27], and b) the equivalent acidic conditions are often chosen for biological activity testing of chitosan and its quaternized derivatives [3, 5,17, 18].

Stating the analysis of experimental data of Table 1, the following remark should be made. Molecular mass of HTPC samples have been determined in 0.33M CH$_3$COOH+0.2M CH$_3$COONa by two methods, founded on the different physical principles, to be sure of the molecular dispersity of the solutions studied. As easy to see from Table 1, the values of $M_w$ and $M_{Dq}$ are not correlated well only for the high molecular sample HTPC-6 that permitted us to be sure in the absence of aggregation of the studied polymers in dilute solutions.

The pairs of chitosan/HTPC samples of the same polymerization degree $P = M/M_0$ (here $M_0$ is the molecular mass of monomer unit) will be a starting point of the discussion.

An experimental fact that immediately manifests itself under comparing the hydrodynamic properties of the pairs chitosan/HTPC is a substantial difference of their intrinsic viscosity values $[\eta]$. For instance, the $[\eta]$ values of HTPC-1, 4, 6, 7 are several times smaller than that of chitosan samples 1, 4, 6 and 7, which were used for the synthesis of quaternized derivatives.

Since the intrinsic viscosity $[\eta]$ depends on the size of macromolecules in solution, the viscometric data indicates a decreasing molecular size of HTPC in solution if compared to chitosan, although their $M_w$ values are significantly higher in relation to the initial chitosan. It should be taken into account here that the mean value of the molecular mass of monomer unit of HTPC with $M_0 = 302$ g/mol at a deacetylation degree of 90% and a DQ of 90% is essentially heavier than that of chitosan, for which $M_0$ is 165 g/mol at a deacetylation degree 90%. And thus, the modification of initial chitosan to its quaternized form – HNPC, nearly twice increases the linear density of the polysaccharide chain.
i.e. molecular mass per the chain unit length \( M_L = M_0/\lambda \), where \( \lambda \) is the projection of the monomer unit onto the direction of the chain propagation. The latter leads to the conclusion that the increasing density of the HTPC coil is observed in solution if the hydrodynamic size of its molecules are less or close to the size of chitosan molecules in 0.33M CH\(_3\)COOH + 0.2M CH\(_3\)COONa.

The plot \( R_h = f(\lg P) \) depicted in Figure 4 shows a more compact in comparison with chitosan hydrodynamic dimension of HTPC molecules at polymerization degree \( P \geq 240 \) (or \( M \geq 80 \times 10^3 \)). The hydrodynamic radius \( R_h \) of molecules was calculated from eq. (3), using the experimental values of their translational diffusion coefficient \( D_0 \) (Table 1).

**Figure 4:** The dependence of hydrodynamic radius \( R_h \) on polymerization degree \( P \) of the samples for chitosan series (a) and HTPC series (b) at \( P < 700 \) in semi logarithmic scale.

The disparity with [\( \eta_0 \)] and \( R_h \) of compared polymers in the same solvent may be caused by different thermodynamic conditions for each of them in this solvent or by a change of conformational properties of HTPC chain as a consequence of chemical modification. Both of these assumptions can be verified based on the received experimental data.

A change in the chemical structure of side-substituents usually affects the molecular properties of a polymer as it was shown, for instance, for multiple cellulose derivatives [23]. In particular, it is known that elongation of side-chain-substituents influences not only the solubility of cellulose derivatives, but also increases their chain rigidity [23]. In contrast to the uncharged polysaccharide cellulose, hydrodynamic behavior of chitosan and its derivatives is additionally complicated by the electrostatic interactions of the charged groups in a polymer chain. As a result, chain rigidity of chitosan consists of two components: the contribution of the equilibrium thermodynamic rigidity of the main polysaccharide chain and the electrostatic contribution, which may be significant in value according to [15, 16, 27].

Chain rigidity is the important conformational parameter of polymer chain which is usually associated with the Kuhn segment length \( A \). The polymerization degree \( P \) of the samples under consideration varies from \( \sim 100 \) to \( \sim 1000 \), that is high enough to be sure of Gaussian properties of their chains in solution [28]. The hydrodynamic characteristics of chitosan and HTPC molecules presented in Table 1 make it possible to determine their equilibrium rigidity, for example, using a theory of translational friction of partially penetrable persistent chains [29]. The theoretical dependence \( D_0/M/RT = f(M^{1/2}) \) (Eq. (7)) of this theory usually describes well the behavior of polysaccharides in solutions [13, 14, 23, 26]. The plot \( D_0/M/RT = f(M^{1/2}) \) for both series of the samples is shown in Figure 5.

\[
D_0 M / RT = \left(1 / P \cdot N_A \eta_0 \right) (M_L / A)^{1/2} M^{1/2} + \left( M_L / 3\pi N_A \eta_0 \right) [\ln (A / d) - Q]
\]

(7)

Here, \( R \) is universal gas constant, \( P_f = 5.11 \) is the Flory hydrodynamic constant, \( N_A \) is the Avogadro number. \( A \) is the length of the statistical Kuhn segment, which is equal to the double persistent length value, \( M_L \) is the molecular mass per the chain unit length, \( d \) is the chain hydrodynamic diameter, and \( Q = 1.056 \) is a numerical coefficient of the theory [29].

**Figure 5:** Dependence of \( D_0 M/RT \) versus \( M^{1/2} \) for chitosan series (a) and HTPC (b) in 0.33M CH\(_3\)COOH + 0.2M CH\(_3\)COONa. Molecular mass determined by static light scattering was used.

According to Eq. (7), the slope of the dependence \( D_0 M/RT = f(M^{1/2}) \) to the X-axis allows the determination
of the equilibrium rigidity of macromolecules $A$, whereas the intercept of this dependence with the $Y$-axis is specified as the $A/d$ ratio. $A = 150 \pm 20$ Å was obtained for chitosan from the corresponding plot in Figure 5. The same characteristic for HTPC made up $A = 160 \pm 20$ Å. The samples with same high degree of deacetylation of 90% and a DQ of 90% were used for this analysis.

It should be noted, however, that despite the proximity of the experimentally determined values of the total chain rigidity of chitosan and HTPC, these polymers must have different contributions of the electrostatic component of their rigidity due to the difference of charged group distribution along their chains. In acidic conditions, positively charged amino groups of chitosan are situated at the backbone of the polysaccharide chains, and positively charged quaternized amino groups of HTPC are out of the backbone and leading to the dissimilar type of charge distribution in these polymers. The latter also may be responsible for the difference in the intramolecular repulsion of charged groups in the initial chitosan and HTPC coils. An absence of any criteria to account for this difference needs to be based on the experimental data for analysis of hydrodynamic properties of HTPC.

Thus, it was shown that the hydrodynamic properties of chitosan and HTPC within the framework of the same model turns out that their total chain rigidity values are very close to each other, and that conformation properties cannot be responsible for the observed difference in $R_h$ and [n] of HTPC and chitosan molecules. By the way, it can be pointed out that the obtained $A$ values for chitosan and HTPC are characteristic for the semi rigid polymers and correspond well to the prior data for chitosans with the deacetylation degrees of 80-90% [13, 14, 16, 26].

Experimental data obtained by the static light scattering method allow the conclusion on the thermodynamic quality of the polymer-solvent systems. The plot $Hc/\theta(0, \theta) = \theta(c)$ depicted in Figure 3 for one of chitosan samples and for one representative of the HTPCs indicates the difference in the slopes of the extrapolation lines and, consequently, the difference in the values of the second virial coefficient for these polymer-solvent systems. The mean value $A_2 = (1.1 \pm 0.1) \times 10^{-4}$ cm$^3$/g mol was obtained for the homologous series of chitosan in 0.33M CH$_3$COOH + 0.2M CH$_3$COONa. This value correlates with the data available in the literature for chitosan – 0.33M CH$_3$COOH – 0.2M CH$_3$COONa system [16]. The mean value of the second virial coefficient for the HTPC series amounted to $A_2 = (0.5 \pm 0.1) \times 10^{-4}$ cm$^3$/g mol, which is nearly an order of magnitude smaller than for initial chitosan. Such a behavior of $A_2$ value may be considered as an indicator of a worsening of thermodynamic conditions for the modified chitosan in 0.33M CH$_3$COOH + 0.2M CH$_3$COONa as compared to initial polysaccharide. Normally, a worsening of thermodynamic quality of the solvent leads to a decreasing of the size of the macromolecules in solution that explains the observed difference in hydrodynamic properties of HTPC and chitosan, in particular, the intrinsic viscosity $[\eta]$ and diffusion coefficient $D_0$ behavior (Table 1).

![Figure 3](image)

Based on the well known Flory equation (Eq. (8)), which describes the dependence between $[\eta]$, $M$ and radius of gyration $R_g$ of the polymer coil in solution, it may be concluded that as a parameter proportional to the cubed size of macromolecule, the intrinsic viscosity appears to be more sensitive than others hydrodynamic parameters to variations of the thermodynamic conditions [28].

$$[\eta] = \Phi_0 <R^2>_f^{3/2}/M,$$

(8)

where $\Phi_0$ is the Flory parameter, $R_g$ is an averaged radius of gyration of the polymer coil.

Thus, it is not surprising that the $[\eta]$ value of HTPC samples varies more significantly in relation to chitosan of the same polymerization degree than the translational diffusion coefficient $D_0$, because $D_0$ is just inversely proportional to the hydrodynamic dimension of macromolecules in solutions (see Eq. (3) and Table 1).

The 0.33M CH$_3$COOH + 0.2M CH$_3$COONa is a thermodynamically good solvent for chitosan where its molecules have an enlarged size, but this not the case for the modified chitosan HTPC. The size of its molecules diminishes due to the worsening of thermodynamic conditions for HTPC in 0.33M CH$_3$COOH + 0.2M CH$_3$COONa, which is accompanied by a decrease of the intrinsic viscosity and the change of the translation diffusion coefficient of HTPC samples if compared to chitosan of the same polymerization degree.

Mark-Kuhn-Houwink (M-K-H) relationships for chitosan and HTPC in 0.33M CH$_3$COOH+0.2M CH$_3$COONa, determined using the data of Table 1, also give a confirmation of the worsening of
thermodynamic conditions for HTPC in comparison with chitosan. The M-K-H equations for intrinsic viscosity \([\eta]\) and translation diffusion coefficient \(D_0\), obtained as the extrapolation lines of the dependencies \(\eta = f(M_w)\) and \(D = f(M_w)\) (Suppl. data, Figure 2), are shown below:

\[
\eta = (5.0 \pm 0.1) \times 10^{-3} M^{0.99 \pm 0.09} \text{ (chitosan)} \tag{9}
\]

\[
D_0 = (4.9 \pm 0.1) \times 10^{-4} M^{-0.70 \pm 0.06} \text{ (chitosan)}
\]

\[
\eta = (29 \pm 1) \times 10^{-3} M^{0.7 \pm 0.1} \text{ (HTPC)} \tag{10}
\]

\[
D_0 = (10.0 \pm 0.5) \times 10^{-4} M^{-0.54 \pm 0.06} \text{ (HTPC)}
\]

A decrease of the exponent values and significant change of \(K_\eta\) and \(K_D\) coefficients can be concluded for HTPC when comparing Eqs. (9) and (10). The numerical coefficient \(K_\eta\) and the exponent value \(a\) of the relationship \([\eta] = K_\eta M^a\) for HTPC (Eq. (10)) differ from those for chitosan (Eq. (9)) and indicate a change of thermodynamic conditions for HTPC in an acidic solvent as well as the parameters of the relationship \(D = K_D M^b\) for these polymers. Difference of the dependencies of \(D_0\) as a function of \(M\) for chitosan and HTPC in acidic solvent is also responsible for a view of the plot \(R_h = f(P)\) presented in Figure 4, which was discussed earlier.

Usually, the reduction of the exponent value \(a\) in the M-K-H relationship \([\eta] = K_\eta M^a\) is observed if a polymer is replaced from the thermodynamically good solvent to a thermodynamically poor solvent [28]. This is a common rule for different classes of polymers. HTPC and chitosan can be considered as similarly structured polymers, and thus the observed tendency of the change of their exponent \(a\) in M-K-H relationship for intrinsic viscosity corresponds to the common rule.

### 3.2. Hydrodynamic Properties of HTPC in 0.2M NaCl

In contrast to chitosan, HTPC easily dissolves in water and salted aqueous solvents that is very useful for practical application of this polysaccharide derivative. But the hydrodynamic behavior of the HTPC molecules in 0.2M NaCl revealed itself in a number of specificities.

First of all it was detected that 0.2M NaCl, a neutral pH solvent, is not a buffer for HTPC. Monitoring of pH under a serial dilutions of HTPC solutions in 0.2 M NaCl with the same solvent had shown a marked reduction in pH value, which was not observed for HTPC solutions in 0.33 M CH\(_3\)COOH + 0.2M CH\(_3\)COONa at the acidic conditions (see Figure 6b). This phenomenon could be connected with the start of a protonation process of the secondary amino groups of HTPC under conditions corresponding to the infinite dilution. The secondary amino groups in the compact coils of HTPC molecules are the inner groups in its content that are screened by the positively charged quaternary amine groups at relatively high concentration of the solute. Their protonation becomes possible only with the decreasing of solute concentration in 0.2M NaCl.

![Figure 6: Variation of pH value in solutions of HTPC-1 in 0.2M NaCl (a) and in 0.33M CH\(_3\)COOH+ 0.2M CH\(_3\)COONa (b) under the consequent dilutions.](image)

Usually, the reduction of the exponent value \(a\) in the M-K-H relationship \([\eta] = K_\eta M^a\) is observed if a polymer is replaced from the thermodynamically good solvent to a thermodynamically poor solvent [28]. This is a common rule for different classes of polymers. HTPC and chitosan can be considered as similarly structured polymers, and thus the observed tendency of the change of their exponent \(a\) in M-K-H relationship for intrinsic viscosity corresponds to the common rule.

It was detected that the change of pH in HTPC solutions in 0.2M NaCl depends on a critical manner of the molecular mass of the samples and their concentration in solutions (Figure 7). For determination of the threshold concentration value \(c_{cr}\) at which the secondary amino group protonation begins, the absorbance spectra of HTPC in 0.2M NaCl have been utilized.

HTPC absorbs light in the near visible area owing to difference of its chemical structure from chitosan (Figure 7a). This property of HTPC permitted us to determine carefully the threshold concentration of the protonation start up for HTPC molecules in 0.2M NaCl. As shown in Figure 7b, the transition to another state of HTPC-6 molecules in 0.2M NaCl takes place at a concentration of \((0.24 \pm 0.1) \times 10^{-2} \text{ g/cm}^3\) (middle point of the decay curve), at which the shift of the absorbance maximum to the smaller wavelengths is clearly observed. A shift of the position of absorbance maximum for two HTPC samples presented at Figure 7b showing a value of ~5 nm, which is not large, but sufficient for spectral measurements. The threshold concentration depends on polymerization degree of...
HTPC. It is clear from Figure 7b that high molecular mass sample HTPC-6 possesses a lower threshold concentration than HTPC-4 due to these samples having different numbers of the secondary amino groups. Table 2 presents the values of the critical solute concentrations of the protonation start up for HTPC samples, obtained from the concentration dependencies of the maximum position of their spectra in 0.2M NaCl. Above $c_{cr}$ the thermodynamic and electrostatic conditions are constant for HTPC molecules in 0.2M NaCl, but lower than $c_{cr}$ the conditions will change with the dilution.

**Table 2: Critical Solute Concentration $c_{cr}$ of HTPC Samples in 0.2M NaCl**

<table>
<thead>
<tr>
<th>Sample</th>
<th>$c_{cr} \times 10^2$, g/cm$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTPC-1</td>
<td>0.20±0.1</td>
</tr>
<tr>
<td>HTPC-5</td>
<td>0.35</td>
</tr>
<tr>
<td>HTPC-6</td>
<td>0.13</td>
</tr>
<tr>
<td>HTPC-7</td>
<td>0.24</td>
</tr>
</tbody>
</table>

The observed behavior of HTPC in aqueous solvent, containing Na$^+$ and Cl$^-$ as the counterions, also indicates that this chitosan derivative will be highly sensitive to the counterions’ charge and structure, if the solvent will be not a buffer. Due to a possible additional protonation of the secondary amino groups and the presence of the quaternary amino groups bearing a positive charge, HTPC molecules in aqueous solvents differ from that of chitosan as a strongly charged type of a polyelectrolyte. Concentration dependent transitions of solution properties are specific for the strongly charged polyelectrolytes for which a competition of thermodynamic and electrostatic factors should be taken into account for analyzing their behavior in aqueous media at the presence of counterions [32].

**4. CONCLUSION**

The performed study of hydrodynamic properties of quaternized chitosan HTPC in 0.33M CH$_3$COOH+0.2M CH$_3$COONa and in 0.2M NaCl highlights the different types of behavior of its molecules at different pH values. Diluted solutions of HTPC in the acidic media are molecular dispersed, which gives an opportunity to determine the molecular characteristics of HTPC by hydrodynamic methods and light scattering techniques. The comparison of hydrodynamic properties of HTPC and initial chitosan performed in 0.33M CH$_3$COOH + 0.2M CH$_3$COONa, has shown that HTPC molecules with $M_w$ above 80x10$^3$ g/mol can be more compact in size if compared with chitosan of the same polymerization degree due to the difference of thermodynamic quality of solvent. Thermodynamic conditions for HTPC in 0.33M CH$_3$COOH + 0.2M CH$_3$COONa are not the same (worse) than that for chitosan.

Solution properties of HTPC in 0.2M NaCl at neutral pH are concentration dependent. The existence of the critical solute concentration for HTPC samples was detected in 0.2M NaCl at which a protonation of the secondary amino groups of quaternized chitosan starts.
This phenomenon means that neutral pH solvents cannot be used for the determination of the molecular parameters of HTPC, because traditional extrapolation of hydrodynamic properties to the infinite dilution will not be correct in that case. The threshold solute concentrations for the studied HTPC samples depend on their molecular mass and locate at interval (0.1-0.4) x10^2 g/cm^3. The possibility for the determination of the threshold solute concentration for the HTPC protonation in 0.2M NaCl using the absorbance spectra has been demonstrated. The detected concentration dependent behavior of HTPC molecules in dilute salted solutions at neutral pH need to be taken into account in the experiments with the quaternized chitosans in water-salt systems.

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