AQUEOUS SOLUTIONS OF HYDROCHLORIC ACID AS SIMPLE SOLVENTS OF CHITOSAN FOR VISCOSITY- AND LIGHT-SCATTERING-BASED MOLECULAR WEIGHT DETERMINATION

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1. Introduction

Basic studies on chitosan and practical applications of this biopolymer require knowledge of its major physicochemical properties, especially the molecular weight and degree of deacetylation (DA). These parameters influence not only on simple properties of chitosan like solubility and viscosity of solutions but also its biological activity. Measurements of the average molecular weight by viscosity, HPLC-GPC or the light scattering methods require suitable solvents for molecular dissolution of chitosan. In previous studies, a variety of solvents has been applied to dissolve chitosans, most of them consisting of three components: water, acid and salt [1 - 4]. Addition of salt causes screening of charges on polymer chains and thus provides coiled conformation of macromolecules desired for the above mentioned measurements.

The aim of this study is to test whether the salt, usually applied as the third component of a chitosan solvent, can be replaced by an excess of acid, so that the three-component solvent could be replaced with a simpler, two-component system (water + acid) to provide coiled conformation of chitosan chain. Application of such a two-component solvent could simplify measurements and eliminate at least one source of errors (salt concentration). In this work we tested the suitability of water - hydrochloric acid system. The tests were performed on 1×10^{-2} M chitosan solutions (chitosan molar concentration based on the average molecular weight of a repeating unit), with an excess of HCl over the stoichiometric amount required to protonate the chitosan amino groups. To prove that structure of chitosan in aqueous solution of hydrochloric acid is similar as in acid + salt

system, comparative studies were carried out on the influence of ionic strength of solutions containing hydrochloric acid alone and containing the stoichiometric amount of HCl with relevant amount of NaCl added to provide the same ionic strength on macromolecular size (determined as the average radius of gyration R_g) and reduced viscosity. Ionic strength of the solutions was calculated from the equation:

$$I = \frac{1}{2}\sum_{i=1}^{n} c_i z_i^2 ,$$

(1)

where c_i is the molar concentration of ion i, z_i is the charge of that ion and the sum includes all low-molecular-weight ions in the solution.

To confirm that chitosan does not undergo pronounced acid hydrolysis in 0.2 M HCl during the time between samples preparation and analysis (up to a few days), measurements were carried out on changes of the reduced viscosity in time, for solutions stored at two temperatures: 4 and 25 °C.

2. Materials and methods

Chitosan used in this study as the starting material was synthesized at the Sea Fisheries Institute, Gdynia, Poland. It was an average viscosity grade preparation with weight-average molecular weight of ca. 450 kDa and deacetylation degree of 85%, as determined by the potentiometric titration [5, 6]. Hydrochloric acid was purchased from Polish Chemical Reagents (POCh). Aqueous solutions were made up in water purified by Nanopure II system (Barnstead, USA). All other chemicals were of analytical grade and were used as received. Viscosity and static light scattering (SLS) measurements of 10^{-2} M chitosan solutions were made in 0.01 - 0.3 M HCl solution and in 0.01 M HCl with 0.01, 0.04, 0.09, 0.19, 0.29 NaCl at a temperature of 25.0 ± 0.1 °C.

Weight-average molecular weight, second viral coefficient (A_2), and radius of gyration (R_g) of chitosan were determined by multi-angle laser light-scattering setup (Brookhaven Instruments BI - SM 200) equipped with an Innova 90C argon ion laser (λ = 514.5 nm). Directly before the light-scattering measurements, solutions were passed three times through filters of 0.45 μm pore size (Sartorius).

Intensity of scattered light was measured for at least 5 dilutions of each sample in the angular range 30 ÷ 120°. Zimm-plot algorithm was applied for the evaluation of results [7]. The refractive index increment (dn/dc) for chitosan in 0.1 M HCl was determined using a differential refractometer (SLS-Systemtechnic, λ = 543 nm), for all remaining systems (HCl + HCl and NaCl) dn/dc was assumed as 0.189 cm³/g [8]. AVS-350 setup (Schott Geräte) equipped with an Ubbelohde viscometer was used for viscosity measurements.

Acid hydrolysis was carried out on two 10^{-2} M solutions of chitosan in 0.2 M HCl. Before preparation of solutions, 250 ml flasks were sterilized by gamma rays (25 kGy, ^{60}Co source) to eliminate microorganisms. Solutions were stored for 55 days at 5 °C and 25 °C. At chosen time intervals, solution samples were collected and their reduced viscosity was measured.

3. Results and discussion

3.1. Viscosity measurements

The observations on hydrodynamic behaviour of chitosan in solutions containing different concentrations of HCl or HCl + NaCl were carried out by viscosity measurements. Changes in reduced viscosity, $((\eta-\eta_0)/(\eta_0 c))$, (where η, η_0 is the viscosity of the solution and solvent, respectively, and c is the polymer concentration) of chitosan in these two kinds of solvents: are shown in Figure 1. Both curves have a similar shape. The reduced viscosity decreases with increasing ionic strength and becomes approximately constant for ionic strength of 0.2 - 0.3. The observed drop in viscosity of the polymer solution with increasing concentration of solvent (HCl) is a result of contraction of chitosan chains from rod-like to coiled conformation and, in consequence, the lower hydrodynamic size of macromolecules. The reduced viscosity for the system containing hydrochloric acid alone is consistent with the data obtained for the solvents containing stoichiometric amount of hydrochloric acid in relation to chitosan and sodium chloride.

Our results of reduced viscosity in function of hydrochloric acid concentration are comparable with data obtained earlier by Rinaudo et al [9]. Authors examined the protonation of chitosan in acetic and hydrochloric aqueous solutions. In both systems the reduced viscosities of chitosan solutions were measured at different acid concentrations (from 2×10^{-4} up to 16 M for acetic acid, and 3.2×10^{-5} to 0.1 M hydrochloric acid). Similarly as in our data, in the case of HCl, they observed a strong decrease in viscosity, corresponding to the rod–to–coil conformational transition. Acetic acid did not exert such a strong effect because it is a weak acid and its dissociation degree decreases with increasing concentration. Therefore, increasing acetic acid concentration does not lead to a proportional increase in the concentration of ions which could be screening charges on the polyelectrolyte chain.
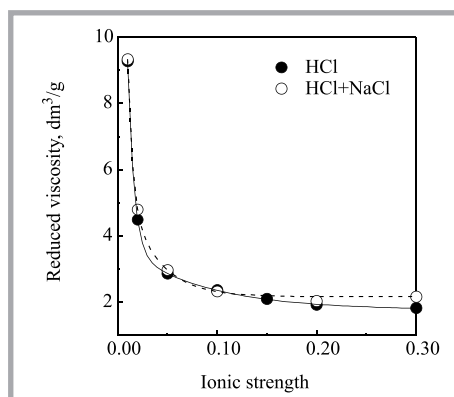

Figure 1. *Reduced viscosity of aqueous chitosan solutions containing HCl alone or HCl (10^{-2} M) and NaCl as a function of ionic strength. Values measured for 10^{-2} M chitosan solutions at 25.0 °C.*
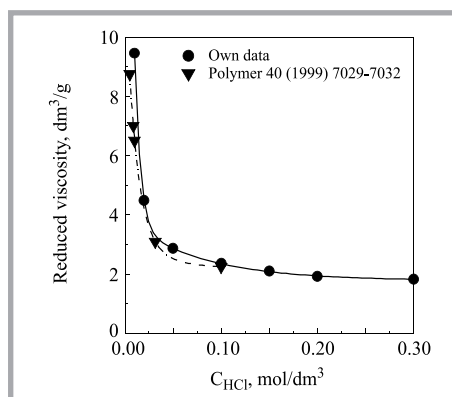
Figure 2. *Reduced viscosity of chitosan solutions at 25 °C as a function of hydrochloric acid concentration. Comparison between own data and from M. Rinaudo et al [9] (M_v = 285 kDa, DA = 88).*

As one of the consequences, the authors did not observe salting-out effect of concentrated solution of acetic acid, while it occurs for HCl above 1 M concentration.

3.2. Static light scattering study

The static light scattering method is a universal and absolute method for the molecular weight determination of the macromolecular substances in a solution and it gives additional information about the thermodynamic interaction between a solvent and the macromolecules.

The intensity of the scattered light from polymer solutions can be characterized by the equation [7]:

$$\lim_{\substack{\theta \to 0 \\ c \to 0}} \frac{Kc}{R(\theta)} = \frac{1}{M_w} + \frac{16\pi^2}{3\lambda^2 M_W} \langle R_g^2 \rangle \sin\frac{\theta}{2} + 2A_2 c \qquad (2)$$

where: $R(\theta)$ is the difference Rayleigh ratio for a solution and a solvent at the scattering angle θ, λ is the wavelength of the light in vacuum, M_w – the weight average molecular weight, A_2 – the second virial coefficient, $\langle R_g^2 \rangle$ - the mean square radius of gyration of polymer, c is the polymer concentration, K – an optical constant, it is given by[7]:

$$K = \frac{2\pi n_0^2 \left(\frac{dn}{dc}\right)^2}{N_A \lambda^4} \qquad (3)$$

where n_o is refractive index of a solvent, dn/dc – the specific refractive index increment of a solution describes the change of the refractive index of a system under consideration with its concentration c, N_A – the Avogadro constant.

R_θ denotes intensity of the light scattered by the polymer in a unit solution volume in the direction described by the observation angle (θ) to the incident (primary) beam, it is given by [7]:

$$R_\theta = \frac{i_{u,\theta} r^2}{I_0 (1 + \cos^2 \theta)} \qquad (4)$$

$i_{u,\theta}$ is the intensity of the scattered light, I_0 – is the intensity of the incident beam, r – the distance between the scattering volume and the detector, and $1 + \cos 2\theta$ is the geometrical correction.

$\langle R_g^2 \rangle$ - is a parameter which characterizes the particle dimension, it follows from the classical definition of the radius of gyration, that it is the square root of the average square distance of a mass element from the centre of a mass [7]:

$$\langle R_g^2 \rangle^{0.5} = \left(\frac{1}{N} \sum_{i=1}^{N} < r_i^2 >\right)^{0.5} \qquad (5)$$

where: N – is the number of mass elements on the chain, r_i – a distance of the i-th mass element within a chain from the centre of mass. The bracket in equation 5 determines the averaging over all possible conformations of macromolecules.

The second virial coefficient (A_2) is an important parameter quantitatively characterizing the thermodynamic interaction among the solute molecules at a given temperature. When $A_2 > 0$ then polymer prefers to contacts with solvent molecules more than with other polymer molecules. In case of $A_2 = 0$ polymer-polymer and polymer-solvent interaction are energetically equivalent and solvent is called theta solvent. For $A_2 < 0$, polymer-polymer contact is preferred to polymer-solvent and the solvent is a poor one for the given polymer [7]. With R_θ measured at a series of c and angles, we can determine M_w, R_g, and A_2 by use of a Zimm method, which, according to Equation 1, allows for both angle and concentration extrapolation to their zero values.

SLS measurement were carried out for 10^{-2} M solution of chitosan with 0.01 - 0.3 ionic strengths. The first group was chitosan solutions in hydrochloric acid, the second - chitosan solution in stiochiometric amount of HCl and added sodium chloride. The variation of radius of gyration (R_g) and the second virial coefficient (A_2) with the ionic strength are shown in Figure 3 and 4, respectively (in case of R_g it was difficult to obtain reliable data for the ionic strength values below 0.1 due to the prevailing rod-like conformation). In the examined range of the ionic strength we cannot observe significant difference between the results of hydrochloric acid alone and with addition of salt.

Data shown in Figures 3 and 4 may be useful for selecting an optimum concentration of HCl. The concentration should be high enough to provide coiled conformation of macromolecules, suitable for light-scattering and viscosity measurements. On the other hand, too high acid concentration is not suitable, because when the screening of chain-bound charges becomes too strong, segment-segment interactions may become too high in comparison to segment-solvent forces, potentially leading to the onset of aggregation of chitosan macromolecules, or, in an extreme case, to precipitation. Based on Figures 1 and 2, HCl concentrations lower than 0.1 M do not seem to be appropriate, because they do not provide a
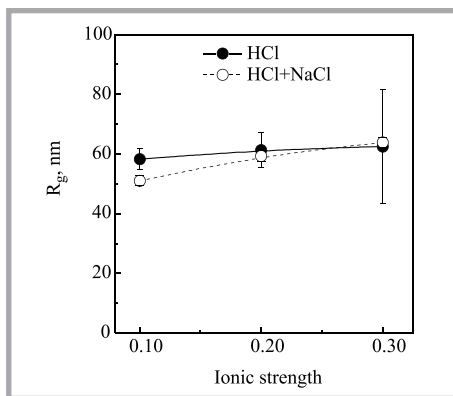

Figure 3. *Radius of gyration of chitosan as a function of an ionic strength in solutions containing HCl alone or HCl (10^{-2} M) and NaCl.*
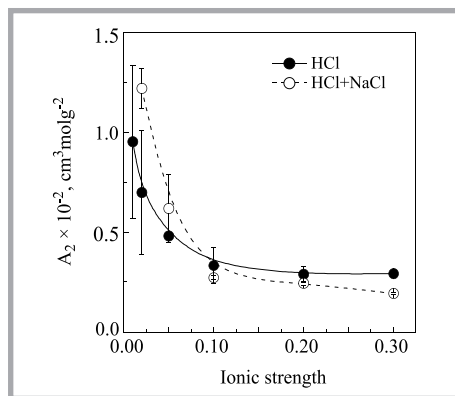
Figure 4. *Dependence of the second virial coefficient (A_2) of 10^{-2} M chitosan solutions on ionic strength in solutions containing HCl alone or HCl (10^{-2} M) and NaCl.*

coiled conformation, and, since the slope of viscosity vs. acid concentration in this range is steep, a small error in HCl concentration could lead to a large error in viscosity determination. Above 0.1 M of HCl the further decrease in viscosity is relatively mild, indicating that there are no strong changes in conformation. In principle, R_g values should follow the same trend as viscosity (i.e., a slight decrease), but actually there is rather some weak opposite tendency. Combining this fact with the ongoing decrease in A_2 (when A_2 tends to zero, the solvent turns from a thermodynamically good one to a thermodynamically poor one), these data may be interpreted as an indication of an onset of aggregation (R_g measured by SLS, in contrast to viscosity values, is very sensitive to the presence of even a tiny fraction of aggregates). Thus, we conclude that under our conditions (chitosan of 85 % DA, 10^{-2} M), the most suitable HCl concentration is 0.1 M.

A similar tendency in radius of gyration (R_g) was observed for acetic acid aqueous solutions by Pa et al. [10]. Their results showed R_g increase with increasing concentration of acid, indicating a possibility of aggregation tendencies in strongly acidic solutions.

In order to check whether light scattering measurements in our solvent lead to similar M_w values as obtain in typical tree-component solvents, we have determined M_w of the same chitosan sample in four solvents [Table 1].

Taking into consideration the assumptions and uncertainties inherent to light scattering measurements (e.g. dn/dc values), the M_w values seem to be in relatively good agreement.

3.3. Hydrolysis in 0.2 M HCl

It is common knowledge that chitosan in acidic aqueous solution undergoes hydrolysis. Some laboratories use acids to reach a higher degree of deacetylation and sometimes to synthesize chitosan oligomers. However, typical conditions of such reaction are relatively drastic: high concentration of acid and high temperature [13 - 15]. Less is known about the rate of hydrolysis under much milder conditions.

In order to confirm that chitosan does not undergo pronounced acid hydrolysis in dilute HCl during the typical time between samples preparation and analysis (1 - 2 days), we carried out time-dependent measurements of intrinsic viscosity of chitosan in 0.2 M HCl (twice as high as the proposed optimum concentration), for solutions stored at two temperatures: 4 and 25 °C. As expected, hydrolysis is faster at 25 °C [Figure 5]. However, up to five days the decrease in viscosity was within the limits of error, while after 55 days it was equal to

Table 1. *Values of M_w for our chitosan samples in the various solvents obtained by the light scattering method.*

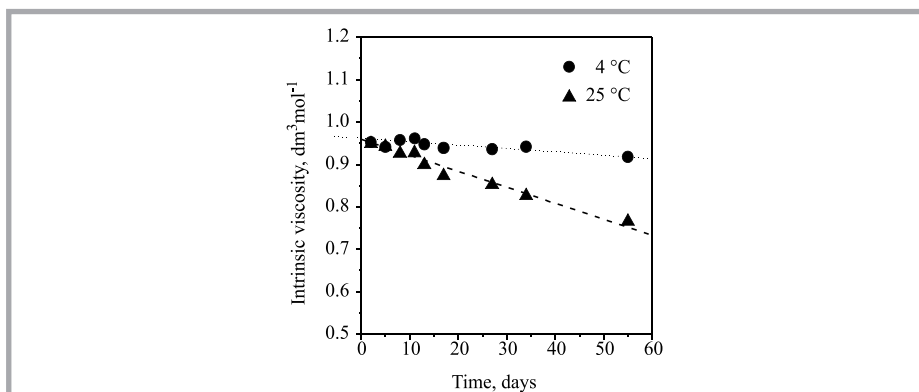| Solvent composition	M_w obtained by LSI, kDa	dn/dc, mL/g	Source of dn/dc value
0.1 M HCl	534	0.1460	This work
8.5% HCOOH/ 0.5 M HCOONa	463	0.1740	R. Muzzarelli, et al.[11]
HClO$_4$ / 0.25 M NaClO$_4$, pH=3	430	0.1870	P. Ulanski, et al.[12]
0.2 M AcOH / 0.15 M AcONH$_4$	460	0.1870	P. Sorlier, et al.[3]

Figure 5. *Dependence of intrinsic viscosity of chitosan as a function of time in 0.2 M HCl.*

7% and 22% at the two tested storage temperatures, respectively. Rate of hydrolysis is so low that its influence on results can be omitted in typical analytical conditions. However, to eliminate hydrolysis as a potential source of error, prolonged storage at RT should be avoided.

4. Conclusions

Our studies on aqueous chitosan solutions show that hydrochloric acid, when used in excess over the stoichiometric amount, behaves like a neutral electrolyte (salt) and leads to a partial screening of repulsive interactions between ionic groups of chitosan. Physico-chemical properties of such solutions (viscosity, radius of gyration of chitosan chains, ratio of segment-segment to segment-solvent interactions) are comparable to a system containing stoichiometric amount of HCl and relevant amount of NaCl to provide the same ionic strength. We suggest that aqueous HCl solutions may be applied for light-scattering and/or viscometry measurements, e.g. intended for determination of average molecular weight of chitosan, as convenient two-component solvents instead of the three-component solvents comprising of water, acid and salt. Analysis of our data indicates that for 10^{-2} M chitosan of DA = 85 %, the optimum HCl concentration is ca. 0.1 M. Rate of chitosan hydrolysis in even more acidic conditions (0.2 M HCl) is so low that its influence on the results can be neglected in typical analytical conditions.

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