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SOME APPROACHES TO VISCOMETRIC STUDY OF CHITOSAN IN ACETIC ACID SOLUTION

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Abstract. Some ways of estimating the values of the intrinsic viscosity of chitosan were analyzed. It was shown that the method of Irzhak and Baranov for estimating the current value of the intrinsic viscosity allows to adequately estimates the conformational state of the macromolecular coil and its degree of swelling.

Keywords: chitosan, polyelectrolyte, viscosimetry, conformation.

1. Introduction

Recently emergence of a large number of researches connected with creation and studying of film materials on the basis of chitosan for use in a number of areas [1-6] has been noted. According to modern views, the supramolecular structure of polymer in the films created from solution is defined by a conformational state of polymer in initial solution, which, in turn, predetermines a complex of physical and chemical properties of a material.

One of the most available and informative methods of research of a conformational state of polymers in solution is viscosimetry. However, in case of using viscosimetry for research of solutions of polyelectrolytes, reliable determination of characteristic viscosity for the purpose of assessment of their conformational state, encounters certain difficulties. In this work an attempt of the analysis of some ways of assessment of values of characteristic viscosity of polymer of a natural origin – chitosan, which at dissolution in sour water environments (for example, in acetic acid) gains properties of polyelectrolyte, is made.

2. Experimental

We used a sample of chitosan (ChT) manufactured at Bioprogress ZAO (Russia) by alkaline deacetylation of crab chitin (degree of deacetylation ~ 84 %). The molecular weight of initial chitosan was determined by the method combining sedimentation velocity method and viscosimetry, by the formula [7]:

$$M_{sh} = \left(\frac{S_0 h_0 [h]^{1/3} N_A}{A_{hi}(1 - \bar{n}r_0)} \right)^{3/2}$$

where S_0 –constant of sedimentation; η_0 – dynamic viscosity of the solvent equal to $1.2269 \cdot 10^{-2}$ PP; $[\eta]$ – intrinsic viscosity of polymer defined in the acetate buffer with pH = 4.5, dl/g; N_A – Avogadro's number equal to $6.023 \cdot 10^{23}$ mol^{-1}; $1 - \bar{n}r_0$ – Archimedes factor or buoyancy factor, v – the partial specific volume, cm^3/g, ρ_0 – density of solvent, g/cm^3; A_{hi} – hydrodynamic invariant equal to $2.71 \cdot 10^6$. Constants of sedimentation of samples of ChT were defined on the analytical ultracentrifuge MEGOHM– 3180, equipped with Filpota-Svensson's optics.

The experiments were carried out in a two-sector 12 mm aluminum cell at 298 K. Thermostating was carried out with an accuracy of ± 0.1 K. Constant of sedimentation was calculated by measuring the boundary position and its offset in time by means of optical schemes by the formula [7]:

$$S_0 = \frac{1}{w^2 X_{max}} \cdot \frac{dX_{max}}{dt}$$

Archimedes factor was determined pycnometrically by standard methods [7] and calculated using the following formula:

$$n = n_0 \left[\frac{100}{m_0 r} \left(\frac{1}{m_0} - \frac{1}{m} \right) \right]$$

where v_0 – volume of the pycnometer; m_0 – weight of the solvent in the pycnometer; m – weight of the solution in the pycnometer; $\rho = 100g/m_0$; (g – solution concentration in g/ml).

The value of M_{sh} for the sample of ChT used in work was 113000 Da.

For the purpose of the constants K and α determination in Mark-Houwink-Kuhn's equation the sample of initial chitosan was fractionated to 10 fractions, for each of which value $M_{s\eta}$ and value of characteristic viscosity was defined. Fractionation of ChT was carried out a by the method of consecutive sedimentation [8]. Acetone was used as the precipitator. Viscometric investigations were conducted according to a standard technique on Ubbelohde's viscometer at 298 K [9].

Acetic acid with the concentration of 1, 10 and 70 g/dl was used as the solvent. Value of intrinsic viscosity of the initial ChT sample and its fractions in solutions of acetic acid was determined in two ways – by the method of Fuoss [10] and the method of Irzhak and Baranov [11, 12]. For calculation of intrinsic viscosity by Huggins, Kremer and Irzhak-Baranov's equations organic solvent was used as a diluter, while for Fuoss method application water was used.

3. Results and Discussion

It is well known that connection between the viscosity of the polymer and its concentration in solution can be expressed in the form of extrapolation formulas [7]:

$$h_{sp} = [h]c + k_1[h]^2 c^2 + k_2[h]^3 c^3 + ... \qquad (1)$$

$$\text{or } \ln h_{rel} = [h]c - K_1[h]^2 c^2 - K_2[h]^3 c^3 - ... \qquad (2)$$

where c – concentration of the polymer in the solution, g/dl; k_1 and k_2, K_1 and K_2 – constants reflecting the interaction of the polymer with a solvent; h_{sp} – specific viscosity of polymer solution equal to: $h_{sp} = h_{rel}-1$. Relative viscosity h_{rel} is the ratio of viscosity of the polymer solution (h_p) to the viscosity of the solvent (h_0).

A special case of Eq. (1) is Huggin's equation (3) [13], and the Eq. (2) is Kremer's equation (4) [14], for which the main condition of applicability is preservation of linear dependence of reduced viscosity (h_{red}/c) or ($\ln h_{rel}$) from concentration of polymer in solution and lack of strong intermolecular interactions between macrochain links:

$$\frac{h_{sp}}{c} = [h] + k_1[h]^2 c \qquad (3)$$

$$\frac{\ln h_{rel}}{c} = [h] + K_1[h]^2 c \qquad (4)$$

In this case, by extrapolation on zero concentration the value of the intrinsic viscosity is calculated, using which, by the known for this polymer-solvent couple Mark-Kuhn-Hauvinka's equation, the molecular mass of polymer is defined.

To estimate viscometrically the molecular weight in the case of solutions of polyelectrolyte, to which ChT concerns also, measurements need to be carried out in a buffer solution compensating charge, available on the macromolecule and providing submission of regularity of change of the reduced viscosity from concentration to Huggins's equation (Fig. 1, *curve* 1).

If the purpose of viscometric research is not determination of molecular weight, namely the evaluation of the conformational state of the polyelectrolyte in the studied (non-buffered) solvent, the determination of the [η] becomes not unambiguous as experimental data for viscosities for ChT solution, for example in acetic acid in the field of dilute solutions does not comply with the Huggin's equation (Fig. 1, *curve* 2). As seen from the *curve* 2 of Fig. 1 in the field of dilute solution (with concentration of ChT in solution less than 0.5 g/l) the reduced viscosity decreases with increasing of polymer concentration, whereas, according to Eq. (3) growth of the viscosity had to be observed.
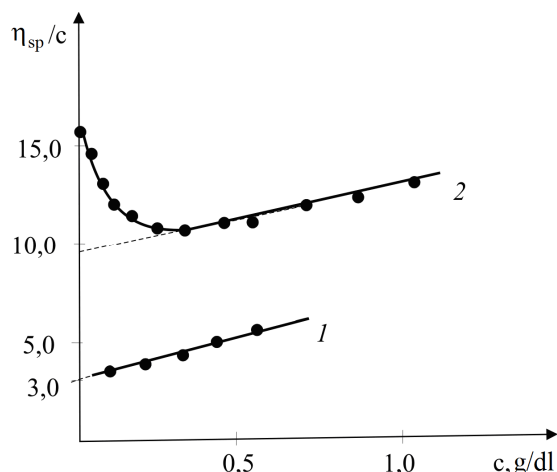

Fig. 1. Concentration dependence of the reduced viscosity of the solution of ChT in acetate buffer (1) and 1 % acetic acid (2)

The reason of observed effect in the field of the diluted solutions is well known effect of polyelectrolyte swelling [15]. This effect is caused by the fact that at dilution of solution of polyelectrolyte concentration of counterions decreases, which in turn, causes the unfolding of macroion due to repulsion of similarly charged chains. In this case the determination of the intrinsic viscosity according to Eqs. (3) and (4) by extrapolating to zero concentration normally used for neutral polymers, is impossible.

In principle it is possible to determine the value of the intrinsic viscosity of the polyelectrolyte of ChT using the Huggin's or Kraemer's equations investigating solutions of higher concentration, which from the formal point of view comply with these equations (solutions with a concentration higher than 0.5 g/dl). However, in the field of semi-dilute solutions, ChT solutions are associated [16-19] and exhibiting strong intermolecular interaction. Therefore, the value of the intrinsic viscosity defined in this area of concentration (Fig. 1, *curve 2*), reflects rather the size of some associate than the size of a separate macromolecular coil.

For determination of intrinsic viscosity of polyelectrolytes and evaluation of their conformational state the following empirical equation was suggested by Fuoss and Strauss [10]:

$$\frac{h_{sp}}{c} = \frac{[h]}{1 + B\sqrt{c}} \qquad (5)$$

where B – coefficient characterizing the electrostatic interaction of the polyion with simple ions.

When using approach of Fuoss and Strauss, dilution of solution of the polymer of initial concentration is made by water instead of solvent. In that case when a strong electrolyte is used as the solvent (*e.g.*, hydrochloric acid), dilution by water does not change the degree of dissociation of the solvent and does not change the ionic strength of the solution. Therefore, the value of the intrinsic viscosity determined by extrapolation to zero concentration will indeed reflect the size of the coil in the solvent being. In our case, acetic acid is used as the solvent. It is weak and its dilution with water is accompanied by an increase in its degree of dissociation. Increasing the degree of dissociation of acetic acid leads to additional protonation of ChT macromolecules in solution and causes unfolding of the macromolecular coil. As a result, the value of intrinsic viscosity of ChT (Table 1) determined by Fuoss's equation will not reflect the conformation of macromolecules in 1 % solution of acetic acid, but will reflect conformation of ChT in solution of infinitely diluted acetic acid.

A more reasonable method for determining the intrinsic viscosity of polyelectrolytes in order to assess conformational state of the coil is considered the reception of isoionic dilution [20]. At the same time, dilution of the polyelectrolyte solution is made by low-molecular salt. This method is very time consuming because it requires optimization. Meanwhile, in [11, 12], it was suggested that adequate alternative to the method of isoionic dilution is the method of determining the intrinsic viscosity of Irzhak and Baranov (method of assessing the current intrinsic viscosity), by the equation:

$$[h] = \frac{\partial \ln h_{rel}}{\partial c} \qquad (6)$$

Using Eq. (6) the intrinsic viscosity can be determined by the initial slope of the $\ln h_{rel} - c$ (Fig. 2). In this case, we can estimate the $[h]$ of polyelectrolyte avoiding both extrapolation to zero concentration (definition of $[h]$ by Eqs. (3) and (4)), and "overstating" of $[h]$ values, concomitant to determination of the intrinsic viscosity according to Eq. (5).

It should be noted that the values of intrinsic viscosity determined in a buffered solution according to Eqs. (3) and (4) coincide with the value obtained by Eq. (6) (Table 1), thus confirming the adequacy of using of the Irzhak and Baranov's equation for the systems we have studied.

Meanwhile, with increase in concentration of the acetic acid used as solvent, there is a reduction of value of characteristic viscosity of ChT in the solution (Table 1), testifying to reduction of the sizes taken by the isolated macrocoil. This fact becomes clear if to consider that increase of concentration of acetic acid is accompanied by reduction of degree of its dissociation that, in turn, leads to reduction of extent of protonation of macromolecules of ChT in solution and to natural reduction of the sizes of a coil. Moreover, there is a correlation between the value of the intrinsic viscosity of the macromolecular coil in the investigated solvent and the degree of its protonation, as it is defined in [21, 22] (Fig. 3). It is also important that when extrapolating the straight line (Fig. 3) to zero degree of protonation the obtained value of intrinsic viscosity coincides with the value $[h]$, defined in the acetate buffer.

Table 1

The intrinsic viscosity of the chitosan defined by formulas analyzed in the work

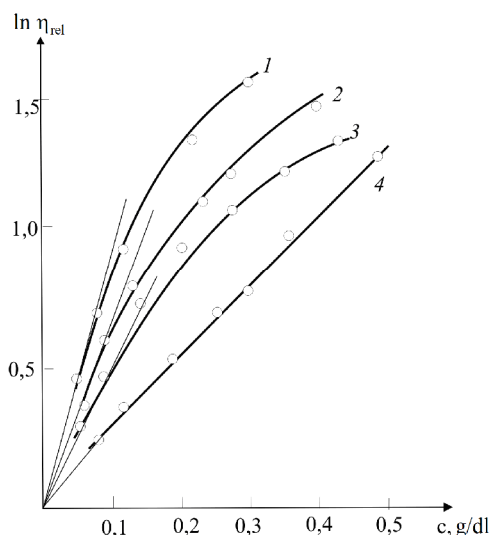| The equation used to calculate the $[h]$		The solvent	$[h]$, dl/g
(3)		1 % acetic acid	-
(3)		acetate buffer	3.06
(4)		1 % acetic acid	-
(4)		acetate buffer	3.00
(5)		1 % acetic acid	11.20
(6)		1 % acetic acid	7.79
(6)		5 % acetic acid	7.05
(6)		15 % acetic acid	5.52
(6)		50 % acetic acid	5.30
(6)		70 % acetic acid	5.01
(6)		acetate buffer	3.02

Fig. 2. Dependence of relative viscosity on the concentration of chitosan in solution of 1% (1), 15% (2) and 70% (3) acetic acid and acetate buffer (4)
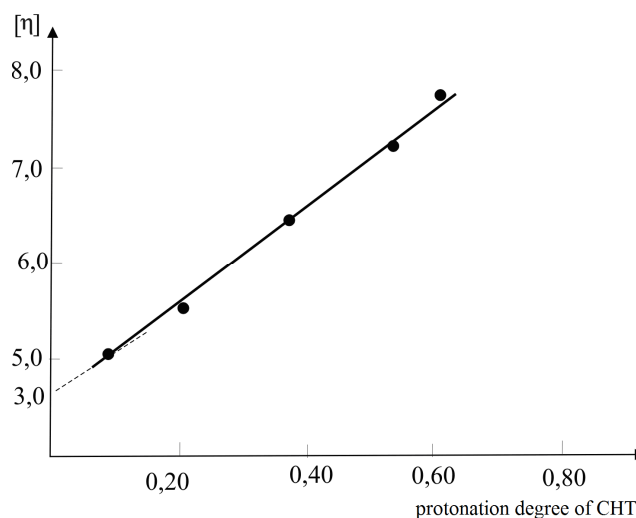

Fig. 3. The dependence of intrinsic viscosity of ChT on the protonation degree of the chitosan in the acetic acid solution

Table 2

Some characteristics of chitosan calculated using Fuoss and Irzhak-Baranov's methods

Concentration of acetic acid, %	Fuoss method				Irzhak-Baranov method			
	α	$K \cdot 10^5$	$[h]$, g/dl	M^*, kDa	α	$K \cdot 10^5$	$[h]$, g/dl	M^*, kDa
1	1.15	2.89	12.20	77.9	1.02	5.53	7.79	111.6
10	1.03	10.11	11.30	79.7	0.93	13.91	6.89	113.5
70	0.90	37.41	10.09	83.8	0.81	41.39	5.25	116.3

* *M* is calculated by the Mark-Houwink-Kuhn's equation.

Comparison of values of the intrinsic viscosity of the initial sample ChT defined by Fuoss's method and Irzhak-Baranov's method in acetic acid solution of 1, 10 and 70 % indicates that the value $[h]$, defined by Eq. (3) is in all cases less than values defined by Eq. (2) (Table 1).

After fractionation of the initial sample of ChT and determining the molecular weight of each fraction the values of intrinsic viscosity of fractions in 1, 10, and 70 % acetic acid were determined by two analyzed methods – by Fuoss and by Irzhak and Baranov. This made it possible to estimate the parameters K and α in Mark-Houwink-Kuhn's equation, testifying to the conformational state of ChT macromolecules in solution. Comparison of the values α in Mark-Houwink-Kuhn equation defined by two analyzed methods shows that for all the studied concentrations of acetic acid the macromolecular coil "by Fuoss" has more comprehensive conformation than the coil "by Irzhak." The reason for the discrepancy in the values of K and α, defined using two methods of determining the viscosity is obviously related to the fact that when using the Fuoss's method the coefficients in the Mark-Kuhn-Houwink's equation does not reflect ChT conformation in solution of acetic acid

concentrations studied but reflects ChT conformation in a dilute solution of acetic acid. Moreover, as it can be seen from the Table, the molecular weight value calculated from the values $[h]$, α and K according to the Irzhak and Baranov's equation satisfactorily coincide with ChT molecular weight obtained by the absolute method in acetate buffer, $M_{sh} = 113000$ Da.

4. Conclusions

1. Using a weak electrolyte – acetic acid – as a solvent for ChT leads to certain difficulties in determining the values of its intrinsic viscosity in order to assess its conformational state. Thus, using the Fuoss's method for determining intrinsic viscosity of polyelectrolyte leads to overstated values of intrinsic viscosity due to the increased degree of ChT protonation and unfolding of the macromolecular coil at decreasing concentration of acetic acid.

2. It is assumed that more correct method for determining the intrinsic viscosity of ChT in solution of acetic acid is the method of Irzhak and Baranov. The

values of intrinsic viscosity of ChT in acetate buffer obtained by this method coincide with the values defined by the method of Huggins and Kraemer in similar conditions, indicating the adequacy of the Irzhak and Baranov approach for the systems we have analyzed.

Acknowledgements

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ДЕЯКІ ПІДХОДИ ДО ВІСКОЗИМЕТРИЧНОГО ДОСЛІДЖЕННЯ ХІТОЗАНУ В РОЗЧИНІ ОЦТОВОЇ КИСЛОТИ

Анотація. Проаналізовані деякі способи оцінки значення характеристичної в'язкості хітозану. Показано, що метод Іржака і Баранова для оцінки поточного значення характеристичної в'язкості дає можливість адекватно оцінювати конформаційний стан клубка макромолекул і його ступінь набухання.

Ключові слова: хітозан, поліелектроліт, віскозиметрія, конформація.