

INTRINSIC VISCOSITY AND CONFORMATIONAL PARAMETERS OF CHITOSAN CHAINS

Vera Halabalova^{1,*}, Lubomir Simek¹ and Pavel Mokrejs²

¹Tomas Bata University, Faculty of Technology, Department of Food Biochemistry and Analysis, Nam. TGM 275, 762 72 Zlin, the Czech Republic

² Tomas Bata University, Faculty of Technology, Department of Polymeric Engineering, nam. TGM 275, 762 72 Zlin, the Czech Republic

*E-mail: halabalova@ft.utb.cz

ABSTRACT

The dependence of the intrinsic viscosity $[\eta]$ on the weight average degree of polymerization P_w of chitosans in aqueous acid media is discussed in terms of the Yamakawa-Fujii theory for the worm-like cylinder model. The conformational characteristics (Kuhn statistical segment length and Flory characteristic ratio) are estimated. They increase linearly with the reciprocal of the square root of salt concentration. The "intrinsic" values for uncharged chains are comparable to those for cellulose and lower than those for chitin.

Keywords: Chitosan; Conformational parameters; Intrinsic viscosity; Worm-like cylinder model.

© 2011 RASĀYAN. All rights reserved.

INTRODUCTION

Chitosans, i.e. products of deacetylation of chitin, may be viewed as copolymers consisting of (1→4) linked 2-acetamide-2-deoxy-β-D-glycopyranose (N-acetyl glucosamine) and 2-amino-2-deoxy-β-D-glycopyranose (glucosamine) units. The composition depends on the deacetylation process and is characterized by the degree of acetylation F_A (mole fraction of 2-acetamido-β-D-glycopyranose units). In aqueous acid media the amino groups are protonated and the polymer is transformed into a polycation where electrostatic repulsion of charged groups affects the chain conformation¹⁻⁷. Therefore, the solution properties (e.g., chain dimensions, transport coefficients, second virial coefficient) depend not only on the polymer molecular weight, molecular weight distribution, and polymer-solvent interaction, but also on the content and distribution of amino groups, pH and/or the ionic strength of solution (concentration and type of low-molecular-weight electrolytes).

This also holds for the intrinsic viscosity $[\eta]$ and the parameters of the Mark-Houwink- Kuhn-Sakurada (MHKS) equation

$$[\eta] = K \cdot M^a \quad (1)$$

$$[\eta] = K' \cdot P^a \quad (1a)$$

Where, M and P is, respectively, the molecular weight and the degree of polymerization.

The a values have recently been reviewed⁸. As they cover a surprisingly broad range (0.15-1.26) some authors believe that, depending on the degree of acetylation and composition of the solvent, chitosan chains can assume largely different shapes from compact spheres through random coils to rods. Maghami and Roberts have shown that the lowest value is an artefact^{9,10}. Other authors have explained the variation of a values as another artefact brought about by the impact of supermolecular particles (microgel, aggregates) on the weight average degree of polymerization P_w determined by light scattering¹¹⁻¹⁴. This factor has recently been discussed in some detail¹⁵. Nevertheless, good agreement has been established in correlations of $[\eta]$ vs M_w and $[\eta]$ vs M_{SD} where the M_w values were estimated by light scattering and the

M_{SD} averages by combination of the sedimentation and diffusion coefficients according to Svedberg^{11, 12, 15-17}. The plots are non-linear and the quotient $\ln [\eta] / \ln M$ decreases with increasing M .

The MHKS exponent alone can hardly be a reliable criterion of conformation as it is a composite quantity resulting from superposition of several effects (expansion due to the excluded volume effect, chain stiffness, and strength of hydrodynamic interaction) and, particularly with stiff polymer chains, need not be constant in a broad span of molecular weights¹⁸. By means of procedures based on modern theories of hydrodynamic properties, these effects can be resolved and the parameters characterizing the chain stiffness (Kuhn statistical segment length l_k , Flory characteristic ratio C_∞ and the strength of the hydrodynamic interaction can be evaluated¹⁹⁻²¹.

Estimation of these parameters from reported intrinsic viscosity data is subject of the present paper^{11, 12, 16}. Following the suggestion by Domard and Rinaudo, we characterize the samples by the degree of polymerization P , instead of molecular weight²². It will be seen that, in this way, the effect of differences in the degree of acetylation is reduced, thus simplifying the analysis and discussion of results. In the second part of the paper the methods of estimation of the conformational parameters from the $[\eta]$ data are described, including a novel procedure to be used if reliable values of P are not available. In the third part the conformational parameters of chitosans are estimated and compared with those of chitin and cellulose, and their dependences on the degree of acetylation and the ionic strength of solutions are discussed. The fourth part is devoted to the problems of the excluded-volume effect and strength of hydrodynamic interaction, and to estimation of the range of P values where chitosan chains assume the random coil conformation. In Appendices the estimation of conformational parameters of chitin and cellulose from existing $[\eta]$ data is described.

Methods of estimation of conformational parameters

The conformational characteristics (Kuhn statistical segment length l_k or the persistence length $q = l_k / 2$, Flory characteristic ratio C_∞) are computed from the ratio $(\langle R^2 \rangle_0 / M)_\infty$ which can be estimated from the intrinsic viscosity by an appropriate procedure¹⁹⁻²¹. The symbol $\langle R^2 \rangle_0$ stands for the mean-square end-to-end distance of the chain unperturbed by the excluded-volume effect, M is the polymer molecular weight, and the subscript ∞ refers to the random-coil conformation ($M \rightarrow \infty$). The Kuhn segment length is related to this ratio by equation:

$$l_k = (\langle R^2 \rangle_0 / M)_\infty M_L \quad (2)$$

$$\text{where} \quad M_L = m_0 / l_0 \quad (3)$$

and M_L is the shift factor that is usually set equal to the molecular weight per unit contour length at full extension, and m_0 and l_0 are, respectively, the molecular weight and length of the chain repeating unit^{19, 20}. The Flory characteristic ratio C_∞ (i.e., the ratio of the unperturbed mean square end-to-end distance of the real and freely rotating model chain at the same molecular weight in the random coil limit) is calculated as²¹

$$C_\infty = (\langle R^2 \rangle_0 / M)_\infty (M_b / l_b^2) \quad (3a)$$

Here, M_b and l_b^2 are, respectively, the mean molecular weight and the mean square length of the main chain bond. With cellulose and glycosans the main chain bonds and the chain repeating units are identical ($M_b = m_0$), hence the characteristic ratio and the Kuhn segment length are related by

$$C_\infty = l_k / l_b \quad (3b)$$

where $l_b = 5.15 \text{ \AA}$ is the length of the anhydroglucose unit²². While the Kuhn segment length measures the length of chain segments long enough to behave as though they were connected to one another by universal joints and, therefore, are uncorrelated in direction, the characteristic ratio reflects hindrances to rotation around the main chain bonds which are due to fixed bond angles and steric and/or energetic interactions of main chain units.

If chitosan chains assumed the conformation of non-draining random coils expanded by the excluded-volume effect, the conformational parameters could be obtained from the intercept K_0 of the Burchard-Stockmayer-Fixman (BSF) plot, $[\eta] / M^{1/2}$ vs $M^{1/2}$, according to the equations^{23, 24}

$$[\eta]/M^{1/2} = K_0 + C_\eta z \dots \quad (4)$$

$$K_0 = \Phi_{0,\infty} \left(\langle R^2 \rangle_0 / M \right)_\infty^{3/2} \quad (5)$$

$$\alpha_\eta^3 = [\eta]/K_0 M^{1/2} \quad (6)$$

where $\Phi_{0,\infty}$ is the Flory viscosity function in the limit of non-draining random coil ($\Phi_{0,\infty} = 2.5 \times 10^{21}$ for $[\eta]$ expressed in dl/g), C_η is a numerical coefficient ($C_\eta = 1.14$ for the same limit), z is the excluded-volume variable (proportional to $M^{1/2}$), and α_η^3 is the viscosity expansion factor^{19, 20}. Eqn (4) is known to be valid in a limited range of z or α_η^3 values²⁰. At $\alpha_\eta^3 > 2.5$ the BSF plot bends downwards and the extrapolation becomes unreliable. This rule is valid for systems where the MHKS exponent is $a < 0.8$ ¹⁸⁻²⁰. On the other hand, linear BSF plots have been found with some systems where the exponent a was higher than 0.8. In these cases the BSF method yields too low values of the intercept and conformational parameters derived there from¹⁸.

To avoid errors following from an inappropriate use of the BSF method, it is recommended to compare, in logarithmic scale, the plot of $[\eta]/M^{1/2}$ vs $M^{1/2}$ with the theoretical dependence of α_η^3 vs z . The function derived by Barrett²⁵

$$\alpha_\eta^3 = (1 + 3.8z + 1.9z^2)^{0.3} \quad (7)$$

seems to be suitable for this purpose as it has been obtained by combining the results of a numerical solution of the Kirkwood-Riseman hydrodynamic equations with the first-order perturbation calculations.

As follows from Eqns (7) and (4), the quotient $d \ln \alpha_\eta^3 / d \ln z$ is equivalent to $d \ln ([\eta]/M^{1/2}) / d \ln M^{1/2}$. It should increase with increasing z to the asymptotic value 0.6. Higher values of the slope are indicative of a more involved hydrodynamic behaviour. Then it is recommended to apply the theories of hydrodynamic properties where the polymer chains are represented by the continuous worm-like cylinder model^{26, 27}.

This is characterized by the contour length L , the effective (cross-sectional) hydrodynamic diameter d and the Kuhn statistical segment length l_k . The limit for $L \rightarrow \infty$ is the random flight chain. The chains are assumed to be unperturbed by the excluded-volume effect (subscript 0 with $[\eta]_0$).

The result of the theory can be written²⁶

$$[\eta]_0 = \left[\Phi_{0,\infty} (l_k / M_L)^{3/2} M^{1/2} \right] F_1 \quad (8)$$

where F_1 is a function of the reduced contour length L_r (number of Kuhn segments per chain) and the reduced cross-sectional chain diameter d_r defined by

$$d_r = d / l_k \quad (9)$$

Bushin et al. and Bohdanecky have independently found a simple approximation to the F_1 function^{26, 28, 29}

$$F_1 = (B_0 + A_0 / L_r^{-1/2})^{-3} \quad (10)$$

and proposed a simple procedure ("BB method") of treating experimental data according to the equation¹⁹

$$(M^2 / [\eta]_0)^{1/3} = A_\eta + B_\eta M^{1/2} \quad (11)$$

By replacing the molecular weight by the degree of polymerization $P = M/m_0$, Eqn (11) is transformed into

$$(P^2 / [\eta]_0)^{1/3} = (A_\eta / m_0^{2/3}) + (B_\eta / m_0^{1/6}) P^{1/2} \quad (12)$$

The parameters A_η and B_η are determined, respectively, from the intercept and slope of the plot $(P^2 / [\eta]_0)^{1/3}$ vs $P^{1/2}$. They are related to the characteristic parameters of the worm-like chain model by Eqns (5), (8) and

$$B_\eta = K_0^{-1/3} \quad (13)$$

$$M_k = M_L / l_k \quad (14)$$

where M_k is the molecular weight per Kuhn segment. The values of d_r and M_L can be estimated by means of equations²⁹

$$d_r^2 / A_0 = 4.54 \times 10^{-3} v_2 (B_\eta^4 / A_\eta) \quad (15)$$

$$\log d_r = [\log(d_r^2 / A_0) + 0.173] / 2.15 \quad (16)$$

$$A_0 = 0.46 - 0.53 \log d_r \quad (17)$$

$$M_L = 1.375 \times 10^7 (A_\eta / A_0) \quad (18)$$

where v_2 is the partial specific polymer volume. The shift factor M_L thus obtained is checked against the theoretical value M'_L . For chitosan the latter is calculated by the formulae:

$$M'_L = m_0 / l_b \quad (19)$$

$$\text{with } m_0 = 161 + 42F_A \quad (20)$$

The Yamakawa-Fujii theory and the BB method have been developed for unperturbed polymer chains, i.e., for systems where the second virial coefficient is zero (Θ condition)^{26, 28, 29}. If solutions are not at Θ condition, the intrinsic viscosity $[\eta]$ differs from $[\eta]_0$. The dependence of the viscosity expansion factor α_η^3 on the excluded-volume variable z for stiff chains is more involved than for non-draining random coils. As the chain stiffness prevents the repeating units from having contact with one another, there exists a certain chain length below which the excluded-volume effect on chain dimensions and hydrodynamic properties is negligible. Norisuye et al. have recently found that this effect can satisfactorily be explained in the framework of the Yamakawa-Stockmayer-Shimada theory³⁰⁻³³. The theory predicts that the radius-of-gyration expansion factor α_s and the viscosity expansion factor α_η^3 are universal functions of the scaled excluded-volume variable z :

$$z = (3/4)K(L_r)z \quad (21)$$

$$\text{with } z = (3/2\pi)^{3/2} (B/l_k) L_r^{1/2} \quad (22)$$

$$\text{and } K(L_r) = (4/3) - 2.711 L_r^{-1/2} + (7/6) L_r^{-1} \quad (23)$$

(for $L_r > 6$), or:

$$K(L_r) = L_r^{-1} \exp[-6.61 L_r^{-1} + 0.9198 + 0.03516 L_r] \quad (24)$$

(for $L_r < 6$)

where B is characteristic of the excluded-volume strength (polymer - solvent interaction). The $K(L_r)$ function is zero for $L_r < 1$ and approaches the random-coil value $4/3$ at infinite L_r . In this limit the scaled z parameter is equal to z .

The plot according to Eqn (12) should be linear if the viscosity expansion factor α_η^3 is equal or very close to unity. Expansion by the excluded-volume effect is expected to manifest itself by downward deviation from linearity of the BB plot at high chain lengths. However, as $[\eta]^{1/3}$ is used, the BB plot is less sensitive to expansion than plots using $[\eta]$ values²⁹. Thus, small deviations from linearity may escape observation, particularly if the scatter of data points is significant or the samples differ in composition (e.g., degree of acetylation).

A crucial question in studies of polyelectrolytes is the impact of external conditions on the stiffness characteristics. There are numerous detailed studies of the intrinsic viscosity of chitosans which could be exploited for this purpose, e.g., by applying the BB method. In many of them, however, the samples were characterized by number-average degrees of polymerization P_n , which are not suitable for correlation of the intrinsic viscosity unless the polymolecularity index is very low and similar for all samples, or by the viscosity-average or weight-average degrees of polymerization (P_v or P_w) which do not seem reliable. In order to exploit these data we have developed an approximate method where the need of

P values is limited though not fully eliminated. We do not deny that the method is merely a substitute for a more reliable procedure such as based on Eqn (12).

The method relates the intrinsic viscosity $[\eta]_{0,a}$ in a reference solvent system to the $[\eta]_0$ values for other solvents. The former is given by:

$$[\eta]_{0,a} = \Phi_{0,\infty} (l_k / M_L)^{3/2} M^{1/2} (B_{0,a} + A_{0,a} M_L^{1/2} l_k^{1/2} / M^{1/2})^{-3} \quad (25)$$

By eliminating the degree of polymerization from Eqns (8) and (25) we obtain:

$$[\eta]_0 = [\eta]_{0,a} (l_k / l_{k,a})^{3/2} (B_0 + A_{0,a} / L_{r,a}^{1/2})^3 / (B_0 + A_0 / L_r^{1/2})^3 \quad (26)$$

where $l_{k,a}$, $A_{0,a}$ and $L_{r,a}$ are the respective quantities for the reference system. Variation in the Kuhn segment length with composition of the solvent (e.g., ionic strength, degree of dissociation) automatically implies variation in the reduced cross-sectional chain diameter d_r and, consequently, in the parameter A_0 . On the contrary, the values of B_0 , d and M_L may be taken as independent of external conditions. We admit that this assumption may be a source of inaccuracy the magnitude of which cannot be assessed.

The assumption that the chains are unperturbed by the excluded-volume effect imposes restrictions on the use of Eqn (26). While it may be justified with stiff-chain polyelectrolytes, where the excluded-volume effect has been proven to be very weak, its use is prohibited or, at least, limited with polyelectrolytes derived from flexible chains such as vinyl polymers or polyacrylics where the excluded-volume effect is usually far from being negligible¹⁵.

As follows from Eqn (26), the ratio $r = (l_k / l_{k,a})$ is a relative measure of stiffness. In order to estimate it we have to extrapolate the values of $[\eta]_0 / [\eta]_{0,a}$ to $1 / L_r^{1/2} = 0$. However, the form of Eqns (25) and (26) makes this step difficult because the convergence is slow, particularly at high r values. This is illustrated by some calculated examples (Fig. 1) where the basic parameters approximately correspond to chitosans. We have found, however, that conditions for estimating the r ratio become better if $[\eta]_0^{1/3}$ is plotted against $[\eta]_{0,a}^{1/3}$ (Figs. 2a and 2b). It should be linear if $A_0 \cong 0$ ($d_r \cong 0.4$) for both systems. In other cases the plots are non-linear at low L_r values but are nearly linear at high ones, and their slopes asymptotically approach $r^{1/2}$. The asymptotic behaviour is attained the sooner the lower the A_0 value. Fig. 2a presents such plots for some systems selected from Fig. 1. It is seen that, already at $[\eta] > 1$ dl/g, calculated points can very well be fitted by straight lines with asymptotic slopes. The same holds for chains with a larger cross-sectional diameter (Fig. 2b). Having estimated r we can convert it into the Kuhn segment length by comparison with the l_k value estimated in the standard manner, e.g., using Eqn (12).

The ionic strength dependence of the intrinsic viscosity of polyelectrolytes usually obeys the empirical equation^{15, 34-36}:

$$[\eta]_s = [\eta]_\infty + S C_s^{-1/2} \quad (27)$$

where symbols $[\eta]_s$ and $[\eta]_\infty$ stand, respectively, for the intrinsic viscosity at salt concentration C_s and $C_s = \infty$. The slope S , referred to as the salt tolerance, is assumed to be related to the chain stiffness and expansion, and $[\eta]_\infty$ to correspond to conformations unaffected by electrostatic interaction³⁴. The plot of $[\eta]_s$ vs $C_s^{-1/2}$ is used to estimate $[\eta]_\infty$ (Fig. 3).

Estimation of conformational parameters

Plot according to Eqn (27)

Fig. 3 shows good correspondence of the plots according to Eqn (27) of data for $0.02 < F_A < 0.2$ from several papers. The intercepts and slopes decrease with an increase in the degree of polymerization. The same rule holds for $0.09 < F_A < 0.6$. The effect of monovalent anions (Cl, NO₃, ClO₄) in the neutral electrolyte has recently been shown to be weak³⁶.

Plots according to Eqns (4) and (12)

Figs. 4a and 4b present the BSF plot (according to Eqns (4) and (8)) in normal and logarithmic scales. The former tends to the origin. The initial part of the latter is much steeper than is the theoretical dependence of the viscosity expansion factor α_η^3 on the excluded-volume variable z for the model of non-draining random coils expanded by the excluded-volume effect. This is schematically represented by curve 1. This model is obviously not suitable for interpreting the hydrodynamic behaviour of chitosan. Therefore, we make use of the worm-like cylinder model²⁶.

The plot of $(P_w^2 / [\eta])^{1/3}$ vs $P_w^{1/2}$ according to Eqn (12) of data (Fig. 5) is remarkably smooth with no detectable effect of the degree of acetylation^{12, 15-17}. Slight deviation from linearity at $P^{1/2} > 50$ may indicate the chain expansion caused by the excluded-volume effect becoming noticeable. To illustrate this effect we computed curves 3 and 4 for two values of B / l_k using Eqns (7), (8), (13), (21) to (23) and the parameters A_η and B_η corresponding to curve 1. Values B / l_k lower than 0.1 have negligible impact on linearity of the plot. Noticeable deviation can be expected at $P > 400$ and 200 with systems characterized by $B / l_k = 0.1$ and 0.4, respectively. Approximate linear fitting of curves 3 and 4 would yield too high values of A_η and conformational parameters.

The conformational characteristics derived from the intercept and slope of curve 1 by means of Eqns (12) to (20), (2) and (3b) are listed in Table 1. The d value is similar to that estimated by Pogodina et al. using Eqn (19) and the solid state density of the polymer¹⁷. The M_L value differs from M'_L by less than 20%.

Curves 2 to 4 in Fig. 4a have been drawn to illustrate the sensitivity on the excluded-volume effect of the BSF plot. Comparison with curves 3 and 4 in Fig. 5 shows that application of the BSF and BB methods to stiff-chain polymers can provide complementary information. While the latter should yield a quantitative estimate of the conformational parameters and merely a very qualitative information on the interaction parameter B / l_k , the former provides no information on the conformational characteristics but allows the B / l_k value to be assessed.

Plot according to Eqn (26)

In this part we analyse the intrinsic viscosity data $[\eta]_s$ measured at various salt concentrations C_s as reported by several authors^{13, 15, 32}. Included are also $[\eta]_\infty$ values obtained in the respective papers by linearly extrapolating the plots of $[\eta]_s$ vs $C_s^{-1/2}$ according to Eqn (27). Data in these papers do not refer to exactly identical degrees of ionization α . Despite that, we combined them because, as has been shown by Kienzle-Sterzer, the effect of the degree of ionization on the parameters of Eqn (27) does not exceed 5% in $[\eta]_\infty$ and 20% in S^{35} .

Fig. 6 presents plots of $[\eta]_s^{1/3}$ against $[\eta]_a^{1/3}$ according to Eqn (26). The reference concentration is $C_{s,a} = 0.1$ M. In the theoretical part we have shown that the slope of this plot for chitosan at $[\eta] > 1$ dl/g is close to $r^{1/2} = (l_k / l_{k,a})^{1/2}$. The r values estimated in Fig. 6 are plotted against $C_s^{-1/2}$ in Fig. 7. No systematic effect of the degree of acetylation ($0 < F_A < 0.6$) is detected and the dependence can be fitted by straight line.

Effect of solvent and degree of acetylation on conformation

Most intrinsic viscosity data used for the estimation of the r values originate from the paper by Anthonsen et al.¹³. As the r values display no significant effect of the degree of acetylation (Fig. 7), the value of $l_k = 14.1$ nm estimated from the BB plot (Fig. 5) for $F_A = 0.02 - 0.2$ and $C_s = 0.2$ M is used to convert the r values into l_k and C_∞ with no respect to the degree of acetylation. By interpolation in Fig. 7 the r value corresponding to $C_s = 0.2$ M is $r \approx 0.9$. Then the values of $l_{k,a}$ (at the reference concentration $C_{s,a} = 0.1$ M) is estimated to be 15.7 nm and the values of l_k and C_∞ for other salt concentration are readily computed (Fig. 8). The "intrinsic" values, $l_{k,\infty} = 10 \pm 2$ nm and $(C_\infty)_\infty = 19.3 \pm 1.4$, corresponding to $C_s^{-1/2} = 0$ are characteristic of chain stiffness which arises solely from the chain backbone and the steric effects of the lateral groups. Differences (Fig. 8) are within the limits of accuracy of estimation. By another treatment of the $[\eta]$ data Rinaudo et al. obtained $l_{k,\infty} = 8.4$ to 10 nm¹⁵.

By analyzing the $[\eta]$ data published by Terbojevich et al. we assessed the conformational characteristics of chitin (Appendix I)³⁷. As the amino groups in chitin are completely acetylated and, therefore, practically uncharged, the effect of the electrostatic interaction on the chain stiffness is very weak, if any. Hence, the conformational characteristics for chitin chains can be compared with those for uncharged chitosan chains. The former turn out to be stiffer (Table 1).

As follows from the definitions, the characteristic ratio, unlike the Kuhn statistical segment length, depends only on the rotational hindrances of the units around the main chain bonds. High values of C_∞ for chitin and $(C_\infty)_\infty$ for chitosan are indicative of strong hindrances. The value of $(C_\infty)_\infty = 19$ for chitosan amounts to about 30% of that for chitin ($C_\infty \cong 62$). As the valence angles in the main chains of both polymers are the same, the difference in C_∞ , is to be attributed to stronger steric and/or energetic interactions in chitin.

Chitosans are copolymers of acetylated and non-acetylated glucosamine units. For chains consisting of two different kinds of statistically independent chain elements and conforming to the random flight-chain model, the ratio $(\langle R^2 \rangle_0 / P)_\infty$ is a linear function of composition.¹⁸

$$(\langle R^2 \rangle_0 / P)_\infty = (\langle R^2 \rangle_0 / P)_\infty^A w_A + (\langle R^2 \rangle_0 / P)_\infty^B (1 - w_A) \quad (28)$$

where w_A is the weight fraction of monomer units A in the copolymer and the superscripts A and B to $(\langle R^2 \rangle_0 / P)_\infty$ denote the values for parent homopolymers. If the average sequence lengths are small, as with random or alternating copolymers, additional short-range interactions between unlike units take place and positive or negative deviations from additivity appear. This is the case of chitosans. For a qualitative discussion it is possible to neglect the differences in M_L values for comonomers and make direct use of $(C_\infty)_\infty$. These values are almost constant at $F_A < 0.6$ but lower than those calculated on the assumption of additivity of values for homopolymers. This finding suggests short range interactions of unlike monomer units. High value of the characteristic ratio for chitin, indicating high stiffness, can be attributed to strong steric interferences brought about by the size of acetyl groups and/or to hydrogen bonding between acetyl amino and hydroxy groups of neighboring glucosamine units^{3, 16, 38}. Both types of interaction are decreased if sequences of acetylated units are interrupted by non-acetylated ones. This may be the reason of negative deviations of C_∞ of chitosans from additivity.

As can be seen in Fig. 8 repulsive interaction of charged amino groups raises the Kuhn segment length of chitosan chains up to $l_k \approx 30$ nm at $C_s = 10^{-2}$ M. Linear dependence of l_k vs $C_s^{-1/2}$ is in agreement with most experimental results obtained with other polyelectrolytes and with theories predicting that, because of electrostatic interaction of charged groups, the Kuhn length increases linearly with $C_s^{-1/2}$ at higher salt concentrations³⁹⁻⁴¹. It is remarkable that, contrary to expectation, no significant effect of the degree of acetylation can be detected.

Fig. 8 also contains the results of the other authors. Higher value of the Kuhn segment length reported by Pogodina et al. at $C_s^{-1/2} = 0.16$ has been obtained from the BB plot fitting the data¹⁷. Our value results from line 1 in Fig. 5, fitting a broader range of P values, including those data. This example illustrates the sensitivity of estimation of the conformational parameters on the range and scatter of data points used in the BB method.

Kienzle-Sterzer et al. obtained lower values of the conformational parameters from the $[\eta]$ data for one chitosan sample ($P_w = 1.3 \times 10^3$)³⁵. The difference is probably due to a different method of evaluation of experimental data. Working with one polymer sample only the authors made use of the Yamakawa-Fujii theory, assuming the chains to be random coils in the non-draining limit ($d_r = 0.4$), and calculated l_k using Eqn (8) with $F_1 = 1$ ²⁶. However, the present analysis shows that the reduced cross-sectional chain diameter is $d_r \approx 0.034$. Then the F_1 value calculated by means of Eqns (12) to (18) for $L_r = 34$ (corresponding to $P_w = 1.3 \times 10^3$) is equal to 0.56. Hence, the Kuhn statistical segment length estimated by the above authors should be multiplied by $0.56^{-2/3} = 1.47$. As shown by the dashed line in

Fig. 8, this operation shifts the values by Kienzle-Sterzer et al. into the range of values estimated in this study³⁵.

At another place the same authors made use of the plot of $[\eta]$ vs $M^{-1/2}$, the slope A_1 of which is approximately equal to K_0^{-1} ^{35, 42}. However, applying this method to stiff-chain polymers, Dondos and Staikos obtained too low K_0 values⁴³. The reason is that the method originally proposed to eliminate the excluded-volume effect from data for non-draining random coils cannot eliminate the effect of weak hydrodynamic interaction^{42, 44}. With stiff chain polymers the former effect is usually weak, whereas the latter is strong.

Let us compare the l_k values derived from the intrinsic viscosity data with those calculated from the radii of gyration $\langle S^2 \rangle^{1/2}$ estimated by light scattering. A remarkable agreement is found with the values of $l_k = 12$ nm (for commercial samples of $F_A \cong 0.25$) and 10 nm (for homogeneous samples with $F_A = 0.01$ to 0.71)^{11, 45}. Other estimates are high: $l_k = 20$ to 30 nm (homogeneous samples, $F_A = 0.02$ to 0.6) and 42 nm (commercial samples, $F_A = 0.15$ to 0.42)^{46, 47}.

Using $l_k = 42$ nm, $M'_L = 420$ nm⁻¹, $d = 0.66$ nm and Eqns (8) to (18) we constructed the dependence of $[\eta]_0$ vs P_w (curve 2 in Fig. 9) and compared it with curve 1, calculated with the parameter values in Table 1. At $P < 50$ the difference is small. At $P > 300$ the $[\eta]_0$ values on curve 2 are twice those on curve 1. The difference might be attributed to chain expansion by the excluded-volume effect. However, as the radius of gyration expansion factor α was low ($\alpha = 1.05$) this explanation cannot be accepted⁴⁷. The high l_k value is probably overestimated. As is well known the accuracy of radii of gyration and the l_k values derived there from depends on the effect of residual supermolecular particles and polymolecularity on the radii of gyration, and on the procedures used to take account of polymolecularity and excluded volume effect when calculating l_k .

Comparison of conformational parameters of chitosans and cellulose

As the repeating units of chitosans and cellulose chains are 1,4- β -D linked it is interesting to compare their conformational characteristics. The parameters of cellulose were evaluated from the reported intrinsic viscosity data in Appendix II and are listed in Table 2⁴⁸⁻⁵³. The values of the characteristic ratio are in the range $19 < C_\infty < 28$ and compare well with those estimated by Saalwachter et al. from the radii of gyration ($20 < C_\infty < 25$)⁵⁴. The differences in C_∞ are, at least partly, related with specific interaction of cellulose and solvents (complex formation). All values are lower than that calculated theoretically ($C_\infty \cong 100$) in the isomeric state approximation^{55, 56}. As similarly low values have been found with cellulose derivatives (e.g., $C_\infty = 38$ for cellulose acetate), strong difference from the theoretical value appears to be connected with the conformation of cellulose chains. As an explanation, Brant and Goebel postulated occurrence of a small fraction (3–5 %) of alternate conformers disrupting the strong directional persistence of dominating sequences⁵⁶.

The $(C_\infty)_\infty$ value for uncharged chitosan chains ($C_\infty = 19$) is similar to those for cellulose. Though the value estimated for chitin ($C_\infty \cong 50$) is higher, it is still below the theoretical value for cellulose. This situation leads to the hypothesis that the factor suggested by Brant and Goebel also affects the conformation of chitin and chitosans⁵⁸.

Random –Coil conformation, draining and excluded-volume effects with Chitosan chains

In this part we are concerned with the following questions: (i) Under which conditions do chitosan chains assume the random-coil conformation? (ii) Is negligible excluded-volume effect on $[\eta]$ compatible with high value of the second virial coefficient? (iii) Can variation in external conditions bring about transition from weak hydrodynamic interaction to the non-draining regime?

Ad (i). Typical values of the MHKS exponent for the model of random coils in the non-draining limit are in the range $0.5 < a < 0.8$. As these values were found in some papers on chitosan, the authors concluded that this model can well describe the conformation and hydrodynamic properties of this polymer in dilute solutions. The discussion in Part 3 of the present paper has shown that the model of the worm-like cylinder model is more adequate.

Nevertheless, it is useful to establish the range where the ratio $\langle R^2 \rangle_0 / P$ is independent of the chain length, as is typical for random coils. Theoretically, this behaviour is attained at infinite chain lengths but, basing on the theory of the worm-like chain model, we assume that the random-coil conformation is approached at the reduced chain length of $L_r \cong 10$ at which this ratio differs from the random coil value $(\langle R^2 \rangle_0 / P)_\infty$ by less than 10%¹⁹⁻²¹. Using $M_L \cong 420 \text{ nm}^{-1}$, $M_0 = 170$ (mean value for $F_A < 0.5$) and the l_k values in Fig. 11 we computed the degree of polymerization per Kuhn segmented corresponding to $L_r \cong 10$ to be $P_k \approx 50$ and 15 at $C_s = 0.01 \text{ M}$ and ∞ , respectively. The above criterion ($L_r \cong 10$) is fulfilled with $P > 500$ at low salt concentration and with $P > 150$ at high ones.

Ad (ii). We have shown in Figs. 4 and 5 that the B / l_k parameter at $C_s = 0.2 - 0.3 \text{ M}$ is very low (≈ 0.01) and, consequently, the chain expansion due to the excluded-volume effect is weak. Very low value of the interaction parameter B seems to be at variance with the second virial coefficients A_2 being of the order of $10^{-3} \text{ cm}^3 \text{ mol/g}^2$, which usually are indicative of strong polymer-solvent interaction¹³. Three effects must be considered when interpreting the second virial coefficient of polyelectrolytes: the ideal Donnan term, the reduction of the effective charge of the macroions due to the counterion condensation, and the excluded-volume effect resulting from the macromolecular character of the polyelectrolyte⁵⁷. While the former two contributions are independent of the molecular weight, the third causes a decrease of A_2 in good solvent systems with increasing M . As no molecular-weight dependence of the second virial coefficient has been observed with chitosans, a possible explanation of the apparent contradiction of B and A_2 values is that high values of A_2 are mainly caused by the first and second effects¹³. These can be expressed by⁵⁷:

$$A'_{2,D} = (1000 / \mu)(Z / M)^2 \left[(\gamma_c^0)^2 / \gamma_s^0 \right] \quad (29)$$

where $A'_{2,D}$ is the reduced Donnan virial coefficient, μ is the ionic strength (mol / l), Z is the number of charges on the chain, γ_c^0 is the activity coefficient of added salt in the absence of polyelectrolyte, and γ_s^0 is the counterion activity coefficient in the salt-free polyelectrolyte solutions. As Z / M is constant, the reduced Donnan term is independent of the molecular weight. Since γ_s^0 is of the order of unity and γ_c^0 is in the range from 0.1 to 0.2 we obtain, assuming $M / Z = 165$, an estimate of $A_{2,D} \times 10^3$ to be 1.8 to 7 $\text{cm}^3 \text{ mol/g}^2$ which compares well with reported A_2 values^{13, 57}.

Ad (iii). The preceding points show that chitosan molecules can be represented by the model of the unperturbed random flight chain in almost the whole range of degrees of polymerization of practical interest ($P > 200$) at high salt concentrations and at $P > 600$ at low ones. Despite that, as born out by low value of the reduced cross-sectional chain diameter ($d_r = 0.034$ at $C_s = 0.25 \text{ M}$), the hydrodynamic behaviour at $C_s \cong 0.1 - 0.3 \text{ M}$ is far from the non-draining regime. If this regime should set on at high salt concentrations and at finite P values, the reduced diameter d_r and the cross-sectional chain diameter d would have to be, respectively, 0.4 and 7 nm. Such an increase is not probable. Contrary to that, assuming (at least in the first approximation) the diameter and the shift factor M_L to be independent of external conditions and using $l_k = 14 \text{ nm}$, $d_r = 0.034$ for $C_s = 0.25 \text{ M}$ (Table 1) and $l_{k,\infty}$ value (cf. Fig. 8), we obtain $d_r = 0.06$ at $C_s = \infty$. With these chain characteristics we calculated the values of A_0 , A_η and B_η and by substituting them into Eqns (12) and (8), we constructed theoretical dependences of $[\eta]_0$ vs P for $C_s = 0.01 \text{ M}$, 0.25 M and ∞ (Fig. 10). It can be seen that the quotient $d \ln [\eta]_0 / d \ln P$ is higher than unity at $P < 100$ and approaches 0.5 at very high P values and at $C_s = \infty$ only.

CONCLUSION

There are some other topics where the information in literature is contradictory, e.g. the effect of anions or urea on $[\eta]$. While no effect was detected by Rodriguez-Sanchez with several 1:1 electrolytes and a weak one (less than 10%) was reported by Trzcinski et al. with chlorides, nitrates and perchlorates, Domard and Rinaudo found a difference of about 20% between chlorides and bromides^{22, 36, 58}.

The paper of Lyubina et al. indicates a decrease of $[\eta]$ on addition of urea, whereas an increase of about 5% on transition from 0.1 M NaCl to 0.1 M NaCl / 4 M urea has been found by Domard and Rinaudo^{22, 59}. According to Tsai and Chen, the intrinsic viscosities in 4M urea are higher by 100 to 300% than those in 0.1 M salt solutions⁶⁰.

Table-1: Characteristics of chitosan and chitin chains ^a.

A'_η	B'_η	$(\langle R^2 \rangle_0 / M)_\infty$ (nm ²)	M_L (nm ⁻¹)	M'_L	l_k (nm)	d_r	d (nm)
Chitosan ($0.02 < F_A < 0.6$)							
8.0 ^b	1.37 ^b	0.053	270 ^c	320 ^d	14.1 ^c , 16.5 ^d	0.034	0.48
Chitin ($F_A = 1$)							
5.0 ^b	1.22 ^b	0.072	320 ^c	384 ^d	23 ^c , 28 ^d , 36 ^e	0.027	0.62, 0.66 ^f

a. $A'_\eta = A_\eta / m_0^{2/3}$, $B'_\eta = B_\eta / m_0^{1/6}$

b. estimated from Fig. 5 (chitosan) or Fig. 11 (chitin)

c. calculated from A'_η values

d. calculated according to Eqn (2) with $M_0 = 165$ and 203 (for chitosan and chitin, respectively) and $l_0 = 0.515$ nm

e. calculated from radii of gyration

f. calculated from solid state density

Table-2: Characteristics of cellulose chains ^{a, b}.

Solvent	B'_η	$(\langle R^2 \rangle_0 / M)_\infty$ (nm ²)	l_k (nm)	C_∞
Cadoxene	1.7	0.033	12.4	20
Fe-Na-tartrate	1.4	0.046	14.1	28
LiCl - DMAc	1.2	0.069	19.7	37
Cu-oxam	1.8	0.030	9.9, 13 ^c	18
Cu-en	1.65	0.037	11.5	22

a. for definition of A'_η and B'_η cf. footnote a. in Table 1

b. estimated from Fig. 12 (with $A'_\eta = 10$ for all systems) with $M_L = 315$ nm⁻¹ and $M_0 = 162$ c. from Saalwachter et al. where $C_\infty = 24.7$ and 20.4 for solutions in Cd-tren and Ni-tren complexes can also be found⁵⁴

Appendix I

It is remarkable that (i) the $[\eta]$ values of chitin in LiCl-dimethyl acetamide solutions are almost twice those for chitosan at similar P_w values ($443 < P_w < 2510$), (ii) the MHKS exponent ($a = 0.66$) is lower than 0.8, (iii) the logarithmic plot of $[\eta]$ vs P_w runs parallel to the upper part of the plot for chitosan at $C_s = 0.25$ M (Fig. 11)⁵⁸. The second point could suggest that the chitin chains are non-draining random coils expanded by the excluded-volume effect and that, therefore, the conformational parameters could be estimated by means of the BSF method. This treatment (Fig. 11) would yield $K_0 = 14.5 \times 10^{-3}$ dl/g and $l_k = 15.5$ nm. This value would amount to less than half that estimated from the radii of gyration (30–80 nm), indicating the stiffness of chitin chains to be similar to that of uncharged chitosan. However, the viscosity expansion factor α_η^3 ($1.3 < \alpha_\eta^3 < 2$) would be at variance with the radius-of-gyration expansion factors $\alpha_R^3 \cong 1$ ³⁷. Furthermore, the number of Kuhn segments L_r of studied samples would be $12 < L_r < 60$. At such chain length the non-draining regime would be attained with $d_r \cong 0.4$ only¹⁸. However, a much lower value of d_r obtains from $d = 0.66$ nm and the above values of l_k . With regard to these contradictions we decided to apply Eqns (12)–(18), Fig. 11.

Using the parameters of the BB plot ($A_\eta = 230$, $B_\eta = 2.8$, Fig. 11) and the reciprocal value of the solid state density instead of the partial specific volume we obtained the conformational characteristics listed in Table 1. The shift factor M_L calculated from A_η is lower than M'_L computed from the ratio of m_0 / l_0 . Using either of them we obtain the Kuhn segment length comparable to the lowest value estimated by Terbojevich et al. from the radii of gyration³⁷. The cross-sectional diameter is in good agreement with that computed from the solid-state density.

All of the l_k values computed from both the $[\eta]$ data and the radii of gyration give evidence of a higher stiffness of chitin chains as compared with those of chitosan. The difference between those estimated from $[\eta]$ and radii of gyration may be due to the effect of polymolecularity on the latter. Though the interpretation of hydrodynamic data and the parameters derived there from the worm-like cylinder model appears to be more consistent, a reliable conclusion could be gained with $[\eta]$ values covering a broader range of molecular weights, in particular, lower ones.

Appendix II

Cellulose is dissolved by aqueous solutions of metal complexes (Cu, Cd, Pd, Ni, Fe) with amines [ammonia, ethylene diamine, tris-2-aminoethylamine (tren)] or with salts of hydroxyacids (Fe-Na-tartrate). It is also soluble if solutions of LiCl in N, N-dimethyl acetamide (DMAc). Fig. 12 presents the plot according to Eqn (12) of data for solutions in cadoxen (Cd-ammonia), Fe-Na-tartrate and LiCl / DMAc⁵¹⁻⁵⁵. Lines 1 and 2 have been calculated from the MHKS parameters for Cu-en (copper-ethylenediamine) and Cu-oxam (copper-ammonia) estimated by Marx⁴⁸. Data for cadoxen originate from three papers where the molecular weights were determined by different methods (combination of diffusion and sedimentation coefficients, light scattering)⁵¹⁻⁵³. As they are in remarkably good agreement, the conformational parameters derived there from (Table 2) can be regarded as reliable.

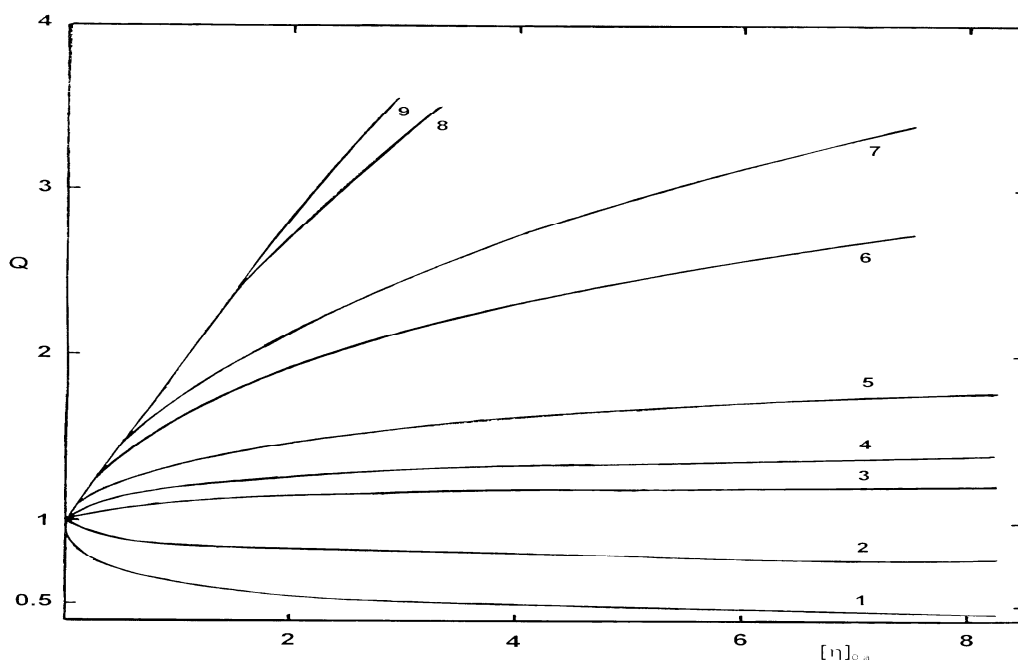


Fig.-1: Plot of theoretical values of $Q = [\eta]_0 / [\eta]_{0,a}$ vs $[\eta]_{0,a}$. Calculations according to Eqn (25) with $d = 0.88$ nm, $l_{k,a} = 22$ nm, $A_{0,a} = 1.20$, $M_L = 420$ nm⁻¹. Curves 1 to 9 correspond to $l_k / l_{k,a} = 0.5, 0.8, 1.2, 1.4, 1.8, 3, 4, 10$ and 15 .

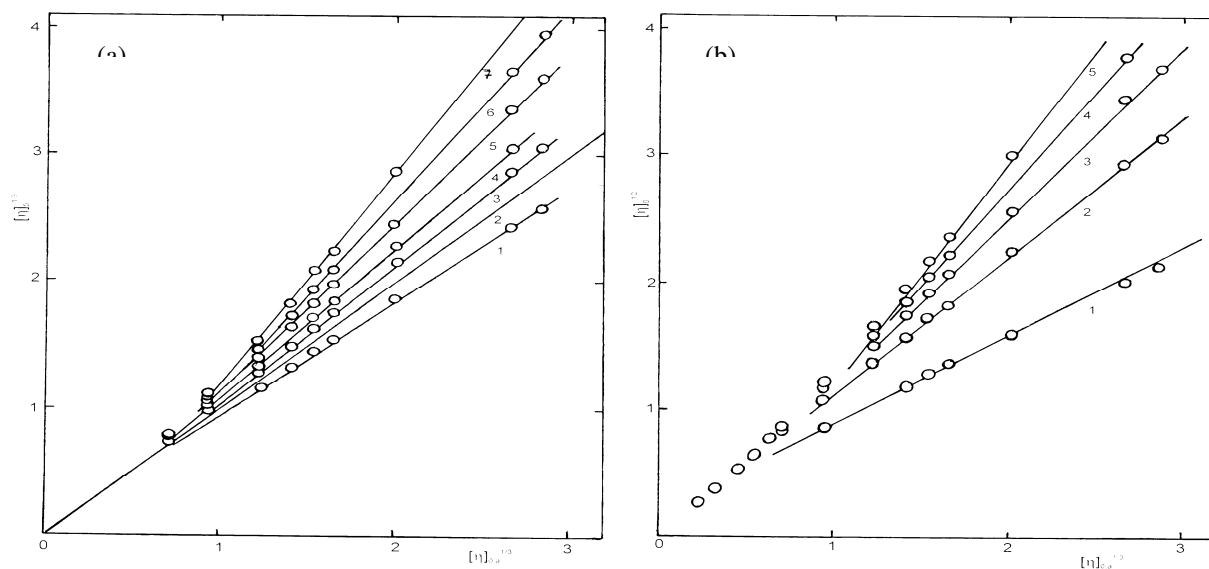


Fig.-2: Plot of $[\eta]_0^{1/3}$ vs $[\eta]_{0,a}^{1/3}$.

- (a) Result of theoretical calculations with parameter values ($d, A_{0,a}, M_L$) as in Fig. 1 and $l_k / l_{k,a} = 0.8, 1, 1.2, 1.4, 1.8, 2.3$ and 3 (curves 1 to 7).
(b) Results of similar calculations with $d = 2$ nm and $l_k / l_{k,a} = 0.5, 1.2, 1.8, 2.3$ and 3 (curves 1 to 5).
Calculated values are denoted by open rings, lines are drawn with asymptotic slopes equal to $(l_k / l_{k,a})^{1/3}$.

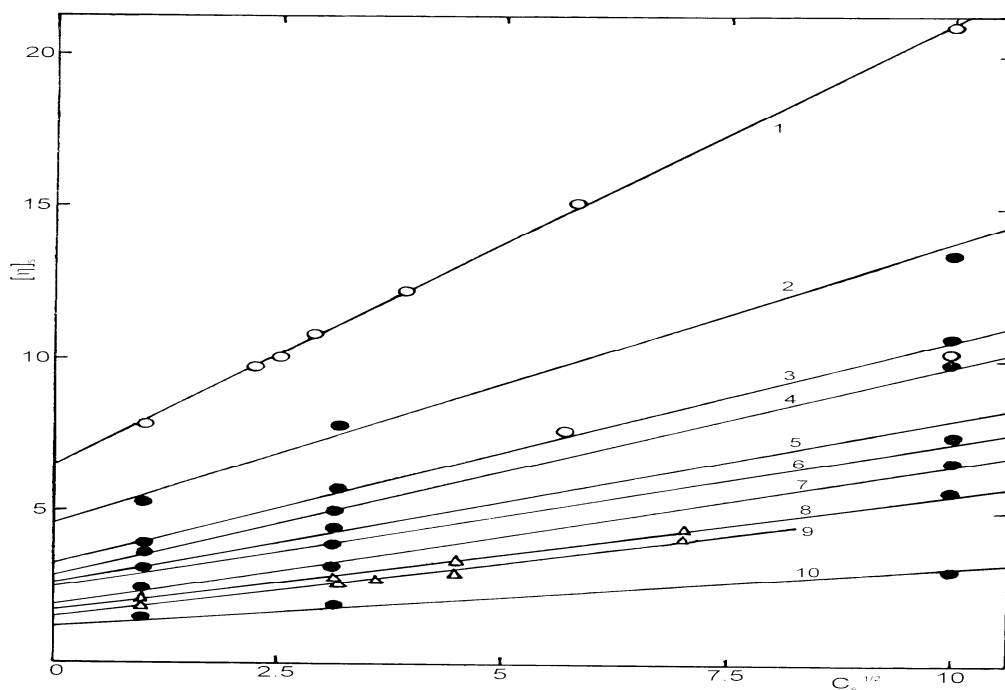


Fig.-3: Plot of $[\eta]_s$ vs $C_s^{-1/2}$ for chitosans according to Eqn (27).

Data points ($0.02 < F_A < 0.2$): $\bullet^{13}, \circ^{16}, \triangle^{37}$.

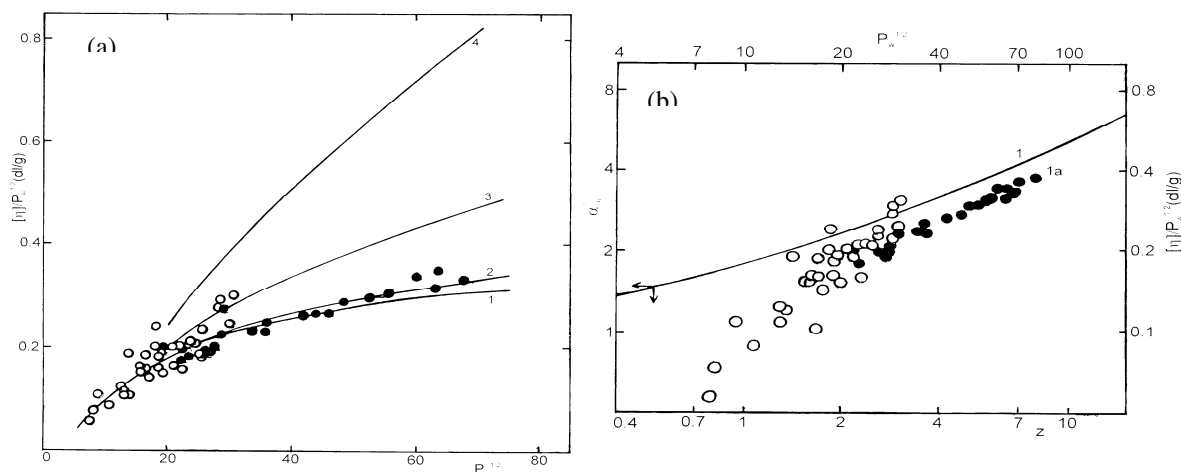


Fig.-4: Burchard-Stockmayer-Fixman plot of data for chitosans in normal (a) and logarithmic scales (b).
Panel (a): Experimental data: $\circ$ ¹⁸, $\bullet$ ¹⁶. Theoretical curve 1 ($[\eta]_0 / P^{1/2}$ for $B / l_k = 0$) was calculated using Eqns (9), (2), (6), (13) with A_η and B_η values listed in Table 1. Curves 2 to 4 ($[\eta] / P^{1/2}$) were computed as $[\eta]_0 \alpha_\eta^3 / P^{1/2}$ where α_η^3 were calculated using Eqns (23), (22), (21) and (8) with $B / l_k = 0.01, 0.1$ and 0.4 .
Panel (b): Theoretical curve (left and bottom scales) were calculated according to Eqn (8), experimental data (right and top scales). Notation of experimental points as in panel (a).

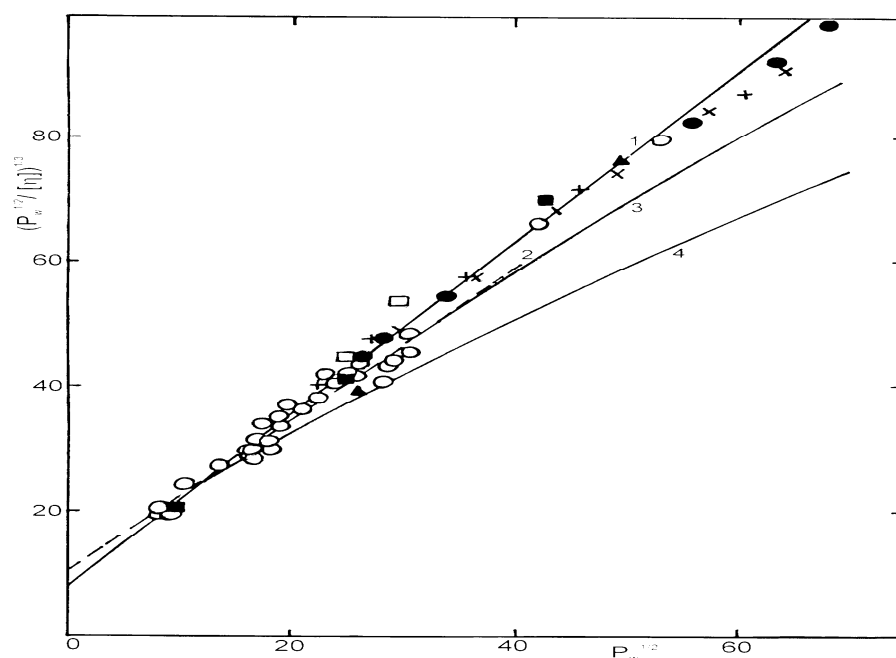


Fig.-5: Plot according to Eqn (12) of data for chitosans.
Experimental data: $\blacksquare$ (0.02 M acetate buffer pH 4.3 / 0.1 M NaCl, $0.07 < F_A < 0.25$)¹², $+$ $\times$ $\bullet$ (0.3M HAc / 0.2M NaCl, $F_A = 0.02, 0.11$ and 0.22)¹⁶, $\blacktriangle$ (0.1M HAc / 0.2 M NaAc, $F_A = 0.15$ and 0.42)¹⁷, $\circ$ (0.33 M HAc / 0.3M NaCl, $F_A = 0.18$)¹⁸. Lines 1 and 2 calculated with Eqn (12) and parameters listed in Table 1 or published by Pogodina et al. respectively¹⁷. Curves 3 and 4 were calculated for expanded chains with the same parameters as curves 3 and 4 in Fig. 4.

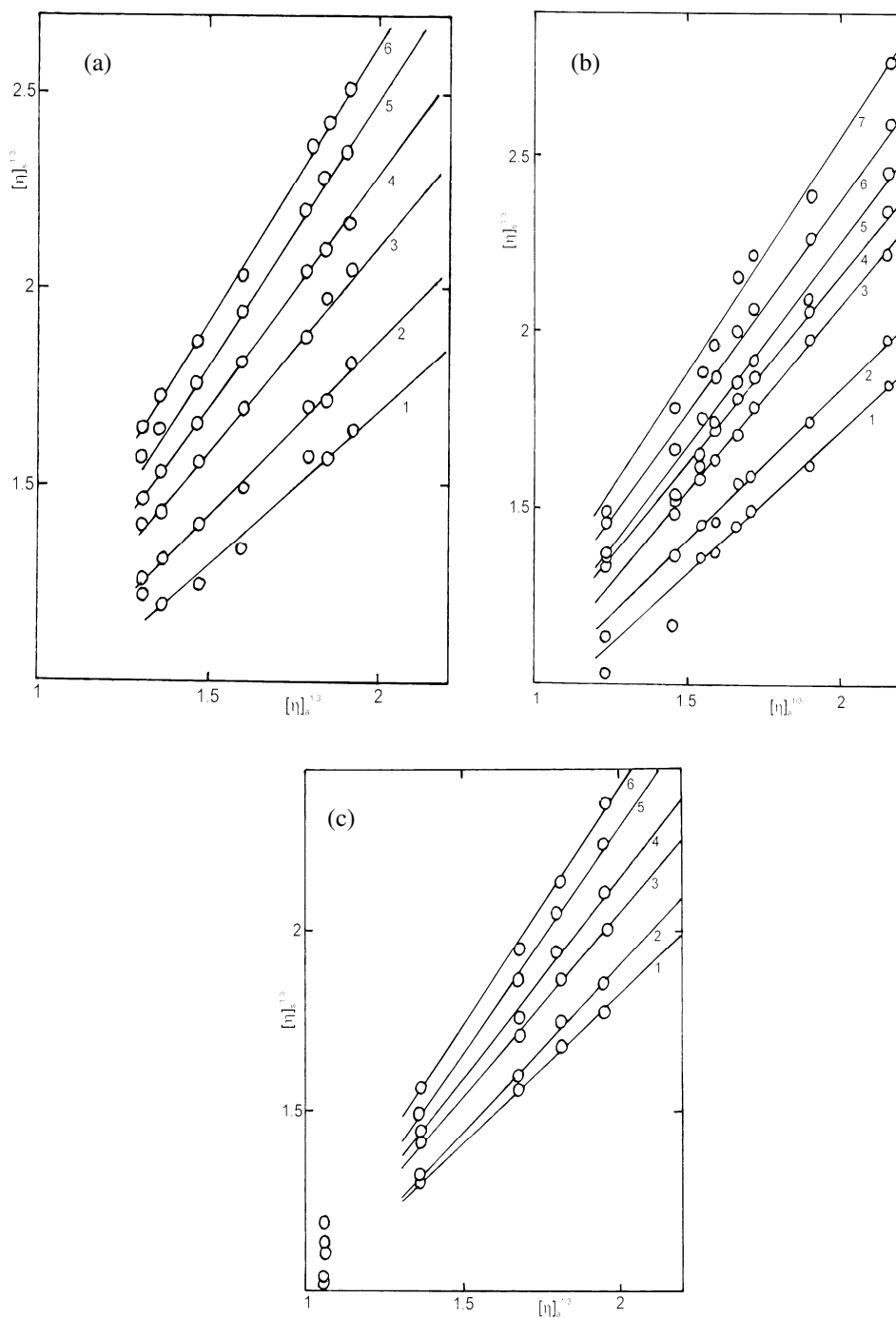


Fig.-6: Plot according to Eqn (26) of data for chitosans with different F_A values. The values of $[\eta]_\infty$ and $[\eta]$ at $C_s^{-1/2} = 7.5$ and 5 were obtained by linear extrapolation or interpolation of plots of $[\eta]$ vs $C_s^{-1/2}$.

Panel (a): $F_A = 0 - 0.02$. Lines 1 to 6 for $C_s^{-1/2} = 0, 1, 3.16, 5, 7.5$ and 10 .

Panel (b): $F_A = 0.09 - 0.21$. Lines 1 to 7 for $C_s^{-1/2} = 0, 1, 3.16, 4.5, 5, 7.5$ and 10 .

Panel (c): $F_A = 0.6$. Lines 1 to 6 for the same salt concentrations as in panel (a).

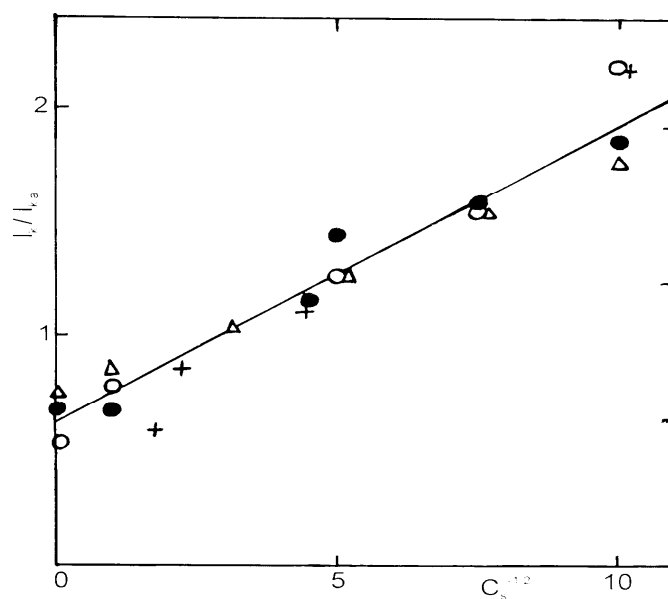


Fig.-7: Effect of salt concentration on the ratio $r = l_k / l_{k,a}$. Values of r estimated from Fig. 6: $\circ$ ($F_A = 0 - 0.02$), $\bullet$ (0.09 - 0.21), $\triangle$ (0.6). Crosses $+$ denote results estimated from data by Rinaudo et al.¹⁵.

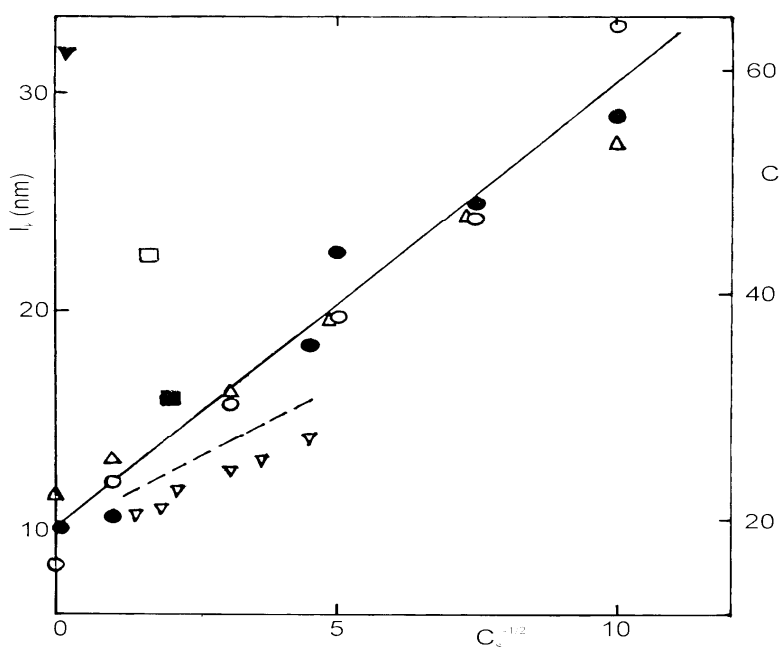


Fig.-8: Effect of salt concentration on conformational parameters of chitosans. Kuhn segment length l_k (left hand scale), Flory characteristic ratio C_∞ (right hand scale). Notation of data points as in Fig. 6 and Fig. 7 ($\bullet$ $\triangle$). Literature values $\square$ $\circ$ ∇ ^{18, 36}, $\blacktriangledown$ ¹⁷. Value estimated in Fig. 5 (BB plot) $\blacksquare$. Dashed curve represents data according to Trzcinski corrected as described in text³⁶.

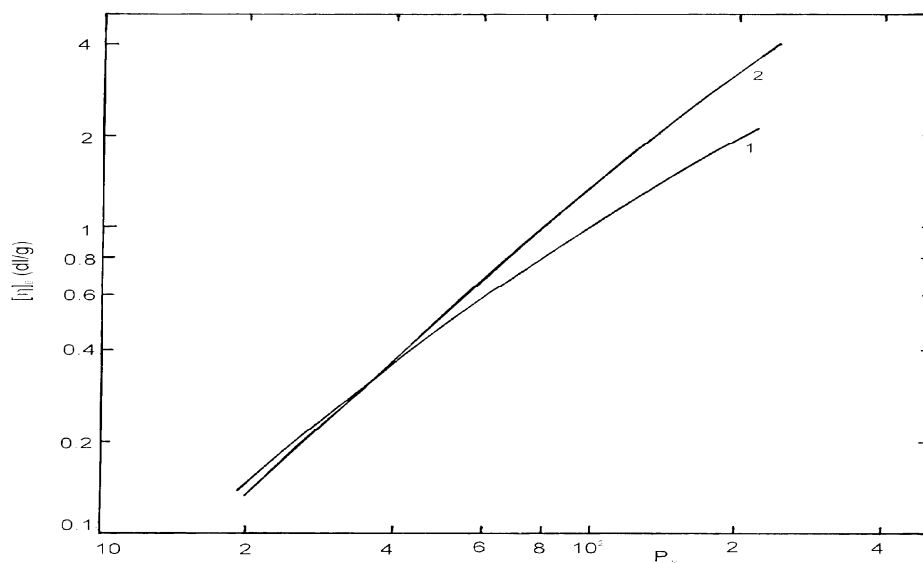


Fig.-9: Comparison of theoretical dependences of $[\eta]_0$ vs P_w using Eqns (8) to (18). Curve 1 was calculated with parameters listed in Table 1, curve 2 with parameters reported Brugnerotto et al.⁴⁷.

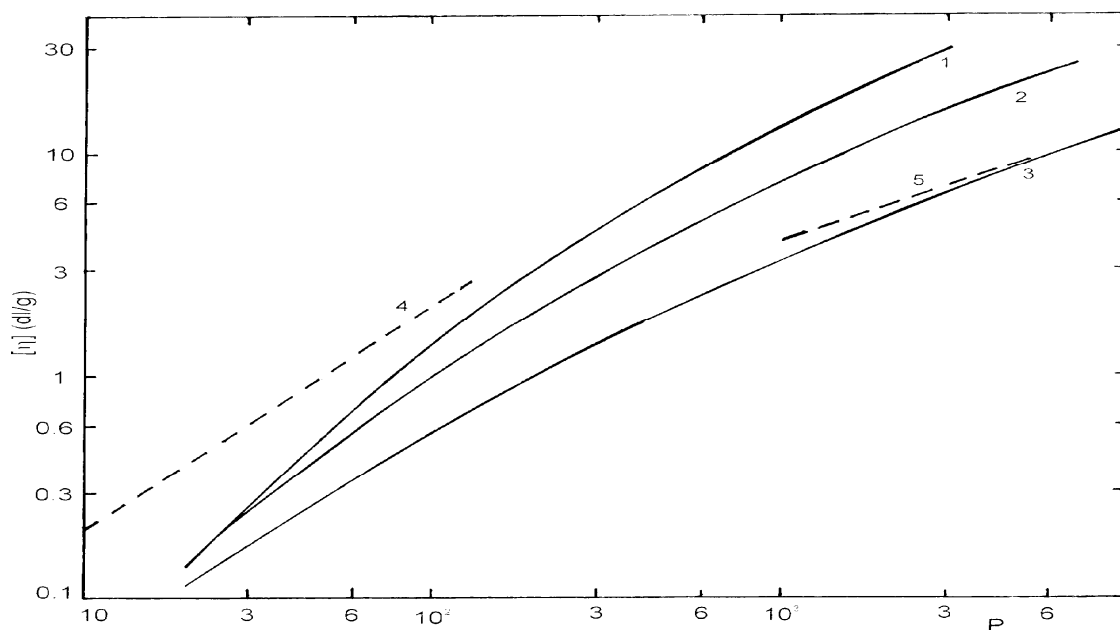


Fig.-10: Prediction of plots of $[\eta]_0$ vs P for chitosans at different salt concentrations. Curves 1 to 3 were calculated using Eqns (8) to (18) and $d_r = 0.017, 0.034, 0.06$ at $C_s = 0.01 \text{ M}, 0.025 \text{ M}$ and ∞ , respectively. Curves 4 and 5 are schematically drawn with $d \ln[\eta]_0 / d \ln P = 1$ and $1/2$, respectively.

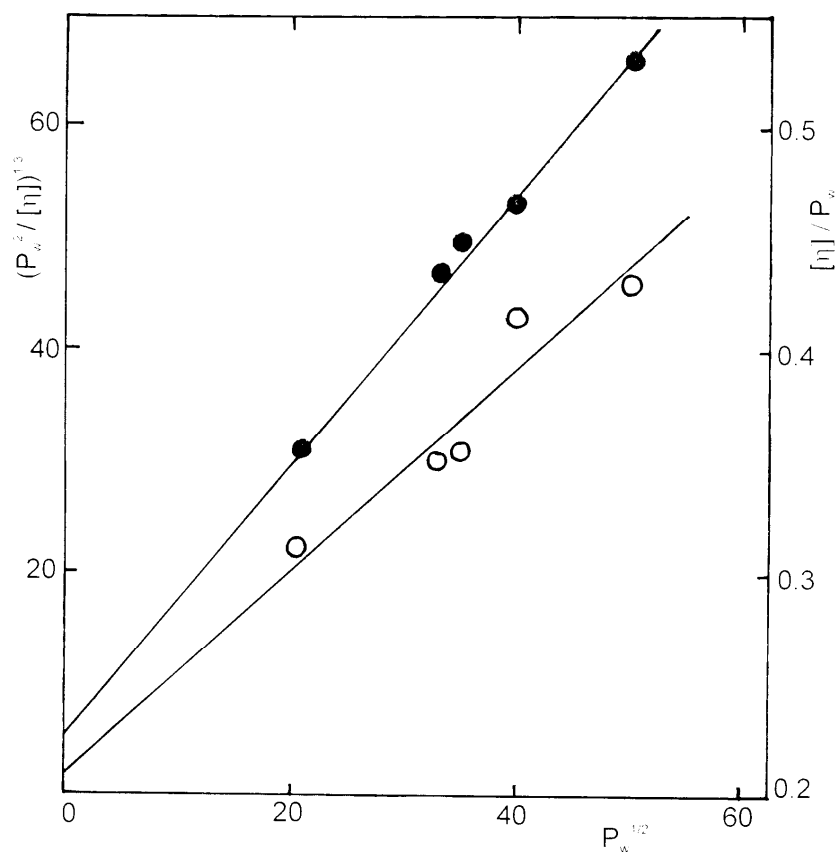


Fig.-11: Plots according to Eqns (5) and (12) of experimental data for chitin⁵⁸.
Solvent: 0.5M LiCl / N,N-dimethylamide. ● estimated by “BSF” method^{23,24}, ○ estimated by “BB” method^{28,29}.

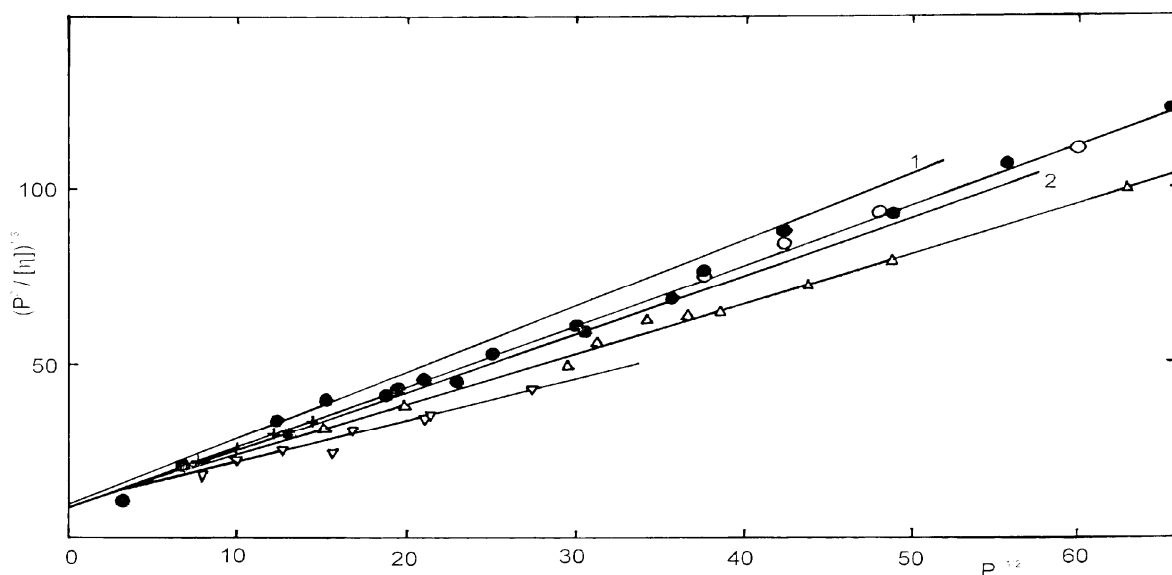


Fig.-12: Plot according to Eqn (12) of experimental data for cellulose.
Solvents: + ○ ● cadoxen⁵¹⁻⁵³, △ Fe-Na-tartrate⁵⁴, ▽ LiCl / dimethylacetamide⁵⁵. Calculated lines 1 and 2 for Cu-oxam and Cu-en (details in text)⁴⁸.

ACKNOWLEDGEMENTS

The authors devotes this paper to remembrance of †Dr. Miloslav Bohdanecky from Academy of Sciences of the Czech Republic.

REFERENCES

1. C.J. Brine, P.A. Sanford and J.P. Zikakis, *Advances in chitin and chitosan*, Elsevier Applied Science, London, p. 206-215 (1992).
2. K.G. Gupta and M.N.V. Ravi Kumar, *J. Macromol. Sci.-Rev. Macromol. Chem. Phys. C*, **40**, 293 (2000).
3. G.A.F. Roberts, *Chitin Chemistry*, McMillan Press Ltd., London, p. 102-115, 274-331 (1992).
4. D.K. Singh and A.J. Ray, *J. Macromol. Sci.-Rev. Macromol. Chem. Phys. C*, **40**, 69 (2000).
5. Y. Shigemisa and S. Minami, *Biotechnol. Genetic. Eng. Rev.*, **13**, 383 (1995).
6. G. Skjak-Braek, T. Anthonsen and P. Stanford, *Chitin and Chitosan: Sources, Chemistry, Biochemistry, Physical Properties and Applications*, Elsevier Applied Science, London, p. 749 (1989).
7. M.H. Struszczyk, *Polimery* (in Polish), **47**, 316 (2002).
8. M.R. Kasaai, J. Arul and J. Charlet, *J. Polym. Sci. Pt. B-Polym. Phys.*, **38**, 2591 (2000).
9. O.G. Maghami and G.A.F. Roberts, *Makromol. Chem.*, **189**, 195 (1988).
10. L.A. Berkovich, G.I. Timofeyeva, M.P. Tiurupa and W.A. Davankov, *Polym. Sci. USSR*, **22**, 1834 (1980).
11. G. Berth, H. Dautzenberg and M.C. Peter, *Carbohydr. Polym.*, **36**, 205 (1998).
12. G. Berth and H. Dautzenberg, *Carbohydr. Polym.*, **47**, 39 (2002).
13. M.W. Anthonsen, K.M. Varum and O. Smidsrød, *Carbohydr. Polym.*, **22**, 193 (1993).
14. H.M. Ottoy, K.M. Varum, B.E. Christensen, H.M. Anthonsen and O. Smidsrød, *Carbohydr. Polym.*, **31**, 253 (1996).
15. M. Rinaudo, M. Milas and P. Le Dung, *Int. J. Biol. Macromol.*, **15**, 281 (1993).
16. M. Terbojevich, A. Cosani, M. Scandola and A. Fornasa, *Solution Properties and Mesophase Formation of Chitosan*. In: R. Muzzarelli, C. Jeuniaux and G. W. Gooday, *Chitin in Nature and Technology*. Proceedings of the 3rd International Conference on Chitin and Chitosan, Plenum Press, New York, p. 348 (1986).
17. N.V. Pogodina, G.M. Pavlov, S.V. Bushin, A.B. Melnikov, Ye.B. Lysenko, L.A. Nudga, V.N. Marsheva, G.N., Marchenko V.N. Tsvetkov, *Polym. Vysokomolekulyarnyye Soedineniya* (in Russian), **A 28**, 231 (1986).
18. M. Bohdanecky and J. Kovar, *Viscosity of Polymer Solutions*, Elsevier, Amsterdam, p. 104-108 (1982).
19. H. Fujita, *Polymer Solutions*, Elsevier, Amsterdam, vol. 9 (1990).
20. H. Yamakawa, *Modern Theory of Polymer Solutions*, Harper and Row, New York, p. 52-57, 269-275 (1971).
21. P.J. Flory, *Statistical Mechanics of Chain Molecules*, Interscience, New York, p. 115-156 (1969).
22. A. Domard and M. Rinaudo, *Int. J. Biol. Macromol.*, **5**, 49 (1983).
23. W. Burchard, *Makromol. Chem.*, **50**, 20 (1961).
24. W.H. Stockmayer and M. Fixman, *J. Polym. Sci. Pt. C*, **1**, 137 (1963).
25. A.J. Barrett, *Macromolecules*, **17**, 1561 (1963).
26. H. Yamakawa and M. Fujii, *Macromolecules*, **7**, 128 (1974).
27. H. Yamakawa and T. Yoshizaki, *Macromolecules*, **13**, 633 (1980).
28. S.V. Bushin, V.N. Tsvetkov, Ye.B. Lysenko and V.N. Yemelyanov, *Vysokomolekulyarnyye Soedineniya* (in Russian), **A 23**, 2494 (1981).
29. M. Bohdanecky, *Macromolecules*, **16**, 1483 (1983).
30. T. Norisuye, A. Tsuboi and A. Teramoto, *Polym. J.*, **28**, 357 (1996).
31. H. Yamakawa and W.H. Stockmayer, *J. Chem. Phys.*, **57**, 2483 (1972).

32. T. Norisuye and H. Fujita, *Polym. J.*, **14**, 143 (1982).
33. H. Yamakawa and J. Shimada, *J. Chem. Phys.*, **83**, 2607 (1985).
34. O. Smidsrød and A. Haug, *Biopolymers*, **1**, 1213 (1971).
35. C. Kienzle-Sterzer, D. Rodriguez-Sanchez and C. Rha, Solution Properties of Chitosan: Chain Conformation. In: J.P. Zikakis, Chitin, Chitosan and Related Enzymes, Academic Press, New York, p. 383-393 (1984).
36. S. Trzcinski, K.M. Varum, D.U. Staszewska, O. Smidsrød and M. Bohdanecky, *Carbohydr. Polym.*, **48**, 171 (2002).
37. M. Terbojevich, C. Carraro, A. Cosani and E. Marsano, *Carbohydr. Res.*, **180**, 73 (1988).
38. R. Minke and J. Blackwell, *J. Mol. Biol.*, **120**, 167 (1978).
39. M. Tricot, *Macromolecules*, **17**, 1698 (1984).
40. M. Le Bret, *J. Chem. Phys.*, **76**, 6243 (1982).
41. M. Fixman, *J. Chem. Phys.*, **76**, 6346 (1982).
42. A. Dondos and H. Benoit, *Polymer*, **18**, 1161 (1977).
43. G. Staikos and A. Dondos, *Europ. Polym. J.*, **19**, 555 (1983).
44. J. Horsky and M. Bohdanecky, *Macromol. Theory Simul.*, **7**, 317 (1998).
45. C. Schatz, C. Viton, T. Delair, C. Pichot and A. Domard, *Biomacromolecules*, **4**, 641 (2003).
46. M. Terbojevich, A. Cosani, G. Conio, E. Marsano and E. Bianchi, *Carbohydr. Res.*, **209**, 251 (1991).
47. J. Brugnerotto, J. Desbrieres, G. Roberts and M. Rinaudo, *Polymer*, **42**, 9921 (2001).
48. M. Marx, *Makromol. Chem.*, **16**, 157 (2001).
49. S. Ya. Ljubina, C.I. Klenin, I.A. Strelina, A.V. Toyskaya, A.V. Chripusev and E.Ya. Urinov, *Polym. Sci. USSR Ser. A* (in Russian), **19**, 244 (1977).
50. D. Henley, *Ark. Kem.*, **18**, 327 (1961).
51. W. Brown and R. Wickstrom, *Europ. Polym. J.*, **1**, 1 (1965).
52. L. Valtasaari, *Makromol. Chem.*, **150**, 117 (1971).
53. E. Bianchi, A. Ciferri, G. Conio, G. Cosani and M. Terbojevich, *Macromolecules*, **18**, 646 (1985).
54. K. Saalwachter, W. Burchard, P. Kluffers, G. Kettenbach, P. Mayer, D. Klemm and S. Dugarman, *Macromolecules*, **33**, 4994 (2000).
55. D.A. Brant and K.D. Goebel, *Macromolecules*, **5**, 536 (1972).
56. D.A. Brant and K.D. Goebel, *Macromolecules*, **8**, 522 (1975).
57. H. Dautzenberg, W. Jaeger, J. Kotz, Ch. Seidel and D. Stscherbina, Polyelectrolytes: Formation, Characterization and Application, C. Hanser Publ., Munich, p. 196 (1994).
58. D. Rodriguez-Sanchez, C.A. Kienzle-Sterzer and C. Rha, Proceedings 2nd Int. Conf. Chitin/Chitosan, Tottori, The Japanese Society of Chitin and Chitosan, p. 30 (1982).
59. S.Ya. Lyubina, I.N. Strelina, L.A. Nudga, Ye.A. Plisko and I.N. Bogatova, *Polym. Sci. USSR Ser. A* (in Russian), **25**, 1467 (1983).
60. M.L. Tsai and R.H. Chen, *Int. J. Biol. Macromol.*, **20**, 233 (1997).

[RJC-762/2011]