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Estimation of the Relative Stiffness of the Molecular Chain in Polyelectrolytes From Measurements of Viscosity at Different Ionic Strengths

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Synopsis

A method was developed that allowed comparison of the stiffness of the chain in different polyelectrolytes from measurements of the intrinsic viscosity at different concentrations of added monovalent (sodium) salt. The response to salt was quantitatively expressed as the slope of straight lines relating the intrinsic viscosity to the reciprocal of the square-root of the ionic strength. This slope increased considerably with increasing molecular weight of the polyelectrolyte, and could serve to characterize the response to salt of different substances only when comparison was made at a constant molecular weight. An empirical parameter, *B*, which is the slope corresponding to an intrinsic viscosity of 1.0 at an ionic strength of 0.1 M could be correlated to the unperturbed dimensions of the molecules. A method of extrapolation, enabling the determination of *B* from measurements of viscosity on only one sample of unknown molecular weight, was evaluated. The empirically found correlation between *B* and some well established parameters of stiffness did not contrast predictions from the "fuzzy-sphere model" of Fixman, provided that reasonable assumptions regarding ion-binding and the interaction between polymer and solvent were made.

INTRODUCTION

During a study of the solubility of anionic polysaccharides in the presence of inorganic salts, it was observed that the decrease in viscosity that occurred when salt was added to solutions of different polysaccharides varied considerably with the type of polysaccharide present. For example, the ratio between the viscosity without added salt and the viscosity at a high salt concentration was as much as ten times higher for dextran sulphate than for alginate.[1] Such effects are experimentally very easily detectable, and it was thought that a study of the dependence of viscosity upon ionic strength might provide useful information about the solution properties of anionic polysaccharides. Although some information concerning the dependence of of the intrinsic viscosity of different polyelectrolytes upon ionic strength has already been collected and compared,[2] the possibility of using such data for characterizing the physical properties of the polymeric chains in solution seems not to have been fully explored. The intention in this paper is first to study the effect of adding salt to alginate and to find a way of presenting the results that allows an easy comparison with data for other polyelectrolytes. A series of polyelectrolytes are then compared in this way, using data taken partly from the literature and partly from our own experiments. The most important factors that are responsible for the observed differences are then evaluated.

Theory

A general criticism of much work on the solution properties of polysaccharides is that comparison has been made of the unperturbed-chain dimensions of different substances when the theoretical methods by which they were calculated have been of limited validity.[3-5] The conclusions that are drawn then rest, not only upon the experimental results, but also upon the validity of the theories that have been used. In this paper we shall try to avoid any direct reliance upon theories of viscosity in comparing viscosity data for different substances, but we must nevertheless seek some theoretical guidance, and we select somewhat arbitrarily, the Fixman theory[6] for this purpose. Fixman's expression for the intrinsic viscosity of a polyelectrolyte reads:

$$[\eta] = K_\theta M^{1/2}[1 + Cn^{1/2} + D_0 \cdot q(2D\epsilon z_c b_\theta)^{-1} n^{1/2} \varkappa^{-1}] \tag{1}$$

where K_θ is the constant in Flory's[7] "equivalent sphere" viscosity theory:

$$[\eta] = K_\theta M^{1/2} \alpha_\eta^3 \tag{2}$$

The sum of the terms inside the square brackets is recognized as equivalent to α_η^3 where α_η is the viscosity-expansion factor.[7] For further explanation of the physical meaning of the terms inside the square bracket, comparison with the Fixman[8] expression for the expansion of non-ionic polymers may be made. In Fixman's equation:

$$\alpha_\eta^3 = 1 + C_1 \cdot n^{1/2}, \tag{3}$$

C_1 is a constant depending on the degree of interaction between the chain segments and the solvent molecules (and is zero in a θ-solvent), and n is the degree of polymerization. The last term in eq. (1) takes into account the polyelectrolytic character of the molecules. In that term, $\varkappa$ is the Debye-Hückel parameter;[9] the product, $\epsilon \cdot z_c$, is the charge of the counterions; D is the dielectric constant of the solution; D_0 is "an effective dielectric constant" operating near the polyionic chain; q is the charge per monomer residue; and b_θ is the effective bond length, which in θ-solutions is related to the root-mean-square end-to-end distance $(\bar{r}^2)^{1/2}$, and the Kuhn statistical segment length, A_m, by:

$$(\bar{r}_\theta^2)^{1/2} = b_\theta \cdot n^{1/2} = (A_m \cdot b_0 \cdot n)^{1/2} \tag{3}$$

where b_0 is the length of the monomer unit.

Equation (1) predicts a linear relationship between the intrinsic viscosity and the reciprocal of the square-root of the ionic strength, which is in agreement with much experimental evidence.[2,10-14] If this theory is correct, and providing that q, D_0 and n could be determined independently, the stiffness parameter, b_θ, could be calculated from measurements of $[\eta]$ at different ionic strengths, I, with the aid of eq. (1). Unfortunately, the parameters q and D_0 cannot both be measured directly, and therefore the slope of $[\eta]$ against $1/\sqrt{I}^{1/2}$ cannot be used directly as a quantitative measure of stiffness. In the physical situation, D_0 is probably smaller than D, and q is also smaller than that corresponding to the presence of a full, unit charge on every ionizable fixed group. This type of behavior has led to the concept of ion-pair formation between the polyion and the counterions,[10,15] and in Fixman's theory, it could be taken into account by replacing q with $q(1 - f)$, where f is the fraction of the fixed charges that are bound in ion-pairs. Fixman rejected this possibility because ion-binding and viscosity could not be discussed with the same theoretical model. He therefore kept q equal to the stoichiometric charge and let the "effective dielectric constant" be lower than that of pure water. The ratio D/D_0 was then regarded as an adjustable parameter.

When testing the theory on measurements with sodium polyacrylate, Fixman found that D/D_0 was much larger than unity, and, more important, that the ratio decreased considerably with decreasing pH. This may be explained if the fraction of the fixed charges bound in ion-pairs decreases when the polyion is being discharged. Such a behavior would be in agreement with a considerable amount of evidence obtained both indirectly[10,15,16] and from direct determinations of cation-activity coefficients in solutions of polyanions.[17]

One may study the effect of changing the stoichiometric charge-density of a polymer, not only by changing the pH in solutions of weak polyacids, but also by using polymers with different spacings between the fixed charges. Liquori et al.[18] determined, for example, the osmotic coefficients of a series of polyanions and found a close correlation between the distance between the charged groups and the amount of ion-pair formation; the larger the distance, the smaller was the fraction of ion-pairs. The chemical nature of the polymer chain seemed to be of only limited importance. It has also been shown that changes in the ionic strength of the solution cause only minor changes in the amount of ion-pair formation.[19–22] It seems, therefore, that the "effective charge" of a polymer, i.e., the charge that causes the polyelectrolyte to expand, is only slightly dependent upon the stoichiometric charge of the polymer over a rather wide range of stoichiometric charge densities. If this is true, the dependence of the intrinsic viscosity upon the ionic strength is, according to eq. (1), dominated by the values of the parameter b_θ and the degree of polymerization, and might, therefore, be used for evaluating the stiffness of the polyelectrolyte molecule at any given degree of polymerization.

Having in mind that Fixman's theory is only an approximation to the real situation, it is clear that such measurements of viscosity can yield quantitative information on stiffness only if the effect can be empirically correlated to well-established parameters of stiffness.

MATERIALS AND METHODS

The samples of alginate were prepared, as previously described,[14] from *Laminaria digitata* harvested at Tarva, August 29, 1961. The ratio[23] between mannuronic- and guluronic-acid residues (M/G) in this alginate was 1.6. A guluronic acid-rich fragment (M/G = 0.1) of $DP_n = 80$ was prepared as previously described.[24]

Five different samples of pectin were used. Samples I, III, IV, and VI were kindly supplied by Dr. R. Kohn, Slovak Academy of Sciences, Bratislava, and have been characterized by him.[25] Sample II was a commercial preparation from Fluka A/G, Switzerland.

The dextran sulfate sample had a degree of substitution of 1.6 and was a commercial preparation from Pharmacia, Uppsala, Sweden.

Intrinsic viscosities were determined by isoionic dilution at 20°C in a Zimm-Crothers Model A, low-shear, rotating cylinder viscometer, as described previously.[14] The salt used for obtaining different ionic strengths was in every case sodium chloride.

RESULTS AND DISCUSSION

Dependence of the Intrinsic Viscosity Upon the Ionic Strength

The intrinsic viscosity of ten samples of alginate, all with known molecular weights[14,26] was determined at several ionic strengths, I. The results, plotted as $[\eta]$ (100 ml/g) against $1/\sqrt{I}$, are shown in Figure 1. It is seen that all the curves are straight lines. The slopes of the curves, $S = \Delta[\eta]/\Delta\left(\frac{1}{\sqrt{I}}\right)$, are given in Table I, together with the corresponding molecular weights, M_w, and the intrinsic viscosity at $I = 0.1$ M. In Figure 1 and

TABLE I

Dependence Upon Ionic Strength (I) of the Intrinsic Viscosity, $[\eta]$, of Alginates of Different Molecular Weights (M_w)

Sample No.	$M_w \times 10^{-5}$	$[\eta]_{0.1}$	$S = \Delta[\eta]/\Delta\left(\frac{1}{\sqrt{I}}\right)$
1	27.0	55	9.3
2	16.5	33	4.8
3	15.5	31	4.18
4	9.7	19.5	2.56
5	6.5	13.0	1.44
6	4.7	9.4	0.88
7	2.95	5.9	0.42
8	2.48	4.95	0.32
9	1.44	2.88	0.165
10	1.12	2.25	0.120
11	0.15*	0.30	0.008 ± 0.0025

* Guluronic acid-rich fragment. The error in S is, as indicated, rather high because of the low intrinsic viscosities of this sample.

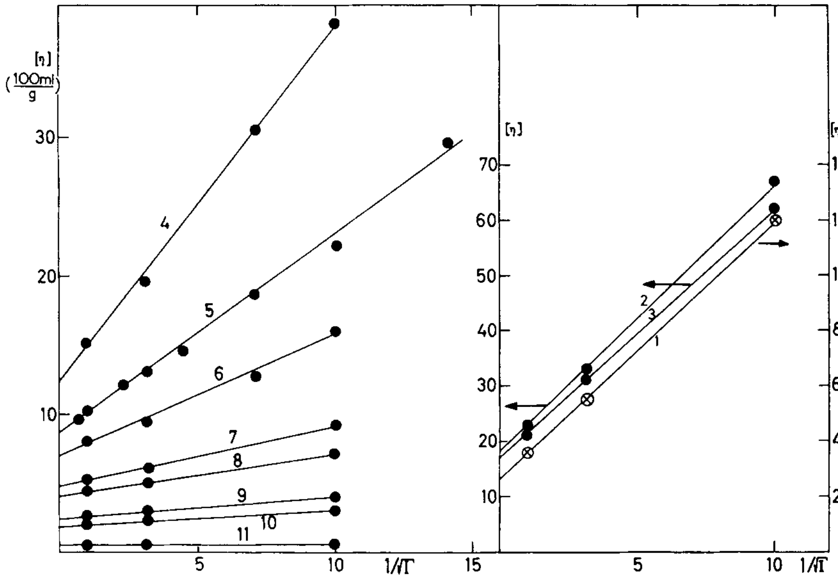

Fig. 1. The intrinsic viscosity of alginate, plotted against the reciprocal of the square root of the ionic strength. The numbers represent the samples given in Table I.

Table I are also included results for a fragment of alginate containing 90% guluronic-acid residues.

According to Fixman's theory (eq. [1]), S should be proportional to the molecular weight of the polyelectrolyte. A double logarithmic plot of S against M_w should therefore yield a straight line with a slope of unity. Such a plot is shown in Figure 2. The linearity is seen to be very good, but the slope is 1.37. This deviation from Fixman's theory may be due to partial free drainage[14,26] of the alginate molecules in aqueous salt solutions.

Evaluation of a Measure of the Salt Effect that is Independent of Molecular Weight

Because of the observed strong molecular-weight dependence of S, it can serve as a parameter of stiffness only if comparison is made at a certain molecular weight. It is not easy to prepare different polysaccharides of exactly the same molecular weight, and a means of comparison that eliminates this difficulty was therefore sought. Instead of comparing values of S corresponding to a particular molecular weight, one may compare such values corresponding to a certain intrinsic viscosity obtained at one particular ionic strength. If the values of S obtained in this way could be empirically correlated with any of the known parameters of stiffness, one would have the great advantage that the molecular weight of the polyelectrolyte need not be determined before comparison of chain stiffness could be made. Since $[\eta]$ is linear in the reciprocal of the square-root of the ionic strength, one may choose $[\eta]$-values obtained at any ionic strength for this compari-

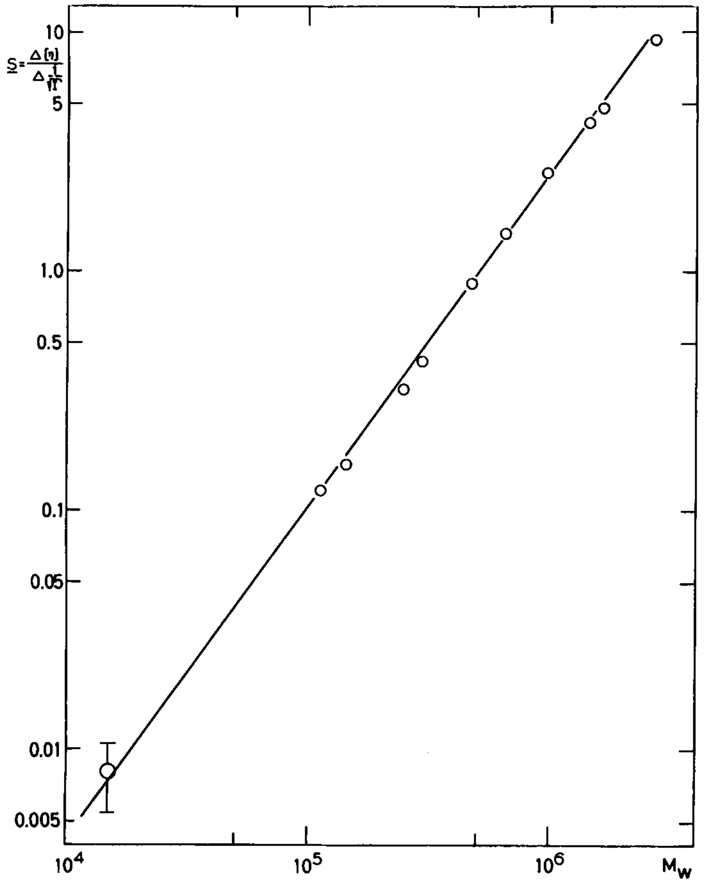

Fig. 2. The ionic-strength dependence of $[\eta]$ plotted against the molecular weight of the alginate.

son. Because the ionic strength, 0.1, is included in most of the relevant published work, we have chosen the intrinsic viscosities measured at this ionic strength as a basis for comparison.

In Figure 3, a double-logarithmic plot of S versus $[\eta]_{I=0.1}$ for alginate (Curve 5) is shown. The points again fall on a straight line having the same slope (1.37) as in Figure 2. This is to be expected, since the exponent a in the Mark-Houwink equation

$$[\eta] = K \cdot M^a \tag{5}$$

is 1.0 at ionic strength 0.1 (Ref. 14).

In the same figure, data taken from the literature are given for the sodium salts of polyphosphate,[27,28] polyacrylate,[11] carboxymethyl amylose,[29] carboxymethyl cellulose[31] and for native, double-stranded DNA.[33] The values of S were found by plotting the experimental results as $[\eta]$ against $1/\sqrt{I}$ and measuring the slopes of the resultant straight lines.

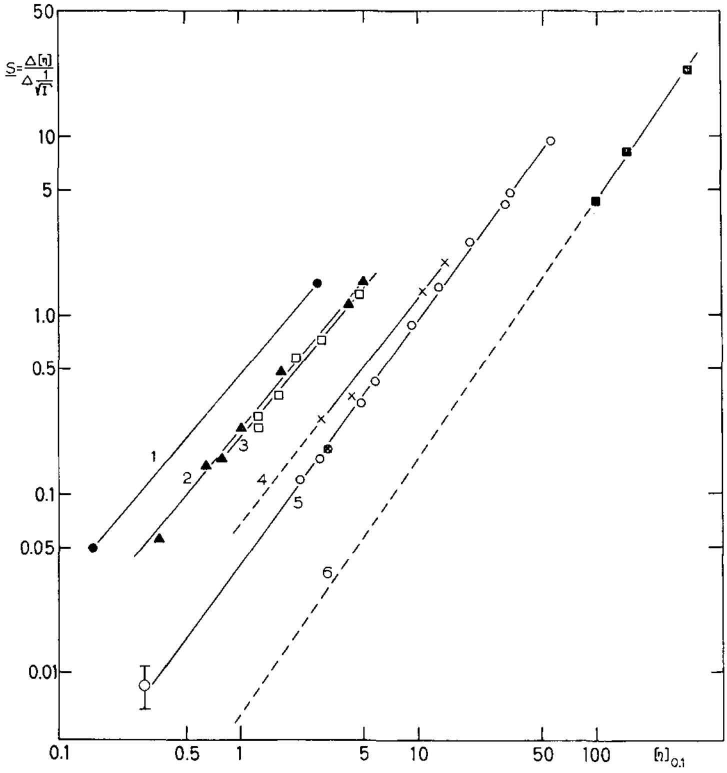

Fig. 3. The ionic strength dependence of $|\eta|$ plotted against the intrinsic viscosity in 0.1 M sodium chloride solution.

●	Curve 1.	Polyphosphate.
▲	Curve 2.	Polyacrylate.
□	Curve 3.	Carboxymethyl amylose, degree of substitution 0.8.
×	Curve 4.	Carboxymethyl cellulose, degree of substitution 1.06.
○	Curve 5.	Alginate.
■	Curve 6.	Double-stranded DNA.
⊗		Acetylated alginate.

In Figure 3, no tendency for deviation from linearity was observed for any of the substances. This should justify the straight line drawn between the two available points for polyphosphate. The lines are nearly parallel, and the results for each polyelectrolyte can be expressed by the relation

$$S = B\cdot([\eta]_{0.1})^{v} \qquad (6)$$

where v was found to have its lowest value of 1.2 for polyphosphate and its highest of 1.4 for DNA. The difference between these exponents is of doubtful significance and they are in any case sufficiently similar to justify the use of B as a characteristic measure for the response of $[\eta]$ to the addition of salt.

It is important to note that the value of B may be obtained from only one set of S versus $[\eta]_{0.1}$ data. Using eq. (6) and an average value of 1.3 for the exponent v, B may be calculated directly. Better accuracy may be obtained, however, by plotting the observed point in Figure 3 and extrapolating graphically to $[\eta]_{0.1} = 1.0$, using the same slope as the closest experimental curve.

Empirical Correlation Between *B* and Known Parameters of Stiffness

The values of B determined from Figure 3 are given in Table II, together with several parameters of stiffness taken from the literature. According to Flory's theory the following relation between these parameters are valid under θ-conditions:

$$K_\theta = \Phi\left(\frac{\overline{r_\theta^2}}{M}\right)^{3/2} = \Phi\left(A_m\cdot\frac{b_0}{M_0}\right)^{3/2} = \Phi\cdot b_\theta^3\cdot M_0^{-3/2} = \Phi\left(\frac{\sigma^2\cdot\overline{r_f^2}}{M}\right)^{3/2} \qquad (7)$$

In this equation, Φ is the Flory viscosity-constant, M_0 is the weight of the monomeric unit, and σ is the steric factor, equal to $\left(\frac{\overline{r_\theta^2}}{\overline{r_f^2}}\right)^{1/2}$ where $\left(\overline{r_f^2}\right)^{1/2}$ is the root-mean-square end-to-end distance for the case of free rotation around all single bonds.

Table II shows that the value of B is in some way inversely related to K_θ, b_θ, A_m and σ. The parameters of stiffness are, in the case of polyphosphate, polyacrylate and carboxymethyl amylose, obtained from viscosity measurements in θ-solvents. The data for carboxymethyl cellulose (CMC) and alginate are obtained by using the Burchard-Stockmayer-Fixman method of extrapolation. The given values for CMC are taken from Brown and Henley[31] (see footnote to Table II), discarding one measurement on a low molecular-weight sample, and performing the extrapolation in harmony with results on alginate. To our knowledge, no unperturbed dimensions are reported for DNA (see footnote to Table II). However, the reported value of A_m in 0.2 M salt should be sufficiently high compared to that for alginate, to allow the conclusions that the mechanical inflexibility in the DNA-chain is higher than in alginate. The difference in B-value between these two substances is marked.

Figure 4 gives a double-logarithmic plot of B against K_θ and b_θ, respectively. The plot against K_θ is linear, whereas in that against b_θ, the points for polyphosphate and polyacrylate lie below the straight line drawn through the points for the three polysaccharides. The two straight lines follow the equations:

$$B = \text{Const.}\cdot\left(\frac{1}{K_\theta}\right)^{0.78} \qquad (8)$$

$$B = \text{Const.}\cdot\left(\frac{1}{b_\theta}\right)^{2} \qquad (9)$$

TABLE II
The Characteristic Parameter, B, for the Dependence Upon Ionic Strength of $[\eta]$, Compared With Accepted Parameters of Stiffness for Different Polyelectrolytes

	B	K_θ	b_θ (Å)	A_m (Å)	σ	Ref.
Polyphosphate	0.44	4.94×10^{-4}	6.2		1.68	2, 8
Polyacrylate	0.23	1.24×10^{-3}	6.7	(10)	2.38 (1.96)	11 (7)
Carboxymethylamylose	0.20	1.35×10^{-3}	11.5	25.6	2.7 (3.0)	29 (30)
Carboxymethylcellulose*	0.065	6.0×10^{-3}	20.5	82	2.65 (3.8)	31 (32)
Alginate	0.04	1.1×10^{-2}	26	130	3.1	14
DNA	0.0055			540**		34

* Brown and Henley[31] reported $K_\theta = 4.2 \times 10^{-3}$, $b_\theta = 19.3$, $A_m = 72$ and $\sigma = 2.5$.

** This value is determined by the Bloomfield-Zimm[35] viscosity theory from measurements in 0.2 M NaCl. Ross and Scruggs[33] have shown that the dimensions in 0.2 M NaCl are not far from those at infinite ionic strength. A_m values have been reported for DNA being as high as 900 Å (Ref. 36).

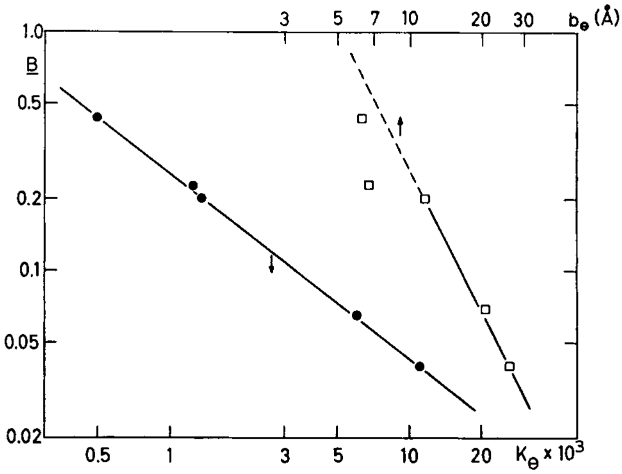

Fig. 4. Values of B plotted against accepted parameters of stiffness.
●: Against K_θ □: Against b_θ

Using the relationship between b_θ and K_θ given in eq. (7), eq. (8) can be written

$$B = \text{Const.} \cdot M_0^{1.17} \left(\frac{1}{b_\theta}\right)^{2.3} \tag{10}$$

In view of the limited number of experimental results, the difference between the exponents in eq. (9) and eq. (10), correlating B and $1/b_\theta$, should not be regarded as significant. The polyphosphate and the polyacrylate probably fall outside the K_θ-b_θ relationship because they have lower monomeric weights than the polysaccharides (see eq. [10]).

Plots of B against A_m or σ showed much more scatter, and it was therefore concluded that the best applicable correlation between B and any parameter for chain extension is that given by eqs. (8) or (10).

Examination of the Empirically Found Correlation Between B and b_θ in Terms of Fixman's Theory

Fixman's theoretical equation (eq. [1]) may be simplified to give the following expression for S:

$$S = \text{Const.} \cdot \frac{M}{b_\theta \cdot M_0} \tag{11}$$

where the constant includes the parameters describing the effect upon S of the charge-density of the polyelectrolyte. If comparison of S had been made for molecules of identical stoichiometric charge density, the theory, therefore, predicts proportionality with the reciprocal of the effective bond length. By comparison with the expression for B:

$$B = \frac{S}{([\eta]_{0.1})^{\nu}}$$

it is seen that B is also dependent upon how $[\eta_{0.1}]$ varies with b_θ. The intrinsic viscosity at ionic strength 0.1 is, according to eq. (1), dependent upon K_θ, the solute-solvent interaction parameter C, and the electrostatic term. Knowledge of the magnitude of all three terms is therefore needed for calculating the exact theoretical relationship between B and b_θ. Such knowledge is lacking, but it is clear that $[\eta]_{0.1}$ must increase with K_θ, and therefore with b_θ (eq. [7]), and a stronger dependence of b_θ upon B than that corresponding to inverse proportionality is to be expected. The strong dependence observed (eq. [10]) does not, therefore, contradict predictions from Fixman's theory.

Since $[\eta]_{0.1}$ (and therefore, B) should be dependent upon the solute-solvent interaction parameter C, it is rather surprising that the plot of B against K_θ for the chemically very different molecules in Figure 4 gives so little scatter. This suggests that the $C \cdot n^{1/2}$-term in eq. (1) is small compared to the two other terms, so that a variation in the solute-solvent interaction among the different substances does not show up as a variation in $[\eta]_{0.1}$.

To test this point, one of the alginate samples was acetylated to diminish the interaction between the water and the alginate. Viscosity measurements yielded $[\eta]_{0.1} = 3.2$, $S = 0.18$ and $B = 0.38$. The data are plotted in Figure 3, and it is seen that the result is not significantly different from that given by unsubstituted alginate. Since alginate is a very stiff molecule, it should be able to accommodate substituents without much change in stiffness, as does cellulose,[29] and the identical B-values of alginate and acetylated alginate therefore support the view that B is not very sensitive to changes in the degree of solvation of the chains.

Sensitivity of *B* Towards Changes in Stoichiometric Charge Density of the Polymers

The correlation obtained between B and K_θ suggests that B is not very sensitive to differences in the stoichiometric charge densities of the polymers. More evidence was obtained by studying the effect of the addition of salt to pectin samples of different degrees of esterification (Table III). The results for Sample V in Table III are taken from the work of Pals and Hermans;[13] the other results are obtained by our own experimentation. Table III shows that only for the samples with degrees of esterification of 78% and 89% does a change in the stoichiometric charge density result in a change in B.

The low values of B for the pectin samples indicate that these chains are

TABLE III

Dependence of $[\eta]$ Upon Ionic Strength (I) for Sodium Pectinates of Varying Degrees of Esterification

Sample No.	Degree of esterification	$[\eta]_{0.1}$	S	B
I	0	0.60	0.028	0.044
II	0	0.78	0.030	0.034
III	27.3	0.90	0.040	0.044
IV	58.3	2.20	0.15	0.052
V	78	3.20	0.12	0.026
VI	89	3.0	0.02	0.005

TABLE IV

Dependence of $[\eta]$ Upon Ionic Strength (I) for Sodium Polyacrylate of Varying Degrees of Dissociation

Degree of dissociation %	$[\eta]_{0.1}$	S	B
100	8.8	3.15	0.26
33	6.32	2.47	0.27
10	1.8	1.04	0.47

very inflexible. It was therefore decided to test the effect of changing the charge density in the more flexible polyacrylate molecule.

Flory and Osterheld[37] measured the intrinsic viscosity of a sample of polyacrylic acid at various degrees of dissociation of the carboxyl group, in each case at a series of different sodium chloride concentrations. From their results, the data in Table IV were obtained. The value of B at the lowest degree of dissociation is not as accurate as the others, because experimental results in the range of ionic strength around 0.1 were lacking. No tendency for a decrease in B with a decrease in the degree of dissociation is seen in the Table.

The results for pectin and polyacrylate support the idea that some variation in the stoichiometric charge density of the polyelectrolytes does not lead to a variation in B. One should therefore be able to use B as a parameter of stiffness for polyelectrolytes with fixed charged groups corresponding to a broad range of stoichiometric charge densities.

Use of *B* as a Parameter of Stiffness

More viscosity data are available than reliable estimates of unperturbed dimensions. Some of these data will be given below with a short discussion for each substance.

The values of B for pectin already given in Table III are lower than those for carboxymethyl cellulose (CMC) (Table II). This is in agreement with Pals and Hermans[38] who found from viscosity data that the end-to-end distance for pectin at I = ∞ was about 10% higher than those for CMC of the same molecular weight.

Data for the dependence of $[\eta]$ upon I for a series of different substances are collected in Table V. Not always did the data give an exact linearity between $[\eta]$ and $1/\sqrt{I}$ over the whole range of ionic strengths. The linearity around I = 0.1 was always good, however, and the best straight line in this range of ionic strength was used to determine the S-values.

Cleland[39] determined the radius of gyration of sodium hyaluronate by light scattering, and his value ($[R_G]_Z$ = 613 Å, M_w = 300.000, I = 0.1) is somewhat lower than the corresponding figure for alginate ($(R_G)_Z$ = 700 Å).[26] This agrees well with the relative values of B for these two substances (B = 0.04 for alginate [Table II]).

TABLE V

Dependence of $[\eta]$ Upon Ionic Strength for Different Polyelectrolytes

Substance	$[\eta]_{0.1}$*	S	B	Reference
Hyaluronate	8.1	0.90	0.065	39
Dextran sulfate	1.27	0.30	0.23	[a]
Amylose xanthate	0.80	0.18	0.22	42
Poly-vinyl alcohol sulfate DS = 0.65	2.22	0.60	0.24	43
CMC, Pals et Hermans DS = 0.4	3.83	0.275	0.044	38
" " DS = 0.56	4.80	0.38	0.045	38
" " DS = 0.73	8.7	0.85	0.045	38
" Schneider & Doty DS = 1.0	9.0	1.04	0.065	32

* Sodium chloride was in all cases used for obtaining different ionic strengths.

[a] Own experimentation.

No determination of stiffness on dextran sulfate is available. Senti et al.[40] found K_θ to be 9.8×10^{-4} for an unsubstituted dextran sample. Granath[41] found the effective bond-length of dextran to increase with decreasing degree of branching. Values of b_θ from 6.25 to 10 Å were reported. Assuming that the introduction of sulfate groups into the dextran molecule does not drastically change its flexibility, the high value of B for dextran sulfate in Table V agrees with the indications from Senti's and Granath's works that the dextran molecule is very flexible.

The value of B for amylose xanthate in Table V is very similar to that for carboxymethyl amylose in Table II, indicating a similar degree of flexibility in these two substances. Pramanik and Chondhury[42] reported an effective bond-length of 6.24 Å for amylose xanthate, i.e., considerably lower than that given previously for carboxymethyl amylose (b_θ = 11.5 Å). Such a difference is not indicated by their B-values.

The identical B-values of polyvinyl alcohol sulfate and polyacrylic acid (Tables II and V) are in agreement with much work on vinyl polymers,[7] which indicates a similar degree of flexibility within this group of substances.

The results for carboxymethyl cellulose in Table V are given to show that the values of B obtained by different experimentators are very similar. The results of Pals and Hermans on CMC-samples with different degrees of substitution (DS) also show, in agreement with results in the preceeding paragraph, that a variation in the stoichiometric charge density has only a very small effect upon B.

In conclusion to this and the preceeding paragraphs it may be said that, as judged from our own experimentation and from a survey of literature data, no exception has been found to the rule that the values of B can serve as an independent parameter of stiffness. This seems to hold true regardless of the chemical nature of the charged groups and of the chain skeleton.

It may be pointed out that the B-value should be regarded as an operational quantity, and that its determination requires only that straight lines are obtained in two data-plots $\left([\eta] \text{ versus } \frac{1}{\sqrt{I}} \text{ and } \log S \text{ versus } \log([\eta]_{0.1})\right)$; this condition has hitherto been invariably satisfied in practice.

The quantitative correlation obtained here between B and unperturbed dimensions should not be regarded as the final one, and when more data about stiffness are available, some modification may be needed. An assumption implicit in Fixman's theory is that the effective bond length, b_θ, is independent of the ionic strength. This implies that the short-range electrostatic interaction between neighboring monomers does not vary sufficiently with ionic strength to cause any rotational or conformational changes that affect b_θ. If this is not the case in the physical situation, such changes would give a contribution to the B-value.

Because of these possible limitations to the validity of B as a parameter of stiffness, we suggest it should be used mainly as a preliminary measure of the relative stiffness in different polymer chains. Its use on acidic algal polysaccharides will be reported later. One great advantage is that it is rapid and, since it rests only upon viscosity measurements, very accurate. In this connection, it is instructive to see that the B-values for CMC from the viscosity data of Brown and Henley[31] and Scheider and Doty[32] are identical (Table II and V), whereas their reported values for the Kuhn statistical segment length differ by a factor of 3. In a very recent paper Cleland[44] is trying to estimate the unperturbed dimensions of hyaluronic acid from hydrodynamic measurements in good solvents. His conclusion, "The present order-of-magnitude estimates of σ are not sufficiently accurate to permit meaningful comparison between the different ionic polysaccharides," suggests that the determination of B-values may become a useful approach.

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