

A Light Scattering Study of Alginate

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Samples of alginate with intrinsic viscosities ranging from 0.7 to 30 (100 ml/g) were examined with light scattering techniques. The samples were found to be contaminated with approximately spherical particles of a diameter of 400–1200 Å, probably originating from the plant material. The presence of these particles was primarily revealed by a low value of the second virial coefficient, and they could be removed by prolonged centrifugation of the alginate solutions. We obtained the following relations between intrinsic viscosity, molecular weight, and radius of gyration of alginate in solutions containing 0.09 M sodium chloride and 0.01 M sodium fluoride:

$$\begin{aligned} [\eta] \text{ (100 ml/g)} &= 2.0 \times 10^{-5} M_w \\ R_G \text{ (Å)} &= 0.95 \cdot M_w^{0.54} \quad (M_w > 65\,000) \end{aligned}$$

Based on these results it was concluded that the alginate molecules in solution could best be described as very extended random coils with partially free drainage.

The modified Staudinger equation, $[\eta] = K \cdot M_v^a$ may be used for calculating the molecular weight of alginic acid. For a polydisperse system the equation yields the viscosity average (M_v) of the molecular weight, which, according to the definition

$$M_v = (\sum M_i^a \cdot c_i / \sum c_i)^{1/a}$$

equals the weight average when the exponent, a , in the Staudinger equation equals 1. The indices in the Staudinger equation are most commonly obtained by determining the viscosity and the weight average molecular weight by sedimentation or light scattering measurements on samples of different molecular weight. The number average molecular weight, as obtained by osmotic pressure measurements or end group determinations, are also often correlated to the intrinsic viscosity. If the indices in the Staudinger equation shall be obtained from such measurements, the molecular weight distribution either has to be known or has to be the same for each sample. The molecular weights to be calculated from such an equation are correct only for samples with the same molecular weight distribution as in the samples used in the original experiment. The intrinsic viscosity of a poly-

electrolyte like alginate is dependent on the ionic strength, the pH, and the type of inorganic salts present in the solution. We have previously shown¹ that the viscosity of alginate solutions is independent of pH between 4 and 12, and only slightly dependent of both the ionic strength between 0.1 and 1.0, and of the anion for potassium and sodium salts. The indices in the modified Staudinger equation for alginate have been obtained by a number of authors. The ionic strength of the solutions was in all cases kept above 0.1, and the results may therefore be compared. Donnan and Rose² determined the intrinsic viscosity and osmotic pressure of seven commercial samples and found the linear relationship: $[\eta]$ (100 ml/g) = $8 \times 10^{-5} \cdot M_{\text{osm}}$. Säverborn³ determined the intrinsic viscosity and molecular weight by sedimentation velocity and diffusion and by sedimentation equilibrium on two samples — one commercial, and one prepared in the laboratory. He assumed $a = 1$ and found the relation: $[\eta]$ (100 ml/g) = $11-15 \times 10^{-5} \cdot M_{\text{sed}}$. Cook and Smith⁴ determined the molecular weight by sedimentation of two commercial and three laboratory prepared samples ranging from 3.1 to 17.5 (100 ml/g) in intrinsic viscosity. They found the errors in the diffusion experiments too high to allow the calculation of the molecular weight in the usual way, and used the viscosity data for calculating the frictional coefficient. Assuming an ellipsoid model for alginate (Simha-Perrin equation) they got $[\eta] = 10.9 \times 10^{-5} \cdot M_{\text{sed}}$. The random coil model (Mandelkern-Flory equation) gave $[\eta] = 7.0 \times 10^{-5} \cdot M_{\text{sed}}$. The authors favoured the latter model. Wassermann *et al.*⁵ determined the molecular weight by means of light scattering experiments, obtaining the following equation: $[\eta] = 10^{-4.8 \pm 1.0} \cdot M_{\text{L.S.}}^{1.1 \pm 0.3}$. Their results are clearly not accurate enough to have practical value. To all the above results the criticism may be made that the alginate samples were insufficiently characterized both with respect to the molecular weight distribution and chemical composition. The intrinsic viscosity was in all cases determined in capillary viscometers without making any extrapolation to zero rate of shear. We have found¹ that extrapolation to zero rate of shear is necessary for obtaining the correct intrinsic viscosity of alginate. It is of practical and theoretical importance to know the indices in the modified Staudinger equation with some degree of accuracy: the object of this paper is therefore to determine the molecular weight both with end group determinations and with light scattering measurement and to correlate the results to the intrinsic viscosity obtained at zero rate of shear. The conclusions which may be drawn concerning the shape of the alginate molecules in solution are also discussed.

RESULTS

a. *Preparation of optically clean solutions.* Light scattering is a unique tool for obtaining information about the weight and size of macromolecules, but the difficulties in preparing optically clean solutions have limited both the use of the method and the validity of the conclusions that may be drawn from the experiments.^{6,7} As pointed out by Holzer *et al.*,⁸ publication of experimental details is necessary to make the results useful in a field like determination of molecular weights of polymeric substances where no routine procedures

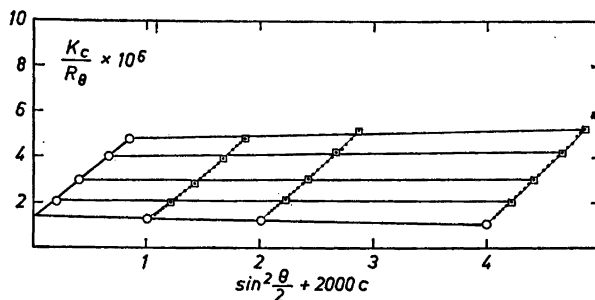


Fig. 1. Light scattering data for alginate ($[\eta] = 13.7$), purified only by filtration.

yet have been adopted. This is particularly so with respect to light scattering measurements. In the present work a certain type of particles has been found to be present in all alginate preparations investigated. The effect of these particles and their removal is discussed in some detail.

An alginate sample of intrinsic viscosity 13.7 (100 ml/g) was dissolved in an aqueous solution of 0.01 M sodium fluoride and 0.09 M sodium chloride to give a concentration of 0.2 g/100 ml. The sodium fluoride was included in order to prevent bacterial contamination. The solution was filtered through the membrane filter MF100 into the cylindrical light scattering cell. The scattering was measured at every five degrees of angle from 30° to 135° . The solution was diluted by filtering salt solution into the cell, the scattering measurements being repeated at different concentrations. The Zimm-plot of the results is shown in Fig. 1. The molecular weight, the radius of gyration, and the second virial coefficient were calculated in the usual way,⁹ the results being:

$$M_w = 800\,000, \quad R_g = 1100 \text{ \AA}, \quad B = -0.1 \times 10^{-3}$$

The values for both the molecular weight and the radius of gyration seem reasonable, but the negative second virial coefficient can not be accepted for the highly charged poly-electrolyte alginate. Schneider and Doty¹⁰ found for a sample of carboxy methyl cellulose having approximately the same charge density a value of the second virial coefficient $B = 3.6 \times 10^{-3}$ in 0.1 M salt solution. The alginate solution may, therefore, possibly contain an impurity which gives a negative contribution to the second virial coefficient. Experiments with the filter MF50 (pore size = 5000 \AA) failed to change the Zimm-plot significantly. Filtration through MF10 reduced the turbidity considerably, but by analyzing the filtrate for carbohydrate it was found that only ca. 10 % of the alginate molecules had passed the filter.

The impurity therefore seemed to be of approximately the same size as the alginate molecules, hence attempts to purify the solutions by centrifugation were carried out. Solutions of alginates (0.2 %) with approximately the same intrinsic viscosity were centrifuged for different periods of time at 28 000 g and 140 000 g. The scattering was measured in the decanted solutions as described above. The Zimm-plot of one of the solutions (centrifuged for 1/2 h

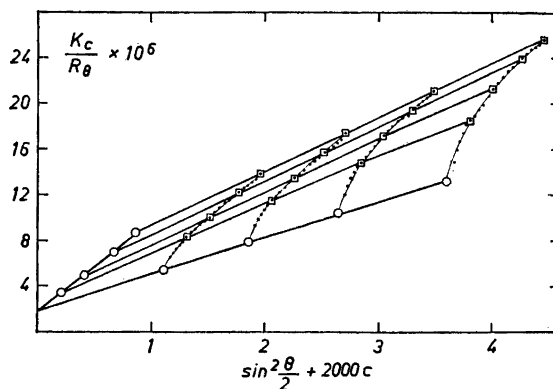


Fig. 2. Light scattering data for the same alginate sample, highly purified by centrifugation.

at 28 000 g , followed by 2 h at 140 000 g and, after decanting, 2 h more at 140 000 g) is shown in Fig. 2. The diagram shows that the second virial coefficient now has a high positive value. In Table 1 the molecular weights, the radii of gyration, and the second virial coefficients obtained from the centrifugation experiments are given. With increasing centrifugation the ratio $M_w/[\eta]$ scatters around a constant value, the ratio $R_G/[\eta]$ increases slightly, whereas the second virial coefficient shows a very marked increase.

Fig. 3 shows the second virial coefficients plotted as a function of the degree of centrifugation expressed as $t \times G$, where t is the centrifugation time in hours, and G is the centrifugal force. Although the scattering of the points is considerable, a good correlation between the second virial coefficient

Table 1. Molecular weights, radii of gyration, and second virial coefficients for alginate solutions centrifuged for different periods of time.

Centrifugation condition	$[\eta]$	M_w $\times 10^{-3}$	$M_w/[\eta]$ $\times 10^{-4}$	R_G	$R_G/[\eta]$	B $\times 10^3$
No centrifugation	13.7	790	5.75	1070	85.5	-0.1
1/2 h at 28 000 g	12.6	666	5.3	960	76.2	0.1
1 h » 28 000 g	11.8	625	5.3	1030	87.5	0.25
2 h » 28 000 g	11.5	625	5.43	985	85.6	0.5
1 h at 28 000 g + 1 h at 140 000 g	12.3	660	5.35	1050	85.5	1.0
2 h at 140 000 g	12.7	715	5.63	1230	97	1.85
2 h at 140 000 g	12.3	690	5.61	1230	100	2.0
1/2 h at 28 000 g + 2 h at 140 000 g	11.8	625	5.3	1130	96	2.3
1/2 h at 28 000 g + 4 h at 140 000 g	11.2	625	5.59	1230	110	2.6
1/2 h at 28 000 g + 2 \times 2 h at 140 000 g	11.0	556	5.06	1380	125	3.4
1/2 h at 28 000 g + 2 \times 2 h at 140 000 g	10.1	525	5.2	1220	121	3.1

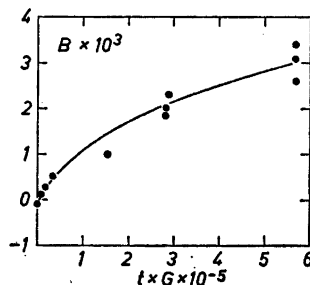


Fig. 3. Second virial coefficient as a function of the degree of centrifugation.

and the degree of centrifugation is clearly indicated. In every centrifugation tube a small amount of a compact precipitate could be observed.

From Table 1 it is seen that the effect of the impurity on the determined molecular weight and radius of gyration of alginate is small compared to the effect on the second virial coefficient. The relative error in the molecular weights will, of course, be higher for alginate samples of lower molecular weights. In order to assure correct determinations in these cases also, all solutions were centrifuged half an hour at 28 000 g , followed by two times two hours centrifugation at 140 000 g . The second virial coefficient was in all the experiments used as a criterion for the purity of the solutions.

b. *Characterization of the impurity.* When compared at the alginate concentration 0.2 % the scattering at 90° of the highly purified solution in Fig. 2 is only about 10 % of the scattering of a solution which has not been subjected to centrifugation (Fig. 1). A quantitative comparison of the scattering of the precipitate with the decrease in scattering of alginate solutions upon centrifugation was attempted. The scattering of a 0.2 % alginate solution was measured after centrifugation for half an hour at 28 000 g . The solution was then centrifugated for 2 h at 140 000 g . After decanting, the precipitate was washed with a small amount of water to remove traces of alginate and then suspended

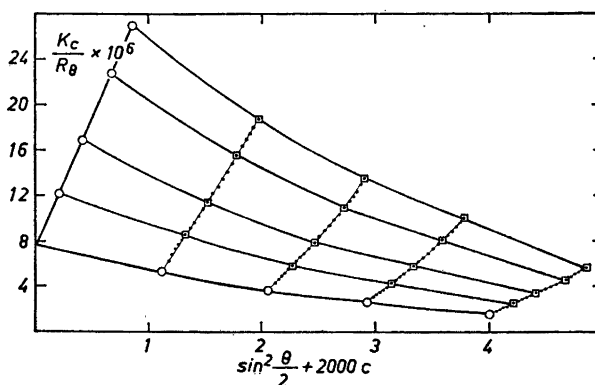


Fig. 4. Light scattering data of particle suspension. Concentration of undiluted solution arbitrarily set to 0.2 %.

in a salt solution of the same volume as the original alginate solution. The scattering was measured in this particle suspension and in the decanted alginate solution. Compared at 90° the scattering of the particle suspension corresponded to 70 % of the decrease in scattering observed in the alginate solution upon centrifugation.

This particle suspension was considered representative for the impurity in the alginate. Light scattering measurements of this material were performed and the results are given as a Zimm-plot in Fig. 4. The concentration of the particles was not known, thus the concentration of the undiluted solution was arbitrarily set equal to 0.2 %. The real concentration was most probably several orders of magnitude lower than this. Without the knowledge of this concentration it is, of course, not possible to determine the molecular weight of the particles. The second virial coefficient may, however, be found as seen from the formula:

$$\frac{2B}{K_1} = \lim \left[\frac{d(Kc/R_\theta)_{\theta=0}}{dc} \right]_{c=0}$$

where the symbols have their usual meaning (*cf.* Experimental) and K_1 is the constant used in the Zimm diagram ($K_1 = 2000$ in Fig. 4). The same applies to the radius of gyration:

$$R_G^2 = M_w \cdot \frac{3 \lambda^2}{16 \pi^2} \cdot \lim \left[\frac{d(Kc/R_\theta)_{c=0}}{d\left(\sin^2 \frac{\theta}{2}\right)} \right]_{\theta=0}$$

where

$$\frac{1}{M_w} = \lim \left[\left(\frac{Kc}{R_\theta} \right)_{\theta=0} \right]_{c=0}$$

A value of the refractive index increment of 0.2 (ml/g) was used in the calculations. If the particles consist mainly of protein, this should be a good approximation. The Zimm diagram yielded $R_G = 800 \text{ \AA}$ and $B^2 = -2 \times 10^{-3}$. The shape of the diagram indicates⁹ that the particle suspension consists of aggregates dissociating to some extent by dilution.

Electronic micrographs of the suspension showed the existence of spherical and slightly ellipsoidal particles of diameter from 400 to 1200 Å. As judged by several micrographs, the main material seemed to consist of aggregates of spheres of 400 Å in diameter. One micrograph is shown in Fig. 5. The average size of the particles as measured by light scattering is high compared to the particle sizes seen in the micrographs, but, since the R_G is a Z -average and since no information on the extent of dissociation of the aggregates by dilution has been obtained, the two results can be regarded as being in reasonable agreement with each other.

The origin and the chemical nature of the particles were not investigated. Turbidity measurements of a series of alginate samples, both commercial and those prepared at the laboratory, revealed the existence of the particles in all the samples. It is, therefore, reasonable to assume that their origin is the plant material used for preparation of the alginate, and that contamination

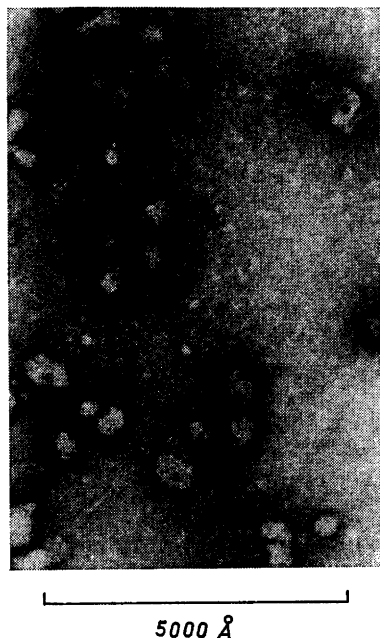


Fig. 5. Electron micrograph of particle suspension (negative staining technique).

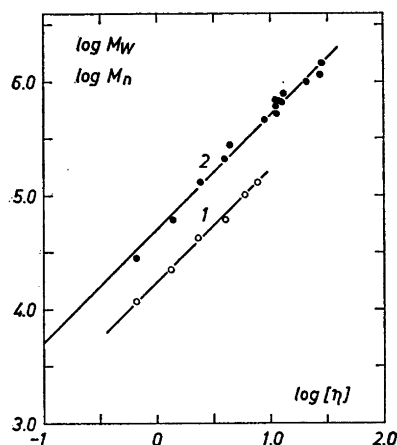


Fig. 6. Correlation between molecular weight and intrinsic viscosity. 1) Number average molecular weights from end-group determinations. 2) Weight average molecular weights from light scattering.

by particles of this type always must be taken into account in light scattering studies of alginate.

c. *Characterization of alginate samples of different molecular weights.* Alginate samples of a series of different intrinsic viscosities were prepared by degrading an alginate solution of high intrinsic viscosity (27.2) at pH 4. The number average molecular weight of some of the lower molecular weight samples was determined by measuring the reducing power of the solution. The logarithm of the molecular weights is plotted against the logarithm of the intrinsic viscosity in Fig. 6, curve 1, resulting in the following relationship:

$$[\eta] = 6.0 \times 10^{-5} \cdot M_n$$

Light scattering experiments were carried out on a number of samples prepared by the same degradation technique. The second virial coefficient was in all experiments found to have a high positive value (2 to 3.5×10^{-3}), showing that the particles had been effectively removed. The results of the molecular weight determinations are given in Fig. 6, curve 2. The best straight line to be drawn through the points as calculated by the least square method followed the equation:

$$[\eta] = 2.44 \times 10^{-5} \cdot M_w^{0.982}$$

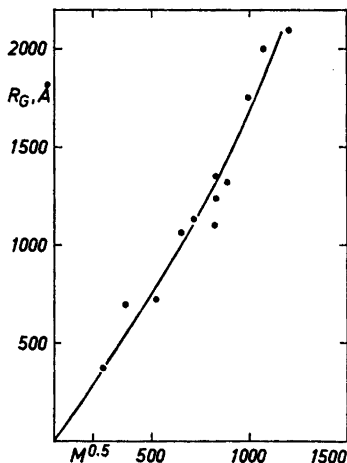


Fig. 7. Radius of gyration as a function of the square root of the molecular weight.

Because of the scattering of the points we do not regard the exponent in the equation significantly different from 1, thus the best straight line where $a = 1$ gave the equation:

$$[\eta] = 2.0 \times 10^{-5} \cdot M_w$$

This line is drawn in Fig. 6. It should be kept in mind that this equation applies to alginate in solutions of alkali metal salts of ionic strength 0.1. Work is in progress to determine the exponent in the Staudinger equation at other values of the ionic strength.

Except for the samples of the lowest intrinsic viscosity ($[\eta] = 0.66$), all the scattering envelopes were markedly asymmetric and the radii of gyration could be determined. The R_G -values are plotted in Fig. 7 as a function of $M_w^{0.5}$. The points are grouped around a slightly curved line; by plotting the logarithm of R_G against the logarithm of M_w the following equation was obtained:

$$R_G (\text{Å}) = 0.95 \cdot M_w^{0.54}$$

DISCUSSION

Both the number and the weight average molecular weight determinations revealed that the exponent in the modified Staudinger equation is close to 1 for alginate in solutions containing 0.1 M sodium salt. This is in agreement with previous molecular weight determinations.^{2,4} The viscosity average of the molecular weight for alginate is therefore equal to the weight average. Consequently, when methods giving the weight average molecular weight are used, the constant K in the modified Staudinger equation is independent of the molecular weight distribution. The results of the present light scattering measurements agree with the results of Wassermann *et al.*,⁵ but the molecular weights are high compared to Säverborn's³ and Cook and Smith's⁴ results.

Säverborn used diffusion measurements for obtaining frictional coefficients, a method shown by Cook and Smith to be highly inaccurate for alginate. As the latter authors had to assign a certain shape to the alginate molecules for calculating the frictional coefficient from the intrinsic viscosity, their values can only be regarded as estimates.

The number average¹ molecular weights found in this work are about 25 % higher than those reported by Donnan and Rose² ($K = 6.0 \times 10^{-5}$ compared to $K = 8.0 \times 10^{-5}$). Such a difference may well be caused by a difference in molecular weight distribution. It is not unlikely that the commercial preparations used by Donnan and Rose had a wider molecular weight distribution than the samples used by us; our number average molecular weight determinations may, therefore, be regarded as being in reasonable agreement with those of Donnan and Rose.

In the molecular weight range where both the weight and the number average molecular weights are determined the ratio, λ , between the two averages is 3 ($\lambda = M_w/M_n = K_n/K_w$ when a in the modified Staudinger equation is 1). For random degradation of a long linear polymer chain, λ approaches the value 2 regardless of the original molecular weight distribution.¹¹ The samples used in this work were degraded at pH 4, where the alginate is soluble, and where the linkage between two mannuronic acid residues are split 2.5 times faster than the linkage between two guluronic acid residues.¹² The two monomers have been shown to exist to a large extent in long homopolymer "blocks":^{13,14} a molecular weight distribution with a λ -value well above 2 seems therefore reasonable. In addition, the molecular weight of the undegraded sample is not known. Values of λ close to 3 have been found for different cellulosic materials.^{10,15} For branched molecules λ is usually very high, a value of 267 has, *e.g.*, been found for an amylopectine sample.¹⁶ The present value of λ is therefore sufficiently low to strongly indicate that the alginate molecule must be linear, in agreement with recent chemical evidence.³³

The value of the exponent, a , in the modified Staudinger equation is usually taken as an indication of the stiffness of the molecules. According to the most commonly applied statistical and hydrodynamical treatment of polymers⁹ a value of a between 0.5 and 0.8 indicates a randomly coiled molecule, a value between 0.8 and 1.8 indicates a "stiff coil", and the value 1.8 indicates a rigid rod. With this definition the alginate molecule should clearly be a "stiff coil".

The statistical treatment of random coil molecule shows that the radius of gyration should increase with the molecular weight in the power of 0.5–0.6. This is in good agreement with our experimentally determined value (0.54), which is lower than what should be expected for "stiff coils"⁹ (0.6–0.99). It seems therefore reasonable to assume that the alginate molecule follows the statistics of a random coil molecule and that the high exponent in the Staudinger equation is due to a deviation from the behaviour predicted by the commonly accepted hydrodynamical theories.^{9,34}

The extension of random coil molecules may be described according to Kuhn *et al.*^{17,18} by a parameter A_m which is the length of the statistical segment, defined by the equation:

$$(\bar{h}^2)^{1/2} = (A_m \cdot b_0 \cdot P)^{1/2}$$

where $(\bar{h}^2)^{1/2}$ is the end-to-end distance, b_0 is the length of the monomer unit, and P is the degree of polymerization. The relation between the radius of gyration and the end-to-end distance for randomly coiled molecules is:⁹

$$R_G^2 = \bar{h}^2/6$$

A_m may be calculated from the obtained relationship between R_G and M_w , using 198 as the weight and 5.15 Å as the length of a monomer unit.¹⁹

The above equation is based on monodisperse systems, hence the same type of average of end-to-end distance and degree of polymerization must be used in our case. Since light scattering yields the Z -average of the radius of gyration and the weight average of the molecular weight, some assumptions regarding the molecular weight distribution must be made. Zimm²⁰ has given a distribution formula whose breadth is determined by a variable parameter Z . This formula describes a single peak distribution and has been used by different authors for averaging data on cellulosic materials.^{10,15,21} When $Z = 1$, the most probable distribution ($M_z:M_w:M_n = 3:2:1$) is obtained; when $Z = \infty$ the system is monodisperse. In our case the ratio between M_w and M_n is 3, which corresponds to $Z = 0.5$, and the ratios $M_z:M_w:M_n = 5:3:1$. Using a value of 500 000 for M_w we obtain $(R_G)_z = 1090$ Å and $M_z = 833$ 000, which gives $A_m = 340$ Å. This value is extremely high compared to usual values for vinyl polymers (*e.g.* $A_m = 16$ Å for polyvinylalcohol¹⁸) and to amylose in aqueous solution²² ($A_m = 21$ Å), but compares well with values for the very extended carboxy methyl cellulose ($A_m = 335$ Å at infinite ionic strength¹⁰). In spite of the somewhat arbitrary way of accounting for the polydispersity of alginate, it should therefore be safe to conclude that the alginate molecule in 0.1 M salt solution has an extended form.

Because of the scattering of the points relating R_G to M_w , and because of the uncertainty regarding the polydispersity, a detailed comparison of the viscosity with the hydrodynamic theories (for reviews see Refs. 9, 23–25) will not be made. However, the marked disagreement between the experimentally observed exponent in the Staudinger equation¹⁰ and the exponent predicted for a random coil molecule by the commonly accepted Fox and Flory theory³⁴ requires some comment, and some conclusions regarding the drainage of the coil may be reached. As the volume of the polymer segments in a randomly coiled molecule is low compared to the total volume of the coil, the resistance to flow (*i.e.* the viscosity) will depend on whether the solvent molecules are “trapped” inside the coil or not. In the extreme case, where there is no hydrodynamic interaction between the chain segments (free-drained molecules), the theory predicts^{23,26} that $[\eta]$ is proportional to the square of the radius of gyration (weight average):

$$[\eta] = A \cdot (R_G)_w^2$$

Using the experimentally obtained relationship between $(R_G)_Z$ and M_w , and the assumption that the ratios between M_z , M_w , and M_n are the same for all the molecular weights, we find that $[\eta]$ should be proportional to $M^{1.08}$. This exponent is not far removed from the experimentally observed value. The free-drained model, however, also predicts²³ that the sedimentation rate should be independent of M ; this has been shown by Cook and Smith not to be the case for alginate.⁴

At the other extreme, the hydrodynamic interaction between the chain segments is so pronounced that the interior part of the molecule behaves essentially as impermeable to the flow of liquid around it. This model is often referred to as "equivalent particle"²⁵ or "equivalent sphere".^{9,23} For a sphere the intrinsic viscosity is proportional to R^3/M , and for a randomly coiled molecule (spherical symmetry) the radius of the sphere may be assumed proportional to R_G hence we may write according to Hermans:²⁵

$$[\eta] = K'(R_G)_n^3/M_n$$

In this case the number averages must be used in a polydisperse system. By accounting for the polydispersity in the same way as above we get

$$[\eta] = K' \cdot M_n^{0.62}$$

This exponent is very low compared to the experimentally obtained value ($a = 1$). The "constant" K' must therefore increase considerably with increasing molecular weight. Such an increase is, as pointed out by Kurata and Yamakawa,²⁷ indicative of partial free drainage. The same behaviour has been observed for many cellulosic materials²⁵ and also for amylose in dimethyl sulphoxide,²⁸ *i.e.* for macromolecules having an extended conformation in solution.

It may tentatively be concluded therefore that alginate behaves in 0.1 M salt solution like a partially free-drained random coil with a very extended form. Whether the high degree of extension is due to the charged groups on the polymer chain or to "stiffness" in the chain backbone can not be decided from the results presented here, and further work is in progress.

EXPERIMENTAL

a. *Cleaning procedures.* Membrane filters (Membranefiltergesellschaft, Göttingen) were used to remove dust from water and aqueous solutions. The solutions were filtered into a vacuum desiccator containing the light scattering cell. All the filters originally contained some particles that were liberated when water was filtered through them. It was necessary to pass 2–5 liters of water through the filters before the water was optically clean. After this washing procedure one filtration was sufficient to give clean water with all the filters of pore sizes lower than 10 000 Å (MF100). Some alginate solutions of high viscosity had to be filtered 2 or 3 times before the dust was sufficiently removed. It was found important in these cases to use a very low filtration rate (100–200 ml/h). The cleaning procedure for the light scattering cells involved washing in detergent solution followed by rinsing, first in distilled water and finally 6 to 8 times with water free of dust.

For cleaning the alginate solutions two different centrifuges were used (MSE, Type MR65, max. speed 16 000 rpm, corresponding to 28 000 *g*, and a Spinco Model 2 prepara-

tive ultracentrifuge, max. speed 40 000 rpm, corresponding to 140 000 g). The last step in all the cleaning procedures was the filtration through the membrane filter directly into the light scattering cell.

b. *Light scattering measurements.* All the measurements were carried out with blue light ($\lambda = 436 \text{ m}\mu$) in a Brice Phoenix light scattering photometer. A cylindrical cell, model C-101, was used. The calibration of the apparatus was made by using an opal glass reference standard which had its transmittance determined by Phoenix Precision Instrument Company. The scattering intensity was corrected for reflections in the cell according to the empirical formula given by Tomimatsu and Palmer.²⁹ In this formula it is necessary to know the scattering intensity at the angle $(180-\theta)$ for correcting the intensity at the angle θ . As the scattering at angles higher than 135° could not be measured, the correction for the angles $30, 35,$ and 40° was made by assuming that the scattering intensity at $150, 145,$ and 140° was equal to the intensity at 135° .

A typical experiment for determining molecular weight, radius of gyration, and second virial coefficient of a sample was done in the following way. The cleaning of the filter, of the light scattering cell and of the solvent (0.09 M NaCl + 0.01 M NaF) was controlled by measuring the intensity of the solvent at every fifth degree of angle from 30 to 135° . The alginate solution (usually 0.2 %) containing the proper amount of salt (0.09 M NaCl + 0.01 M NaF) was filtered into the empty cell. The intensity measurements were performed as with the solvent. The solution was diluted by filtering the solvent directly into the cell. The new alginate concentration was found by weighing and the intensity measurements were repeated. Four alginate concentrations from 0.03 to 0.2 % were usually measured.

From the intensity measurements the expression Kc/R_θ was calculated by a digital computer for every value of the angle and every value of the concentration, c . The quantities K and R_θ are given by the equations:

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

$$K = \frac{2 \cdot \pi^2}{\lambda^4 N} \cdot \left(n_0 \frac{dn}{dc} \right)^2$$

where r^2 is the distance from the scattering element to the detector, i_θ is the difference in intensity between solution and solvent at the angle θ , λ is the wave length, N the Avogadro number, n_0 the refractive index of the solvent, and dn/dc the refractive index increment of alginate. By plotting Kc/R_θ as a function of $(\sin^2 \frac{\theta}{2} + \text{const. } c)$ according to Zimm, the values of $(Kc/R_\theta)_{\theta=0}$ and $(K_\theta/R_\theta)_{c=0}$ were found by extrapolation, and the molecular weight, M , and the second virial coefficient, B , found from the equation:

$$\left(\frac{Kc}{R_\theta} \right)_{\theta=0} = \frac{1}{M} + 2Bc + \dots$$

The radius of gyration, R_G , was determined from the equation:

$$\left(\frac{Kc}{R_\theta} \right)_{c=0} = \frac{1}{M} \left(1 + 1/3 \cdot R_G^2 \cdot \left(\left(\frac{4\pi}{\lambda} \cdot \sin^2 \frac{\theta}{2} \right)^2 + \dots \right) \right)$$

c. *Refractive index increment measurements.* The refractive index increment is a constant at low concentrations of polymeric material.⁹ It is usually determined by measuring the difference in refractive index of the solvent and a solution with a known concentration. For a polyelectrolyte like alginate the solvent and the solution must be brought to equilibrium through dialysis prior to the measurements.³⁰ The refractive index increment for alginate containing 0.1 M NaCl, relative to a 0.1 M NaCl-solution has been determined by Buchner, Cooper and Wassermann. At $\lambda = 436 \text{ m}\mu$ the result was 0.165 ml/g. This value was used by us to calibrate a differential refractometer with white light as source. The refractive index increment determined by dialysing alginate against

an aqueous solution of 0.09 M NaCl and 0.01 M NaF was 0.157 ml/g. This value was used in all the calculations.

d. *Chemical characterization of alginate.* The alginate was prepared from *Laminaria digitata* harvested at Tarva 29/8. The ratio between mannuronic and guluronic acid residues in the alginate was 1.60.³¹ The reducing power was measured by the method of Nelson,³² using calibration curves obtained with mannuronic and guluronic acid.

Degradation experiment. An alginate sample of intrinsic viscosity 27.2 (100 ml/g) was dissolved to 1% in water. The pH was adjusted with hydrochloric acid to 4. The hydrolysis was carried out at 100°C and samples were removed at intervals. The intrinsic viscosity and the reducing power were determined in the cooled solution after neutralization with NaOH. In the range of intrinsic viscosities lower than 4 the viscosity was determined in an Ubbelohde viscometer and the intrinsic viscosity found by extrapolation. For values higher than 4 the intrinsic viscosity at zero rate of shear was determined using correlation curves relating the intrinsic viscosity to the viscosity at a certain concentration in the Ubbelohde viscometer.¹ In all cases a salt concentration of 0.1 M (0.09 M NaCl + 0.01 M NaF) was used. The samples to be used in the light scattering measurements were precipitated with a large excess of ethanol and prepared in the dry state in the usual way.³¹ The dry alginate was in all cases considered to contain 10% of water;³¹ all the alginate concentrations were corrected according to this figure. In order to eliminate errors due to slight degradation during the preparation and the cleaning of the alginate solutions, the intrinsic viscosity was in all cases determined in the centrifuged solutions used in the light scattering measurements.

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REFERENCES

1. Haug, A. and Smidsrød, O. *Acta Chem. Scand.* **16** (1962) 1569.
2. Donnan, F. G. and Rose, R. C. *Can. J. Research B* **28** (1950) 105.
3. Säverborn, S. *A contribution to the knowledge of the acid polyuronides*, Uppsala 1945.
4. Cook, W. H. and Smith, D. B. *Can. J. Biochem. Physiol.* **32** (1954) 227.
5. Buchner, P., Cooper, R. E. and Wassermann, H. *J. Chem. Soc.* **1961** 3974.
6. Kratochvil, J. P. *Anal. Chem.* **36** (1964) 459 R.
7. Kratochvil, J. P. *Anal. Chem.* **38** (1966) 517 R.
8. Holzer, A., Lowey, S. and Schuster, T. M. *The Molecular Basis of Neoplasia*, (Symp. Fundamental Cancer Res. 15th); Univ. Texas Press, Austin 1961, p. 259.
9. Tanford, C. *Physical Chemistry of Macromolecules*, Wiley, New York, London 1961.
10. Schneider, N. S. and Doty, P. *J. Phys. Chem.* **58** (1954) 762.
11. Charlesby, A. *Proc. Roy. Soc. (London), Ser. A.* **224** (1954) 120.
12. Unpublished results from this Institute.
13. Haug, A., Larsen, B. and Smidsrød, O. *Acta Chem. Scand.* **20** (1966) 183.
14. Haug, A., Larsen, B. and Smidsrød, O. *Acta Chem. Scand.* **21** (1967) 691.
15. Holzer, A. M., Benoit, H. and Doty, P. *J. Phys. Chem.* **58** (1954) 624.
16. Stacy, C. J. and Foster, J. F. *J. Polymer Sci.* **20** (1956) 57; **25** (1957) 39.
17. Kuhn, W. and Kuhn, H. *Helv. Chim. Acta* **26** (1943) 1394.
18. Kuhn, W., Kuhn, H. and Silberberg, A. *J. Polymer Sci.* **14** (1954) 193.
19. Astbury, W. T. *Nature* **155** (1945) 667.
20. Zimm, B. H. *J. Chem. Phys.* **16** (1948) 1099.
21. Benoit, H., Holzer, M. A. and Doty, P. *J. Phys. Chem.* **58** (1954) 635.
22. Banks, W. and Greenwood, C. T. *Makromol. Chem.* **67** (1963) 49.
23. Flory, P. J. *Principles of Polymer Chemistry*, Cornell University Press, Ithaca, New York 1953.
24. Banks, W. and Greenwood, C. T. *Advan. Carbohydrate Chem.* **18** (1963) 357.
25. Hermans, J. J. *J. Polymer Sci. Part C* **1** (1963) 117.
26. Debye, P. *J. Chem. Phys.* **14** (1946) 636.
27. Kurata, M. and Yamakawa, H. *J. Chem. Phys.* **29** (1958) 311.

28. Cowie, J. M. G. *Makromol. Chem.* **42** (1961) 230.
29. Tomimatsu, Y. and Palmer, K. J. *J. Phys. Chem.* **67** (1963) 1720.
30. Overbeck, J. Th. G., Vrij, A. and Huisman, H. F. *Proc. Interdisciplinary Conf. Electromagnetic Scattering*. Pergamon, New York 1963, p. 321.
31. Haug, A. Report No. 30, Norwegian Institute of Seaweed Research (1964).
32. Nelson, N. *J. Biol. Chem.* **153** (1944) 375.
33. Rees, D. A. and Samuel, J. W. B. *J. Chem. Soc.* **1967** 2295.
34. Flory, P. J. and Fox, T. G., Jr. *J. Am. Chem. Soc.* **73** (1951) 1904.

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