Comparison of Some Physicochemical Properties of Alginic Acids of Differing Composition

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SUMMARY

Because of the intriguing rheological behavior of alginates and unanswered questions concerning the solution conformation of these biopolymers, some of the physicochemical properties of alginates were investigated by means of light-scattering, rheology, optical rotation, nuclear magnetic resonance, and near infrared spectroscopy studies.

Alginic acid from Macrocystis pyrifera was fractionated into L-guluronate and D-mannuronate rich fractions. Light scattering and viscosity studies indicated that the fractionated materials had lower weight-average molecular weights, root-mean-square end-to-end distances, and intrinsic viscosities. Our data suggest that the L-guluronate rich polymers may have a more rigid solution conformation, in the presence of 0.1 M NaCl at 20°, than do those polymers more abundant in D-mannuronic acid. In addition, the alginate

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fractions described above differed from one another in that the L-guluronate rich polymers were precipitated by MnCl$_2$ at concentrations $\geqslant 2.5$ mM, whereas the D-mannuronate rich polymers were soluble at even higher MnCl$_2$ concentrations.

The possibility that the solution properties of alginates might be due to extensive alginate-water interactions was investigated by nuclear magnetic resonance, near infrared spectroscopy, and rheology studies on unfractionated alginate solutions. It was observed that alginate had only a slight effect on the position of the water O-H peak at 1.443 μ, no effect on the nuclear magnetic resonance spectrum of water, and that the specific viscosities of alginate solutions, to which neutral salts had been added, were identical even when the added salts had opposing effects on the ordered structure of water. Thus it was concluded that alginates do not extensively orient the structure of water, nor do salt-induced changes in the ordered structure of water measurably affect the solution conformation of alginates. The results of preliminary optical rotation studies on aqueous alginate solutions, perturbed in a variety of manners, did not suggest that alginates were characterized by an ordered secondary structure.

INTRODUCTION

Alginic acid is believed to be a linear [1], polydisperse [2], block copolymer [3]. The monomeric components, D-mannuronic acid and L-guluronic acid [4], are connected via β- and α-1,4-glycosidic linkages, respectively [5]. The chemical compositions of alginates (ratios of the two component uronic acids) are variable and dependent both upon the material from which the polysaccharide was obtained and the method of extraction [6]. Haug and co-workers have correlated changes in the chemical composition of alginates with variations in the following physical properties: solubility in acidic solutions [7], ion exchange properties [8], and the acid dissociation of alginates [9].

Thus it was of interest to examine several physical and molecular parameters of alginates differing in chemical compositions. To this end, light-scattering and viscosity studies were carried out on chemically distinct alginates. A comparison between fractions was made with respect to weight-average molecular weights, z-average root-mean-square end-to-end distances, and intrinsic viscosities. In addition, we examined the rheological behavior of these alginates in the presence of a variety of netural salts

in order to further characterize the hydrodynamic properties of these fractions.

In recent years much attention has been directed towards macromolecule-water interactions and the effect of these interactions on macromolecular conformations in aqueous solutions [10-13]. As a consequence, the denaturation of proteins and nucleic acids can in part by explained in terms of changes in water-macromolecule interactions [13]. While it now seems clear that secondary and tertiary structures may be stabilized or disrupted by such interactions, it follows that some of the properties of random coil polymers may also be attributed to water-polymer interactions. Because of the intriguing physical characteristics of aqueous alginate solutions, i.e., their ability to form gels, and rheological behavior at low ionic strengths [14], we felt it would be of interest to determine whether this physical behavior was related to extensive water-alginate interactions. Data from nuclear magnetic resonance, near infrared, and viscosity studies concerning the effect of water on the conformation of alginate and the reciprocal effect of alginate on the ordered structure of water are presented and discussed.

Although most of the physicochemical properties of aqueous alginate solutions indicate that these polymers are in a stiff random coil configuration [15], there is some evidence suggesting that alginates may have an ordered or partially ordered secondary structure [1, 16]. The optical rotations of aqueous alginate solutions at several wavelengths, perturbed by a variety of agents, were determined to further investigate the possibility of alginates having some degree of ordered structure. This preliminary study supports the premise that alginates are random coil polymers.

METHODS

Sodium alginates (Kelco-Gel HV and Keltone) were obtained as a gift from Kelco Co., Los Angeles, California. D-Mannuronate rich and L-guluronate rich fractions were prepared by the method of Haug [17]. Determination of the uronic acid composition was accomplished in the manner described by Haug and Larsen [18]. The ratios of mannuronic to guluronic acids are given in Table 1.

Rheology experiments were conducted using a Brookfield Syncho-Lectric Viscometer with UL adaptor. The standard procedure for determining viscosities of alginate solutions was to measure the viscosities of 25 ml aliquots of test solution after 20 min of equilibration at 20° at

Table 1. Comparison of Some Physical and Chemical Properties of Various Alginate Samples

Alginate sample	Mannuronate Guluronate	$\bar{M}_w \times 10^5$	$\langle \bar{r}^2 \rangle^{1/2}$	$A_2 \times 10^3$	$[\eta]$ (dl/g)	M.E.L. $\langle r^2 \rangle^{1/2}$
Mannuronate rich fraction	2.72	2.570	1810	6.82	8.8	3.55
Guluronate rich fraction	.82	1.780	1485	8.30	10.0	2.95
Unfractionated	1.68	4.075	2410	10.30	11.0	-

spindle speeds of 6, 12, 30, and 60 rpm. All viscosities presented in this paper were obtained by extrapolation to zero rate of shear. Intrinsic viscosities were calculated by extrapolating the viscosities of alginate solutions differing in concentration (g/dl) and at constant ionic strength (0.1 M) to both zero rate of shear and $C = 0$. Intrinsic viscosities were also determined with No. 1 and 2 Ubbelohde-type kinematic viscometers under the same conditions as outlined above. Temperatures were maintained to within $\pm 0.02°$. Salts used in this study included NaCl, KCl, $MnCl_2$, LiCl, NH_4Cl, Tris-Cl, CH_3NH_3Cl, and $(CH_3)_4NCl$. In addition, one set of experiments was run at $37.5°$. In this instance the procedure was exactly as described above except the equilibration took place at elevated temperature.

Light-scattering studies and associated differential refractometry were conducted under the supervision of Dr. David A. Brant. A SOFICA light-scattering instrument and a Brice-Phoenix differential refractometer were used in this work. The caluclations, correction factors, and references were as described by Chiang [19]. Readings were made at 546 mμ from 30 to $150°$. The instrument was calibrated with benzene as a standard $(T = 25°)$ for converting the phototube reading to absolute scattering.

Solutions for light-scattering studies were prepared as follows. Stock solutions of Na alginate (dried in vacuo) were dissolved in 0.1 M NaCl. Corrections for water of hydration were made. All solvents and solutions were passed through millipore filters in order to eliminate particulate matter. Solutions were then dialyzed versus solvent for 4 days. No obvious bacterial growth was apparent at the end of dialysis. Concentrations were measured by comparing the dry weights of stock solutions and their solvent per unit weight. Densities and specific gravities were measured to convert grams of sodium alginate/gram of solution to grams of sodium alginate/milliliter of solution. In making dilutions, we assumed that no change in volume occurred on mixing stock solution and solvent. Dilutions were made on the basis of weight. Just prior to light-scattering measurements, 30 ml of each solvent and solution were centrifuged at 36,000 $\times$ g for 20 min, the middle 10 ml removed by syringe, a millipore filter attached (type HA with a 0.45-micropore size), 4 ml used to rinse the filter, and the remaining 6 ml added to the light-scattering cell. The cells were centrifuged for 1 hr at 18,000 rpm in a SW 25.1 rotor in a Spinco Model L centrifuge. The same solutions were used for light-scattering measurements and differential refractometry.

Optical rotations were determined using a Perkin-Elmer Spectropolarimeter at $25°$. In the optical rotation studies involving change in pH (over the

range 3.0 to 9.0), citrate (pH 3.0 to 5.2) and Tris succinate (pH 4.5 to 9.0) buffered alginate solutions (0.2 M NaCl, and 0.05 M buffer) were examined.

A Varian A60 was used for nuclear magnetic resonance studies.

The near infrared spectrum of water was examined using a Beckman DK-2A spectrophotometer with infrasil cells (light path, 1mm). Constant temperature was maintained using a FSB Haake circulator. Samples were allowed to equilibrate for 30 min.

RESULTS

Zimm plots of unfractionated alginic acid (Keltone), L-guluronate, and D-mannuronate rich fractions are shown in Figs. 1, 2, and 3, respectively. Table 1 contains calculated values for weight-average molecular weights ($\bar{M}_w$), intrinsic viscosities ($[\eta]$), z-average root-mean-square end-to-end distances ($\langle \bar{r}^2 \rangle_z^{1/2}$), and the mannuronic:guluronic acid ratios of the respective samples. Note that the salt fractionated alginates have smaller molecular weights; Keltone having a $\bar{M}_w$ value of 4.07 $\times$ 10^5 while the value for the L-guluronate rich fraction was 1.78 $\times$ 10^5 and for the D-mannuronate rich fraction 2.57 $\times$ 10^5. This would indicate that even under very mild conditions some of the alginate bonds may have been split, as the fractionation procedure involved only aqueous salt and ethanol treatment at ambient temperatures or lower. Sedimentation and diffusion studies have shown that alginates are polymolecular [2]. Thus the possibility is raised that these fractions are not fragmentation products but naturally occurring polymers. The lower molecular weights of the fractionated alginates are reflected in lower intrinsic viscosities and root-mean-square end-to-end distances.

From the mannuronate to guluronate ratio and the weight-average molecular weights ($\bar{M}_w$), an average contour or maximum extended length (M.E.L.) can be calculated by assuming that each β-linked mannuronate residue has a unit length of 5.15 Å and that each α-linked guluronate residue has a unit length of 4.35 Å [20, 21].* The maximum extended lengths (M.E.L.) were

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*X-ray fiber repeat data has been published for α-L-(1→4) polyguluronic acid [21], but not for β-D-(1→4) polymannuronic acid; however, it is highly probable that it is somewhat similar to that of β-D-(1→4) mannan for which x-ray fiber data is available [20]. For this reason a monomeric unit length value of 5.15 Å for β-linked mannuronic acid residue has been used in calculations of average contour lengths.
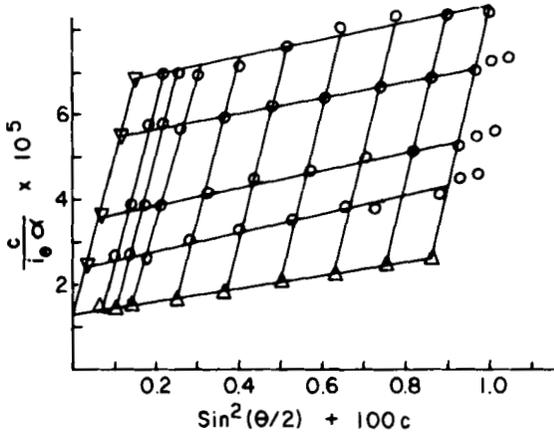
Fig. 1. Zimm plot of unfractionated Na alginate. $(\Delta n/\Delta c)_{av} = 0.180$ ml/g.

Fig. 2. Zimm plot of L-guluronic acid rich fraction of Na alginate. $(\Delta n/\Delta c)_{av} = 0.153$ ml/g.

calculated as follows:

$$M.E.L. = \bar{M}_w/198 \times f_m \times 5.15 + \bar{M}_w/198 \times f_g \times 4.35$$

where f_m and f_g refer to the fraction of β-linked mannuronic acid and α-linked guluronic acid residues per chain, respectively. The M.E.L. to

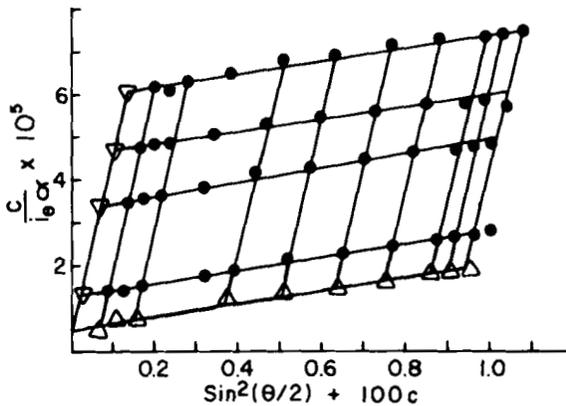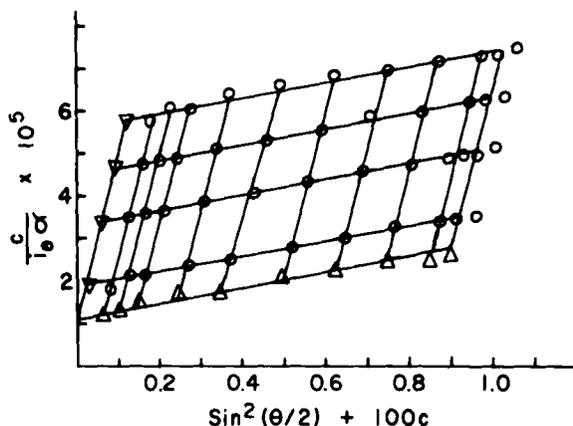Fig. 3. Zimm plot of D-mannuronic acid rich fraction of Na alginate.
$(\Delta n/\Delta c)_{av}$ = 0.168 ml/g.

$(\langle \bar{r}^2 \rangle_z^{1/2})$ ratios indicate that the L-guluronic acid rich fraction was stiffer than the D-mannuronic acid rich fraction. For as may be noted in Table 1, the guluronate rich fraction has the lower ratio (2.95 as compared to 3.55). Also, note that the intrinsic viscosity of the L-guluronic acid rich fraction was higher than that of the D-mannuronic acid rich fraction; whereas the $\bar{M}_w$ and $(\langle \bar{r}^2 \rangle_z^{1/2})$ values were lower.

Viscosity changes in solutions of unfractionated alginate (Keltone), mannuronate, and guluronate rich fractions as a function of increasing ionic strengths are shown in Figs. 4, 5, and 6. As can be seen in Fig. 4, all monovalent cations examined (Na^+, Li^+, K^+, $Tris^+$, NH_4^+, and $CH_3NH_3^+$) had indistinguishable effects on the viscosity of unfractionated alginate. Thus under the conditions employed in these experiments, the ionic radius or base strength of the monovalent cation has no effect on Keltone viscosity. Magnesium ions, on the other hand, caused a sharper decrease in viscosity than did the monovalent cations; however, at higher molar concentrations the viscosity was not significantly different than that found with monovalent counter ions. Initially, manganous ions had the same effect upon unfractionated alginate viscosity as magnesium ions. However, at ionic strengths $\geqslant 2.5$ mM, manganous ions caused an extremely sharp rise in the viscosity of the unfractionated alginate solution. This increase in viscosity was coincidental with an increase in solution turbidity and is attributed to precipitation of manganous alginate.
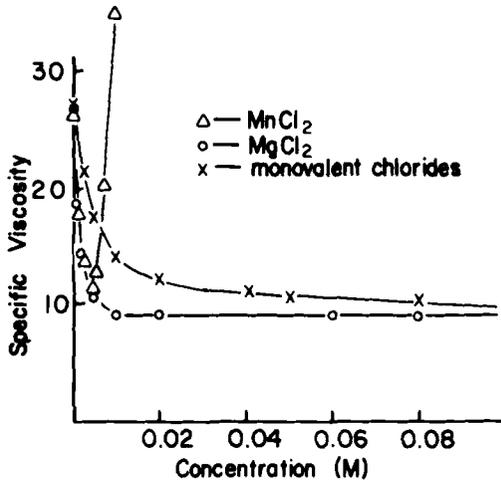
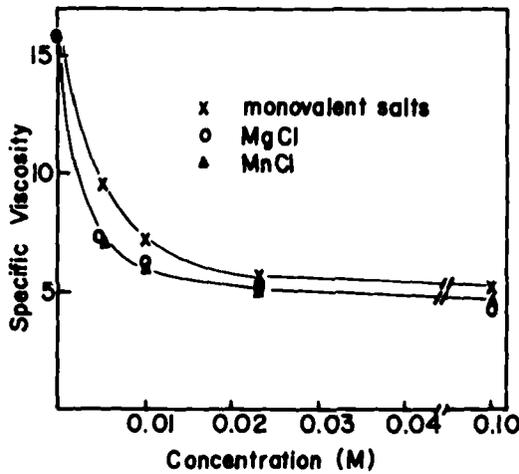**Fig. 4.** Effect of salts on unfractionated alginate viscosity. T = 20°, Na alginate concentration = 0.25%.

Fig. 5. Effect of salts on D-mannuronic acid rich fraction of Na alginate. T = 20°, Na alginate concentration = 0.25%.
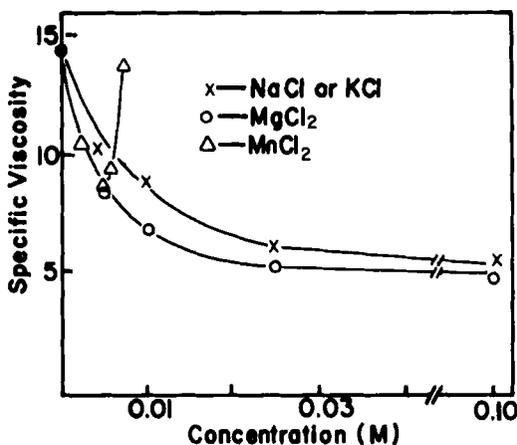
Fig. 6.   Effect of salts on L-guluronic acid rich fraction of Na alginate.
T = 20°, Na alginate concentration = 0.25%.

The effects of Na^+, K^+, Mn^{+2}, and Mg^{+2} ions on the viscosity of the man-
nuronate and guluronate rich fractions (Figs. 5 and 6) reveal a major difference
in rheological behavior between alginate fractions. As may be seen from the
data (Fig. 6), manganous ions precipitated the alginates of the L-guluronate
rich fraction, yet at concentrations not sufficient to cause precipitation the
effect on viscosity was identical to that of magnesium ions. In addition, the
effects of K^+, Na^+, and Mg^{+2} on the viscosity of the L-guluronate rich fraction
are similar to those observed with the unfractionated alginate. From Fig. 5
it was apparent that the effects of K^+, Na^+, and Mg^{+2} on the viscosity of the
D-mannuronate rich fraction were, as with the L-guluronate rich fraction,
similar to the effects these cations had on unfractionated alginate. In con-
trast to L-guluronate rich and unfractionated alginate, manganous ions did
not cause D-mannuronate rich alginate precipitation. With this fraction, the
effects of manganous and magnesium ions were indistinguishable even at
high ionic strengths.

Though the majority of their physical properties indicate a stiff random
coil structure [15], the stiffness, as reflected by the high intrinsic viscosities
of alginates, suggested that additional structural features might be involved.
Because of the effect changes in ionic strength have on alginate viscosity, we
felt a study of change in optical rotation as a function of change in ionic
strength might give an indication of a possible ordered secondary structure.

No changes in the optical rotation of alginate solutions, over a wide range of salt concentration, were detected when measured at five wavelengths between 365 and 598 mμ. The effect of pH (3.0 to 9.0) on the optical rotation of alginate was investigated, and again no changes were observed. Thus even the rather drastic change from the protonated to unprotonated form did not alter the optical rotation. This behavior would be expected of a polymer with a random coil and not of one with an ordered conformation; however, as will be pointed out in later discussion, these observations do not unequivocally rule out the latter conformation.

Another possible explanation for the high viscosity of alginate solutions may involve their interactions with icelike water clusters of the Nemethy-Scheraga type [22] to form large extensively hydrated macroions. Or perhaps the structure of water is modified in the vicinity of alginate polymers to give rise to ordered lattices in a manner similar to that discussed by Bernal [23]. This possibility was investigated by examination of the infrared absorption of aqueous alginate solutions, as well as the water protons by nuclear magnetic resonance studies.

Nuclear magnetic resonance has been employed as a technique for the study of water-polymer interaction by several workers [12, 24, 25]. In our nuclear magnetic resonance studies on 3% alginate solutions, in the presence and absence of NaCl (0.1 M), no noticeable change in the water proton peak could be detected.

Klotz has pointed out that the absorption of the O-H peak in the near infrared region of 1.4-1.5 μ, in the presence of various solutes, may be used as an indication of water-solute interaction [10]. Peak shifts towards lower wavelengths were observed for less oriented (less hydrogen bonded) systems. Tetramethylammonium chloride (TMA-Cl) has been reported to shift the near infrared peak to higher wavelengths, indicative of an ordering effect on the structure of water [10]. We have confirmed the above effect, with respect to TMA-Cl as well as the disruptive effect of KCl on the oriented structure of water [10], as reflected by a shift towards lower wavelengths (Figure 7). In addition, a small change in the position of the O-H peak was observed with aqueous alginate solutions. At 23° the O-H peak shifts from 1.443 to 1.446 μ in the presence of 4 M TMA, in the presence of 4 M KCl the peak shifts to 1.430, and in the case of a 3% alginate solution the peak was located at 1.444 μ.

Results of associated viscosity studies comparing the effect of KCl and TMA on alginate viscosity are found in Fig. 8. As discussed above, monovalent cations give very similar curves when specific viscosity is plotted against increasing ionic strength. If the degree of water orientation is of
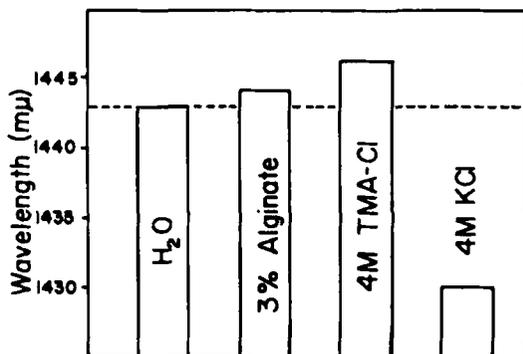
Fig. 7. Effect of KCl and guanidine hydrochloride on the infrared absorption peak at 1.43 μ.
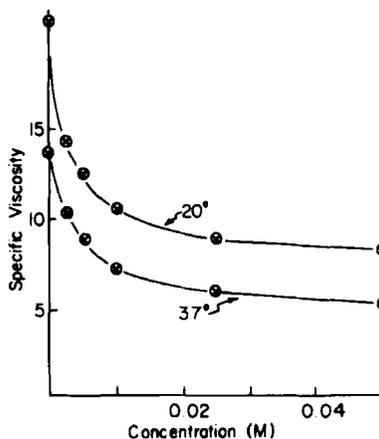
Fig. 8. Effect of temperature and salts on unfractionated Na alginate. Na alginate concentration = 0.25%.

dominant importance with respect to the structure of alginates, one would expect to detect differences between the KCl-alginate and TMA-alginate viscosity profiles. Separate studies were made at 20 and 37.5°. As may be seen from these data, the KCl and TMA curves are identical to one another at both temperatures. Thus, our viscosity, near infrared, and nuclear magnetic resonance studies indicate that alginates do not appreciably effect the degree to which the structure of water is oriented, nor is the viscosity of alginate solutions measurably affected by changes in this orientation.

DISCUSSION

Recent work has clarified a number of the important structural features of alginates. Analysis of the hydrolysis products of reduced methylated alginates have indicated that they are linear polymers [1]. Also, it has been demonstrated that at least some alginates are block copolymers in that they are composed of the following three structural units: a guluronic acid block, a mannuronic acid block, and a heterogenous block [3]. It was reported that the proportion of homopolymer blocks varies among alginates extracted from different sources [26]. Unfortunately, little else is known about the detailed structure of alginates in aqueous solutions.

Light-scattering studies on an alginate sample extracted from <u>Laminaria</u> <u>digitata</u> have been reported by Smidsrød and Haug [15]. The difficulties encountered by these workers in obtaining optically clean alginate solutions were avoided by the use of extreme care in the preparation and handling of all light-scattering solutions. It is evident from a comparison of results that the alginates used in their studies were significantly different from those employed in our own. For example, they experimentally obtain weight-average molecular weights of ~5.0 to 7.0 X 10^5 for alginate samples with intrinsic viscosities of ~10.0 to 13.0 (dl/g), whereas, under equivalent ionic conditions, our unfractionated alginate sample was characterized by $\bar{M}_w$ = 4.07 X 10^5 and $[\eta]$ - 11.0 (dl/g). These discrepancies are not totally un-expected, for as has been pointed out by Haug [6], alginates extracted from different sources and by different methods often have variant chemical com-postions. Also, it is reasonable to expect that these fractions would differ in molecular weight distributions. In addition to having determined the molecular weights and intrinsic viscosities mentioned above, these same work-ers acid degraded an alginate sample into a series of fractions with lower molecular weights and intrinsic viscosities. This allowed them to calculate a value of 1.0 for the exponent "a" of the Mark-Houwink equation, $[\eta]$ = KM^a. An exponent value of unity is generally interpreted as being indicative of a polymer with a "stiff coil," solution conformation [15]. In the case at hand, a similar interpretation would be valid only if the various alignate fractions were part of a homogenous series with respect to chemical com-position; or alternatively, if variations in mannuronic:guluronic acid ratios did not appreciably alter the physical and hydrodynamic properties of alginates. As may be seen from our data (Table 1), the physical and hydro-dynamic properties of alginates do vary with changes in the mannuronic:guluronic acid ratio. The chemical composition of the individual alginate fractions used by Smidsrød and Haug to experimentally calculate the value

of the exponent of the Mark-Houwink equation were not determined. Thus conclusions concerning the "stiffness" or shape of alginate polymers based upon this exponent value are premature. Especially in light of the fact that the method chosen to fractionate alginate into lower molecular weight samples, mild acid degradation would be expected to yield fractions with dissimilar chemical compositions [3].

A consideration of several physical and molecular parameters determined for the L-guluronate rich fraction as compared to the corresponding values of the D-mannuronate rich fraction (Table 1) strongly suggests, under the conditions employed in the determination of these parameters, that the guluronate rich fraction is characterized by a more rigid, extended conformation. For example, the guluronate rich fraction has the lower M.E.L.: $\langle \overline{r^2} \rangle_z^{1/2}$ ratio, indicating that this fraction contains the less flexible polymers, and although the guluronate rich fraction has a lower weight-average molecular weight than does the mannuronate rich fraction, the former is characterized by a higher intrinsic viscosity. Due to the fact that weight-average molecular weights were used in calculating theoretical extended lengths, the validity of our conclusion, with regard to the relative stiffness of the two alginates, is dependent upon the assumption that the polydispersities of the fractions are similar.

It may be postulated that the extra stiffness associated with the L-guluronate rich fraction is attributable to either stronger carboxylate-carboxylate repulsions between adjacent α-linked guluronic acid residues, or the decreased flexibility of this glycosidic linkage due to more extensive steric restrictions than in the case of β-linked mannuronic acid residues. The inference from available x-ray crystallographic data is that carboxylate groups of adjacent, α-linked guluronic acid residues are situated closer together than are those of β-linked mannuronic acid residues [20, 21]. If this is the case, the electrostatic repulsive force between carboxylate groups of neighboring guluronic acid residues would be greater than between contiguous mannuronic acid units, and therefore the guluronic acid rich polymers would be expected to be the more rigid. However, it should be noted that our light-scattering and intrinsic viscosity studies were carried out in the presence of 0.1 M NaCl. As indicated by Figs. 4, 5, and 6, at this salt concentration alginates assume a less extended conformation than in the complete absence of neutral salts. Presumably the sharp decrease in specific viscosity of aqueous alginate solutions concomitant with the addition of neutral salts is due to a collapse of the rigid, extended alginate structure as maintained by charge-charge repulsions between carboxylate groups. Charge-charge repulsions should be minimized under the experimental conditions of our studies by the charge

shielding effect of sodium ions. Thus the more attractive explanation for
the extra rigidity of the guluronic acid rich polymers would be one involving
more extensive steric hinderences to the flexibility of the glycosidic bond
between guluronic acid residues in contrast to the steric restrictions imposed
on the β-linkage connecting D-mannuronic acid residues.

The rheological behavior of the alginate fractions, in the presence of
varying concentrations of a variety of cations, was found to be similar in all
but one instance. The guluronic acid rich fraction was precipitated by slight
concentrations of manganous ions, whereas the mannuronic acid rich fraction
was not precipitated even at concentrations up to 0.1 M (Figs. 5 and 6). It
is known that alginates are precipitated by manganous ions through the
formation of cation complexes involving two carboxyl groups of one macro-
molecule and two vicinal hydroxl groups of another [27]. Since both hy-
droxyl groups involved in the complex are on the same uronic acid residue,
it is probable that the specificity of the manganous ion facilitated precipitation
is determined by the orientation of the two carboxyl groups involved in the
cross-linkage. Given the difference in chemical composition of the two
fractions, one may speculate that the two carboxyl groups involved in the
alginate-manganous ion complex are located on adjacent guluronic residues.
Regardless of the exact mechanism whereby polymers rich in guluronic acid
are selectively precipitated by manganous ions, the fact that the two fractions
are differentially soluble in the presence of manganous ions serves to further
emphasize the dependence of the physical properties of alginates upon their
chemical compositions.

The question arises, especially in light of the high intrinsic viscosities of
alginates and their ability to form gels, as to whether the rather rigid, extended
structure attributed to alginates [15] is determined solely by charge-charge
interactions. For this reason it was of interest to investigate both the effect
of alginates on the structure of water and the role alginate-solvent interactions
play in maintaining the macromolecular configuration of this polymer.

The effects of alginates on the O-H absorption peak of water in the near
infrared (1.4-5 μ) were used as a criteria for evaluating the effects of these
polymers on the "ordered" structure of water. Surprisingly, an extremely
viscous alginate solution (3%) had only a slight effect upon the position of
this peak. In nuclear magnetic resonance studies on aqueous nucleic acid
[24] and starch solutions [25], as well as agar gels [12], a broadening of
the resonance signal of water protons has been interpreted as being indicative
of a polymer-induced increase in the structural "order" of water. In agree-
ment with the observations of others on the effect of pectic acid [12] (a
polygalacturonic acid polymer) and hyaluronic acid [28] on the water proton

signal, a broadening of this peak was not observed with aqueous alginate solutions. Thus our nuclear magnetic resonance and infrared results suggest that alginates at best have only a slight "ordering" effect on the structure of water.

As mentioned previously, alginate viscosity profiles in the presence of an ordering or disruptive agent $[(CH_3)_4N^+, K^+]$ with respect to the hydrogen bonded structure of water are identical. Furthermore, if a comparison of the reduced viscosities of these alginate solutions were extended to higher ionic strengths, conditions under which the effect of these agents on the "ordered" structure of water would be more pronounced, still no differences were observed. Consequently, it may be tentatively concluded that changes in the macromolecular configuration of this polyelectrolyte are due primarily to charge interference (either charge shielding or ion binding) by the cations used in this study.

A number of interesting observations suggest that alginates may be characterized by an ordered secondary structure. In addition, it has recently been reported that a certain percentage of mannuronic acid residues are protected by conformational features of the polysaccharide chain from cleavage by an enzyme, specific for β-1,4-mannuronic acid linkages [16]. Our observation that the optical rotation of alginates remained constant between the wavelengths 365 to 586 mμ over a wide salt concentration and pH range is inconsistent with the hypothesis that alginates have an ordered or partially ordered secondary structure. However, this evidence is certainly insufficient to categorically rule out this possibility. It is conceivable that the conditions used were not sufficiently rigorous to disrupt this hypothetical ordered structure; or alternatively, that disruption of this secondary structure does not alter the optical rotation of alginate solutions at the five wavelengths studies. This question is being further investigated by means of optical rotatory dispersion and circular dichroism studies, the results of which will be published elsewhere at a later date.

The studies described in the present paper do not support the hypothesis that the solution conformations of alginates involve an ordered secondary structure or are due to extensive water-alginate interactions. We have suggested that alginates rich in guluronic acid are characterized by a more rigid structure, in the presence of sodium cations, than are those abundant in mannuronic acid. It is evident from the $\bar{M}_w$, $[\eta]$, $(\langle \bar{r}^2 \rangle_z^{1/2})$ values, and rheological behavior (under various ionic conditions) that the alginate fractions of differing composition used in our studies vary with respect to their physical and hydrodynamic properties. This heterogeniety makes it difficult to draw general conclusions on the structure of alginates.

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