A Study of the Chain Stiffness and Extension of Alginates, in Vitro Epimerized Alginates, and Periodate-Oxidized Alginates Using Size-Exclusion Chromatography Combined with Light Scattering and Viscosity Detectors

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A series of alginates isolated from the stem and leaf of a brown algae (*Laminaria hyperborea*), bacterial mannuronan, in vitro epimerized mannuronans, and periodate oxidized alginates were analyzed by size-exclusion chromatography (SEC) combined with online multiangle laser light scattering (MALS) and viscometry (collectively abbreviated SMV). Selected samples were also analyzed off-line using low-angle laser light scattering and capillary viscometry. Excellent agreement between the two methods was obtained for properly purified samples. In contrast, abnormal results were obtained for some industrial samples due to the presence of particulate material. Naturally occurring alginates and in vitro epimerized mannuronans were found to obey essentially the same R_G-M and $[\eta]-M$ relations, and hence, the same Mark−Houwink−Sakurada (MHS) equations (valid for $I = 0.10$ M): 20 000 g/mol $< M <$ 100 000 g/mol, $[\eta] = 0.0054 \cdot M^{1.00}$; 100 000 g/mol $< M <$ 1 000 000 g/mol, $[\eta] = 0.071 \cdot M^{0.89}$. Application of the wormlike chain model to the $[\eta]-M$ data obtained by SMV yielded persistence lengths (q) of 15 nm for all alginates at an ionic strength of 0.17 M. Intrinsic viscosities corresponding to infinite ionic strength were estimated on the basis of Smidsrød's *B*-parameter, and the wormlike chain model then yielded $q = 12$ nm. Periodate oxidized alginates showed, in contrast, a pronounced decrease in persistence length with increasing degree of oxidation, reaching values below 4 nm at 44% oxidation. Periodate oxidation also resulted in some depolymerization, even in the presence of a free-radical scavenger.

Introduction

Alginates are linear (unbranched) polysaccharides which are produced by brown algae (*Laminarales*)[1] and some bacteria (*Azotobacter vinelandii*, *Pseudomonas aeruginosa*, and others). The monomers are residues of 4-linked β-D-mannuronic acid (M) and its 5-epimer, 4-linked α-L-guluronic acid (G) (Figure 1).

The relative content and intrachain arrangement (sequence) of M and G varies considerably, depending on the action of enzymes (C5-epimerases) which convert homopolymeric mannuronan into functional alginates. Different epimerases give rise to different epimerization patterns, varying from the formation of contiguous G-blocks (...GGGGG...) to elements of strictly alternating sequences (...MGMGMGMG...), as demonstrated for the enzymes AlgE1−AlgE7 from *A. vinelandii*.[2,3]

Different alginates have very different physical and biological properties.[4] The G-blocks are the primary structural elements involved in the cooperative binding of Ca^{2+} which induces chain dimerization and subsequent gelation to give Ca-alginate gels. Alginates with a high content of long G-blocks, such as alginate from the stipe of *L. hyperborea*, are generally preferred when high gel strength is needed. Moreover, high-G alginates are relatively non-immunogenic and are preferred as immobilization materials in, e.g., cell therapy or tissue engineering.[5] In contrast, alginate with a very low content of randomly distributed G-residues ($F_G < 0.1$, $N_{G>1} = 0$), such as alginate from *P. aeruginosa*, is highly immunogenic. The role of M-blocks and alternating sequences in the gelling process is not entirely clear. In Ca-alginate gels, they are assumed to function as non-gelling segments. However, conversion of M-blocks to poly-MG-blocks by in vitro epimerization of gelling alginates leads to pronounced changes in the gelling behavior with calcium, primarily by increasing the degree of syneresis. This has been attributed to the higher flexibility of MG-blocks.[6] Recent findings[7] indicate

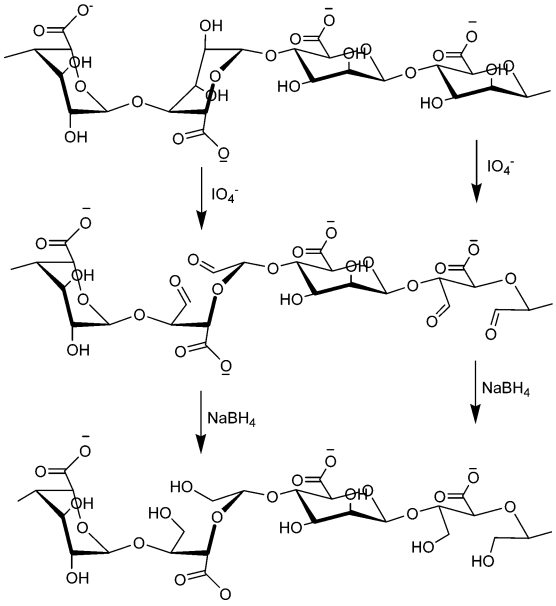

Figure 1. Chemical structure of an alginate fragment (..GGMM..) before and after partial periodate oxidation (oxidation occurs randomly) and the corresponding structure obtained after aldehyde reduction. Abbreviations: M, β-D-mannuronic acid; G, α-L-guluronic acid.

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that MG-blocks also bind calcium and may form junctions with G-blocks as well as secondary MG/MG junctions, accounting for the observed syneresis.

Experimental studies of the physical properties of alginates with different chemical compositions have mostly been restricted to samples that can be obtained directly from algal or bacterial sources or by time-consuming fractionation steps. Recently, mannuronan devoid of any G-residues has become available from C5-epimerase negative mutants of *Pseudomonas*.[8] Moreover, novel alginates may be produced from mannuronan (or any alginate) by in vitro epimerization by one or several of the seven epimerases (AlgE1−AlgE7) from *A. vinelandii*, which have been closed and expressed in *E. coli*.[2,3] In the present study, we study pure mannuronan, a polyalternating alginate (F_G = 0.45) obtained by epimerization with AlgE4, and high-G alginates (F_G = 0.86 and $F_G \geq 0.95$) obtained by epimerization with AlgE6.

The dilute solution properties of alginates reflect the flexibility and extension of the chains, which are usually considered to be semiflexible, with reported unpertubed dimensions (Kuhn lengths) in the range 14−16 nm.[9] It was established by Smidsrød about 30 years ago that the relative extension of the three types of "blocks" in alginate increased in the order "MG-blocks" < "MM-blocks" < "GG-blocks".[9] This conclusion was first reached on the basis of classical light scattering measurements, by comparing the *z*-average radius of gyration ($R_{G,z}$) with the weight average molecular weight (M_w) for alginates of different chemical compositions (Table 1 of ref 9). Alginates with a high G-content and long G-blocks (e.g., alginate from *L. hyperborea* stipe) appeared to be more extended than alginates with intermediate compositions. Apart from this, there exists no systematic study of the R_G-M relationships in different alginates.

A second and independent experimental approach to the study of chain stiffness in polyelectrolytes was introduced by Smidsrød and Haug.[10] On the basis of studies of the ionic strength (I) dependence of the intrinsic viscosity of polyelectrolytes, they introduced the empirical *B*-parameter. A linear correlation between log B and log l_K (Kuhn length) was observed for a wide variety of water-soluble polyelectrolytes ranging from polyphosphate to DNA. When applied to alginates, the method confirmed the general picture obtained by light scattering. Of particular interest was the relatively high *B*-parameter (and hence, high flexibility) of an acid-soluble alginate fraction with intermediate G-content (F_G = 0.36) and with a high content of MG-blocks. Recently, *B*-values for a series of in vitro epimerized alginates was reported.[8] The findings were generally in agreement with those of Smidsrød and Haug, with the notable exception that the *B*-parameter of an essentially polyalternating alginate was only slightly higher (0.038) than mannuronan (0.033) or alginates with long G-blocks (B = 0.032−0.036). This contrasts a value of 0.065 obtained for an acid-soluble alginate fraction dominated by polyalternating structures.[10]

A third approach to the question of relative chain extensions is computer modeling studies. The initial calculations by Smidsrød and Whittington[11] again confirmed that the relative flexibility of the glycosidic linkages in alginates decreased in the order MG < MM < GG. Stokke et al.[12] extended these calculations in a Monte Carlo model which incorporated the sequence of monomers in naturally occurring alginates according to a second-order Markov model. The predicted chain extensions, expressed as the characteristic ratio (C_∞), were also in this case qualitatively consistent with the data obtained by other methods. The model predicted a C_∞ of about 70 for pure mannuronan (F_G = 0), increasing to about 100 for typical *L. hyperborea* stipe alginate and finally a steep increase to a value of about 175 for a (hypothetical) alginate with F_G = 1. It also predicted a relatively low value of about 50 for a strictly polyalternating alginate. More recently, Braccini et al.[13] performed simulations on polyuronides, including mannuronan (β-D-(1→4)-polymannuronic acid) and guluronan (α-(1→4)-polyguluronic acid). The calculated characteristic ratios were 43 and 91, respectively. The corresponding persistence lengths (l_p) were 11.9 and 21 nm. The C_∞ values are about half of those obtained by Stokke et al., presumably reflecting different calculation parameters (force fields).

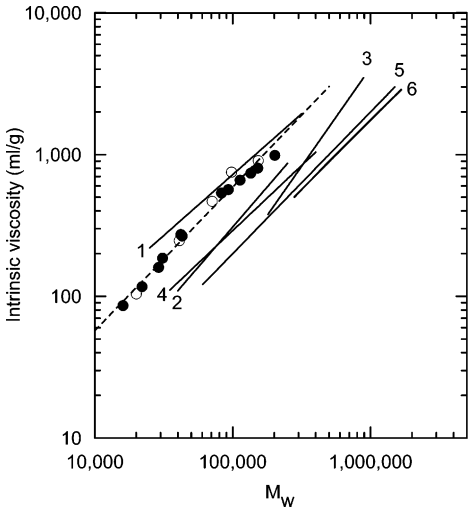

Figure 2. Molecular weight dependence of the intrinsic viscosity for various alginates−literature data: 1, ref 16; 2, ref 14 (*L. hyperborea* alginates); 3, ref 15 (G-fractions); 4, ref 14 (*M. pyrifera* alginates); 5, ref 18; 6, ref 15 (G-fractions). Present study, results obtained by static LALLS and conventional viscometry for alginates from *L. hyperborea* stipe (●) and leaf (○).

Analyses of the relationship between the intrinsic viscosity ([η]) and the molecular weight represent, together with the R_G-M relationship, a classic basis for studies of solution properties, from which stiffness analyses can be carried out on the basis of a variety of theoretical models. However, a survey of the literature shows that determination of the molecular weight and/or the intrinsic viscosity of various alginates is often far from trivial. Figure 2 reproduces a series of [η]−M_w relationships of different alginates that have been presented in the literature.[14−18] [η] was generally obtained by conventional capillary viscometry, and M_w was determined by classical light scattering or sedimentation equilibrium. The literature data vary considerably, and readily identifiable consensus relationships cannot be obtained. In practice, calculated molecular weights may vary within a factor of 3, depending on which set of data is being used. The data do not allow a good basis for determining the influence of the chemical composition on the [η]−M_w relationship. In some of the light scattering studies, the presence of particulate impurities was observed.[15,18] Their influence on the measurements could be partially eliminated by a combination of ultracentrifugation and filtration. Still, a curvature in the angular dependence of $(K_c/R_\theta)^{-1}$ was observed at low angles due to remaining particles, making the precise extrapolation to zero angle more uncertain. In another study, apparently normal light scattering behavior of unaggregated alginates was reported, provided the samples were thoroughly purified by membrane filtration.[16] Thus, variable literature data reflect differences in sample quality and purification, as well as data processing. Slightly different analysis conditions (temperature, ionic strengths,

shear rates) were further used, although they were generally within ranges where such differences are expected to play only minor, or even negligible, roles. An additional goal of the present study is to determine the $[\eta]-M_w$ relationships for a wide range of alginates under identical experimental conditions, preferentially without the influence of aggregates.

Periodate oxidation is a well-established method for introducing additional flexibility in polysaccharides. In (1→4)-linked polysaccharides such as alginates, the periodate ion cleaves the C2−C3 linkage forming the corresponding dialdehyde (Figure 1).[19] Due to intramolecular hemiacetal formation, an oxidation limit of about 44% occurs in alginates,[20] although subsequent reduction of the hemiacetals allows further oxidation. The C2−C3 cleavage results in a dramatic increase in the flexibility of the oxidized residues because of the ring opening. As a consequence, the alginate becomes more flexible, as demonstrated experimentally by a large increase in the *B*-parameter.[21] An additional feature of partially periodate oxidized alginates is that, following aldehyde reduction with $NaBH_4$, the linkages of oxidized residues become very sensitive to acid hydrolysis. This is in fact the general basis for the classical Smith degradation traditionally used in the structural analysis of complex carbohydrates[22] but may also be used to obtain alginates which are more rapidly degraded in vivo.[23] In a recent study, Lee et al.[24] reported the partial (0−85.6%) periodate oxidation of a G-rich, low M_w fragment obtained by partial hydrolysis of a commercial alginate followed by precipitation at low pH. The samples (M_w ranging from 7100 to 5900 Da) were studied by SEC combined with on-line light scattering and viscometry. Although the estimates of the degree of oxidation were not consistent with the existence of an oxidation limit, the estimates of the persistence length (based on the wormlike chain model) showed a marked decrease with increasing degrees of oxidation. However, the reported low estimates of the persistence lengths (5.2−1.9 nm) have recently been questioned.[7]

The combination of SEC with multiple detectors has several advantages over classical approaches such as "stand-alone" light scattering or viscometry, and has in recent years been widely used for a variety of water-soluble polysaccharides. In addition to the experimental simplicity allowing multiple, automated analyses, it combines fractionation and analysis. "Slice-by-slice" processing of polydisperse samples provides both the $R_{G,i}-M_i$ relationship (provided R_G is sufficiently large, generally $>\lambda/20$) and the $[\eta]_i-M_i$ relationships, where *i* refers to the elution slice number. Another advantage is that particles, aggregates, or other inhomogeneities may be excluded from the data analysis provided they elute in a confined region, usually near the exclusion limit or close to the salt peak. If the data are to be compared with results from static measurements, some additional criteria must be met. First, the columns should separate effectively across the entire chain length distribution and avoid material eluting in the void volume or overlapping with the salt peak, allowing complete sample recovery. For linear polymers such as alginates, it is possible to adjust the molecular weight distribution by partial depolymerization to fit a selected column set. Second, sample loadings must be appropriately determined to avoid overloading effects (viscous tunneling) but allowing the best possible signal-to-noise ratio, which in any case may be low in the low molecular weight tail. Interdetector volumes must be accurately determined, and band-broadening arising from multiple detectors should be corrected for, although these effects are usually only important for samples of low polydispersity. The method for fitting and extrapolating the angular dependence of the scattered light should be critically evaluated in each case.[25] The estimates of the molecular weight distributions and averages may sometimes depend strongly on the method selected fit of the experimental log M_i-V_i relationships to a standard function (e.g., linear or experimental fits), which is used to extrapolate M_i in the low molecular weight tail where the intensity of the scattered light becomes too weak for precise measurements. This influences, in particular, the determination of M_n. It seems from the literature that most investigators choose a linear (first-order polynomial) fit. SEC combined with on-line light scattering and viscosity detectors has previously been used to study solution properties of polysaccharides such as cereal β-glucans,[26] hyaluronan,[27] or scleroglucans (linear and cyclic species),[28] and the method is therefore in many cases well-established.

The present study involves an experimental reexamination of the $M-[\eta]$ and $M-R_G$ relations of a wide range of alginate standards, in vitro epimerized alginates, as well as periodate-oxidized alginates. The standards include alginate PT-180, which is a preparation of ultrapure alginate isolated from the stem of *L. hyperborea*. It has been used as an immobilization agent in clinical trials with human pancreatic islets for treatment of diabetes.[29] Because of its purity (no particulate material), its intermediate range of molecular weights (M_w ca. 200 000 Da), and its availability, it was considered ideally suited for SMV analysis. Further standards covering different molecular weight ranges are obtained by partial acid hydrolysis of the parent samples. These were used to validate the analyses by comparison to independent results obtained by static LALLS (obtaining M_w and A_2) and conventional intrinsic viscosity measurements.

Pullulan and scleroglucan were included as reference materials, because they represent examples of well-defined structures with well-understood solution properties which contrast those of alginates. Pullulans, consisting of maltotriose repeating units linked by α-1,6 linkages, have been extensively studied in the literature[30−34] and are also much used as calibration standards in SEC. These linkages lead to locally high flexibility, resulting in typically random coil behavior in solution with an estimated characteristic ratio (C_∞) of 4.3, corresponding to a persistence length of 1.3 nm.[31] Scleroglucan consists of tetrasaccharide repeating units, each unit consisting of three (1→3)-linked β-D-glucose residues with a single (1→6)-linked β-D-glucose residue side chain. Scleroglucan forms an extremely stiff triple-stranded structure in solution, with a persistence length in the range of 150 nm.[28]

Experimental Section

Materials. Pullulan standard P-1390 was obtained from Hayashibara Biochemical Laboratories, Japan. All alginates were prepared in our laboratories according to standard methods. Partially periodate oxidized alginates were prepared, analyzed for residual periodate (after oxidation), and reduced essentially as described by Smidsrød and Painter. [21]

Intrinsic Viscosity Measurements. Measurements were performed at 20.0 °C in a Ubbelohde capillary viscometer (Schott-Geräte capillary no. 0a) equipped with an AVS 310 control unit and PC-operated titrator for automatic and sequential dilution with solvent, as well as for automatic data acquisition and calculations. The solvent flow-through time was 212 s, corresponding to an average shear rate of 110 s^{-1}.

Static Low-Angle Laser Light Scattering (LALLS). Measurements were carried out on a Chromatix KMX-6 light scattering photometer as described earlier,[26,35] using 0.05 M Na_2SO_4 containing 0.01 M EDTA, pH 6, as solvent.

Refractive Index Increment ($dn/dc)_\mu$. $(dn/dc)_\mu$ was determined for a purified sample of mannuronan and an alginate from *L. hyperborea*

stipe. The samples (concentration series) were extensively dialyzed against the Na_2SO_4/EDTA solvent (described above) prior to analyses, which were carried out at $\lambda_0 = 633$ nm on an Optilab DSP differential refractometer (Wyatt, U.S.A.). The alginate concentration was determined by the phenol−sulfuric acid method.[36] $(dn/dc)_\mu$ was found to be 0.151 ± 0.003 mL/g and 0.149 ± 0.005 mL/g for the two samples. An average value of 0.150 mL/g was therefore used in all light scattering measurements. This value is in excellent agreement with literature data for other types of alginates.[18]

Size-Exclusion Chromatography (SEC) with Online Multiangle Laser Light Scattering (MALS) and Viscometry (VISC). Measurements were carried out at ambient temperature on an HPLC system consisting of a solvent reservoir, on-line degasser, HPLA isocratic pump, autoinjector, precolumn, and three columns (serially connected) of TSK G-6000PWXL, 5000 PWXL, and 4000 PWXL. In some experiments, only one or two of these columns were used. The column outlet was connected to a Dawn DSP multiangle laser light scattering photometer (Wyatt, U.S.A.) ($\lambda_0 = 633$ nm) followed by Optilab DSP differential refractometer (P-10 cell) and finally a Viscotek TDA 301 viscosity detector. The flow rate was 0.4 mL/min when the viscometer was connected; otherwise, it was 0.5 mL/min. The injection volume was 100−250 μL, and the sample concentration was adjusted to obtain the best possible light scattering signal without influencing the RI profile (overloading). Samples were filtered (pore size 0.22 or 0.45 μm) prior to injection. Data from the light scattering and the differential refractometers were collected and processed using Astra (v. 4.70.07) software (Wyatt, U.S.A.), whereas data from the viscometer were collected and processed by the TriSec (v. 3.0) software. Data from both software packages (slice results) were exported to an Excel spreadsheet for further processing.

Results

Static LALLS and Capillary Viscometry for Alginates from the Leaf and Stem of *L. hyperborea.* A series of alginate standards were prepared by partial acid hydrolysis, starting with purified alginates obtained from *Laminaria hyperborea* stipe and leaf, respectively. These were first analyzed by static LALLS to obtain the weight average molecular weight (M_w) and the second virial coefficient (A_2) and by conventional capillary viscometry to obtain the intrinsic viscosity and Huggins' constant (k'). For LALLS, we used the same solvent as in SEC-MALS (0.05 M Na_2SO_4 containing 0.01 M EDTA, pH 6), whereas the viscometry was carried out with 0.1 M NaCl as solvent ($T = 20$ °C), which has traditionally been used in most previous investigations. A double-logarithmic plot of $[\eta]$ as a function of M_w is included in Figure 2. The tendency for differences between alginate from the stipe and leaf of *L. hyperborea* is most likely insignificant, and for practical purposes, the data were fitted to a single MHS equation

$$[\eta] = 0.00504 \cdot M_w^{1.01} \quad (1)$$

The average value of A_2 in these experiments was 6.7 $(\pm 1.0)\cdot 10^{-3}$ mL·mol·g^{-2}. No molecular weight dependence could be accurately determined due to the scatter in the data.

Developing the SEC−MALS−VISC (SMV) Method for Alginates. Initial SMV studies were carried out on four of the alginate standards and, for the sake of comparison, a pullulan with high molecular weight (P-1390, polydisperse). Results obtained in the same experimental setup and identical analysis conditions have previously been reported for three barley β-glucans[26] and linear and cyclic scleroglucan.[28] All samples displayed normal chromatographic properties and provided good experimental data for M, R_G, and $[\eta]$ over a wide range of molecular weights. They all gave rise to linear plots of K_c/R_θ versus $\sin^2(\theta/2)$ through the entire chromatographic profile, enabling data processing according to the Zimm formalism (first-order linear fit).

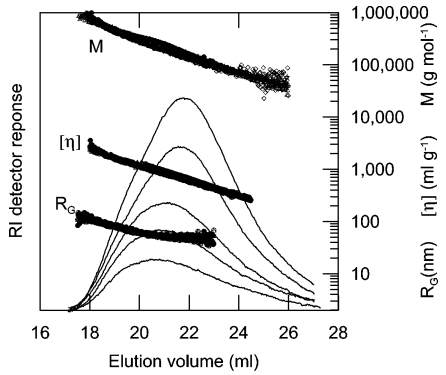

Figure 3. SMV analysis of an ultrapure alginate from *L. hyperborea* stipe (PT-180) at different concentrations.

Figure 3 shows results obtained for different concentrations of PT-180. The distorted peaks at high concentrations are ascribed to a general overloading of the columns. However, a slight shift toward higher elution volumes with increasing sample loading was observed also for the lowest concentrations. This phenomenon was actually observed for a wide range of different polyelectrolytes (data not shown) but was not observed for neutral polysaccharides within the same range of concentrations and molecular weights.[26] We found, however, that with light scattering detection the calculated weight average molecular weight (M_w) was essentially independent of the sample concentration and even the degree of peak distortion. The figure also illustrates that for $V > 25$ mL no reliable data could be obtained except for the RI detector. This is a general phenomenon in SEC-LS and is generally circumvented by extrapolation to obtain estimates of molecular weights in this region. This is particularly important for the determination of M_n.

In some other samples, an upturn in the log $M-V$ curve was observed near the void volume (e.g., Figure 6). This may be due to either the incomplete separation of the largest molecules (elution in, or close to, the void volume) or the presence of aggregates, or both. In most cases, it could be assumed that the upturn was due primarily to incomplete separation, as no signs of deviating geometries were indicated in the R_G-M curve. As the commercial software could not fit data to models which incorporated both the upturn and at the same time extrapolated properly in the low-M region, a "split-peak" fitting procedure was therefore adapted. The elution volume (V') below which the log $M-V$ curve is linear was first identified, typically $V' = 19$ mL. For $V < V'$, unfitted data were used, whereas for $V > V'$, data were fitted to a straight line. This procedure was generally used unless otherwise stated. For PT-180, we then obtained $M_w = 200\,000 \pm 3\,300$ Da, $M_n = 99\,000 \pm 3000$, and $M_w/M_n = 2.0 \pm 0.1$ ($n = 4$). The molecular weight is in perfect agreement with the value obtained by static LALLS (202 000 Da).

Figure 4 shows corresponding results for the three other standards obtained from *L. hyperborea* stipe, which had been partially acid-hydrolyzed to obtain samples of identical chemical compositions but with different molecular weight distributions, with M_w of 297 000, 161 000, and 36 000, respectively. Increasing sample concentrations were employed with decreasing molecular weights to maintain as high a light scattering signal as possible without entering a regime with distorted peaks. Generally, this procedure extended the range of molecular weights and intrinsic viscosities available for subsequent

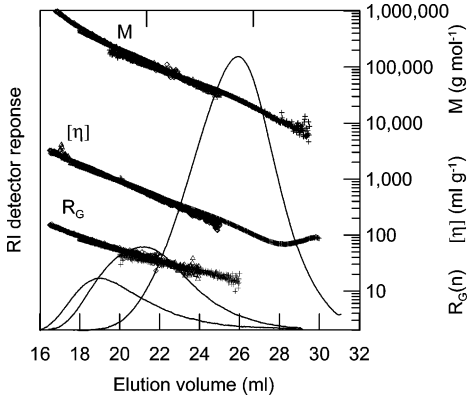

Figure 4. SMV analysis of an alginate from *L. hyperborea* stipe (S-series) that has been partially hydrolyzed to M_w of 297 000, 161 000, and 36 000.

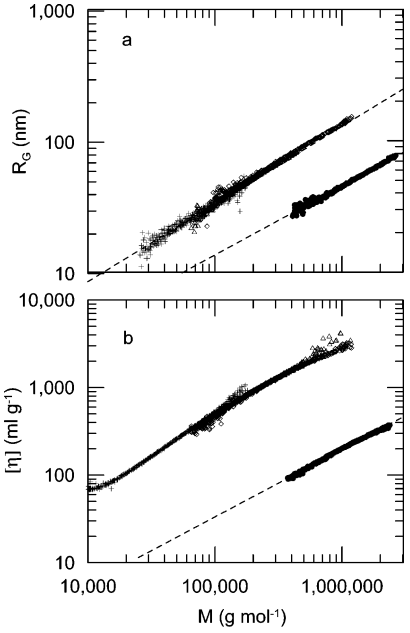

Figure 5. Molecular weight dependence of R_G (a) and [η] (b) for alginates (data from Figure 4) and pullulan P-1390. Results obtained independently by static LALLS (M_v, see text) and conventional capillary viscometry are included for comparison (○).

analysis. In contrast, the range of R_G values was only marginally extended due to the inherent limitation in light scattering (R_G approaching $\lambda/20$).

Figure 5a shows double logarithmic plots of $R_{G,i}$ versus M_i (unfitted data) for the *L. hyperborea* stipe alginates. Corresponding results obtained for pullulan P-1390 are included for comparison.

The plots were essentially linear for both polysaccharides. The linear parts of the figure were fitted according to the standard scaling relation, obtaining

$$\text{Alginate: } R_G = 0.0352 \cdot M^{0.60} \quad (2)$$

$$\text{Pullulan: } R_G = 0.0133 \cdot M^{0.58} \quad (3)$$

The exponent obtained for pullulan is in good agreement with the literature.[31−34]

Figure 5b gives the corresponding double logarithmic plots of [η] versus M. Due to the use of alginates in different molecular weight ranges, acceptable data in the molecular weight range from 20 000 g/mol to about 1 000 000 g/mol is obtained.

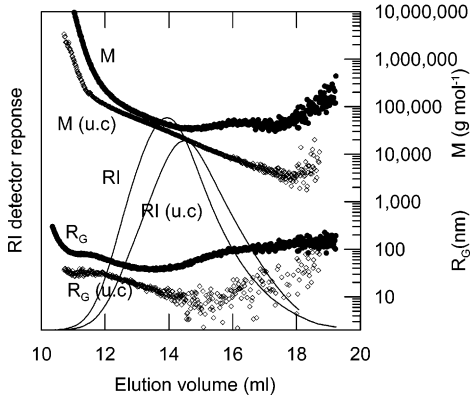

Figure 6. SEC-MALS analysis of a low molecular weight industrial alginate, before and after ultracentrifugation (u.c). Data: RI detector signal, molecular weight, and R_G (nm).

The results are in fair agreement with those obtained by static LALLS, but data from the extended molecular weight range clearly demonstrate that the curves are not strictly linear. In addition, the intrinsic viscosity was observed to level off toward the lowest molecular weights (M < 20 000). This observation could be reproduced in parallel analyses of the partially hydrolyzed *L. hyperborea* stipe alginates but was not observed with other samples. Due to the curvature above a molecular weight of 20 000, a single MHS relation could not be used. This observation is indeed similar to that obtained for hyaluronan,[27] and for practical purposes, we divide the curve into two regions, obtaining the MHS relationships

$$20\,000 < M < 100\,000\text{: } [\eta] = 0.0054 \cdot M^{1.00} \quad (4)$$

$$100\,000 < M < 1\,000\,000\text{: } [\eta] = 0.071 \cdot M^{0.89} \quad (5)$$

The curvature at high molecular weight may in part be ascribed to the influence of non-Newtonian behavior, since the on-line viscometer operates at rather high shear rates, but is possibly also the effect of changes in the degree of draining.[27]

Alginate-Containing Particulate Impurities. Figure 6 shows elution profiles and corresponding M and R_G data obtained with an industrial alginate sample originating from *L. hyperborea* stipe. We included this sample because it displays a feature sometimes seen in polysaccharides, namely, an apparent increase in M and R_G with increasing elution volume.

Clearly, the material eluting late differs from normal, dispersed alginate chains. Most likely, the sample contains small amounts of particulate material which elute together with the low molecular weight tail. By treating such samples by ultracentrifugation (100 000 *g* for 2.3 h), this artifact in most cases disappeared, indicating that the particles were denser than the alginate. The presence of such particles has been described earlier.[18] Careful purification is thus a prerequisite for studying alginates as well as other polymers using SEC-MALS.

Mannuronan and in Vitro Epimerized Mannuronans. Homopolymeric mannuronan was studied by SMV together with mannuronan subjected to in vitro epimerization using the recombinant mannuronan C5-epimerases. The processive enzyme AlgE4 was used to obtain an almost strictly polyalternating alginate (F_G = 0.46),[37] and the enzyme AlgE6 was used to produce two alginates with exceptionally high contents of L-guluronic acid (F_G = 0.86 and ≥0.95) and extremely long G-blocks.[37] In addition, some samples were partially hydrolyzed to obtain additional data in the lower molecular weight range. The elution properties and basic data (M, R_G, and [η] as a function of elution volume) were qualitatively quite similar to

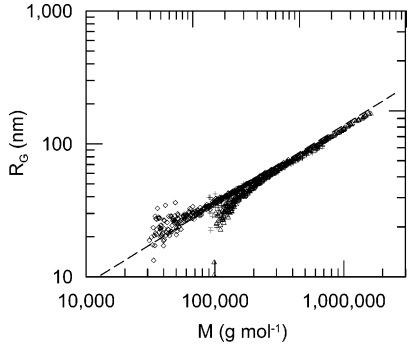

Figure 7. Molecular weight dependence of R_G obtained by SEC-MALS for mannuronan (●), polyalternating alginate (AlgE4 epimerized mannuronan, $F_G = 0.45$) (◇), and mannuronan epimerized by AlgE6 to obtain $F_G \geq 0.85$ (△). The line represents fitted data for alginates from *L. hyperborea* stipe (S-series) taken from Figure 5a (eq 2).

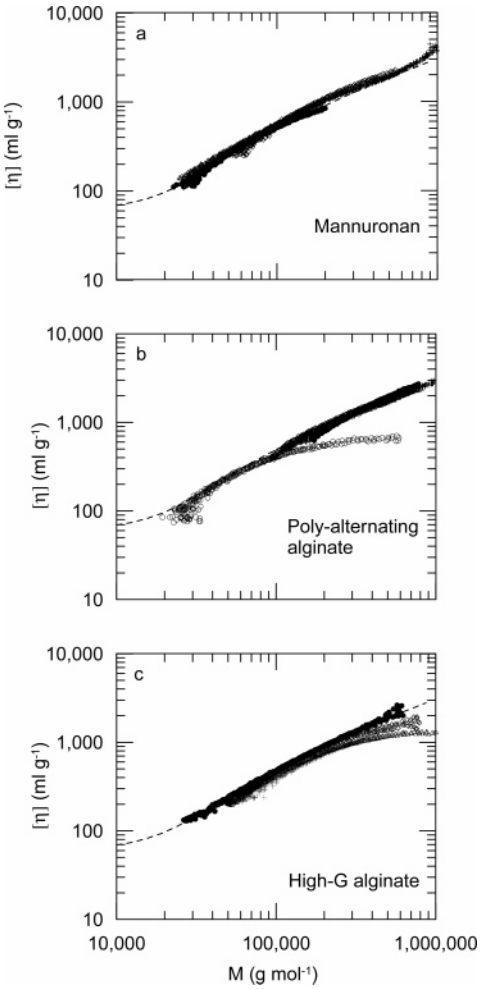

Figure 8. Molecular weight dependence of [η] (MHS plot) obtained in different experiments for mannuronan (a), polyalternating alginate (AlgE4 epimerized mannuronan, $F_G = 0.45$) (b), and mannuronan epimerized by AlgE6 to obtain $F_G \geq 0.85$ (c). The dotted line corresponds to fitted data from Figure 5b (*L. hyperborea* stipe (S-series)). Different symbols represent different analyses.

those of the *L. hyperborea* alginates and are therefore not shown. The corresponding R_G-M and [η]$-M$ plots are given in Figures 7 and 8, respectively.

The range of R_G data is somewhat limited due to the accessible molecular weight range. Moreover, the R_G-M data for epimerized mannuronans tended to vary slightly between different experiments. The reason for this variability is tentatively ascribed to insufficient purity. Nevertheless, it is immediately noted that the data for different alginates are more or less overlapping. For some of the partially degraded samples, a leveling of the R_G data at high M can be observed, suggesting partial aggregation. Otherwise, the data roughly overlay those shown in Figure 5a, and eq 2 most likely applies in all cases.

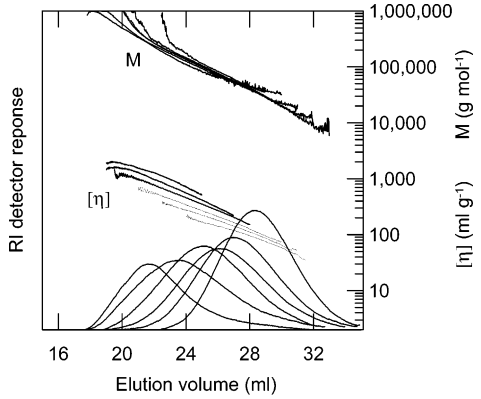

Figure 9. SMV analysis of periodate oxidized alginate. $P_0 = 0\%$, 5%, 10%, 20%, and 44%.

The [η]$-M$ relations (Figure 8) also include results from different experiments for each sample, illustrating the variability of the method. Again, the results are not quite as reproducible as those obtained for the *L. hyperborea* stipe alginates. The largest variation is found for the high-G alginates, especially at high molecular weights. Partial hydrolysis of the polyalternating alginate extended the range of experimental data in the low-M region, but for this sample, [η] leveled off at high M tail (corresponding to less than 10% of the material). This artifact is probably caused by some aggregation. Nevertheless, all data except the deviating tails overlapped fairly well those of the *L. hyperborea* alginates (dotted line included in the figures), suggesting that eq 3 applies to all these alginates. A possible exception may be seen for the high-G alginate. Data corresponding to the average of four different experiments are 10−20% below those of *L. hyperborea* stipe.

Periodate-Oxidized Alginates. An alginate isolated from the leaf of *L. hyperborea* ($F_G = 0.55$) and mannuronan were both subjected to oxidation with periodate to various extents by varying the periodate−alginate ratio. The effective degree of oxidation (D_{ox} = fraction of oxidized residues) was determined by titration of residual periodate. After reduction with $NaBH_4$ to convert the aldehydes/hemiacetals to the corresponding alcohols, they were subjected to SMV analysis. The results (elution profile, M, and [η]) are given in Figure 9.

It is immediately noted by the shift in elution profiles and the corresponding M_w values (decreasing from 260 000 g/mol before oxidation to 48 000 after 44% oxidation) that periodate oxidation is accompanied by depolymerization. This phenomenon is well-known and is ascribed to side-reactions involving free radicals produced by decomposition of periodate.[38] The presence of 10% *n*-propanol during the oxidation was in this case not sufficient to eliminate the depolymerization. Because of the depolymerization, the chains became too short for the determination of R_G, and further analysis resided solely on the [η]$-M$ relationship shown in Figure 10.

In contrast to the epimerized samples, large differences in the intrinsic viscosity were observed. For example, at a molecular weight of 100 000, a 44% oxidation led to an approximately threefold decrease in intrinsic viscosity. The slope of the curves, corresponding to the MHS exponent, also decreased from 1.00 before oxidation to 0.65 for 44% oxidation.

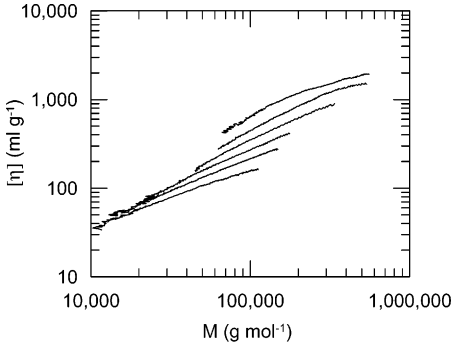

Figure 10. The molecular weight dependence of [η] for periodate oxidized alginates. P_0 = 0%, 5%, 10%, 20%, and 44% (top/down).

Analyses at Higher Ionic Strength (1.01 M). Because of the pronounced effects of the ionic strength on the shape and extension of charged polymers in general, attempts were made to carry out SMV analysis in 0.33 M Na_2SO_4, corresponding to an ionic strength of 1.0 M, as compared to 0.17 M in all other SEC analyses presented here. Although alginates tend to precipitate at very high ionic strengths, no signs of precipitation were indeed observed. Qualitatively, there was little difference in the results obtained at 1.0 and 0.17 M. The ratio between the RI detector response and sample concentration was the same at both ionic strengths, indicating d*n*/d*c* was also independent of the ionic strength (within this range), and the same value (0.150) was therefore used in the processing of data. On the other hand, the second virial coefficient (A_2) of polyelectrolytes is generally very sensitive to ionic strength. However, due to the low concentrations used in SEC, the error in the M_w estimate obtained by using the same A_2 for I = 0.17 and 1.01 M is 5% or less, which could be easily shown by systematically varying A_2 in the data processing. Besides the effect of A_2, we still observed a slight decrease (about 7−11%) in M_w when increasing the ionic strength. The reason for this is not understood at present. A roughly 10% decrease in intrinsic viscosity was observed at the same time, which qualitatively is to be expected for a semiflexible polyelectrolyte. Figure 11 shows the R_G−M and [η]−M relationships obtained for an alginate standard (LVG) at the two ionic strengths.

Due to the simultaneous decrease in M_w and [η], the effect of increasing the ionic strength is almost, but not entirely, canceled out. The R_G−M data are also essentially overlapping at high molecular weight (M > ca. 200 000) but suggest a slight difference in the low M-range, where, contrary to normal behavior, the intrinsic viscosity appeared to increase slightly with increasing ionic strength.

***B*-Parameter.** To theoretically extrapolate the intrinsic viscosity to infinite (or, in principle, any) ionic strength, the *B*-parameter of two alginates (*L. hyperborea* stipe and leaf, respectively) was determined by the conventional method[8,10] except that the SEC eluent salt Na_2SO_4 was used instead of NaCl. Results (0.032 *L. hyperborea* stipe and 0.039 for *L. hyperborea* leaf) are identical to literature data obtained in aqueous NaCl,[10] showing that the change of salt from NaCl to Na_2SO_4 has no effect on the *B*-parameter, and literature data obtained with NaCl can therefore be used as a basis for recalculating (by interpolation or extrapolation) intrinsic viscosities to other ionic strengths also in Na_2SO_4.

Discussion

The Mark−Houwink−Sakurada Relation. The initial results obtained for *L. hyperborea* stipe and leaf alginates by static LALLS and conventional intrinsic viscosity measurements are within the range reported in the literature (Figure 2). In particular, the exponent of 1.01 conforms well in comparison to most other studies. Our data are in the best agreement with those of Berth,[16] which are shifted to lower molecular weights (or higher intrinsic viscosities) by a factor of 2−3 compared to older studies.[14−18] The reason for this difference is presumably the problems associated with particulate impurities in the samples studied earlier.[15,18]

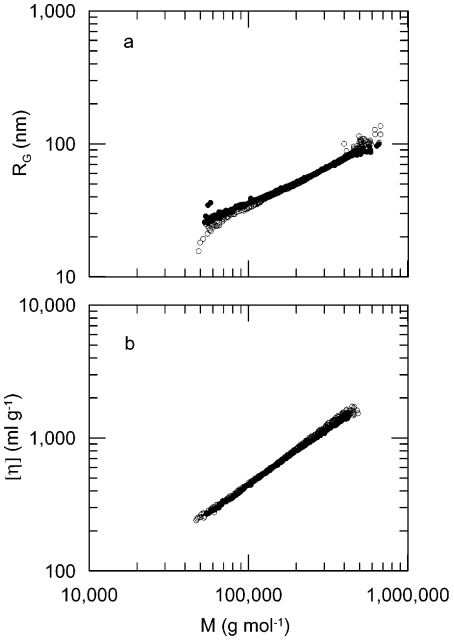

Figure 11. The molecular weight dependence of R_G (a) and [η] (b) for an alginate analyzed at I = 0.17 M (○) and 1.0 M (●).

According to all of the results of the present study (both static measurements and SMV), the corresponding MHS equation (eq 1) can, for practical purposes, indeed be used for all alginates, irrespective of their chemical composition. This strongly facilitates the use of intrinsic viscosity (obtained in 0.1 M NaCl) as a convenient method to monitor the molecular weight of alginates.

SMV−Methodological Aspects. The combination of size-exclusion chromatography with multiple detectors is a remarkably powerful—yet simple—method to study and compare the chain extension and flexibility of polymers. It is also ideally suited for alginates and other water-soluble polysaccharides. Results obtained for alginates were in excellent agreement with those obtained independently by static LALLS and conventional viscometry. However, atypical SEC behavior is sometimes encountered. The concentration dependence of the elution profile of polyelectrolytes (Figure 3) appears not to be recognized or discussed much in the literature. It will certainly influence results where calculations are based on calibration with standards, a method that is still used in many laboratories. Another feature pertaining to alginates is the variable presence of aggregates or particulate material, which is well-known to occur in many alginates and which strongly influences results obtained by conventional light scattering. Whereas well-purified samples show no such impurities, the high-G sample (epimerized with AlgE6) probably contained a small amount of aggregate-like material eluting near the exclusion limit and showed a larger variability between different injections than most other alginates. The reason is unclear but is tentatively ascribed to some aggregation. On the other hand, an industrial, low-M_w sample displayed abnormal results with an apparent increase in both

molecular weight and R_G with increasing elution volume (Figure 6). Normal results could be obtained only after extensive ultracentrifugation. Hence, high-M_w impurities do not always elute near the exclusion limit but can be revealed by abnormal R_G values at high elution volumes. These results demonstrate that SEC-MALS must be critically analyzed, and analysis of unknown alginates should always be accompanied by coanalysis of purified alginates with normal SEC behavior.

For samples with normal polydispersity, i.e., M_w/M_n near 2, the current method provides acceptable data for analysis of the R_G-M and $[\eta]-M$ relations over a molecular weight range of about 1.5 decades. The range can be extended to lower molecular weights by simple partial degradation (Figure 4). For intrinsic viscosities above ca. 1000−2000 mL/g, the influence of shear thinning generally appears. In the low molecular weight range, the R_G determination is limited by the wavelength of the light scattering instrument.

The solution behavior of polyelectrolytes is generally dependent on ionic strength. In SEC, a high ionic strength is preferable to suppress ionic effects. In our experience, the use of 0.05 M Na_2SO_4 supplied with 0.01 M EDTA (pH 6.0) works well not only for alginates but also uncharged polymers such as pullulan, scleroglucan,[28] and cereal β-glucans.[26] The addition of EDTA is used routinely in our laboratory to prevent aggregation in Ca-binding polymers. Higher ionic strength (0.33 M Na_2SO_4) gave slightly different results and represents a practical limit to SEC studies of alginates.

For carefully purified standards, we obtained acceptable agreement between results obtained by SMV and by conventional static measurements (Figure 5b). It should be noted that $[\eta]$ was determined at two different ionic strengths in the two setups. For better comparison, the data obtained by the conventional method (at $I = 0.1$ M) were recalculated to SEC conditions ($I = 0.17$ M) by using the known *B*-parameter, which quantifies the ionic strength dependence of $[\eta]$ (see below). In addition, the effect of polydispersity must in principle be accounted for. SEC elution slices are in practice considered monodisperse (in fact, they are not, but it is inherently assumed that band broadening effects effectively canceled by equal contributions from neighboring slices). For a proper comparison with the $[\eta]-M$ data obtained by SEC, the intrinsic viscosity obtained by the conventional ("batch") measurement should be compared to the viscosity average molecular weight (M_v) rather than M_w, each defined as

$$M_w = \frac{\sum_i w_i M_i}{\sum_i w_i} \tag{6}$$

$$M_v = \left(\frac{\sum_i w_i M_i^a}{\sum_i w_i} \right)^{1/a} \tag{7}$$

Here, w_i is the weight fraction of each *i*-mer and M_i is the corresponding molecular weight, whereas the exponent *a* is the exponent of the MHS relation

$$[\eta] = KM^a \tag{8}$$

The samples used in static LALLS and "batch" viscometry were all obtained by random depolymerization, and the polydispersities (M_w/M_n) were in all cases close to 2.0. For samples with such distributions, w_i is given by[39]

$$w_i = i\alpha^2(1-\alpha)^{i-1} \tag{9}$$

where the degree of scission (fraction of broken linkages) $\alpha = DP_n^{-1} = M_0/M_n$. On the basis of these equations, the ratio M_w/M_v could easily be calculated for any combination of M_n and MHS exponent. For alginates, having MHS exponents in the range 0.8−1, we calculated that M_w/M_v was in the range 1.00−1.05 for the M_w range 20 000−400 000. Thus, the difference in M_w and M_v is below the general uncertainty in the M_w determination in LALLS (5−10%). We therefore conclude, in agreement with previous literature[14] that results from SEC-MALS generally agree well with static light scattering, provided that the samples are pure, stable, and do not aggregate.

Comparison to Other Polysaccharides. The R_G-M and $[\eta]-M$ relations obtained for alginates nicely demonstrate the coiled nature of these polysaccharides in solution. The exponents in the corresponding scaling relations are within the ranges found in other studies. In comparison, the much more flexible pullulan has much lower intrinsic viscosities and radii of gyration (at the same molecular weight), as expected.

Stiffness and Extension of Alginates. A major objective of the present investigation was to systematically investigate the influence of the content and distribution of α-L-guluronic acid residues in alginates on the chain stiffness and extension. Surprisingly, both R_G-M and $[\eta]-M$ relationships of all alginates, from pure mannuronan via PT-180 to polyguluronan, and in particular the polyalternating alginate, were essentially independent of the chemical compositions. Before accepting these results, we decided to include a corresponding study of partially periodate-oxidized alginates, which have long been known to possess much higher chain flexibilities due to the ring opening. The data thus obtained were qualitatively in good agreement with published data,[21] and they certainly contrasted the data obtained for in vitro epimerized alginates by showing a pronounced decrease in $[\eta]$ (at constant M_w) with increasing degree of oxidation. Thus, we must conclude that our data obtained with in vitro epimerized alginates truly reflect a novel and rather surprising finding, namely, that all alginates—at least under the conditions used in this study—have essentially the same chain extensions, irrespective of their chemical composition.

This finding is clearly at variance with the experimental result reported for an acid-soluble fragment of alginate whose composition is close to polyalternating.[9] It is further at variance with the theoretical calculations of alginate chain extension based on the local chemistry of alginate the monomers and the geometry of the four possible glycosidic linkages (MM, MG, GM, GG).[12] On the other hand, our results are corroborated by recent studies[8] showing also that the *B*-parameter of in vitro epimerized alginates (identical or close to those studied here) is largely independent of the chemical composition. As the samples used in the studies by Smidsrød et al. more that 30 years ago no longer exist, a reinvestigation, including a direct experimental comparison with in vitro epimerized alginates, could not be carried out. Most likely, the acid-soluble fragment which originally gave rise to a high *B*-parameter contained some kind of impurities which affected its solution properties.

Polyalternating sequences have been considered unable to cooperatively bind Ca^{2+} ions and form junction zones in the same way as G-blocks. Moreover, their contribution to the *overall* chain extension and flexibility is, according to our results, the same as for mannuronan. This does not preclude

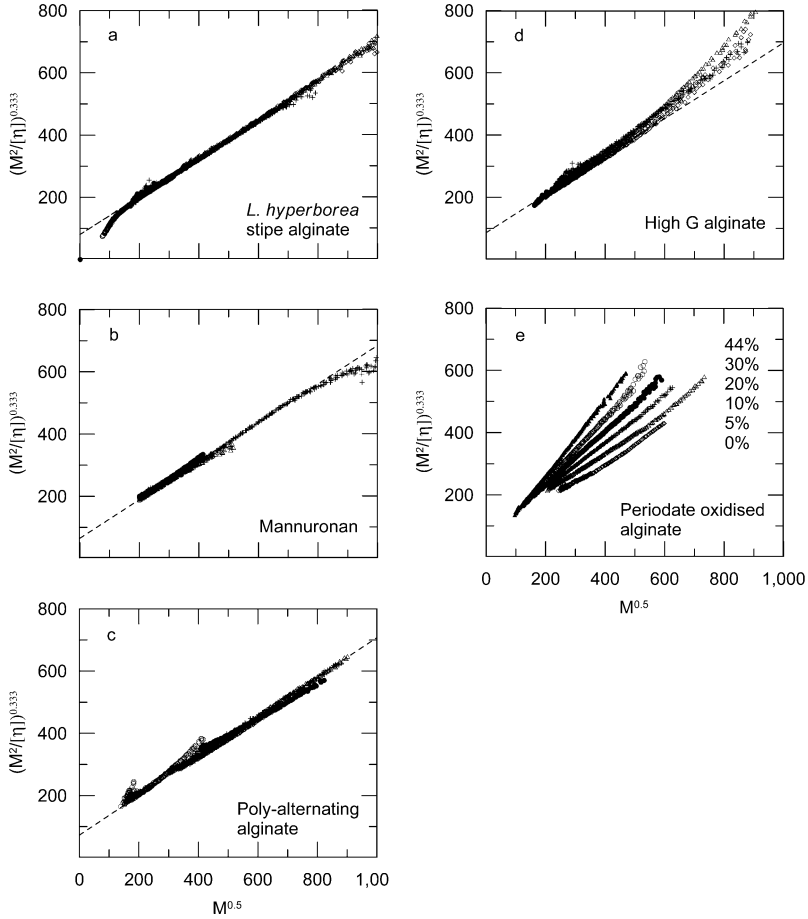

Figure 12. Bohdanecky plots for alginate from *L. hyperborea* stipe (a), mannuronan (b), polyalternating alginate (AlgE4 epimerized mannuronan, $F_G = 0.45$) (c), mannuronan epimerized by AlgE6 to obtain $F_G \geq 0.85$ (d), and periodate oxidized alginates (e).

polyalternating sequences from behaving differently from other sequences, as the M−G and G−M on a *local* scale certainly give rise to "kinks" in the chains, thereby changing the local directionality. The introduction of polyalternating sequences by in vitro epimerization of M-blocks has been found to have a pronounced effect on the gelling properties of alginate. For example, epimerization of mannuronan using AlgE6 to obtain functional G-blocks (i.e., a gelling alginate), followed by a second epimerization using AlgE4 to convert residual mannuronan sequences to polyalternating sequences, resulted in an alginate which formed nonsyneretic gels with calcium.[6] Recently, Donati et al.[7] formulated a novel hypothesis on the direct involvement of polyalternating sequences in calcium−alginate gels.

Persistence Lengths ($I = 0.17$ M). Both the R_G-M and $[\eta]-M$ relationships contain quantitative information about the shape of polymers in solution. In general, the scaling relations conform what is already well-accepted for alginates, namely, their behavior as slightly expanded random coils. The R_G-M exponent (b) is 0.6, with no significant variation with varying sequence including PT-180/S-series, pure mannuronan, and in vitro epimerized alginates. For the same samples, the MHS exponent is close to 1.0, again with no significant dependence on the structure of the samples. In contrast to alginates, periodate-oxidized alginates show a marked decrease in the MHS exponent with increasing degree of oxidation (from 1.0 to 0.5), reflecting the gradual decrease in chain expansion resulting in more typical random coil behavior due to the increase in local flexibility.

For further analysis of these changes, we decided to analyze the $[\eta]-M$ data by means of the wormlike chain model. We follow essentially the analysis performed by Mendichi et al.[27] on hyaluronan based on the same experimental method. Hyaluronan has approximately the same stiffness as alginates, besides being polyanionic and unbranched. To simplify the calculation procedures, we used the approximation of the wormlike chain model introduced by Bohdanecký,[40] in line with previous analyses of, for example, hyaluronan,[27] viilinan,[41] cereal β-glucan,[42] and xanthan.[43] According to the model, $(M^2/[\eta])^{1/3}$ is a linear function of $M^{1/2}$

$$\left(\frac{M^2}{[\eta]}\right)^{1/3} = A_\eta + B_\eta M^{1/2} \tag{10}$$

$$A_\eta = A_0 M_L \Phi_{0,\infty}{}^{-1/3} \tag{11}$$

$$B_\eta = B_0 \Phi_{0,\infty}{}^{-1/3} \left(\frac{2q}{M_L}\right)^{-1/2} \tag{12}$$

$\Phi_{0,\infty}$ is the limiting value of the Flory viscosity constant and equals $2.86 \cdot 10^{23}$. A_0 and B_0 are known functions of the reduced hydrodynamic diameter (d_r), and B_0 can in practice be replaced by a mean value (1.05). q is the persistence length, and M_L is the molar mass per unit of contour length. Plots of $(M^2/[\eta])^{1/3}$ as a function of $M^{1/2}$ are given in Figure 12 for a selection of samples (alginates) studied here.

The M and $[\eta]$ data were obtained directly from SMV elution slices without prior fitting of data. In general, linear plots were

obtained, from which the intercepts (A_η) and slopes (B_η) were determined following linear regression. For the high-G alginates, differences were observed at high molecular weight, as also seen in Figure 8. However, the data seemed to converge with decreasing molecular weight toward the fitted line shown in the figure.

Provided the partial hydrodynamic volume (ν) is known, the hydrodynamic diameter (d) can be calculated according to the relation proposed by Bohdanecký[40]

$$d = [4\nu M_L/(\pi N_A)]^{1/2} \quad (13)$$

M_L and q can then be directly calculated. Martin et al.[44] reported $\nu = 0.54 \pm 0.06$ mL/g for alginate, whereas Wedlock et al.[17] reported $\nu = 0.49$ mL/g for alginates from *L. hyperborea* (in 0.2 M NaCl). For the *L. hyperborea* S-series, which covered the largest molecular weight range besides having very good raw data (little noise and smallest deviation from linearity in the Bohdanecký plots), we obtain $M_L = 440 \pm 10$ nm^{-1} and $q = 15.3 \pm 0.3$ nm (average of six injections) using $\nu = 0.54$ mL/g. The corresponding values obtained for $\nu = 0.49$ mL/g are essentially identical ($M_L = 437$ nm^{-1} and $q = 15.2$ nm). The M_L value is in reasonable agreement with the value (424 nm^{-1}) which can be obtained directly from the chemical structure, according to the relation

$$M_L = \frac{F_G M_{0,G} + (1 - F_G) M_{0,M}}{F_G b_G + (1 - F_G) b_M} \quad (14)$$

F_G is the fraction of L-guluronic acid residues. $M_{0,G}$ and $M_{0,M}$ are the molar masses of the two monomers (both equalling 198 g/mol for the Na^+ form). The bond distances are 0.515 nm (b_M) and 0.435 nm (b_G), respectively, with the first referring to the M in the $^4C_1$ form, and G in the $^1C_4$ form.[9] For periodate-oxidized residues, the average bond distance was estimated to 0.424 nm based on the Kirkwood−Riseman freely rotating chain model.[45] If $M_L = 424$ nm^{-1} was used as fixed value for *L. hyperborea* S-series, the corresponding persistence length was calculated to 14.7 nm. The small difference in the calculated persistence length indicates that the latter method can be generally used, which is particularly useful in those cases where the quality of the raw data did not allow sufficiently precise estimates of A_η and, hence, in the calculated M_L. Using this method, we obtained $q = 14.5 \pm 1.8$ nm for mannuronan, $q = 14.9 \pm 1.2$ nm for polyalternating alginate, and $q = 16.5 \pm 0.5$ nm for an alginate with $F_G > 0.85$. These values are slightly lower than those reported by Gamini et al.[46] (15 and 20 nm for alginates from *L. hyperborea* ($F_G = 0.72$) and *Macrocystis pyrifera* ($F_G = 0.42$), respectively) using the same model and where the $[\eta]-M$ relationship was obtained from MHS parameters by a special fitting routine of SEC-LALLS data (molecular weight distribution).[14] The difference clearly reflects differences in the primary $[\eta]-M$ relationship underlying the calculations.

For periodate-oxidized alginates, the calculated persistence lengths decreased from 14.7 nm before oxidation to below 4 nm at 44% oxidation (Figure 13).

The use of the wormlike chain model in the absence of excluded volume effects may be somewhat questionable for the most oxidized samples, which approach the behavior of random coils, thereby overestimating the chain stiffness. In any case, the pronounced decrease in chain flexibility as a consequence of oxidation is clearly demonstrated and certainly contrasts the marginal influence of epimerization on the flexibility.

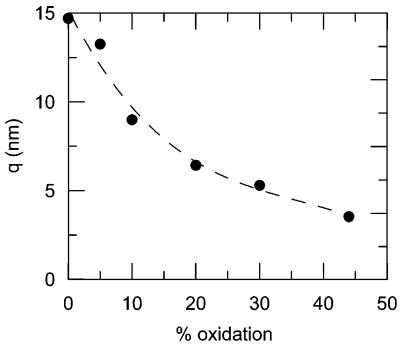

Figure 13. The dependence of the degree of periodate oxidation on the calculated persistence lengths of alginates.

It may be noted our values for the persistence lengths are well above 5.2 nm reported by Lee et al.[24] for a G-rich, low-M_w sample ($M_w = 7100$ Da) and support the conclusion[7] that 5.2 nm is an underestimation. A possible reason may be that data selected for further analysis in Bohdanecký plots ($M >$ 10 000; Figure 6 of ref 24) correspond only to the first part of the chromatographic peak eluting near the void volume. The possibility for incomplete separation near the void volume together with a narrow range of molecular weights may possibly account for the systematically lower q-values observed. This emphasizes the importance of employing broad molecular weight distributions, and preferentially a combination of samples covering different molecular weight ranges, when analyzing polymer chain stiffness and extension by means of SMV.

Unperturbed Dimensions ($I = \infty$). It should be noted that all SMV data discussed up to this point refer to an ionic strength of 0.17 M. Due to the polyelectrolytic character of alginates, both $[\eta]$ and R_G depend strongly on the ionic strength. Of particular interest are the unperturbed dimensions, corresponding to conditions where excluded volume effects and local charge effects are eliminated. For charged polymers, this corresponds to infinite ionic strength, a condition where no physical studies can actually be carried out. In the case of alginates, an ionic strength of 1.0 M seems to be above the practical limit as judged from the SMV results (Figure 11). As demonstrated by Smidsrød et al.,[10] the intrinsic viscosity of high molecular weight alginates may differ considerably between $I = 1$ M and $I = \infty$ (extrapolated value). Thus, other approaches are needed. Several authors have used theoretical models to estimate the electrostatic contribution to the persistence lengths, most commonly on the basis of R_G-M data, but also from $[\eta]-M$ data.[27,41] As an alternative, we introduce here another, quite simple approach, taking advantage of the empirically observed linear relationship between the intrinsic viscosity and the $I^{1/2}$ through B parameters[10]

$$[\eta]_I = [\eta]_\infty + B[\eta]_{0.1}{}^{\nu}(I^{-1/2})$$

The B-parameter and the exponent ν (usually set to 1.3) are known for a series of alginates,[8,10] including periodate-oxidized alginates.[21] We used this relation to extrapolate our $[\eta]$ values obtained by SMV (for each elution slice) to infinite ionic strength ($[\eta]_\infty$). These values were again analyzed using Bohdanecký's model to yield persistence lengths at infinite ionic strength, q_∞. For the *L. hyperborea* stipe alginate (S-series), this procedure reduced the persistence length from 15.3 to 12.0 nm or, by the method of fixed M_L, from 14.7 to 12.1 nm. We thus conclude that the intrinsic persistence length (in the absence of electrostatic effects) of naturally occurring alginates and in vitro epimerized mannuronans is 12 nm.

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