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Carbohydrate Polymers 54 (2003) 27–32
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

A physico-chemical comparative study on extracellular carbohydrate polymers from five desert algae

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Received 23 September 2002; accepted 4 April 2003

Abstract

Hydrodynamic properties of five newly isolated algal extracellular polysaccharides with putative adhesive properties are described, using a combination of size exclusion chromatography, total or 'multi-angle' laser light scattering and analytical ultracentrifugation. The respective polysaccharides had been extracted from four filamentous cyanobacteria: *Microcoleus vaginatus*, *Scytonema javanicum*, *Phormidium tenue* and *Nostoc* sp. and a coccoid single-cell green algae *Desmococcus olivaceus* that had been separated from desert algal crusts of the Chinese Tegger Desert. SEC/MALLS experiments showed that the saccharides had diverse weight average molecular weights ranging from 4000 to 250,000 g/mol and all five showed either bi-modal or tri-modal molecular weight distribution profiles. Use of the Mark–Houwink–Kuhn–Sakurada (MHKS) scaling relationship between sedimentation coefficient and (weight average) molecular weight for the five samples, assuming a homologous conformation series revealed an MHKS b exponent of (0.33 ± 0.04), suggesting a conformation between that of a stiff rod ($b \sim 0.18$) and a random coil ($b \sim 0.4$–0.5), i.e. a ' flexible rod' or 'stiff coil'.

Keywords: Desert algal polysaccharide; Hydrodynamic characterization

1. Introduction

Algal polysaccharides are currently widely used commercially (Graham & Wilcox, 2000). Generally, the algae which produce these materials occur in fresh water and marine habitats but they have adapted to life on land in a variety of terrestrial environments. They commonly grow either on the surface or at a depth of up to several centimeters in soil. The activities of soil algae are thought to enhance soil formation and water retention, stabilize soil, increase the availability of nutrients of plant growing nearby and reduce soil erosion (Johansen, 1993). Because of their benefit to agriculture, they have been introduced as soil conditioners in many countries (Metting, 1981) and they have also been suggested for use as biofertilizers (Painter, 1993).

Soil algae are known to excrete a variety of extracellular polymeric substances (EPS) especially polysaccharides which may play an important role for their vital function as suggested by many authours (Mazor, Kidron, Vonshak, & Abeliovich, 1996). A physico-chemical investigation would appear to provide underpinning information concerning the function and uses of these materials. The algal exopolysaccharides investigated in this article were obtained from the media cultures of *Nostoc* sp., *Microcoleus vaginatus*, *Desmococcus olivaceus*, *Phormidium tenue* and *Scystonema javanicum* which have been separated from desert algal crusts of the Tegger Desert of China as described in Hu, Paulsen, Petersen, and Klaveness (2003).

To our knowledge isolation and purification of exopolysaccharides from desert soil algae and subsequent characterization by chemical and physical methods has not been performed. The aim of the study was to further purify the algal exopolysaccharides from 1 M NaCl fractions previously fractionated by Hu et al. (2003) and determine the composition, hydrodynamic properties (namely molecular weight and sedimentation velocity coefficients) of the purified carbohydrate polymers. These

Abbreviations: IEC, ion exchange chromatography; M_w, weight average molecular weight; s, sedimentation coefficient; S, Svedberg unit ($= 10^{-13}$ s); SEC, size exclusion chromatography; MALLS, multi angle laser light scattering.

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0144-8617/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0144-8617(03)00136-X

techniques have the advantages of requiring only small amounts of purified material.

2. Materials and methods

1.0 M NaCl fractions containing polysaccharides from five desert soil algae, i.e. *Nostoc* sp., *M. vaginatus*, *D. olivaceus*, *P. tenue* and *S. javanicum* previously fractionated by anion exchange column chromatography was supplied by Hu et al. (2003).

2.1. Further fractionation of 1.0 M NaCl fractions

Each sample was dissolved in distilled water by mechanical stirring overnight. The solution was filtered through 0.45 μm membrane filter. The solution was then applied to a DEAE-Sepharose fast flow column (50 cm × 5 cm, coupled to a fraction collector, a LKB SupraFrac) with chloride as counter ion. The column was first eluted with distilled water for 1 l, followed by a NaCl gradient (0 → 1 M) at a flow rate of 2 ml/min regulated by a P-3 peristaltic pump. Fractions (8 ml) from linear gradient were monitored for carbohydrate using phenol–sulphuric assay (Chaplin, 1994). Fractions containing polysaccharide were pooled, dialysed against running tap water followed by distilled water in a Spectrapor dialysis tubing (MWCO = 3500) and lyophilized.

2.2. Monosaccharide composition analysis of purified biopolymers

The monosaccharide composition analysis was determined by methanolysis. Briefly, the polysaccharide samples were subjected to methanolysis with 4 M hydrochloric acid in anhydrous methanol for approximately 24 h at 80 °C. Mannitol was added as an internal standard followed by trimethylsilylation. The trimethylsilylated samples were subjected to gas chromatographic analysis (Barsett & Paulsen, 1992).

2.3. Hydrodynamic characterization

The polysaccharides were dissolved overnight in a phosphate buffered saline, pH 6.8, $I = 0.1$ 'Paley' buffer (Green, 1933) prior to performing hydrodynamic characterization.

2.3.1. Molecular weight determination of polysaccharides using size-exclusion chromatography coupled to multi-angle laser light scattering (SEC/MALLS)

SEC/MALLS allows on-line light scattering of a heterogeneous solute separating molecules according to their hydrodynamic volumes by size exclusion chromatography and the absolute weight average molecular weight (M_w) can be obtained. (Jumel, 1994; Wyatt, 1992). The chromatography system consisted of a HPLC pump (Model PU-1580, Jasco Corp., Tokyo, Japan). A Rheodyne injection valve (Model 7125, Rheodyne, St Louis, MS) fitted with a 100 μl loop and the following column system: Phenomenex guard column, TSK G6000PW and TSK G5000PW connected in series. Eluent (phosphate buffered saline) was monitored using a Dawn DSP multi-angle laser light scattering photometer and an Optilab 903 interferometric refractometer (both instruments from Wyatt Technology, Santa Barbara, CA). Signals from the light scattering photometer and the refractometer were captured and analysed on a PC using the dedicated ASTRA™ software. Eluent was pumped at a flow rate of 0.8 ml/min. Injections of samples (injection volume 100 μl) were performed at room temperature.

2.3.2. Sedimentation velocity

Sedimentation velocity experiments were performed using the Optima XL-I analytical ultracentrifuge (Beckman Instruments, Palo Alto, CA) equipped with refractometric (Rayleigh interference) optics. Solutions and solvents were filled into their respective channels into 12 mm optical path length double sector cells and run at 20 °C and at a rotor speeds of 55,000 rpm (polysaccharide from *P. tenue*) and 50,000 rpm (polysaccharide from *Nostoc* sp.), 35,000 rpm (polysaccharides from *M. vaginatus* and *D. olivaceus*). Data was captured from Rayleigh interference optical system and apparent sedimentation coefficient distributions $g(s^*)$, were obtained using the time derivative procedure as described by Stafford (1992) and implemented in the DCDT + software of Philo (2000). Apparent sedimentation coefficients (s^*) were converted to standard conditions according to the standard equation (van Holde, Johnson, & Shing Ho, 1998).

$$s_{20,w} = s^* \frac{(1 - \bar{v}\rho_{20,w})\eta_{T,b}}{(1 - \bar{v}\rho_{T,b})\eta_{20,w}}$$

where $s_{20,w}$ is the sedimentation coefficient expressed in terms of the standard solvent water at 20 °C; s^* is the measured sedimentation coefficient at experimental conditions (i.e. T, b); $\eta_{T,b}$, $\eta_{20,w}$ are, the viscosities of solvent (b) at temperature T and water at 20.0 °C respectively and $\rho_{T,b}$, $\rho_{20,w}$ are the corresponding densities. The $s_{20,w}$ values were evaluated at various concentrations ranging from 0.3 to 1.0 mg/ml. In order to remove the effects of non-ideality, plots of $s_{20,w}$ versus concentration were extrapolated to zero concentration to give $s^0_{20,w}$.

3. Results and discussion

3.1. Yields of the carbohydrate polymer

According to the elution profile of a 0 → 1 M NaCl gradient elution, *Nostoc* sp., *D. olivaceus*, *P. tenue* and *S. javanicum* contain one carbohydrate polymer, designated

Table 1
Appearances and yields of biopolymers from desert algae after ion exchange column chromatography

Sample	Amount of starting material (mg)	Amount of purified material (mg)	Appearance of purified polymer
Nostoc sp.	178	97	White solidified
M. vaginatus	97	10.8 (M1)	White solidified
		6.3 (M2)	White solidified
D. olivaceus	156	74	Brown solidified
P. tenue	45	12	White solidified
S. javanicum	49	2	White solidified

as biopolymers N, D, P and S respectively. Whereas, *M. vaginatus* was separated into two polymers, designated M1 and M2 respectively. M1 eluted earlier than M2.

The appearances and yields of the polymers after 1.0 M NaCl fractions had been subjected to anion exchange column chromatography, are shown in Table 1. The recovery amount of polymer from *S. javanicum* was very low, due to very poor solubility of its 1.0 M NaCl fraction in water and this led to a very small amount being loaded onto the column.

3.2. Monosaccharide composition of the carbohydrate polymers after ion exchange chromatography

The results on the composition of the carbohydrate part of the purified polymer from methanolysis are shown in Table 2.

The carbohydrate polymer from *Nostoc* sp. contained approximately glucose 42%, xylose 22% and galactose 20% along with the minor components such as 2-*O*-methyl glucose 9%, galacturonic acid 3% and glucuronic acid 4%. This polymer possesses the highest carbohydrate content among the polymers separated from five species.

As mentioned above, 1 M NaCl fraction of *M. vaginatus* was separated into two biopolymers, M1 and M2. The major sugars present in the carbohydrate moiety of the biopolymer M1 were mannose 42%, 2-*O*-methyl rhamnose 20%, glucose 14% and xylose 10% while rhamnose, fucose and galactose were present as minor components.

The carbohydrate moiety of the biopolymer M2 consisted of galactose, glucose and arabinose as the major sugars along with a substantial amount of acidic sugars i.e. galacturonic acid 10% and glucuronic acid 14%. Rhamnose, xylose and mannose were present as minor components.

In comparison between the biopolymer M1 and M2—isolated from the same origin, it was found that there was a great difference in sugar composition. Notably, mannose was a major sugar found in M1, but only as a minor sugar in M2. Conversely, galactose was a major sugar in M2, but it was present as a minor sugar for M1. Additionally, two acidic sugars were only found in M2 as mentioned above, but none of these were detected in M1.

The carbohydrate moiety of the biopolymer D consisted mainly of glucose 30%, galactose 20% and xylose 15%. Arabinose, rhamnose, mannose, 2-*O*-methyl glucose, galacturonic acid and glucuronic acid were present as minor sugars in comparable amounts.

The carbohydrate moiety of the biopolymer P was the least complex among the polymers investigated here as it consisted principally of arabinose 53% and glucose 45% with mannose and galactose appearing to be present in negligible amount.

The carbohydrate moiety of the biopolymer S contained mainly mannose 36% and glucose 24% along with rhamnose 13%, 2-*O*-methyl rhamnose 11% and galactose

Table 2
Monosaccharide composition (% of total carbohydrate content) and total carbohydrate content of biopolymers before and after ion exchange column chromatography

	Nostoc sp.		*M. vaginatus*			*D. olivaceus*		*P. tenue*		*S. javanicum*	
	Before	After	Before	After		Before	After	Before	After	Before	After
				M1	M2						
Arabinose	–	–	9.7	–	11.4	13.1	6.5	43.9	52.6	9.6	–
Rhamnose	3.5	–	5.8	5.9	4.0	7.0	5.1	10.4	–	7.4	12.9
2-*O*-methyl rhamnose	–	–	4.8	19.8	–	–	–	–	–		10.7
Fucose	–	–	4.7	3.3	–	1.4	–	2.3	–	tr	–
Xylose	20.9	21.5	8.8	9.6	7.2	12.4	15.3	4.7	–	6.0	–
Mannose	1.6	1.4	21.5	42.4	3.5	5.9	4.8	2.9	1.0	22.9	35.6
Galactose	21.5	19.6	18.6	5.4	31.1	28.8	20.2	1.3	1.4	23.4	8.1
Glucose	44.0	41.7	20.4	13.7	19.5	27.6	30.0	32.5	45.0	24.8	24.4
2-*O*-methyl glucose	8.6	8.9	–	–	–	3.9	5.5	–	–	–	3.5
Galacturonic acid	–	3.3	4.9	–	10.2	tr	4.5	tr	–	tr	4.8
Glucuronic acid	–	3.6	3.7	–	13.6	tr	8.1	–	–	tr	–
N-acetyl glucosamine	–	–	2.1	–	–	–	–	1.3	–	tr	–
Total carbohydrate	40.5	92.8	27.6	54.9	41.6	16.2	41.5	36.1	62.1	16.6	33.1

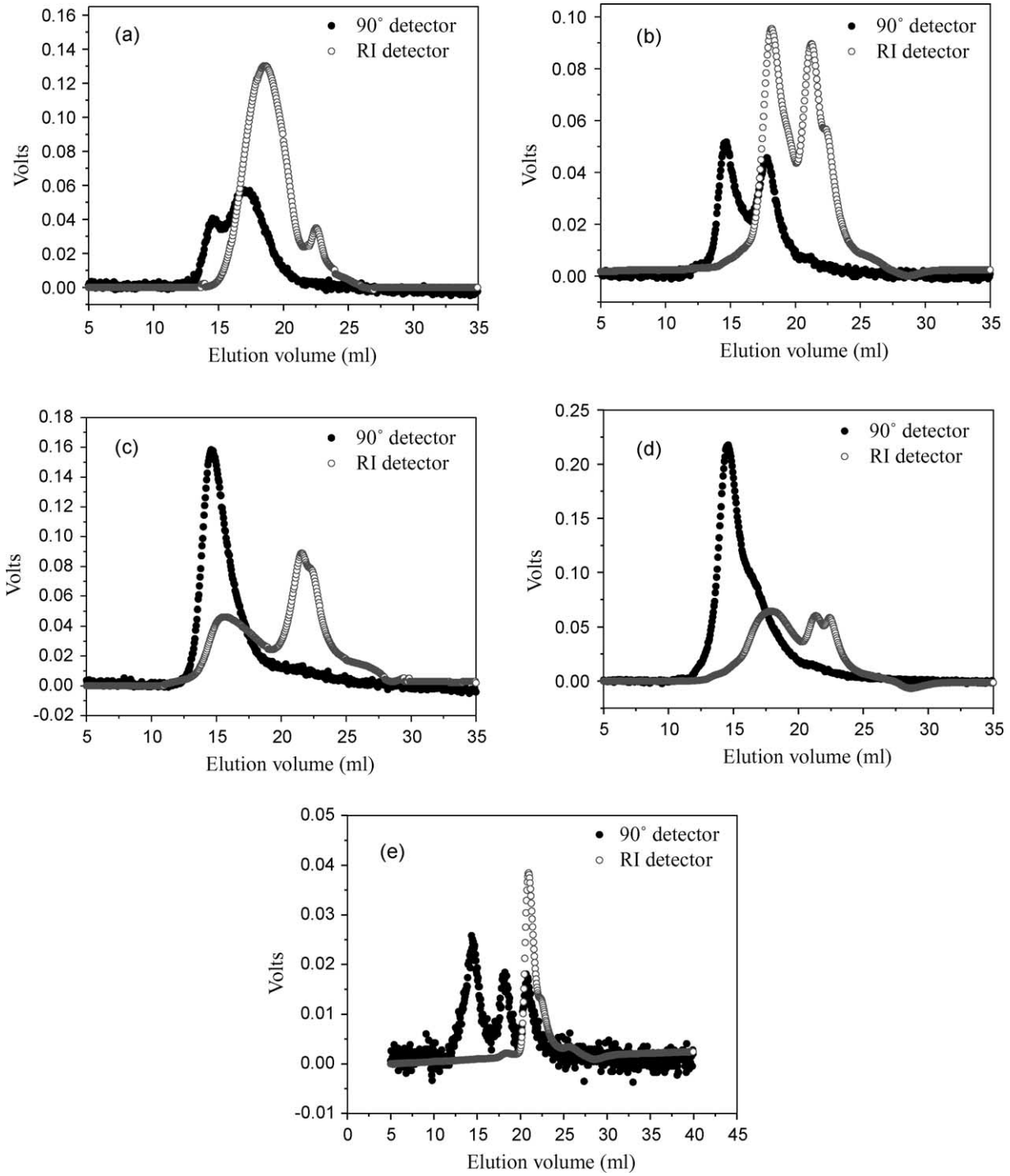

Fig. 1. Light scattering and refractive index profiles from SEC/MALLS experiments on the algal exopolysaccharides: (a) N; (b) M1; (c) M2; (d) D; (e) P.

8%. Galacturonic acid and 2-*O*-methyl glucose were present as minor sugars. Its total carbohydrate content present in the polymer was the lowest compared to others.

According to the results, it would indicate that all the polymer separated from 1 M NaCl fractions were heteropolysaccharides which were probably associated with other macromolecule, more specifically proteins. Results from the previous study revealed that all of these fraction contained considerable amounts of protein for *M. vaginatus* and *S. javanicum* (~50%) and moderate amounts in *P. tenue* and *D. olivaceus* 22% and 14% respectively. As for *Nostoc* sp., the protein content in the 1 M NaCl fraction was about 7% which was the lowest among the five samples (Hu et al., 2002). The high carbohydrate content in the polymer N was obtained after ion exchange column chromatography.

Table 3
Hydrodynamic data for biopolymers from desert algae

Sample	M_w obtained from SEC/MALLS of main component	$s^0_{20,w}$ (S)	f/f_0
N	33,000 ± 4000	1.8	3.2
M1	38,000 ± 3000	1.5	4.2
M2	250,000 ± 30,000	3.5	6.3
D	97,000 ± 2000	2.5	4.7
P	4000 ± 1000	0.9	1.5

3.3. Homogeneity and molecular weight determination

Homogeneity and molecular weights of the polymers were investigated using SEC/MALLS. The chromatograms for all samples (excluding the polymer from *S. javanicum* due to insufficient amount obtained) are shown in Fig. 1 and their molecular weights are shown in Table 3. We would like to stress the values in Table 3 are only estimates and may be affected by the trace amounts of large molecular weight material at low elution volume.

3.3.1. Biopolymer N

The RI chromatogram (Fig. 1a) shows one large relatively symmetrical peak (the small peak is most likely due to salt at the total permeation volume) and the light scattering chromatogram also shows one large peak, although there is a large shoulder on the high molecular side. However, there is no RI signal associated with this which indicates that there is a very high molecular weight component present in a very small amount. The RI peak eluted at ~15.3–21.0 ml was considered to be a main macromolecular component.

3.3.2. Biopolymer M1

Both RI and light scattering chromatograms suggest that the sample contains more than one species and this time there is a corresponding RI trace for the high molecular weight material, however, most of the material is of low molecular weight.

3.3.3. Biopolymer M2

Both RI and light scattering chromatogram indicate that there is only one macromolecular component present, RI trace on the low molecular weight side is most likely to be very small molecules eluting at the total permeation volume, at ~22–25 ml.

3.3.4. Biopolymer D

Both light scattering and RI traces show that the sample contains more than one species and these species were not well separated with the chosen column system. However, material eluting between ~15.9–19.9 ml was considered to be a main polymer in this sample, and the weight average molecular weight over this region was determined.

3.3.5. Biopolymer P

The light scattering chromatogram indicates that the sample contains at least three macromolecular species with very small amounts of the two relatively highest molecular weight species (the first two peaks). Most of the sample eluted at the lower molecular weight end.

3.4. Sedimentation velocity

The sedimentation coefficients ($s^0_{20,w}$) for all samples excluding *S. javanicum* are shown in Table 3. There appears to be a direct relationship between $s^0_{20,w}$ and molecular weight, despite the fact that the polymers from the four algae investigated here were not chemically identical.

In theory, the double logarithmic plot of $s^0_{20,w}$ against M_w of a homologous series (different molecular weight samples of the same macromolecule) can give information regarding the gross conformation of the molecule through the Mark–Houwink–Kuhn–Sakurada (MHKS) relationship (Tombs & Harding, 1998)

$$s^0_{20,w} = k'' M^b$$

The exponent b is obtained from the slope, which can be used to probe the gross conformation of macromolecule in solution between the extremes represented by the apices of the popular 'Haug Triangle' (Smidsrød & Andresen, 1979) representation: compact sphere ($b = 0.67$), random coil ($b = 0.4$-0.5) and rigid rod ($b = 0.15$).

Due to the limited amount of each biopolymer, a depolymerization procedure to obtain a homologous series of samples was not possible. It was however possible to use this procedure to see whether all polymers investigated here adopt a similar conformation. If this was the case, the double logarithmic plot of $s^0_{20,w}$ against M_w of different polymers should be ~linear. Fortunately, as can be seen in Fig. 2, the relationship between $s^0_{20,w}$ and M_w from the plot was reasonably linear and the exponent b obtained from the slope was 0.33 ± 0.04. This suggests that all the biopolymers investigated here might adopt the same

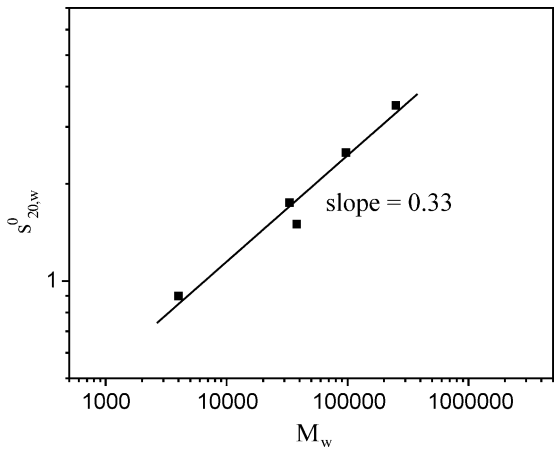

Fig. 2. Double logarithmic plot of $s^0_{20,w}$ against M_w of the polysaccharides. Slope (b) = (0.33 ± 0.04).

conformation, between a random coil and a rigid rod. However, the linearity of the plot from different species of the biopolymer does not confirm the similarity of the conformation of the molecules.

To obtain further information regarding the conformation of the macromolecules, the translational frictional ratio, f/f_0, a parameter which depends on conformation and molecular hydration was calculated due to the following equation (Tanford, 1961)

$$f/f_0 = M_w(1 - \bar{v}\rho_{20,w})/[(N_A 6\pi\eta_{20,w} s^0_{20,w})(4\pi N_A/3\bar{v}M_w)^{1/3}]$$

where N_A is Avogadro's number, $\eta_{20,w}$ and $\rho_{20,w}$ are the viscosity and density of water at 20.00 °C, respectively. The values of f/f_0 for all polymers are shown in Table 3. The large values for the frictional ratio could be due either to a large degree of swelling through water association or 'hydration' of the polysaccharides, or due to strong departure from spherical or coiled asymmetry, or perhaps a combination of both. It is impossible to distinguish the two on the basis of ultracentrifuge measurements alone, but the data are at least consistent with a stiffish random coil conformation as mentioned above in relation to the MHKS data, which is also consistent with the chemical composition of these polymers which do not appear to have significant amounts of charged residues (Hu et al., 2003).

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