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^{1}H-NMR Study of Na Alginates Extracted from *Sargassum* spp. in Relation to Metal Biosorption

Thomas A. Davis,[1,2] Francisco Llanes,[3] Bohumil Volesky,[2] Guillermo Diaz-Pulido,[4] Laurence McCook,[4] and Alfonso Mucci*,[1]

Departments of [1]Earth and Planetary Sciences and [2]Chemical Engineering, McGill University, Montreal, Quebec, Canada; [3]Biomaterials Center, University of Havana, Havana, Cuba; and [4]Australian Institute of Marine Science & CRC Reef Research, Townsville MC, Queensland, Australia

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Abstract

The use of a number of species of marine brown algae in the implementation of bioremediation strategies for toxic heavy metals is being considered and evaluated. The biosorption capacity of these algae for heavy metals resides mainly in a group of linear polysaccharides known as alginates that occur as a gel in the algal thallus. The potential for selective metal binding by the biomass of two species of *Sargassum* was evaluated by ^{1}H-NMR (nuclear magnetic resonance) following a high temperature, alkaline extraction and purification of their alginate polysaccharide. The alkaline extraction protocol applied to *Sargassum fluitans* and *Sargassum siliquosum* yielded alginate samples of low viscosity, suitable for direct acquisition of well-resolved spectra. Estimates of both the ratio of β-D-mannopyranuronosyl (M) and α-L-gulopyranuronosyl (G) residues along the polymer chain and the frequencies of occurrence of diad uronic acid residue pairs were obtained. Guluronic acid (G) was the major component in all extracts and the GG diads accounted for more than 49% of the polymer diads. Whereas the performance of *Sargassum* spp. in the metal biosorption process is a function of both its alginate content and composition, the occurrence of "G-blocks" in both purified alginates and in the raw brown seaweed is critical because it results in a well-established selectivity for divalent ions, potentially increasing the commercial effectiveness of targeted biosorption as a means of remediation.

*Author to whom all correspondence and reprint requests should be addressed.

Index Entries: Alginate; brown seaweed; biosorption; extraction; heavy metals; nuclear magnetic resonance; uronic acid; *Sargassum*.

Introduction

The contamination of aquatic environments by toxic heavy metals has become a major source of concern over the last several decades. The metals originate both from anthropogenic point source release (e.g., from mining or electroplating industry) as well as long-range atmospheric transport and deposition (e.g., refs. *1–3*). In response to more stringent government regulations, there is a strong demand for economic remediation technologies. Bioremediation is a viable alternative, especially if the sorbent can be recycled and the heavy metals recovered, with clear economic benefits. This has led to the concept of utilizing naturally occurring biomass as a substrate for metal-ion chelation, by means of a passive or, rather, nonmetabolically mediated process. The term *biosorption (4)* has been applied to such a process and numerous articles have been published in which the metal-binding properties of a range of *biosorbents*, including fungi, yeast, bacteria, and algae *(5–12)* were characterized.

In preparation for the implementation of a remediation program, the elucidation of the binding mechanism(s) of heavy metals, particularly by biomaterials with high metal-uptake capacities, is a critical first step. Furthermore, biomaterials that also bind metals selectively may be particularly advantageous, because metals are usually present as a mixture of competing cations and anions in a given aqueous solution. It is now well established that the brown algae possess a high metal-uptake capacity *(13)* and that certain raw brown algal tissues are selective *(14)* when exposed to aqueous solutions containing several metal cations. Much of the uptake capacity and selective binding are attributed to the presence of the alginate biopolymer contained within the brown algal thallus.

Sargassum spp., a widespread and common genus of marine brown algae, has considerable potential for use in remediation schemes *(15)*. It has a high metal-uptake capacity *(16–18)* and leaches less of its extracellular polysaccharide matrix *(15)* than other brown algae. The cohesion of the polysaccharide-containing biomass substrate is critical for implementation, because the high levels of alginate in the algal thallus are responsible for most of the binding of cationic heavy metals to the biomass *(19)*. Thus, loss of this polysaccharide to the aqueous phase would limit its effectiveness.

This study was conducted as an extension of the work of Fourest and Volesky *(19)* that focused on the role of alginate in heavy-metal remediation by brown seaweed. Their work revealed that cadmium binding by the isolated alginate and bulk *Sargassum fluitans* biomass arises from bridging or bidentate complex formation with the carboxylic groups of the alginate (*see* Fig. 1). One attempt was made *(15)* to investigate the relationship between heavy-metal uptake and the alginate macromolecular conforma-

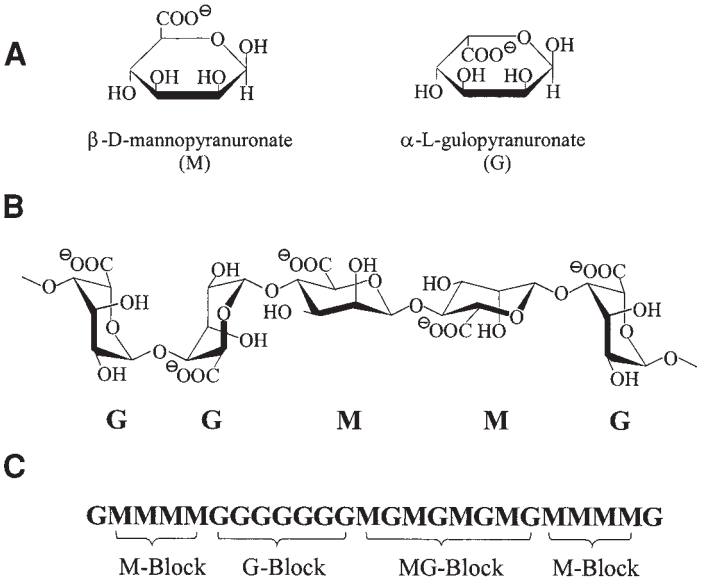

Fig. 1. Alginate structural data: **(A)** alginate monomers (M versus G); **(B)** the macromolecular conformation of the alginate polymer; **(C)** chain sequences. (After ref. *20*.)

tion, but this study was limited to single-metal systems (Ca, Zn, Cd, Cu, and Pb) at fixed pH. No correlation was found and this was probably the result of the absence of competing ions of varying affinities for the alginate within each system. The sulfonic group of fucoidan has also been identified as a ligand capable of contributing to metal sequestration in *S. fluitans (19)*. However, fucoidan is present in much lower quantities than alginate (e.g., < 10% of titratable binding sites) *(19)* and, consequently, contributes much less to the total metal binding capacity of the raw algae.

Alginic acid or alginate, the salt of alginic acid, is the common name given to a family of linear polysaccharides containing 1,4-linked β-D-mannuronic (M) and α-L-guluronic (G) acid residues arranged in a nonregular, blockwise order along the chain (*see* Fig. 1). The relative abundance of the M and G residues and their macromolecular conformation determine the physical properties and the affinity of the alginate for divalent metals *(21)*. Polymannuronic acid is a flat ribbonlike chain, its molecular repeat is 10.35 Å, and it contains two diequatorially (1e,4e) linked β-D-mannuronic acid residues in the chair form *(22)*. In contrast, polyguluronic acid contains two diaxially (1a,4a) linked α-L-guluronic acid residues in the chair form, which produce a rodlike conformation with a molecular repeat of 8.7 Å *(23)* (*see* Fig. 1b). The difference in conformation between the two homopolymeric blocks is believed to be chiefly responsible for their variable affinity for heavy metals. The affinity of some divalent metals varies among alginates with different M:G ratios. Haug *(24)* showed that the affinity of alginates for divalent cations such as Pb^{2+}, Cu^{2+}, Cd^{2+}, Zn^{2+}, and Ca^{2+} increases with the guluronic acid content. In addition, the selectivity coefficients for

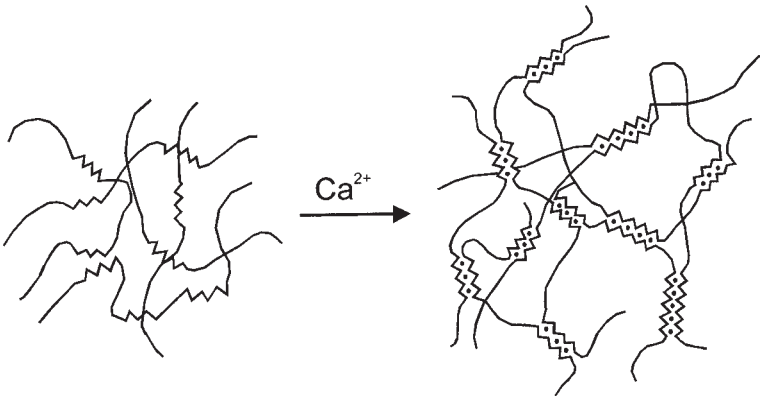

Fig. 2. Schematic representation of the calcium-induced gelation of alginate in accordance with the "egg-box" structural model. (From ref. *26*.)

the ion-exchange reaction between sodium and divalent metals for two alginates *(25)* further support the higher affinity of guluronic acid for divalent metals.

The higher specificity for divalent metals is explained by the "zigzag" structure of polyguluronic acid, which can accommodate the Ca^{2+} (and other divalent cations) ion more easily. The alginates are thought to adopt an ordered solution conformation, through dimerization of the polyguluronate sequences in the presence of calcium or other divalent cations of similar size (*see* Fig. 2). The rigid and buckled shape of the poly-G sections results in an alignment of two chain sections, yielding an array of coordination sites with cavities favorable to divalent cations because they are lined with carboxylate and other electronegative oxygen atoms. This description is known as the "egg-box" model *(27–29)*. The regions of dimerization are terminated by chain sequences of polymannuronate. As a result, several different chains may become interconnected and this promotes gel network formation.

The detailed composition of alginates *(30–32)* derived from brown algae such as the kelps (order Laminariales) *Laminaria digitata*, *Laminaria hyperborea*, and *Macrocystis pyrifera* has been the focus of considerable research effort. However, relatively little detailed information exists in the literature on the composition of alginates from species of "rockweeds" (order Fucales), such as *Sargassum*. Together, the Laminariales and Fucales are probably the largest, most abundant, and widespread macroalgae, making them highly suited for potential remediation applications. Consequently, the main objective of this study was to characterize the detailed uronic acid composition (e.g., F_{GG} versus F_{MM} and F_{MG}) of alginates extracted from two abundant species of *Sargassum*.

In this article, we present ^{1}H-NMR (nuclear magnetic resonance) spectra (500 MHz) of alginates extracted from *S. fluitans* and *S. siliquosum* following an alkaline extraction protocol that yielded sufficiently degraded

and low-viscosity samples to allow acquisition of well-resolved spectra without further treatment.

Materials and Methods

Study Specimens

Sargassum fluitans, one of the few known pelagic species of *Sargassum*, originates from the Sargasso Sea of the northwest Atlantic Ocean. It is carried by wind and tides to the shores of Cuba, where it accumulates in copious quantities along the beaches. The biomass used in this study was collected fresh at Guanabo Beach, 30 km east of Havana. *Sargassum siliquosum* and *S. oligocystum* were collected from the reef flat, 1–4 m below low-tide level, on the fringing reef at Goold Island (18° 10.9' S, 146° 10.2'E) on the inshore, central Great Barrier Reef, Australia. These benthic species are found as part of mixed-species assemblages that dominate the reef flat of many inshore reefs on the Great Barrier Reef *(33–35)*.

Alkaline Extraction

Alginate was extracted both from bulk samples and from the easily separable frond, stipe, and air bladder of *S. fluitans* and *S. siliquosum* in a 2% solution of Na_2CO_3 according to a slight modification of the method of Percival and McDowell *(36)*. The extraction was carried out at 80°C instead of room temperature in order to reduce the viscosity (for NMR work) and ensure the complete extraction of the alginate. In the presence of excess Na_2CO_3, the alginic acid is converted to a Na alginate and is solubilized. The resulting Na alginate solution was separated from the solid phase by filtration. This step is followed by the precipitation of the alginic acid by addition of dilute hydrochloric acid and conversion of the sodium salt to the insoluble acid (pH < 1.0). The alginic acid precipitate was pelleted by centrifugation and washed with a 95% aqueous ethanol solution prior to conversion back to the sodium salt upon the addition of a concentrated sodium carbonate solution.

Neutral Extraction

Alginate was also extracted from the bulk samples of *S. fluitans* and *S. oligocystum* according to the protocol described by Haug *(37)* and Rivera-Carro *(38)*. The ground algal material was extracted three times in 0.2 *N* HCl prior to filtration and washing with water. The residue was resuspended in distilled water and sufficient sodium hydroxide was added to neutralize the alginic acid and maintain the pH between 6.5 and 7.5. The suspension was gently stirred overnight and then filtered and extracted once more. All filtrates were pooled and sodium chloride was added to the solution to obtain a 1% (w/w) final concentration. An equal volume of ethanol was added to precipitate the alginate, which was then washed first with a 60% aqueous ethanol solution, then twice with ethanol, and three times

with ethyl ether. The alginate was dried at 30–40°C. In contrast to the alkaline extraction, the standard neutral extraction yields viscous alginates and increases the time necessary for the preparation of the NMR samples by requiring an additional prehydrolysis step.

Analytical Procedures

Intrinsic Viscosity

A Schott Gerate viscometer Type 53610/I (Appl. no. 29743; K = 0.009 cS/s) was used to measure the relative viscosities. The apparatus was submerged in a water bath (20°C) during automated flow-time determinations. Solutions of 0.2%, 0.4%, and 1.0% sodium alginate in a 0.1 *M* sodium chloride buffer were used and the intrinsic viscosity [η], was determined by extrapolation to zero concentration according to the method of Haug and Smidsrød *(39)*. Ten measurements were made for each solution and the resulting averages used to calculate the relative viscosities.

^{1}H-NMR

The freeze-dried Na alginates were dissolved in D_2O and dried several times prior to NMR spectrum acquisition. The ^{1}H spectra were recorded with a Varian Unity 500 spectrometer at a temperature of 70°C, a sweep width of 5999.7 Hz, an 80° pulse, and an acquisition time of 2.048 s. Typically, 128 or 256 repetitive scans were acquired and the data were processed with a line broadening of 0.6 Hz. Sodium 3-trimethylsilylpropionate-2,2,3,3-d_4 (TSP) (Aldrich) was used as an internal reference. We initially added disodic ethylene dinitrilotetraacetic acid (EDTA) to the solution to avoid signal broadening by divalent cations, but we discontinued this addition because there was no discernable difference between spectra obtained with or without the EDTA. The solvent peak (HDO) was partly eliminated using a decoupler with a 5.0 second delay period. We also recorded several spectra at 90°C, without the decoupler because the HDO peak was shifted further upfield, away from the peaks of interest. Results of the integration of the peaks of interest was not affected by the change in temperature or the use of the decoupler. Consequently, and for greater convenience, the final spectra were recorded at 70°C with the decoupler and in the absence of EDTA. Multiple-peak deconvolutions were performed with the software Origin® (OriginLab™ Corp.) using the multipeaks function and a Lorentzian line shape.

Application of ^{1}H-NMR to the Present Study

The approach of Grasdalen et al. *(40)* was adopted to determine the M:G ratio and block distribution of the alginates extracted in this study. Because our spectra were acquired at a high field strength (500 MHz), further resolution of the low-field region of the alginate was obtained and additional peaks for the G-centered triads (GGM, MGM) and M-neighbor-

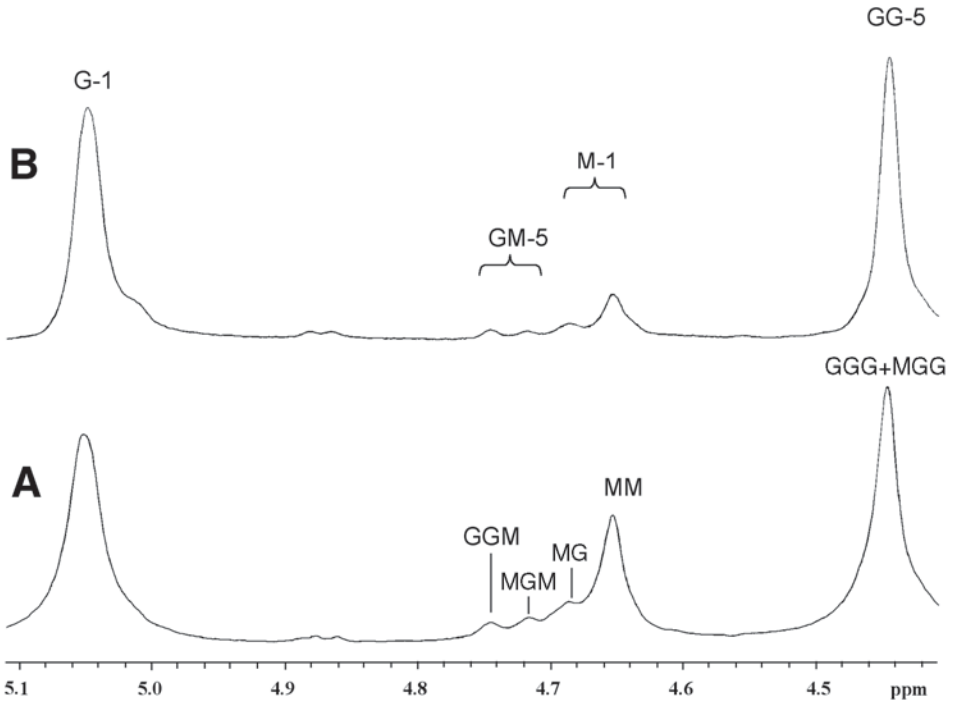

Fig. 3. Low-field region of the ^{1}H-NMR spectra (500 MHz) for solutions of sodium alginate extracted from **(A)** *S. siliquosum* (whole) and **(B)** *S. fluitans* (whole; in D_2O at 70°C). Samples (i.e., those reported in Table 1) were untreated (i.e., were not subjected to an acid prehydrolysis step). Doublets refer to the signal associated with the first monomer (e.g., M in MG-1) and triplets refer to the signal associated with the middle monomer (e.g., G in MGM-5). G-1 designates the proton on carbon-1 of guluronic acid; M-1 designates the proton on carbon-1 of mannuronic acid; similarly GM-5 refers to the proton on carbon-5 of guluronic acid. Peak assignments from refs. *31* and *40*.

ing diads (MG, MM) were partly resolved (*see* Fig. 3). These peaks were assigned by Grasdalen *(31)* based on high-field spectra obtained at 400 MHz. Our spectra are very similar to those published by Grasdalen *(31)* except that his spectra display a greater resolution, presumably because of an extensive and controlled depolymerization pretreatment of his alginate.

The two p*D*-dependent peaks (i.e., the GG-5 and GM-5) were further resolved into four peaks by Grasdalen *(31)* at p*D* = 4. Two of these are assigned to the GGM and MGM triads and result from a splitting of the GM-5 peak (*see* Fig. 3A). The other two correspond to the GGG and MGG triads and arise from the previously assigned GG-5 peak (*see* Fig. 3A). The M-1 peak was also further resolved into two peaks corresponding to the MG and MM diads (*see* Fig. 3A).

The mole fraction of M can be calculated from the condition

$$F_G + F_M = 1 \quad (1)$$

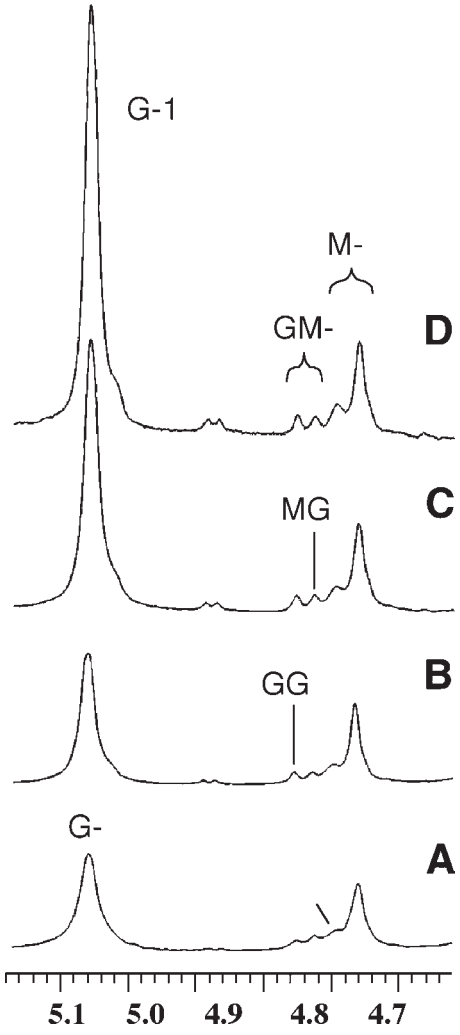

Fig. 4. Low-field region of the ^{1}H-NMR spectra (500 MHz) for solutions of sodium alginate extracted from *S. fluitans*: curve A: air bladders; curve B: stipes; curve C: whole; curve D: fronds (in D_2O at 70°C). Compositional data are reported in Table 1. Peak assignments from refs. *31* and *40*.

Furthermore, it was pointed out *(40)* that for long chains, corrections for the reducing-end residues (i.e., a hemiacetal; or, in other words, the anomeric carbon not in a glycosidic bond) may be neglected, so that $F_{MG} = F_{GM}$. This can be confirmed by calculating the average degree of polymerization by comparing the results of peak integration of the reducing-end units (e.g., δ 4.88 in Fig. 4) to the total spectrum signal intensity. Thus, with the relations for the doublet frequencies being

$$F_{GG} + F_{GM} = F_G \quad (2)$$

$$F_{MM} + F_{MG} = F_M \quad (3)$$

the M:G ratio is defined explicitly as

$$\frac{M}{G} = \frac{1 - F_G}{F_G} \tag{4}$$

following substitution of F_G (determined directly from integration of the spectra). The frequency relationships are given as follows:

$$F_G = F_{GGG} + F_{MGG} + F_{GGM} + F_{MGM} \tag{5}$$

$$F_{MG} = F_{GM} = F_{GGM} + F_{MGM} \tag{6}$$

$$F_{MGG} = F_{GGM} \tag{7}$$

Figure 3A,B show spectra of alginates extracted from whole *S. siliquosum* and *S. fluitans*, respectively. The cluster of peaks that are partly resolved and lie between δ 4.63 and 4.76 corresponds to the GGM and MGM triads that arise from splitting of the GM-5 peak, and the MG and MM diads that arise from splitting of the M-1 peak (*see* Fig. 3A). The GG-5 peak should be resolved into the GGG and MGG triads, but only one broad peak centered at δ 4.45 is observed. The latter may reflect the predominance of GGG over MGG triads (discussed in more detail in the next section). Hence, because the G-centered triads are poorly resolved and the GGG peak overlaps the MGG peak (because of line broadening), we used the approach originally proposed by Grasdalen et al. *(40)* for the low-field spectra to calculate the M:G ratio and diad frequencies. This was achieved following the combined integration of the two GM-5 triad peaks and the two M-1 diad peaks as well as the individual integration of the GG-5 and G-1 peaks.

Results and Discussion

Determination of the intrinsic viscosity of the alginate samples revealed a dramatic difference between those extracted by the standard neutral technique *(37,38)* and the alkaline method performed at 80°C. The intrinsic viscosities, [η], of the alginates extracted from *S. fluitans* and *S. oligocystum* by the neutral technique are 11.6 and 10.0 dL/g, respectively. These viscous extracts cannot be readily characterized by solution NMR spectroscopic techniques *(31,40)* without a prior, controlled depolymerization in order to reduce their viscosity. In contrast, the intrinsic viscosities of the alginates extracted from *S. fluitans* and *S. siliquosum* (the samples herein characterized by ^{1}H-NMR) by the alkaline method are 0.57 and 0.84 dL/g, respectively. The alkaline extraction method, when performed at room temperature, may lead to degradation in cases where the seaweed contains reducing substances, but this can be overcome by adding formaldehyde to the seaweed suspension before it is made alkaline *(41)*. It is clear that the elevated temperature (80°C) at which the extraction was carried out in this work contributed to enhanced degradation, but this was desirable in order to obtain well-resolved spectra. The clear advantage

Table 1
Compositional Data of Alginates Extracted from Whole and Individual Parts of *Sargassum* spp.

Source	Composition, fractions		Doublet frequencies				M/G
	F_M	F_G	F_{MM}	F_{MG}	F_{GM}	F_{GG}	
S. fluitans, frond	0.13	0.87	0.02	0.11	0.11	0.76	0.15
S. fluitans, whole	0.16	0.84	0.13	0.03	0.03	0.81	0.19
S. fluitans, stipe	0.37	0.63	0.34	0.03	0.03	0.60	0.60
S. fluitans, bladder	0.41	0.59	0.37	0.03	0.03	0.56	0.69
S. siliquosum, frond	0.41	0.59	0.38	0.03	0.03	0.56	0.70
S. siliquosum, whole	0.42	0.58	0.41	0.01	0.01	0.57	0.72
S. siliquosum, stipe	0.48	0.52	0.46	0.03	0.03	0.49	0.94
S. siliquosum, bladder	0.42	0.58	0.41	0.01	0.01	0.57	0.72

of using the alkaline extraction method is the elimination of the hydrolysis step prior to NMR acquisition.

The efficiencies of the two alginate extraction methods employed in this work were comparable, and their efficiencies relative to an alkaline extraction performed with NaOH is under investigation. These results, in addition to the relative cadmium-binding performance and polymer composition (^{1}H-NMR) of the extracts obtained by the different extraction methods, are the subject of a forthcoming publication *(42)*.

The ^{1}H-NMR results are summarized in Table 1. The M:G ratio of the Na alginates extracted from *S. fluitans* varied from 0.15 to 0.69, with alginate derived from the fronds having the highest guluronic acid content. Guluronic acid is the major component in all extracts and the GG diads (F_{GG}) account for more than 55% of the polymer diads. The F_{MM} in the fronds is unusually low and the alternating blocks (e.g., F_{MG} and F_{GM}) are more abundant than for the other extracts of *S. fluitans*. The fact that the composition of the alginate from the whole sample closely matches that of the alginate from the frond, with a slightly lower guluronic acid content, is the result of the predominance of the fronds (dry weight) relative to the stipes and air bladders in the seaweed's natural state.

The range of M:G ratios (i.e., 0.70–0.94) among the various parts of *S. siliquosum* is much narrower than for *S. fluitans*. The composition of the alginates extracted from the stipes and air bladders of *S. fluitans* is very similar to that of the bulk *S. siliquosum* as well as its air bladders and fronds. The F_{GG} of all the *S. siliquosum* samples ranges from 0.49 to 0.57. In all cases, except for the fronds of *S. fluitans*, the alternating block sequence frequencies are very low and reflect the dominance of homopolymeric blocks of guluronic and mannuronic acid in these alginates. As outlined in the following subsection, our estimated M:G ratios are in close agreement with those of other samples.

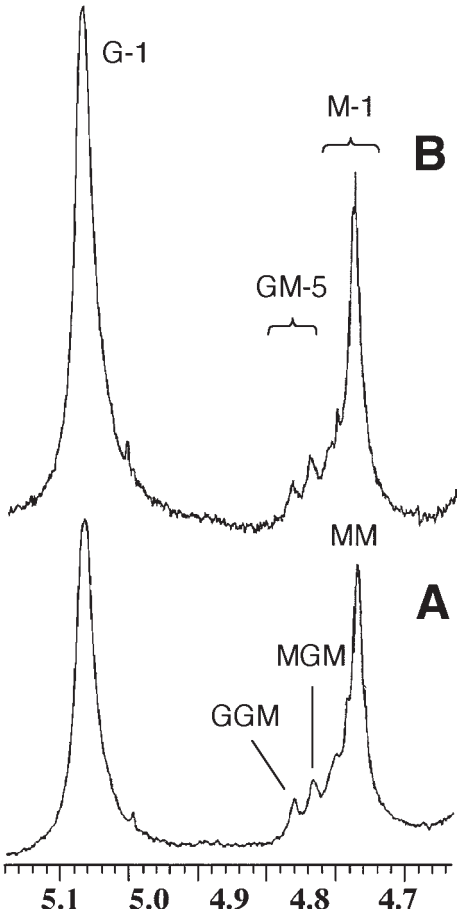

Fig. 5. Low-field region of the ^{1}H-NMR spectra (500 MHz) for solutions of sodium alginate extracted from *S. siliquosum*: curve A: stipes; curve B: air bladders (in D_2O at 70°C). Compositional data are reported in Table 1. Peak assignments from refs. *31* and *40*.

Deconvolution and Test of Peak Relationships

The above-described results are represented by the low-field region of the spectra in Figs. 4 and 5. As indicated in Fig. 3 and discussed in the previous section, the GM-5 and M-1 peaks are both split into their respective diad and triad peaks (e.g., GGM, MGM, MG, and MM; *see* Fig. 3A). The diad peak (MM) is the strongest of these four peaks in all of the spectra (*see* Figs. 4 and 5). The MG peak is poorly resolved in the spectra of the air bladders of *S. fluitans*, as well as in those of the air bladders and stipes of *S. siliquosum*, but it is partly resolved in spectra of the alginate of the whole, stipes, and fronds of *S. fluitans* (*see* Fig. 4B,C,D). We used the latter three spectra to test the relationship described by Eq. (6). The equation relates the sum of the mixed triad frequencies F_{GGM} and F_{MGM} to the mixed diad frequencies F_{MG}, or F_{GM}. Furthermore, according to Eq. (7), the triad (MGG and GGM) peak intensities and hence, their frequencies, should be equal.

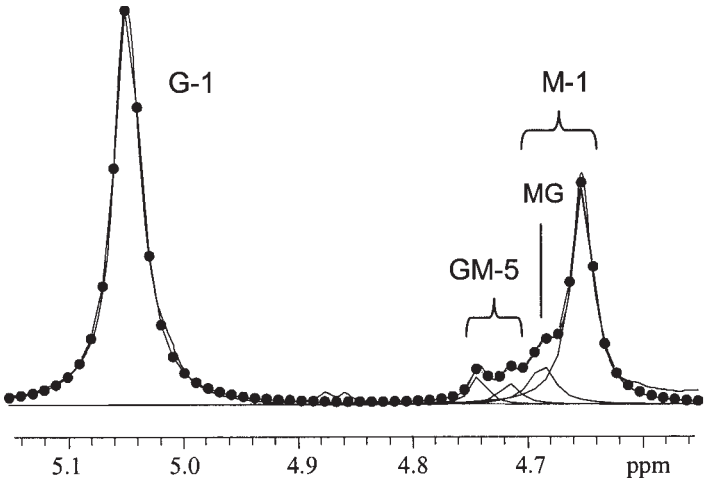

Fig. 6. Deconvolution of the low-field region of the ^{1}H-NMR spectra (500 MHz, in D_2O, 70°C) of sodium alginate extracted from *S. fluitans* (whole sample).

Deconvolution of these peaks, including the G-1 peak, afforded reasonable curve fitting (*see* Fig. 6). We performed deconvolutions of each of the three spectra (*see* Fig. 4B,C,D) and the results conformed to Eq. (6). The areas corresponding to the two triad (GGM and MGM) frequencies consistently sum up to within ±10% of the area corresponding to the MG diad. This relationship applies to all of the spectra and supports our interpretation that the mixed sequences (MG) are less abundant than the homopolymeric diads. This can be further confirmed by Eq. (7), whereby the relatively small peak intensity of GGM (δ4.75) should correspond to the peak intensity of the MGG resonance (δ 4.44). The large GG-5 signal in all our spectra is the combined signal of both the GGG and MGG resonances and thus, given that the relative contribution of MGG to this peak is very small, it reflects the high proportion of GGG sequences in our samples. This would imply that for these untreated Na alginate samples, the homopolymeric guluronic diad frequency may approximate the homopolymeric guluronic triad frequency.

Other Alginates

The M:G ratios of samples extracted from both *S. fluitans* and *S. siliquosum* ranged from 0.15 to 0.94. Five out of the eight sample values are consistent with the M:G ratio (approximately 0.6) obtained by Llanes and others *(43)* for alginates extracted from a mixture of *Sargassum* species (e.g., whole *S. fluitans* and *S. natans*). Two alginate samples extracted from *S. fluitans* (i.e., frond and whole) were more enriched in guluronic acid than the rest of the samples and represent, to the best of the authors' knowledge, the lowest M:G ratios documented for *Sargassum* species.

M:G ratios for alginates extracted from other brown algae are reported in Table 2. For *Laminaria digitata* and *L. hyperborea* (Table 2), the stipes are most enriched in guluronic acid, as reflected by their relatively low M:G

Table 2
Alginate Data from Previous Studies

Source of alginate	Tissue	M/G
Laminaria digitata	New fronds	2.3
after Haug and others *(44)*	Old fronds	1.35
	Stipes	1.15
Laminaria hyperborea	New fronds	1.9
after Haug and others *(44)*	Old fronds	1.25
	Stipes	0.6
Sargassum siliquastrum	Fronds	0.85
after Minghou and others *(45)*	Stipes	0.78
	Air bladder	1.01
Sargassum hemiphyllum	Fronds	0.88
after Minghou and others *(45)*	Stipes	1.04

ratio. This contrasts with the trend observed for the alginate samples of *S. fluitans* reported here. A similar trend *(45)*, however, was observed for *S. hemiphyllum* with the alginate from the fronds representing the more guluronic acid-rich sample. Furthermore, the *Laminaria* species exhibit a much wider range in alginate composition for the different parts and growth stages of the plant than was observed for our *Sargassum* alginate samples. Although variability may be expected for different thalli of the same plant, the *Sargassum* alginate samples are all richer in guluronic acid than samples from species of *Laminaria*. The same is true of *S. siliquastrum* and *S. hemiphyllum* (*see* Table 2) and a more detailed compositional analysis of these species may reveal the same high frequency of guluronic acid blocks (F_{GG}) as for *S. fluitans* and *S. siliquosum*.

It has been reported *(45)* that most *Sargassum* alginates have M:G ratios ranging from 0.8 to 1.5, whereas alginates from species such as *Ascophyllum nodosum* (approx 1.2; order Fucales) and *L. japonica* (approx 2.2; order Laminariales) possess relatively high M:G ratios *(28,30–32,46,47)*. Our results support these findings and show that the alginates examined here display a high proportion of homopolymeric guluronic acid blocks ($F_{GG} > 0.49$) along the polymer chain. Furthermore, far less variability in M:G ratio is observed for the various parts of the plant than for species of *Laminaria*. Similarly, Llanes and others *(43)* used solid-state ^{13}C-NMR spectroscopy to show that the alginate extracted from a mixed *Sargassum* sample contained guluronic acid, which mainly occurred as homopolymeric blocks. Their solid-state analysis of the alginate extract powder and our aqueous solution ^{1}H-NMR data are consistent and emphasize the predominance of G homopolymeric blocks in *Sargassum*.

Significance for Applied Biosorption

Differential Properties of the Purified Alginate and Raw Biomass

The relevance of these results in the context of heavy-metal remediation derives from the premise that the macromolecular composition,

monomer sequencing, and conformation of the alginate biopolymer are key factors in determining the differential affinity of various metals for the biomass substrate. The influence of composition on metal affinity has been previously demonstrated for raw samples of brown algae for the strontium–calcium and strontium–magnesium metal pair systems *(14)*. In that study, it was found that raw brown algal thallus displays a marked selectivity for strontium over magnesium or calcium as the guluronic acid content of the alginate in the thallus increased. Those results compare remarkably well with the selectivity measurements performed in the same study *(14)* with extracted alginate for the same divalent ion pairs.

In another study *(48)*, which investigated the role of alginate as a structural component in brown seaweeds, Andresen and others also reported on the differential metal selectivities of the raw biomass and extracted alginate. The calcium–magnesium ion-exchange reaction was used as a "probe" to test whether the network structure was different in the gel and in the plant. In that study, alginate rich in G-blocks was extracted from *L. hyberborea* stipes. The sample was dialyzed extensively, first in seawater and then against different calcium–magnesium chloride solutions in order to determine selectivity coefficients. Andresen and others then applied the same experimental protocol to the raw *L. hyperborea* thallus (stipe) and obtained comparable, relative selectivities for both systems. The algal thallus did, however, display a markedly higher selectivity for calcium over magnesium than the extracted alginate at low calcium contents. Measurements of the modulus of rigidity of the gels and the algal thalli, reported in the same study *(48)*, reveal that the alginate's conformation is likely different in the plant than in the extracted, alginate gels. The difference in structural arrangement may account for the higher selectivity of calcium over magnesium in the raw algal thallus than by the extracted alginate.

Other Considerations

The alginate content, normally expressed as percent dry weight of the native plant, directly correlates with the metal-uptake capacity of brown algal species such as *Sargassum*. The alginate content of *S. fluitans* has been reported *(19)* to account for 45% of the dry weight of the biomass following stripping of its sea salts and conversion to the protonated form by soaking in a 0.1 *N* HCl solution and extensive rinsing with deionized water. Figueira et al. *(49)* have shown that for a variety of brown algae, between approx 40% and 50% of the original biomass' dry weight is lost during this acid treatment. The alginate yields of *S. fluitans* and *S. oligocystum* (*S. siliquosum* not determined) extracted for this work were approximately 45% and 37%, respectively. The alginate content of *Sargassum* varies from species to species and depends on such factors as the growth stage *(36,50,51)*. In summary, both the alginate content and its conformation (discussed earlier) determine the metal-binding behavior of *Sargassum* spp.

It is now clear that the presence of guluronic acid blocks (most easily characterized by F_{GG}) in alginates within the thallus of any brown seaweed will significantly influence the relative affinity of the biomass substrate for

different divalent ions. In the context of heavy-metal remediation applications, where competing ions will likely be present, the enhanced selectivity of the G-blocks, for toxic divalent ions such as cadmium and copper, may be critical to its intended implementation. Apart from the *Sargassum* species discussed in this work and those referred to in the discussion, the only other known source of commercially available alginates rich in guluronic acid is the stipes of *L. hyperborea*. In contrast to samples of *Laminaria* (Table 2), which display a wide range of M:G ratios, our *Sargassum* samples were, for the most part, uniformly rich in guluronic acid (Table 1). If *Sargassum* spp. prove to consistently have a lower degree of compositional variability or perhaps contain alginate that does not exceed an M:G ratio of 1, it would not be necessary to separate the stipes from the fronds in order to use the guluronic acid-rich algal thallus. This would add cost-effectiveness to the implementation by eliminating a labor-intensive step in the preparation of the substrate for bioremediation applications. In addition to the narrower compositional range (among parts of the plant) of alginate in the *Sargassum* species used in this study, its resistance to leaching *(15)* is also an important consideration in the design of remediation strategies.

Future studies should focus on a careful characterization of the alginate composition of a variety of brown algal tissues, coupled with a comparison of their performance in competitive metal-binding experiments over a range of ionic strengths. The objective would be to relate the percent alginate content and F_{GG} to their overall multimetal uptake performance.

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