

Extraction and Characterization of Alginate from *Sargassum angustifolium* collected from northern coasts of Persian Gulf, Bushehr



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Abstract

Alginates, unbranched polymers of polysaccharide with gel forming properties made up by blocks of beta-(1-4)-d-mannuronic acid (M) and alpha-(1-4)-L-guluronic acid (G), have emerged as valuable biomaterials with interesting biomedical and biotechnological applications. In brown seaweeds, alginates are the most abundant polysaccharides reaching up to 40% of the dry weight. The extraction of alginate may be regarded as a process in two steps: transformation of insoluble alginate into a soluble form namely sodium alginate, followed by diffusion of the soluble glycuronan into solution. Alginate fractions from *Sargassum angustifolium* brown seaweed were extracted after collection, washing, drying and grinding of seaweeds into powder, and then characterized by ¹HNMR and rheological measurements. ¹HNMR spectroscopy is most suitable for characterizing both the composition and the distribution sequence of the two uronate residues in algal samples. The alginate extraction conditions were investigated. In the optimized condition, samples extracted for 3×3h at 50°C were further purified by re-precipitation with ethanol. The M/G ratio value was 0.89, higher than the ratio for most *Sargassum* species alginates. The homopolymeric blocks F_{GG} and F_{MM} of these fractions characterized by ¹HNMR spectroscopy were 0.39 and 0.33, respectively ($\eta < 1$). Therefore, the alginate from *S. angustifolium* is much richer in mannuronic block structures than those from other *Sargassum* species. Values of M_w for alginate samples were also calculated using intrinsic viscosity data. The M_w value was 702.6 kDa.

Key words: Alginate, *Sargassum angustifolium*, Mannuronic acid, Guluronic acid

Introducton

Alginate is a family of unbranched binary copolymers of linked β -D-mannuronic acid (M) and α -L-guluronic acid (G) residues (Fig. 1(a) and (b)) of widely varying composition and sequence. The first information about the sequential structure of alginates came from the work by Haug et al. (Haug, A. 1964, 1965, 1966, 1967). By partial acidic hydrolysis and fractionation, they were able to separate alginate into three fractions of widely differing composition. Two of these contained almost homopolymeric molecules of guluronic and mannuronic acid, respectively, while a third fraction consisted of nearly equal proportions of both monomers, and was shown to contain a large number of MG dimer residues. It was concluded that alginate was a true block copolymer composed of homopolymeric regions of M and G, termed M- and G-blocks, respectively, interspersed with regions of alternating structure (MG-blocks; Fig. 1(c)) (Painter, T.J. 1968, Larsen, B. 1970, Smidsrød, O. 1969).

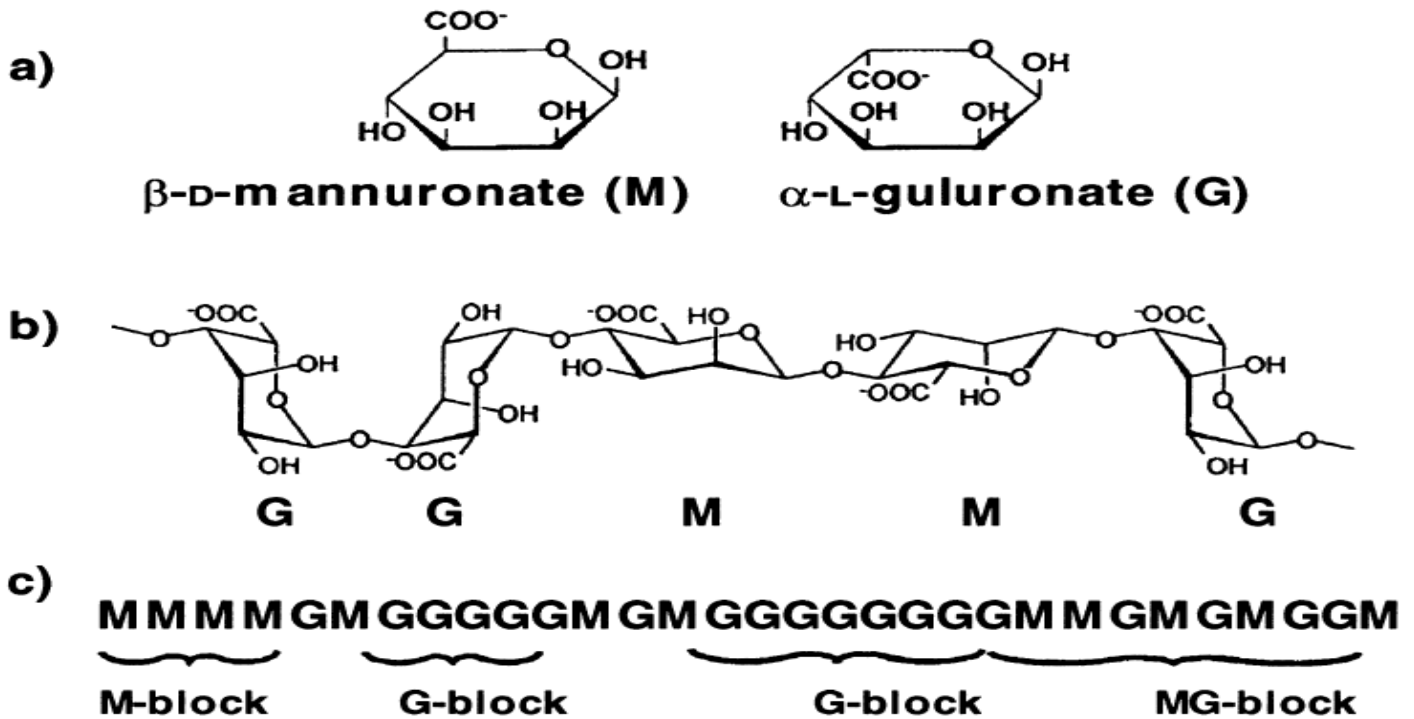


Figure 1- Structural characteristics of alginates: (a) alginate monomers, (b) chain conformation, (c) block distribution (Draget et al., 2005).

This polysaccharide was recognized as a structural component of marine brown algae (Phaeophyceae), where it constitutes up to 40% of the dry matter and occurs mainly in the intercellular mucilage and algal cell wall as an insoluble mixture of calcium, magnesium, potassium, and sodium salts (Haug and Smidsrød 1967). The presence of alginate provides the mechanical strength and flexibility of the seaweed and, additionally, acts as water reservoir preventing dehydration once part of the seaweed has been exposed to air. Alginates well meet all the requirements for their use in pharmaceutical and medical applications. They have been largely used in wound dressings, dental impression, and formulations for preventing gastric reflux. (Smidsrød and Skjåk-Bræk 1990)

The main industrial applications of alginate as a natural polymeric material are linked to its stabilizing, viscosifying, and gelling properties and its ability to retain water (Cottrell and Kovacs 1980 ; Littlecott 1982 ; Sime 1990). The main focus of the present study was to determine the alginate yield, alginate viscosity, molecular weight and monomer fractions of alginate extracted from *Sargassum angustifolium*, commonly found at Port Bushehr, Iran.

Materials and methods

Extraction and purification of alginates

Sargassum angustifolium was collected in coastal waters of Bushehr (North Persian Gulf) in August 2011. The seaweed samples were washed with water to remove impurities such as sand and air-dried for at least 2 days. Then, the seaweed samples were cut into small pieces and subsequently kept in bottles and stored in the refrigerator. Alginates were extracted according to the procedure of Calumpong et al. (1999). Twenty-five grams of dried algae were soaked in 800 mL of 2% formaldehyde during 24 h at room temperature, washed with water and then added to 0.2 M HCl (800 mL) and left for 24 h. After this time, the samples were washed again with deionized water before extraction with 2% sodium carbonate during 3 h at 50°C. So, the soluble fraction was collected by centrifugation (3500rpm, 20 min) and polysaccharides were precipitated by ethanol 95%. Sodium alginate collected was washed by acetone, dried at 40°C and dissolved in 100 mL of deionized water. It was then precipitated again with ethanol and dried at 40°C.

To determine the ash content, three sets of dry samples (5 g each) were placed in crucibles and dried in the oven at 105°C for 30 min. The dry samples were weighed again and then calcined in the muffle furnace at 450°C for 3 h. Calcined samples were cooled in a desiccator and weighed again to determine the ash content according to the following equation:

$$\text{Ash content(\%)} = \left(\frac{\text{weight of ash}}{\text{weight of dry algae}} \right) \times 100 \quad (1)$$

Dried polysaccharides samples (1 mg) were dispersed in 100 mg of anhydrous KBr and pressed. The IR spectra were recorded at room temperature in the wavenumber range of 400– 4,000 cm^{-1} and referenced against air with a Bruker, Vector22 FT- IR instrument. The ^1H spectra analyses were achieved at 90°C with a Bruker 400 NMR spectrometer and 64 repetitive scans. Sample were prepared for NMR analysis by the partial acidhydrolysis method (Davis et al. 2004).

Results and Discussion

Alginate from *Sargassum angustifolium* was extracted successfully. After drying, the yield of extraction was $40.78 \pm 4.4\%$ (w/w). There average ash content was 30.8%.

The FT-IR spectrum of sodiualginate from *S. angustifolium* is presented in the Fig. 2. A broad band at 3443 cm^{-1} was assigned to hydrogen bonded O–H stretching vibrations and two other at 2928 and 1627 cm^{-1} were attributed to C–H stretching and to carboxylate O–C–O asymmetric stretching vibrations, respectively. According to Mathlouti and Koenig (1986) and to Silverstein et al. (1991), the absorption at 1421 cm^{-1} was assigned to C–OH deformation vibration with contribution of O–C–O symmetric stretching vibration of carboxylate group. The bands measured at 1321 (shoulder peak), 1096 and 1030 cm^{-1} may be attributed to C–C–H (and O–C–H) deformation, to C–O stretching vibrations and C–O (and C–C) stretching vibrations of pyranose rings, respectively.

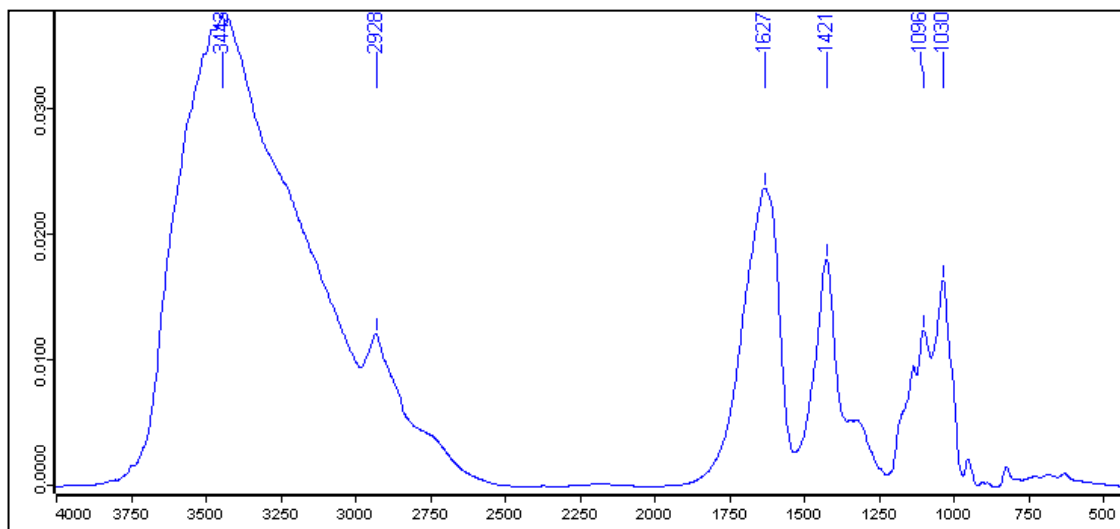


Figure 2- FT-IR spectrum

^1H NMR spectroscopy is considered to be the most reliable method for determining both composition and much of the detailed block structure of the alginate, giving the two monad values (F_M and F_G) and the four diad frequencies (F_{GG} , F_{MM} , F_{MG} , F_{GM} ; Draget et al. 2002). The values obtained were compared with data collected by Torres et al. (2007) about other *Sargassum* species producing alginates (Table 1). The η parameter

$$\eta = \frac{F_{MG}}{F_M + F_G} \quad (2)$$

gives an information about the abundance of homopolymeric block when its value is inferior to 1 according to Grasdalen et al. (1979). The sodium alginate extracted from *S. angustifolium* had an M/G ratio of 0.89 and a $\eta = 0.56$. Viscosity of obtained alginate was 14.55 dl g^{-1} . The calculated M_w was in kDa before it was converted to g mol^{-1} .

Table 1- Compositional data of alginates extracted from *Sargassum* species.

Sargassum Species	M/G	F_M	F_G	*F_{MG}	F_{GG}	F_{MM}	η	References
S.vulgare (Brazil)	1.27	0.56	0.44	0.02	0.43	0.55	0.04	Torres et al.2007
S.fluitans (Florida)	1.18	0.54	0.46	0.36	0.28	0.36	0.72	Davis et al. 2004
S.turbiniarioides Grunow(Madagascar)	0.94	0.48	0.52	0.25	0.39	0.36	0.50	Fenoradosoa et al.2010
S. angustifolium(Iran)	0.89	0.47	0.53	0.14	0.39	0.33	0.56	This study
S.latifolium (Egypt)	0.82	0.45	0.55	0.08	0.51	0.41	0.16	Larsen et al.2003
S.asperifolium (Egypt)	0.69	0.41	0.59	0.22	0.48	0.30	0.45	Larsen et al.2003
S.oligocystum (Australia)	0.62	0.38	0.62	0.14	0.55	0.31	0.29	Davis et al. 2004
S.thunbergii (Korea)	0.53	0.34	0.66	0.34	0.48	0.17	0.75	Davis et al. 2003
S.dentifolium (Egypt)	0.52	0.34	0.66	0.22	0.55	0.23	0.49	Larsen et al. 2003
S.fluitans (Cuba)	0.52	0.34	0.66	0.18	0.57	0.25	0.40	Davis et al. 2004
S.muticum (England)	0.31	0.24	0.76	0.34	0.59	0.07	0.93	Davis et al. 2003
S.polycystum	0.21	0.18	0.82	0.10	0.77	0.12	0.34	Davis et al. 2003
S.filipendula	0.19	0.16	0.84	0.16	0.76	0.07	0.59	Davis et al. 2003

*F_{MG}=F_{GM}

Except for *s.vulgare* and *S. fluitans*, the rest of mentioned species have more G blocks in their alginate. As table 1 shows, the extracted alginate is very similar to that extracted from *S. turbiniarioides Grunow* (Fenoradosoa et al. 2010).

Conclusion

Water-soluble alginate was obtained from an aqueous extract of *S. angustifolium*. FT-IR and NMR spectra analysis of the whole alginate, suggested that the alginate obtained in the present work contained acid residues by M/G ratio of about 0.89. The molecular weight of the alginate obtained from this work is generally comparable to those obtained from Egypt, Brazil and Malaysia (Torres et al. 2007). Thus, the alginate extracted from Persian Gulf species has the potential to be used in relevant industries according to its viscosity range. To our knowledge, this is the first report relative to the characterisation of a polysaccharide from *S. angustifolium*.

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