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Carbohydrate Research 343 (2008) 308–316
Carbohydrate RESEARCH

# FT-IR spectra of alginic acid block fractions in three species of brown seaweeds

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Received 23 August 2007; received in revised form 12 October 2007; accepted 23 October 2007

**Abstract**—Sodium alginates obtained by alkaline extraction of *Lessonia flavicans*, *Desmarestia ligulata* and *Desmarestia distans* (Phaeophyta) from southern Chile were partially hydrolyzed with HCl. Each alginate gave three fractions that were characterized using FT-IR spectroscopy. The fractions soluble in 0.3 M HCl presented in the fingerprint region four vibrations at around 960, 911, 890 and 815 $cm^{-1}$ that were assigned to heteropolymeric blocks. The fractions soluble at pH 2.85 showed bands at around 948, 888 and 820 $cm^{-1}$ attributed to homopolymannuronic acid blocks, the first band is resolved in the second-derivative spectra into two bands at 951 and 936 $cm^{-1}$. The fractions insoluble at pH 2.85 presented four bands at around 947, 903, 812 and 781 $cm^{-1}$, which were assigned to homopolyguluronic acid blocks. For some samples, the second derivative FT-IR spectra showed new bands indicating the presence of other structures, in low proportions. Structures deduced by FT-IR spectroscopy were corroborated by solution $^{1}H$ and $^{13}C$ NMR spectroscopy. Two-dimensional spectra were collected to confirm the fine structure of the hetero- and homopolymeric fractions. A geometrically optimized model for the disaccharide α-L-gulopyranuronosyl-(1→4)-α-L-gulopyranuronic acid was calculated using density functional theory; good agreement was obtained between its corresponding calculated vibrations and the experimental bands assigned to homopolyguluronic acid blocks.


*Keywords:* Alginic acids; Block composition; FT-IR characteristic bands; DFT-calculated vibrations

## 1. Introduction

The major structural polysaccharide of brown seaweeds (Phaeophyta) is alginic acid, a linear copolymer of (1→4)-linked β-D-mannopyranuronic acid (M) and (1→4)-linked α-L-gulopyranuronic acid (G) residues, arranged in heteropolymeric and homopolymeric blocks.[1,2] The content of uronic acids varies with species and tissues types, and partial acid hydrolysis of alginic acids allows the preparation of fractions enriched in hetero- and homopolymeric blocks.[3,4] Earlier studies done in our laboratory showed no relation between M/G values and block composition in alginic acids from *Lessonia*.[5,6] We also found that the homopolymannuronic acid fraction stimulated defence mechanisms in wheat and tobacco plants.[7,8]

We now report the fine chemical structures in the fractions obtained by partial hydrolysis of sodium alginate from three brown seaweed species using solid state FT-IR. Results were compared with solution $^{1}H$ and $^{13}C$ NMR spectroscopic studies. To interpret some FT-IR assignments, the disaccharide α-L-gulopyranuronosyl-(1→4)-α-L-gulopyranuronic acid (GG) was used as a structural model; the GG arrangement was calculated using density functional theory. The aim of this work was the study of the fine structure of alginic acid block fractions from different sources by FT-IR spectroscopy. In addition, the results obtained by this technique may contribute to the structural elucidation of industrial polysaccharides.

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doi:10.1016/j.carres.2007.10.016

## 2. Experimental

### 2.1. Materials and methods

*Lessonia flavicans* Bory de Saint-Vicent, *Desmarestia ligulata* (Stackhouse) Lamouroux and *Desmarestia distans* (C. Agardh) J. Agardh, were collected in Fuerte Bulnes (53°37′55.6″S, 70°55′17.9″W), Chile, in autumn and identified by Dr. Andrés Mansilla. Samples were deposited in Sala de Colecciones, Departamento de Ciencias y Recursos Naturales, Universidad de Magallanes, Punta Arenas, Chile. D-Galacturonic acid and D-glucuronolactone from Sigma Chemical Co., St. Louis, MO, USA were used as standards. One and two-dimensional NMR spectra were recorded at 70 °C on a Bruker Avance 400 spectrometer equipped with a *z*-gradient inverse broad band probe of 5 mm diameter, operating at 400.13 MHz ($^1$H) and 100.62 MHz ($^{13}$C) after isotopic exchange with $D_2O$ ($3 \times 0.75$ mL) using $D_2O$ as solvent and $CH_3OH$ as the internal reference ($\delta^{13}$C: 49.50 ppm, $\delta^1$H: 3.340 ppm). All two-dimensional experiments were acquired using a pulse field gradient incorporated into NMR pulse sequences. The two-dimensional homonuclear $^1$H–$^1$H correlation spectroscopy (COSY) was acquired with $128 \times 2040$ data points having a spectral width of 1200 Hz and processed in a $1024 \times 1024$ matrix to give a final resolution close to 2.3 Hz/point in the two-dimensions. The two-dimensional heteronuclear-single quantum coherence correlation (2D $^1$H–$^{13}$C HSQC) spectra were acquired with $128 \times 1024$ data point and processed in a $1024 \times 1024$ matrix to give a final resolution close to 2.3 Hz/point in $^1$H and close to 2.4 Hz/point in $^{13}$C. The number of scans (ns) in each experiment was dependent on the sample concentrations.

### 2.2. FT-IR spectroscopy

Samples of sodium alginates and fractions were powdered under liquid nitrogen and dried for 8 h at 56 °C in vacuo. The FT-IR spectra of the samples in KBr pellets (10% w/w) were recorded in the 4000–400 cm$^{-1}$ region using a Bruker IFS 66*v* instrument; 32 scans were taken with a resolution of 4 cm$^{-1}$. Derivation including Savitzky-Golay algorithm with 23 smoothing points was performed using the OPUS/I.R. version 1.44 software incorporated into the hardware of the instrument.[9]

### 2.3. Extraction and purification

Ground blades (~2 mm size) of algal samples (100 g) were stirred for 10 min in 1 L petroleum ether (bp 40–60 °C). The supernatant was concentrated in vacuo and the extraction process was repeated (10 times) until no more solid residue was obtained from the concentrate. The residual petroleum ether was evaporated at room temperature for 72 h and the algae were treated with 1.6 L of 96% ethanol and 0.4 L of 37% aqueous formaldehyde. After 72 h, the solid was decanted and dried at room temperature. Extraction was performed with 3% sodium carbonate solution (2 L) at 50 °C for 4 h. The mixture was centrifuged (4000*g*) and the supernatant solution was dialyzed against distilled water using Spectra/Por membrane (MWCO 3500). The resulting solution was concentrated in vacuo, poured over 0.5 L of 96% ethanol and centrifuged. The solid obtained was dried in an oven at 60 °C for 48 h. The extracts were pooled and purified as previously described.[5]

### 2.4. Total hydrolysis

The sodium alginate sample (5 mg) and 4.5 mL of 90% formic acid were placed in a sealed tube and heated for 6 h at 100 °C in an oven. The resulting solution was diluted with 20 mL of distilled water, heated at reflux for 2 h and concentrated in vacuo.[6] The residue was dissolved in distilled water and the M/G ratio determined by a Merck-Hitachi L-6000 HPLC with a Whatman Partisil 10-Sax column ($250 \times 4.6$ mm) and a L-4000 A UV detector.[10,11]

### 2.5. Fractionation of sodium alginate

Purified sodium alginate (0.250 g) in 25 mL of water was heated at reflux with 0.75 mL of 3.0 M HCl under $N_2$ for 0.3 h. After cooling, the mixture was centrifuged (3000*g*) and the supernatant solution was neutralized with 1.0 M NaOH and poured over 100 mL of ethanol yielding a precipitate that was dissolved in distilled water and freeze-dried (Fraction 1). The insoluble material obtained by centrifugation was heated at reflux with 0.3 M HCl for 2 h under $N_2$. The precipitate was separated by centrifugation and neutralized with 1.0 M NaOH; the pH was decreased to 2.85 by the addition of 1.0 M HCl. The soluble fraction was neutralized, dialyzed against distilled water and freeze-dried (Fraction 2). The precipitate was dissolved by neutralization, dialyzed and freeze-dried (Fraction 3).

### 2.6. Theoretical calculations

The structural features of α-L-gulopyranuronosyl-(1→4)-α-L-gulopyranuronic acid (GG) were analyzed with theoretical methods (density functional theory, DFT) using the Accelrys program CERIUS2 4.6, subroutine DMol3 on an Octane SGI computer.[12] Initial coordinates were obtained from the build routine incorporated in the DMol3 package. Geometry optimization was performed until convergence was reached. This converged structure was used to calculate the IR spectrum. Specific bands were animated with the same program. Local density was the Perdew and Wang

(PWC) functional using a double numeric basis set with polarization functions (DNP) on all atoms.[13,14]

## 3. Results and discussion

### 3.1. Preparation of the fractions

The isolation of white solids by alkaline extraction of blades of *L. flavicans*, *D. ligulata* and *D. distans* was achieved after previous treatment of seaweed samples with petroleum ether to remove fats, followed by polymerization of phenolic compounds with formadehyde. The extracts were purified by precipitation first with $CaCl_2$, followed by treatment with HCl. Dissolution in alkali gave sodium alginates in 11.0%, 27.2% and 15.2% yields, respectively. Alginates from *D. ligulata* and *D. distans* were richer in guluronic acid (M/G ratio 0.77 and 0.58, respectively) than the alginate from *L. flavicans* (M/G ratio 1.03) but less than the alginic acids from *D. ligulata* and *Desmarestia firma* studied by Calberg et al.[15] The M/G values of alginates from *Lessonia vadosa* blades collected at the same locality in winter and spring varied between 0.79 and 0.33.[7] According to Marritt, *L. flavicans* from Australia is rich in homoguluronic acid.[16]

Partial acid hydrolysis of each alginate afforded three fractions with ~75% of recovery yields (Table 1). Although the M/G ratio for the alginates samples varies from 0.58 to 1.03, the proportion of soluble fraction in 0.3 M HCl is quite low as shown in Table 1. According to Haug et al., the first fraction obtained by partial hydrolysis with 0.3 M HCl of sodium alginate is mainly composed of heteropolymeric blocks (MG), the second fraction soluble at pH 2.85 is enriched in polymannuronic acid (MM) and the third fraction insoluble at pH 2.85 is enriched in polyguluronic acid (GG).[3]

It is important to establish the content of guluronic acid in homopolymeric blocks because the gel-forming capacity of alginates depends on the presence of homopolyguluronic acid chains.[17] Larsen et al. studied block distribution of alginates from *Cystoseria trinide* and *Sargassum latifolium* by $^1$H NMR spectroscopy and found that both alginates contained the same proportions of homoguluronic fractions even though the M/G values were 0.48 and 0.80, respectively.[2] These findings and the results previously found in block compositions of *Lessonia* alginates support the assumption that no relation between M/G ratio and block composition can be established.

### 3.2. Vibrational assignments

The FT-IR spectrum of sodium alginate from *D. ligulata* (DLA) is presented in Figure 1. In the 3600–1600 $cm^{-1}$ region three bands appear: a broad band centred at 3427.5 $cm^{-1}$ assigned to hydrogen bonded O–H stretching vibrations, the weak signal at 2927.0 $cm^{-1}$ due to C–H stretching vibrations, and the asymmetric stretching of carboxylate O–C–O vibration at 1615.6 $cm^{-1}$. The band at 1415.3 $cm^{-1}$ may be due to C–OH deformation vibration with contribution of O–C–O symmetric stretching vibration of carboxylate group.[18,19] The weak bands at 1301.1, 1125.3 and 1094.1 $cm^{-1}$ may be assigned to C–C–H and O–C–H deformation, C–O stretching, and C–O and C–C stretching vibrations of pyranose rings, respectively; the band at 1035.6 $cm^{-1}$ may be also due to C–O stretching vibrations. The fingerprint, or anomeric, region (950–750 $cm^{-1}$) is the most discussed in carbohydrates.[18,20] The spectrum shows a band at 948.5 $cm^{-1}$, which was assigned to the C–O stretching vibration of uronic acid residues, and one at 888.3 $cm^{-1}$ assigned to the C1–H deformation vibration of β-mannuronic acid residues. The band at 820.0 $cm^{-1}$ seems to be characteristic of mannuronic acid residues.[6,7]

The FT-IR spectra and the second-derivative spectra give more information than classical IR for polysaccharides and biomolecules contained in organisms such as red seaweeds, fungi and bacteria.[9,21–25] Recently, the glucose content in blood was quantitatively determined by second-derivative FT-IR.[26]

**Table 1.** Yields of the fractions obtained by partial hydrolysis of sodium alginates from *Lessonia flavicans*, *Desmarestia ligulata* and *D. distans*, and signals assigned in the FT-IR spectra

| Alginate | Fraction | Yield[a] (%) | Wavenumber ($cm^{-1}$) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *L. flavicans* | $F_1$ | 8.5 | 960.7 | | 911.5 | | 890.3 | (822.9)[b] | 815.1 | | |
| | $F_2$ | 41.3 | | 948.8 (951.8, 936.7) | | | 888.8 | (822.2) | 820.3 | | (780.7) |
| | $F_3$ | 22.2 | | 947.3 | | 903.6 | | | | 812.5 | 781.1 |
| *D. ligulata* | $F_1$ | 3.5 | 964.0 | | 910.7 | | 889.1 | (815.9) | 813.6 | | (774.9) |
| | $F_2$ | 37.0 | | 946.4 (951.3, 936.2) | | | 883.9 | (824.0) | 820.7 | | |
| | $F_3$ | 47.1 | | 947.0 | | 902.7 | (878.7) | (822.1, 814.0) | | 813.9 | (781.7) |
| *D. distans* | $F_1$ | 3.7 | 963.9 | | 911.8 | | 890.6 | (818.6) | 815.4 | | (776.1) |
| | $F_2$ | 25.1 | | 939.4 (953.9, 937.7) | | | 886.3 | (822.5) | 820.2 | | |
| | $F_3$ | 56.4 | | 947.4 | | 903.7 | | | | 811.0 | 781.4 |

[a] On sodium alginate basis.
[b] Values in parentheses correspond to those found in the second derivative spectra.

The second-derivative spectrum (Fig. 1B) has more bands than the normal spectrum, among these there is one at 994.9 $cm^{-1}$ which may be due to C–O and C–C stretching vibrations, another signal at 902.4 $cm^{-1}$ is assigned to C1–H deformation vibration of α-L-guluronic acid residues and a clear signal at 781.3 $cm^{-1}$ that is seen as a very weak band at 781.1 $cm^{-1}$ in the normal spectrum. Very similar results were found in the analysis of the FT-IR spectra of sodium alginates from *L. flavicans* (LFA) and *D. distans* (DDA) (figures not shown).

The fractions obtained by partial hydrolysis of sodium alginates were characterized by FT-IR spectroscopy (Table 1). In Figure 2 the normal and second-derivative FT-IR spectra in the region 1250–700 $cm^{-1}$ of the three fractions obtained by partial hydrolysis of sodium alginate from *L. flavicans* (LFA) are shown.

According to Mackie alginates show two characteristic bands in the IR spectra; they are at 808 $cm^{-1}$, assigned to mannuronic acid residues, and at 787 $cm^{-1}$, assigned to guluronic acid residues.[27] FT-IR spectra of

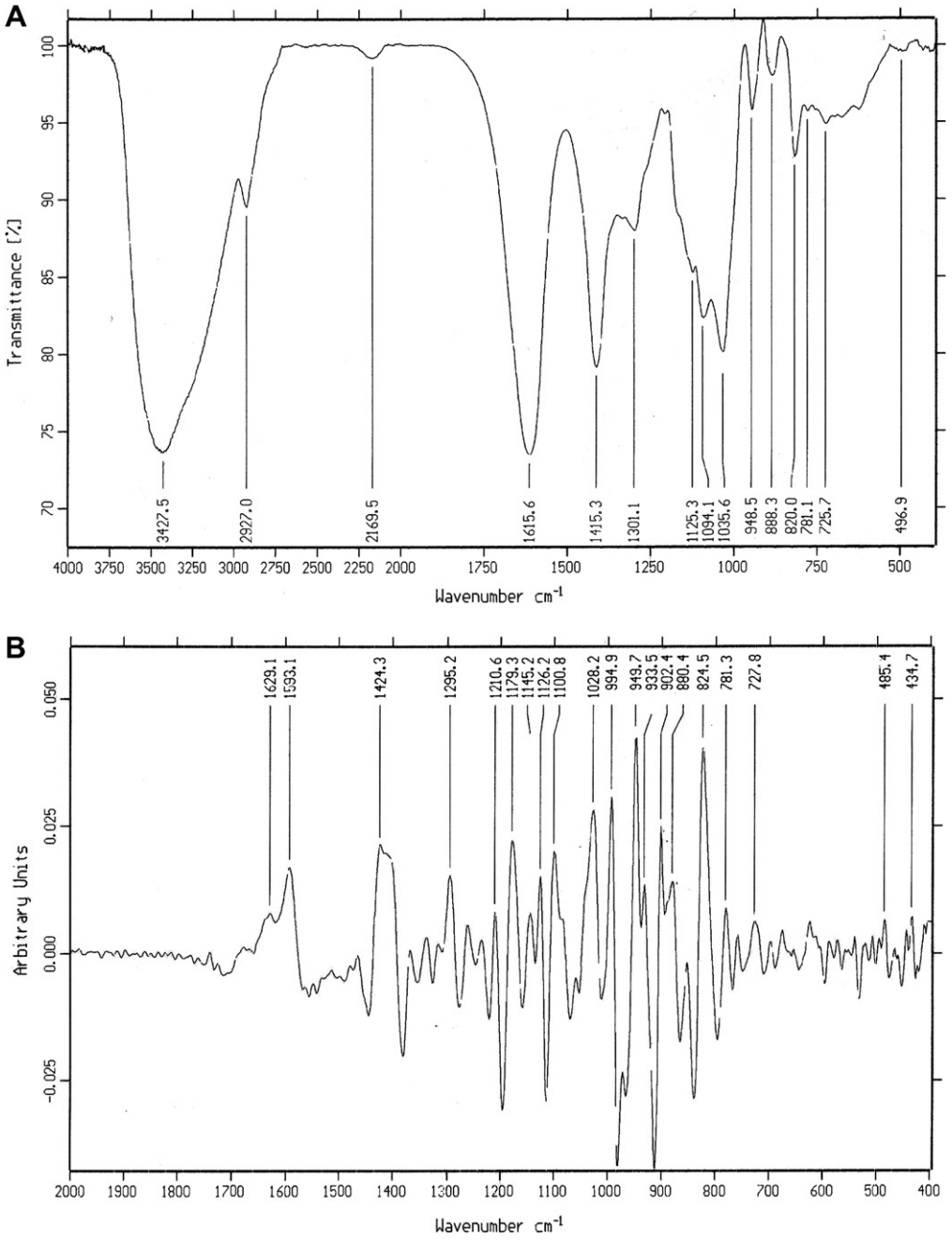


**Figure 1.** FT-IR spectra of sodium alginate from *Desmarestia ligulata*. (A) Normal spectrum and (B) second-derivative spectrum.

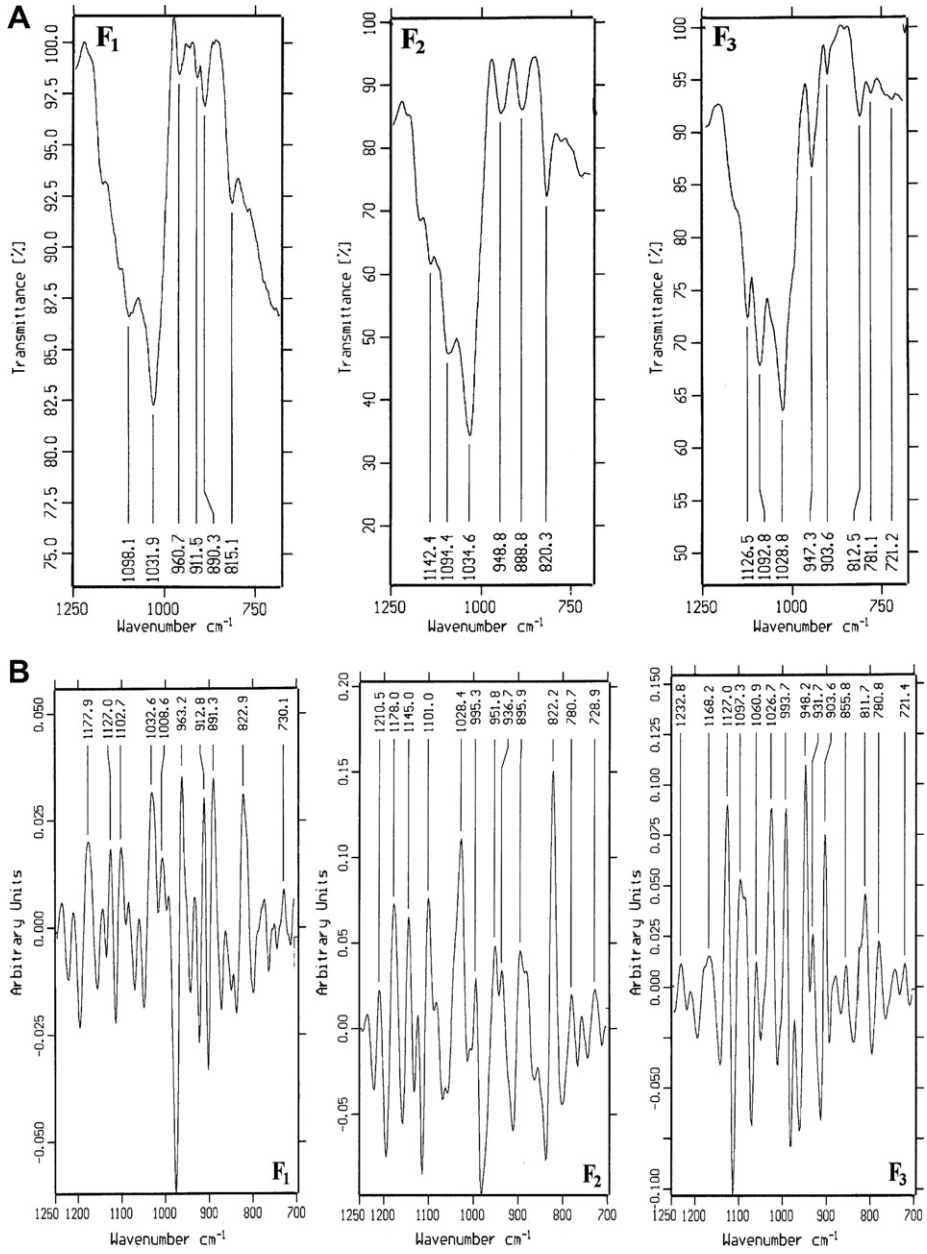


**Figure 2.** (A) FT-IR spectra in the 1250–700 $cm^{-1}$ region of fractions obtained by partial hydrolysis of sodium alginate from *Lessonia flavicans*; (B) second-derivative spectra in the same region.

commercial alginate and samples obtained in this laboratory showed both bands, which may be assigned to guluronic acid residue, plus a new band at 822 $cm^{-1}$ that seemed to correspond to mannuronic acid residues.[6,7] The FT-IR spectrum of fraction 1 ($F_1$) of LFA presents very similar bands to those of sodium alginate in the 3500–1300 $cm^{-1}$ region while the fingerprint region has two bands, located at 960.7 and 815.1 $cm^{-1}$ (Fig. 2A). The former was assigned to C–O stretching vibrations with contributions from C–C stretching and C–C–O deformation vibrations of the uronic acids residues in heteropolymeric blocks.[18] The latter appeared as a broad band at 822.9 $cm^{-1}$ in the second derivative spectrum. The same band is present in the FT-IR spectrum of fraction 2 ($F_2$) of LFA, and was assigned to deformation vibrations of β-D-mannopyranuronic acid residues. It is noteworthy that the C–O stretching vibration of the carboxylate group is shifted down to 948.8 $cm^{-1}$ in relation to the value for $F_1$. This band is resolved in the second-derivative spectrum into two signals. In the anomeric region the spectrum presents the band assigned to β-D-mannopyranuronic C1–H deformation vibration at 888.8 $cm^{-1}$. Furthermore, Figure 2B shows a band at 780.7 $cm^{-1}$ in the second derivative spectrum of $F_2$, which may indicate the presence of α-L-guluronic acid residues in the polymannuronic acid enriched frac-

tion. The FT-IR spectrum of fraction 3 ($F_3$) from LFA has the C–O stretching vibration at 947.3 $cm^{-1}$ (Fig. 2A), a band at 903.6 $cm^{-1}$ that is assigned to anomeric C–H deformation vibration of α-L-guluronic acids residues, and bands at 812.5 and 781.1 $cm^{-1}$ that are probably due to deformation vibrations of COH, CCH and OCH moieties in the α-L-guluronic acid residues with contributions of the bending deformation vibration of the C–O–C glycosidic linkage in homopolymeric blocks.[6,7] As can be seen in Table 1, bands in the FT-IR spectra of fractions obtained from DLA and DDA are very similar to those described above. It is noteworthy that the 813.9 $cm^{-1}$ band found in the FT-IR spectrum of fraction 3 from DLA is shown in the second-derivative spectrum as a broad band between 822.1 and 814 $cm^{-1}$, which may indicate the presence of mannuronic acid residues in the homopolyguluronic fraction. The M/G value could not be determined by HPLC because only one main peak with small amount of impurities was shown. All FT-IR spectra of the $F_1$, $F_2$ and $F_3$ fractions of alginates from the three seaweed species present different characteristic bands in the fingerprint region that may be applied for the identification of MG, MM and GG fractions in alginates.

### 3.3. $^1H$ and $^{13}C$ NMR assignments

Structures deduced by FT-IR were corroborated with $^1H$ and $^{13}C$ NMR spectroscopy. Two-dimensional NMR spectra were collected to confirm the fine structure of the hetero- and homo-polymeric fractions. Assignments of the $^{13}C$ and $^1H$ NMR spectra (Tables 2 and 3) were accomplished with the aid of 2D NMR experiments, and literature data.[28–33] Figure 3 shows the $^{13}C$–$^1H$ HSQC NMR spectrum of fraction from DLA. The major $^{13}C$ signals were assigned to mannuronic acid and guluronic acid in the sequence GMG and MGM in heteropolymeric blocks. In the anomeric region connectivities between two $^{13}C$–$^1H$ systems were observed. The 102.13/4.70 ppm correlation was assigned to C1/H1 of β-D-mannuronic acid residues in the triad GMG and the 100.51/5.06 ppm correlation was assigned to C1/H1 of α-L-guluronic acid residue in the MGM triad. Correlations for the rest of $^{13}C/^1H$ systems, with the exception of C4/H4 in mannuronic acid residues, were obtained as shown in Figure 3. Values are very similar to those obtained for fraction 1 of sodium alginate from *Lessonia vadosa*.[34] In addition, the $^{13}C$ NMR spectrum presents small signals, which may be assigned to mannuronic acid in the GMM or MMM triad. Results are in agreement with the M/G ratio of 1.2 found for the fraction and with the presence in the FT-IR second-derivative spectrum of a signal assigned to homopolymannuronic acid. The two-dimensional $^{13}C$–$^1H$ HSQC spectrum of $F_2$ fraction (not shown) of DDA allowed the full identification of the $^{13}C$–$^1H$ systems of a homopolymannuronic acid fraction as indicated in Tables 2 and 3. Furthermore, the spectrum showed signals in minor proportions that were assigned to α-L-guluronic acid in GGM and GGG triads. These results are in agreement with those obtained from FT-IR spectroscopy. A M/G ratio value of 5.7 was found for this fraction by total hydrolysis and HPLC analysis.

The $^{13}C$ NMR spectra (not shown) of $F_3$ fractions of LFA and DDA presented six signals that were assigned to regular homopolyguluronic acid (Table 3) and are in good agreement with those reported recently for alginate fractions.[34] The $^{13}C$ NMR spectrum of fraction 3 from DLA showed small peaks attributed to the presence in minor proportions of mannuronic acid residues in GMM and MMM triads. This result agrees with the interpretation found by FT-IR. It can be seen in Tables 2 and 3 that the chemical shifts of β-D-mannuronic and α-L-guluronic acids residues in GMG and MGM triads, and MMM and GGG triads of alginate fractions from three different seaweeds give very similar values.

In conclusion, $^1H$ and $^{13}C$ NMR spectroscopy in solution corroborates block structures deduced by solid state FT-IR spectroscopy. Vibrational spectroscopy is a good technique for the study of block structures in alginic acids, with the second-derivative FT-IR identifying minor components in hetero- and homopolymeric blocks.

The seaweeds *L. flavicans* and *D. ligulata* are very good producers of homopolymannuronic acid blocks while *D. distans* is rich in homopolyguluronic acid fraction. Characterization of brown seaweeds phycocolloids by this technique will help to find homopolymeric fractions with specific properties and new industrial and biological applications.

**Table 2.** Assignments of chemical shifts (ppm) in the $^1H$ NMR spectra of fractions obtained by partial hydrolysis of sodium alginate from *Lessonia flavicans* (L.F.), *Desmarestia ligulata* (D.L.) and *Desmarestia distans* (D.D.)

| Fraction | δ (ppm) | | | | |
|---|---|---|---|---|---|
| | H-1 | H-2 | H-3 | H-4 | H-5 |
| $F_1$ (L.F.)[a] | 4.70 | 3.94 | 3.75 | 3.99 | 3.76 |
| $F_1$ (L.F.)[b] | 5.06 | 3.99 | 4.17 | 4.23 | 4.72 |
| $F_2$ (L.F.) | 4.65 | 4.02 | 3.73 | 3.89 | 3.73 |
| $F_3$ (L.F.) | 5.08 | 3.91 | 4.04 | 4.15 | 4.47 |
| $F_1$ (D.L.)[a] | 4.70 | 3.94 | 3.74 | 3.99 | 3.77 |
| $F_1$ (D.L.)[b] | 5.06 | 3.99 | 4.17 | 4.23 | 4.73 |
| $F_2$ (D.L.) | 4.64 | 4.01 | 3.72 | 3.88 | 3.72 |
| $F_3$ (D.L.) | 5.09 | 3.92 | 4.05 | 4.16 | 4.48 |
| $F_1$ (D.D.)[a] | 4.69 | 3.94 | 3.75 | 3.98 | 3.76 |
| $F_1$ (D.D.)[b] | 5.07 | 3.98 | 4.17 | 4.22 | 4.70 |
| $F_2$ (D.D.) | 4.62 | 3.99 | 3.70 | 3.86 | 3.70 |
| $F_3$ (D.D.) | 5.03 | 3.88 | 4.00 | 4.11 | 4.43 |

[a] Chemical shifts for mannuronic acid residue in the heteropolymeric alternate sequence.

[b] Chemical shifts for guluronic acid residue in the heteropolymeric alternate sequence.

**Table 3.** Assignments of chemical shifts (ppm) in the $^{13}C$ NMR spectra of fractions obtained by partial hydrolysis of sodium alginates from *Lessonia flavicans* (L.F.), *Desmarestia ligulata* (D.L.) and *Desmarestia distans* (D.D.)

| Fraction | δ (ppm) | | | | | |
|---|---|---|---|---|---|---|
| | C-1 | C-2 | C-3 | C-4 | C-5 | C-6 |
| $F_1$ (L.F.)[a] | 102.10 | 71.65 | 72.69 | 78.59 | 77.15 | 176.32 |
| $F_1$ (L.F.)[b] | 100.52 | 65.82 | 70.38 | 80.85 | 68.59 | 176.32 |
| $F_2$ (L.F.) | 100.90 | 70.78 | 72.23 | 78.89 | 76.93 | 175.55 |
| $F_3$ (L.F.) | 101.40 | 66.04 | 70.01 | 80.70 | 68.12 | 175.77 |
| $F_1$ (D.L.)[a] | 102.13 | 71.68 | 72.69 | 78.57 | 77.17 | 176.40 |
| $F_1$ (D.L.)[b] | 100.51 | 65.82 | 70.39 | 80.89 | 68.69 | 176.40 |
| $F_2$ (D.L.) | 100.85 | 70.75 | 72.18 | 78.81 | 76.88 | 175.56 |
| $F_3$ (D.L.) | 101.42 | 66.03 | 70.00 | 80.72 | 68.13 | 175.78 |
| $F_1$ (D.D.)[a] | 102.13 | 71.69 | 72.69 | 78.56 | 77.18 | 176.24 |
| $F_1$ (D.D.)[b] | 100.52 | 65.84 | 70.40 | 80.88 | 68.70 | 176.24 |
| $F_2$ (D.D.) | 100.83 | 70.73 | 72.16 | 78.79 | 76.86 | 175.55 |
| $F_3$ (D.D.) | 101.37 | 65.93 | 69.92 | 80.66 | 68.03 | 175.76 |

[a] Chemical shifts for mannuronic acid residue in the heteropolymeric alternate sequence.
[b] Chemical shifts for guluronic acid residue in the heteropolymeric alternate sequence.

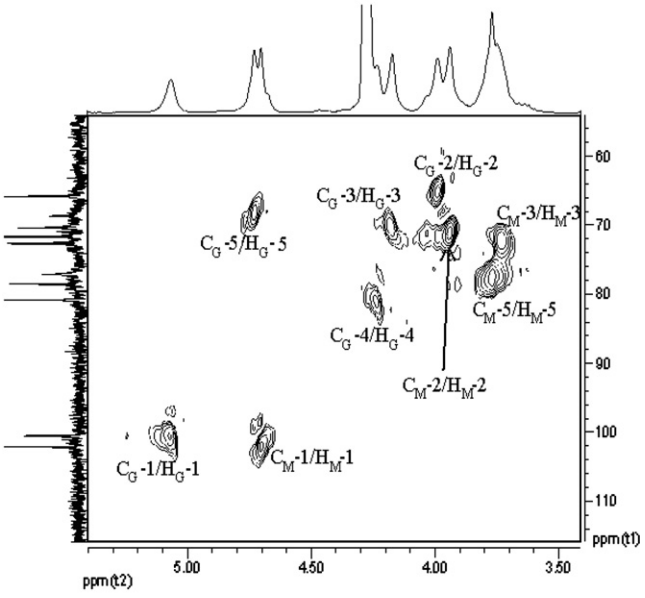


**Figure 3.** $^{13}C/^{1}H$ HSQC NMR spectrum (ns 64) in $D_2O$ at 70 °C of $F_1$, fraction soluble in 0.3 M HCl obtained by partial hydrolysis of sodium alginate from *Desmarestia ligulata*. $C_G$-1: carbon 1 of guluronic acid residue, $H_G$-1: hydrogen linked to carbon 1 of guluronic acid, and so on.

### 3.4. Theoretical calculations

The α-L-gulopyranuronosyl-(1→4)-α-L-gulopyranuronic acid (GG) structural arrangement was studied with density functional theory. The highest level of theory implemented in the program DMol3 was used. An ab initio model with both pyranose rings having the expected $^{1}C_4$ conformation was geometry-optimized through energy minimization as depicted in Figure 4; only 2H-bond interactions are shown for clarity whereas the complete set is seen in Figures 5 and 6. These H-bonds are largely responsible for the long calculation times.

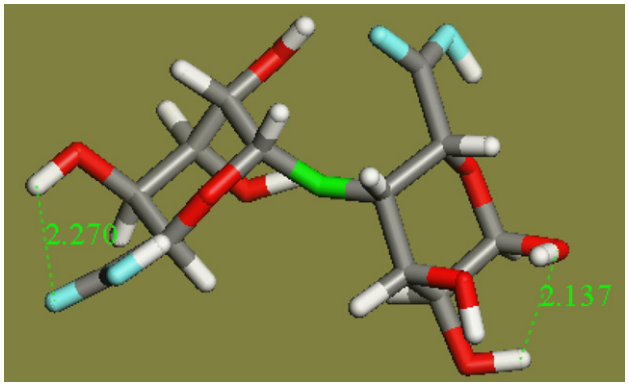


**Figure 4.** Geometrically optimized GG model. Carboxylate oxygens are light blue and the anomeric oxygen is green for clarity; only two H-bonds are shown.

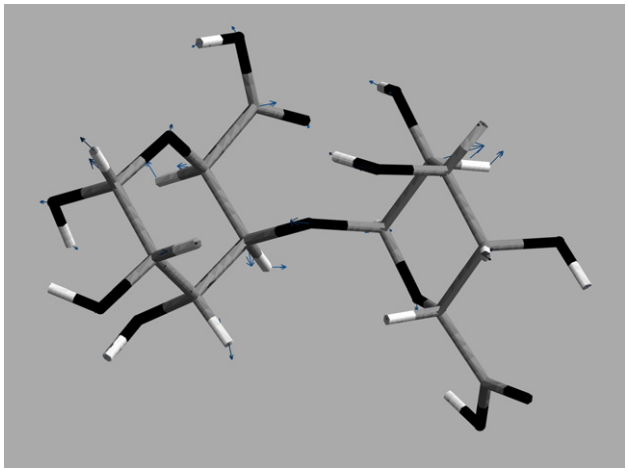

**Figure 5.** Displacement of atoms in the calculated IR spectrum band of 794 $cm^{-1}$.

The corresponding IR spectrum was calculated and attention was mainly focused on the zone where the

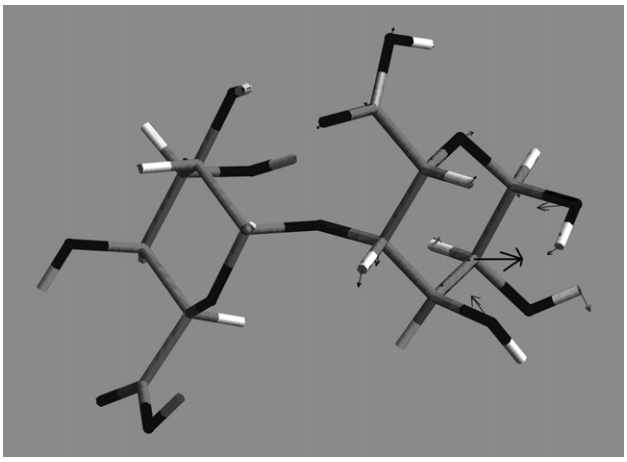

**Figure 6.** Displacement of atoms in the calculated IR spectrum band of 818 $cm^{-1}$.

characteristic absorptions around 800 $cm^{-1}$ related to the homopolyguluronic acid fraction were previously found. Theoretical studies of cellulose and cellobiose vibrational spectra did not show calculated frequencies in this area nor were they observed in the FT-IR measurements of amylose and cellulose oligomers.[18,35,36] Other DFT calculations for different glycosidic linkages have been successfully compared with experimental IR data in some disaccharides from structures based on X-ray data.[37,38] From the calculated IR spectrum we identified two bands in this area at 794 and 818 $cm^{-1}$. According to the literature frequencies below 1500 $cm^{-1}$ in carbohydrates are due to mixed modes, and every band results from the contribution of more than one kind of motion.[36] The 794 $cm^{-1}$ wavelength shows a high degree of mixing with an important contribution from the C1–O–C4′ glycosidic deformation vibration, with atomic displacements equally distributed on both pyranose rings, see Figure 5. In contrast, displacements on the glycosylated pyranose ring are more marked in the 818 $cm^{-1}$ band; this is shown to the right in Figure 6. The modes related to C–O–H, C–C–H and O–C–H are observed and confirmed the assignments attributed to the experimental IR spectra of homopolyguluronic fractions. The calculated IR spectrum is depicted in Figure 7 and the intensity of the 794 and 818 $cm^{-1}$ bands is weak agreeing with the experimental FT-IR spectra. Therefore, the DFT model supports the assignments made from the experimental FT-IR data of homopolyguluronic fractions. Related reliability for DFT calculations, non-based on crystal structures, has been recently shown for glucose and fructose.[39]

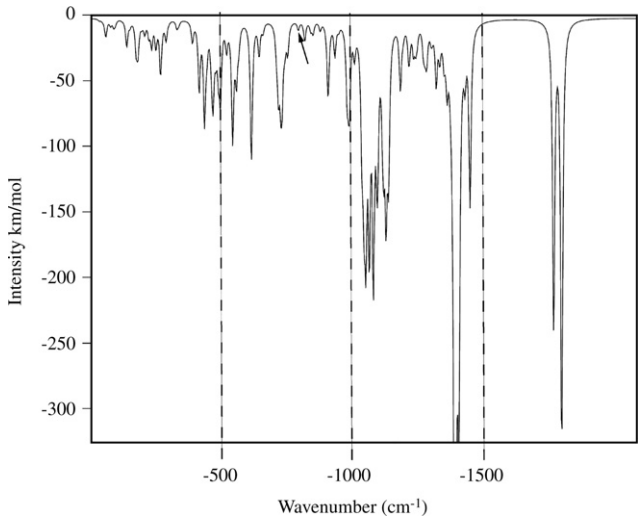


**Figure 7.** Calculated IR spectrum for the GG model, the arrow shows the 794 $cm^{-1}$ band, the 818 $cm^{-1}$ band is the next one and slightly stronger.

## Acknowledgements

The financial support of Dirección de Investigaciones Científicas y Tecnológicas, Universidad de Santiago de Chile is gratefully acknowledged. The authors thank Grant MECESUP USA-0007 for the NMR equipment facilities, Dr. Andrés Mansilla (Universidad de Magallanes, Chile) for the collection and identification of the algal material and Cristian Opazo at Vassar College SCIVIS lab for assistance with DMol program.

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