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Isolation and characterization of soluble sulfated polysaccharide from the red seaweed *Gracilaria cornea*

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Abstract

The composition, structure and rheological properties of a soluble sulfated polysaccharide from *Gracilaria cornea* (Brazilian red marine alga) were investigated. Agarocolloid yield, intrinsic viscosity, monosaccharide composition, sulfate and cation content as well as molecular weight were determined. The main polysaccharide components were 3,6-anhydrogalactose (24.7%) and galactose (64.6%). In addition, minor components such as 6-*O*-methyl-galactose (8.5%), glucose (1.5%), xylose (0.7%) and sulfated groups (4.8%) were detected. Comparison between sulfate contents determined by Fourier transform IR (FT-IR) spectroscopy and microelemental analysis was made. Data from ^{13}C NMR and FT-IR provided evidence of sulfation in C-4 and C-6 of galactose. Sodium, calcium, magnesium and potassium cations were detected in the agarocolloid. The intrinsic viscosities were lower than typical values for agar in the same experimental conditions. No gelation in 1.5, 2.0 and 3.0% (w/v) aqueous solution was observed, even by cooling up to 4 °C. Gel permeation chromatography indicated two major polysaccharide fractions of M_{pk} 7.4×10^4 and 1.8×10^4 g/mol and a minor fraction of M_{pk} 2.1×10^6 g/mol, probably a protein–polysaccharide complex.

Keywords: *Gracilaria cornea*; Sulfated polysaccharide; Sulfation degree; Viscosity

1. Introduction

Sulfated polysaccharides are widespread in nature, occurring in marine algae and in a great variety of other organisms. In marine algae, they are present as sulfate fucose (fucoidans) and as sulfate galactans (carrageenans and agars; Painter, 1983). Recently, there has been an increasing interest in systematic screening of biological activity of sulfated polysaccharide isolated from marine algae (Caceres, Carlucci, Damonte, Matsuhiro, & Zuniga, 2000; Pereira, Mulloy, & Mourão, 1999; Shanmugan & Mody, 2000; Xue, Fan, Lin, Chen Li, Deng, & Lu, 2001; Yamada, Ogamo, Saito, Uchiyama, & Nakagawa, 2000). Anticoagulant and antithrombotic activities are among the most widely studied properties.

Anticoagulant activity has been found for sulfated polysaccharides extracted from brown marine alga (Pereira et al., 1999; Shanmugan & Mody, 2000) and red marine alga (Caceres et al., 2000; Carlucci et al., 1997; Farias, Valente, Pereira, & Mourão, 2000; Yamada et al., 2000). Marine brown alga has also exhibited antithrombin activity (Nishino, Fukuna, Nagumo, Fujihara, & Kaji, 1999; Pereira et al., 1999; Shanmugan & Mody, 2000; Church et al., 1989). Antiviral effects have been reported for sulfated polysaccharides from marine alga, from red species against herpes simplex virus (Caceres et al., 2000; Carlucci et al., 1997) and against human immunodeficiency virus (HIV) types 1 and 2 (Carlucci et al., 1997; Schaeffer & Krylov, 2000; Yamada et al., 2000).

In general, biological activity of sulfated polysaccharide from marine algae is related to the molecular size, type of sugar and sulfate content. Sulfate position, type of linkage and molecular geometry are also known to have a role in activity (Shanmugan & Mody, 2000). Sulfated homopolysaccharides, for example, are more potent as anti-HIV agents than heteropolysaccharides. The presence of the sulfate group is necessary for anti-HIV activity, and potency increase with increasing degree of sulfation (Schaeffer & Krylov, 2000). Different structural features determine not only the biological activity but also the mechanism by which they exert this activity (Pereira et al., 1999). Reviews on the chemistry, physico-chemistry, structure of algal galactans have been recently published by Lahaye (2001a,b).

Red algal galactans are sulfated polysaccharides that usually have a linear backbone built on alternating 3-linked

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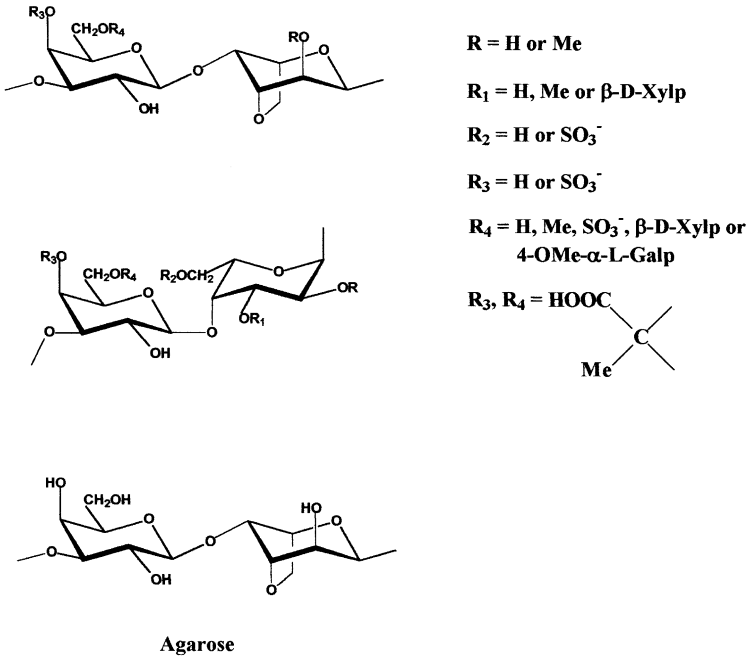

Fig. 1. Structural features of the agar group of polysaccharides (Usov, 1998).

β-D-galactopyranose and 4-linked α-galactopyranose residues. The β-galactose residues always belong to the D-series, whereas the α-galactose residues are D in carrageenans and L in agars. A substantial part or even all the α-galactose residues may exist in the form of 3,6-anhydro derivative. Various hydroxyl groups may be substituted by ester sulfate, methyl groups, pyruvic acid acetal and sometimes by additional monosaccharides (Usov, 1998). Structural features of the agar group of polysaccharides is shown in Fig. 1. They were found to have an agarose type structure. In general, some C-6 of the 3,6-anhydro-α-L-galactose (3,6-AG) are substituted with sulfate groups resulting in α-L-galactose-6-sulfate, which is known as a biogenetic precursor of 3,6-AG (Rees, 1961).

Gracilaria spp. that belongs to the Gracilariaceae family, is a red seaweed widely distributed in tropical Atlantic waters and one of the principal sources of commercial agars (Lai & Lii, 1997). The agar extracted by alkali treatment from *Gracilaria cornea* from Yucatan, Mexico, was studied in relation to the effect of season on the agar content and chemical characteristics (Freile-Pelegrin & Robledo, 1997a) and to the influence of alkali treatment (Freile-Pelegrin & Robledo, 1997b). Seasonal variation in the biomass and agar yield from *Hydropuntia cornea* (J. Agardh) Wynne (= *G. cornea* J. Agardh; Bird, Oliveira, & McLachlan, 1986) was studied by Marinho-Soriano, Silva, and Moreira (2001).

This work is concerned with the isolation and characterization of a sulfated polysaccharide from the red alga *G. cornea*, obtained without alkaline hydrolysis of sulfates. The properties of this polysaccharide are compared with *Gracilaria* agar. This will facilitate a future screening of the biological properties of sulfated polysaccharides and elucidate the relationship between these properties and the macromolecule structure of agars or agaroids.

2. Experimental

2.1. Isolation of soluble sulfated polysaccharide

Specimens of the red seaweed *G. cornea* were collected in April, 2000 on the Atlantic coast of Brazil (Fleixeira Beach, Trairi, Ceará) cleaned of epiphytes, washed with distilled water and stored at −20 °C. The sulfated polysaccharide was extracted from the dried tissue (100 g) by papain digestion, and partially purified by cetylpyridinium chloride, as described by Farias et al. (2000). About 21.4 g (dry weight) of crude sulfated polysaccharide, denoted as crudeSP, was obtained after these procedures. The crudeSP (1 g) was dispersed in 100 ml of distilled water and stirred for 15 h at room temperature (25–28 °C). The water-soluble fraction (designated as solSP) was separated from the insoluble one (insolSP) by filtration in a sintered-glass plate of fine grade (4–5.5 μm pore size). The fraction was lyophilized, weighed (0.51 g) and stored.

2.2. Composition

The monosaccharide composition was quantified by GC–MS as their alditol acetate derivatives using reductive hydrolysis method of Stevenson and Furneaux (1991). A Varian 3300 chromatograph and a Finnigan Mat ITD spectrometer was utilized. Helium was the carrier gas (1 ml/min).

Protein content was calculated from %N determined by elemental microanalysis, using the correction factor of 6.25, as proposed by Marks, Baum, and Swain (1985). Moisture was obtained by heating 0.5 g of samples at 105 °C for 24 h. Samples of 0.5 g were also heated at 600 °C for ash determination.

The sample preparation for the determination of cation content was made by the solSP digestion in a microwave furnace equipped with PTFA closed vessels (Milestone, Ethos 1600). All solutions were prepared from analytical grade reagents, using Milli-Q water with a conductivity lower than 18 mΩ/cm. Concentrated solutions of analytical grade HNO_3 and H_2O_2 (2:1 v/v) were used for sample decomposition. The analysis of calcium, magnesium and potassium were performed in a Carl Zeiss, model AAF 5FL, atomic absorption spectrophotomer. Sodium was analyzed by flame photometry, using Micronal B-22 equipment.

2.3. Sulfation degree

The total content of sulfate are determined either by infrared spectroscopy, as described by Rochas, Lahaye, and Yaphe (1986) and by S% from elemental analysis. The Fourier transform IR spectra (FT-IR) were recorded with a Shimadzu IR spectrophotomer (model 8300) between 400 and 4000 cm^{-1}. The samples were analyzed as KBr pellet.

2.4. Molar mass distribution

The peak molar masses (M_{pk}) were estimated by gel permeation chromatography (GPC) with Shimadzu equipment at room temperature using an Ultrahydrogel linear column (7.8 × 300 mm), flow 0.5 ml/min, 0.5% polysaccharide concentration and 0.1 M $NaNO_3$ as solvent. A differential refractometer and an ultraviolet photometer (at 280 nm) was used as detectors and the elution volume corrected to the internal marker of ethylene glycol at 11.25 ml. Pullulan samples (Shodex Denko) of M_w 5.9×10^3, 1.18×10^4, 4.73×10^4, 2.12×10^5 and 7.88×10^5 g/mol were used as standard.

2.5. Nuclear magnetic resonance spectroscopy

^{13}C NMR spectra of 2.5% w/v solutions in D_2O at room temperature were recorded in a Bruker advance DRX 500 spectrometer at 40 °C. Chemical shifts are given in values relative to internal acetone at 31.07 ppm.

2.6. Viscosity measurement

The measurements were performed in an Ubbelohde viscometer with capillary diameter 0.5 mm and a flow time for 0.1 M NaCl of 208 s at 45 °C and for 0.1 M KCl of 153 s at 65 °C. All solutions were prepared by mixing with magnetic stirring for at least 2 h at room temperature (25–28 °C) followed by filtration in a sintered-glass plate of fine grade. The intrinsic viscosity determination was made in the presence of 0.1 M NaCl (temperature 45 °C) and of 0.1 M KCl (temperature 65 °C) by the use of Huggins equation. The maximum temperature variation during each experiment was 0.1 °C. All flow times were averages of at least five replicates.

2.7. Gelation

An attempt was made to measure gelling and melting temperatures by visual observation of the onset of the fluidity of the gel on cooling and heating, respectively, as previously described (Oliveira, Silva, de Paula, Feitosa, & Paula, 2001). Samples of 1 ml of aqueous solution in 1.5, 2.0 and 3.0% (w/v) concentration were kept in a water bath at 80 °C for 30 min and cooled to 4 °C at a rate of 1 °C/min and observed at each 5 °C after 5 min at constant temperature. The solutions were also kept at this temperature for 40 min prior to the final observation. The experiments were performed twice for each solution concentration.

3. Results and discussion

The crudeSP account for 21.4% of the seaweeds dried weight. The yield of the water-soluble fraction (solSP) was 51.3% of the crudeSP, which represents 11.0% of the seaweed dried weight. The agar yield of *G. cornea* from Mexico and from Brazil was found to range from 35.6 to 42.1% (Freile-Pelegrin & Robledo, 1997a) and from 29 to 41% (Marinho-Soriano et al., 2001), respectively, depending on the season. The low temperature employed in sulfated polysaccharide isolation (25–28 °C) in comparison with the high temperature in agar extraction (85 °C) could be one of the reasons for the low crudeSP yield. When the comparison was made between sulfated polysaccharide isolated by the same conditions, the yield of *G. cornea* was higher than the value obtained to *Botryocladia occidentalis* red alga (yield ≅ 4%; Farias et al., 2000).

The moisture and protein content of sulfated polysaccharide fractions were shown in Table 1. There are no significant differences in moisture among the fractions. These results indicate a little higher protein content in the soluble fraction than in the other fractions.

3.1. Composition

Analysis of sulfated polysaccharide hydrolysates from *G. cornea* by GC–MS indicates the presence of galactose

Table 1
Moisture and protein content of sulfated polysaccharide fractions from *G. cornea*

Fraction	Moisture (%)	Nitrogen (%)	Protein (% in dried weight)
crudeSP	11.6	0.44	2.8
solSP	11.1	0.47	3.0
insolSP	11.4	0.41	2.6

Table 2
Comparison between native agar from different *Gracilaria* species and sulfated polysaccharide from *G. cornea* (AG: anhydrous galactose; gal: galactose)

Polysaccharide	4-*O*-Me-L-gal (%), I	6-*O*-Me-L-gal (%), II	Galactose (%), III	3,6-AG (%), IV	2-*O*-Me-3,6-AG (%), V	Glucose (%), VI	Molar ratio[a] (II + III)/(IV + V)	$NaSO_3$ (%)	Reference
Native agar									
G. tikvahiae	6.2	30.4	12.4	36.3	–	4.1	1.00	4.0	Quemener and Lahaye (1998)
G. eucheumoides	–	2.5	40.4	2.2	36.6	0.5	1.07	5.4	Quemener and Lahaye (1998)
G. sjoestedtii	–	6.8	52.6	46.0	–	–	0.94	2.3	Craigie et al. (1984)
G. textorii	–	9.9	32.6	40.2	–	–	1.06	13.9	Craigie et al. (1984)
G. verrucosa	–	14.6	43.2	48.3	–	–	1.69	2.3	Craigie et al. (1984)
G. domingeusis	n.d.	54.8[b]		27.6	n.d.	n.d.	1.27	7.6	Valiente et al. (1992)
G. mammillaris	n.d.	54.5[b]		36.7	n.d.	n.d.	n.d.	8.9	Valiente et al. (1992)
G. cornea—Mexico	n.d.	n.d.	n.d.	31.6–32.5[c]	n.d.	n.d.	n.d.	4.8–5.5[c]	Freile-Pelegrin and Robledo (1997a)
Soluble sulphated									
G. cornea—Brazil[d]	–	8.5	64.6	24.7	–	1.5	2.96	4.8	This work

[a] As suggested by Quemener and Lahaye (1998).
[b] Galactose + 6-*O*-Me-gal.
[c] Depending on season.
[d] Contains also 0.7% xylose.

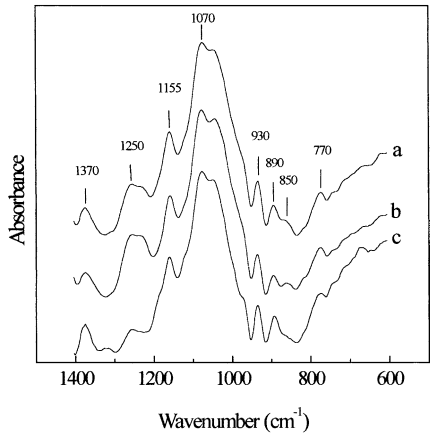

Fig. 2. FT-IR spectra in KBr pellet of *G. cornea* sulfated polysaccharide fractions: (a) crudeSP; (b) solSP; (c) insolSP. Wavenumbers range from 1400 to 600 cm^{-1}.

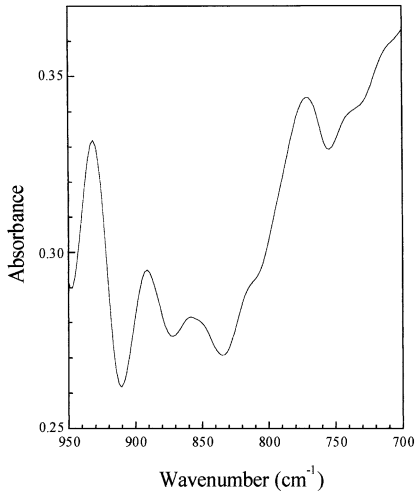

Fig. 3. FT-IR spectrum in KBr pellet of the soluble fraction from *G. cornea* sulfated polysaccharide in the wavenumber range from 950 to 700 cm^{-1}.

(64.6%), 3,6-anhydrogalactose (24.7%), 6-*O*-methyl-galactose (8.5%), glucose (1.5%) and xylose (0.7%). Comparison between solSP composition and native agar from different *Gracilaria* species is shown in Table 2. *G. cornea* sulfated polysaccharide has the highest galactose content and the lowest anhydrogalactose (3,6-AG and 2-*O*-Me-3,6-AG) content. The ratio between galactose and anhydrogalactose (2.96) is very far from the ideal agarose ratio (1.0).

The presence of sodium, potassium, calcium and magnesium was detected in solSP. The content of Na^+ (2.33%), Ca^{2+} (0.21%) and Mg^{2+} (0.09%) ranged between the values determined for agars: 0.01–4.2, 0.02–0.9 and 0.02–0.62%, respectively (Meer, 1980). There is no value in the literature to compare with the potassium content (0.26%).

3.2. FT-IR analysis and sulfated content

The FT-IR spectra of crudeSP and its fractions (Fig. 2) were very similar. Comparison between the spectra and data from Table 3 reveals the presence of most characteristic bands of agarocolloids (at 1370, 1250, 1070, 930, 890, 740 and 716 cm^{-1}). Mollet, Rahaoui, and Lemoine (1998) also found bands at 1150 and 770 cm^{-1}, not assigned. In order to identify the position of the sulfate group, a specific region of the FT-IR spectra of soluble fraction was amplified (Fig. 3). The band close to 850 cm^{-1} could be assigned to the sulfate in C-4 from galactose. The presence of a shoulder close to 820 cm^{-1} could indicate a small degree of substitution on C-6. As no bands or shoulders have been detected at 830 and 805 cm^{-1}, the 2-sulfate galactose and sulfate on C-2 of 3,6-anhydrogalactose were not present.

Table 3
Assignment of most important bands in agarocolloids (Mollet et al., 1998)

Wavenumber (cm^{-1})	Assignment
1370	Ester sulfate
1250	ν_{as} S=O (ester sulfate)
1070	Skeletal mode of the galactan
930	Vibrations of the C–O–C of 3,6-anhydro-L-galactose
890	Agar specific band
845	4-Sulfate galactose
830	2-Sulfate galactose
820	6-Sulfate galactose
805	Sulfate on C-2 of 3,6-anhydro-L-galactose
740	C–O–C bending mode in glycosidic linkages
716	C–O–C bending mode in glycosidic linkages

The absorbance of the asymmetric stretching of S=O band (1250 cm^{-1}) was used by Rochas et al. (1986) for calculating total ester sulfate content of carrageenan and agar. The authors determined a correlation between the DS sulfate (degree of substitution with sulfate of disaccharide repeat unit) and the ratio of absorbance 1250/2920 cm^{-1}. The shape of the curve indicates that this estimation of sulfate is precise only for polymers having a DS sulfate of less than 0.8.

Based on Rochas et al. (1986) proposition, the DS sulfate was found to be 0.19, 0.21, 0.10 for crude SP, solSP and insolSP, respectively. The DS sulfate was also determined by S% and C% using Eq. (1) below. The proposed equation is based on the agarobiose structure (Fig. 1), considering that DS sulfate is defined as the number of OSO_3^-, or sulfur atoms, per disaccharide repeat unit, which possess 12 carbon atoms. So, the number 12 multiplied by atomic mass of carbon corresponds to the number of carbon atoms per repeat unit. These assumptions could be applied even if the gal/3,6-AG ratio was higher than 1 as the number of carbon atoms in galactose and in 3,6-anhydrogalactose are the same.

$$(S\%/\text{atomic mass of S})/(C\%/\text{atomic mass of C} \times 12) = 4.5(S\%/C\%) \quad (1)$$

A comparison between sulfate content by the two different methods are made in Table 4. The agreement between the

Table 4
Comparison between sulfate content of *G. cornea* polysaccharide fractions

Sample	DS sulfate from		S%	C%
	IR	Microanalysis		
crudeSP	0.15	0.19	1.29	29.71
solSP	0.21	0.22	1.26	26.01
insolSP	0.07	0.13	0.93	32.35

DS sulfate for crude and soluble polysaccharide fraction was very good (difference: <5%). The agreement for the insoluble sample was not so good. In fact, as the DS sulfate becomes lower, the 1250 cm^{-1} band intensity decreases; the determinations by infrared spectrometry are progressively less accurate. The difference between the DS sulfate estimated from the methods in crude and insoluble samples are 5 and 59%, respectively. In the latter case, the value determined by elemental microanalysis could be more appropriate.

Table 2 shows sulfate content, expressed as $NaSO_3$ (% in dried weight), in agar from some *Gracilaria* species. The *Gracilaria* polysaccharides represent a typical agar, with low sulfate content (2.3–8.9%). The galactan from *G. textorii*, which undoubtedly is of the agar family, shows an unusual behavior (Craigie, Wen, & van der Meer, 1984). The *G. cornea* solSP has a sulfate content (4.8%) in the observed range and is similar to the values obtained for agar isolated from the Mexican species, 4.8–5.5%, respectively for the dry and rainy seasons (Freile-Pelegrin & Robledo, 1997a).

3.3. Molar mass distribution

The GPC chromatogram of solSP fraction is shown in Fig. 4. A shoulder at 7.10 ml (peak I) and two main peaks at 8.64 and 9.29 ml (peaks II and III, respectively) were detected by refraction index measurements. Two peaks, corresponding to I and III appeared when ultraviolet detection was used. The *G. cornea* solSP behaves as a heterogeneous system similar to other natural polysaccharides: those from *Anacardium occidentale* (de Paula & Rodrigues, 1995) and *Albizia lebbeck* (de Paula, Santana, & Rodrigues, 2001) exudates and from *B. occidentalis* red alga (Farias et al., 2000).

In order to estimate the peak molar mass (M_{pk}) for solSP *G. cornea*, a calibration plot was obtained by use of pullulan fractions. The equation obtained from this calibration plot was $\log M_w = 13.039 - 0.9449V_e$, where V_e is the elution volume in ml. The linear correlation coefficient was 0.9980. Based on this equation, the peaks I, II and III correspond to M_{pk} values of 2.1×10^6, 7.4×10^4 and 1.8×10^4 g/mol, respectively. Weight-average molecular weight (M_w) of six agar samples ranged from 3.0×10^4 to 1.9×10^5 g/mol (Tashiro, Mochizuki, Ogawa, Mizuno, & Iso, 1996). Considering that $M_w \geq M_{pk}$ for the same sample, the values

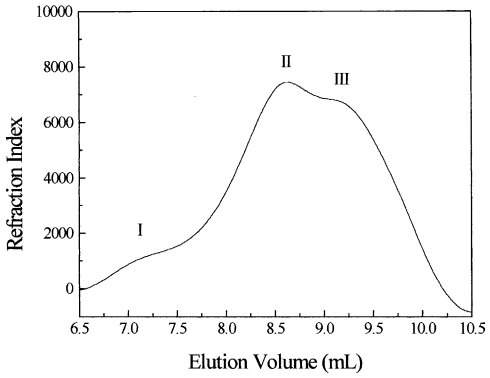

Fig. 4. GPC curve for *G. cornea* soluble sulfated polysaccharide solution in 0.1 M $NaNO_3$.

related to peaks II and III were in agreement with this result. The M_{pk} of fraction I may be due to very high molecular weight macromolecules or aggregation between macromolecules.

Assuming that the refraction index is proportional to the content of material, fractions I, II and III contain 5, 68 and 27% of the whole solSP, respectively. Considering the absorbance at 280 nm, fractions I and III contain 1.8 and 1.2% of protein material, respectively. Fraction I is probably a polysaccharide–protein complex of $M_{pk} = 2.1 \times 10^6$ g/mol, composed of 60% polysaccharide and 40% protein. Fraction II, the main component of solSP, is a polysaccharide of $M_{pk} = 7.4 \times 10^4$ g/mol. Fraction III is a polysaccharide of M_{pk} 1.8×10^4 g/mol with a small amount of protein residues (4%), rather than a protein–polysaccharide complex.

3.4. ^{13}C NMR of the soluble sulfated polysaccharide

^{13}C NMR spectrum of sulfated polysaccharide from *G. cornea* is shown in Fig. 5. The signal assignments (Table 5) were made on the basis of comparison with spectra of model compounds, agarose and other related polysaccharides (Lahaye, Yaphe, Viet, & Rochas, 1989; Lai & Lii, 1998; Miller & Furneaux, 1997; Usov, Yarotsky, & Shashkov, 1980; Valiente, Fernandez, Perez, Marquina, & Velez, 1992).

The main signals are due to β-D-galactose and 3,6-anhydrogalactose units. The presence of a (→3)-β-D-galactose (1→4)6-*O*-SO_3-α-L-galactose 1→) repeat unit was inferred by distinct peaks at 103.1 due to an anomeric carbon of β-D-galactose linked to α-L-galactose (Cl in 100.8 ppm; Lai & Lii, 1998; Valiente et al., 1992). The downfield displacement of the galactose primary group from 61.2 to 67.2 ppm is characteristic of 6-*O* substitution of α-L-galactose by sulfate groups (Lahaye et al., 1989; Miller & Furneaux, 1997). Additional peaks due to 6-*O*-methyl-galactose-4-sulfate residue were also detected (Table 5).

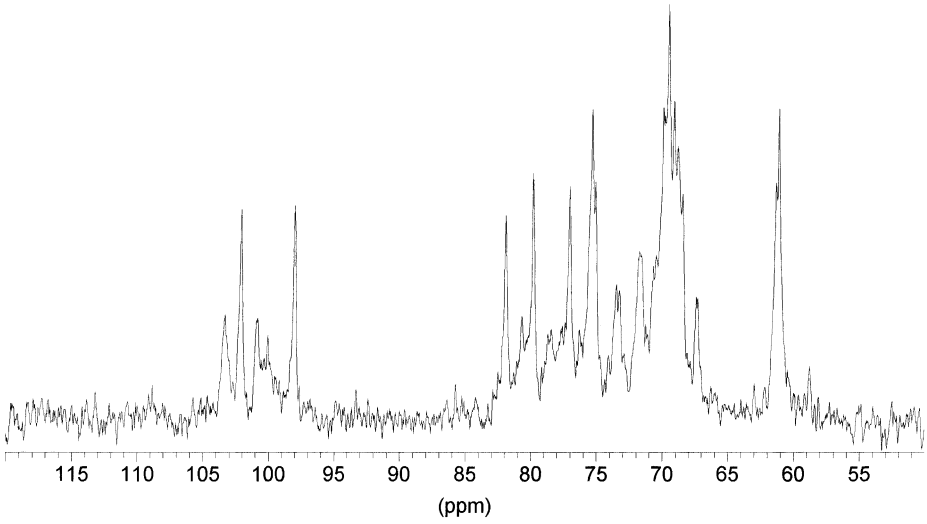

Fig. 5. ^{13}C NMR spectrum of *G. cornea* soluble sulfated polysaccharide in D_2O.

3.5. Intrinsic viscosity

Intrinsic viscosity of six agar samples in 0.1 M KCl at 65 °C ranged from 0.97 to 3.40 dl/g (Tashiro et al., 1996). The corresponding value for *G. cornea* solSP was 0.72 dl/g, lower than the lowest value in the above range. The $[\eta]$ determination made in 0.1 M NaCl at 45 °C also provided a low value (0.80 dl/g). Lai and Lii (1998) found $[\eta]$ values from 1.05 to 2.75 dl/g under the same experimental conditions and determined lowest $[\eta]$ value for agar which contains the smallest amount of 3,6-AG (35.4%). As the 3,6-AG content of *G. cornea* solSP was still lower (24.7%), the determined $[\eta]$ value was expected (Fig. 6).

As the polysaccharide concentration increases, the effect of the interaction between the macromolecules over the reduced viscosity becomes predominant. These changes in interaction are reflected in changes in the Huggins viscosity slope parameter, k_H. Usual value of k_H range between 0.33 and 0.80 (Elias, 1997). The Huggins constant of 0.83 and 0.84 calculated for sulfated polysaccharide in the presence of NaCl and KCl is just outside this range. High values of 1.9 and 6.2 were determined for anionic polysaccharide in the presence of cross-linking agents, in this case, Ca^{2+} and Al^{3+}, respectively (de Paula et al., 2001). Sodium and potassium cations are not cross-linking agents which explains the lower Huggins constant obtained for *G. cornea* polysaccharide.

Large interactions between macromolecular chains were

Table 5
Chemical shift assignments for ^{13}C NMR spectra of soluble sulfate polysaccharide from *G. cornea* (G: (1 → 3) linked β-D-galactose; A: (1 → 4) linked 3,6-anhydrogalactose; G6M-4S: (6-*O*-methyl-D-galactose-4 sulfate; G′-L6S: (→ 3) β-D-galactose (1 → 4)-α-L-galactose-6 sulfate)

Residue unit		^{13}Carbon chemical shift						
		C1	C2	C3	C4	C5	C6	Me
Usov et al. (1980)								
$(G–A)_n$	G	102.3	70.1	82.2	68.6	75.2	61.3	
	A	98.2	69.7	80.0	77.2	75.5	69.7	
G6M-4S		102.4	70.0	80.0	71.5	70.8	70.0	59.0
Lahaye et al. (1989)								
$(G–A)_n$	G	102.4	70.2	82.2	68.8	75.3	61.4	
	A	98.3	69.9	80.1	77.4	75.7	69.4	
(G′-L6S)	G′	103.7	69.8	81.2	69.1	75.9	61.8	
	L	101.3	69.3	71.1	79.1	70.3	67.9	
Miller and Furneaux (1997)								
(G6M-A)	G6M	102.3	70.1	82.1	69.0	73.5	71.7	59.1
	A	98.2	69.9	80.1	77.3	75.5	69.3	
Lai and Lii (1998)								
$(G–A)_n$	G	102.4	70.2	82.3	68.7	75.3	61.4	
	A	98.2	69.9	80.1	77.3	75.6	69.4	
Valiente et al. (1992)								
$(G–A)_n$	G	102.4	70.1	82.1	68.4	75.6	61.4	
	A	98.3	69.8	80.1	77.4	75.6	69.3	
Observed spectrum								
$(G–A)_n$	G	102.0	69.8	81.8	68.4	75.0	61.0	
	A	98.0	69.4	79.7	77.0	75.2	69.3	
	G6M-4S	102.0	70.1	80.2	71.4	70.1	68.6	58.7
(G′-L6S)	G′	103.1	69.9	81.8	69.0	75.5	61.3	
	L6S	100.8	69.5	71.4	79.9	70.4	67.2	

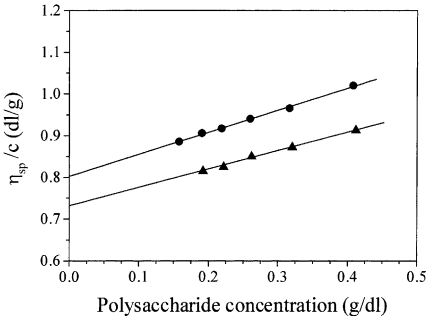

Fig. 6. Reduced viscosity of *G. cornea* soluble sulfated polysaccharide versus polymer concentration in the presence of 0.1 M NaCl at temperature = 45 °C (●) and 0.1 M KCl at temperature = 65 °C (▲).

expected for agarose molecules (Yenigül, 1993) because of the intense hydrogen bonding formation between disaccharide repeat units (Tashiro et al., 1996). This interaction may provide favorable gelling properties (high melting temperatures, high gelling temperatures) and also a high Huggins constant value.

No gelation was observed in 1.5, 2.0 and 3.0% (w/v) aqueous solution of *G. cornea* polysaccharide, even when cooled at 4 °C. The gatactose/3,6-AG ratio found for the *G. cornea* polysaccharide (2.96) was very different from the ideal agarose ratio (1.0). Their structure may also be different. This structure deviation and the high sulfate content may explain the absence of gelling behavior of solSP solution. Gelling and melting temperatures for native agar from Mexican *G. cornea* were 32.4–36.3 and 70.0–74.7 °C, respectively, depending on the season (Freile-Pelegrin & Robledo, 1997a). The solSP from Brazilian *G. cornea* seaweed (isolation carried out by a different method) and the agar from Mexican species probably have a different sugar composition and structure which explains the differences in the gelling behavior.

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