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Fractionation of extracted Madagascan *Gracilaria corticata* polysaccharides: Structure and properties

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Abstract

Polysaccharides were sequentially extracted from *Gracilaria corticata* which is collected from south-west coast of Madagascar. Chemical analysis combined with ^{1}H, ^{13}C NMR and Fourier transform infrared spectroscopies showed that the fraction extracted with water/ethanol 60% (v/v) as solvent has low methoxyl and pyruvate contents and a great ability to form relatively strong physical gels in the presence of KCl. Rheological properties of extracted fractions are discussed as well as the ionic selectivity.

Keywords: *Gracilaria corticata*; Polysaccharides; Step-by-step extraction; Agar; Chemical composition; Rheology

1. Introduction

Many structural studies of polysaccharides from *Rhodophyta* seaweeds (red algae) have been developed. These seaweeds mainly contain agar and carrageenan polysaccharides characterized by a backbone of alternating 3-linked β-D-galactopyranosyl and 4-linked α-L or α-D-galactopyranosyl residues, respectively (Araki & Arai, 1959).

In Madagascar, *Gelidium madagascariense* and *Eucheuma denticulatum* have been studied by Mollion, Andriantsiferana, and Sekkal (1990), particularly because they have been industrially exploited.

The chemical structures of polysaccharides extracted from Madagascan *Gracilaria corticata* are complex as shown by Andriamanantoanina (1992). Later, Mazumder et al. (2002) obtained similar results from analysis of polysaccharides from *G. corticata* collected in China. Aqueous extraction of *G. corticata* gives sulfated, methylated and pyruvated galactan of the agarose type. Sulfate groups are located at C-4 of D-galactose units and C-6 of L-galactose units; methoxyl groups are located at C-6 of D-galactose and C-2 of L-galactose residues (Fig. 1).

Several studies on polysaccharides from *Gracilaria* genus indicated that the extracted agars are composed of complex series of polysaccharides which range from neutral molecules to a highly charged galactans as it was found in the following species: *G. pseudoverrucosa* (Lahaye & Yaphe, 1988), *G. dominguensis* (Fernandez et al., 1989), *G. gracilis*, *G. dura* and *G. bursa-pastoris* (Marinho-Soriano, 2001), *G. lemaneiformis* (Chirapart, Ohno, Ukeda, Sawamura, & Kusunose, 1995) or *G. tikvahiae* (Craigie & Wen, 1984).

Gracilaria corticata is a predominant red seaweed species of west coast of Madagascan south island, belonging to the family *Gracilariacea* and order *Gracilariales*. This species is also found along the east coast of Madagascar particularly at coast of Mahambo.

The purpose of the present investigation was to confirm structural characterization of polysaccharides isolated from Madagascan *G. corticata*, and to determine the main

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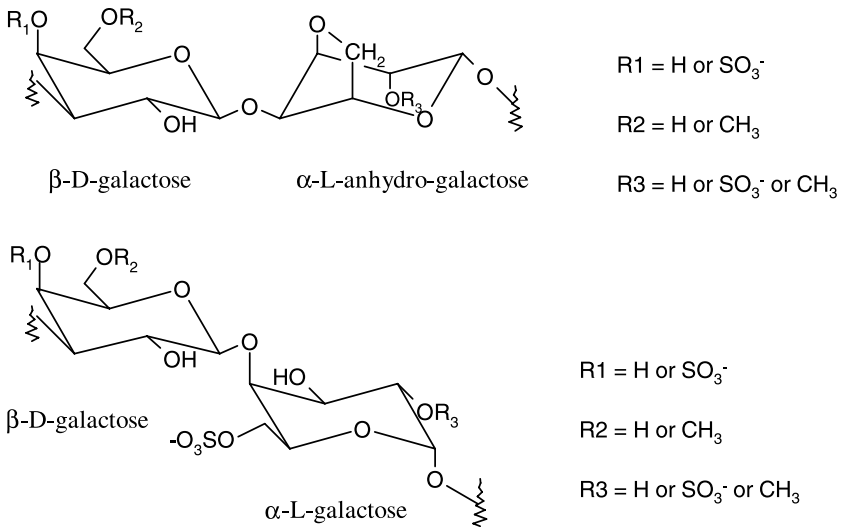

Fig. 1. Chemical structure of the repeat unit of agar type molecules with the different types of sugar units and the different substituents.

physical properties of extracted polysaccharides and especially their rheological behavior in aqueous solution in the presence of NaCl or KCl as it was never published before. Indeed, the gelling property of agar is due to the three equatorial hydrogen atoms on the 3,6-anhydro-L-galactopyranose residues which constrain the molecule such as to form a double helix (Duckworth, Hong, & Yaphe, 1971). The conformational analysis of agarose also predicts single and double helices (Jimenez-Barbero, Bouffar-Roupe, Rochas, & Perez, 1989; Kouwijver & Pérez, 1998).

The current study is part of a program on polysaccharides from Madagascar seaweeds investigation.

2. Experimental

2.1. Materials

Our sample was growing attached on the Ambotsibotsiky coast (Toliara) of south-west of Madagascar and was collected in May 2003. A specimen of this species is stored in the "Centre National de Recherches sur l'Environnement," CNRE/Madagascar.

It was first cleaned from their epiphytes, washed with distilled water, dried and weighted.

A sample of commercial pure agarose (Agarose Kalys-6; lot H070905 delivered by Kalys, France) was used for comparison of physical properties; this sample is characterized by a melting temperature at 70 °C and a gelling temperature at 32.1 °C.

2.2. Extraction of agar

Before extraction of polysaccharides, dried grinded seaweeds were preliminary washed with acetone and hot 94% ethanol to eliminate the pigments (Chinkichi, 1962). The protocol adopted for selective extraction of polysaccharides is shown in Fig. 2 and summarized as followed.

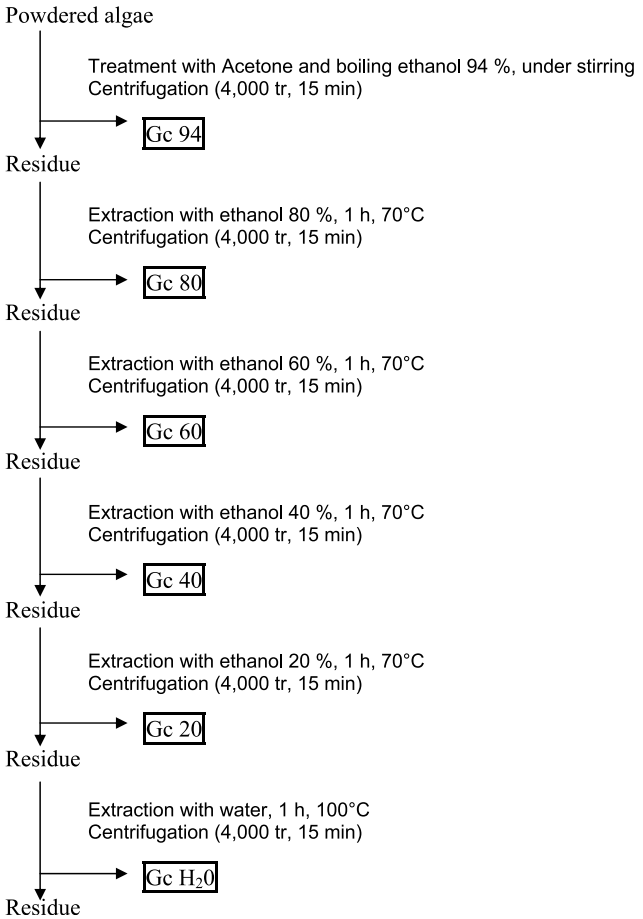

Fig. 2. Sequential extraction with ethanol/water solvents of polysaccharides from Madagascan *G. corticata*.

The moistened seaweeds were stirred successively and three times for each condition in ethanol/water 80, 60, 40, 20% (v/v) at 70 °C for 1 h, and later in distilled water

at 100 °C for 1 h. The insoluble fraction after each extraction was separated from the crude extract by centrifugation (8000*g*, 15 min)

2.3. Purification of polysaccharides

The aqueous solutions of agar type molecules (around 1 g/L) from the different extractions were precipitated with ethanol around 50% (v/v water/ethanol) in the presence of salt excess (NaCl 0.5 M) to get the sodium salt form of the polysaccharides when they contain charged monomeric units (Milas & Rinaudo, 1997). The precipitated polysaccharides after 12 h at 4 °C were recovered by centrifugation (8000*g*, 15 min), washed three times with ethanol and dried at room temperature.

2.4. Alkali modification of polysaccharides

The procedure for alkaline treatment used to increase the gelling properties of the agar type molecules was inspired by the work of Ciancia, Noseda, Matulewicz, and Cerezo (1993): Sodium borohydride (25 mg) was added in 200 mL of aqueous solution containing about 500 mg polysaccharides. After stirring 24 h at room temperature, 6 M sodium hydroxide (40 mL) and sodium borohydride (15 mg) were added to give a final concentration of about 1 M sodium hydroxide. The solution was stirred at 80 °C for 1 h. The reaction was stopped by cooling in an ice bath. Then the solution was neutralized with 6 N hydrochloric acid (to a pH around 7). The modified polysaccharides were dialyzed and precipitated with ethanol up to 50% (v/v water/ethanol); the precipitate was redissolved in water and reprecipitated with ethanol. The precipitate was washed three times with ethanol and dried at room temperature.

2.5. NMR analysis

^{1}H NMR spectra of different extracted fractions were recorded at 85 °C on 10 mg/mL solution in D_2O. ^{1}H NMR experiments were performed using a Bruker AC300 spectrometer operating at 300 MHz. ^{1}H NMR spectra were collected using 8K data points. Chemical shifts are given relative to external tetramethylsilane (TMS = 0 ppm) and calibration was performed using the signal of the residual protons of the solvent as a secondary reference. Deuterium oxide was obtained from SDS (Vitry, France). ^{13}C NMR spectrum was obtained in the same experimental conditions but at 60 mg/mL and 75.47 MHz with a delay time of 0.5 s and 8K data points.

2.6. FTIR analysis

Fourier transform infrared (FTIR) spectra of KBr pellets of the polysaccharides (1%, w/w) were recorded in a Perkin–Elmer FTIR spectrum RXI scanning between 4000 and 400 cm^{-1}.

2.7. GC analysis

Polysaccharides were hydrolyzed to monomeric units and transformed in their alditol acetates. Each polysaccharide (~1 mg) was placed in a tube, aqueous trifluoroacetic acid (TFA, 2 N, 0.5 mL containing 1 mg/mL of inositol as internal standard) was added for hydrolysis and the tube was heated (2 h, 100 °C). The solvent was evaporated and two portions of distillated water (~2 mL) were added and evaporated to remove residual trace of TFA. Aqueous NH_4OH (two drops, 4%, v/v) was added before $NaBH_4$ (~20 mg) for reduction and the tube was allowed at room temperature for 7 h. Excess reducing reagent ($NaBH_4$) was quenched with aqueous AcOH (50%) and the solution was evaporated to dryness. Boric acid was removed by evaporation with three or four portions of MeOH containing 1% HCl (v/v). The alditols were acetylated in acetic anhydride (0.5 mL) and pyridine (0.5 mL) at room temperature for one night. Water was added and the solution was evaporated to dryness. The residue was then dissolved in CH_3Cl (~2 mL) and analyzed by g.l.c. on a 30 m × 0.25 mm, SP 2380 fused silica column programed to hold for 2 min at 195 °C, increase at 2.5–225 °C, and hold for 5 min. Inositol is used as internal standard for the quantitative determination of the sugar composition.

2.8. Molecular weight distribution

Purified polymers under their sodium salt form were characterized by steric exclusion chromatography (SEC) using a Waters Alliance GPCV2000 (USA) equipped with three detectors on line: A differential refractometer, a viscometric detector, and a multi angle laser light scattering (MALLS) detector from Wyatt (USA). The concentration injected was 0.5 g/L, with an injection volume of 108 μL using two columns in series (Shodex OH-pack 805 and 806). All the samples were filtrated on a 0.2 μm pore diameter membrane (Sartorius AG; cellulose acetate filter) before injection, in order to retain large aggregates. The eluent used was 0.1 M $NaNO_3$, with an elution temperature of 30 °C and a flow rate of 0.5 mL/min; the molecular weight distribution, weight-average molecular weight (M_w), polydispersity index (M_w/M_n), and intrinsic viscosity (η, mL/g) of the eluted polymers were obtained as characteristics of the biopolymers.

2.9. Rheology

The rheological behavior was studied using an AR 1000 rheometer from TA instruments at 25 °C, when not precised. For viscoelastic solution, a plane-cone geometry is used with a 3°59′ and 4 cm diameter; for strong gel, plane–plane geometry is used with a 2-cm diameter plane. Dynamic experiments were performed in the linear domain. The storage modulus (G') and the loss modulus (G'') (Pa) as well as the complex viscosity $|\eta^*|$ (Pa s) were determined as a function of the frequency (f (Hz)). The

temperature dependence was imposed at a given rate (5 °C/min) using the Peltier plate with a film of silicone to avoid evaporation and the modulus is measured at constant frequency (1 Hz).

2.10. Differential scanning calorimetry

DSC experiments were realized on a DSC III from Setaram (France) with a heating and cooling rate of 0.4 °C/min. The reference cell was filled with solvent and the sample compartment with the polymeric solution. The polymer concentration was 10 g/L dissolved in 0.1 M KCl.

3. Results and discussion

3.1. Sequential extraction

The algae were fractionated following the process described in Fig. 2. Each crude extract was repurified and characterized. Progressively, the solvent is enriched with water to increase the solvent quality for polysaccharides. Then, each fraction after repurification was characterized by spectroscopy and macromolecular techniques.

The sequential extraction method is supposed to allow the isolation of homogeneous polysaccharides with similar structure (Lahaye, Rochas, & Yaphe, 1986).

The yields of agar type fractions from *G. corticata* from Madagascar in 94, 80, 60, 40 and 20% ethanol at 70 °C and water at 100 °C are illustrated in Table 1. Except for the first extraction (ethanol 94%) used to take off some pigments, the different crude fractions are found in the same range of weight fraction values. Around 50% of weight was recovered after purification of the more important fractions of polysaccharides extracted by 80, 40 and 20% ethanol, respectively, but less is obtained for GC60, the fraction extracted by 60% ethanol; only 28% is obtained due of difficulties during precipitation at 50% ethanol; in addition, it will be shown that the structure and the physical properties of this polymeric fraction are very different from that of the others.

Compared with the results obtained previously on *G. corticata* by Mazumder et al. (2002) and Andriamanantoanina (1992), there is an important difference: In these cases, the polysaccharides were successively extracted with water (cold and hot). From Table 1, it is demonstrated in our work that the yields of extraction by water/ethanol mixtures are relatively low but more selective.

3.2. Structure analysis

3.2.1. Gas chromatography

The main results obtained on alditol acetates after complete hydrolysis are given in Table 2. Analysis by gas chromatography showed that 6-*O*-methyl-D-galactose was the major constituent in fractions GC20, GC40 and GC80, while for fraction GC60 D-galactose is the main constituent. The proportion of 6-*O*-methyl-D-galactose on the basis of the total D-galactose unit is clearly higher in GC80 fraction. In addition, the experimental conditions (TFA 2 N, 2 h, 100 °C) for total hydrolysis allowed to detect the presence of 2-*O*-methyl-3,6 anhydro-L-galactose which was confirmed by mass spectroscopy. Nevertheless, the quantitative yield of this unit is not representative taking into account the instability of 3,6-anhydrogalactose residues during acid hydrolysis. In relation with this mechanism, the fraction of monosaccharides obtained after acid hydrolysis was always lower than 50% (w/w) of the initial polymer.

3.2.2. NMR spectroscopy

The ^{13}C NMR spectrum of the fraction extracted with ethanol 80% (Fig. 3) showed 12 main signals attributed to the repeat unit of 3-linked-6-*O*-methyl-β-D-galactose 4-linked-2-*O*-methyl-3,6-anhydro-α-L-galactose in a ratio around 58% (this value corresponds to the ratio integral of the signal for C-1 of 2-*O*-methyl-3,6-anhydro-α-L-galactose at 99.04 ppm) (A1, Fig. 3) and the integral of total anomeric carbons of L-galactose (97.51 ppm, C-1 of 2-*O*-methyl-3,6-anhydro-α-L-galactose with 4-*O*-sulfate-β-D-galactose; 98.67 ppm, C-1 of 2-*O*-methyl-3,6-anhydro-α-L-galactose with (4,6)-carboxylethylidene-β-D-galactose; 99.04 ppm, C-1 of 2-*O*-methyl-3,6-anhydro-α-L-galactose with 6-*O*-methyl-β-D-galactose; 99.47 ppm, C-1 of 2-*O*-methyl-3,6-anhydro-α-L-galactose with 4-*O*-sulfate-β-D-galactose).

We can notice the presence of a large signal at 59.84 ppm attributed to the carbon of —O—CH_3 substituents.

Table 1
Polysaccharide yields after ethanolic successive extractions

Extraction condition (by EtOH/H_2O mixture)	Crude extract	Yields[a] (%)	Relative yield (%)[b]	Purified extract yields (%)
EtOH 94%, boiling, 5 min	GC94	1.1	8.3	
EtOH 80%, 70 °C, 1 h	GC80	2.5	18.8	51
EtOH 60%, 70 °C, 1 h	GC60	2.1	15.8	28
EtOH 40%, 70 °C, 1 h	GC40	3.0	22.5	47
EtOH 20%, 70 °C, 1 h	GC20	1.7	12.8	44
H_2O boiling, 1 h	GC H_2O	2.9	21.8	

[a] Percentage weight of dried algae weight.
[b] Percentage among the isolated crude fractions.

Table 2
Analysis of the different fractions by gas chromatography

$t_{R(min)}$	Compounds	% (w/w)			
		GC80	GC60	GC40	GC20
(a) Identification of monosaccharide contents					
4.7	2-*O*-methyl-3,6-anhydro-α-L-galactose	0.6	–	1.5	0.7
7.8	6-*O*-methyl-β-D-galactose	27.2	8.0	31.3	19.0
8.4	Xylose	–	1.1	0.7	1.9
11.9	Galactose	4.2	21.2	8.5	16.3
13.1	Glucose	0.6	2.1	1.4	0.3
(b) Percentage of 6-O-methyl-β-D-galactose contents					
Ratio	6-*O*-methyl-β-D-gal/6-*O*-methyl-β-D-gal + D-gal	87	27	79	54

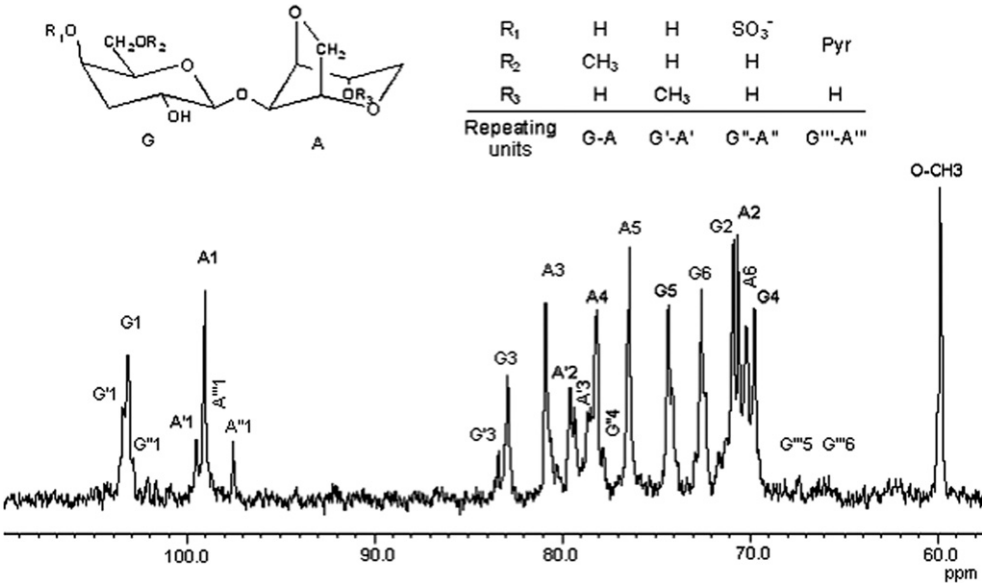

Fig. 3. ^{13}C NMR spectra of fraction extract with 80% ethanol (GC80) (G = D-galactose unit and A = anhydro-L-galactose unit; A1 is the carbon in the 1 position of unit A).

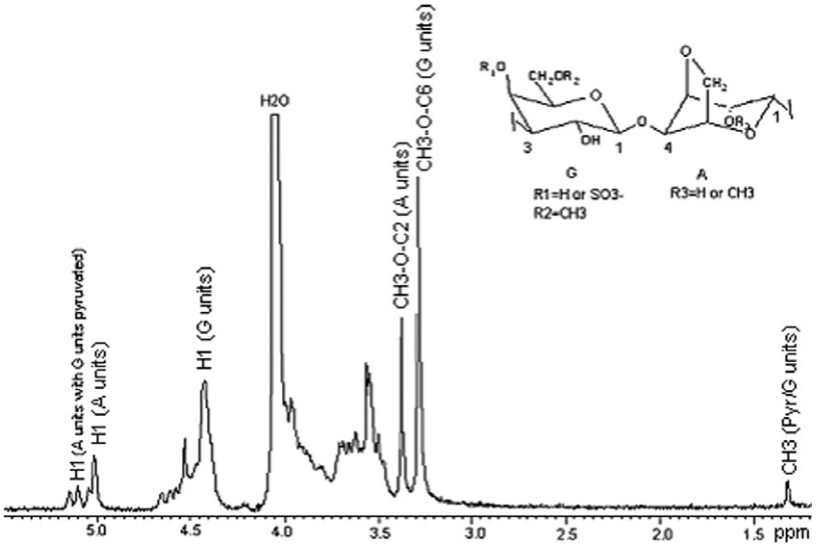

Fig. 4. ^{1}H NMR spectra of fraction extracted with 80% ethanol (GC80)(G = D-galactose unit and A = anhydro-α-L-galactose unit).

The ratio of integrals for protons [CH_3—O—C_2 of A units]/[H_1 of G units] (Fig. 4) indicated that GC80 fraction contained more 2-*O*-methyl group than GC20 and GC40 fractions.

Indeed, except the fraction GC60, ^{1}H NMR spectra of the different fractions were characterized by large signals at 3.28 and 3.37 ppm attributed to —CH_3 of 6-*O*-methyl-β-D-galactose and 2-*O*-methyl-3,6-anhydro-α-L-galactose, respectively (David, 1977) (Fig. 4).

The anomeric proton resonances at 4.98–5.13 ppm are attributed to α-(1,4)-linked-L-galactopyranose from which one fraction is shift by the presence of a connected pyruvylated G unit; the signals between 4.42 and 4.62 ppm are attributed to β-(1,3)-linked-D-galactopyranose.

The ^{1}H NMR signal at 1.32 ppm attributed to the methyl group of (4,6)-carboxylethylidene-β-D-galactopyranose was shown in spectra of successive GC20, 40 and 80 fractions which have the same type of spectrum as given

Fig. 5. FTIR spectra (1600–750 cm^{-1}) for sequentially extracted fractions GC80, GC60, GC40 and GC20.

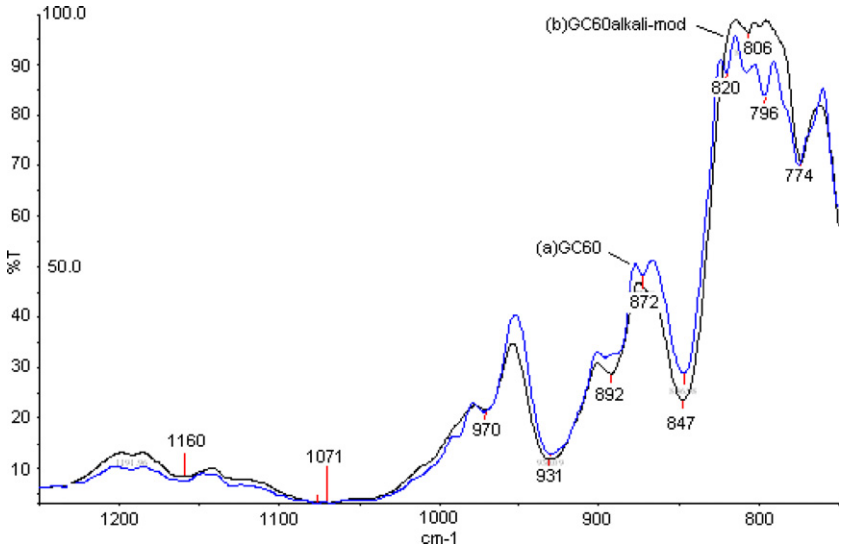

Fig. 6. Comparison of FTIR spectra of extracted fractions GC60 (a), GC60 alkali-modified (b).

Table 3
DSC characteristics for the different fractions

Fraction extracts	[Conc] (g/L)	[KCl] (mol/L)	Thermal cycles	Temperature of transition (°C)	ΔH (J/g)
GC20	10	0.1	Heating	44.66	6.32
			Cooling	42.62	5.32
				50.54	3.67
GC40	10	0.1	Heating	45.81	4.59
			Cooling	40.41	9.27
GC60	10	0.1	Heating	70.37	21.49
			Cooling	50.07	22.85
GC80	10	0.1	Heating	44.31	10.25
			Cooling	41.72	12.98

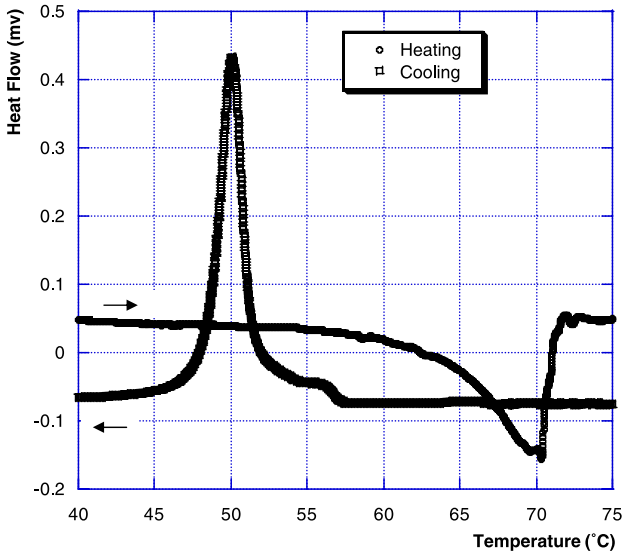

Fig. 7. DSC heating (1) and cooling (2) curves for GC60 in 0.1 M KCl. Cp = 10 g/L.

Table 4
SEC chromatogram analysis

Fraction extracts	M_w	η (mL/g)	% soluble	M_v
GC20	1.000×10^6	343	37.5	0.688×10^6
GC40	0.990×10^6	319	17.1	0.775×10^6
GC60	0.696×10^6	515	48	0.743×10^6
GC80	0.852×10^6	255	21.0	–

in Fig. 4; for the extraction with ethanol 60%, one notices the absence of this signal at 1.32 ppm.

The existence of many signals attributed to anomeric protons indicated the complexity of polysaccharides structure from *G. corticata* from Madagascar.

3.2.3. FTIR

IR spectra of the sequential fractions extracted from *G. corticata* showed typical absorption of agar type polysaccharides (Christiaen & Bodard, 1983; Rochas, Lahaye, & Yaphe, 1986) (Fig. 5). The absorbance at 930 cm^{-1} which has been assigned to 3,6-anhydro-α-L-galactose (C—O vibration) is large in all the fractions. A broad absorption at 1240 cm^{-1} (—S=O antisymmetric stretching vibration) reported to be representative of the amount of sulfate present as substituents in galactans is present in all the samples (Fournet et al., 1997; Stancioff & Stanley, 1969). The presence of signals characterizing sulfation on several carbons, between 800 and 900 cm^{-1}, confirms the complexity of these extracted polysaccharides. The intense peak at 846–850 cm^{-1} attributed to axial sulfate ester at O—C-4 of 3-linked-β-D-galactose residues is observed in the spectra of all fractions; in GC60 fraction, two low absorbance bands at 820 and 805 cm^{-1}, respectively, are characteristics of 6-sulfate-α-L-galactose, and an axial sulfate ester at O—C-2 of 3,6-anhydro-α-L-galactose (Lloyd, Dogson, Price, & Rose, 1961). We can suggest that the sulfate groups occur principally at C-4 of β-D-galactose in all fractions.

The alkaline treatment of fraction GC60 does not removed sulfate substituents at O—C-2 of 3,6-anhydro-α-L-galactose units nor at O—C-4 of 3-linked-β-D-galactose identified at 805 and 850 cm^{-1}, respectively.

The differences observed for GC60 fraction was confirmed from the ^{13}C NMR results:

(i) Absence signal at ~61.5 ppm attributed to C-6 of β-D-galactose linked to a 6-*O*-sulfate-α-L-galactose (the biological precursor).
(ii) Presence of signals attributed to repeat units containing β-D-galactose-4-sulfate (A1: 97.51 ppm, G1: 102.87 ppm, G4: 77.77 ppm).

Compared with GC20, GC40 and GC80, the fraction GC60 has less 6-*O*-methyl-β-D-galactose (from g.l.c. and ^1H NMR data) and has no pyruvic acid (from ^1H NMR).

GC20, GC40 and GC80 contain a large amount of 6-*O*-methyl-β-D-galactose and pyruvic acid which will play a role on the physical properties preventing aggregation (Christiaen & Bodard, 1983; Guisley, 1970).

From comparison of our data obtained by ^1H NMR, ^{13}C NMR and FTIR, it comes that the structures given for fractions GC20, GC40 and GC80 are similar to those proposed by Mazumder et al. (2002) on *G. corticata* produced in India and by Andriamanantoanina (1992) for *G. corticata* collected at Madagascar when the materials are extracted with hot water (Fig. 6).

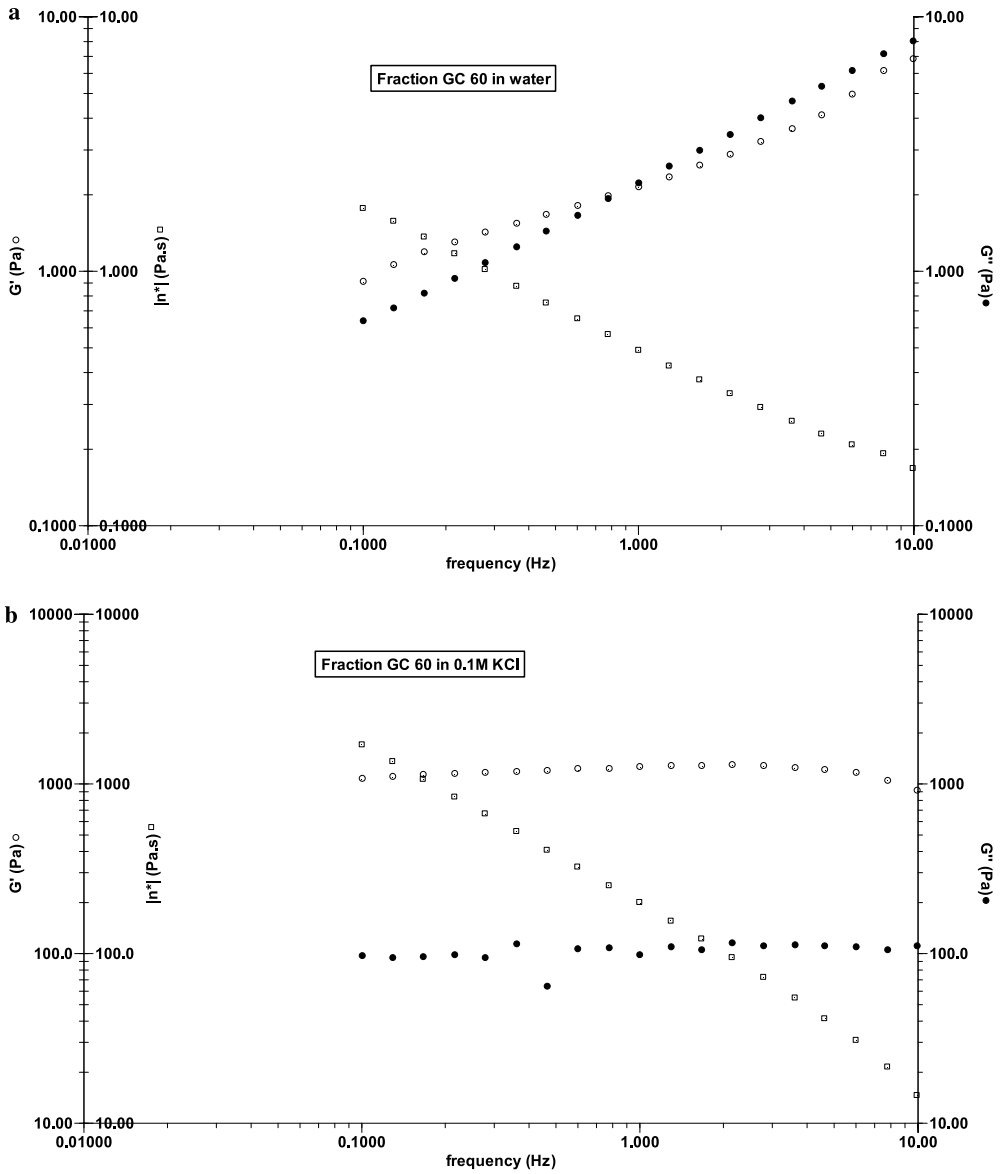

Fig. 8. Rheological properties of GC60 (a) in water G'(■), G''(□), [η^*] (∇); (b) in 0.1 M KCl G'(●), G''(○), [η^*] (▼); $T = 20$ °C; cp = 10 g/L. (c). Comparison between GC60 (10 g/L in KCl) (○,●) and agarose (◇, ◆) in water at 5 g/L.

3.3. Physical properties

The polysaccharides obtained were dissolved in aqueous solution of 0.1 M KCl to screen the probable electrostatic repulsions and characterized by rheology and DSC to examine the conformational stability in relation with rheological behavior.

DSC is a convenient technique to determine the enthalpy of conformational change in stereoregular polysaccharides such as gellan (Mazen, Milas, & Rinaudo, 1999) or carrageenan (Rochas & Rinaudo, 1982, 1984); for these polymers, the conformation is usually a double helix at low temperature and goes to a disordered coil at higher temperature in direct relation with the salt concentration. The conformational change is characterized by Tm, the helix-coil melting temperature determined for half the transition, which position depends on the stability of the double helix. When a gel is formed with such polymers in given thermodynamic conditions, based on aggregation of double helices, an additional small contribution is obtained in enthalpy and the conformational transition occurs at a melting temperature (T_F) higher than the reconformational temperature corresponding to the gel formation (T_G); an hysteresis characteristic of the degree of aggregation can be demonstrated from the difference ($T_F - T_G$). Our main DSC experimental data obtained in 0.1 M KCl are given in Table 3. On a general point of view, these results indicate that the conformational transition is relatively large with a peak maximum located at 45.8 °C on heating but at 40.4 °C on cooling for GC40. This small hysteresis (~5 °C) and the large peak mean a small cooperativity of the interactions in such system; the same trend is also obtained for GC20 and GC80 which have also nearly the same chemical structure (from NMR and from IR).

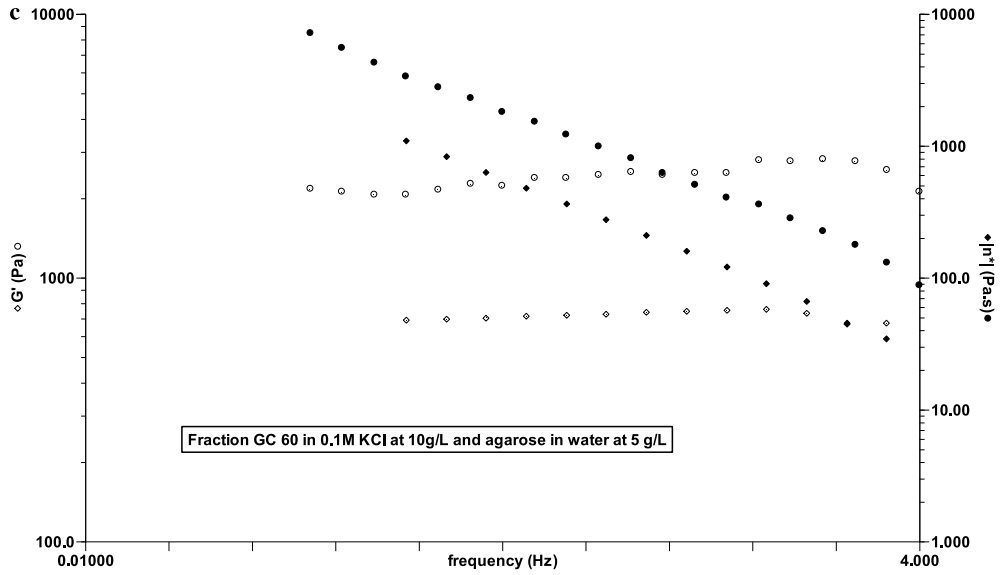

Fig. 8. *(continued)*

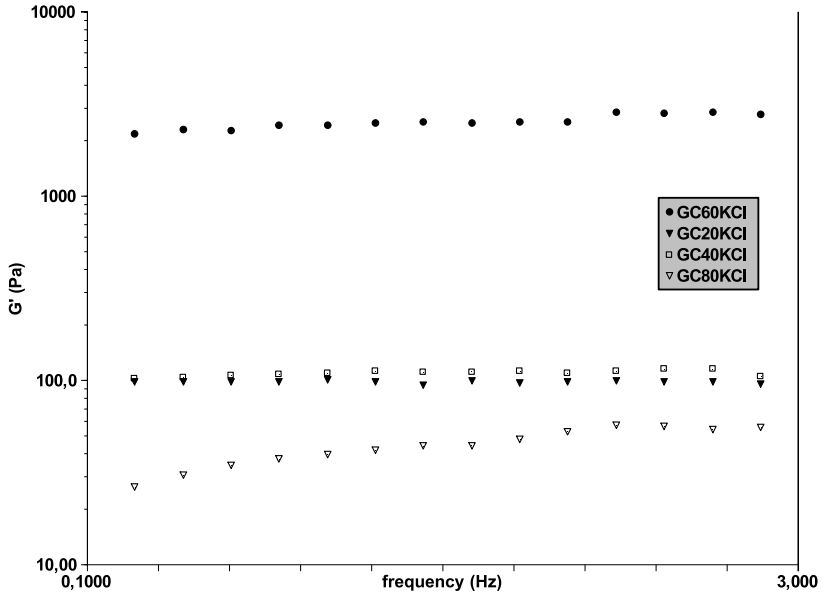

Fig. 9. Elastic modulus G' as a function of the frequency (in Hz) for the different samples in 0.1 M KCl, $T = 25$ °C, Cp = 10 g/L. (▽ GC80, ● GC60, □ GC40, ▼ GC20).

A complex behavior is observed on GC20 on cooling; it is assumed that it contains a mixture of two components: One has the same behavior than GC40 and GC80 and an other component looks similar to GC60. The sample GC60 is completely different; a much larger enthalpy (~20 J/g) for the sol–gel transition is obtained in the same way as a larger hysteresis and a larger cooperativity and can be compared with the sample of agarose tested (Table 3, Fig. 7). Nevertheless, the dependence of the hysteresis with external salt concentration indicates that the polymers are charged (as observed with carrageenans).

From SEC experiments, one gets the weight-average molecular weight and the intrinsic viscosity. The original data obtained are given in Table 4. A general trend is that molecular weight is nearly independent on the water content in the extraction solvent. From the intrinsic viscosity, the viscometric-average molecular weights were calculated. A relatively low intrinsic viscosity is obtained and also a low solubility estimated from the fraction eluted in SEC. The molecular weight values are certainly overestimated due to aggregation in relation with the low solubility of these polysaccharides. The better results are obtained for

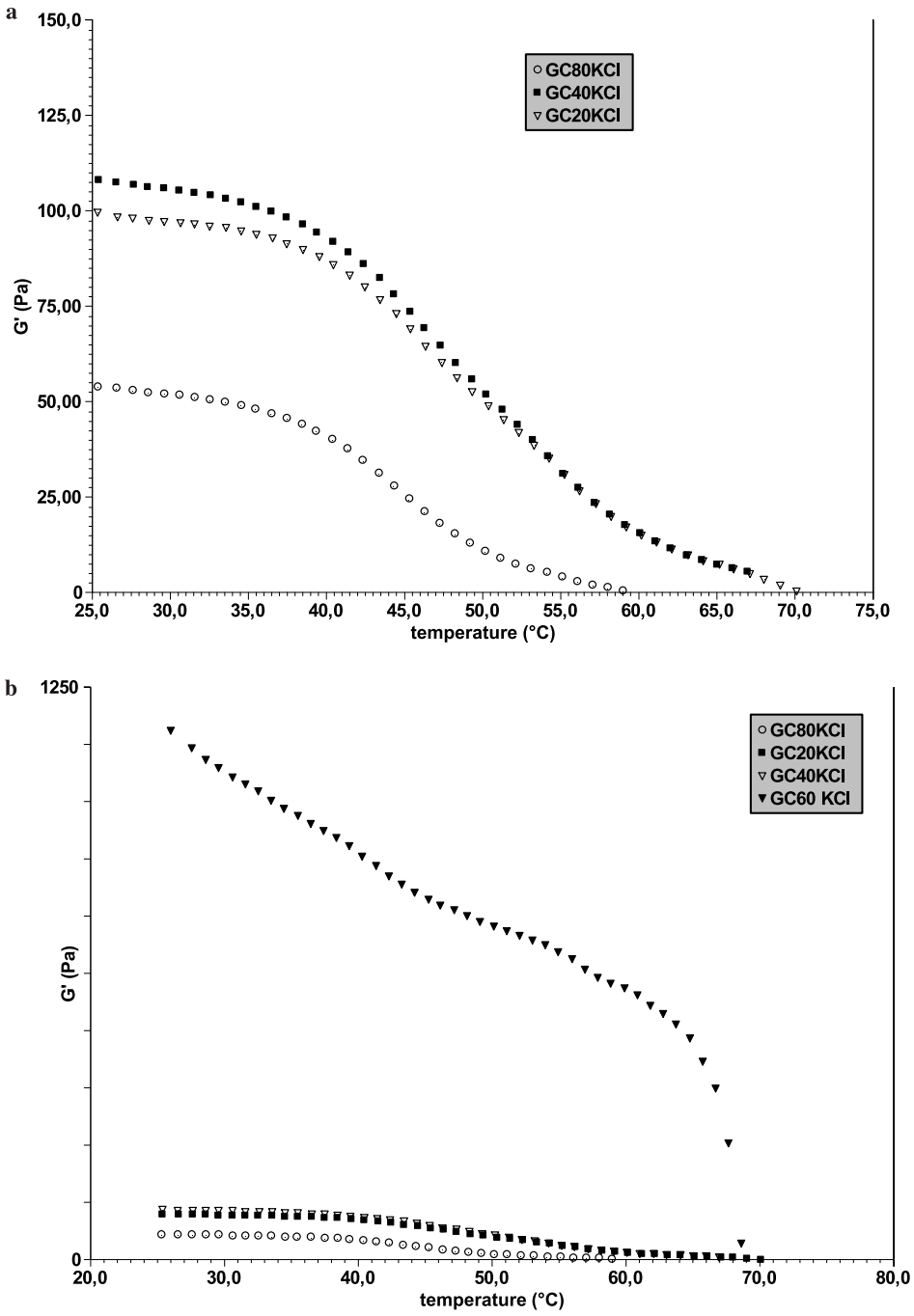

Fig. 10. Influence of the temperature on the G' modulus at 1 Hz for the different samples. (a) ▽ GC20, ■ GC40, ○ GC80; (b) ■ GC20, ▽ GC40, ○ GC80 and ▼ GC60. Cp = 10g /L, in 0.1 M KCl.

GC60 in the coil conformation in the SEC conditions; as a first hypothesis, it is related with a higher charge density.

Then, the rheological behavior of the different fractions was examined to estimate their performance in relation with their chemical structure. All the samples directly dissolved in water behave as a viscous solution ($G' < G''$ in all the range of frequencies). The data obtained for GC60 are given in Fig. 8. In water, $G'' > G'$ for frequency lower than $\omega_0 < 0.85$ Hz indicating a viscoelastic solution behavior (Fig. 8a). When KCl is added (in 0.1 M KCl), it induces a gel-like behavior ($G' > G''$ in all the range of frequencies covered) with G' larger than the values obtained in water as shown in Fig. 8b. Fig. 8c represents the rheological behavior of agarose in water (at 5 g/L) compared with that of GC60 (10 g/L in 0.1 M KCl); the G' moduli are in the same range and that of GC60 is higher due to the higher polymer concentration. The gel-like behavior of GC60 is promoted by external salt meaning that it is charged and may be compared with the data obtained with carrageenans (Rochas & Rinaudo, 1984).

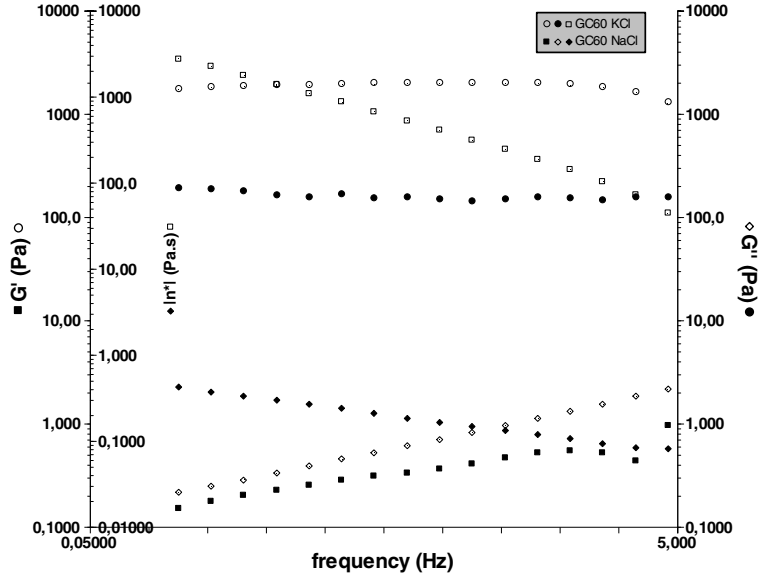

Fig. 11. Influence of the external salt on the rheological behavior of GC60 sample at 25 °C in 0.1 M NaCl (G' ■; G'' ◇; $|\eta*|$ ♦) compared with 0.1 M KCl (G' ○; G'' ●; $|\eta^*|$ □).

The rheological behavior giving the elastic G' modulus in 0.1 M KCl as a function of the frequency (in Hz) is shown in Fig. 9 for the different samples. From these data, it is clear that GC20 and GC40 look like each other; GC80 has the lower modulus even if the DSC enthalpy was higher. This seems to be related to the large yield in methylated units preventing chain aggregation and gelation. The substituents may hinder the aggregation of the double helices which seem to exist in these samples. For all samples, the presence of external salt favors the gel formation even if it is not efficient for GC80.

The stability of these gels was characterized from the dependence of G' with temperature (Fig. 10). The G' modulus at 1 Hz for GC20, GC40 and GC80 starts to decrease very early (around 30 °C) corresponding with the DSC thermogram; here also GC20 and GC40 are very similar. With GC60, it seems that a two-step mechanism is observed: G' decreases from 25 to 45 °C with no well-defined signal observed in DSC. Then a second step, with a sharp decrease of the G' modulus is obtained around 65 °C corresponding to the sharp peak in DSC. GC60 is clearly a gelling polymer with $G' > G''$ in all the range of frequency covered in presence of salt excess. A good agreement is obtained between the different techniques used.

Considering the gelation observed, the ionic selectivity known for κ-carrageenan was searched; no ionic selectivity on the gel-like modulus was observed on the fractions GC20, GC40, GC80 in the presence of salt (KCl or NaCl; data not shown). On the opposite, for GC60, the potassium counterions are needed for gelation ($G' > G''$) while sodium gives a viscoelastic solution ($G'' > G'$ for $\omega_0 < 0.85$ Hz) as shown in Fig. 11; such selectivity was previously observed with κ-carrageenans (Rochas & Rinaudo, 1984).

4. Conclusion

As a first conclusion, it is demonstrated that the protocol proposed for successive extractions of polymers from algae does not allow to get well-defined fractions of polysaccharides with different chemical structures and/or molecular weights.

The majority of the polysaccharides extracted from *G. corticata* have a similar structure of agarose type but with much 6-*O*-methyl-β-D-galactose units and sulfate ionic groups.

For the first time, the molecular weight distribution and the rheological properties of these polymers were experimented and discussed. These polymers have a loose gel-like behavior in the presence of salt excess (KCl). An original fraction GC60, among all the fractions tested, forms a stronger gel with an ionic selectivity (K^+ being preferred to Na^+) as it was observed for κ-carrageenans. It has also a lower yield in methyl substituents and no pyruvate groups than the other fractions. The behavior of this fraction is compared with that of agarose; it is demonstrated that the gel modulus G' of GC60 fraction are in the same range as that obtained with agarose.

The successive extraction in presence of ethanol/water mixture allows to evidence the presence of a small fraction in *G. corticata* (GC60) with a gelling character and responsible of the low gelling potential of the polysaccharides extracted from this algae.

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