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Caesalpinia ferrea var. *ferrea* seeds as a new source of partially substituted galactomannan

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ABSTRACT

The hydrocolloid extracted from *Caesalpinia ferrea* var. *ferrea* seeds purified by membrane filtration was denominated GMPF (14% yield) and contained 75% total carbohydrate and 9% protein, and the mannose to galactose (Man:Gal) ratio was around to 2.1:1.0. The periodate oxidation, methylation and 1D/2D NMR spectra results indicated galactomannan (GM) with a (1 → 4)-linked β-D-mannopyranose backbone, partially substituted at *O*-6 with single-unit α-D-galactopyranose side-chains. The splitting of three ^{13}C signals in the regions of the 4-*O*-Man*p* units revealed that the α-D-Gal*p* units were irregularly (54%) distributed at the main chain. By rheology, GPC-coupled to multi-detectors, static and dynamic light scattering it was observed that the GM in nitrite solution presented an intrinsic viscosity of 860 mL/g, a polydispersity of 1.55, and a structural parameter ρ (R_g/R_h) of 1.77, showing a random coil and flexible conformation, which was confirmed by the Mark–Houwink constant α (0.63). The results suggest that GMPF can be a new source of GM.

1. Introduction

Caesalpinia ferrea var. *ferrea*, ironwood, is a leguminous plant that grows in many regions of Brazil, with fruit formation occurring at different times of the year according to the region of cultivation. It is known in Brazil as pau-ferro, and the bark, seeds, roots and fruits are used in Brazilian folk medicine, where its pharmacological properties have been previously investigated (Carvalho et al., 1996; Nakamura et al., 2002).

In many legumes, the seeds are composed principally of lipids and carbohydrates; galactomannan is the main carbohydrate in the endosperm and is an energy source, participating in the germination process (Buckeridge, Santos, & Tiné, 2000). Dirk, van der Krol, Vreugdenhil, Hilhorst, and Bewley (1999) proposed that galactomannan degradation and starch synthesis may be related. Another function of this polymer is water retention by solvation, preventing complete drying of the seeds and protein denaturation, especially of the enzymes responsible for the germination process (Srivastava & Kapoor, 2005).

Seed galactomannan has a linear main chain of (1 → 4)-linked β-D-mannopyranosyl units, partially substituted at *O*-6 with α-D-galactopyranosyl units. The degree of substitution is dependent on the source of the galactomannan (Cunha, Castro, Rocha, de Paula, & Feitosa, 2008; Dea & Morrison, 1975; Sierakowski, Milas, Desbrières, & Rinaudo, 2000).

The main commercial sources of galactomannans are guar gum (*Cyamoposis tetragonolobus*), locust beans (*Ceratonia siliqua*) and tara gum (*Caesalpinia spinosa*), which differ in their mannose:galactose (Man:Gal) ratio and the distribution of galactose residues linked to the mannose main chain (Andrade, Azero, Luciano, & Gonçalves, 1999; Maier, Anderson, Karl, Magnuson, & Whistler, 1993).

The structural study of galactomannans is essential for industrial applications. The main property of these hydrocolloids is that they form very viscous solutions at relatively low concentrations. Other properties of galactomannans include their use as emulsifiers, thickeners and stabilising agents. All such properties are dependent on the chemical, physical and structural properties of the polymer, such as molar mass, anomeric configuration, conformation in solution, and branching position of the polysaccharide (Dakia, Wathelet, & Paquot, 2007; Garti, Madar, Aserin, & Sternheim, 1997).

It follows that a structural analysis of a galactomannan is essential to gain an understanding of its physical properties, particularly the rheological and gelling behaviour, and also synergistic interactions with other polysaccharides (Chaubey & Kapoor, 2001).

The physico–chemical parameters are important to determine and establish the macromolecular characteristics of the polysaccharide for applications, and linking size-exclusion chromatography to laser light scattering is an excellent option because

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it is fast and requires only small amounts of the sample. Light scattering measurements are the most important techniques for determining dilute polymer solutions (Li, Cui, & Wang, 2006). Static and dynamic light scattering are two different techniques for measuring the patterns of light scattered from a polymer in solution (Burchard, 1994, 2008; Kato, Katsuki, & Takahashi, 1984; Li, Wang, Cui, Huang & Kakuda, 2006; Pinder, Nash, Hemar, & Singh, 2003; Wang, Huang, Nakamura, Burchard, & Hallett, 2005; Zulfiqar, Lieberwirth, & Sarwar, 2008). Static light scattering (SLS) measures light intensity as a function of scattering angle and solute concentration. This allows for the determination of the molar mass, radius of gyration, and shape information of the solute. Dynamic light scattering (DLS) utilises the intensity and polarisation of light scattered from a solution to characterise the size, shape, and interaction of the particles in solution (Zulfiqar et al., 2008). Both SLS and DLS provide a basis for studying the solution properties of polysaccharides (Li, Cui, et al., 2006; Li, Wang, et al., 2006).

We report herein the composition, structural analyses, and physico-chemical properties of galactomannan extracted from *C. ferrea* var. *ferrea* seeds. The structure–conformation relationship of the polysaccharide will be discussed by comparing our results to those of other studies in the literature.

2. Materials and methods

2.1. Isolation and purification of the polysaccharide

Seeds of *C. ferrea* var. *ferrea* were provided by St. Helena Agropecuária Com. Emp. Ltda – Projeto Matas Nativa, Itatinga, São Paulo, Brazil. The seed flour (50 g) was boiled with 99% (v/v) ethyl ether for 4 h under reflux to remove ethyl ether-soluble substances (lipids and pigments, among others). The residue was air-dried at 25 °C and submitted to aqueous extraction five times followed by centrifugation for 20 min at 8243 × *g* (Sigma 4K 15 centrifuge) to remove the insoluble fraction. The combined supernatants were concentrated, and the polysaccharide was precipitated with two volumes of commercial ethanol in the presence of NaCl (0.1 M) and dried at 25 °C (Lucyszyn, Quoirin, Koehler, Reicher, & Sierakowski, 2006). To reduce seed coat residues and protein, the polysaccharide was resolubilised in water at 30 °C, filtered under pressure through cellulose acetate membranes with 0.8, 0.45 and 0.22-μm pore diameters (Millipore®) and precipitated with ethanol to give the GMPF sample.

2.2. General chemical analysis of GMPF

For the determination of the total carbohydrate content of GMPF, the colorimetric phenol–sulphuric acid method was employed (Dubois, Gilles, Rebers, & Smith, 1956), using glucose as the standard (Sigma–Aldrich®). Protein determination was performed with the Folin-Ciocalteau reagent (Merck®) (Hartree, 1972) using bovine serum albumin (BSA) (Sigma–Aldrich®) as the standard. The moisture and residual mass content was obtained by thermogravimetric analysis (TGA) in the range of 30–800 °C, at 10 °C/min, under O_2 atmosphere with a Mettler Toledo TGA/SDTA851 analyser, using 10 mg of sample in an alumina crucible. Prior to the analysis, the polysaccharide was kept for 48 h at 25 °C under an atmosphere with 40% moisture.

2.3. Monosaccharide and structural composition

The monosaccharide composition was obtained by the alditol acetate derivatives method (Wolfrom & Thompson, 1963a, 1963b), and linkage types in the GMPF sample were determined according to the per-*O*-methylated method using powdered NaOH in Me_2SO–MeI, as described by Ciucanu and Kerek (1984) with modifications. After the methylation process the derived GMPF was successively hydrolysed, reduced with $NaBD_4$ and acetylated according to the Wolfrom and Thompson (1963a, 1963b) method, producing a mixture of partial *O*-methylated alditol acetates, which were analysed by GC/GC–MS using a Varian gas chromatograph, model 3300, coupled to a Finnigan Ion-Trap mass spectrometer, model 810 R-12, equipped with a DB-225 capillary column (30 m × 0.25 mm i.d.), with He as the carrier gas. Analysis was carried out from 50 to 220 °C at 40 °C/min and maintained at 220 °C until the end of the analysis (18 min). For periodate oxidation, the polysaccharide (0.1 g) was dissolved in water (25 mL), and the solution was cooled to 0 °C. A cold solution of sodium metaperiodate (0.15 M, 30 mL) was added to the solution, and the volume was made up to 100 mL. The reaction was conducted at 4 °C to avoid overoxidation, and the amounts of periodate consumed and formic acid liberated were estimated at different time intervals. The periodate oxidation was completed in 144 h.

2.4. Nuclear magnetic resonance analysis of GMPF

Nuclear magnetic resonance (NMR) spectra of GMPF were obtained using a 400-MHz Bruker model DRX AVANCE spectrometer with a 5-mm inverse probe. ^{13}C NMR (100.6 MHz) and ^{1}H NMR (400.13 MHz) analyses were performed at 70 °C with the samples dissolved in D_2O (25 mg/mL). For the ^{1}H NMR, the OH groups were exchanged with D_2O followed by freeze-drying. Chemical shifts were referred, in ppm (δ), to the corresponding acetone (δ_C 30.20) or DOH (δ_H 4.70) signals for ^{13}C and ^{1}H signals, respectively. The ^{13}C NMR coupled pulse of 90° was performed with a delay time of 0.1 s and acquisition time of 0.6 s for decoupled spectra. Correlation spectroscopy, HMQC and COSY were performed with parameters described in the Bruker manual.

2.5. Rheological measurement

The Huggins equation was used to determine the intrinsic viscosity ($[\eta]$) of polysaccharide by extrapolation of the reduced viscosity (η_{red}) to the limit at zero concentration ($c \rightarrow 0$), where the linear coefficient is represented by $[\eta]$ (Huggins, 1942). The polysaccharide (0.2–1.0 mg/mL) was solubilised in aqueous 0.1 M $NaNO_2$ with 0.02% (w/w) NaN_3. The apparent viscosity value of the sample was obtained using a RheoStress1 rheometer (Haake GmbH, Germany) equipped with a DG43 spindle. A Haake DC30 bath and a thermostatic Universal Temperature Controller (UTC) were used to maintain a constant temperature of 25 °C.

2.6. Light scattering measurements

2.6.1. Gel permeation chromatography (GPC) coupled to RI–LALS–RALS–viscosimeter detectors

For GPC analysis, the experiments were carried out at 30 °C using a Viscotek-GPC multi-detector with refractive index (RI), light scattering (LS) and viscometer systems (Malvern Co., USA), with sequentially coupled TSK PWxl (Tosoh, Japan) column models 6000, 4000 and 2500 with size-exclusion limits of 8×10^6, 3×10^5 and 3×10^3 Da, respectively. The samples (1 mg/mL) were solubilised in aqueous 0.1 mol/L sodium nitrite containing 0.02% (w/w) sodium azide and filtered through a 0.45-μm pore-diameter membrane. The solutions (100 μL) were measured using GPC to calculate the polydispersity index (M_w/M_n) and the molar mass, M_w, relative to PEO 22k (polyethylene oxide), which was used as a standard to determination of instrument constants (calibration). The results were compiled in OmniSEC Software (Malvern Co., USA) using a refractive-index increment (dn/dc) of 0.146.

2.6.2. Static light scattering (SLS)

The SLS measurements were performed with a multi-angle laser light scattering apparatus from Brookhaven Instruments Co. (New York, USA) BI 9000 equipped with a He–Ne laser (632.8 nm) as the light source. Instrument alignment was checked to be satisfactory, with an error less than 0.3%, using well-filtered toluene (0.22 μm cellulose acetate filter). Toluene was also used for the calibration of the apparatus to ensure that there was no angular dependence of the scattered light from toluene, and a value of 1.40×10^{-5} cm^{-1} was used as the Rayleigh ratio.

The sample in sodium nitrite 0.1 M solution containing 0.02% (w/w) sodium azide was filtered through 0.22 μm cellulose acetate directly into a cylindrical quartz cell (25 mm in diameter), which was immersed in a decalin bath at 25 °C. The measurements were carried out over an angular range of 30–150° at appropriate intervals, and the data were analysed by the Zimm plot method. Each Zimm plot was made with seven spaced concentrations (0.19, 0.36, 0.5, 0.62, 0.83 and 1.0 mg/mL). The molar mass, M_w, radius of gyration, R_g, and second virial coefficient, A_2, were calculated by the usual method.

2.6.3. Dynamic light scattering (DLS)

The DLS study was performed on the same apparatus as the SLS measurements. The measurements of the scattering angle were fixed at 90°, and a temperature of 25 °C was maintained using a decalin bath. The preparation of the sample was the same as the static light scattering, and the polysaccharide concentration was fixed to 1 mg/mL in 0.1 M sodium nitrite solution containing 0.02% (w/w) sodium azide solution.

Because of the polydisperse nature of the polysaccharide, the auto-correlation functions were analysed by a number of approaches using software provided by Brookhaven Instruments (New York, USA). These include the commonly used CONTIN method as well as the non-negatively constrained least squares (NNLS) and the double exponential (DE) methods (Wang et al., 2005). Hydrodynamic diameter, D_h, distributions (histograms) were obtained with a CONTIN routine, and the value referred to as R_h was the mean value of the radii distribution. The dn/dc value used was determined by GPC analysis (Viscotek instrument), which was equal to 0.146 mL/g in nitrite solution. The results were evaluated by dynamic light scattering software (Brookhaven Instruments).

3. Results and discussion

3.1. Chemical characterisation of GMPF

The aqueous extract of the seed, submitted to a process of membrane filtration using filters with different pore diameters and ethanol precipitation, gave a more purified polysaccharide (GMPF) with a yield of 14% (w/w), a residual mass of 2.7% (w/w), a moisture content of 14% (w/w), a protein content of 8.3% (w/w) and a total carbohydrate content of 75% (w/w). These values are similar to those of the galactomannan obtained from other seeds such as *Leucaena leucocephela* and *Prosopis juliflora*, as reported by Ono et al. (2003) and Vieira, Mendes, Gallão, and Brito (2007), respectively. The moisture and residual mass of GMPF obtained by TGA curve (Fig. 1) were in agreement with values reported for purified commercial guar (Cunha, de Paula, & Feitosa, 2007), locust and tara gum (Sittikijyothin, Torres, & Gonçalves, 2005).

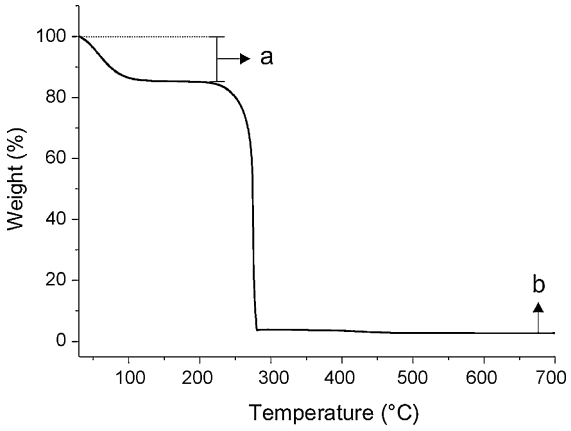

Fig. 1. TGA curve from *C. ferrea* galactomannan (GMPF) for moisture (region a) and residual mass (region b) measurements under O_2 atmosphere at 10 °C/min.

3.2. Monosaccharide and structural composition

The gas chromatography analysis of GMPF as alditol acetates derivatives was shown to give rise to D-mannose (64.2%) and D-galactose (30.2%) in a Man:Gal ratio of 2.1:1.0. Methylation-GC–MS analysis of GPMF showed the high formation of 2,3,4,6-tetra-*O*-methylgalactopyranose (30 mol%), 2,3,6-trio-*O*-methylmannopyranose (39 mol%) and 2,3-di-*O*-methylmannopyranose (30 mol%) and therefore a Man:Gal ratio of 2.3:1.0, which was very close to the results of the GC analysis. The results indicate that the backbone is composed of (1→4)-D-mannopyranosyl residues and carries a single (1→6) linked D-galactopyranosyl residue, as deduced by the presence of the 2,3-di-*O*-methylmannopyranose derivatives. In periodate oxidation the polysaccharide consumed 1.37 mol of periodate and liberated 0.31 mol of formic acid per hexosyl unit. The periodate oxidation results are in good agreement with the theoretical values (1.33 mol of periodate and 0.27 mol of formic acid) of the proposed galactomannan structure based on methylation data.

The Man:Gal ratio by GC and GC–MS analysis were confirmed by integration of the anomeric region of ^{1}H NMR (Fig. 2a) and ^{13}C NMR (data not shown) spectra at low field, arising from non-substituted (δ 5.21/100.7) and *O*-substituted β-mannopyranosyl (δ 5.26/100.5) units and also α-galactopyranosyl units (δ 5.50/99.4). This Man:Gal ratio is consistent with a highly branched structure similar to those of commercial guar gum, according to Srivastava and Kapoor (2005) and Cunha et al. (2008). Assignments of other ^{1}H NMR chemical shifts were based on literature data and HMQC correlation analysis (Fig. 2c), as shown in Table 1. The COSY correlation (data not shown) confirmed, by the coupling of vicinal protons, the assignments of the HMQC analysis. The distribution of Gal*p* units along the main chain, based on the study of Dea and Morrison (1975), was determined using the splitting of *O*-substituted C-4 signals of β-Man*p* in the ^{13}C NMR spectrum of GMPF. The Man C-4 resonance region (Fig. 2b) was split into three signals, the first, denoted I, at δ 77.5, corresponding to two continuous *O*-substituted β-D-mannopyranose units; the second, denoted II, at δ 77.3, due to dyads in which only one of the two β-D-mannopyranose units is *O*-substituted and the third, denoted III, at δ 77.0, was assigned to non-substituted β-mannopyranosyl units that are adjacent to other units of the same monosaccharide. This showed a dependence on the nearest neighbour probabilities of the α-galactopyranosyl groups distribution along the β-mannan chain. By comparing the signal intensities it can be noted that peak II on the spectrum is much more intense (54%) than peak III (24%) and peak I (22%), and therefore galactosyl units (based on a Man:Gal ratio about 2.1:1.0) for GMPF were not regularly distributed along the main chain. Thus, the galactose units had randomised distribution along the mannan chain (Fig. 2d).

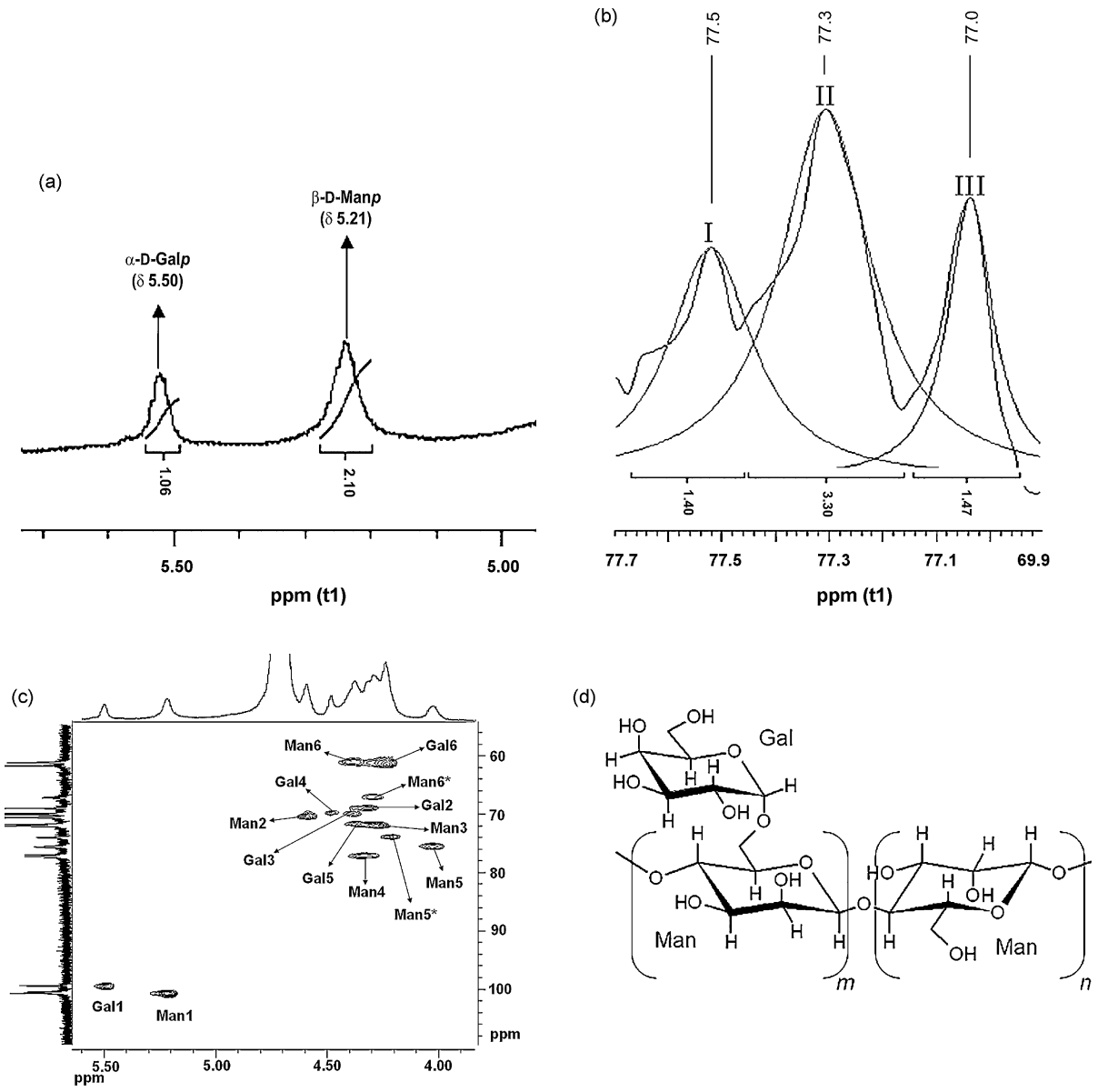

Fig. 2. Anomeric region in ^{1}H NMR (a), split of Man C-4 resonance (b), HMQC correlation (c) and chemical parcial structure (d) for galactomannan (GMPF) from *C. ferrea* var. *ferrea* seeds.

3.3. Physico-chemical properties of GMPF

3.3.1. Rheological measurement

The intrinsic viscosity [η] value of *C. ferrea* galactomannan, which measures the hydrodynamic volume occupied by macromolecules in a dilute solution, was determined graphically (η_{red} versus *c*, data not shown) as 860 mL/g (Table 2). This value is lower than the value determined in seed galactomannans of *C. siliqua* (1150 mL/g) (Dakia et al., 2007) but was similar to that from *Cassia grandis* (848 mL/g) (Joshi & Kapoor, 2003). The differences in the values can be related to the different extraction processes used and to the values of the molar mass of the polysaccharides. The Huggins constant (Huggins, 1942), k', was calculated to be 0.49 for GMPF, where the solvent used can be considered favourable to polymer–solvent interactions ($0.8 < k' < 0.3$) (Azero & Andrade, 1999).

Table 1
^{1}H NMR and ^{13}C NMR shifts for galactomannan (GMPF) from *C. ferrea* var. *ferrea* seeds.

Unities	Chemical shifts (δ, ppm)[a]					
	C-l/H-l	C-2/H-2	C-3/H-3	C-4/H-4	C-5/H-5	C-6/H-6
α-D-Galactopyranosyl	99.4/5.51	69.1/4.34	70.1/4.40	69.9/4.47	71.8/4.38	61.7/4.24
4-Linked-β-D-manopyranosyl	100.7/5.21	70.5/4.60	72.1/4.29	77.0/4.37	75.6/4.05	61.2/4.29
4,6-Linked-β-D-manopyranosyl	100.5/5.26	70.5/4.60	72.1/4.29	77.5/4.37 77.3/4.37	74.1/4.22	67.2/4.39

[a] In D_2O, at 70 °C, based on HMQC and COSY correlations.

Table 2
The solution properties of galactomannan measured by GPC analysis.

Sample	M_w[a] (g/mol)	M_n[a] (g/mol)	M_w/M_n[a]	$[\eta]$[a] (mL/g)	$[\eta]$[b] (mL/g)	α[b]	k'[a]
GMPF	604,000 ± 0.912	389,500 ± 0.590	1.55	1051	860	0.63	0.49

Symbols used: molar mass ± S.D. (M_w), numeric ± S.D. (M_n), the intrinsic viscosity (η) and the Mark–Houwink constant (α). Huggins constant (k'); standard deviation (S.D.).
[a] By gel permeation chromatography (GPC) coupled to RI–LALS–RALS–viscosimeter detectors.
[b] Measured using the Rheometer, Haake RS 1, sensor DG 43, at 25 °C.

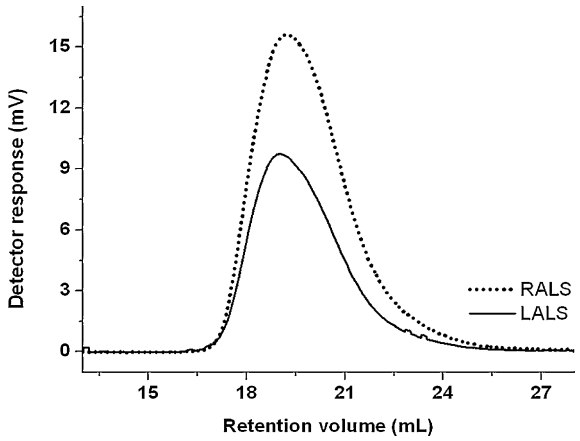

Fig. 3. GPC analyses of galactomannan (1 mg/mL) from *C. ferrea* seeds.

3.3.2. GPC analysis

The molar mass (M_w) and numeric mass (M_n), conformation parameters and intrinsic viscosity (IV) of GMPF were elucidated by triple detectors (RI, LALS–RALS and viscometer) coupled to GPC system and used OmniSEC Software. These analyses provided pertinent information on the structure and polymer conformation with only small amounts of the sample (Beer, Wood, & Weisz, 1999). The results of the GPC analysis for GMPF are given in Table 2. After solubilisation and ultrafiltration the galactomannan, eluted between 17 and 25 mL, showed a homogeneous profile according to the single RALS and LALS (Fig. 3) but a polydisperse profile as calculated by the M_w/M_n index (1.55). The mean molar mass value of galactomannan was $6.04 \times 10^5 \pm 0.912$ g/mol. Furthermore, the intrinsic viscosity values obtained from GPC measurements (1051 mL/g) are in good agreement with the result obtained from rheology analysis (Table 2). The results are close to those of other leguminous seed galactomannans, as reported for *Cyamopsis tetragonolobus* (Lucyszyn et al., 2006) and *C. siliqua* (Dakia et al., 2007).

The constant α in the Mark–Houwink–Sakurada equation is related to the molar mass and intrinsic viscosity as well as to conformational information on the polymers in solution (Beer et al., 1999). The α value determined for GMPF was 0.63, showing that this polysaccharide in solution has a flexible random coil conformation, which is in good agreement with data previously published for other galactomannans presented in literature such as guar gum (Beer et al., 1999; Cheng, Brown, & Prud'home, 2002; Robinson, Ross-Murphy, & Morris, 1982), tara gum and locust beans (Picout, Ross-Murphy, Jumel, & Harding, 2002) with values of $0.5 < \alpha < 0.8$.

3.3.3. Static light scattering

Static light scattering measurements were carried out on the aggregate-free solutions after consecutives filtrations. It is well known that polysaccharides tend to form aggregates in aqueous systems (Li, Cui, et al., 2006; Li, Wang, et al., 2006). The existence of these large particles causes problems for the determination of the molecular weight and size of the polysaccharide by static light scattering. In the current paper, using consecutive filtrations, we successfully removed the large particles from the dilute solutions of galactomannan. Fig. 4 illustrates the Zimm plot obtained from *C. ferrea* galactomannan. The molar mass, radius of gyration and second virial coefficient calculated from the Zimm plots for the sample are summarised in Table 3. The Zimm plots show that the angular dependence of the scattered light did not change systematically with decreasing concentration of the polymer, indicating no detectable conformation change of the molecules upon dilution. Furthermore, the statistical analysis by Student's t-test with $p = 0.05$ demonstrated that the value of M_w obtained from static light scattering measurement ($5.28 \times 10^5 \pm 0.440$ g/mol) showed good agreement with the results obtained from GPC ($t = 1.201 < t_{0.05} = 2.776$).

In the extremely dilute solution, the second virial coefficient (A_2) is a quantitative indicator of the affinity between the polymer and the solvent, i.e., the thermodynamic quality of the solvent for the given polymer (Li, Cui, et al., 2006; Li, Wang, et al., 2006; Wohlfarth, 2004). The value of A_2 obtained suggests that the 0.1 M sodium nitrite solution containing 0.02% (w/w) sodium azide was a good solvent for galactomannan from *C. ferrea* seeds. The radius of gyration (R_g), a geometrical parameter of a polymer chain, was calculated by a sum over all distances of the scattering elements from the centre of mass or the sum over all intramolecular distances in

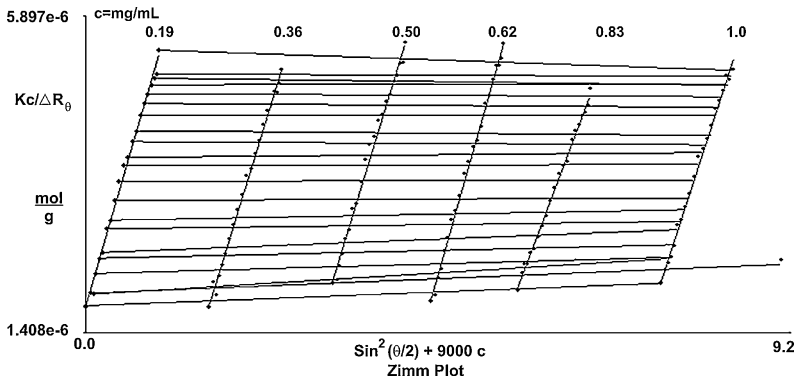

Fig. 4. Static light scattering data presented in a Zimm plot obtained from *C. ferrea* galactomannan (1 mg/ml) in 0.1 M sodium nitrite solution containing 0.02% (w/w) sodium azide.

Table 3
Experimental data of static and dynamic light scattering from *C. ferrea* galactomannan.

Sample	M_w[a] (g/mol)	A_2 ($\times 10^{-4}$ [a] cm^3 mol/g^2)	R_g[a] (nm)	R_h[b] (nm)	R_g/R_h	(Γ) (s^{-1})[b]		
						NNLS	CONTIN	DE
GMPF	528,000 ± 0.440	2.0	94.9	53.5	1.77	1979	2758	1667

Symbols used: molar mass ± S.D. (M_w), second virial coefficient (A_2), radius of gyration (R_g), hydrodynamic radius (R_h), mean decay rate (Γ); standard deviation (S.D.).
[a] Measured by static light scattering.
[b] Measured by dynamic light scattering.

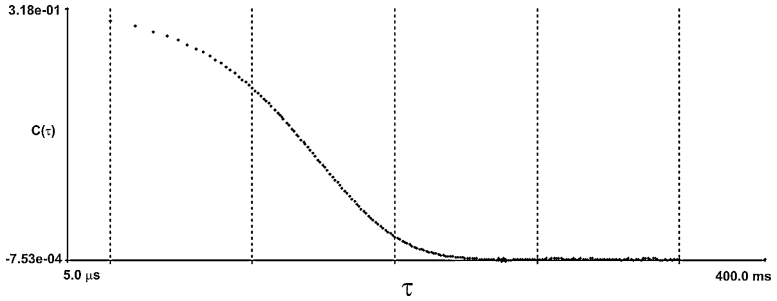

Fig. 5. Typical auto-correlation function obtained from dynamic light scattering measurements of *C. ferrea* seeds galactomannan (1 mg/mL) in 0.1 M sodium nitrite solution containing 0.02% (w/w) sodium azide. Scattering angle $\theta = 90^\circ$.

the macromolecule (Burchard, 1994, 2008) and was determined to be 94.9 nm. This value is similar to that obtained for other galactomannans such as guar gum (Picout, Ross-Murphy, Errington, & Harding, 2001) and locust bean and carob gum (Picout et al., 2002).

3.3.4. Dynamic light scattering

The aggregate-free solution of galactomannan was characterised by dynamic light scattering (DLS) measurements. A typical auto-correlation function obtained from DLS of dilute solutions of native *C. ferrea* polysaccharide is shown in Fig. 5. The relaxation processes for the sample took place over a wide range of times, indicating a wide distribution of decay rates (Γ). The auto-correlation function of the polysaccharide was analysed by several approaches including NNLS, CONTIN and double exponential (DE) methods, and the results are summarised in Table 2. All these methods fit the auto-correlation functions reasonably well, yielding small root-mean-square errors (data not shown). The mean decay rates obtained by NNLS and DE methods were in the same range. However, these results were significantly different from those obtained from CONTIN analysis. The CONTIN method seemed to give a better resolution of the distribution of the decay rate compared to the NNLS method.

By using the CONTIN method, the hydrodynamic radius of galactomannan at a specific angle of 90° was calculated as 53.5 nm and is shown in Table 3.

Static and hydrodynamic dimensions vary characteristically with the structure of the macromolecules, and a combination of the two may provide qualitative information on the architecture of the macromolecules (Burchard, 1994, 2003; Wang et al., 2005). The structure-sensitive parameter ρ is defined as the ratio of the radius of gyration R_g obtained from static light scattering to the hydrodynamic radius R_h from dynamic light scattering (Burchard, Schmidt, & Stockmayert, 1980; Burchard, 1994, 2003, 2008). Generally, the value of ρ decreases with increasing branching density, but an increase in polydispersity counteracts the effect of branching. A significantly higher value of $\rho = 1.77$ was obtained for *C. ferrea* galactomannan, which is compatible with a random coil conformation in good solvent (Burchard, 1994, 2003), and the result is in agreement with the GPC analysis (Section 3.3.2).

4. Conclusions

The chemical and physico-chemical analysis of polysaccharide extracted from the leguminous seeds, *C. ferrea* var. *ferrea*, indicated that it is rich in galactomannan. The partial structural elucidation showed that biopolymer is composed of D-mannose and D-galactose ratio around 2.1:1.0, with a main chain of (1 → 4) linked β-D-mannopyranosyl units, where some are attached at *O*-6 with α-D-galactopyranosyl single-unit as side-chains. The composition and the structure were similar to partially substituted galactomannans, whose galactose units are distributed in randomised form. The homogeneity, low degree of contaminants such as proteins, high intrinsic viscosity and molecular mass revealed a polysaccharide with potential for industrial uses, such as thickening and could be promising source to substitute the commercial polysaccharide from *C. tetragonolobus* (guar gum).

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