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Cassia spectabilis DC seed galactomannan: Structural, crystallographical and rheological studies

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Abstract

The seeds of *Cassia spectabilis* DC (family: Leguminoseae), an Indian fast growing spreading tree, contain about 40% of endosperm and possess the characteristics of becoming a potential source of commercial gum. The purified galactomannan shows Mw 1.1×10^6, intrinsic viscosity $[\eta]$ 615 mL/g with $k' = 1.706 \times 10^{-1}$, and a mannose to galactose ratio of 2.65. The hydrolysis of the fully methylated polysaccharide reveals clearly the expected structure of legume galactomannans. The orthorhombic lattice constants of the hydrated gums are as follows: $a = 9.12$ Å, $b = 25.63$ Å and $c = 10.28$ Å. The results of X-ray fiber studies show that the b dimension of the unit cell is very sensitive to relative humidity (RH), galactose substitution and orientation of the films. The probable space group symmetry of the unit cell is $P2_12_12$. Rheological studies of the galactomannan have shown that the transition from semi-dilute to dilute regime occurs at a critical concentration $C_c^* = 2.75$. The slope of the log–log plot of specific viscosity versus C at zero shear rate is 5.87 in the more concentrated regime. The viscoelastic and critical shear rate behavior indicate the characteristics of a coil polymer. The large dependence of the viscosity on the coil overlap parameter is probably due to polymer–polymer interactions and peculiarity of the galactose distribution along the chain. Above 20 g/L concentration, the rheological behavior of the gum is like the one of a weak-gel.

Keywords: *Cassia spectabilis*; Galactomannan; Structure; Crystallography; Rheology

1. Introduction

Seed galactomannans [1,2], commonly known as seed gums, have widespread industrial applications [3] in food, paper, textile, petroleum, pharmaceuticals, cosmetics, etc. They are mostly found in the endosperm of leguminous seeds as cell wall storage component and energy reserve. Galactomannans have the fundamental structure consisting of a main chain of β-(1 → 4)-D-mannopyranose units substituted by

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single α-D-galactopyranose units at O-6, although there are few deviations from this basic structure. They differ from each other in mannose:galactose ratio and fine structure regarding distribution of single galactose branches on the main chain, thereby causing variations in solubility, rheology and other properties.

Cassia spectabilis DC, Local name: Archibald's Cassia and Calceolaria Cassia, is a fast growing spreading tree, up to 20 m in height, native from tropical America and introduced in some parts of India [4]. The plant is reported to be a source of senna and may prove to be a cheaper substitute for the true senna. According to Hussain and Kapoor [5], it is enumerated as *Senna spectabilis* (DC) Irwin and Barneby sp. *C. spectabilis* DC. The seeds are dicotyledonous, brown, more or less quadrate and medium sized (wt. of 100 seeds; 2.84 g) having about 40% endosperm. In view of this high content of endosperm gum, and to test its characteristics of becoming a potential source of commercial gum, *C. spectabilis* seed galactomannan has been studied in terms of structural, crystallographical and rheological aspects.

2. Experimental

The crude gum, isolated through a dry/wet milling process, was purified by dispersing the gum in water and precipitation with EtOH. The resulting polysaccharide was complexed with $Ba(OH)_2$ followed by decomplexing with an excess of 2 N AcOH, regeneration of the polymer with EtOH and continuous stirring with aq EtOH followed by centrifugation. It was successively washed with 70, 80, 90 and 95% EtOH. The sample was finally purified by dialysis and filtration through various Millipore membranes.

The constituent sugar analysis was carried out after total hydrolysis with 2 M H_2SO_4 at 100 °C for 18 h by HPLC for neutral sugars and by GLC for the corresponding alditol acetates using a Supelco SP 2380 (30 m × 0.53 mm) column with a temperature programme from 195 °C (4 min) to 225 °C at a rate of 2.5 °C/min. GC–MS was performed on a Nermag R-1010C Spectrometer equipped with a Delsi Chromatograph model DI-700 and a Digital Equipment PDP-1173 Computer using a SP 2380 (25 m × 0.32 mm) column with a temperature programme of 180–220 °C at a rate of 3 °C/min.

Average molecular weights and polydispersity of the galactomannans were determined on a multi-angle laser light scattering apparatus (DAWN-DSP-F from WYATT Technology) on line with a Waters 150-C ALC/GPC at 25 °C, using $\lambda_\circ = 632.8$ nm for 1 g/L initial solution after filtration through 3.0, 1.0, 0.5, and 0.2 μm Millipore filters. The system contained two Shodex columns OH-PAC 804 and 805 in series. The eluent used was 0.1 mol/dm^3 NH_4NO_5 with 0.5 g/L NaN_3 as preservative. The dn/dc was determined in the same solvent using a Brice-Phoenix Differential Refractometer.

For ^{13}C NMR analysis, spectra were obtained on either AM 300 or AM 400 Bruker Spectrometers, both equipped with a process controller. A galactomannan sample (20 mg/mL) was dissolved in D_2O at 70–75 °C with continuous stirring for 5 h, followed by sonication for 15 min, and filtration. Spectra were recorded at 80 °C under conditions of inverse gated decoupling (proton decoupled spectrum without NOE). Peak integrals were performed using the Bruker software and assignments were made with reference to results already published [6]. For ^1H NMR analysis, the galactomannan was firstly exchanged in D_2O by repeated evaporations of 1 g/L solution and finally dissolved in high quality D_2O (99.96% D). The spectra were obtained at 80 °C using a relaxation delay of 5 s and a pulse width of 45° to reach the conditions of quantitative analysis.

For methylation analysis, the gum was suspended in freshly distilled Me_2SO, and extensively stirred for 22 h at room temperature, followed by low intensity sonication for 20 min and stirring for another 32 h. The partially dispersed product was methylated according to the procedure of Hakomori [7]. The resulting product was again subjected to Hakomori methylation followed by two successive treatments of Purdie methylation [8]. The completely methylated polysaccharide, having no –OH absorption in IR at 3600–3400 cm^{-1}, was boiled under reflux with HCOOH. The formed partially methylated monosaccharides were converted to corresponding alditol acetate derivatives, and analyzed by GLC and GC–MS using a Supelco SP 2380 column (30 m × 0.53 mm) with a temperature programme from 195 °C (4 min) to 225 °C at a rate of 2.5 °C/min.

About 1% galactomannan solution was used for the preparation of films by slow evaporation for 30–32 h. On drying, the films show appreciable orientation and were cut into strips of about 1.8 mm × 8 mm. The resulting strips were placed between clamps and hung with weight (15–20 g) in a closely tight jar conditioned at 95% relative humidity for 5 days until elongation of about 350–400% was

obtained. The crystallinity of the resulting stretched film was enhanced by annealing in a sealed high pressure bomb at 100 °C over aqueous $CuSO_4$ sulfate for 4 h. The resulting film was mounted onto a 0.20 mm diameter collimator and exposed in vacuo to CuK_α ($\lambda = 1.5418$ Å) X-ray in a Warhus camera using a Philips PW 4720 generator for 20–22 h.

Intrinsic viscosity $[\eta]$ of the 1 g/L solution in pure water was determined with a Fica Capillary Viscometer of capillary diameter 0.46 mm at 25 °C. No shear effect was observed. The rheological measurements were determined on two viscometers. A Contraves Low-Shear Viscometer was used for shear rate, $\dot{\gamma}$, from 10^{-2} to 10^{1} s^{-1}, and a Carri-Med Controlled stress 50 Rheometer equipped with a Rheo 1000 C system and a 5.0 software for 10^{-3} $\langle \dot{\gamma} \rangle$ 10^{3} s^{-1}. The different cones used for Carri-Med had a diameter of 2, 4, 6 cm (cone angle 4°) and also 6 cm diameter with a cone angle of 1°. The shear stress and shear rate can be varied depending upon the geometry used and experimental conditions. All experiments were carried out at 25 °C.

3. Results and discussion

Isolation and purification.—The endosperm of the seeds contains the water-soluble galactomannan, while the rest of the seed meal mainly contains pentoses. The analysis of seed components is presented in Table 1. The endosperm was separated from the seeds by dry and wet milling processes using various mixers, sieves and grinders. The yield of the crude gum was 31–34% in the dry process compared to 22–25% in the wet process. This is due to the fact that during dry milling of seeds, small portions of seed coat and hull contaminate the endosperm and are thereby responsible for the pentose (xylose mainly) impurities in the gum. On the contrary, the use of organic solvent during the wet process affords purer gum than the dry process. The comparative analysis of both gums is given in Table 2. These parameters are almost at par with the standard specifications required for commercial gums [3] in various industrial applications. The rest of the seed meal (approx. 60–64%) contains appreciable amounts of proteins, pentose polysaccharides and crude fiber and can be utilized for cattle and poultry feed.

Galactomannan characterization.—The purified galactomannan was a white amorphous powder, found to be homogeneous. The intrinsic viscosity of the gum, determined in the Newtonian regime, is 615.0 mL/g. The average molecular weight (Mw) is about 1.1×10^6, having a polydispersity $Mw/Mn = 1.21$ with a $dn/dc = 0.150$ mL/g. The results of capillary viscometry and GPC studies are presented in Table 3.

The ratio of constituent monosaccharides (Table 4) were determined by HPLC and GLC. Monosaccharide characterization was performed by GC–MS and their *m/z* values have been found identical with literature [11,12]. The ratio of D-galactose and D-mannose, as determined by ^1H and ^{13}C NMR of the relative areas of the anomeric galactose and mannose signals (substituted and non-substituted) is in agreement with the chemical analysis results (Table 4). During ^{13}C NMR studies, a comparative low proportion of mannose/galactose is observed which may be due to an incomplete dissolution of some portions of the galactomannan in deuterium oxide and the presence of aggregation at the polymer concentration used.

Structural studies.—For the methylation, galactomannans are firstly converted to acetate derivatives [9,10] and then subjected to Purdie [8] and Hakomori [7] methylation procedures. In this case, the galactomannan which is insoluble in Me_2SO_4 due to its high mannose content was suspended directly in Me_2SO, slightly depolymerized by mild sonication, extensively stirred and subjected to methylation. GLC analysis showed the presence of 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,3,6-tri-*O*-methyl-D-mannose and 2,3-di-*O*-methyl-D-mannose in molar proportions of 1.00:1.58:1.02. These partially methylated sugars were confirmed by GC–MS and their *m/z* values

Table 1
Composition of the components of *C. spectabilis* seeds

Seed part	Protein %	Moisture %	Ether extract %	Ash %	Crude fiber %	Type of carbohydrates %
Seed coat (26–30%)	10.24	5.5	2.25	3.52	28.9	Pentose
Endosperm (39–44%)	8.41	7.9	0.65	0.68	1.48	Galactomannan
Germ (30–34%)	32.38	6.9	3.42	4.12	15.2	Pentose (xylose mainly)

Table 2
Parameters of *C. spectabilis* seed gum isolated by dry and wet processes

	Dry process gum	Wet process gum
Yield on seed basis (%)	31–34	22–25
Protein (%)	6.8	4.6
Moisture (%)	7.3	7.9
Pentosan (%)	5.6	0.3
Ash loss on drying (%)	1.2	0.4
Loss on drying (%)	< 10.0	< 10.0
Starch	nil	nil
Constituent carbohydrate	Galactose Mannose Pentoses (traces)	Galactose Mannose

Table 3
Preliminary analysis of *C. spectabilis* seed galactomannan

Average molecular weight	Polydispersity	dn/dc (mL/g)	RMS Radius moment (nm)	Intrinsic viscosity
Mn 9.2×10^5 Mw 1.1×10^6 Mz 2.26×10^6	Mw/Mn = 1.21	0.150	Number average 55.9 Weight average 58.5 *Z*-average 60.8	615.0 mL/g $k' = 1.7 \times 10^{-1}$

have been found identical with those in the literature [11,12]. The methylation analysis data indicate that the Man:Gal ratio of the polymer is 2.60, which is in good agreement with chemical analysis results.

The anomeric configuration of the galactose and mannose residues was determined by the CrO_3 oxidation method [13]. It indicates that the D-mannopyranosyl units are β-linked (oxidized more rapidly) and the D-galactopyranosyl residues are α-linked. This was also confirmed by NMR analysis.

As the galactomannan at 20 mg/mL concentration yielded a viscous solution, successful spectroscopy was only possible after diminishing the viscosity by partial acid hydrolysis or sonication. In this case, sonication was performed for better resolved NMR spectra. During ^{13}C NMR studies of the galactomannan, all the carbon lines were well-resolved and the chemical shifts were found in accordance with those in the literature [6,14]; the chemical shifts are reported in Table 5. The Man C-4 resonance splits into

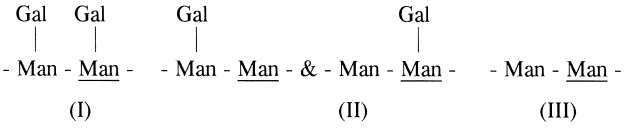
Table 4
Monosaccharide composition in *C. spectabilis* seed galactomannan

Monosaccharides	Methods				
	GLC	HPLC	^{13}C NMR	^{1}H NMR	Methylation
D-Galactose	1.00	1.00	1.00	1.00	1.00
D-Mannose	2.65	2.51	2.41	2.46	2.60

Table 5
Assignment of peaks in the ^{13}C NMR spectrum of *C. spectabilis* seed galactomannan

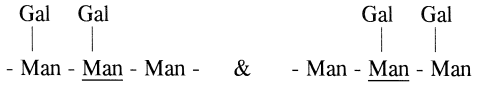
Type of unit	Chemical shifts (δ, ppm)					
	C-1	C-2	C-3	C-4	C-5	C-6
α-D-Galactopyranosyl	99.65	69.38	70.42	72.23	72.02	62.05
β-D-Mannopyranosyl	100.88	70.89	72.37	77.23	75.89	61.51
Unbranched at O-6				77.46		
β-D-Mannopyranosyl	100.76	70.89	72.37	77.46	74.26	67.55
Branched at O-6				77.69		67.41
						67.25

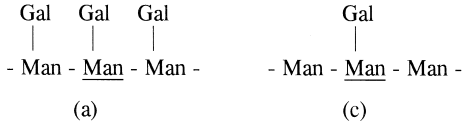
three signals, I, II and III, respectively, in evident dependence upon the nearest neighbor probabilities of D-galactosyl groups along the D-mannan chains, as previously reported [15].



In the spectrum, peaks II (40%) and III (50%) are much more intense than peak I (10%) suggesting larger proportions of the regions having single substituted galactose in two contiguous mannose units along with non-substituted regions. For comparison, a random distribution of galactose would give the following abundances: I (14%), II (48%) and III (38%).

Manzi et al. [14] have carried out ^{13}C NMR studies of depolymerized *Gleditsia tricanthos* galactomannan having different Man:Gal ratios and have considered the resonances of C-6 of substituted mannose. Accordingly, the peak at the lowest field originated from the C-6 resonance of the intermediate unit from groups of three contiguous substituted mannose units as in triad (a). The signal at higher field is due to blocks of three contiguous mannose units where only the intermediate sugar is substituted (triad c). The last peak represents the superimposition of signals from triads (b) where only two contiguous units are substituted. In the case of *C. spectabilis* seed gum, the C-6 (substituted Man) signals are well resolved. Triad (b) (about 75%) dominates the two other triads (a and c) and gives some insight into the kind of arrangements of the galactose units.





This result could be compared to the result given by a random distribution of galactose, as one should get (a) 14%, (b) 48%, and (c) 38%. Of course, more detailed characterization and interpretation are needed at this point using for example enzyme digestion [16], the model for which has been validated by biosynthetic studies [17].

Crystallographic studies.—The X-ray diffraction pattern in vacuo of the well-oriented and crystalline film of *C. spectabilis* galactomannan, extended to 350% under 95% RH, is shown in Fig. 1. This typical diffraction pattern has much resemblance with *C. siamea* [18] (Man/Gal = 2.55) and Tara gum [19] (Man/Gal = 3.0) but differs from the patterns obtained for highly substituted galactomannans like *Medicago sativa* and *Trifolium alexandrium* [18]. Interplanar spacings, *hkl* indices, of the diffraction diagram are reported in Table 6. It suggests an orthorhombic unit cell having $a = 9.12$, $b = 25.63$, and $c = 10.28$. These lattice constants are very close to those of Tara gum with $a = 8.91$, $b = 24.17$, and $c = 10.46$, and of *C. siamea* gum having $a = 9.00$, $b = 24.62$, and $c = 10.30$. The lattice constants of *C. spectabilis* and other seed galactomannans are summarized in Table 7.

The orthorhombic unit cell dimensions of *C. spectabilis* seed galactomannan show a relative constancy of *a* and *c* dimensions, however, the *b* dimension is sensitive to the degree of galactose substitution and hydration conditions. Chien and Winter [19] have found that it decreases with decreasing galactose content. According to the results of Kapoor et al. [18], it ranges from 30.66–30.80 Å in highly substituted gums but decreases to 24.81–24.75 Å for galactomannans having about 30% galactose. Song et al. [20] have found variations of more than 15% for samples maintained at constant RH, compared to those recorded under vacuum. It is to point out here that the X-ray diffraction of the sample was carried out under vacuum.

The diffraction pattern similarities which have been found among mannans and galactomannans indicate a pronounced similarity in both molecular conformation and crystalline packing as already reported [20]. The near constancy of the fiber repeat (*c*) and of the lateral dimensions (*a*) suggest a packing model having mannan–mannan interaction and permitting also galactose substitution on mannose residues in such a way that there is little alterations of the main chain organization. The third dimension (*b*) relates to the distance between mannan sheets, although not constant in magnitude and varying with the degree of galactose substitution. The rigid sheets of mannan chains are stabilized by hydrogen bonding and the single galactose units are placed between these sheets. Any gaps created by the absence of galactose substitution are expected to be filled with water at high humidities [20].

The diffraction diagram of *C. spectabilis* seed

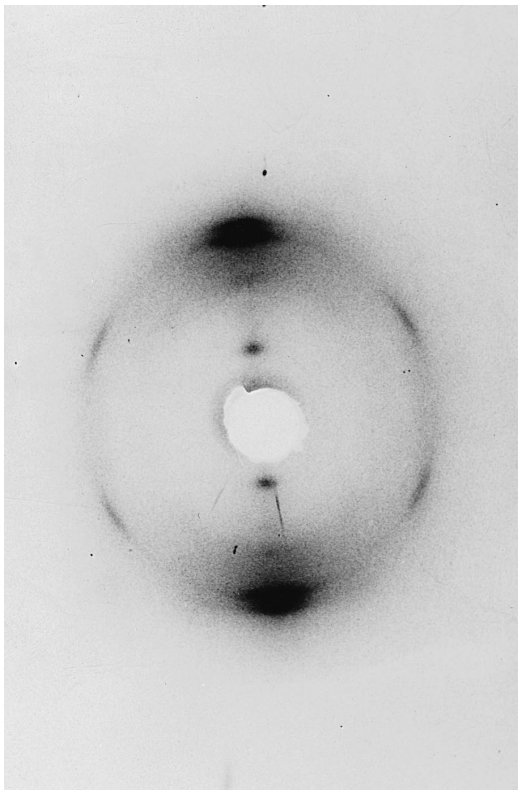


Fig. 1. Typical X-ray diffraction pattern under vacuum at room temperature of *C. spectabilis* seed galactomannan elongated at 95% relative humidity.

galactomannan has a layer line separation of 10.28 Å in agreement with what is found for other gums (Table 7). The meridional reflections are missing on the first layer line but appear on the second layer. The tilting of the specimen by 17° from the normal X-ray beam does not cause resolved splitting of the third layer intensity across the meridian. This is consistent with a crystallographic space group which does not have a 2_1 axis parallel to the fiber axis. It suggests that the probable space group symmetry of the unit cell is $P2_12_12$.

Rheological studies.—The flow behavior of dilute, semi-dilute and concentrated solutions of the galactomannan were determined over a wide range of shear rates. A Contraves Viscometer was used for dilute and semi-dilute solutions, whereas semi-dilute and concentrated solutions were investigated on a Carri-Med Rheometer. The variation of specific viscosity as a function of the shear rate for galactomannan solutions having a wide range of concentration from 0.2 g/L to 50 g/L are presented in a log–log plot in Fig. 2. The figure illustrates that for the shear rate range investigated the flow behavior of solutions at lower concentrations (0.2–2.0 g/L) is newtonian but it becomes shear-thinning for 3 g/L or higher. The shear rate ($\dot{\gamma}_c$) corresponding to the transition

Table 6
Interplanar spacings (Å) for *C. spectabilis* seed galactomannan

hkl	Interplanar spacing (Å)	*hkl*	Interplanar spacing (Å)
020	12.82	141	4.67
110	8.59	102	4.48
120	7.43	220	4.30
111	6.59	042	4.01
040	6.41	132	3.97
140	5.24	240	3.72
002	5.14	241	3.49
022	4.77	242	3.01

Table 7
Orthorhombic unit cell dimensions of *C. spectabilis* and other seed galactomannans

Seed galactomannan	Man:Gal ratio	Unit cell parameters (Å)		
		a	*b*	*c*
C. spectabilis	2.51	9.12	25.63	10.28
C. siamea [18]	2.55	9.00	24.81	10.30
C. saltiana [18]	2.29	8.99	24.75	10.30
S. didymobotrya [32]	3.04	9.00	24.62	10.30
Tara [19]	2.56	8.91	24.17	10.46
M. sativa [18]	1.16	9.00	30.66	10.24
Fenugreek [20]	1.08	8.94	29.50	10.27
Mannan I [33,34]	0	8.92	7.21	10.27

from newtonian to viscoelastic behavior moves to lower values with increasing concentration as found usually for polymer solutions.

Fig. 3 shows the concentration dependence of the specific viscosity at zero shear rate ($\eta_{sp} \rightarrow 0$) in a logarithmic plot as a function of (a) the overlap parameter $C[\eta]$ or (b) as a function of the modified Huggins relation $C[\eta] + k'(C[\eta])^2$. The critical overlap parameter $C_c^*[\eta]$, from which deviation from the Huggins relation appears, determined from these curves, is equal to 1.7. It corresponds to a value of the critical concentration $C_c^* = 2.75$ g/L for our sample. These values indicate the transition from dilute to semi-dilute regime. During previous studies, $C_c^*[\eta] = 2.2$ was found for *C. siamea* [21] seed galactomannan having a Man:Gal ratio of 2.55 and an intrinsic viscosity of 1166 mL/g, whereas in the case of *C. nodosa* [22] (Man/Gal = 3.5; $[\eta] = 1210$ mL/g) the experimental value was 2.5. In literature [23,24], most of the randomly coiled polysaccharides have a reported critical overlap parameter between 2 and 4. The galactomannan from *Mimosa scabrella* [25] having a high galactose content (Man/Gal = 1.1) and $[\eta] = 900$ mL/g as well as some other flexible polymers like polystyrene and polystyrenesulfonate

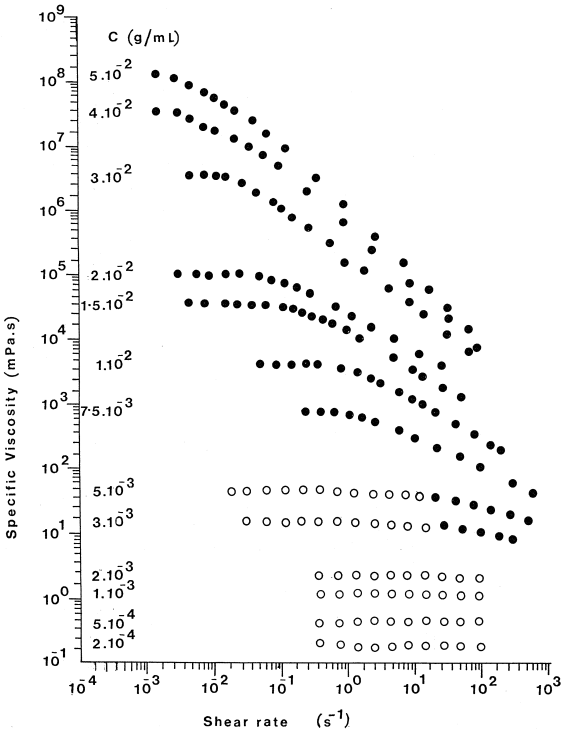


Fig. 2. Variation of the specific viscosity as a function of the shear rate for different galactomannan concentrations in water at 25 °C with Contraves Low-Shear (-○-○-) and Carri-Med (-●-●-).

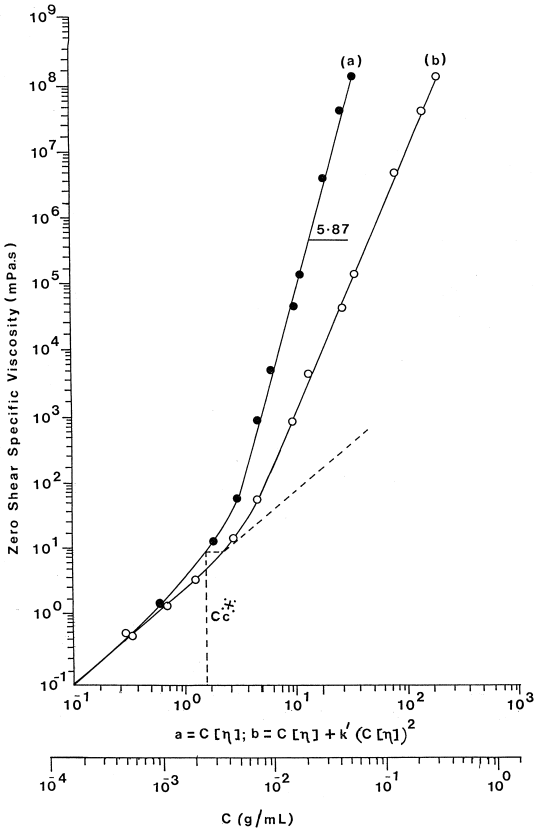


Fig. 3. Dependence of specific viscosity in the newtonian plateau η_{spo} as a function of the overlap parameter; (a) $C[\eta]$ and (b) $C[\eta] + k'(C[\eta])^2$ and galactomannan concentration (C).

[25] have $C_c^*[\eta] \sim 2.6$. It is to be pointed out here that the comparative lower value of $C_c^*[\eta]$ for our polymer could be due to its low galactose content and the resulting appearance of chain associations when the polymer concentration increases. Some of the stiffed hydrocolloids like xanthan [26], succinoglycan [27] and welan [28] also show lower $C_c^*[\eta]$ values with interchain associations.

Fig. 3 represents the variation of log η_{spo} as a function of log $C[\eta]$. This curve can be fitted with the equation:

$$\eta_{spo} = C[\eta] + k'(C[\eta])^2 + B(C[\eta])^n \quad (1)$$

and allows polymers with different intrinsic viscosities to be compared. The log–log plot of η_{spo} versus $C[\eta]$ becomes linear at higher concentration with a slope of 5.87. Consequently, the following equation is obtained for higher concentration:

$$\eta_{spo} \sim (c[\eta])^{5.87} \sim C^{5.87}M^{4.3} \text{ or } C^{5.87}M^{5.75} \quad (2)$$

For the determination of the above equation, the value of the Mark–Houwink parameter $a = 0.723$

[29] or 0.98 [30] is taken for calculation. In the literature, different values for the exponent are reported depending upon the source of the galactomannans, their Man:Gal ratio and intrinsic viscosity. Doublier and Launay [30] and Kapoor et al. [22] have found $\eta_{spo} \sim C^{6.6}$ for galactomannans with a Man:Gal ratio of 4.0 and 3.5, respectively. However, in the case of *M. scabrella* seed galactomannan (Man/Gal = 1.1), the exponent value [25] was 4.2. The value of this exponent is directly related to the galactose content. It increases when the galactose content decreases, so when a large number of interchain associations is promoted in the semi-dilute regime. It may also account for the lower $C_c^*[\eta]$ value from which the dilute behavior is lost for *C. spectabilis* (Table 8).

The galactomannan shows a tendency for a sharp increase of specific viscosity with polymer concentration: it exerts $\eta_{spo} = 1200$ mPa s at $C[\eta] = 5$ ($C = 8.3$ g/L), = 10,000 mPa s at $C[\eta] = 7$ ($C = 11.7$ g/L), and = 55,000 mPa s at $C[\eta] = 10$ ($C = 16.7$). Similarly, the value of η_{spo} at $C = 10$ g/L is about 5000 mPa s which is in the range of the reported values (Table 8) of 9500 and 4600 mPa s for *C. nodosa* and *C. siamea* seed galactomannans, respectively, having Man:Gal ratios > 2.5. Once again, the higher value found for *C. nodosa* can be explained by its high Man:Gal ratio, leading to interaction phenomena at higher polymer concentrations.

The behavior of the galactomannan becomes viscoelastic above a critical shear rate and polymer concentration. The relationship between viscosity and shear rate in this domain can be expressed by $\eta \sim \dot{\gamma}^n$. Fig. 4 represents a plot of n versus $C[\eta]$ for different galactomannans (Table 8), with varying Man:Gal ratio, already published in the literature. Like Guar [30], the maximum value of n is reached when $C[\eta] = 30$, whereas in *C. nodosa* and *C. siamea*, this value is obtained at $C[\eta] = 60$. In the case of *M. scabrella* seed galactomannan, the value of n is also reached at $C[\eta] = 30$, but its value is lower (> 0.6). Again, the higher absolute value of n obtained for *C. spectabilis* confirms the presence of interchain associations in the semi-dilute regime, while the lower value for *C. siamea* can be related to its large polydispersity in molecular weight.

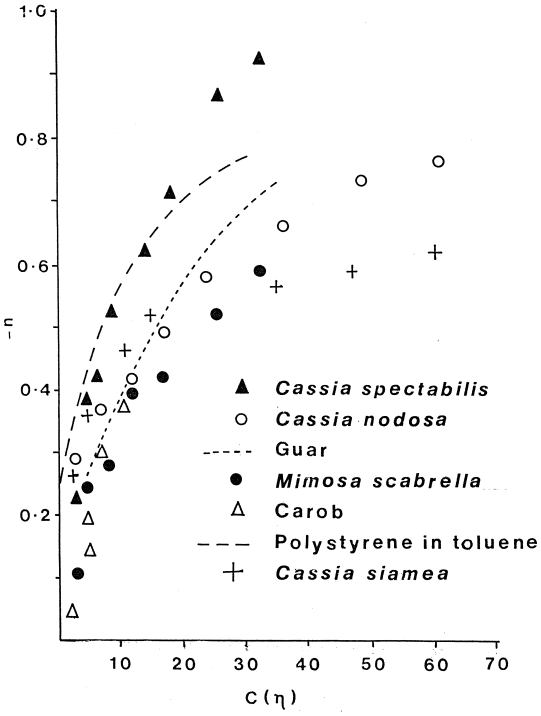


Fig. 4. Limiting slope (n) as a function of overlap parameter $C[\eta]$ for *C. spectabilis* and other seed galactomannans and polymers.

The viscoelastic behavior of the galactomannan with respect to different polymer concentrations and critical shear rate is presented as a log–log plot in

Table 8
Critical overlap parameter, specific viscosity for $C = 10$ g/L, slope S of log η_{spo} versus log $C[\eta]$ for the highest $C[\eta]$ values, limit slope (n) of viscosity versus shear rate in the non-newtonian region of *C. spectabilis* and other seed galactomannans with different Man:Gal ratio and $[\eta]$

	Galactomannan species			
	a	b	c	d
Man:Gal ratio	2.65	3.5	2.55	1.1
Mw	1.11×10^6	7.01×10^5	8.41×10^5	–
Mw/Mn	1.21	1.48	2.5	–
$[\eta]$ mL/g	615	1210	1166	900
η_{spo} ($C = 10$ g/L)	5000	9500	4600	300
$C_c^*[\eta]$	1.69	2.5	2.2	2.61
Slope (S)	5.9	6.4	4.2	4.2
Limit slope (n)	−0.95	−0.81	−0.65	−0.65

(a) Present work; (b) *C. nodosa* [22]; (c) *C. siamea* [21]; (d) *M. scabrella* [25].

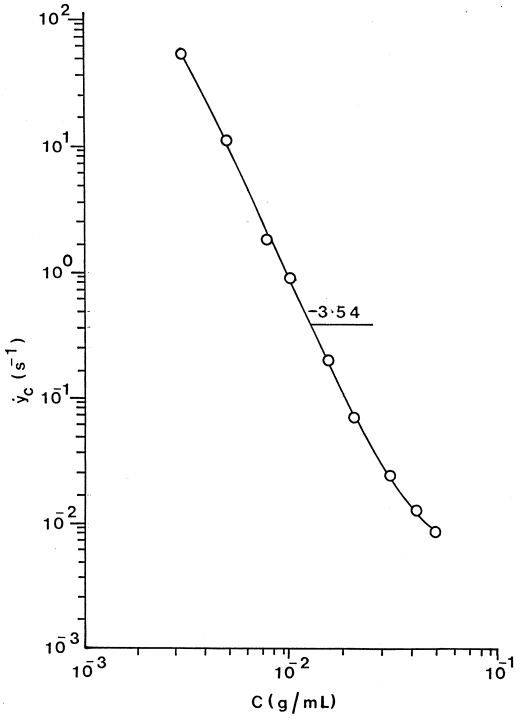


Fig. 5. Critical value of shear rate ($\dot{\gamma}_c$) of the onset of viscoelastic behavior as a function of galactomannan concentration (C).

Fig. 5. The plot reaches a slope of -3.54 and becomes a little bit curved at about 20 g/L and higher concentrations. It represents three domains. Below the concentration of 3 g/L, where the Huggins law applies and $\dot{\gamma}_c$ cannot be determined with the rheometers used in this work, the first domain corresponds to the dilute regime. It is characterized by $C_c^* = 2.75$ g/L and below. Then, there is a sharp decrease of $\dot{\gamma}_c$ with the increase of polymer concentration (slope of -3.54). This region between 2.7 to 20 g/L is characterized as the semi-dilute regime. In the concentrated domain (20 g/L and above), $\dot{\gamma}_c$ becomes less dependent on the concentration at $C_c^{**} = 20$ g/L. This behavior can be related to that obtained on the diffusion coefficient in xanthan [31] solutions in which a critical concentration C_c^{**} is introduced for the entrance in the concentrated regime (related to the semi-flexible character of this polymer).

Dynamic measurements.—Studies regarding the comparison of the elastic and loss moduli, G' and G'', have been carried out for 3–50 g/L solutions at 10^{-3} to 10^{1} frequencies (s^{-1}). At concentrations below 10 g/L, the viscosities of the galactomannan solutions

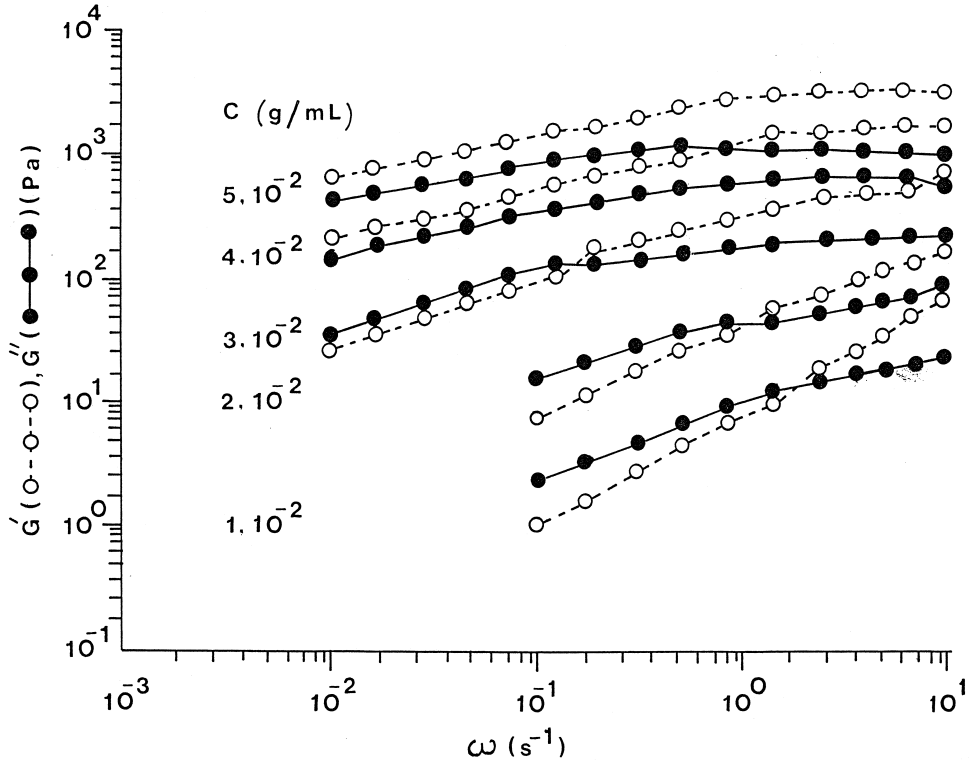


Fig. 6. G' (Pa) (-○-○-) and G'' (Pa) (-●-●-) moduli as a function of the frequency (ω), for different concentrations in water at 25 °C.

were too low to yield reliable values of G' and G''. The log–log plot of G' and G'' (Pa) versus ω (s^{-1}) obtained for 10, 20, 30, 40, and 50 g/L solutions are presented in Fig. 6. The maximum G' value is obtained for a 50 g/L solution (3.71×10^3 Pa). The modulus values and the shape of the curves are compatible with a 'gel-like' behavior and confirm, in the semi-dilute and concentrated regimes, the presence of larger interchain interactions in this sample than expected from its Man:Gal ratio. From this value of G' at 50 g/L concentration, the mass between entanglements is calculated by the relationship $M_e = CRT/G'$. The value obtained ($M_e = 34{,}000$) corresponds to the formation of a pseudonetwork. In other seed galactomannans from *Cassia* species, the reported values [21,22] of M_e at 50 g/L concentration are between 31,000 and 34,000 which corresponds to the value found for *C. spectabilis* inspite of different Man:Gal ratios (see Table 8).

4. Conclusion

The galactomannan obtained from the seeds of *C. spectabilis* is characterized by a low galactose content (Man/Gal = 2.65). Its Man:Gal ratio lies between those of commercial Guar (1.63) and locust bean (3.12) gums. Methylation and ^{13}C NMR studies reveal that the gum has the basic structure of legume galactomannans. X-ray structural studies show that the *b* dimension of the unit cell is sensitive to relative humidity (RH), galactose substitution and orientation of the films. The probable space group symmetry of the unit cell is $P2_12_12$. The galactomannan solutions show a viscoelastic behavior above the critical shear rate which can be represented by $\eta \sim \dot{\gamma}^n$. The high value of the specific viscosity and its dependence on the polymeric concentration seems to be a characteristic of galactomannans with low galactose content. The value of the critical concentration is in agreement with results obtained for most coiled hydrocolloids. At high concentration, the polymer behavior becomes that of a pseudonetwork. The studies reveal that single side chains of galactose units are attached to the main mannan chain according to a particular pattern, leaving many 'free zones' of unsubstituted mannose residues for self association or aggregations. One way to test for such mannan–mannan interactions being responsible for the observed behavior would be to monitor the effect of alkali treatment and neutralization [35]. This constitutes a track for future works.

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