



Hydrodynamic and rheological properties of *Irvingia gabonensis* gum



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ABSTRACT

The polysaccharide component of *Irvingia gabonensis* endosperm was isolated and its hydrodynamic and rheological properties investigated. The polysaccharide is an arabinogalactan and contains small amounts of rhamnose, galacturonic acid, glucose and glucuronic acid.

The polysaccharide displayed typical polyelectrolyte behaviour in solution. The intrinsic viscosity at infinite ionic strength, a measure of the hydrodynamic volume of the uncharged polysaccharide molecule, was obtained as 4.9 dl/g. The macromolecules have a semi flexible backbone with a Smidsrod stiffness parameter of 0.085. The polysaccharide exhibited non-Newtonian behaviour at all the concentrations (0.2% to 3.0% (w/v)) investigated. Cox–Merz plots showed that $\eta(\dot{\gamma})$ and $\eta^*(\omega)$ were closely superimposable except at low shear rates and higher concentrations, where $\eta^* > \eta$. The high M_w (1.56×10^6 g/mol) and its random coil conformation show *I. gabonensis* polysaccharide has potential for application as a thickener.

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1. Introduction

Polysaccharide gums are widely used in the Food Industry because of their ability to modify the rheology of food products. The high molecular weight of the polysaccharide molecules and their ability to absorb water and raise viscosity of the medium make them suitable for use as thickeners, suspending agents, stabilizers, binders and sugar crystal growth inhibitors. Examples include tree exudates gums such as gum Arabic and gum tragacanth, guar gum and locust bean gum, from seed endosperm, carrageenans and alginates from seaweeds, xanthan and gellan gums from bacteria and chitin and chitosan from the shells of crustaceans (Phillips & Williams, 2009; Izydorczyk, Cui, & Wang, 2005).

Irvingia gabonensis gum is obtained from the seed endosperm of *I. gabonensis* nut. The powdered endosperm is employed as a thickener in sub-Saharan African cuisines (Akusu & Kiin-Kabari, 2013). Although *I. gabonensis* gum is a promising commercially important polysaccharide, there is scanty information on the rheology of this potential source of seed gum, apart from a report by Ndjouenkeu, Goycoolea, Morris, and Akingbala (1996). This paper reports on the monosaccharide composition, molecular weight and rheological properties of the gum using capillary viscometry together with steady shear and small angle deformation oscillation rheometry.

2. Materials and methods

2.1. Isolation of gum from *Irvingia gabonensis* seed

The *I. gabonensis* seeds obtained after cracking the nuts were weighed and soaked in water at room temperature for 1 h. The hydrated seed coat detached cleanly at minimal pressure on rubbing with the finger. The endosperm was air-dried for one week. The resultant seed endosperm was pulverized and extracted in a soxhlet extractor with hexane for 8 h. The defatted powder was further extracted with 95% ethanol to remove soluble sugars and organic pigments for 10 h. The *I. gabonensis* seed powder, 2% (w/v) was dispersed in distilled water by means of a magnetic stirrer maintained at 50 °C and stirred continuously by means of a magnetic bar for 6 h. The sample was transferred into centrifuge tubes and centrifuged at 2500 rpm for 2 h with the temperature maintained at 25 °C. The supernatant was collected and subjected to a second centrifugation. The supernatant was collected in a round-bottomed flask and freeze dried. The resultant *I. gabonensis* gum powder was stored in an airtight container.

2.2. Elemental analysis

The elemental analysis was done with a Carlo-Erba CHN analyzer (Milan, Italy). The sample was initially combusted in oxygen, subjected to chromatography (GC–MS) and the component elements quantified with a thermal conductivity detector. Calibration was done using least squares to linear fit.

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2.3. Sugar composition

The sugar composition was determined by HPAEC-PAD using a Dionex ICS-2500 system. Sugar analysis was by methanolysis followed by TFA hydrolysis using myo-inositol as internal standard as reported previously (Kokubun, Madhav, Moreau, & Williams, 2014).

2.4. Fourier transform infra-red spectroscopy

The Fourier transform infra-red (FTIR) spectroscopic analysis of *I. gabonensis* polysaccharide was obtained on a KBr disc using an FTIR spectrophotometer (Perkin Elmer Spectrum Two, USA).

2.5. Molecular weight determination

The molecular weight was determined using gel permeation chromatography coupled to multiangle laser light scattering and refractive index detectors (Optilab DSP, Wyatt Technology Corporation, Santa Barbara Ca93103). The polysaccharide solution (20 ml) containing 4.001×10^{-4} g/ml was microwaved in a high pressure vessel for 40 s to ensure complete disaggregation (Ratcliffe, Williams, Viebke, & Meadows, 2005), filtered through a 0.45 μ m syringe filter and injected into a 200 μ l loop through a rheodyne and passed through a combination of Suprema columns (100 Å, 3000 Å and 30,000 Å) packed with 10 μ m beads of poly-hydroxymethacrylate copolymer. The total injected mass was 8.002×10^{-5} g. The solvent (0.1 M NaNO₃ + 10^{-6} M NaN₃ solution) was pumped (Waters: 515 HPLC Pump, Milford, MA 01757, USA) through a degasser (CSI 6150, Cambridge Scientific Instruments, England) at a flow rate of 0.5 ml/min. The M_w and R_g were determined from the Berry first-order equation

$$\frac{K_c}{R_\theta} = \frac{1}{M_w} + \frac{16\pi^2}{3\lambda^2} \times R_g^2 \times \sin^2\left(\frac{\theta}{2}\right) \quad (1)$$

2.6. Intrinsic viscosity measurements

The intrinsic viscosity of *I. gabonensis* gum was determined in NaCl solutions of different ionic strengths (0.06–0.16 M). 6.5 ml of the gum solution (0.1% (w/v)) was transferred into a Canon-Ubbelohde capillary viscometer (No. 75, J 349), which was immersed in a precision water bath to maintain the temperature at 25.0 ± 0.1 °C. After equilibration for 10 min, the flow time was determined between the two etched marks. Serial isoionic dilution was performed in situ and three readings were taken for each dilution and averaged. The relative viscosity, η_{rel} , of the polymer solution was obtained by dividing the flow time of the solution by that of the solvent. The specific viscosity, η_{sp} , is given by $\eta_{rel} - 1$. The intrinsic viscosity, $[\eta]$, was obtained by combined Huggins and Kraemer extrapolation.

2.7. Rheological measurements

The *Irvingia* gum solutions were prepared in distilled water. Five concentrations of the gum solutions (0.2%, 0.5%, 1.0%, 2.0% and 3.0%) were prepared by mixing the desired amount of dry gum powder in distilled water while continuously dispersing the gum overnight at ambient temperature by means of a roller mixer (SRT2, Stuart Scientific, UK). The rheological data were obtained using a Controlled Stress Rheometer (AR 2000, TA Instruments, DE, USA) at 25 °C with standard steel cone (40 mm 2°, ser no. 982525) for concentrations 0.5 to 3.0% and standard-size recessed end concentric cylinders (Stator inner radius 15 mm, rotor outer radius 14 mm, cylinder immersed height 42 mm, gap 4000 μ m) for 0.2% solution and analyzed using TA Data Analysis Software. The flow properties

Table 1

Some properties of *Irvingia gabonensis* seed and polysaccharide.

(a) Physical composition of <i>Irvingia gabonensis</i> seed ^a	
Hull (%)	11.0 $\pm$ 3.8
Endosperm (%)	88.90 $\pm$ 3.8
Germ (%)	Negligible
Yield (%)	95.5
(b) Elemental composition (%)	
Nitrogen	1.950
Carbon	36.829
Hydrogen	5.893
(c) Sugar composition of <i>Irvingia gabonensis</i> polysaccharide (relative mole%)	
Rha	8.70
Ara	18.82
Gal	61.72
Glc	1.12
GalA	9.11
GlcA	0.54
(d) Molecular characteristics of <i>Irvingia gabonensis</i> polysaccharide ^b	
Weight-average molecular weight, M_w (g/mol)	$1.56 \pm 0.11 \times 10^6$
Weight-average radius of gyration, R_w (nm)	53.15 ± 1.20
z-average molecular weight, R_z (nm)	64.7 ± 0.9
Polydispersity, M_w/M_n	1.68 ± 0.07
Mass recovery (%)	79.0 ± 0.2

^a Based on weight of defatted flour.

^b Mean for nineteen seeds $\pm$ SD. Rha = rhamnose, Ara = arabinose, Gal = galactose, Glc = glucose, Man = mannose, GalA = galacturonic acid, GlcA = glucuronic acid.

^c Mean $\pm$ STD of two determinations.

were obtained by subjecting the samples to a stepped-flow procedure at shear rates of 10^{-3} to 10^3 s⁻¹ after equilibration for 2 min. In the oscillation procedure, a frequency sweep was performed on the gum solutions in the region of 10^{-1} to 120 rad/s at an amplitude strain within the linear viscoelastic region. A stress sweep was performed on each sample to locate the linear viscoelastic region.

3. Results and discussion

3.1. Seed composition and yield of polysaccharide

The composition of *I. gabonensis* seed is reported in Table 1a, which indicates that it consists of 89% endosperm, 11% hull and negligible germ. Although oil is the major constituent of the seed $\sim$ 73.8% (Matos et al., 2009), the defatted endosperm yielded $\sim$ 95.5% of the polysaccharide, an indication that the defatted endosperm is essentially the polysaccharide with <5% non polysaccharide constituents. For most seeds the polysaccharide is mainly contained in the endosperm and the ratio of endosperm to the whole seed becomes a measure of the polysaccharide content. In *Mucuna flagellipes* seed, the endosperm constitutes 67.2% of the whole seed and yields 32.6% of polysaccharide based on defatted endosperm flour (Nwokocho & Williams, 2009). The seed of *Cyamopsis tetragonolobus* is composed of 40% of endosperm with the endosperm containing 75–85% of the polysaccharide (Sharma, Dhuldhoya, Merchant, & Merchant, 2014). From Table 1b, the *I. gabonensis* polysaccharide is composed of nitrogen (1.950%), carbon (36.829%) and hydrogen (5.893%). The *I. gabonensis* polysaccharide contained higher amount of nitrogen than reported for *M. flagellipes* polysaccharide (1.87%) (Nwokocho & Williams, 2009).

The *I. gabonensis* polysaccharide (Table 1c) was found to consist of galactose (61.72%), arabinose (18.8%), rhamnose (8.7%), galacturonic acid (9.1%) and small amounts of glucose (1.1%) and glucuronic acid (0.5%). The FTIR spectrum (Fig. 1) of *I. gabonensis* polysaccharide showed the presence of peaks at 1640–1727 cm⁻¹ (COO⁻, asymmetric stretching) and 1413.4 cm⁻¹ (COO⁻, symmetric stretching) which are most pronounced in spectra of polysaccharides containing uronic acid groups (Akdama et al., 2009; Cui, Phillips, Blackwell, & Nikiforuk, 2007).

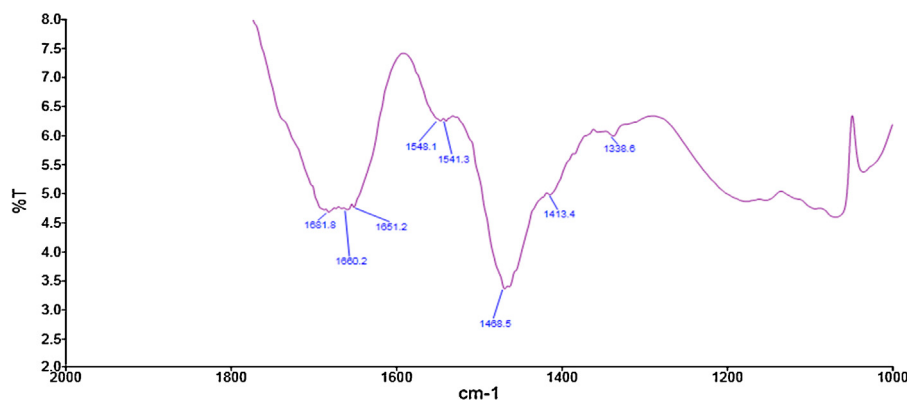


Fig. 1. FTIR spectrum of fingerprint region of *Irvingia gabonensis* polysaccharide.

3.2. Molecular weight

Fig. 2 shows the refractive index GPC elution profile of the *I. gabonensis* polysaccharide gum together with the molecular weight of the eluting species. The weight average molecular weight of the polysaccharide was found to be 1.56×10^6 g/mol at a mass recovery of 79% (Table 1d). The polysaccharide coils were found to have a radius of gyration of 60.75 nm. We have not found any report on the M_w of *I. gabonensis* polysaccharide for comparison. However, several data are available on other seed polysaccharides: *Detarium senegalense* [2.75×10^6 g/mol] (Wang, Ellis, Ross-Murphy, & Reid, 1996), *Copaifera langsdorfii* [7.82×10^5 g/mol] (Stupp et al., 2008), *Cassia siamea* [8.4×10^5 g/mol] (Kapoor, Milas, Taravel, & Rinaudo, 1996). The radius of gyration, R_g , and the degree of polydispersity are similar to values ($R_g = 65$ nm, $M_w/M_n = 1.7$) reported for *Copaifera l.* seed polysaccharide (Stupp et al., 2008).

The shape of *I. gabonensis* polysaccharide chains was estimated from the plot of the radius of gyration (R_g) versus molecular weight (M_w) according to the following equation

$$R_g \approx M_w^\alpha \quad (2)$$

where the exponent α is related to the shape of the polymer coil. The exponent α is 0.5–0.6 for random coil polymers and 0.33 for spheres (Andersson, Wittgren, & Wahlund, 2003). From Fig. 2b, α for *I. gabonensis* polysaccharide was obtained to be 0.53 ± 0.02 suggesting the polysaccharide consists of random coils.

3.3. Intrinsic viscosity

Fig. 3a shows the Huggins and Kraemer plots for *I. gabonensis* gum in 0.1 M NaCl and the intrinsic viscosity determined from the

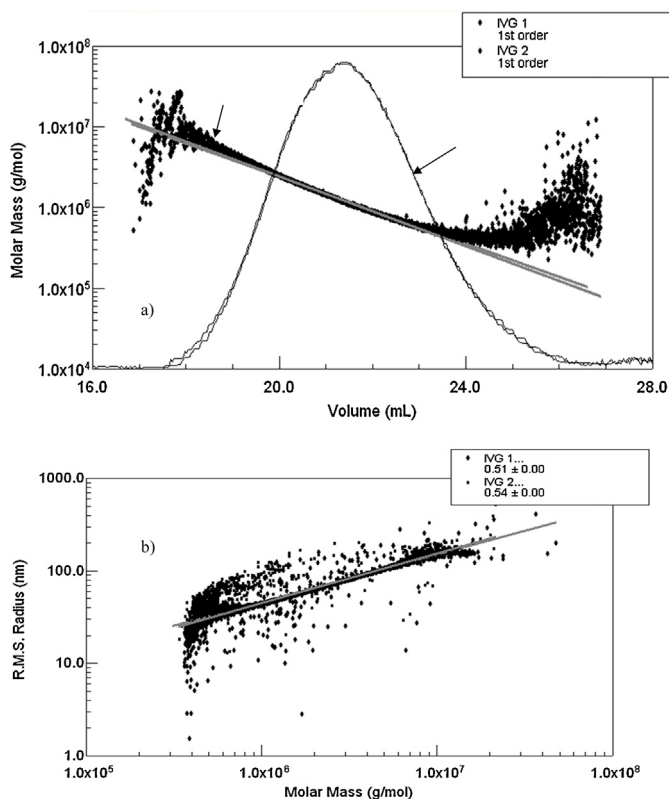


Fig. 2. GPC elution profile of *Irvingia gabonensis* polysaccharide showing (a) M_w versus volume with concentration overlay (two measurements). (b) Log–log plot of RMS radius versus molar mass.

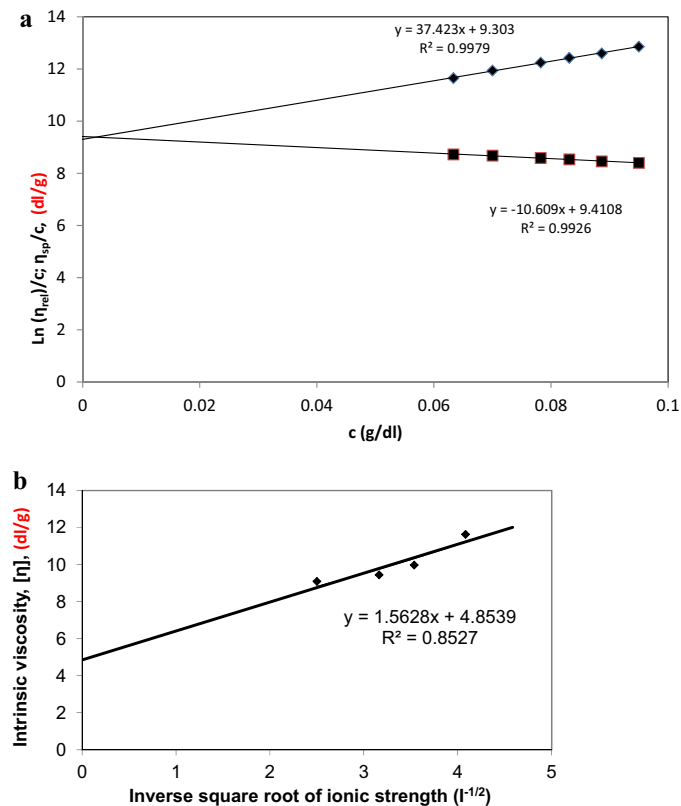


Fig. 3. (a). Intrinsic viscosity of *Irvingia gabonensis* polysaccharide in 0.1 M NaCl by combined Huggins and Kraemer extrapolation; (b). Intrinsic viscosity as a function of inverse of ionic strength.

Table 2A comparison with the stiffness parameter, B , of other polyelectrolytes.

Polymer	B	References
<i>Irvingia gabonensis</i>	0.085	This paper
<i>Enterolobium contortisiliquum</i>	0.090	Oliveira et al. (2001)
Carboxymethyl cellulose	0.065–0.078	Trivedi and Patel (1982)
Sodium polyacrylate	0.27	Smidsrod and Haug (1971)
Xanthan	0.005	Smidsrod and Haug (1971)
Hsian-Tsao	0.026–0.030	Lai et al. (2000)
Maize bran heteroxylan	0.024	Chanliaud et al. (1997)
Colanic acid	0.42	Ren et al. (2003)

intercept was found to be 9.35 dl/g. This is larger than the value of 4.4 dl/g reported by Ndjouenkeu et al. (1996).

The intrinsic viscosities of the *I. gabonensis* gum were determined in the presence of various concentrations of NaCl and the results are plotted against the inverse square root of the electrolyte concentration, $I^{-1/2}$, in Fig. 3b. The gum behaves as a polyelectrolyte due to the presence of uronic acid residues. For polyelectrolytes in solution, the following relationship exists (Eq. (3)) between intrinsic viscosity and ionic strength (Smidsrod & Haug, 1971).

$$[\eta] = [\eta]^\infty + SI^{-1/2} \quad (3)$$

where $[\eta]$, $[\eta]^\infty$ are the intrinsic viscosity and intrinsic viscosity at infinite ionic strength, and S is a parameter related to the stiffness of polymers. The Smidsrod stiffness parameter, B is given by the following equation.

$$S = B \times ([\eta]_{I=0.1})^\nu \quad (4)$$

where $[\eta]_{I=0.1}$ is the intrinsic viscosity in 0.1 M NaCl (determined as 9.35 dl/g), and ν has a value of 1.3 (Ren, Ellis, Sutherland, & Ross-Murphy, 2003; Smidsrod & Haug, 1971). From the plot of $[\eta]$ against $I^{-1/2}$, $S = 1.56$, giving a stiffness parameter B , of 0.085. The use of a linear fit to the data in Fig. 3b is reasonable approach to test the B -parameter concept. Table 2 gives a comparison of the stiffness parameter of *I. gabonensis* polysaccharide and some other polysaccharides. *I. gabonensis* with a chain flexibility of 0.085 is comparable in flexibility to *Enterolobium contortisiliquum* (Oliveira, Silva, de Paula, Feitosa, & Paula, 2001) and carboxymethylcellulose (Trivedi & Patel, 1982) but more flexible than xanthan (Smidsrod & Haug, 1971), hsian-tsao leaf gum (Lai, Tang, & Lin, 2000) and maize bran heteroxylan (Chanliaud, Saulnier, & Thibault, 1997) and less flexible than sodium polyacrylate (Smidsrod & Haug, 1971) and colanic acid (Ren et al., 2003).

The $[\eta]$ of the uncharged polysaccharide coil obtained by extrapolating to $I^{-1/2} = 0$ was 4.9 dl/g, the intrinsic viscosity at infinite ionic strength. This is a measure of the hydrodynamic volume of the uncharged individual polymer coils of *I. gabonensis* gum.

3.4. Steady shear viscosity

Fig. 4 shows the viscosity as a function of shear rate for *Irvingia* polysaccharide solutions at varying concentrations in water and with no added salt. The *I. gabonensis* gum was non-Newtonian at all the concentrations (0.2% to 3.0% (w/v)). The Cross model (Eq. (5)) was fitted to the viscosity–shear rate profiles to determine the flow characteristics.

$$\text{Cross model: } \eta = \eta_\infty + \frac{\eta_0 - \eta_\infty}{1 + (\tau\dot{\gamma})^m} \quad (5)$$

where η , η_0 , η_∞ are the shear viscosity, zero shear viscosity and infinite shear viscosity (Pa s), respectively; τ is Cross relaxation time (s), $\dot{\gamma}$ is the shear rate (1/s) and m is rate index (dimensionless).

The zero shear viscosity (η_0) ranged from 0.08 to 388.4 Pa s. The η_0 (388.4 Pa s) at 3% is much larger than reported for some other polysaccharides at the same concentration: 0.84 Pa s for *Leucaena leucocephala* (Nwokocho & Williams, 2009) and 53.3 Pa s for *M. flagellipes* (Nwokocho & Williams, 2009). This indicates a high thickening potential of the polysaccharide. The relaxation time, τ , increased with increase in polysaccharide concentration. τ is related to the critical shear rate, $\dot{\gamma}_{\text{crit}} (\tau = 1/\dot{\gamma}_{\text{crit}})$, which marks the onset of transition from linear response ($\eta = \eta_0$) at low shear rate to shear thinning response ($\eta \sim \dot{\gamma}^{-m}$) at higher shear rate. The rate index, $0.6183 < m < 0.8323$, is a measure of the degree of dependence of viscosity on shear rate in the shear thinning region for the *I. gabonensis* polysaccharide solution. For Newtonian solutions, $m = 0$, while it approaches unity for increasing shear thinning.

3.5. Viscoelastic properties

The variation of the storage, G' and loss G'' moduli with frequency of oscillation for *I. gabonensis* gum solutions is presented in Fig. 5. At 0.2% (w/w), both moduli showed strong frequency dependence with $G'' > G'$ at all frequencies, which is a typical characteristic of polymer solutions in the dilute regime. For the 0.5% and 1.0% solutions G'' was less than G' at low ω but became higher at high ω indicating that the concentration is above coil overlap concentration. For polymer concentrations of 2.0% and 3.0%, G' and G'' were both frequency dependent but G' was greater than G'' at all

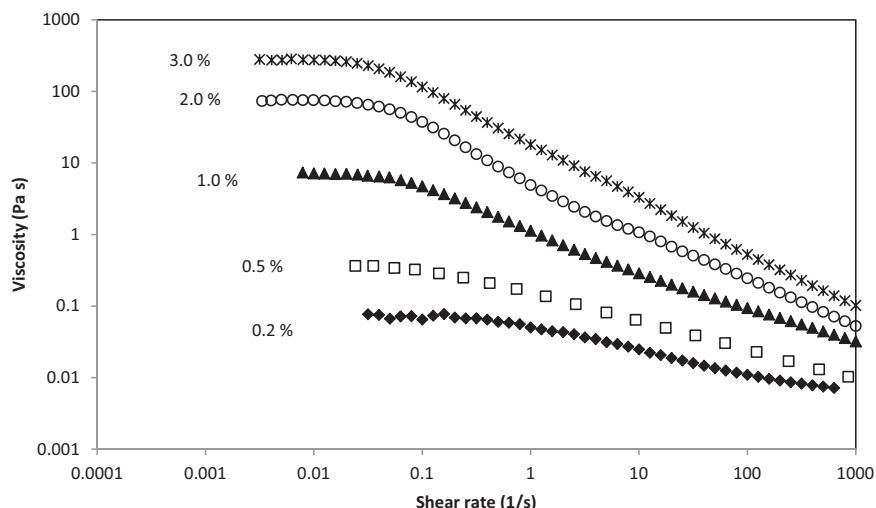


Fig. 4. Dependence of viscosity on shear rate and concentration for *Irvingia* polysaccharide in water with no added salt at 25 °C.

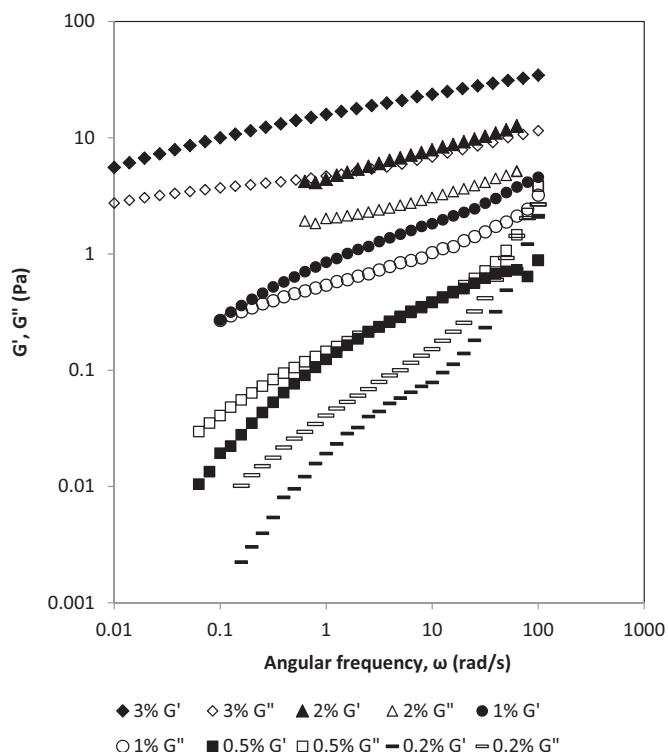


Fig. 5. G' , G'' versus angular frequency for different concentrations of *Irvingia gabonensis* polysaccharide in water with no added salt at 25 °C.

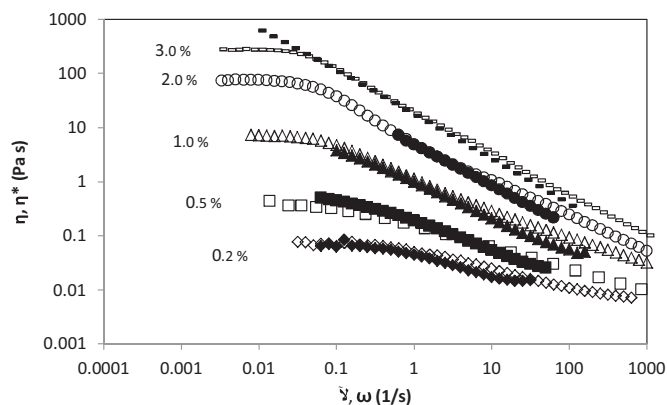


Fig. 6. Cox–Merz superimposition: Steady shear viscosity (η) versus shear rate ($\dot{\gamma}$) (opened symbols); dynamic viscosity (η^*) versus angular frequency (ω) (filled symbols) of *I. gabonensis* polysaccharide in water with no added salt at 25 °C.

angular frequencies investigated. This is typical behaviour of weak gels.

Cox–Merz plots were used to investigate the interrelationship between the data from steady shear and that from small angle deformation oscillations within the linear viscoelastic region (Fig. 6). Close superimposition was observed for plots of ($\log \eta(\dot{\gamma})$ and $\log \eta^*(\omega)$) for $\dot{\gamma} = \omega$. Deviation was observed at low shear rate and angular frequency, at higher concentrations (3.0%) due to enthalpic associations that survived low-amplitude oscillation but were broken down by shear, giving rise to $\eta^* > \eta$.

4. Conclusion

The gum isolated from *I. gabonensis* endosperm has M_w of 1.56×10^6 g/mol. It is an arabinogalactan but also contains a small proportion of neutral sugars and uronic acids. The latter give rise

to polyelectrolyte properties. The gum has an intrinsic viscosity at zero ionic strength of 4.9 dl/g. It showed non-Newtonian behaviour at concentrations from 0.2 to 3.0%. Oscillation frequency sweeps showed that *I. gabonensis* gum solutions were dominated by a viscous response at concentrations less than 1.0% and by an elastic response at higher concentrations. Application of Cox–Merz rule showed that $\eta(\dot{\gamma})$ and $\eta^*(\omega)$ were closely superimposable.

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