
Journal Article

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Isolation and characterization of a novel polysaccharide from seeds of *Peltophorum pterocarpum*

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Abstract

Peltophorum pterocarpum seeds yielded ~20% water soluble polysaccharide. The polysaccharide is a galactomannan with mannose:galactose ratio of 4.4:1. The polysaccharide had an intrinsic viscosity of 3.14 dl/g and weight average molecular weight of 2.49 x 10^5 g/mol. The polysaccharide solutions were non-Newtonian at concentrations above 1%. A double logarithmic plot of the zero shear specific viscosity versus volume concentration gave a coil overlap concentration, c* of 2.6/[η], with slope $c^{1.4}$ in the dilute regime and $c^{4.3}$ in the concentrated regime. The experimental data were fitted to different viscosity models with the Martin model giving the best fit. The Cox-Merz empirical rule gave close superimposition of the data from steady shear viscosity $\eta(\dot{\gamma})$ and complex viscosity $\eta^*(\omega)$.

Keywords: *Peltophorum pterocarpum* seed polysaccharide, molecular weight, monosaccharide composition, solution rheology

Introduction

Plants of the *leguminosae* family continue to dominate the food industry as a source of water soluble polysaccharides. The polysaccharide is located mainly in the endosperm and regulates the physiological functions of the seeds by preventing the seed from completely drying out thus avoiding the denaturation of essential enzymes (Srivastava & Kapoor, 2005). Polysaccharides are safe, replenishable and biodegradable. Hence they are ideal for application in food uses. They are employed as viscosity modifiers, gelling agents, suspending agents, encapsulating agents, water crystal and sugar crystal growth inhibitors, in flavour release control and to reduce syneresis when added to starch products.

Water soluble plant polysaccharides from guar and locust bean are among the most commonly applied industrially. They are also among the most studied and their properties are well documented in the literature (Labeau, 2012; Gong, Liu, Chen, Han, Gao, & Zhang, 2012; Prabaharan, 2011; Patel, Ranjan, & Patel, 1987; Kök, 2007; Mao, & Chen, 2006; Fernandes, Gonçalves, Doublier, 1993). However, there are other plants of the leguminosae family which show potential for application but little is known about their polysaccharide properties, hence they have remained largely unexplored. *Peltophorum pterocarpum* is one of these plants. *Peltophorum pterocarpum* is a legume of the subfamily: *Fabaceae. Peltophorum pterocarpum* is used as an ornamental plant; it is widely referred to as flamboyant tree. It produces yellow flowers and short flat elongated pods that contain the seeds. Mayworm, Salatino & Buckeridge (2004), who worked on *Peltophorum dubium* have reported that the seed endosperm contains a galactomannan with mannose:galactose ratio of 4.5:1. We have not found any report on the molecular characteristics and rheology of the seed polysaccharide. In this study we reported the molecular characteristics and the solution properties of *Peltophorum pterocarpum* polysaccharide investigated in the dilute and concentrated regimes. The potential of this polysaccharide has been highlighted elsewhere (Nwokocha & Williams, 2012a).

2.0 Materials and Methods

2.1 Seed composition and polysaccharide isolation

Whole seeds were selected and the weights determined. These were soaked in water to hydrate and the swollen seeds cut through with a razor, the hull, germ and endosperm were carefully separated and left to air dry to a constant weight. These were weighed and the weights reported as percentages of the whole seed. Since the seeds were small in size, polysaccharide isolation was based on defatted whole seed flour prepared as follows: Whole seed was coarsely crushed in a Warring blender and defatted in a soxhlet by extracting with hexane for 8 h. The residue was left in a fume chamber to get rid of residual hexane. A known weight of sample (~20 g) was dispersed in 400 ml of water and left in a water bath maintained at 60°C to fully hydrate overnight. This was blended in a Warring blender, poured into a centrifuge bottle and centrifuged at 2500 rpm for 2 h. The supernatant was collected. The residue was mixed with 200 ml water and blended and the blender rinsed with some water and both transferred into a centrifuge bottle and centrifuged. The supernatants were pooled, concentrated using a Rotavapor and the polysaccharide precipitated with isopropanol. The polysaccharide was purified by dispersing in water and re-precipitating with isopropanol. The sample was reconstituted in a small amount of water and freeze dried.

2.2 Molecular weight determination

The molecular weight was determined using gel permeation chromatography coupled to multiangle laser light scattering and refractive index and UV detectors (Optilab DSP, Wyatt Technology Corporation, Santa Barbara Ca93103). The polysaccharide solution (20 ml) containing 3.9985 x 10^{-3} g/ml was subjected to microwave bomb treatment for 40 s to ensure complete disaggregation (Ratcliffe *et al.*, 2005), filtered through a 0.45 μm syringe and injected through a rheodyne into a 200 µl loop connected to a combination of Suprema columns (100Å, 3000Å and 30000Å) packed with 10 µm beads of polyhydroxymethacrylate copolymer network. The solvent (0.1M $NaNO_3$ + 10^{-6}M NaN_3 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 total injected mass was 7.997 x 10^{-4} g. The chromatogram was analyzed with Astra software using a Zimm first order polynomial and a predetermined dn/dc value of 0.140 ml/g .

2.3 Intrinsic viscosity

A sample for intrinsic viscosity measurement was prepared by dispersing 0.4 % (w/w) polysaccharide powder in distilled water overnight on a roller mixer (SRT2, Stuart Scientific, UK) maintained at 25°C. The polysaccharide solution was passed through a 0.8 micron Nylon filter and 7 ml of the solution transferred into a Canon-Ubbelohde capillary viscometer (75, J379). The viscometer was immersed in a precision water bath maintained at 25.0±0.1 °C and the flow time between the two etched marks determined after equilibrating for 15 minutes. Serial dilution was performed in situ and three readings were taken for each dilution after equilibration and averaged. The relative viscosity, η_r, was calculated as the ratio of the flow time of the polymer solution to that of water. The specific viscosity, η_{sp}, was obtained as η_r-1. The intrinsic viscosity, [η], was evaluated by Fedors equations (eq. 1) and combined extrapolation of the Huggins and Kraemer

$$\frac{1}{2(\eta_r^{\frac{1}{2}}-1)}=\frac{1}{[\eta]c}-\frac{1}{c_m[\eta]} \qquad \text{(eq. 1)}$$

Where c is the polymer concentration, c_m is the upper limit of Fedors concentration.

2.4 Rheological measurements

Polysaccharide solutions (0.49 % to 5%, w/w) were characterized rheologically using a steady shear stepped flow procedure at shear rates from 0.001/s to 1000/s (AR2000, TA Instruments, Newcastle, UK). Small angle deformation oscillatory measurements were used to study the visco-elastic properties at angular frequency from 0. 01 rad/s to 625.8 rad/s at a percentage strain within the linear viscoelastic range. The percentage strain was determined by carrying out an oscillation stress sweep at an angular frequency of 6.283 rad/s. Standard-size recessed end concentric cylinders (gap 4 mm, rotor outer radius 14 mm) was used for 0.49 %; 60mm acrylic plate (ser no 70408, gap 1000 micro m) was used for 1% to 1.5%, and 40mm steel plate hatched (ser no 994387, gap 1000 micro m) for 2% and higher concentrations. A solvent trap was employed to reduce loss of moisture. The measurements were conducted at a temperature of 25°C.

3.0 Results and Discussion

3.1 Physical composition of seed and polysaccharide yield

Peltophorum pterocarpum seeds (Figure 1) consist of 42.1% coat, 29.3 % endosperm and 28.6 % germ (Tab 1a). The endosperm is translucent and rubbery in appearance indicating a high content of polysaccharide. The polysaccharide in most seeds is located in the endosperm; hence a high content of endosperm may indicate a high content of the polysaccharide. The polysaccharide yield from *Peltophorum pterocarpum* seed was 20. 4 %. This yield is in the range of 14- 30% reported for *Leucaena leucocephala* (Pamplona & Zerrudo, 2008; Unrau, 1961; Soni & Varshney, 2003), but less than 32.6 % reported for *Mucuna flagellipes* with 67.15 % endosperm (Nwokocha & Williams, 2009) and 59.8% for *Detarium senegalense* (Wang, Ellis, Ross-Murphy, & Reid, 1996)). The *Peltophorum pterocarpum* seed polysaccharide was found to contain mannose (80.07%), galactose (18.08%), and very small amounts of glucose (0.51%), arabinose (0.29%), rhamnose (0.22%), glucuronic acid (0.46%) and galacturonic acid (0.37%). Therefore *Peltophorum pterocarpum* polysaccharide is a galactomannan with mannose:galactose (M/G) ratio of 4.4:1. The M/G ratio is similar to the 4.5:1 reported for *Peltophorum dubium* galactomannan (Mayworm et al., 2004) but higher than M/G of: locust bean gum, 4:1; Tara gum, 3:1; guar gum, 2:1 (Picout, Ross-Murphy, Jumel, & Harding, 2002; Mathur & Mathur, 2005). The M/G ratio has been reported to affect the functionality of galactomannans. Galactomannans with high percentage galactose have high cold water dispersibility and high viscosity but poor gelling properties (Mathur & Mathur, 2005). The high M/G ratio of *Peltophorum pterocarpum* galactomannan indicates that it has potential application in combination with kappa carrageenan to modify to enhance its gel characteristics.

3.2 Molecular weight

Figure 2 is the refractive index and molecular weight elution profile of *Peltophorum pterocarpum,* and the molecular characteristics are presented in Tab. 1c. The mass recovery of the polysaccharide was 91.5 %. The number average molecular weight M_n and weight average molecular weight M_w determined for the polysaccharide were 1.23×10^5 g/mol and 2.49×10^5 g/mol, respectively, giving a polydispersity index M_w/M_n of 2.02. The weight-average radius of gyration was 39 nm. Galactomannans in solutions have been reported to have an extended rod like conformation (Mathur & Mathur, 2005). The M_w of *Peltophorum pterocarpum* is lower than 8.4×10^5 g/mol reported for ***Cassia siamea*** polysaccharide (Kapoor, Milas, Taravel, Rinaudo, 1996) and 7.82×10^5 g/mol for *Copaifera langsdorfii* seed polysaccharide (Stupp, de Freitas, Sierakowski, Deschamps, Wisniewski, & Biavatti, 2008)).

3.3 Intrinsic viscosity

The $[\eta]$ of *Peltophorum pterocarpum* polysaccharide was determined by capillary viscometry in the relative viscosity range of $1.8 < \eta_r < 2.5$ corresponding to polysaccharide concentrations of 0.2% to 0.4% (w/w), and experiments at higher concentrations in rheometric steady shear measurements indicated that the polysaccharide solutions were Newtonian in this concentration range. The intrinsic viscosity of *Peltophorum pterocarpum* polysaccharide obtained by combined Huggins and Kraemer extrapolation (Figure 3a) was 3.14±0.04 dl/g. This was comparable to the value of 3.2 dl/g obtained from the slope (Slope = $1/[\eta]$) of Fedors plot (Figure 3b), which has a c_{max} of 0.84 g/dl obtained from the intercept (Intercept = $1/c_m[\eta]$). The Huggins constant, K_h, calculated from the slope ($4.1747 = k_h[\eta]^2$) was 0.42. This is in good agreement with K_h of 0.4 reported in literature (Masuoka & Cowman, 2002). The Kraemer constant, k_k obtained from the slope ($-0.3693 = k_k[\eta]^2$) was -0.04. Values of $k_h < 0.5$ are indicative of dilute polymer solutions in good solvents, so also negative values of k_k indicate good polymer solvation (Mello, Delpech, Coutinho, & Fernanda, Albino, 2006). The intrinsic viscosity of *Peltophorum pterocarpum* polysaccharide is significantly lower than 7.9 dl/g reported for *Mucuna flagellipes* (Nwokocha & Williams, 2009), 8.9 dl/g reported for *Detarium senegalense* polysaccharide (Wang et al, 1996) and 11.3 dl/g for *Cassia japonica* (Azero, Lopes & Andrade , 1997). The low value of $[\eta]$ indicates a relatively high value of c* which implies a solution of *Peltophorum pterocarpum* will exert significant effect on the viscosity only at relatively high concentrations.

3.4 Shear rate dependence of viscosity

The shear rate dependence of viscosity in the moderately concentrated solution is demonstrated in the concentration range 0.49% to 5% (w/w) (Figure 4A). Solutions less than 1% were independent of shear rate hence they exhibited Newtonian flow. However concentrations of 1.5%

and higher exhibited non-Newtonian flow with obvious shear thinning which became more pronounced as concentration increased. Several models have been employed in describing the flow properties of polysaccharide systems of moderately concentrated solutions. One of such models is the Cross model (eq. 2), which we have found suitable for describing the flow properties of *Peltophorum pterocarpum* polysaccharide; solutions of 1.0% and lower were described by the Newtonian model (eq. 3)

$$Cross: \frac{\eta - \eta_\infty}{\eta_o - \eta_\infty} = \frac{1}{1 + (\tau * \dot{\gamma})^m} \quad \text{(eq. 2)}$$

$$Newtonian: \eta = \sigma / \dot{\gamma} \quad \text{(eq. 3)}$$

In these models η, η_o, η_∞ are the shear, zero shear and infinite shear viscosities (Pa s), τ is the Cross relaxation time (s), m is Cross rate index (dimensionless), $\dot{\gamma}$ is shear rate (1/s) and σ is shear stress (Pa). The η_o is concentration dependent as shown by the increase in viscosity from 0.004 Pa s at 0.49 % to 204.4 Pa s at 5 % (w/w). The τ increased with concentration, from 3.391 x 10^{-3} s at 1.5% to 1172 s at 5%. The increase in τ can be explained from increasing degree of chain entanglement as concentration increased. This indicates a corresponding increase in the time required to relax the stresses imposed on the systems by the applied force. The rate index was in the range of $0.39 < m < 0.89$, and is a measure of the degree of dependence of viscosity on shear rate in the shear thinning region for the *Peltophorum pterocarpum*. The value of $m = 0$ for Newtonian solutions, while it approaches unity for increasing shear thinning. Figure 4B shows that the flow curves can be reduced to a simple master curve by plotting the ratio of viscosity η and zero shear viscosity η_o (η/η_o) versus the product of time constant τ and shear rate $\dot{\gamma}$ ($\tau\dot{\gamma}$). The profile shows that $\eta/\eta_o \sim 1$ as $\tau\dot{\gamma} \sim 0$. Also the polysaccharide concentrations from 3 % to 5 % were highly shear thinning in the shear rate range covered. The complete profile displayed two distinct viscosity-shear rate regions- a Newtonian region at low $\tau\dot{\gamma}$ and a shear thinning region at higher $\tau\dot{\gamma}$.

3.5 Coil overlap parameter

A complete treatment of the viscosity of *Peltophorum pterocarpum* in the entire concentration range, the dilute and moderately concentrated, was done by plotting on a log-log scale the zero shear specific viscosity, $\eta_{sp,o}$, versus volume concentration, c[η] (Figure 5A). The experimental data was fitted to the Bueche model by drawing two linear curves, one describing the dilute regime and the other the semi concentrated regime to determine the point of intersection of the two lines. This point corresponds to the point of coil overlap, c*[η], that is, the physical contact point of polymers. c* is the critical concentration and is a measure of molecular size and conformation of a polymer: the higher the molecular weight and more rigid the conformation, the lower the c*(Hwang & Shin, 2000). Intermolecular entanglements predominate the overall molecular motion of polymers at c[η] > c*[η], while individual molecules are statistically separated from other molecules at c[η] < c*[η] (Hwang & Shin, 2000). The slope of the line below c*[η] was found to be $c^{1.4}$. The region above c*[η] could not be fitted to a single linear line without excluding some points. However, if the points at 0.49% and 5 % were excluded, a fit with a slope of $c^{4.3}$ in the semi concentrated region was obtained. Hence the Bueche model could not give a complete treatment of the experimental data. We have also fitted the experimental data to some other models proposed for viscosities covering the dilute and semi dilute concentrations (Figure 5B). One of such models is the Martin model (Ren, Sutherland, & Ross-Murphy, 2003), represented by Eq. 4,

$$\eta_{sp,0} = c[\eta]\exp(k_m c[\eta]) \qquad \text{(Eq. 4)}$$

in which k_m is the Martin parameter and expresses the balance between polymer-polymer and polymer-solvent interactions. The Martin model gave a fairly good fitting with $k_m = 0.25$. For most polysaccharides, the Martin model has been reported to deviate at high values of c[η] (Masuoka & Cowman, 2002; Ratcliffe *et al.*, 2005; Nwokocha & Williams, 2012b). We have also fitted our experimental data to Eq. 5, a modified Kulicke (Ren *et al.*, 2003) and Eq. 6, an expanded Martin model for tetrahedral packing (Masuoka & Cowman, 2002), with $k_h = 0.4$ (and $n' = 3.4$ for Eq. 5). Good fitting was obtained in the region of dilute concentration but deviation at higher concentrations. The Huggins parameter, K_h, represents polymer-polymer interactions. Experimental values of K_h have been reported to range from 0.3 to 0.5 (Masuoka & Cowman, 2002).

$$\eta_{sp,o} = c[\eta] + k_h (c[\eta])^{n'} \qquad \text{(Eq. 5)}$$

$$\eta_{sp,o} = c[\eta]\{1 + k_h c[\eta] + \frac{1}{2!} k_h (c[\eta])^2 + \frac{1}{3!} k_h (c[\eta])^3\} \qquad \text{(Eq. 6)}$$

3.6 Complex viscosity

Figure 6 shows a plot of storage modulus (G′) and loss modulus (G″) versus angular frequency (ω) for 1.5% and 2% solutions of *Peltophoum pterocarpum* seed polysaccharide. At polysaccharide concentration of 1.5%, G″ > G′ and both increased with ω but no crossover occurred throughout the whole frequency range. This is entirely a viscous response of the polysaccharide solution. At polysaccharide concentration of 2%, G′ was independent of ω at low ω; this indicates a solid-like response of the polysaccharide solution. However G″ increased with increase in ω resulting in G″ crossing over G′ at ω = 0.01463 rad/s. This transition from G′ > G″ to G″ > G′ indicates a change from solid-like response to liquid-like response of the polysaccharide solution. Figure 7 shows the Cox-Merz superimposition of the data from steady shear viscosity $\eta(\dot{\gamma})$ and complex viscosity $\eta^*(\omega)$ measurements plotted at equivalent values of shear rate ($\dot{\gamma}$) and angular frequency (ω) for 1.5% and 2% polysaccharide concentrations. Both curves showed close superimposition, the deviations ($\eta^* > \eta$) observed at low shear rates are attributed to enthalpic association in which some bonds broken down under steady shear survived oscillatory shear. Disordered polymer coils normally meet the Cox-Merz empirical rule where interactions between chains in the entangled network are merely topological (Cárdenas, Higuera-Ciapara, & Goycoolea, 1997).

4.0 Conclusions

Peltophorum pterocarpum seed yielded 20.4% of a galactomannan with mannose:galactose ratio of 4.4:1. The polysaccharide had intrinsic viscosity of 3.14 dl/g and weight average molecular weight of 2.49 x 10^5 g/mol. The polysaccharide solution was Newtonian below 1% and at 4% to 5%, exhibited properties of a weak gel and has potential as a viscosifying agent in food and related products.

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Figure 1. *Peltophorum pterocarpum* seeds

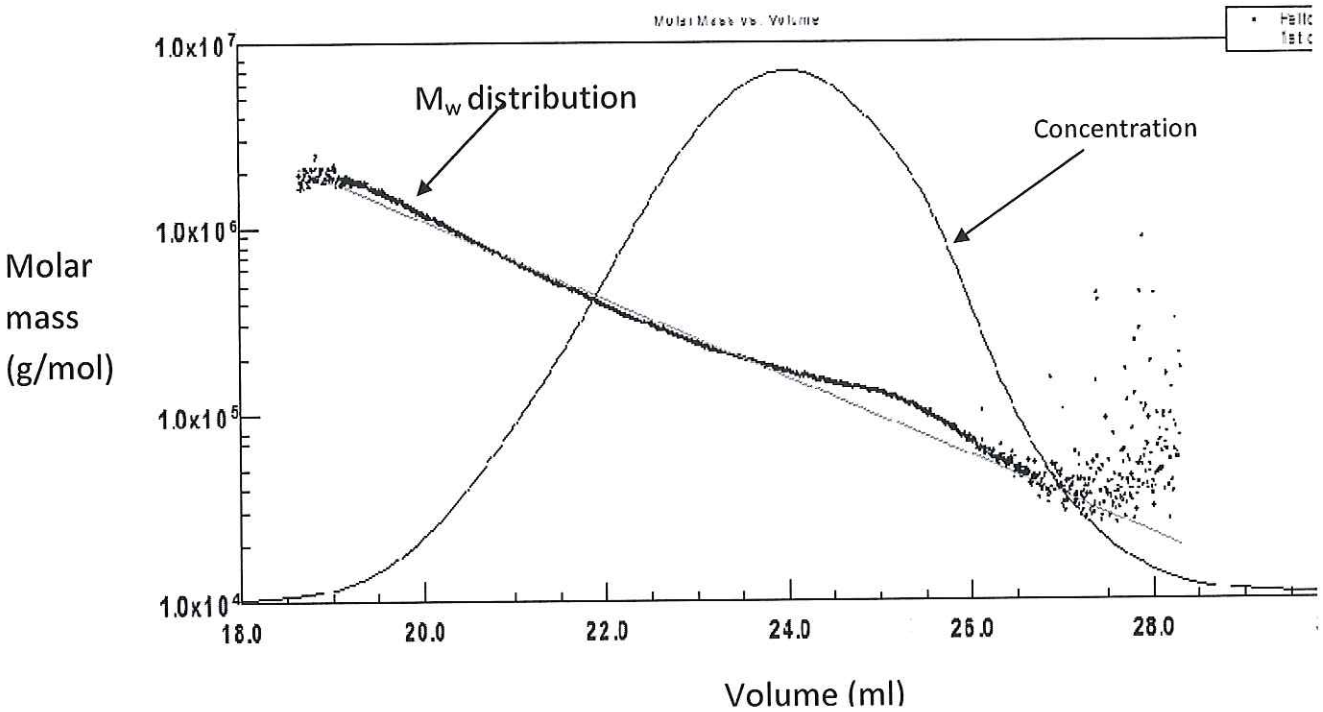

Figure 2. Molecular weight vs. elution volume for *Peltophorum pterocarpum* polysaccharide with concentration overlay. The M_w distribution was fitted to a Zimm first order polynomial.

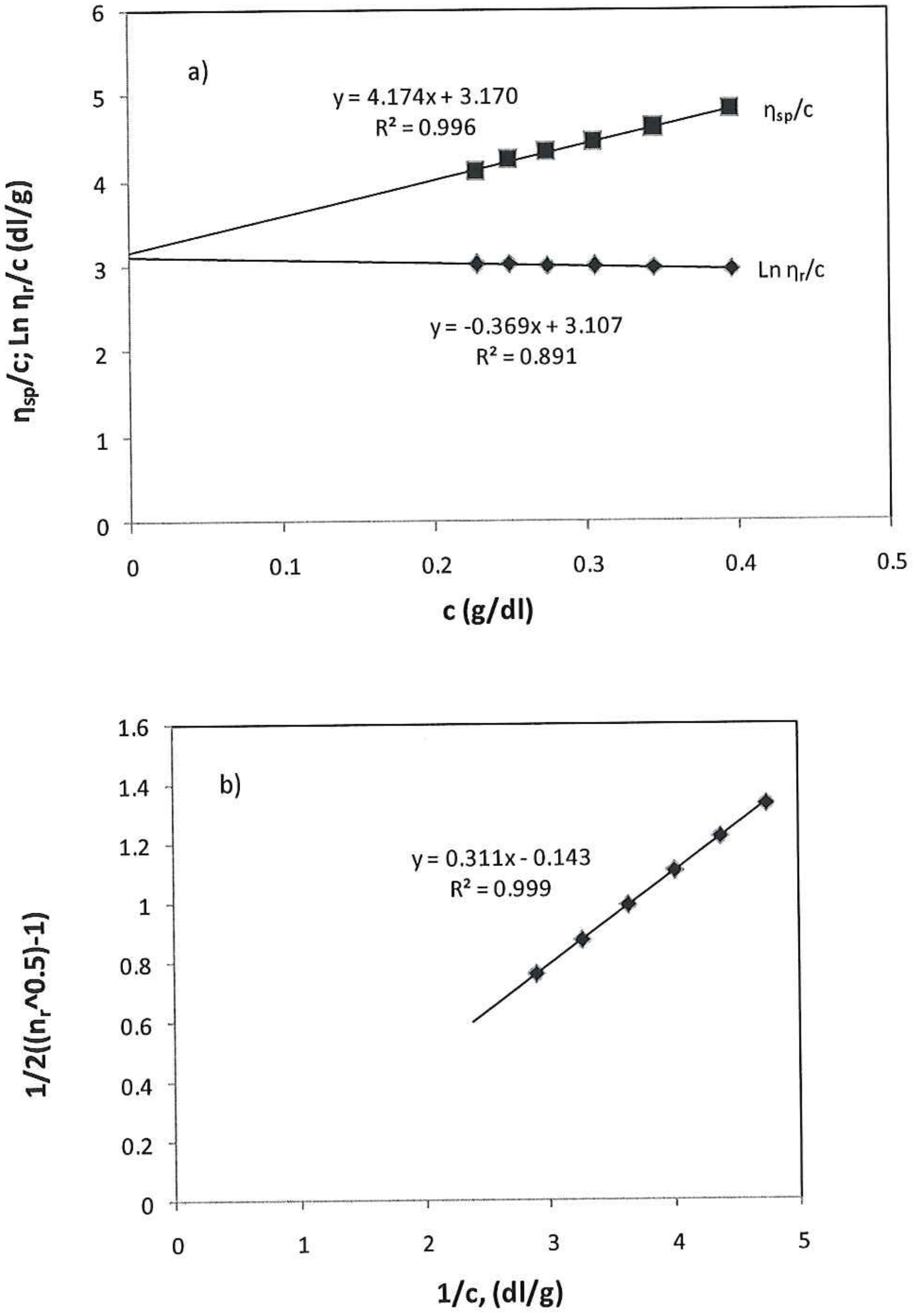

Figure 3. Determination of intrinsic viscosity of *Peltophorum pterocarpum* polysaccharide by: (a). Combined Huggins and Kraemer equations, (b). Fedors equation.

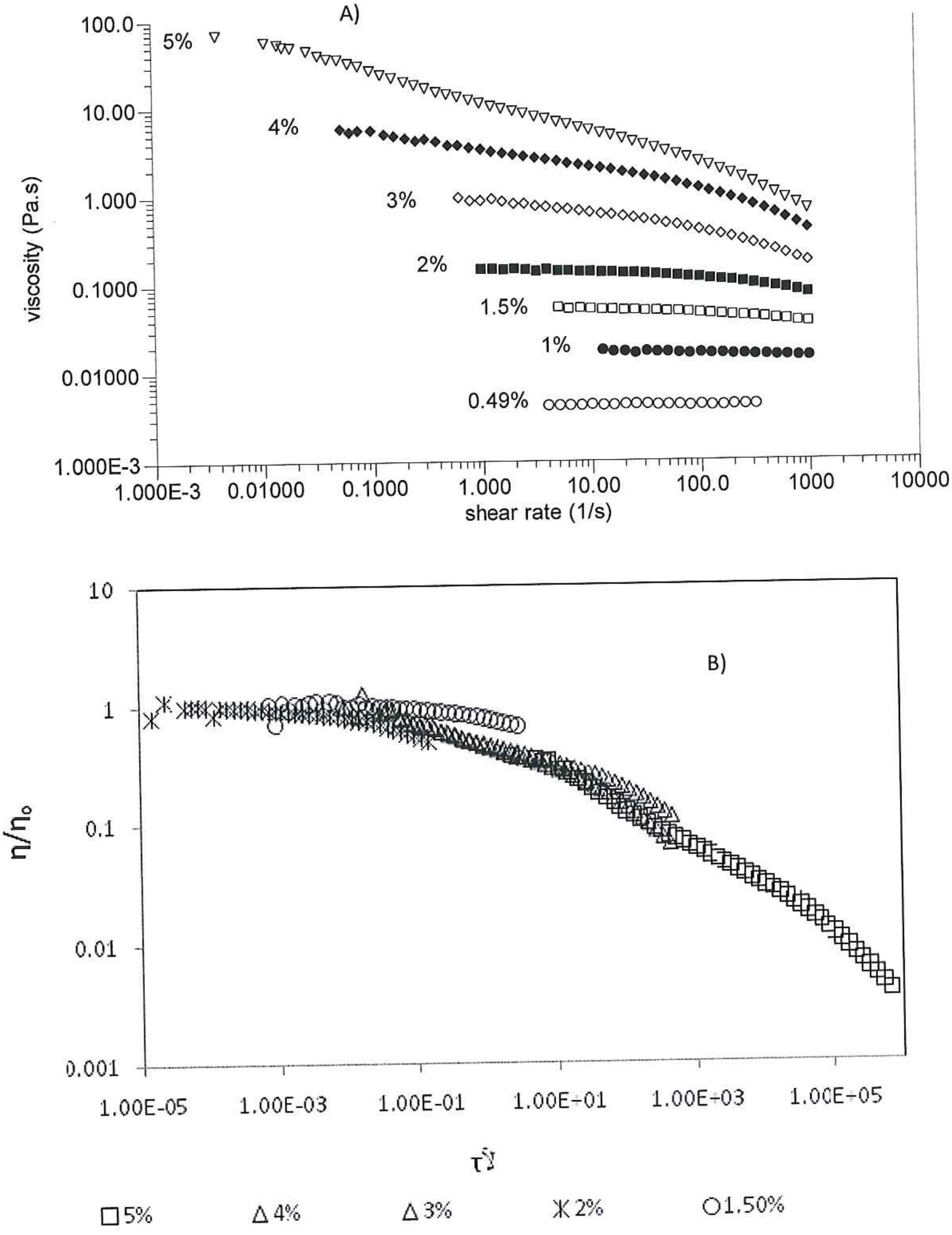

Figure 4. A). Viscosity dependence of shear rate and concentration; B). Generalized flow curves of viscosity dependence of shear rate for different concentrations *of Peltophorum pterocarpum* polysaccharide in water at 25°C.

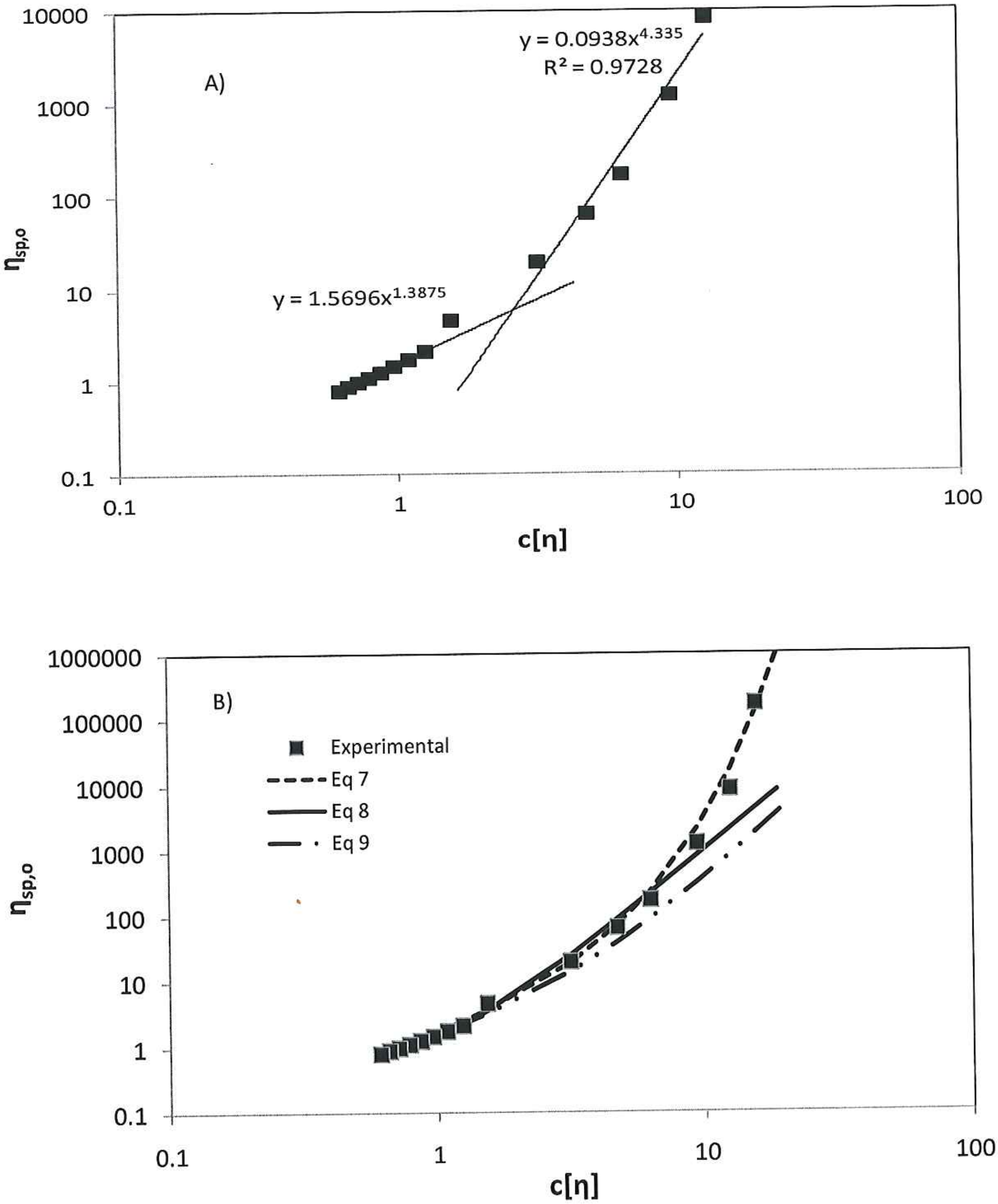

Figure 5. A). Zero shear specific viscosity versus volume concentration; B) Experimental data fitted to various viscosity models for different concentrations *of Peltophorum pterocarpum* polysaccharide in water at 25°C

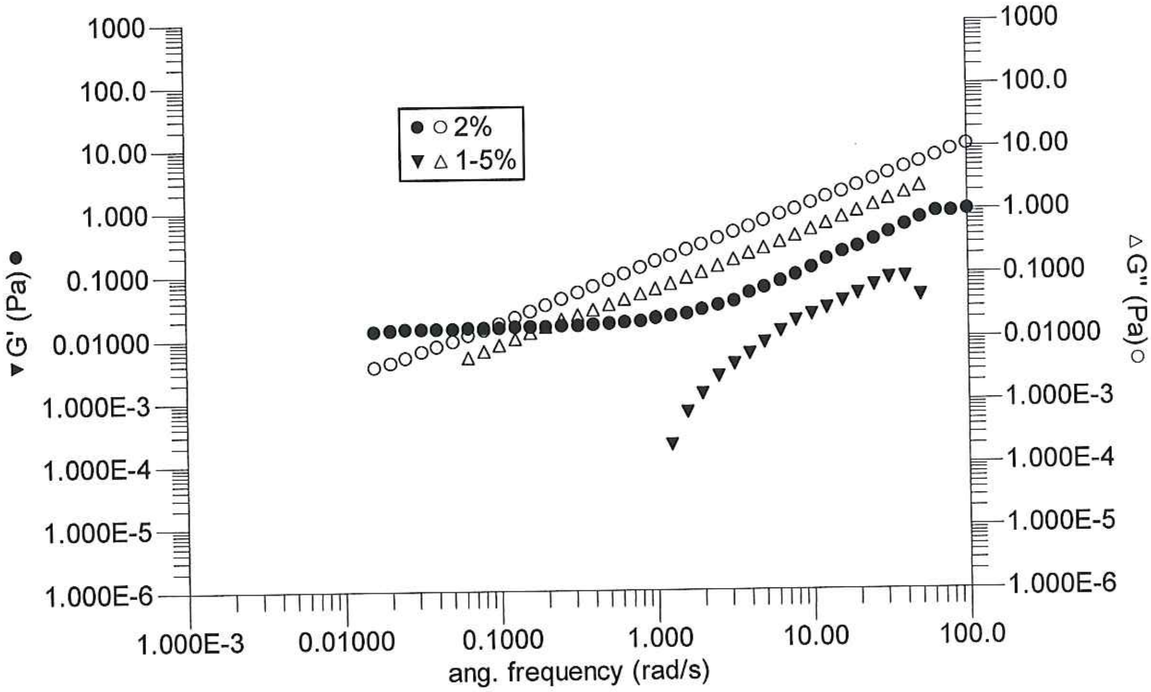

Figure 6. Elastic modulus (G′) and loss modulus (G″) as a function of angular frequency (ω) for 1.5% and 2% concentrations of *Peltophorum pterocarpum* polysaccharide at 25°C

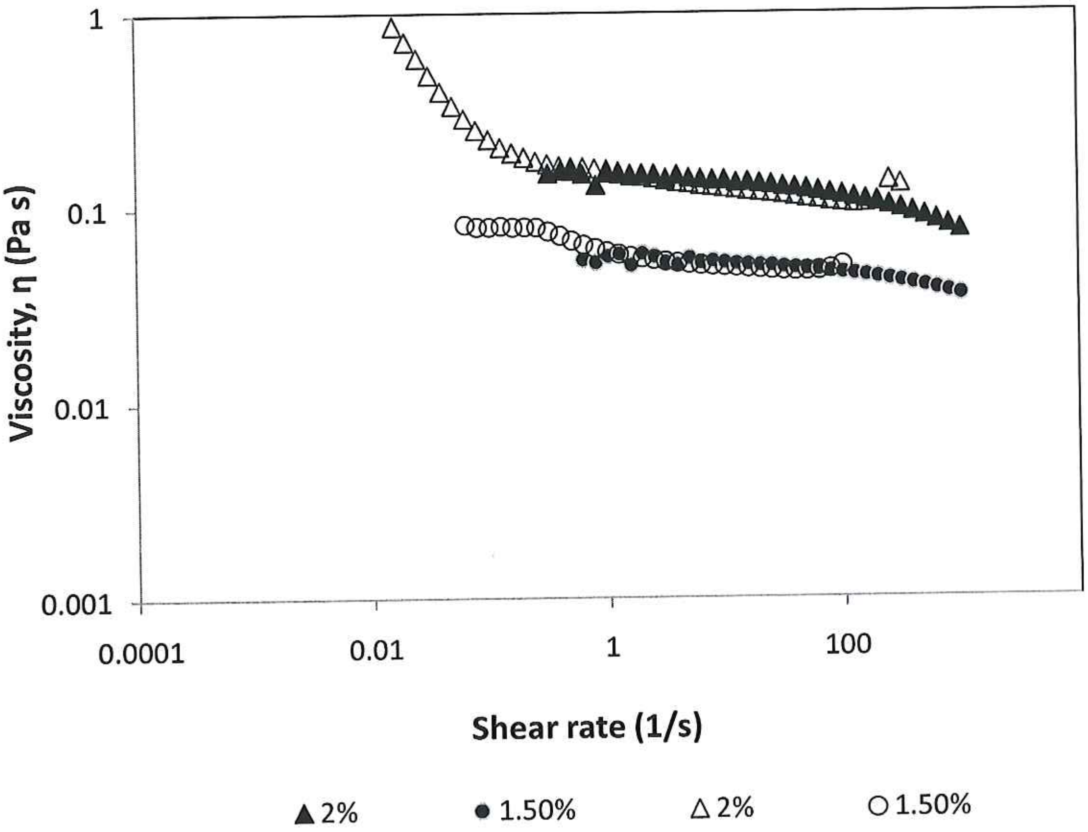

Figure 7. Cox-Merz plots: Steady shear viscosity (η) versus shear rate ($\dot{\gamma}$) (closed symbols); complex viscosity (η*) versus angular frequency (ω) (opened symbols) for 1.5% and 2% solutions of *Peltophorum pterocarpum* polysaccharide at 25°C.

Tab 1. Some properties of *Peltophorum pterocarpum* seed and polysaccharide

a). Physical composition of seed and polysaccharide yield

Parameter	Range	Mean ± SD
Coat (%)	34.7-53.5	42.06±8.05
Endosperm (%)	26.8-30.6	29.32±1.74
Germ (%)	16.3-34.7	28.62±8.36
*Yield (%)		20.4±3.1

b) Monosaccharide composition of *Peltophorum pterocarpum* polysaccharide (Relative mole %)

Rha	Ara	Gal	Glc	Man	GalA	GlcA
0.22	0.29	18.08	0.51	80.07	0.37	0.46

c). Molecular characteristics

M_n (g/mol)	1.23×10^5 (0.5%)
M_w (g/mol)	2.49×10^5 (0.29 %)
R_g (nm)	39.0 (0.22 %)
M_w/M_n	2.023 (0.6 %)
Recovery (%)	91.5

Means ± SD based on four seeds; *Mean ± SD for two determinations.

Rha= rhamnose, Ara= arabinose, Gal= galactose, Glc= glucose, Man= mannose, GalA= galacturonic acid, GlcA= glucuronic acid

R_g = weight-average radius of gyration, M_w/M_n = polydispersity index