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Rheological characterization of the galactomannan from *Leucaena leucocephala* seed

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ABSTRACT

A water soluble galactomannan isolated from *Leucaena leucocephala* seeds gave an intrinsic viscosity of 3.5 dl/g and viscosity average molecular mass, M_v, of 6.98×10^5 g/mol. This was in reasonably good agreement with the value of the weight average molecular mass, M_w, of $5.44 \pm 0.20 \times 10^5$ g/mol determined by GPC-MALLS coupled to RI. The onset of polymer coil overlap occurred at $c^*[\eta]$ of 2.1, with slope of 3.0 above and 1.3 below the point of polymer coil overlap. The shear viscosity of the polysaccharide was temperature dependent and decreased with increasing temperature. The activation energy for viscous flow of 3.0% polysaccharide concentration obtained by Arrhenius plot of zero shear viscosity as a function of temperature was 26.4 kJ/mol. Both the storage modulus (G') and loss modulus (G'') showed strong dependence on frequency indicating the presence of entangled coils. The Cox–Merz plot gave close superimposition of the complex and shear viscosities.

1. Introduction

Galactomannans from carob (*Ceratonia siliqua*) and guar (*Cyamopsis tetragonoloba*) seeds are commonly used in food products because of their good thickening properties and have been extensively studied (Closs, Conde-Petit, Roberts, Tolstoguzo, & Escher, 1999; Juszczak, Fortuna, Witczak, & Dymel, 2004).

Leucaena leucocephala (Lam.) de Wit (synonym: *Leucaena glauca*) is a lesser known plant and belongs to the legume family *Fabaceae* and subfamily *Mimosoidaeae*. It is native to America although wide spread in Africa (Shelton & Brewbaker, 1998). The plant finds use as a source of timber, fibre, and animal fodder. It is also used as an ornamental and hedgerow plant. The plant bears fairly flat pods that can contain about 20–30 seeds per pod, with seeds about 0.7 cm long in the main axis (Fig. 1). The fresh pods are used as vegetable. The seeds have potential as a source of water soluble polysaccharide. Research reports show the seeds contain about 20–25% of a galactomannan, consisting of 57% mannose and 43% galactose (Unrau, 1961) located mainly in the endosperm. The use of *Leucaena* polysaccharide as a binder in tabletting has been reported (Deodhar, Paradkar, & Purohit, 1998). In this work we isolated *L. leucocephala* polysaccharide gum and determined its physicochemical characteristics and rheological properties.

2. Materials and methods

2.1. Seed characteristics and isolation of polysaccharide

The *L. leucocephala* seeds were obtained from trees grown in the Botanical Gardens of the University of Ibadan, Nigeria. The seeds were swollen in water overnight and cut through with a thin blade to extract the endosperm. The hull and endosperm were air dried for a week to constant weight and weighed. The hull and endosperm were calculated as a percentage of the whole seed. Pulverized whole seed was defatted by extracting with n-hexane for 8 h and later extracted with methanol for 8 h to remove soluble sugars and pigments. The seed flour was dispersed in distilled water for 12 h by means of a magnetic stirrer and the solubilized polysaccharide recovered by centrifugation. The polysaccharide solution was treated with excess isopropanol during which the polysaccharide precipitated. The polysaccharide was purified by re-dissolution in distilled water, centrifugation and re-precipitation. The polysaccharide powder was recovered by freeze drying.

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. A polysaccharide solution containing 1.009×10^{-3} g/ml was filtered through a 0.45 μm syringe filter and injected into a 200 μl loop, passed through a combination of Suprema columns (100 Å, 3000 Å and 30,000 Å) packed with

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Fig. 1. *Leucaena leucocephala* seeds.

10 µm beads of polyhydroxymethacrylate copolymer network. The flow rate was 0.5 ml/min and the eluent was 0.1 M $NaNO_3$ solution containing 0.005% of sodium azide. The chromatogram was analysed with Astra software with a predetermined d*n*/d*c* value of 0.140 (Li & Xie, 2006).

2.3. Intrinsic viscosity

The intrinsic viscosity of *L. leucocephala* seed polysaccharide was determined in distilled water using a stock solution containing 0.37 g of the polysaccharide (dry basis) in 100 ml of the solution. The polysaccharide powder was dispersed at room temperature by placing on a roller mixer (SRT2, Staurt Scientific, UK). 7 ml of solution was transferred into a Cannon–Ubbelohde capillary viscometer (75, J349); the viscometer was immersed in a precision water bath to maintain the temperature at 25.0 ± 0.1 °C. The sample was left to equilibrate for 15 min and the flow time was determined between the two etched marks. Serial dilution was performed in situ and after equilibration three readings were taken for each dilution and averaged. The flow time measured in this way was converted to relative viscosity (η_r) by dividing by the flow time of water and the intrinsic viscosity, [η], evaluated by applying Fedors equation (Eq. (1)).

$$\text{Fedors equation}: \quad \frac{1}{2(\eta_r^{1/2}-1)} = \frac{1}{[\eta]c} - \frac{1}{c_m[\eta]} \qquad (1)$$

where c is concentration of polymer and c_m is a concentration factor.

2.4. Rheological characterization

Different concentrations of the *L. leucocephala* seed polysaccharide solutions (0.5–5.0%) were prepared by dispersing the desired amount of polysaccharide powder in distilled water while continuously solubilizing it overnight or more at ambient temperature by means of a roller mixer (SRT2, Stuart Scientific, UK). The rheological data were generated on a Controlled Stress Rheometer (AR 2000, TA Instruments, Newcastle, UK) at 25 °C using cone and plate geometry (6 cm diameter, truncation gap 50 µm) for sample concentrations 2–5% and standard-size recessed end geometry (rotor outer radius 14 mm, gap 4 mm) for concentrations 0.5 and 1%. Low viscosity oil, polysalylsilicone, was used to coat the periphery in the case of cone and plate geometry. The resulting data was analysed using TA Data Analysis Software.

2.4.1. Effect of concentration

The flow properties were obtained by subjecting the polysaccharide solutions to a stepped-flow procedure at shear rates from 10^{-2} to $10^3\ s^{-1}$. In the oscillation procedure, strain sweep was performed on each gum solution at a frequency of 1 Hz to locate the linear viscoelastic region. A frequency sweep was performed on the gum solutions in the region of 0.1–120 rad/s at an amplitude strain within the linear viscoelastic region.

Table 1
Physical composition of *Leucaena leucoephala* seed and molecular characteristics of the polysaccharide.

(a) Physical composition of *Leucaena leucocephala* seed[a]

Composition	Range	Mean
Weight (g)	0.037–0.045	0.042 ± 0.003
Hull (%)	43.48–48.89	46.48 ± 1.96
Endosperm (%)	51.11–56.52	53.52 ± 1.96
Germ (%)	Negligible	–
Yield	(%)	9.0

(b) Molecular characteristics of *Leucaena leucocephala* polysaccharide[b]

M_w (g/mol)	$5.444 \pm 0.020 \times 10^5$
R_g (nm)	57.6 ± 1.4
Polydipersity, M_w/M_n	1.834 ± 0.013
Mass recovery (%)	80.4 ± 0.04
M_v (g/mol)	6.98×10^5

[a] Mean for nineteen seeds ± SD.
[b] Mean + STD of two determinations. R_g = weight-average radius of gyration.

2.4.2. Effect of temperature

Effect of temperature on shear viscosity was determined at temperatures ranging from 5 °C to 65 °C on 3% polysaccharide concentration in the range of shear rates 10^{-2} to $10^3\ s^{-1}$. The effect of temperature on the viscosity of the polysaccharide solution was evaluated by fitting Arrhenius equation (Eq. (2)) to the viscosity–temperature relationship.

$$\text{Arrhenius equation for viscous flow}: \quad \eta = A \exp \frac{E_a}{RT} \qquad (2)$$

where η is the viscosity, A the pre-exponential factor (Pa s), E_a the activation energy for viscous flow (J/mol), R the gas constant (8.314 J/mol K), T is the temperature (K).

3. Results and discussion

3.1. Composition

L. leucocephala seeds were small and with an average weight of 0.04 g. The hull constituted 43.48–48.89% and endosperm 51.11–56.52% of the seed while the germ was negligible (Table 1a). Legumes generally have been reported to have moderately thick seed coat (Riahi & Ramaswamy, 2003). *Mucuna flagellipes* seed contains 32.85% hull, 67.15% endosperm and negligible germ, with the endosperm yielding 32.6% gum (Nwokocha & Williams, 2009). The seed of *Sesbania aculeata* has been reported to consist of coat (6.9–18.9%), endosperm (30–42%) and germ (51.1%) (Faroogi & Sharma, 1972) while the seed of *Cyamopsis tetragonolobus* is composed of hull (15%), germ (45%), and endosperm (40%) with the endosperm containing 75–85% of the hydrocolloid (Sharma, Dhuldhoya, Merchant, & Merchant, 2006). The yield of the water soluble polysaccharide from *Leucaena* seed was 9%. This is less than the yield of 14.9–30% reported in the literature (Pamplona & Zerrudo, 2008; Soni & Varshney, 2003; Unrau, 1961). The low yield might have resulted from losses during the purification process. Azero, Lopes, and Andrade (1997) isolated 26% of gum from *Cassia japonica* seeds.

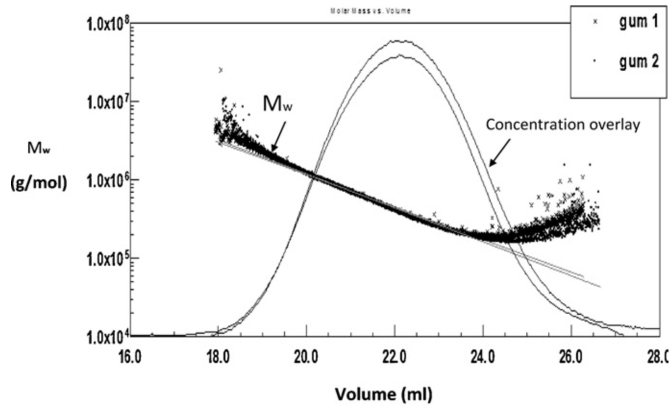

Fig. 2. GPC elution profile of *Leucaena leucocephala* polysaccharide showing M_w distribution overlaid with concentration (duplicate measurements).

3.2. Molecular weight distribution

The plot of molar mass versus elution volume is given in Fig. 2 while the molecular characteristics are presented in Table 1b. The average molecular weight (M_w) obtained from GPC-MALLS for *L. leucocephala* polysaccharide was 5.44×10^5 g/mol at a mass recovery of 80.4%. We have not found any report on the molecular weight of *L. leucocephala* polysaccharide; however, weight average molecular weights reported for some other tropical galactomannans are: 7.01×10^5 g/mol for *Cassia nodosa* seed polysaccharide (Kapoor, Milas, Taravel, & Rinaudo, 1994), 3.23×10^5 g/mol for fenugreek gum (Jiang, Zhu, Zhang, & Sun, 2007) and $0.86–1.1 \times 10^6$ g/mol for locust bean gum (Pollard et al., 2008). The radius of gyration (R_g) and polydispersity (M_w/M_n) obtained for *L. leucocephala* polysaccharide were 57.6 nm and 1.834, respectively. The polydispersity of *L. leucocephala* was higher than 1.55 reported for *Caesalpinia ferrea* var. *ferrea* (de Souza, Lucyszyn, Ferraz, & Sierakowski, 2010), and 1.35 for *Cassia grandis* (Joshi & Kapoor, 2005).

3.3. Intrinsic viscosity

The intrinsic viscosity, $[\eta]$, of *L. leucocephala* polysaccharide calculated from the slope (slope = $1/[\eta]$) of Fedors equation (Fig. 3) was 3.5 dl/g and the Fedors concentration factor, c_m, obtained from the intercept (intercept = $1/c_m[\eta]$) was 1.15 g/dl. This value is lower than 7.7 dl/g previously reported for *L. leucocephala* polysaccharide by Pamplona and Zerrudo (2008). Rincon, Clamens, Guerrero, De Pinto, and Martínez (2007) reported an intrinsic viscosity of 0.25 dl/g for the tree exudates of *L. leucocephala*, this is far too low compared with our report. In related works, intrinsic viscosity of 11.3 dl/g was reported for *Cassia japonica* (Azero et al., 1997); 8.9 dl/g for Detarium senegalense (Wang, Ellis, Ross-Murphy, & Reid, 1996); 9.0 dl/g for *Mimosa scabrella* (Ganter, Milas, Correa, Reicher, & Rinaudo, 1992); 12.1 dl/g for *Cassia nodosa* seed polysaccharide (Kapoor et al., 1994) and 8.6 dl/g for *Caesalpinia ferrea* seed polysaccharide (de Souza et al., 2010). The viscosity average molecular weight (M_v) was calculated from the intrinsic viscosity using the modified Mark–Houwink equation (Eq. (3)) for guar gum which had been applied to some other galactomannans (Lazaridou, Biladeris, & Izydorczyk, 2000,). This gave a value of M_v, 6.98×10^5 g/mol, which was slightly higher than that obtained by GPC-MALLS.

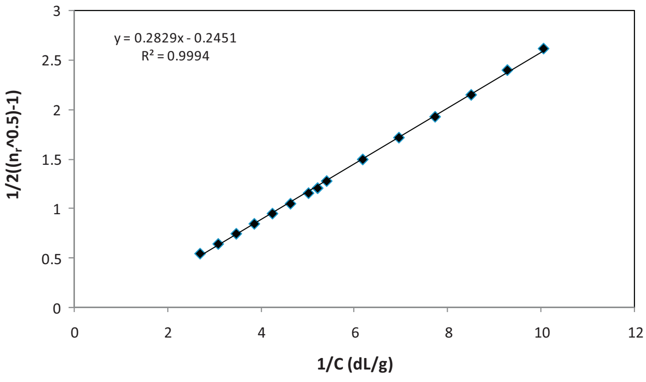

Fig. 3. Intrinsic viscosity of *Leucaena leucocephala* polysaccharide according to Fedors equation.

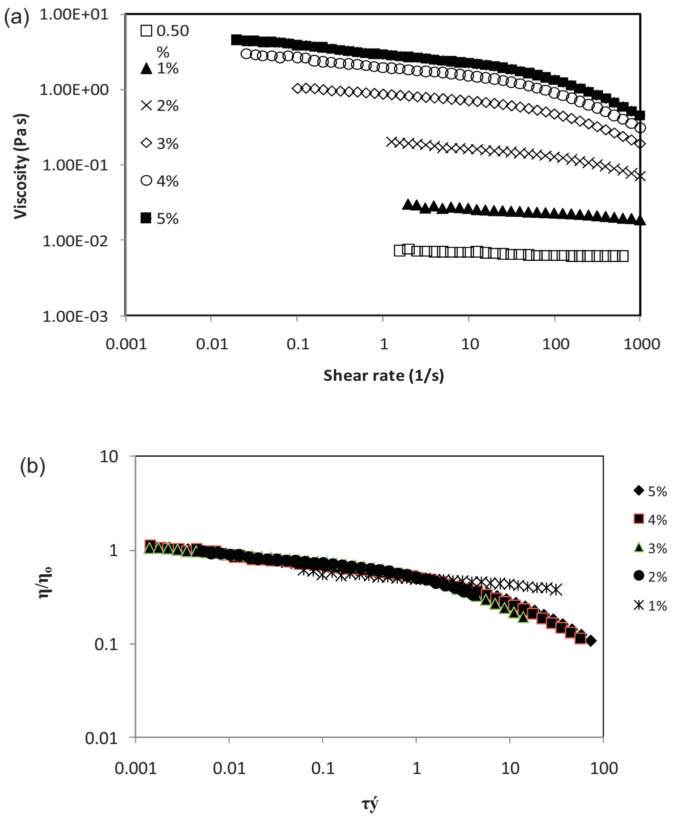

Fig. 4. (a) Viscosity–shear rate profiles of different concentrations; (b) generalized flow curves: plot of η/η_0 versus $\tau\dot{\gamma}$, at different concentrations of *Leucaena leucocephala* polysaccharide at 25 °C.

$$[\eta] = 80.2 \times 10^{-6}\ M_v^{0.79} \tag{3}$$

3.4. Shear rate dependence of viscosity and viscoelastic properties

Fig. 4a shows the shear rate dependence of viscosity of *L. leucocephala* polysaccharide solution. The polysaccharide displayed shear thinning behaviour at concentrations above 1% with a well defined Newtonian plateau at 2% and above. The flow curves at 1% and above could best be fitted to the Cross model (Eq. (4)) and 0.5% to the Newtonian model (Eq. (5)). The rheological characteristics are presented in Table 2.

$$\text{Cross model}: \quad \frac{\eta - \eta_\infty}{\eta_0 - \eta_\infty} - \frac{1}{1 + (\tau \times \dot{\gamma})^m} \tag{4}$$

$$\text{Newtonian model}: \quad \eta = \frac{\sigma}{\dot{\gamma}} \tag{5}$$

where η is the viscosity, σ the shear stress, $\dot{\gamma}$ the shear rate, and m is the rate index.

Table 2
Rheological model-fitted characteristics of *Leucaena leucocephala* polysaccharide.

Polysaccharide conc. % (w/v)	Rheological model	η_0 (Pa s)	η_∞ (Pa s)	τ (s)	m	S.E.
0.5	Newtonian	6.000E−3	–	–	–	1.998
1.0	Cross	0.02875	1.48E−3	1.835E−4	0.3157	16.68
2.0	Cross	0.2344	8.69E−8	3.116E−3	0.5955	7.451
3.0	Cross	0.8367	5.397E−8	6.957E−3	0.6318	4.263
4.0	Cross	1.862	2.434E−7	1.149E−2	0.6573	3.528
5.0	Cross	2.899	1.346E−6	1.270E−2	0.6493	10.16

η_0, η_∞ = zero and infinite shear viscosities, respectively; τ = cross relaxation time, m = rate index, S.E. = standard error.

The zero shear viscosity (η_0), infinite shear viscosity (η_∞) and Cross relaxation time (τ) increased with the increase in polysaccharide concentration. The rate index at 1% was near Newtonian. The Cross relaxation time (τ) is related to the critical shear rate ($\dot{\gamma}_{\text{crit}}$) by $1/\dot{\gamma}_{\text{crit}} = \tau$. The $\dot{\gamma}_{\text{crit}}$ marks the onset of shear thinning of the concentrated polysaccharide solution. The onset of shear thinning shifted to lower shear rates as polysaccharide concentration increased. From Fig. 4b, plots of η/η_0 versus $\tau\dot{\gamma}$ at different polysaccharide concentrations superimposed to give a master curve. At very low shear rates, the master curve approached unity. Viscosity profiles with no defined shear thinning (for example at 1%) show deviation from the master curve at shear rates approaching infinity.

From Fig. 5a, both the storage modulus, G', and loss modulus, G'', for polysaccharide concentrations 2–5% increased with increase in angular frequency, ω. At 4.0% and 5.0% the polysaccharide exhibited predominantly viscous behaviour with $G'' > G'$ in the frequency range investigated. However, at 2.0% and 3.0%, there was a transition from predominantly viscous response at short timescales of oscillation ($G'' > G'$) to a predominantly elastic response at long timescales ($G' > G''$). The cross over point occurred at $\omega = 0.269$ rad/s and $G' = 0.083$ Pa in 2%, and $\omega = 0.012$ rad/s and $G' = 0.016$ Pa in 3%. The average time for microstructural coupling, τ' (s) = $1/\omega$ (Nwokocha & Williams, 2009), estimated from the cross over point (where $G' = G''$) was 3.7 s for 2% and 84.9 s for 3%. This indicates the time for microstructural coupling was concentration dependent and increased with concentration. The interrelationship between the rotational and viscoelastic properties of *L. leucocephala* polysaccharide solutions was tested by applying Cox–Merz plots in which double logarithmic plots of shear viscosity (η) versus shear rate ($\dot{\gamma}$) and complex dynamic viscosity (η^*) versus angular frequency (ω), gave close superimposition (Fig. 5b) in which a close superimposition was obtained.

3.5. Coil overlap concentration

To estimate the coil overlap concentration, c^*, there was the need to combine the zero shear specific viscosities, $\eta_{\text{sp,o}}$, from rotational and capillary viscometry measurements by carrying out a double logarithmic plot of $\eta_{\text{sp,o}}$, versus space occupancy, $c[\eta]$. This was determined by drawing two linear curves, one describing the dilute regime and the other the concentrated regime (Fig. 6a). The intersection of the two lines provides the point of transition from the dilute regime to the semi dilute regime occurred at a critical coil overlap concentration, c^*, of $2.1/[\eta]$, and the slopes of the lines below and above c^* were 1.3 and 3.0, respectively. c^* of $2.6/[\eta]$ and slope of 4.2 in the concentrated domain was reported for *Mimosa scabrella* seed polysaccharide (Ganter et al., 1992) and c^* of $2.5/[\eta]$ and slope of 6.5 in the concentrated regime for *Cassia nodosa* (Kapoor et al., 1994). Random coil polysaccharides have values of $c^* \sim 4/[\eta]$, with the slopes of 1.4 and 3.3 below and above c^*, respectively (Morris, Cutler, Ross-Murphy, Rees, & Price, 1981).

Our experimental data was also fitted to known equations which describe the viscosity of polymers over the entire concentration range (Fig. 6b). The first of these equations fitted to the data was Martin equation (Ren, Sutherland, & Ross-Murphy, 2003) (Eq. (6)),

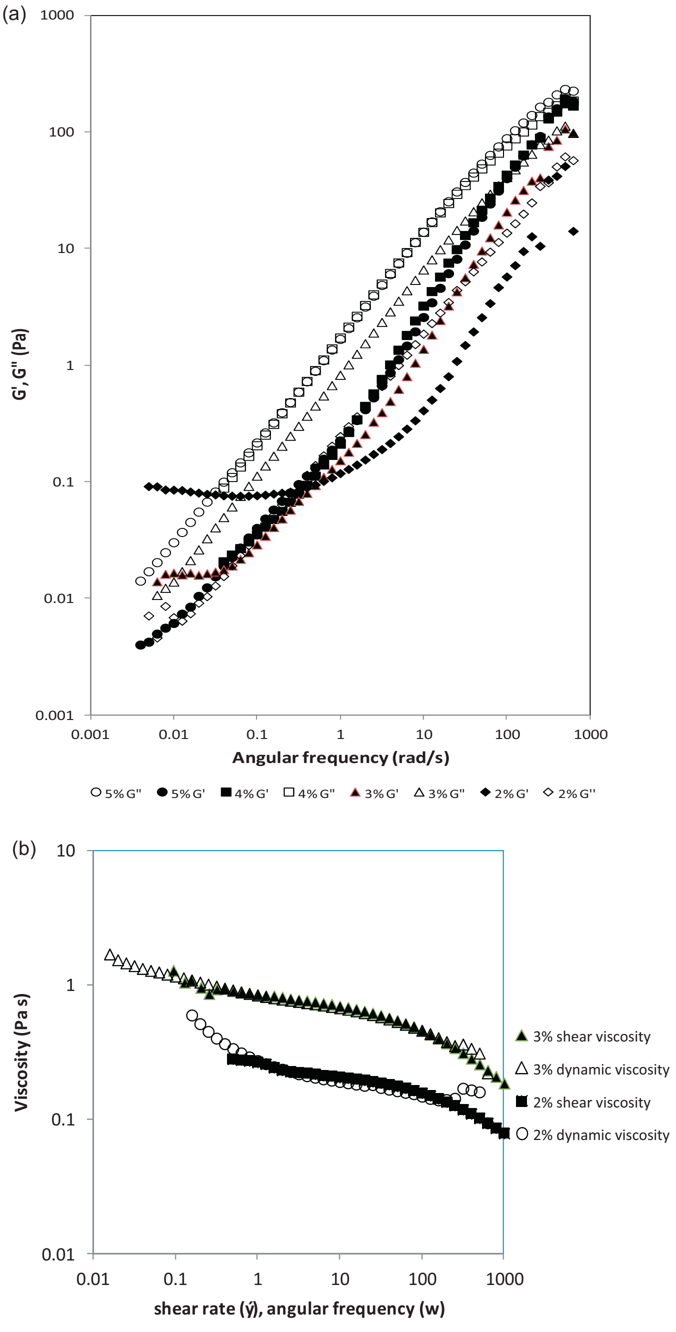

Fig. 5. (a) Frequency sweep showing G' and G'' at different concentrations of *Leucaena leucocephala* polysaccharide at 25 °C; (b) Cox–Merz plots: superimposition of $\eta(\dot{\gamma})$ and $\eta^*(\omega)$.

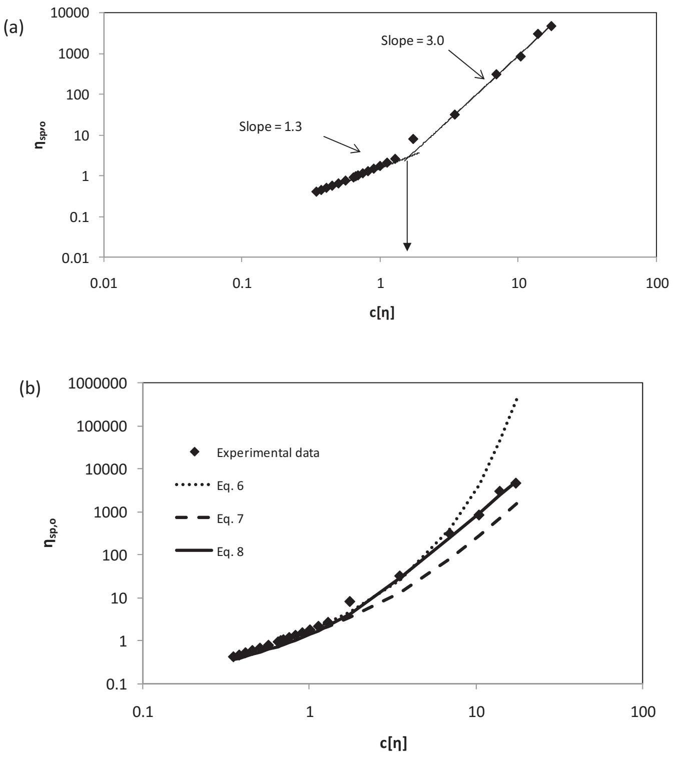

Fig. 6. (a) Plot of zero shear specific viscosity, $\eta_{sp,o}$, versus space occupancy, $c[\eta]$, showing point of polymer overlap for *Leucaena leucocephala* polysaccharide at 25 °C (b). Experimental data fitted to Eqs. (6)–(8).

where k_m is Martin parameter and expresses the balance between polymer–polymer and polymer–solvent interactions.

$$\eta_{sp,o} = c[\eta]\exp(k_m c[\eta]) \tag{6}$$

Martin equation did not give a particularly good fit to the experimental data. Close fitting was observed at $k_m = 0.25$ with $c[\eta] < 10$. Similar deviations at high $c[\eta]$ have been reported for hyaluronan at $k_m = 0.4$ (Masuoka & Cowman, 2002). Ratcliffe, Williams, Viebke, and Meadows (2005) have also reported similar deviations with konjac glucomannan.

Another commonly fitted equation is Eq. (7) (Masuoka & Cowman, 2002).

$$\eta_{sp,o} = c[\eta]\left\{1 + k_H c[\eta] + \frac{1}{2!}k_H(c[\eta])^2 + \frac{1}{3!}k_H(c[\eta])^3\right\} \tag{7}$$

where k_H is Huggins parameter and expresses polymer–polymer and polymer–solvent interactions. This equation with $k_H = 0.4$ fitted the experimental data in the region of $c[\eta] < 2$ but deviated at high $c[\eta]$. However curve-fitting of data with Eq. (8) (modified form of Kulicke equation, Ren et al., 2003) gave an excellent fit with $k_H = 0.4$ and $n = 3.3$. Similar good fits have been reported for some other polysaccharides (Ren et al., 2003; Xu, Liu, & Zhang, 2006).

$$\eta_{sp,o} = c[\eta] + k_H(c[\eta])^n \tag{8}$$

3.6. Effect of temperature

Fig. 7a shows the temperature dependence of shear viscosity of 3% *L. leucocephala* polysaccharide. The viscosity–shear rate profiles showed a decrease in viscosity with increasing temperature. This is attributed to increased kinetic motion of the molecules which resulted in a weakening of the intra- and intermolecular attractions as temperature increased. Plot of natural logarithm of zero shear viscosity (η_0) versus inverse of absolute temperature ($1/T$) (Fig. 7b) fitted into the Arrhenius equation for viscous flow with a pre-exponential factor, $A = 2.0 \times 10^5$ Pa s. The E_a was evaluated from the slope (slope = E_a/R); when the slope was multiplied by R (8.314 J mol^{-1} K^{-1}), the E_a obtained was 26.4 kJ/mol. This is the minimum energy required to effect flow in 3% *Leucaena* polysaccharide and it is a measure of the energy required to overcome the intra- and intermolecular forces of the polysaccharide solution at rest. Xu, Chen, and Zhang (2007) estimated the activation energy for viscous flow of Aeromonas gum to be 45.2 kJ/mol and reported it was independent of concentration while Bae, Oh, Lee, Yoo, and Lee (2007) working on levan polysaccharides from *Microbacterium laevaniformans* reported that the activation energy of flow decreased with increasing concentrations.

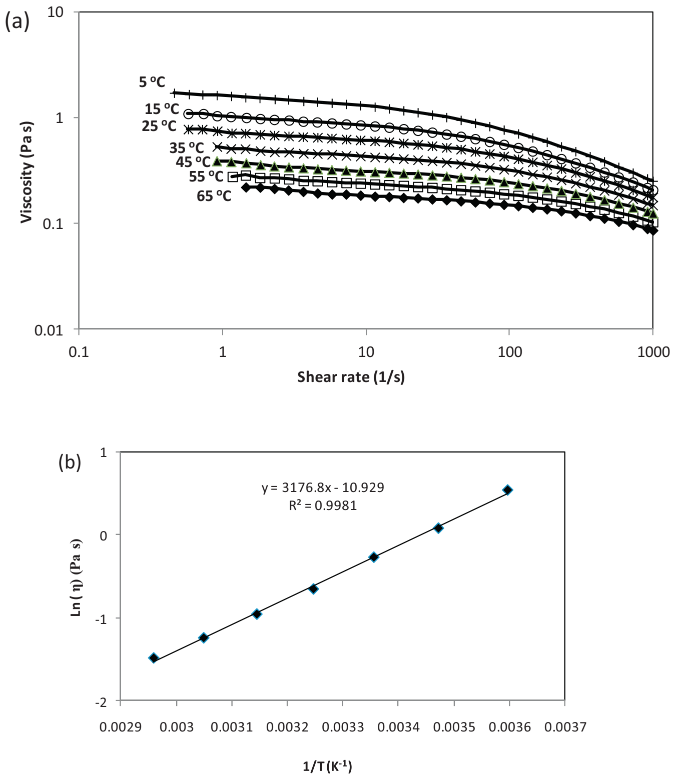

Fig. 7. (a) Effect of temperature on the viscosity–shear rate profile of 3% *Leucaena* polysaccharide, (b) Arrhenius plot: zero shear viscosity vs. temperature.

4. Conclusions

A water soluble polysaccharide isolated from *L. leucocephala* seeds had an intrinsic viscosity of 3.5 dl/g and average molecular weight of $5.44 \pm 0.020 \times 10^5$ g/mol. The solution properties were non-Newtonian at concentrations above 1% polysaccharide concentration with a coil overlap concentration, c^*, of $2.1/[\eta]$. Cox–Merz rule was closely obeyed as shown by the superimposition of the complex and shear viscosities.

Acknowledgements

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