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**Solution characteristics and thermorheology of *Prosopis africana* seed
polysaccharide**

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Abstract

Of about forty four species of the *Prosopis* family known, only *Prosopis africana* is indigenous to Africa. We have isolated the polysaccharide constituent of *Prosopis africana* seed and studied its solution properties. The polysaccharide had a weight-average molecular weight of 1.72×10^6 g/mol and intrinsic viscosity of 11.6 dL/g. The effect of concentration and temperature on the polysaccharide viscosity and microstructure were investigated under steady and dynamic shear. The polysaccharide showed typical shear thinning characteristics and the critical angular frequency at the G-crossover points ($G' = G''$) was concentration and temperature dependent and shifted to lower frequencies at higher polysaccharide concentrations. The onset of polymer overlap occurred at a critical concentration, $c^* = 0.186$ g/dL with slope of 1.4 below and 4.4 above the point of polymer overlap concentration. The rheological behavior was studied as a function of temperature and the activation energy for viscous flow of 2% solution of the polysaccharide was calculated to be 24.3 kJ/mol. The

rheological properties were shown to be similar to guar gum and hence it has potential use as a thickener in foods.

Key words: *Prosopis africana* seed polysaccharide; molecular weight; rheological properties

1.0 Introduction

Prosopis africana (Guill., Perrott. & Rich.) Taub. is a perennial leguminous tree of the subfamily Mimosoidae and is found growing in the savanna, especially Senegal and Nigeria (Agboola, 2004). There are about 44 species of the *Prosopis* family of which *P. africana* is the only species indigenous to Africa (Geesing, Al-Khawlani & Abba, 2004). The tree bears dark brown indehiscent pods 10-20 cm long and 2-3 cm in diameter. The pods are fleshy when immature, but dry at maturity leaving the seeds loose 'rattling'. Each pod contains about 10 seeds. The seeds which are 8-10 mm long and 4-9 mm across are glossy, dark brown and ellipsoid, (Vautier, Sacande & Schmidt, 2007). Bhatia, Gupta & Soni (2013) reported endosperm content of 22% for *Prosopis juliflora* seeds while Escobar et al (1987) in their work reported a polysaccharide yield of 30% from the seeds. *Prosopis* seed polysaccharide is a galactomannan with linear (1, 4) - linked β -D-mannopyranosyl units and single unit α -D-galactopyranosyl residue branch points at the O-6 position of the mannopyranosyl residues. The *Prosopis* galactomannan has been reported to have M/G ratio of 1.4 - 1.9 (Cruz Alcedo, 1999; Azero & Andrade, 2006; Matsuhira, Presle, Saenz & Urzua, 2006; López-Franco et al., 2013; Rincón, Muñoz, Ramírez, Galán & Alfaro, 2014). Intrinsic viscosity values in the range of 5.9 - 12.6

dL/g have been reported depending on the method of isolation (Cruz Alcedo, 1999). The similarity in M/G ratio for the *Prosopis* species compared to those of commercial galactomannans, notably guar and locust bean gum, suggests that they could have application in the Food and related industries as a rheological modifier (Cruz Alcedo, 1999, Azero & Andrade, 2006). A few of the properties of *P. africana* studied have been highlighted elsewhere (Nwokocha & Williams, 2012).

2.1 Isolation of polysaccharide

Cleaned whole seeds were heated in boiling water for about 30 min and the water decanted. The swollen seeds were rubbed and the translucent endosperms were separated from the hulls and germs. The endosperms were air dried to ambient moisture for over a period of one week. Approximately 10 g of the endosperm were placed in 500 mL of water and kept in a thermostated water bath at 60°C to fully hydrate overnight. This was blended in a Waring Blender, poured into centrifuge cups and centrifuged at 2500 rpm for 2 h. The supernatant was decanted and the residue reconstituted in water, blended and centrifuged. The pooled supernatant was centrifuged again and the supernatant treated with excess isopropanol to precipitate the polysaccharide. The precipitate was collected, centrifuged to expel trapped solvent and reconstituted in a small amount of water and freeze dried. The yield of polysaccharide based on endosperm was 53.6%. The polysaccharide powder was stored in a dry container.

2.2 Determination of the molecular mass

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 at ~0.01%) 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 (100 \AA , 3000 \AA and 30000 \AA) 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 data was analyzed using Astra software and it was found that the Berry second-order polynomial gave the best fit. A dn/dc value of 0.126 mL/g was used.

2.3 Intrinsic viscosity

The intrinsic viscosity of *P. africana* was determined using a Canon-Ubbelohde viscometer (75 J379). The polysaccharide powder (0.1258 g/dL, dry basis) was dissolved in water by placing on a roller mixer (SRT2, Staurt Scientific, UK) overnight. The polysaccharide solution was passed through a 0.8 micron Nylon filter and 7 mL of the solution transferred into the Canon-Ubbelohde capillary viscometer. The viscometer was immersed in a precision water bath maintained at $25.0 \pm 0.1^\circ\text{C}$ 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 , the ratio of the flow time of the polymer solution to water was calculated and used to obtain the intrinsic viscosity, $[\eta]$, according to Fedors equation (Eq. 1).

$$\text{Fedors Equation: } \frac{1}{2(\eta_r^{\frac{1}{2}} - 1)} = \frac{1}{[\eta]c} - \frac{1}{c_m[\eta]} \quad (\text{Eq. 1})$$

where c is concentration of polymer and c_m is a concentration factor and indicates the upper limit of polymer concentration in the Fedors equation.

2.4 Shear flow and oscillation studies

Shear flow and oscillation characteristics of *P. africana* polysaccharide were investigated on Advanced Rheometer (AR2000, TA Instruments, Newcastle, UK) using 0.25-2% (w/w), polysaccharide solutions in water. The geometry used was 60 mm acrylic parallel plate (serial no. 70408, gap 1000 μm). Low viscosity oil, polysilylsilicone, was used as solvent trap to prevent moisture loss. The sample was allowed to equilibrate for 2 min before measurement.

2.4.1 Effect of concentration

The samples were subjected to stepped-flow procedure at shear rates of 0.001 to 1000/s. The oscillation properties were obtained at a frequency sweep of 0.01 to 268.3 rad/s at an oscillation stress within the linear viscoelastic region. The linear viscoelastic region was determined by carrying out a strain sweep at an angular frequency of 6.283 rad/s.

2.4.2 Effect of temperature

Effect of temperature on shear viscosity was determined in the temperature range of 15°C to 75°C on 2% polysaccharide solutions in the range of shear rates 10^{-2} to 10^3 s^{-1} . In the oscillation procedure, a frequency sweep was carried from 0.01 to 268.3 rad/s at an oscillation stress within the linear viscoelastic region. A constant time of 10 s was allowed in-between measurements for temperature equilibration. The zero shear viscosity values were plotted as a function of inverse absolute temperature and fitted to the Arrhenius-type equation (Eq. 2) for viscous flow (Rincón, Muñoz, Ramírez, Galán & Alfaro, 2014) to estimate the effect of temperature on the viscous stability of the polysaccharide solution.

$$\eta_o = \eta_{o,T_\infty} \exp \frac{E_a}{RT} \quad (\text{Eq. 2})$$

η_o = zero shear viscosity (Pas), η_{o,T_∞} zero shear viscosity (Pas) at infinite temperature, E_a = activation energy for viscous flow (J/mol), R= gas constant (8.314 J/mol K), T= temperature (K).

Since η_{o,T_∞} corresponds to the zero shear viscosity at infinite temperature, the same exponential equation can be written by choosing a reference temperature (Eq. 3), in this case 333 K.

$$\eta_o = \eta_{o,333} \exp \frac{E_a}{R} \left[\frac{1}{T} - \frac{1}{333} \right] \quad (\text{Eq. 3})$$

3. Results and Discussion

3.1 Molecular mass

Figure 1 shows the GPC refractive index and corresponding molecular mass elution profiles of the *P. africana* polysaccharide. The mass recovery was 96.4% and the molecular parameters obtained by fitting to Berry second-order polynomial are presented in Table 1. The weight average molecular weight of *P. africana* polysaccharide is in the range of values (1.2×10^6 - 1.8×10^6 g/mol) reported for polysaccharides of other *Prosopis* species (Cruz Alcedo, 1999; López-Franco, Cervantes-Montaño, Martínez-Robinson, Lizardi-Mendoza & Robles-Ozuna, 2013). It is also within the range of M_w reported for guar gum (5.0×10^5 - 8.0×10^6 g/mol) (Rodge, Sonkamble, Salve, Hashmi, 2012) and locust bean gum, 4×10^5 - 1.0×10^6 g/mol (Kuntz, 2009; Haddarah, Bassal, Ismail, Gaiani, Ioannou, Charbonnel, Hamieh & Ghoul, 2014) indicating that *P. africana* polysaccharide should be effective as a thickener.

3.2 Intrinsic viscosity

Figure 2 shows the Fedors plot used to determine the intrinsic viscosity of *P. africana* seed polysaccharide. The intrinsic viscosity of *P. africana* polysaccharide was 11.6 dL/g, and the Fedors concentration parameter, c_m , 0.446 g/dL. The intrinsic viscosity of *P. africana* polysaccharide is in the range of 9.4 – 12.36 dL/g reported for polysaccharides from other *Prosopis species* (Azero & Andrade, 2006; López-Franco et al., 2013; Rincón, Muñoz, Ramírez, Galán & Alfaro, 2014). The intrinsic viscosity of *P. africana* is also in the range of values reported for guar polysaccharide (10.3 - 12.5 dL/g) (Azero & Andrade, 2006; Frollini, Reed, Milas & Rinaudo, 1995) and for locust bean polysaccharide (15.2 dL/g) (Andrade, Azero, Luciano & Goncalves, 1999). It is higher than values reported for some other seed polysaccharides: 4.7 dL/g for tamarind (Khounvilay and Sittikijyothin, 2012), 4.3 dL/g for *Brachystegia eurycoma* (Nwokocha & Williams, 2014).

3.3 Dependence of viscosity on concentration and shear rate

Fig 3a shows the viscosity-shear rate profiles for different concentrations of *P. africana* seed polysaccharide and the flow characteristics obtained by fitting to the Williamson model (Eq. 4) is presented in Table 2a.

$$\text{Williamson model: } \frac{\eta}{\eta_0} = \frac{1}{(1 + (\tau * \dot{\gamma})^N)} \quad (\text{Eq. 4})$$

where η , and η_0 are shear and zero shear viscosities, respectively, and $\dot{\gamma}$, τ , and N are shear rate, Williamson relaxation time and Williamson rate index, respectively.

The polysaccharide solutions exhibited typical polysaccharide solution behavior with a Newtonian plateau (η_0) at low shear rates and a shear thinning region at higher shear rates

(Nwokocha & Williams, 2014; Xu, Liu, & Zhang, 2006; Hwang & Shin, 2000; Wang, Ellis, Ross-Murphy & Burchard, 1997). The viscosity of *P. africana* increased with increase in concentration as indicated by increase in zero shear viscosity (0.0287 Pa s at 0.25% to 216.6 Pa s at 2%). The Williamson relaxation time, τ , increased with increase in concentration from 2.361e-3 s at 0.25% to 2.302 s at 2%. The relaxation time is related to the critical shear rate, $\dot{\gamma}_{crit}$, ($\dot{\gamma}_{crit} = 1/\tau$) which marks the onset of shear thinning. The onset of shear thinning shifted to lower shear rates at higher polymer concentration. The polysaccharide solutions exhibited non-Newtonian properties in the range of concentrations studied with the Williamson rate index, N , of $0.5608 < N < 0.7998$. N is a measure of the shear thinning properties of the solutions. $N = 0$ for a Newtonian fluid and approaches unity for high shear thinning fluids. This is reflected in the increase in values of N as concentration of polysaccharide solutions increased. In Figure 3b, the flow profiles were reduced to a single master curve by plotting the reduced viscosity, η/η_0 , against the product of shear rate and the time constant, $(\tau \dot{\gamma})$. The curve shows that $\eta/\eta_0 \sim 1$ as $\tau \dot{\gamma} \sim 0$. For Newtonian fluids, $\tau \dot{\gamma} = 0$ but infinity for a perfect solid.

3.4 Coil overlap parameter

Fig 4 shows a double logarithmic plot of the zero shear specific viscosity, $\eta_{sp,o}$, versus coil overlap parameter, $c[\eta]$, for *P. africana* polysaccharide. The data can be fitted to two linear lines, one describing the dilute solution regime and the other the concentrated regime. The polymer concentration at this point of intersection is the critical overlap concentration, $c^* = 0.186$ g/dL, ($c^* = 2.2/[\eta]$), and corresponds to the physical point of polymer contact. At $c < c^*$, the polysaccharide chains are statistically separated from each other while at $c > c^*$, the polysaccharide chains interpenetrate each other (Hwang & Shin, 2000). The viscosity

dependence of concentration is seen in the change in exponent from $c^{1.4}$ (in the dilute regime) to $c^{4.4}$ (in the concentrated regime). The value $c^{4.4}$ obtained for *P africana* in this work is higher than $c^{-3.3}$ obtained by Morris, Cutler, Ross-Murphy, Rees, & Price (1981) in the concentrated regime for conformational disordered polysaccharides. Similar deviations have been reported for some other polysaccharides and Rincon et al (2014) have provided a good review of the literature. These included fenugreek (4.6), guar (3.9) locust bean gum (4.2), tara (4.3) and *P. juliflora* (3.7). The deviation has been explained to arise from occurrence of chain-chain interactions (hyper-entanglements) in addition to physical interaction of overlapping polymer coils (Rincon et al, 2014).

3.5 Dependence of dynamic properties on concentration and shear rate

Fig 5 shows the mechanical spectra for different concentrations of polysaccharide solution in water and the microstructural parameters are presented in Table 2b. At 0.5% polymer concentration, G'' was higher than G' at all the angular frequencies with both G' and G'' increasing with increase in angular frequency. This is typical for polymer solutions at concentrations below the critical polymer coil overlap concentration. For polymer concentrations of 0.75% to 2%, at lower angular frequencies, G'' was greater than G' but G' became greater than G'' at higher frequencies and the crossover occurred at a critical angular frequency, ω_{crit} of 28 rad/s in 0.75%, 11 rad/s in 1%, 4 rad/s in 1.5% and 2.0 rad/s in 2%. This behavior is indicative of polymer solutions above coil overlap (Ren, Ellis, Sutherland, Ross-Murphy, 2003). The characteristic time for microstructural coupling ($\tau' = 1/\omega_{crit}$, (s)) increased as polymer concentration increased. The overall response of *P. africana* polysaccharide can be described in terms of the complex modulus, G^* , and the complex viscosity, η^* , where $|\eta^*| = |G^*|/\omega$ and $|G^*| = (|G'|^2 + |G''|^2)^{1/2}$. Figure 6 shows the correlation of the shear viscosity, η , and the complex

viscosity, η^* , for polymer concentrations 1% and 1.5%; tested by superimposing $\eta(\dot{\gamma})$ on $|\eta^*(\omega)|$ for $\dot{\gamma} = \omega$. The complex viscosity, η^* , is the oscillatory analogue of shear viscosity, η , such that $\eta^*(\omega)$ should exhibit a similar profile with changes in ω as $\eta(\dot{\gamma})$ with $\dot{\gamma}$. The comparison of $\eta^*(\omega)$ and $\eta(\dot{\gamma})$ (Cox-Merz plots) gave a good superimposition for the *P. africana* polysaccharide as reported for some other seed polysaccharides (Wang et al., 1997; Nwokocha and Williams, 2014).

The high intrinsic viscosity of *P. africana* which is in the range of values reported for guar (Frollini et al., 1995; Azero & Andrade, 2006) has necessitated a comparison of their shear and dynamic properties to see if they can replace each other in industrial application. Figure 7a showed the comparison of the shear viscosity of *P. africana* and guar polysaccharides at 2% concentration and the flow curves fitted to the Williamson model. Guar polysaccharide exhibited higher η_0 (567.5 Pa s) and relaxation time (43.84 s) than *P. africana* (339.3 Pa s; 5.485 s), however, *P. africana* ($N= 0.7766$) exhibited better stability to shear than guar polysaccharide ($N= 0.8510$). Figure 7b compared the G' , G'' and η^* of 2% concentrations of the polysaccharides as a function of angular frequency. For guar polysaccharide, $G' > G''$ throughout the range of frequency sweep used indicating an elastic response (gel-like properties) of the polysaccharide. *P. africana* exhibited an overall viscoelastic response: a viscous response ($G'' > G'$) below the crossover point ($\omega = 1.994$ rad/s) and an elastic response ($G' > G''$) above this point. Guar polysaccharide exhibited superior thickening properties but less shear stability than *P. africana* polysaccharide.

3.6 Effect of temperature on shear viscosity and dynamic properties

The viscosity-shear rate profiles for 2% solutions of *P. africana* polysaccharide at different temperatures are shown in Figure 8a. The relaxation times obtained by fitting the shear viscosity curves to the Williamson model were temperature-dependent and decreased from 6.44 s at 15°C to 1.80 s at 75°C. The shear viscosities at low shear rates decreased as temperature increased. The higher viscosity observed at lower temperatures is attributed to increased relaxation time for molecular entanglements. The E_a obtained from the plot of log zero shear viscosity against inverse absolute temperature for 2% solutions of *P. africana* polysaccharide (Figure 8b) was 24.3 kJ/mol ($R^2 = 0.993$). This is the minimum energy required to initiate flow in the solution of the polysaccharide at rest. This value is slightly higher than activation energies of flow in the range of 19.2-22.8 kJ/mol for 2% and 3% gum solutions of *P. africana* reported by Achi & Okolo (2004) while E_a of 24.17 kJ/mol was reported for 1% solution of *P. juliflora* by Rincon et al. (2014). The activation energy for viscous flow is dependent on concentration (Nwokocha & Williams, 2014), ionic strength, polymer physicochemical characteristics and on the shear stress applied (Rincon et al., 2014).

Table 3 shows the parameters of frequency sweep at different temperatures. From the table, an increase in temperature shifted the crossover point to higher frequencies on the other hand the time for microstructural coupling (denoted by $1/\omega$) decreased. At 15°C, the time for microstructural coupling was 1.2488 s but as temperature increased to 55°C it decreased to 0.2313 s. The polysaccharide microstructure exhibited temperature sensitivity. At lower temperatures, that is, low vibrational energy the polymer coils exhibited strong inter- and intra-molecular association. As temperature increased, the increases in vibrational energy of the molecules resulted in the weakening of the intra- and inter- molecular bonds. This explains why the time for microstructural coupling decreased as temperature increased.

4.0 Conclusion

The polysaccharide isolated from *P. africana* exhibited a high intrinsic viscosity of 11.6 dL/g and weight average molecular weight of 1.72×10^6 g/mol. The *P. africana* polysaccharide exhibited high viscosity at moderately low concentrations and the solution was non-Newtonian at concentration as low as 0.25%. Under oscillatory shear, polysaccharide solutions less than 0.5% gave purely viscous response while higher concentrations up to 2% gave viscoelastic response. The polysaccharide solutions were temperature dependent and the viscosity decreased with increase in temperature. The activation energy of viscous flow of a 2% polysaccharide solution was 24.3 kJ/mol. The polysaccharide has similar rheological characteristics to guar gum and could have potential application as a thickener in food systems.

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Table 1. Molecular characteristics of *Prosopis africana* seed polysaccharide

Molar mass moments (g/mol)	
M_n	1.059 E+06 (0.7%)
M_w	1.720 E+06 (0.5%)
M_z	2.807E+06 (1.3%)
Polydispersity	
M_w/M_n	1.623±0.013 (0.8%)
M_z/M_n	2.650±0.038 (1.4%)
R.M.S radius moments (nm)	
R_n	86.5 (2.9%)
R_w	105.5 (1.8%)
R_z	130.4 (1.6%)
Mass recovery (%)	96.4

M_n , M_w , M_z = number average, weight average, z-average molecular weights, respectively; R_n , R_w , R_z = number average, weight average and z-average root mean radius moments, respectively.

Table 2a. Rheological characteristics obtained by fitting the Williamson model to different concentrations of *Prosopis african* seed polysaccharide at 25°C

Conc. (g/dL)	η_0 (Pa s)	τ (s)	$\dot{\gamma}_{crit}= 1/\tau$ (1/s)	N	se
2.0	216.6	2.302	0.4344	0.7998	17.44
1.5	55.75	0.9613	1.040	0.7826	11.53
1.0	9.642	0.3453	2.896	0.7538	4.734
0.75	2.423	0.1581	6.325	0.6995	8.096
0.5	0.4150	0.0463	21.598	0.6329	12.96
0.4	0.1618	0.0200	50.000	0.6344	8.28
0.3	0.0548	6.794e-3	147.189	0.5668	12.88
0.25	0.0287	2.361e-3	423.549	0.5608	17.24

η = shear viscosity, η_0 = zero shear viscosity, $\dot{\gamma}$ = shear rate, τ = Williamson relaxation time, N = Williamson rate index, se = standard error

Table 2b. Effect of concentration of *Prosopis africana* polysaccharide solution on the microstructural parameters of frequency sweep at 25°C

Conc (g/dL)	G' crossover (Pa)	ω_{crit} (rad/s)	$1/\omega_{crit}$ (s)
2.0	72.37	1.994	0.5015
1.5	41.79	4.034	0.2479
1.0	20.02	11.76	0.0850
0.75	12.07	28.88	0.0346

G' = storage modulus, ω_{crit} = angular frequency at crossover point, $1/\omega_{crit}$ = time for microstructural coupling

Table 3. Effect of temperature on the microstructural parameters of frequency sweep on 2% *Prosopis africana* polysaccharide solution

Temperature (°C)	G' cross over (Pas)	ω_{crit} (rad/s)	1/ ω_{crit} (s)
15	83.74	0.8008	1.2488
25	72.37	1.994	0.5015
45	87.45	2.893	0.3457
55	89.55	4.323	0.2313

G' = storage modulus, ω_{crit} = angular frequency at crossover point, 1/ ω = time for microstructural coupling

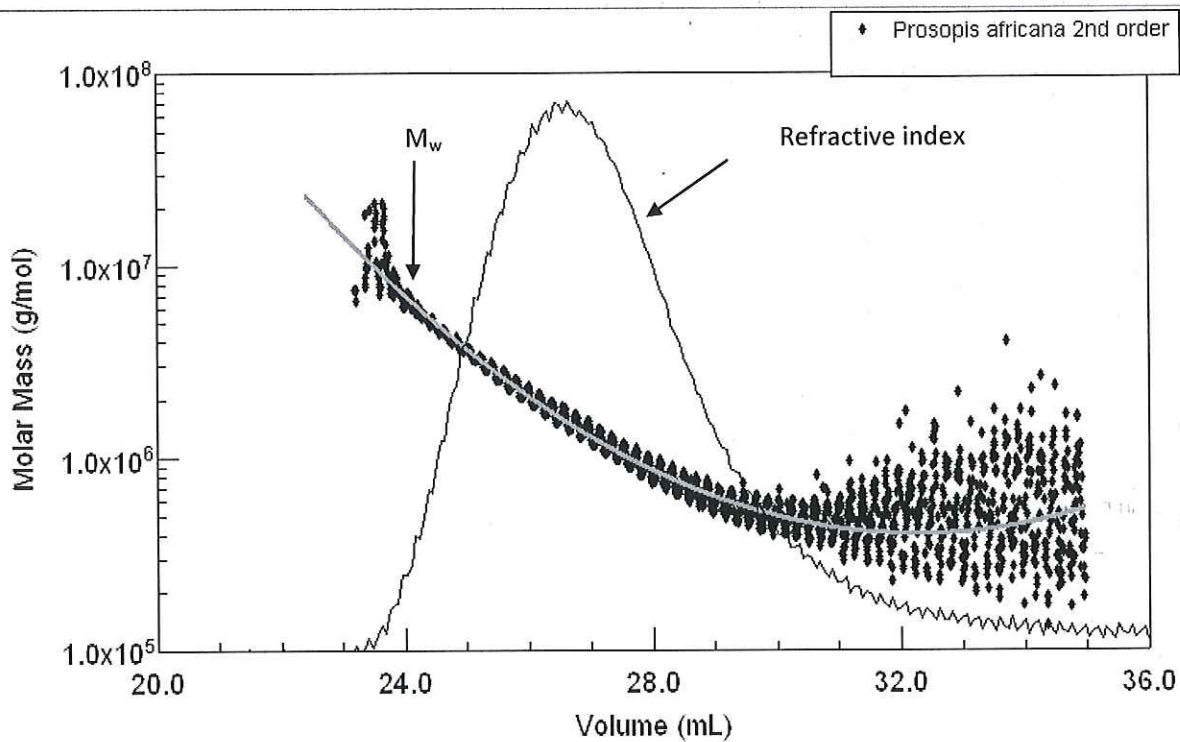


Figure 1: The GPC/MALLS RI and Mw elution profiles of *Prosopis africana* seed polysaccharide

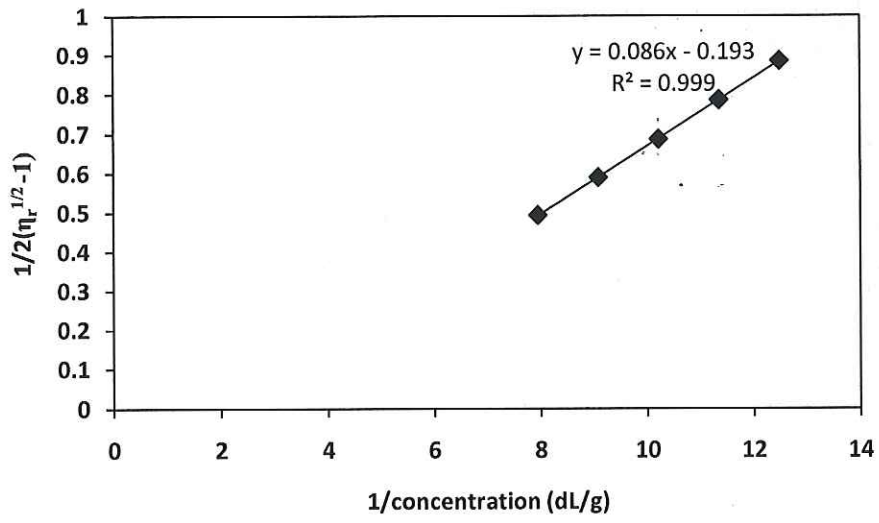
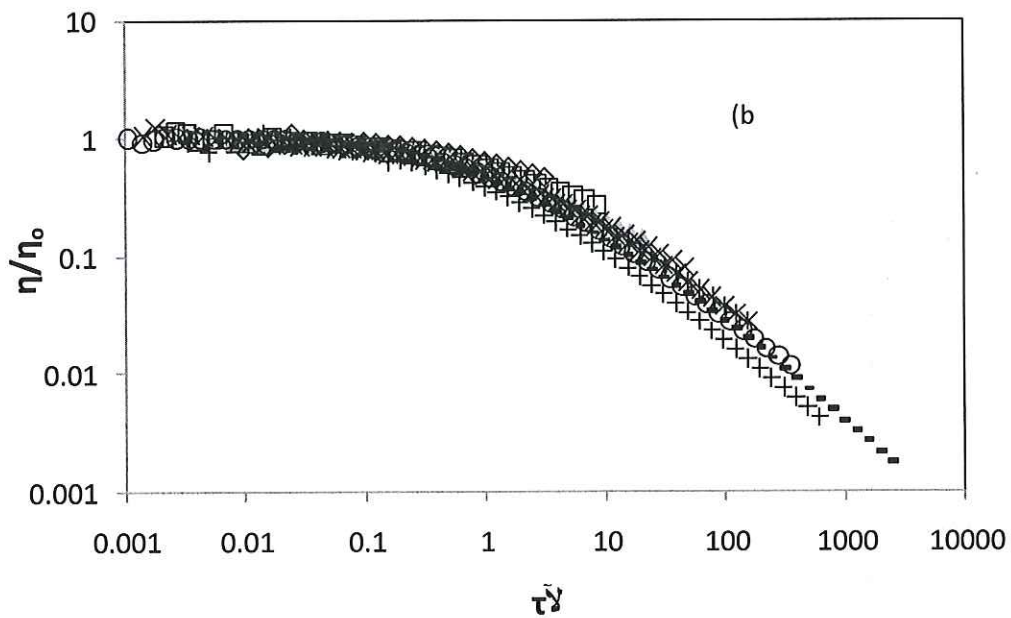
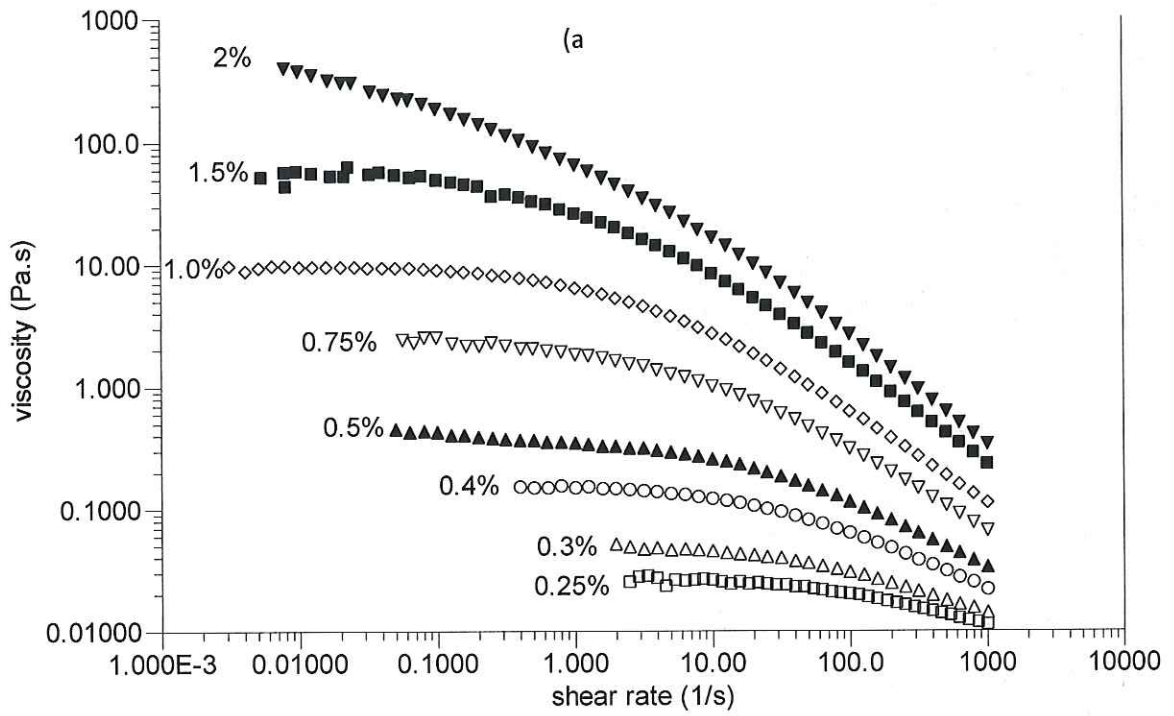


Figure 2. Fedors plot for determination of intrinsic viscosity of *P. africana* polysaccharide



◇ 0.25% □ 0.30% ▲ 0.40% × 0.50% ✖ 0.75% ○ 1% + 1.50% - 2%

Figure 3 a). Viscosity-shear rate profiles for different concentrations of *Prosopis africana* seed polysaccharide in water at 25°C fitted to Williamson model. b). Generalized flow curve:

Reduced viscosity (η/η_0) versus Deborah Number ($\tau\lambda$)

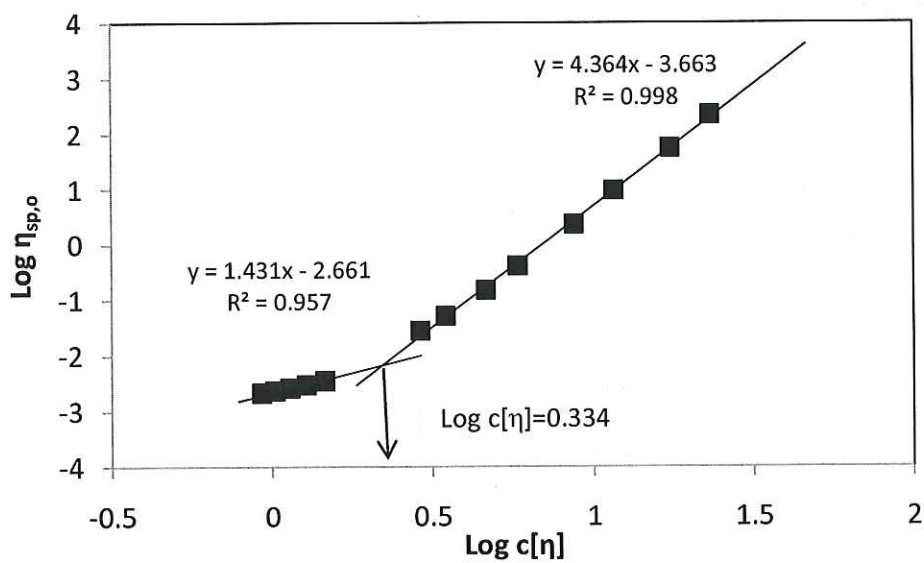


Figure 4. Log zero shear specific viscosity ($\eta_{sp,0}$) versus coil overlap concentration ($c[\eta]$).

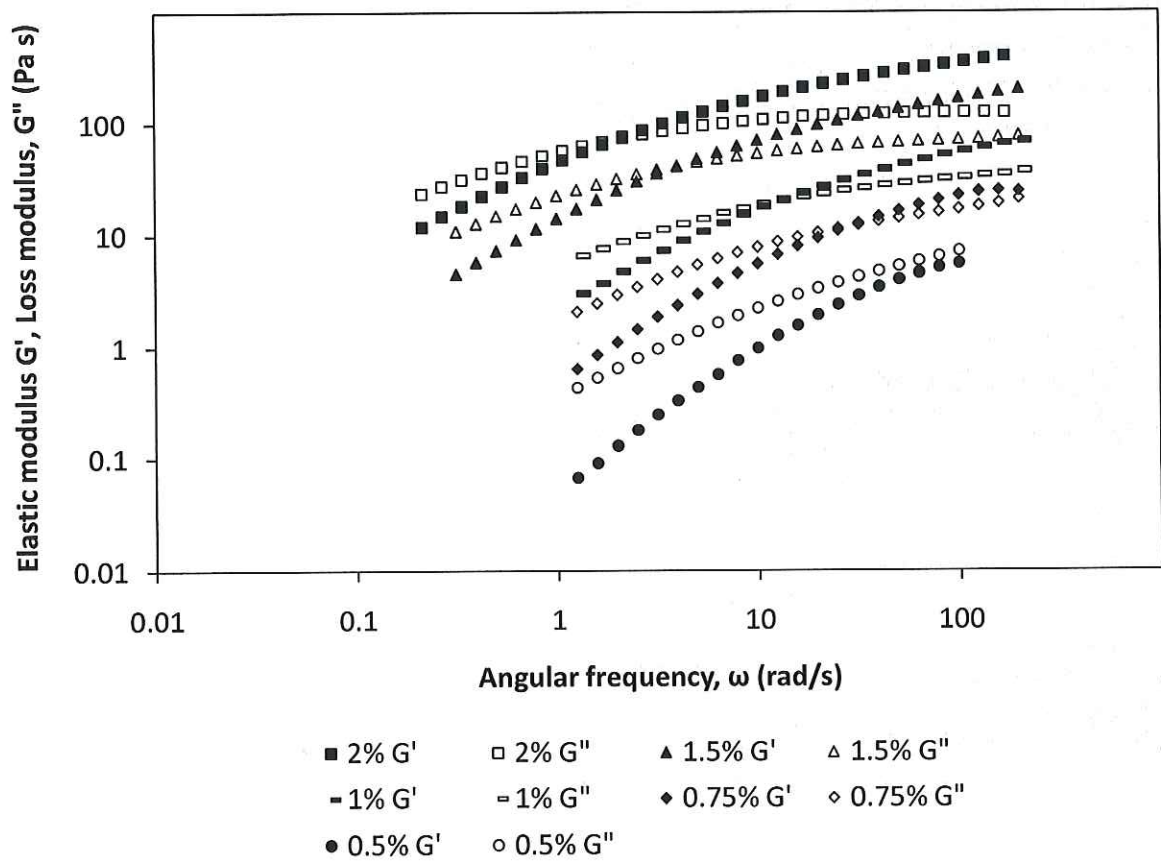


Figure 5 . G' , G'' as a function of angular frequency for different concentrations of *Prosopis africana* polysaccharide at 25°C

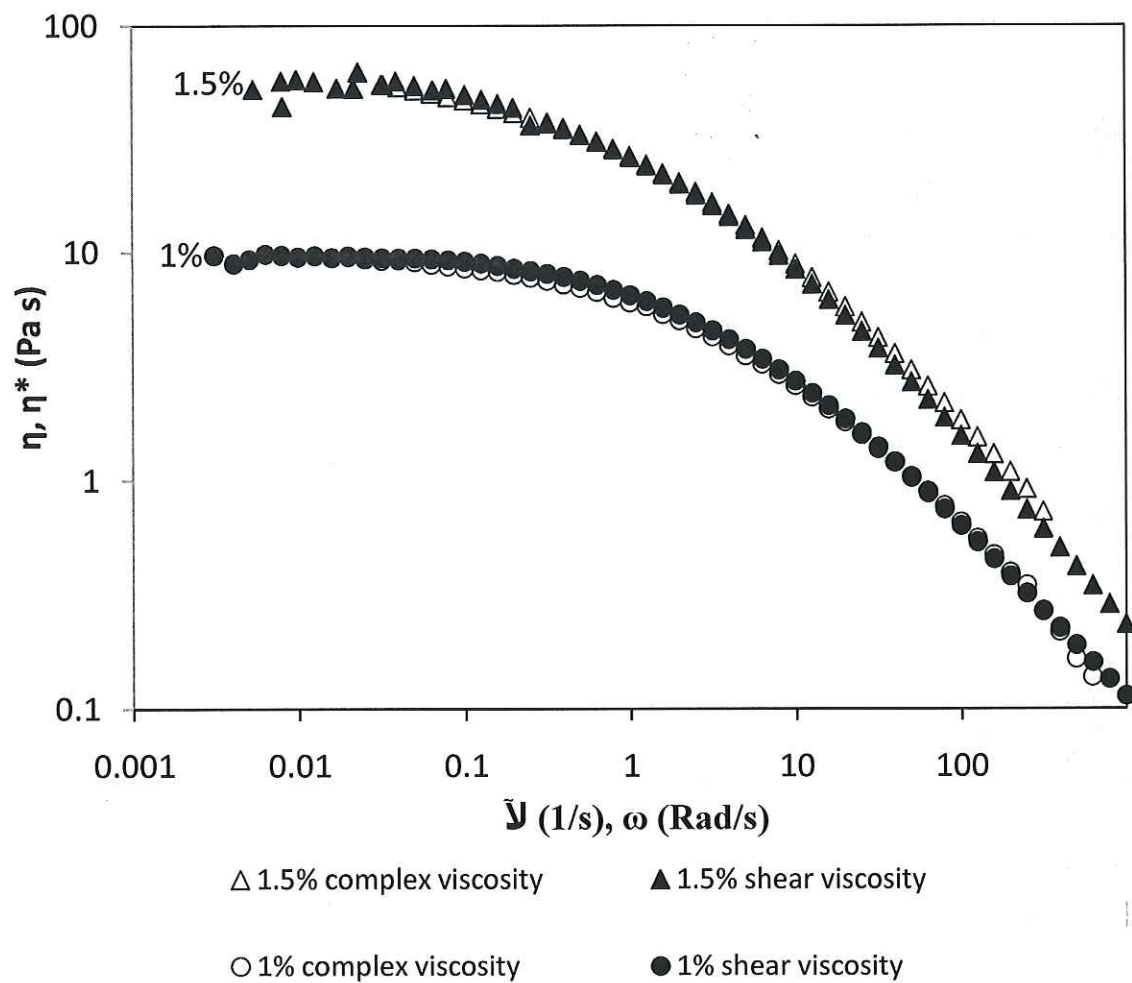


Figure. 6 Cox-Merz plot: Superimposition of $\eta^*(\omega)$ and $\eta(\dot{\gamma})$.

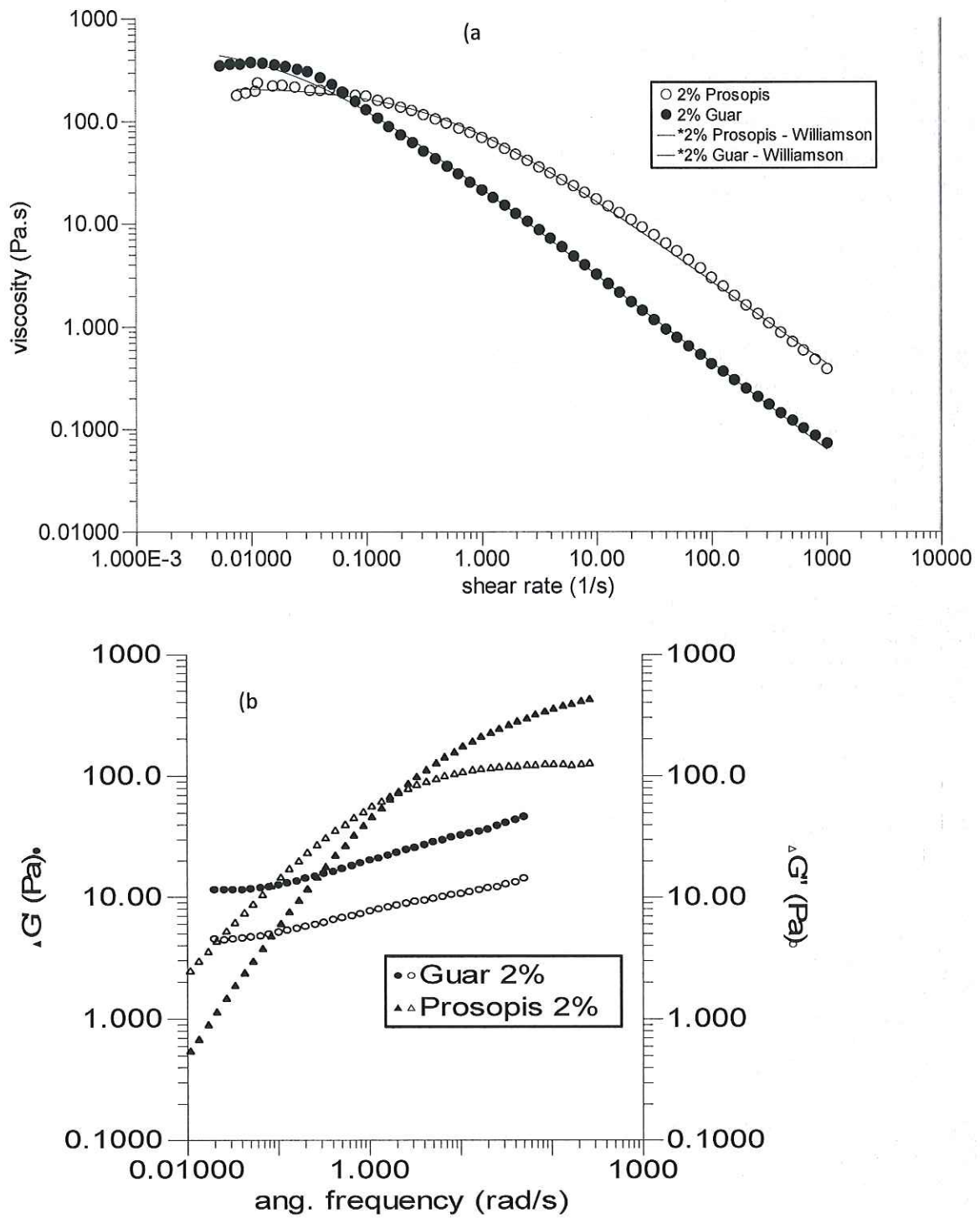


Figure 7. a).Viscosity-shear rate profiles; b). G' , G'' versus angular frequency for 2% aqueous solutions of *Prosopis africana* polysaccharide and guar gum at 25°C

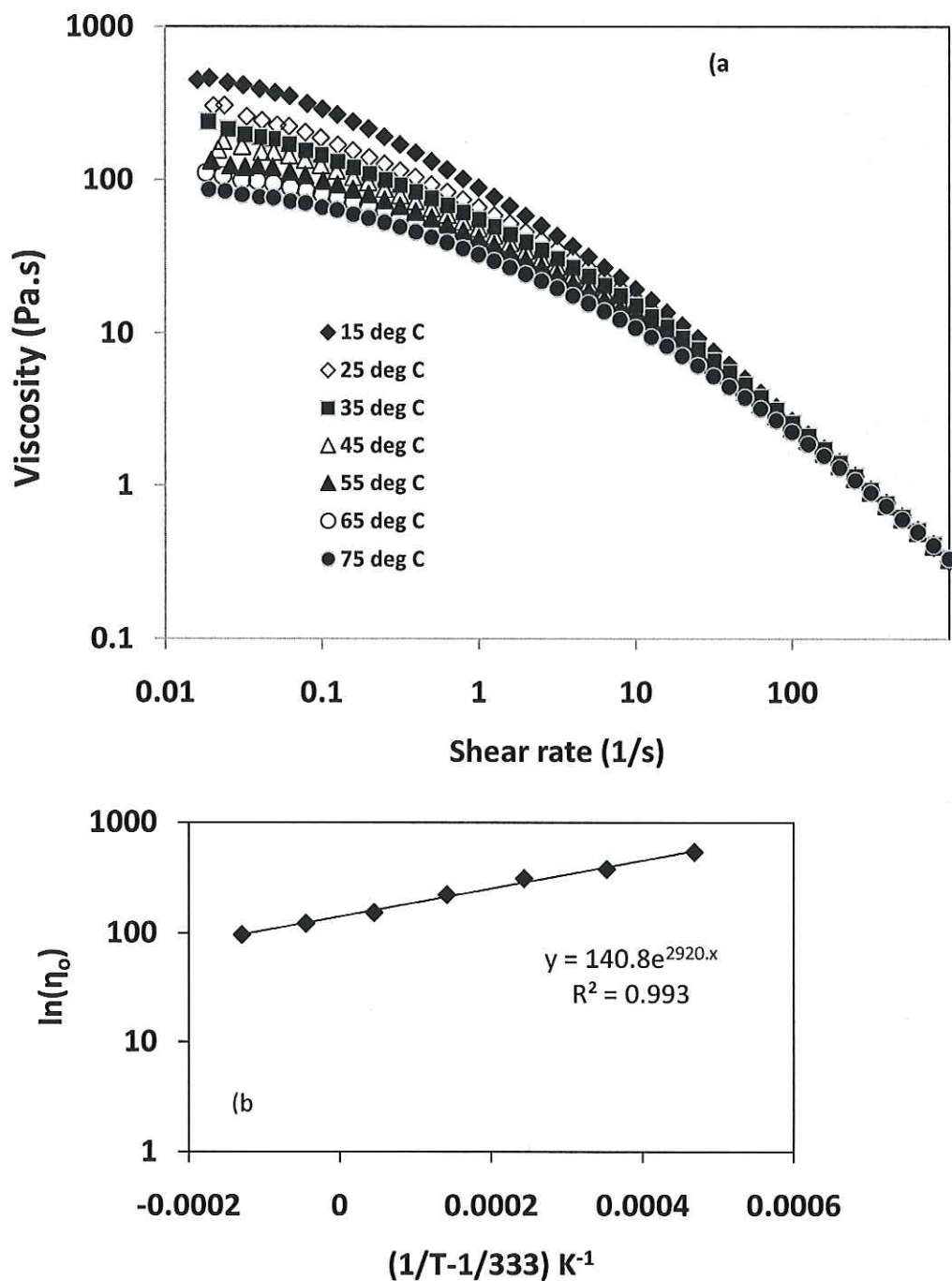


Figure 8. a). Viscosity –shear rate profiles for 2% solution of *Prosopis africana* polysaccharide at different temperature; b). Arrhenius plot: Log zero shear viscosities versus inverse of absolute temperature.