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**Rheological properties of the polysaccharide from *Adansonia digitata* leaves**  
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**Abstract**

The rheological properties of the polysaccharide from *Adansonia digitata* leaved were studied in dilute and semi-dilute solutions. The intrinsic viscosity of the polysaccharide obtained by the combined Huggins and Kraemer extrapolations was 3.27 dl/g. The polysaccharide solution was sensitive to heat and the viscosity decreased with increase in temperature. The minimum energy to initiate flow in 4.0% polysaccharide solution calculated from Arrhenius plot of zero shear viscosity as a function of temperature was 43.4 kJ/mol. The polysaccharide contained random coil macromolecules with  $M_w$  of  $4.1 \times 10^5$  g/mol.

**1.0 Introduction**

Many of the hydrocolloids currently available as industrial gums were first used in an empirical way in domestic cookery (Ndjouenkeu, Goycoolea, Morris, & Akingbala, 1996). There are many others, however, that are not yet exploited commercially but are extensively used in local recipes, particularly in Nigeria and other tropical regions of the world. These materials are obtained from plants that grow wild or are cultivated only on a limited scale, and their functional properties as hydrocolloids remain largely unexplored. The performance of these plants in local cookery indicates they have potential for exploitation; however, there is a lack of information on the properties of the hydrocolloids. Recently, some of them are receiving attention: *Afzelia africana* (Ren, Picout, Ellis, & Ross-Murphy, 2003; Ren, Picout, Ellis, Ross-Murphy, & Grant Reid, 2005), *Irvingia gabonensis* (Ndjouenkeu *et al.*, 1996; Uzomah & Ahiligwo, 1999), *Brachystegia eurycoma* (Nwokocha & Williams, In press; Uzomah, & Ahiligwo, 1999), *Corchorus olitorius* (Yamazaki, Kurita, & Matsumura, 2009), *Detarium microcarpum* (Wang *et al.*, 1996), *Mucuna flagellipes* (Nwokocha & Williams, 2009) and *Hibiscus esculentum* (Ndjouenkeu *et al.*, 1996).

*Adansonia digitata* L.(*Bombacaceae*) is an African plant known as baobab tree. The baobab tree grows in most parts of West Africa as well as in parts of north and east Africa where the leaves are used as a vegetable and as a soup thickener (Builders, Okeke, Egieye, 2007; Woolfe, Chaplin, & Otchere, 1977). The mucilage has been reported to contain mainly galacturonic and glucuronic acids with minor quantities of galactose, rhamnose, glucose and arabinose (Woolfe *et al.*, 1977). In this work, we studied the molecular characteristics of the polysaccharide isolated from the leaves of *Adansonia digitata* using GPC-MALLS coupled to RI and UV detectors and the rheological properties using capillary viscometry in the dilute regime, and steady shear and small angle deformation oscillatory measurements in the semi dilute regime.

## **2.0 Materials and methods**

### **2.1 Sample preparation and isolation of polysaccharide**

Fresh leaves were collected from *Adansonia digitata* tree and air dried. The leaves were pulverized and defatted in a soxhlet extractor using hexane as solvent. The defatted flour was rid of the solvent by leaving to dry in a vacuum chamber. The calculated amount of flour, 10 g/l (w/v) was dispersed in deionised water by means of a mechanical stirrer for 3 h. The resulting dispersion was poured into centrifuge bottles and centrifuged at 2500 rpm for 2 h at 25°C. The supernatant was pooled together. The residue was reconstituted with fresh water and dispersed again and centrifuged. The extraction was repeated again. The pooled supernatant was purified further by centrifuging for 3 h. The resulting supernatant was treated with excess isopropanol to precipitate the polysaccharide. The powdered polysaccharide 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 (Optilab

DSP, Wyatt Technology Corporation, Santa Barbara Ca93103). The polysaccharide solution (20 ml) containing  $4.001 \times 10^{-4}$  g/ml (w/w) was subjected to microwave bomb treatment for 40 s to ensure complete disaggregation (Ratcliffe, Williams, Viebke, & Meadows, 2005), filtered through a 0.45  $\mu\text{m}$  syringe filter and injected through a rheodyne into a 200  $\mu\text{l}$  loop connected to a combination of Suprema columns (100 $\text{\AA}$ , 3000 $\text{\AA}$  and 30000 $\text{\AA}$ ) packed with 10  $\mu\text{m}$  beads of polyhydroxymethacrylate copolymer network through which the degassed (CSI 6150, Cambridge Scientific Instruments, England) eluent (0.1M NaNO<sub>3</sub> + 10<sup>-6</sup>M NaN<sub>3</sub> solution) was pumped (Waters: 515 HPLC Pump, Milford, MA 01757, USA) at a flow rate of 0.5 ml/min. The total injected mass was  $8.002 \times 10^{-5}$  g. The chromatogram was analyzed with Astra software with a predetermined dn/dc value of 0.140 ml/g.

### 2.3 Determination of intrinsic viscosity

0.2 % (dry basis, w/w) *Adansonia digitata* polysaccharide powder was dispersed in 0.1M NaCl solution at room temperature by placing on a roller mixer (SRT2, Stuart Scientific, UK) overnight. 7 ml of solution was transferred into a Cannon-Ubbelohde capillary viscometer (No 75), which was immersed in a precision water bath to maintain the temperature at  $25.0 \pm 0.1$  °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,  $\eta_r$ , was calculated as flow time of the solution,  $t$ , divided by the flow time of the solvent,  $t_s$ . The intrinsic viscosity,  $[\eta]$ , was determined by applying Fedor's equation (Eq. 1).

$$\frac{1}{2(\eta_r^{1/2} - 1)} = \frac{1}{[\eta]C} - \frac{1}{[\eta]C_{\max}} \quad (\text{Eq. 1})$$

Where  $C$  is the concentration of polymer (g/dl),  $C_{\max}$  is a factor showing Fedor's concentration limit.

The specific viscosity,  $\eta_{sp}$ , is related to  $\eta_r$  by  $\eta_{sp} = \eta_r - 1$ . The  $[\eta]$  was also evaluated by the combined Huggins and Kraemer plots.

#### **2.4 Rheological measurements**

Different concentrations of the *Adansonia digitata* polysaccharide solutions (0.5 - 6.0%) were prepared by dispersing the desired amount of the 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 (40 mm 2° steel cone, ser no 982525, truncation gap 53  $\mu\text{m}$ ) for sample concentrations 3 to 6% and standard-size recessed end geometry (rotor outer radius 14, gap 4000  $\mu\text{m}$ ) for concentrations 0.5 and 2.0%. Low viscosity oil, polysilylsilicone, was used to coat the edge of the cone in the case of cone and plate geometry. The flow properties were obtained by subjecting the polysaccharide solutions to a stepped-flow procedure at a shear rate of 0.01 to 1000  $\text{s}^{-1}$ . In the oscillation procedure, strain sweep was performed on each gum solution from 0.003 to 100% at an angular frequency of 1 rad/s to locate the linear viscoelastic region. A frequency sweep was performed on the gum solutions in the region of 0.1 to 120 rad/s at an amplitude strain within the linear viscoelastic region. The resulting data was analyzed using TA Data Analysis Software.

#### **2.5 Effect of temperature**

The effect of temperature on the viscosity of *Adansonia digitata* polysaccharide was investigated in water by subjecting a 4% polysaccharide solution to a stepped flow procedure in the temperature range 5° to 65°C at 10° interval. The Cross model was fitted to the flow curves to determine the zero shear viscosities. The activation energy

for viscous flow of 4% polysaccharide in water was determined from the Arrhenius plot of zero shear viscosities ( $\eta_0$ ) versus the inverse of absolute temperature (T) (Eq 2).

$$\eta_0 = A \exp E_a / RT \quad (\text{Eq. 2})$$

Where A= pre-exponential factor (Pas),  $E_a$ = activation energy for viscous flow (J/mol), R= gas constant (8.314 J/mol K), T= temperature (K).

### 3.0 Results and Discussion

#### 3.1 Molecular weight

The refractive index elution profile of *Adansonia digitata* polysaccharide and the molecular weight distribution presented in Figure 1a. The result of the analysis (Table 1) shows that *Adansonia digitata* polysaccharide is characterized by a number-average molecular weight,  $M_n$ , of  $3.647 \times 10^6$  g/mol, weight-average molecular weight,  $M_w$ , of  $4.01 \times 10^6$  g/mol and z-average molecular weight,  $M_z$ , of  $4.33 \times 10^6$  g/mol. Yamazaki *et al.*, (2009) have reported a molecular weight of  $9.4 \times 10^5$  g/mol for the hydrocolloid from *Corchorus olitorius* leaves. The weight-average radius of gyration,  $R_g$ , of *Adansonia digitata* polysaccharide was 172.7 nm. The root mean square (RMS) radius or radius of gyration ( $R_g$ ) is related to the molar mass (M) by  $R_g \propto M^v$  (Eq. 3)

The log-log plot of  $R_g$  vs M is linear from which the exponent  $v$  is obtained as the slope. The value of the parameter  $v$  gives information about the conformation of the polysaccharide macromolecules. It has a value of 0.33 for spheres and 0.5-0.6 for random coils (Andersson, Wittgren, & Wahlund, 2003). Figure 1b shows the conformation plot for *Adansonia digitata* polysaccharide in aqueous solution. From the slope,  $v$  had an average value of  $0.575 \pm 0.05$ ; this is consistent with values reported for random coil polymers in a good solvent.

#### 3.2 Intrinsic viscosity

The intrinsic viscosity was determined in 0.1M NaCl using isoionic dilution of the polysaccharide solution in the concentration range 0.19% to 0.065% (w/w) and relative viscosity,  $\eta_r$ , of  $1.2 < \eta_r < 2.0$ . Sometimes, the intercept of the combined Huggins and Kraemer extrapolations do not meet at  $C = 0$ , which is the situation in this case, thus  $[\eta]$  was presented as the average of the Huggins and Kraemer intercepts. The combined Huggins and Kraemer plots (Fig. 2a) gave an intrinsic viscosity of  $3.27 \pm 0.07$  dl/g which is in good agreement with the value of 3.28 dl/g obtained from the slope of Fedors plot (slope =  $1/[\eta]$ ) (Fig. 2b). Fedors equation has been reported to give the same intrinsic viscosity values for both native and modified dextrans as the Huggins equation when applied in the dilute domain (Rotureau *et al.*, 2006). The limiting concentration of a Fedors plot of *Adansonia digitata* polysaccharide calculated from the intercept (intercept =  $1/C_m[\eta]$ ) was 1.245 g/dl. The Huggins constant,  $h_c$ , calculated from the slope (slope =  $h_c[\eta]^2$ ) of the Huggins plot was 0.63. The value of the Kraemer constant from the slope (slope =  $-k_c[\eta]^2$ ) was 0.02. Both  $h_c$  and  $k_c$  are constants related to polymer-solvent interactions. In a solvent where polymer-polymer interactions counter balances polymer-solvent interactions, both  $h_c$  and  $k_c$  are related through:  $h_c = k_c + 0.5$ . The intrinsic viscosity of *Adansonia digitata* polysaccharide is less than 4.4 dl/g and 7.6 dl/g reported for *Irvingia gabonensis* and okra, respectively (Ndjouenkeu *et al.*, 1996), and 19.8 dl/g reported for the exopolysaccharide from *Escherichia coli* strain S61 (Ren *et al.*, 2003).

### 3.3 Viscosity dependence of shear rate

The dependence of viscosity on shear rate was investigated in the semi dilute regime at shear rates from  $10^{-3} - 10^3$  s<sup>-1</sup> in the range of concentrations 1.0 to 6.0% (w/w) (Fig. 3). Two distinct flow regimes were observed- a low shear Newtonian plateau in which the viscosity remained constant ( $\eta_0$ ) and a shear thinning or power law regime in which viscosity decreased at relatively higher shear rates. According to Graessley

(1974), a constant viscosity is maintained in the low shear rate region because the rate of intermolecular disentanglements brought about by shearing forces is nearly the same as that of entanglements newly formed. On the other hand, the decrease in viscosity in the power law region occurs because the rate of disentanglements is higher than the rate of formation of new entanglements. The magnitude of the zero shear viscosity is a macroscopic representation of the micro structural nature of the biopolymer (Hwang and Shin, 2000). Both the magnitudes of the zero shear viscosity and viscosity in the power law region are important in formulation of thixotropic fluids.

Different types of models have been employed in describing the flow properties of biopolymer solutions in the semi dilute concentrations. One of such models which we have found useful in this case is the Cross model (Eq. 4) which we have used to obtain the flow characteristics of *Adansonia digitata* polysaccharide.

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

where  $\eta$ ,  $\eta_0$ ,  $\eta_{\infty}$  are the shear, zero shear and infinite shear viscosity (Pa s), respectively;  $\tau$  is Cross relaxation time (s),  $\dot{\gamma}$  is the shear rate (1/s) and  $m$  is the Cross rate index (dimensionless).

The viscosity exhibited shear rate dependence for the concentrations 0.5 % to 6.0%, with the degree of shear thinning becoming more pronounced as polysaccharide concentration increased. The Cross model was used to calculate the  $\eta_0$ ,  $\eta_{\infty}$ ,  $\tau$  and  $m$ . Values of  $\eta_0$  and  $\tau$  increased with increase in concentration. The relaxation time,  $\tau$ , is related to the critical shear rate ( $\dot{\gamma}_{\text{crit}}$ ) through  $1/\dot{\gamma}_{\text{crit}} = \tau$ . The  $\dot{\gamma}_{\text{crit}}$  marks the onset of shear thinning of the polysaccharide solution and the result indicates that the onset of shear thinning shifted to lower shear rates as polysaccharide concentration increased.



This has been explained in terms of the degree of chain entanglements. At high polymer concentration there is restriction of movement of the individual chains as a result of the corresponding increase in entanglements which results in an increase in time to replace the entanglements disrupted by the imposed deformation (Graessley, 1974). The Cross rate index,  $m$ , fell within the range  $0.57 < m < 0.85$  and indicates the degree of dependence of viscosity on shear rate in the shear thinning region for *Adansonia digitata* polysaccharide.

### 3.4 Small angle deformation oscillation studies

Fig. 4 shows a plot of storage modulus  $G'$  and loss modulus  $G''$  versus angular frequency  $\omega$  for polysaccharide solutions of 3, 4, and 5%.  $G'$  and  $G''$  showed dependence on  $\omega$  and concentration, with the dependence decreasing with increase in concentration. At low angular frequency (i.e.  $\omega < 0.186$  rad/s),  $G'' > G'$  for the three polymer solutions indicating a predominantly liquid-like response. As  $\omega$  increased,  $G'$  increased faster than  $G''$  so that a crossover occurred to a predominantly elastic response with  $G' > G''$ . The crossover points occurred at lower angular frequencies of 1.697, 0.2294 and 0.168 rad/s for 3, 4, and 5% polymer concentration, respectively. The angular frequency at crossover point decreased to lower values while the storage modulus increased with increasing polymer concentration. Similar observation has been reported for colanic acid (Ren *et al.*, 2003) and *Detarium* gum (Wang *et al.*, 1996). In Fig. 5, the Cox-Merz rule was tested by the superimposition of the plots of  $\log \eta$  versus  $\log \dot{\gamma}$  and  $\log \eta^*$  versus  $\log \omega$  for 3, 4 and 5% polymer concentrations. A close superimposition was observed expect that  $\eta^* > \eta$  at low angular frequencies. This deviation occurred because some structures which were ruptured under steady shear survived oscillatory shear.

### 3.5 Effect of temperature

Fig. 6a, b shows the effect of temperature on the viscosity of 4% *Adansonia digitata* polysaccharide in water. The steady shear flow profiles had two distinct regions- zero shear and shear thinning regions similar to those obtained for most polysaccharides in this concentration regime. A progressive decrease in viscosity was observed as temperature increased. This is because an increase in temperature increases the kinetic motion of the macromolecules and thus promotes disentanglement of the chains leading to a decrease in viscosity. A similar loss of viscosity with increase in temperature was reported by Woolfe *et al.*, 1977). The zero shear viscosities were determined by fitting the flow curves to the Cross model. The Arrhenius plot of the zero shear viscosities versus inverse of absolute temperature (Fig. 6b) was used to determine the activation energy ( $E_a$ ) of viscous flow in water. The  $E_a$  required to initiate flow in 4% polysaccharide in water was 43.4kJ/mol and the pre-exponential factor (A) was  $1.0 \times 10^{-6}$  Pa.s. The activation energy of flow has been reported to be influenced by polysaccharide concentration (Nwokocha & Williams, In press), nature of intra and inter chain interactions and the molecular weight of the polysaccharide. A cashew tree exudates polysaccharide with molecular weight of  $1.6 \times 10^4$  g/mol has  $E_a$  of flow  $\sim 16$  kJ/mol at 3% (de Paula & Rodrigues, 1995).

#### **4.0 Conclusion**

The polysaccharide constituent of *Adansonia digitata* leaves, a popular food thickener in tropical Africa was isolated and studied. The polysaccharide contained random coil macromolecules with intrinsic viscosity of 3.27 dl/g and weight-average molecular weight of  $4.01 \times 10^6$  g/mol. The polysaccharide solutions exhibited shear thinning properties in the concentration range of 1.0 – 6.0 g/dl (w/w).

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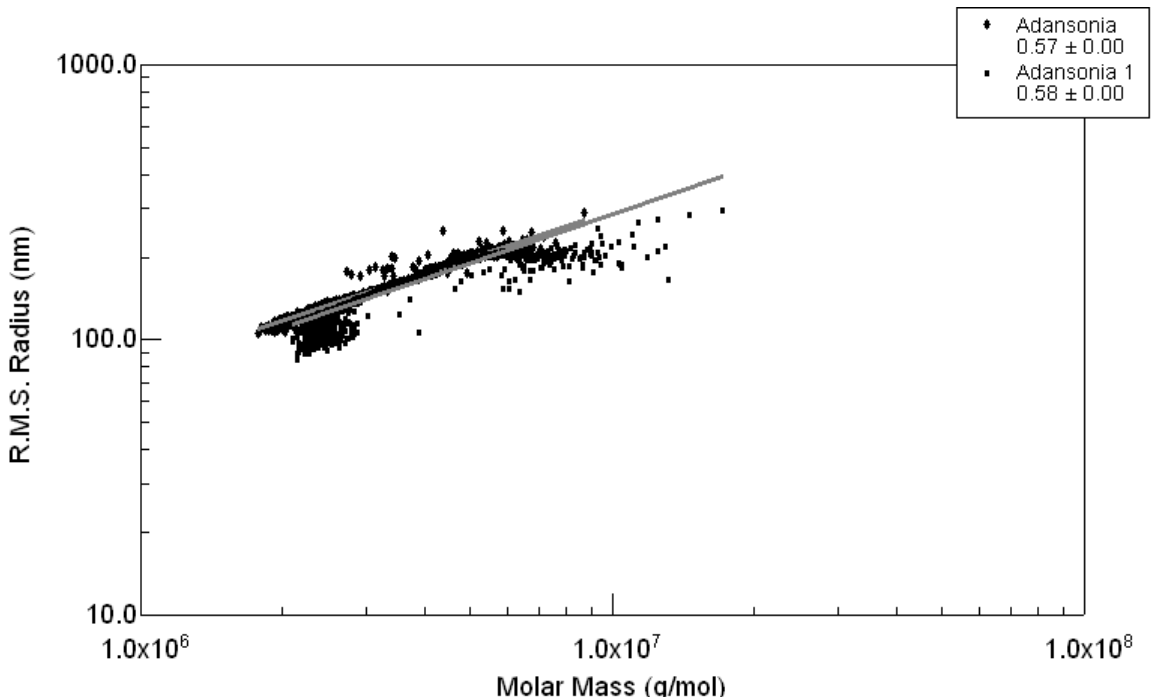
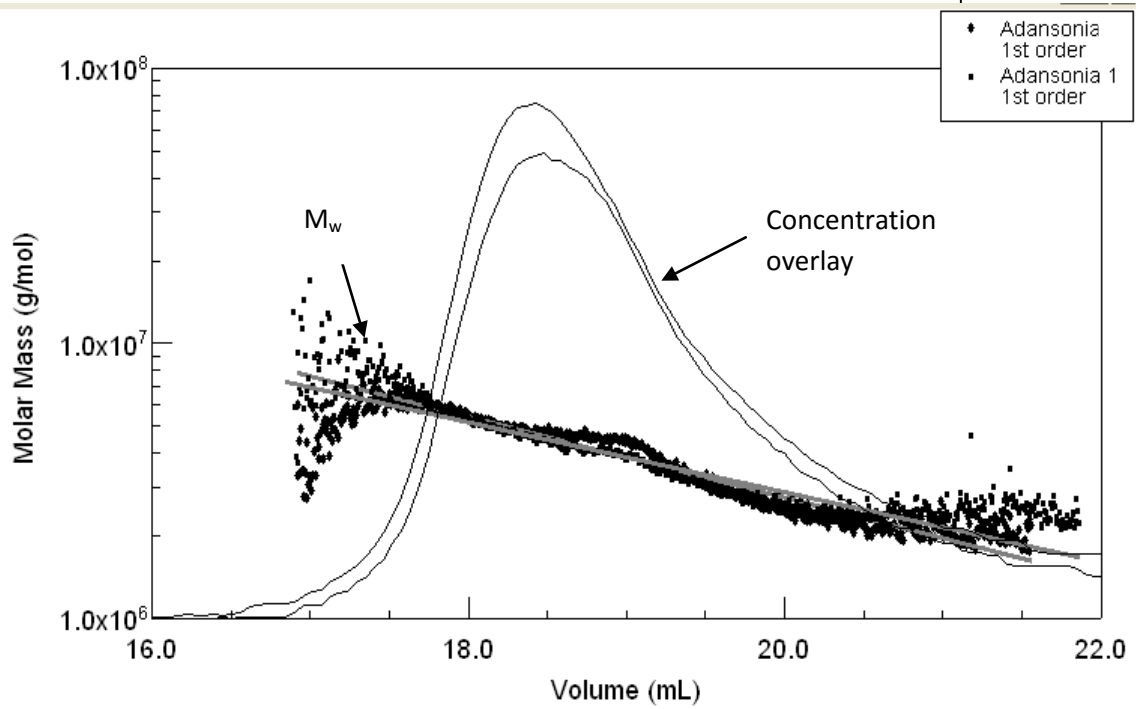


Figure 1. a). GPC elution profile of *Adansonia digitata* showing Mw distribution versus time (duplicate determination)

b). Log-log plot of root mean square radius versus molecular weight of *Adansonia digitata* polysaccharide.

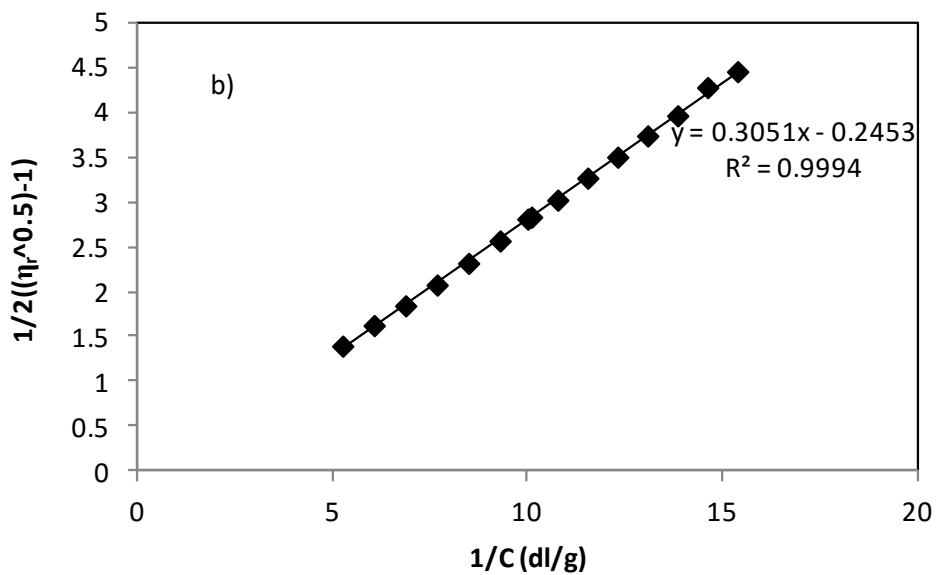
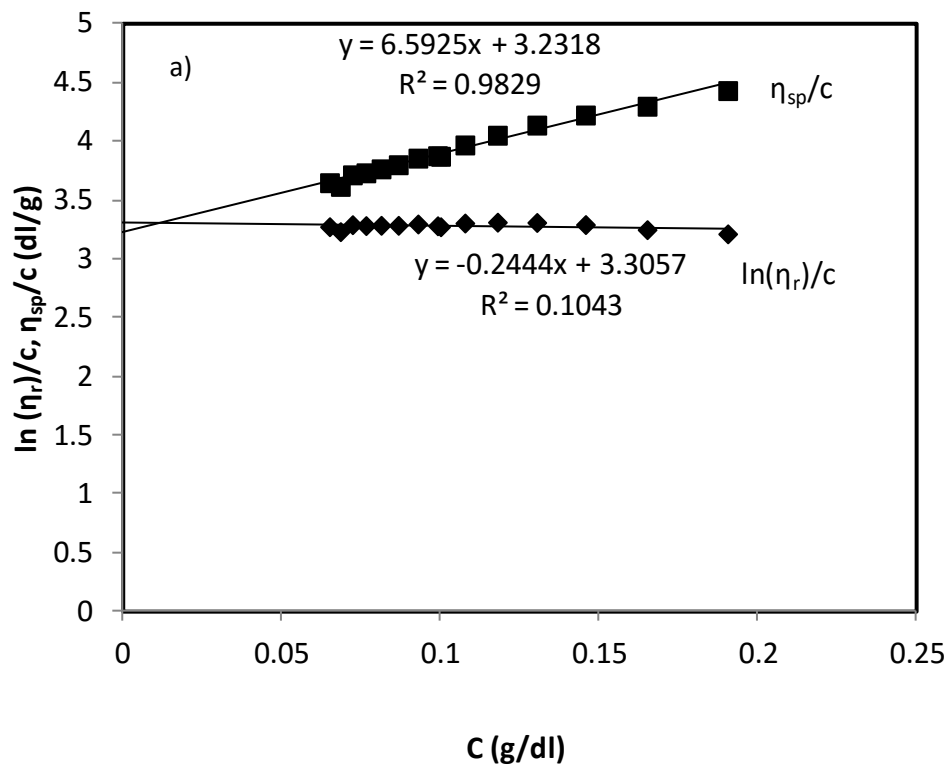


Fig. 2: Intrinsic viscosity of *Adansonia digitata* polysaccharide in 0.1M NaCl solution by (a) Combined Huggins and Kraemer extrapolations; (b) Fedor's method

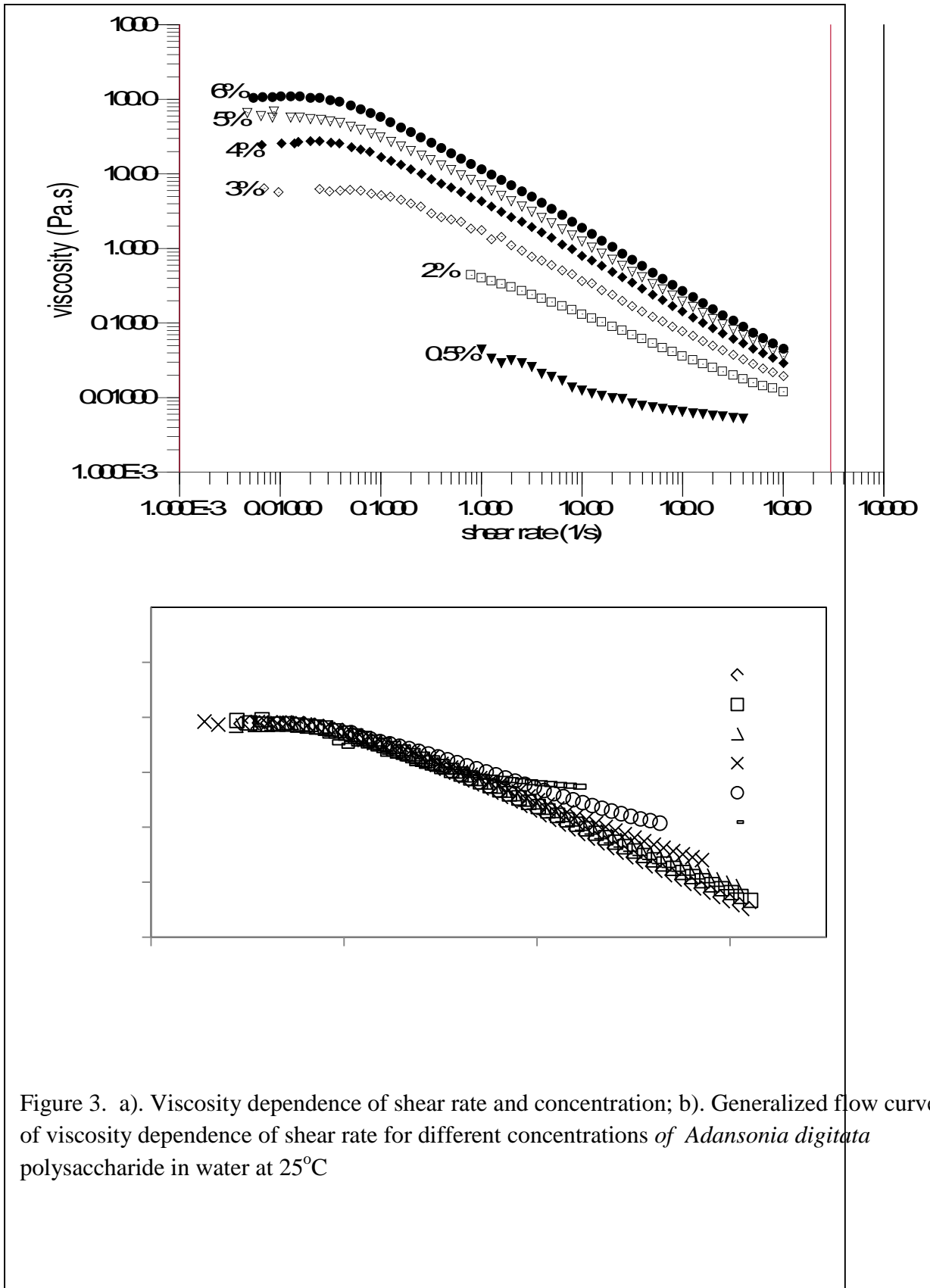


Figure 3. a). Viscosity dependence of shear rate and concentration; b). Generalized flow curves of viscosity dependence of shear rate for different concentrations of *Adansonia digitata* polysaccharide in water at 25°C

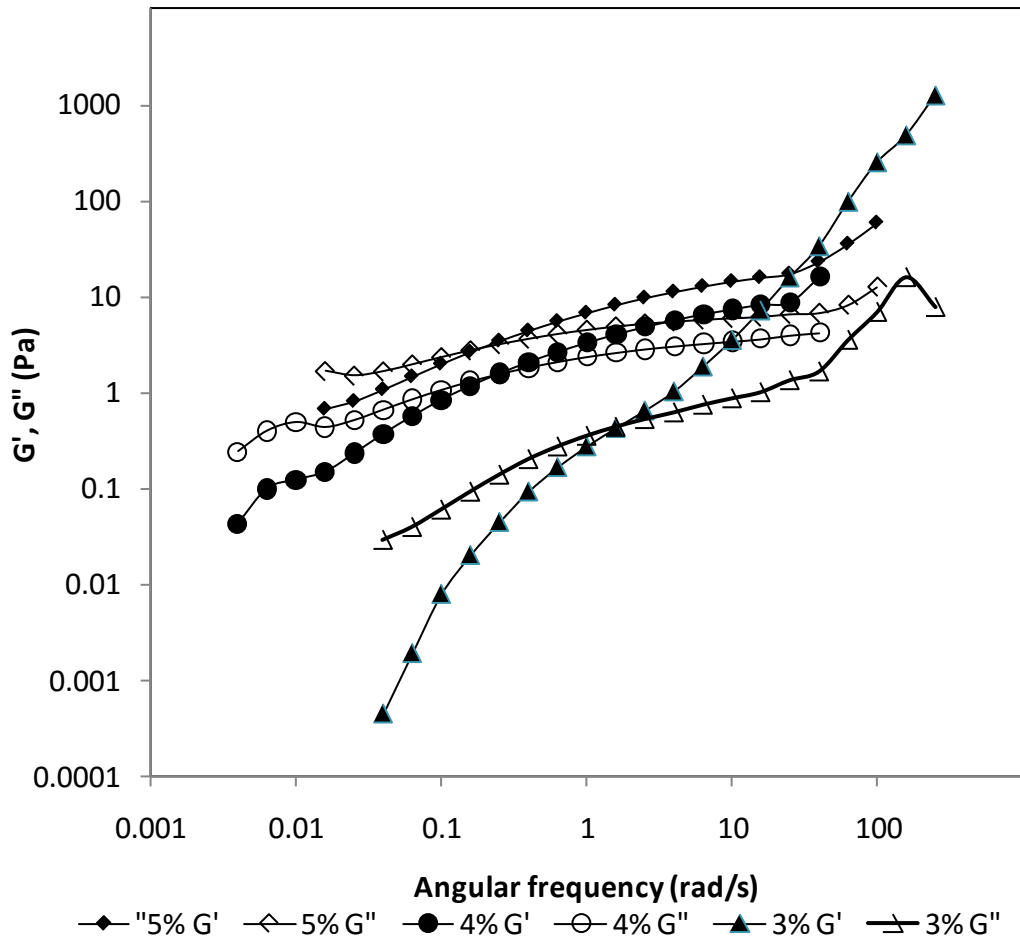


Fig. 4. Frequency sweep showing  $G'$ ,  $G''$  versus angular frequency



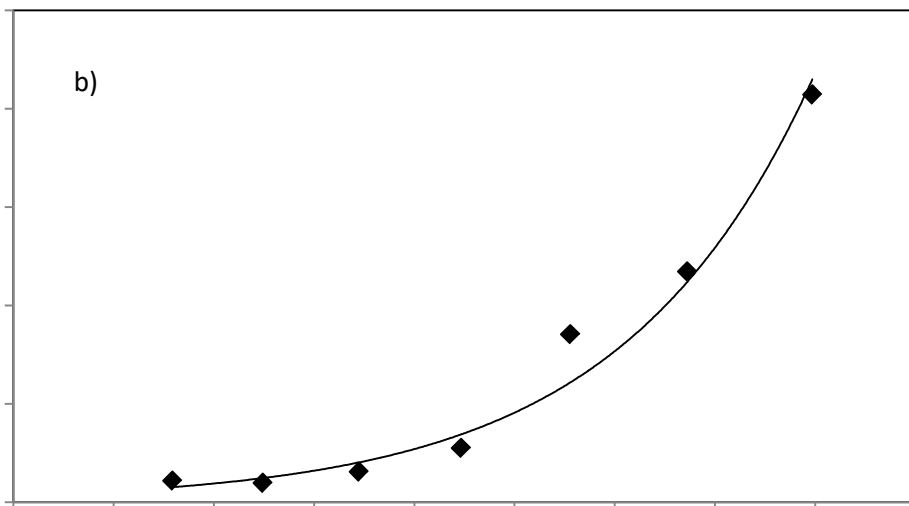
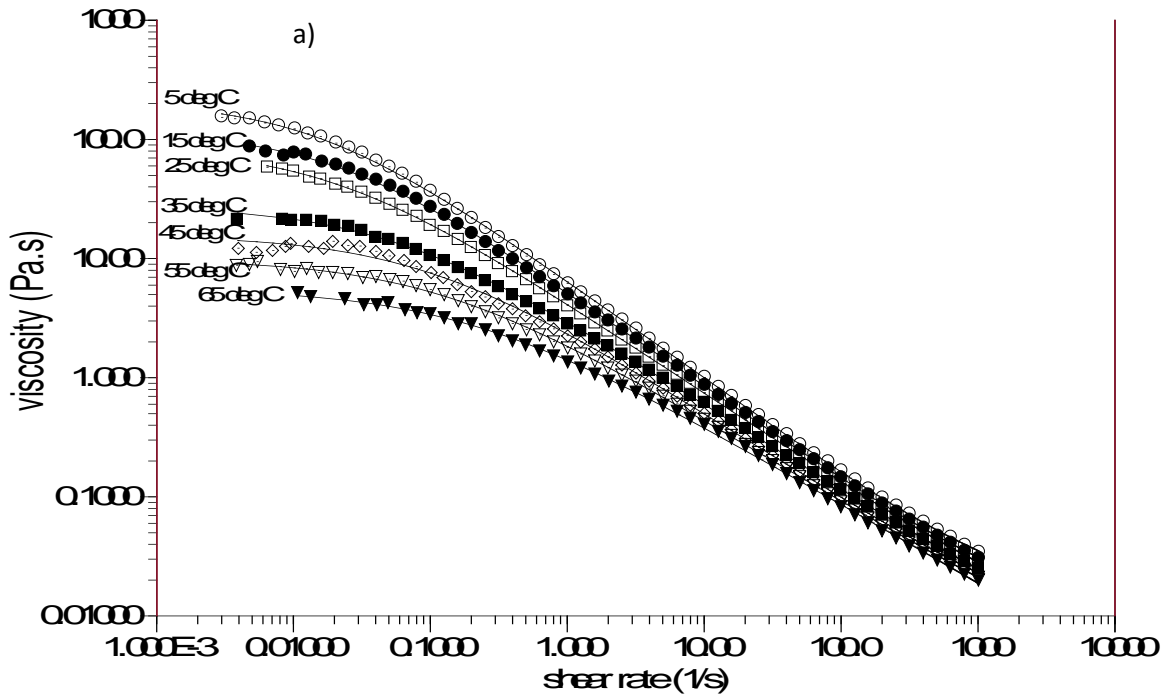


Figure 6. a) Effect of temperature on viscosity-shear rate profiles of 4% *Adansonia digitata* polysaccharide in water; b) Arrhenius plot of zero shear viscosity versus inverse of absolute temperature

Table 1. Molecular characteristics

$M_n$ ( $\times 10^{-6}$ ), g/mol	$3.647 \pm 0.01$ (0.8%)
$M_w$ ( $\times 10^{-6}$ ), g/mol	$4.01 \pm 0.08$ (0.5%)
$M_z$ ( $\times 10^{-6}$ ), g/mol	$4.33 \pm 0.15$ (1.1%)
$R_n$ , nm	$163.0 \pm 4.2$ (0.28%)
$R_w$ , nm	$172.7 \pm 5.1$ (0.28%)
$R_z$ , nm	$180.9 \pm 5.9$ (0.29%)
$M_w/M_n$	$1.1 \pm 0.02$ (0.7%)
$M_n/M_z$	$1.2 \pm 0.04$ (1.2%)

Data are mean of two determinations  $\pm$  standard deviation

Table 2. Flow characteristics of different concentrations of *Adansonia digitata* polysaccharide at 25°C fitted to Cross model

Conc. g/dl	$\eta_0$ (Pa s)	$\eta_\infty$ (Pa s)	$\tau$ (s)	m	s.e
6.0	153.7	5.083E-3	19.97	0.8382	4.196
5.0	75.76	5.572E-3	16.34	0.8078	4.539
4.0	34.38	7.134E-3	16.03	0.7825	7.025
3.0	7.654	6.125E-3	5.514	0.7322	11.93
2.0	1.001	4.863E-3	1.875	0.6556	1.195
0.5	0.09435	4.948E-3	1.657	0.8108	26.50