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Isolation and Rheological Characterization of *Mucuna flagellipes* Seed Gum

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ABSTRACT

The gum from *M. flagellipes* seed endosperm has been isolated and characterised using capillary viscometry and steady shear and small deformation oscillatory rheometry. The endosperm was found to constitute 67.15% of the whole seed and yielded 32.6% of gum. The Huggins and Kraemer plots obtained by capillary viscometry gave an intrinsic viscosity of 7.9 dL/g and viscosity average molecular mass was calculated to be 2.1 x 10^6 using the Mark Houwink relationship. The zero shear viscosity was plotted against the coil overlap parameter, C[η], and the slopes of the lines in the dilute and semi-dilute regions were found to be ~ 1.0 and 4.6 respectively. The curves were fitted to the Tuinier and Martin equations and showed only qualitative agreement. The shear flow viscosity profiles indicated that *M. flagellipes* gum did not exhibit significant shear thinning at polymer concentrations less than 0.5%, however, at higher concentrations, pronounced shear thinning was observed with the relaxation time (τ) increasing with increase in polymer concentration. The dynamic viscosity profiles showed that at all polymer concentrations examined, a Newtonian plateau was obtained at low frequencies indicating that the loss modulus was the dominant response. Plots of log η versus log $\dot{\gamma}$ and log η* versus log ω were not superimposible and hence did not obey the Cox-Merz rule.

Key words: *Mucuna flagellipes*, seed gum, isolation, rheological properties

INTRODUCTION

Vegetable gums are commonly used in food manufacturing processes as viscosifying agents. Many plants have been chemically analyzed for their potential as sources of seed gums. These include guar with 19-43% gum (Undersander, et al, 1991), Cassia brewsteri with 33.7±0.4% gum (Cunningham and Walsh, 2002) and mesquite with 24.9% gum (Estevez et al, 2004). *Mucuna flagellipes* is indigenous to West Africa and belongs to the family leguminosae and subfamily papilionaceae. The plant is an annual crop and a climber and can be cultivated more than once a year. It is high yielding; and bears pods which contain usually three to four seeds per pod. The seeds are usually dark brown to black, sometimes speckled, depending on variety. Among the natives, the endosperm which is rich in gum is pulverized and used as thickener in many traditional food preparations. Several authors have reported on its suitability for application in processed foods including use as a rheology modifier (Onweluzo et al, 1999), stabilizer (Onweluzo et al,1995, Onweluzo et al, 2004) and film former (Ojile et al, 2000). Mucuna gum is a galactomannan and has D-galactose and D-mannose as the main sugars (Onweluzo et al, 1995; Srivastava and Kapoor, 2005). However, we are yet to find reports on the proportion of gum in the seed or on its intrinsic viscosity, molecular mass and rheological properties.

MATERIALS AND METHODS

Isolation of seed gum: *M. flagellipes* seeds were dehulled to obtain the seed endosperms (Figure 1). The endosperms were pulverized by means of a hammer mill and the flour obtained by passing through a 500μm sieve and dried in the oven at 80°C for 6 hr. The flour was defatted by extracting with hexane in a soxhlet extractor for 6 hr. 5 g of sample was dispersed in 400 ml distilled water and hydrated continuously by means of a magnetic stirrer (FBI 15001, Fischer Scientific, UK) for 6 hr. This was poured into centrifuge tubes and centrifuged at 2500rpm for 30min. The supernatant was poured into a large beaker. The residue was reconstituted repeatedly with fresh distilled water, stirred and centrifuged again. The supernatant was pooled together and treated with 2-propanol when the gum spooled out. The clear liquor was decanted while the trapped solvent was removed by filtering under suction in a Buchner funnel. The crude gum was re-dissolved in fresh distilled water and re-precipitated with 2-propanol. The gum sample was dried in a convention oven at 60°C overnight and cooled in a desiccator. This was pulverized in a mortar and stored in a sealed container. The gum preparation was carried out in triplicate.

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

Intrinsic viscosity: The intrinsic viscosity of *M. flagellipes* gum was determined in distilled water. The gum solution was prepared by dispersing 50 mg of the gum (db, dry basis) in 100 mL of the distilled water at room temperature and placing on a roller mixer (SRT2, Staurt Scientific) overnight. 2 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±0.1 ^{o}C and after equilibration for 10 minutes, the flow time was determined between the two etched marks. Serial dilution was performed in situ and three readings were taken for each dilution and averaged. The viscosity average molecular mass, M_w was evaluated using the Mark-Houwink equation (Lazaridou et al, 2000) (equation 1).

$$[\eta] = 80.2 \times 10^{-6} \times M_w^{0.79} \quad (1)$$

Rheological characterization of *M. flagellipes* gum: Different concentrations of the *M. flagellipes* gum solutions (0.1%, 0.2%, 0.5%, 1.0%, 2.0% and 3.0%) were prepared by dispersing the desired amount of dry gum powder in distilled water and leaving to tumble overnight at ambient temperature using a roller mixer to ensure complete hydration (SRT2, Stuart Scientific, UK). Steady shear viscosity and small deformation oscillation experiments were performed using a Controlled Stress Rheometer (AR 2000, TA Instruments) fitted with cone and plate geometry (60mm 2^{o} steel cone, 50µm gap). The flow properties were obtained at 25^{o}C by subjecting the gum solutions to stepped-flow at a shear rate of 10^{-3} to $10^{3}s^{-1}$ after pre-conditioning. In the small deformation oscillation experiments, stress sweeps were performed on each gum solution to locate the linear viscoelastic region. A frequency sweep was performed on the gum solutions in the region of 10^{-1} to 120 rad/s at an amplitude strain within the linear viscoelastic region.

RESULTS AND DISCUSSION

Composition: Table 1 shows the physical composition of *M. flagellipes* seed. The endosperm constituted 46.73 - 74.76 % of whole seed with mean of 67.15%. The hull was easily separable from the endosperm by mechanical means. There was no visible germ. The defatted endosperm yielded 32.6±1.97% of gum (Table 1). Elemental analysis showed the gum contained 1.87 % nitrogen, 39.25% carbon and 6.43% hydrogen. This is the first available data on *M. flagellipes* gum and values are in the range of those reported for other sources of galactomannan (Undersander, et al, 1991, Cunningham and Walsh, 2002).

Intrinsic viscosity: Figure 2 shows the Huggins and Kraemer plot for *M. flagellipes* gum and the intrinsic viscosity of *M. flagellipes* obtained from the intercept was 7.9 dL/g. Application of the Mark-Houwink equation gave a viscosity average molecular mass of 2.1×10^6 which is similar to the viscosity average molecular mass values reported for other galactomannans such as carob gum (Lazaridou et al, 2000).

The double logarithmic plot of zero shear specific viscosity, $\eta_{sp,o,}$ against the coil overlap parameter, C[η], gave a critical overlap concentration, C*, of 3.98/[η] and the slopes of the lines below and above C* were ~1.0 and 4.6 respectively . This compares to values of ~4/[η] for C* and 1.4 and to 3.3 for the slopes of the lines for a range of random coil polysaccharides (Morris et al, 1981). Slopes of ~1 in the dilute region have been reported for *Aeromonas* polysaccharide (Xu et al, 2006), the exopolysaccharide from *Escherichia coli* strain S61 (Ren et al, 2003) and acid hydrolyzed amioca starch (Chamberlain and Rao, 2000). A higher value for the slope in the semi-dilute region has been reported for other polysaccharides. Kapoor et al (1998) have reported slope of 5.87 for *Cassia spectabilis* galactomannan . Andrade et al (1999) reported C* =3.3/[η] for galactomannans from *Caesalpinia pulcherrima* and *Cassia javanica*. The data were also fitted to the equations proposed by Tuinier et al, (equation 2) and Martin (equation 3) and curves obtained are shown in Figure 3b.

$$\eta_{sp,o} = c[\eta]_o + \frac{1}{25}([\eta]_o c)^{\frac{7}{2}} \qquad (2)$$

$$\eta_{sp,o} = c[\eta]_o \exp(k_m c[\eta]_o) \qquad (3)$$

where k_m is Martin polymer-polymer interaction parameter.

Both models showed only qualitative agreement as has been reported by Ratcliffe et al, (2005) for glucomannan.

Steady shear viscosity and viscoelastic properties

Figure 4 shows the steady shear viscosity as a function of shear rate for different concentrations of *M. flagellipes* gum. The flow profiles indicate that *M. flagellipes* gum exhibited Newtonian behaviour at polymer concentrations less than 0.5%. At higher concentrations, above C*, the polymer solutions became more viscous and exhibited non-Newtonian behaviour with marked shear thinning observed. Table 3 shows the values of the various parameters obtained by fitting the data to the Cross (equation 4) and Newtonian models (equation 5)

$$Cross: \frac{\eta - \eta_\infty}{\eta_o - \eta_\infty} = \frac{1}{1 + (\tau * \dot{\gamma})^m} \qquad (4)$$

$$Newtonian: \eta = \sigma / \dot{\gamma} \qquad (5)$$

Where η, η_o and η_∞ are viscosity (Pa.s), zero shear viscosity (Pa.s) and infinite shear viscosity (Pa.s) respectively, $\dot{\gamma}$ is shear rate (s^{-1}), τ is relaxation time (s) and m is the rate index (dimensionless) and σ is shear stress (Pa). The relaxation time, τ ($\tau = 1/\dot{\gamma}_{crit}$, $\dot{\gamma}_{crit}$ = critical shear rate, marks the onset of shear thinning), increased with increase in polymer concentration.

The variation of the storage modulus (G'), loss modulus (G") and complex shear viscosity (η*) with the frequency of oscillation (ω) for the gum solutions are shown in Figures 5 and 6. In Figure 5, the gum at 1.0% concentration gave a predominantly viscous response (G" > G'), however at concentrations of 2.0% and 3.0%, there was a transition from a predominantly viscous response at long timescales of measurement (G" > G') to a predominantly elastic response at shorter timescales (G' > G"). This cross over point occurred at ω = 9.087 rad/s in 3.0% and ω = 30.62 rad/s in 2.0% while the corresponding G' are 72.24 Pa and 41.96 Pa respectively. The average timescales for microstructural coupling estimated from the crossover point of the moduli (given by 1/ ω, where G'= G") for 2% and 3% gum solutions were 0.03s and 0.11s respectively. In Figure 6, η* exhibited a Newtonian plateau at low ω indicating that the loss modulus was the dominant response at all gum concentrations.

Figure 7 shows the steady shear viscosity (log η versus log $\dot{\gamma}$) and dynamic viscosity (log η* versus log ω) of the solutions at varying concentration. It was noted that the two sets of curves did not superimpose at any of the concentrations in contradiction to the Cox-Merz rule. Such behaviours has been observed for other polysaccharides including konjac glucamannan (Ratcliffe et al, 2005) and *Aeromonas* gum (Xu et al, 2006) and has been attributed to weak association between the polymer chains, so-called hyperentanglements.

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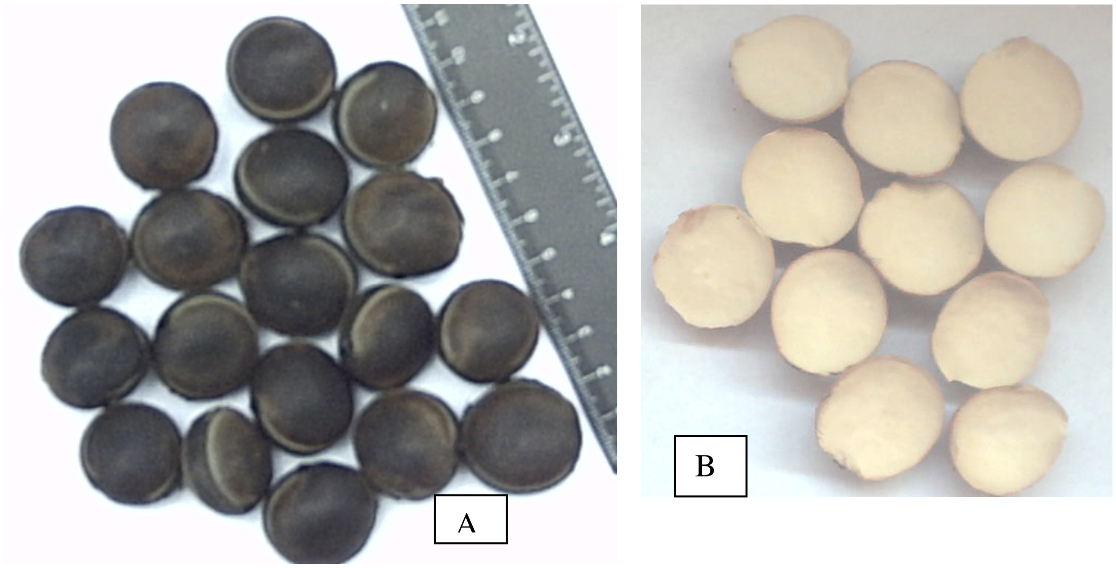

Fig. 1: ***M. flagellipes*** **seeds (A), endosperm cotyledons (B)**

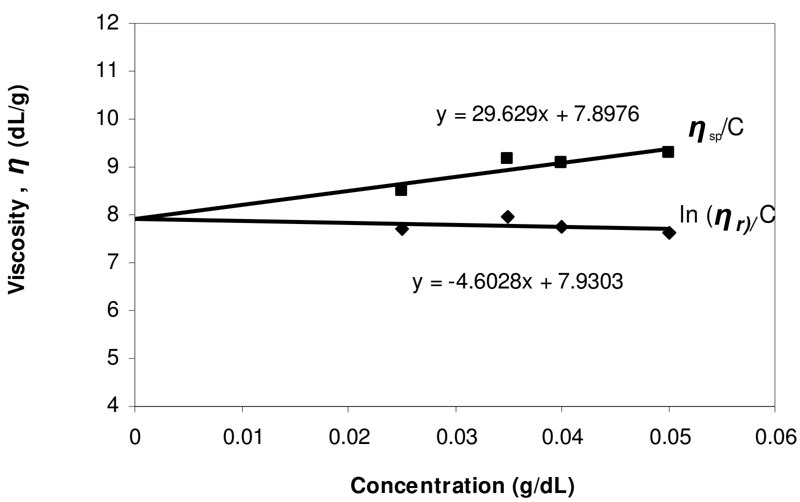

Fig.2. Determination of the intrinsic viscosity of *M. flagellipes* gum

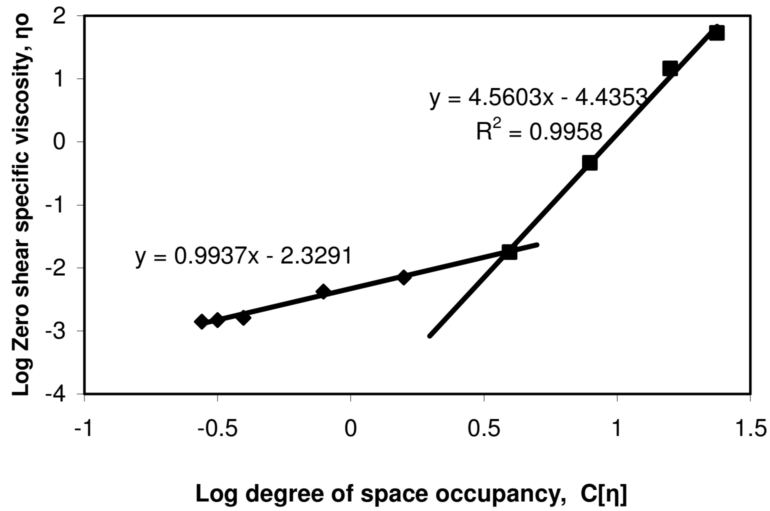

Fig.3a: Zero shear pecific viscosity versus Degree of space-occupancy of *M. flagellipes* gum

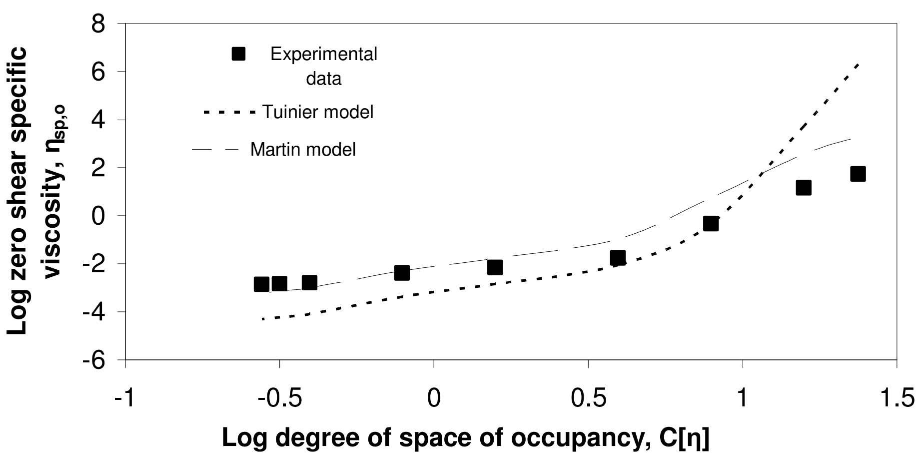

Figure 3: Log zero shear specific viscosity $\eta_{sp,o}$ versus Log degree of occupancy, C[η]

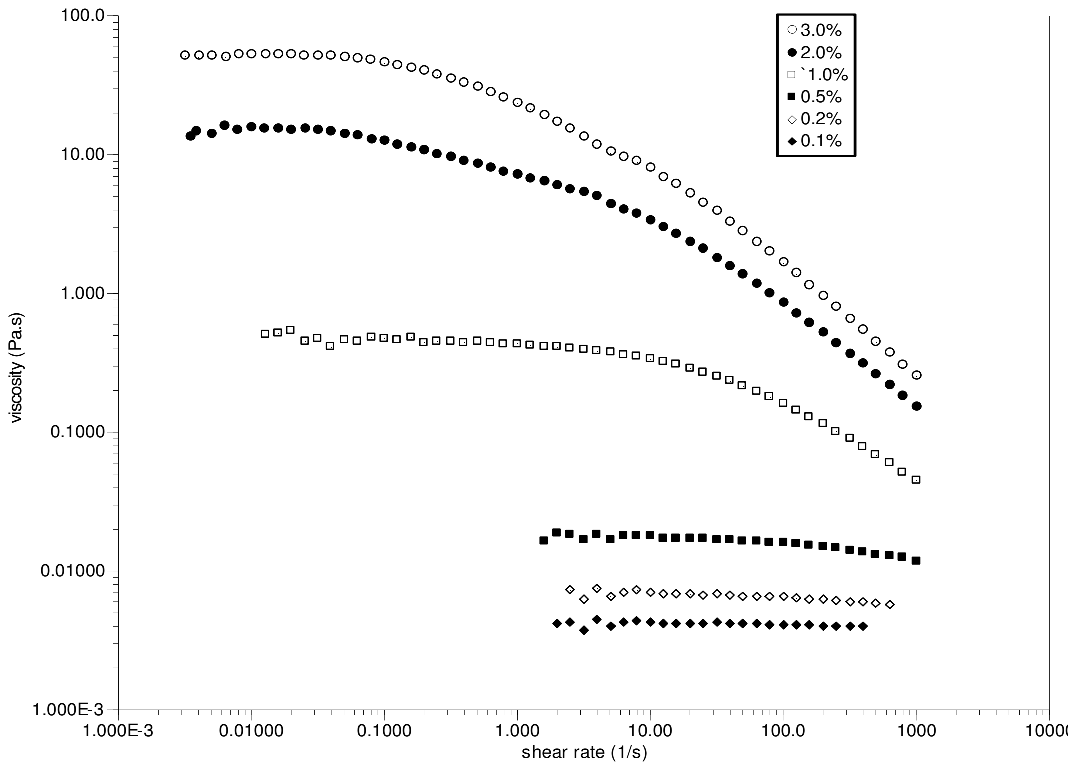

Fig.4: Viscosity-shear rate profiles for different concentrations of *M. flagellipes* gum

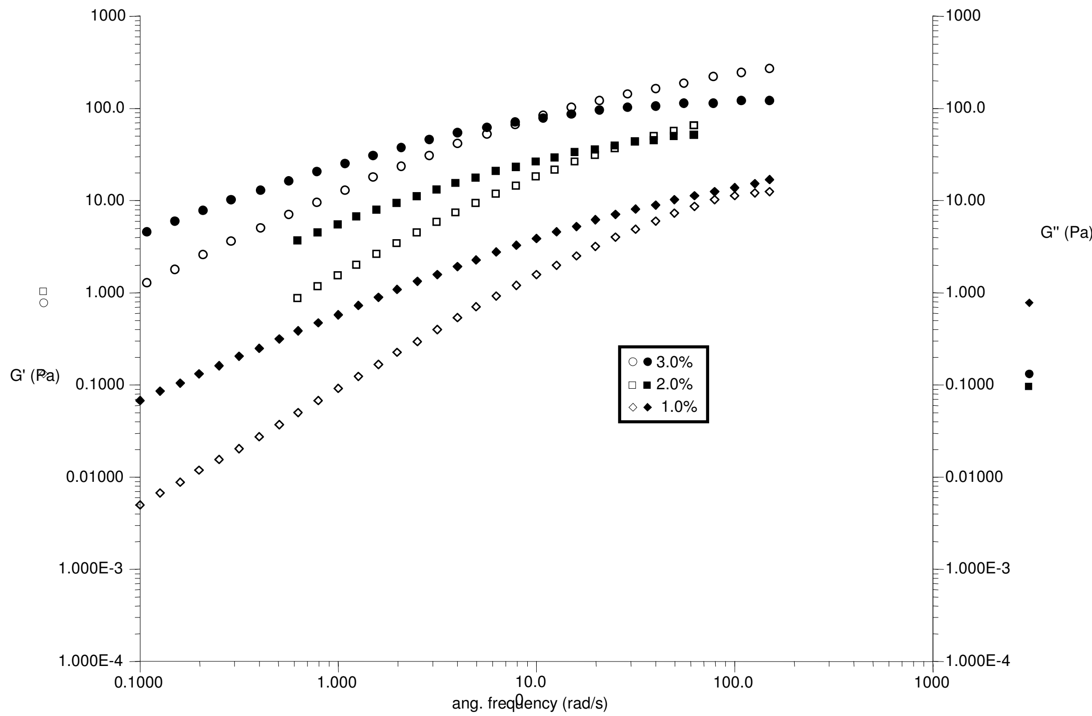

Fig. 5: Frequency sweep showing G' and G'' for different concentrations of *M. flagellipes* gum

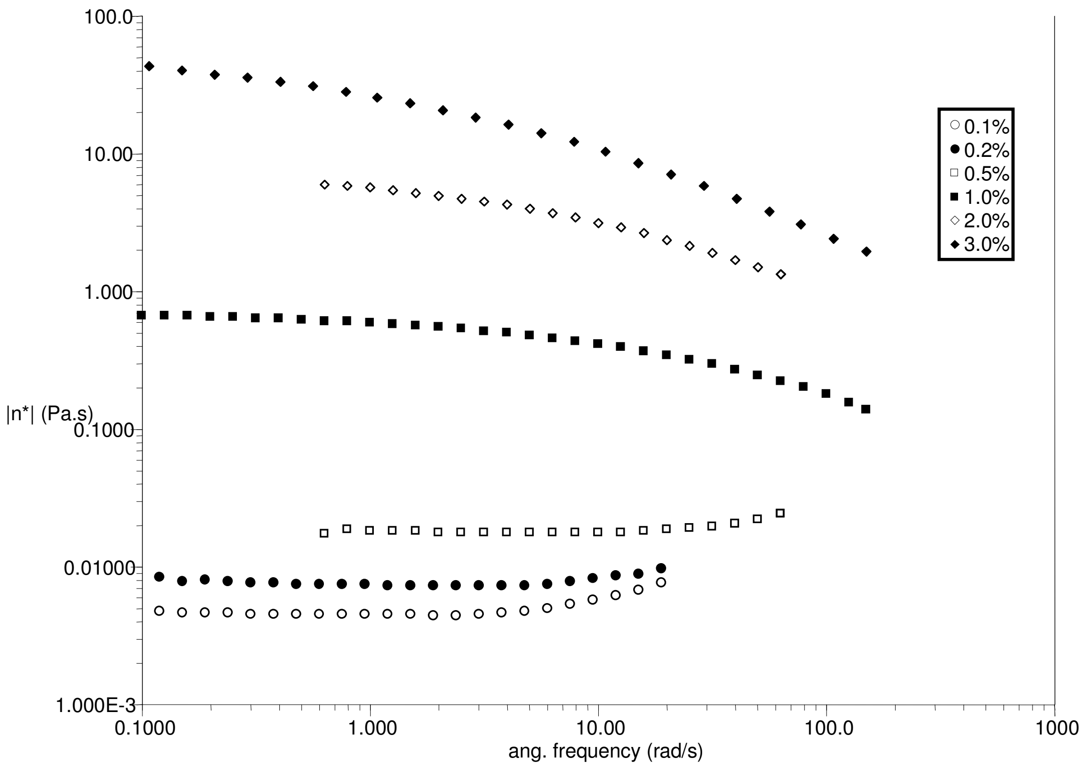

Fig. 6: Frequency sweep showing dynamic viscosity /n*/ for different concentrations of *M. flagellipes* gum

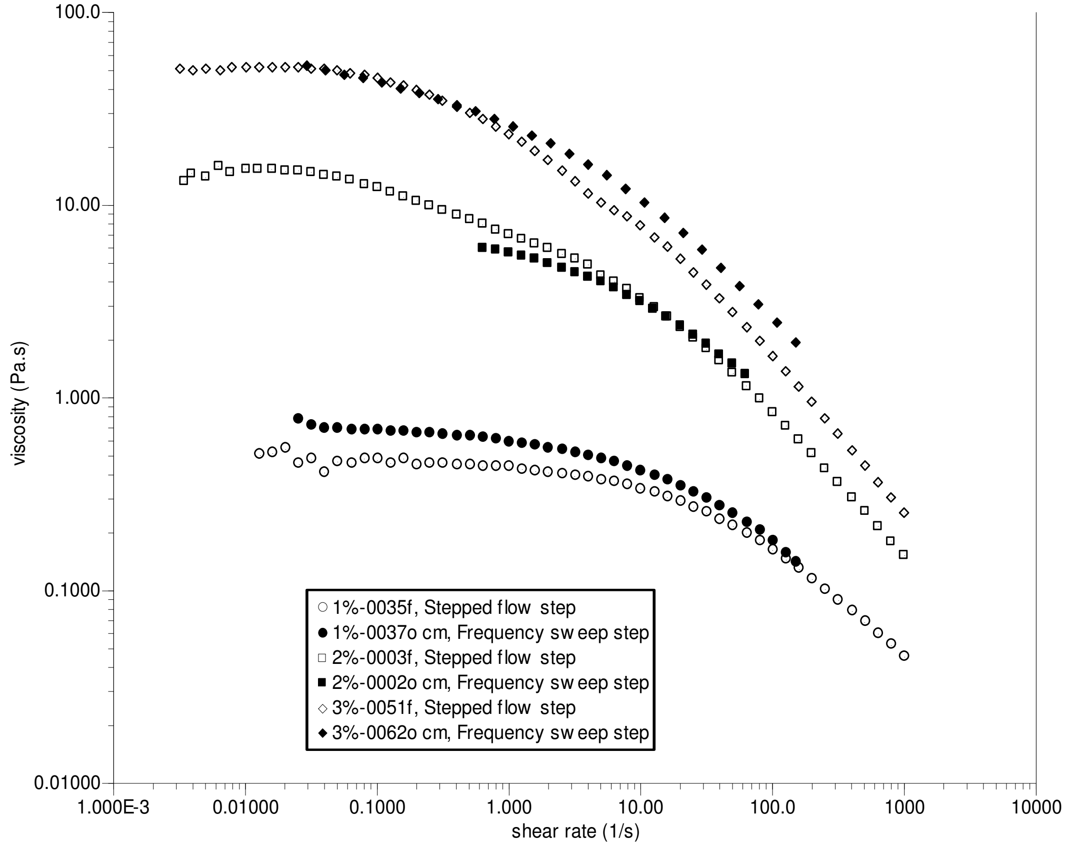

Figure 7: Cox-Merz plots, superimposition of log η (Ỹ) and log η*(ω)

Table 1: Physical composition of *M. flagellipes* seed[a]

Composition	Range	Mean
Hull (%)	25.24-53.27	32.85±10.15
Endosperm (%)	46.73-74.76	67.15±10.15
Germ (%)	-	-

[a]= mean for nine seeds ± SD

Table 2: Yield and composition of *M. flagellipes* gum

Yield (%)[a]	32.6±1.97
Carbon (%)	39.253
Hydrogen (%)	6.425
Nitrogen (%)	1.873

[a]= mean of triplicate determinations ± SD

Table 3: Rheological model-fitted characteristics of *M. flagellipes* gum.

Gum conc. % (w/v)	Rheological Models	η_o (Pa.s)	η_∞ (Pa.s)	τ (s)	κ	S.E
3.0	Cross	53.30	1.868E-7	1.80	0.7370	12.41
2.0	Cross	14.62	8.709E-8	0.7196	0.6689	18.89
1.0	Cross	0.12413	4.356E-3	4.33E-3	0.7202	20.16
0.5	Cross	0.01775	8.359E-3	1.756E-3	0.8417	57.10
0.2	Newtonian	5.862E-3	-	-	-	12.2
0.1	Newtonian	4.001E-3	-	-	-	4.12

Table 4: Analysis result of curve of Log η_{sp} versus Log C[η] for *M. flagellipes* gum concentrations

[η] (dL/g)	7.9
Log C*[η]	0.6
C*[η]	3.98
C* (g/dL)	0.50
Log η_{sp} at C*	-1.75
η_{sp} at C* (Pa.s)	0.018
Slope below C*	1.0
Slope above C*	4.6