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Rheological characterization of galactomannans extracted from seeds of *Caesalpinia pulcherrima*

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ABSTRACT

The galactomannan from the seeds of *Caesalpinia pulcherrima* L. was isolated and purified by precipitation method using alcohol to get *C. pulcherrima* (CP) gum. CP gum was studied for its physicochemical and rheological properties. The composition of CP gum was found to contain mannose:galactose:glucose:xylose in a proportion of 2.8:1:0.1:0.08, with M/G ratio 2.80. The molecular weight (M_w) for CP gum was obtained to be approximately 2.72×10^6 Da by static light scattering measurements and 2.79×10^6 Da using Mark–Houwink relationship. The intrinsic viscosity by Huggins and Kraemer plots using capillary viscometry was obtained as 12–12.5 dl/g. The rheological behavior of aqueous galactomannan solutions was studied at 25 °C, using steady-shear and dynamic oscillatory measurements. The various concentrations of CP gum exhibits shear thinning non Newtonian behavior at high shear rate and Newtonian flow at low shear rate. Experimental data in steady shear has been correlated and found a better fitting with the Cross and Carreau models. A graph of the specific viscosity at zero shear rate (η_{sp0}) against coil overlap parameter ($C[\eta]$) was plotted and the slope of the lines in dilute and semi-dilute regions were found to be 1.23 and 4.1 respectively. The critical concentration (C^*) was found to be about 3.8/[η]. The linear viscoelastic region for CP gum solutions presented nature as that of macromolecular solutions. At all shear rates and frequencies, η_{ap} and η^* had almost similar magnitudes, which shows its reasonable agreement with the Cox–Merz rule. The present investigation shows the suitability of CP gum as a pharmaceutical aid application like viscosity modifier, release retardants, binders.

1. Introduction

Caesalpinia pulcherrima (CP) belongs to Leguminosae (family: Fabaceae and subfamily: Caesalpinioideae) is a traditional medicine plant with thorny bushy legume locally known as Dwarf Poinciana, Dwarf Flamboyan, Pride of Barbados, Barbados Pride, Barbados Flower-fence, Peacock Flower, Paradise Poinciana, Red Bird-of-Paradise is widely distributed in tropical and sub-tropical regions like India, Myanmar, Vietnam, Sri Lanka, and Malay Peninsula. Various pharmacological activities of *C. pulcherrima* L. have been reported such as analgesic and anti-inflammatory (Patel, Verma, Chatterjee, & Gauthaman, 2010; Rao, Fang, & Tzeng, 2005; Sharma & Rajani, 2011; Yodsaoue, Karalai, Ponglimanont, Tewtrakul, & Chantrapromma, 2011), antiulcer (Sharma & Rajani, 2011), antimicrobial activity (Das et al., 2010), antibacterial and antifungal activity (Srinivas et al., 2003), antitumor (Mcpherson, Cordell, Soejarto, Pezzuto, & Harry, 1983; Srinivas et al., 2003), cytotoxic activity (Das et al., 2010; Mcpherson et al., 1983), astringent, abortifacient, emmenagogue (Awasthi, Kumar, & Misra, 1980), selective activity against DNA Repair-Deficient Yeast Mutants (Patil et al., 1997).

Galactomannans (GM) are polysaccharides with (1 → 4)-β-D-mannopyranosyl residues decorated with (1 → 6) linked α-D-galactopyranosyl residues (Carmen, Petkowicz Sierakowski, Lba, Ganter, & Reicher, 1998; Cerqueira, Souza, & Simões, 2011). Galactomannans (often called "Pharaoh's Polysaccharides") are water soluble hydrocolloids, high molar mass, water-soluble, non-ionic polysaccharides forming highly viscous, stable aqueous solutions (Cerqueira et al., 2011; Magdel-Din, Helmy, & Salem, 1998; Pollard, Eder, Fischer, & Windhab, 2010). Galactomannans are widely useful in the various industry mainly as thickening, stabilizing agents in a range of applications (Sierakowski, Milas, Desbrie'res, & Rinaudo, 2000); in the development of edible films or coatings for food applications (Cerqueira et al., 2011); gelling agents (Morris, Cutler, Ross-Murphy, & Rees, 1981), excellent thickeners and stabilizers of emulsions, and the nontoxic nature allows their use in the textile, pharmaceutical, biomedical, cosmetics, food industries (Srivastava & Kapoor, 2005; Vieira, Mendes, Gallao, & de Brito, 2007); mass-efficient aqueous thickeners, co-gellants, and nutritional supplements (Gidley & Reid, 1995; Pollard et al., 2010). Galactomannans has an advantage to form very viscous solutions at relatively

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low concentrations that are only slightly affected by pH, Ionic strength and heat processing (Sittikijyothin, Torres, & Goncalves, 2005).

The functional ability of GM is related to their molar mass and structural features: the solution viscosity (hence rheology) depends mainly on the molar mass while the synergistic interactions seem to be determined by the mannose/galactose ratio (M/G) and fine structure of a galactomannan chain (Bourbon, Pinheiro, Ribeiro, Miranda, & Maia, 2010; Fernandes, Gonçalves, & Doublier, 1991; Schorsch, Gamier, & Doublier, 1997). The M/G ratio of GM and its distribution pattern plays an important role on the chain conformation which decides its functionality (Wu, Li, Cui, Eskin, & Goff, 2012). GM with a different M/G ratio has different behaviors including mechanical and thermal properties from different film formulations (containing Guar Gum and Locust Bean Gum with glycerol and sorbitol), pointing at a promising use of biobased-galactomannan films and coatings as edible packaging materials (Cerqueira et al., 2011; Mikkonen et al., 2007). The rheological studies of polysaccharide gum in aqueous solutions are useful to understand the polysaccharide structure and its function as its potentiality is extended day by day in the pharmaceutical industry for controlling drug release formulations (Sittikijyothin et al., 2005; Srivastava & Kapoor, 2005; Sumathi & Alok, 2002; Xu, Liu, & Zhang, 2006). Galactomannans from various sources may have resembled the contents of galactose; their interaction with other materials can be different due to the difference in the fine structure of the GM. Many properties of galactomannans are M/G ratio dependant. The higher M/G ratio of GM reduces its solubility and the galactose side chains prevent mannan backbone from forming hydrogen bonded aggregates. A Galactomannan solution usually displays a non-Newtonian behavior, where the viscosity reduces with the rise of shear rate (Garti, Madar, Aserin, & Sternheim, 1997). The extent of substitution in galactomannans greatly affects their solution properties (Cerqueira et al., 2011).

In the present work, non-traditional galactomannans were isolated from the seeds of *C. pulcherrima* leguminous species growing in the central western region of India. The major points to be investigated includes the steady shear and dynamic viscoelastic properties of *C. pulcherrima* GM in aqueous solutions, in the range from dilute to concentrated solutions were carried out and are reported.

2. Methodology

2.1. Plant material

Pods of *C. pulcherrima* were collected from selected shrubs, growing in Nasik, Maharashtra, India. The seeds were isolated from the pods, cleaned and suspended in 99% ethanol in ratio 1:3 (seeds:ethanol by volume) at 60–80 °C for 30 min to inactivate the enzymes and eliminate low-molecular-weight compounds. Then ethanol was decanted. The treated seeds were mechanically grinded. The endosperms were manually separated from the germ and the husk of the seeds. The distilled water was added in a proportion of 1:5 (endosperm:water in terms of weight) and kept or approximately 24 h. Further distilled water was added in a double proportion and continued to mix in a blender for 5 min. The suspension was filtered through a nylon net followed by a centrifugation step at 25,000 × *g* (Remi Labs, India) for 30 min at 25 ± 0.5 °C to remove insoluble matter. The galactomannan was precipitated by the addition of 99% ethanol. The ethanol was decanted and the precipitated galactomannan was lyophilized and kept in a dry place (desiccators) till further use.

2.2. Determination of the chemical composition

The moisture content of *C. Pulcherrima* (CP) gum was calculated from the weight loss upon overnight heating at 105 °C in an oven according to the American Association of Clinical Chemistry (AACC, 1995) and the ash content of the GM sample was determined according to AOAC Official Method 923.03. Protein content was determined by obtaining the total nitrogen content by the Kjeldahl method, as described in the Association of Analytical Communities (AOAC, 2000) Official Method of Analysis 981.10. The factor $N \times 5.7$ was used for the estimation of the total protein content of the sample. The elemental analyses were carried out with an elemental analyzer (Elementar Vario El, Germany) instrument for the determination of carbon, nitrogen, sulfur, oxygen (C, S, N, O) at National Institute of Pharmaceutical Education & Research (NIPER), Mohali, Chandigarh, Punjab, India. The main monosaccharide components were analyzed by HPTLC (CAMAG Technologies, Switzerland).

2.3. Development of HPTLC method for estimation of monosaccharide composition

Monosaccharide components were determined according to Shirakawa, Yamotoya, and Nishinari (1998). Briefly, exact 50 mg crude CP was hydrolyzed in 2 N hydrochloric acid at 100 °C for 3 h at 0.1 MPa. The hydrolysates were converted to the corresponding aditol chlorides. Then HPTLC was performed using CAMAG Automatic TLC Sampler 4 (ATS4).

Standard monosaccharides such as galactose, mannose, xylose, glucose of 5 μg/mL was prepared in ethanol. 10 μL each of the different concentrations of standard monosaccharide solutions were spotted (band width: 6 mm) in triplicate on HPTLC plates precoated with 200 μm layer of Si-gel Si60F254 (E. Merck) using an automatic applicator Linomat-5. Before use, the plates were prewashed with 0.02 M sodium acetate and activated at 110 °C for 5 min. Chromatograms were developed for 9.0 cm using acetone–water (9:1 v/v) as mobile phase. Chromatography was performed in a twin trough chamber (CAMAG, Switzerland). On development of chromatogram, the plates were sprayed with 0.5% phosphomolybdic acid reagent and dried the plate at 105–110 °C for 5 min. The developed plates were subjected for scanning in UV spectrum region between 190 and 400 nm to record the peak areas.

CP gum solution (10 μL) was spotted on precoated HPTLC plates with 200 μm layers of Si-gel Si60F254 using an automatic applicator Linomat-5. Chromatograms were developed, scanned and the peak areas recorded. The quantitative estimation of monosaccharide components like galactose, mannose, xylose, glucose in the sample was detected by using the calibration curve of monosaccharides like galactose, mannose, xylose, glucose from 2000 to 10000 ng/μL.

2.4. Preparation of CP gum solutions

To study the effect of the concentration on the viscosity, different concentrations of the CP gum solutions were prepared (0.5%, 1%, 2%, 3%, 4%, 5% w/v). The dispersions of the CP gum were prepared in a distilled water containing sodium azide (0.02% w/w) to prevent bacterial degradation. Further the solutions were stirred continuously at 25 ± 0.5 °C for 24 h at 100 rpm to dissolve the CP gum completely in the solution. The sample solutions were then centrifuged at the 3000 × *g* for 1 h, at 25 ± 0.5 °C prior to the experimentation to remove the air bubbles and the impurities, if present.

2.5. Molecular weight determination

The molecular weight was determined by static light scattering method and viscosity method as follows.

2.5.1. Static light scattering method

The molecular weight of an unknown polymer is quickly obtainable using a static light scattering (SLS) measurement. The intensity of scattered light (I) that a macromolecule produces is proportional to the product of the weight average molecular weight (M_w) and the concentration of the macromolecule (C). The SLS method involves the measurement of the intensity of a polymer particle with a known Rayleigh ratio, usually toluene, followed by the measurement of the scattering intensity of a number of known concentrations of a solution of the polymer sample. The instrument measures the intensity of scattered light (KC/R_θ) of various concentrations (C) of sample at one angle which is compared with the scattering produced from a standard (Toluene). The graphical presentation of the same is called as Debye plot and allows for the determination of both absolute molecular weight and 2nd virial coefficient. The weight average molecular weight (M_w) is determined from the intercept at zero concentration, *i.e.* $KC/R_\theta = 1/M_w$ (for $c \to 0$) where M_w is expressed in Dalton (or g/mol). The 2nd virial coefficient (A_2) is determined from the gradient of the Debye plot. Each plot and molecular weight determination are performed by several individual measurements; from the solvent used (zero concentration measurement), through sample preparation at various concentrations. Intercept at zero concentration will be $1/M_w$ of a plot of KC/R_θ against C and the gradient is proportional to A_2. The molecular weight (as weight average molecular weight, M_w) was determined using the Rayleigh equation for the polymer given by Dollinger, Kunitani, Johnson, and Jones (1992).

$$\frac{KC}{R_\theta} = \left(\frac{1}{M} + 2A_2C\right) P_\theta \quad (1)$$

where R_θ is the Rayleigh ratio – the ratio of scattered light to incident light of the sample; M, sample molecular weight; A_2, 2nd virial coefficient; P_θ, angular dependence of the sample scattering intensity; C, concentration; K, optical constant.

Static light scattering measurements were performed on a Malvern Zeta Sizer Nano Series Nano-ZS 90 instrument (UK). The experiments were performed at a temperature of $25 \pm 0.5\,^\circ$C. CP gum samples were prepared at 1 mg/mL and allowed to dissolve in the distilled water overnight to ensure adequate solvation and centrifuged at the 3000 g for 1 h at $25 \pm 0.5\,^\circ$C to remove any immiscible contents. The prepared samples were diluted with distilled water as per the requirement at the time of evaluation (0.0001–0.0005 g/mL) followed by filtration through 0.22 μ cellulose nitrate filter (membrane filter) and collected directly into clean cuvette. The solvent scattering was measured for various concentrations and Debye plot was generated of variation in the average intensity of scattering light (KC/R_θ) against concentration (C). The intercept of extrapolation to zero concentration was calculated and the 2nd virial coefficient (A_2) was determined from the gradient of the Debye plot. The weight average molecular weight, M_w was calculated using the Rayleigh equation for the CP gum. Similarly standard preparation of the CP gum was prepared in Toluene separately for SLS measurements.

2.5.2. Viscosity method

The molecular weight (as viscosity average molecular weight, M_w) was determined using the Mark–Houwink equation for GM (Beer, Wood, & Weisz, 1999; Doublier & Launay, 1981; Picout & Ross-Murphy, 2007; Cerqueira).

$$[\eta] = KM_w^\alpha \quad (2)$$

where $[\eta]$ is intrinsic viscosity, k and α are constant for the specific polymer system, and M_w is the molecular weight (molar mass) of the polymer.

2.6. Intrinsic viscosity

The viscometric measurements such as relative viscosity (η_{rel}), specific viscosity (η_{sp}), reduced viscosity (η_{red}) and intrinsic viscosity (η) of dilute solutions of CP gum (in the concentration range of 0.01–0.1 g dL^{-1}) were measured at $25 \pm 0.5\,^\circ$C with a Cannon-Ubbelohde Viscometer (semi-micro Viscometer nr 50, Cannon Instrument Co., PA, USA) using exactly 10 mL of solution samples. The densities of the solutions were measured by using the pycnometer. The effect of the concentration of the CP gum on the viscosity of dilute gum solutions was determined using the Huggins and Kraemers equations:

$$\frac{\eta_{sp}}{C} = [\eta] + k'[\eta]^2 C \quad \text{(Huggins equation)} \quad (3)$$

$$\frac{\ln \eta_{rel}}{C} = [\eta] + k''[\eta]^2 C \quad \text{(Kraemers equation)} \quad (4)$$

where η_{sp} and η_{rel} are the (dimensionless) specific and relative viscosities respectively, C is the concentration of the solution; k' and k'' are the Huggins and the Kraemer's coefficients viscosities, respectively.

2.7. Rheological measurements

Rheological measurements of the CP gum solutions were performed using a Controlled Stress Rheometer (Viscotech Rheometer, Rheologica Instruments AB, Lund, Sweden). Data analysis was done with Stress Rheologic Basic software, version 5.0. A cone and plate geometry was used with 25 mm diameter and cone of 1.0°. The samples were exposed to increasing shear rate (0.01–100 s^{-1}) and the relation between shear stress and shear rate was studied. The effect of increasing shear on the viscosity was also analyzed.

Viscosity flow curves were obtained at $25 \pm 0.5\,^\circ$C with the operating shear rate ranging from 0.01 to 100 s^{-1}. The curves were fitted and extrapolated to lower shear rates by Cross (1965) as per Eq. (5). Linear viscoelastic region (LVR) was determined in the stress range of 0.1–100 Pa at a constant frequency (5 Hz). The ability of the CP gum solution to resist the deformation with applied stress was recorded in terms of trends of elastic modulus (G') and loss modulus (G''). The oscillation frequency sweep was determined in the frequency range of 0.1–100 Hz at a constant stress in LVR. The effect of frequency on G' and G'' was analyzed. The rheological tests were performed in triplicate, in each time a fresh sample of the CP gum solution was used and the results were expressed as an average of these three determinations.

3. Results and discussion

3.1. Characterization of C. pulcherrima gum

The extraction and purification processes in the present work were found to be effective to obtain the purified galactomannans at the laboratory scale. The purified galactomannan was found to be homogeneous with $29 \pm 3\%$ yield of the total weight of the seeds. The obtained values are in good agreement with those reported by Andrade, Azero, Luciano, and Goncalves (1999) and Cerqueira et al. (2011) while found to be less as per Pollard et al. (2010).

The physicochemical characteristics of purified CP gum were analyzed for moisture content, ash value, and protein ($N \times 5.7$) content and were found to be $10 \pm 1\%$, 0.056% and 0.016% respectively. It has been observed that, the purification process was sufficient to eliminate ash and proteins in CP gum. The elements

Table 1
Quantification of monosaccharides in CP gum as per HPTLC–UV analysis.

Name of the monosaccharide	*Rf* value of monosaccharides	*Area at Rf*	Monosaccharide content in the CP gum (% w/w)
Mannose	0.45	9180.885	2.8
Galactose	0.34	1562.062	1.0
Xylose	0.40	185.98	0.1
Glucose	0.60	70.84	0.08

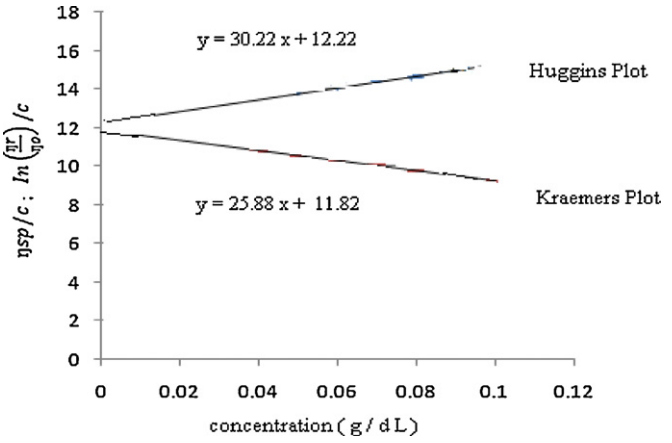

Fig. 1. Determination of intrinsic viscosity based on Huggins and Kraemer plots, η_{sp}/C and $(\ln \eta_{sp})/C$ against concentration for CP gum solution at 25 °C.

proportions from the CP gum were confirmed presence of carbon (39.61 ± 1.48%), hydrogen (6.44 ± 0.4243%), nitrogen (0.002802%), and sulphur (0.03573%). The monosaccharide contents of the *CP gum* were analyzed by HPTLC method and the ratio of mannose, galactose, glucose, and xylose was found to be 2.8:1:0.0.1:0.08 with M/G ratio 2.80, which is in good agreement with previous reports by Cerqueira et al. (2009) as per Table 1. Thus, the CP gum was found to be rich in carbohydrate as per the elemental analysis and HPTLC study.

The viscometric measurement such as relative viscosity (η_{rel}), specific viscosity (η_{sp}), reduced viscosity (η_{red}) and intrinsic viscosity (η) of dilute solutions of CP gum (0.01–0.1 g dL^{-1}) was described in Table 2. An extrapolation of Huggins' and Kraemer's equations (Eqs. (3) and (4)) was helped to determine intrinsic viscosity [η] of a CP gum solution at 25 °C and represented the hydrodynamic volume of individual molecules in a solution as shown in Fig. 1. The intrinsic viscosity depends mainly on the molecular structure and molecular weight of the polysaccharides as well as on the solvent quality. Intrinsic viscosity for CP gum was obtained using very dilute gum solutions (0.1–1%, w/w) and was observed to be 12.22 dl/g as per Huggins' extrapolation, and 11.82 dl/g as per Kraemer extrapolation.

The good linearity was observed in the CP gum samples up to infinite dilution. The intrinsic viscosity value of Huggins' extrapolation, $[\eta]_H$ at 25 °C (dl/g) was found to be 12.22 and Kraemer extrapolation, $[\eta]_K$ at 25 °C (dl/g) was found to be 11.82. The intrinsic viscosity value of Huggins' extrapolation was found to be closer to that the Kraemer extrapolation. Sittikijyothin et al. (2005) was reported that a higher value of the intrinsic viscosity was obtained for purified samples with a decrease in the magnitude of Huggins' coefficient ($k_{H'}$). The calculated value of $k_{H'}$ of our sample was found to be 0.88 which is quiet consistent with others previously reported. The solute–solvent interactions and the state of aggregation of macromolecules of the gum will decides the $k_{H'}$ value. The entanglement, unentanglement and configurational relaxation of the polymeric chain determine $k_{H'}$ value. From the present study, one can predict that $k_{H'}$ and [η] are quite consistent.

A polymer is generally a mixture of molecules of identical or near identical chemical structure and composition, but differing in degree of polymerization (DP) or molecular weight. It is necessary to dissolve the polymer in an appropriate solvent and begin with a dilute solution for molecular weight determination. Malvern Zeta Sizer Nano Series Nano-ZS 90 instrument detected 2nd virial coefficient, A_2 (mL mol/g^2) and correlation coefficient (R^2) as 0.0353 ± 0.00482 and 0.982 respectively.

Debye plot (Fig. 2) of variation in the average intensity of scattering light (KC/R_θ) against concentration (C) obtain the straight line (correlation coefficient 0.982) whose intercept at zero yield the value of weight average molecular weight of 272 ± 160 kDa. Slope of the straight line was 0.0353 ± 0.00482 yield 2nd virial coefficient, A_2 (ml mol/g^2) as 0.982. The value of A_2 reveals that that water acts a theta solvent for CP and molecules tend to stay in solution.

The weight average molecular weight (M_w) by static light scattering measurements using Rayleigh equation (Eq. (1)) was found to be 2.72×10^6 Da. The weight average molecular weight (M_V) using Mark–Houwink viscosity relationship was found to be 2.79×10^6 Da which is quite consistent with M_w obtained by the Malvern Zeta Sizer instrument.

3.2. Rheological behavior

Fig. 3 predicted the flow behavior of the CP gum solutions at different concentrations. CP gum exhibited shear thinning behavior as the viscosity decreased with increasing shear rate and revealed as a Newtonian region in the low shear rate range. The dilute solutions of polymers showed viscosity independence nature on shear rate as the polymer molecules are widely separated and the polymer chains are not in contact with each other. With rise in concentration of the polymer, the viscosity increases and the flow behavior became nonNewtonian as the polymeric chains begin to overlap and the physical contact changes the flow behavior. Extent of chain overlapping with further rise in concentration of CP gum will decide

Table 2
Viscometric measurement of CP gum.

Sr. no.	Conc. (g/mL)	Time (min)	Density (gm/cm^3)	Viscosity (η)	Relative viscosity (η_r)	Specific viscosity (η_{sp})	Reduced viscosity η_{red} (η_{sp}/C)	$\ln(\eta/\eta_0)/C$
1	0.01	0.383	1.0710	0.9895	1.1071	0.1071	10.71	10.17
2	0.02	0.439	1.0735	1.1368	1.2720	0.2720	13.60	12.02
3	0.03	0.486	1.0755	1.2609	1.4108	0.4108	13.70	11.47
4	0.04	0.537	1.0777	1.3931	1.5610	0.5610	14.02	11.13
5	0.05	0.584	1.0796	1.5209	1.7018	0.7018	14.03	10.63
6	0.06	0.636	1.0825	1.6608	1.8583	0.8583	14.30	10.32
7	0.07	0.688	1.0864	1.8030	2.0174	1.0174	14.53	10.02
8	0.08	0.735	1.0899	1.9324	2.1622	1.1622	14.62	9.63
9	0.09	0.789	1.0922	2.0788	2.3260	1.3260	14.73	9.37
10	0.10	0.833	1.0987	2.2078	2.4704	1.4704	14.70	9.04

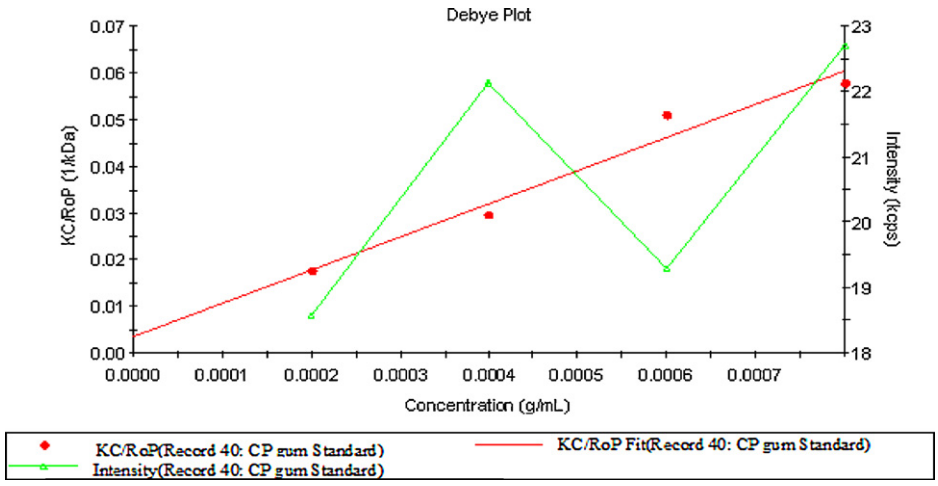

Fig. 2. Debye plot of variation in the average intensity of scattering light (KC/R_θ) against concentration (C) with intercept of extrapolation to zero concentration and the 2nd virial coefficient (A_2).

the viscosity of the gum solution which can be characterized by the coil overlap parameter, $c\,[\eta]$. Coil overlap parameter indicates the volume occupied by the polymer molecule in the solution (Rao, 2007). The Cross model was used to fit the flow curves using Eq. (5) and the zero-shear viscosity of concentrated solutions was determined (Cross, 1965). The data for the dilute solutions were obtained by viscometric measurements, whereas the data for the concentrated solutions were estimated by Eq. (6) using data from flow measurements (Carreau, 1972).

$$\eta_a = \eta_\infty + \frac{(\eta_0 - \eta_\infty)}{[1 + \tau\gamma^m]} \tag{5}$$

$$\eta_a = \eta_\infty + \frac{(\eta_0 - \eta_\infty)}{[1 + (\lambda\gamma)^2]^N} \tag{6}$$

where γ is the shear rate (s^{-1}), η_a is the apparent viscosity (Pa s), η_0 is the zero-shear rate viscosity (Pa s), η_∞ is the infinite shear rate viscosity (Pa s), τ (s) and λ (s) are time constants, and m and N are dimensionless constants related to the power law exponent α by $m = 1 - \alpha$ and $N = (1 - \alpha)/2$ ($0 \le N < 0.5$), for the case $\eta_\infty \ll \eta_a \ll \eta_0$. Since at high shear rate Newtonian viscosity was never approached in our study, the above equations were simplified (only three adjustable parameters), assuming $\eta_0 \gg \eta_\infty$.

Table 3 provided η_0 and other constants of the galactomannan are in agreement with the model predictions, as described by magnitudes of relative deviation error (RE). The rate of entanglement of the polymer reduces with the reduction in concentration of the polymer and exhibits shear thinning nonNewtonian behavior.

Low concentrated polymeric solution exhibits slight shear rate dependence due to the wide separation of the polymeric molecules with a scattered distribution of polymeric chain. With a rise in the concentration, a point is reached when the chains begin to overlap and the flow behavior changes due to changes in physical contact. A further rise in the polymer concentration, viscosity depends upon the extent of overlapping which can be characterized by the coil overlap parameter, $C\,[\eta]$ that indicates the volume occupied by the polymer molecule in the solution (Rao, 2007). A plot of $C[\eta]$ *vs.* "zero-shear viscosity" (η_{sp0}) is shown in Fig. 4, where two concentration regions can be observed. From Fig. 4 it can be predicted that the domain with a slope of 1.23 represents the dilute solution, and the other domain had a slope of 4.1 represents semi-dilute solutions, which represents the specific intermolecular associations (Morris et al., 1981; Sittikijyothin et al., 2005). In the present work, a critical concentration, C^*, was obtained at $C^*[\eta] = 3.8$, considering only two main concentration domains which are in good agreement with previous results obtained for galactomannans.

3.3. Viscoelastic properties

Analysis of viscoelastic materials is designed not to destroy the structure, so that measurements can provide information on the intermolecular and interparticle forces in the material. Hence,

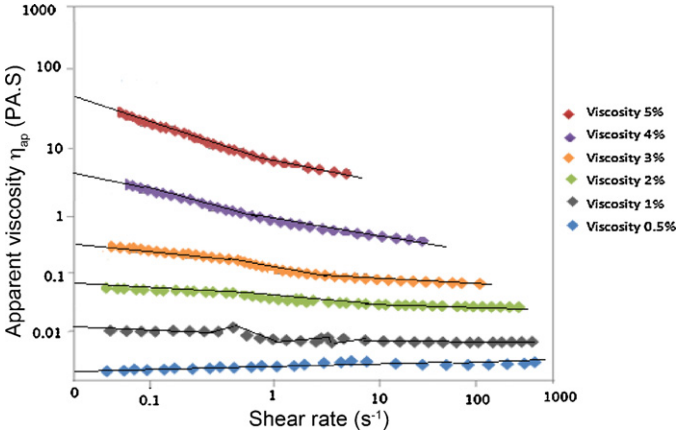

Fig. 3. Flow curves of CP gum solutions, at 25 °C, at different concentrations (wt %): 0.5%, 1%, 2%, 3%, 4%, and 5%.

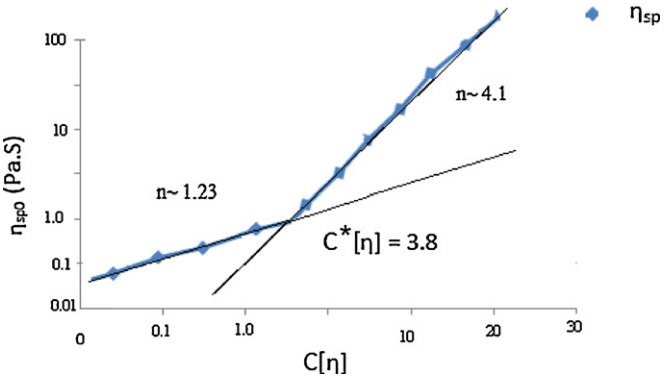

Fig. 4. Illustration of dilute and concentrated domains for CP gum in terms of $C[\eta]$ (coil overlap parameter) against specific viscosity at zero shear rate η_{sp0}; with a slope of 1.23 and 4.1, respectively.

Table 3
Magnitudes of the Cross model parameters and Carreau model parameters for steady simple shearing, obtained for the aqueous solutions of CP gum.

Concentration (wt %)	Cross model parameters				Carreau model parameters			
	η_0 (Pa s)	τ (s)	m	RE[a]	η_0 (Pa s)	λ (s)	N	RE[a]
0.5	0.684	0.0017	0.121	0.062	0.341	0.008	0.060	0.066
1.0	0.8462	0.174	0.242	0.041	0.4231	0.669	0.121	0.043
2.0	0.9942	0.412	0.540	0.032	0.4923	0.206	0.270	0.031
3.0	4.68	0.326	0.824	0.047	2.79	0.163	0.412	0.040
4.0	8.42	0.4015	0.856	0.0681	5.62	0.2011	0.428	0.064
5.0	99.68	0.541	0.982	0.074	47.62	0.254	0.491	0.022

[a] RE, relative deviation error = $\sum_{i=2}^{n}(|(X_{exp,i} - X_{cal,i})/X_{exp,i}|)/n$.

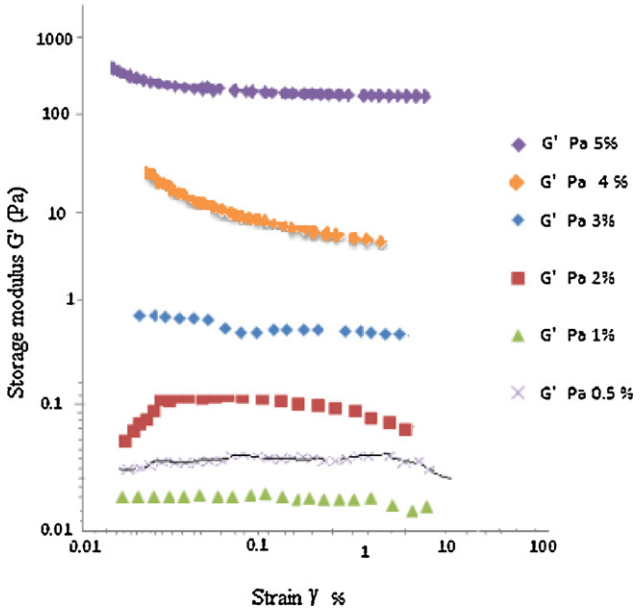

Fig. 5. Strain dependence of the storage modulus of CP gum solutions, at 25 °C, at different concentrations (wt %): 0.5%, 1%, 2%, 3%, 4%, and 5%.

the oscillation stress sweep was conducted to find out the linear viscoelastic region (LVR). The relationship between G' on strain (γ) for sample solutions was determined and is presented in Fig. 5. LVR was found in the strain range of 0.01–1% for the studied range of the sample solutions.

The frequency dependence of the G' and G'' was helped to predict the valuable information about the structure of a polymer solution.

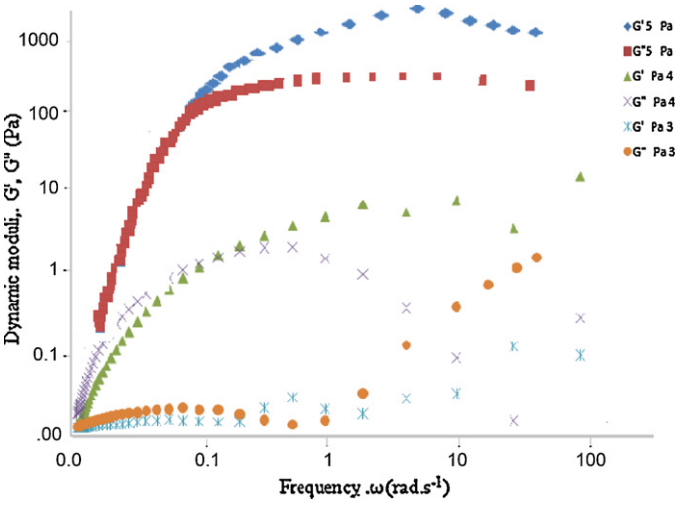

Fig. 6. Effect of CP gum concentration on the storage modulus (G'') and loss modulus (G') measured during frequency sweeps (3%, 4%, and 5%).

An oscillation stress sweep test is a dynamic test, where the G' (elastic or storage modulus) component and G'' (viscous or loss modulus) component were measured at a constant frequency and a range of stress. The range of stress over which G' is independent of the applied stress amplitude is called the LVR. G' is a measure of energy stored and recovered per cycle of deformation and reflects the solid like component of the viscoelastic material. It is closely related to the connectivity of the polymer network and is found to be directly proportional to the number of entities, which can support stress including the physical entanglements and chemical bonds (McLeish, 2002). The frequency sweeps for CP gum solutions

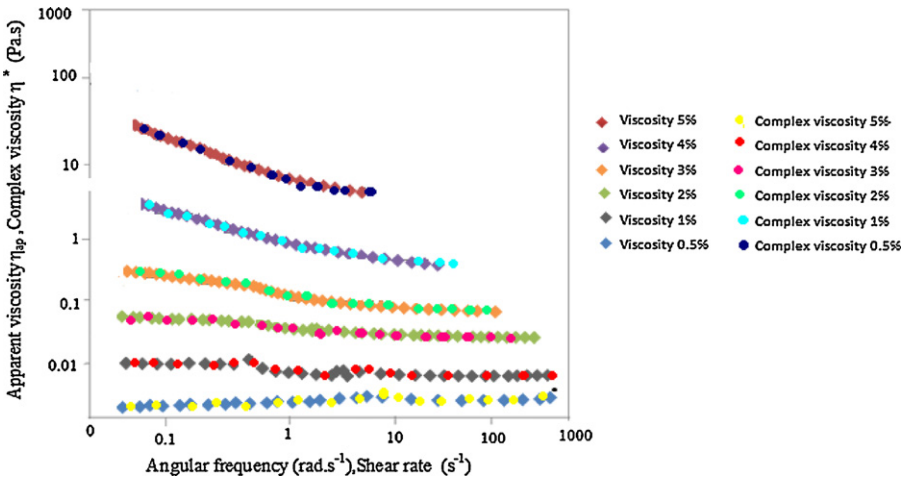

Fig. 7. Superimposition of shear rate dependence of apparent viscosity (η_{ap}) determined by flow measurements and of frequency dependence of complex viscosity (η^*) determined by oscillatory measurements, for CP gum solutions at different concentrations: 0.5%, 1%, 2%, 3%, 4%, and 5%.

presented typical shape of macromolecular solutions as shown in Fig. 6. The G'' is higher at low frequencies when the sample is predominantly viscous while at high frequencies G' is predominantly elastic (due to the highly structured nature of the polymer). Crossover of the moduli was observed at the lowest concentration indicating the commencement of chain entanglement at low concentrations. The obtained results for our CP gum are in good agreement with those reported by Andrade et al. (1999) and Pollard et al. (2010).

The Cox–Merz rule explains a relationship between apparent viscosity, η_{ap} in steady shear and the complex viscosity, η^*, in an oscillatory frequency sweep. The validity of the Cox–Merz rule entails an agreement between the linear viscoelastic response measured in small amplitude oscillatory shear and the nonlinear response measured in steady shear flow measurements. Thus, the Cox–Merz correlation allows use of data from the linear region in the study of nonlinear viscoelastic behavior. The apparent viscosity, η_{ap}, in steady shear and the complex viscosity, η^*, in oscillatory shear, were plotted at equal values of shear range and frequency for CP gum at several concentrations. As per Fig. 7, good superimposition of η^* and η at lower oscillatory frequency sweep and small deviation was observed at higher frequency which showed its reasonable agreement with the Cox–Merz rule (Eq. (7)) (Kaur, Singh, & Singh, 2009).

$$\eta*(\omega) = \eta_{ap}(\gamma) \quad (7)$$

For CP gum solutions, at all shear rates and frequencies, η_{ap} and η^* had almost similar magnitudes. The Cox–Merz rule has been found to be applicable to GM with structural homogeneity guar gum and locust bean gum while variation from the Cox–Merz rule could be an indication for heterogeneous polysaccharides, in which the rheological behavior is controlled by physical entanglements (Rao, 2007).

4. Conclusions

A galactomannan can be easily obtained from mature seeds of *C. pulcherrima*, Leguminosae. The isolated galactomannans shows M/G value (2.80) which closest to the available commercial galactomannans like commercial Tara Gum, and *Sophora japonica*, with a high Man monosaccharide content (leading to M/G over 5), guar gum (1.6–1.8), locust bean gum (3.5–3.8) and *Cassia javanica* (3.23–3.25). *C. pulcherrima* Gum was found to be a highly viscous, high molecular weight polysaccharide that exhibited shear thinning nonNewtonian flow behavior in all the concentrations studied due to random-coil polymer in the range of concentration and shear rates studied. The intrinsic viscosity of the isolated galactomannan is 12–12.5 dl/g, found nearer to the available commercial galactomannans. The G'' is higher at low frequencies when the sample is predominantly viscous while at high frequencies G' is predominantly an elastic nature. The rheological characteristics of the CP gum demonstrate that it can be used as a viscosity modifier, thickening agent in food industry as well as in the pharmaceutical industry.

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