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Rheological Properties of Polysaccharide-Protein complex from Longan (*Dimocarpus longan Lour.*) Pulp

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Abstract: Crude longan polysaccharide (CLP) was extracted from Longan (*Dimocarpus longan Lour.*) pulp. Its chemical composition was determined by chemical analysis, High Performance Liquid Chromatography (HPLC) and Gel Permeation Chromatography (GPC). The flow and viscoelastic behavior of CLP solution was investigated by steady shear and small amplitude oscillatory shear (SAOS) experiments, respectively. The result showed that the CLP solution was a polysaccharide-protein complex. The rheology experiments show that it was a pseudoplastic flow in a range of shear rate (0.1-100s^{-1}). Both flow behavior and viscoelastic behavior of CLP were influenced by cations such as Na^{+} and Ca^{2+}. An increase of apparent viscosity, *G'* and *G"* was accompanied with addition of Na^{+} and Ca^{2+}. Polysaccharide which named LPB-2-M was obtained from isolation and purification of CLP, which was a single component due to the narrow molecular weight distribution, which was evident by the polydispersity index (PDI=Mw/Mn). The average molecular weight (Mw) of LPB-2-M was 80 kDa.

Key words: Longan polysaccharide; rheological property; monosaccharide constitution; molecular weight

1. Introduction

Bioactivity polysaccharides recently have attracted much attentions due to their broad spectra of therapeutic properties including anti-oxidation, immunomoulation, anti-tumour and hypoglycemic activity [1] and relatively low toxicity. Polysaccharides from animal, plant and microbial fermentation extracts are an interesting source of additives for high value healthy food and pharmaceutical industry. They can promote the healthy growth and development of living organisms, and have been widely studied in recent years from their biological, chemical and physical properties [2].

Longan (*Dimocarpus longan* Lour.) belonging to the Sapindaceae family, is an important tropical fruit and mainly planted in Southeast Asia, such as south of China, Vietnam and Thailand [3-7]. Its taste, nutrition and flavor are welcomed by consumers in the world. The fresh seed and pericarp of Longan have been used as a traditional Chinese medicine (TCM) since ancient times. Many reports show their great health effects such as anti-oxidation [7, 8], anti-tumor [8, 9], the activity of anti-glycation [3], immunomodulatory activity [5, 10] and anticancer activity [11]. The enhancement of extraction by physical fields such as ultrasonic-assisted [12], microwave-assisted [13], and pressure-assisted extract [1] has been widely used. The chemical structure of polysaccharides has also been discussed. Yang et al [14] used gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) to get the chemical structure of longan fruit pericarp polysaccharides, they comprised of L-arabinofuranose (L-Ara*f*, 32.8%), D-glucopyranose (D-Glc*p*, 17.6%), D-galactopyranose (D-Gal*p,*-33.7%) and D-galacturonic acid (GalpA, 15.9%), the backbone consisted of →5)-L-Ara*f*-(1→,→6)-D-Glc*p*-(1→,→3)-D-Gal*p*-(1→,→3)-D-GalpA-(1→and→6)-D-Gal*p*-(1→ with a molar proportion of 2:1:1:1:1. Nuclear magnetic resonance (NMR) spectra indicated that the configuration of anomeric carbon in glucose residual was α-form and confirmed to be (1→6)- α-D-glucan by chemcial shift of C6 [11].

Rheological properties have been used to evaluate thickeners, stabilizers, gelling agents, emulsifiers, flavor fixation agents and texture modifiers of polysaccharide [15], which performs a key role for the application in food systems. The study of rheological properties of Longan polysaccharides solution can be used to stabilize Longan drinks quality, which is important to Longan drinks development and quality control. Iagher et al [16] have studied the rheological properties of polysaccharides from mango (*Mangifera indica* L.) pulp, which shows a weak gel behavior. Guo et al [17] studied the rheological behavior of alkaline extracted gel fraction (AEG) of psyllium polysaccharides and found that Ca^{2+} have a significant influence on the gel properties. The rheological properties of acidic polysaccharide from boat-fruited sterculia seeds were also discussed by Wu et al [18]. Among those natural polysaccharides, the molecular structure, such as molecular weight, degree of branches, and functional groups [19] are main factors to affect the rheological behavior of polysaccharides. Furthermore, temperature, concentration of polysaccharide and presence of sugars or salts also affect the rheological properties of polysaccharide [20-22].

To the best of our knowledge, there is no paper on rheological properties study on Longan polysaccharides. So the aim of this work is to study the rheological properties of Longan polysaccharides for further application, such as Jelly. In order to prepare the polysaccharides, we used ethanol precipitate to prepare water-soluble polysaccharides. The rheology properties of polysaccharide were studied by dynamic shear rheometer, and some structure information was also discussed to explain this phenomenon.

2. Materials and methods

2.1. Plant materials and sample preparation

The hot air dried Shixia Longan (*Dimocarpus longan Lour.* harvested from Guangdong Province) pulp were soaked into four-fold 80% (v/v) ethanol for 48 hours, and then dried before grinding into 200 mesh powder which was subject to further analysis.

2.2. Chemicals

Glucose, phenol and sulphuric acid were obtained from Guangzhou Reagent Co. (Guangzhou, China). Standards of xylose (Xyl), arabinose (Ara), glucose (Glc), galactose (Gal), fructose (Fru), mannose (Man), galacturonic acid (GalA) and glucuronic acid (GlcA) were purchased from Sigma Chemical Co (St. Louis, MO, USA). All the other chemicals used were of analytical grade.

2.3. Extraction of quantification of Longan polysaccharide

The method of extract polysaccharides by hot water and alcohol precipitation was slightly modified according Yi's methods [23]. In brief, 10 g of Longan pulp powder were dispersed in 100 mL of distilled water and heated to 90°C for two hours once for three times. The extract was filtered through four-fold gauze, and then centrifuged at 3000 rpm for 10 minutes. The supernatant was concentrated to 100 mL using rotatory evaporator (LR4002, Heidolph, Germany) at 65 °C under vacuum. Anhydrate ethanol (400mL) was added in the concentration extract to precipitate polysaccharides for overnight at 4°C. The pellet was gathered after centrifugation, and successively washed by anhydrate ethanol, absolute ether and acetone and then dried at 45 °C under vacuum. The content of total polysaccharide was determined by the phenol-sulphuric acid method and expressed as glucose equivalents. The content of hexuronic acid was determined by the method of Blumenkrantz and Asboe-Hansen and expressed as glycuronic acid equivalents. The protein content was estimated using Folin-Ciocalteu's reagent and expressed as bovine serum albumin equivalents [23]**.**

2.4. Fractionation of polysaccharide

The proteins in the CLP were removed using the Sevag reagent [24] and decolored by H_2O_2, after removal of the Sevag reagent. Two kinds of chromatography (anion-exchange DEAE-cellulose chromatography and Sephacryxl S-400 HR gel chromatography) were used to purify CLP. The major fraction named LPB-2-M was obtained based on the elution curve for the further analysis. The yields of LPB-2-M were 46.7%.

2.5. Measurement of molecular weight

The relative molecular weight of the purified polysaccharide was determined by an Agilent HP-GPC 220 system, which is equipped with three X-stream columns, and a differential refractive index (RID) detector. All columns were maintained at constant 40 °C with a flow rate of 1 mL/min. The sample of LPB-2-M was dissolved in pure water then filtered through a 0.45μm membrane under pressure. A series of dextran standards (with molecular weight with 10 kDa, 40 kDa, 70 kDa, and 500 kDa) were used to determine the sample.

2.6. Monosaccharide measurement

LPB-2-M (10 mg) was hydrolyzed by 5mL of 3 M trifluoroacetic acid at 100 °C for 6 h. Derivatization of the released monosaccharides was then carried out by the 1-phenyl-3-methyl-5-pyrazolone reagent (PMP). The PMP derivatives were loaded onto a HPLC-2010 chromatography system (Shimadzu, Shanghai, China) equipped with an ODS-C18 capillary column and a SPD detector. The following program was adopted for HPLC chromatography analysis: column temperature 40°C, wavelength of detector 245nm, injection 10 μL, mobile phase was mix solution of acetonitrile and phosphate buffer (pH 6.8) at the ratio of 15 and 85 at a flow rate of 1mL/min [25].

2.7. Rheological measurement

2.7.1. Preparation of samples

Water-soluble polysaccharide solutions at different concentrations (3.0%, 5.0%, 10%, w/v) were prepared by dissolving the dried CLP powder in distilled water and samples (5%, w/v) dissolved in different Na^+ concentration solutions (0 mM, 50 mM, 150 mM, 250 mM) and different Ca^{2+} concentration solutions (0 mM, 50 mM, 150 mM, 250 mM). All the samples were stirred for 2h.

2.7.2. Rheological experiment

Dynamic shear rheological properties such as storage modulus (G'), loss modulus (G'') and complex viscosity (η^*) of the gels were measured under small-amplitude oscillatory shear using a parallel geometry with diameter of 50 mm in an MCR 302 Rheometer (Anton paar, Austria, DE). The dynamic measurements were performed at a strain value of 0.02 (2%) (within the linear viscoelastic regime) in a frequency range of 0.1-100 rad/s. Steady shear tests were also carried out to assess the flow behavior of the polysaccharide samples using the same apparatus described above. Flow curves were measured at increasing shear rate from 0.1 to 100 s^{-1} at 25°C.

3. Results and discussion

3.1. Chemical composition of Longan polysaccharide

Water-soluble polysaccharides were extracted from Longan pulp by hot water and alcohol precipitation in this work. The crude Longan polysaccharides (CLP) was determined to contain 80.72% of polysaccharides, 5.27% of uronic acids and 13.10% of proteins, which is similar to previous report [5]. Yi et al have studied this polysaccharide-protein complexes, and found that exhibit good immunomodulatory activities [23]. Different carbohydrate/protein ratios may affect the bioactivity, for example, polysaccharide-protein complex from L. barbarum (LBP) with high protein content are able to activate T cells, whereas high carbohydrate content could not [26]. The effect of immunomodulatory activities by different carbohydrate/protein ratios of Longan polysaccharide-protein were also discussed by Yi et al.

3.2. Some major components of CLP

The CLP have been purified named LPB-2-M.. The monosaccharide component and molecular weight distribution of LPB-2-M have been seen Fig.1 and Fig.2. From Fig.1, it could be seen that the composition of monosaccharide of LPB-2-M was tested, which contains ribose (21 min), rhamnose (23 min), fructose (24 min), glucose (39 min), arabinose(43 min), galactose (51min) and xylose (55 min). This result is different from other reports [27], LPS-N consist of xylose and glucose, LPS-A1 was composed of rhamnose, xylose, arabinose and galactose, LPS-A2 only contained rhamnose, because of different method of extraction and fractionation.

In Fig.2, a single peak at 24 min of LPB-2-M was observed, which means that it is single component with constant molecular weight. The range of molecular weight distributed from 23 kDa to 410 kDa, and polydispersity (Mw/Mn) obtained for LPB-2-M was 1.03. The average molecular weight (Mw) was 80 kDa, which account for 90% approximately. Some other reports on molecular weight of polysaccharide extracted from Longan were 13.8 kDa for neutral Longan polysaccharide LPS-N, 1382 kDa and 571 kDa for two acid polysaccharide (LPS-A1 and LPS-A2) from Longan pulp [27]. It was also reported that the weight-average molar mass of LPI was 14 kDa, and its polydispersity (Mw/Mn) was 1.36 [5, 6].

3.3. Rheological properties

3.3.1 Effect of CLP concentrations

Steady shear and SAOS measurements at 25°C were performed to study the rheological behavior of CLP at the concentrations of 3, 5 and 10% (w/v). The dispersion at low concentration always shows erratic signals because of its very low viscosity or due to the parallel plate geometry limit. The mechanical spectrum (frequency dependence of *G'*, *G"* and η*) of crude longan polysaccharides (CLP) (5% w/w) at 25°C is shown in Fig.3. For this sample, The complex viscosity η* decreases linearly with increasing frequency with a slope of -0.92 on a double logarithmic scale, showing a strong shear thinning behavior with a power-law index of 0.08. The slope is deeper than the value of -0.76 described by Morris [28] and it was used to describe "weak gel" characteristics of a polysaccharide gel formed by overlapping and entangled flexible random coil chains. *G'* is always approximately 5 times higher than *G"* in the frequency range studied, and both the moduli show little frequency dependency ($G' \sim \omega^{0.07}$, $G'' \sim \omega^{0.17}$), exhibiting a weak gel-like behavior. Steady shear viscosity (η) from rotational measurements is substantially lower than η* from SAOS measurements. This violation of the Cox-Merz rule [29] implies a structure that remains intact under small-amplitude oscillation, but may be broken down by the shear applied in rotational measurements, however, this should be further verified by recovery experiments in future.

The samples at lower (3%) and higher (10%) concentration exhibit qualitatively similar rheological behavior, but large quantitative difference in viscosity and the two moduli. Fig. 4 shows the double logarithmic curve of log *G*' and log *G″* versus log C. Comparisons are made at 1 rad s^{-1} (log frequency = 0). From Fig.4, it shows that *G'* and *G"* increase linearly with C on a double logarithmic scale. The slope of *G'* is higher than that of *G"*, leading to an increase in the separation between *G'* and *G"* with increasing concentration. Tan δ represents *G"* normalized by *G'* (i.e. *G"*/*G'*), which is a sensitive indicator of crosslinking. For an ideal network, tan δ = 0, and the decrease in tan δ indicates the proceeding of the network formation and the elimination of in homogeneities [30]. In fact, the value of tan δ=*G"*/*G'* decreases from 0.42 to 0.15 with increasing concentration for the concentrations measured, which indicates a better network is forming. This is due to the stronger interaction between the molecules with increasing the CLP concentration. In addition, both slopes are much higher than the typical values of normal gelling polysaccharides [31].

The double logarithmic curve of log η* and log η versus log C is shown in Fig.5. The separation between log η* and log η decreases with decreasing concentration, extrapolating to zero at ~3% (i.e. progressively smaller departure from Cox-Merz superposition as concentration is lowered). Although the slope of log η versus log C (~4.9) is much lower than the corresponding value of ~6.7 for η*, it is still substantially higher than the slope of ~3.4 normally observed for solutions of entangled polysaccharide coils [32].

3.3.2 Effect of Na^+/Ca^{2+} concentrations

Salt addition to 5% CLP can increase both the moduli depending on salt concentration and type (Fig. 6). As a monovalent ion, Na^+ can increase *G'* and *G"* when the concentration is above 50 mM, but decrease the moduli slightly when the concentration is below 50 mM. However, this is not the case for divalent ions. Immediate sharp increase in the moduli is observed when Ca^{2+} concentration is low (<50 mM), and they continue to increase with Ca^{2+} concentration (>50 mM), but more gradually than those at low Ca^{2+} concentration. The moduli are higher with Ca^{2+} than those with Na+ at any given concentration, and the moduli at 250 mM are two orders of magnitude lower than those with Ca^{2+}. Interestingly, tan δ almost didn't change with salt concentration and type, indicating that network structure doesn't change or at least not significantly with salt addition.

Considering the components of CLP, it is reasonable to say that the related polysaccharide is negatively charged due to the presence of uronic acids. The anionic polysaccharide may have a strong interaction with salt, which cause the transition of polysaccharides conformation, however, this is beyond the topic of this paper and the detailed mechanism will be further studied in another paper by dynamic light scattering and Zetasizer. As inferred from the effects of salt on rheological behavior, CLP in solution may have ordered helical structures, as many polysaccharides (xanthan, gellan, carrageenan, etc) can adopt [33]. Since for disordered polymers, addition of salt would decrease the dimensions of the coils, leading to reduction in viscosity and the dynamic moduli [31]. It is well known that charged polysaccharides and proteins could form complexes by electrostatic interactions. The properties, such as gelling properties, can be improved by the interactions between the two categories of biopolymers [34-40]. This could be the reason that the increase in the moduli is much higher than some reported polysaccharides, as was mentioned in last section. On the other hand, the electrostatic repulsion between the polysaccharide molecules can act as a barrier preventing the association between the molecules. This barrier, however, can be destroyed by charge screening of salt addition, thus promoting the association between the polysaccharide molecules. But in the meantime, the electrostatic interactions between polysaccharides and proteins are also weakened due to the charge screening, which explains the slight decrease in the moduli when NaCl was added (<50 mM). Further increase of NaCl concentration will promote the association between the polysaccharides, leading to the increase in the moduli. As a divalent ions, Ca^{2+} could link the molecule structure by possible ion bridging, making the association between the polysaccharide molecules stronger. As a result, the addition of Ca^{2+} leads to higher moduli and viscosity than Na^{+}.

The response to salt is broadly similar to that of gellan [31], which is anionic and forms double helices at room temperature. Monovalent (Group I) metal ions promote gelation by binding to individual double helices and thus suppressing the electrostatic barrier to helix–helix association. Binding occurs only above a minimum critical cation concentration, which for Na^{+} is ~65 mM (the concentration at which the moduli and viscosity of CLP increase steeply on progressive addition of NaCl). Ca^{2+} (and other divalent metal ions) promotes gelation by binding between helices, and there is an immediate sharp increase in moduli on progressive addition of $CaCl_2$ to gellan solutions (as seems also to occur with CLP).

These apparent similarities may be coincidental, but it would be worth exploring the effect of salts on CLP in greater detail, by studying a few more concentrations in and around the regions of steep increase and inflexions in Fig. 6.

4. Conclusion

Crude longan polysaccharide (CLP) was extracted from Longan (Dimocarpus longan Lour.) pulp. In order to use the CLP widely, the chemical composition and rheological properties have been discussed. The chemical composition was determined by chemical analysis, High Performance Liquid Chromatography (HPLC) and Gel Permeation Chromatography (GPC). The flow and viscoelastic behavior of CLP solution was investigated by steady shear and small amplitude oscillatory shear (SAOS) experiments, respectively.

Water-soluble polysaccharides (CLP, and LPB-2-M) were isolated from Dimocarpus longan Lour. All of them contain major sugars and uronic acid while CLP consists of protein in particular. The monosaccharide analysis confirmed that LPB-2-M consist of monosaccharide such as ribose, rhamnose, fructose, glucose, arobinose, galactose and xylose. The CLP solution show a weak gel-like behavior, but the structure can be broken down by the steady shear. An ordered structure was inferred based on the response of salt to the rheological properties. The Na^+ and Ca^{2+} can increase both moduli and viscosity, Ca^{2+} but works more efficiently.

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Figures

Fig.1 HPLC chromatograph of derivate of mixed monosaccharide and LPB-2-M

Fig. 2 GPC chromatography of LPB-2-M

Fig.3 Frequency-dependence of G'(■), G''(●) and η* (▲) and shear-rate dependence of η (Δ) for 5% CLP in water at 25°C.

Fig. 4 Variation of log G' (■) and log G'' (●), measured at 1 rad s^{-1} and 25°C, with log C for CLP in water at concentrations of C = 3, 5 and 10%.

Fig. 5 Variation of log η * (▲) at 1 rad s^{-1} and log η (Δ) at 1rad s^{-1} with log C for CLP in water (25°C) at concentrations of C = 3, 5 and 10%.

Fig. 6 Variation of log G' (squares) and log G'' (circles), measured at 1 rad s^{-1}, with molar concentrations of added NaCl (open symbols) or $CaCl_2$ (filled symbols) for 5% CLP at 25°C.

Fig. 7 Variation of log η (measured at 1 rad s^{-1}) with molar concentrations of added NaCl (Δ) or $CaCl_2$ (▲) for 5% CLP at 25°C.

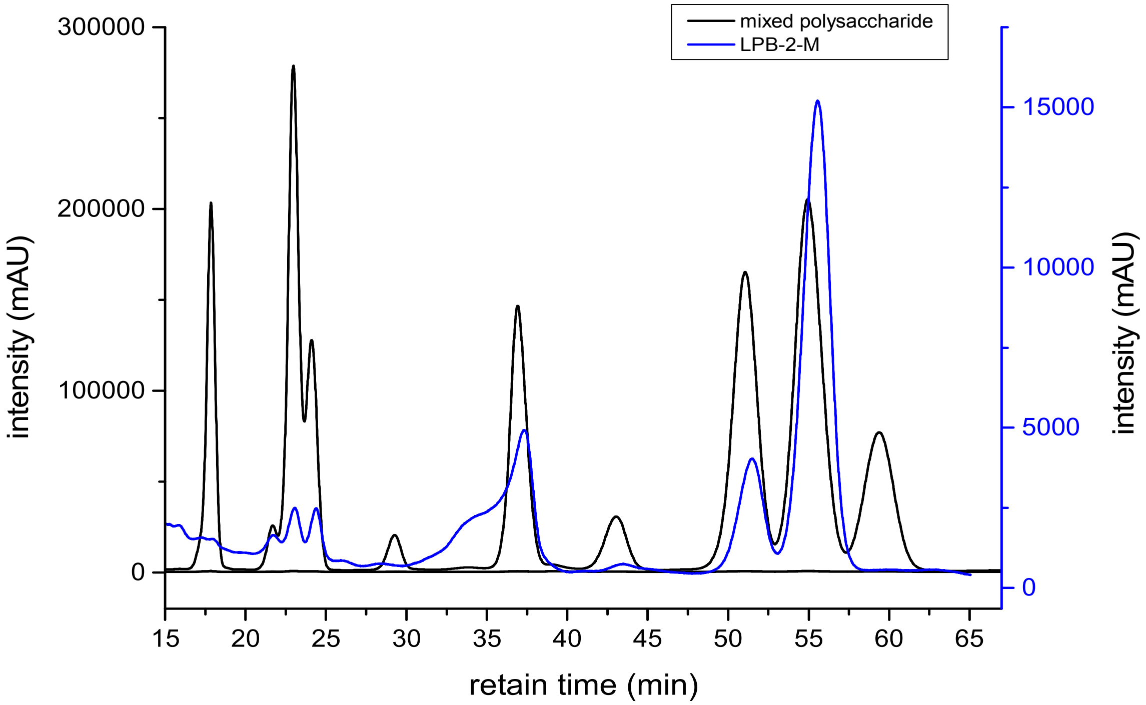
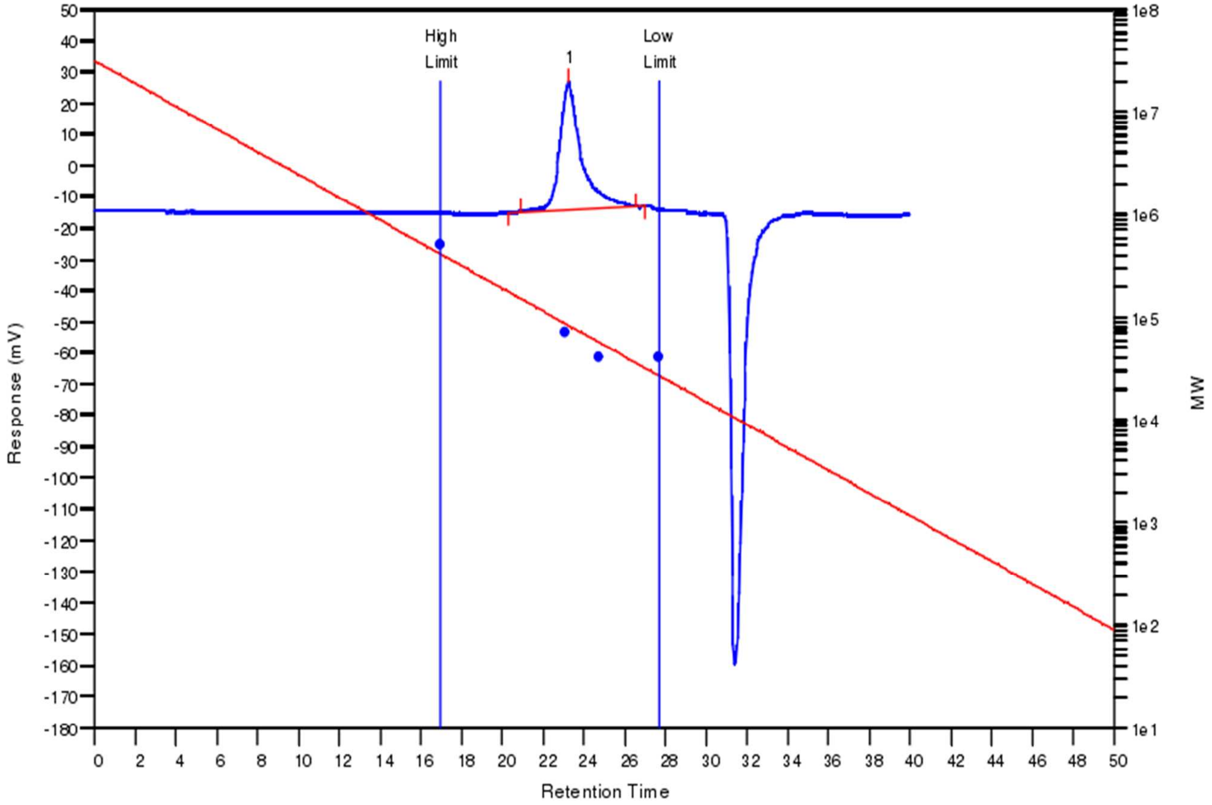


Fig.1 HPLC chromatograph of derivate of mixed monosaccharide and LPB-2-M



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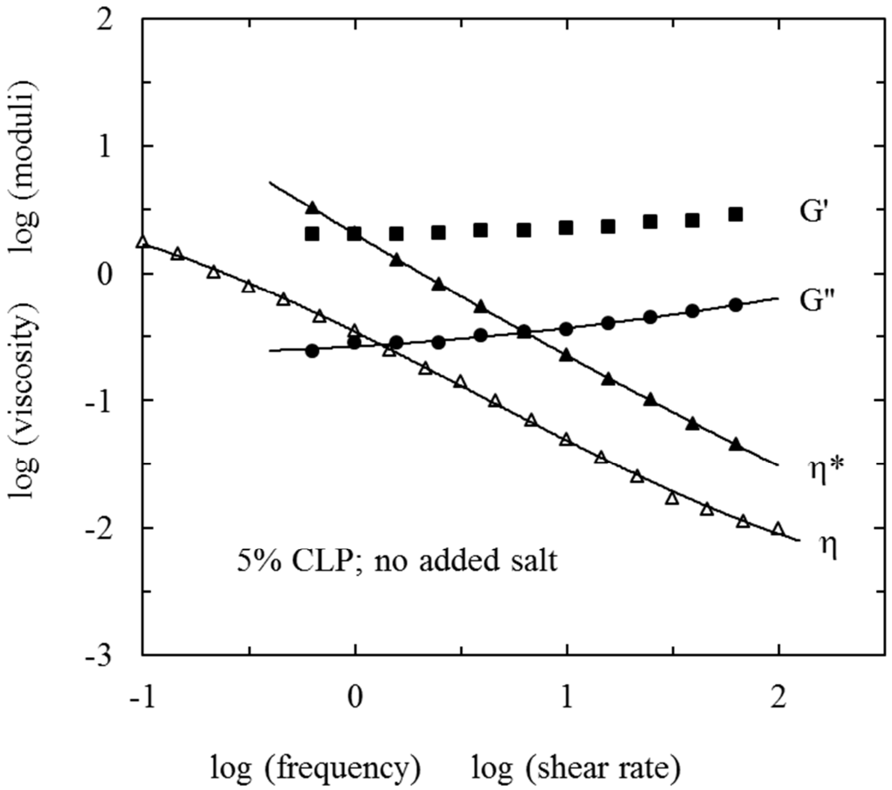

Fig.3 Frequency-dependence of G'(■), G"(●) and η* (▲) and shear-rate dependence of η (Δ) for 5% CLP in water at 25°C.

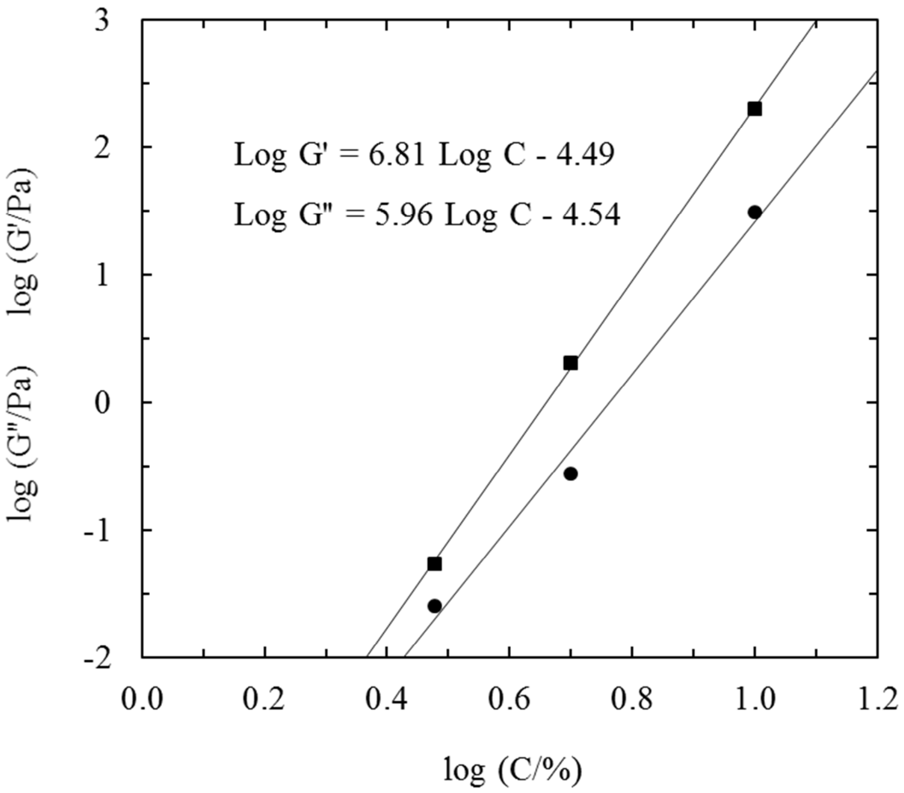

Fig. 4 Variation of log G' (■) and log G" (●), measured at 1 rad s^{-1} and 25°C, with log C for CLP in water at concentrations of C = 3, 5 and 10%.

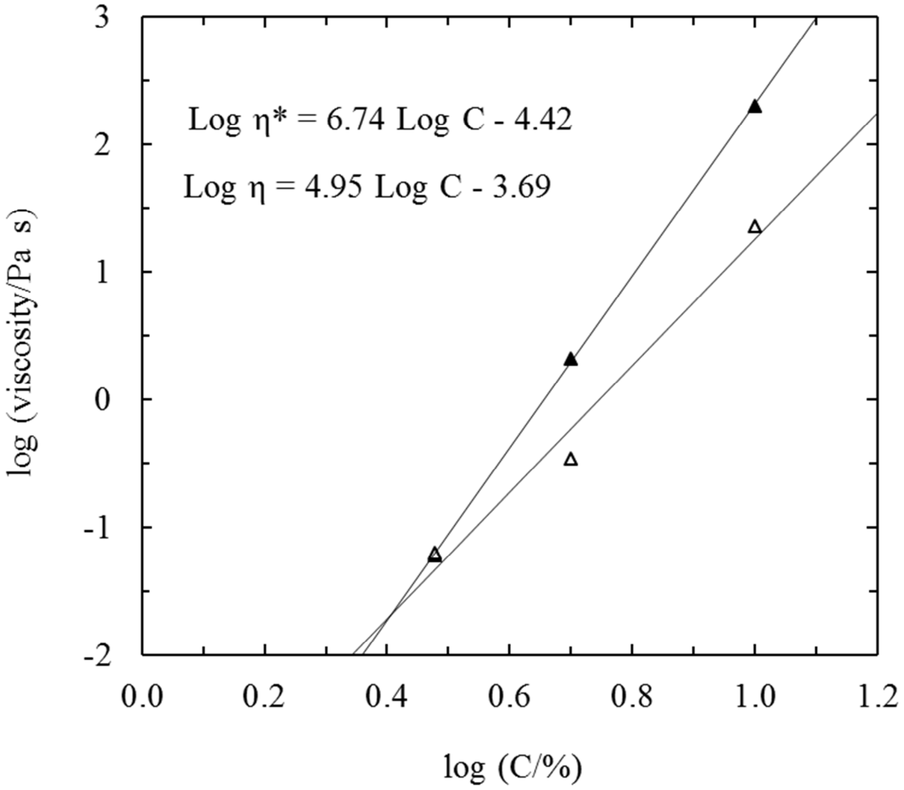

Fig. 5 Variation of log η* (▲) at 1 rad s^{-1} and log η (Δ) at 1rad s^{-1} with log C for CLP in water (25°C) at concentrations of C = 3, 5 and 10%.

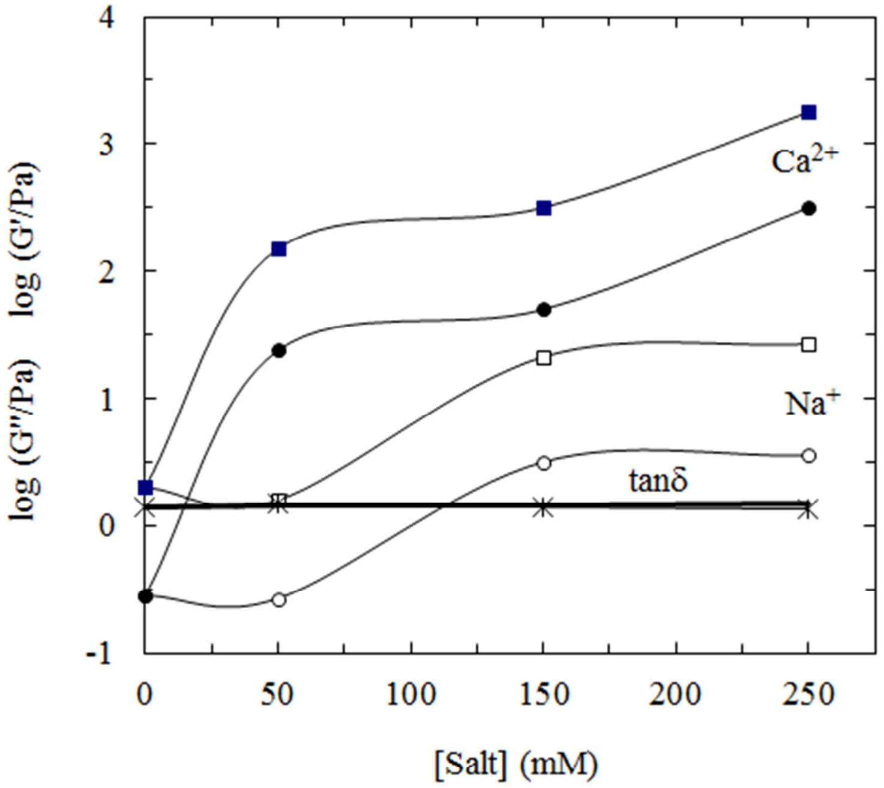

Fig. 6 (A)Variation of tan δ, log G' (squares) and log G" (circles), measured at 1 rad s^{-1}, with molar concentrations of added NaCl (open symbols) or $CaCl_2$ (filled symbols) for 5% CLP at 25°C.

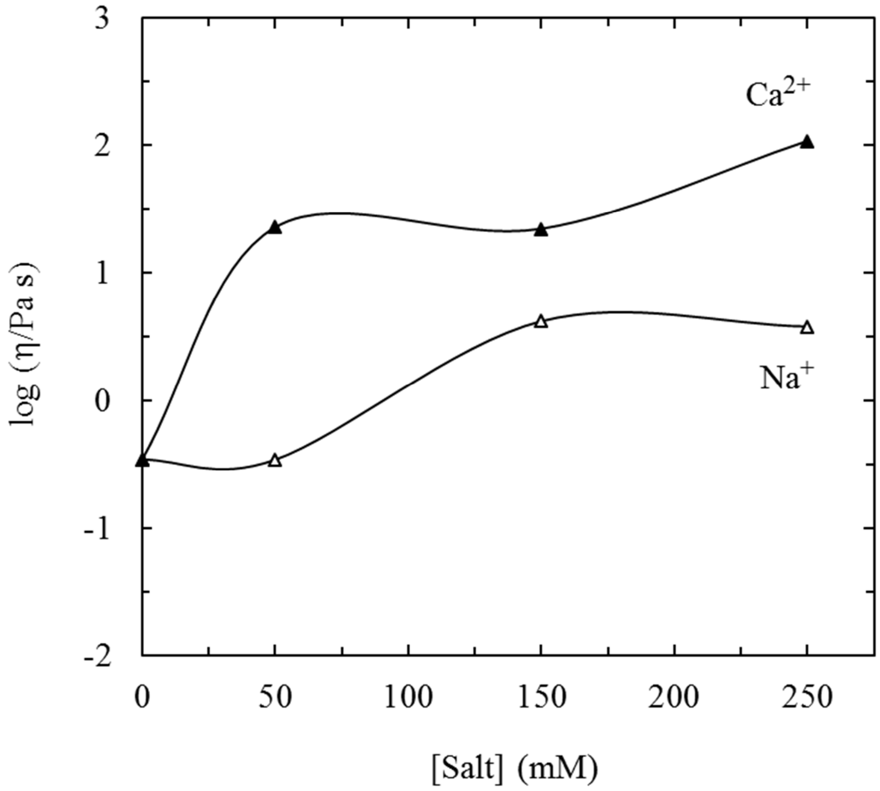

Fig. 6 (B) Variation of log η (measured at 1 rad s^{-1}) with molar concentrations of added NaCl (Δ) or CaCl$_2$ (▲) for 5% CLP at 25°C.