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A natural shear-thickening water-soluble polymer from the fronds of the black tree fern, *Cyathea medullaris*: Influence of salt, pH and temperature

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ABSTRACT

A water-soluble polymer was extracted from the fronds of the black tree fern (*Cyathea medullaris* or "Mamaku" in Māori) and characterized under various physico-chemical conditions. The rheological properties (in the semi-dilute region) and particle size and charge (in the dilute region) were investigated under various salt (0–1 M NaCl), pH (1–12) and temperature conditions (5–80 °C), using rheometry and dynamic light scattering techniques. A 7% (w/w) Mamaku crude extract showed strong shear-thickening properties at high salinity levels (1 M NaCl) and over the whole range of pH (1–12). However, the thickening properties disappeared above 50 °C. Apparent viscosity remained constant over the pH range 3–9, although the particle size systematically decreased with increasing pH. Overall, Mamaku solutions showed very good salt and pH resistance and exhibited strong temperature dependency. Hydrophobic and electrostatic interactions are not likely to be the cause of the shear-thickening phenomenon observed. Other forces such as hydrogen bonding may play a dominant role on the formation of shear-induced associations.

1. Introduction

Polysaccharides from plant extracts are important additives especially in industries such as the pharmaceutical, cosmetic, nutraceutical and food industry. The demand for novel natural hydrocolloids with specific functionality is very high. Here we study a water-soluble polymer extracted from the fronds of New Zealand's tallest tree fern: the Mamaku in Māori. The pith of the trunk and fronds of this black tree fern (*Cyathea medullaris*) has been traditionally used for treating wounds, boils, eruptions and gastrointestinal problems (Brooker, Cambie, & Cooper, 1987). The brownish-red, mucus-like fluid present in the trunks and fronds maybe involved in these curative properties. In fact, some leading cosmetic brands in the market currently use Mamaku extract in their formulations. Anti-inflammatory and antioxidant activities have been traced to water-soluble polysaccharides from ferns and other plant species (Ananthi et al., 2010; Cambie & Ferguson, 2003; Xu et al., 2009). Given its potential for future applications, the study of physicochemical properties of Mamaku is of high importance.

The non-starch polysaccharide (NSP) fraction of Mamaku extract consists of *ca.* 73% uronic acid, *ca.* 14.3% galactose, *ca.* 7.1% xylose and *ca.* 3.1% arabinose. The crude extract is rich in sugars (*ca.* 44%) and has a high mineral content (*ca.* 16%) and *ca.* 2% protein (Goh, Matia-Merino, Hall, Moughan, & Singh, 2007). We have previously shown the unusual rheological properties of Mamaku crude extract (Goh et al., 2007), where we examined the dependency of its shear-thickening behaviour and the normal stress response, on polymer concentration. The shear-thickening phenomenon is not commonly encountered in natural polymers but more in synthetic associating polymers, in surfactant solutions and concentrated dispersions. Shear-thickening has been reported in starch (Kim, Willett, Carriere, & Felker, 2002; White, Chellamuthu, & Rothstein, 2010), pectin solutions (Kjoniksen, Hiorth, & Nystrom, 2005) and modified alginates (Burckbuchler et al., 2006) amongst other biopolymers. The anomaly of shear-thickening has been frequently modelled in associating polymer solutions (Indei, 2007; Jiang, Keffer, Edwards, & Allred, 2003; Tanaka & Koga, 2006). However, its molecular origin is still under debate. The two main mechanisms proposed for shear-thickening are: (i) a nonlinear high tension along stretched chains beyond the Gaussian range (Serero, Jacobsen, Berret, & May, 2000) and (ii) an association amongst the different polymer chains following shear-induced transition from intra- to intermolecular association (Witten & Cohen, 1985).

The shear-thickening phenomenon has also been explained (Indei, 2007; Indei, Koga, & Tanaka, 2005) on the basis of the transient network theory (Tanaka & Edwards, 1992).

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The possible temporary junctions of functional groups on a polymer can have different origins. Temporal bonds cannot be explained in terms of *Van der Waals* type contact energy alone, since the binding energy is insufficient (Tanaka, 2002). Some associative forces could be: (i) *hydrophobic* like in neutral or charged telechelic polymers which carry hydrophobic associative functional groups at their both chain ends (Tanaka, 2002) or in “hydrophobically modified alkali-soluble emulsion” (HASE) physical gels with hydrophobic groups distributed along the backbone (Mewis & Biebaut, 2001), (ii) *electrostatic* like in charged polymers: polyelectrolytes and polyampholytes. The shear-thickening phenomenon has been described as the result of repulsive forces between negative charges pushed together under shear in “hydrolyzed polyacrylamide” (HPAM) solutions (Lewandowska, 2007). Intermolecular electrostatically driven interactions can also occur between opposite charges along the macromolecular chain during the shear-thickening process in the case of polyampholytes (Bossard, Sfika, & Tsitsilianis, 2004), or between two oppositely charged polyelectrolytes (Liu, Morishima, & Winnik, 2001). (iii) *Hydrogen bonding* like in the case of pectin solutions where the alignment and stretching of the polymer chains due to shear forces give rise to more sites becoming available for the formation of intermolecular hydrogen-bonded complexes (Kjoniksen et al., 2005).

Rheological studies of a polymer under different solvent conditions can help to understand the possible mechanism behind the thickening phenomenon. Numerous studies exist in this area: the effect of salt addition (NaCl) is normally used to provide information about the role of electrostatic interactions on the intermolecular associations under shear flow (Kjoniksen et al., 2005). Studies on synthetic polymers (Feng, Grassl, Billon, Khoukh, & Francois, 2002) and mixtures of surfactants and polyelectrolytes (Noda, Hashidzume, & Morishima, 2000) have shown that addition of salt can induce strong shear-thickening. Also addition of salt to an already dissolved polymer can lead to a maximum in viscosity (Ma, Huang, Zhao, & Xi, 2005; Maia, Costa, Borsali, & Garcia, 2005) or to a consistently decreased viscosity if the polymer is dissolved directly in the salt solution. Effects of temperature on the shear-thickening region have been previously studied in waxy maize starch (Carriere & Loffredo, 1998). Different temperature dependencies of synthetic poly (N-isopropylacrylamide) polymers showing shear-thickening behaviour have been attributed to the degree of hydrogen bonding and solubility of the polymer (Yu, Lu, Liu, Lin, & Chiu, 2004). Finally, studies have also reported a strong viscosity maximum at intermediate pH (4–6) (Li & Kwak, 2004) and at pH (6–9) (Ma et al., 2005) in shear-thickening hydrophobically modified polymers in various ways.

Temporary junctions of functional groups at intermediate shear rates has been the proposed explanation given for the shear-thickening behaviour observed in our Mamaku fern extract (Goh et al., 2007). The objective of the current study is to provide some further knowledge on the rheological behaviour, molecular size and charge of this peculiar biopolymer under various physicochemical conditions (salt, pH and temperature), attempting to infer possible interactions which could govern the polymer association process in aqueous solution under shear. This is the first study on Mamaku fern extract describing such effects.

2. Materials and methods

2.1. Isolation of Mamaku gum

As reported in our earlier study (Goh et al., 2007), the fronds of the tree fern were obtained from the Massey University campus in Palmerston North, New Zealand. The leaves on the fronds were removed and the fronds were washed, cut into 5 mm thick slices and blended with warm water (~50 °C) in a wet disintegrator (Jeffco, Dry Creek, Australia) for 5 min. This step removed the gum from the fronds into the continuous phase of the mixture. Using muslin cloths, the crude gum solution was separated and then centrifuged at 13,600 × *g* for 30 min at 20 °C (Sorvall RC5C centrifuge and GS-3 rotor, Dupont Instrument, DE, USA) to remove any insoluble materials. The supernatant was then freeze-dried.

2.2. Rheological characterization

Freeze-dried Mamaku samples (7%, w/w) were re-dispersed always using Milli-Q water ($\geq$17.5 MΩ cm) containing 0.02% (w/w) sodium azide. The effect of ionic strength was tested by dissolving the samples in 0.05–1 M NaCl solutions. Evaluation of the effect of various salts involved dissolving the freeze-dried Mamaku samples in 0.5 M of each of the following salt solutions: NaCl, $CaCl_2$, $MgCl_2$ or KCl. In all the cases, the samples were allowed to hydrate for ~24 h at room temperature and then centrifuged at 3100 × *g* for 30 min at 20 °C (Centra MP4R and Rotor 224, International Equipment Company, Needham Heights, MA, USA) to remove any large aggregates, before using them for rheological characterization.

In order to evaluate the effect of pH, 8.5% (w/w) freeze-dried Mamaku solutions were prepared in Milli-Q water ($\geq$17.5 MΩ cm) with 0.02% sodium azide and then left to hydrate overnight as indicated above. The samples were then centrifuged (as above) and the pH was adjusted ranging from 1 to 11 using 0.5 –1 M NaOH or HCl solutions. All the samples were adjusted to 7% (w/w) Mamaku concentration before rheological testing.

Oscillatory and rotational rheological measurements were performed in a control-stress rheometer (Paar Physica MCR 301, Anton-Paar, GmbH, Germany) using a double-gap measuring system DG 26-7 (bob: 26.68 mm o.d., 24.68 mm i.d.) with a Peltier heating unit (C-PTD 200) at constant temperature of 20 ± 0.1 °C. In rotational mode, viscosity curves were obtained at shear rates between 0.01 and 1000 s^{-1}. For the oscillatory measurements, the linear viscoelastic region was first established by running an amplitude sweep test (1.0 Hz, strain between 0.1 and 100%). A constant strain of 5% within the linear region was fixed for a subsequent frequency sweep test in the range of 0.01–10 Hz. The viscoelastic properties of the samples were quantified by analysing the data for the following dynamic rheological parameters: storage or elastic modulus (G'), loss or viscous modulus (G''), loss tangent ($\tan\delta$), and complex viscosity (η^*). The simplified Cross equation (1) shown below was used to fit the complex viscosity curves (Microcal^{TM} Origin^{TM} software, version 5.0) assuming the Cox–Merz rule where $|\eta^*(\omega)| = \eta(\dot{\gamma})$:

$$\eta = \frac{\eta_0}{1+(\lambda\dot{\gamma})^{1-n}} \tag{1}$$

where η is the apparent viscosity, η_0 is the zero shear rate viscosity, $\dot{\gamma}$ is the shear rate, λ is the relaxation time and *n* the proportionality constant. The fitting of the Cross equation is dependent on the initial estimates of the three fitting parameters (η_0, λ and n).

Rheological properties were also evaluated at fixed temperatures between 5 °C and 80 °C on 7% (w/w) Mamaku solutions prepared as described above. After loading the sample onto the rheometer, enough time was given to ensure that the sample had reached the temperature equilibrium. As a quantifying method, the measured temperature dependence was fitted by the Arrhenius equation (Rubinstein & Colby, 2003) given by

$$\eta(T) = \eta_0 e^{E_a/RT} \tag{2}$$

where E_a is the activation energy (kJ mol^{-1}), $R = 8.31 \times 10^{-3}$ kJ mol^{-1} K^{-1} is the gas constant, T is the absolute temperature (K) and η_0 is a pre-exponential constant.

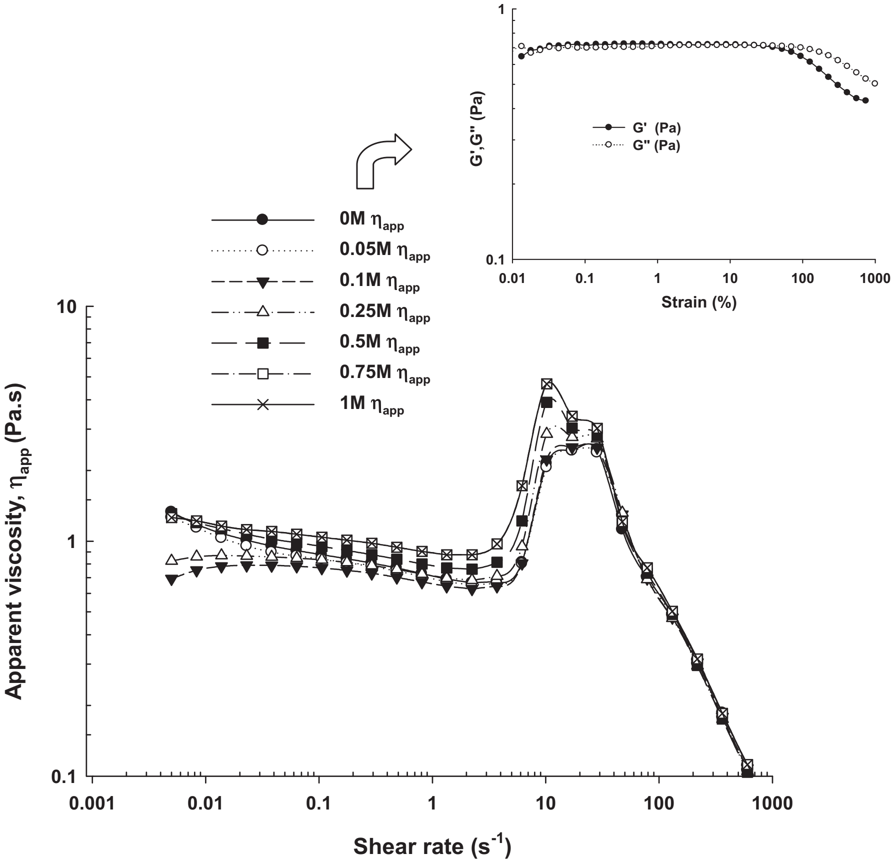

Fig. 1. Apparent viscosity (Pa s) of 7% (w/w) Mamaku extract at various NaCl concentrations (0–1 M) at 20 °C using double gap geometry. Inset corresponds to an amplitude sweep oscillatory test obtained at 1 Hz and 20 °C of 7% (w/w) Mamaku solution at 0.1 M NaCl.

2.3. *Particle size and ζ-potential measurements*

Particle size measurements were carried out by photon correlation spectroscopy in a Malvern Zetasizer Nano ZS instrument (Malvern Instruments Ltd., Malvern, Worcs, UK) with a non-invasive back scatter technology (NIBS®) using polystyrene disposable cuvettes. Data analysis was performed by the Dispersion Technology Software™ (version 4.20, Malvern, Worcestershire, UK) obtaining distributions of particle sizes based on *z*-average (also known as the "cumulants mean"). This mean size is the intensity mean calculated from the signal intensity related to the diffusion of the particle. ζ-Potential measurements were obtained in the same instrument using a DTS1060 folded capillary cell. The Malvern Zetasizer uses laser Doppler velocimetry and phase analysis light scattering technique (PALS) to obtain ζ-potential readings.

Freeze-dried Mamaku samples (1%, w/w) were re-dispersed in Milli-Q water (≥17.5 MΩ cm) with 0.02% (w/w) sodium azide overnight. The samples were dialysed at 20 °C for 48 h (12,000 Da molecular weight cut-off dialysis tubing) against different salts concentrations (0.01–1 M NaCl). Both dialysed sample and dialysate were retained in each case. Dialyzed samples were centrifuged at 3100 × *g* for 30 min at 20 °C (Centra MP4R and Rotor 224, International Equipment Company, Needham Heights, MA, USA) and the dialysates were filtered through a 0.22 μm membrane filter (Millipore Corp., MA, USA) prior to their use in light scattering measurements. Similarly, 1% (w/w) Mamaku solutions were dialyzed against 0.05 M NaCl solution and both sample and dialysate were subjected to the same procedure as above with a final pH adjustment between 1.5 and 12 by the slow addition of 1 M HCl or 1 M NaOH to well-stirred solutions.

Dialysed Mamaku samples were diluted using the filtered dialysates as diluents to obtain a range of polymer concentrations between 9.8×10^{-5} and 5.46×10^{-4} g/mL for size and ζ-potential measurements. For these concentrations the count rates ranged from ~120 to 470 kcps and the polydispersity varied between 0.2 and 0.4 for all measurements. The concentrations of all Mamaku samples were determined using the phenol-sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The total amount of carbohydrate was determined based on a standard calibration curve prepared using glucose and an absorbance of 485 nm.

3. Results and discussion

3.1. *Effect of ionic strength*

The crude Mamaku freeze-dried samples contain *ca.* 10% (w/w) of non-starch polysaccharide (NSP) fraction as previously reported (Goh et al., 2007). All rheological data shown here (7%, w/w, extract) represent therefore around 0.7% (w/w) of NSP. We believe this is the fraction responsible for the unusual shear-thickening rheological behaviour of Mamaku extract initially reported.

The steady state shear viscosity of 7% (w/w) Mamaku solution at different NaCl concentrations (0.0–1.0 M) is illustrated in Fig. 1 and the inset graph (discussed later on) corresponds to the amplitude sweep oscillatory profile at 0.1 M NaCl. Mamaku solution exhibited a Newtonian (0.1–0.25 M NaCl) or slightly shear thinning region (≥0.5 M NaCl) at low shear rates, followed by a pronounced shear-thickening region at *ca.* 10 s^{-1} (possible temporary bonds) with a typical shear thinning behaviour above 30 s^{-1} in all cases, corresponding to the disruption and alignment of the possible "associated" chains. High salt concentrations above 0.5 M NaCl

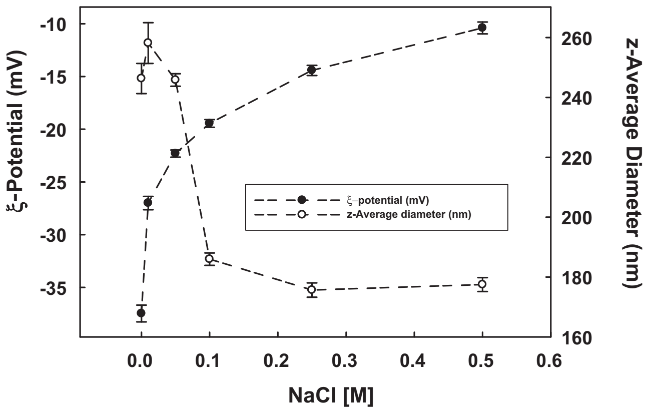

Fig. 2. Zeta potential (mV) and *z*-average diameter (nm) measurements at 20 °C of diluted Mamaku samples (between 9.8×10^{-5} and 5.46×10^{-4} g/mL) dialyzed against 0.0–0.5 M NaCl solutions.

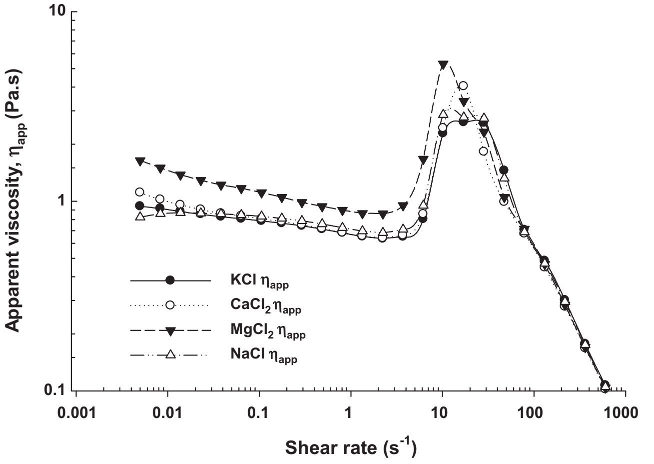

Fig. 3. Apparent viscosity (Pa s) of 7% (w/w) Mamaku extract in the presence of 0.5 M of various salts: KCl, $CaCl_2$, $MgCl_2$, and NaCl and measured at 20 °C and using double gap geometry.

seemed to enhance the viscosity and the shear-thickening effect (or maximum viscosity during shear). For example, Mamaku at 1 M NaCl was twice as viscous (*ca.* 4.5 Pa s at 10 s^{-1}) compared to 0.05 M NaCl (*ca.* 2.0 Pa s at 10 s^{-1}).

Mamaku samples were also studied under dilute conditions. Fig. 2 shows the charge (ζ-potential) and the size (*z*-average diameter) of Mamaku polymer with increasing ionic strength up to 0.5 M NaCl. There was a steep reduction in the charge density of the biopolymer with increasing amount of salt (it became less negatively charged) from −37.5 mV with no added salt to −19.4 mV at 0.1 M and −10.3 mV at 0.5 M. However the *z*-average diameter was only significantly reduced at ionic strengths ≥0.1 M NaCl (*i.e.* from *ca.* 245 nm *z*-average diameter at natural conditions to *ca.* 186 nm at 0.1 M NaCl). Further increase in ionic strength only slightly reduced the size (178 nm at 0.5 M NaCl). It was also observed that at 1 M NaCl (data not shown), some precipitates were formed after leaving the solution unperturbed for 48 h at room temperature.

The NSP fraction of Mamaku consists of ∼73% uronic acid (Goh et al., 2007) so the carboxylic acid function on the sugars is likely to be the origin of the overall negative charge. Its polyelectrolyte character was confirmed by the screening of charges with the addition of salt as shown by the gradual decrease in size and charge (Fig. 2). This so-called salting out effect involves the electrostatic screening of the charged groups by the counter-ions (Na^+ in this case) decreasing the intra-molecular repulsive electrostatic forces which leads to the shrinkage of the polysaccharide while adopting a more entropically favoured “compact” conformation (Munk, 1989). The marked reduction of the hydrodynamic volume and therefore of the intrinsic viscosity of polymers after addition of salt has been repeatedly reported in literature (Feng et al., 2002; Mohammadifar, Musavi, Kiumarsi, & Williams, 2006).

If the shear-thickening effect of a polymer is based on other forces such as hydrogen bonding or hydrophobic interactions rather than electrostatic interactions, a partial screening of the electrostatic forces by addition of certain amount of salt, can result in a more pronounced shear-thickening effect. Higher viscosities and a thickening effect occurring at lower shear rates (i) by promoting the formation of interpolymer hydrogen bonds in pectin systems with addition up to 0.1 M NaCl (Kjoniksen et al., 2005) and (ii) by promoting hydrophobic interactions in synthetic polymers above 1.5 M NaCl (Feng et al., 2002) have been reported earlier. An enhancement of hydrophobic intermolecular interactions under shear with increasing salt content (Tan, Tam, & Jenkins, 2000) has also been the explanation for a more pronounced peak in the thickening region detected at higher shear rates in associative polymers. In this latter case, the viscosity of the solution was markedly decreased with the addition of salt (0.2–1 M NaCl) due to the initial collapse of the polymer chains. Clearly the addition of salt increases the polarity of the solvent, having two opposite effects on the polymer chain: it shields the intramolecular repulsion and enhances the intermolecular interactions so an increase of, for example hydrophobic forces, allows the junctions formed under shear to support the stress before rupturing.

In the case of Mamaku solutions, the addition of salt up to 0.25 M NaCl hardly had any effect on the apparent viscosity. High salinity levels (1 M NaCl) if anything, seemed to increase the maximum viscosity during the shear-thickening process (Fig. 1). Under dilute conditions the polymer chains become clearly more compact with addition of salt (Fig. 2), however, at semi-dilute Mamaku concentration the viscosity is hardly affected by the salt levels tested, showing that this polymer exhibits very good salt resistance. The two opposite effects of salt described above may counteract each other resulting in only slight changes in viscosity. The shear-thickening did not disappear with the addition of salt, if anything, was promoted, which could indicate that interactions other than electrostatic may be involved during the shearing process.

The inset graph in Fig. 1 shows the amplitude sweep oscillatory profile of 7% (w/w) Mamaku solution at 0.1 M NaCl. Some authors (Bossard et al., 2004) have found that shear-thickening (shear-induced viscosity enhancement) is related to a strain hardening of the *G*′ modulus. This was not the case for our polysaccharide as observed in Fig. 1. From this graph it can be appreciated the strain-weakening nature of the material with a long viscoelastic linear region (up to 40% deformation), typical of elastic-rubbery type of polymers. Also under the described conditions, the elastic portion was equal to the viscous portion ($G' = G''$). In summary, the high elastic component of Mamaku that had given rise to the appearance of normal stresses under shear and thickening behaviour (Goh et al., 2007) did not lead to a strain-hardening behaviour under an increasing strain applied in oscillatory mode.

Fig. 3 shows the apparent viscosity of 7% (w/w) Mamaku solution in the presence of 0.5 M of each of the following salts: KCl, $CaCl_2$, $MgCl_2$ and NaCl. Interestingly only $MgCl_2$ seemed to increase slightly the viscosity of the Mamaku solution and consequently the peak viscosity at 10 s^{-1}. The viscosity profiles for the other salts were similar.

Compared to monovalent ions such as sodium, magnesium has been found to be much more efficient at promoting shear-thickening – at a lower shear rate and resulting in higher increases in viscosity – in halato-telechelic polymers (Maus, Fayt, Jerome,

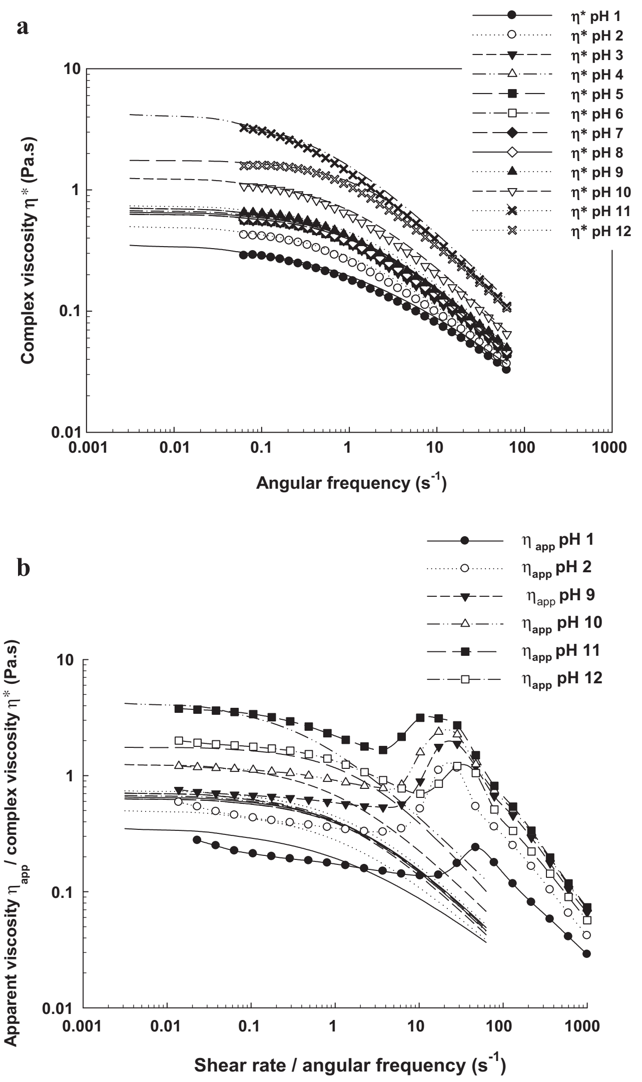

Fig. 4. Superposition of complex viscosity (lines) corresponding to fits from the Cross equation and: (a) experimental complex viscosity at 5% strain and (b) experimental apparent viscosity of 7% (w/w) Mamaku solution at 20 °C and at various pH values (1–12) as a function of shear rate and angular frequency (for clarity of the figure only the viscosity curve at pH 9 is plotted).

& Teyssie, 1995). This has been attributed to the lower thermal stability of the complexes of monovalent ions with the polymer compared to divalent ions. When comparing Mg^{2+} against Ca^{2+}, a higher efficiency of Mg^{2+} (Maus et al., 1995) has been related to a smaller critical concentration for gelation found for telechelic polymers end-capped with Mg carboxylate rather than with Ca carboxylate (Broze, Jerome, & Teyssie, 1981). This may explain why we observed an increased viscosity in the Mamaku extract, in the presence of Mg^{2+} ions.

3.2. *Effect of pH*

Fig. 4a shows the complex viscosity measured under oscillation between 6.28×10^{-2} and $62.8\,s^{-1}$ and the fitted viscosity curves obtained using the simplified Cross equation of 7% (w/w) Mamaku solution between pH 1 and 12. Experimental and fitted values based on the Cross equation are in very good agreement. The good fits using the Cross model were shown on the corresponding coefficients, $R^2 > 0.99$ and $\chi^2 \leq 10^{-4}$. It is clear that only very acidic conditions (pH 1–2) or very alkaline pH's (pH 10–12) changed the rheological behaviour of the solution. The shear-thickening effect was detected through the whole range of pH (Fig. 4b). The apparent viscosity was similar between pH 3 and pH 9 (for clarity of the figure only the viscosity curve at pH 9 is plotted). The solution exhibited higher apparent viscosity at pH ≥ pH 10 and lower apparent viscosity at pH ≤ pH 2.0, with the onset of the thickening occurring at lower shear rate $\sim 6.2\,s^{-1}$ at pH 11, compared to $\sim 28\,s^{-1}$ at pH 1 where the thickening effect was less pronounced. Also in this Fig. 4b data from oscillatory measurements (lines) are plotted together with the rotational viscosity. Like for other polymer solutions, the Cox–Merz rule where $|\eta^*(\omega)| = \eta(\dot{\gamma})$, applied only at low shear rate/angular frequency where both viscosities coincide.

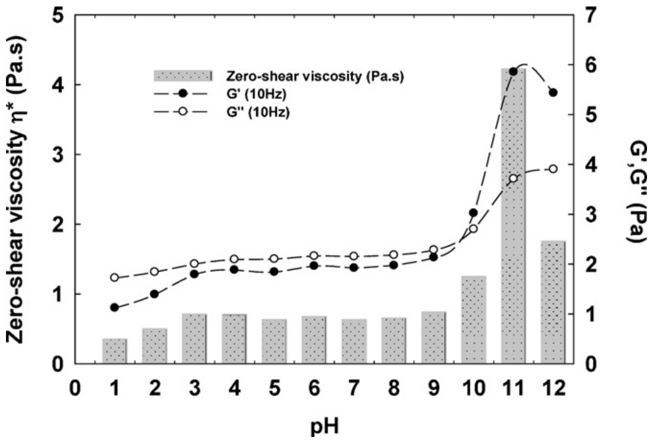

Fig. 5. Zero-shear viscosity (Pa s) obtained by the Cross equation and viscoelastic parameters, G' and G'' (Pa) obtained at 10 Hz and 5% strain of 7% (w/w) Mamaku solution measured at 20 °C and pH 1–12.

The zero-shear rate viscosity (η_0) obtained from the simplified Cross equation by fitting the complex viscosity curves is plotted in Fig. 5 together with the viscoelastic parameters G' and G'' at 10 Hz to further summarize the rheological behaviour of the solution with changing pH. In general, the relaxation time ($G' = G''$) of the polymer observed from the frequency sweep increased only above pH 9. When comparing the viscoelastic properties at a fixed frequency (10 Hz) clearly the loss modulus G” dominated over the elastic modulus G' between pH 1 to pH 9 (Fig. 5). Above pH 9, the higher zero-shear viscosities values (η_0) especially at pH 11 (~4 Pa s) correspond to the elasticity G' being greater than the loss modulus G'' and showing higher values ($G' \sim 6$ Pa at pH 11 compared to $G' \sim 2$ Pa at natural pH 5.0) due to the development of a network structure. The similar rheology obtained in such a wide range of pH 3–9 shows the high pH tolerance of Mamaku solutions.

The change in colour undergone by the Mamaku solution when varying the pH is illustrated in Fig. 6. From the natural Mamaku pH around 5.0, the solution became lighter brown at acidic pH and darkened when increasing pH, showing a maximum dark chocolate brown at pH 11, probably corresponding well with the observed maximum viscosity and perhaps reflecting chemical reactions happening in the excess of NaOH.

Clearly at the natural pH of Mamaku around 5.0, the polymer is negatively charged as shown by the ξ-potential values (Fig. 7). Between pH 5 and 12 the negative charge density on the polymer did not significantly change oscillating between −29 and −31 mV. Only below pH 5, the ξ-potential decreased considerably (from *ca.* −30 mV at pH 5 to around −18 mV at pH 3.0). It is likely that the pK_a value of Mamaku polymer is below pH 3 (readings at lower pH were not obtained). Focusing on the molecular size, the polymer became consistently smaller with increasing pH; from a *z*-average diameter of *ca.* 370 nm at pH 1.5 to around 200 nm at pH 12. Generally Coulombic repulsions between charged groups

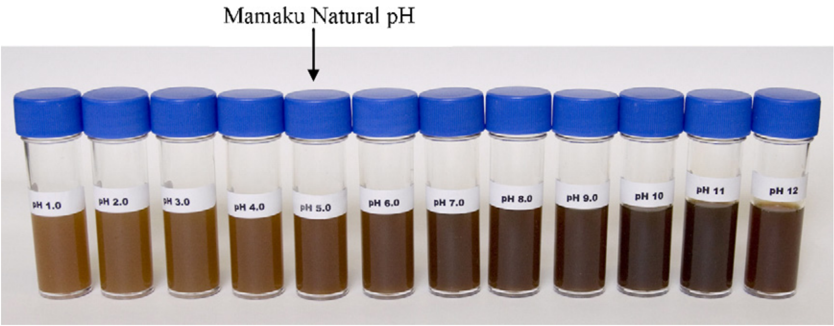

Fig. 6. Visual appearance of 7% (w/w) Mamaku solutions with changing pH from pH 1.0 (left) to pH 12 (right).

along a polymer chain would tend to extend the hydrodynamic radius of a polysaccharide in dilution. However in this case, the size (*z*-average) became systematically smaller from acidic pH 3.0 (less charged) to pH >5.0 (more charged). This may indicate that some sort of disaggregation may be taking place with increasing pH under dilute conditions. It could be possible that some remaining insoluble part of the gum may become dissolved in the presence of sodium hydroxide as shown for arabinogalactans (Tischer, Iacomini, & Gorin, 2002). We are currently investigating further the molecular structure of Mamaku polymer. As mentioned earlier, so far we know that the main fraction of the polymer is uronic acid followed by galactose, xylose and arabinose. Other small monosaccharides present in small quantities are rhamnose, fucose, mannose and glucose.

A strong viscosity maximum at intermediate pH (4–6) (Li & Kwak, 2004) and at pH (6–9) (Ma et al., 2005) has been reported in shear-thickening hydrophobically modified polymers. However, beyond these pH's, the excessive Na$^+$ and OH$^-$ has been shown to reduce the viscosity by shielding the repulsion and destroying intermolecular hydrophobic interactions. Xu, Jamieson, Wang, and Qutubuddin (1996) related an increase in surface potential with an increase in interparticle repulsion and therefore higher zero-shear viscosity and higher critical shear rate $\dot{\gamma}_c$ for the thickening in polymer latex dispersions. A decrease in the average size of polymer aggregates has been shown to decrease the viscosity and shift the shear-thickening towards higher shear rates (Maus et al., 1995). In our Mamaku solutions, no significant changes in viscosity or in the thickening behaviour were observed between pH 3 and pH 9.0 even though there was a reduction in the molecular size and an increase in charge density under dilute conditions. The lower viscosity at pH < 3.0 maybe related to the complete loss of charge. A number of hydrocolloids such as galactomannans, CMC, gum karaya and other seed gums seem to lose their viscosity at low pH (Glicksman, 1982). Increasing pH from 3 to 7 augmented the apparent viscosity of *Alyssum homolocarpum* seed gum and mucilage isolated from *Opuntia ficus* explained by an induction of electrostatic repulsion of ionized carboxylic groups (Koocheki, Mortazavi, Shahidi, Razavi, & Taherian, 2009; Medina-Torres, Brito-De La Fuente, Torrestiana-Sanchez, & Katthain, 2000). No significant difference in viscosity of the seed gum was observed between pH 5 and 9 by these authors. In the present Mamaku solutions the pH tolerance is wide ranging from pH 3 to 9. The increase in viscosity above pH 9 as a result of the formation of a weak gel observed in the excess of NaOH may be related to chemical degradation. Possible gel inhomogeneities at the extreme pH of 12 may explain a further drop in viscosity as compared to pH 11. In summary, the thickening behaviour was not affected by changes in pH. Let us now examine possible hydrophobic interactions by changing the temperature of the solution.

3.3. Effect of temperature

Fig. 8 shows the apparent viscosity of 7% (w/w) Mamaku solution versus shear rate at temperatures between 5 and 80 °C. Clearly the increase in temperature decreased the viscosity as expected and also delayed the onset of the thickening behaviour. It seemed that the higher the viscosity, the lower the critical shear rate for shear-thickening. The shear rate at the maximum viscosity during thickening shifted from *ca.* 2 s^{-1} at 5 °C to *ca.* 200 s^{-1} at 40 °C. From 50 °C onwards, the thickening phenomenon was no longer observed over the shear rate tested.

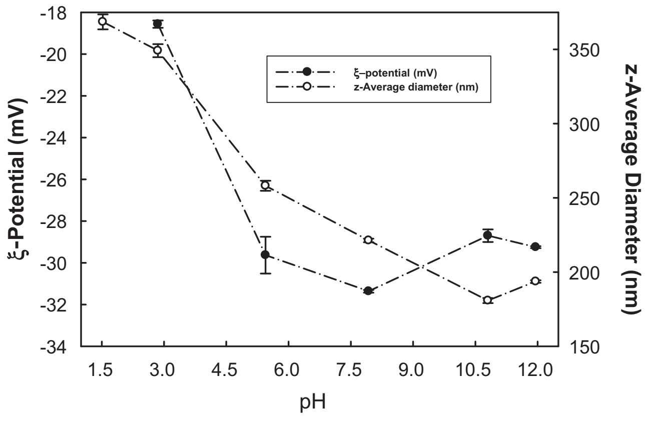

Fig. 7. Zeta potential (mV) and *z*-average diameter (nm) measurements at 20 °C of diluted Mamaku samples (between 9.8×10^{-5} and 5.46×10^{-4} g/mL) dialyzed against 0.05 M NaCl solutions and with pH adjusted between 1.5 and 12.

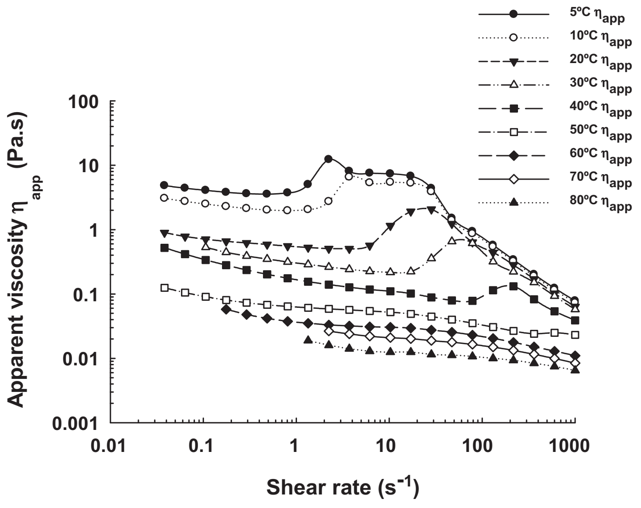

Fig. 8. Apparent viscosity (Pa s) of 7% (w/w) Mamaku solution versus shear rate measured at temperatures between 5 and 80 °C.

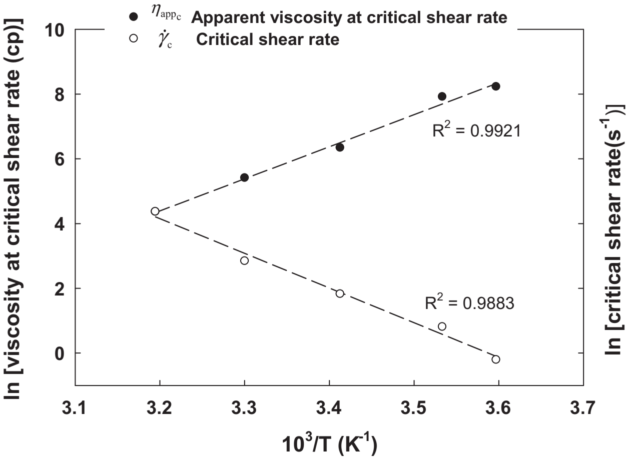

Fig. 9. Arrhenius plot of the apparent viscosity at the critical shear rate (full symbols) and the critical shear rates (empty symbols). The activation energy E_a was calculated from the slope of the apparent viscosity linear fit.

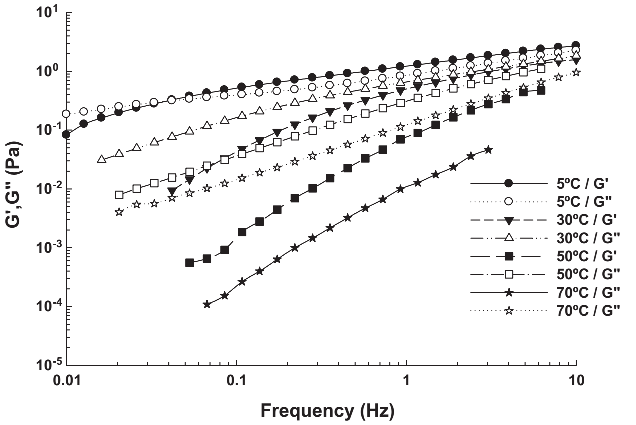

Fig. 10. Mechanical spectrum of 7% (w/w) Mamaku solution at 5, 30, 50 and 70 °C obtained at 1 Hz using double gap geometry.

The critical shear rate ($\dot{\gamma}_c$) or shear rate at the onset of the thickening, as well as the apparent viscosity, varied strongly with temperature according to an Arrhenius behaviour (Fig. 9). Both viscosity and shear rate exhibited a similar temperature dependency as shown on the slope of the lines, but with opposite signs (+10 and −11, respectively). From the viscosity measurements, the activation energy (E_a) was calculated following Eq. (2) obtaining a value of 82.37 kJ mol^{-1}. This is in the same order of magnitude as other associating polymers such as "hydrophobic ethoxylated urethane" (HEUR) associative thickeners (71 kJ mol^{-1}) (Annable, Buscall, Ettelaie, & Whittlestone, 1993) and HEUR with 16 carbons in the end chain (67 kJ mol^{-1}) (Tanaka, 2002). In general, low activation energy indicates few inter- and intra-interactions between polysaccharide chains at a given concentration (Mohammadifar et al., 2006). The high magnitude of activation energy (E_a) in Mamaku solutions indicates the high sensitivity of the solution towards temperature and reflects its influence on the intermolecular interactions.

The shift of the critical shear rate for the shear-thickening transition towards higher shear rates with increasing temperatures has been previously observed in halato-telechelic polymers (Maus et al., 1995), in polyacrylic ester dispersions and poly(acrylamide) solutions (Briscoe, Luckham, & Zhu, 1999; Xu et al., 1996), in dilute surfactant solutions (Gamez-Corrales, Berret, Walker, & Oberdisse, 1999), in concentrated colloidal dispersions (Shenoy & Wagner, 2005) and, in modified alginates (Burckbuchler et al., 2006) amongst others. Xu et al. (1996) concluded that the change in solvent viscosity over a temperature range (10–30 °C) was insufficient to explain the shift in critical shear rate observed. Higher shearing needed to induce thickening with increasing temperature has been interpreted as intramolecular hydrophobic aggregates being reinforced while heating and therefore needing higher shears to unravel the polymer chains (Bokias, Hourdet, & Iliopoulos, 2000). In our case, the viscosity of Mamaku solution was strongly dependent on temperature. A plausible explanation for the disappearance of the thickening effect at elevated temperatures is the enhanced thermal mobility of the polymer chains. The increased mobility seems to somehow restrain the intermolecular associations preventing the shear-thickening phenomenon from happening at ≥50 °C at intermediate shear rates. If hydrophobic forces were the key interactions involved in the transient bonding during the shear-thickening process, the peak viscosity at intermediate shear rates would get more pronounced with increasing temperature as observed for example for modified alginates between 5 and 45 °C (Burckbuchler et al., 2006). In our case the opposite is true, so it is very unlikely that hydrophobic interactions are involved.

Fig. 10 shows the mechanical spectrum of 7% (w/w) Mamaku solution from 5 °C to 70 °C. The cross-over of G' and G'' was shifted from low frequency 3×10^{-2} Hz at 5 °C to higher frequencies with increasing temperature. At 30 °C the viscoelastic parameters were equal ($G' = G''$) over the range (2–10 Hz) and at ≥50 °C the viscous component or G'' was greater than the elastic component or G' over the whole range of frequencies. This indicated that the relaxation time of the sample (inverse of the frequency at the cross-over point) decreased with increasing temperature and no crossover was detected above 30 °C at the frequency range investigated.

The shear-thickening properties of Mamaku did not disappear after heating a 7% (w/w) Mamaku solution at 80 °C for 30 min. Upon cooling, the solution reverted to nearly its original viscosity indicating that the elevated temperature does not degrade the macromolecular structure. The apparent viscosity profile completely overlapped when measuring the viscosity before and after heat treatment at 20 °C (Fig. 11). The up-down viscosity curves showed that the flow behaviours of the Mamaku solution previously discussed (Goh et al., 2007), the shear-thickening and thinning, the thixotropy, and antithixotropy were present after such a heat treatment.

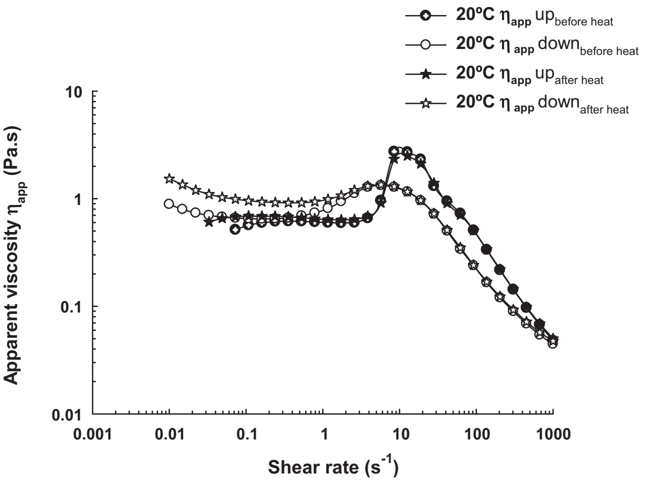

Fig. 11. Apparent viscosity (Pa s) of 7% (w/w) Mamaku solution versus shear rate measured before and after treating mamaku samples at 80 °C for 30 mins. Viscosity curves were obtained at 20 °C.

4. Conclusions

Mamaku polymer is a polyelectrolyte very tolerant to salt and pH changes but very sensitive to temperature variation. The shear-thickening properties of a 7% (w/w) Mamaku solution disappear above 50 °C but remain in the presence of high levels of salt (1 M NaCl) and over the whole range of pH (1–12). The high tolerance of viscosity to salt and pH changes opens up a wide range of possibilities for this novel "smart" fluid. Applications such as in coatings, in cosmetics, as well as in food systems aiming to control satiety by controlling stomach motility, are possible. Hydrophobic and electrostatic interactions are not likely to be the cause of the shear-thickening phenomenon observed in the Mamaku solutions. Future work involves the investigation of possible hydrogen bonding associations under shear by the addition of urea.

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