

Effect of Poly(ethylene glycol) on the Hydrophobic Interactions and Rheology of Proanthocyanidin Biopolymers from *Pinus radiata*

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ABSTRACT: The rheology of solutions of extracts from the bark of *Pinus radiata* was investigated in the presence of poly(ethylene glycol)s (PEGs) of different molecular weights. PEG with a molecular weight of 4600 (1% w/w) was sufficient to reduce the viscosity of a concentrated (40% w/w) pine tannin extract by one order of magnitude. The reduction of the viscosity was due to the inhibition of molecular association via hydrogen bonding and hydrophobic

interactions between tannin and PEG and depended on the molecular weight of PEG. PEG effectively reduced the viscosity of polyphenolic tannins but retained high reactivity toward paraformaldehyde for adhesive formulations. © 2005 Wiley Periodicals, Inc. *J Appl Polym Sci* 97: 1254–1260, 2005

Key words: additives; adhesives; rheology

INTRODUCTION

Condensed polyflavonoid tannins continue to be investigated as important components in adhesives for wood products, providing an opportunity for reductions in formaldehyde emissions from synthetic thermosetting resins and the utilization of renewable resources.^{1,2} Proanthocyanidins (PAs) as polymeric flavon-3-ols are also significant components in important plants used as animal feed stock,³ for which the ability of condensed tannins to interact with dietary and enzymic proteins can limit ruminant nutrition.⁴ Additionally, such interactions provide insoluble complexes, which also limit many biotechnological processes, and recently there has been growing recognition of the role of PAs in providing antinutritive and putative protective effects.⁵ The extraction conditions play a significant role in the stability and viscosity of polyflavonoid tannin solutions. Pizzi and Stephaou⁶ studied many of the condensation mechanisms, including those of *Pinus radiata*, which frequently lead to processing difficulties at the higher concentrations required for adhesive formulations. Interactions due to colloidal self-associations^{7–9} and secondary interactions¹⁰ have been shown to dictate their detailed rheological response. Strong nucleophiles such as phloroglucinol, *m*-phenylene diamine, and

urea have been shown to block the mechanisms of self-condensation^{8,11} and colloidal or molecular association^{12,13} in solution.

Sequential extraction with solvents of increasing polarity separate tannin extracts into fractions with different phenolic characteristics and hydrophobicity levels;¹⁴ moieties with low phenolic concentrations show enhanced hydrophobic properties. The evidence for the hydrophobic properties of tannins is significant.^{15–17} Gray¹⁶ found that a hydrophobic polystyrene resin that did not contain polar groups adsorbed low-molecular-weight phenolic substances, whereas highly polymerized condensed PAs were not adsorbed. It was concluded that the adsorption of low-molecular-weight phenolic components by such polystyrene resins was predominantly due to hydrophobic interactions via the aromatic rings of these polyphenols.

Studies have been carried out to investigate the broad nature of the interactions associated with these polyphenolic PA compounds. Oh et al.¹⁵ studied the formation of wattle and quebracho tannin complexes with proteins, poly(vinylpyrrolidone) (PVP), and poly(ethylene glycol) (PEG; molecular weight = 20,000). Under all pH conditions, such tannins have shown a strong affinity for PVP and PEG, in contrast to their behavior with proteins.³ For PEG, the tannin fraction with the greatest hydrophobic character forms the strongest complex, and this can be attributed to direct hydrophobic interactions with PEG, which is known to exhibit relatively strong hydrophobic properties.¹⁵ Ravindra⁴ also employed hydrophobic inter-

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actions to account for the ability of PEG with a molecular weight of 4000 to inhibit complexation between condensed tannins and protein enzymes. Tannins often suppress feed intake by reducing nutrient availability or by causing malaise.¹⁸ Several studies have been conducted to show how this type of negative effect can be minimized by PEG.^{18–20} PEG also contains weakly hydrogen-bonding ether linkages, which allow highly phenolic PA biopolymers to also interact with PEG through hydrogen-bonding mechanisms.

Importantly, both modes of association in polyphenol-PEG systems are solvent-dependent phenomena. Thus, hydrogen bonding between solutes is favored to occur in hydrophobic solvents such as carbon tetrachloride, whereas hydrophobic bonding between solutes primarily occurs in hydrophilic or polar solvents such as water. This study was carried out to investigate the influence of PEG on the molecular aggregation behavior and flow properties of a PA extract of *P. radiata*.

EXPERIMENTAL

Sample preparation

Tannin samples were extracted with hot water. Distilled water (1 L) at about 95°C was added to 200 g of dried bark of *P. radiata* bark powder, and the mixture was stirred vigorously for 10 min and filtered with a Buchner funnel and Whatman no. 41 filter paper to remove insoluble materials; this yielded a hot-water extract (HWE). Various quantities of PEG (Aldrich Chemical Co., St. Louis, MO), calculated on the basis of the total tannin content (PA) in the extracts, were added immediately (unless otherwise noted) after the filtration. Dilute extract solutions (3 wt % PA at pH 3.3) were also concentrated to 40% PA in a Buchi rotary evaporator (Flawil, Switzerland) at 60°C to minimize the thermal treatment. In this study, chemical modifications of the extract, such as the addition of sulfite,⁹ were avoided so that the original PA ring structure and functionality were maintained, but we recognize that industrial applications such as adhesive extraction may use such modifying techniques.

Extracted tannins were isolated with gel permeation chromatography. A sample of the dried extract, dissolved in 95% ethanol (45 mg/0.4 mL), was applied to a column of Sephadex LH-20 (1.9 × 20 cm) equilibrated with 95% ethanol (225 mL) at a flow rate of 31 mL/h, and 4-mL fractions of the eluted non-tannin extract were collected;²¹ the absorbance at 280 nm was determined. The column was then eluted with 50% acetone/water (150 mL) at a flow rate of 35 mL/h. Tannin fractions (5 mL) were collected, and the absorbance at 400 nm was determined²¹ with a Varian Cary-3 ultraviolet-visible spectrophotometer (Palo Alto, CA). The tannin content was 95%, and the remainder (5%) was designated as non-tannins.

The complex carbohydrate hydrocolloid content was also estimated from a sugar determination. A dried sample (100 mg) was hydrolyzed with 0.5M H₂SO₄ (5.0 mL) for 2 h. After the removal of the precipitate, the aqueous hydrolysate was passed through a column of Amberlite IR-45 (Philadelphia, PA), and the column was washed free of sugars.²² The eluent and water washing were combined, dried, and weighed. This indicated that the concentration of sugars was 27.6%.

The reactivity of the tannin extract was examined with the Stiasny reaction. Dried samples were further dried over CaCl₂ in a high vacuum at 55°C for 2 h. The sample (100 mg) was dissolved in conductivity water (10 mL), 10M hydrochloric acid (1.0 mL), and 40% formaldehyde (2.0 mL) and heated under reflux for 30 min.²² The reaction mixture was filtered while hot through a sintered glass filter (no. 3). The precipitate was washed with hot water (5 × 10 mL) and dried over CaCl₂ in a high vacuum at 55°C for 2 h. The yield was expressed as a percentage of the weight of the initial dried sample. The Stiasny value obtained for HWE was 92.1.

The tannin content (%) and the Stiasny value illustrated the extent and subsequent reactivity of tannin extraction under these HWE conditions, whereas the high sugar fraction indicated the importance of the molecular association of some of the tannins with complex carbohydrates.^{7,10,23}

Characterization

Capillary viscometry was carried out at high degrees of dilution, at which hydrodynamic interactions between soluble species were eliminated and molecular association and size effects could be probed. Capillary viscometry measurements were carried out, after centrifugation for the removal of any small amounts of solids present, with an Ubbelohde dilution viscometer. The samples were initial dilute HWE and concentrated HWE diluted to the appropriate concentration range. The viscometer and sample were maintained at 25 ± 0.1°C. The efflux time was measured for a set volume to flow through the capillary and was repeated at least five times. The flow time for the conductivity water was 149.34 s, and the kinetic energy correction was therefore neglected.

The rheological behavior and viscosity were determined at 25 ± 0.1°C with a Rheometrics RFS II fluid spectrometer (Rheometrics Inc., Piscataway, NJ) equipped with parallel-plate geometry (plate diameter = 50 mm). Throughout the steady-shear measurements, the shear rate was gradually increased from 2.5 × 10⁻² to 1.0 × 10³ s⁻¹. The dynamic time-sweep measurements were carried out to study the reaction rate and curing behavior. Paraformaldehyde (8% w/w tannin; BDH Chemicals, Poole, England) was added to

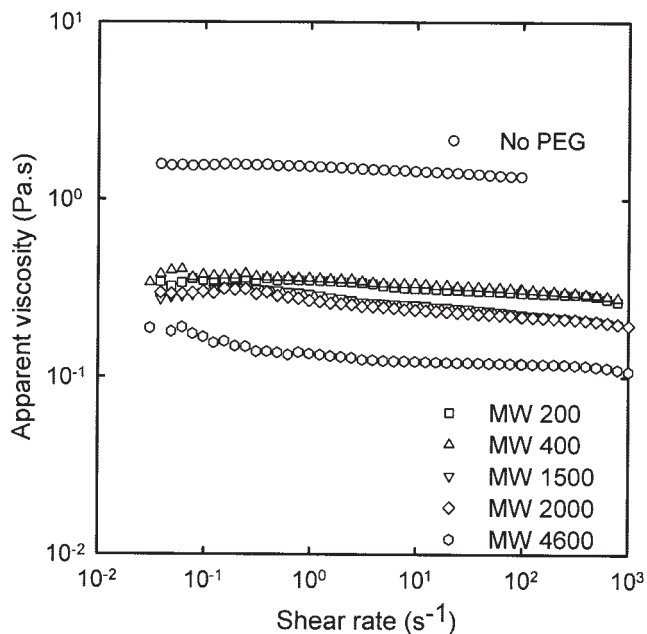


Figure 1 Apparent viscosities of HWEs (40% w/w) in the presence of PEGs (1% w/w) of various molecular weights at 25°C (with PEGs added before the HWE concentration).

a 40% (w/w) aqueous solution of HWE at room temperature. The dynamic modulus was recorded as the time taken for the mixture to cure after it was placed in the parallel-plate geometry of the RFS II at 40, 60, and 80°C. Solvent evaporation was avoided by the edge of the sample being covered with low-viscosity silicon oil at a high temperature.

Fourier transform infrared (FTIR) spectra were obtained with potassium bromide discs made as follows. Each sample (1.0 mg), previously dried at 40°C, was ground with 200 mg of predried potassium bromide in an agate mortar for 10 min and was then pressed at 8 tons on 5.38 cm *in vacuo* for 5 min. The obtained disc (13 mm in diameter) was dried in a vacuum oven at 105°C for 1 day. Dried discs were examined on a PerkinElmer 1710 FTIR spectrometer (Boston, MA). Three scans were made for each run to obtain the spectra.

RESULTS AND DISCUSSION

Figure 1 shows the steady-shear apparent viscosity of HWE with a 40% tannin concentration and a natural pH of 3.5 in the presence of PEGs (1% w/w) of various molecular weights; PEG was added immediately after the initial filtration stage. The 40% HWE solution without PEG yielded a near-Newtonian fluid with an apparent viscosity of approximately 2 Pa s;⁸ with the addition of PEG, the viscosity of this Newtonian liquid was reduced further by about one order of magnitude. This ability of HWE to form low-viscosity Newtonian

liquids at relatively high PA concentrations by a self-stabilization mechanism appears to be an important feature of the rheology of PA biopolymers.⁸ Considering the diversity of the molecular structures of the functional groups of HWE and the different molecular weights of PEG shown here, we believe that both hydrogen bonding and hydrophobic interactions may, in principle, take place as tannins consist of both hydrophobic (the aromatic nuclei) and hydrophilic (polar phenolic hydroxyl groups) regions. The PEG polymer chains also show both hydrophobic and hydrophilic characteristics. Ionic interactions do not dominate because PA polymers only become charged at high pHs by the dissociation of the phenolic hydroxyl groups.¹⁵ For PEGs of various molecular weights investigated here, a progressive reduction in the viscosity of HWE can be seen in Figures 1 and 2 as the PEG molecular weight increases. This was probably due to the enhanced hydrophobic characteristics of the longer PEG chains, which enabled their hydrophobic interactions with the PA chain to become more significant. Hydrophobicity is, according to Tanford,²⁴ a linear function of the length of the hydrocarbon chain for a homologous series. Longer PEG chain lengths may also become more severely entwined, and this can result in a greater hindrance for the PA polymer molecules to interdiffuse and become more self-associated. This can be illustrated by PEG with a molecular weight of 4600, which consists of 104 monomer units; PEG with a molecular weight of 200 consists of only 4 monomer units. Lower molecular weight PEG

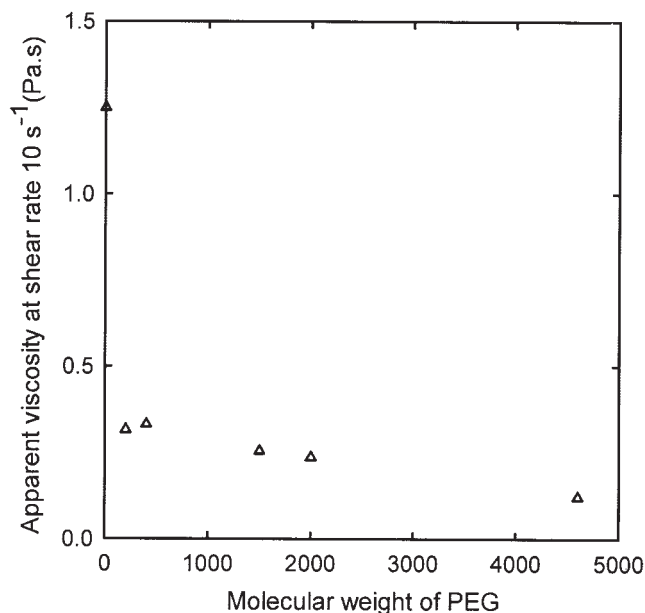


Figure 2 Apparent viscosities of HWEs (40% w/w) at a shear rate of 10 s⁻¹ with and without the presence of PEGs (1% w/w) of various molecular weights at 25°C (with PEGs added before the HWE concentration).

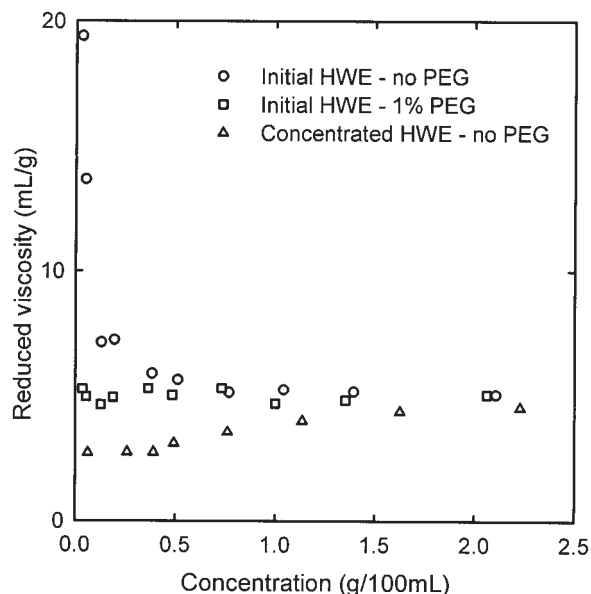


Figure 3 Reduced viscosity of HWEs with and without 1% (w/w) PEG (molecular weight = 4600) at 25°C.

can interact through intermolecular hydrogen bonding between PEG and PA polymers, whereas higher molecular weight PEG can interact through this intermolecular hydrogen bonding as well as PEG–PA hydrophobic interactions. This, in turn, lessens the degree of direct PA–PA interactions via either self-condensation or self-association.

Figure 1 clearly shows that the addition of PEG to the initial PA extract before low-temperature concentration reduced the Newtonian viscosity of 40% HWE by about an order of magnitude and that PEG with a molecular weight of 4600 showed the greatest reduction in the steady-shear viscosity (to ca. 0.1 Pa s). The reduction of the high-shear viscosity (10 s^{-1}) of 40% HWE solutions is shown in Figure 2 as a function of the PEG molecular weight. Increasing the molecular weight and hence the PEG hydrophobicity progressively reduced the high-shear viscosity in a linear manner for the same PEG concentration.

The FTIR spectra of the PA tannin with and without PEGs with molecular weights of 200 and 4600 were almost identical, and this indicated the absence of covalent or ionic bonding between the tannin and PEGs. The absence of this type of bonding was consistent with the reduced viscosity data for very dilute HWE with and without PEG, as shown in Figure 3. When PEG was added to HWE (3% HWE), the PA polymers could interact with PEG via hydrogen bonding and hydrophobic interactions, as noted previously, and this blocked the possible dissociable sites on the PA polymers. This is confirmed by Figure 3, which shows that the single-chain polyelectrolyte behavior of PA,¹³ observed as intrachain repulsion and expansion upon dilution, was eliminated in the pres-

ence of PEG. The high degree of hydrophobic bonding between PEG and single-chain PA polymers significantly influenced the polar functionality (Fig. 3) and prevented molecular association and condensation; this was observed as a progressive reduction in the viscosity at a high tannin concentration (40%; Figs. 1 and 2).

This mechanism is more evident in Figure 4: PEG with a molecular weight of 4600 not only reduced the at-rest and low-shear viscosities of a 40% PA solution but further impeded, at higher PEG concentrations (5 and 10%), molecular association between the PA chains so that non-Newtonian shear-thinning solutions resulted. This yielded high-shear viscosities that were reduced to 0.08 and 0.02 Pa s for 5 and 10% PEG, respectively. Through contributions from both hydrophobic and hydrogen bonding, PEG acted as an adjunct to the PA molecule, shielding the charge potential and making the macromolecular interactions weaker. These macromolecular aggregates were then disturbed at shear rates as low as 10^{-1} s^{-1} , as shown in Figure 4, and this resulted in shear-thinning non-Newtonian flow behavior. Although the lower molecular weight PEG (molecular weight = 200) progressively reduced the Newtonian viscosities with increasing PEG content, it was unable to shield the macromolecular interactions to a sufficient degree to allow shear flow to break the molecular aggregates and produce a shear-thinning fluid.

When PEG was added (1% w/w) after HWE was concentrated to a 40% tannin concentration, a reduction of the viscosity was not observed with any of the

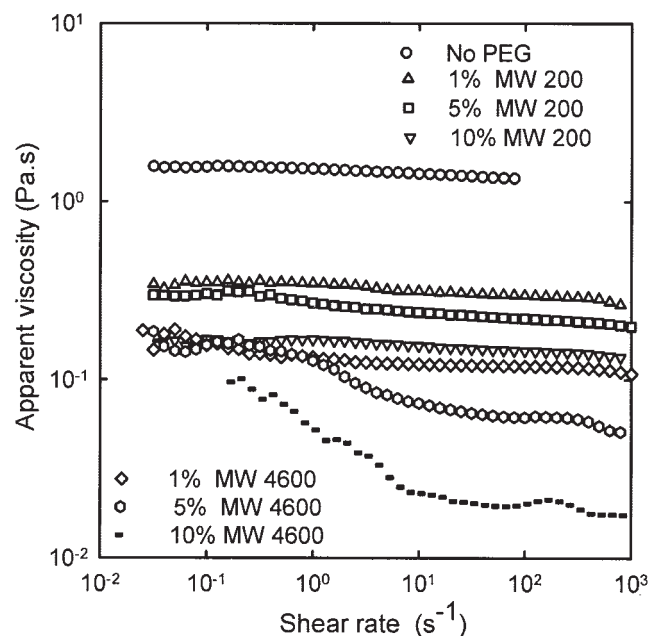


Figure 4 Apparent viscosity data for various concentrations of PEGs with molecular weights of 200 and 4600 at 25°C.

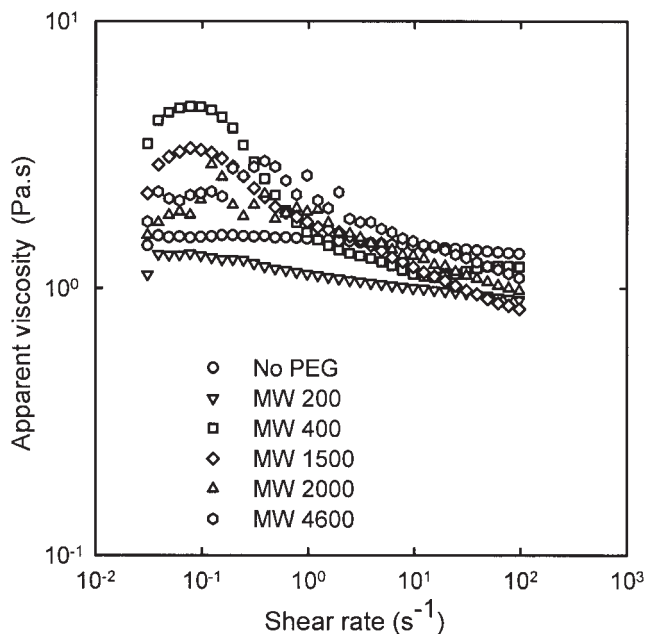


Figure 5 Apparent viscosities of HWEs (40% w/w) in the presence of PEGs (1% w/w) of various molecular weights at 25°C [with PEGs added after the HWE concentration (40%)].

PEG molecular weights examined, as shown in Figure 5. The PEGs under these conditions were not present and thus were not able to block the reactive sites of the PA polymers; this resulted in autocondensation and molecular association during the concentration process. The dominant form of association of PEG with PA is suggested here to be hydrophobic bonding; hydrophobic moieties are accessible during the concentration stage but not after such concentration once the PA-PA interactions have formed. The apparent viscosities of HWE with PEG added after the concentration were higher at low shear rates than those of HWE with no PEG because of the formation of additional secondary PEG-PA aggregates via hydrophobic interactions and hydrogen bonding. When the shear rate was increased continuously, these secondary molecular aggregates reduced into primary flow units, and this resulted in the slight shear-thinning behavior seen in the high-shear region.

This mechanism, contributing to the autocondensation of the PA polymers during the low-temperature concentration stage, was examined further by a comparison of HWE before and after concentration by dilute solution viscometry, in which single-chain intramolecular conformation dictated reduced viscosity. Figure 3 shows the reduced viscosities of HWE (initial HWE) immediately diluted to 2.5% (w/v) and concentrated HWE diluted to this concentration immediately after the concentration process. The initial unconcentrated HWE showed polyelectrolyte chain-expansion behavior upon dilution, whereas the HWE that underwent the concentration process did not demonstrate

such chain expansion because of the irreversible autocondensation of functional sites even under the mild temperature conditions used in the concentration. The presence of a phloroglucinol A-ring of high nucleophilicity may explain the high sensitivity of *P. radiata* tannin to autocondensation.¹¹ At concentrations greater than approximately 0.75 g/100 mL, the reduced viscosity was a little greater in the initial HWE because the PA chains were more extended than in the HWE derived from the concentrated HWE material.

Even though concentration at a low temperature produced stable low-viscosity Newtonian solutions at polymer concentrations of 40% in this article and 68% in a previous study,²³ molecular aggregation processes induced a degree of autocondensation that was sufficient to inhibit chain expansion of the free PA chains in solution. In the presence of a nonionic surfactant, *iso*-octylphenoxypolyethoxyethanol (Triton X100), Figure 6 shows that the interaction with PEG was effectively inhibited because of the surfactant preferentially interacting with PEG and thereby resulting in fewer PEG species available to associate with PA and a reduction of the HWE viscosity to about 10^{-1} Pa s. Figure 6 shows that Triton X100 itself did not affect the apparent viscosity of HWE, thereby indicating the selectivity of the nonionic surfactant to interact with the PEG component.

When Triton X100 was replaced by a strong nucleophile such as urea capable of breaking hydrogen bonding,²⁵ the effectiveness of PEG for reducing viscosity was also inhibited because of the preferential hydrogen bonding between urea and PEG; that is, fewer PEG species were available for interaction with

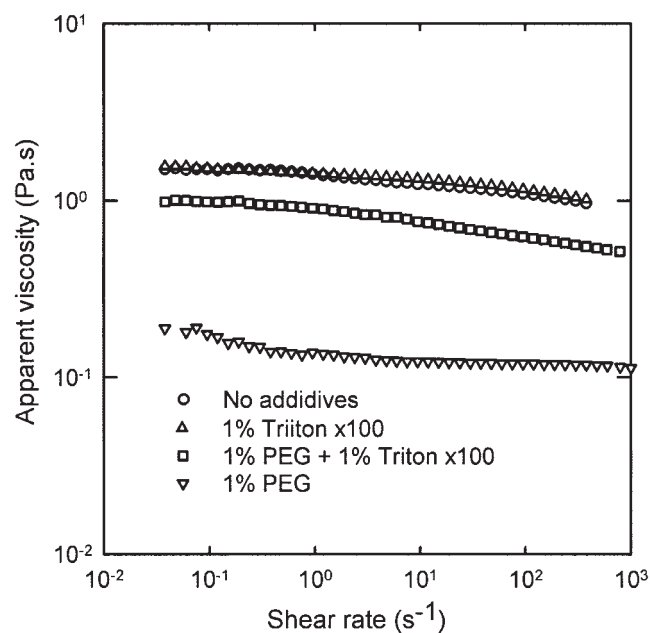


Figure 6 Steady-shear data of HWEs (40% w/w) at 25°C in the presence of PEG and nonionic surfactant Triton X100.

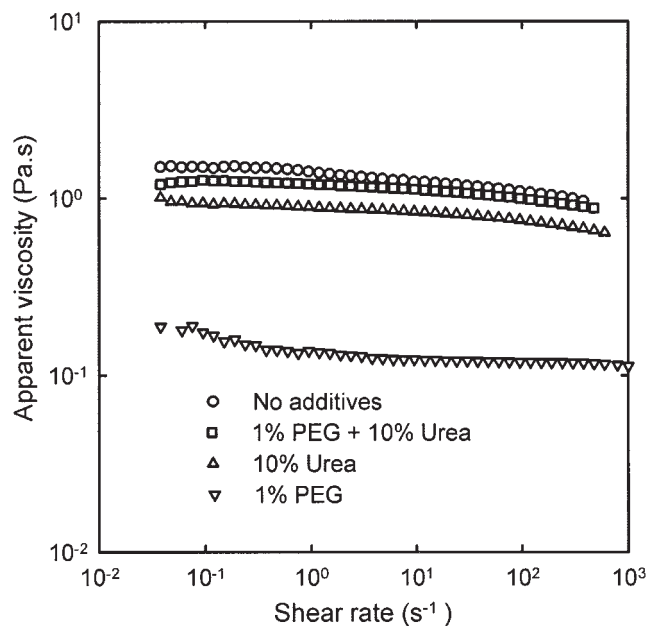


Figure 7 Steady-shear data of HWEs (40% w/w) at 25°C in the presence of PEG and urea.

HWE (Fig. 7). Urea itself tends to reduce the viscosity of PA macromolecules in aqueous solutions by breaking the hydrogen bonding that leads to molecular and colloidal interactions.^{8,12,13} Comparing the degrees of apparent viscosity reduction due to urea and PEG with a molecular weight of 4600, we found that PEG reduced the viscosity by almost one order of magnitude more at moderate shear rates greater than 0.5 s^{-1} . Figures 6 and 7 strongly indicate that both hydrophobic interactions and hydrogen bonding could take place between the PA chains and PEG chains because the nonionic surfactant was well established as disrupting hydrophobic bonding and urea was established as a hydrogen-bond breaker; both inhibited the ability of PEG with a molecular weight of 4600 to reduce the apparent viscosity to about 10^{-1} Pa s .

The reactivity of these polyphenolic PA polymers with formaldehyde is a key parameter in their utilization as potential adhesives. This reactivity has been assessed with a range of techniques, including thermomechanical methods^{26–28} and the evolution of the viscoelasticity.²⁹ Here we examined the impact of PEG on the reactivity of these PA HWEs with formaldehyde through the evolution of the viscoelastic crosslinking. Figure 8(a–c) shows plots of the elastic modulus (G') and viscous modulus (G'') of the reaction mixtures formed from HWE (with and without PEG) and paraformaldehyde at different temperatures; the figures shows an increase in the reactivity as the temperature increased. Figure 8(a) shows that HWEs with no PEG and with PEGs with molecular weights of 200 and 4600 reacted very slowly at 40°C. HWE with PEG with a molecular weight of 200 showed the highest

degree of reactivity at 40°C and also showed the highest moduli in the long-time region. This was because of the presence of smaller PA polymers due to PEG with a molecular weight of 200 inhibiting the interaction between PA molecules, as shown by the reduction

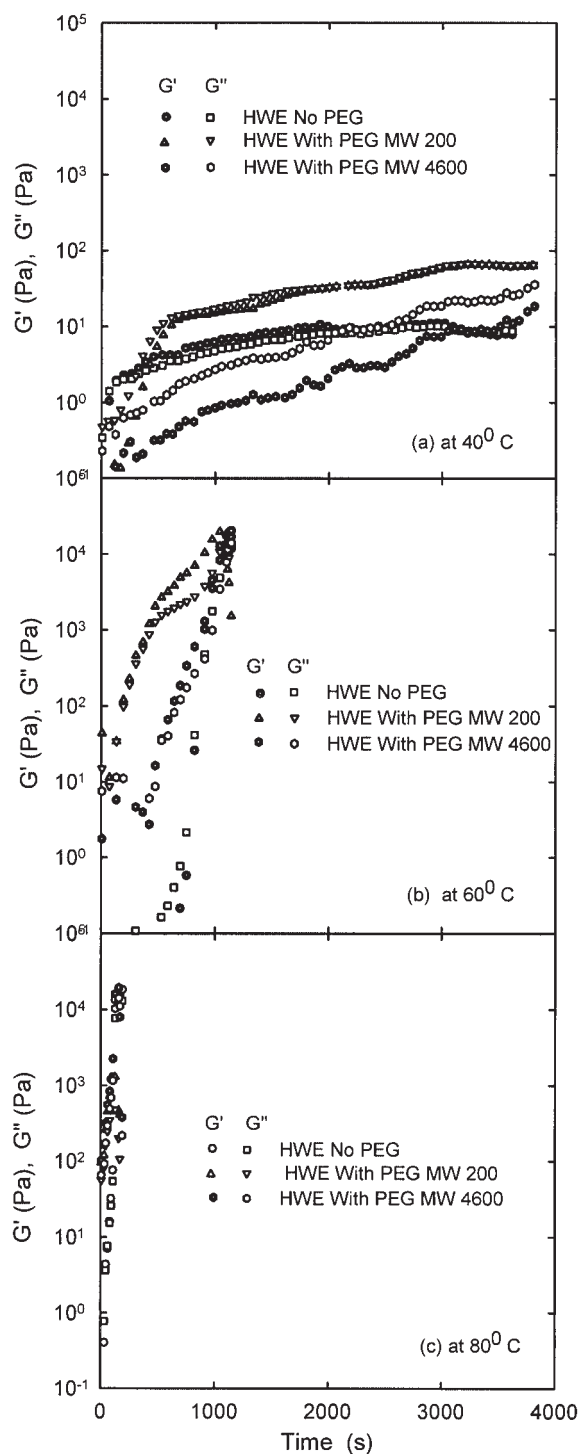


Figure 8 Time-sweep data for reaction mixtures of HWE (40%) and paraformaldehyde in the presence of PEGs with molecular weights of 200 and 4600 at (a) 40, (b) 60, and (c) 80°C.

in the solution apparent viscosity, and therefore providing a higher number of reactive groups with access to paraformaldehyde. HWE with PEG with a molecular weight of 4600 showed a relatively lower degree of reactivity because the PEG molecules could act as more complete adjuncts to PA molecules shielding the reactive sites, as indicated by the greater reduction of the solution viscosity. When the temperature was increased to 80°C, the reaction occurred very rapidly for all the samples studied, including those with PEG [Fig. 8(c)].

CONCLUSIONS

The concentration of *P. radiata* HWE under controlled temperature conditions resulted in a near-Newtonian fluid with an apparent viscosity of only about 2 Pa s at a 40% (w/w) concentration. This viscosity was further reduced considerably by the inhibition of molecular association between the PA polymer chains during concentration by the blocking of the reactive sites through interactions with high-molecular-weight PEG. PEG with a molecular weight of 4600 (1% w/w) was sufficient to reduce the viscosity of a concentrated (40% w/w) pine tannin extract by one order of magnitude to 0.1 Pa s. The viscosity reduction with PEG was only achieved by the addition of PEG before the concentration process. Two modes of association between the PA polymer and PEG were found, and both were dependent on the molecular weight of the PEG employed. Hydrogen bonding between the PEG-PA complex was favored with low-molecular-weight PEG (e.g., molecular weight = 200), whereas hydrophobic bonding between PEG-PA primarily occurred in the presence of high-molecular-weight PEG (e.g., molecular weight = 4600).

HWE with PEG with a molecular weight of 4600 showed a lower degree of reactivity at 40°C toward paraformaldehyde because the PEG molecules were acting as adjuncts with the PA molecules, shielding the reactive sites from the crosslinking agent. When the temperature was increased to 80°C, the reaction occurred very rapidly for all the HWE samples, including those with PEGs (molecular weight = 200 or 4600) as viscosity-reducing additives. The molecular interactions of these PA tannins with PEG, involving

both hydrogen bonding and hydrophobic bonding, influenced both the modes of molecular association and the single-chain conformation in solution. These mechanisms, leading to PEG acting as an adjunct, influenced interactions between PAs and their interactions with complex carbohydrates and proteins, which are being increasingly explored for their effects on industrial processing and nutrition.

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