

# Effect of Ion-Binding on the Formation of Temporary Viscoelastic Networks of Proanthocyanidin Biopolymers

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**ABSTRACT:** The gel formation of the alkali salts of proanthocyanidin polymers from *Pinus radiata* bark with calcium(II) and aluminum(III) has been investigated. The calcium- and aluminum-induced gelation of the hot water extract (HWE) has been studied by UV spectroscopy as well as through conductivity and rheological measurements. The ability of an HWE to complex was observed to increase significantly as the solution's pH became more alkaline, and the conductivity data are consistent with UV spectra with respect to structural change as metal binding occurs. The addition of the cationic species  $\text{Ca}^{2+}$  and  $\text{Al}^{3+}$  markedly increased the elasticity of the extracts, even at low extract concentration. Rheological measurements of metal binding in the HWE paralleled the UV spectral and conductivity behavior, thus indicating that the HWE binds more readily with calcium than with aluminum. In the concentrated HWE system, maximum association of the HWE with metal ions was found to appear at the same molar ratio of 3.6. The addition of such cationic species to the extracts is thought to reduce their fluidity primarily by increasing the number of physical electrostatic crosslinks. Such an increase results, in turn, in an increase in the apparent molecular weight of extract polymers and a decrease in fluidity. © 1997 John Wiley & Sons, Inc. *J Appl Polym Sci* **65**: 1795–1805, 1997

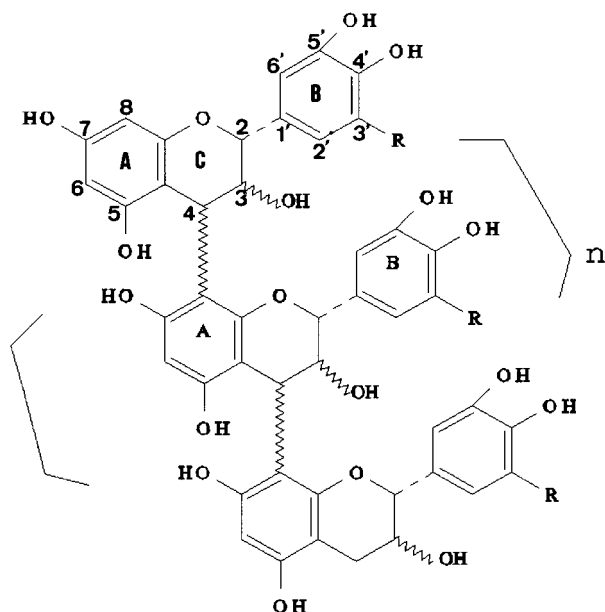
**Key words:** tannin; biopolymer; viscoelastic; ion binding; networks

## INTRODUCTION

Recently the hot water extract (HWE) of *Pinus radiata* (*P.r.*) bark was shown to display significant polyelectrolytic behavior resulting from electrostatic intrachain repulsion and a purely viscous flow behavior.<sup>1</sup> The alkaline sequential extract of these proanthocyanidin tannins demonstrated a highly viscoelastic behavior with the persistence time characteristic of a structured liquid or gel state.<sup>2</sup> The marked viscoelasticity of the alkaline sequen-

tial extract appeared to arise from a major contribution by the more highly polymerized water insoluble fraction enhanced by colloidal interactions, leading to molecular clusters that could be significantly reduced in the presence of strong nucleophiles such as urea.<sup>3</sup> Since such different characteristics of the extracts are due to the diversity of molecular structures and functional groups present in various components, a study of the physicochemical evidence that probes the presence of these multicharge molecules was undertaken by examining (1) the physicochemical properties of HWE polyelectrolytes, which can impart various charge densities to the molecular chain

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- (a) R=H, procyanidin units (PC)  
 (b) R=OH, prodelphinidin units (PD)

**Figure 1** Structure of proanthocyanidins.

and thereby change the specific mode of interaction, and (2) the physical association that occurs between extract molecules in the presence of metal ions such as  $K^+$ ,  $Ca^{2+}$ , and  $Al^{3+}$  at pH values at which full dissociation of the HWE polyelectrolyte species is occurring.

#### Proanthocyanidin Polymers from *Pinus radiata*

Proanthocyanidin polymers (condensed polyphenol tannins) occur in the bark of all conifers and hardwoods examined to date, and they are frequently present in the wood itself.<sup>4</sup> Proanthocyanidin polymers consist of linear chains of flavan-3-ol units that have undergone varying degrees of condensation,<sup>5</sup> as illustrated in Figure 1.

Although the majority of proanthocyanidin polymers consist of both procyanidins and prodelphinidins, Czochanska et al.<sup>5</sup> have shown that *P.r.* bark contains these respective analogs in a ratio of 90 : 10.

#### Metal Complexation

In contrast to the covalent crosslinking present in many synthetic polymer networks, biopolymers

usually form physical gels in which the intermolecular forces involved in the junction zones are often sufficiently weak to allow the system to gel reversibly. Physical gels consist of chains "physically" crosslinked into networks, with the crosslinks themselves being of small but finite energy and lifetime.<sup>6</sup> One type of such physical association is the ionic complexation that occurs between polyelectrolyte molecules and metal ions. Even though the interactions of metal ions with polyflavonoids have not been researched in detail, considerable information is available in the literature<sup>7</sup> on the complexing of model monomeric flavonoids, particularly flavonols, with such metal ions.

Three possible metal complexing sites within a flavonol containing hydroxyls at C-3, 5, 3', and 4' may be envisaged (Fig. 1). These are between the C-3 hydroxyl and the carbonyl, the C-5 hydroxyl and the carbonyl, and the ortho hydroxyls in the B-ring. It has been determined that the first of these sites is normally the first occupied, with the latter two sites having about the same complexing ability under neutral conditions.<sup>8</sup> The complexing ability of a catechol-type B-ring is known to increase as the pH becomes more alkaline.<sup>8,9</sup> Most studies have been carried out on the monomeric model compounds involved in polyphenol autocondensation; however, there is a relatively large ill-defined area regarding the basic properties of natural polyphenolic biopolymeric extracts. Recently, polyflavonoid tannins have been found by Pizzi et al.<sup>10,11</sup> to autocondense and gel in the presence of small amounts of  $SiO_2$ ,  $H_3BO_3$ , and  $AlCl_3$  at high pH. The mechanism of tannin autocondensation and hardening was found to depend on the Lewis acid behavior of the additives used.

## EXPERIMENTAL

#### Sample Preparation

The principal isolation and purification methods used to extract the plant materials were similar to those described previously.<sup>3</sup> Distilled water (500 mL) at 100°C was added to 100 g of dried *P.r.* bark powder. The mixture was stirred vigorously for 10 min prior to filtering with a Büchner funnel and No. 41 Whatman filter paper to remove insoluble materials and yield the HWE. The pH was adjusted with concentrated sodium hydroxide solution. The dilute extract solutions were concen-

trated in a Buchi rotary evaporator at about 70°C. The aluminum, potassium, and calcium ions were introduced into the extracts as the respective chlorides.

### Measurement of Conductance

#### *Conductometric Titrations of HWE*

The total number of acidic groups in the HWE was determined by conductometric titrations. For this purpose a 200-mL (0.3% w/v) HWE solution was placed in a reaction cell and mechanically stirred at 20°C. Nitrogen was continuously bubbled through the system to eliminate carbon dioxide. After reaching thermal equilibrium, the HWE solution was titrated with a volumetric standard of 2.14*N* KOH solution in 0.25 mL increments such that the total volume finally added was 9 mL. The conductivity of the solution was measured with a Metrohm 644 conductometer. The molecular weight of the HWE was assumed to be 2600 for the calculation of phenolic groups in the extract.<sup>12</sup> Since the added volume of titrant was less than 2.5% of the total, volumetric corrections were unnecessary.

#### *Degree of Complex Formation of Polyflavonoid Tannin*

The conductance of HWE solutions (at constant concentration) in the presence of AlCl<sub>3</sub> and CaCl<sub>2</sub> at different pH values was determined by titration. The concentration of aqueous HWE solution was 0.04% (w/v), and the titrants were 0.1*M* AlCl<sub>3</sub> and 0.1*M* CaCl<sub>2</sub>. The pH adjustment of the HWE solution and the diluent was made with 0.05*M* NaOH solution. The titrant was added in 10 μL increments, and the conductivity was recorded after equilibrium.

#### *Degree of Polyflavonoid Tannin Dissociation*

The conductance of HWE solutions (at different dilutions) in the presence of AlCl<sub>3</sub> and CaCl<sub>2</sub> at different pH values was determined. The concentrations of aqueous HWE solution were 0.02% (w/v) to 0.10%, and the titrants were 0.1*M* AlCl<sub>3</sub> and CaCl<sub>2</sub>. The pH adjustment of the HWE solution and the diluent was made with 0.05*M* NaOH solution.

### Spectrophotometric Measurements

The concentration of aqueous HWE solution used was 0.04% (w/v), and the titrants were 0.1*M*

AlCl<sub>3</sub> and 0.1*M* CaCl<sub>2</sub>. The pH adjustment of the HWE solution and the diluent was made with 0.05*M* NaOH solution. The solution to be titrated (2.9 mL) was pipetted into a specially sealed spectrophotometric cell,<sup>13</sup> and the spectrum was recorded using a Varian Cary 3 UV/Vis spectrophotometer. The titrant was then added in 1 μL increments, and the solution was thoroughly mixed before measurement was made.

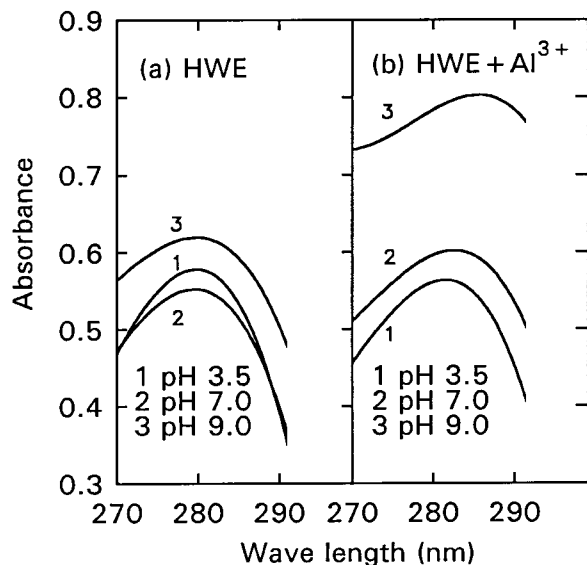
### Rheological Measurements

Rheological parameters were measured at 20°C with a Rheometrics Fluid Spectrometer RFS II (Rheometrics, Piscataway, NJ) equipped with a parallel plate geometry (radius: 25 mm). Solvent evaporation was avoided by covering the edge of the sample with a low-viscosity silicon oil.

## RESULTS AND DISCUSSION

The impact of pH, valence, and the ratio of metal ions to HWE on the UV absorption spectra was determined. There were noticeable differences in the mode of the absorption spectra in aqueous solution [HWE (pH 3.5) spectra had λ<sub>max</sub> at 279 nm] and in methanol, which has already been used for the study of model polyflavonoid spectral shifts.<sup>14</sup> No spectral shift took place for the HWE–CaCl<sub>2</sub> and HWE–AlCl<sub>3</sub> systems in the methanol medium. Thus, all spectral measurements of HWE in the presence of metal ions were made in aqueous medium. For the aqueous HWE–CaCl<sub>2</sub> system, there was no difference in the spectra compared with the HWE spectra when the measurements were made with pH values of 3.5 and 9.0; whereas, the HWE–AlCl<sub>3</sub> system produced substantial spectral shifts with pH variation.

The pH titration of HWE in water caused a vertical shift in the absorbance band with a marginal horizontal shift of λ<sub>max</sub> (281 nm, 283 nm, and 286 nm at pH values of 2.0, 3.2, and 9.0, respectively), as shown in Figure 2(a). This may occur as the HWE undergoes limited rearrangement or irreversible decomposition under increasing alkaline conditions,<sup>14,15</sup> but notably it does not influence the UV absorbance as much as formation of the HWE–AlCl<sub>3</sub> complex [Fig. 2(b)]. Titration of the HWE in the presence of AlCl<sub>3</sub> caused a more pronounced monotonic vertical shift with increasing pH, as shown in Figure 2(b). This finding indicates that the formation of the metal



**Figure 2** The spectra of (a) HWE ( $4.14 \times 10^{-5}$  g/ml) and (b) HWE +  $0.1M$   $AlCl_3$  at pH 3.5 (281 nm), pH 7.0 (283 nm), and pH 9.0 (286 nm).

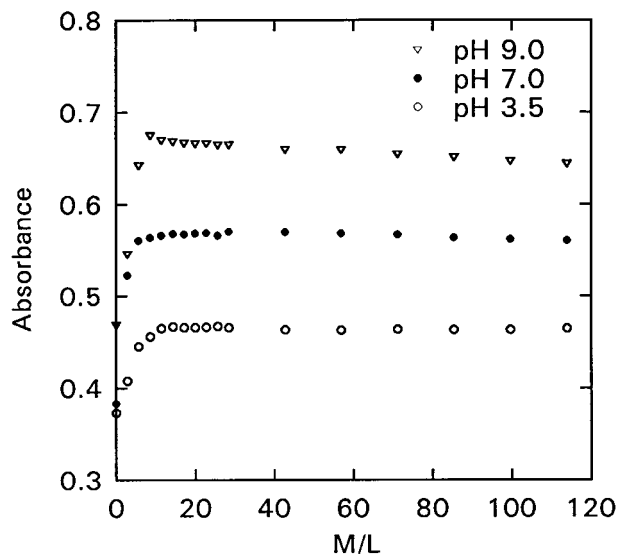
complex is associated with the ionization of at least one of the dissociable sites shown in Figure 1, such as the phenolic one, and that the stabilization of the complex results in the loss of a proton in the chelation step. Figure 2(a,b) indicates that high pH is a favorable condition for the formation of the HWE-metal complex (i.e., the vertical absorbance shift with the addition of  $AlCl_3$  was most significant at pH 9.0).

Figure 3 shows a metal-to-ligand molar ratio M/L (molarity of  $Al^{3+}$ /molarity of HWE employing a molecular weight of 2600 for the HWE<sup>11</sup>) plot for titration of HWE with  $AlCl_3$  at different pH values. At the low pH of 3.5, the plot has an inflection near the M/L value of 6.75, indicating the maximum formation of complex. In aqueous solutions under different pH conditions, the HWE appears to form a maximum amount of complex at a lower molar ratio of 4.34 for both pH 7.0 and 9.0. The difference in the inflection points of the metal-to-ligand plots (4.34 at pH 7.0 and 9.0, and 6.75 at pH 3.5) indicates that the stoichiometry of the complex favored in the presence of excess metal varies with solution pH in such a way that high pH conditions will yield a medium that increases the degree of dissociation and forms a more extensive HWE- $AlCl_3$  complex.

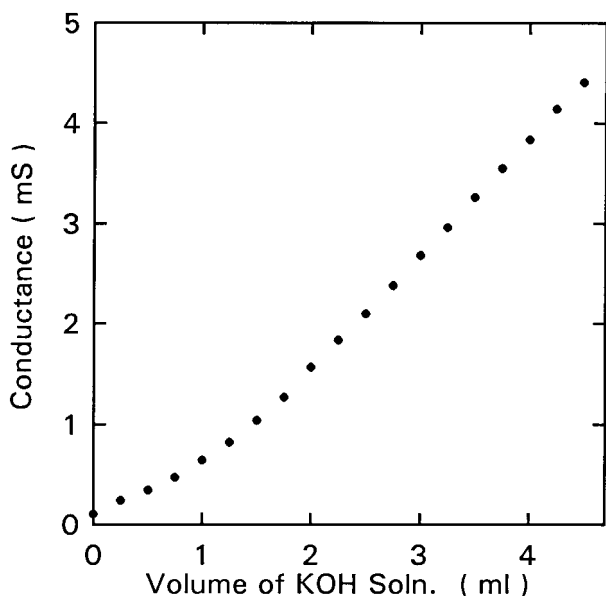
The UV spectra at different pH conditions clearly indicate the formation of a metal complex that is closely related to the dissociation of func-

tional sites in the HWE. Thus, conductometric measurements were carried out to obtain information on the number and nature of ions and charged groups that take part in metal ion binding. The deprotonation of phenolic groups was studied by titration of KOH into deoxygenated solutions of the HWE, which acts as the ligand.

The conductometric titration curve of HWE in water is shown in Figure 4, where the KOH titration curve demonstrates a distinct endpoint. Figure 4 shows an inflection point (1.35 mL of 2.14N KOH) corresponding to completion of the deprotonation of the phenolic groups in HWE. The phenolic groups per monomeric unit of HWE corresponded to 12.5 when the molecular weight of HWE was assumed to be 2600.<sup>12</sup> This value is about a half of the 23.0 phenolic groups of *P.r.* D. Don bark tannin determined in a water and organic solvent mixture (1 : 1  $Me_2SO-H_2O$ ) by Baeza et al.<sup>12</sup> On the basis of an average relative molecular mass of 320 for the monomeric unit ( $C_{16}H_{16}O_7$  for elemental analysis), a value of only 1.54 OH groups per monomeric unit for HWE was found. This may be caused by the OH groups in different units being favorably oriented to produce internal hydrogen bonding, which causes one of the hydrogen atoms to become more acidic while the other decreases in acid strength to the point that it cannot be titrated, as suggested by Baeza et al.<sup>12</sup>



**Figure 3** Molar ratio (M/L) plots for the titration of a  $4.14 \times 10^{-5}$  g/ml HWE solution with  $AlCl_3$ . Plots of data at 281 nm for pH 3.5, 283 nm for pH 7.0, and 286 nm for pH 9.0.

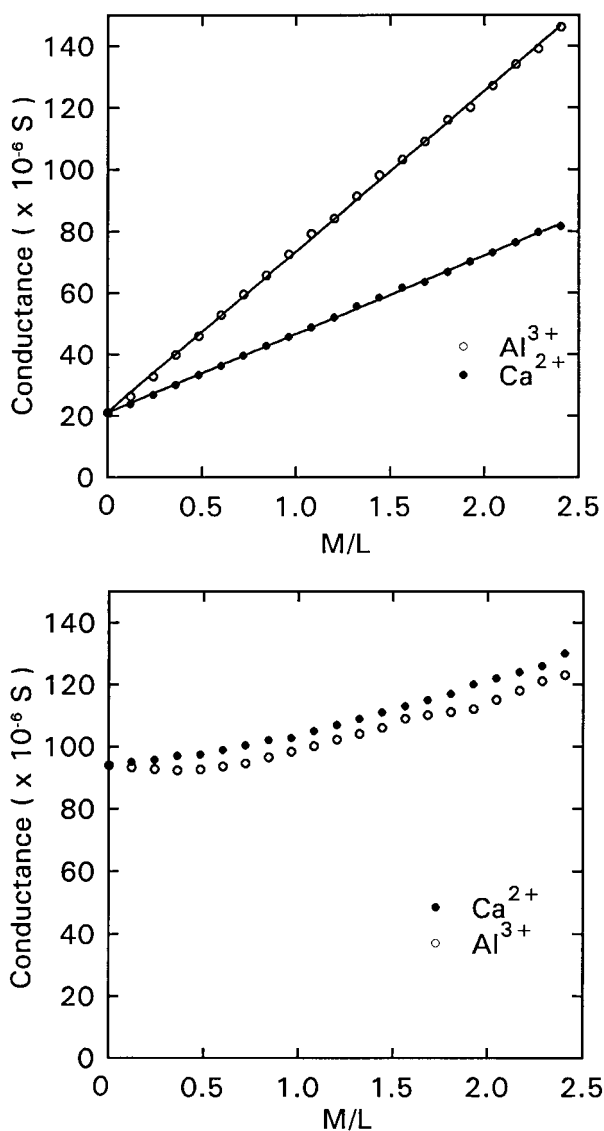


**Figure 4** A conductometric curve for HWE in an aqueous medium.

Figure 5(a,b) shows conductance versus the metal-to-ligand molar ratio for HWE at pH values of 3.5 and 9.0. As the proportion of metal increases at the natural HWE pH of 3.5, no inflection in conductance is observed. The linear increase of conductance upon the addition of  $\text{AlCl}_3$  and  $\text{CaCl}_2$  may only be caused by the increasing ionic strength of the electrolyte, whereas the conductance curve in Figure 5(b) shows clear inflection points for HWE with  $\text{CaCl}_2$  and  $\text{AlCl}_3$  when the pH is increased to 9.0. The  $\text{CaCl}_2$ -HWE system produces a higher conductance with a higher inflection point at a molar ratio of metal to ligand of 0.77. Figure 5(a,b) again suggests that a medium of high pH increases the degree of dissociation of HWE and thus favors complex formation through full dissolution of HWE in this pH region. The  $\text{CaCl}_2$ -HWE system shows an inflection point of 0.77, whereas the  $\text{AlCl}_3$ -HWE system shows an inflection point of 0.58, suggesting that the stoichiometric molar ratio of metal to ligand for HWE is much lower for  $\text{AlCl}_3$  than for  $\text{CaCl}_2$ . The higher conductance value for the  $\text{CaCl}_2$ -HWE system shown in Figure 5 (b) suggests that the free ion concentration is much greater in the  $\text{CaCl}_2$ -HWE system at pH 9.0, since an ionic mobility of  $\text{Al}^{3+}$  ion, itself at the natural pH of 3.5, seems to be much higher than that of  $\text{Ca}^{2+}$  in the system, as demonstrated in Figure 5 (a) (i.e., for the conductance values at a molar ratio of 1 the

conductance is  $72 \mu\text{S}$  for the  $\text{AlCl}_3$ -HWE system and  $45 \mu\text{S}$  for the  $\text{CaCl}_2$ -HWE system).

The conductance of HWE solution was also measured at different dilutions in water at a constant metal ion concentration at the pH values of 3.5 and 9.0 to determine the degree of dissociation ( $\alpha$ ) of the HWE polyelectrolyte. These results are recorded in Tables I through IV in which the calculated values of equivalent conductance ( $\Lambda$ ) as well as the specific conductance ( $\kappa$ ) of the HWE solutions are shown. The deviation from the theoretical values of specific conductance calculated from the separate tannin and background blank



**Figure 5** Molar ratio (M/L) plots for the conductometric titration of HWE solution with  $\text{CaCl}_2$  and  $\text{AlCl}_3$  at (a) pH 3.5 and (b) pH 9.0.

**Table I Conductivity of HWE in Water and 3.3M CaCl<sub>2</sub> Solution at pH 3.5**

Concentration of HWE (w/w %)	$\kappa$ of HWE ( $\mu\text{S m}^{-1}$ )	$\kappa$ of HWE and CaCl <sub>2</sub> Solution ( $\mu\text{S m}^{-1}$ ) <sup>a</sup>	Sum of $\kappa$ of HWE and CaCl <sub>2</sub> Solution ( $\mu\text{S m}^{-1}$ ) <sup>b</sup>	Lowering of $\kappa$ (%)	Equivalent Conductance ( $\Lambda$ ) of HWE ( $\mu\text{S m}^{-1}$ )	$\alpha = (\Lambda/\Lambda_0)$ <sup>c</sup>
0.02	12.78	58.72	82.34	28.68	763.17	0.37
0.04	17.59	61.81	87.15	29.08	401.73	0.19
0.06	24.21	68.35	93.77	27.11	296.17	0.14
0.08	28.87	72.33	98.43	26.51	235.08	0.11
0.10	32.63	74.59	102.19	27.01	193.92	0.09

Specific conductivity ( $\kappa$ ) of 3.3M CaCl<sub>2</sub> blank solution = 69.6  $\mu\text{S m}^{-1}$ .

<sup>a</sup> Observed.

<sup>b</sup> Calculated.

<sup>c</sup>  $\Lambda_0 = 2080 \mu\text{S m}^{-1}$ .

electrolyte solution containing calcium or aluminium chloride is also shown as the percentage variation. When two simple electrolytes are mixed, the conductivity of the mixture is very nearly equal to the sum of the conductivities of the individual components, provided the solution is dilute. However, the calculated sum of the conductivities of the individual electrolytes (HWE + CaCl<sub>2</sub> or AlCl<sub>3</sub>) is found (Tables I–IV) to be greater than the conductance of the mixture, indicating metal ion binding to the tannin sites and that this ion binding is more significant at higher pH. The conductance characteristics ( $\Lambda$  versus  $c^{1/2}$ ) of the HWE given in Tables I through IV are shown to be hyperbolic. For strong electrolytes, the relation between  $\Lambda$  and  $c^{1/2}$  is linear, and for weak electrolytes  $\Lambda$  tends to show steep hyperbolic curves at low molar concentrations of tannin (c). From all of the tables, it can be seen that in the beginning there is a slow rise in the value of

equivalent conductance with progressive dilution of the HWE, whereas towards the end, in the very dilute range, equivalent conductance increases rapidly.

The conductance of a solution depends on the charge, number, and mobility of the ions present. The equivalent conductance depends upon the concentration of HWE, since the extent of the dissociation of an HWE polyelectrolyte is also dependent on the concentration of HWE. At a certain concentration, a polyelectrolyte may only be partly dissociated, whereas at infinite dilution it may be completely dissociated.<sup>16</sup> Here, the degree of dissociation ( $\alpha$ ) of a polyelectrolyte can be calculated by the simple relation  $\alpha = \Lambda/\Lambda_0$ , where  $\Lambda_0$  is the equivalent conductance at infinite dilution, and the results are presented in Figure 6(a,b). The variation of the degree of dissociation of HWE with concentration shows that HWE contains multicharge molecules that increase in charge at

**Table II Conductivity of HWE in Water and 3.3M AlCl<sub>3</sub> Solution at pH 3.5**

Concentration of HWE (w/w %)	$\kappa$ of HWE ( $\mu\text{S m}^{-1}$ )	$\kappa$ of HWE and CaCl <sub>2</sub> Solution ( $\mu\text{S m}^{-1}$ ) <sup>a</sup>	Sum of $\kappa$ of HWE and CaCl <sub>2</sub> Solution ( $\mu\text{S m}^{-1}$ ) <sup>b</sup>	Lower of $\kappa$ (%)	Equivalent Conductance ( $\Lambda$ ) of HWE ( $\mu\text{S m}^{-1}$ )	$\alpha = (\Lambda/\Lambda_0)$ <sup>c</sup>
0.02	12.78	94.74	127.16	25.50	1231.58	0.334
0.04	17.59	86.47	131.97	34.48	562.03	0.15
0.06	24.21	84.96	138.59	38.70	368.20	0.10
0.08	28.87	84.21	143.25	41.21	273.69	0.07
0.10	32.63	90.98	147.01	38.12	236.54	0.06

Specific conductivity ( $\kappa$ ) of 3.3M AlCl<sub>3</sub> blank solution = 114.38  $\mu\text{S m}^{-1}$ .

<sup>a</sup> Observed.

<sup>b</sup> Calculated.

<sup>c</sup>  $\Lambda_0 = 3800 \mu\text{S m}^{-1}$ .

**Table III Conductivity of HWE in Water and 3.3M CaCl<sub>2</sub> Solution at pH 9.0**

Concentration of HWE (w/w %)	$\kappa$ of HWE ( $\mu\text{S m}^{-1}$ )	$\kappa$ of HWE and CaCl <sub>2</sub> Solution ( $\mu\text{S m}^{-1}$ ) <sup>a</sup>	Sum of $\kappa$ of HWE and CaCl <sub>2</sub> Solution ( $\mu\text{S m}^{-1}$ ) <sup>b</sup>	Lowering of $\kappa$ (%)	Equivalent Conductance ( $\Lambda$ ) of HWE ( $\mu\text{S m}^{-1}$ )	$\alpha = (\Lambda/\Lambda_0)$ <sup>c</sup>
0.02	38.50	66.92	137.29	51.26	869.92	0.41
0.04	57.07	77.44	155.87	50.31	503.39	0.24
0.06	83.50	101.50	182.26	44.31	439.85	0.21
0.08	106.02	121.81	204.81	40.53	392.62	0.19
0.10	111.28	129.32	210.08	38.44	336.24	0.16

Specific conductivity ( $\kappa$ ) of 3.3M CaCl<sub>2</sub> blank solution = 98.80  $\mu\text{S m}^{-1}$ .

<sup>a</sup> Observed.

<sup>b</sup> Calculated.

<sup>c</sup>  $\Lambda_0 = 2100 \mu\text{S m}^{-1}$ .

alkaline pH [Fig. 6(b)]. The dissolved polyelectrolytes have a high charge density on the molecules, which can potentially influence both the inter- and intramolecular interactions.

Figure 6(a) shows that even without the addition of NaOH, HWE in solution undergoes a degree of dissociation that indicates the presence of a significant charge on the HWE chains, as previously found.<sup>1</sup> The HWE solution at the zero concentration limit (intrinsic dilution) has an intrinsic dissociation constant  $\text{p}K_a$  of 8.81 based on the data of Figure 6(b), since full dissolution of HWE is only possible under such alkaline conditions. Thus, half the total HWE reactive sites will have dissociated at pH 9.81. At a pH of 7.53, only 5% of the total functional groups are dissociated, whereas at a pH of 10.80, 99% of the HWE is dissociated.

In polyelectrolyte systems such as HWE, in the presence of AlCl<sub>3</sub> and CaCl<sub>2</sub>, the conductance will

be primarily determined by the number of unbound ions present at any particular dilution. The Ca–HWE and the Al–HWE systems at pH values of 3.5 and 9.0 show a similar significant reduction in the degree of dissociation compared with HWE across the whole concentration range. The overall degree of dissociation of the tannin and the tannin metal complex at pH 9.0 [Fig. 6(b)] was significantly higher than at the natural extraction pH of 3.5. At moderate HWE concentrations, the degree of dissociation of the HWE in the presence of Ca<sup>2+</sup> or Al<sup>3+</sup> will not be appreciably affected by dilution, and, as a result, the conductance will rise only slowly with dilution. At high dilutions, however, the metal ions will escape more easily from the electrical double layer of charged HWE polymer and contribute proportionally to the overall conductance of the system.

It is well known that many linear biopolymeric polyelectrolytes have a tendency to form physical

**Table IV Conductivity of HWE in Water and 3.3M AlCl<sub>3</sub> Solution at pH 9.0**

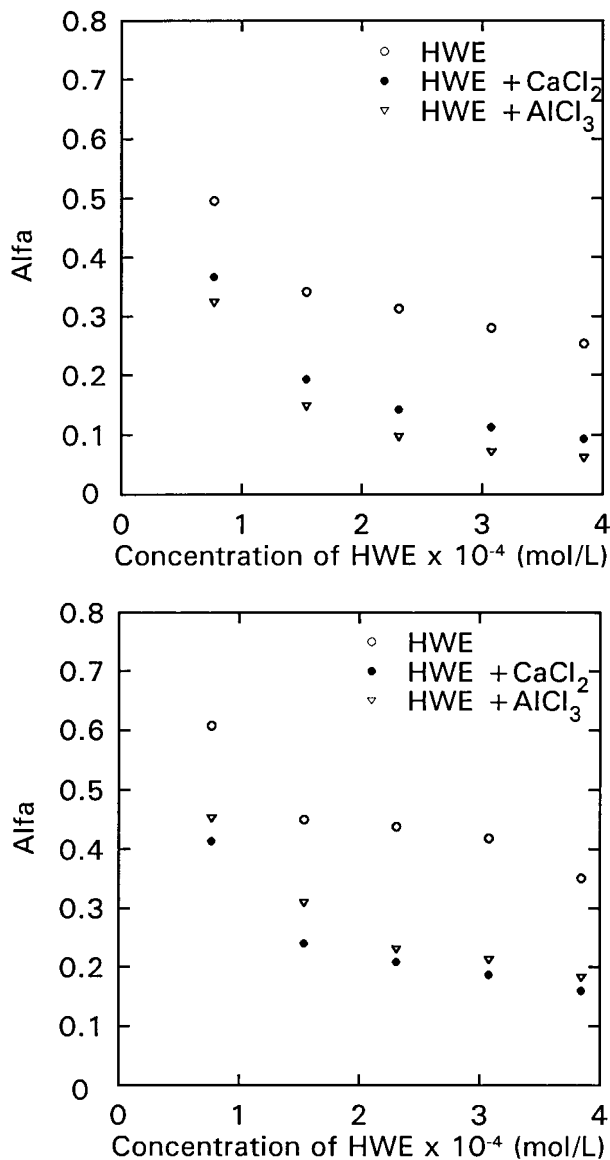
Concentration of HWE (w/w %)	$\kappa$ of HWE ( $\mu\text{S m}^{-1}$ )	$\kappa$ of HWE and CaCl <sub>2</sub> Solution ( $\mu\text{S m}^{-1}$ ) <sup>a</sup>	Sum of $\kappa$ of HWE and CaCl <sub>2</sub> Solution ( $\mu\text{S m}^{-1}$ ) <sup>b</sup>	Lowering of $\kappa$ (%)	Equivalent Conductance ( $\Lambda$ ) of HWE ( $\mu\text{S m}^{-1}$ )	$\alpha = (\Lambda/\Lambda_0)$ <sup>c</sup>
0.02	38.50	59.93	118.57	49.46	779.03	0.45
0.04	57.07	81.96	137.14	40.24	532.71	0.31
0.06	83.50	91.73	163.53	43.91	397.49	0.23
0.08	106.02	112.78	186.09	39.39	366.54	0.21
0.10	111.28	121.05	191.35	36.74	314.74	0.18

Specific conductivity ( $\kappa$ ) of 3.3M AlCl<sub>3</sub> blank solution = 80.08  $\mu\text{S m}^{-1}$ .

<sup>a</sup> Observed.

<sup>b</sup> Calculated.

<sup>c</sup>  $\Lambda_0 = 1720 \mu\text{S m}^{-1}$ .



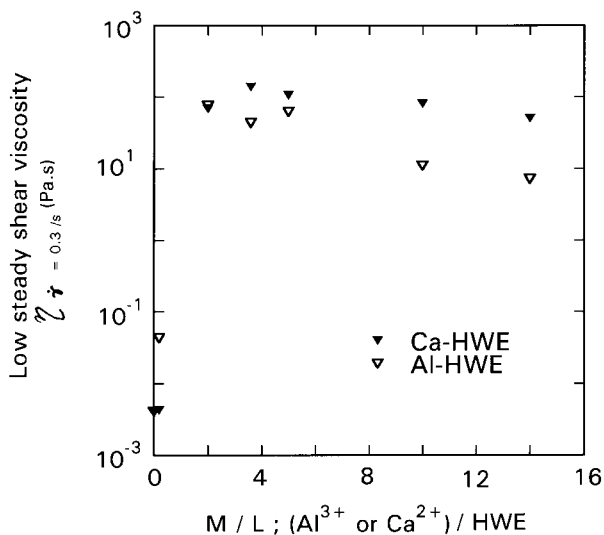
**Figure 6** Degree of dissociation ( $\alpha$ ) of HWE at different dilutions in water and in a constant concentration of  $\text{CaCl}_2$  and  $\text{AlCl}_3$  at (a) pH 3.5 and (b) pH 9.0.

gels owing to the occurrence of ionic complexation between polyelectrolyte molecules and multivalent ions. Solutions of HWE at high pH (pH 11, where it was found that full dissociation of HWE occurred) to which  $\text{AlCl}_3$  is added gelled upon shearing within seconds at ambient temperature. Steady shear or large deformation breaks HWE networks and results in shear thinning. The effect of  $\text{CaCl}_2$  and  $\text{AlCl}_3$  on the steady low-shear equilibrium viscosity (equilibrium viscosity at  $0.3 \text{ s}^{-1}$ ) of HWE is shown in Figure 7. Here, samples are

reported at 13% (w/v) HWE solution at pH 11 in the presence of  $\text{CaCl}_2$  and  $\text{AlCl}_3$ .

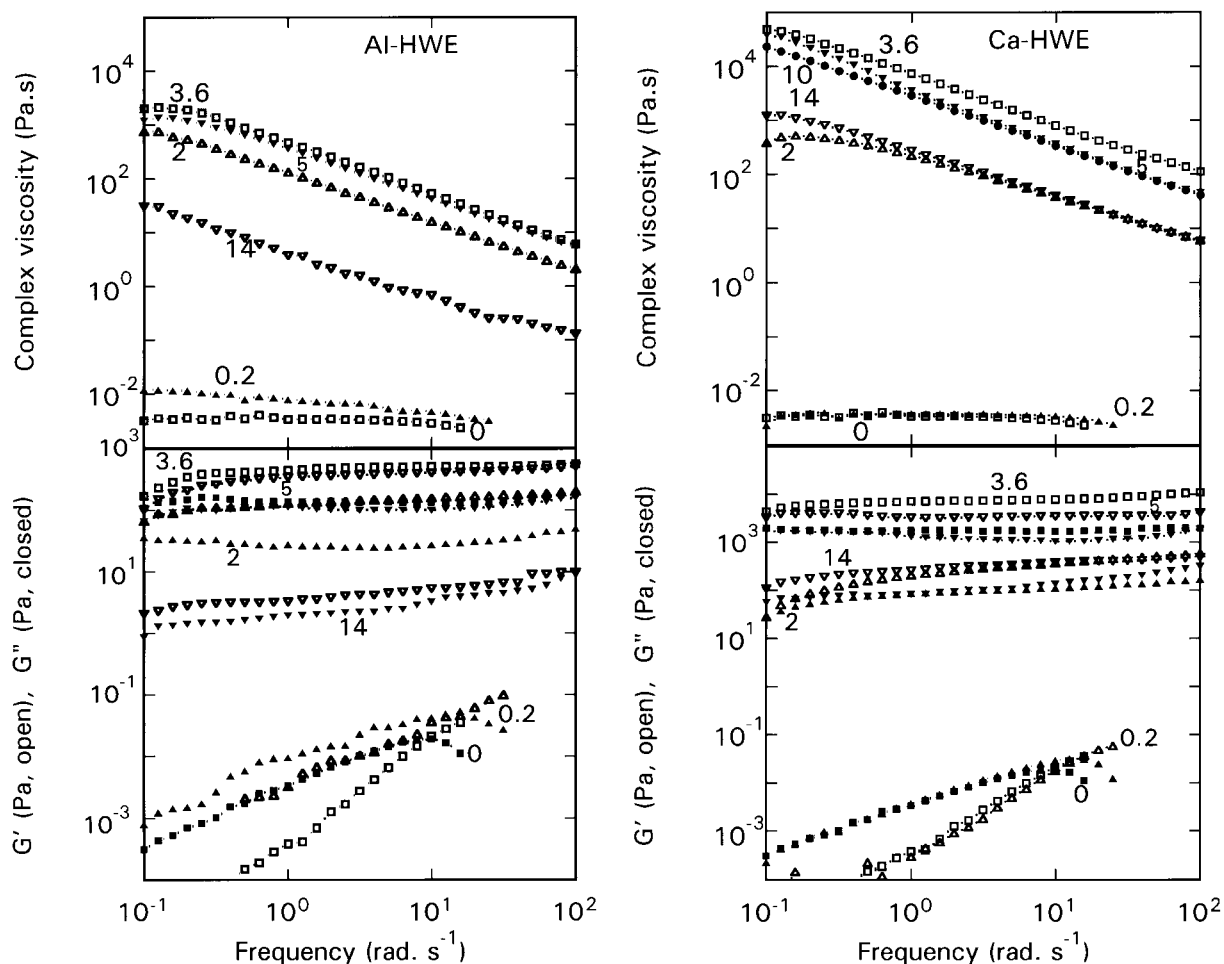
Solutions of HWE were transformed into a strongly gelled fluid by addition of a critical metal ion concentration at a similar molar ratio (3.6) for the  $\text{Ca}$ -HWE and  $\text{Al}$ -HWE systems. The low-shear equilibrium viscosity increased by more than four orders of magnitude and manifested a maximum at this critical value, as shown in Figure 7. At this molar ratio of 3.6, the gel characteristics were dominant, which may have been due to the full complexation of metal ions in regular arrays of electronegative sites formed by HWE chains. With further increases in molar ratio, the viscosity decreased marginally for the  $\text{CaCl}_2$ -HWE system but decreased more significantly for the  $\text{AlCl}_3$ -HWE system (i.e.,  $\text{Ca}^{2+}$  is more efficient than  $\text{Al}^{3+}$  in the association of cations with the HWE chain, which is consistent with the decreased dissociation of the  $\text{Ca}$ -HWE system compared with the  $\text{AlCl}_3$ -HWE shown at high pH in Fig. 6[b]).

To obtain further information about structural differences between gels made at various  $\text{CaCl}_2$  and  $\text{AlCl}_3$  concentrations, a microstructural analysis was made by monitoring the elastic storage and loss shear modulus ( $G'$  and  $G''$ , respectively) from oscillatory deformation at various angular frequencies  $\omega$  in the linear viscoelastic region. The  $\text{CaCl}_2$ -HWE and  $\text{AlCl}_3$ -HWE systems (at molar ratio of 3.6) showed a frequency behavior typical of a true gel (i.e. the storage modulus dominated



**Figure 7** Steady low-shear viscosity of 13% HWE (pH 11) with various molar ratios ( $M/L$ ) for  $\text{Ca}^{2+}$  and  $\text{Al}^{3+}$ .





**Figure 8** Frequency dependence of the magnitude of the elastic storage modulus  $G'$ , the loss shear modulus  $G''$ , and complex viscosity for 13% HWE (pH 11) with (a)  $\text{AlCl}_3$  and (b)  $\text{CaCl}_2$ .

the viscous modulus all over the frequency region studied [ $0.1 \sim 100 \text{ rad s}^{-1}$ ], and both moduli were largely independent of frequency, whereas the dynamic complex viscosity was seen to nearly obey a power law, as demonstrated in Fig. 8[a,b]).

Figure 9 shows the dependence of the complex viscosity  $\eta^*$  and the storage modulus  $G'$  ( $\omega = 0.16 \text{ rad s}^{-1}$ ) at various molar ratios of the metal ions. It can be seen that  $G'$  and  $\eta^*$  of the Ca-HWE and Al-HWE gels pass through a maximum consistent with the steady shear data of Figure 7, suggesting this maximum may be attributed to the maximum extent of the network structure forming at the same molar ratio of 3.6. As the M/L is increased to 0.2, the  $G'$  and  $\eta^*$  of the Ca-HWE system are similar to HWE, whereas the  $G'$  and  $\eta^*$  of the Al-HWE system exceed those of HWE. The maximum strength of the 13% Ca-

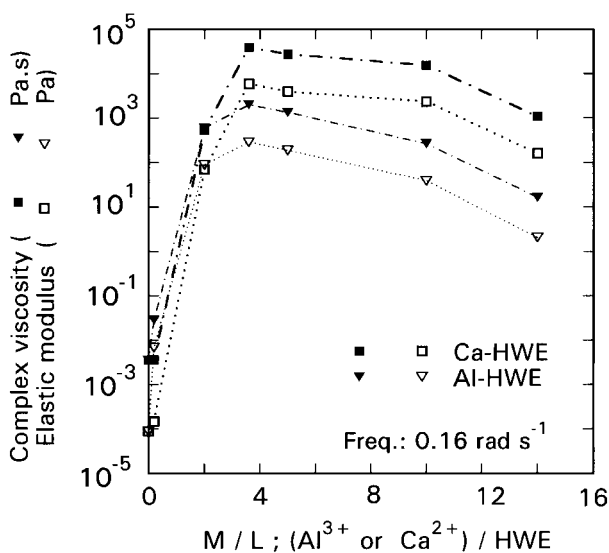
HWE gel (pH 11) exceeded that of the Al-HWE gel, as shown by the elastic modulus and complex viscosity being about one order of magnitude higher at same molar ratio, whereas further addition of  $\text{CaCl}_2$  induced weakening (perhaps syneresis) of the gel structure. Differences in the structural ion-binding between the Ca-HWE and Al-HWE systems may be responsible for this different gel network strength. Here, the ability of HWE polyelectrolyte chains to associate and form gels is determined by two main chemical parameters: (1) the number of successive negative sites required to form a junction zone (and hence the number of such zones within a given HWE chain) and (2) the relative amount of calcium and aluminum cations to HWE present in the system to form these junctions. The first parameters (intrinsic parameters) are known to include the molecu-

lar weight of the polymer and the number and distribution pattern of free dissociable functional groups in the macromolecules. The second parameters (extrinsic parameters) are known to include the polymer concentration, the metal ion concentration, the pH, and the background ionic strength.<sup>17</sup>

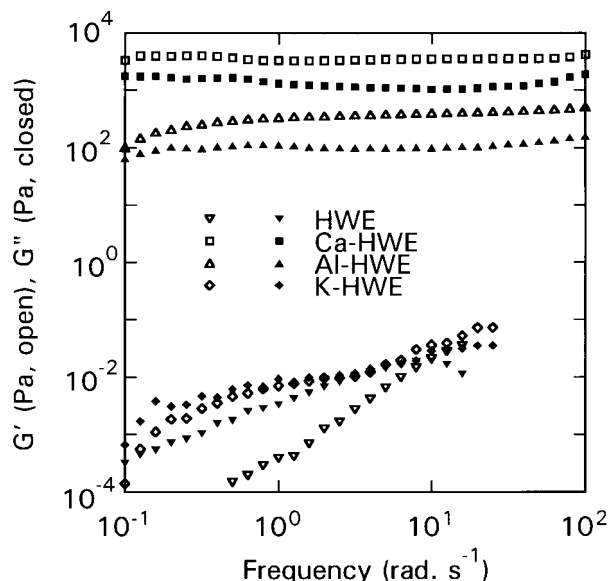
Cations of different valences were compared in the formation of HWE complexes at the 13% (w/v) concentration and pH 11 at an M/L ratio of 5, which are conditions at which gels of high rigidity are formed, as shown in Figure 10. This finding indicates that monovalent metal ions play very little role in structuring the HWE and remain as background electrolyte in the HWE solution, which then acts as a purely viscous liquid with a frequency dependent storage modulus.

## CONCLUSIONS

Ultraviolet spectral studies at different pH values have clearly indicated that the formation of a metal complex is closely associated with the dissociation of functional dissociable sites in the HWE solution from *P.r.* bark. The HWE solution at intrinsic dilution had an intrinsic dissociation constant  $pK_a$  of 8.81, with most of HWE functional groups dissociated at a pH close to 11. Hence, the gel formation of the alkali salts of concentrated HWE from *P.r.* bark with calcium(II) and alumin-



**Figure 9** Complex viscosity and elastic storage modulus versus M/L of  $\text{CaCl}_2$  and  $\text{AlCl}_3$  for 13% HWE (pH 11) at a frequency of  $0.16 \text{ rad s}^{-1}$ .



**Figure 10** Frequency dependence of the magnitude of the storage modulus and the loss modulus for 13% HWE (pH 11) with cations of different valences (M/L = 5).

ium(III) has been investigated at pH 11, where full dissociation of HWE polyelectrolyte is achieved. The addition of the cationic salts  $\text{Ca}^{2+}$  and  $\text{Al}^{3+}$  markedly increased the elasticity of the extracts, even at low concentration. Rheological measurements of metal binding in the HWE solution showed that aluminum binds less readily than calcium. In the concentrated HWE system, maximum association with  $\text{Ca}^{2+}$  and  $\text{Al}^{3+}$  ions was found at the similar molar ratio of 3.6. The addition of such cationic species to the extracts is thought to reduce their fluidity primarily by increasing the number of physical crosslinks. Such an increase results, in turn, in an increase in the apparent molecular weight of extract polymers and a decrease in fluidity.

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## REFERENCES

1. S. Kim and D. E. Mainwaring, *J. Appl. Polym. Sci.*, **56**, 905 (1995).
2. S. Kim and D. E. Mainwaring, *J. Appl. Polym. Sci.*, **56**, 915 (1995).

3. S. Kim, D. Sarathchandra, and D. E. Mainwaring, *J. Appl. Polym. Sci.*, **59**, 1979 (1996).
4. H. L. Hergert, in *Adhesives from Renewable Resources*, R. W. Hemingway, A. H. Conner, and S. J. Branham, Eds., ACS Symposium Series 385, American Chemical Society, Washington DC, 1989, p. 155.
5. Z. Czochanska, L. Y. Foo, R. H. Newman, and L. J. Porter, *J. Chem. Soc., Perkin Trans. 1*, 2278 (1980).
6. S. B. Ross-Murphy and K. P. Shatwell, *Biorheology*, **30**, 217 (1993).
7. P. E. Laks, in *Chemistry and Significance of Condensed Tannins*, R. D. Hemingway and J. J. Karchesy, Eds., Plenum Press, New York, 1989, p. 249.
8. L. J. Porter and K. R. Markham, *J. Chem. Soc. (C)*, 344 (1970).
9. L. Jurd and T. A. Geissman, *J. Org. Chem.*, **21**, 1395 (1956).
10. N. Meikleham, A. Pizzi, and A. Stephanou, *J. Appl. Polym. Sci.*, **56**, 1827 (1995).
11. A. Pizzi and N. Meikleham, *J. Appl. Polym. Sci.*, **55**, 1265 (1995).
12. J. Baeza, J. Freer, N. Rojas, and N. Durán, *Analyst*, **110**, 1407 (1985).
13. H. K. J. Powell and M. C. Taylor, *Aust. J. Chem.*, **35**, 739 (1982).
14. J. B. Harborne, in *Methods in Polyphenol Chemistry*, J. B. Pridham, Ed., Pergamon Press, New York, 1964, p. 13.
15. P. E. Laks and R. W. Hemingway, *J. Chem. Soc. Perkin Trans I*, **465**, 1875 (1987).
16. F. Oosawa, *Polyelectrolytes*, Marcel Dekker, New York, 1971.
17. D. Durand, C. Bertrand, J.-P. Busnel, J. R. Emery, M. A. V. Axelos, J. F. Thibault, J. Lefebvre, J. L. Doublier, A. H. Clark, and A. Lips, in *Physical Networks—Polymers and Gels*, W. Burchard and S. B. Ross-Murphy, Eds., Elsevier Applied Science, London and New York, 1990, pp. 283–300.