Interaction in Xanthan–Glucomannan Mixtures and the Influence of Electrolyte

P. Annable and P. A. Williams*

The North East Wales Institute, Deeside, Clwyd CH5 4BR, U.K.

K. Nishinari

National Food Research Institute, Tsukuba, Ibaraki 305, Japan

Received February 28, 1994; Revised Manuscript Received May 6, 1994®

ABSTRACT: The interaction of xanthan and glucomannan (konjac mannan, KM) has been monitored using differential scanning calorimetry (DSC), electron spin resonance (ESR), and mechanical spectroscopy. The DSC and ESR results indicate that interaction occurs in water immediately following xanthan side chain–backbone association. The process is thermodynamically driven and occurs to reduce xanthan–water contacts. In the presence of electrolyte, both techniques show that the xanthan conformational change shifts to higher temperatures and depends on the nature of the cation. Divalent cations give rise to a greater shift than monovalent cations. The results indicate that xanthan/KM interaction occurs at temperatures much lower than the conformational transition. The rheological properties (i.e., storage (G') and loss (G'') moduli and gelation temperature, T_{gel}) are greatly influenced by the presence of electrolyte. In all instances, G', G'', and gelation temperature are reduced when electrolyte is present, with divalent cations having a greater effect than monovalent cations. The reduction in G', G'', and T_{gel} follows the lyotropic series with increasing effects in the order $K^+ \sim Cs^+ < Na^+ \sim NH_4^+ \sim Ba^{2+} \ll Mg^{2+} \sim Ca^{2+}$. These observations are explained by the fact that electrolyte promotes xanthan self-association at the expense of xanthan/KM interaction.

Introduction

Konjac mannan (KM) is a β-1,4-linked glucomannan with branches consisting of about 16 sugar units linked to C-3 of the glucose and mannose at approximately every 10 residues along the chain.[1] The mannose–glucose ratio is 1.6:1, and it is thought that no block mannan or cellulosic structures are present. Native KM is acetylated (1 acetyl group every 17 sugar residues). It does not gel in water; however, on deacetylation in the presence of alkali, a thermally irreversible gel is formed.[2]

Xanthan is a charged bacterial polysaccharide consisting of a β-1,4-linked glucopyranose backbone with a trisaccharide side chain linked to every second glucose residue. The side chain consists of two mannose units separated by a glucuronic acid residue. The mannose residue attached to the cellulosic backbone is variably acetylated, and the terminal mannose may contain a pyruvate group. The proportion of acetyl and pyruvate substituents is determined by the culture and postfermentation conditions. Xanthan gum does not gel at any concentration; however, it undergoes a temperature-induced conformational transition from an ordered helical structure, where the side chains are folded in and associated with the backbone, to a disordered structure, where the side chains project away from the backbone. The transition is dependent on the ionic strength of the solution, the pH, and the nature of the electrolyte.[3] At present, there is still some debate as to the nature of the ordered form, with arguments presented for both single- and double-stranded structures.[3-10]

Norton et al.,[5] for example, have proposed that, on cooling in solutions of high ionic strength, a single 5-fold helix is formed in which the side chains pack along the backbone, and this is followed by side-by-side association of the xanthan chains. They also calculated the average length of the stable ordered species in salt solution using the Zimm–Bragg helix → coil transition theory and suggested that ordered and disordered regions may exist within a single xanthan molecule. Milas and Rinaudo[6] also support the single-stranded model and argue that xanthan can exist in three molecular conformations. Native xanthan, i.e., xanthan that has never been heated or subjected to low ionic strength, has a completely ordered structure (form I). On heating, this transforms to a completely disordered form (form III), but on cooling a denatured ordered form (form II) is obtained. Form II is more expanded than form I and occurs as a consequence of an alternative pattern of side chain–backbone interactions which are governed more by kinetic than thermodynamic factors. Liu et al.,[7] on the other hand, have proposed a double-stranded model for the ordered species in which the double helix melts from both ends on heating but does not completely unwind, thus resulting in the formation of an expanded dimer in which wormlike chains are joined by a short helical section. Interestingly, however, Kawakami et al.[10] have shown recently that on prolonged heating at high temperature (95 °C, 9 h) the chains can completely unwind and separate. More recent NMR studies by Gamini et al.[11] support the expanded dimer concept. Their work also provides evidence for the side-chain involvement in the transition since they showed that, on melting the double helix, a substantial increase was observed in side-chain mobility before there was any significant change in the backbone mobility.

Mixtures of xanthan and galactomannans or glucomannans form thermally reversible gels which most workers agree are due to molecular association.[13-25] For galactomannans, the gel strength is related to the ratio of mannose–galactose, with increasing interaction encountered as galactose substitution decreases, i.e., guar < tara < locust bean gum. Dea et al.[12] proposed that association takes place between the ordered xanthan helix and unsubstituted regions of the galactomannan backbone. McCleary[13] later modified this model and suggested that interaction could occur between the ordered xanthan chains and sequences along the mannan backbone where the galactose residues are positioned only on one side.

---

* To whom correspondence should be addressed.

® Abstract published in *Advance ACS Abstracts,* June 1, 1994.

Brownsey et al.[14,15] confirmed that molecular binding occurred from X-ray diffraction studies, and since gels were only formed when the solutions were mixed at temperatures above the temperature expected for the xanthan conformational transition, they concluded that the xanthan molecules must interact in the disordered form. Tako et al.,[16,17] however, proposed that the interaction takes place with the side chains of the xanthan molecule with the xanthan in its ordered helical arrangement according to a lock and key effect. They found that the gels containing deacetylated xanthan were much stronger and concluded that deacetylation enhanced the side-chain mobility and thus led to increased interaction. Other workers have also shown that deacetylation enhances xanthan reactivity.[18-20] Williams et al.[21,22] proposed that xanthan/KM gelation could occur with xanthan in both the ordered and the disordered conformation depending on the ionic strength of the solution. Using differential scanning calorimetry (DSC) they showed that in water the interaction appeared to occur a few degrees above that expected for the conformational transition of the xanthan molecules in solution alone, while in 0.04 mol dm^{-3} NaCl the interaction occurred at a much lower temperature (~42 °C) than the disorder → order conformation (~82 °C). Gels formed in water were stronger than those in electrolyte, with the optimum mixing ratio giving maximum gel strength shifted from 1:1 in water to 2:1 in electrolyte. Lopes et al.[20] also concluded that xanthan could interact with guar gum in both ordered and disordered forms and that interaction was stronger in the latter.

Cheetham and Mashimba[23] carried out an extensive series of experiments on xanthan–galactomannan mixtures and concluded that xanthan interaction occurs through disordered sequences along the xanthan chain. They argued that even in electrolyte, where the ordered helical structure exists, there are still sequences of disordered xanthan segments which will interact initially at room temperature to form "gel islands" and that a completely homogeneous gel will only form after heating to above the xanthan conformational transition. This model is in keeping with more recent findings.[24,25]

This paper presents some recent work that we have undertaken to further elucidate the mechanism of interaction in xanthan–glucomannan mixtures. In addition to rheological and DSC experiments we have used the nitroxide spin label technique which is able to monitor the molecular motion of polymer chains in solution within the 10^{-7}–10^{-11} s time regime. In particular, using this technique, we have been able to follow the xanthan conformational change and KM association as a function of temperature and to correlate these processes with gelation. The paper also describes the influence of various cations on the interaction which is an aspect that has hitherto been ignored.

Experimental Section

Materials. A sample of hydroprocessed konjac mannan (KM) was supplied by Marine Colloids Division of FMC Corp. It was found to have an average molecular mass of 480 000 from laser light scattering studies and had an acetyl group on 1 out of every 17 sugar residues, determined using the procedure of Maekaji.[2] Analysis showed that the sample contained only trace amounts of metal ions (i.e., 4.3 × 10^{-6} mol of Na, 3.6 × 10^{-5} mol of K, 8.2 × 10^{-6} mol of Mg, and 1.8 × 10^{-5} mol of Ca per g of konjac) which for the purposes of our experiments could be neglected. The KM was spin-labeled with 4-amino-TEMPO through hydroxyl groups along the polymer chain as previously described.[22]

Xanthan was supplied by Kelco International Ltd. and was coded Keltrol T. After dialysis it was shown to contain 0.7 × 10^{-3}, 0.35 × 10^{-3}, <10^{-5}, and 0.17 × 10^{-3} mol/g of xanthan of K, Na, Mg, and Ca, respectively. The intrinsic viscosity was found to be 29.3 dL g^{-1} in 0.04 mol dm^{-3} NaCl at 25 °C as determined by capillary flow viscometry. The acetyl content was determined by back-titration.[2] It was found that 2 out of every 3 pentasaccharide repeat units contained an acetyl group. The pyruvate content was calculated using the method of Sloneker and Orentas,[26] and it was found that 1 in 3 xanthan repeat units were substituted.

Spin-labeled xanthan was prepared according to the method of Takagami et al.[27] by attaching the spin label through the carboxylic acid groups on the xanthan side chains. The xanthan was then dialyzed and freeze dried.

Rheological Measurements. All stock polymer solutions were prepared using doubly distilled water. In order to obtain reproducible results, the following procedure was adopted to prepare the mixed polymer solutions.

KM was added to water in a preweighed beaker heated in a water bath (95 °C) and mixed vigorously using an overhead stirrer for 2 h. The contents were corrected for water evaporation, and the covered beaker was left to cool overnight.

Xanthan solutions were prepared by heating in a water bath (80 °C) for 30 min before being allowed to cool to room temperature.

Xanthan–KM mixtures were prepared by mixing a 1:1 ratio of solutions of xanthan (0.6 wt %) and KM (0.6 wt %) to a total polysaccharide concentration of 0.6 wt %. To study the effect of various salts, the exact weight of solid was added to give an ionic strength of 0.04 mol dm^{-3} and the mixtures were heated in a water bath for 10 min to ensure homogeneity.

The storage and loss moduli (G' and G'') of xanthan and xanthan–KM mixtures in the presence of various electrolytes at a constant ionic strength of 0.04 mol dm^{-3} were monitored by small deformation oscillation measurements at 25 °C using a Carrimed controlled stress rheometer. Measurements were recorded using a 5 cm/2° steel cone and plate at a displacement amplitude of 6 mrad, a value known to be in the linear viscoelastic region.

G' was also measured as a function of temperature. Samples (1.8 cm^3) were loaded hot onto the preheated rheometer plate, and G' was determined on cooling from 75 to 25 °C in 5 °C steps. Five min of equilibration was allowed before the first oscillation measurement was performed, and each result was carried out in triplicate at each temperature. Measurements were made using a displacement amplitude of 6 mrad and a frequency of 3 Hz.

The effect of the temperature of mixing xanthan and KM was also investigated. Xanthan stock solution was prepared to a concentration of 0.6 wt % in 0.04 mol dm^{-3} NaCl by heating to ~80 °C for 30 min. A KM stock solution (0.6 wt %) was prepared as described previously and NaCl added to a concentration of 0.04 mol dm^{-3}. The solutions were left to cool to room temperature prior to use. Mixed samples of xanthan–KM were prepared by adding 1:1 ratios of the stock solutions at room temperature. The samples were mechanically shaken for 5 min and aliquots (1.8 cm^3) loaded onto the rheometer as before. G' was measured as a function of frequency at an amplitude of 6 mrad. A program was initiated to heat the samples successively to a number of preset temperatures at a steady rate prior to cooling to 25 °C where G' was subsequently determined as a function of frequency.

Differential Scanning Calorimetry (DSC). DSC measurements were made on xanthan and xanthan–KM mixtures using a Setaram Micro DSC equipped with 1 cm^3 batch vessels. Mixtures were prepared to a total polysaccharide concentration of 1.2 wt % with and without added electrolyte as for the rheological experiments.

The DSC heating and cooling curves were carried out at a scan rate of 1 °C min^{-1} over the temperature range 25–95 °C and the samples subjected to an initial heating and cooling cycle to ensure the same thermal history for each.

Electron Spin Resonance (ESR). Spin-labeled xanthan solutions were prepared to a total solids concentration of 1.2 wt % in water or electrolyte as above. The solutions were placed in a flat quartz cell, and spectra were recorded as a function of temperature using a Bruker 300E ESR spectrometer over the range 90–20 °C, with measurements at 5 °C intervals and allowing 5 min of equilibration between each temperature.

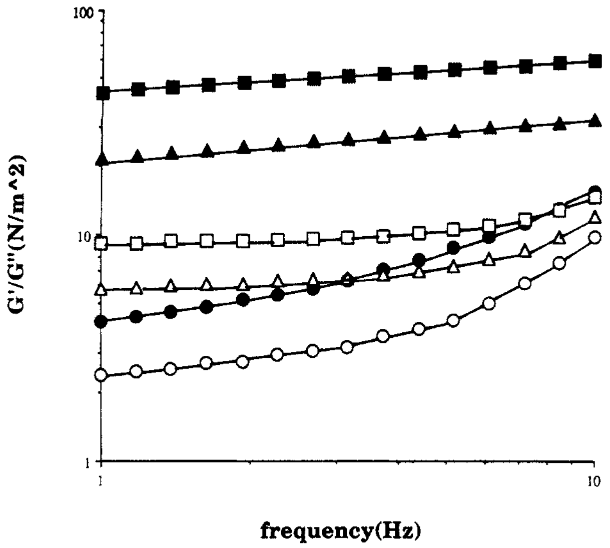

Figure 1. log/log plot of G' (filled symbols) and G'' (open symbols) as a function of frequency for xanthan solutions (0.6 wt %) in H_2O (●,○), 0.04 mol dm^{-3} NaCl (▲,△), and 0.02 mol dm^{-3} $CaCl_2$(■,□).

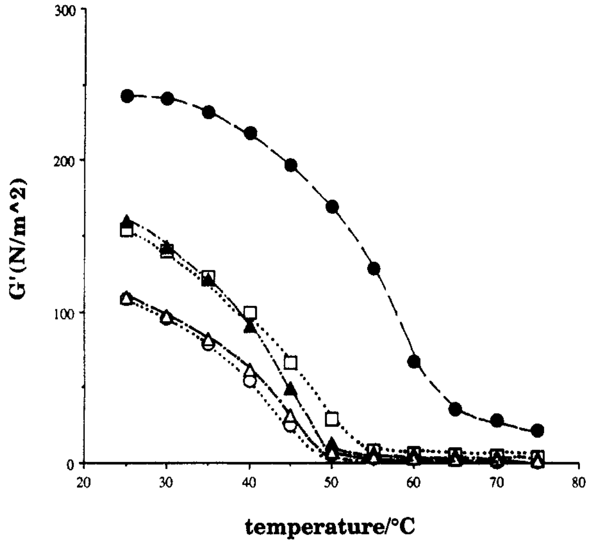

Figure 2. G' as a function of temperature for xanthan-KM mixtures (1:1, 0.6 wt %) in the presence and absence of monovalent cations (0.04 mol dm^{-3}): H_2O (●); NaCl (○); KCl (□); CsCl (▲); NH_4Cl (△).

Measurements were also performed in the same manner for xanthan-KM mixtures in which either the xanthan or the KM was spin-labeled.

Results

Rheology. G' and G'' for 0.6% xanthan solutions at 25 °C in both the presence and absence of electrolyte are shown in Figure 1. G' is seen to be greater than G'' over the entire frequency range studied. Values obtained decreased in the order divalent cations > monovalent cations > water. G' and G'' showed a weak frequency dependence which became less marked in the presence of electrolyte, indicating increased gel characteristics.

The values of G', obtained as a function of temperature, on cooling 1:1 mixtures of xanthan-KM in water and in the presence of monovalent cations are given in Figure 2 and in the presence of divalent cations in Figure 3. The onset of gelation, as noted by the sudden rise in G', occurs at a higher temperature in water than in the presence of electrolyte and is sensitive to the nature of the cation.

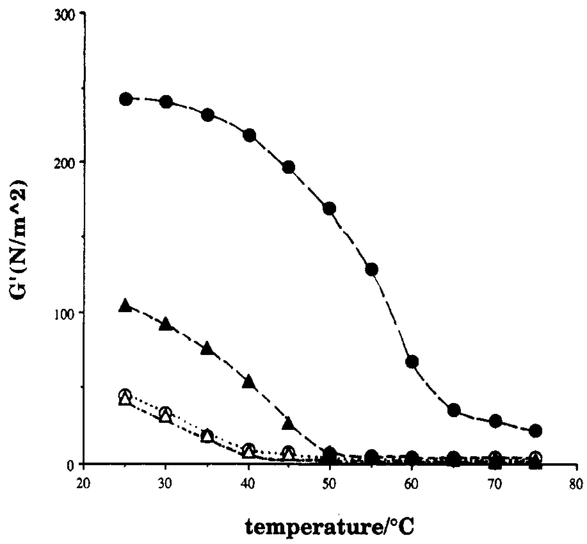

Figure 3. G' as a function of temperature for xanthan-KM mixtures (1:1, 0.6 wt %) in the presence and absence of divalent cations (0.02 mol dm^{-3}): H_2O (●); $BaCl_2$ (▲); $CaCl_2$ (○); $MgCl_2$ (△).

Table 1. Temperature for the Onset of Gelation of Xanthan-KM Mixtures in the Presence of Various Electrolytes

electrolyte	gelation temp/°C	electrolyte	gelation temp/°C
no electrolyte	65	NH_4Cl	50
KCl	54	$BaCl_2$	50
CsCl	53	$MgCl_2$	39
NaCl	50	$CaCl_2$	39

Gelation temperatures for the various systems are given in Table 1.

It is seen that gelation generally tends to occur at a higher temperature in the absence of electrolyte and at higher temperatures in the presence of monovalent cations compared to divalent cations, although Ba^{2+} appears to be something of an anomaly. Increasing the electrolyte concentration did not significantly change the gelation temperature. It is interesting to note that the maximum value obtained for G' (at 25 °C) is also very dependent on the nature of the electrolyte and shows the exact trend as for the gelation temperatures in that G' decreases in the order no electrolyte > $K^+ \sim Cs^+ > NH_4^+ \sim Na^+ \sim Ba^{2+} > Ca^{2+} \sim Mg^{2+}$.

G' for a 1:1 mixture of xanthan-KM in 0.04 mol dm^{-3} NaCl prepared at 25 °C and then heated to various temperatures are given as a function of frequency in Figure 4. It is seen that G' increases when the mixture is subjected to higher temperatures and becomes less frequency dependent, indicating increased gel characteristics.

Differential Scanning Calorimetry. The DSC cooling curves for xanthan and a 1:1 xanthan-KM mixture in water are given in Figure 5. The cooling curve of xanthan alone (curve a) shows an exothermic peak with a midpoint transition temperature, T_m, at 51 °C. This is attributed to the xanthan disorder → order conformational transition.[3] The actual temperature for the onset of the transition is difficult to establish accurately from the curve. The corresponding curve for the xanthan-KM mixture (curve b) shows an exothermic peak with T_m at 57 °C. The onset of the peak occurs at ~63 °C which corresponds to the onset of gelation as measured rheologically.

Figure 6 shows the DSC cooling curves for xanthan alone in the presence of NaCl, KCl, and NH_4Cl at a fixed concentration of 0.04 mol dm^{-3}. The exothermic peak corresponding to the xanthan conformational transition

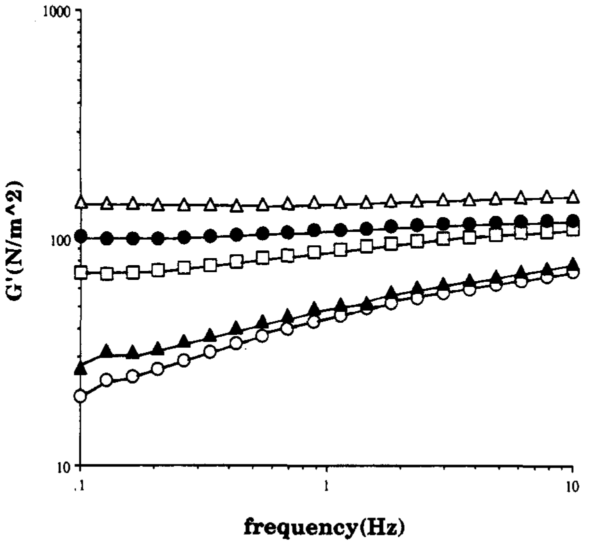

Figure 4. log/log plot of G' as a function of frequency for xanthan–KM (1:1) in NaCl (0.04 mol dm^{-3}). Data accumulated at 25 °C after heat treatment to the following temperatures: 25 (○); 35 (▲); 45 (□); 55 (●); 65 °C (△).

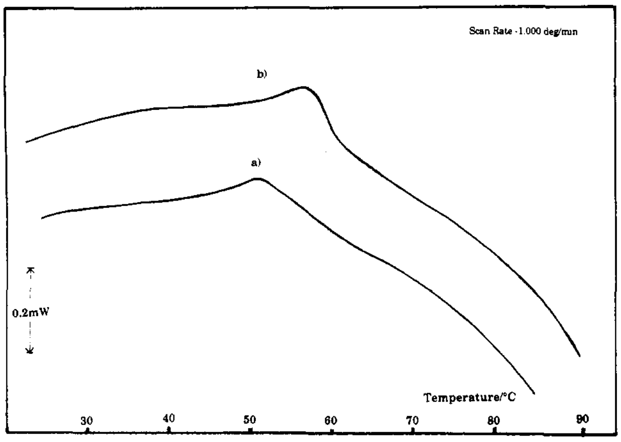

Figure 5. DSC cooling traces of (a) xanthan (1.2 wt %) in H_2O and (b) xanthan–KM (1:1, 1.2 wt % total polysaccharide) in H_2O.

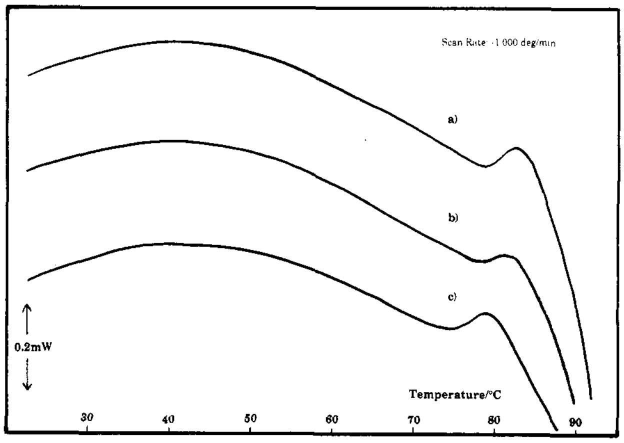

Figure 6. DSC cooling traces of xanthan (1.2 wt %) in monovalent electrolytes (0.04 mol dm^{-3}): (a) KCl; (b) NH_4Cl; (c) NaCl.

now occurs about 30 °C higher than for xanthan in water, showing an increase in the stability of the xanthan ordered conformation at higher temperatures. T_m for the transitions are 83, 82, and 80 °C respectively for the K^+, NH_4^+, and Na^+ salts.

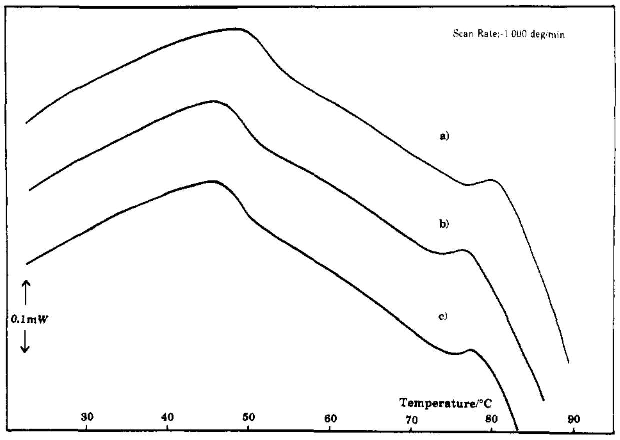

Figure 7. DSC cooling traces for xanthan–KM mixtures (1.2 wt %) in monovalent electrolytes (0.04 mol dm^{-3}): (a) KCl; (b) NH_4Cl; (c) NaCl.

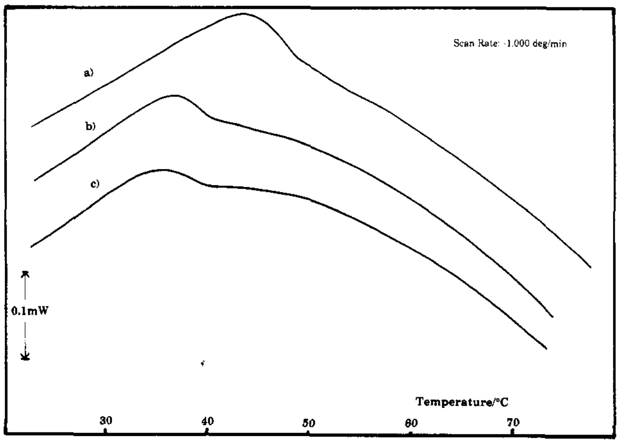

Figure 8. DSC cooling traces for xanthan–KM mixtures (1.2 wt %) in divalent electrolytes (0.02 mol dm^{-3}): (a) $BaCl_2$; (b) $MgCl_2$; (c) $CaCl_2$.

The DSC cooling curves for xanthan–KM mixtures, in the presence of the same monovalent cations, are given in Figure 7 and show two exothermic peaks. The higher temperature peaks at $T_m \sim 80$ °C correspond closely to the xanthan conformational transition (Figure 6), and the lower temperature peaks correspond to the temperature of gelation monitored rheologically (Table 1). The differences in gelation temperature between the monovalent electrolytes is small but still follows the trend $K^+ > Na^+ \sim NH_4^+$ as noted from the rheological measurements.

The DSC cooling curves for xanthan–KM mixtures in the presence of divalent cations are given in Figure 8 and show only one exothermic peak. The onset temperature corresponds very closely to the gelation temperature found rheologically with $Ba^{2+} > Mg^{2+} \sim Ca^{2+}$ (Table 1). The conformational transition of xanthan in the presence of such concentrations of divalent cations is above 100 °C,[6] and hence an exothermic peak is not expected.

ESR Spectroscopy. The ESR spectra of spin-labeled xanthan alone in water are shown in Figure 9 at various temperatures. At high temperatures (spectra c and d) the spectra are essentially isotropic, and since the spin label is attached to the xanthan side chains rather than the backbone, it indicates a high degree of xanthan side-chain mobility. At lower temperatures (spectra a and b) composite spectra containing a significant proportion of an anisotropic component are observed. The presence of the anisotropic component indicates a substantial loss of the xanthan side-chain mobility and is in keeping with the concept that the side chains associate with the xanthan cellulosic backbone on forming the ordered structure.[5,6] The spectra were resolved into two components by computer analysis, and these are presented in Figure 10.

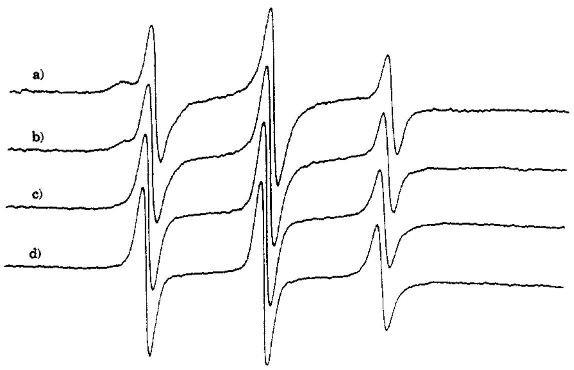

Figure 9. ESR spectra of spin-labeled xanthan (1.2 wt %) in H_2O at various temperatures: (a) 30 °C; (b) 50 °C; (c) 70 °C; (d) 90 °C.

Figure 10. Computer manipulation of composite spectrum (a) into two extreme components: (b) isotropic, mobile spectrum; (c) anisotropic, immobile spectrum.

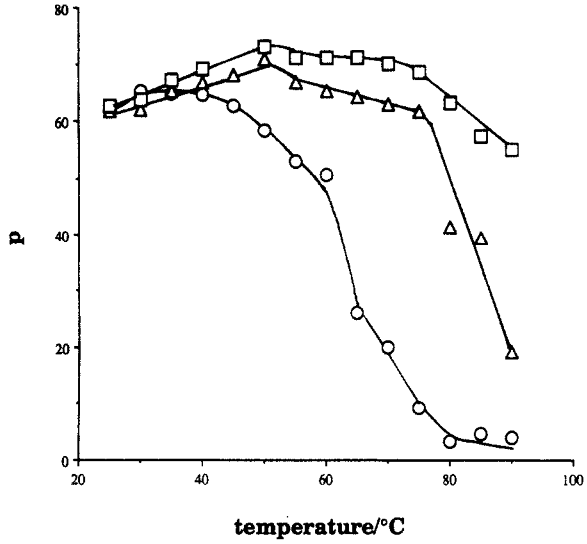

Figure 11. p as a function of temperature for xanthan solutions in the presence and absence of electrolyte; H_2O (○); 0.04 mol dm^{-3} NaCl (△); 0.02 mol dm^{-3} $CaCl_2$ (□).

The rotational correlation times of the isotropic and anisotropic components were calculated to be ~7 × 10^{-10} and 8 × 10^{-9} s, respectively.[28,29] The proportion of anisotropic component, p, is plotted as a function of temperature in Figure 11 (circles) and is seen to increase rapidly on cooling to temperatures below ~80 °C, reaching a maximum at ~50 °C, indicating, therefore, that the xanthan molecules are ordering over this broad temperature range. As noted earlier it was not possible to establish the onset temperature of the conformational transition from the DSC data, and so direct comparison between the two techniques cannot be made. However, the T_m obtained from DSC corresponds closely to the temperature where p reaches its maximum (~50 °C). At this temperature, p represents about 60–65% of the total signal, indicating that this proportion of the side chains has restricted mobility. Figure 11 also shows the values obtained for p for xanthan in the presence of 0.04 mol dm^{-3} NaCl (triangles) and 0.02 mol dm^{-3} $CaCl_2$ (squares). It is readily seen that, in the presence of 0.04 mol dm^{-3} NaCl, the conformational change occurs over a narrower temperature range compared to water, with p starting to increase on cooling from below 90 °C, and reaches a maximum at ~80 °C which corresponds to the T_m of the conformational transition obtained from DSC measurements. For the xanthan–$CaCl_2$ system, p was high over the whole temperature range, indicating an ordered conformation and supporting the view that the xanthan conformational transition is >100 °C.

Figure 12 shows the variation of p for spin-labeled xanthan in the presence of KM as a function of temperature. For mixtures in the absence of electrolyte (Figure 12a) it is evident that p starts to increase at slightly higher temperatures compared to solutions of xanthan alone, indicating that the xanthan ordering process has shifted to slightly higher temperatures. In the presence of 0.04 mol dm^{-3} NaCl (Figure 12b) the variation of p with temperature is the same for xanthan alone and in admixture with KM, indicating that the ordering process is not affected by the presence of the KM. For solutions containing $CaCl_2$ (Figure 12c), p was high in both the presence and absence of KM, indicating that the xanthan chains are always ordered over this temperature range.

ESR spectra obtained using spin-labeled KM in a 1:1 admixture with non-spin-labeled xanthan in water are shown as a function of temperature in Figure 13. At high temperatures (Figure 13c,d), the spectra are essentially isotropic, but at lower temperatures (spectra a and b) an anisotropic component becomes evident. The values of p obtained on cooling are given as a function of temperature in Figure 14, and it is noted that p starts to increase below ~65 °C which corresponds closely to the temperature noted for the onset of gelation. As KM itself does not undergo a conformational transition, the increase in the proportion of the anisotropic component is believed to be a result of the association between KM and xanthan chains, resulting in a decrease of KM mobility in solution. Although it is possible that the decrease in mobility may occur from KM self-aggregation, this is believed not to be the case since the process is completely reversible and KM disaggregation is usually very difficult.[22] Also, no anisotropy is detected on cooling solutions of KM alone.

The ESR spectra obtained for spin-labeled KM in KM–xanthan mixtures at different ratios in water are shown in Figure 15, and the values for p, for the same systems in both water and electrolyte, are presented in Table 2.

As the ratio of KM in the mixture decreases, the value of p increases, indicating a greater proportion of the KM chain segments are interacting. The corresponding data for mixtures in the presence of 0.04 mol dm^{-3} NaCl are also reported and show that p is lower than in water, indicating that a lower proportion of KM chain segments are interacting.

Discussion

In the studies on solutions of xanthan only, the presence of the anisotropic component in the ESR spectra at low temperatures for spin-labeled xanthan (Figure 11) provides

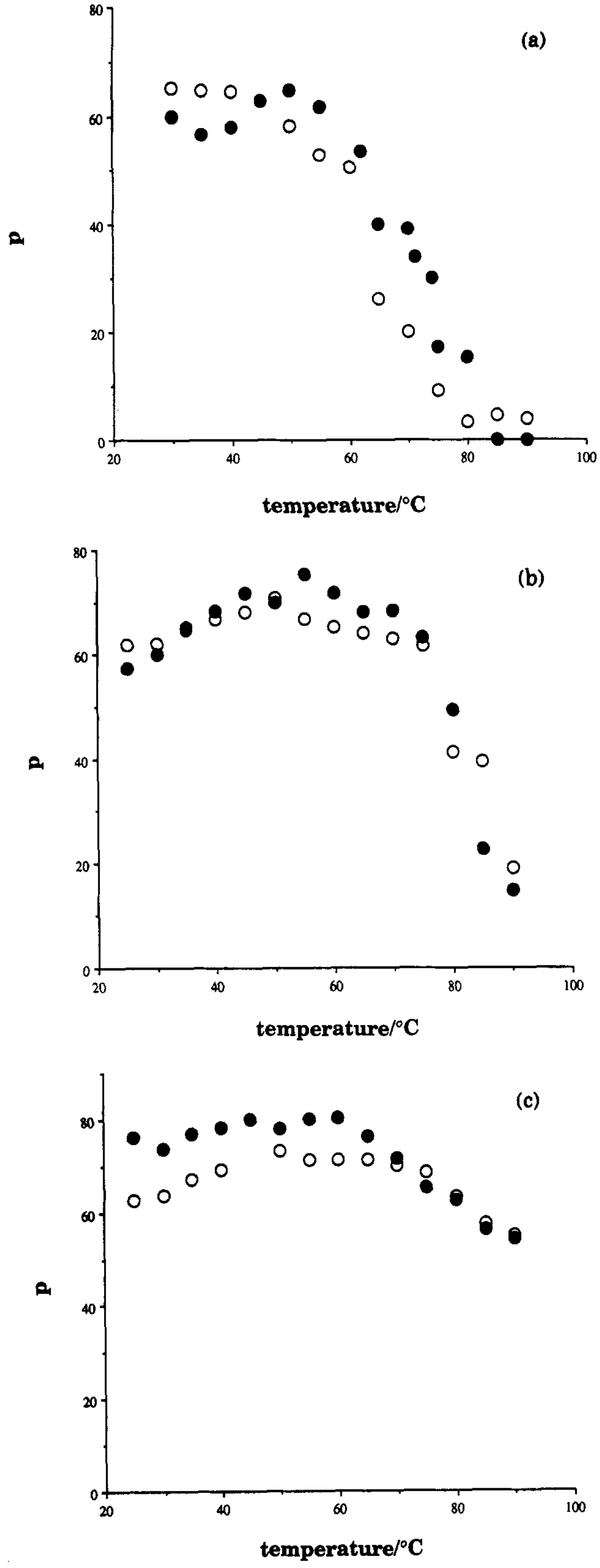

Figure 12. (a) p as a function of temperature for xanthan (○) and xanthan–KM (●) in the absence of electrolyte. (b) p as a function of temperature for xanthan (○) and xanthan–KM (●) in NaCl (0.04 mol dm^{-3}). (c) p as a function of temperature for xanthan (○) and xanthan–KM (●) in $CaCl_2$ (0.02 mol dm^{-3}).

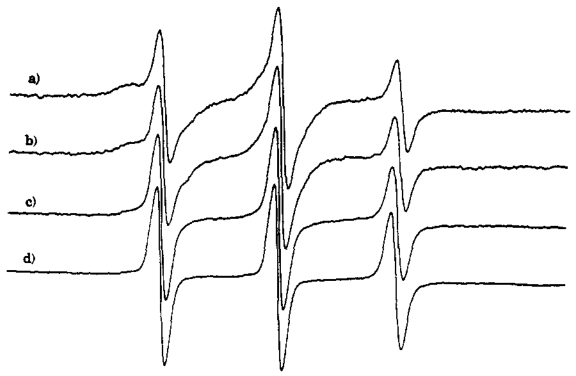

Figure 13. ESR spectra of spin-labeled KM–xanthan (2:3, 0.44 wt %) in H_2O as a function of temperature: (a) 25; (b) 40; (c) 55; (d) 70 °C.

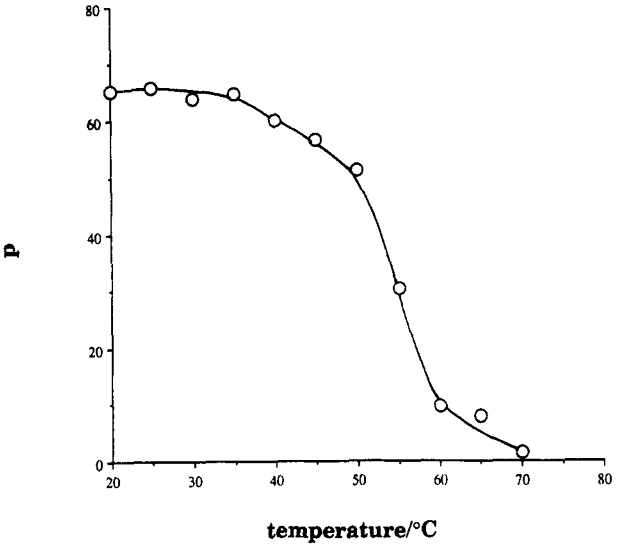

Figure 14. p as a function of temperature for spin-labeled KM in an admixture with xanthan (1:1, 0.44 wt %) in the absence of electrolyte.

Figure 15. ESR spectra of spin-labeled KM–xanthan mixtures at 25 °C at different KM–xanthan mixing ratios: (a) 100:0; (b) 50:50; (c) 40:60; (d) 10:90.

direct evidence that the side chains associate with the backbone during the ordering process in keeping with current models. A more detailed discussion has been reported on this aspect previously.[27] Since the xanthan has been subjected to heat and low ionic strength, the ordered form is expected to be denatured compared to the native material, corresponding to form II as described by Milas and Rinaudo.[6] The loss in side-chain mobility is similar for xanthan in water and electrolyte, suggesting that the same proportion of side chains associate (i.e., ~60–70%).

Our experiments have shown that the temperature of the xanthan conformational change is sensitive to both the presence and nature of the added electrolyte and this behavior is typical of many macromolecules.[30] The effectiveness of the various ions to induce the transition,

Table 2. p for Spin-Labeled KM as a Function of KM–Xanthan Mixing Ratio at 25 °C in Water and in the Presence of 0.04 mol dm^{-3} NaCl

KM:xanthan mixing ratio	p	
	H_2O	NaCl
10:90	98	75
40:60	67	32
50:50	62	28
100:0	0	0

i.e., $Ca^{2+} \sim Mg^{2+} \sim Ba^{2+} \gg K^+ > NH_4^+ > Na^+$, is in accordance with the so-called lyotropic series. This is believed to arise as a consequence of the adsorption or exclusion of the ions from the polymer–water interface and their influence on the interfacial energy.[31] In general, ordered helical structures are favored by nonadsorbing (salting out) ions, while denatured structures are favored by adsorbing (salting in) ions.

For xanthan–KM mixtures in the absence of electrolyte, we have observed that the conformational transition of the spin-labeled xanthan molecules as monitored by ESR shifted to a slightly higher temperature compared to xanthan alone. This is consistent with earlier observations of Dea et al.,[12] who studied xanthan–galactomannan systems using optical rotation. In the ESR experiments using mixtures of spin-labeled KM and xanthan in water (Figure 13) we observed that KM association (i.e., the appearance of the anisotropic component) only occurred below ~65 °C. This corresponds closely to the temperature of gelation (Figure 2) and is lower than the temperature (<80 °C) at which ESR indicates that association of xanthan side chains with the backbone begins. These experiments follow both the gelation process and the xanthan conformational change as a function of temperature and clearly demonstrate that association only occurs after xanthan chain ordering. The proportion of xanthan side chains immobilized after ordering is shown to be similar for both xanthan alone and xanthan–KM in water, indicating that the KM has little effect on the xanthan ordering process. The proportion of KM chain segments which associate with the xanthan molecules decreases as the proportion of KM in the mixture increases (Table 2 and Figure 15). This implies that at higher KM concentrations all of the available xanthan interaction sites are occupied and that the "excess" KM molecules may not interact but are free in solution. This is consistent with the rheological and DSC data we reported previously[21,22] and that of Goycoolea et al.[25] which shows that G' or the enthalpy of gelation does not increase above a 1:1 mixing ratio.

For xanthan–KM systems containing electrolyte it is clearly evident from both the DSC and ESR data that interaction also occurs after the xanthan chains have ordered. The xanthan coil → helix transition occurs at ~80 °C in the presence of the monovalent cations and above 100 °C in the presence of the divalent cations, while gelation occurs at 53 °C or less, depending on the electrolyte present. As noted from ESR studies, the proportion of KM segments interacting (Table 2) increases as the KM content in the mixture decreases but is less in the presence of electrolyte (0.04 mol dm^{-3} NaCl) compared to water. The fact that reduced interaction occurs when electrolyte is present implies that there are fewer interaction sites.

Thus interaction of xanthan and KM occurs in both the presence and absence of electrolyte only after ordering of the xanthan chains. As far as we are aware there have not been any reports published to date which show that xanthan interacts with KM (or galactomannans) when *fully* disordered, i.e., above the helix to coil transition temperature, and hence the suggestion that interaction involves purely disordered regions present within predominantly ordered xanthan chains[23-25] is far from convincing. Of greater significance is that this explanation also assumes it is a complete coincidence that the association of xanthan and KM in the absence of electrolyte occurs immediately following xanthan backbone–side chain association. It is difficult to give a precise description of the interaction because of the uncertainty in the nature of the xanthan ordered and disordered structures; however, we propose the following scheme of events. On cooling, the xanthan molecules are thermodynamically driven to adopt a predominantly ordered structure. The side chains which are directed away from the xanthan cellulosic main chain of the disordered xanthan molecules (single coils or expanded dimers) begin to associate with the backbone, thus reducing polymer–solvent contacts and giving rise to chain stiffening. In the absence of KM (or galactomannans) this leads to either double-helix formation or xanthan self-association, whereas in the presence of KM ordered and/or disordered sequences within the xanthan molecules prefer to interact with KM chains rather than other xanthan chains. One of the most likely reasons why this is the case is that KM is uncharged, while xanthan is highly charged. The addition of electrolyte serves to promote xanthan/xanthan association at the expense of xanthan/KM association by a charge screening effect. The cations which are most effective at promoting xanthan ordering (and promote association of the xanthan chains) give rise to the weakest interaction with KM as noted by the lower values for G' and gelation temperature. There is a strong resemblance between the interaction of KM with xanthan, in the absence of electrolyte, and KM/κ-carrageenan interaction. We have recently presented data indicating that KM also shifts the conformational transition of carrageenan to a slightly higher temperature and that association occurs immediately following the ordering process.[32]

The rheological data are also consistent with this concept. Whereas G' for xanthan in solution alone is enhanced by the presence of monovalent cations and to an even greater extent divalent cations in agreement with the earlier findings of Tako et al.,[33] the exact reverse is true for xanthan–KM mixtures. This arises from the fact that the presence of electrolyte leads to an increase in xanthan ordering and self-association[6] (more so for divalent cations than monovalent cations) at the expense of xanthan/KM interaction. Furthermore, in the heating experiments (Figure 4) in 0.04 mol dm^{-3} NaCl the conformational transition from the ordered to disordered structure occurs at ~80 °C. For the mixtures prepared at 25 °C, therefore, the KM must interact with xanthan molecules present in a predominantly ordered form. Nevertheless, interaction with KM is enhanced by heating to temperatures well below the helix → coil transition and this is explained by the fact that heating disrupts xanthan self-association, thus making the chains more accessible to interaction on raising the temperature.

In conclusion, our results indicate that xanthan/KM interaction occurs only following association of the xanthan side chains and backbone and the driving force is to reduce xanthan–water contacts. The interaction may involve ordered and/or disordered xanthan sequences. The rheological properties and gelation temperatures of mixed xanthan–KM gels decreases in the presence of electrolyte because xanthan self-association is promoted at the expense of xanthan–KM association. Divalent cations have a greater influence than monovalent cations because they are more effective at promoting xanthan ordering/aggregation.

References and Notes

(1) Nishinari, K.; Williams, P. A.; Phillips, G. O. *Food Hydrocolloids* **1992**, *6*, 199.
(2) Maekaji, K. *Agric. Biol. Chem.* **1974**, *38*, 315.
(3) Holzwarth, G. *Biochemistry* **1976**, *15*, 4333.
(4) Holzwarth, G. *Carbohydr. Res.* **1978**, *66*, 173.
(5) Norton, I. T.; Goodall, D. M.; Frangou, S. A.; Morris, E. R.; Rees, D. A. *J. Mol. Biol.* **1984**, *175*, 371.
(6) Milas, M.; Rinaudo, M. *Carbohydr. Res.* **1986**, *156*, 191.
(7) Liu, W.; Norisuye, T. *Int. J. Biol. Macromol.* **1988**, *10*, 44.
(8) Paradossi, G.; Brant, D. A. *Macromolecules* **1982**, *15*, 874.
(9) Jones, S. A.; Goodall, D. A.; Cutler, A. N.; Norton, I. T. *Eur. Biophys. J.* **1987**, *15*, 185.
(10) Kawakami, K.; Okabe, Y.; Norisuye, T. *Carbohydr. Polym.* **1991**, *14*, 189.
(11) Gamini, A.; de Bleijser, J.; Leyte, J. C. *Carbohydr. Res.* **1991**, *220*, 33.
(12) Dea, I. C. M.; Morris, E. R.; Rees, D. A.; Welsh, E. J. *Carbohydr. Res.* **1977**, *57*, 249.
(13) McCleary, B. V. *Carbohydr. Res.* **1979**, *71*, 205.
(14) Cairns, P.; Miles, M. J.; Morris, V. J. *Nature* **1986**, *322*, 89.
(15) Brownsey, G. J.; Cairns, P.; Miles, M. J.; Morris, V. J. *Carbohydr. Res.* **1988**, *176*, 329.
(16) Tako, M.; Asato, A.; Nakamura, S. *Agric. Biol. Chem.* **1984**, *48*, 2995.
(17) Tako, M. *J. Carbohydr. Chem.* **1991**, *10*, 619.
(18) Shatwell, K. P.; Sutherland, I. W.; Ross-Murphy, S. B.; Dea, I. C. M. *Carbohydr. Polym.* **1991**, *14*, 29.
(19) Shatwell, K. P.; Sutherland, I. W.; Ross-Murphy, S. B.; Dea, I. C. M. *Carbohydr. Polym.* **1991**, *14*, 131.
(20) Lopes, L.; Andrade, C. T.; Milas, M.; Rinaudo, M. *Carbohydr. Polym.* **1992**, *17*, 121.
(21) Williams, P. A.; Day, D. H.; Langdon, M. J.; Phillips, G. O.; Nishinari, K. *Food Hydrocolloids* **1991**, *4*, 4891.
(22) Williams, P. A.; Clegg, S. M.; Day, D. H.; Phillips, G. O. In *Food Polymers, Gels and Colloids*; Dickinson, E., Ed.; R.S.C. Publication No. 82; Royal Society of Chemistry: Herts, U.K., 1991; p 339.
(23) Cheetham, N. W. H.; Mashimba, E. N. M. *Carbohydr. Polym.* **1988**, *9*, 195.
(24) Zhan, D. F.; Ridout, M. J.; Brownsey, G. J.; Morris, V. J. *Carbohydr. Polym.* **1993**, *21*, 53.
(25) Goycoolea, F. M.; Foster, T. J.; Richardson, R. K.; Morris, E. R.; Gidley, M. J. In *Gums and Stabilisers for the Food Industry 7*; Phillips, G. O., Williams, P. A., Wedlock, D. J., Eds.; Oxford University Press: Oxford, U.K., 1994; pp 333–344.
(26) Sloneker, J. H.; Orentas, D. G. *Nature* **1962**, *194*, 4827.
(27) Takigami, S.; Shimada, M.; Williams, P. A.; Phillips, G. O. *Int. J. Biol. Macromol.* **1993**, *15*, 367.
(28) Zhao, F.; Rosen, M. T.; Yong, N.-L. *Colloids Surf.* **1984**, *11*, 97.
(29) Freed, J. H. In *Molecular Biology Spin Labelling Theory and Applications*; Berliner, L. J., Ed.; Academic Press: London, 1976.
(30) von Hippel, P. H.; Scheich, T. In *Structure and Stability of Biological Macromolecules*; Timasheff, S. N., Fasman, G. D., Eds.; Marcel Dekker: New York, 1969; p 417.
(31) Piculell, L.; Nilsson, S. *Prog. Colloid Polym. Sci.* **1990**, *82*, 198.
(32) Williams, P. A.; Clegg, S. M.; Langdon, M. J.; Nishinari, K.; Piculell, L. *Macromolecules* **1993**, *26*, 5441.
(33) Tako, M.; Nagahama, T.; Nomura, D. *Nippon Nogei Kagaku Kaishi* **1977**, *51*, 513.