Conformational Role of Xanthan in its Interaction with Locust Bean Gum

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ABSTRACT: Texture analysis and Ubbelohde capillary viscometry were used to investigate the effects of chain conformational changes of xanthan or deacetylated xanthan on its interaction with locust bean gum (LBG). The stability of xanthan helical structure or xanthan chain flexibility played a critical role in interacting with LBG. Destabilization of xanthan helical structure by deacetylation and heating facilitated the intermolecular binding between xanthan and LBG. Xanthan conformational change was noted in the presence of LBG, even when its helical structure was stabilized by salt. However, this conformational change was not observed for deacetylated xanthan, presuming deacetylated xanthan was in the exact conformation to bind LBG.

Keywords: xanthan, locust bean gum, synergistic interaction, intermolecular binding, conformational change

Introduction

THE SYNERGISTIC INTERACTION IN VISCOSITY BETWEEN XANTHAN gum and galactomannans (guar gum and locust bean gum) was first reported by Rocks (1971), who stated that xanthan gum formed thermally reversible gels with locust bean gum (LBG) but not with guar gum. Since then, much effort has been made to elucidate this synergistic interaction. At present, a considerable body of evidence supports an intermolecular binding between xanthan and galactomannans (Morris 1996), although some researchers described the interaction as incompatibility (Kovacs 1973; Doublier and others 1993; Schorsch and others 1995). Nevertheless, this intermolecular binding mechanism is still under debate. Early work suggested that a specific interaction occurred between the ordered xanthan molecule and the galactomannan chain (Dea and Morrison 1975; Dea and others 1977; Morris and others 1977; McCleary 1979). This interaction depended on the mannose/galactose ratio, as well as on the fine structure of the galactomannan. Tako and others (1984), Tako and Nakamura (1985), and Tako (1991) reported that the intermolecular interaction between xanthan and galactomannans occurred between the side chains of xanthan and the backbone of galactomannans, as in a lock-and-key model. Cairns and co-authors (1986, 1987), by means of X-ray fiber diffraction, suggested that gelation occurred only if xanthan was first denatured by heating above the order-disorder transition temperature and the interaction occurred between the xanthan backbone in an extended 2-fold cellulose-like conformation and LBG in a similar 2-fold conformation. Recent evidence strongly suggested that destabilization of the xanthan helix facilitated xanthan and galactomannan binding (Cheetham and Mashimba 1988, 1991; Zhan and others 1993; Foster and Morris 1994; Goycoolea and others 1994). It has been shown that gelation could occur when xanthan and LBG were mixed at temperatures below the xanthan helix-coil transition (Williams and others 1991; Mannion and others 1992; Foster and Morris 1994; Goycoolea and others 1994); the melting temperatures of the mixed gels were independent of ionic strength (Zhan and others 1993) and xanthan conformation (Goycoolea and others 1994); the modulus of the mixed gels increased with increasing disorder of the xanthan helix (Zhan and others 1993). Hence, it has been suggested that galactomannan acts like a denaturant to disturb the helix-coil equilibrium of xanthan and to displace the ordered conformation of xanthan to the conformation required for efficient binding to it within the heterotypic junctions (Zhan and others 1993; Goycoolea and others 1994; Morris and others 1994; Morris ER 1996; Morris VJ 1996).

It is well established that the stability of xanthan-ordered conformation (helix) in aqueous solution increases in the order of acetate-free xanthan < native xanthan < pyruvate-free xanthan (Holzwarth 1976; Smith and others 1981; Crescenzi and others 1986; Foster and Morris 1994); thus acetate groups stabilize the ordered conformation, whereas pyruvate groups reduce the thermal stability of the ordered state. In this article, xanthan samples with different stability of ordered structures, including native and deacetylated xanthan in aqueous solution or in 0.04 *M* NaCl solution, were used to investigate how the stability of xanthan-ordered structure influenced its interaction with LBG.

Materials and Methods

Materials

Xanthan gum and LBG were obtained from Kelco (Chicago, Ill., U.S.A.) and Gumix International, Inc. (Fort Lee, N.J., U.S.A.), respectively. Xanthan gum was used without further purification.

Locust bean gum purification

Two grams of LBG was dispersed in 1000-mL deionized water, heated at 90 °C with stirring for 30 min, cooled down, and then centrifuged at 3800 × *g*. After the supernatant was filtered through a 5-μm membrane, 1000 mL of ethanol was added to the filtrate to precipitate the LBG. The purified LBG was recovered by centrifugation and ready for solution preparation after the ethanol was completely evaporated under a hood.

Preparation of deacetylated xanthan

This sample was prepared by first dissolving the native xanthan (0.2%) in water and then adding KOH and KCl (previously dissolved in a small amount of water) to obtain a solution of 0.025 *M* KOH and 0.1% KCl with stirring at room temperature for 2 h under nitrogen (Holzwarth and Ogletree 1979). The solution was neutralized with 0.05 *M* HCl, and then a 2-fold volume of ethanol was added. The precipitate was washed with ethanol/

Table 1—Structural parameters and intrinsic viscosity of native xanthan, deacetylated xanthan, and locust bean gum

				Intrinsic viscosity (dL/g)	
Sample	**Pyruvate % (w/w)**	**Acetate % (w/w)**	**Molecular weight**	**In water**	**In 0.04 *M* NaCl**
Native xanthan	0.24	5.14	1143000	75.2	16.9
Deacetylated xanthan	0.12	0.15	1025000	43.3	–
LBG	-	-	1409000	7.6	8.0

deionized water (80/20, v/v) until no chloride was detected in the washing solvent by 1% silver nitrate. The sample was ready for solution preparation after the ethanol was completely evaporated.

Preparation of solutions for Ubbelohde viscometry measurement

Approximately 0.10 to 0.15 g of native xanthan, deacetylated xanthan, or LBG from the above procedures was dispersed in 100 mL of deionized water, stirred, and heated at 90 °C with stirring for 30 min. After cooling and filtration through a 5-μm membrane, 0.05 g of sodium azide was added to prevent microbial growth. For the study of salt effect on the interaction, an appropriate amount of NaCl was added and completely dissolved to make 0.04 *M* NaCl solutions. The concentration of each solution was determined by the phenol-sulfuric acid method (Dubois and others 1956).

Blends of different ratios (20:80, 40:60, 60:40, and 80:20, w/w, dry basis) of native xanthan or deacetylated xanthan to LBG were prepared by pipetting the calculated volume of native xanthan or deacetylated xanthan and LBG solutions obtained above, respectively, and mixing them by stirring at room temperature for 2 min.

Determination of acetyl and pyruvate contents of native xanthan or deacetylated xanthan

The acetyl and pyruvate contents of xanthan were measured by following the methods of McComb and McCready (1957) and Sloneker and Orentas (1962), respectively.

Molecular weight determination of native xanthan, deacetylated xanthan, and locust bean gum by gel permeation chromatography

The gel permeation chromatography (GPC) system consisted of 2 peristaltic pumps with low flow rate (0.03 to 8.2 mL/min, VWR Scientific Products Corp.), a fraction collector (RediFrac, Amersham Pharmacia Biotech, Piscataway, N.J., U.S.A.), and a 1000 × 26-mm column packed with Sepharose CL-6B gel (Amersham Pharmacia Biotech). The eluent was 0.2% (w/v) sodium chloride and 0.1% (w/v) sodium azide at a rate of 0.56 mL/min. The concentration of native xanthan, deacetylated xanthan and LBG solutions was 0.05% (w/v). Dextran standards, ranging from 9900 to 695000 of peak molecular weight, and blue dextrin, peak molecular weight of 2000000 (PSS Polymer Standards Service-USA, Inc., Silver Spring, Md., U.S.A.), were used to construct the regression line for molecular weight determination. The concentration of the standards was 0.1% (w/v). The phenol-sulfuric acid method (Dubois and others 1956) was used to measure the concentration of polysaccharide in the eluent.

Gel strength analysis with a texture analyzer

The solution of native xanthan, deacetylated xanthan, or LBG (about 0.3%, w/v) was prepared by dissolving an appropriate amount of native xanthan, deacetylated xanthan, or LBG in deionized water with stirring and heating above 90 °C for 30 min. After cooling, the sample was centrifuged at 3800 × g for 60 min to remove the insoluble particles and its concentration was determined by the phenol-sulfuric acid method (Dubois and others 1956). The solutions were then diluted to 0.2% (w/v) accordingly, and NaCl was added, if necessary, to make the final concentration of the solution 0.04 *M* NaCl. The cold mixed blends (20:80, 40:60, 60:40, and 80:20, v/v, dry basis) were prepared by mixing appropriate amounts of xanthan and LBG solutions at room temperature and stirring for 2 min. The hot mixed blends were prepared by mixing appropriate amounts of reheated xanthan and LBG solutions (above 90 °C) and stirring for 2 min.

For blends with gel formation, the gel strength measurements were carried out at room temperature using a TA-XT2i texture analyzer (Texture Technologies, Scarsdale, N.Y., U.S.A.) equipped with a PC-compatible Texture Expert software (version 1.22, Stable Micro Systems, Ltd., London, UK). Penetration measurements were carried out at a speed of pre-test 3.0 mm/s, test 1.0 mm/s, to a penetration distance of 3.0 mm with a 2.54-mm-dia cylindrical probe. The gel strength was defined as the work (g·s) required to penetrate the gel and calculated from the area under the texture profile.

Viscosity measurement with an Ubbelohde viscometer

The viscosity of native xanthan solution, deacetylated xanthan solution, LBG solution, and blends of native xanthan- or deacetylated xanthan-LBG solution with different ratios in either water or 0.04 *M* NaCl were measured by using an Ubbelohde capillary viscometer (Size 1, Constant = 0.01055, Technical Glass Products, Inc., Dover, N.J., U.S.A.) immersed in a water bath maintained at 30.0 ± 0.1 °C. Each concentration was measured at least in duplicate.

The concentration dependence of the viscosity of each polymer solution and their blends was analyzed by using the classical Huggins equation: $\eta_{sp}/C = [\eta] + bC$, where η_{sp} is the specific viscosity, $[\eta]$ is the intrinsic viscosity, and b is the Huggins parameter. For each concentration, the specific viscosity was determined using the equation $\eta_{sp} = (\eta - \eta s)/\eta s$, where h is the solution viscosity and η_s is the solvent viscosity. The relationships of η_{sp}/C and concentration were assessed by using the nonlinear fitting of the statistics software JMP for SAS (1999).

Results and Discussion

Characterization of stability of xanthan-ordered structure

Table 1 summarizes the structural parameters of native xanthan, deacetylated xanthan, and LBG. LBG had a larger molecular weight (1409000) than xanthan (1143000) and deacetylated xanthan (1025000). Deacetylation clearly reduced the molecular weight of xanthan. The initial concentration of xanthan bearing

sodium ions was 0.10524 g/dL and the acetate and pyruvate contents of xanthan were 5.14 and 0.24%, respectively. Therefore, there were 0.00541 g or 0.000126 mole acetyl and 0.000253 g pyruvic acid or 0.000000337 mole in the xanthan sample. Assuming that x of the inner mannose units and y of the terminal mannose units of the side chain bore an acetyl group and a pyruvic acid group, respectively, the molecular weight of the repeating unit of xanthan was 846 + 42x + 92y and the number (N) of the repeat units was 0.10524/(846 + 42x + 92y). Because xN and yN were 0.000126 mole and 0.00000337 mole, respectively, x was calculated to be 1.07, which was more than 1 because of measurement errors, and y was 0.029. This indicates that 100% of the inner mannoses bore the acetyl groups and 2.9% of the terminal mannoses bore the pyruvate groups. Deacetylation removed approximately 97% of the acetyl groups and 50% of the pyruvate groups.

The intrinsic viscosity of native xanthan in 0.04 *M* NaCl was 16.9 dL/g, which was well below that in the aqueous solution of 75.2 dL/g. This behavior was typical of polyelectrolytes. The electrostatic shielding of the counter ions from NaCl to the charges in xanthan chain affected the contraction of xanthan chain, resulting in a significantly smaller hydrodynamic radius at a high ionic strength. In contrast, LBG, a non-polyelectrolyte, had intrinsic viscosities in aqueous solution and 0.04 *M* NaCl of 7.6 and 8.0 dL/g, respectively. Salt exerted little effect on its intrinsic viscosity.

The intrinsic viscosity of deacetylated xanthan was 43.3 dL/g, about 42% of native xanthan. The molecular weight of deacetylated xanthan is 91% of that of native xanthan. The relationship between molecular weight and intrinsic viscosity can be characterized by the Mark-Houwink equation: $[\eta] = K'M^{\alpha}$, where M is the molecular weight and K' and α are constants depending on both the polymer and the solvent (Flory 1953). The value of α does not fall below 0.5 in any case and seldom exceeds 0.8. Exceptions occur in the case of polyelectrolytes where, in the absence of added salts, α may approach 2 (Flory 1953). Assuming that α ranged from 0.5 to 2, if xanthan had the same molecular weight as the deacetylated xanthan (1025000), native xanthan should have an intrinsic viscosity of 61 to 71 dL/g, based on the equation, which was much higher than that of deacetylated xanthan. Since the intrinsic viscosity of a polymer is proportional to its chain dimension for a constant molecular weight (Flory 1953), a reduction in chain dimension will decrease its intrinsic viscosity. Removing the acetyl groups destabilized the xanthan helix and rendered the deacetylated xanthan chain more flexible, thus resulting in a decrease of chain dimension. Therefore, the flexibility of xanthan chain increased in the order of native xanthan in salt solution < native xanthan < acetate-free xanthan, consistent with previous studies (Holzwarth 1976; Smith and others 1981; Crescenzi and others 1986; Foster and Morris 1994).

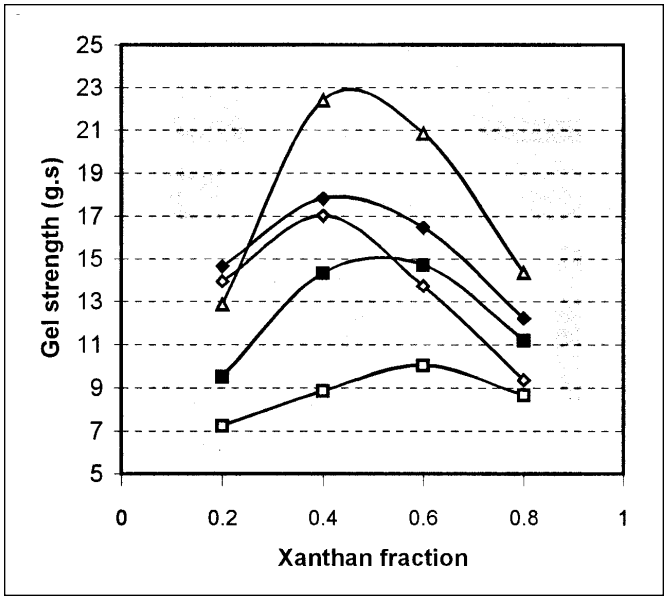

Figure 1—Plots of gel strength against xanthan fraction at a polymer concentration of 0.2% (w/v) by texture analysis. ❑, native xanthan/LBG in 0.04 *M* NaCl solution; ◇, native xanthan/LBG in aqueous solution ; △, deacetylated xanthan/LBG in aqueous solution. Filled symbol: hot mixing; unfilled symbol: cold-mixing.

Gel strength study by a texture analyzer

Gel strength is a reflection of the work of cohesion within the gel as the resistance to the insertion of a probe into a sample under a defined set of conditions (Craig and others 1997). Gel strength as a function of xanthan fraction at a polymer concentration of 0.2% (w/v) by texture analysis is shown in Figure 1. The plots of individual polymers are not shown because each alone did not form gel but formed a viscous solution. Strong synergistic interactions were observed in all blends and clear differences were noted between the different ratios of xanthan and LBG and among xanthan samples with different stability of helical structures. Cold-mixed deacetylated xanthan/LBG in aqueous solution exhibited the strongest synergistic interactions, followed by hot-mixed native xanthan/LBG and cold-mixed native xanthan/LBG in aqueous solution, and hot-mixed native xanthan/LBG and cold-mixed native xanthan/LBG in 0.04 *M* NaCl, consistent with previous studies (Shatwell and others 1991; Tako 1991; Zhan and others 1993). The cold-mixed native xanthan/LBG (4:1) in aqueous solution, the hot-mixed native xanthan/LBG (1:4) in 0.04 *M* NaCl, and all cold-mixed native xanthan/LBG in 0.04 *M* NaCl were viscous gel-like pourable fluids, while others formed nonpourable gels according to the inverting tube method described by Brownsey and others (1988). Deacetylated xanthan and LBG formed the strongest gels. Deacetylation enhanced the intermolecular binding by destabilizing the xanthan helical structure (Foster and Morris 1994) and rendering a more flexible xanthan chain, thus facilitating formation of heterotypic junctions with LBG (Zhan and others 1993; Goycoolea and others 1994; Morris and others 1994; Morris ER 1996; Morris VJ 1996; Ojinnaka and others 1998).

The hot-mixed native xanthan/LBG/water systems formed stronger gels than did the cold-mixed ones. Mixing at a higher temperature significantly enhanced the binding because the mixing temperature (> 90 °C) was well above the order-disorder transition (T_m) of native xanthan in the aqueous solution, 55 °C (Norton and others 1984). The disordered xanthan was capable of directly interacting with LBG to form the heterotypic structures (Zhan and others 1993; Goycoolea and others 1994; Morris and others 1994; Morris ER 1996; Morris VJ 1996). This similar trend was also observed in the native xanthan/guar/water systems, although they did not form gels (Wang 2001).

The hot-mixed native xanthan/LBG blends in 0.04 *M* NaCl solution formed gels, while cold-mixed did not, because the mixing temperature for hot-mixed samples was above the T_m of native xanthan in 0.04 *M* NaCl solution, 84 °C (Williams and others 1991). Since the synergistic interaction between native xanthan

and LBG in aqueous solution was stronger than that in 0.04 *M* NaCl, the addition of NaCl decreased the synergistic interaction between native xanthan and LBG, supporting the suggestion that the highly stable xanthan helix structure from the addition of salt would further block the synergistic interaction (Foster and Morris 1994).

The deacetylated xanthan or native xanthan and LBG mixtures in aqueous solution formed the strongest gels at a ratio of 2:3, whereas the native xanthan and LBG mixtures in 0.04 *M* NaCl solution formed the strongest gels at a ratio of 3:2. The contraction of xanthan molecules from the electrostatic shielding of counterions reduced the binding opportunity of xanthan to LBG; therefore a higher ratio of xanthan was required to achieve the maximum binding, thus maximum synergistic interaction.

Interaction study by Ubbelohde viscometry

The results of reduced viscosity (η_{sp}/C) against concentration of native xanthan, LBG and their mixtures in aqueous or 0.04 *M* NaCl solution, and deacetylated xanthan and its mixtures with LBG in aqueous solution by Ubbelohde viscometry are shown in Figure 2, 3, and 4, respectively. When the concentrations were above the overlap concentration (C*) of native xanthan in aqueous solution, 0.04 g/dL, the synergistic interactions were found for native xanthan/LBG ratios of 2:3 and 3:2 in aqueous solution with the ratio of 2:3 showing the strongest interaction, consistent with the texture study. In native xanthan/LBG/0.04 *M* NaCl systems, the synergistic interactions were observed in the blends with ratios of 4:1 and 3:2, and the ratio of 4:1 had the strongest interaction. For deacetylated xanthan/LBG/water, all mixtures, except for the ratio of 1:4, showed the synergistic interaction, and the ratio of 2:3 had the strongest interaction, in agreement with the texture study.

When the concentrations were below the C*, not only the individual polymer but also their blends followed the Huggins equation. Figure 5 shows the plots of intrinsic viscosities against xanthan fraction for native xanthan and LBG blends in either aqueous or 0.04 *M* NaCl solution. The intrinsic viscosities of the xanthan/LBG blends in aqueous solutions were well below those calculated from the weight averages of the 2 individual ones, only about 25 to 60% of the weight average values. This phenomenon is similar to what was observed in aqueous native xanthan and guar blends (Wang 2001), except that the intrinsic viscosities of xanthan and LBG blends were even lower. Since xanthan plays a critical role in controlling the viscosity of xanthan and LBG blends, the decrease in the intrinsic viscosities of the blends can be attributed to the conformational change of xanthan from a helical state to a much more flexible state because of binding. LBG, a stronger denaturant than guar, can easily disturb the order-disorder equilibrium of xanthan and displaced xanthan from a stiff ordered helix to a rather flexible conformation, where xanthan and LBG formed a more stable heterotypic structure (Morris ER 1996; Morris VJ 1996). This xanthan conformation may be even more flexible than that of xanthan mixed with guar. This is why the intrinsic viscosities of xanthan/LBG blends were even lower than those of xanthan/guar blends. However, the intrinsic viscosities of these blends should be above those calculated from the weight averages of the blends if there was intermolecular binding between xanthan and LBG (Wang 2001). The reason why we did not observe this increase in intrinsic viscosities was because LBG with a flexible coil conformation had a much smaller hydrodynamic radius than that of xanthan, a rigid stiff molecule. Thus binding of xanthan with LBG will not significantly result in the increase of blend-intrinsic viscosities since xanthan conformational change outweighs the increase of intrinsic viscosity from the synergistic binding. Therefore, we observed the dramatic decrease of the intrinsic viscosities of the blends instead.

A similar relationship between the intrinsic viscosities of native xanthan/LBG blends and xanthan fraction was observed in 0.04 *M* NaCl, suggesting the intermolecular binding also occurred. LBG was such a strong denaturant to the xanthan helix that it could disturb xanthan order-disorder transition even when the xanthan helix was stabilized by salt. However, the

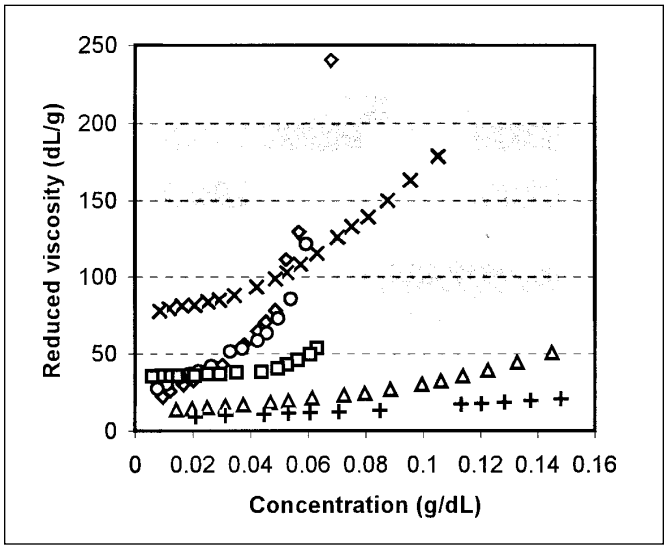

Figure 2—Plots of reduced viscosities against concentration of LBG, xanthan, and their mixtures in aqueous solution by Ubbelohde viscometry. Ratio of xanthan to LBG in mixtures: +, LBG; ×, xanthan; ❑, 4/1; ○, 3/2; ◇, 2/3; △, 1/4.

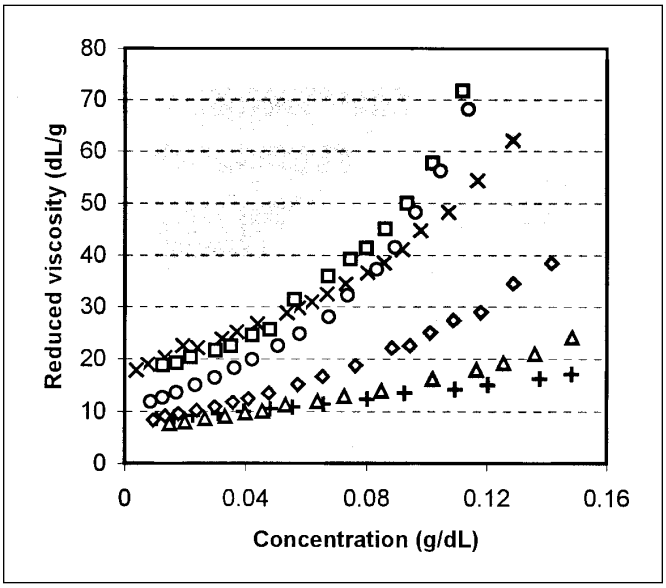

Figure 3—Plots of reduced viscosities against concentration of LBG, xanthan and their mixtures in 0.04 *M* NaCl by Ubbelohde viscometry. Symbols are the same as in Figure 2.

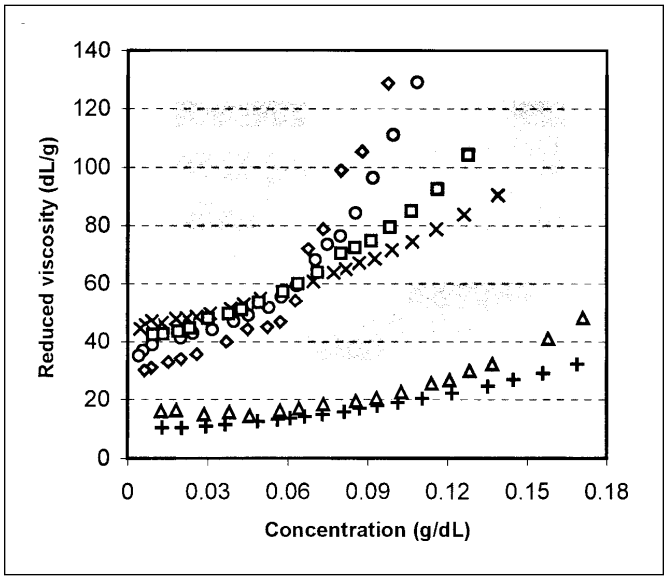

Figure 4—Plots of reduced viscosities against concentration of LBG, deacetylated xanthan, and their mixtures in aqueous solution. Symbols are the same as in Figure 2.

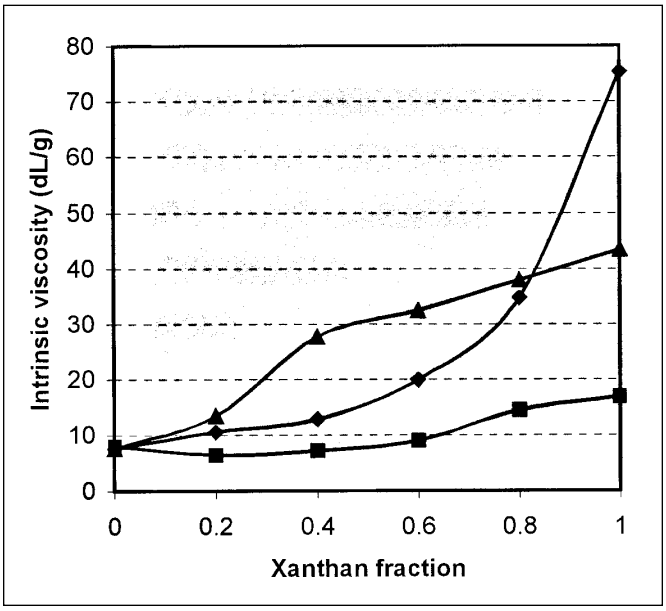

Figure 5—Plots of intrinsic viscosities against xanthan fraction. ■, xanthan/LBG in 0.04 *M* NaCl; ◆, xanthan/LBG in aqueous solution; ▲, deacetylated xanthan/LBG in aqueous solution. The dash lines represent the weight average of the mixtures.

blend-intrinsic viscosities for xanthan:LBG ratios of 1:4 and 2:3 were below that of either xanthan or LBG and the reason is not clear.

The intrinsic viscosities of deacetylated xanthan and LBG blends, however, were higher than those calculated from the weight averages of the 2 individual ones, which was clear evidence of intermolecular binding between xanthan and LBG molecules. Nevertheless, conformational change of deacetylated xanthan may not take place or may not predominate in controlling the intrinsic viscosity. Deacetylated xanthan may be in the exact conformation to bind LBG and the most stable heterotypic structure between deacetylated xanthan and LBG was formed directly without inducing the conformational change of xanthan by LBG. This strong intermolecular binding might also enable portions of deacetylated xanthan chain to associate with LBG so that deacetylated xanthan chain would become rigid. This may be part of the reason why intrinsic viscosities of deacetylated xanthan and LBG blends were higher than those calculated from the weight averages of the 2 individual ones.

The characteristic of flexible polyelectrolytes was not noted in any systems in this study, even in the more flexible deacetylated xanthan systems; whereas in native xanthan/guar/water systems, when xanthan was the main component in the mixture, instead of following the Huggins equation, the reduced viscosity underwent a marked increase with dilution and approached infinity at zero concentration, a characteristic of flexible polyelectrolytes according to the Fuoss empirical equation (Fuoss and Struss 1948; Fuoss and Cathers 1949) and Wang 2001). It is possible that the chain of deacetylated or native xanthan was not flexible enough to exhibit the property of flexible polyelectrolytes under the experimental condition where the ionic strength of solvent from sodium azide was higher than that of the xanthan chain (Wang 2001). The strong intermolecular binding between deacetylated or native xanthan and LBG not only stabilized the current conformation but also stiffened the association zones, resulting in the absence of flexible chain characteristics. As for xanthan and LBG blends in 0.04 *M* NaCl, xanthan helical structure was stabilized by salt, thus no characteristic of flexible polyelectrolytes was observed.

It should be noted from Figure 5 that the intrinsic viscosities of all deacetylated xanthan and LBG blends were higher than those of all native xanthan and LBG blends, even though the intrinsic viscosity of native xanthan was much higher than that of deacetylated xanthan. The possible explanation is that native xanthan went through significant conformational change when bound to LBG while deacetylated xanthan did not.

Conclusions

THE FLEXIBILITY OF XANTHAN CHAIN INCREASED IN THE ORDER OF native xanthan in salt solution < native xanthan < acetate-free xanthan. The stability of xanthan helical structure or xanthan chain flexibility played a critical role in interacting with LBG. Xanthan with a more flexible chain conformation was preferentially bound to LBG, thus resulting in a stronger synergistic interaction. The order-disorder equilibrium of native xanthan can be easily disturbed by LBG, even when xanthan was stabilized by salt. Nevertheless, salt reduced the binding of xanthan to LBG from electrostatic shielding effect. Thus a higher ratio of xanthan was required to achieve the maximum synergistic interaction. The conformational change may not be a prerequisite for deacetylated xanthan to interact with LBG; presumably deacetylated xanthan was in an exact conformation to bind LBG after deacetylation.

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