

**RHEOLOGICAL CHARACTERIZATION OF XANTHAN-GUAR MIXTURES IN
DILUTE SOLUTIONS**

by

HANNA ANTON MICHAEL KHOURYIEH

B.S, Birzeit University, 1997
M.S., Kansas State University, 2003

AN ABSTRACT OF A DISSERTATION

Submitted in partial fulfillment of the
requirements for the degree

DOCTOR OF PHILOSOPHY

Food Science Graduate Program
College of Agriculture

KANSAS STATE UNIVERSITY
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ABSTRACT

Dynamic viscoelastic and intrinsic viscosity properties of native xanthan, deacetylated xanthan, guar, and their mixtures in dilute solutions were investigated by using an oscillating capillary rheometer. Influence of mixing temperature, deacetylation, and salt concentration on xanthan conformation and interaction with guar were studied in order to provide additional evidence that can be used to elucidate the mechanism of the intermolecular interaction between the two biopolymers, and build up a more detailed rheological understanding of molecular interactions between xanthan and guar gum in dilute solutions.

Synergistic interaction was found at mixing temperatures of 25 and 80 °C, but a stronger synergistic interaction was observed at mixing temperature of 80 °C. The differences in viscosity and elasticity measurements between the two mixing temperatures could be attributed to the degree of disordering of xanthan. For both mixing temperatures, the relative viscosity and elasticity of xanthan and guar blends were higher than the relative viscosity and elasticity calculated for blends assuming no interaction, indicating that intermolecular binding occurred between galactomannans backbone and disordered segments of xanthan.

Deacetylated xanthan exhibited a stronger synergistic interaction with guar than native xanthan. The intrinsic viscosities of deacetylated xanthan-guar mixtures were higher than those calculated from the weight averages of the two individually, whereas the intrinsic viscosities of native xanthan-guar mixtures were lower than those calculated from weight averages of the two individually, demonstrating that intermolecular binding occurred between xanthan and guar gum.

Synergistic interaction for both native xanthan-guar mixtures and deacetylated xanthan-guar mixtures in the dilute regime was observed in water and 2 mM NaCl but not in 40 mM

NaCl. The results suggest that intermolecular interaction has occurred between xanthan and guar mixtures in water and 2 mM NaCl, but may not occur in 40 mM NaCl and mutual incompatibility may take place. These results also suggest that degree of disordering of xanthan played a critical role in xanthan-guar interaction and may explain the differences in viscosity, elasticity, and intrinsic viscosity measurements between 2 and 40 mM NaCl, and hence, the intermolecular interaction occurred between the backbone of guar gum and the disordered segments of xanthan.

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DEDICATION

This dissertation is dedicated to the memory of my mother, Helweh Khouryieh, who encouraged me to reach for my dreams. Her happy memory still provides a persistent inspiration for my journey in this life.

Introduction

Engineered food materials are mainly structured by a complex assembly of various food ingredients such as proteins, polysaccharides, lipids, sugars, emulsifiers, minerals, and water (Kilara, 1995). Food proteins and polysaccharides play a key role in the structure and stabilization of food systems through their gelling, thickening, and surface-stabilizing functional properties (Tolstoguzov, 1991). During manufacturing, intrinsic properties of individual components, as well as interactions between the different components determine the final structure, texture, and stability of food materials. Because texture and stability are major criteria of food quality, scientists and food industry are primarily concerned in identifying such interactions in order to provide optimum food quality, design new and attractive food, and food ingredients structure (Sanchez et al., 1997).

The phase behavior of biopolymer-biopolymer-water systems plays an important role in surface and colloid chemistry. For solutions containing two different biopolymers, the phase behavior can be divided into three types depending on the nature of the biopolymers (Williams & Phillips, 1995; Schmitt et al, 1998):

1. Compatibility- the phase behavior results in complete miscibility and the formation of a single homogenous phase.
2. Incompatibility- the phase behavior results in the formation of two liquid polymer layers with each layer enriched in one or other of the polymers. The interactions between the biopolymers are repulsive in nature and the system forms two phases, each of them enriched with one biopolymer. This kind of phase behavior is usually named thermodynamic incompatibility or segregative phase separation.

3. Polymer association- the phase behavior results in co-precipitation of the polymers in the form of a solid coacervate or in some instances to the formation of a gel. The interactions are attractive and the system exhibits a two-phase region with the two biopolymers concentrated in one phase. This type of phase separation is known as complex coacervation or associative phase separation.

In dilute solutions, the individual polymer coils or rods are separate and free to move independently, and their intermolecular interactions are negligible (Roa, 1999). Thus, polymer-polymer interactions can be detected by the increase in solution viscosity. Methods based on viscosity measurements are sensitive and useful methods to investigate polymer-polymer interactions in dilute solutions. More sophisticated rheometers such as Viscostar 3 have the ability to measure the viscoelastic characteristics of biopolymers in very dilute solutions.

Synergistic polysaccharide-polysaccharide interactions are attractive in the food industry because they impart novel and improved texture and rheological characteristics to food products and reduce polymers costs (Williams & Phillips, 2000). A synergistic interaction occurs between xanthan gum and galactomannans in solutions to result in enhanced viscosity or gelation (Dea & Morrison, 1975; Dea, Morris, Rees, Welsh, Barnes, & Price, 1977; Morris, Rees, Young, Walkinshaw, & Darke, 1977).

Although the interactions between xanthan and galactomannans have been intensively studied, the nature of the intermolecular binding mechanism is still controversial, and different models have been proposed. In the literature, much work has been accomplished on the gelling properties of the xanthan-guar mixtures. Studying of the xanthan-guar interactions in dilute solutions has been studied to a lesser extent. Additionally, the high demand for hydrocolloids in the food industry, specifically beverages, requires a comprehensive understanding to the

rheological behavior of these biopolymers in dilute solution systems. Therefore, the main objective of this study was to investigate the interactions between xanthan and guar gum in dilute solutions in order to provide additional evidence that can be used to elucidate the mechanism of the intermolecular interaction between the two biopolymers, and build up a more detailed rheological understanding of molecular interactions between xanthan and guar gum in dilute solutions. To achieve this goal, a sophisticated rheometer was used and a series of experiments were conducted. Thus, the dissertation was divided into four main chapters:

Chapter 1 is the literature review which provides an elaborate background on models describing xanthan-guar interactions, and rheology.

In chapter 2, dynamic viscoelastic and intrinsic viscosity properties of xanthan, guar, and xanthan-guar mixtures in dilute aqueous solutions were investigated by using an oscillating capillary rheometer. Influence of mixing temperature (25 and 80 °C) on xanthan conformation and synergistic interaction with guar in dilute aqueous solutions was studied.

Chapter 3 investigates the effects of xanthan deacetylation on the viscoelastic properties and intrinsic viscosity of xanthan and xanthan-guar mixtures in dilute aqueous solutions. Experimental data of deacetylated xanthan-guar mixtures were compared to those for native xanthan-guar mixtures.

In chapter 4, the role of NaCl concentration (0, 2, and 40 mM NaCl) on xanthan conformation (deacetylated xanthan and native xanthan) and the intermolecular binding with guar gum in dilute solutions was investigated.

Chapter 1

Literature Review

1.1. Food Hydrocolloids

The term 'hydrocolloids' refers to a range of polysaccharides and proteins (Williams & Phillips, 2000). Food hydrocolloids, or more commonly food gums, are usually polysaccharides consisting of long chain of sugar-building units. Because gums dissolve or disperse in water, they are used primarily to thicken and/or gel aqueous solutions and otherwise to modify and/or control the flow properties and textures of liquid food and beverage products and the deformation properties of semisolid foods (Whistler & BeMiller, 1999). Because of their ability to thicken aqueous solutions, hydrocolloids offer many other functions in food and beverages including stabilizing, emulsifying, suspending particulates, foaming, controlling crystallization, syneresis inhibitors, binding, coating, and encapsulation (Dziezak, 1991; Sanderson, 1996; BeMiller & Whistler, 1996).

Hydrocolloids have been extensively used in the beverage industry. The total market for non-alcoholic beverages in 2001 in the United States was approximately \$80 billion (Zammer, 2002). The functional beverages segment accounted for approximately \$ 5.5 billion. In beverages that contain fruit pulp, hydrocolloids slow the rate at which the pulp settles. Anionic hydrocolloids such as xanthan can also prevent a phenomenon called "hard packing" in which the pulp settles out and forms a layer that is a very hard to redisperse into the beverage by shaking the container. In carbonated beverages and powdered drink mixes, the addition of a hydrocolloid at use level of 0.05-0.15% can provide texture and mouthfeel to a low-calorie

beverage that nutritive sweeteners impart to full-calorie beverages. A dilute hydrocolloid solution mimics the Newtonian behavior of a 15% sugar solution (Hoefer, 2004).

Hydrocolloids are usually used in low concentration at usage level less than 1% to accomplish the expected purposes in foods and have a significant influence on the textural and organoleptic properties. Hydrocolloid selection is dictated by the functional characteristics required but is inevitably influenced by price and security of supply. Numerous hydrocolloid products have been developed for use in ready-made meals, novelty foods and low-fat products, which led to an increased demand for hydrocolloids. The world market of hydrocolloids has become huge to reach sales of \$4.4 billions with a total volume of about 260,000 tons (Williams & Phillips, 2000).

The chemical structure of hydrocolloids is either nonionic (neutral) or anionic. Hydrocolloids are generally classified according to their source but can be classified based on the major monosaccharide component. Most of the food hydrocolloids are obtained from land plants, seaweeds, and bacterial and synthetic sources (Table 1.1).

Table 1.1. Source of commercially important hydrocolloids.

Source	Hydrocolloid	
Botanical	Trees <i>cellulose</i>	
	Tree gum extrudates <i>gum Arabic, gum karaya, gum ghatti, gum tragacanth</i>	
	Plants <i>starch, pectin, cellulose</i>	
	Seeds <i>guar gum, locust bean gum, tara gum, tamarind gum</i>	
	Tubers <i>konjac mannan</i>	
	Algal	Red seaweeds <i>agar, carrageenan</i>
		Brown seaweeds <i>alginate</i>
Microbial		<i>xanthan gum, curdlan, dextran, gellan gum, cellulose</i>
	Animal <i>gelatin, caseinate, whey protein, chitosan</i>	

Source: Williams and Phillips (2000)

1.2. Xanthan Gum

1.2.1. Structure

Xanthan gum is an anionic heteropolysaccharide produced by the microorganism *Xanthomonas campestris*. Xanthan's primary structure (Fig.1.1) consist of repeated pentasaccharide units formed by two glucose units, two mannose units, and one glucuronic acid unit, in the molar ratio 2.8:2.0:2.0. Xanthan's backbone consists of (1→4) β-D-glucopyranosyl units. The chemical structure of the main chain is identical to that the chemical structure of cellulose. The backbone is substituted at C-3 on every other glucose residue with a trisaccharide sidechain. The trisaccharide chain consists of a D-glucuronic acid unit between two D-mannose units. Approximately half of the terminal D-mannose unit contain a pyruvic acid residue linked

via keto group to the 4 and 6 positions, with an unknown distribution. The D-mannose linked to the main chain contains an acetyl group at position O-6 (Garcia-Ochoa et al., 2000).

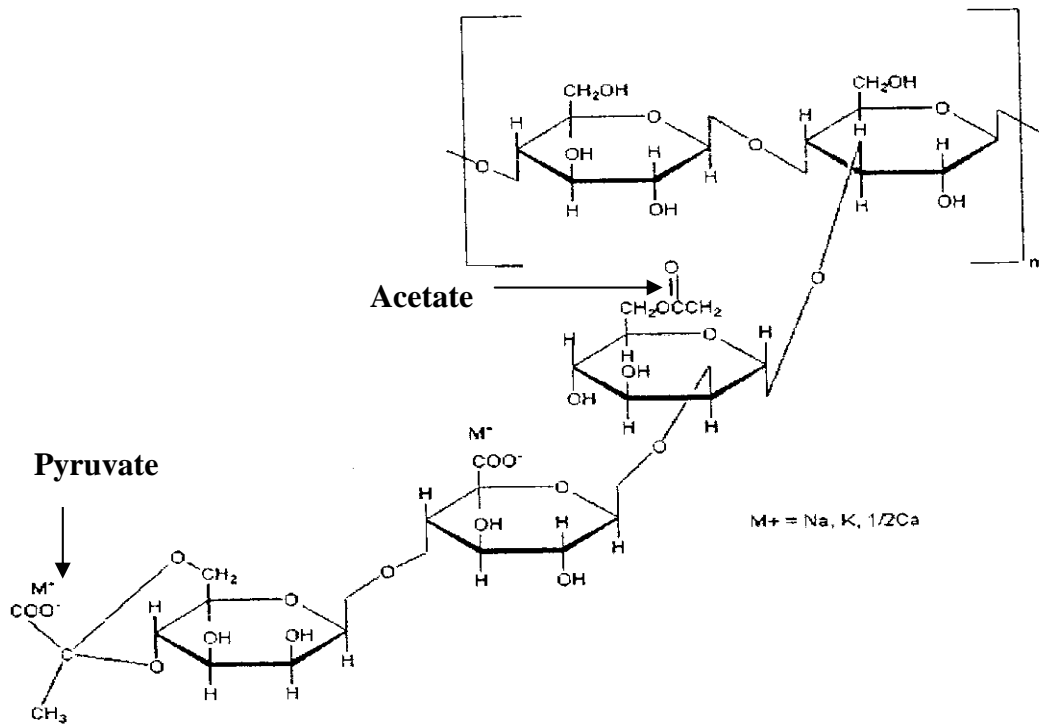


Fig. 1.1. Chemical structure of xanthan (Hoefler, 2004).

The trisaccharide side chains are closely aligned with the polymer backbone causing a single, double, or triple helix stiff chain. The resulting stiff chain interacts with other polymer molecules to form a complex (Morris et al., 1977; Milas & Rinaudo, 1979). The molecular weight distribution of xanthan ranges from 2×10^6 to 20×10^6 , depending on the association between chains, forming aggregates of many individual chains. The variations in the fermentation conditions can influence the structure and molecular weight distribution of xanthan produced (Garcia-Ochoa et al., 2000).

The secondary structure of xanthan undergoes a thermally induced conformational change from a stiff, ordered helical conformation, where the side chains are folded-down and associated with the backbone by non-covalent interactions at lower temperature, to a more

flexible, disordered structure, where the side chains are project away from the backbone (Fig. 1.2 (a) & (b)). This conformational transition depends on ionic strength, nature of electrolyte, pH, and acetyl and pyruvate constituent contents (Holzwarth, 1976; Morris et al., 1977, Baradossi & Brant 1982, Paoletti et al., 1983, Norton et al., 1984).

Rocheffort & Middleman (1987) reported that in distilled water at 25 °C, the backbone is disordered (or partially ordered in the form of a randomly broken helix) but highly extended due to the electrostatic repulsions from the charged groups on the sidechains. Due to the highly extended structure, the molecules may align and associate (due to hydrogen bonding) to form a weakly structured material. Therefore, as the temperature increased, a transition to coil-like configuration occurs, which causes a dissociation of the molecules and a subsequent change in the rheological properties (Rocheffort & Middleman, 1987). When salt is added to the xanthan solution at 25 °C, a disorder-order transition occurs in which the backbone takes on a helical conformation and the charged trisaccharide sidechains collapse down onto the backbone (due to charge screening effects) and stabilize the ordered conformation (Muller et al., 1986).

The structure detail of xanthan's ordered structure, single or double stranded, and the mechanism of transition still remains controversial. Electron microscopy (Milas et al., 1988), optical rotation (Norton et al., 1984), scanning tunneling microscopy (Gunning et al., 1993) studies suggested that the ordered form of xanthan is a single helix. However, there is growing evidence that favors the double-stranded model based on viscometry (Sato et al., 1984a), light scattering (Baradossi & Brant, 1982), electron microscopy (Holzwarth & Prestridge, 1977; Stokke et al., 1986), calorimetry (Paoletti et al., 1983), and combined viscometry and light scattering (Sato et al., 1984b; Liu et al., 1987; Liu & Norisuye, 1988). X-ray diffraction studies suggested that xanthan forms a single-stranded 5-fold helix (Moorhouse et al., 1977).

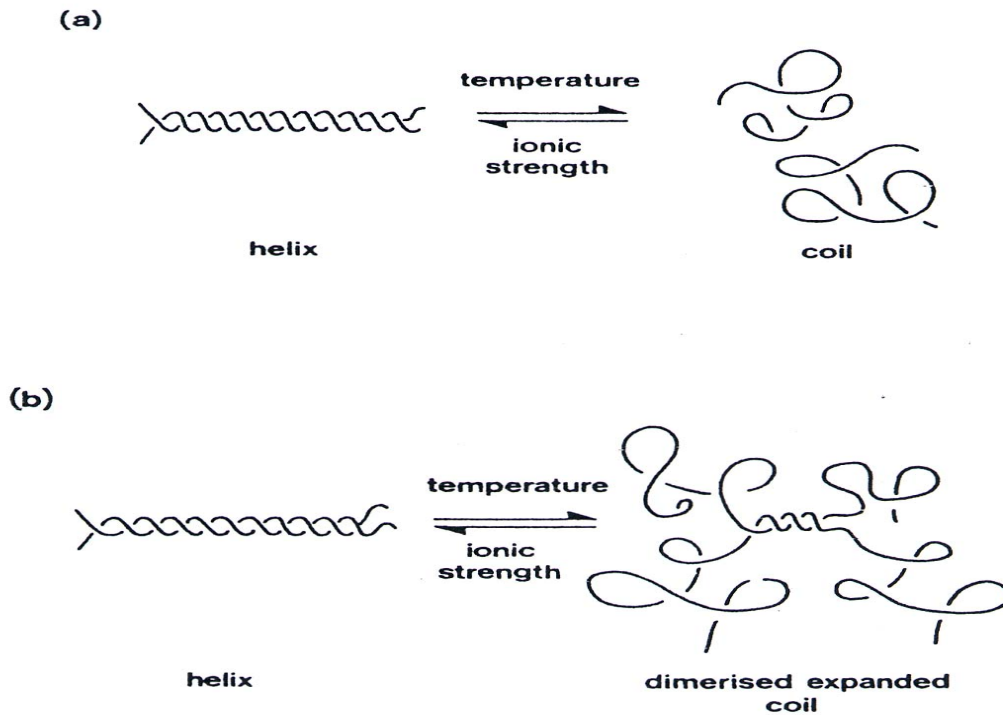


Fig.1.2. Xanthan order-disorder transition. (a) Helix-coil transition; (b) helix-dimerized expanded coil (Morris, 1995).

1.2.2. Properties of Xanthan Solutions

Xanthan gum is very soluble in both cold and hot water due to the polyelectrolyte nature of xanthan molecule. Xanthan solutions are highly viscous even at low concentrations. Xanthan solutions exhibit a pseudoplastic, or shear thinning behavior. Xanthan's viscosity depends on temperature, gum concentration, salt concentration, and pH (Garcia-Ochoa et al., 2000).

Viscosity of xanthan solutions depends on both measurement temperature and dissolution temperature (Garcia-Ochoa et al., 2000). The viscosity decreases as the measurement temperature increases. This behavior is fully reversible between 10 and 80°C. Although, the viscosity decreases as the dissolution temperature is increased up to 40°C. Between 40 and 60°C, the viscosity increases as the temperature is increased. At temperatures above 60°C, the viscosity decreases as the temperature is increased. This unique behavior is associated with the

conformational changes (order-disorder transition) of the xanthan molecule (Garcia-Ochoa et al., 2000). Xanthan conformation shifts from an ordered state at low-dissolution temperature to a disordered state at high dissolution temperature (Milas & Rinaudo, 1979; Garcia-Ochoa & Casas, 1994). The order-disorder transition related to a helix-coil transition of the backbone with simultaneous release of the lateral chains followed by progressive decrease of the rigidity of the (1→4) β-D-glucan chain as the temperature increases between 40 and 60 °C (Milas & Rinaudo, 1979). The transition temperature varies depending on the salt concentration, independently of the polymer concentration (Milas & Rinaudo, 1979). The transition temperature has been reported to be around 55°C in distilled water, and increases with the ionic strength (Norton et al., 1984). Norton et al. (1984) suggested that the helix-coil transition may not take place over a narrow range of temperature, but may be an incremental process taking place a temperature range as wide as 70 °C. Using DSC measurements, Williams et al. (1991) reported that the exothermic peak corresponding to the order-disorder conformational transition for xanthan had a midpoint temperature of 51 °C in water and 84 °C in 0.04M NaCl solution.

The viscosity increases significantly as xanthan concentration is increased. This behavior is attributed to the polymer intermolecular interaction and entanglement which increase the effective macromolecule dimensions and molecular weight. Xanthan viscosity is influenced by salt concentration. At low polymer concentration the viscosity decreases slightly when a small amount of salt is added to the solution (Garcia-Ochoa et al., 2000). This can be related to the reduction in molecular dimensions resulting from diminished intermolecular electrostatic forces (Smith & Pace, 1982). Although, viscosity increases at higher xanthan concentration or when a large amount of salt is added. This behavior is probably due to the increased interaction between the polymer molecules (Smith & Pace, 1982; Milas et al., 1985). Kang & Pettit (1993) reported

that viscosity of xanthan solutions is independent of the salt concentration when the salt concentration more than 0.1% (w/v).

Viscosity of xanthan solutions is independent of pH changes between pH 1 and 13. At pH 9 or higher, xanthan is gradually deacetylated (Tako & Nakamura, 1984), whereas at pH less than 3 xanthan loses the pyruvic acid acetyl groups (Bradshaw et al., 1983)

Xanthan has pyruvate and acetyl substituents on the mannose units in the side chain (Callet et al., 1987). The extent of pyruvate and acetyl substituents depends on the *Xanthomonas* strain, fermentation conditions and post-fermentation process (Rinaudo, 2004). The acetyl groups in xanthan are shown to control the interaction between xanthan and other polysaccharides such as galactomannans (Tako & Nakamura, 1984; Lopes et al., 1992). Removal of pyruvate substituents raises the midpoint temperature of the conformational transition by reducing electrostatic repulsion between chain segments. Acetate substituents, however, appear to contribute to the stability of the ordered structure (Smith et al., 1981), and their removal lowers T_m .

The role of acetal and acyl groups in determining the solution viscosity of xanthan was investigated by Bradshaw et al (1983). Viscosity measurements indicated that the pyruvic acid acetal and o-acetyl contents of xanthan do not affect solution viscosity. The viscosities of native, pyruvate-free and pyruvate/acetate-free xanthan solutions (0.3% w/v) were similar at shear rates $8.8 - 88.3 \text{ s}^{-1}$ in both distilled water and 1% KCl. At the xanthan concentration range 0.2-1.5%, the viscosities of native and pyruvate-free xanthan at 10 s^{-1} were similar. The viscosity increase on addition of 1% KCl to salt-free xanthan solutions was independent of pyruvic acid acetal substitution. They suggested that xanthan samples with various pyruvic acid acetal and o-acetal

contents, prepared under different fermentation conditions should not normally be used for assessing the role of these groups to solution viscosity.

Tako & Nakamura (1984) studied the dynamic viscoelasticity of deacetylated xanthan in aqueous media at various concentrations. The flow properties of deacetylated xanthan solutions exhibited pseudoplastic behavior at concentrations below 0.1% but to plastic behavior above 0.3%. The apparent viscosity of deacetylated xanthan decreased with increasing temperature at concentrations range 0.1 to 0.5%, however, viscosity decreased gradually at 1.0%. Deacetylated xanthan showed a higher dynamic viscoelasticity comparing to native xanthan at high concentrations. Dynamic viscosity and elasticity of deacetylated xanthan showed lower values than those of native xanthan below 0.3%, whereas higher values were observed above 0.5% solution. The higher values at high concentration may be due to the formation of more intense intermolecular association than with native xanthan. The dynamic viscoelasticity of deacetylated xanthan decreased with increasing temperature at various concentrations and by addition of urea (4.0M). The researchers suggested that acetate groups, which attached to the inner mannose residues of the side chains, contribute to the intramolecular association with the backbone. The side chains become more flexible after deacetylation, therefore the intermolecular association between the side chains increases with the increase in concentration, and side chains are more liable to associate with galactomannan.

The influence of acetyl and pyruvate groups on the conformational properties (Dentini et al., 1984) and the viscosity (Callet et al., 1987) of xanthan in dilute aqueous solutions was investigated. Dentini et al. (1984) investigated the Ca^{2+} -induced conformational changes of native and modified xanthan at the same molecular weight using circular dichroism and isothermal microcalorimetric techniques. Both techniques indicated that the acetyl groups

stabilize the ordered conformation of xanthan, which can be induced by increasing the ionic strength of the dilute aqueous solution, whereas pyruvate groups had a strong destabilizing effect on the ordered conformation, which is likely due to the unfavorable electrostatic repulsion. The order of thermal stability of the ordered forms was found to be pyruvate-free xanthan > native xanthan > acetyl and pyruvate-free xanthan > acetyl-free xanthan in both water and $\text{Ca}(\text{ClO}_4)_2$ (Dentini et al., 1984). Callet et al (1987) further studied the effect of acetyl and pyruvate groups on the rheological properties of xanthan in dilute solution depending on viscosity and optical rotation measurements. Acetyl-free, pyruvate-free, and acetyl and pyruvate-free xanthans were prepared by chemical hydrolysis and the viscosities of the different xanthan solutions studied as a function of xanthan concentration (0.02% w/v in 0.1M NaCl) and molecular weight. Conformational transitions of native and modified xanthans were determined by measuring optical rotation as a function of temperature. Values of midpoint transition (T_m) indicated that ordered conformation of acetyl-free xanthan (lower T_m) is less stable than native xanthan whereas the pyruvate-free xanthan has an opposite effect and T_m increases with pyruvate hydrolysis, which is in agreement with those of Holzwarth & Ogletree (1979) and Rinaudo et al., (1983). The acetyl groups stabilize the ordered conformation of xanthan, whereas the electrostatic repulsion of the pyruvate groups was destabilizes it. The viscosity measurements showed that acetyl and pyruvate residues have no influence either on the xanthan dilute solution viscosity or on intrinsic viscosity at a given molecular weight (Callet et al., 1987).

Shatwell et al. (1990) used a intrinsic viscosity at low shear rates ($0-20 \text{ s}^{-1}$) and light scattering measurements to characterize native and modified xanthan, and to study the effect of the different degrees of substitution on chain stiffness and solution behavior. Values obtained

from light scattering measurements indicated that the inherent stiffness of the xanthan macromolecule is not greatly influenced by the pattern of acyl substitution.

1.2.3. Applications

Xanthan is very commonly used in the food industry because of its unique physical, chemical and functional properties. Xanthan gum can be beneficially used in a wide variety of products such as salad dressings, chocolate syrup, relishes, tomato sauces, dry mixes and beverages, candies, gravies, and dairy products (BeMiller & Whistler, 1996; Dziezak, 1991). Xanthan can be used in baked goods as water binder, stabilizer in dressings, and pulp suspensioner at concentration ranges from (0.1 - 0.2%), (0.2 - 0.3%), and (0.02 - 0.06%) respectively (Trudso, 1988).

1.3. Guar Gum

3.1. Structure

Guar gum (Fig.1.3) is a neutral water-soluble polysaccharide obtained from the seed of the guar plant, *Cyanaposis tetragonolobus*, and has the general structure of galactomannans. Guar, the functional polysaccharide in guar gum consists of main chain (1→4)-linked β -D-mannopyranosyl units substituted at O-6 by single-unit side-chains of α -D-galactopyranose. The ratio of mannose to galactose is approximately 1.6:1, depending on the source and method of extraction (Dea & Morrison, 1975).

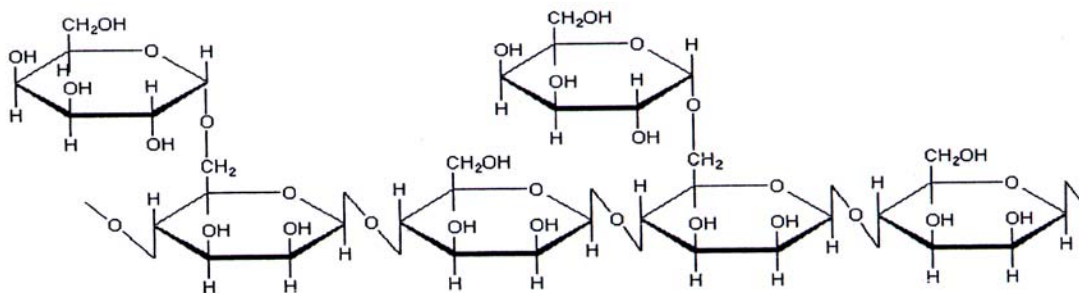


Fig. 1.3. Chemical structure of guar gum (Hoefler, 2004).

1.3.2. Properties of Guar Gum Solutions

Guar gum dissolves in polar solvents that form strong hydrogen bonds. The rate of guar gum dissolution and viscosity development generally increases with decreasing particle size, decreasing pH, and increasing temperature. Guar gum derivatives, such as hydroxypropylguar gum, are more soluble and hydrate faster than guar gum. Dissolution rates are decreased in the presence of dissolved salts and other water-binding materials such as sucrose (Maier et al., 1993).

Guar gum solutions show pseudoplastic or shear thinning behavior. Degree of pseudoplasticity of guar solutions increases with concentration and molecular weight. Guar gum solutions do not exhibit yield stress properties (Elfak et al., 1979; McCleary et al., 1981; McCleary et al., 1984). Guar gum provides thickening and increasing viscosity in aqueous solutions due to its large hydrodynamic volume and the nature of specific intermolecular interactions (Maier et al., 1993). Guar solution viscosities increase proportionally with increases in guar gum concentration (Morris et al., 1981; Robinson et al., 1982). In solutions, guar gum exists as random-coil polymer (Robinson et al., 1982). However, its rheological properties have been found to depart from those typical 'random coil' polysaccharides (Morris et al., 1981). Double-logarithmic plot of 'zero shear' specific viscosity of guar against degree of space-occupancy($c[\eta]$) showed an abrupt change in slope and the onset of the concentrated solutions behavior started at lower values of $c[\eta] \sim 2.5$, in comparison with $c[\eta] \sim 4$ for most other disordered coils. This anomalous behavior has been rationalized in terms of intermolecular associations (hyperentanglement) between unsubstituted regions of mannan chains, in addition to normal topological entanglement (Morris et al., 1981; Goycoolea et al., 1995).

Guar gum tolerate salt well (Maier et al., 1993; Whistler and BeMiller, 1999). Guar gum is soluble in salt solutions that contain up to 70% by weight of monovalent cation salts. Guar's salt tolerance decreases for divalent cations. At high concentrations of calcium ions, guar precipitates from solutions (Maier et al., 1993).

Guar gum is reasonably stable under an acidic environment. Wang et al. (2000) investigated the stability of guar galactomannan in acidic conditions using dilute polymer solutions of 25, 37 and 50°C. The lowest pH values at which guar remained stable were found to be 2.0, 3.0 and 3.5, respectively, at these temperatures. The viscosity of fully hydrated guar gum solutions at acidic pH was slightly lower than at neutral pH even when no degradation occurred.

1.3.3 Applications

Guar gum provides economical thickening for a wide variety of food products (Whistler & BeMiller, 1999). Guar has been extensively applied in dairy products, prepared meals, bakery products, sauces, and pet food. In ice cream and other related dairy products, guar used as a stabilizer. Guar gum prime functions are to bind water, prevent ice crystal growth, improve mouthfeel, reduce the chewiness produced by a combination of carrageenan and locust bean gum, and slow meltdown. Guar gum reduces syneresis in processed cheese. Because of its high viscosity, heat stability, and low cost, guar gum extensively used in canned and intermediate-moisture pet foods. In bakery products such as specialty breads, cakes, and doughnuts, guar improves mixing and recipe tolerance, and shelf life through moisture retention. Guar gum prevents syneresis in frozen foods and pie fillings and controls spreadability in prepared icings (Whistler & BeMiller, 1999).

1.4. Models Describing the Interaction of Xanthan with Galactomannans

A synergistic interaction occurs between xanthan gum and galactomannans such as guar, locust bean gum (LBG), and tara gum. The interaction results in enhanced viscosity or gelation. Rocks (1971) first reported the synergistic interaction between galactomannans and xanthan. Although a few researchers (Kovacs, 1973; Schorsch et al., 1995) have invoked the concept of incompatibility to explain the gelation mechanism between xanthan and galactomannans, there is a considerable body of evidence that supports intermolecular binding between xanthan and galactomannans (McCleary & Neukom, 1982; Cairns et al., 1986; Cairns et al., 1987; Cheetham et al., 1986; Cheetham & Mashimba, 1988, 1991; Cheetham & Punruckrong, 1989; Foster & Morris, 1994; Goycoolea et al., 1994). To date, the intermolecular binding mechanism between xanthan and galactomannans is still controversial, and different models have been proposed.

1.4.1. Unilever (Original) Model: interaction between ordered xanthan and galactomannans

The first model was projected by Morris et al. (1977) and Dea et al. (1977) who proposed the intermolecular binding concept rather than mutual exclusion to explain the gelation mechanism between xanthan and galactomannans. The authors proposed that the synergistic interaction between xanthan and galactomannan is based on a cooperative interaction, depending on the fine structure of the galactomannan. The intermolecular binding involves binding of unsubstituted regions (smooth regions) of the galactomannan to the ordered xanthan helical structure (Fig.1.4). The gel strength of galactomannan-xanthan mixture increased as the galactose content of the galactomannan decreased. Morris et al. (1977), from Unilever's Colworth laboratory, first proposed that gel network is formed by interaction between unsubstituted regions of the galactomannans backbone and the surface of the xanthan 5-fold helix. Dea et al. (1977) reported that xanthan-LBG gelation is thermally reversible, and show

sharp melting and setting behavior over a narrow range of temperature. Gel setting increased as the total polysaccharide increased, and showed a less dependence on the mixing ratio of the two polymers. Substitution of tara gum for LBG substantially formed a weaker gels, but did not significantly alter the gelation temperature, whereas LBG with reduced galactose content resulted in much stronger gels, and guar did not gel at all. Optical rotation measurements (Dea et al., 1977) for xanthan and galactomannans showed maintenance and stabilization of xanthan native conformation in the mixed gels with either tara gum or LBG, and in the presence of guar gum. The order-disorder transition for xanthan was still evident in the synergistic system, but shifted to higher temperature by 10 °C, thus suggesting stabilization of the xanthan helix by interaction with galactomannan.

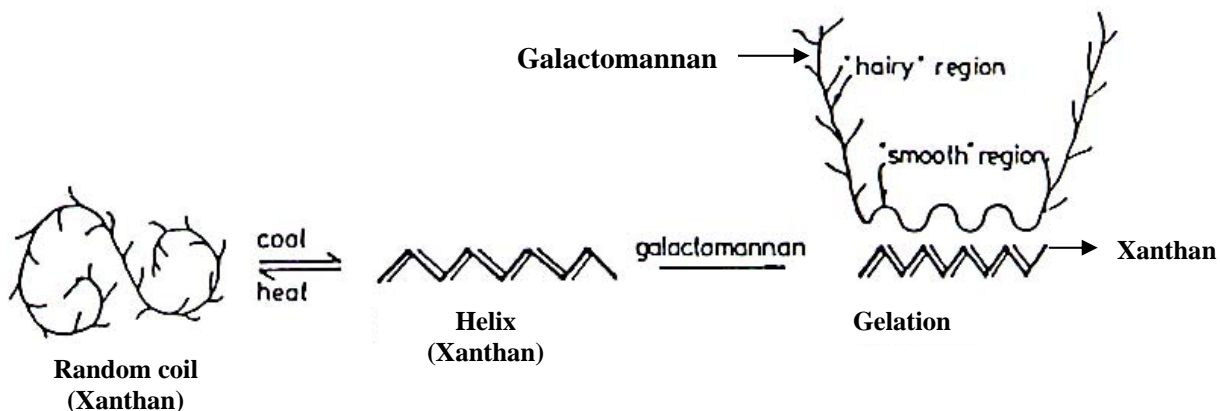


Fig. 1.4. A schematic model to explain xanthan-galactomannans gelation mechanism which involved binding the unsubstituted “smooth” galactomannan backbone with the ordered xanthan helix (modified from Dea et al, 1977).

McCleary et al. (1979) modified the above model in order to explain the strong interaction between xanthan and certain high-galactose galactomannans (Fig. 1.5). A strong interaction was found between xanthan and a galactomannan from *Leucaena leucocephala* which has a relatively high galactose content (M:G 1.6:1). The interaction was greater than would be expected based on galactose content which was similar to guar gum. Approximately one quarter

of the backbone of this galactomannan was composed of regions of alternate galactosyl substitution and was capable of participation in interaction with xanthan. Therefore, they suggested that the interaction involve the ordered xanthan and sequences along the mannan backbone where the galactosyl units are located on one side only. The modified model does not require long unsubstituted regions along the backbone in order to interact with xanthan, but rather regions where the galactosyl units are sited on one side of the mannan backbone.

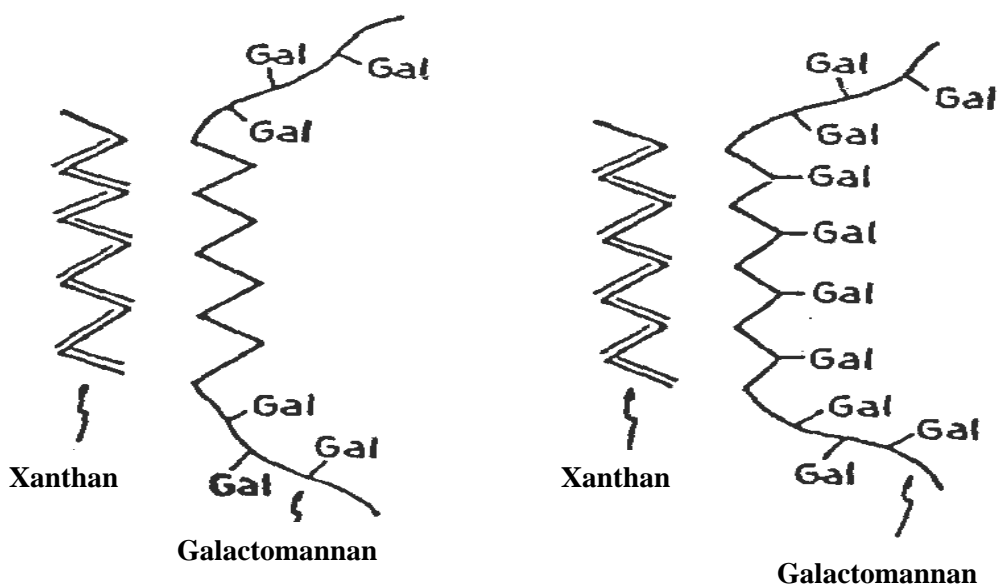


Fig. 1.5. An interaction of ordered xanthan chains with unsubstituted sides of a galactomannan main chain (modified from McCleary et al., 1979).

1.4.2. Norwich Model: Interaction between disordered xanthan and galactomannan

The second model for the gelation of xanthan and galactomannans was proposed based on X-ray diffraction studies (Cairns et al., 1986 & 1987), in which the xanthan helix does not retain an ordered conformation during the interaction with a galactomannan. The association of galactomannan occurs only when the xanthan chain is in the disordered state, and the resulting heterotypic junctions involve direct binding of the galactomannan to the cellulosic backbone of xanthan in an extended conformation, rather than to the 5-fold helix.

Cairns et al. (1986 & 1987) found that mixing of xanthan solution in the helical conformation with LBG solution at room temperature did not form gelation and the x-ray fiber diffraction patterns showed reflections characteristic of xanthan alone with no evidence of LBG crystallization or LBG-xanthan co-crystallization. Gelation occurred only when heated to 95 °C which is above the xanthan helix-coil transition temperature and cooled to room temperature. The x-ray fiber diffraction patterns of the gels formed from heated samples were different from the cold-mixed samples, providing evidence for xanthan-LBG interaction. In order to determine whether heating merely enhanced mixing or was necessary to denature the xanthan helix, xanthan was mixed with LBG in the presence of sufficient calcium chloride to shift xanthan transition temperature to above 100 °C. When xanthan-LBG mixtures containing calcium chloride were heated to 95 °C and cooled to room temperature, no gelation occurred. Therefore, suggesting that denaturation of xanthan helix is necessary in order to intermolecular binding and gelation to occur (Fig. 1.6).

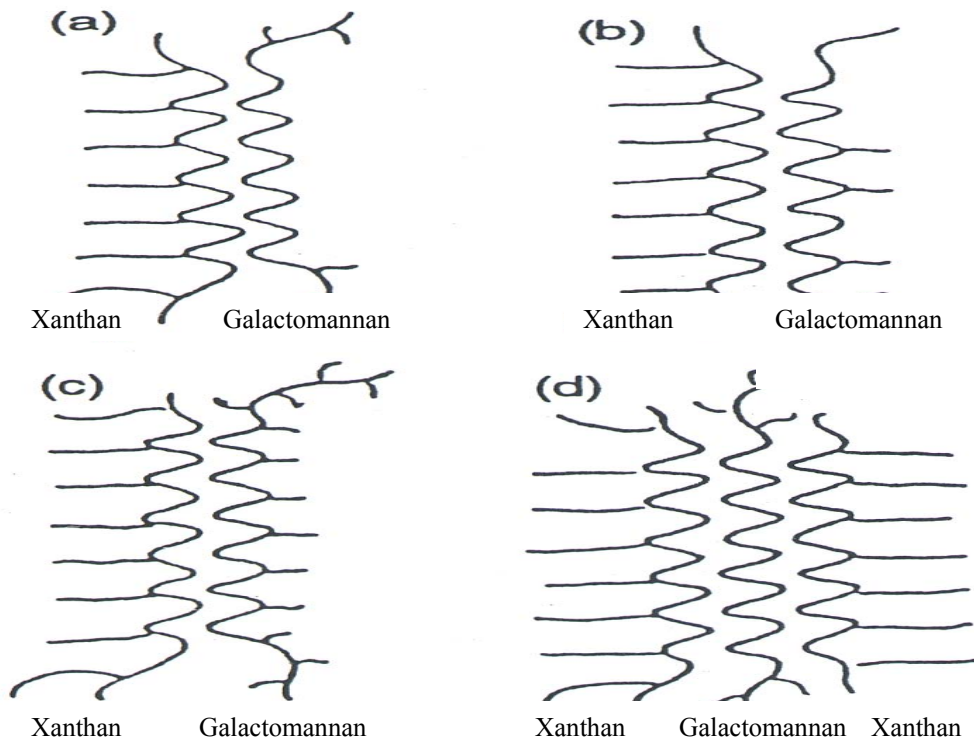


Fig. 1.6. Possible xanthan-galactomannan interactions. Possible binding between a xanthan backbone and (a) bare mannan regions of galactomannan backbone, (b) randomly substituted galactomannan, (c) galactomannan containing galactose on alternate mannose residue, (d) simplest xanthan-galactomannan sandwich structure (modified from Carins et al., 1986).

The authors disputed the original model based on optical rotation studies conducted by Dea et al. (1977). Optical rotation studies of xanthan-galactomannans mixed gels showed substantial recovery of optical rotation on cooling and gelation, suggesting that if the intermolecular binding involved the denatured xanthan, only small parts of the xanthan molecule could be involved in binding and the remainder reformed into a helical structure. X-ray diffraction patterns obtained from the mixed gels indicated that the position of the meridional reflection corresponded to an interplanar spacing of 0.52 nm. This is equivalent to half the axial advance per repeat unit of cellulose and mannan. Other X-ray diffraction patterns would be expected to give an axial advance per repeat unit of 1.04 nm. Therefore, a sandwich structure, in

which the positions of the xanthan side-chains are staggered, could explain the observed repeat distance of 0.52nm. The exact junction zone stoichiometry was not obtained, and several galactomannans molecules may be sandwiched between xanthan backbones.

This model was further supported by enzymatic studies (McCleary & Neukom, 1982), gel permeation studies (Cheetham & others, 1986), gel melting points and/or optical rotation studies (Cheetham & Mashimba, 1988 & 1991). Cheetham et al. (1986) and Cheetham & Mashimba (1988 & 1991) proposed that the interaction occurs between the disordered segments of the xanthan chains and galactomannan. Cheetham & Mashimba (1988) argued that even in the presence of salt where xanthan is in the ordered conformation, there are still disordered regions of xanthan that available to interact with galactomannan. Xanthan molecules in the presence of salt is not as single, largely-disordered chains as in water, but partly as coaxial or side by side helices attached by some single-chain regions which have disordered side chains. Once the interaction occurred, additional salt restored the ordered conformation in the non-junction zones segments of the xanthan chains and locked the junction zones in place. Optical rotation experiments showed that partially dialyzed xanthan against distilled water had less negative rotation than fully dialyzed xanthan, indicating that fully dialyzed xanthan is more disordered. The fully dialyzed xanthan formed a gel at room temperature, whereas the partially dialyzed did not. These results demonstrated that disordering xanthan side chains is critical for strong interaction with LBG to form a gel. LBG must be added when the disordered xanthan conformation is available for interaction, and prior to xanthan chains can self-associate. They reported that salt enhanced xanthan-xanthan interactions via pyruvate (Smith et al., 1981), side-by-side dimerization (Morris et al., 1983) or limited double-helix formation. Pyruvate-free xanthan did not form gel in cold water or even on heating and cooling. Acetate-free xanthan

formed gel with LBG when heated and cooled. These results suggested that pyruvate destabilizes the ordered conformation of xanthan (Holzwarth, 1979), whereas acetate stabilizes the molecule (Smith et al., 1981; Tako & Nakamura, 1984).

To further elucidate the mechanism of gel formation between xanthan and LBG, Cheetham & Mashimba (1991) conducted optical rotation studies to provide further evidence supports the proposal that intermolecular binding to form gel junction zones involves xanthan in the disordered conformation rather than in the ordered, helical form. In water, optical rotation measurements before heating indicated that xanthan-LBG mixture in water was less negative than that of xanthan alone. After heating to 60 °C, the optical rotation measurements were identical to that of xanthan alone. The results were interpreted as xanthan at room temperature is induced to take up the disordered conformation in the presence of LBG. Heating xanthan in presence of LBG and cooling to 25 °C left xanthan with greater amount of the disordered conformation than before heating. These results were maintained on prolonged storage and even after refrigeration. The xanthan-LBG junction zones were locked the polysaccharide chains in their appropriate conformations, and the chain regions that were not involved in the junction-zone formation returned to the more ordered conformation on cooling. In the presence of salt, fewer regions of xanthan were induced into a more disordered conformation as salt stabilized the ordered conformation and form junction zones with LBG.

Zhan et al. (1993), using optical rotation and rheological studies, investigated the effect of mixing temperature upon gelation of xanthan-LBG mixtures. The researchers reported that melting temperatures of gels (T_g) remained constant and were independent of ionic strength, whereas the helix-coil transition temperature (T_m) for xanthan alone increased as the ionic strength increased. When xanthan-LBG solutions were mixed below, above and between both T_g

and T_m , gelation occurred to all mixing temperatures, even when mixing temperature was under T_m . Storage modulus (G') was greater than the loss modulus (G'') at all mixing temperatures. At a certain ionic strength, increasing mixing temperature led to a significant increase in the G' which is attributed to the degree of disordering of the xanthan molecule at the mixing temperature. Based on these conclusions, the researchers suggested that the nature of the gelation determined by the level of disorder induced in xanthan before mixing and the level of interaction between xanthan and LBG would increase as the level of disorder induced in xanthan molecules increased. The order-disorder transition for xanthan was an equilibrium process and the degree of disordering was dependent on the temperature of the xanthan sample relative to T_m . Addition of LBG perturbed the helix-coil equilibrium process and acted as a denaturant for the xanthan helix. Thus, a favorable xanthan-LBG interaction would drive the xanthan coil-helix process to create more disordered xanthan. These results supported previous studies (Cairns et al., 1986, 1987) and rejected that gelation can be explained by two separate mechanisms (Williams et al., 1991; Mannion et al., 1992). Therefore, the researchers suggested a one single mechanism in which intermolecular binding occurred between galactomannan and the disordered segments of the xanthan chains for both hot setting and cold setting gels.

1.4.3. Tako Model: Interaction between xanthan side chains and galactomannans

The third model was proposed by Tako et al. (1984), Tako et al. (1985), and Tako (1991) in which the intermolecular binding occurs between the side chains of xanthan in the helical form and backbone of the galactomannans as in a lock and key effect (Fig. 1.7).

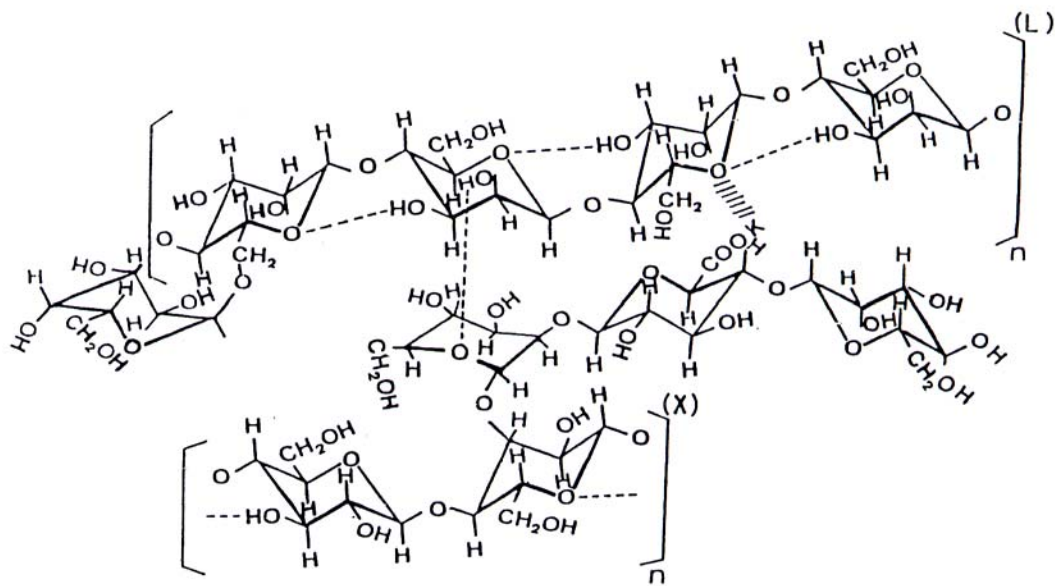


Fig. 1.7. Possible binding sites for D-mannose-specific interaction between deacetylated xanthan (X) and locust bean gum (L). Dotted lines refer to hydrogen bonding and the broken line to electrostatic attraction (Tako, 1991).

The researchers suggested that the side chains of the xanthan inserted into adjacent unsubstituted regions of the galactomannan backbone, which adopted an extended two fold ribbon-like conformation, aligning parallel or antiparallel. In this mechanism, hydrogen bonding played a major role in the interaction and one xanthan chain can associate with two or more backbone of the galactomannan. The hemiacetyl oxygen atom of the inner mannose of the side chain of xanthan interacted with the OH-2 of mannosyl unit of the backbone of the galactomannan via hydrogen bonds. The cation K^+ which associated with the carboxyl oxygen atom on the glucuronic acid residue of the intermediate side-chain of the xanthan took place in the interaction with the hemiacetyl oxygen atom of the mannan backbone via an electrostatic attraction. Deacetylation of xanthan strongly increased the synergistic interaction with galactomannans at high concentration (0.2%) as revealed by dynamic viscoelasticity measurements. The side chains of xanthan become more flexible after deacetylation, thus the

intermolecular association between the side chains increased as the concentration increased, and the side chains were liable to interact with galactomannan. In this model the precise conformation in which xanthan interacts with galactomannans is unknown and results do not agree with X-ray studies.

1.4.4. Mutual Incompatibility

Doublier & Llamas (1991) studied the interaction of xanthan with LBG and guar gum using rheological techniques. They found that xanthan gum, even when present in very low proportions (1% of the total), has a pronounced influence on the rheological properties. Because this influence was the same for both guar gum and LBG, they pointed out that current models could not be used to explain interaction between xanthan and galactomannans. Therefore, they suggested an alternative explanation based on incompatibility and volume exclusion.

Shatwell et al (1991) studied the interaction between xanthan and guar gum using rheological and chiroptical studies. Oscillatory-shear measurements were determined upon mixed systems consisting of 0.5% (w/w) xanthan and 1.0% (w/w) guar gum in de-ionized water. Xanthan-guar mixtures had rheological properties of an entangled network rather than a gel, although low acetyl xanthan samples showed more gel-like characteristics, indicating weak intermolecular association. The enhanced viscosity of xanthan-guar mixture was due solely to the presence of topological entanglements and not to a more specific intermolecular interaction. They made the point that precipitation of one or both polymers at low temperatures as a result of mutual incompatibility could not be ruled out. Furthermore, they suggested that weak interaction might occur between xanthan molecules themselves, since this process would also be favored following deacetylation. Evidence from both rheological and chiroptical results indicated a possible weak interaction between some low-acetyl xanthans and guar. However, the nature of

this interaction whether to be molecular or thermodynamic in origin is still controversial and in active dispute.

Polarization microscopy has been used to study the structure of 1:1 xanthan-galactomannan (guar gum and LBG) mixtures in aqueous solution with total concentration ranging from 0.5 to 4% (Schorsch et al., 1995). Both xanthan solutions and xanthan-galactomannan mixtures exhibited birefringence areas but were more anisotropic than with xanthan gum alone. The results suggested that xanthan molecules organized themselves as liquid crystalline mesophases in definite enriched xanthan areas resulting from a concentration of xanthan inside these birefringent zones. Upon heating, this anisotropy disappeared at a temperature well below the helix-coil transition temperature of xanthan molecules, and the ordered helical structure of xanthan still existed, suggesting that xanthan molecules are no longer concentrated in specific areas but more evenly distributed in the medium. Therefore, the authors suggested that the concept of mutual incompatibility between the polysaccharides could be favored for the formation of a mixed network.

1.4.5. Other Studies

Mannion et al. (1992) suggested that gelation between xanthan and galactomannan may occur at temperatures below T_m and can be explained by two different mechanisms. Mannion et al. (1992) conducted rheological and ultracentrifugation studies on heated and unheated mixtures of xanthan with LBG, and temperature fractions of LBG with different mannose:galactose ratios. Solutions were mixed at room temperature in 0.1M NaCl. Synergistic interaction occurred between xanthan and LBG at room temperature, resulting in G' four times higher than that of xanthan alone. The maximum synergy occurred with mixtures containing 1:1 ratio and the interactions were independent of the galactomannan mannose:galactose ratio. Under such

temperature and ionic strength xanthan molecules are in the helical conformation as confirmed by Morris et al. (1977), and Norton et al. (1984). Thus the authors suggested that xanthan entirely interacted with LBG in the helical conformation, supporting the original model (Dea et al., 1977 and Morris et al., 1977). They also found that gelation occurred when mixtures heated to 60 °C (less than T_m for xanthan at 0.1M NaCl) and cooled to room temperature, where other workers (Cairns et al., 1986) found no gelation. The increase in G' by heating at temperatures below T_m was attributed to heating the mixtures above the melting temperature may allow more complete mixing and the input of the energy would facilitate interactions which are not possible for unheated mixtures. They concluded that xanthan and galactomannans interacted by two different mechanisms. One takes place at room temperature where the interaction with ordered xanthan gives weaker elastic gels with little dependence on the galactose content of the galactomannan. The second requires heating of the polysaccharide mixture and the interaction with denatured segments of the xanthan gives a stronger gel which is highly dependent on the side chains of the galactomannan.

Lopes et al (1992) studied the synergistic interaction between xanthan and guar gum using low shear viscosity measurements as a function of temperature. At a total polymer concentration of 0.5g/L in 0.02 M NaCl, a small synergistic effect was observed when the two polysaccharides were mixed, but a much stronger interaction was noted in the absence of salts. Gelation was observed for temperatures lower than 15°C for native-guar mixture (weight ratio 1/1) in 0.02M NaCl and at 22-24°C for the same mixture in water. For a mixture of deacetylated xanthan and guar gum, gelation was observed at a temperature below 26°C in water. They concluded that there is a stronger interaction between deacetylated xanthan and guar gum

than native xanthan and guar gum because of enhanced deacetylated xanthan-guar gum backbone association as a result of removing the acetyl group from the side chain.

1.5. Rheology of Solutions

Rheology is the science of the deformation and flow of matter (Rao, 1999; Steffe, 1992). Rheological properties of food materials over a wide range of phase behavior can be expressed in terms of viscous, elastic, and viscoelastic functions. In terms of fluid and solid phases, viscous functions are used to relate stress with shear rate in liquid systems, whereas elastic functions are related to the appropriate stress function to strain in solids. Viscoelastic properties cover materials that exhibit both viscous and elastic properties (Rao, 1999).

1.5.1. Viscosity

When applying force on a volume of material, a displacement or deformation occurs (Fig. 1.8). The force per unit cross-sectional area is known as the stress (σ), and has units of Pascal (Pa), and the resulting fractional increase (or decrease) in height is known as the strain (ϵ), and this parameter is dimensionless (Morris, 1995). The rate at which this deformation is made (strain rate) has units of reciprocal time (s^{-1}). Strain is the relative deformation of materials when a stress is applied. When a material encounters a shear stress, a shear strain is observed. If two plates of area A , separated by fluid-filled distance H , are moved at velocity V by a force F relative to each other, Newton's law states that the shear stress (the force divided by area parallel to the force, or F/A) is proportional to the shear strain rate V/H (Hoefer, 2004). The proportionality constant is known as the viscosity (η). The effect of (shear strain) is quantified by the displacement the displacement per unit height (D/H), and the rate of this effect (strain rate) is velocity per unit height (V/H). The viscosity is the internal friction of the fluid or its tendency to resist the flow and is defined by

$$\eta = \text{shear stress} / \text{strain rate} \quad (1)$$

The preferred units for viscosity are Pa s or mPa s.

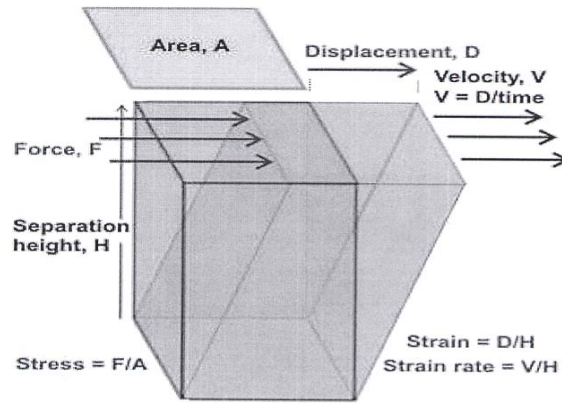


Fig. 1.8. Diagram showing a small cubical volume in shear. The displacement D due to deformation (Hoefler, 2004).

1.5.2. Viscoelasticity

All materials has viscoelastic behavior, but the viscous or elastic component may dominate depending on the situation. Methods to determine linear viscoelastic materials functions can be divided into static methods and dynamic methods (Steffe, 1996). Dynamic testing involves an application of an oscillatory shear stress and static methods involve creep or stress relaxation. Dynamic testing is a common method for studying the viscoelastic behavior of foods.

In dynamic tests, materials are subjected to deformation which varies harmonically with time (Fig. 1.9). When small-amplitude oscillatory shear test is used, the sample is subjected to a sinusoidal shear strain, γ , and the resulting shear stress, σ , is measured. The strain is a function of time defined as:

$$\gamma = \gamma_0 \sin(\omega t) \quad (2)$$

where γ_0 is the amplitude of the shear strain, and ω is the frequency. The input strain varies with the amplitude and frequency. With a small strain amplitude, the resulting shear stress is defined as

$$\sigma = \sigma_0 \sin(\omega t - \delta) \quad (3)$$

where σ_0 is the amplitude of shear stress and δ is the phase lag or phase shift. By decomposing the stress wave into two waves of the same frequency, two dynamic moduli, the storage modulus, G' , and the loss modulus, G'' , are introduced:

$$\sigma = G' \gamma + (G''/\omega) \dot{\gamma} \quad (4)$$

The complex modulus, G^* , can be obtained by taking the complex ratio of the shear stress σ_0 to the shear strain γ_0 . The storage modulus, G' , and the loss modulus, G'' , are the real and the imaginary component of the complex rigidity modulus, G^* , respectively:

$$G^* = \sigma_0/\gamma_0 = G' + iG'' = ((G')^2 + (G'')^2)^{1/2} \quad (5)$$

Similarly the complex viscosity, η^* , can be written as:

$$\eta^* = G^*/\omega = \eta' - i\eta'' = ((\eta')^2 + (\eta'')^2)^{1/2} \quad (6)$$

where η' is the viscous component (viscosity), η'' is the elastic component (elasticity) and i is an imaginary number. The complex viscosity is related to the complex modulus by

$$\eta^* = G^*/i\omega \quad (7)$$

or

$$\eta' = G'/\omega \text{ and } \eta'' = G''/\omega \quad (8)$$

Another function used to describe the viscoelastic behavior is the tangent of the phase shift ($\tan \delta$) which is a function of frequency:

$$\tan(\delta) = G''/G' = \eta'/\eta'' \quad (9)$$

If a material is an ideal elastic material (Hookean solid), the stress and strain are in phase and $\delta = 0$. Hence, G'' and η' are equal to 0 because there is no viscous dissipation of energy. If a material is an ideal viscous substance (an ideal Newtonian), the stress and strain are 90 degrees out of phase and $\delta = \pi/2$. Hence, G' and η'' are zero because the material does not store energy.

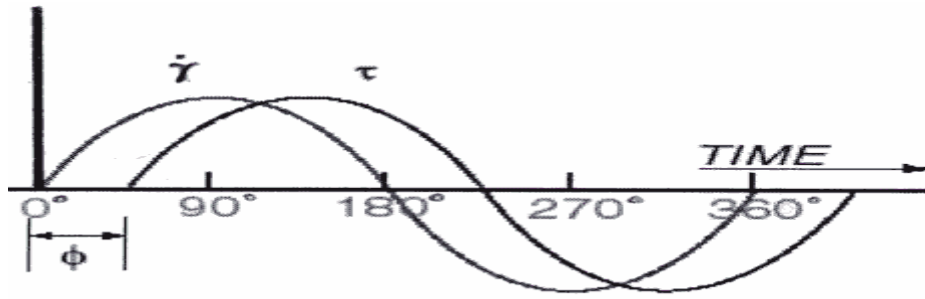


Fig. 1.9. Sinusoidal deformation of a cubical volume of fluid. The Sinusoidal time-varying shear rate and shear stress differ in phase by the angle phi (www.Vilastic.com).

The Deborah number (N_{De}), a dimensionless number, can be used to measure the degree of viscoelasticity. If $N_{De} \ll 1$, the stress is proportional to the viscosity times the shear rate and the materials behave as a viscous liquid. On the contrary, if $N_{De} \gg 1$, the stress is proportional to the modulus of rigidity times the strain and the materials behave like an elastic solid. Material will show viscoelastic behavior if $N_{De} = 1$. (Steffe, 1996).

Deborah number is defined as:

$$De = t_D / t_0 \quad (10)$$

where t_D is time of the deformation (relaxation) and t_0 is time of observation. The time t_D for liquids is very small and is very large for solids. Small Deborah numbers ($t_D < t_0$) mean liquid-like behavior and we can see the material flowing, while large numbers ($t_D > t_0$) mean solid-like behavior (Rao, 1999).

1.5.3. Fluids Flow Behavior

Fluids flow behavior can be divided into Newtonian and Non-Newtonian

1.5.3.1. Newtonian Behavior

In Newtonian fluids, the flow behavior is independent on the shear rate and shear histories, only dependent on both the temperature and composition (Rao 1977a, b). Typical Newtonian foods (Rao, 1999) are those containing compounds of low molecular weight (e.g. sugars) and that do not contain large concentrations of either dissolved polymers (e.g. pectins, proteins, starches) or insoluble solids. Some examples of Newtonian foods include water, milk, filtered juices, sugar syrups, honeys, carbonated beverages, and edible oils (Rao, 1999).

1.5.3.2. Non-Newtonian Behavior

Non-Newtonian flow behavior can be divided into time independent and time dependent. Time independent flow behavior may depend only on shear rate and not on the duration of shear, whereas time dependent flow behavior depends also on the duration of shear (Rao, 1999).

1.5.3.2.1. Time Independent

Shear-Thinning Behavior- Shear-thinning fluids are also referred to as pseudoplastic. With shear-thinning fluids, the curve (Fig. 1.10) begins at the origin of the shear stress-shear rate plot but in concave upward; that is, an increasing shear rate gives a less than proportional increase in shear stress (Rao, 1999). The viscosity (Fig. 1.11) of the fluids decreases with increasing shear rate (Steffe, 1996). This type of fluids can be reversible (Howard, 1991). The reason for shear-thinning may be due to breakdown of structural units in a food due to the hydrodynamic forces generated during shear (Rao, 1999). Most non-Newtonian foods exhibit shear-thinning behavior, including many salad dressings and some concentrated fruit juices.

Shear-Thickening Behavior- This type of behavior is also known as dilatant. In shear-thickening behavior, the curve (Fig. 1.10) also begins at the origin of the shear stress-shear rate plot but in concave downward; the increasing shear stress gives a less than proportional increase in shear rate (Rao, 1999). The viscosity (Fig. 1.11) of a fluid increases with increasing the shear rate

(Steffe, 1996). The reason for the shear-thickening fluids may be due to the increase in the size of the structural units as a result of shear. A typical example of shear-thickening is partially gelatinized starch dispersions (Rao, 1999).

Yield Stress- The flow of some materials does not begin until a threshold value of stress; yield stress, is exceeded (Rao, 1999). Steffe (1996) defined the yield stress as the minimum shear stress that is required to initiate flow. Bingham plastic and Herschel-Bulkley models (Fig. 1.10) are examples of yield stress. When the shear rate-shear stress data follow a straight line with a yield stress, the food is considered to follow Bingham plastic model. Examples of foods that have shear-thinning with yield stress behavior include tomato concentrates, tomato ketchup, mustard, and mayonnaise.

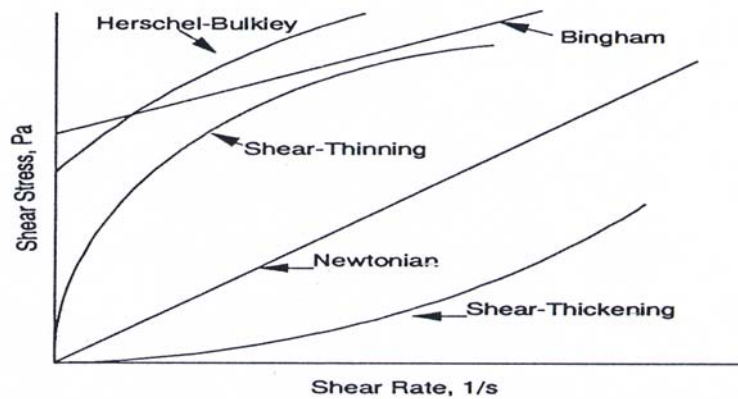


Fig. 1.10. Curves for typical time-independent fluids (Steffe, 1996).

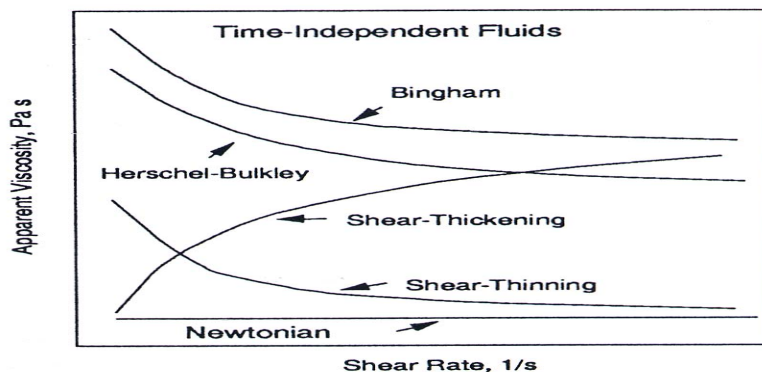


Fig. 1.11. Apparent viscosity of time-independent fluids (Steffe, 1996).

1.5.3.2.2. Time-Dependent Behavior

Time-dependent flow behavior can be divided into thixotropic and antithixotropic (rheopectic) (Fig. 1.12).

Thixotropic Behavior- Foods that exhibit time-dependent shear-thinning behavior are called to have a thixotropic flow behavior (Rao, 1999). In thixotropic behavior, the viscosity decreases as a function of time at a constant temperature and shear rate (Rao, 1977a). When the hydrodynamic forces during shear are sufficiently high, the interparticle linkages are broken, resulting in reduction in the size of the structural units which means lower resistance to flow during shear (Mewis, 1979). Foods that exhibit thixotropic behavior are usually heterogeneous systems containing a dispersed phase (Rao, 1999). Common examples of foods containing this type of behavior are salad dressings and soft cheeses.

Antithixotropic Behavior- Time-dependent shear-thickening behavior is called antithixotropic behavior (Rao, 1999). In antithixotropic behavior, the viscosity increases as a function of time in antithixotropic behavior (Rao, 1977a).

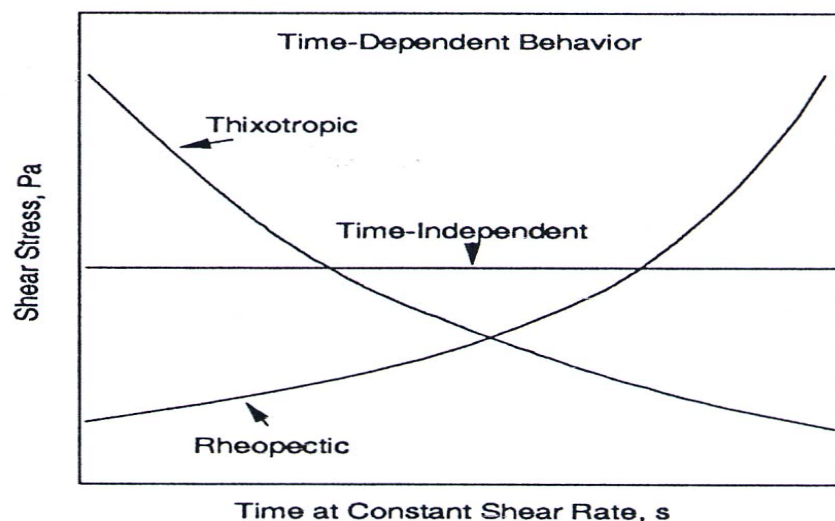


Fig. 1.12. Time-dependent behavior of fluids (Steffe, 1996).

1.5.4. Concentration Regimes

The concentration regimes can be divided into dilute ($C < C^*$), semi-dilute ($C^* < C < C^\ddagger$), and concentrated ($C > C^*$) solutions (Tirrell, 1994). The viscosity of polymer solutions shows a marked increase at critical polymer concentration, commonly referred to as C^* , corresponding to the transition from the dilute region to the semi-dilute region. At low concentrations ($C < C^*$), the polymer chains are not in contact with each other and moving independently without interpenetration, the polymer coils have infinite dilution radii, and the viscosity is relatively low (Fig.1.13). At the overlap threshold concentration ($C = C^*$), the coils begin to overlap without contraction. In the semi-dilute region, the coils contact each other and interpenetration occurs, and polymer chains reach minimum dimensions at a concentration C^\ddagger that is independent of the molecular weight (Rao, 1999).

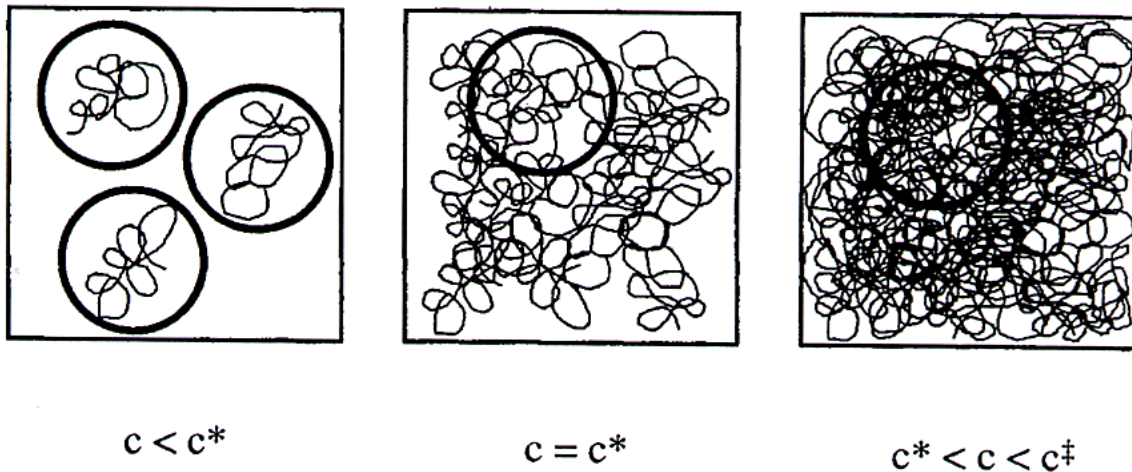


Fig. 1.13. Polymer chain entanglement in dispersions (Macosko, 1994).

Morris et al., (1981) studied the concentration dependence of specific viscosity for many random-coil polysaccharide solutions. From the double logarithmic plots of η_{sp} against C , a pronounced increase was observed above a specific critical or coil overlap concentration (C^*) (Fig. 1.13).

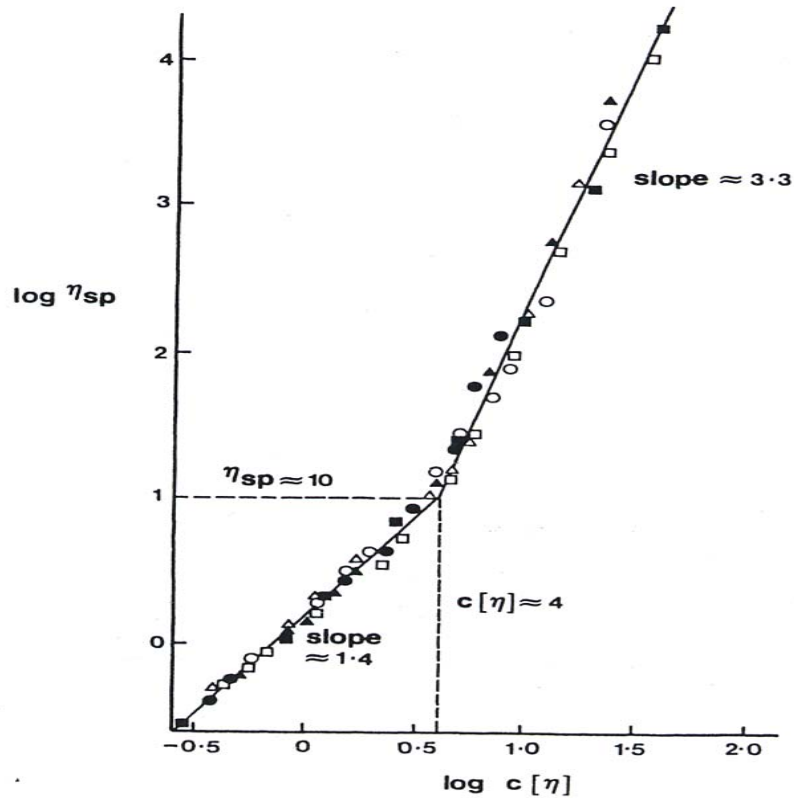


Fig. 1.14. Plot of $\log c[\eta]$ (coil overlap parameter) vs. $\log \eta_{sp}$ (specific viscosity) for random-coil polysaccharide solutions (Morris et al., 1981).

This behavior was attributed to the transition from a dilute regime, in which individual polymer molecules were isolated coils, to a concentrated regime, in which the total hydrodynamic volume of the individual chains exceeded the volume of the solution. Thus, C^* marks the onset of significant coil overlap and interpretation (Morris et al., 1981). Intrinsic viscosity varies with coil dimensions for random-coil polymer solutions, and the hydrodynamic volume is proportional to $[\eta]$ and molecular weight (M_r). Because the total number of coils is proportional to $C/[\eta]$, the degree of occupancy of space can be assessed by the dimensionless coil-overlap parameter ($C[\eta]$) (Morris et al., 1981). For random-coil polysaccharide solutions except for galactomannans, Morris et al. (1981) reported that the slope of double logarithmic plots of η_{sp} against $C[\eta]$ was close to 1.4 in a dilute regime, whereas, in the concentrated regime,

the slope increased to 3.3. The C^* transition occurred at a value of $C[\eta]$ close to 4, and the specific viscosity at this degree of coil overlap was invariably close to 10. LBG and guar gum biopolymers were found to deviate from the above observations. The C^* transition occurred at a lower value of the coil overlap parameter $C[\eta] = 2.5$, and the viscosity showed a higher dependence on concentration with slope of 5.1 instead of 3.3 (Morris et al., 1981). These deviations were attributed to specific intermolecular associations (hyperentanglements) between regular and rigid chain sequences in addition to the simple process of interpretation (Rao, 1999). Launay et al. (1986) suggested that there could be two transitions before the onset of high concentration-viscosity behavior, instead of one transition as suggested by Morris et al. (1981),

1.5.5. Intrinsic Viscosity

Intrinsic viscosity, denoted as $[\eta]$, is a useful experimental parameter in the studies of dilute solutions. It is the volume occupied by the individual polymer molecules in isolation (Richardson and Kasapis, 1998). In dilute solutions, the polymer chains are separate, and the $[\eta]$ of a polymer in solution depends only on the dimensions of the polymer chain. Because the $[\eta]$ indicates the hydrodynamic volume of the polymer molecule and is related to the molecular weight and to the radius of gyration, $[\eta]$ provides deep insights on the molecular characteristics of a biopolymer (Rao, 1999). Dilute solution viscosity data can be used to determine the $[\eta]$ as the zero concentration-limit of specific viscosity (η_{sp}) divided by concentration (C):

$$[\eta] = \lim_{C \rightarrow 0} (\eta_{sp}/C) \tag{11}$$

Where the quantity (η_{sp}/C) is defined as the reduced viscosity; specific viscosity $(\eta_{sp}) = (\eta - \eta_s)/\eta_s$; $\eta_s = \eta_{rel} - 1$; relative viscosity $(\eta_{rel}) = \eta/\eta_s$ and η and η_s is the apparent viscosities of the solution

and the solvent, respectively. Huggins (1942) (2) and Kraemer's (1938) (3) equations are common methods used for determining the $[\eta]$ of food gums from experimental dilute solution viscosity data.

$$\eta_{sp}/c = [\eta] + k' [\eta]^2 c \quad (12)$$

$$\ln (\eta_{rel})/c = [\eta] + k'' [\eta]^2 c \quad (13)$$

where k' and k'' are constants known as Huggin's and Kraemer's constants. The extrapolations to zero concentration may be done by plotting η_{sp}/c versus c and $\ln (\eta_{rel})/c$ versus c which would result in straight lines, respectively. The extrapolation to $[\eta]$ is usually done when the η_{rel} values are between 1.2 and 2.0 and the experimental values of η_{sp} in the range 0.2 to 1.0. The Huggins constant k' , 0.3 in good solvents and 1.0 in theta solvents, indicates the polymer-polymer interaction. The high values are reflects the existence of association between macromolecules (Rao, 1999).

For a flexible-chain polyelectrolyte (charged polymers), Fuoss & Strauss (1948) proposed an empirical expression:

$$\eta_{sp}/c = [\eta] / (1 + Bc^{1/2}) \quad (14)$$

where, B is a constant that accounts for the interaction of polyelectrolytes. By plotting (C/η_{sp}) vs. $C^{1/2}$ a linear relationship was found with an intercept of $1/[\eta]$ and slope of $B/[\eta]$.

Tanglertpaibul & Rao (1987) determined the intrinsic viscosity from the relative viscosity by using the expression:

$$\eta_{rel} = 1 + [\eta] c \quad (15)$$

The $[\eta]$ was obtained from the slope of η_{rel} vs. C plot, which gave straight lines with linear regression correlation coefficients in the range 0.99 to 1.0. Chou & Kokini (1987) suggested a

similar method for polyelectrolyte, in which the interactions between macromolecules in dilute solutions are not existent and the second term of the Huggins equation is negligible; therefore, a plot of η_{sp} vs. C is linear.

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Chapter 2

Influence of mixing temperature on xanthan conformation and interaction of xanthan-guar gum in dilute aqueous solutions

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Abstract

Dynamic viscoelastic and intrinsic viscosity properties of xanthan, guar, and xanthan-guar blends in dilute aqueous solutions were investigated by using an oscillating capillary rheometer. Influence of mixing temperature on xanthan conformation and interaction with guar is discussed. Synergistic interaction occurred at mixing temperatures of 25 and 80 °C, but a stronger synergistic interaction was observed at mixing temperature 80 °C. The viscous component for all gum solutions was greater than that of the elastic component, which indicated a liquid-like behavior in the dilute regime for the polysaccharide solutions. For both mixing temperatures, the relative viscosities and elasticities of xanthan and guar blends were higher than the relative viscosities and elasticities calculated for blends assuming no interaction. The intrinsic viscosities of xanthan and xanthan-guar blends were higher at 80 °C than at 25 °C. The intrinsic viscosities of xanthan and guar blends were lower than those calculated from the weight averages of the two, and significantly decreased as the xanthan fraction decreased, indicating that xanthan was crucial in controlling the blend viscosity, and that the molecular binding occurred between xanthan and guar.

Keywords: Xanthan, guar, synergistic interaction, viscoelastic, intrinsic viscosity, mixing temperature.

2.1. Introduction

Synergistic polysaccharide-polysaccharide interactions are attractive in the food industry because they impart novel and improved texture and rheological characteristics to food products and reduce polymers costs (Williams & Phillips, 2000). A synergistic interaction occurs between xanthan gum and galactomannans in solutions to result in enhanced viscosity or gelation (Dea & Morrison, 1975; Dea, Morris, Rees, Welsh, Barnes, & Price, 1977; Morris, Rees, Young, Walkinshaw, & Darke, 1977).

The synergistic interaction between xanthan and galactomannans was first reported by Rocks (1971), who pointed out that xanthan gum formed thermoreversible gels when mixed with locust bean gum (LBG), but not with guar gum. Although a few researchers (Kovacs, 1973; Schorsch, Garnier, & Doublier, 1995) have invoked the concept of incompatibility to explain the gelation mechanism between xanthan and galactomannans, there is a considerable body of evidence that supports intermolecular binding between xanthan and galactomannans (McCleary & Neukom, 1982; Cairns, Miles, & Morris, 1986; Cairns, Miles, Morris, & Brownsey, 1987; Cheetham, McCleary, Teng, Lum, & Maryanto, 1986; Cheetham & Mashimba, 1988, 1991; Cheetham & Punruckrong, 1989; Foster & Morris, 1994; Goycoolea, Foster, Richardson, Morris, & Gidley, 1994). To date, the intermolecular binding mechanism between xanthan and galactomannans is still controversial, and different models have been proposed.

The original model proposed a synergistic interaction between xanthan gum and galactomannans, based on a cooperative interaction, depending on the fine structure of the galactomannans. The intermolecular binding occurs between the ordered (helix) xanthan and unsubstituted or poorly substituted regions of the galactomannan backbone (Dea et al., 1977; Morris et al., 1977). The synergistic interaction is strongly dependant on the amount of galactose

content (Dea & Morrison, 1975) and its distribution to involve the regions of galactomannans backbone, where the galactose units are substituted on one side (McCleary, 1979).

Tako, Asato, & Nakamura (1984) and Tako (1991) proposed a different model in which the intermolecular interaction occurred between the side chains of xanthan and backbone of the galactomannans. They suggested that the side chains of the xanthan are inserted into adjacent unsubstituted regions of the galactomannan backbone, which adopted an extended, two-fold, ribbon-like conformation.

An alternative model was proposed by Cairns et al. (1986) and Cairns et al. (1987) in which intermolecular binding occurred between the disordered xanthan and galactomannans, and disordering of xanthan helical structure is necessary for gelation. They proposed that xanthan has a disordered, extended, two-fold, cellulose-like conformation, rather than a five-fold helix, when interacting with galactomannan. Cheetham et al. (1986) and Cheetham & Mashimba (1988, 1991) proposed that the interaction occurs between the disordered segments of the xanthan chains and galactomannan.

Mannion et al. (1992) suggested that xanthan and galactomannans gelation can be explained by two different mechanisms. One takes place at room temperature, at which the interaction with ordered xanthan gives weaker elastic gels with little dependence on the galactose content of the galactomannan. The second mechanism requires heating of the polysaccharide mixture, and the interaction with heat-disordered segments of the xanthan gives a stronger gel that is highly dependent on the side chains of the galactomannan. Zhan, Ridout, Brownsey, & Morris (1993) argued that gelation between xanthan and LBG can be explained only by a single mechanism. In agreement with Cheetham et al. (1986) and Cheetham & Mashimba (1988, 1991), they suggested that intermolecular binding involves binding of disordered segments of the

xanthan chain to LBG chains in hot mixing, in which the preparation temperature is higher than the transition temperature of xanthan, and in cold mixing, in which preparation temperature is lower, The authors also suggested that the extent of disorder induced in xanthan before mixing is the main factor in the interaction.

In all these studies, the extent of interaction between xanthan and galactomannan was indicated by the gel strengths, and the authors have used relatively high polymer concentrations ($> 0.1\%$). In dilute solutions, the individual polymer coils or rods are separate and free to move independently, and their intermolecular interactions are negligible. Thus, polymer-polymer interactions can be detected by the increase in solution viscosity and elasticity. In the present study, synergistic interaction between xanthan and guar was investigated in dilute aqueous solutions. An oscillatory capillary rheometer was used to determine viscoelastic parameters for polysaccharides solutions. The influence of mixing temperature on xanthan conformation and synergistic interaction with guar gum is discussed.

2.2. Materials and Methods

2.2.1. Materials

Xanthan gum and guar gum were purchased from Sigma (Sigma-Aldrich, St. Louis, MO).

2.2.2. Stock solutions preparation

The stock solutions of xanthan gum (0.1%, w/v) and guar gum (0.2%, w/v) were prepared by thoroughly dispersing the required amount of gum in deionized distilled water. The gum solutions were continuously stirred with a magnetic stirrer for 3 h at ambient temperature and heated for 30 min at 90 °C in a water bath to completely hydrate the gums. Guar gum was centrifuged at 3500g for 1 h to remove the insoluble molecules. The solutions were dialyzed

against deionized distilled water for 3 d by using a dialyzing tube (Snakeskin TM Pleated Dialysis Tubing, Pierce, Rockford, IL), which was designed to let through compounds with a molecular weight of 10 kDa or less. Stock solutions were refrigerated at 4 °C to minimize bacterial growth.

2.2.3. Preparation of mixed solutions of xanthan and guar gum

To study the interaction between the polysaccharides in dilute domain, the xanthan gum solution was diluted with deionized distilled water to a final concentration of 0.025%, whereas the guar solution was diluted to 0.075%. At these concentrations, we found that xanthan and guar gums are in the dilute domain. The gums were blended at the following ratios: 100% xanthan:0%Guar, 80% xanthan:20% guar, 60% xanthan:40% guar, 40% xanthan:60% guar, 20% xanthan:80% guar, and 0% xanthan:100% guar. Freshly prepared xanthan and guar solutions were mixed at 25 and 80 °C. For blends mixed at 80 °C, the appropriate amounts of the polysaccharide solutions were separately placed in beakers and heated at 80 °C for 30 min in a water bath and were stirred with a magnetic stirrer for 3 min. For blends mixed at 25 °C, the appropriate amounts of freshly prepared xanthan and guar solutions at 25 °C were stirred for 3 min. The concentration of xanthan and guar solutions was confirmed by the phenol-sulfuric method (Dubois, Giles, Hamilton, Rebers, & Smith, 1956).

2.2.4. Molecular weight determination of polysaccharides

The molecular weight of xanthan ($M_w = 2.65 \times 10^6$) and guar ($M_w = 1.45 \times 10^6$) was determined by high performance size-exclusion chromatography (HPSEC) coupled on line with a multiangle laser light scattering detector (MALLS) and a refractive index detector. The MALLS detector was a DAWN DSP-F laser photometer from Wyatt Technology Corp. (Santa Barbara, CA, USA). The Wyatt optilab DPS interferometric refractometer with a measuring

wavelength of 633 nm was used. A PL aquagel-OH Mixed 8 μ m column was used. A sample volume of 100 μ m/ ml was injected at flow rate of 1ml/min using 100 mM NaCl as the mobile phase at a temperature of 30 °C. The dn/dc used for xanthan was 0.145 and for guar was 0.13. The collected data were analyzed using Astra software version 4.70.07.

2.2.5. Density measurement

The densities of the polysaccharide solutions were determined with a standardized 10-mL pycnometer. The mass of the solution was calculated from the weight difference between the empty pycnometer (Kmax®, Kimble Glass Inc, Vineland, NJ) and the filled vessel. The picnometer filled with each respective gum solution was incubated at 20 °C for 1 h (Equatherm, Lab-Line Instruments Inc, Melrose Park, IL) to equilibrate the sample before density determinations (Yaseen, Herald, Aramouni, & Alavi, 2005).

2.2.6. Rheological properties

The viscous (η') and elastic (η'') components of the polysaccharide solutions and their blends were measured as a function of oscillating shear rate by using an oscillating capillary rheometer (Viscoelasticity Analyzer, Vilastic 3, Vilastic Scientific, Inc, Austin, TX). The instrument and theory of measurement have been described elsewhere (Thurston, 1960, 1976). The Viscoelasticity Analyzer is based on the principles of generating oscillatory flow at a selected frequency within a straight, cylindrical, stainless steel tube (0.0504 cm radius and 6.038 cm length). The Vilastic instrument produces an oscillatory flow in a capillary and measures the pressure and volumetric flow rate, allowing the determination of both viscous and elastic components of a fluid sample.

The complex coefficient of viscosity (η^*) consists of viscous (η') and elastic (η'') components, and is defined as:

$$\eta^* = \eta' - i\eta'' \quad (1)$$

where i is an imaginary number. The η' and η'' are related to dissipated and recovered energy, respectively. Similarly complex rigidity (G^*) is defined as:

$$G^* = G' + iG'' \quad (2)$$

Where G' is the storage modulus and G'' is the loss modulus. The complex coefficient of viscosity is related to the complex rigidity modulus by

$$\eta^* = G^* / i\omega \quad (3)$$

or $\eta' = G'' / \omega$ and $\eta'' = G' / \omega$

where the radian frequency $\omega = 2\pi f$, f is the frequency in Hertz.

The η' and η'' of dilute solutions of xanthan and guar were determined in the shear-rate range 0.8 to 30s^{-1} at a frequency of 2 Hz. Morris & Taylor (1982) reported that oral perception of solution viscosity correlated well with viscosity measurements at 10s^{-1} . Thus, all η' and η'' measurements were statistically assessed at 10s^{-1} . Rheological measurements were carried out at $20 \text{ }^\circ\text{C} \pm 0.1$ by using a temperature-controlled circulating water bath (Haake DC5, Gebr. Haake GmbH, Karlsruhe, Germany). The Viscoelasticity Analyzer was calibrated with deionized distilled water at $20 \text{ }^\circ\text{C}$ before the verification procedure to further ensure that the rheometer was operating at optimum accuracy.

2.2.7. Intrinsic viscosity determination

Intrinsic viscosity, denoted as $[\eta]$, is a useful experimental parameter in the study of dilute solutions. Intrinsic viscosity is a measure of the hydrodynamic volume occupied by the individual polymer molecules in isolation (Richardson & Kasapis, 1998). In dilute solutions, the polymer chains are separate, and the $[\eta]$ of a polymer in solution depends only on the dimensions of the polymer chain. Because $[\eta]$ indicates the hydrodynamic volume of the

polymer molecule and is related to the molecular weight, it provides deep insights on the molecular characteristics of a biopolymer (Rao, 1999).

One approach to determine the $[\eta]$ is through extrapolation to infinite dilution according to the Huggins (1942) and Kraemer (1938) empirical expressions below:

$$\frac{\eta_{sp}}{C} = [\eta] + k' [\eta]^2 C \quad (4)$$

$$\frac{\ln \eta_{rel}}{C} = [\eta] + k'' [\eta]^2 C \quad (5)$$

where the specific viscosity (η_{sp}) = $(\eta - \eta_s) / \eta_s = \eta_{rel} - 1$, the relative viscosity (η_{rel}) = η / η_s , and η and η_s are the apparent viscosities of the solution and the solvent, respectively. The extrapolations to zero concentration are done by plotting η_{sp}/C versus C or $\ln(\eta_{rel})/C$ versus C , which would result in straight lines, respectively. Tanglertpaibul & Rao (1987) determined the intrinsic viscosity from the relative viscosity by using the expression:

$$\eta_{rel} = 1 + [\eta]C \quad (6)$$

The $[\eta]$ was obtained from the slope of η_{rel} vs. C plot, which gave straight lines with linear regression correlation coefficients in the range 0.99 to 1.0. Chou & Kokini (1987) suggested a similar method for polyelectrolyte, in which the interactions between macromolecules in dilute solutions are not existent and the second term of the Huggins equation is negligible; therefore, a plot of η_{sp} vs. C is linear.

In this study, the $[\eta]$ was determined for each solution by measuring relative viscosities of polysaccharides solutions within the range $1.2 < \eta_{rel} < 2.0$ at $\gamma = 10s^{-1}$. The intercept of η_{sp}/C vs. C plot in the dilute region gave the first estimation of $[\eta]$ for guar gum, whereas the slope of η_{rel} vs. C plot gave the first estimation of $[\eta]$ for xanthan and xanthan-guar blends.

2. 2.8. Statistical analysis

A two-way factorial design was used for study of rheological properties. Three replications and two subsamples were performed. The analysis of variance (ANOVA) and general linear models procedure (GLM) were conducted with Statistical Analysis System (2002-2003) (version 9.1, SAS Institute, Inc., Cary, NC). Comparisons among treatments were analyzed by using Fisher's least significant difference (LSD), with a significance level of $P < 0.05$.

2.3. Results and Discussion

2.3.1. Flow Properties of Polysaccharides

The η' and η'' behavior as a function of shear rate of xanthan, guar, and their mixtures at 25 and 80 °C in water are shown in Figs. 2.1 through 2.4. In general, at both temperatures, no shear-rate viscosity dependence was observed over a range of shear rates up to 10 s^{-1} for xanthan and for xanthan:guar ratios of 4:1, 3:2, 2:3; Newtonian behavior was reached in the same range. At shear rates in excess of 10 s^{-1} , a pseudoplastic behavior was exhibited. Guar gum and a xanthan:guar ratio of 1:4 had no shear rate dependence over the whole range. Guar viscosity remained constant over the same range of shear rate. The guar behavior was consistent with η' results previously obtained for galactomannans over a larger range of shear rates ($10 < \dot{\gamma} < 350 \text{ s}^{-1}$) (Bresolin, Sander, Reicher, Sierakowski, Rinaudo, & Ganter, 1997). Shear-rate elasticity dependence was observed in the same range of shear rate, except for guar and xanthan:guar at a ratio of 1:4. At 80 °C (Fig. 2.4), guar elasticity was too low to be detected accurately by the viscoelastic analyzer. For all shear rates studied, the η' for polysaccharides was higher than the η'' , indicating that the polysaccharide solutions were in the dilute regime. Steffe (1996) reported that dilute solutions exhibited η' values larger than η'' values over the entire frequency range.

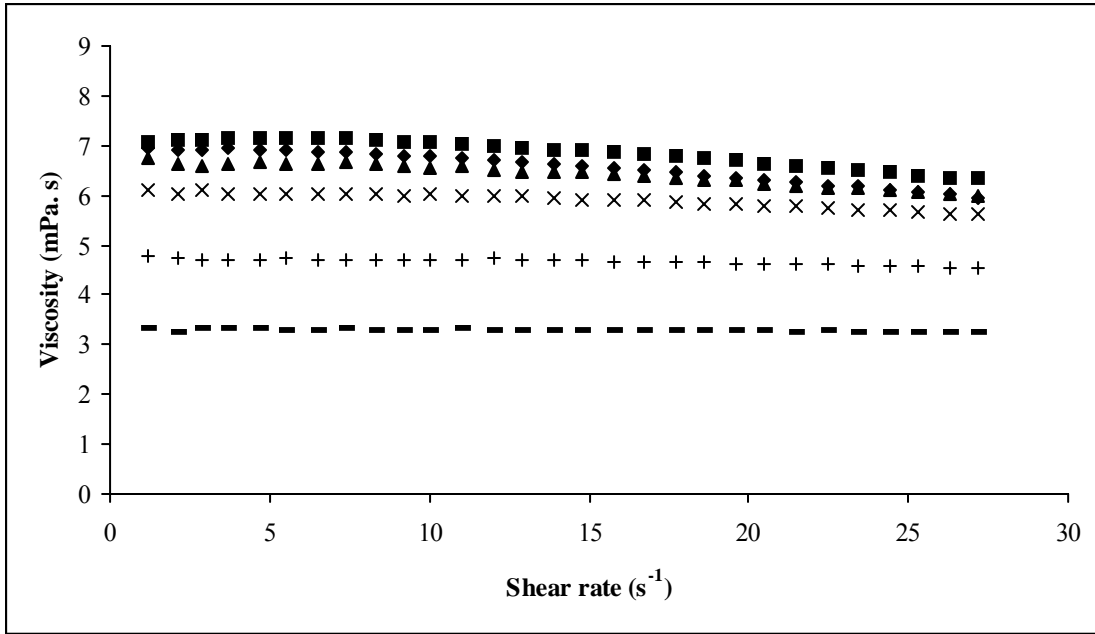


Fig. 2.1. Viscosity of xanthan, guar, and their mixtures against shear rate in water at mixing temperature 25°C. Xanthan:Guar ratio: (◆) xanthan, (-) guar, (■) 4:1, (▲) 3:2, (×) 2:3, (+) 1:4.

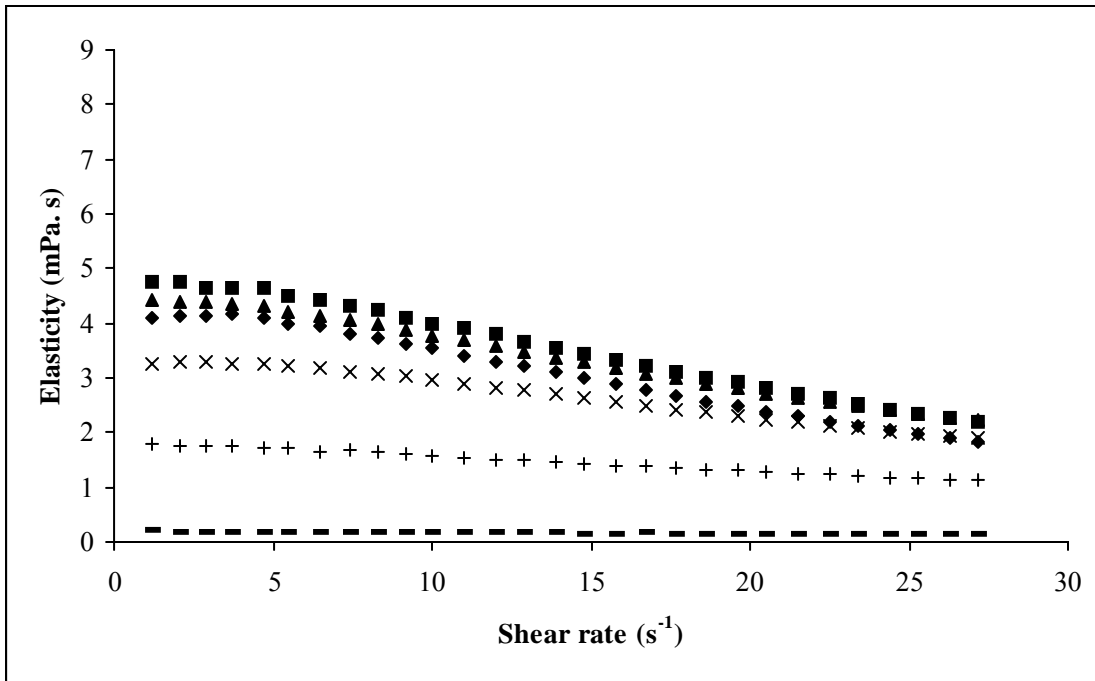


Fig. 2.2. Elasticity of xanthan, guar, and their mixtures against shear rate in water at mixing temperature 25°C. Xanthan:Guar ratio: (◆) xanthan, (-) guar, (■) 4:1, (▲) 3:2, (×) 2:3, (+) 1:4.

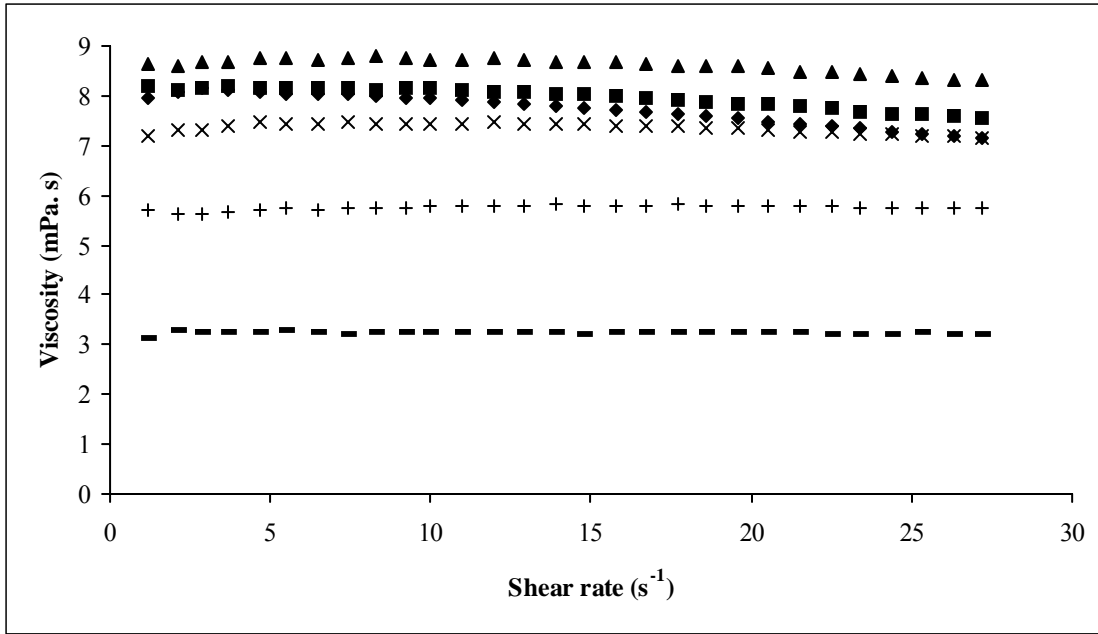


Fig. 2.3. Viscosity of xanthan, guar, and their mixtures against shear rate in water at mixing temperature 80°C. Xanthan:Guar ratio: (◆) xanthan, (-) guar, (■) 4:1, (▲) 3:2, (×) 2:3, (+) 1:4.

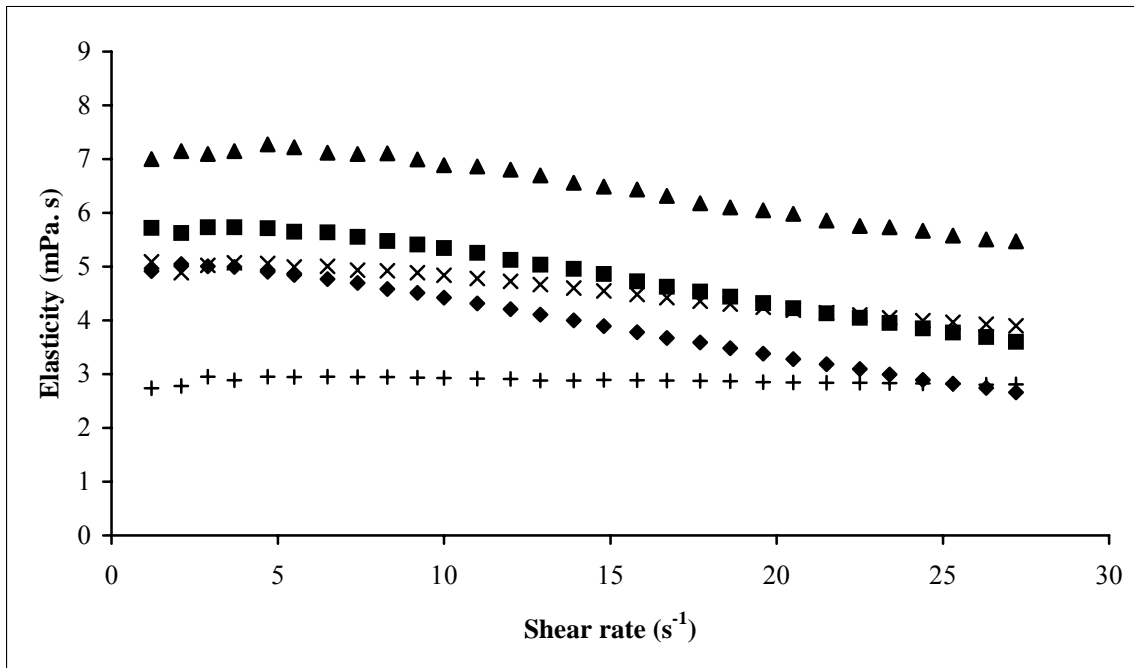


Fig. 2.4. Elasticity of xanthan, guar, and their mixtures against shear rate in water at mixing temperature 80°C. Xanthan:Guar ratio: (◆) xanthan, (■) 4:1, (▲) 3:2, (×) 2:3, (+) 1:4.

2.3.2. Dynamics of Polysaccharides Interactions

Figs. 2.1 through 2.4 depict the dynamic rheological parameters of xanthan, guar, and their blends as a function of shear rate. The synergistic interaction in the dilute domain was indicated by both η' and η'' measurements. Synergistic interaction between xanthan and guar was observed at both mixing temperatures, but the synergistic effect was much stronger at the 80 °C mixing temperature. Significant differences ($p < 0.05$) were found in η' and η'' measurements between the two mixing temperatures (Table 2.1). This is in agreement with previous studies in which hot-mixed blends showed stronger η' synergistic interactions in aqueous solutions (Goycoolea et al., 1994; Morris VJ, 1996; Wang, Wang, & Sun, 2002). Xanthan η' and η'' were higher at 80 °C because it was above the order-disorder temperature of xanthan (51 °C) in water (Williams, Day, Landon, Phillips, & Nishinari, 1991), which is consistent with previous results found by Dea et al. (1977). At a mixing temperature of 25 °C, the η' synergistic interaction was only noted at a xanthan:guar ratio of 4:1, whereas η'' synergistic interaction was observed at xanthan:guar ratios of 4:1 and 3:2. On the other hand, at a mixing temperature of 80 °C, the η' synergistic interaction was noted at xanthan:guar ratios of 4:1 and 3:2, whereas the η'' synergistic interaction was observed at all mixing ratios except for xanthan:guar ratio 1:4. Wang et al. (2002) reported that the η' synergistic interaction was only noted in a cold mixing at a xanthan:guar ratio of 2:3 and below a shear rate of 20 s^{-1} , and in hot mixing at xanthan:guar ratios of 1:4 and 3:2.

Fig. 2.5 shows the relative viscosity behavior of xanthan and guar blends in water at both mixing temperatures (25 and 80 °C). At both mixing temperatures, the relative viscosities of xanthan and guar blends were higher than the relative viscosities calculated for blends assuming

no interaction. These results show the effect of xanthan conformation on the increase of viscosity, reflecting that intermolecular interaction has occurred between xanthan and guar blends. These results are consistent with previous studies (Lopes, Andrade, Milas, & Rinaudo, 1992; Bresolin et al., 1997) that showed viscosities of xanthan and guar blends in water were higher than the calculated values assuming no interaction for xanthan and guar blends, supporting the intermolecular interaction. When xanthan and guar were blended at a mixing temperature of 25 °C, a smaller synergistic interaction was observed with the maximum xanthan:guar ratio of 4:1; at a mixing temperature of 80 °C, a stronger synergy was noted and the maximum synergistic interaction was obtained at a ratio of 3:2. The plot of the η'' component of xanthan-guar blends against polysaccharide ratio is shown in Fig. 2.6. For both mixing temperatures, the η'' differs with the xanthan:guar ratio. A stronger η'' synergy was observed for mixing at 80 °C. The maximum synergistic interaction was noted at a xanthan:guar ratio of 3:2 for blends mixed at 80 °C and at 4:1 for blends mixed at 25 °C.

Our results show the influence of xanthan conformation on the increase of η' and η'' , which are supposed to reflect intermolecular binding. In aqueous solutions, the structure of xanthan undergoes an 'order-disorder' transition from helix to coil structure. This conformational transition depends on temperature, ionic strength of solutions, nature of electrolyte, pH, and acetyl and pyruvate constituent contents (Holzwarth 1976; Morris et al., 1977; Baradossi & Brant, 1982; Paoletti, Cesàro, & Delben, 1983). Xanthan gum is in the disordered conformation at both mixing temperatures.

Table 2.1. Effect of mixing temperature on the dynamic viscoelastic parameters for xanthan, guar, and their blends measured at $\gamma = 10\text{s}^{-1}$.

Xanthan:Guar(%)	η' (mP. s)		η'' (mP. s)	
	25°C	80°C	25°C	80°C
100:0	6.78 ^a ± 0.15	7.94 ^b ± 0.22	3.55 ^a ± 0.11	4.42 ^b ± 0.21
80:20	7.05 ^a ± 0.05	8.14 ^b ± 0.21	3.99 ^a ± 0.21	5.34 ^b ± 0.41
60:40	6.57 ^a ± 0.06	8.72 ^b ± 0.28	3.77 ^a ± 0.28	6.89 ^b ± 0.35
40:60	6.09 ^a ± 0.17	7.44 ^b ± 0.38	2.99 ^a ± 0.09	4.84 ^b ± 0.27
20:80	4.71 ^a ± 0.06	5.79 ^b ± 0.56	1.58 ^a ± 0.02	2.93 ^b ± 0.25
0:100	3.24 ^a ± 0.14	3.24 ^a ± 0.02	0.16 ^a ± 0.03	***

^{a, b} Means followed by the same letters in the same row are not significantly different ($P < 0.05$).

Results are expressed as means ± SD for three replications.

*** Considered not detectable elasticity.

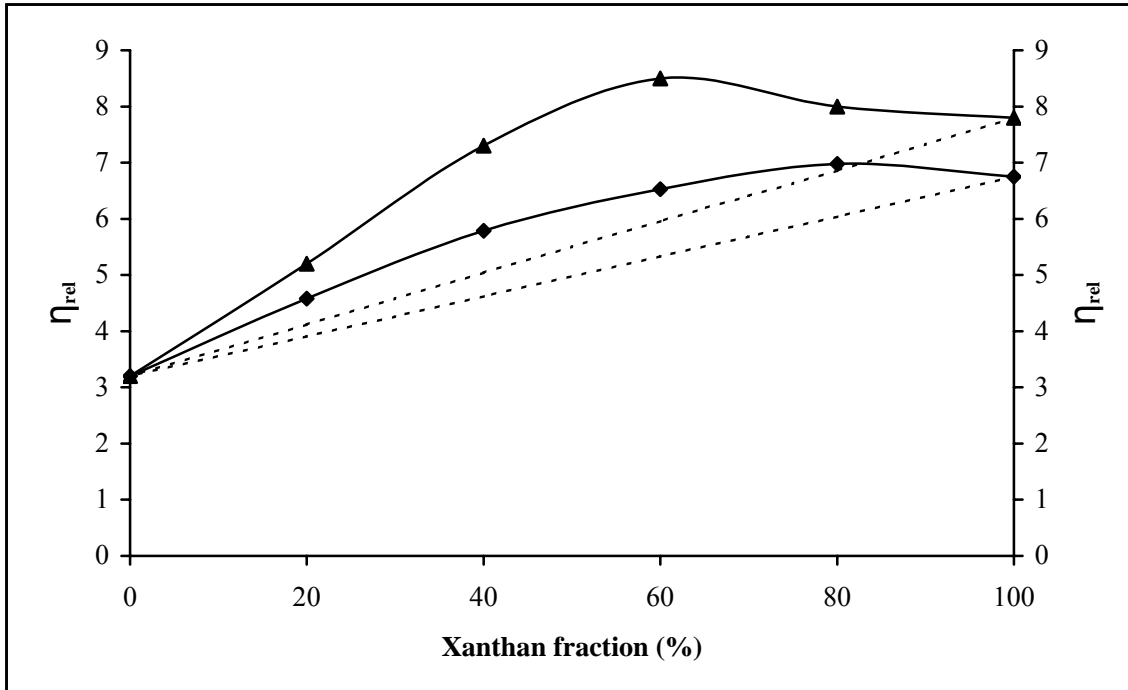


Fig. 2.5. Relative viscosity of xanthan, guar, and their mixtures in water: (♦) mixing at 25°C; (▲) mixing at 80°C; (---) values calculated for mixtures assuming no interaction.

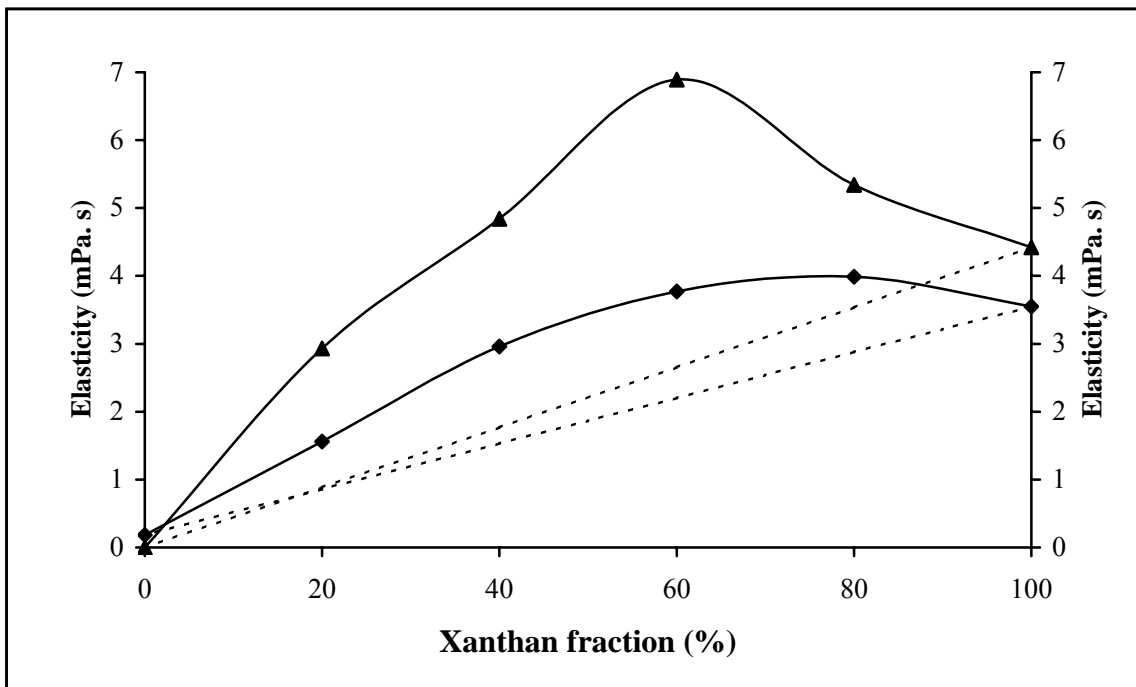


Fig. 2.6. Elasticity of xanthan, guar, and their blends in water: (♦) mixing at 25°C; (▲) mixing at 80°C; (---) values calculated for mixtures assuming no interaction.

Optical-rotation studies (Lecourtier, Chauveteau, & Muller, 1986; Milas & Rinaudo, 1986; Cheetham & Mashimba, 1988, 1991) have confirmed that xanthan is in the disordered conformation in water at room temperature. Electrostatic repulsions that involve glucuronate and pyruvate in the side chains are poorly shielded, thus favoring the disordered conformation (Cheetham & Mashimba, 1991). The increased η' and η'' at the mixing temperature of 25 °C, support a conclusion that the intermolecular interaction between xanthan and guar may have occurred (Morris VJ 1996; Cairns et al., 1986; Cairns et al., 1987; Cheetham & Mashimba, 1988, 1991; Zhan et al., 1993; Foster & Morris, 1994; Goycoolea et al., 1994). Mixing the polymers at 80 °C substantially enhanced the molecular associations between xanthan and guar, as indicated by the increase of the η' and η'' values. Heating xanthan above the order-disorder transition temperature (51 °C) of xanthan in aqueous water (Williams et al., 1991) further disordered xanthan, to a degree higher than that of disordering xanthan at the mixing temperature of 25 °C. Cheetham & Mashimba (1991) showed that heating xanthan and cooling to 25 °C left xanthan with a greater amount of the disordered conformation. Thus, the highly disordered xanthan was capable of directly interacting with guar to form heterotypic structures and a higher synergistic interaction (Zhan et al., 1993; Goycoolea et al., 1994; Morris, Brownsey, & Ridout, 1994; Morris E.R., 1996; Morris V.J., 1996). Because the synergistic interaction was stronger at the mixing temperature of 80 °C, our results suggest that the degree of disordering of xanthan is critical in xanthan-guar interaction in water, which may explain the differences in η' and η'' measurements at both mixing temperatures. These results support previous studies (Cheetham & Mashimba, 1988, 1991; Zhan et al., 1993) in which intermolecular binding occurs between galactomannans and disordered segments of xanthan.

2.3.3. Intrinsic Viscosities of Polysaccharides

Xanthan and some of xanthan-guar blends did not follow the Huggins equation at high dilution series, whereas the Huggins equation successfully fit the guar solutions. For neutral polysaccharide such as guar, the Huggins plot has an advantage of giving linear plots (Morris, 1995). Plots of the reduced viscosity (η_{sp}/C) against the concentration of xanthan and some of the xanthan-guar blends resulted in a nonlinear relationship at low xanthan and low xanthan-guar blend concentrations. Also, the Fuoss empirical equation (Fuoss & Strauss, 1948) that was suggested for a flexible-chain polyelectrolyte did not show the typical viscosity-concentration relationship of flexible polyelectrolytes for xanthan alone. Thus, it was impossible to determine the $[\eta]$ by extrapolation of experimental data, which prompted the use of the slope model (Tanglertpaibul & Rao, 1987) to determine the $[\eta]$ by plotting η_{rel} vs. C (equation 6). Figures 2.7 and 2.8 show relative viscosity as a function of concentration for xanthan, guar, and their mixtures at mixing temperatures of 25 and 80 °C, respectively. Straight-line relationships with large linear regression coefficients were obtained for xanthan and xanthan-guar blends in the relative viscosity range of 1.2 to 2. Similar results were found by Higiro, Herald, & Alavi (2006). Intrinsic viscosities of xanthan, guar, and their blends in water at both mixing temperatures are shown in Table 2.2. Intrinsic viscosity of polymers is dependent on their molecular weight and chain dimension (Flory, 1953). In dilute solutions, the polymer chains are separate, and thus $[\eta]$ of a polymer in solution depends only on the dimensions of the polymer chain (Rao, 1999).

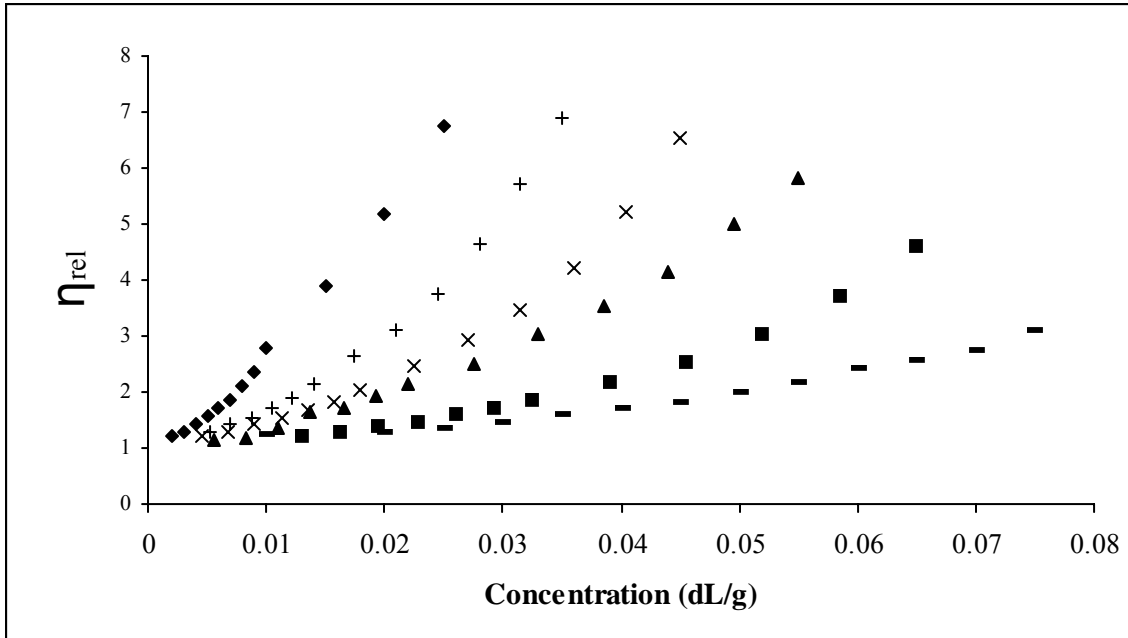


Fig. 2.7. Relative viscosities of xanthan, guar, and their mixtures against concentration in water at mixing temperature 25°C. Xanthan:Guar ratio: (◆) xanthan, (-) guar, (+) 4:1, (×) 3:2, (▲) 2:3, (■) 1:4.

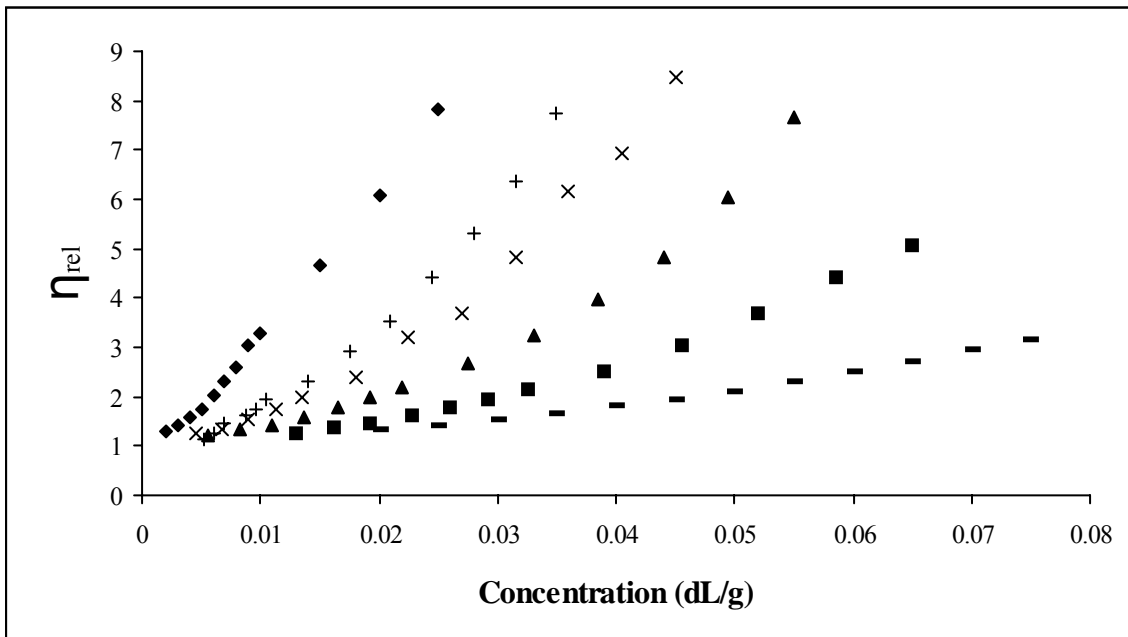


Fig. 2.8. Relative viscosities of xanthan, guar, and their mixtures against concentration in water at mixing temperature 80°C. Xanthan:Guar ratio: (◆) xanthan, (-) guar, (+) 4:1, (×) 3:2, (▲) 2:3, (■) 1:4.

Table 2.2 Effect of mixing temperature on the intrinsic viscosity and slope of the double logarithmic plot of η_{sp} against $c[\eta]$ for xanthan, guar, and their blends in the dilute domain

Xanthan:Guar(%)	Mixing at 25°C		Mixing at 80°C	
	$[\eta]$ (dL/g)	Slope	$[\eta]$ (dL/g)	Slope
100:0	155.7 ^a ± 9.3	1.38 ^d ± 0.02	173.0 ^a ± 6.0	1.32 ^a ± 0.02
80:20	82.2 ^b ± 2.7	1.50 ^b ± 0.01	134.8 ^b ± 4.7	1.68 ^b ± 0.02
60:40	63.1 ^c ± 1.7	1.44 ^c ± 0.02	82.7 ^c ± 5.9	1.55 ^c ± 0.02
40:60	56.9 ^c ± 7.2	1.55 ^b ± 0.02	55.5 ^d ± 2.2	1.51 ^d ± 0.02
20:80	32.8 ^d ± 2.8	1.68 ^a ± 0.04	38.8 ^e ± 2.5	1.60 ^e ± 0.02
0:100	12.0 ^e ± 2.1	1.44 ^{cd} ± 0.06	11.5 ^f ± 0.5	1.44 ^f ± 0.01

^{a, b, c, d, e} Means followed by the same letters in the same column are not significantly different ($P < 0.05$).

Results are expressed as means ± SD for three replications.

The $[\eta]$ of xanthan in water with mixing at 25 and 80 °C was 154 and 173 dl/g, respectively, whereas that of guar was 12 and 11.5 dl/g. Xanthan and guar intrinsic viscosities in water were comparable to those reported by Launay, Cuvelier, & Martinez-Reyes (1984, 1997). Xanthan gum had a much higher $[\eta]$ than guar, which can be attributed to the significant difference in their chain stiffness. Xanthan had a stronger chain stiffness which increased its chain dimensions. The stiff xanthan in water had a larger chain dimension than the flexible random coil conformation of guar, thus a higher $[\eta]$.

The intermolecular binding between xanthan and guar as a result of xanthan conformation can be further supported by the intrinsic viscosities of xanthan and guar blends (Fig. 2.9). At both mixing temperatures, the intrinsic viscosities were lower than those calculated from weight averages of the two individually (Wang, 2001), indicating that molecular binding

occurred between xanthan and guar. The intrinsic viscosities decreased as the xanthan fraction decreased in the blends. The intrinsic viscosities of xanthan-guar blends at a mixing temperature of 80 °C were higher than those at a mixing temperature of 25 °C, which could be attributed to the increased chain dimensions of xanthan. Heating xanthan to 80 °C would further disorder xanthan and increase its chain dimensions, thus a higher $[\eta]$. Because xanthan plays a crucial role in controlling the viscosity of xanthan-guar blends, the dramatic decrease of the intrinsic viscosities of their blends can be attributed to the conformational change of xanthan from a helical form to a more flexible form because of binding. Xanthan conformation change outweighed the increase of $[\eta]$ due to binding; thus, the dramatic decrease of the intrinsic viscosities of the blends was observed (Wang, 2001). These results are in agreement with previous studies in which the intermolecular binding between xanthan and galactomannan occurred (McCleary & Neukom, 1982; Cairns et al., 1986; Cairns et al., 1987; Cheetham et al., 1986; Cheetham & Mashimba, 1988, 1991; Cheetham & Punruckrong, 1989; Foster & Morris, 1994; Goycoolea et al., 1994; Wang, 2001).

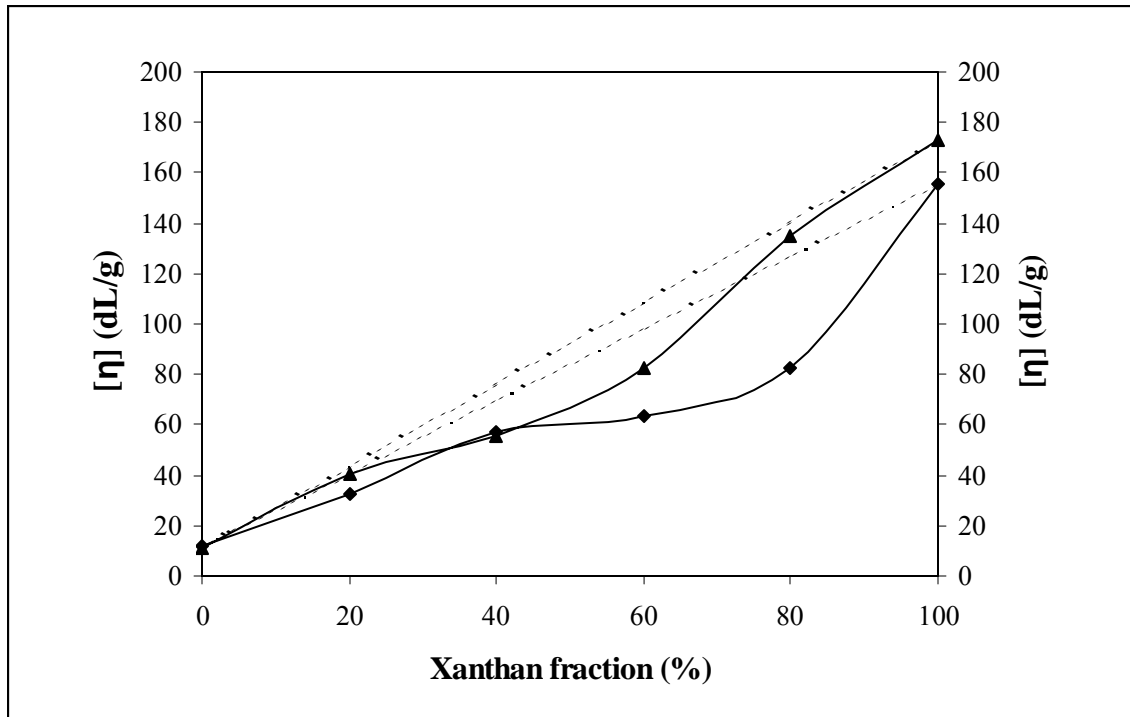


Fig. 2.9. Plots of intrinsic viscosities against xanthan:guar ratio. (▲) xanthan:guar blends mixed at 80°C, (◆) xanthan:guar blends mixed at 25°C, (---) intrinsic viscosity calculated from the weight averages of the two individuals, assuming no interaction.

2.3.4. Coil Overlap Parameter of Polysaccharides

Morris, Cutler, Ross-Murphy, Ress, & Price (1981) studied the concentration dependence of η_{sp} for many random-coil polysaccharide solutions. From the double logarithmic plots of η_{sp} against C , a pronounced increase was observed above a specific critical or coil overlap concentration (C^*). This behavior was attributed to the transition from a dilute regime, in which individual polymer molecules were isolated coils, to a concentrated regime, in which the total hydrodynamic volume of the individual chains exceeded the volume of the solution. Thus, C^* marks the onset of significant coil overlap (Morris et al., 1981). Intrinsic viscosity varies with coil dimensions for random-coil polymer solutions, and the hydrodynamic volume is proportional to $[\eta]$ and molecular weight (M_r). Because the total number of coils is proportional

to $C/[\eta]$, the degree of occupancy of space can be assessed by the dimensionless coil-overlap parameter ($C[\eta]$) (Morris et al., 1981).

To estimate the coil-overlap parameter for xanthan, guar, and their blends, a double logarithmic plot of η_{sp} against $C[\eta]$ was obtained (Figs. 2.10 & 2.11). As the concentration of polysaccharides increased, no change in the slope was obtained, indicating that xanthan, guar, and their blends were in the dilute domain. The plots of xanthan and xanthan-guar blends were significantly shifted to the right when the blends were heated to 80 °C, whereas the plots of guar solutions changed slightly with temperature. Doublier & Launay (1981) reported that master curves of galactomannans solutions were independent of temperature because the intrinsic viscosity of galactomannans did not vary with temperature. Xanthan and guar solutions in the dilute domain exhibited a very similar behavior to those reported by Morris et al. (1981). For random-coil polysaccharide solutions except for galactomannans, Morris et al. (1981) reported that the slope of double logarithmic plots of η_{sp} against $C[\eta]$ was close to 1.4 in a dilute regime, whereas, in the concentrated regime, the slope increased to 3.3 ± 0.3 . The C^* transition occurred at a value of $C[\eta]$ close to 4, and the η_{sp} at this degree of coil overlap was invariably close to 10. For LBG and guar gum, the C^* transition occurred at a lower degree of occupancy ($C[\eta]$ close to 2.5) (Morris et al., 1981). As shown in Table 2.2, the slope of a double logarithmic plot of η_{sp} against $C[\eta]$ for xanthan was 1.38 and 1.32 at 25 and 80°C, respectively, and for guar was 1.44 for both temperatures. Cuvelier & Launay (1986) found the limit of the dilute domain for xanthan at $C^* = 0.03$ g/dl, and the chains begin to overlap at a very low concentration of $C^* = 0.028$ g/dl and $c[\eta] = 1.4$.

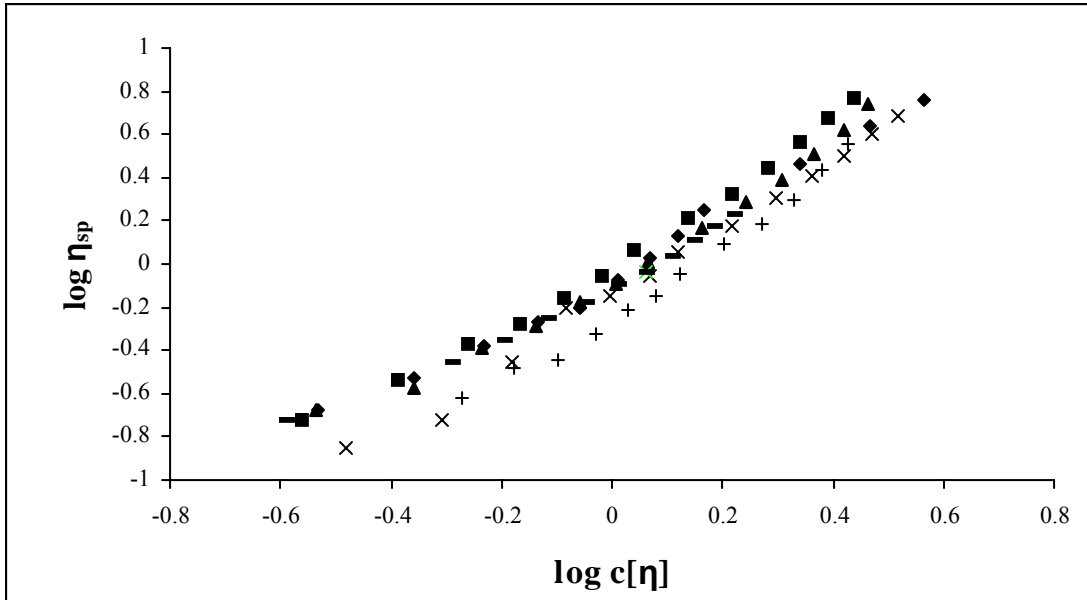


Fig. 2.10. Double logarithmic plot of specific viscosity(η_{sp}) against coil-overlap parameter ($c[\eta]$) for xanthan, guar, and their blends at mixing temperature 25°C. Xanthan:Guar ratio: (◆) xanthan, (■) guar, (▲) 4:1, (△) 3:2, (×) 2:3, (+) 1:4.

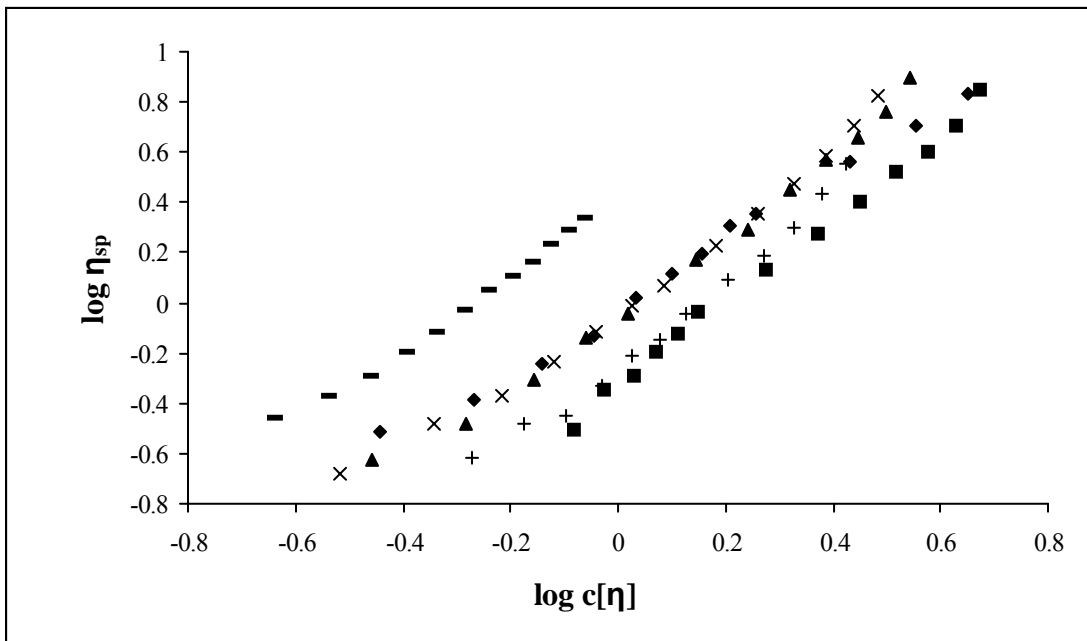


Fig. 2.11. Double logarithmic plot of specific viscosity(η_{sp}) against coil-overlap parameter ($c[\eta]$) for xanthan, guar and their blends at mixing temperature 80°C. Xanthan:Guar ratio: (◆) xanthan, (■) guar, (▲) 4:1, (△) 3:2, (×) 2:3, (+) 1:4.

Launay et al. (1997) showed that the double logarithmic plot for galactomannans gave a slope of 1.43 and $C[\eta]$ of 0.76 in the dilute domain. Our results showed that the slopes of xanthan and guar were lower than those reported by Morris et al. (1981), Cuvelier & Launay (1986), and Launay et al. (1997), demonstrating that both xanthan (0.025%) and guar gum (0.075%) were in the dilute domain ($C < C^*$). The slope of xanthan and guar blends ranged from 1.43 to 1.68 at the mixing temperature of 25 °C, and ranged from 1.51 to 1.7 at the mixing temperature of 80 °C. These slopes were relatively higher than slopes of either xanthan or guar alone, and this possibly could be attributed to the intermolecular binding between xanthan and guar gum.

2. 4. Conclusion

Synergistic interaction between xanthan and guar in water has been found at both mixing temperatures in dilute domain; a stronger synergistic interaction was noted at a 80 °C mixing temperature. The η' for all gum solutions was always greater than the η'' , which indicates liquid-like behavior in the dilute regime for the polysaccharide solutions. The differences in η' and η'' measurements between the two mixing temperatures could be attributed to the degree of disordering of xanthan. For both mixing temperatures, the relative viscosity and η'' of xanthan and guar blends were higher than the relative viscosity and η'' calculated for blends assuming no interaction, indicating that intermolecular binding occurred between galactomannans backbone and disordered segments of xanthan. The intrinsic viscosities of xanthan and xanthan-guar blends were higher at 80 °C than at 25 °C. Double logarithmic plots of η_{sp} against $C[\eta]$ showed only trends in dilute solutions. The slopes of the double logarithmic plots of η_{sp} against $C[\eta]$ of the blends were significantly higher than the slopes of either xanthan or guar alone.

Acknowledgements

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Chapter 3

Influence of deacetylation on the rheological properties of xanthan-guar interactions in dilute aqueous solutions

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Abstract

An oscillating capillary rheometer was used to investigate the effects of xanthan deacetylation on the viscoelastic properties and intrinsic viscosity of xanthan and guar mixtures in dilute aqueous solutions. Deacetylated xanthan exhibited a stronger synergistic interaction with guar than native xanthan did. No gels were observed for all xanthan-guar mixtures. Native xanthan-guar mixtures exhibited a liquid-like behavior, whereas deacetylated xanthan-guar mixtures exhibited a gel-like behavior. The relative viscosity and elasticity of deacetylated xanthan-guar mixtures were much stronger than those for native xanthan-guar mixtures. The intrinsic viscosities of deacetylated xanthan-guar mixtures were higher than those calculated from the weight averages of the two individually, whereas the intrinsic viscosities of native xanthan-guar mixtures were lower than those calculated from weight averages of the two individually, demonstrating that intermolecular binding occurred between xanthan and guar gum in dilute aqueous solutions.

Keywords: Xanthan-guar mixtures, synergistic interaction, viscoelastic properties, intrinsic viscosity, deacetylation

3.1. Introduction

Synergistic polysaccharide-polysaccharide interactions are attractive in the food industry because they impart novel and improved texture and rheological characteristics to food products, and they reduce polymers costs (Williams & Phillips, 2000). Many researchers have observed synergistic interaction between xanthan gum and galactomannans in solutions that resulted in enhanced viscosity or gelation (Rocks, 1971; Dea & Morrison, 1975; Dea, Morris, Rees, Welsh, Barnes, & Price, 1977; Morris, Rees, Young, Walkinshaw, & Darke, 1977). Although a few researchers (Kovacs, 1973; Schorsch, Garnier, & Doublier, 1995) have invoked the concept of mutual incompatibility to explain the interaction mechanism between xanthan and galactomannans, there is increasing evidence that supports intermolecular binding between xanthan and galactomannans (Morris, 1996). To date, the intermolecular binding mechanism between xanthan and galactomannans is still controversial, and different models have been proposed (Dea et al., 1977; Morris et al., 1977; McCleary, 1979; Tako, Asato, & Nakamura, 1984; Tako, 1991; Cairns, Miles, & Morris, 1986; Cairns, Miles, Morris, & Brownsey, 1987; Cheetham, McCleary, Teng, Lum, & Maryanto, 1986; Cheetham & Mashimba, 1988, 1991; Mannion, Melia, Launay, Cuvelier, Hill, Harding, & Mitchell, 1992; Zhan, Ridout, Brownsey, & Morris 1993).

Xanthan gum is an anionic heteropolysaccharide produced by the microorganism *Xanthomonas campestris*. Xanthan's main backbone consists of (1→4) β-D-glucopyranosyl units substituted at C-3 on every other glucose residue with a charged trisaccharide sidechain (Jansson, Kennark, & Lindberg, 1975). The trisaccharide chain consists of a D-glucuronic acid unit between two D-mannose units. Approximately one-half of the terminal D-mannose unit contains a pyruvic acid residue linked via keto groups to the 4 and 6 positions, with an unknown

distribution. The D-mannose linked to the main chain contains an acetyl group at position O-6 (Garcia-Ochoa, Santos, Casas, & Gomez, 2000). The acetate and pyruvate contents are variable on the side chain, and depend on the bacterial strains and on the fermentation conditions used to produce the gum. In aqueous solutions, the secondary structure of xanthan undergoes an 'order-disorder' transition from helix to coil structure. This conformational transition depends on temperature, ionic strength of solutions, nature of electrolyte, pH, and acetyl and pyruvate constituent contents (Holzwarth, 1976; Morris et al., 1977; Baradossi & Brant, 1982; Paoletti, Cesàro, & Delben, 1983; Norton, Goodall, Frangou, Morris, & Rees, 1984).

Guar gum is obtained from the seed of the guar plant, *Cyanaposis tetragonolobus*, and has the general structure of galactomannans. Guar consists of a main chain (1→4) β-D-mannopyranosyl unit substituted at O-6 by single-unit side-chains of α-D-galactopyranose. The ratio of mannose to galactose is approximately 1.6:1, depending on the source and method of extraction used to isolate the gum (Dea & Morrison, 1975).

Tako & Nakamura (1985) reported an enhanced synergistic interaction between deacetylated xanthan and guar gum. They investigated the rheological properties of a series of aqueous mixtures of xanthan and guar gum at a concentration of 0.2% of total gum. A slight synergistic increase in dynamic modulus was observed in the mixture with native xanthan, but the synergistic interaction was much stronger with deacetylated xanthan, indicating that the intermolecular interaction resulted from deacetylation of xanthan. The maximum dynamic modulus was achieved when the ratio of xanthan to guar gum was 2:1. The researchers concluded that the side chains of the guar gum molecule prevented the insertion of the charged trisaccharide side-chains of the xanthan molecule into the backbone of the guar gum molecule.

They proposed that an increase in the flexibility of the xanthan molecule upon deacetylation may facilitate the molecular interaction between xanthan sidechains and galactomannans backbone.

Shatwell, Sutherland, Ross-Murphy, & Dea, (1991) conducted rheological and chiroptical studies on a range of xanthan with various amounts of acetyl and pyruvic acid substitution. Oscillatory-shear measurements were determined upon mixed systems consisting of 0.5% (w/w) xanthan and 1.0% (w/w) guar gum in de-ionized water. The enhanced viscosity of xanthan-guar mixture was due solely to the presence of topological entanglements and not to a more specific intermolecular interaction. Evidence from both rheological and chiroptical results indicated a possible weak interaction between some low-acetyl xanthans and guar, but the nature of this interaction, whether molecular or thermodynamic in origin, is still controversial.

To date, much work has been accomplished on the gelling properties of the polysaccharides. The polysaccharide interactions in dilute solutions have been studied to a lesser extent. In dilute solutions, the individual polymer coils or rods are separate and free to move independently, and their intermolecular interactions are negligible. Thus, polymer-polymer interactions can be detected by the increase in solution viscosity and elasticity. The objective of this study was to investigate the effect of xanthan acyl substituents on the interactions between xanthan and guar gum in dilute aqueous solutions. An oscillatory capillary rheometer was used to determine dynamic viscoelastic properties for the polysaccharide solutions. Intrinsic viscosity and viscoelastic measurements were used to characterize the synergistic interaction between the polymers.

3.2. Materials and Methods

3.2.1. Materials

Xanthan gum and guar gum were purchased from Sigma (Sigma-Aldrich, St. Louis, MO).

3.2.2. Preparation of deacetylated xanthan

Deacetylation of xanthan was achieved by dissolving 0.2% (w/v) of native xanthan in deionized distilled water, and adding 0.025 M KOH and 0.1% (w/v) KCl for 2.5 h at room temperature under an atmosphere of nitrogen. The alkali solution was neutralized with 0.05 M HCl to pH 6.5. The solution was dialyzed against deionized distilled water for 3d by using a dialyzing tube (Snakeskin TM Pleated Dialysis Tubing, Pierce, Rockford, Ill., U.S.A.), and the deacetylated xanthan was recovered by lyophilization (Sloneker & Jeanes, 1962).

3.2.3. Stock solutions preparation

The stock solutions of deacetylated xanthan (0.1%, w/v), native xanthan (0.1%, w/v) and guar gum (0.2%, w/v) were prepared by thoroughly dispersing the required amount of gum in deionized distilled water. The gum solutions were continuously stirred with a magnetic stirrer for 3 h at ambient temperature and heated for 30 min at 90 °C in a water bath to completely hydrate the gums. Guar gum was centrifuged at 3500 g for 1 h to remove the insoluble particles. The solutions were dialyzed against deionized distilled water for 3 d by using a dialyzing tube (Snakeskin TM Pleated Dialysis Tubing, Pierce, Rockford, Ill., U.S.A.), with a molecular weight cutoff of 10 kDa. Stock solutions were refrigerated at 4 °C to minimize bacterial growth.

3.2.4. Preparation of mixed solutions of xanthan and guar gum

To study the interaction between the polysaccharides in the dilute domain, the deacetylated and native xanthan gum solutions were diluted with deionized distilled water to a final concentration of 0.025%, whereas the guar solution was diluted to 0.075%. The gums were blended at the following ratios: 100% xanthan:0%Guar, 80% xanthan:20% guar, 60% xanthan:40% guar, 40% xanthan:60% guar, 20% xanthan:80% guar, and 0% xanthan:100% guar. Freshly prepared xanthan and guar solutions were mixed at 25 °C, and were stirred with a

magnetic stirrer for 3 min. The concentration of xanthan and guar solutions was confirmed by the phenol-sulfuric method (Dubois, Giles, Hamilton, Rebers, & Smith, 1956).

3.2.5. Determination of acetyl and pyruvate contents

The acetyl and pyruvate contents of native xanthan and deacetylated xanthan were determined by the hydroxamic acid (McComb & McCready, 1957) and the 2,4-dinitrophenylhydrazine (Sloneker & Orentas, 1962) methods, respectively.

3.2.6. Molecular weight determination of polysaccharides

The molecular weights of deacetylated xanthan, native xanthan, and guar gum were determined by high performance size-exclusion chromatography (HPSEC), coupled on line with a multiangle laser light scattering detector (MALLS) and a refractive index detector. The MALLS detector was a DAWN DSP laser photometer from Wyatt Technology Corp. (Santa Barbara, Calif., U.S.A.) and the refractive index detector a Wyatt optilab DPS interferometric refractometer. A PL aquagel-OH mixed 8 μm column (Polymer Labs, Amherst, M.A., U.S.A.) was used. A sample volume of 100 μm / mL was injected at flow rate of 1ml/min using 100 mM NaCl as the mobile phase at a temperature of 30 °C. The dn/dc used for xanthan was 0.145 and for guar was 0.13. The data were analyzed by using Astra software version 4.5 (Wyatt Technology Corp., Santa Barbara, Calif., U.S.A.).

3.2.7. Density determination of polysaccharides

The densities of the polysaccharide solutions were determined with a standardized 10-mL pycnometer. The mass of the solution was calculated from the weight difference between the empty pycnometer (Kmax®, Kimble Glass Inc, Vineland, N.J., U.S.A.) and the filled vessel. The pycnometer filled with each respective polysaccharide solution was incubated at 20 °C for 1 h

(Equatherm, Lab-Line Instruments Inc, Melrose Park, Ill. U.S.A.) to equilibrate the sample before density determinations (Yaseen, Herald, Aramouni, & Alavi, 2005).

3.2.8. Determination of rheological measurements

The viscous (η') and elastic (η'') components of the polysaccharide solutions and their mixtures were measured as a function of oscillating shear rate by using an oscillating capillary rheometer (Viscoelasticity Analyzer, Vilastic 3, Vilastic Scientific, Inc, Austin, Tex., U.S.A.). The instrument and theory of measurement are described elsewhere (Thurston, 1960, 1976). The Viscoelasticity Analyzer is based on the principles of generating oscillatory flow at a selected frequency within a straight, cylindrical, stainless steel tube (0.0504 cm radius and 6.038 cm length). The Vilastic instrument produces an oscillatory flow in a capillary, and measures the pressure and volumetric flow rate, allowing the determination of both viscous and elastic components of a fluid sample.

The complex coefficient of viscosity (η^*) consists of viscous (η') and elastic (η'') components, and is defined as:

$$\eta^* = \eta' - i\eta'' \quad (1)$$

where i is an imaginary number. The η' and η'' are related to dissipated and recovered energy, respectively. Similarly, complex rigidity (G^*) is defined as:

$$G^* = G' + iG'' \quad (2)$$

Where G' is the storage modulus and G'' is the loss modulus. The complex coefficient of viscosity is related to the complex rigidity modulus by

$$\eta^* = G^* / i\omega \quad (3)$$

$$\text{or } \eta' = G'' / \omega \text{ and } \eta'' = G' / \omega$$

where the radian frequency $\omega = 2\pi f$, and f is the frequency in Hertz.

The η' and η'' of dilute solutions of xanthan and guar were determined in the shear-rate range 0.8 to 30s⁻¹ at a frequency of 2 Hz. Morris and Taylor (1982) reported that oral perception of solution viscosity correlated well with viscosity measurements at 10s⁻¹. Thus, all η' and η'' measurements were statistically assessed at 10s⁻¹. Rheological measurements were carried out at 20 °C ± 0.1 by using a temperature-controlled circulating water bath (Haake DC5, Gebr. Haake GmbH, Karlsruhe, Germany). The Viscoelasticity Analyzer was calibrated with deionized distilled water at 20 °C before the verification procedure to further ensure that the rheometer was operating at optimum accuracy.

3.2.9. Intrinsic viscosity determination

Intrinsic viscosity, denoted as $[\eta]$, is a useful experimental parameter in the study of dilute solutions. Intrinsic viscosity is a measure of the hydrodynamic volume occupied by the individual polymer molecules in isolation (Richardson & Kasapis, 1998). In dilute solutions, the polymer chains are separate, and the $[\eta]$ of a polymer in solution depends only on the dimensions of the polymer chain. Because $[\eta]$ indicates the hydrodynamic volume of the polymer molecule and is related to the molecular weight, it provides deep insights on the molecular characteristics of a biopolymer (Rao,1999).

One approach to determine the intrinsic viscosity is through extrapolation to infinite dilution, according to the Huggins (1942) empirical expression:

$$\frac{\eta_{sp}}{C} = [\eta] + k'[\eta]^2 C \quad (4)$$

where the specific viscosity (η_{sp}) = ($\eta - \eta_s$) / $\eta_s = \eta_{rel} - 1$, the relative viscosity (η_{rel}) = η / η_s , and η and η_s are the apparent viscosities of the solution and the solvent, respectively. The extrapolations to zero concentration are usually determined by plotting η_{sp} / C versus C or \ln

$(\eta_{rel})/C$ versus C , which would result in straight lines, respectively. Tanglertpaibul and Rao (1987) determined the intrinsic viscosity from the relative viscosity by using the expression:

$$\eta_{rel} = 1 + [\eta]C \quad (5)$$

The $[\eta]$ was obtained from the slope of η_{rel} vs. C plot, which gave straight lines, with linear regression correlation coefficients in the range 0.99 to 1.0. Chou and Kokini (1987) suggested a similar method for polyelectrolyte, in which the interactions between macromolecules in dilute solutions are not existent, and the second term of the Huggins equation is negligible; therefore, a plot of η_{sp} vs. C is linear.

In this study, the $[\eta]$ was determined for each solution by measuring relative viscosities of polysaccharides solutions within the range $1.2 < \eta_{rel} < 2.0$ at $\gamma = 10s^{-1}$. The intercept of η_{sp}/C vs. C plot in the dilute region gave the first estimation of $[\eta]$ for guar gum, whereas the slope of η_{rel} vs. C plot gave the first estimation of $[\eta]$ for xanthan and xanthan-guar mixtures.

3.2.10. Statistical analysis

A two-way factorial design was used for the study of rheological properties. For all polysaccharides samples, three replications and two subsamples were performed. The analysis of variance (ANOVA) and general linear models procedure (GLM) were conducted with Statistical Analysis System (2002-2003) (version 9.1, SAS Institute, Inc., Cary, N.C., U.S.A.). Comparisons among treatments were analyzed by using Fisher's least significant difference (LSD), with a significance level of $P < 0.05$.

3.3. Results and Discussion

3.3.1. Characterization of polysaccharides

Values of acetyl and pyruvate contents, and weight average molecular weights for the polysaccharides, are given in Table 3.1. The molecular weights of native xanthan (2.65×10^6) and deacetylated xanthan (2.4×10^6) were much larger than that of guar gum (1.45×10^6). Some reduction in molecular weight of native xanthan occurred due to the chemical modification. The acetate and pyruvate contents of native xanthan were 3.53 and 0.9%, respectively. Deacetylation of xanthan removed approximately 91% of the acetate content, but it did not affect the pyruvate content of xanthan.

Table 3.1. Chemical composition of native xanthan, deacetylated xanthan, and guar gum.

Polysaccharide	Acetate (%)	Pyruvate (%)	Molecular weight
Native xanthan	3.51	0.9	2.65×10^6
Deacetylated xanthan	0.32	0.9	2.36×10^6
Guar	---	---	1.45×10^6

3.3.2. Dynamics of polysaccharides interactions

Figures 3.1a & b and 3.2 a & b show the changes in η' and η'' as a function of shear rate for native xanthan, deacetylated xanthan, guar, and their mixtures in water. Over the entire range of shear rates, both deacetylated xanthan and native xanthan exhibited a pseudoplastic behavior. For xanthan-guar mixtures, shear-rate dependence was observed for all mixtures, except for xanthan:guar at a ratio of 1:4. No shear-rate dependence was observed over the entire range of shear rates for guar gum. The guar behavior is consistent with viscosity results previously obtained for galactomannans over a larger range of shear rates ($10 < \dot{\gamma} < 350 \text{ s}^{-1}$) (Bresolin, Sander, Reicher, Sierakowski, Rinaudo, & Ganter, 1997). No gels were formed for any of the

xanthan-guar mixtures. For all shear rates studied, the η' of native xanthan and all native xanthan-guar mixtures was higher than the η'' , indicating liquid-like behavior in the dilute regime, whereas η' of deacetylated xanthan and deacetylated xanthan-guar mixtures was lower than the η'' , except for deacetylated xanthan-guar mixture at ratio of 1:4, indicating gel-like behavior in the dilute regime.

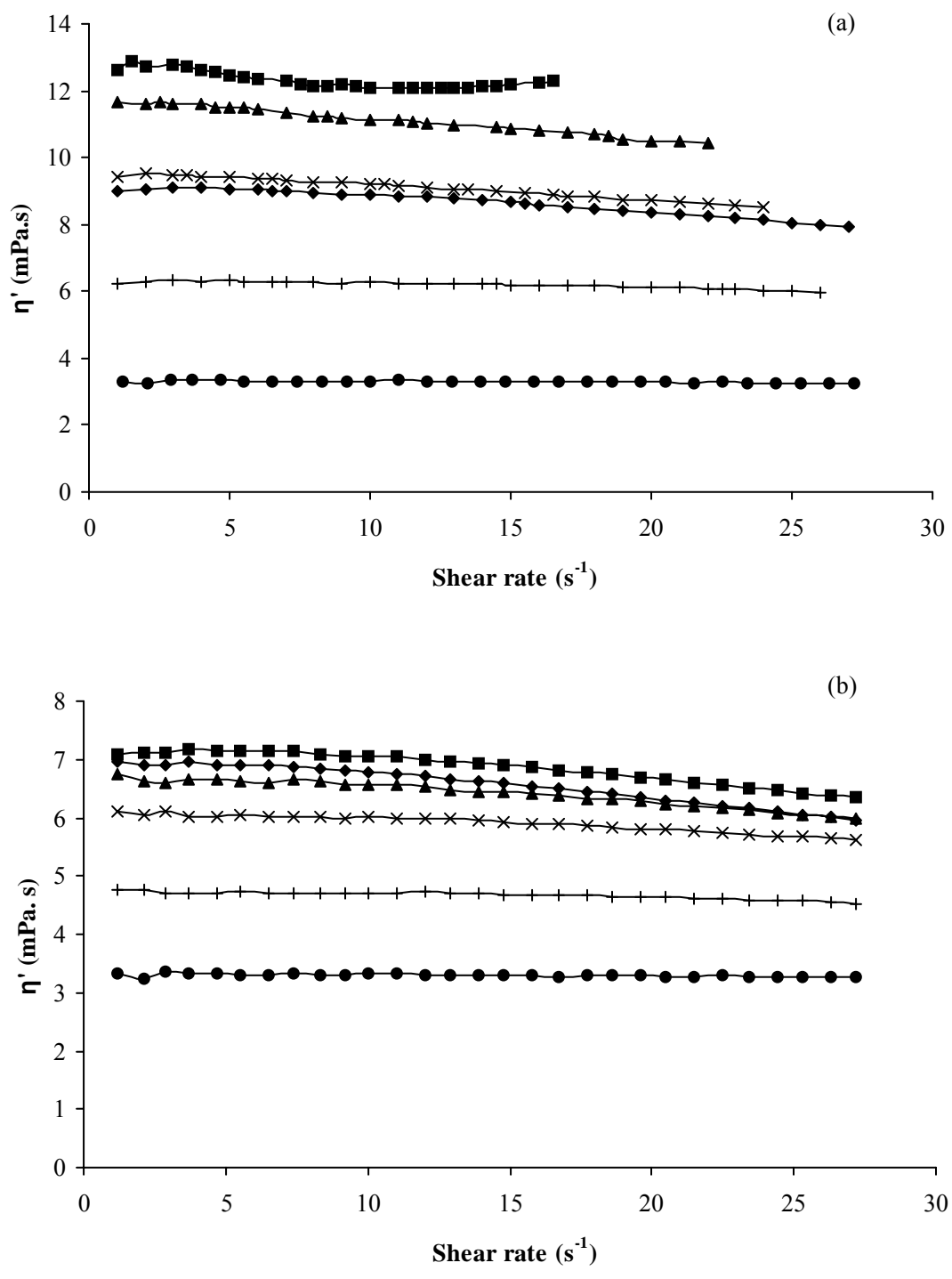


Fig. 3.1. Viscosity of xanthan, guar, and their mixtures as function of shear rate. (a) deacetylated xanthan-guar mixtures, (b) native xanthan-guar mixtures. Xanthan:Guar ratio: (◆) xanthan, (●) guar, (■) 4:1, (▲) 3:2, (×) 2:3, (+) 1:4.

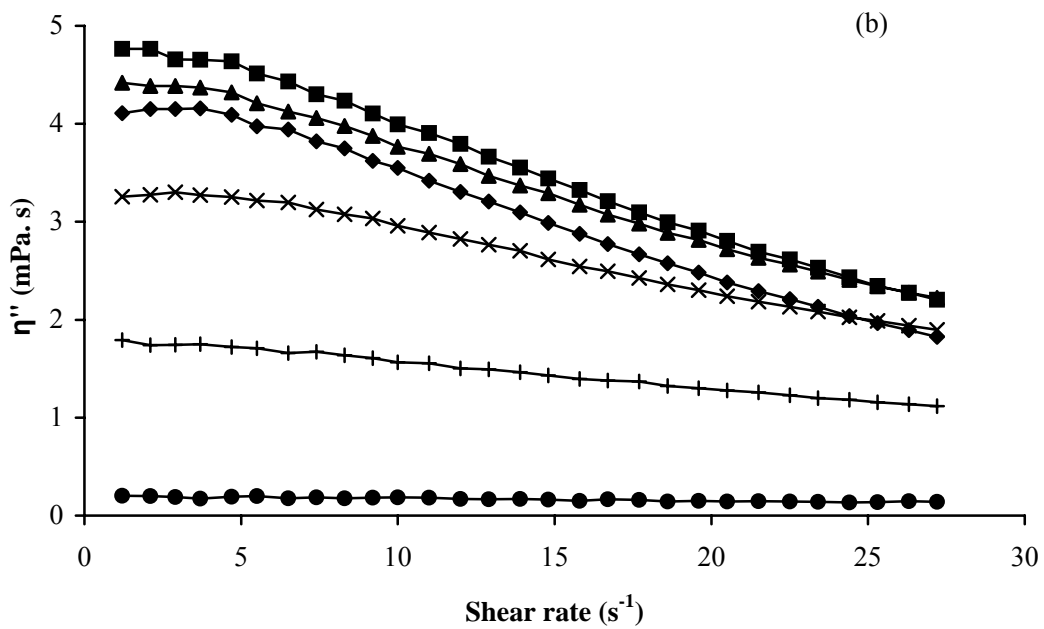
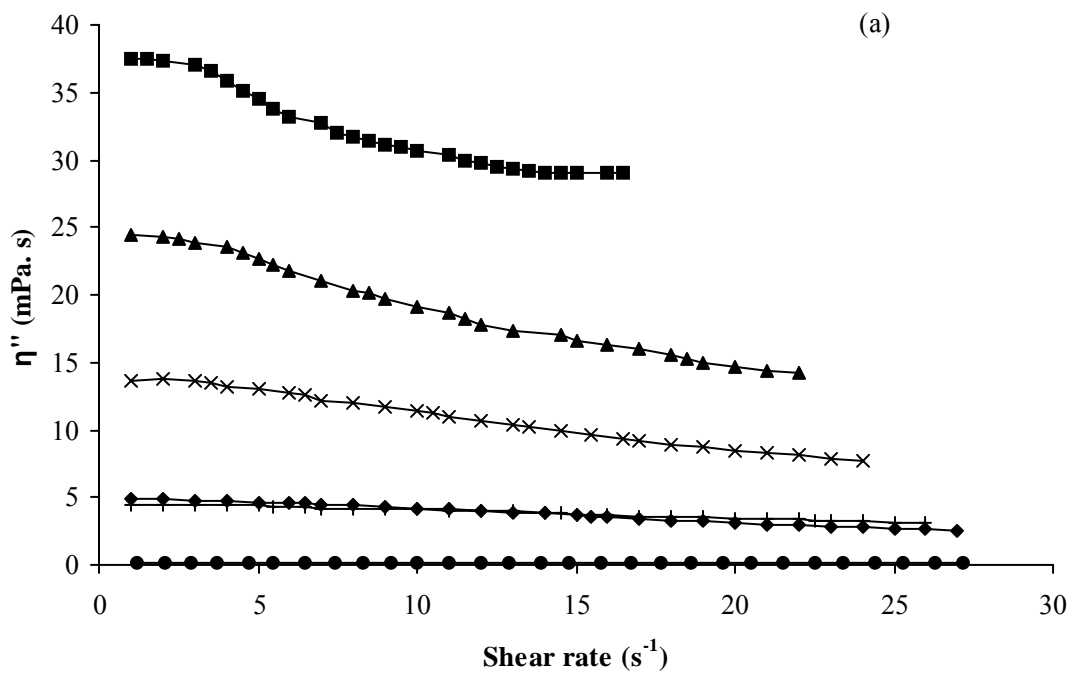


Fig. 3.2. Elasticity of xanthan, guar, and their mixtures as function of shear rate. (a) deacetylated xanthan-guar mixtures, (b) native xanthan-guar mixtures. Xanthan:Guar ratio: (◆) xanthan, (●) guar, (■) 4:1, (▲) 3:2, (×) 2:3, (+) 1:4.

The η' and η'' values of polysaccharides at shear rate 10 s^{-1} are given in Table 3.2. Significant differences ($P < 0.05$) were found between the native xanthan-guar mixtures and deacetylated xanthan-guar mixtures. Deacetylated xanthan-guar mixtures exhibited significantly larger η' and η'' values than did native xanthan-guar mixtures. No significant difference in η' and η'' for guar gum was found.

Table 3.2. Effect of deacetylation on the viscosity (η') and elasticity (η'') parameters[†] for native and deacetylated xanthan, guar, and their mixtures measured at $\dot{\gamma} = 10 \text{ s}^{-1}$.

Xanthan:Guar(%)	η' (mP. s)		η'' (mP. s)	
	Native xanthan:guar	Deacetylated xanthan:guar	Native xanthan:guar	Deacetylated xanthan:guar
100:0	$6.78^a \pm 0.15$	$8.93^b \pm 0.92$	$3.55^a \pm 0.11$	$4.23^a \pm 0.68$
80:20	$7.05^a \pm 0.05$	$12.16^b \pm 1.54$	$3.99^a \pm 0.21$	$30.96^b \pm 2.66$
60:40	$6.57^a \pm 0.06$	$11.81^b \pm 0.46$	$3.77^a \pm 0.28$	$20.75^b \pm 2.25$
40:60	$6.09^a \pm 0.17$	$10.0^b \pm 0.15$	$2.99^a \pm 0.09$	$12.46^b \pm 0.57$
20:80	$4.71^a \pm 0.06$	$6.27^b \pm 0.49$	$1.58^a \pm 0.02$	$4.08^b \pm 0.52$
0:100	$3.24^a \pm 0.14$	$3.24^a \pm 0.14$	$0.16^a \pm 0.03$	$0.16^a \pm 0.03$

^{a, b} Means followed by the same letters in the same row for η' or η'' are not significantly different ($P < 0.05$).

[†]Results are expressed as means \pm SD for three replications.

The η_{rel} of deacetylated and native xanthan, guar, and their mixtures were plotted as a function of the mixing ratio in water, allowing the influence of deacetylation on the intermolecular interaction to be determined (Fig. 3.3). The η_{rel} of deacetylated xanthan-guar mixtures and native xanthan-guar mixtures differed with respect to the polysaccharide ratio, and the maximum synergy was observed at the ratio of 4:1. The relative viscosities of the

polysaccharide mixtures were higher than the relative viscosities calculated for mixtures assuming no interaction, reflecting that intermolecular interaction occurred between xanthan and guar mixtures. These results are consistent with previous studies (Lopes, Andrade, Milas, & Rinaudo, 1992; Bresolin et al., 1997) that showed viscosity of xanthan and guar blends in water was higher than the calculated value assuming no interaction for xanthan and guar mixtures, which support the hypothesis of intermolecular interaction. The synergistic interaction in dilute aqueous solutions was further supported by elasticity measurements. Figure 3.4 shows the η'' of deacetylated xanthan, native xanthan, guar, and their mixtures against polysaccharide ratio. In the mixtures with native xanthan, a small synergistic increase in η'' was observed, whereas the η'' synergistic interaction was significantly enhanced in the mixtures with deacetylated xanthan. The maximum synergistic interaction was noted at a xanthan:guar ratio of 3:2.

Our results demonstrated the influence of deacetylation on the increase of η' and η'' , which may reflect intermolecular binding between the polysaccharides. Tako & Nakamura (1984) and Smith et al. (1981) reported that acetate stabilizes the ordered conformation of xanthan, whereas pyruvate destabilizes the conformation (Holzwarth, 1976) due to the increasing electrostatic repulsions between the side chains.

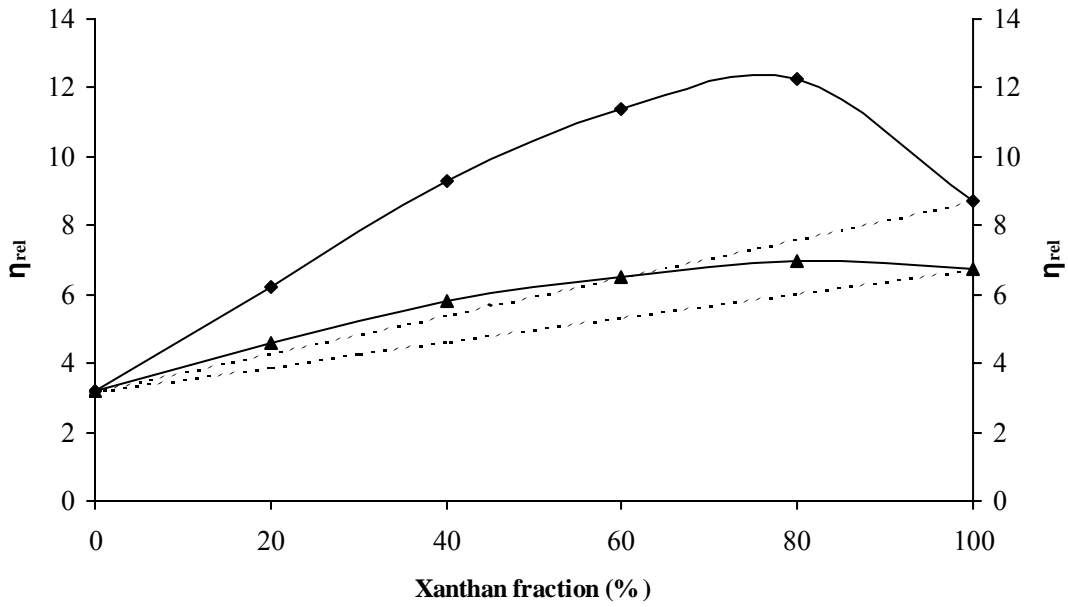


Fig. 3.3. Relative viscosity of xanthan, guar, and their mixtures against xanthan fraction. (\diamond) deacetylated xanthan-guar mixtures; (\blacktriangle) native xanthan-guar mixtures; (---) values calculated for mixtures assuming no interaction.

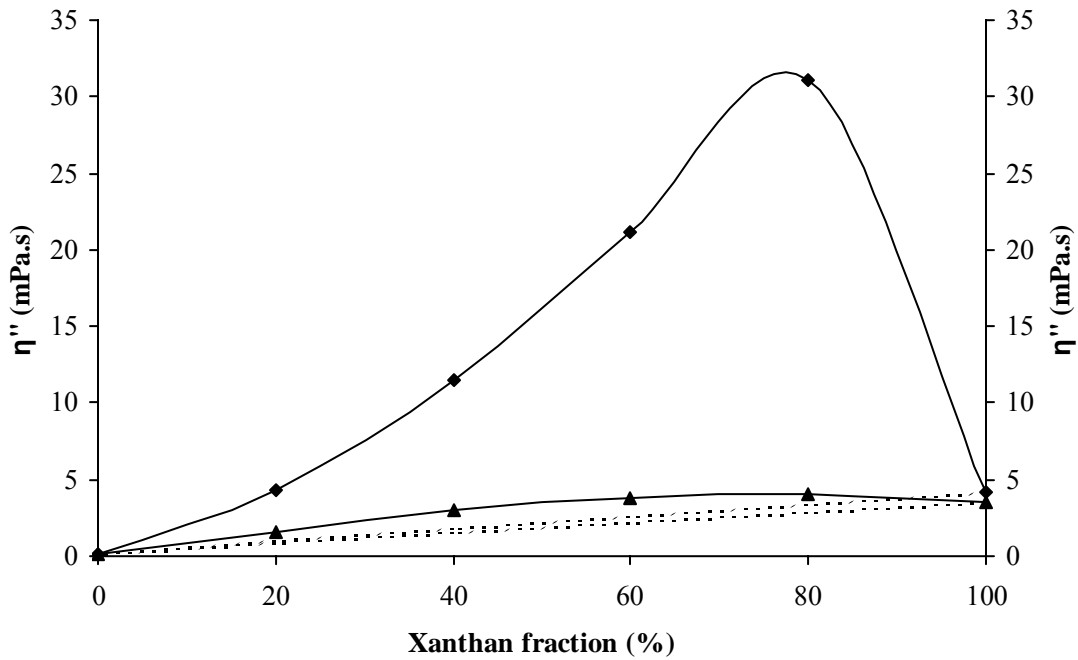


Fig. 3.4. Elasticity of xanthan, guar, and their mixtures. (\diamond) deacetylated xanthan-guar mixtures; (\blacktriangle) native xanthan-guar mixtures; (---) values calculated for mixtures assuming no interaction.

Our results are in agreement with the findings of Tako & Nakamura (1985), who suggested a stronger interaction between deacetylated xanthan molecules than between native xanthan molecules. Removing the hydrophobic acetyl group from the side chain of xanthan significantly enhanced the synergistic interaction with guar gum in dilute aqueous solutions. The η_{rel} of deacetylated xanthan-guar mixtures was much stronger than the relative viscosity of native xanthan-guar mixtures. Deacetylation of xanthan strongly enhanced the synergistic interaction, possibly due to destabilizing the helical structure of xanthan and increasing xanthan chain flexibility, thus facilitating the formation of heterotypic junctions with guar gum. Foster & Morris (1994) reported that interaction of deacetylated xanthan with either carob or konjac mannan in dilute solutions resulted in a pronounced increase in viscosity measurements, suggesting that deacetylation favors intermolecular binding due to destabilization of the helical structure of xanthan.

3.3.3. Intrinsic viscosities of polysaccharides

Intrinsic viscosity of polymers is dependent on their molecular weight and chain dimension (Flory, 1953). In dilute solutions, the polymer chains are separate, so $[\eta]$ of a polymer in solution depends only on the dimensions of the polymer chain (Rao, 1999). The intermolecular binding between xanthan and guar was further supported by the intrinsic viscosities of xanthan and guar mixtures (Fig. 3.5). The intrinsic viscosities of deacetylated xanthan-guar mixtures were higher than those calculated from the weight averages of the two individually, whereas the intrinsic viscosities of native xanthan-guar mixtures were lower than those calculated from weight averages of the two individually, demonstrating that intermolecular binding may have occurred between xanthan and guar.

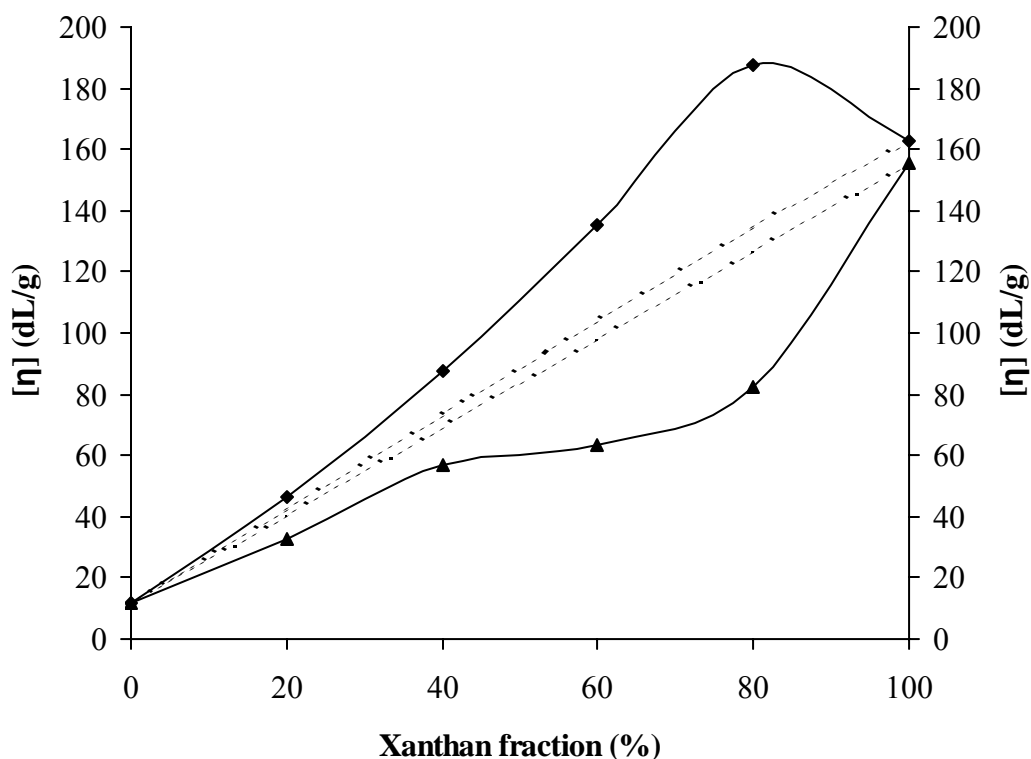


Fig. 3.5. Plots of intrinsic viscosities against xanthan fraction. (◆) deacetylated xanthan-guar mixtures; (▲) native xanthan-guar mixtures; (---) intrinsic viscosity calculated from the weight averages of the two individuals, assuming no interaction.

These results are consistent with a previous study conducted by Wang, Wang, & Sun (2002). The strong intermolecular binding between deacetylated xanthan and guar can be attributed to the increased chain flexibility of deacetylated xanthan, which may be facilitated by the interaction with guar gum. The intrinsic viscosities of native xanthan-guar mixtures decreased as the xanthan fraction decreased in the mixtures. The intrinsic viscosities of native xanthan-guar mixtures were lower than those calculated from weight averages of the two individually. Because xanthan plays a crucial role in controlling the viscosity of xanthan-guar mixtures, the dramatic decrease in the intrinsic viscosities of their mixtures may be attributed to

the conformational change of xanthan from a helical form to a more flexible form due to binding of guar to xanthan. Xanthan conformation change outweighed the increase in intrinsic viscosity due to this binding; thus, the dramatic decrease in the intrinsic viscosities of the mixtures was observed. These results suggest that conformational change of deacetylated xanthan may not predominate in controlling the intrinsic viscosity, and that deacetylated xanthan may require to be in a specific conformation to bind guar.

The η_{rel} as a function of concentration for deacetylated xanthan, native xanthan, guar, and their mixtures is shown in Figs. 3.6 a & b. Straight-line relationships with large linear regression coefficients were obtained for xanthan and xanthan-guar mixtures in the η_{rel} range of 1.2 to 2. Similar results were found by Higiro, Herald, & Alavi (2006). Native and deacetylated xanthan, and some of xanthan-guar mixtures, did not follow the Huggins equation at high dilution series, whereas the Huggins equation successfully fit the guar solutions. For neutral polysaccharide such as guar, the Huggins plot has an advantage of giving linear plots (Morris, 1995). Plots of the reduced viscosity (η_{sp}/C) against the concentration of xanthan and some of the xanthan-guar mixtures resulted in a nonlinear relationship at low xanthan and low xanthan-guar mixture concentrations. Also, the Fuoss empirical equation (Fuoss & Strauss, 1948) that was suggested for a flexible-chain polyelectrolyte did not show the typical viscosity-concentration relationship of flexible polyelectrolytes for xanthan alone, was in agreement with Wang et al. (2002). Thus, this model was not successfully implemented to determine the intrinsic viscosity by extrapolation of experimental data, which prompted the use of the slope model (Tanglertpaibul & Rao, 1987) to determine the $[\eta]$ by plotting relative viscosity vs. C (equation 5). Table 3.2 shows the values of the intrinsic viscosities of deacetylated xanthan, native xanthan, guar, and their mixtures. Xanthan and guar intrinsic viscosities were comparable to those reported by Launay, Cuvelier, &

Martinez-Reyes (1984, 1997). The $[\eta]$ of deacetylated xanthan was higher than the $[\eta]$ of native xanthan. Deacetylated xanthan had a $[\eta]$ of 163 dl/g, whereas the $[\eta]$ of native xanthan was 154dl/g, but the difference was not statistically significant ($P > 0.05$). This is consistent with Callet, Milas, & Rinaudo (1987), who reported that acetyl and pyruvate contents have no influence on the intrinsic viscosity of xanthan in dilute solution. The $[\eta]$ of guar gum was 12 dl/g. Deacetylated and native xanthan had a much higher $[\eta]$ than guar gum, which may be attributed to the significant difference in their chain stiffness. Xanthan may have a stronger chain stiffness than the flexible, random coil conformation of guar, which may increase the chain dimensions, thus, a higher $[\eta]$.

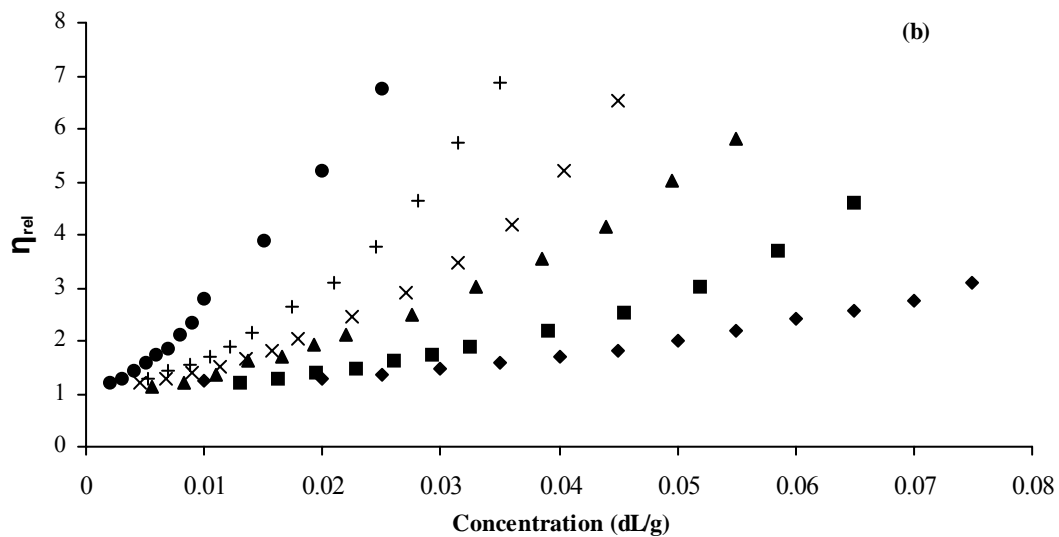
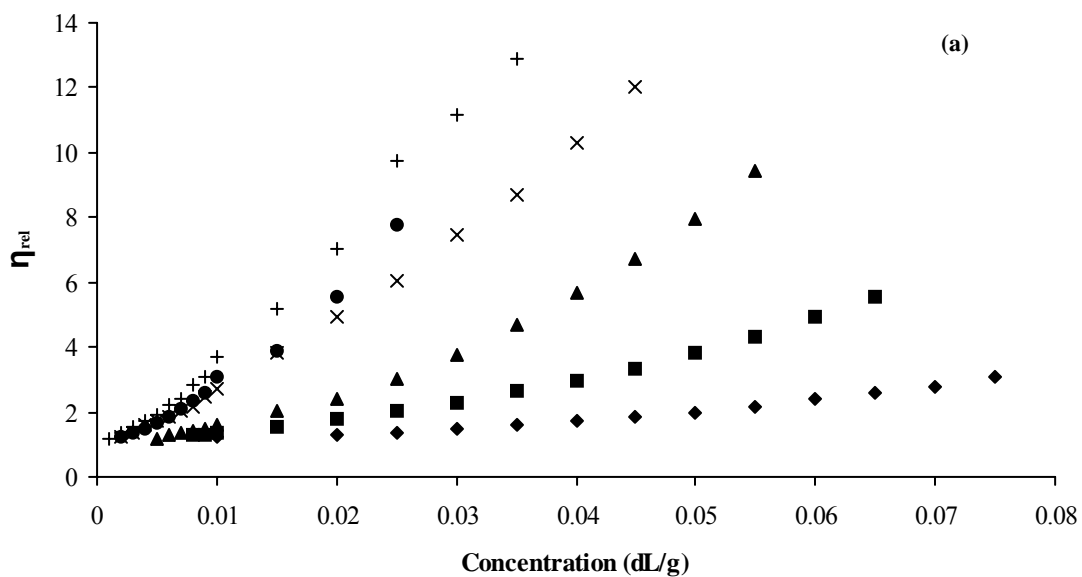


Fig. 3.6. Relative viscosities of xanthan, guar, and their mixtures as function of concentration. (a) deacetylated xanthan-guar mixtures, (b) native xanthan-guar mixtures. Xanthan:Guar ratio: (●) xanthan, (◆) guar, (+) 4:1, (×) 3:2, (▲) 2:3, (■) 1:4.

3.3.4. Coil overlap parameter of polysaccharides

In dilute solutions, the individual polymer coils are separate from each other and are free to move independently. With increasing concentrations, the coils start to overlap and interpenetrate one another. The transition from dilute solutions to concentrated solutions is usually accompanied by a pronounced change in the concentration dependence of solution viscosity (Morris, Cutler, Ross-Murphy, Ress, & Price, 1981; Morris, 1995). The corresponding concentration is called critical, or coil overlap, concentration (C^*). For random-coil polysaccharide solutions, except galactomannans, Morris and others (1981) reported that the slope of double logarithmic plots of η_{sp} against $C[\eta]$ was close to 1.4 in a dilute regime, whereas, in the concentrated regime, the slope increased to 3.3. The C^* transition occurred at a value of $C[\eta]$ close to 4, and the η_{sp} at this degree of coil overlap was invariably close to 10. Guar gum was found to deviate from the above observations. The C^* transition occurred at a smaller value of the coil-overlap parameter, $C[\eta] = 2.5$, and the viscosity showed a higher dependence on concentration with, a slope of 5.1 instead of 3.3 (Morris et al., 1981). In this study, all the polysaccharides systems were studied in dilute solutions. As shown in Fig. 3.7 a & b, no change in the slope of a double logarithmic plot of η_{sp} against the coil-overlap parameter ($C[\eta]$) occurred, indicating that no molecular entanglements were obtained, and that xanthan (deacetylated and native), guar, and their mixtures were in the dilute domain. As shown in Table 3.3, the slope of a double logarithmic plot of η_{sp} against $C[\eta]$ for native xanthan and deacetylated xanthan was 1.38 and 1.32, respectively, and for guar was 1.44. Our results showed that the slopes of xanthan and guar were lower than those reported by Morris et al. (1981), Cuvelier & Launay (1986), and Launay et al. (1997), demonstrating that both xanthan (0.025%) and guar gum (0.075%) were in the dilute domain ($C < C^*$). The slopes for deacetylated xanthan and

deacetylated xanthan-guar mixtures were lower than those for native xanthan and native xanthan-guar mixtures, indicating more flexible xanthan due to the deacetylation.

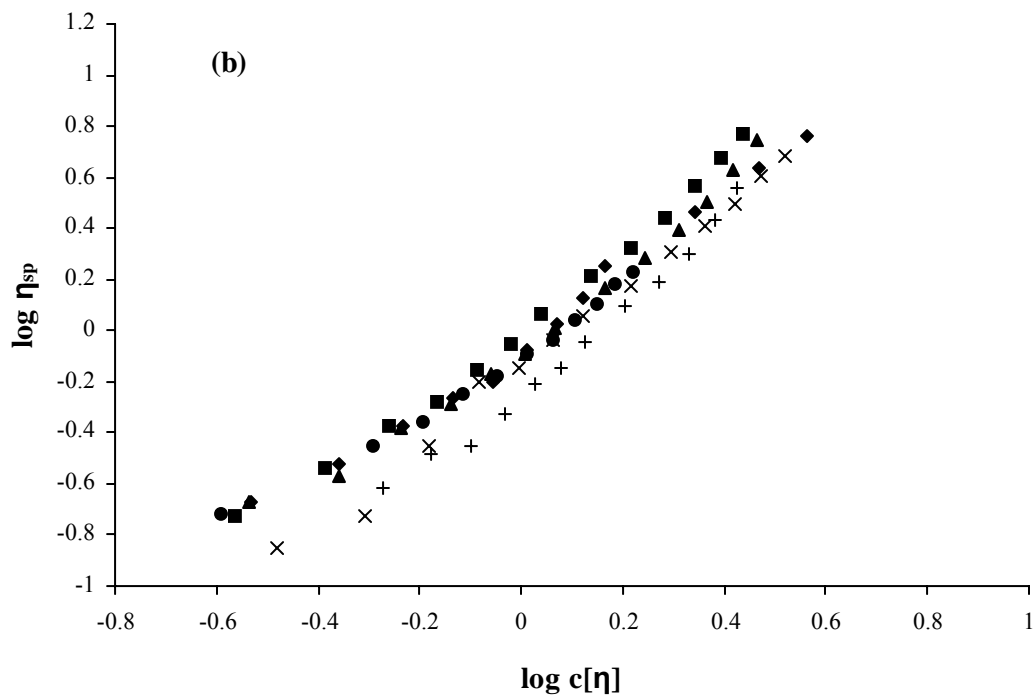
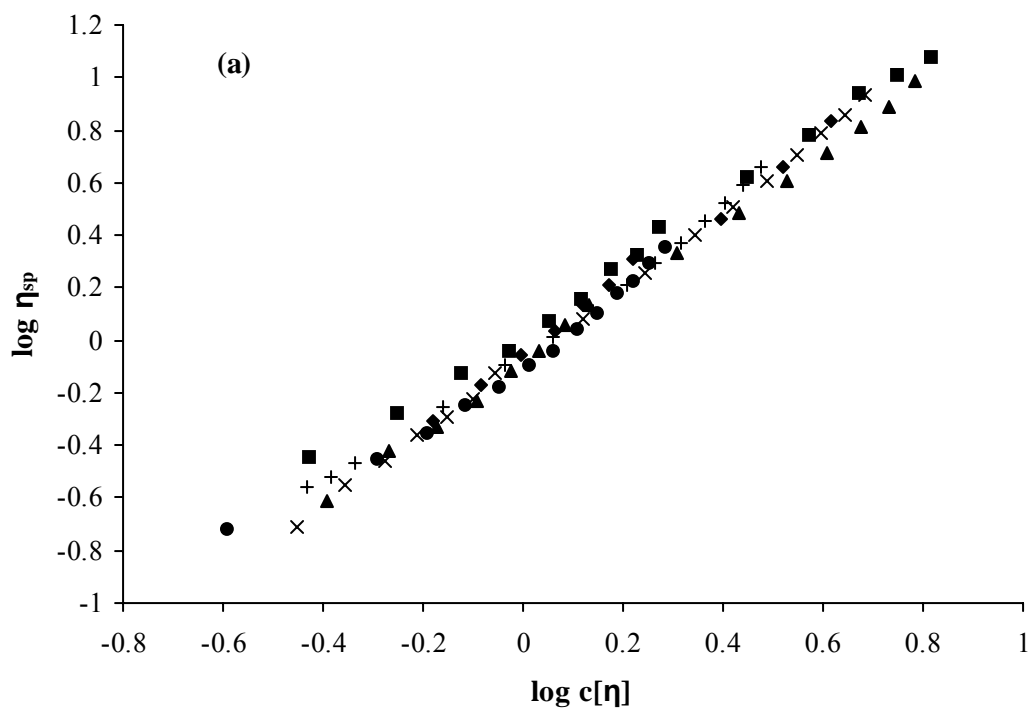


Fig. 3.7. Double logarithmic plot of specific viscosity (η_{sp}) against coil-overlap parameter ($c[\eta]$) for xanthan, guar, and their mixtures. (a) deacetylated xanthan-guar mixtures, (b) native xanthan-guar mixtures. Xanthan:Guar ratio: (●) xanthan, (◆) guar, (■) 4:1, (▲) 3:2, (×) 2:3, (+) 1:4.

Table 3.3. Effect of deacetylation on the intrinsic viscosity and slope[†] of the double logarithmic plot of η_{sp} against $c[\eta]$ for native xanthan, deacetylated xanthan, guar, and their mixtures in the dilute domain.

Xanthan:Guar(%)	$[\eta]$ (dL/g)		Slope	
	Native xanthan:guar	Deacetylated xanthan:guar	Native xanthan:guar	Deacetylated xanthan:guar
100:0	155.7 ^a ± 9.3	163.0 ^a ± 5.0	1.38 ^a ± 0.02	1.32 ^b ± 0.05
80:20	82.2 ^a ± 2.7	187.4 ^b ± 5.8	1.50 ^a ± 0.01	1.22 ^b ± 0.03
60:40	63.1 ^a ± 1.7	135.3 ^b ± 5.9	1.44 ^a ± 0.02	1.36 ^b ± 0.06
40:60	56.9 ^a ± 7.2	87.9 ^b ± 7.3	1.55 ^a ± 0.02	1.43 ^b ± 0.07
20:80	32.8 ^a ± 2.8	46.1 ^b ± 2.8	1.68 ^a ± 0.04	1.33 ^b ± 0.02
0:100	12.0 ^a ± 2.1	12.0 ^a ± 2.1	1.44 ^a ± 0.06	1.44 ^a ± 0.06

^{a, b} Means followed by the same letters in the same row are not significantly different (P < 0.05).

[†]Results are expressed as means ± SD for three replications

3.4. Conclusion

Synergistic interactions for both native xanthan-guar mixtures and deacetylated xanthan-guar mixtures in the dilute aqueous solutions were observed. Destabilizing the helical structure of xanthan, due to deacetylation, played a significant role in its interaction with guar. Deacetylation of xanthan strongly enhanced the synergistic interaction with guar because it destabilized the helical structure and increased chain flexibility of xanthan. Intermolecular binding occurred between xanthan and guar gum in dilute aqueous solutions, as indicated by viscosity and elasticity measurements.

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Chapter 4

Influence of Ionic Strength on Xanthan Conformation and Interaction of Xanthan-Guar Gum in Dilute Solutions

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Abstract

An oscillating capillary rheometer was used to investigate the dynamic viscoelastic and intrinsic viscosity properties of deacetylated xanthan, native xanthan, guar gum, and xanthan-guar mixtures in dilute solutions. Influence of ionic strength on xanthan conformation and interaction with guar was elaborated. As the salt concentration increased, a significant ($P < 0.05$) decrease in viscosity (η') and elasticity (η'') values was observed for both native xanthan-guar mixtures and deacetylated xanthan-guar mixtures. In water and 2 mM NaCl solution, the relative viscosity and η'' of both native xanthan-guar mixtures and deacetylated xanthan-guar mixtures were much higher than of those calculated for mixtures assuming no interaction, whereas no pronounced increase was found for polysaccharide mixtures in 40 mM NaCl. The intrinsic viscosities of deacetylated xanthan-guar mixtures in water and 2 mM NaCl were higher, whereas the intrinsic viscosities of native xanthan-guar mixtures were lower than those calculated from the weight averages of the two individually, assuming no interaction. These results demonstrated that intermolecular interaction has occurred between xanthan and guar mixtures in water and 2 mM NaCl, but may not occur in 40 mM NaCl, and mutual incompatibility may occur. The results suggest that the degree of disordering of xanthan played a critical role in xanthan-guar interaction and may explain the differences in η' , η'' , and intrinsic viscosity measurements between 2 and 40 mM NaCl.

Keywords: deacetylated and native Xanthan, guar, synergistic interaction, ionic strength, viscoelastic, intrinsic viscosity.

4.1. Introduction

Xanthan gum is an anionic heteropolysaccharide produced by the microorganism *Xanthomonas campestris*. Xanthan's main backbone consists of (1→4) β-D-glucopyranosyl units and is substituted at C-3 on every other glucose residue with a charged trisaccharide sidechain (Jansson, Kennark, & Lindberg, 1975). The trisaccharide chain consists of a D-glucuronic acid unit between two D-mannose units. Approximately one-half of the terminal D-mannose unit contains a pyruvic acid residue linked via keto group to the 4 and 6 positions, with an unknown distribution. The D-mannose linked to the main chain contains an acetyl group at position O-6 (Garcia-Ochoa, Santos, Casas, & Gomez, 2000). In aqueous solutions, the structure of xanthan undergoes a thermally induced transition from an ordered (helical) to a disordered conformation. This conformational transition depends on ionic strength, nature of electrolyte, pH, and acetyl and pyruvate constituent contents (Holzwarth 1976; Morris, Rees, Young, Walkinshaw, & Darke, 1977; Baradossi & Brant, 1982; Paoletti, Cesàro, & Delben, 1983; Norton, Goodall, Frangou, Morris, & Rees, 1984). At high temperature and low ionic strength, xanthan exists in solutions as a disordered structure, but reduction in temperature and/or addition of salts induces an ordered structure (Norton et al., 1984). In distilled water at 25 °C, the backbone of xanthan is disordered (or partly ordered in the form of a randomly broken helix) but highly extended due to the electrostatic repulsions from the charged groups on the sidechains (Rocheftort & Middleman, 1987). Because the structure is highly extended, the molecules may align and associate via hydrogen bonding to form a weakly structured material. Rocheftort & Middleman (1987) reported that, as the temperature increased, a transition to coil-like configuration occurs, which causes a dissociation of the molecules and a subsequent change in the rheological properties (Rocheftort & Middleman, 1987). When salt is added to the solution at 25 °C, a disorder-order

transition occurs in which the backbone takes on a helical conformation, and the charged trisaccharide sidechains collapse down onto the backbone (due to charge screening effects) and stabilize the ordered conformation (Muller, Anhourrache, Lecourtier, & Chauveteau, 1986).

Guar gum is obtained from the seed of the guar plant, *Cyanaposis tetragonolobus*, and has the general structure of galactomannans. Guar consists of a main chain (1→4) β-D-mannopyranosyl unit substituted at O-6 by single-unit side-chains of α-D-galactopyranose. The ratio of mannose to galactose is approximately 1.6:1, depending on the source and method of extraction (Dea & Morrison, 1975).

A synergistic interaction occurs between xanthan gum and galactomannans in solutions, which results in enhanced viscosity or gelation (Dea & Morrison, 1975; Dea, Morris, Rees, Welsh, Barnes, & Price, 1977; Morris et al., 1977). Rocks (1971) first reported the synergistic interaction between xanthan and galactomannans. The author pointed out that xanthan gum formed thermoreversible gels when mixed with locust bean gum (LBG), but not with guar gum. Although a few researchers (Kovacs, 1973; Schorsch, Garnier, & Doublier, 1995) invoked the concept of incompatibility to explain the gelation mechanism between xanthan and galactomannans, there is a considerable body of evidence that supports the theory of intermolecular binding between xanthan and galactomannans (McCleary & Neukom, 1982; Cairns, Miles, & Morris, 1986; Cairns, Miles, Morris, & Brownsey, 1987; Cheetham, McCleary, Teng, Lum, & Maryanto, 1986; Cheetham & Mashimba, 1988, 1991; Cheetham & Punruckrong, 1989; Foster & Morris, 1994; Goycoolea, Foster, Richardson, Morris, & Gidley, 1994).

To date, the intermolecular binding mechanism between xanthan and galactomannans is still controversial, and different models have been proposed. The first model was proposed by Dea et al. (1977) and Morris et al. (1977), who reported the synergistic interaction between

xanthan and galactomannans, based on a cooperative interaction, depending on the fine structure of the galactomannans. The intermolecular binding may occur between the ordered xanthan and unsubstituted or poorly substituted regions of the galactomannan backbone (Dea et al., 1977; Morris et al., 1977). The synergistic interaction is strongly dependent on the amount of galactose content (Dea & Morrison, 1975) and galactose distribution to the regions of galactomannans backbone, where the galactose units are substituted on one side (McCleary, 1979).

Tako, Asato, & Nakamura (1984) and Tako (1991) proposed a different model, in which the intermolecular interaction occurs between the side chains of xanthan and the backbone of the galactomannans. The authors suggested that the side chains of the xanthan are inserted into adjacent unsubstituted regions of the galactomannan backbone, which adopts an extended, two-fold, ribbon-like conformation.

The third model was proposed by Cairns et al. (1986) and Cairns et al. (1987), in which intermolecular binding may occur between the disordered xanthan and galactomannans, and disordering of xanthan helical structure is necessary for gelation. They proposed that xanthan has a disordered, extended, two-fold, cellulose-like conformation, rather than a five-fold helix, when interacting with galactomannan. Cheetham et al. (1986) and Cheetham & Mashimba (1988, 1991) proposed that the interaction occurs between the disordered segments of the xanthan chains and galactomannan.

Mannion et al. (1992) suggested that xanthan and galactomannans gelation can be explained by two different mechanisms. One takes place at room temperature, at which the interaction with ordered xanthan gives weaker elastic gels with little dependence on the galactose content of the galactomannan. The second mechanism requires heating of the polysaccharide mixture, and the interaction with heat-disordered segments of the xanthan gives a stronger gel

that is highly dependent on the side chains of the galactomannan. Zhan, Ridout, Brownsey, & Morris (1993) argued that gelation between xanthan and LBG can be explained only by a single mechanism. In agreement with Cheetham et al. (1986) and Cheetham & Mashimba (1988, 1991), they suggested that intermolecular binding involves binding of disordered segments of the xanthan chain to LBG chains in hot mixing, in which the preparation temperature is higher than the transition temperature of xanthan, and in cold mixing, in which preparation temperature is lower. The authors also suggested that the extent of disorder induced in xanthan before mixing is the main factor in the interaction.

To date, much work has been accomplished on the gelling properties of the polysaccharides. The polysaccharide interactions in dilute solutions have been studied to a lesser extent. The objective of this study was to investigate the effect of ionic strength on xanthan conformation and interaction with guar gum in dilute solutions to characterize the interaction mechanism. Intrinsic viscosity and dynamic viscoelastic measurements were used to characterize the synergistic interaction and intermolecular binding for the polysaccharide mixtures.

4.2. Materials and Methods

4.2.1. Materials

Xanthan gum and guar gum were purchased from Sigma (Sigma-Aldrich, St. Louis, Mo., U.S.A.).

4.2.2. Preparation of deacetylated xanthan

Deacetylation of xanthan was achieved by dissolving 0.2% (w/v) of native xanthan in deionized distilled water, and adding 0.025 M KOH and 0.1% (w/v) KCl for 2.5 h at room temperature under an atmosphere of nitrogen. The alkali solution was neutralized with 0.05 M HCl to pH 6.5. The solution was dialyzed against deionized distilled water for 3 d by using a

dialyzing tube (Snakeskin™ Pleated Dialysis Tubing, Pierce, Rockford, Ill., U.S.A.), and the deacetylated xanthan was recovered by lyophilization (Sloneker & Jeanes, 1962).

4.2.3. Stock solutions preparation

The stock solutions of deacetylated xanthan (0.1%, w/v), native xanthan (0.1%, w/v) and guar gum (0.2%, w/v) were prepared by thoroughly dispersing the required amount of gum in deionized distilled water. The gum solutions were continuously stirred with a magnetic stirrer for 3 h at ambient temperature and were heated for 30 min at 90 °C in a water bath to completely hydrate the gums. Guar gum was centrifuged at 3500 g for 1 h to remove the insoluble particles. The solutions were dialyzed against deionized distilled water for 3 d by using a dialyzing tube (Snakeskin™ Pleated Dialysis Tubing, Pierce, Rockford, Ill., U.S.A.), with a molecular weight cutoff of 10 kDa. Stock solutions were refrigerated at 4 °C to minimize bacterial growth.

4.2.4. Preparation of mixed solutions of xanthan and guar gum

To study the interaction between the polysaccharides in dilute domain, the deacetylated and native xanthan gum solutions were diluted with deionized distilled water to a final concentration of 0.025%, whereas the guar solution was diluted to 0.075%. The gums were blended at the following ratios: 100% xanthan:0% guar, 80% xanthan:20% guar, 60% xanthan:40% guar, 40% xanthan:60% guar, 20% xanthan:80% guar, and 0% xanthan:100% guar. Freshly prepared xanthan (deacetylated or native) and guar solutions were mixed at 25 °C, and were stirred with a magnetic stirrer for 3 min. To study the effect of salt on the polysaccharides and their interactions, the appropriate amounts of sodium chloride were added to the diluted solutions of deacetylated xanthan, native xanthan, and guar gum, and were completely dissolved to obtain final concentrations of 2 and 40 mM NaCl. The concentration of each solution was confirmed by the phenol-sulfuric method (Dubois, Giles, Hamilton, Rebers, & Smith, 1956).

4.2.5. Determination of acetyl and pyruvate contents

The acetyl and pyruvate contents of native xanthan and deacetylated xanthan were determined by the hydroxamic acid (McComb & McCready, 1957) and the 2,4-dinitrophenylhydrazine (Sloneker & Orentas, 1962) methods, respectively.

4.2.6. Molecular weight determination of polysaccharides

The molecular weights of deacetylated xanthan, native xanthan, and guar gum were determined by high performance size-exclusion chromatography (HPSEC), coupled on-line with a multi-angle laser light scattering detector (MALLS) and a refractive index detector. The MALLS detector was a DAWN DSP laser photometer from Wyatt Technology Corp. (Santa Barbara, Calif., U.S.A.) and the refractive index detector was a Wyatt optilab DPS interferometric refractometer. A PL aquagel-OH mixed 8- μm column (Polymer Labs, Amherst, Mass., U.S.A.) was used. A sample volume of 100 μm / mL was injected at a flow rate of 1ml/min, using 100 mM NaCl as the mobile phase, at a temperature of 30 °C. The dn/dc used for xanthan was 0.145 and for guar was 0.13. The data were analyzed by using Astra software version 4.5 (Wyatt Technology Corp., Santa Barbara, Calif., U.S.A.).

4.2.7. Density measurement

The densities of the polysaccharide solutions were determined with a standardized 10-mL pycnometer (Kmax®, Kimble Glass Inc, Vineland, N.J., U.S.A.). The mass of the solution was calculated from the weight difference between the empty pycnometer and the filled vessel. The pycnometer was filled with each respective polysaccharide solution was incubated at 20 °C for 1 h (Equatherm, Lab-Line Instruments Inc, Melrose Park, Ill., U.S.A.) to equilibrate the sample before density determinations (Yaseen, Herald, Aramouni, & Alavi, 2005).

4.2.8. Rheological properties

The viscous (η') and elastic (η'') components of the polysaccharide solutions and their mixtures were measured as a function of oscillating shear rate by using an oscillating capillary rheometer (Viscoelasticity Analyzer, Vilastic 3, Vilastic Scientific, Inc, Austin, Tex., U.S.A.). The instrument and theory of measurement are described elsewhere (Thurston, 1960, 1976). The Viscoelasticity Analyzer is based on the principles of generating oscillatory flow at a selected frequency within a straight, cylindrical, stainless steel tube (0.0504 cm radius and 6.038 cm length). The Vilastic instrument produces an oscillatory flow in a capillary, and measures the pressure and volumetric flow rate, allowing the determination of both viscous and elastic components of a fluid sample.

The complex coefficient of viscosity (η^*) consists of viscous (η') and elastic (η'') components, and is defined as:

$$\eta^* = \eta' - i\eta'' \quad (1)$$

where i is an imaginary number. The η' and η'' are related to dissipated and recovered energy, respectively. Similarly, complex rigidity (G^*) is defined as:

$$G^* = G' + iG'' \quad (2)$$

where G' is the storage modulus and G'' is the loss modulus. The complex coefficient of viscosity is related to the complex rigidity modulus by

$$\eta^* = G^* / i\omega \quad (3)$$

$$\text{or } \eta' = G'' / \omega \text{ and } \eta'' = G' / \omega$$

where the radian frequency $\omega = 2\pi f$, and f is the frequency in Hertz.

The η' and η'' of dilute solutions of xanthan and guar were determined in the shear-rate range 0.8 to 30s⁻¹ at a frequency of 2 Hz. Morris and Taylor (1982) reported that oral perception of solution viscosity correlated well with viscosity measurements at 10s⁻¹. Thus, all η' and η''

measurements were statistically assessed at 10s^{-1} . Rheological measurements were carried out at $20\text{ }^{\circ}\text{C} \pm 0.1$ by using a temperature-controlled circulating water bath (Haake DC5, Gebr. Haake GmbH, Karlsruhe, Germany). The Viscoelasticity Analyzer was calibrated with deionized distilled water at $20\text{ }^{\circ}\text{C}$ before the verification procedure to further ensure that the rheometer was operating at optimum accuracy.

4.2.9. Intrinsic viscosity determination

Intrinsic viscosity, denoted as $[\eta]$, is a useful experimental parameter in the study of dilute solutions. Intrinsic viscosity is a measure of the hydrodynamic volume occupied by the individual polymer molecules in isolation (Richardson & Kasapis, 1998). In dilute solutions, the polymer chains are separate, and the $[\eta]$ of a polymer in solution depends only on the dimensions of the polymer chain. Because $[\eta]$ indicates the hydrodynamic volume of the polymer molecule and is related to the molecular weight, it provides deep insights into the molecular characteristics of a biopolymer (Rao, 1999).

One approach to determine the intrinsic viscosity is through extrapolation to infinite dilution, according to the Huggins (1942) empirical expression:

$$\frac{\eta_{sp}}{C} = [\eta] + k'[\eta]^2 C \quad (4)$$

where the specific viscosity (η_{sp}) = $(\eta - \eta_s) / \eta_s = \eta_{rel} - 1$, the relative viscosity (η_{rel}) = η / η_s , and η and η_s are the apparent viscosities of the solution and the solvent, respectively. The extrapolations to zero concentration are usually determined by plotting η_{sp} / C versus C or $\ln(\eta_{rel}) / C$ versus C , which would result in straight lines, respectively. Tanglerpaibul and Rao (1987) determined the intrinsic viscosity from the relative viscosity by using the expression:

$$\eta_{rel} = 1 + [\eta]C \quad (5)$$

The $[\eta]$ was obtained from the slope of η_{rel} vs. C plot, which gave straight lines, with linear regression correlation coefficients in the range 0.99 to 1.0. Chou and Kokini (1987) suggested a similar method for polyelectrolytes, in which the interactions between macromolecules in dilute solutions are not existent, and the second term of the Huggins equation is negligible; therefore, a plot of η_{sp} vs. C is linear.

In this study, the $[\eta]$ was determined for each solution by measuring relative viscosities of polysaccharides solutions within the range $1.2 < \eta_{\text{rel}} < 2.0$ at $\gamma = 10\text{s}^{-1}$. The intercept of η_{sp}/C vs. C plot in the dilute domain gave the first estimation of $[\eta]$ for guar gum, whereas the slope of η_{rel} vs. C plot gave the first estimation of $[\eta]$ for xanthan and xanthan-guar mixtures.

4.2.10. Statistical analysis

A two-way factorial design was used for the study of rheological properties. For all polysaccharides samples, three replications were performed. The analysis of variance (ANOVA) and general linear models procedure (GLM) were conducted with Statistical Analysis System (2002-2003) (version 9.1, SAS Institute, Inc., Cary, N.C., U.S.A.). Comparisons among treatments were analyzed by using Fisher's least significant difference (LSD), with a significance level of $P < 0.05$.

4.3. Results and Discussion

4.3.1. Interaction of polysaccharides

To evaluate the synergistic/ non synergistic effect of the xanthan-guar mixtures in dilute solutions, the η_{rel} of polysaccharides and their mixtures were plotted as a function of xanthan fraction at $\gamma = 10\text{ s}^{-1}$ (Fig. 4.1a & b). Fig. 4.1a shows the η_{rel} behavior of native xanthan, guar, and their mixtures in water, 2 and 40 mM NaCl at $\gamma = 10\text{ s}^{-1}$ and 25°C . Fig. 4.1b depicts η_{rel}

behavior of deacetylated xanthan, guar, and their mixtures. In water and 2 mM NaCl, the relative viscosities of both native xanthan-guar mixtures and deacetylated xanthan-guar mixtures were much higher than the relative viscosities calculated for mixtures assuming no interaction, whereas no pronounced increase was found for polysaccharide mixtures in 40 mM NaCl. Such deviations from the values calculated for mixtures assuming no interaction may be explained in terms of specific molecular interactions between xanthan and guar gum. In

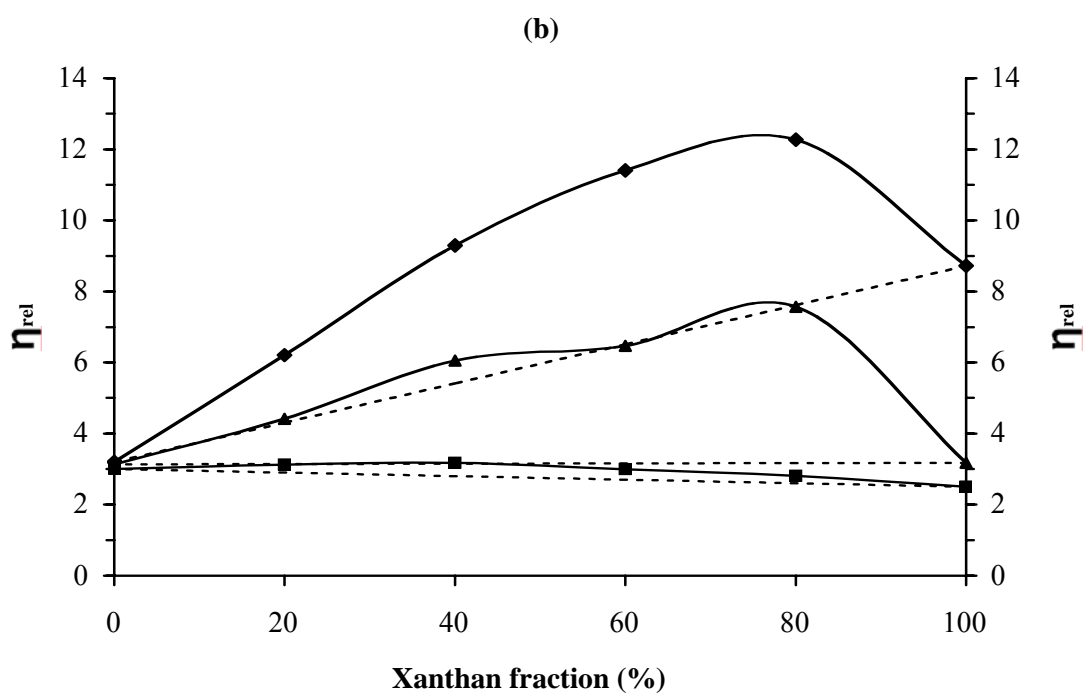
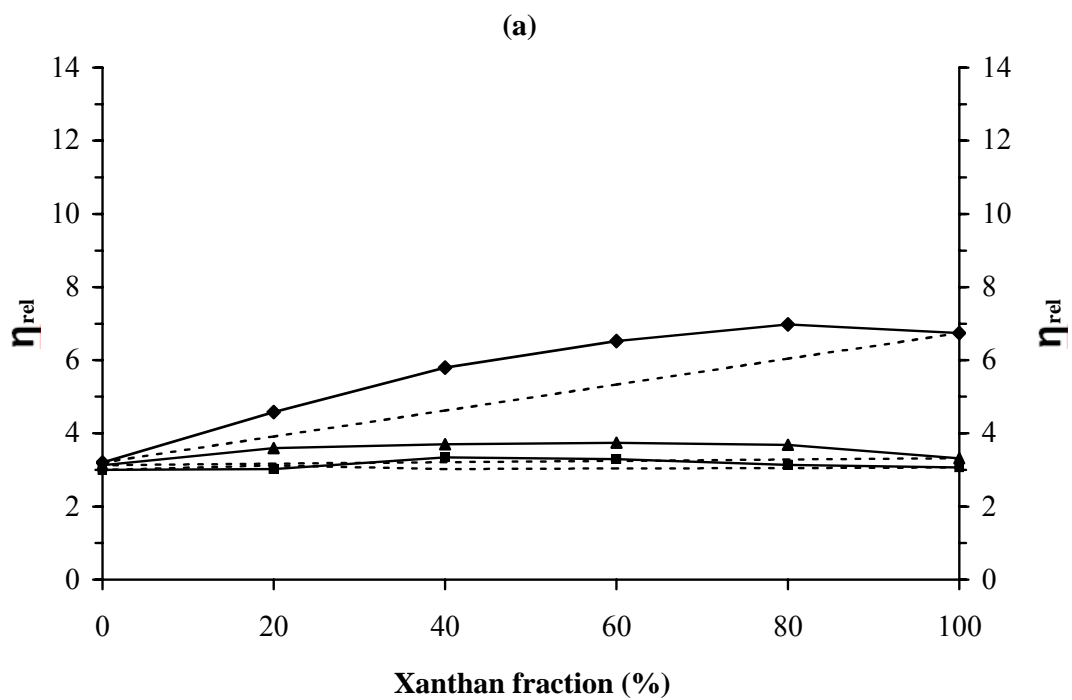


Fig. 4.1. Relative viscosity (η_{rel}) against xanthan fraction for (a) native xanthan-guar mixtures and (b) deacetylated xanthan-guar mixtures. (◆) water; (▲) 2 mM NaCl; (■) 40 mM NaCl; (---) values calculated for mixtures assuming no interaction.

the 2 mM NaCl, a smaller synergistic interaction was observed, whereas a stronger synergy in the absence of salt was noted. No synergistic interaction was found in 40 mM NaCl. The η_{rel} of native xanthan-guar mixtures and deacetylated xanthan-guar mixtures varied with respect to polysaccharide ratio. The maximum viscosity synergistic interaction for native xanthan-guar mixtures was observed at a ratio of 3:2, whereas the maximum viscosity synergistic interaction for deacetylated xanthan-guar mixtures was obtained at a ratio of 4:1. These results show the influence of xanthan conformation on the increase of viscosity for the polysaccharide mixtures, reflecting that intermolecular interaction occurred between xanthan and guar mixtures in water and 2 mM NaCl, but not in 40 mM NaCl solution.

The viscosity measurements in dilute solutions were further supported by η'' measurements determined by capillary viscometry (Fig. 4.2a & b). Fig. 4.2a & b depict the η'' behavior as a function of mixing ratio of native xanthan-guar mixtures and deacetylated xanthan-guar mixtures, respectively, in water and 2 and 40 mM NaCl at $\gamma = 10 \text{ s}^{-1}$. There was a very pronounced increase in η'' for both native xanthan-guar mixtures and deacetylated xanthan-guar mixtures in water and 2 mM NaCl, whereas the η'' of the polysaccharide mixtures was diminished in 40 mM NaCl, confirming the viscosity measurements.

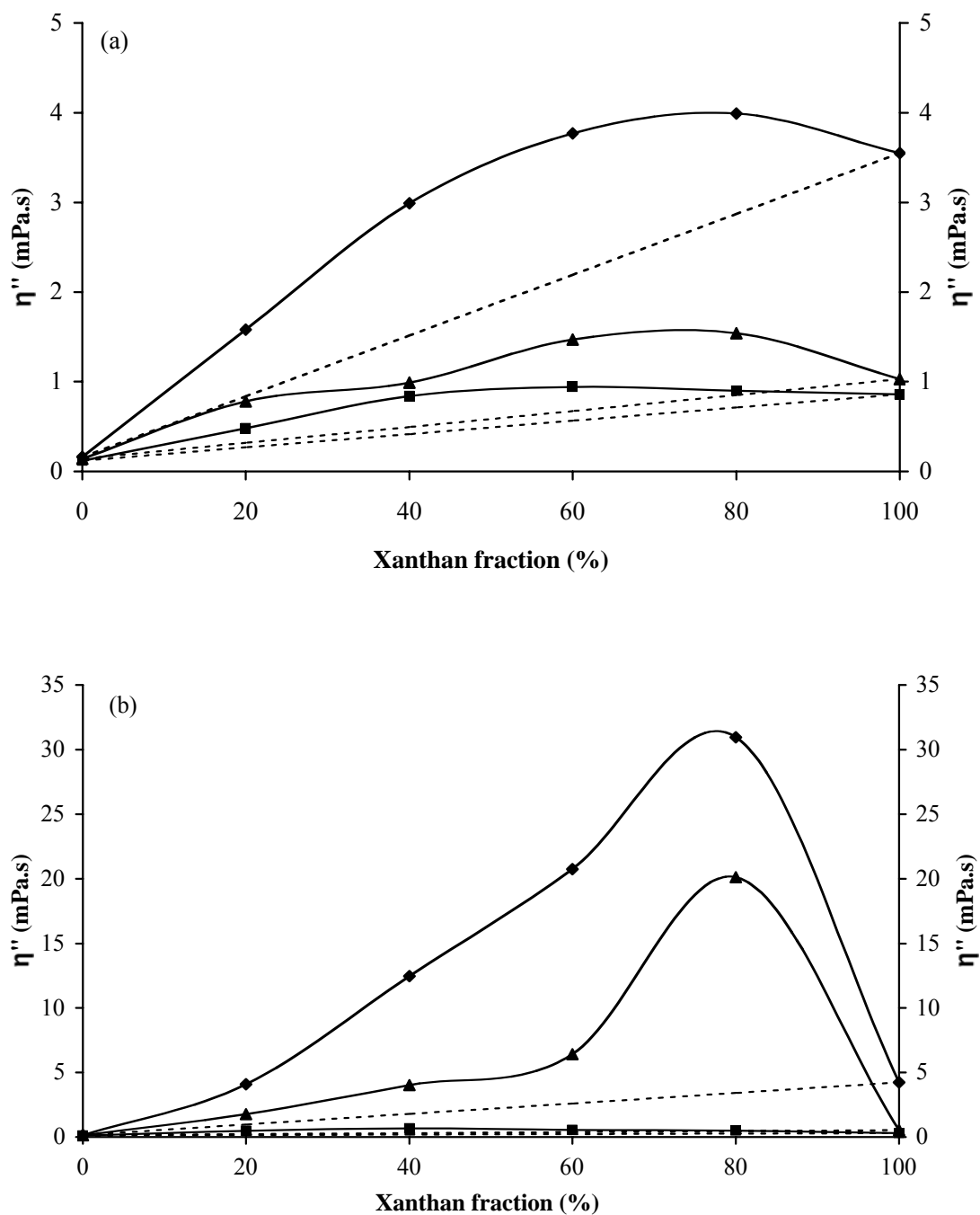


Fig. 4.2. Elasticity (η'') against xanthan fraction for (a) native xanthan-guar mixtures and (b) deacetylated xanthan-guar mixtures. (♦) water; (▲) 2 mM NaCl; (■) 40 mM NaCl; (---) values calculated for mixtures assuming no interaction.

To further characterize the intermolecular interaction between the polysaccharides in water and salt solutions, the η' and η'' values in dilute solutions at $\gamma = 10 \text{ s}^{-1}$ were statistically analyzed (Table 4.1). As shown in Table 4.1, significant differences ($P < 0.05$) in η' and η'' values were found between water and salt solutions for the native xanthan-guar mixtures and for the deacetylated xanthan-guar mixtures. As the salt concentration increased, a significant ($P < 0.05$) decrease in η' and η'' values was observed for both native xanthan-guar mixtures and deacetylated xanthan-guar mixtures. The η' of native xanthan significantly decreased as the salt concentration increased, whereas no significant difference was found in the η'' values between 2 and 40 mM NaCl. The η' and η'' values of deacetylated xanthan significantly decreased in salts, compared with values in water solutions, but no significant decrease was found between 2 and 40 mM NaCl. The decrease in η' and η'' values in salt exhibited a typical polyelectrolyte behavior. This decrease with the addition of salt was due to the charge screening for electrostatic repulsions of the trisaccharide sidechains (Muller et al., 1986; Rochefort & Middleman, 1987). The charge screening led to a more compact conformation and caused a reduction in hydrodynamic size of the molecule (Rochefort & Middleman, 1987), hence lowering the viscosities and elasticities. In contrast, guar gum, which is a neutral polysaccharide, exhibited no significant difference in η' and η'' values between water and salt solutions.

In aqueous solutions, the structure of xanthan undergoes an 'order-disorder' transition from helix to coil structure. This conformational transition depends on temperature, ionic strength of solutions, nature of electrolyte, pH, and acetate and pyruvate contents (Holzwarth 1976; Morris et al., 1977; Baradossi & Brant, 1982;

Table 4.1. Effect of ionic strength on the dynamic viscoelastic parameters of native xanthan-guar blends and deacetylated xanthan-guar blends measured at 20 °C and 10 s⁻¹.

Native Xanthan:Guar (%)	Viscosity (mP. s)			Elasticity (mP. s)		
	0 mM NaCl	2 mM NaCl	40 mM NaCl	0 mM NaCl	2 mM NaCl	40 mM NaCl
100:0	6.78 ^a ± 0.15	3.33 ^b ± 0.11	3.11 ^c ± 0.03	3.55 ^a ± 0.11	1.03 ^b ± 0.08	0.86 ^b ± 0.01
80:20	7.05 ^a ± 0.05	3.92 ^b ± 0.18	3.23 ^c ± 0.06	3.99 ^a ± 0.21	1.54 ^b ± 0.25	0.90 ^c ± 0.02
60:40	6.57 ^a ± 0.06	3.94 ^b ± 0.18	3.34 ^c ± 0.03	3.77 ^a ± 0.28	1.47 ^b ± 0.32	0.94 ^c ± 0.01
40:60	6.09 ^a ± 0.17	3.72 ^b ± 0.04	3.44 ^c ± 0.04	2.99 ^a ± 0.09	0.99 ^b ± 0.07	0.84 ^b ± 0.05
20:80	4.71 ^a ± 0.06	3.64 ^b ± 0.18	3.20 ^c ± 0.22	1.58 ^a ± 0.02	0.78 ^b ± 0.09	0.48 ^c ± 0.04
0:100	3.24 ^a ± 0.14	3.16 ^{ab} ± 0.14	3.04 ^b ± 0.03	0.16 ^a ± 0.03	0.14 ^a ± 0.02	0.12 ^a ± 0.02
Deacetylated Xanthan:Guar (%)						
100:0	8.93 ^a ± 0.92	3.25 ^b ± 0.06	2.56 ^b ± 0.05	4.23 ^a ± 0.68	0.54 ^b ± 0.06	0.31 ^b ± 0.03
80:20	12.16 ^a ± 1.54	7.97 ^b ± 0.23	2.88 ^c ± 0.09	30.96 ^a ± 2.66	20.13 ^b ± 2.88	0.49 ^c ± 0.03
60:40	11.81 ^a ± 0.46	6.88 ^b ± 0.51	3.04 ^c ± 0.05	20.75 ^a ± 2.25	6.41 ^a ± 1.35	0.56 ^c ± 0.05
40:60	10.0 ^a ± 0.15	6.11 ^b ± 0.31	3.37 ^c ± 0.01	12.46 ^a ± 0.57	4.03 ^b ± 0.85	0.67 ^c ± 0.01
20:80	6.27 ^a ± 0.49	4.81 ^b ± 0.29	3.17 ^c ± 0.15	4.08 ^a ± 0.52	1.78 ^b ± 0.44	0.47 ^b ± 0.06
0:100	3.24 ^a ± 0.14	3.16 ^a ± 0.14	3.04 ^a ± 0.03	0.16 ^a ± 0.03	0.14 ^a ± 0.02	0.12 ^a ± 0.02

^{a, b, c} Means followed by the same letters in the same row are not significantly different ($P \leq 0.05$)
 Results are expressed as means ± SD for three replications

Cesàro, & Delben, 1983; Norton et al., 1984). Our results show the influence of xanthan conformation on the increase of η' and η'' in water and 2 mM NaCl, reflecting that intermolecular binding between xanthan (native and deacetylated) and guar has occurred in dilute solutions. Xanthan is in the disordered conformation in distilled, deionized water at 25 °C, whereas, in salt at 25 °C, xanthan starts to take on an ordered conformation due to charge screening effects. In distilled water at 25 °C, the backbone of xanthan is disordered, but highly extended, due to the electrostatic repulsions from the charged groups on the trisaccharide sidechains (Rochefort & Middleman, 1987). Because the structure is highly extended, the molecules may align and associate by hydrogen bonding to form a weakly structured material. Therefore, as the temperature increases, a transition to coil-like configuration occurs, which causes a dissociation of the molecules and a subsequent change in the rheological properties (Rochefort & Middleman, 1987). When salt is added to the solution at 25 °C, a disorder-order transition occurs in which the backbone takes on a helical conformation, and the charged trisaccharide sidechains collapse down onto the backbone (due to charge screening effects) and stabilize the ordered conformation (Muller et al., 1986). Mixing the polysaccharides in 40 mM NaCl substantially diminished the molecular associations between xanthan and guar gum, as indicated by the pronounced decreases in the η' and η'' values. The η' and η'' differences in water, 2 mM NaCl, and 40 mM NaCl can be explained in terms of disordering degree of xanthan structure. In water, xanthan is in the disordered conformation. Optical-rotation studies (Lecourtier, Chauveteau, & Muller, 1986; Milas & Rinaudo, 1986; Cheetham & Mashimba, 1988, 1991) have confirmed that xanthan is in the disordered conformation in water at room temperature. Electrostatic repulsions that involve glucuronate and pyruvate in the side chains are poorly shielded, thus favoring the disordered conformation (Cheetham & Mashimba, 1991). In 2

mM NaCl, xanthan is partly ordered, whereas the molecule is completely ordered in 40 mM NaCl. Muller et al., (1986), using optical rotation studies, noted a sharp transition in xanthan structure at 10 mM NaCl. Norton et al., (1984) reported that a complete conformational ordering would occur at 25 °C only for salt concentrations in excess of 35 mM. Rochefort & Middleman (1987) observed a gradual transition in the shear viscosity of a 250-ppm xanthan solution, confirming the idea of co-existence of ordered and disordered regions in a single chain. They concluded that a complete ordered conformation can be obtained for salt concentrations > 10 mM NaCl. Thus, the highly disordered xanthan was capable of directly interacting with guar to form heterotypic structures and a higher synergistic interaction (Zhan et al., 1993; Goycoolea et al., 1994; Morris, Brownsey, & Ridout, 1994; Morris E.R., 1996; Morris V.J., 1996).

Because the synergistic interaction decreased as the salt concentration increased, our results suggest that the degree of disordering of xanthan is critical in xanthan-guar interaction, which may explain the differences in η' and η'' measurements between 2 and 40 mM NaCl. These results support previous studies (Cheetham & Mashimba, 1988, 1991; Zhan et al., 1993) proposing that intermolecular binding occurred between galactomannans and disordered segments of xanthan.

4.3.2. Intrinsic viscosities of polysaccharides

The intermolecular binding between xanthan and guar was further supported by the intrinsic viscosities of xanthan and guar mixtures (Fig. 4.3a & b). Fig. 4.3a & b

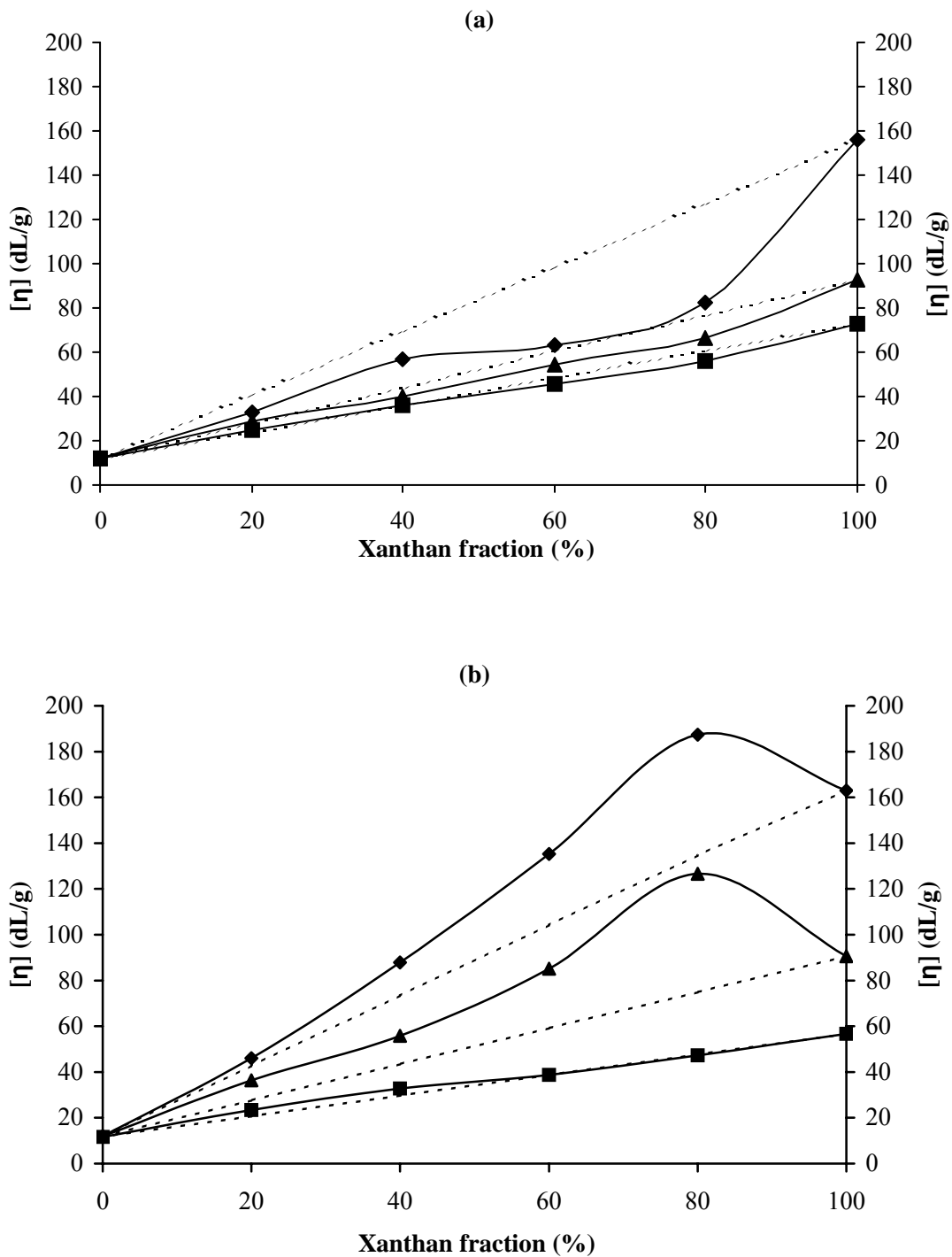


Fig. 4.3. Plots of intrinsic viscosities against xanthan fraction. (a) native xanthan-guar mixtures and (b) deacetylated xanthan-guar mixtures. (♦) water; (▲) 2 mM NaCl; (■) 40 mM NaCl; (---) values calculated for mixtures assuming no interaction.

depict the intrinsic viscosities against the percentage of xanthan fraction at different ionic strengths for native xanthan-guar mixtures and deacetylated xanthan-guar mixtures, respectively. The intrinsic viscosities of deacetylated xanthan-guar mixtures in water and 2 mM NaCl were higher than those calculated from the weight averages of the two individually assuming no interaction, whereas the intrinsic viscosities of native xanthan-guar mixtures were lower than those calculated from weight averages of the two individually, demonstrating that intermolecular interaction occurred between xanthan and guar gum in water and 2 mM NaCl. If no interaction existed between the two polymers in the mixtures, a linear relationship between intrinsic viscosity and xanthan fraction would be observed, and the intrinsic viscosities for the mixtures would be only the weight averages of the two polymers. Instead, the intrinsic viscosities of the mixtures was out of linearity, suggesting that specific attraction forces were present between the xanthan and guar molecules in water and 2 mM NaCl. In 40 mM NaCl, however, a linear relationship was found between the intrinsic viscosities and xanthan fraction for both deacetylated xanthan-guar mixtures and native xanthan-guar mixtures, suggesting that the intrinsic viscosities of the mixtures were only the weight averages of the two polymers, and that no specific interaction forces were present between the two polymer molecules that would encourage aggregation. The ordered, helical structure of xanthan was stabilized in 40 mM NaCl; thus, the interaction between xanthan and guar gum diminished. The results suggest that the intermolecular binding between xanthan and guar gum may not occur in 40 mM NaCl, and mutual incompatibility may take place under these conditions. These results are in agreement with a previous study conducted by Wang, Wang, & Sun (2002). The discrepancy in the intrinsic viscosities behavior of both deacetylated xanthan-guar mixtures and native xanthan-guar mixtures in water and salts solutions can be explained again in terms of disordering degree of

xanthan structure. In 2 mM NaCl, xanthan is partly ordered, whereas the molecule is completely ordered in 40 mM NaCl.

Intrinsic viscosity of polymers is dependent on their molecular weight and chain dimension (Flory, 1953). In dilute solutions, the polymer chains are separate, so $[\eta]$ of a polymer in solution depends only on the dimensions of the polymer chain (Rao, 1999). The strong intermolecular binding between deacetylated xanthan and guar can be attributed to the increased chain flexibility of deacetylated xanthan, which may facilitate the interaction with guar gum. The intrinsic viscosities of native xanthan-guar mixtures increased as the xanthan fraction increased in the mixtures. The intrinsic viscosities of native xanthan-guar mixtures were lower than those calculated from weight averages of the two individually. Because xanthan plays a crucial role in controlling the viscosity of xanthan-guar mixtures, the decrease in intrinsic viscosities of their mixtures can be attributed to the conformational change of xanthan from a helical form to a more flexible form because of binding. Xanthan conformation change outweighed the increase of intrinsic viscosity due to binding; thus, the dramatic decrease of the intrinsic viscosities of the mixtures was observed. These results suggest that conformational change of deacetylated xanthan may not predominate in controlling the intrinsic viscosity, and that deacetylated xanthan may be in an exact conformation to bind guar.

Table 4.2 shows the values of the intrinsic viscosities of deacetylated xanthan, native xanthan, guar, and their mixtures in water and salt solutions. The $[\eta]$ of deacetylated and native xanthan decreased significantly ($p < 0.05$) as the salt concentration increased, showing a typical behavior of polyelectrolytes. The charge

Table 4.2. Effect of ionic strength on the intrinsic viscosity of native xanthan-guar blends and deacetylated xanthan-guar blends measured at 20 °C and 10 s⁻¹.

Native Xanthan:Guar (%)	[η] (dL/g)		
	0 mM NaCl	2 mM NaCl	40 mM NaCl
100:0	155.7 ^a ± 9.3	92.9 ^b ± 2.7	73.2 ^c ± 1.1
80:20	82.2 ^a ± 2.8	66.0 ^b ± 1.3	56.1 ^c ± 2.2
60:40	63.3 ^a ± 1.4	54.3 ^b ± 0.9	45.3 ^c ± 1.8
40:60	56.9 ^a ± 7.2	39.8 ^b ± 1.4	36.0 ^b ± 1.5
20:80	32.8 ^a ± 2.86	29.2 ^{ab} ± 0.7	25.0 ^b ± 2.5
0:100	12.0 ^a ± 2.1	11.9 ^a ± 0.6	11.7 ^a ± 0.6
Deacetylated Xanthan:Guar (%)			
100:0	163.0 ^a ± 5.0	90.6 ^b ± 2.3	56.7 ^c ± 3.3
80:20	187.4 ^a ± 5.8	126.6 ^b ± 5.2	47.2 ^c ± 2.5
60:40	135.0 ^a ± 7.3	85.2 ^b ± 1.4	38.7 ^c ± 0.8
40:60	88.0 ^a ± 5.6	55.8 ^b ± 1.1	32.7 ^c ± 0.2
20:80	46.1 ^a ± 2.8	36.4 ^b ± 2.2	23.4 ^c ± 1.6
0:100	12.0 ^a ± 2.1	11.9 ^a ± 0.6	11.7 ^a ± 0.6

^{a, b, c} Means followed by the same letters in the same row are not significantly different ($P \leq 0.05$)

Results are expressed as means ± SD for three replications

screening for electrostatic repulsions of the trisaccharide sidechains from NaCl led to a more compact conformation and caused a significant reduction in hydrodynamic size of the molecule. Thus, the [η] was decreased. On the other hand, salt exhibited little effect on the [η] guar gum due to the nonionic nature of guar. The [η] of guar gum decreased slightly with no significant difference as the salt concentration increased.

The [η] of deacetylated xanthan in water was higher than the [η] of native xanthan. Deacetylated xanthan had a [η] of 163 dl/g, whereas the [η] of native xanthan was 154dl/g; however, the difference not statistically significant ($P > 0.05$). This is consistent with findings reported by Callet, Milas, & Rinaudo (1987), who showed that acetyl and pyruvate contents have

no influence on the intrinsic viscosity of xanthan in dilute solution. The $[\eta]$ of guar gum was 12 dl/g. Deacetylated and native xanthan had a much higher $[\eta]$ than guar gum, which can be attributed to the significant difference in their chain stiffness. Xanthan has a stronger chain stiffness than the flexible random-coil conformation of guar, which may increase the chain dimensions, resulting in a higher $[\eta]$.

4.3.3. Coil overlap parameter of polysaccharides

In dilute solutions, the individual polymers coils are separate from each other and are free to move independently. With increasing concentrations, the coils start to overlap and interpenetrate one another. The transition from dilute solutions to concentrated solutions is usually accompanied by a pronounced change in the concentration dependence of solution viscosity (Morris, Cutler, Ross-Murphy, Ress, & Price, 1981; Morris, 1995). The corresponding concentration is called critical or coil-overlap concentration (C^*). For random-coil polysaccharide solutions except for galactomannans, Morris et al. (1981) reported that the slope of double logarithmic plots of η_{sp} against $C[\eta]$ was close to 1.4 in a dilute regime, whereas, in the concentrated regime, the slope increased to 3.3. The C^* transition occurred at a value of $C[\eta]$ close to 4, and the η_{sp} at this degree of coil overlap was invariably close to 10. Guar gum was found to deviate from those observations. The C^* transition of guar occurred at a lower value of the coil-overlap parameter $C[\eta] = 2.5$, and the viscosity showed a higher dependence on concentration, with a slope of 5.1 instead of 3.3 (Morris et al., 1981).

In this study, all the polysaccharides systems were studied in the dilute regime. Fig. 4.4a & b and Fig. 4.5a & b show the double logarithmic plot of η_{sp} against coil-overlap parameter ($C[\eta]$) for native xanthan-guar mixtures and deacetylated xanthan-guar mixtures in 2 and 40 mM NaCl, respectively. There was no change in the slope of the double logarithmic plot of η_{sp}

against $C[\eta]$, indicating that no molecular entanglements were obtained, and polysaccharide mixtures in 2 and 40 mM NaCl were in the dilute domain. The slope values of the double logarithmic plot of η_{sp} against $C[\eta]$ are given in Table 4.3. As shown in Table 4.3, the slope of native xanthan and deacetylated xanthan in salt solutions significantly decreased, comparing with the slope in water solutions, whereas no significant change was observed for guar solutions. Our results showed that the slopes of xanthan and guar were lower than those reported by Morris et al. (1981), Cuvelier & Launay (1986), and Launay, Cuvelier, & Martinez-Reyes (1997), demonstrating that both xanthan (0.025%) and guar gum (0.075%) were in the dilute domain ($C < C^*$). The slopes for deacetylated xanthan and deacetylated xanthan-guar mixtures were lower than those for native xanthan and native xanthan-guar mixtures, indicating more flexible xanthan due to the deacetylation.

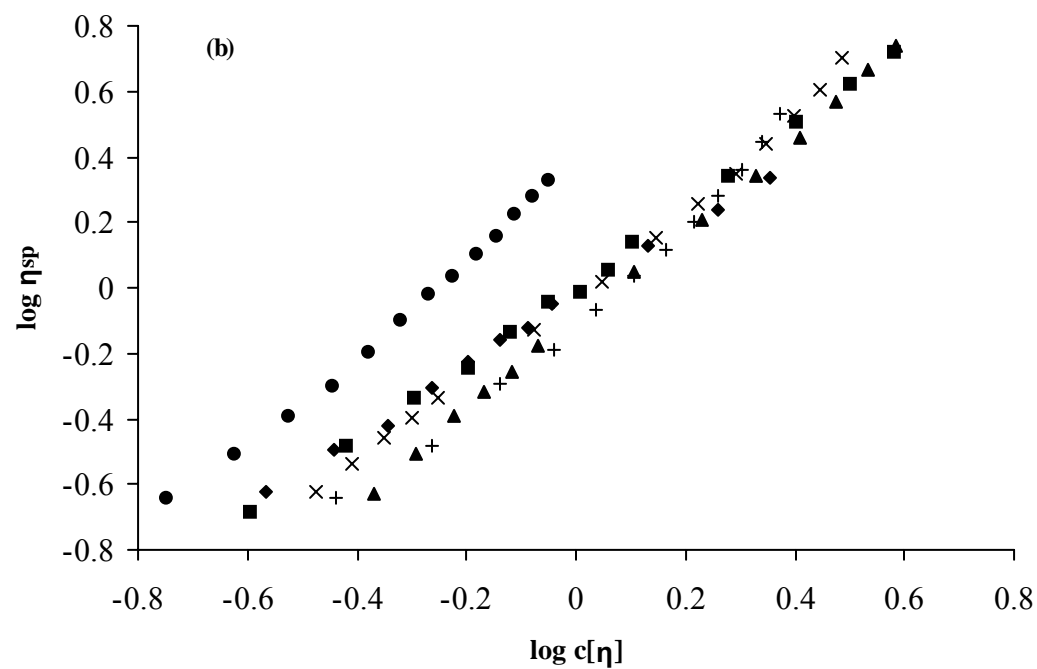
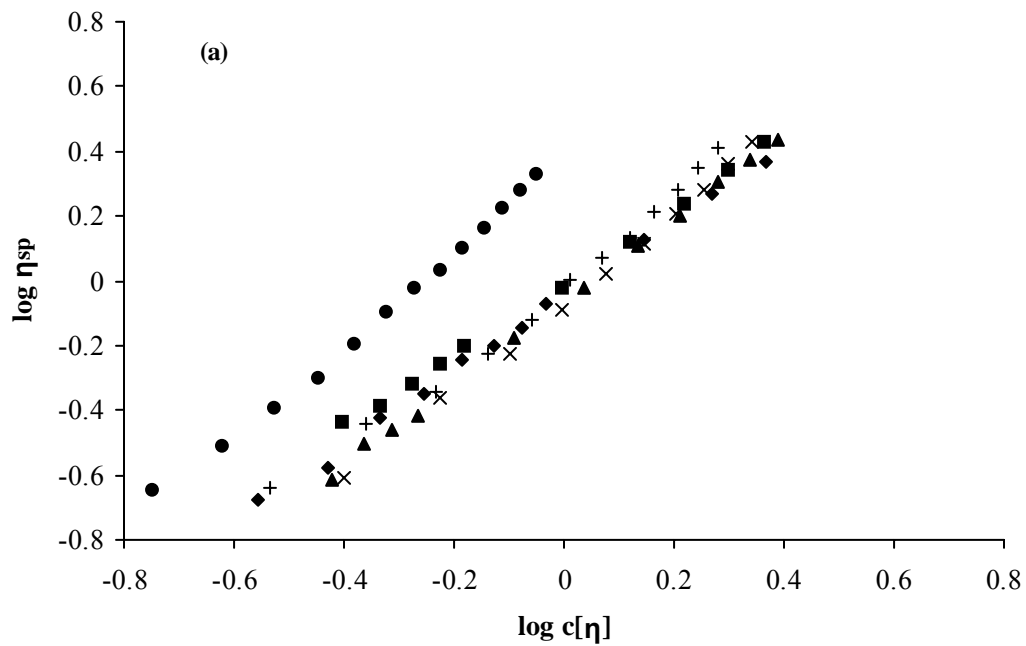


Fig. 4.4. Double logarithmic plot of specific viscosity (η_{sp}) against coil-overlap parameter ($c[\eta]$) in 2 mM NaCl for (a) deacetylated xanthan-guar mixtures, (b) native xanthan-guar mixtures. Xanthan:Guar ratio: (◆) xanthan, (●) guar, (■) 4:1, (▲) 3:2, (×) 2:3, (+) 1:4.

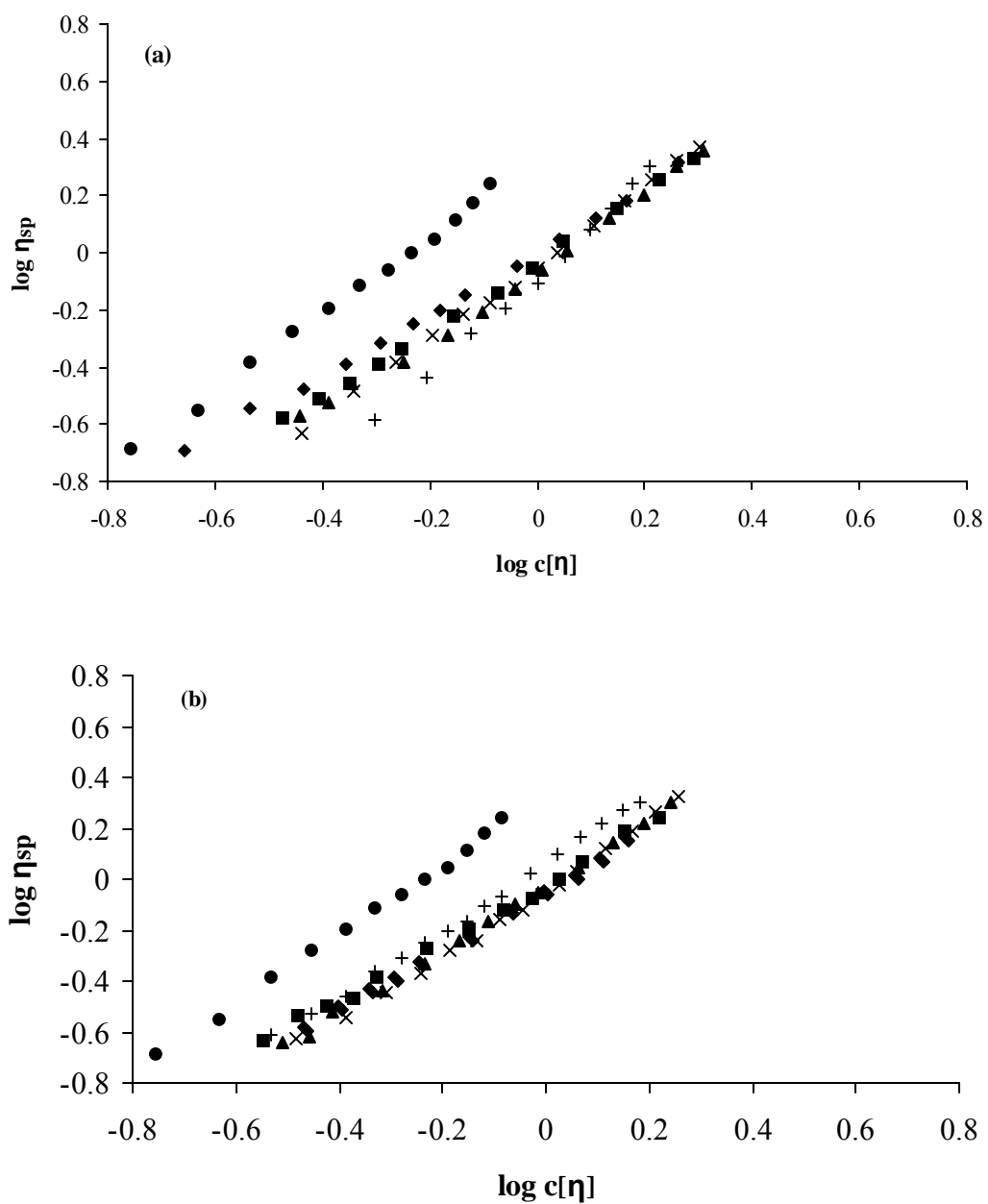


Fig. 4.5. Double logarithmic plot of specific viscosity (η_{sp}) against coil-overlap parameter ($c[\eta]$) in 40 mM NaCl for (a) deacetylated xanthan-guar mixtures, (b) native xanthan-guar mixtures. Xanthan:Guar ratio: (◆) xanthan, (●) guar, (■) 4:1, (▲) 3:2, (×) 2:3, (+) 1:4.

Table 4.3. Effect of ionic strength on the slope of native xanthan-guar blends and deacetylated xanthan-guar blends measured at 20 °C and 10 s⁻¹.

Native Xanthan:Guar (%)	Slope		
	0 mM NaCl	2 mM NaCl	40 mM NaCl
100:0	1.38 ^a ± 0.02	1.17 ^b ± 0.03	1.11 ^b ± 0.03
80:20	1.51 ^a ± 0.01	1.17 ^a ± 0.04	1.20 ^a ± 0.02
60:40	1.34 ^a ± 0.16	1.28 ^a ± 0.02	1.27 ^a ± 0.02
40:60	1.55 ^a ± 0.02	1.38 ^b ± 0.02	1.36 ^b ± 0.02
20:80	1.69 ^a ± 0.02	1.31 ^b ± 0.04	1.33 ^b ± 0.08
0:100	1.44 ^a ± 0.06	1.41 ^a ± 0.01	1.39 ^a ± 0.01
Deacetylated Xanthan:Guar (%)			
100:0	1.32 ^a ± 0.05	1.09 ^b ± 0.03	1.17 ^c ± 0.02
80:20	1.22 ^{ab} ± 0.03	1.25 ^a ± 0.05	1.18 ^b ± 0.03
60:40	1.33 ^{ab} ± 0.03	1.38 ^a ± 0.02	1.28 ^b ± 0.03
40:60	1.43 ^a ± 0.07	1.36 ^b ± 0.04	1.33 ^b ± 0.03
20:80	1.35 ^{ab} ± 0.06	1.41 ^a ± 0.04	1.32 ^b ± 0.02
0:100	1.44 ^a ± 0.06	1.41 ^a ± 0.01	1.39 ^a ± 0.01

^{a, b, c} Means followed by the same letters in the same row are not significantly different ($P \leq 0.05$)

Results are expressed as means ± SD for three replications

4.4. Conclusion

Synergistic interactions for both native xanthan-guar mixtures and deacetylated xanthan-guar mixtures in the dilute regime were observed in water and 2 mM NaCl, but not in 40 mM NaCl. A stronger synergistic interaction was noted for deacetylated xanthan-guar mixtures. The results suggest that intermolecular interaction has occurred between xanthan and guar mixtures in water and 2 mM NaCl, but may not occur in 40 mM NaCl, and mutual incompatibility may occur. The results suggest that the degree of disordering of xanthan played a critical role in xanthan-guar interaction and may explain the differences in η' , η'' , and $[\eta]$ measurements between 2 and 40 mM NaCl, and hence, the intermolecular interaction that occurred between the backbone of guar gum and the disordered segments of xanthan.

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Future Recommendations

Although rheological methods provided an absolute evidence for the intermolecular interaction between xanthan and guar gum in dilute solutions, the interaction between the two polysaccharides in dilute solutions can be further studied by other analytical methods.

Light scattering methods can be used to study the interaction between the two polysaccharides. Preliminary work revealed that high performance size-exclusion chromatography (HPSEC), coupled on line with a multiangle laser light scattering detector (MALLS) and a refractive index detector can be a useful method to characterize xanthan-guar interaction. However, much attention needs to be paid to the sample aging of the polysaccharides. Xanthan's molecular weight significantly increased as the sample age increased due to polymer aggregations.

Xanthan-guar interaction can be investigated by using atomic force microscopy (AFM) technique. AFM can be used to characterize the conformations of individual macromolecules of xanthan and guar gum and to provide images of xanthan-guar mixture structure in dilute solution.

Combinations of xanthan-guar gum can be applied to food systems to improve texture and stability of finished products. The xanthan-guar system can be used in milk-juice beverages to prevent casein micelles from clumping together and precipitation. Chocolate and flavored milks are examples where this system can be applied to suspend cocoa powder. In low-calorie beverages such as soda, a combination of xanthan-guar gum can be used to improve texture and mouthfeel without adding any significant calories. In beverages that contain fruit pulp, xanthan-guar gum system can be used to slow the rate at which the pulp settles.

APPENDIX A



13 September 2006

Our ref: CT/mm/sept 06.J026

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Dear Mr. Khouryieh

FOOD RESEARCH INTERNATIONAL, In Press, 2006, Khouryieh et al, “Influence of mixing...”

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