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Gelation and rheology of xanthan/enzyme-modified guar blends

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Abstract

The rheological behavior and synergistic character of mixed polysaccharide systems are examined for blends of xanthan with enzymatically-modified guar. In particular, the enzyme α-galactosidase is used to selectively cleave off the galactose side chains of guar in order to obtain galactomannans with tailored molecular architecture: EMG1 with a relatively high galactose content of 33.6% and a mannose (M) to galactose (G) ratio of 1.85, and EMG2 with a lower galactose content (25.2%) and an M/G ratio of 2.86. Blends of xanthan with enzymatically-modified guar gum samples are examined in terms of their dynamic rheological properties and compared to those of xanthan — locust bean gum blends. The extent of synergism, illustrated by the gel elastic modulus G' and yield stress τ_c, is found to increase with increasing extent of enzymatic modification. At constant ionic strength, the EMG2 and locust bean blends behave similarly, with increasing extent of synergy as the temperature of mixing is increased. Additionally, at a fixed mixing temperature, the blends made in water have a higher elastic modulus than those made in salt. In contrast, the EMG1 blends are weaker and the dynamic moduli are unaffected by changes in the mixing temperature or ionic strength. These results are consistent with those of other researchers and are directly related to both the level of disorder in the xanthan molecule as well as the galactose content and fine structure of the galactomannan.

Keywords: Rheology; Modulus; Yield stress; Guar; Xanthan; Mixed polysaccharide

1. Introduction

Natural biopolymers are used extensively in many applications, from hydraulic fracturing fluids in enhanced oil/gas production to coatings and food additives, because of their ability to modulate rheological properties. In particular, galactomannans such as guar and locust bean gums are used as rheology modifiers in food products ranging from dressings, soups and sauces to gelated desserts and baked goods to impart desirable textures, modulus, mouthfeel and shelf-stability (Fox, 1992; Maier, Anderson, Karl & Magnuson, 1992). The water binding properties of these gums are used to advantage in preventing ice crystal growth and synerisis in dairy and frozen dessert products (Morris, 1990, 1995; Urlacher & Dalbe, 1992). Galactomannans can synergistically bind with the helices of other biopolymers such as xanthan and carrageenans, hence bringing about gelation of two components which are individually non-gelling, resulting in improved product quality and reduced production costs (Dea, Morris, Rees, Welsh, Barnes & Price, 1977). In addition to the above, low cost and abundance are major factors contributing to the popularity of galactomannans in the food industry.

At present, guar gum and locust bean gum are the only two galactomannans that are utilized on an industrial scale. Although the chemical structures of the two gums are the same, differences in the mannose to galactose ratios and the fine structures bring about significant differences in their properties. For example, guar undergoes rapid hydration in cold water to produce highly viscous solutions whereas locust bean gum can be dissolved only after heating to high temperatures (Fox, 1992; Maier et al., 1992). In addition, due to the greater proportion of galactose side chains, guar is unable to bind with the helices of xanthan or carrageenans to form synergistic gels (like locust bean gum) and can only produce an increase in viscosity (Dea & Morrison, 1975). Hence, the potential applications of guar gum are limited. This is unfortunate in light of the high costs and diminishing world supply of locust bean gum owing to factors such as long maturation period and competition from other cash crops (Bulpin, Gidley, Jeffocat & Underwood, 1990).

An alternative lies in tailoring the fine structure of guar gum using enzymes to produce a range of galactomannans with varying functionality. In particular, α-galactosidase from germinating legume seeds is effective in cleaving off

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the galactose side chains only, producing guar with a range of mannose to galactose ratios including that comparable to locust bean gum (Bulpin et al., 1990; McCleary, Amado, Weibel & Neukom, 1981; McCleary, Dea, Windust & Cooke, 1984). Previous research in our laboratory has dealt with rheological changes, molecular weight distributions and diffusion issues arising due to enzymatic action on guar solutions and gels (Burke, Park, Srinivasarao & Khan, 2000; Tayal & Khan, 2000; Tayal, Pai, Kelly & Khan, 1998; Tayal, Kelly & Khan, 1999a; Tayal, Pai & Khan, 1999b). The primary interests in these studies have been on the utilization of β-mannanase, a back-bone cleaving enzyme, and on investigating the properties of guar by itself. In this work, in contrast, we focus on understanding the rheological properties of guar, enzymatically modified using a side-chain cleaving α-galactosidase, in synergistic blends with xanthan.

D-galacto-D-mannans are reserve carbohydrates found in the endosperms of some legume seeds. They consist of a β-D-(1 → 4)-mannan backbone which is partially substituted by single (1 → 6)-α-D-galactopyranosyl groups (McCleary, 1979; McCleary, Clark, Dea & Rees, 1985). Well-known galactomannans, guar and locust bean gum, have galactose substitution degrees of 38 and 23%, respectively. Chemical procedures and hydrolysis techniques using highly purified enzymes indicate that the D-galactosyl groups in guar are arranged mainly in pairs and triplets. However, locust bean has a non-regular distribution of D-galactose units, with a high proportion of substituted couplets, lesser amount of triplets and regions of unsubstituted D-mannosyl residues (Hoffman & Svensson, 1978; Hoffman, Londberg & Painter, 1975; McCleary, Nurthen, Taravel & Joseleau, 1983; McCleary et al., 1985; Painter, Gonzalez & Hemmer, 1979). Evidence from NMR spectroscopy is also consistent with a random arrangement of D-galactosyl groups in the two galactomannans (Grasdalen & Painter, 1980).

Xanthan gum is an extracellular polysaccharide obtained from the bacterium *Xanthomonas campestris*. The primary structure of xanthan consists of a (1 → 4)-linked β-D-glucopyranosyl backbone, alternate units of which are substituted at the O-3 position by a trisaccharide side chain containing a D-glucoronosyl unit between two D-mannosyl units (Jansson, Kenne & Lindberg, 1975; Melton, Mindt, Rees & Sanderson, 1976). The terminal β-D-mannopyranosyl unit is glycosidically linked to the O-4 position of the β-D-glucopyranosyluronic acid unit, which in turn is glycosidically linked to the O-2 position of an α-D-mannopyranosyl unit. Approximately half of the terminal D-mannosyl units contain a pyruvic acid residue as a 4,6-cyclic acetal. Finally, the nonterminal D-mannosyl unit is stoichiometrically substituted at O-6 with an acetyl group (Jansson et al., 1975; Melton et al., 1976). The secondary structure of xanthan can undergo a unique transition from a disordered random coil configuration where the side chains are oriented away from the backbone to a rigid, helical structure where the side chains are folded along the backbone. The transition is dependent on temperature and ionic strength of the solution with the ordered form being stabilized at low temperatures and high ionic strengths (Morris, Rees, Young, Walkinshaw & Darke, 1977; Norton, Goodall, Frangou, Morris & Rees, 1984).

In this study, we use rheology to investigate synergistic effects in blends formed using xanthan and enzymatically-modified guar. In this regard, the structure of guar gum is tailored using highly specific α-galactosidase, a side-chain cleaving enzyme, to obtain two samples, one (EMG1) with a relatively high galactose content (33.6%) and an M/G ratio of 1.85 and the other (EMG2) with a lower galactose content (25.2%) and an M/G ratio of 2.86. We use dynamic rheology, a sensitive probe for material microstructure to probe gel formation and interactions of these enzymatically-modified guar with xanthan (Raghavan, Hou, Baker & Khan, 2000a; Raghavan, Walls & Khan, 2000b). Previous studies in this area have revealed that enzymatic modification of guar with α-galactosidase (McCleary et al., 1981) enhances its interaction with xanthan, agarose (McCleary et al., 1984) and κ-carrageenan (Bulpin et al., 1990). In particular, increases in compressive yield stress, compressive fracture strain and elastic modulus (measured at a single frequency) have been observed in these blends (McCleary et al., 1981, 1984). However, no detailed rheological studies have been undertaken for any of these systems.

The present study expands the scope of previous research and addresses some unresolved issues that include the effect of temperature of preparation (T_p), xanthan to galactomannan ratio and ionic strength of xanthan solutions on the viscoelastic behavior of blends containing enzyme-modified guar and xanthan. In particular, our study reveals that enzymatic modification produces blends with significantly higher elastic modulus as compared to native guar blends, with gel strength increasing with decreasing galactose content. EMG1 blends containing galactomannan with a high galactose content (33.6%) are unaffected by changes in temperature of preparation or changes in solvent environment produced by addition of salt. In contrast, at a fixed ionic strength, EMG2 blends containing galactomannan with a low galactose content (25.2%) exhibit a higher elasticity (G') with increasing temperature of mixing. However, at a fixed temperature, gel strength decreases when ionic strength of the solution is increased. These results are explained in terms of the change in xanthan conformation in response to temperature or ionic strength and/or the galactose content and fine structure of the galactomannan.

2. Experimental

2.1. Materials

Samples of Uniguar 150 (*Cyamopsis tetragonolobus*) and locust bean gum (*Ceratonia siliqua*) were obtained from Rhodia Food Ingredients, Cranbury, New Jersey. These

Table 1
Mannose to Galactose ratios of galactomannans determined by the alditol acetate method

Galactomannan	Mannose (mol%)	Galactose (mol%)	M/G ratio
Native guar	57.9	39.1	1.48
EMG1	62.1	33.6	1.85
EMG2	72.0	25.2	2.86
Locust bean gum	72.9	25.8	2.82

commercial samples were found to contain insoluble impurities like seed husks and other cellulosic material, which produced hazy solutions upon dissolution of the gums in water. Hence, an ethanol extraction procedure was used to purify the galactomannans to remove insoluble impurities (Mannion, Melia, Launay, Cuvelier, Hill, Harding & Mitchell, 1992). A commercial sample of xanthan gum obtained from Aldrich Chemical Company was used in all experiments.

2.2. Purification of galactomannans

The desired amount of Uniguar 150 needed to produce a 0.8% (w/w) solution was measured and slowly dispersed in deionized water at 25°C. Sodium thiosulfate (0.5% w/w) and sodium azide (0.05% w/w) were added to guar to prevent thermal and bacterial degradation, respectively. A Dyna-mix model mixer from Fisher Scientific with an impeller having two straight blades mounted on the shaft was used to homogenize the solutions. The required amount of water was taken in a beaker and the mixer speed was adjusted so as to form a shallow vortex. The guar was then sprinkled slowly onto the free surface of the vortex to produce a uniform dispersion with minimum formation of clumps commonly called 'fish eyes'. The mixer speed was set to 6000 rpm, the beaker sealed with Parafilm and the solution was allowed to hydrate for 24 h to allow optimum viscosity development.

Locust bean solutions were made at 80°C as the gum is insoluble in water at room temperature due to its higher mannan content (Maier et al., 1992). Locust bean gum (0.8% w/w), sodium thiosulfate (0.5% w/w), sodium azide (0.02% w/w) and water were measured out into a Waring blender. The jar was tightly capped, sealed with Parafilm to prevent evaporation and the contents were heated to 80°C. When the desired temperature was reached, the solution was sheared at high speed for 20 min to obtain a homogeneous, viscous solution. Both the galactomannan dispersions were centrifuged (RC5C, Sorvall Instruments, Dupont) at 4000 rpm for 20 min to separate out impurities. The supernatant (clear, viscous solution) containing the purified polymer was saved for extraction while the pellet, mainly containing impurities, was discarded. The solubilized galactomannan was precipitated by pouring into twice the volume of ethanol. This process was continued until addition of ethanol to the supernatant did not result in further precipitation of the biopolymer. The precipitate was washed with ethanol and lyophilized for 24 h. The dried sample was pulverized to a fine powder and stored (Mannion et al., 1992).

2.2.1. Preparation of solutions

Solutions of the purified guar and locust bean gum were prepared in the same way as described above at 25 and 80°C, respectively. All guar solutions, unmodified and enzymatically-modified (see Section 2.2.2 for procedure), were soluble in water at room temperature and showed the same consistency in solutions. Xanthan solutions were prepared by dispersing the gum in deionized water at room temperature and shearing in a Waring blender for 15 min. The solution was then centrifuged at 3000 rpm for 10 min to remove air bubbles produced during shearing. Sodium thiosulfate (0.5% w/w) was added to the galactomannan solutions to prevent thermal degradation. Sodium azide (0.05% w/w) was added to all the solutions to prevent bacterial degradation. All solutions and dilutions of stock solutions were made on a weight basis.

2.2.2. Degradation of galactomannan solutions

Guar gum was hydrolyzed using α-galactosidase from guar seed obtained from Megazyme International, Ireland. Guar solutions (0.8% w/w) were incubated with 0.4 U/ml guar of α-galactosidase in a constant temperature gyrotory water bath shaker. (One unit of activity is the amount of enzyme required to release one micromole of product (e.g. *p*-nitrophenyl) per minute at pH 4.5 and 40°C). Incubation times were set at 2.5 and 5 h to obtain two modified guar samples. Enzymatic hydrolysis was carried out at 35°C so that enzyme activity was at its optimum. The enzyme was deactivated at the end of the incubation time interval by heating the solution at 85°C for 10 min. Mannose to galactose ratios of native guar, locust bean gum and the enzyme-modified guar samples were determined by conversion to alditol acetates and analysis by GC–MS. The samples were hydrolyzed in 2 M TFA at 121°C for 2 h and the hydrolyzed carbohydrate was reduced with sodium borodeuteride at room temperature. The product was then acetylated using acetic anhydride at 120°C for 3 h. The derivatized sample was analyzed by GC–MS using Sp2330 Supelco column. Internal standard (myo-inositol) was added to the sample prior to the reduction step. The results obtained are summarized in Table 1.

2.2.3. Preparation of blends

Blends of galactomannan with xanthan gum (0.8 wt% total biopolymer concentration) were prepared by first mixing appropriate amounts of xanthan and galactomannan solutions at 25°C. The mixtures were then homogenized at the desired preparation temperature T_p for 60 s. The samples were finally centrifuged at 2500 rpm for 10 min (at 25°C) to remove any entrapped air bubbles. All samples were allowed to stand for 24 h at room temperature before any further rheological testing.

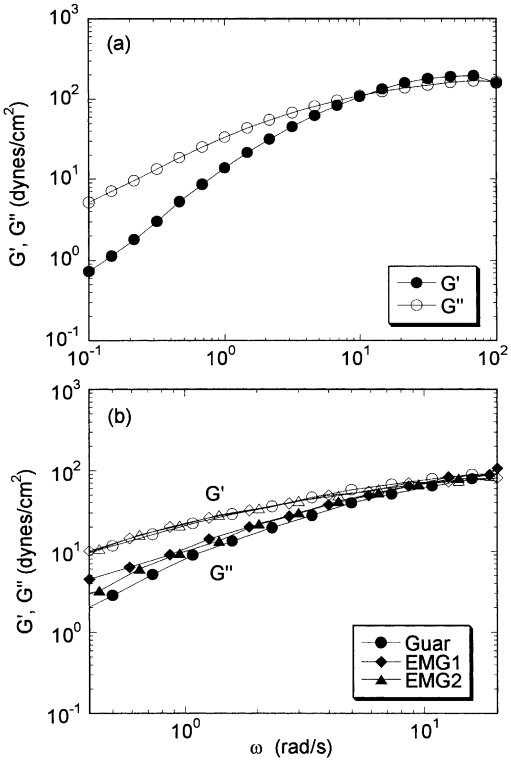

Fig. 1. (a) Dynamic elastic (G') and viscous moduli (G'') of a 0.8% w/w guar galactomannan solution as function of frequency. (b) Overlay of frequency sweeps of 0.8% w/w native guar, EMG1 and EMG2 solutions. EMG1 and EMG2 correspond to enzymatically-modified guar with 33.6 mol% galactose (M/G ratio of 1.85) and 25.2 mol% galactose (M/G ratio of 2.86), respectively.

2.3. Rheological characterization

Dynamic rheological measurements were conducted using a Rheometrics Dynamic Stress Rheometer (DSR II) fitted with 40 mm diameter parallel plates. Plates with radial grooves were chosen in order to prevent slippage of the gels. These experiments allowed us to probe material microstructure through measurement of the elastic (G') and viscous (G'') moduli as a function of frequency or stress. Dynamic frequency sweeps were performed at a constant strain amplitude of 1%. Dynamic strain sweeps were conducted prior to performing the frequency sweeps to ensure operation within the linear viscoelastic region. All experiments were conducted at 25°C although the mixing temperature of the blends might have been varied. The samples were covered with a thin layer of silicone oil to prevent evaporation of water from the solutions. Experiments were repeated to ensure that the addition of oil did not affect the data.

3. Results and discussion

3.1. Viscoelastic behavior of xanthan–galactomannan blends

3.1.1. Solution rheology

We begin by investigating the rheological behavior of each of the biopolymers — guar, locust bean and xanthan when dissolved in water. The concentration of each biopolymer was maintained at 0.8% w/w in every case. Fig. 1a depicts the frequency spectrum of 0.8% w/w guar solution alone. We observe that guar behaves like a typical macromolecular entangled biopolymer in solution, with the elastic modulus G' dominating over the viscous modulus G'' in the high frequency range. A crossover point is observed at intermediate frequencies and at lower frequencies or longer relaxation times, the viscous modulus predominates, with G' and G'' traces showing characteristic slopes of -2 and -1, respectively. Enzymatic modification does not change the solution rheology as can be seen from the overlay of frequency sweeps of EMG1 and EMG2 solutions with native guar solutions in Fig. 1b. This is consistent with the fact that the enzyme only clips off the galactose side chains and hence does not effectively change the hydrodynamic radius of the guar molecule, which would cause changes in viscosity.

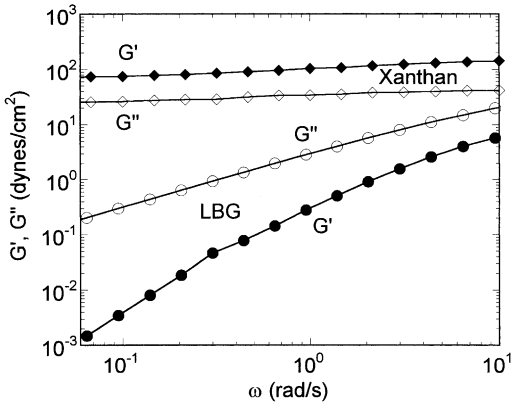

Fig. 2. Frequency spectra of the dynamic elastic (G') and viscous (G'') moduli of locust bean and xanthan gum solutions. Total polysaccharide concentration was maintained at 0.8% w/w. (Open symbols — G''; closed symbols — G'.)

In contrast to guar gum, the viscous modulus G'' is greater than the elastic modulus G' over the entire frequency range tested for a freshly prepared locust bean gum solution (Fig. 2). The moduli are highly frequency dependent and we see no evidence of a cross-over point, suggesting that locust bean behaves merely like a dilute polymer solution. These differences between guar and locust bean solutions are due to the fact that the molecular weight of locust bean is almost an order of magnitude lower, hence requiring higher concentrations to achieve significant degree of overlap between the molecules. Fig. 2 also contrasts the dynamic response of xanthan solutions with that of galactomannan solutions. We observe that xanthan shows a weak gel-like behavior when subjected to small oscillatory deformations with the elastic modulus G' being higher than the viscous modulus G'' over the entire frequency range studied. We also note that the G' of xanthan is higher than locust bean gum, particularly at low frequencies, suggestive of a relatively stronger network. In solution at low temperatures, the

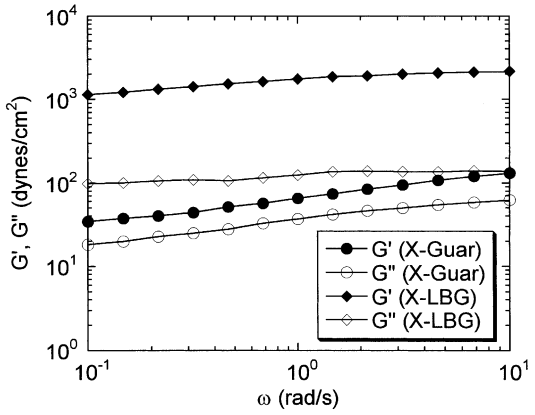

Fig. 3. Comparison of frequency spectra of the elastic (G') and viscous (G'') moduli of blends of xanthan with native guar, X–Guar (○), and xanthan with locust bean gum, X-LBG (◆). Total polysaccharide concentration was maintained at 0.8% w/w. (Open symbols — G''; closed symbols — G'.)

xanthan molecule is disordered (or partially ordered), but has an extended structure due to the electrostatic repulsions from the charged groups on the side chains. As a result, weak, non-covalent interactions develop between the molecules and they align and stack together building up a tenuous, gel-like structure (Richardson & Ross-Murphy, 1987; Rochefort & Middleman, 1987). These unusual rheological characteristics are responsible for the widespread use of xanthan as a thickening and suspending agent.

3.1.2. Blend rheology

We now proceed to examine the interactions that develop when xanthan and the galactomannans are blended together. Fig. 3 compares the dynamic frequency spectra of two blends, one containing xanthan and locust bean and the other containing xanthan and native guar. The total concentration was maintained at 0.8% w/w and the two biopolymers were mixed in a 1:1 ratio. We observe that the xanthan–locust bean system shows a highly elastic behavior, with the elastic modulus G' significantly larger than the viscous modulus, G'' with both moduli being effectively frequency independent over the entire range of experimental conditions. Such a response is indicative of the presence of a three-dimensional network in the system. In addition, the elastic modulus of the gel is at least one or two orders of magnitude higher than the xanthan and locust bean solution moduli, respectively, indicating the presence of synergistic behavior.

In contrast, the xanthan–guar blend data in Fig. 3 exhibits no such synergism. The spectrum possesses characteristics and values similar to that of xanthan only, indicating that the role of xanthan is predominant. Thus, the addition of xanthan to guar brings about a transition from an entangled polymer solution (Fig. 1) to a weakly associated network. The synergistic interactions of xanthan with locust bean gum can be explained in terms of junction zones formed by the binding of the ordered xanthan helix with the smooth regions on the galactomannan backbone (Dea & Morrison,

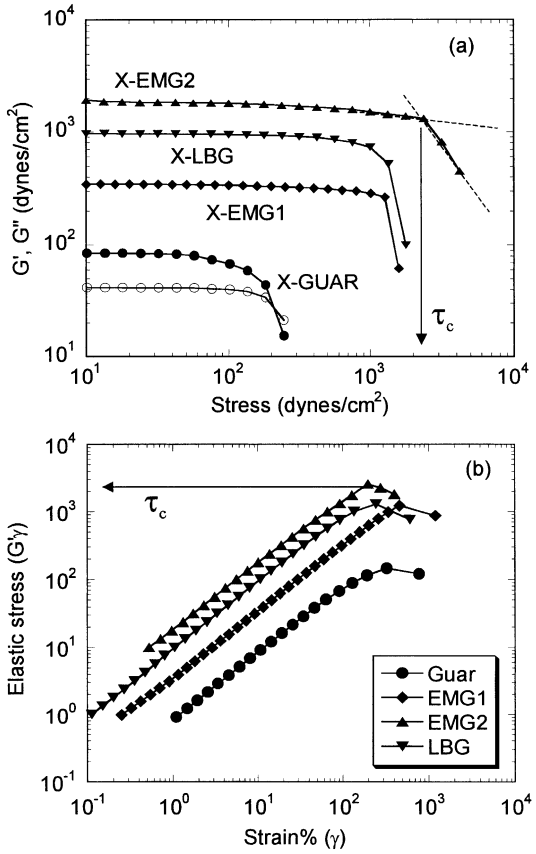

Fig. 4. (a) Elastic modulus G' as a function of increasing oscillatory stress, τ, shown for blends of xanthan (X) with native guar, enzyme-modified guar1 (EMG1, 33.6% galactose), enzyme-modified guar2 (EMG2, 25.2% galactose) and locust bean gum (LBG). Representative viscous moduli (G'') are shown for native guar and EMG2 blends only for the sake of clarity. (Open symbols — G''; closed symbols — G'). (b) The product of elastic modulus and strain ($G'\gamma$) as a function of strain shown for X–Guar, X–EMG1, X–EMG2 and X–LBG blends, with the maximum corresponding to the critical stress.

1975; Dea et al., 1977) and/or with sections of the galactomannan where the galactose residues were positioned on one side of the chain (McCleary, 1979). The formation of such junction zones is facilitated in locust bean gums by the presence of fewer galactose residues, and leads to an increase in viscosity or development of a gel-like network. In the case of guar-xanthan blends, it is possible that the presence of numerous galactose side chains attached to the mannan backbone in guar hinders the formation of junction zones and prevents the development of a three-dimensional network (McCleary, 1979).

3.1.3. Effect of enzymatic modification of guar

We proceed to compare and contrast the rheological characteristics of the two enzymatically modified guar samples, EMG1 (33.6% galactose; M/G ratio of 1.85) and EMG2 (25.2% galactose; M/G ratio of 2.86) when blended with xanthan. The biopolymers were mixed in a 1:1 ratio and the total concentration was maintained at 0.8% w/w. Fig. 4a compares the elastic (G') and viscous (G'') moduli

Table 2
Comparison of the yield stresses of blends of xanthan with guar, EMG1, EMG2 and locust bean gum

Blend	X–GUAR	X–EMG1	X–EMG2	X–LBG
τ_c (dynes/cm^2) from Method 1	130 ± 10	1250 ± 80	2250 ± 100	1250 ± 80
τ_c (dynes/cm^2) from Method 2	142 ± 10	1230 ± 50	2531 ± 50	1250 ± 50

behavior of blends containing native guar, EMG1, EMG2 and locust bean gum under the influence of increasing oscillatory shear stress. For the sake of clarity, we have plotted loss moduli for the native guar and EMG2 blends only. All the blends show an initial linear viscoelastic region where the moduli are independent of the stress. The elastic modulus is larger than the viscous modulus indicating the presence of a network structure. As the applied stress is increased, the bonds holding the network together begin to rupture. At a critical value of the stress (τ_c), the network structure breaks down leading to a sharp decrease in the value of G'. This critical stress, which is typically obtained from the intersection of the two asymptotic lines drawn through the initial and post-breakdown modulus data (shown in Fig. 4a), can be considered to be an approximate measure of the yield stress of the material. Table 2 summarizes the critical stresses of the different blends obtained using this method (Method 1). Values of the critical stress can also be pinpointed using an alternative approach that involves plotting the product of the elastic modulus and strain amplitude ($G'\gamma$) as a function of strain (γ). A maximum in such a plot (Fig. 4b) corresponds to the presence of a yield stress (Yang, Scriven & Macosko, 1986). Values of the yield stress obtained using such an approach are also shown in Table 2 (Method 2) and are quite consistent with the other method.

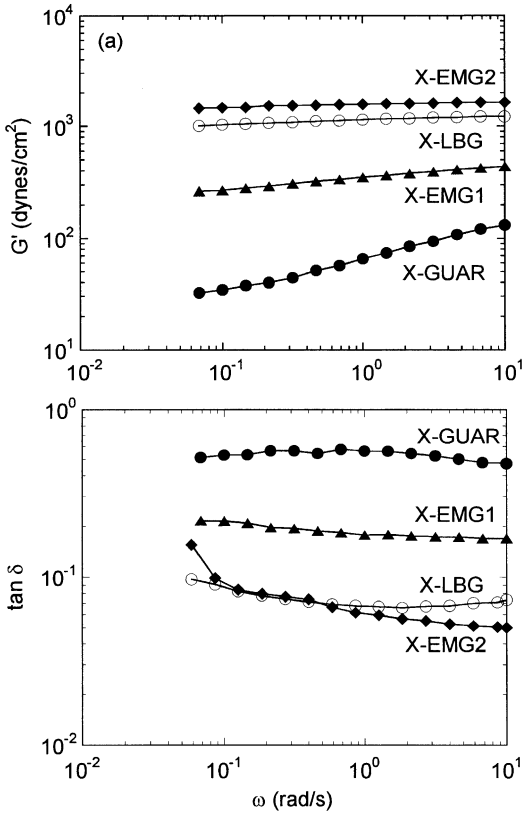

Fig. 5. Elastic modulus G' (a) and loss tangent tan δ. (b) as a function of frequency shown for blends of xanthan with native guar, EMG1 (33.6% galactose), EMG2 (25.2% galactose) and locust bean gum. Here, EMG stands for enzymatically-modified guar.

We observe from Fig. 4 and Table 2 that the blend made with native guar is the least elastic and yields at the lowest stress. Enzymatic modification of guar increases the elasticity of the resulting blends with the EMG2 blend having the highest critical stress, even exceeding that of the locust bean–xanthan blend. This increase in the strength of the blends (G') as guar is modified can be attributed to the progressive exposure of contiguous sequences of unsubstituted mannan regions, which are then free to interact with xanthan to form junction zones. In other words, as the galactose content of guar decreases due to enzymatic hydrolysis, it interacts to a greater extent with xanthan producing stronger and more elastic gels. Enhanced moduli and yield stresses of modified guar and xanthan blends are significant from the standpoint of commercial applicability where these parameters play an important role in determining the textural qualities of food. A comparison of the results for blends containing EMG2 and locust bean gum indicates that as a result of enzymatic action, modified guar can show rheological properties comparable to or better than those of locust bean gum. It is interesting to note that the values of the critical stress obtained using both methods are quite similar, albeit not exact. However, the relative trends in both cases stay the same.

Fig. 5a and b show the variation of the elastic modulus (G') and loss tangent (tan δ) as a function of frequency for the different blends. In all cases, we find the elastic modulus to be greater than the viscous modulus over the entire range of experimental frequencies, as evidenced from tan $\delta < 1$. The guar blend has an elastic modulus that is most frequency dependent and the largest tan δ, indicating that it possesses the weakest physical associations. Both the loss tangent and the frequency dependence of G' decrease with increasing extent of enzymatic modification. These results, together with a corresponding increase in G' suggest that the blends become more elastic as guar is modified, with G' values of the EMG2 blends being almost two orders of magnitude higher than the modulus of the native guar blend. Note also that the EMG2 blend shows a G' higher than that of the locust bean blend.

The effect of varying xanthan to biopolymer ratio is

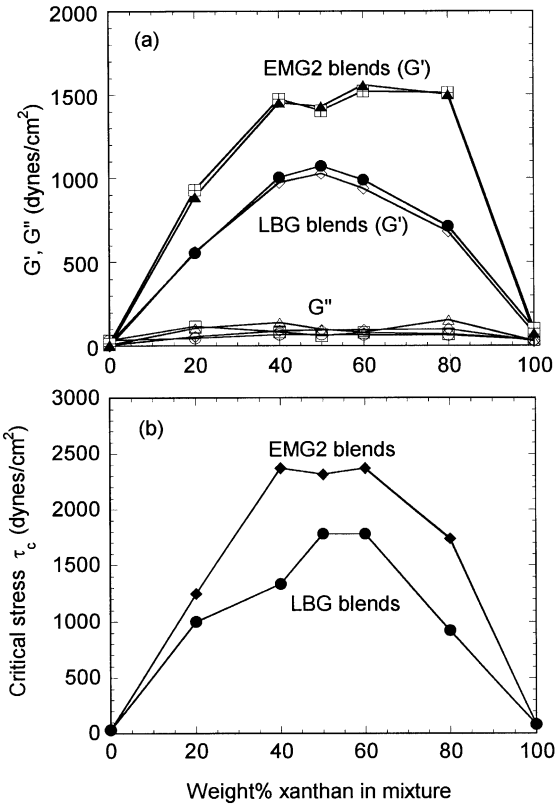

Fig. 6. Variation of gel moduli (a) and critical stress (b) as a function of xanthan content in blends of xanthan with locust bean gum and EMG2, respectively. Data for two different frequencies, 1 and 0.1 rad/s are shown in (a). Critical stresses in (b) were measured at a frequency of 1 rad/s.

examined in Fig. 6a and b. The values of elastic moduli of locust bean/xanthan and EMG2/xanthan blends are compared in Fig. 6a. We use two different frequencies because the rheological data was observed to be slightly dependent on frequency. Both the galactomannans exhibit similar trends in behavior with the elastic modulus passing through a broad maximum with increasing xanthan content (40–60%) in the mixture. It is important to note that the loss modulus G'' remains effectively unchanged with blend composition. This suggests that the addition of xanthan affects only the elasticity of the system through an increase in either the quantity or duration of the cross-links between the two biopolymers. (Mannion et al., 1992). The failure characteristics of the gels under the influence of large stresses are shown in Fig. 6b. We see that the critical stress also passes through a broad maximum depending on the xanthan content of the mixture. Thus, at varying levels of xanthan content, modified guar gels exhibit higher elastic moduli and break down at significantly higher levels of stress as compared to locust bean gels. These results have important ramifications in practical applications where different functionalities can be used to create a range of textures, including that of locust bean gels. It is interesting to note that the M/G ratio of both EMG2 and LBG are similar. The enhanced modulus and yield stress of the EMG2 blends compared to the LBG blends could thus be due to a difference in the distribution pattern of the galactose units along the mannose backbone.

3.2. Mechanisms of interaction

We focus, in this part, on understanding the mechanisms responsible for the interaction properties of blends of xanthan and enzyme-modified guar. In particular, the effects of order–disorder transitions in the xanthan molecule on blend rheology are examined by varying two parameters — temperature of mixing (T_p) of the blend and ionic strength of the xanthan solution. It is known that xanthan exists in the ordered conformation at low temperatures and becomes increasingly disordered as the temperature of the solution is increased. The helix-coil transition temperature of xanthan solutions in water was found to be approximately 53°C (data not shown) from differential scanning calorimetry (DSC) measurements. Hence, three different temperatures of mixing (T_p) — 25, 40 and 85°C — were chosen for preparing the blends. At 25 and 40°C, xanthan molecules exist mainly in the ordered conformation, although some sequences along the chain length can be disordered, while at 85°C the chains are completely disordered. It is also possible to increase the xanthan transition temperature by addition of salts since they shield electrostatic repulsions between charged side groups on the xanthan side chains and thus stabilize the ordered conformation (Morris et al., 1977; Norton et al., 1984). In accordance with this, DSC measurements on a 0.8% w/w xanthan solution dissolved in 0.5 M NaCl showed that the transition temperature was shifted to 125°C, consistent with the results obtained by Rinaudo, Milas, Bresolin and Ganter (1999) and Zhan, Ridout, Brownsey and Morris (1993). Modified guar samples were blended with xanthan solutions made in water and in 0.5 M NaCl at the three temperatures chosen previously and the results were compared. It should be noted that although the temperatures chosen lie below the helix-coil transition temperature in the presence of salt, the xanthan molecules still possess disordered regions, the number of which is dependent on the temperature.

3.2.1. Effect of temperature of mixing (T_p)

Fig. 7a–c show the effect of variation of temperature of mixing (T_p) on the resulting dynamic moduli (measured at 25°C) of blends of xanthan with locust bean gum, EMG1 and EMG2, respectively. Deionized water was used as the solvent and the total biopolymer concentration was maintained at 0.8% w/w in all the cases. We find the elastic modulus to be higher than the viscous modulus and effectively independent of frequency for all samples, indicating the systems possess gel-like characteristics. The elastic moduli of EMG2 gels (Fig. 7c) are almost an order of magnitude higher than the EMG1 gels (Fig. 7b) at any particular mixing temperature. The galactose content of modified guar also plays an important role in influencing

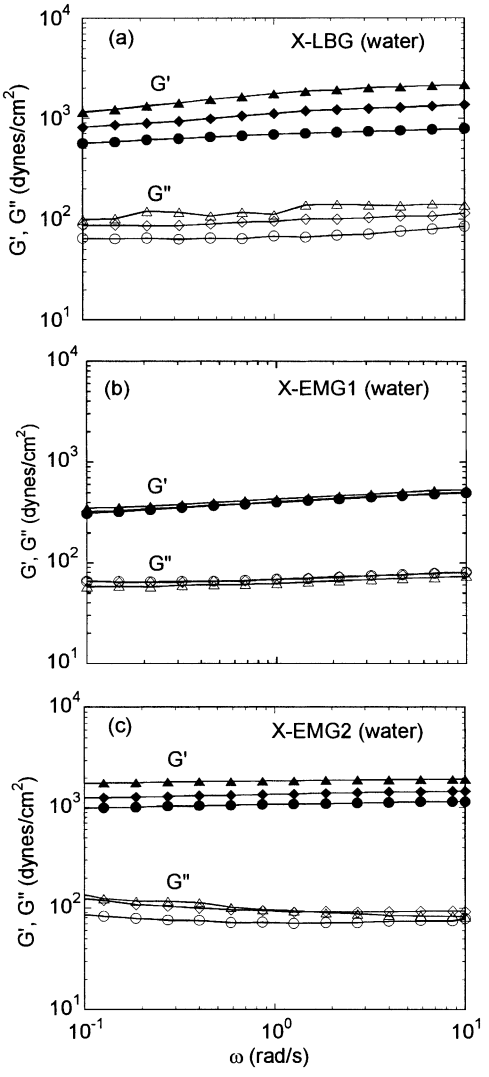

Fig. 7. Effect of mixing temperature on elastic (G') and viscous (G'') moduli of blends of xanthan with (a) locust bean gum, (b) EMG1 (33.6% galactose) and (c) EMG2 (25.2% galactose) made in water. Here, the temperatures of mixing T_p are 25 (○), 40 (◆) and 85°C (△). All rheological measurements were made at 25°C.

the effect of temperature on the elastic modulus of the blends. Gel strength of EMG2 blends (low galactose content) varies strongly with mixing temperature and synergistic effects are directly related to temperature of mixing. In contrast, dynamic moduli of EMG1 blends (high galactose content) remain constant and are insensitive to changes in T_p. Upon comparing Fig. 7a and c, we see that the EMG2 blend shows characteristics similar to those of the locust bean blend. It is important to note that at any given temperature EMG2 gels are more elastic (higher G') than the locust bean gels. In both cases, synergism increases as the mixing temperature T_p is increased from 25 to 85°C. Fig. 8 shows a representative frequency sweep for an EMG2 blend made in 0.5 M NaCl. All experimental conditions were similar to those described above. As mentioned earlier, the helix-coil transition temperature of xanthan is shifted to 125°C in the

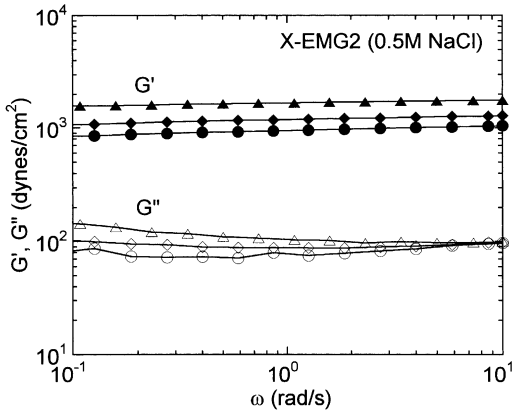

Fig. 8. Effect of mixing temperature on elastic (G') and viscous (G'') moduli of a blend of xanthan with EMG2 (25.2% galactose) made in 0.5 M NaCl. Here, the temperatures of mixing T_p are 25 (○), 40 (◆) and 85°C (△). All rheological measurements were made at 25°C.

presence of 0.5 M NaCl. Thus, the xanthan molecule exists in the ordered form at all the three temperatures, albeit with varying proportions of disordered regions. Elastic moduli are higher than viscous moduli and they are relatively frequency independent, indicative of the presence of a network system. As before, elastic moduli of EMG2 blends increase with an increase in the temperature of mixing. Trends similar to those seen in Fig. 7a and c are observed (data not shown) for blends of xanthan with locust bean gum and EMG1. Locust bean blends are as elastic as the EMG2 blends and show increasing G' with increasing T_p whereas the EMG1 blends are weaker than the EMG2 blends by almost an order of magnitude and are unaffected by changes in T_p.

Fig. 9a and b summarize the effects of solvent conditions and mixing temperature on the resultant elastic moduli of the blends of the three galactomannans. We have chosen a frequency of 1 rad/s to make the comparison. Similar trends are seen irrespective of the frequency chosen since the moduli are relatively frequency independent. From Fig. 9a, it is clear that the elastic moduli of EMG1 blends are about an order of magnitude lower than the EMG2 blends and remain effectively constant irrespective of the temperature of mixing. Changing the solvent environment by addition of salt also does not affect the gel modulus, which is comparable to that obtained in the absence of salt. Since increase in temperature and/or salt affects xanthan conformation, the relative insensitivity of EMG1 blend rheology to temperature of mixing and solvent suggests that the interaction between xanthan and modified guar is dictated primarily by the guar structure. This is to be contrasted with EMG2 blends where xanthan conformation plays a dominant role in blend interaction, as discussed later. In the EMG1 blends, the low level of modification results in only a slightly higher percentage of exposed mannan regions compared to native guar. Although this enhances binding with xanthan (as evidenced by a higher G'), the maximum number of junction zones possible are formed

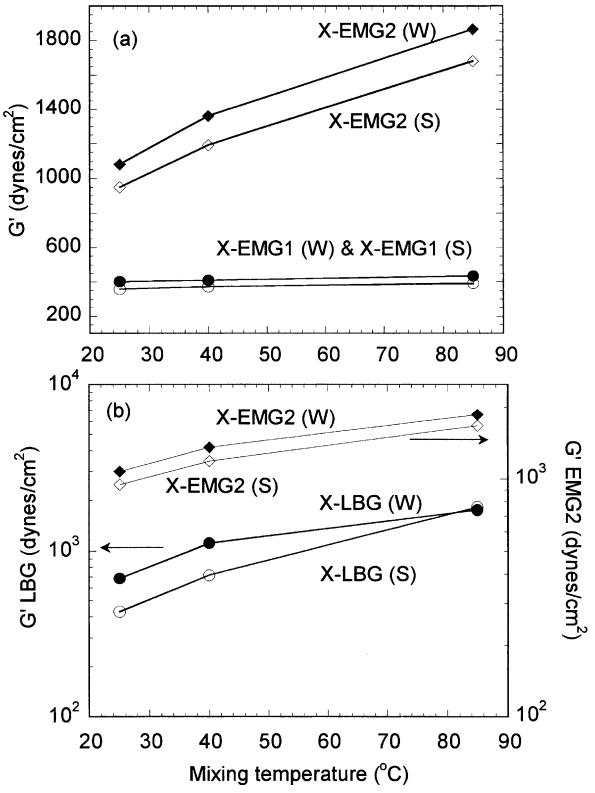

Fig. 9. Effect of added salt and mixing temperature (T_p) on elastic modulus G' for (a) EMG1 and EMG2 blends and (b) EMG2 and LBG blends. G' values were measured at a frequency of 1 rad/s. Here W represent samples prepared in water only whereas S stands for solutions containing added salt (0.5 M NaCl).

at 25°C only. Since altering temperature/ionic strength changes xanthan conformation only and does not produce additional galactose free regions, the extent of binding does not change, resulting in a constant gel modulus.

Fig. 9b compares the moduli of xanthan–locust bean blends with xanthan–EMG2 blends. For all temperatures of mixing, locust bean blends show a lower modulus and are more sensitive to changes in temperature of mixing than the EMG2 blends. In both systems, blends made in 0.5 M NaCl are weaker than those made in water at any particular temperature of comparison. These synergistic effects can be best explained on the basis of interaction of the galactose-depleted regions along the galactomannan backbone with disordered sequences of the xanthan molecule (Tako, 1991; Tako, Asato & Nakamura, 1984; Zhan et al., 1993). As the mixing temperature T_p is increased from 25 to 85°C, not only do the xanthan aggregates dissociate, but more and more segments of the xanthan molecule become disordered in response to increased energy input into the system (Norton et al., 1984). This enables the galactomannan to interact better with xanthan, producing stronger gels. The fact that gelation occurs even when the xanthan transition temperature is raised to 125°C by the addition of salt provides evidence for this mechanism. The reduction in magnitude of the gel modulus in the presence of salt further supports this mechanism since addition of salt stabilizes the ordered structure of xanthan reducing the availability of disordered regions for junction zone formation. Thus, complete disordering of the xanthan molecule is not a prerequisite for gelation as concluded by some authors (Brownsey, Cairns, Miles & Morris, 1988; Cairns, Miles & Morris, 1986; Cairns, Miles, Morris & Brownsey, 1987). The above results are consistent with those obtained using GPC, polarimetry, (Cheetham & Mashimba, 1988; Cheetham, McCleary, Teng, Lum & Maryanto, 1986) viscometry, (Lopes, Andrade & Milas, 1992) and optical rotation (Zhan et al., 1993) which provide evidence for xanthan–galactomannan interactions via disordered segments of the xanthan molecule.

4. Summary

We use dynamic rheology to investigate synergistic effects in blends formed using xanthan and enzymatically-modified guar. In particular, the structure of guar gum is tailored using α-galactosidase, a side chain cleaving enzyme, to obtain two samples, one with a high (33.6%) galactose content (EMG1) and the other with a low (25.2%) galactose content (EMG2). We find that at high extents of enzymatic modification, guar (EMG2) can interact synergistically with xanthan to produce gels with elastic modulus and yield stress similar to or exceeding those of locust bean gum. This is significant in applications where guar gum is chosen to function as a replacement for locust bean gum. The rheological behavior of the blends is however is dependent on the resulting mannose to galactose ratio of the modified guar, with the elastic moduli of EMG1–xanthan blends being relatively insensitive to changes in temperature of mixing. In contrast, at constant ionic strength, the EMG2–xanthan blends become progressively more elastic as the temperature of mixing is increased. At any particular temperature of mixing, EMG1 blends are unaffected by the presence of salt whereas EMG2 blends made in salt are found to be weaker than the corresponding blends made in water. This is in keeping with the mechanism in which the galactomannan interacts with disordered sequences along the xanthan molecule. We propose that at low extents of modification, guar structure dictates rheology whereas at higher extents of modification, the rheology is strongly affected by xanthan conformation.

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References

Brownsey, G. J., Cairns, P., Miles, M. J., & Morris, V. J. (1988). *Carbohydrate Research*, *176*, 329–349.

Bulpin, P. V., Gidley, M. J., Jeffocat, R., & Underwood, D. R. (1990). *Carbohydrate Polymers*, *12*, 155–168.

Burke, M. D., Park, J. O., Srinivasarao, M., & Khan, S. A. (2000). *Macromolecules*, *33*, 7500–7507.

Cairns, P., Miles, M. J., & Morris, V. J. (1986). *Nature*, *322*, 89–90.

Cairns, P., Miles, M. J., Morris, V. J., & Brownsey, G. J. (1987). *Carbohydrate Research*, *160*, 411–423.

Cheetham, N. W. H., & Mashimba, E. N. M. (1988). *Carbohydrate Polymers*, *9*, 195–212.

Cheetham, N. W. H., McCleary, B. V., Teng, G., Lum, F., & Maryanto, (1986). *Carbohydrate Polymers*, *6*, 257–268.

Dea, I. C. M., & Morrison, A. (1975). *Advances in Carbohydrate Chemistry and Biochemistry*, *31*, 241–312.

Dea, I. C. M., Morris, E. R., Rees, D. A., Welsh, E. J., Barnes, H. A., & Price, J. (1977). *Carbohydrate Research*, *57*, 249–272.

Fox, J. E. (1992). In A. Imeson, *Thickening and gelling agents for food* (pp. 153–171). London: Blackie Academic and Professional.

Grasdalen, H., & Painter, T. J. (1980). *Carbohydrate Research*, *81*, 59–66.

Hoffman, J., & Svensson, S. (1978). *Carbohydrate Research*, *65*, 65–71.

Hoffman, J., Londberg, B., & Painter, T. J. (1975). *Acta Chemica Scandinavica Series B*, *29*, 137–140.

Jansson, P. E., Kenne, L., & Lindberg, B. (1975). *Carbohydrate Research*, *45*, 275–282.

Lopes, L., Andrade, C. T., & Milas, M. (1992). *Carbohydrate Polymers*, *17*, 121–126.

Maier, H., Anderson, M., Karl, C., & Magnuson, K. (1992). In R. L. Whistler & J. N. BeMiller, *Industrial gums — Polysaccharides and their derivatives* (pp. 181–227). San Diego: Academic Press.

Mannion, R. O., Melia, C. D., Launay, B., Cuvelier, G., Hill, S. E., Harding, S. E., & Mitchell, J. R. (1992). *Carbohydrate Polymers*, *19*, 91–97.

McCleary, B. V. (1979). *Carbohydrate Research*, *71*, 205–230.

McCleary, B. V., Amado, R., Weibel, R., & Neukom, H. (1981). *Carbohydrate Research*, *92*, 269–285.

McCleary, B. V., Nurthen, E., Taravel, F. R., & Joseleau, J. (1983). *Carbohydrate Research*, *118*, 91–109.

McCleary, B. V., Dea, I. C. M., Windust, J., & Cooke, D. (1984). *Carbohydrate Polymers*, *4*, 253–270.

McCleary, B. V., Clark, A. H., Dea, I. C. M., & Rees, D. A. (1985). *Carbohydrate Research*, *139*, 237–260.

Melton, L. D., Mindt, L., Rees, D. A., & Sanderson, G. R. (1976). *Carbohydrate Research*, *46*, 245–261.

Morris, E. R. (1990). In P. Harris, *Food gels* (pp. 291–357). Amsterdam: Elsevier.

Morris, V. J. (1995). M. S. A. editor. *Food Polysaccharides and their Applications*. New York: Marcel Dekker (pp.341–375).

Morris, E. R., Rees, D. A., Young, G., Walkinshaw, M. D., & Darke, A. (1977). *Journal of Molecular Biology*, *110*, 1–16.

Norton, I. T., Goodall, D. M., Frangou, S. A., Morris, E. R., & Rees, D. A. (1984). *Journal of Molecular Biology*, *175*, 371–394.

Painter, T. J., Gonzalez, J. J., & Hemmer, P. C. (1979). *Carbohydrate Research*, *69*, 217–226.

Raghavan, S. R., Hou, J., Baker, G. L., & Khan, S. A. (2000a). *Langmuir*, *16*, 1066–1077.

Raghavan, S. R., Walls, H. J., & Khan, S. A. (2000b). *Langmuir*, *16*, 7920–7930.

Richardson, R. K., & Ross-Murphy, S. B. (1987). *International Journal of Biological Macromolecules*, *9*, 257–264.

Rinaudo, M., Milas, M., Bresolin, T., & Ganter, J. L. M. S. (1999). *Macromolecular Symposia*, *140*, 115–124.

Rochefort, W. E., & Middleman, S. (1987). *Journal of Rheology*, *31*, 337–369.

Tako, M. (1991). *Journal of Carbohydrate Chemistry*, *10*, 619–633.

Tako, M., Asato, A., & Nakamura, S. (1984). *Agricultural and Biological Chemistry*, *48*, 2995–3000.

Tayal, A., & Khan, S. A. (2000). *Macromolecules*, *33*, 9488–9493.

Tayal, A., Pai, V. B., Kelly, R. M., & Khan, S. A. (1998). In Amjad, *Water soluble polymers* (pp. 41–49). New York: Plenum Press.

Tayal, A., Kelly, R. M., & Khan, S. A. (1999a). *Macromolecules*, *32*, 294–300.

Tayal, A., Pai, V. B., & Khan, S. A. (1999b). *Macromolecules*, *32*, 5567–5574.

Urlacher, B., & Dalbe, B. (1992). In A. Imeson, *Thickening and gelling agents for food* (pp. 202–227). London: Blackie Academic and Professional.

Yang, M. C., Scriven, L. E., & Macosko, C. W. (1986). *Journal of Rheology*, *30*, 1015–1029.

Zhan, D. F., Ridout, M. J., Brownsey, G. J., & Morris, V. J. (1993). *Carbohydrate Polymers*, *21*, 53–58.