Evolution of Microstructure and Rheology in Mixed Polysaccharide Systems

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Received August 29, 2001

ABSTRACT: Synergistic biopolymer blends composed of xanthan and enzymatically modified guar galactomannan are investigated in terms of their time-dependent properties. In particular, a side-chain cleaving enzyme, α-galactosidase, is used to cleave off galactose sugar units from guar to produce modified galactomannans with varying galactose contents of 25.2 and 16.2%. Laser scanning confocal microscopy and dynamic rheology are used to monitor the properties of each of these two modified guar gum in *solution* as well as in *blends* with xanthan as they are allowed to age over a period of 3 weeks. Our results indicate that solutions of guar with a higher galactose (25.2%) content undergo no rheological change over the period of observation and show a constant gel elastic modulus (G') in blends with xanthan. Confocal images of the solutions and the blends also indicate that the systems are stable over a period of 3 weeks. In contrast, guar gum with a lower galactose content (16.2%) forms interchain associations in *solution,* developing aggregates that convert it from a macromolecular solution to a gel. This is reflected in its dynamic moduli which increase significantly with time and show a transition from frequency-dependent behavior with G'' (viscous modulus) > G' (elastic modulus) to a frequency-independent character with $G' > G''$. This process of association and phase separation is directly observed in confocal images of the modified guar as well as in its blend, though not to the same extent in the latter. The presence of a second component thus seems to retard the association process. Interestingly, the blend moduli remain unchanged in magnitude and show gellike features even though the mode of association and concomitant microstructure changes.

Introduction

Mixed polysaccharide systems are used in a wide range of applications because of their ability to interact synergistically and render materials with controlled properties.[1,2] In particular, blends of galactomannans such as guar and locust bean gums are used by themselves or in combination with xanthan in a range of applications that include coatings, drug delivery, oil/gas production, and food additives in common products like dressings, sauces, gelated desserts, and frozen and baked products.[3−9] In this regard, locust bean can induce a gelling synergy in mixtures with xanthan whereas guar produces only a synergistic increase in viscosity. Such differences in behavior can be attributed to variation in the chemical structure of guar and locust bean gum.[10−13] Guar is composed of approximately 38% galactose units with a mannose-to-galactose ratio of ∼1.5 whereas locust bean gum has a galactose content of approximately 25% with a mannose-to-galactose ratio of ∼2.8. In addition, the distribution of the galactose units along the mannan backbone is different for the two.[3−6,14−16] Research studies on guar galactomannan have demonstrated that the fine structure can be altered using highly specific enzymes to achieve a reduced galactose content and subsequently enhance its synergistic interaction properties with xanthan gum.[2,11,17−22] Recently, texture profile analysis has been used to compare the properties of locust bean and enzyme-modified guar blends with xanthan and κ-carrageenan.[23] The results indicate that modified guar with galactose content comparable to that of locust bean gum shows similar textural characteristics when blended with xanthan or κ-carrageenan. More importantly, the new textures created using modified guar with different galactose contents assume great significance in view of product development requirements of the food and other industries.

Although enzymatic modification provides a powerful alternative for developing new materials and enhancing the applicability of galactomannans, reduction of galactose content below a critical level can promote noncovalent, self-associations among the exposed mannan regions, thus imposing limits on extent of modification practically achievable.[12,13] At low galactose contents, solubility and viscosity of guar solutions decrease due to the alignment of β-D-mannan residues after removal of galactose side chains. This process, which is also dependent on the concentration of galactomannan in solution, leads to the formation of aggregates that increase the turbidity of the solutions. When almost all the galactose chains are removed, the β-D-mannan aggregates become insoluble and precipitate out of solution.[11,12,17,18] Such self-associations between mannan segments can also be promoted by successive freeze−thaw cycles which raises the effective concentration of the polymer in solution due to ice formation. Dea et al.[12] found that locust bean gum formed a weak, cohesive gel network at concentration as low as 0.5% after freeze−thaw cycles whereas another galactomannan, tara gum, formed gels only at concentrations equal to or higher than 0.75%, and guar gum showed no evidence of association. In a later study,[24] it was shown that the presence of totally unsubstituted regions of mannan

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10.1021/ma0115545 CCC: $22.00
Published on Web 02/01/2002

backbone with weight-average mannan block lengths of six monomer units or higher was required for self-association and precipitation to occur. These observations are further supported by Hatakeyama et al.,[25] who showed that the viscosity of 3% locust bean solutions and gels increased upon storage at low temperatures for a couple of days. In addition, the authors observed that the strength of hydrogels prepared from locust bean solutions increased after repeated freeze−thaw cycles. The tendency of concentrated locust bean solutions to form associations and change from macromolecular solutions to gels upon storage without freeze−thaw processing has also been reported.[26,27] Change of solvent quality can also promote gelation as has been seen in systems containing galactomannans and solvents like aqueous ethylene glycol, glycerol, and sucrose.[12,28]

While time-dependent behavior has been observed at the macroscopic level for various systems, no attempt has been made to investigate this phenomenon in terms of both microstructural and macroscopic changes. Our effort focuses on probing at both these length scales using rheology and laser scanning confocal microscopy (LSCM). LSCM is a relatively new technique that can be used to follow structural changes by using fluorescent probes or attaching fluorescent tags to the molecules of interest. This approach has recently been used effectively in probing microstructures of dextran/locust bean gum mixtures,[26] locust bean solutions,[27] and micellar casein−galactomannan mixtures[29] as well as in investigating ice recrystallization processes in sucrose−skim milk solutions containing varying amounts of locust bean/guar.[30]

In this study, we focus on investigating the characteristics of enzyme-modified guar at very low galactose contents (15−17%) as it is allowed to age over a period of time. Structural rearrangements and aggregation phenomena in guar solutions as well as in blends with xanthan are monitored using a combination of LSCM and dynamic rheology. The results are compared with data for samples containing guar that has an intermediate galactose content (25%) in order to understand the role of galactose content on microstructure and rheology during the aging process.

Experimental Methods

Materials. Samples of Uniguar 150 (*Cyamopsis tetragonolobus*) were obtained from Rhodia Food Ingredients, Cranbury, NJ. These 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, described below, was used to purify the galactomannans to remove insoluble impurities.[31] A commercial sample of xanthan gum obtained from Aldrich Chemical Co., Inc., was used in all experiments.

The desired amount of Uniguar 150 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 was sealed with Parafilm, and the solution was allowed to hydrate for 24 h to allow optimum viscosity development. The galactomannan dispersion was then 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.

Preparation of Guar and Xanthan Solutions. Solutions of purified guar were prepared following the procedure (excluding the impurity removal and extraction step) described above. 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.

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 the appropriate volume of enzyme (activity known) 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.[32]) The 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 using the standard method of denaturing it through heating the solution at 85 °C for 10 min.

Fluorescent Labeling Protocols. Fluorescein isothiocyanate (FITC), tetramethyl rhodamine isothiocyanate (TRITC), and fluoresceinamine were purchased from Sigma Chemicals, St. Louis, MO. The wavelengths of absorption maxima (λ_{max}) and emission maxima (λ_{emis}) were 492 and 518 nm for FITC and fluoresceinamine and 555 and 580 nm for TRITC, respectively.

Guar gum was labeled with FITC or TRITC following the protocol developed by de Belder and Granath.[33] 1 g of guar gum (Uniguar 150, Rhodia Food Ingredients, Cranbury, NJ) was dissolved in 10 mL of dimethyl sulfoxide (DMSO) containing a few drops of pyridine. FITC (0.1 g) was added, and the reaction was catalyzed by 20 mg of dibutyltin dilaurate. The reaction was carried out in a constant temperature water bath maintained at 65 °C with continuous stirring using a magnetic stirrer for 2 h. The solution was then cooled, and the polysaccharide was precipitated using ethanol and filtered. The precipitate was washed repeatedly with ethanol until all the free dye was removed; i.e., the ethanol filtrate was clear. The labeled guar gum was then lyophilized to obtain a fine powder, which was then used in further experiments.

Multiple Labeling Experiments. A new dual tagging procedure involving attachment of fluorescent molecules to each of the polysaccharides, guar and xanthan, was used to simultaneously image both molecules as the structural features of the blends changed. Instead of FITC, a similar molecule, rhodamine isothiocyanate (RITC), was attached to guar using a procedure analogous to the one described above. Xanthan derivatives were prepared by isocyanide coupling of fluoresceinamine to the carboxyl groups of xanthan following the procedure outlined by Holzwarth.[34] 5-Aminofluorescein, acetaldehyde, dimethyl sulfoxide, and cyclohexyl isocyanide were purchased from the Sigma Chemical Co. Xanthan (5−25 mg) was dissolved in 100 mL of 2 mM sodium chloride, pH 7. To this solution was added 100 mL of solution containing 1 part DMSO and 2 parts water. Acetaldehyde (30 μL), cyclohexyl isocyanide (25 μL), and dye (3 mg in 3 mL of DMSO) were then added and allowed to react for 3 h at room temperature. The labeled polysaccharide was then purified by several cycles of precipitation with ethanol until all the residual free dye was washed off. The polymer was subsequently freeze-dried, powdered, and stored until further use.

The fluorescent probes were chosen to match individual Ar ion and Kr ion laser lines which allowed imaging of one species independent of the other. Care was taken to adjust the individual laser intensities to prevent saturation or photobleaching of the probes.

Preparation of the Blends. Blends of galactomannan with xanthan gum (0.8% w/w total polysaccharide) were prepared homogenizing the two polysaccharides in a 1:1 ratio at 80 °C for 60 s. The samples were then kept in a quiescent state for 2 min to allow escape of some of some of the air bubbles produced by the mixing process. All samples were allowed to stand for 24 h at room temperature before further testing.

Laser Scanning Confocal Microscopy. The samples were placed on an ESCO frosted microscope slide with Grace Bio-Lab incubation chamber cover[7] and imaged on a Leica TCS NT microscope equipped with an Ar ion (488 and 514 nm), Kr ion (568 nm), and a He−Ne (633 nm) laser. For the purposes of imaging the fluorescein molecules, the 488 nm line of the Ar ion laser was used in conjunction with a 10 × 0.3NA dry PL Fluotar objective. The rhodamine molecules were imaged similarly using the Kr ion laser. Several samples were imaged to ascertain that the data are reproducible, and typical results are reported in this paper.

Rheological Measurements. Dynamic rheological measurements were conducted using a stress-controlled rheometer (Rheometrics DSR II) fitted with 40 mm diameter serrated parallel plates to prevent the gel from slipping. Dynamic frequency sweeps were performed at 25 °C at a constant strain amplitude of 1%. Dynamic stress sweeps were conducted prior to the frequency sweeps to ensure operation within the linear viscoelastic region. All samples were covered with a thin layer of silicone oil and enclosed within a solvent trap to prevent evaporation of water. Experiments were repeated to ensure that the addition of oil did not affect the data. Syneresis was seen in the highly modified guar solutions and gels a few days after they were prepared. Rheological measurements on these samples were taken by carefully blotting the gels to remove the surface water layer and ensure good contact with the geometry chosen.

Results and Discussion

We begin by characterizing the rheology of blends containing xanthan and guar of varying galactose content. Guar solutions were modified by hydrolyzing the biopolymer with 0.4 U/mL α-galactosidase at 35 °C for different time intervals (5, 10, 15, 20, and 25 h). Blends were then made by homogenizing the modified guar with xanthan at two different temperatures, 40 and 80 °C. The dynamic moduli of all the blends were measured (at 25 °C) by performing frequency sweeps in the linear viscoelastic region. Figure 1 shows representative frequency sweeps for two blends made at 80 °C containing guar that had been subjected to enzymatic action for 5 and 20 h, respectively. Gels are formed in both cases, as indicated by the dominance of the elastic modulus G' over the viscous modulus and the relative frequency independence of the moduli. Additionally, the elastic modulus increases with extent of hydrolysis by the enzyme. Gelation was seen in all samples, irrespective of the hydrolysis time (or galactose content of guar) and the mixing temperature chosen.

The effects of hydrolysis time and temperature of mixing on rheology of the blends are illustrated in Figure 2a,b, where the dynamic moduli are plotted as a function of enzyme hydrolysis time for the two mixing temperatures. The values of the dynamic moduli were read off the corresponding frequency sweeps at two values of 1 and 10 rad/s to account for the slight frequency dependence observed. Gels are formed in all cases, with the elastic modulus increasing with increasing incubation time. However, for both temperatures of

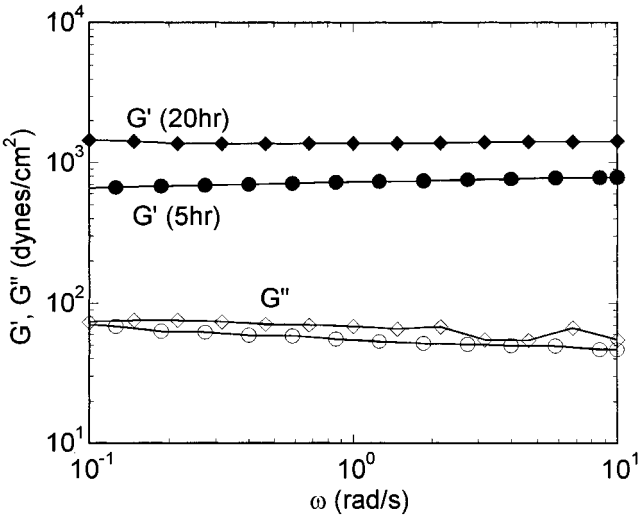

Figure 1. Frequency spectrum of the elastic (G') and viscous (G'') moduli of blends containing xanthan and guar modified by enzymatic hydrolysis with α-galactosidase for 5 h (circle) and 20 h (diamond), respectively.

mixing, the elastic modulus G' passes through a maximum and then decreases. The observed increase in gel modulus is due to the progressive increase in the number of junction zones formed between the xanthan molecules and the unsubstituted mannan regions created by the action of the enzyme.[18] However, as the substituent galactose side chains are cleaved off by α-galactosidase, exposed mannan units which are adjacent begin to associate with each other. This process of self-gelation now competes with the process of formation of mixed junction zones and hence the contribution of guar chains to the overall mixed gel modulus reduces, producing weaker gels. The self-gelation becomes increasingly dominant as more and more exposed mannan chains are produced by enzymatic action, especially at long incubation times, resulting in weaker gels. Previous research in our laboratory[2] has shown that at 80 °C xanthan molecules are in the preferred coil conformation which allows for better binding with the modified guar, as opposed to the conformation at 40 °C. As a result, the aggregation process is hindered in blends made at 80 °C and begins at lower galactose contents as compared to the blends made at 40 °C, which explains the shift of the maximum toward higher incubation times (Figure 2b).

This phenomenon of self-association has also been observed in the case of gels containing locust bean gum (23% galactose) and xanthan when they were subjected to freeze−thaw cycles over a period of time.[12,25] The time-dependent behavior observed in these studies together with our results from Figure 2 suggests that two parameters—the galactose content of the galactomannan as well as the time interval that the system is subject to observation—are important in understanding the behavior of galactomannans at very low galactose contents in relation to long-term stability issues. Further experiments were designed by modifying guar to two different galactose contents (25.2% and 16.2%) and then monitoring the properties over an extended period of time. Blends of these two samples made at 80 °C were also monitored over a period of time using a combination of rheology and fluorescence confocal scanning laser microscopy.

Figure 3 depicts an overlay of the dynamic frequency sweeps of a blend containing enzyme-modified guar

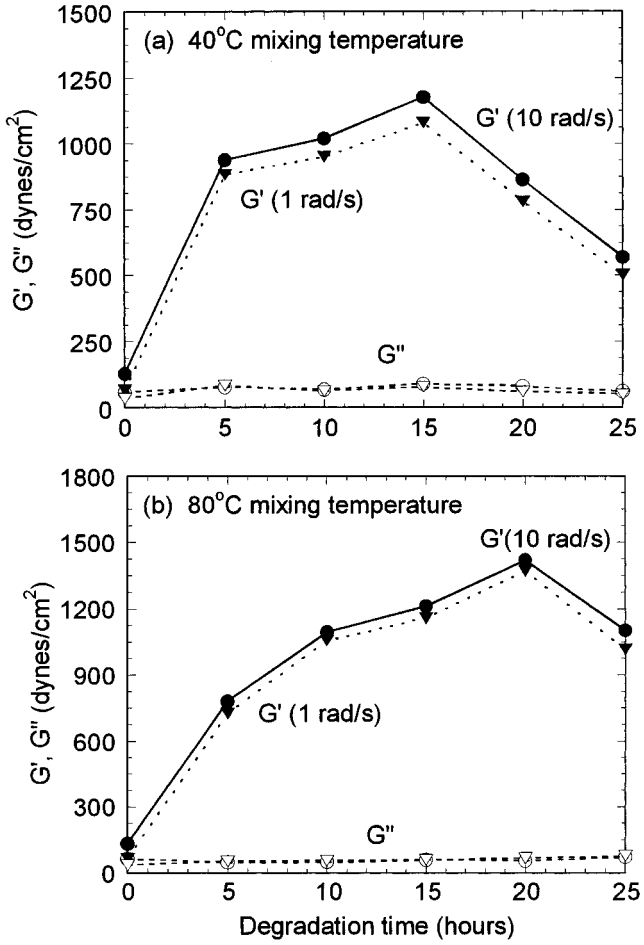

Figure 2. Variation in the dynamic elastic moduli of blends degraded with α-galactosidase for varying intervals of time. Temperatures of mixing were (a) 40 and (b) 80 °C. Rheological measurements were made at 25 °C.

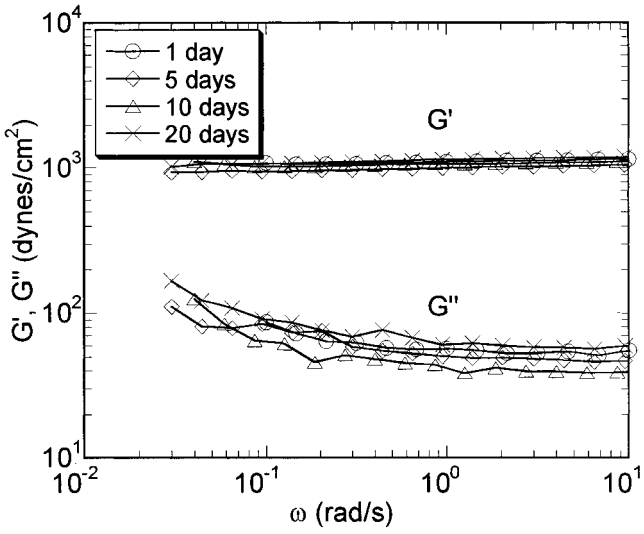

Figure 3. Dynamic moduli vs frequency behavior of a blend containing enzyme-modified guar (25.2% galactose) and xanthan, measured on days 1, 5, 10, and 20 following its preparation.

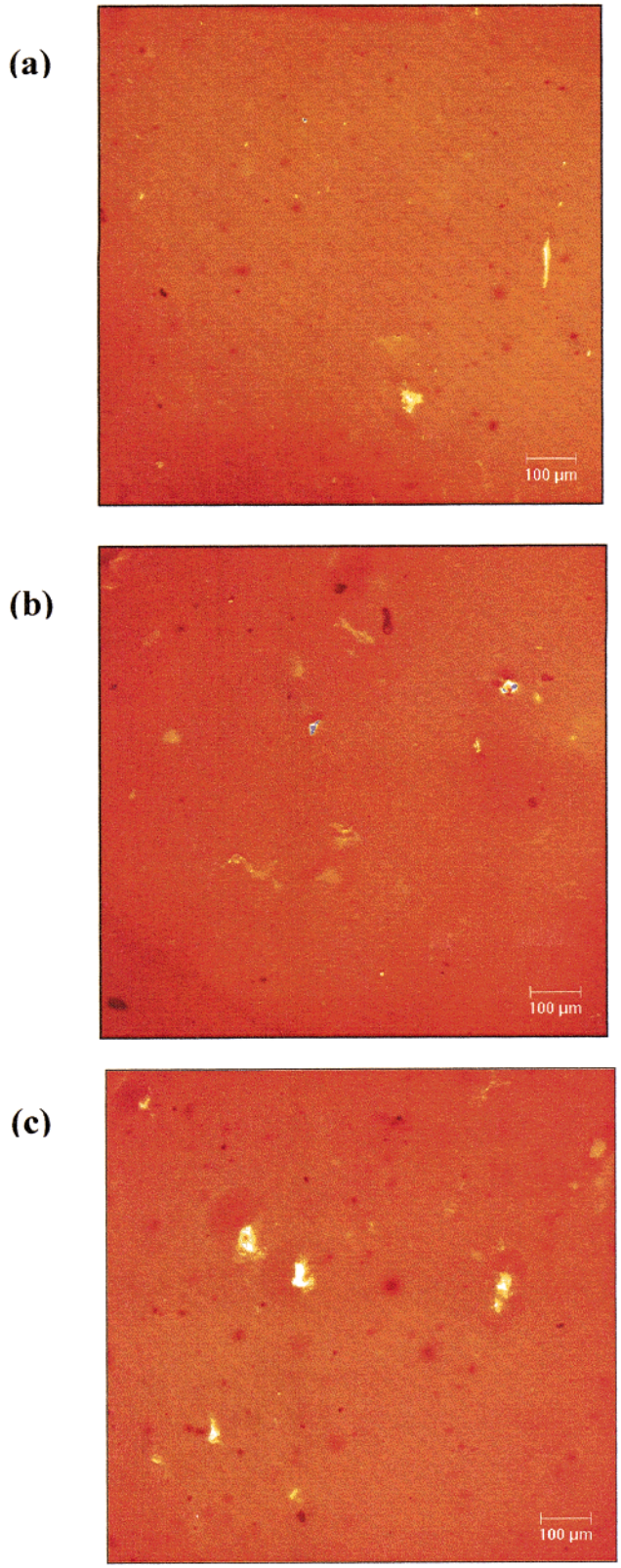

Figure 4. Fluorescent confocal images of a xanthan/enzyme-modified guar (25.2% galactose) blend taken on days (a) 1, (b) 10, and (c) 20 following its preparation.

(25.2% galactose) with xanthan measured at different time intervals following its preparation. Representative sweeps corresponding to days 1, 5, 10, and 20 only after sample preparation have been overlaid to maintain clarity. (Measurements corresponding to day 1 were actually taken after the gels were allowed to set for 24 h after blending.) The process of gelation is very rapid, and an equilibrium gel modulus is attained by day 1. There are no significant changes in the dynamic moduli even over a period of 20 days, which suggests that the junction zones giving rise to the gel network are time-invariant and there are no further rearrangements at

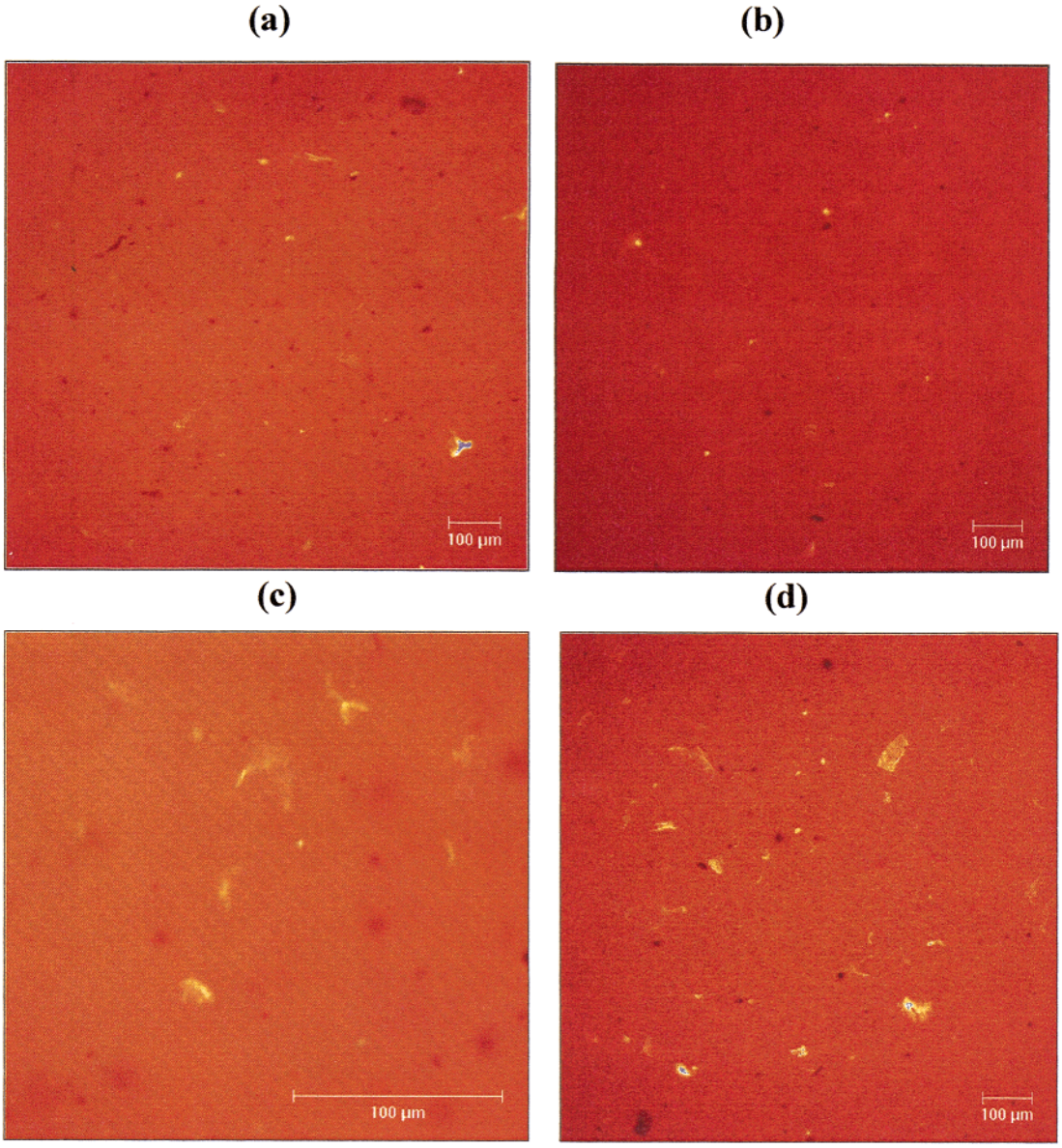

Figure 5. Fluorescent confocal images of enzyme-modified guar solutions (25.2% galactose) shown for (a) day of enzyme modification (day 0), and (b) 2, (c) 10, and (d) 20 days following it.

the molecular level. To support the rheological results, the actual microstructure of the gels was imaged concurrently using laser scanning confocal microscopy. A fluorescent molecule, fluorescein isothiocyanate (FITC), was attached to modified guar, which allowed easy identification of guar-rich domains within the blend. Figure 4 shows a series of images of the above blend taken on day1, day 10, and day 20 following its preparation. A uniform background fluorescence is observed which is offset by a couple of bright inclusions which may correspond to guar-rich domains. It is of importance to note that the qualitative nature of the microstructures remains constant over time, and there is no evidence of development of any sort of structure which would been indicative of the self-association phenomenon between guar molecules. These results suggest that at 25.2% galactose the level of modification achieved is insufficient to produce contiguous exposed mannan regions which are a prerequisite for self-association.

However, it is possible that the presence of a second component, namely xanthan, which binds to the guar chains, retards, or altogether prevents the aggregation and subsequent self-association of guar. To account for this possibility, confocal images of fluorescently tagged modified *guar solutions* were also monitored over a period of 20 days. Figure 5 illustrates the microstructure of enzyme-modified guar solution (25.2% galactose) imaged on day 1, day 10, and day 20 following enzyme modification. These images are similar to those of the guar blends and also show a uniform background fluorescence with a few bright inclusions. The rheological behavior of these solutions was monitored simultaneously, and an overlay of the frequency sweeps on day 1 and day 20 is shown in Figure 6. The moduli remain essentially unchanged with the elastic modulus dominating over the viscous modulus at high frequencies, a crossover point at intermediate frequencies beyond which the trends are reversed. The absence of any increase in moduli or significant shift in crossover frequency again points to the fact that there is no development of structure. Thus, modified guar with 25.2% galactose is found to be stable at room temperature and does not aggregate and undergo syneresis.

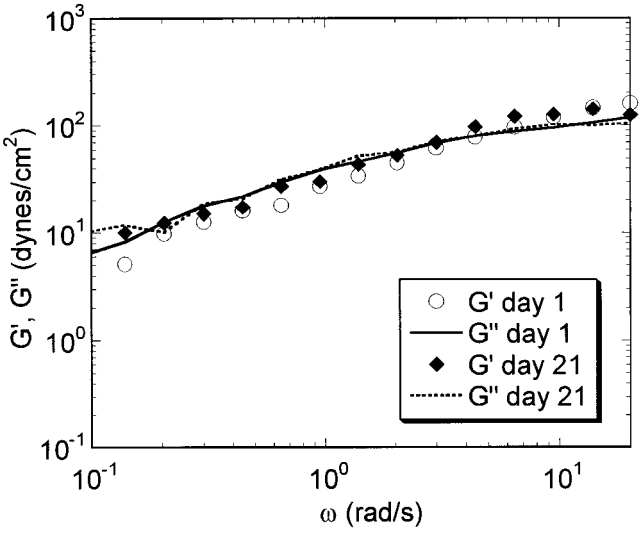

Figure 6. Dynamic moduli vs frequency behavior of an enzyme-modified guar solution (25.2% galactose) measured after 1 and 21 days following its preparation.

Although a quantification of the galactose content of guar at various incubation times shown in Figure 2 has not been carried out, it is evident that aggregation starts below a certain galactose content. To reach these low galactose contents, guar gum was further incubated with α-galactosidase at a concentration of 0.75 U/mL of guar for 9 h. It has been shown that at very low galactose contents self-association can even cause the galactomannan to precipitate out of solution.[11,12,17,18,25] Hence, care was taken to see that the solutions remained clear and transparent during and after incubation which ensured that the galactomannan remained in solution. The galactose content of the modified sample was determined to be 16.2% from analysis of the alditol acetate derivatives.[2]

The rheological changes exhibited by the enzyme-modified guar *solution* (16.2% galactose) over a period of 20 days are illustrated in Figure 7a,b. The frequency sweep of guar measured immediately after it has been modified (Figure 7a) is typical of that of a macromolecular polymer solution. The viscous component G'' is greater than the elastic component G' for most of the frequency range measured, and both moduli show characteristic frequency dependence at low frequencies, G' scaling with ω^2 and G'' varying with ω. A crossover point into the rubbery plateau region is seen at high frequencies beyond which solidlike behavior is observed with G' dominant over G''. The rheology of the solutions after a period of 2 days, during which they were left undisturbed, differs considerably from the earlier traces. The absolute values of G' and G'' show a substantial increase over day 2. More importantly, G' is larger than G'' for all frequencies measured, and the frequency dependence of the moduli also decreases. Thus, the crossover point shifts to a much higher frequency, indicating that the relaxation of the guar chains is slower. This suggests that the chains are no longer independent of each other and now possess long-range connectivity. An examination of the frequency sweeps on days 10 and 20 indicates a progressive increase in the dynamic moduli with frequency-independent behavior and relative increase of G' over G'', features characteristic of gel formation. Thus, our rheological data allow us to capture the slow transition of modified guar from a macromolecular solution into a weakly associated

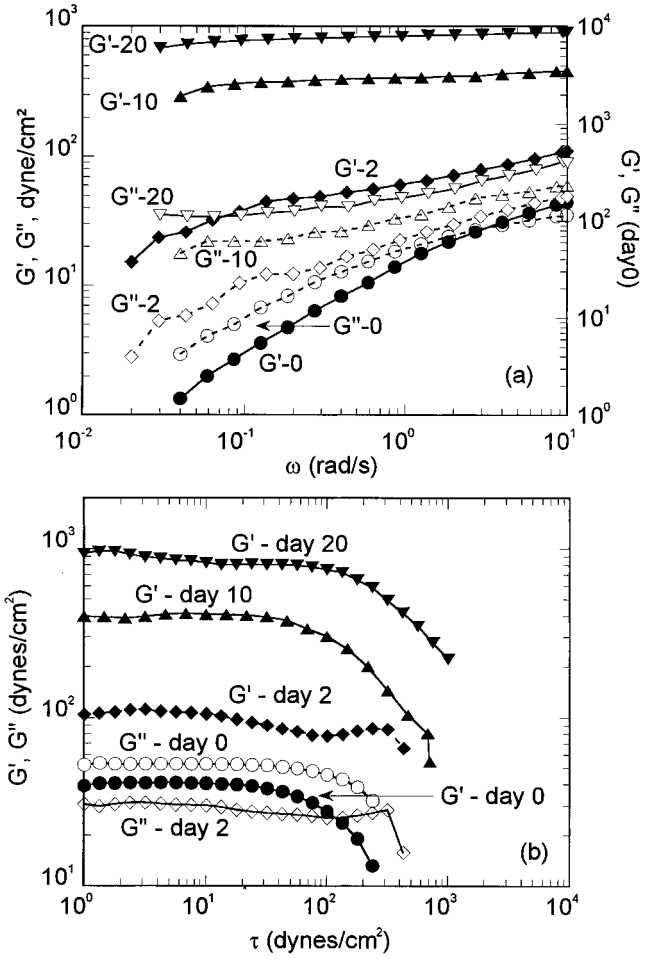

Figure 7. Dynamic moduli as a function of (a) frequency and (b) stress amplitude of an enzyme-modified guar (16.2% galactose) shown at different time intervals following its preparation. The numbers in (a) correspond to days following sample preparation.

system and finally to the formation of a gel network.

Dynamic stress sweeps shown in Figure 7b indicate that guar samples remain linearly viscoelastic until a critical stress is reached, beyond which there is a sharp decrease in the moduli. Immediately following enzyme modification (day 0), the sample is solution-like with the viscous modulus G'' larger than the elastic modulus G'. As the solutions undergo molecular rearrangement and the exposed mannan regions of guar chains form weak aggregates via hydrogen bonding, substantial changes in rheological behavior are observed. For instance, the sample of day 2 in contrast to the fresh sample shows the elastic modulus to dominate the viscous modulus, suggesting network formation. As the association phenomenon progresses with time, the gel modulus increase by more than an order of magnitude, suggesting that a long-range network structure has been established between the guar aggregates.

A series of fluorescent confocal images of FITC-tagged guar solutions (16.2% galactose) taken concurrently are shown in Figure 8 and clearly support the rheological data and the interpretation above. Confocal images of the structure of guar solutions immediately after modification (day 0) show a uniform background fluorescence and a few localized bright spots which are enriched in guar. On day 2 there is a distinct change with the regions of fluorescent intensity increasing in both size and number. Strandlike features interconnecting dif-

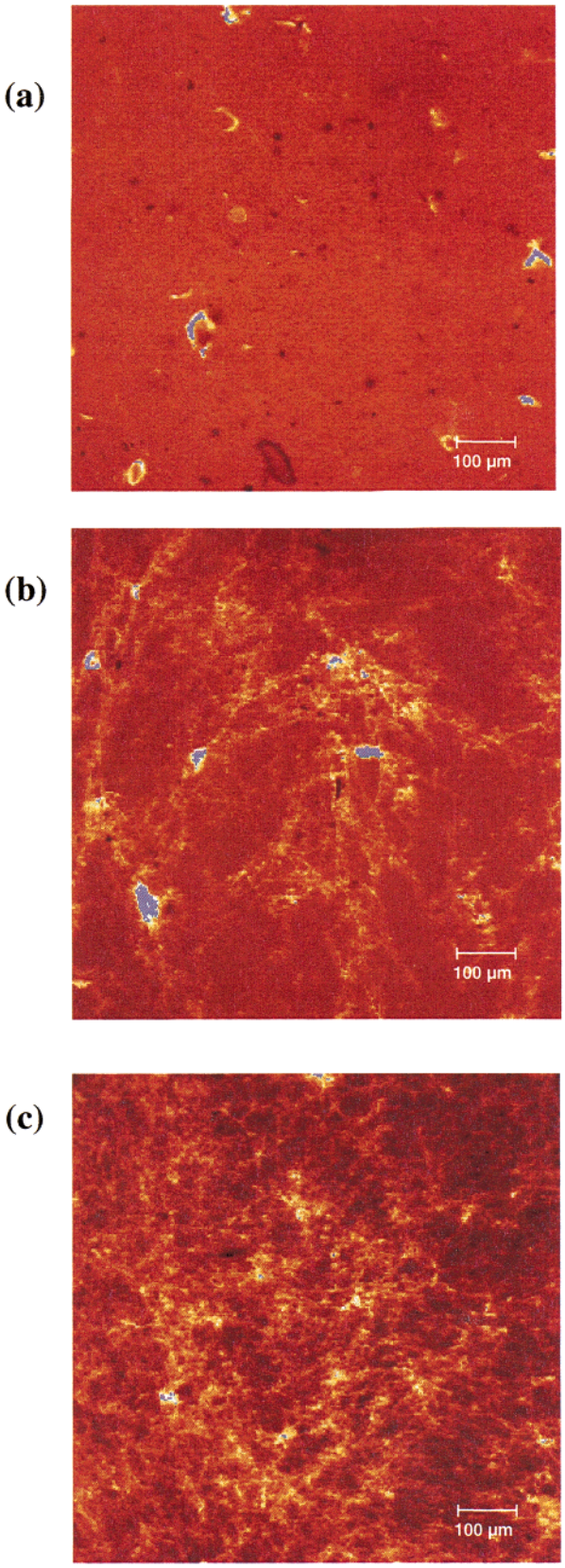

Figure 8. Fluorescent confocal images of enzyme-modified guar solutions (16.2% galactose) taken on days (a) 0 (i.e., day sample prepared), (b) 10, and (c) 20 following its preparation, showing progressive phase separation with time.

ferent areas are also seen, suggesting that some structural characteristics are beginning to develop. These features become more prominent by day 10, and a clear network structure can be identified. However, the

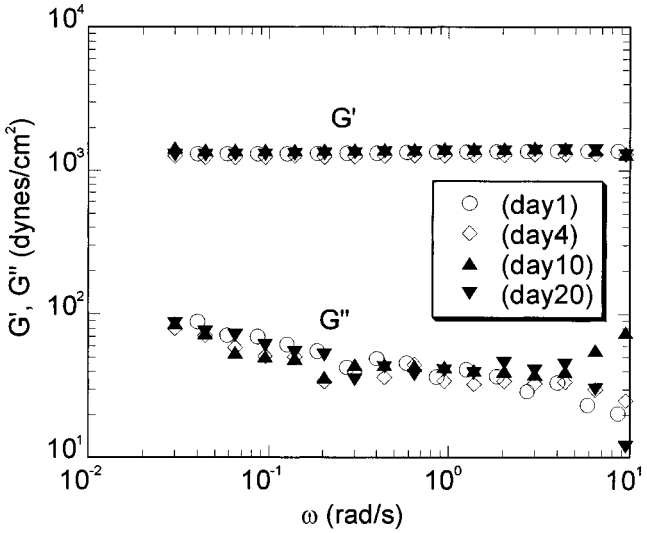

Figure 9. Overlay of dynamic moduli vs frequency behavior of a blend containing enzyme-modified guar (16.2% galactose) and xanthan measured on days 1, 4, 10, and 20 following its preparation.

network is not yet spatially uniform and continues to undergo rearrangements which convert the coarse network to a fine meshed, self-similar one as shown in the image on day 20. The forces responsible for the formation of an interconnected network also cause the solvent to be progressively expelled out of the continuous network phase. This phase separation results in a loss of uniformity of the background fluorescence and creation of dark regions within the network (day 10 and 20) which correspond to solvent-rich regions.

It is of considerable interest to determine whether the aggregation and phase separation phenomenon observed in guar solutions extends to the mixed blends also. Preliminary experiments conducted on the blends containing guar with 16.2% galactose content showed evidence of syneresis upon storage for 3 weeks, in contrast to the blends made earlier with guar containing 25.2% galactose. A combination of rheology and microscopy was again used to investigate aging effects in these blends. Figure 9 shows an overlay of the dynamic frequency sweeps of the mixed systems conducted on day 1, day 4, day 10, and day 20. The elastic modulus dominates over the viscous modulus, and the frequency sweeps remain unchanged with time, suggesting that the blends are stable as before. A comparison of the dynamic rheology of guar blends (Figures 3 and 9) with different galactose contents seems to further highlight the similarity of their macroscopic behavior. It is interesting to note that the moduli of the blends do not change even when guar of different galactose contents is used. This is probably due to the fact that a 1:1 xanthan-to-guar ratio was maintained in all cases which limits the number of xanthan molecules available for interaction. As the galactose content is reduced from 25.2% to 16.2%, the number of unsubstituted mannan regions increases; however, no additional junction zones are formed due to shortage of xanthan molecules, and the gel modulus remains unchanged.

Although the macroscopic rheology of the blends is unchanged, the appearance of syneresis suggests that microscopic phenomena in the two samples (containing guar with 16.2% and 25.2% galactose) differ. A multiple labeling approach for fluorescence microscopy was employed to investigate these phenomena and evaluate

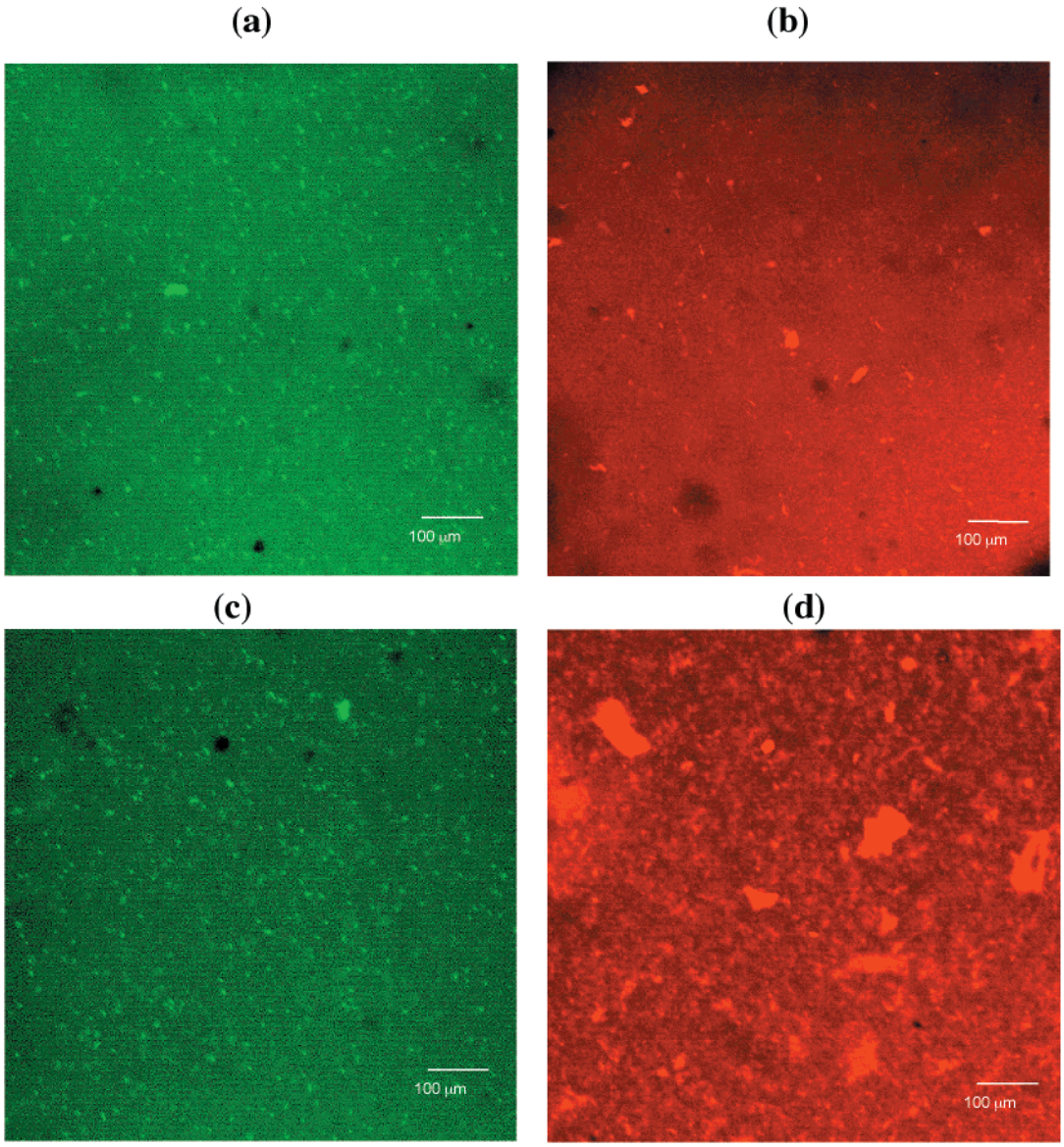

Figure 10. Fluorescent confocal images of a xanthan/guar (16.2% galactose) blend taken on day 0 (a, b) and day 20 (c, d) following its preparation. Images a and c correspond to the fluoresceinamine-labeled xanthan whereas images b and d correspond to TRITC-labeled guar molecules.

the contribution of each biopolymer to the overall structural changes in the blend. Guar gum was tagged with tetramethylrhodamine isothiocyanate (TRITC) while xanthan was tagged with fluoresceinamine. These fluorophores have different excitation and emission wavelengths and a system of argon and krypton lasers can be used to image each separately. Some representative images of the blends taken on day 0 and day 20 are shown in Figure 10. Figure 10b, which corresponds to the TRITC-tagged guar species in the blend on day 0, shows a few bright specks, suggesting that guar has begun to aggregate. The aggregates increase in size and number and form interconnections with each other. This eventually leads to phase separation as seen in the image taken on day 20 (Figure 10d). However, the extent of separation is not as pronounced as in the solutions, and expulsion of the solvent is not seen as clearly. The images of the fluoresceinamine-tagged xanthan species taken concurrently on day 0 (Figure 10a) and day 20 (Figure 10c) show the presence of a number of fluorescent specks uniformly distributed throughout the solution. The nature of the image does not change over the period of observation, suggesting that xanthan molecules remain unaffected by the structural rearrangements in guar. Although a more extensive study is necessary to make conclusive statements about the role of xanthan, these results indicate that phase separation does not affect the xanthan−guar mixed junction zones to a significant extent. On the contrary, comparison of the images of the gels with those of solutions (Figure 8) indicates that the process of mixed junction zone formation competes with self-association of guar and retards phase separation.

Summary

In this study, we compare the time-dependent behavior of modified guar, the galactose side chains of which have been enzymatically cleaved to render galactomannan with two different galactose contents of 25.2% and 16.2%. At low extents of modification (25.2% galactose), we find the galactomannan to remain as a stable solution for a period of 3 weeks; the corresponding blend with xanthan exhibits gellike features that also remain invariant with time. These results are consistent with

confocal images of both the solution and blend, which reveal uniform microstructures that remain unchanged with time. In contrast, guar with 16.2% galactose forms noncovalent self-associations in solution leading to aggregation and eventual phase separation. This slow transition from an entangled macromolecular solution to a weak gel and the final phase separation has been monitored using a combination of rheology and fluorescent laser scanning confocal microscopy. Confocal images of blends of this modified guar with xanthan reveal that the phase separation process is retarded by the addition of a second component, possibly due to the mixed junction zone formation between guar and xanthan competing with the self-association of guar.

Acknowledgment. The authors gratefully acknowledge the U.S. Department of Agriculture and the National Science Foundation for partial support of this work.

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MA0115545