Transmission Electron Microscopy of Gel Network Morphology: Relating Network Microstructure to Mechanical Properties

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ABSTRACT: Freeze–fracture TEM images of polymer network structure in aqueous (hydroxypropyl)guar gels are presented. With these images correlations between observed gel morphology and macroscopic chemical and mechanical properties can be directly determined. The images show that guar molecules associate, even without titanate cross-linker, to form an extended network, the structure of which depends on the electrolyte concentration. The titanate cross-linker does not appear to influence the microstructure but rather appears to weld together polymer molecules already associated by hydrogen bonding. Mechanical shear degrades the gel by breaking up the network inhomogeneously to form "fractured gel particles", small domains of well-cross-linked gel separated by fluid domains. The TEM images confirm and extend rheological measurements.

Introduction

Considerable interest in the physics of polymer gels has been motivated by the results of Tanaka and co-workers[1] on the phase behavior of covalently cross-linked polyacrylimide gels. Covalently bonded gels find application as electrophoresis media, gel permeation chromatography packings, and absorbents. Gels can also be formed by the association of polymer molecules. The major application of these gels is found in oil field operations. Metal ions are used to cross-link water-soluble polymers to produce high-viscosity gels that are used to support and transport solid particles[2] and to block flow channels in underground formations.[3] Our work concerns these metal ion cross-linked gels.

The rheological and mechanical properties of cross-linked polymer gels arise from the structure of the gel network. In turn, the structure of the gel network results from (1) thermodynamically determined interactions between the polymer chain segments, (2) the interactions of the cross-linking metal ion with the polymer, and (3) the deformation history of the network. In this work, we show direct images of aqueous polymer gel microstructure using freeze–fracture transmission electron microscopy (TEM) and relate the observed network microstructure to the macroscopic chemical, rheological, and mechanical properties of the gel.

Interpretations of mechanical and rheological measurements on polymer gels invariably begin with a conceptual model of the gel network. Often, models derived from polymer kinetic theory are invoked to try to relate the observed bulk properties to the underlying molecular structures.[4] The complexity of the rheology of titanium cross-linked (hydroxypropyl)guars (i.e., shear rate dependence, shear history dependence, and time dependence) suggests that the gel structure is itself complex.[5,6] Inferences about this gel structure based on observations of rheological properties have never been definitive. Fortunately, freeze–fracture techniques, developed by researchers in the life sciences to study cell physiology, can be extended to directly image aqueous gel structure.

Until recently, however, microstructural investigations of aqueous systems by TEM were hampered by a lack of simple and reliable sample preparation techniques. In earlier freeze–fracture investigations, the cooling rates employed were too slow to prevent structural rearrangements caused by ice crystal formation and phase separation.[7] If water crystallizes as large grains of ice, the advancing crystal front expels solute particles and dispersed species to the crystalline grain boundaries, thereby disrupting the structure and spatial organization of the dispersed particles. The key to the higher freezing rates employed in this study is the jet-freeze technique (commercially available from Balzers Union, Hudson, NH, as the Cryojet QFD 020). In this technique, a 10–50-μm-thick layer of sample liquid trapped between two thin copper plates is rapidly cooled by spraying high-velocity liquid propane at −180 °C onto both sides of the sample. Cooling rates of up to 30 000 °C/s have been measured; these rates are adequate to freeze water in a vitreous state and preclude rearrangements of even small structures.[8] The technique has been used successfully to preserve and image cell structure,[9] surfactant–water dispersions,[10] and liquid crystal structure.[8] Nonaqueous gel networks have also been observed with freeze–fracture by Tohyama and Miller.[11]

Materials and Methods

Guar is a polysaccharide consisting of D-mannose residues with pendant D-galactosyl units with the ratio of mannose to galactose residues reported to be 1.6:1 to 1.8:1.[12] To produce (hydroxypropyl)guar (HPG), the hydroxyl group on the pendant $-CH_2OH$ units can be reacted with propylene oxide to form $-CH_2OCH_2(OH)CHCH_3$.[13] This substitution reduces crystallinity in the guar, thereby reducing the insoluble residue in guar solutions. A polymer composed of just the mannose backbone is insoluble in water because of hydrogen bonding between mannose chains. Even relatively short regions of galactose-free mannose backbone are able to act a sites for interchain associations by hydrogen bonds. The added hydroxypropyl groups sterically prevent crystallization of the sugar residues. The HPG sample used in this study contained 0.4 mol of hydroxypropyl groups per mole of sugar (mannose + galactose) residues.

A series of six samples was prepared to study the effects of salt (i.e., extent of hydration of the polymer), cross-linking agent, and mechanical shear on the morphology of aqueous (hydroxypropyl)guar gel networks. The samples were designated as follows:

S1. A 0.48 wt % solution of commercial (hydroxypropyl)guar (Celanese Inc., SCN 9574) of average molecular weight 1–2 million in water with no added salt.

S2. As S1 but cross-linked with a 0.04 vol % solution of 1:9 parts by volume of titanium acetyl acetonate (Tyzor AA, Du Pont Inc.) in isopropyl alcohol. The gel was prepared by mixing 0.04 mL of titanate–alcohol solution into 10 mL of guar (S1) by hand.

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The gel was allowed to sit without agitation for 20 min, and then the samples were immediately processed for freeze–fracture as described below.

S3. As S2, but subjected to shear degradation. A sample of 250 mL of guar solution (S1) was put into a blender and subjected to low shear to form a shallow vortex; into this 1 mL of titanate solution was injected. The speed of the blender was increased, and the gel was sheared for 160 min. During this period, the blender was turned off for 10 min every 30–40 min to avoid overheating. This length of shear degradation was chosen to reduce the gel viscosity to about the viscosity of the initial un-cross-linked guar solution (S1). The time between this shear degradation and the processing for freeze–fracture was about 20 h. During this period the gel appeared to stiffen somewhat. Just before freezing, the gel was sheared for 10 min, after which time the gel again appeared to be fluid.

S4. As S1, but with 2 wt % added potassium chloride (KCl, Fisher Chemical Co.).

S5. As S2, but with 2 wt % added KCl.

S6. As S3, but with 2 wt % added KCl. Only 1 h of shear in the blender was required to reduce the viscosity of the cross-linked gel with 2 wt % KCl to the original un-cross-linked guar solution viscosity. The sample with KCl did not stiffen and regain its viscosity even after 15 h between the shear and the freeze–fracture experiments as did the similar sample without salt (S3).

To prepare the gels for observation in the TEM, drops of the samples were placed on small sheets of Parafilm (SPI, West Chester, PA) with a pipet. A much smaller droplet (0.1–0.5 μL) of each sample was transferred to the surface of a nitric acid etched copper freeze–fracture planchette (Balzers) by gently touching the copper surface to the large droplet on the Parafilm. This method of creating the necessary small droplets avoided the shear associated with drawing the sample liquid into a micropipet. A second planchette was gently placed on top of the first, and the sample liquid slowly spread itself by capillary action to cover the sheets and form a layer 10–50 μM thick. The samples were annealed for 3–5 min in a humidity-controlled glovebox to further reduce any effects of shear-induced alignment. The apparatus used for freezing the copper-sandwiched samples was a modified version of the Balzers Cryojet QFD 020 jet-freeze device. The sample sandwiches were mounted on a Teflon support arm that was dropped by a solenoid-actuated release mechanism between the opposed high-velocity jets of liquid propane at −180 °C in the Balzers cryojet device. The impinging high-velocity liquid propane provides a minimum cooling rate of 10 000 °C/s. The frozen copper-sandwich samples were stored in liquid nitrogen until transfer into a spring-loaded "mousetrap" sample carrier. The loaded sample carrier was mounted onto a liquid nitrogen cooled coldfinger in a Balzers 400 freeze–etch device, and the vacuum chamber was evacuated to better than 10^{-7} Torr. The temperature of the sample carrier and coldfinger was adjusted to −100 °C, and the spring mechanism was externally actuated, causing the samples to fracture. The fractured samples were exposed to the vacuum for 120 s at −100 °C to remove about 150 nm of frozen water by sublimation,[14] thereby exposing the gel network. The fracture surfaces were then replicated by evaporating 1.5 nm of a platinum–carbon mixture from an electrode at a 45° angle to the fracture surface, followed by a 15-nm-thick film of carbon deposited at normal incidence to increase the mechanical strength of the replica. The samples and replicas were removed from the vacuum chamber, and the samples and copper planchettes were dissolved in chromic acid, leaving the platinum–carbon replicas behind.[9] The replicas were washed in distilled water and collected on Formvar-coated 50-mesh gold electron microscope grids (Pelco; Tustin, CA). The replicas were examined in a JEOL 100CX scanning-transmission electron microscope (STEM) in the conventional transmission mode using 80-kV electrons. Images were recorded on Kodak electron image film. An intermediate negative was made from each image recorded with the STEM, and prints were made from the intermediate negatives. This was done so that "shadows", which are caused by an absence of platinum in the replica, appear dark in the prints.

Observations and Discussion

Figures 1–6 show the electron micrographs of replicas of samples S1–S6, respectively. In each micrograph, the polymer strands are light lines on a mottled gray background of water. The polymer network stands out above the background because the water surrounding the network has been sublimed away to a depth of more than 100 nm. The network structure of the polymer remains because its vapor pressure is negligible at −100 °C. The network appears as if it were unidirectionally illuminated with a strong planar light source, and the three-dimensional structure apparent in the replicas is real. There is at least 100 nm from the highest point in the replica to the

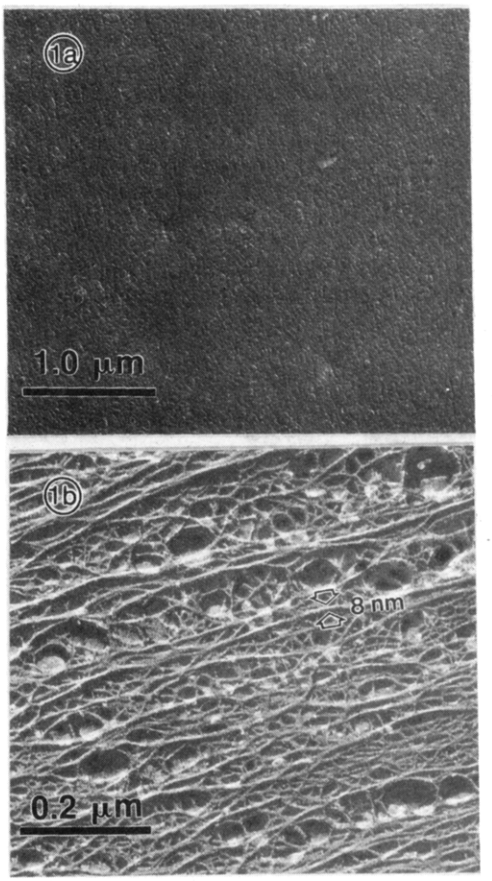

Figure 1. (a) TEM image of freeze–fracture replica of un-cross-linked guar solution S1. Polymer strands appear light on a gray water background. Note the extended linear configuration of the polymer chains and the elliptical water cells. (b) High-magnification view of S1 polymer chains. Maximum diameter of strands is 8 nm. Extended entangled configuration is evident.

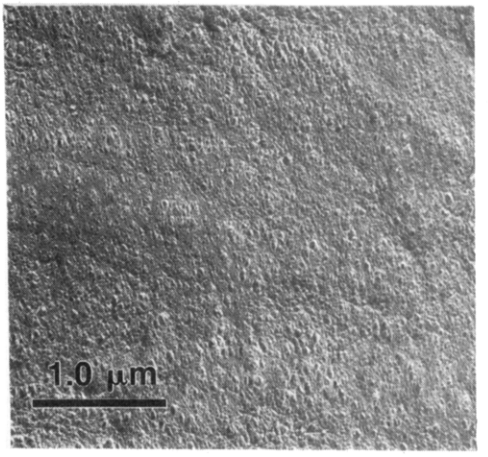

Figure 2. Guar solution after cross-linking with titanium ions (sample S2). The network morphology is similar to that in the un-cross-linked network in Figure 1. The titanate ions seem to weld together polymer chains already associated by hydrogen bonding.

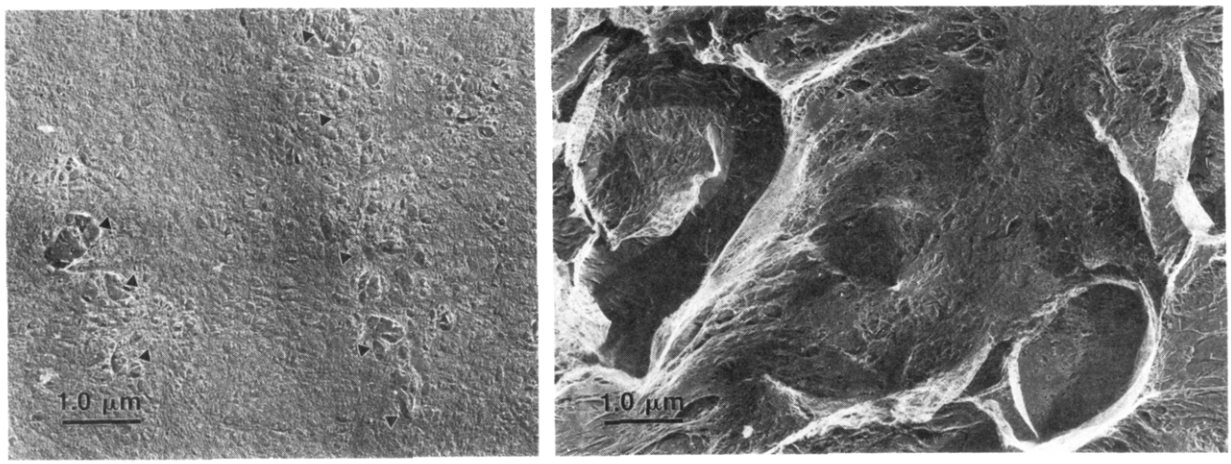

Figure 3. (a) Effect of shear on cross-linked polymer network (sample S3). Shear appears to fracture the network along weak zones (arrows), producing "fractured gel particles". The network within the particles seems to be unchanged. (b) Fractured gel particles. Highly degraded regions show the nonhomogeneous morphology of sheared gels.

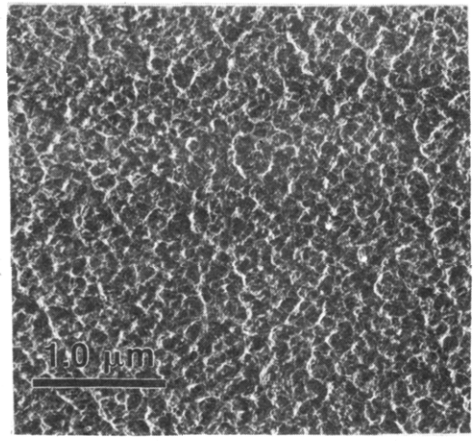

Figure 4. Effect of added KCl on guar solution (sample S4). Added electrolyte strengthens hydrogen bonds, making the polymer chains condense and appear to be thicker. The water cells are more irregular. Compare to Figure 1.

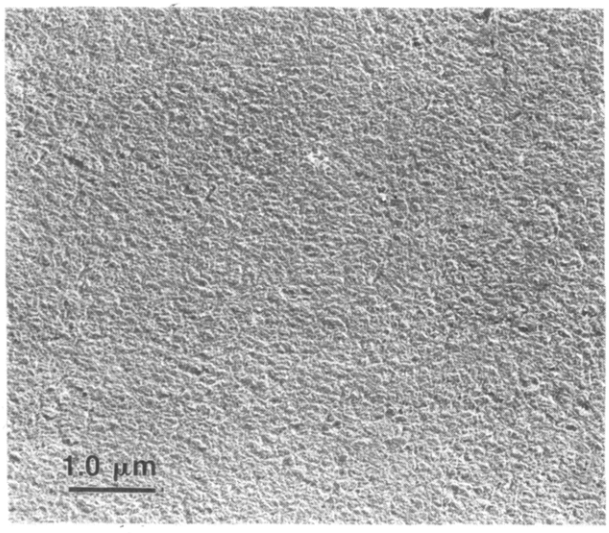

Figure 5. KCl-containing guar solution cross-linked with titanate (sample S5). Note that the network morphology is similar to that of the un-cross-linked, KCl-containing sample (Figure 4), but different from that of the salt-free samples. Compare to Figure 2.

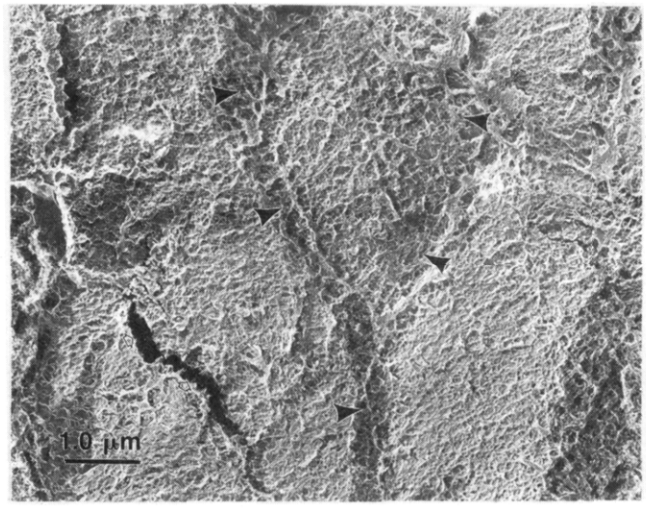

Figure 6. Sheared cross-linked gel containing salt. As in Figure 3, the gel appears to have broken up along weak zones (arrows), producing "fractured gel particles", as in the salt-free samples (See Figures 2 and 3). The network structure within each particle is unchanged. Compare to Figure 5. Open arrows mark tears in replica film.

lowest; because of the larger depth of field in TEM, the entire replica is in focus. There is no indication of any reorganization or disruption caused by ice-crystal growth or phase separation. This is consistent with previous work on dispersions of small virus molecules[8] frozen and replicated in a similar way. Bright white patches appear on some of the replicas of salt-containing polymer gels (Figures 4–6); these are residues of the KCl that were not completely removed from the replicas before imaging. These white spots are purely cosmetic artifacts and do not influence the organization or structure apparent in the replicas.

The salt concentration of the guar solution plays a dramatic role in determining the microstructure of the polymer network. Without salt, both the un-cross-linked guar (Figure 1) and the titanate-cross-linked gel (Figure 2) show a fine-grained texture; the polymer strands appear to be in an extended linear configuration, and the water cells defined by the network are elliptical in shape. The polymer strands themselves appear to be fine and uniform with diameters less than about 8 nm (Figure 1b). As the sampling technique was designed to minimize shear during preparation and the individual samples were annealed prior to freezing, we believe the extended configuration of the un-cross-linked polymer strands in Figure 1 is real and not a result of shear-induced alignment. This is confirmed

by the similar appearance of the more rigid cross-linked gel (Figure 2) and the greatly different appearance of the polymer solution when KCl was added (Figure 4).

The guar molecules, even in the uncomplexed state, are overlapped and entangled. The extent of overlap can be assessed by considering the ratio of the actual polymer concentration to the critical concentration at which chain overlap first occurs. This critical concentration is given the symbol c^* and can be determined experimentally by the relation[3]

$$c^* \approx 0.7/[\eta]$$

in which $[\eta]$ is the intrinsic viscosity. The intrinsic viscosity of HPG solution in 2 wt % KCl is 16.4 dL/g at shear rates between 0.5 and 1.0 s^{-1}. The critical overlap concentration is, therefore, 0.07 wt %. The solutions used in this study had concentrations of 0.48 wt %, about 7 times the critical overlap concentration. Consequently, the differences between a HPG solution and a gel is not the presence or absence of chain contacts, but the strength and order of these contacts.

With salt, both the guar solution (Figure 4) and the titanate-cross-linked gel (Figure 5) show a coarser network structure. The polymer strands appear to have a more condensed structure, and the water cells are more irregular in shape. The polymer strands appear to be much thicker, most being greater than 10 nm in diameter. The degree of association of polymer molecules is clearly increased by the added salt.

Salt increases the intermolecular attractions between polysaccharide chains by changing the hydrogen bonding between hydroxyl groups on the sugar residues.[15] (Hydroxypropyl)guar is based on a random copolymer of galactose and mannose residues. The electrolyte competes for waters of hydration with the hydroxyl groups, causing them to become somewhat less hydrated. These dehydrated sugar residues are then free to associate with other polymer chains by hydrogen bonding. The extreme example of these hydrogen-bonding interactions is cellulose, which hydrogen bonds so strongly that it becomes insoluble in water, even though the individual monomeric sugar residues that comprise cellulose (glucopyranose sugars) are water soluble. The weak hydrogen bonding between guar molecules in solution appears to have a major effect on the microtructure of the network formed, even after cross-linking with titanium ions. These differences in microstructure are manifested in the mechanical properties of the gels. Guar gels with added salt are more susceptible to mechanical shear degradation than gels containing no salt. For example, in our sample preparation, it took roughly 3 times longer to degrade a guar gel made without salt (S3) than one with 2 wt % KCl (S6).

There is little difference between the network structure present in the polymer solutions before (Figures 1 and 4) and after (Figures 2 and 5) adding titanium ions. Rather than joining two polymer chains that would otherwise be separated in solution, the metal ions appear to reinforce or weld together chains that already tend to aggregate in solution. There are other indications in the literature that this is the mechanism of gel formation for polysaccharides. For example, xanthan gum and locust bean gum individually remain in solution, but when mixed, crystallize to form an insoluble gel.[16] Also, substituted cellulosics form reversible gels when the solvent quality is reduced by the addition of salt or an increase in temperature.[17]

Shear affects the morphology of the cross-linked guar gels both with and without added salt in similar ways. The gel network does not appear to be degraded homogeneously (see Figures 3a and 6). Rather, there appear to be regions

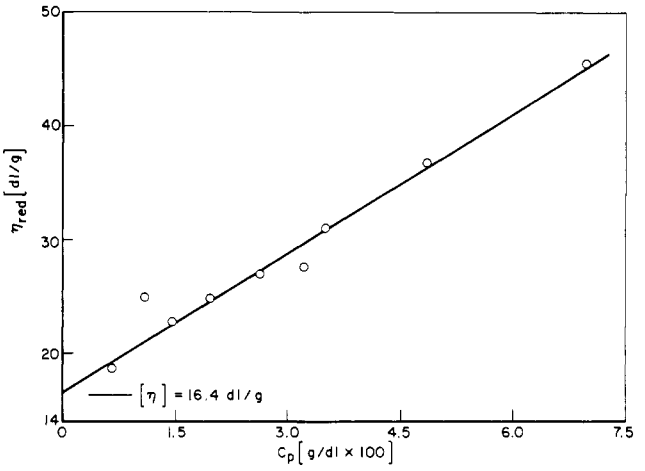

Figure 7. Relative viscosity vs. polymer concentration for HPG sample in 2 wt % KCl buffered with sodium diacetate to pH 4.8. From the data, the intrinsic viscosity is 16.4 dL/g.

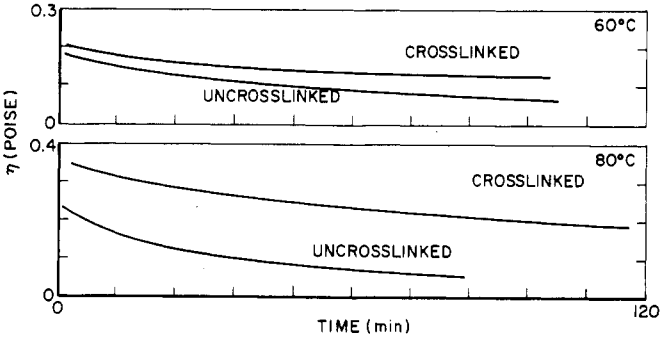

Figure 8. Viscosity vs. time for 0.48 wt % HPG solutions and gels at 60 and 80 °C. As S5, but buffered with sodium diacetate to pH 4.8. Data taken on a computer-controlled reciprocating capillary viscometer. See text for explanation.

that have the same network density as the unsheared gels, surrounded by regions of more open, degraded structure (arrowed). This picture of "fractured gel particles" (Figure 3b) had been inferred previously from rheological measurements of the strain sensitivity of dynamic oscillatory moduli of shear-degraded gels.[5]

When polymer gels are subjected to sufficient shear, they do not flow, but fracture along weak points in the network. The resulting gel particles are surrounded by fluid regions with fewer polymer bridges (arrows, Figures 3 and 6). Still, the microstructure of the network is unchanged within the gel particles. Ideally, the gel could eventually recover its original viscosity by repairing the gaps between gel particles with new polymer bridges. This process of repair would be favored by low salt concentrations because the guar molecules are more soluble without salt and have a more extended configuration. The salt-free gel samples we prepared (S3) recovered much more of their initial viscosity on standing than did the gels containing salt (S6). A second important factor to note is that although S3 recovered a portion of its original viscosity on standing, it was quickly degraded by shearing a second time. This suggests that even though the gaps between gel particles can be repaired by new polymer bridges, the network density and strength are still less along these gaps and will fracture again on shear.

The observed morphology of the gel particles shown in Figures 3 and 6 is consistent with the greater temperature stability of the viscosity of gels compared to polymer solutions. Figure 8 shows viscosity vs. time data for a guar solution and gel taken in a continuous reciprocating ca-

pillary viscometer at two different temperatures. Details of the apparatus are given elsewhere.[18] At 60 °C, the viscosity decrease over 2 h for the gel and that for the polymer solution kept under continuous shear are about equal. No significant backbone chain scission of the guar molecules should occur under these conditions. At 80 °C, the viscosity of the gel decreases only slightly, but the viscosity of the gel solution decreases much more rapidly. The rapid decrease in the polymer solution viscosity is caused by chain scission; the decrease in average molecular weight results in a decrease in viscosity. For the gel, on the other hand, the basic flow unit is a gel particle, not an isolated molecule. It requires many more chain scissions to decrease the size of the gel particle. Hence, the cross-linked gel particles shown in Figures 3 and 6 lead to an increased stability of the fluid viscosity.

Conclusions

Freeze–fracture replication TEM provides direct artifact-free images of polymer network structure in aqueous (hydroxypropyl)guar gels. With these images, we can easily determine correlations between the observed gel morphology and the macroscopic physical properties as a function of added salt, cross-linking agent, and mechanical shear.

The images show that guar molecules associate, most likely via hydrogen bonding, even without titanate cross-liner, to form an extended network, the structure of which depends on the electrolyte concentration. The association of polymer molecules is increased by added salt, which enhances hydrogen bonding between the sugar residues, causing the polymer chains to contract from an extended linear configuration without KCl to a more compact condensed state with added KCl.

The titanate cross-linker, the macroscopic effect of which is to transform the guar solution from a thin syrup to a stiff gel, has little effect on the microstructure of the polymer network, regardless of the electrolyte concentration. The titanate seems to weld together polymer chains already associated by hydrogen bonding. The network does not change, only the strength of the polymer bonds.

Mechanical shear affects gel with and without salt in similar ways by causing breaks in the network resulting in "fractured gel particles", regions of well-cross-linked polymer-rich networks separated by narrow zones of polymer-poor fluid domains. Although the bulk of the network remains intact, its three-dimensional connectivity is lost, and its viscosity approaches that of the continuous aqueous phase. The breaks in the network can be repaired by establishing new bridges across the gaps, a process favored by low salt concentrations. However, these bridged gaps are weaker than the original gel, and less shear is required to degrade the gel viscosity.

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