Linear Viscoelastic Behavior of Enzymatically Modified Guar Gum Solutions: Structure, Relaxations, and Gel Formation

R. H. W. Wientjes, M. H. G. Duits,* J. W. P. Bakker, R. J. J. Jongschaap, and J. Mellema

Rheology Group, Department of Applied Physics (member Twente Institute of Mechanics and J.M. Burgerscentre), University of Twente, PO Box 217, 7500 AE Enschede, The Netherlands

Received February 20, 2001; Revised Manuscript Received June 1, 2001

ABSTRACT: To gain more insight into the mechanisms of stress relaxation in aqueous guar gum solutions, we investigated the effect of chemical modifications of the polymer and of the solvent on the linear viscoelastic behavior in different regions of the frequency domain. Interchain bonding could be ruled out as the origin for the high-frequency relaxation behavior, while it was corroborated that such a bonding must be (directly or indirectly) involved in the relaxations at low frequencies. In the enzymatic modifications, galactose side groups were removed in different fractions f (0.30−0.57) of the available amount. Moduli were measured as a function of frequency (0.003−20 Hz) and temperature (283−323 K). On increasing f, a transition from a liquid to a gel was found at $f = f_c$. Below f_c the changes in the relaxation behavior were very modest, and changing the solvent had little effect. Above f_c gels were formed. The low-frequency storage moduli strongly increased whereas the high-frequency moduli remained essentially unchanged. For both modified and unmodified guar solutions, we attribute the viscoelastic response at high frequencies to conformational relaxations of multichain structures and at low frequencies to interchain bonding effects. More detailed mechanistic information is hard to obtain. Yet we propose a microscopic picture that can explain most of our observations. It would merit a further study with other techniques. The possibility to make weak gels of α-D-galactosidase-treated guar was demonstrated, and also this deserves further study.

1. Introduction

Guar gum is a polysaccharide originating from the seed endosperm of the guar plant (*Cyamopsis tetragonolobus*), also known as cluster bean. It is a member of the class of galactomannans, which all consist of a (1→4)-linked β-D-mannopyranosyl backbone partially substituted at O-6 with α-D-galactopyranosyl side groups.[1] The different galactomannans are distinguished by their natural source and by their mannose/galactose ratio, which is approximately 2 for guar, 3 for tara gum, and 4 for locust bean gum (LBG). Several of them are used in the food industry as thickeners and/or as gelling agents. The M/G ratio plays an important role in such applications. Galactomannans have a poorer solubility in water for higher M/G ratios, and for M/G > 5 the polymer is no longer soluble. Related to this solubility issue, it is also easier to make a gel from a galactomannan with a higher M/G. For example, whereas LBG forms a gel after freeze−thawing,[2,3] guar gum does not. However, guar gum is still interesting for industrial applications because of its availability at lower cost, its use as a food thickener, and its potential to replace LBG after enzymatic modification (to increase M/G). In the present paper we shall study the linear rheological behavior of solutions of guar gum as well as modifications at higher M/G, obtained via the enzymatic removal of side groups. One of our aims was to explore the potential use of thus modified guar for making weak gels. Encouraging results were obtained, and these will be presented in section 3.

However to obtain these, more was needed than just a manipulation of the structure at the *molecular* level. As a consequence of bonding between the associative sites on the chains, also structures and processes at *mesoscopic* length and time scales come into play. These (not precisely known) structures and processes can have a profound influence on the material properties of the final product. To maximally exploit the potential for making weak gels from modified guar gum, they should be identified. This is a complex task, but progress along this line can be achieved by analyzing the viscoelastic behavior. Our second (and main) aim of this study has therefore been to analyze the linear rheological behavior of (native and modified) guar gum solutions, with a focus on understanding the relaxation mechanisms.

For this, one should first consider the state of the art in understanding in the rheology of native guar solutions. Several research groups have investigated the rheology of guar gum solutions.[4−9] Comparing the different studies, it becomes clear that the rheological behavior is still incompletely understood and that specific polymer−polymer interactions might play a role in the observed rheological behavior as well as reptation phenomena. This motivated us previously[10] to perform a systematic study on the frequency, temperature, concentration, and molecular weight dependence of the linear viscoelastic moduli. The main findings are summarized here. We found two storage modulus plateau zones: one at low frequencies (<0.03 Hz) and one at high frequencies (>10 Hz). The storage modulus plateau at low frequencies was in the decipascal range and appeared to be sensitive to strain. Its magnitude was found to increase with temperature and showed no dependence on thermal history. On varying the concentration, it remained more or less constant. The storage modulus plateau at high frequencies was preceded by a broad relaxation spectrum, which is not related to polydispersity. The mean relaxation time of the spectrum showed an Arrhenius-like temperature dependence and a strong concentration dependence. The magnitude of the high-frequency storage modulus increased

10.1021/ma0103019 CCC: $20.00
Published on Web 07/13/2001

with concentration while it was insensitive to temperature.

From comparisons with existing microrheological models, it was concluded that a recently developed transient network model,[11,12] in which the chains are supposed to contain two different types of stickers (weak and strong), gave the best consistency with experimental results. Using a "chemical equilibrium" description for the formation and annihilation of bonds, a predicted relaxation spectrum featuring two $G'(\omega)$ plateaus was obtained. Also, the experimentally observed temperature dependences of the plateaus could be reproduced within the model by inserting suitable numbers for the bond (activation) energies. Dependences on molecular weight were qualitatively confirmed. In this interpretation, the weak bonds (apparent activation energy $10k_BT$) were associated with hydrogen bridges. The strong bonds would be due to hydrophobic interactions between the free backbone parts.[13,14] In line with our mentioned goals, we want to further investigate the validity of these concepts, both for native guar gum and for enzymatic modifications thereof. We shall consider in the present paper three issues:

1. Weak physical bonds as the origin for the high-frequency behavior. In the case of guar, the only conceivable weak physical bonds would be hydrogen bridges. Exploiting the sensitivity of these bonds to their chemical environment, we have investigated this issue by modifying the solvent.

2. Strong hydrophobic bonds as the origin for the low-frequency behavior. The local hydrophobicity of galactomannan chains is generally believed to be related to the number of adjacent bare mannose backbone units. We have investigated the role of hydrophobicity by enzymatically removing galactose side groups.

3. Thermodynamic equilibrium. To what extent the structure formed by the associating guar chains (and probed by the linear measurements) corresponds to an equilibrium state was studied by considering the dependence of the moduli on the thermal treatment of the samples.

This paper is further organized as follows: The details of the enzymatic modifications and of the rheological experiments are given in section 2. Here it will also be shown that the making of gels takes more than just increasing the M/G ratio. In section 3 the experimental results are presented, including the temperature-dependent behavior of the gels, which may provide interesting perspectives for applications. In section 4 various aspects of the results will be discussed, covering stress relaxations, structure (formation), and gel properties. Also, a tentative picture for the structure and relaxations will be proposed. Here we have aimed for the simplest picture that would be in accordance with our observations. In section 5 the main conclusions will be summarized.

2. Experimental Section

2.1. Materials. Guar gum [Meyhall] was purified by adding 10.0 g of guar to 400 g of acetate buffer of pH 4.66 [Merck]. The slurry was homogenized for 75 s with a food blender (500 W) and centrifuged at 22 000*g* (15 000 rpm) for 5 h at room temperature. The supernatant, containing 80% of the suspended mass, was diluted by a factor of 2, thus yielding a 1% w/w stock solution. This solution was stored in a refrigerator. The molecular weight was determined earlier[10] as 910 kDa, while the M/G ratio was found to be 1.59. Galactose side groups were removed from the guar chains by using α-D-galactosidase [Megazyme]. The activity of the enzyme was specified as 47 U/mg, with 1 U defined as the amount of enzyme that releases 1 mmol of *p*-nitrophenol per minute (substrate: *p*-nitrophenyl-α-galactoside) at pH 4.5 and 313 K. The activity of the enzyme for cleaving the side groups of guar was optimized by setting the temperature at 310 K and the pH at 4.7. All modifications were done in 1% w/w guar stock solution.

For our enzymatic modifications of guar, a suitable protocol had to be developed. Preliminary modification experiments in which the enzyme was used at low concentration (order: 1 U/g guar) and without terminating its activity showed a phase separation after a few hours. Following the viscoelastic moduli in time, a strong decrease was observed, leading to immeasurably small signals after 2 h. Visual inspection of the solution afterward confirmed the phase separation.

This phenomenon was not mentioned in another study,[15] where similar degrees of modification (i.e., the fraction of side groups removed) had been reached, but using higher galactosidase concentrations (order: 10 U/g of guar). Assuming that α-D-galactosidase-treated guar chains are adequately characterized by their degree of modification, this would suggest that the structure built up by the modified guar chains is not an equilibrium structure. We think that the phase separation is the result of two interfering processes: the ongoing creation of (hydrophobic) stickers on the chains and the aggregation of the chains themselves via the formation of bonds. It is conceivable that distinguishable aggregates are formed in the early stages of the modification, which subsequently consolidate via an increase in the number of intraaggregate bonds. Such a consolidation will only be favored by the ongoing creation of stickers. Then if the number of interaggregate bonds is sufficiently small, a phase separation will result.

This consideration led us to adapt our protocol. By choosing enzyme concentrations now of order 10^3 U/g of guar gum and killing the enzyme after a well-defined short reaction time, we aimed to complete the molecular modification before any mesoscopic restructuring (i.e., aggregation) could occur. To estimate the time needed for mesoscopic restructuring, we have used our previous observation that the low-frequency storage modulus needs about 15 min to rebuild after being destroyed by shear.[10] In the final protocol, the degree of modification was varied by changing the enzyme concentration, while keeping the reaction time constant. The required amount of enzyme was added to 50 mL of the 1% w/w guar gum solution. The solution was mixed for 30 s by using a hand blender (Braun, MR 500 CA, 300 W). The thus obtained homogeneous solution was poured in a preheated glass bottle at 310 K and kept at this temperature under stirring for 15 min. Hereafter, the modification was stopped instantly by putting the sample in a microwave oven [Sharp R222] at 240 W for 40 s. Subsequently, the solution was placed for 15 min in a water bath at 365 K to ensure inactivation of all the enzyme. Any boiling of the sample was avoided: preliminary experiments with guar solutions had shown that boiling could cause irreversible aggregation. In the end samples were stored at either 277 or 248 K or placed directly in a rheometer, depending on the type of experiment. The amount of galactose liberated by the enzyme was determined using a lactose/D-galactose test kit [Boehringer Mannheim]. With this kit the galactose concentration in a solution can be established via a quantitative conversion reaction in which NADH is formed. The NADH absorption (peak at 240 nm) was measured in a [Shimadzu UV-2102] UV spectrophotometer and converted to a concentration using the Lambert−Beer law. Results are shown in Figure 1.

To verify that only mannose−galactose bonds had been cleaved by the enzyme, and no mannose−mannose bonds (as has been reported[15]), the intrinsic viscosity of a strongly modified guar gum (50% galactose removal) was determined. The results are shown in Figure 2.

The intrinsic viscosity corresponds fairly well to that of the unmodified guar, thus confirming that no significant scission of the mannose backbone had occurred. Further on we checked the influence of the thermal and mechanical treatments of the procedure by taking a sample through the modification

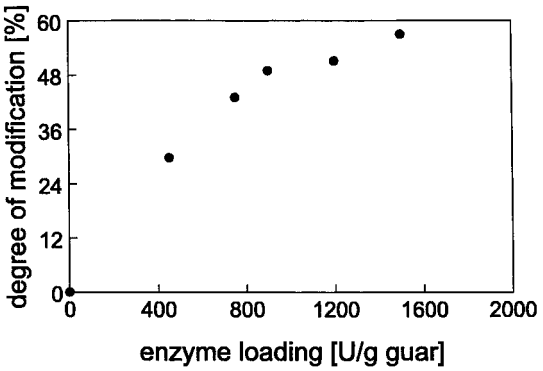

Figure 1. Degree of modification (fraction of galactose side groups removed) as a function of the enzyme loading.

protocol as described above, but without adding enzyme. The moduli of the thus obtained "reference solution" are compared with those of the original stock solution in Figure 2. Although a detectable offset between supposedly coinciding moduli was found, the differences are still very small, and the shapes of the relaxation spectra are unchanged. In the following, all enzymatically modified systems will be compared to the reference solution.

2.2. Rheological Measurements. Linear viscoelastic moduli measurements between 10^{-3} and 20 Hz and as a function of temperature were performed using a Bohlin VOR with a cone and plate geometry (cone angle of 1 deg and 60 mm plate diameter). A Haake RS 150 with a plate−plate geometry (60 mm diameter) was used to measure the storage moduli of the systems that had formed gels. Measurements were conducted at temperatures between 274.5 and 323 K. With the Bohlin, a correction was made for the temperature dependence (−19 μm/10 K) of the gap width between cone and plate. To avoid effects of solvent evaporation, homemade vapor locks, filled with paraffin oil, were used for both rheometers.

Solutions were inserted in the Bohlin by using a 10 mL syringe. After insertion, the sample was presheared at 80 s^{-1} for 1 min to obtain a homogeneously filled gap and to define the mechanical history of the sample. Then the sample was allowed to relax for 30 min before the measurements were started. This protocol was chosen on the basis of our observation that already a short preshearing was sufficient to make the low-frequency storage modulus disappear for 15 min. Given the preshear in the rheometer, it did not make a difference whether the solution was introduced via a syringe or by pouring. The behavior for frequencies >0.03 Hz did not show any dependence on mechanical history.

Gels were cautiously placed upon the lower plate of the Haake RS150 geometry at a temperature of 278 K. The gap was then set to 2 mm with exponentially decreasing speed. This was done to keep the gel structure intact. For the stronger gels that showed syneresis, the expelled acetate buffer was collected and measured in order to quantify the extent of syneresis. Before measurements were started, moduli measurements were performed between 0.1 and 0.5 Pa at 1 Hz in order to check the limits of linearity. It turned out that all gels showed linear behavior up to the highest stress.

3. Results

3.1. Enzymatic Modifications. Unless stated otherwise, samples were stored at 277 K for 1 week to equilibrate before the rheological behavior was investigated. In Figure 3 the storage moduli as a function of frequency are shown for the different degrees of modification (0, 30, 43, 49, 51, and 57% of available galactose side groups removed).

The measurement temperature for all these samples was set at 283 K to allow comparison with earlier obtained results.[10] The results show clearly two regimes as a function of the degree of modification.

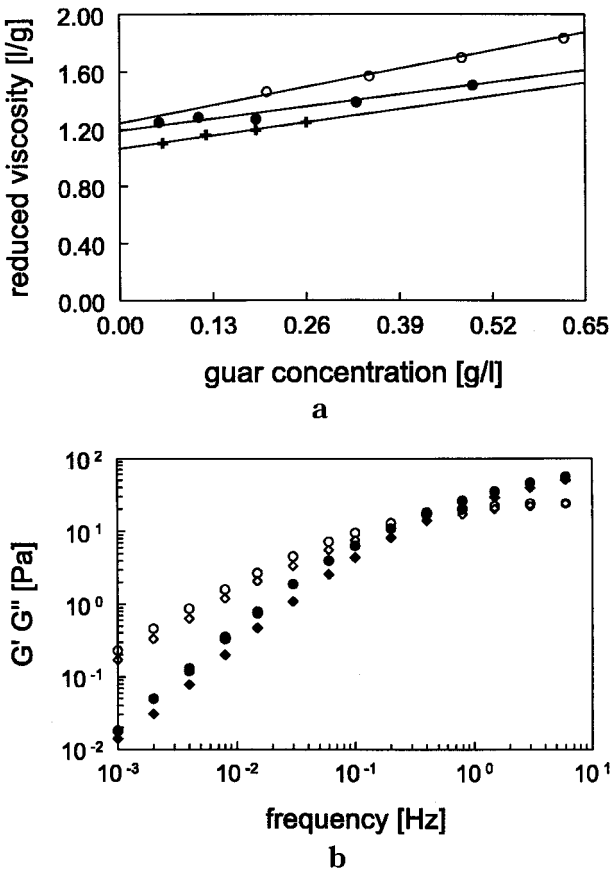

Figure 2. (top) Intrinsic viscosities (*y*-axis values) before and after enzymatic modification: (●, ○) unmodified guar (duplo measurement); (+) modified guar. (bottom) Linear viscoelastic moduli (filled, G'; open, G'') before and after the thermal and mechanical steps of the enzymatic modification procedure: (○, ●) before, (◇, ◆) after.

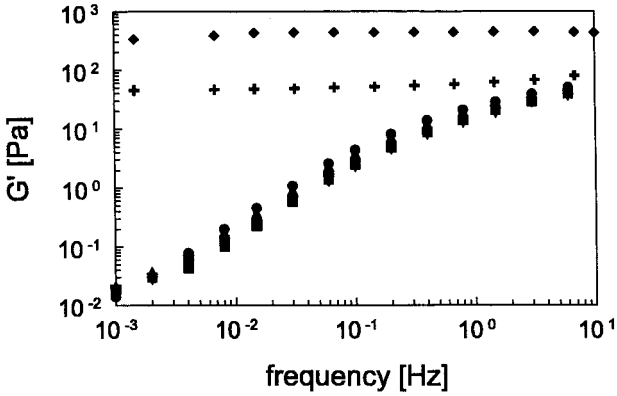

Figure 3. Storage moduli as a function of frequency for different degrees of modification: ●, 0; ▲, 30; ■, 43; ▼, 49; +, 51; and ◆, 57% of galactose removed. Measurement temperature was 283 K.

3.1.1. Nongelling Systems. At modifications up to 50% removal of the galactose, only minor changes in the linear rheological behavior are observed. In the high-frequency regime, the shapes of the $G'(\omega)$ and $G''(\omega)$ curves remain unchanged, and also the magnitude of $G'(\omega)$ remains practically constant. The mean relaxation time (defined as the time corresponding to the frequency where $G' = G''$) changes less than a factor of 2. Also, the temperature dependence of this time does not change: Arrhenius plots showed again a good linearity, with activation energies of $(13 \pm 1)k_BT$ (with $T = 283$

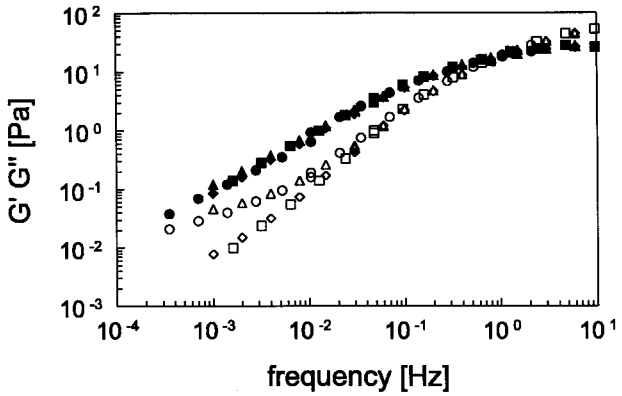

Figure 4. Low-frequency moduli of modified guar solutions. Curves have been scaled along the frequency axis to obtain superposition. G', open symbols; G'', filled symbols; △, reference solution; ○, 43% of galactose removed; ◇, 0.1 M Na_2CO_3 pH = 10 solution; □, 8 M urea solution.

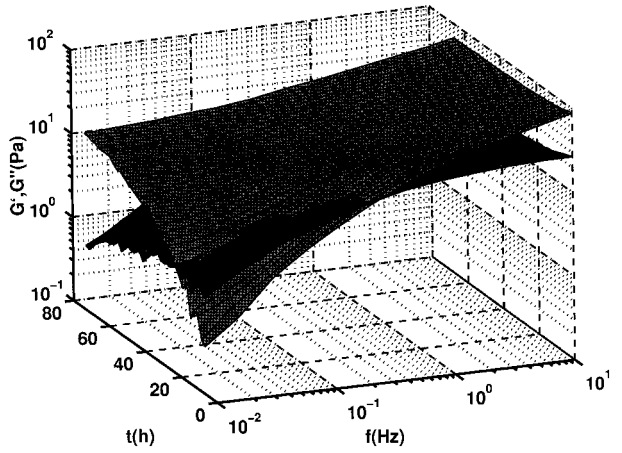

Figure 5. Gel formation measured on-line. Gray, G'; black, G''. Measurements were started directly after an enzymatic modification with a duration of 15 min (see section 2.1). Note that the characteristic time scale for gelation is about 100 times larger.

K) for both modified and unmodified guar gum solutions. The discrepancy between this value and the earlier found energy[10] of $10k_BT$ is likely due to some differences in the experimental protocols.[16] Also, in the low-frequency regime the differences between unmodified and 43% modified guar solutions are modest. These results are also shown in Figure 4.

3.1.2. Gelling Systems. *Gel Formation.* When more than 50% of the available galactose side groups was removed, gel formation occurred, as evidenced by the frequency-independent storage moduli in Figure 3. In a side experiment, an enzyme-treated guar solution was introduced in the rheometer directly after the modification, so that gel formation could be observed on-line. The result is shown in Figure 5.

It makes clear that the gel formation manifests itself by a gradual increase of the low-frequency storage modulus. Note that G' at high frequencies is conserved in this process. These observations corroborate our previous assumption that the low-frequency G' in unmodified guar solutions is related to strong (i.e., long living) reversible bonds. Creating more and longer bare mannose backbone parts leads to an increase in the number and strength of these bonds and hence a higher G' in the frequency range corresponding to the average lifetime of the bond. The finding that only the relaxation

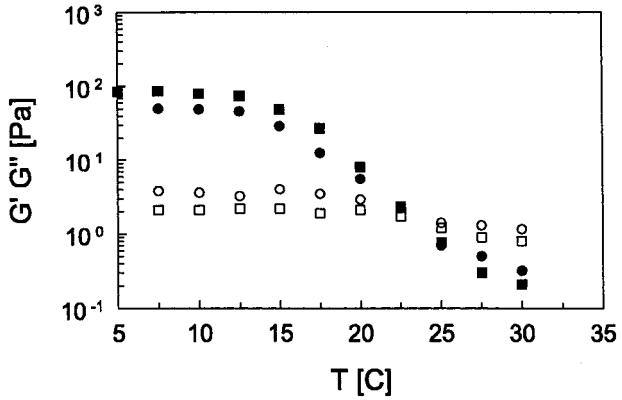

Figure 6. Temperature dependence of the moduli of a gel-forming 51% modified guar solution at a frequency of 0.0316 Hz. Measurements were done in duplo: (■, □) and (●, ○) = (G', G'').

processes at the lowest frequencies are influenced by these bonds is remarkable. Apparently, there is not a strong coupling between the relaxation mechanisms at low and high frequencies. The time needed for gel formation was confirmed to be much longer than the enzymatic modification time and short enough compared to the storage time (see section 2.1).

Melting Behavior. The gelled systems showed a distinct temperature dependence in their rheology. In Figure 6 the temperature dependence of the moduli at a frequency of 0.0316 Hz is shown, and in Figure 7 the storage modulus as a function of the frequency is shown for three different temperatures (that were increased with time).

It can be seen from Figure 6 that the gel starts to melt at 288 K (15 °C) and becomes fluidlike at 296 K. The distinction between "fluid" and "solid" is in the rheological sense always somewhat arbitrary, since it depends on the time scale considered. We have chosen 0.0316 Hz as a reference frequency and defined systems with $G'' > G'$ at this frequency as fluidlike. The melting behavior turned out to be thermoreversible: lowering temperature from 296 to 278 K resulted after a few hours in a gel showing the very same temperature-dependent behavior. The reproducibility of the initial gel formation was also investigated by redoing the enzymatic modification with a different sample. The melting curve for this system is also presented in Figure 6 and shows that reproducibility is fairly good. Changing the degree of enzymatic modification resulted only in slight differences in the melting behavior. For all gels, it was observed that melting starts at 288 K.

Influence of Storage Temperature. The dependence on the thermal history of the sample was investigated by changing the storage temperature. Besides samples that were stored at 277 K for 1 week, also a series was stored at 248 K for 6 days and subsequently placed at 277 K for 1 day to allow thawing. The rheological behavior of these freeze−thawed samples was similar, in that a transition from a liquid to a gel was observed above a certain degree of modification. However, in a quantitative sense there was an important difference: now the required degree of modification amounted approximately 40% instead of 50% (see Figure 8). This corresponds with results from McCleary et al.,[17] who found qualitatively that the storage temperature influences gel formation of modified guar gum solutions.

This clear influence of freeze−thawing on the gelation behavior illustrates that the obtained mesoscopic

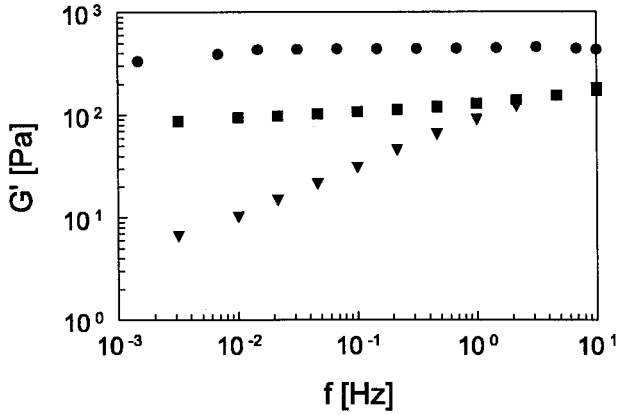

Figure 7. Storage modulus of the 51% modified guar solution, as a function of frequency, for different temperatures: ●, 283 K; ■, 295 K; ▼, 305 K.

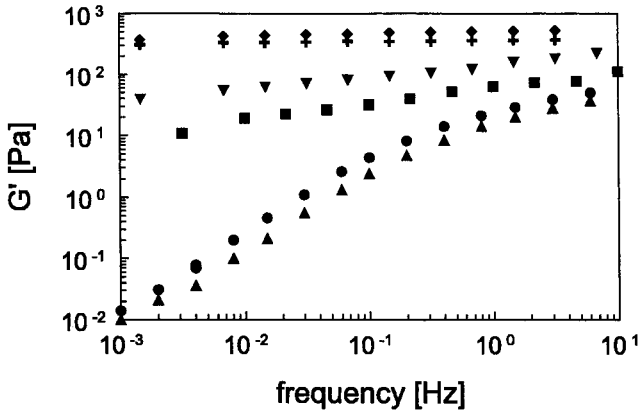

Figure 8. Storage moduli of freeze−thawed solutions, as a function of frequency and for different degrees of modification: ●, 0; ▲, 30; ■, 43; ▼, 49; +, 51; and ◆, 57% galactose removal. Storage temperature was 248 K. Measurement temperature was 278 K.

Table 1. Properties of Modified 1% (w/w) Guar Gum Gels

modification deg (%)	storage temp (K)	concn (% w/w) after syneresis	storage modulus (Pa) after syneresis
51	277	1.0	52
57	277	1.6	450
43	248	1.2	23
49	248	1.3	80
51	248	1.4	350
57	248	1.5	400

structure (as reflected via the moduli curves) depends on the aggregation kinetics. Apparently, during freeze−thawing different aggregation processes are favored than during storage at 277 K. This will lead to correspondingly different aggregate structures, which will presumably grow until they become kinetically trapped in some state.

In all cases, the final state (i.e., after a week) of the gel was attained via a syneresis process. The freeze−thawing also turned out to have an influence on the degree of syneresis. The comparison between freeze−thawed and non-freeze−thawed gels is summarized in Table 1. The different concentrations after syneresis for samples with 51% degree of modification confirm the presence of nonequilibrium structures in the gels.

3.2. Solvent Modifications. To investigate what is the contribution to the rheological behavior of interchain hydrogen bonding, we have solved the guar gum also in an 8 M urea solution and in an aqueous 0.1 M

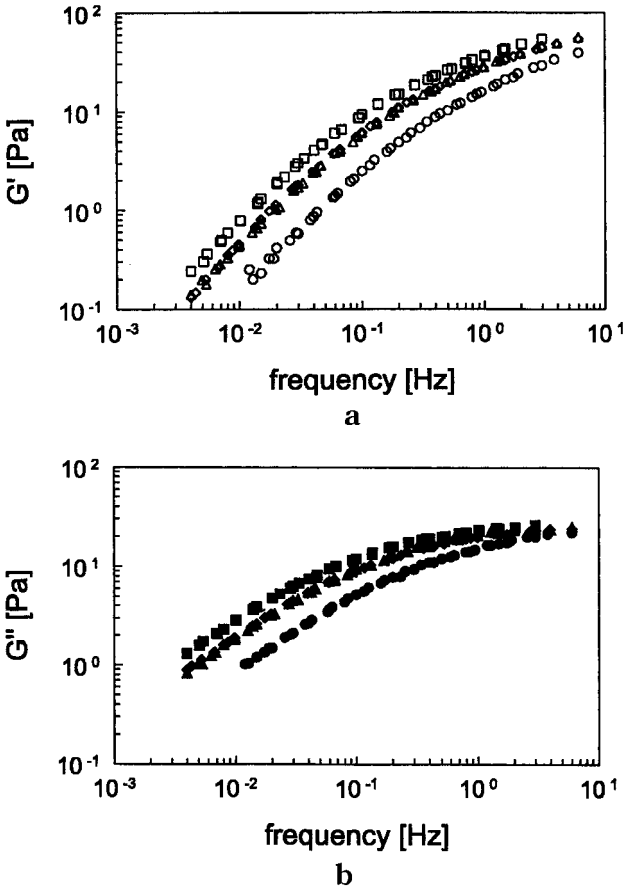

Figure 9. Influence of different sample modifications on the high-frequency behavior of guar gum: *G′*, open symbols; *G″*, filled symbols; Δ, reference solution; ○, 43% galactose removal; ◇, 0.1 M Na_2CO_3 pH = 10 solution; □, 8 M urea solution.

Na_2CO_3 buffer with pH = 10. Urea is well-known as a depressant of hydrogen bonds. This functionality is ascribed to the energetically more favorable hydrogen bonding of water with urea than with other compounds. In solutions of guar gum, the addition of urea should therefore produce a decreased chance of hydrogen bridging between mannose backbone units.[18,19] To maximize its influence, we added the urea to a concentration of 8 mol/L. The effect on the viscoelastic properties at high frequencies is shown in Figure 9.

A marked decrease in the magnitude of the high-frequency storage modulus should occur if thermal breakup of guar−guar hydrogen bonds would be responsible for the stress relaxations. In contrast, only a small frequency shift to the lower frequencies (multiplication factor 0.6) was observed. This finding indicates that hydrogen bonds between polymers do not play a role in the high-frequency regime. The small change in characteristic relaxation frequency might be due to the increased solvent viscosity. The effect of adding urea on the viscoelastic behavior at low frequencies was already shown in Figure 4. The decrease of the storage modulus in the low-frequency range is probably caused by the bare mannose backbone units becoming less lyophobic. In literature it has been mentioned[20] that for protein−protein interactions urea diminishes the hydrophobic effect by displacing water in the solvating shell. This might also apply for guar gum.

A second experiment was done to influence the hydrogen-bonding affinity between the guar chains. Here the acetate buffer at pH 4.7 was replaced by a 0.1

M Na_2CO_3 solution, titrated with HCl to obtain a buffer with pH 10. At this higher pH the number of hydrogen bonds should drop due to the decreased availability of hydrogen species. The effects on the high- and low-frequency relaxation behavior are demonstrated in Figures 4 and 9 and are seen to be small like in the case of urea. The characteristic relaxation time at high frequencies is now completely unaffected, in line with the unchanged solvent viscosity. This finding again indicates that hydrogen bonding does not play a role in the stress relaxation at high frequencies. We believe that more generally it can be said that these relaxations are not related to physical interchain bonding.

In a more general sense, replacing the acetate buffer with urea or Na_2CO_3 can be seen as experiments in which the solvent quality is changed. Removing galactose side groups from the guar chains could be regarded as an "inverse method" for changing the solvent quality: here the molecular properties of the polymer are changed, while the solvent remains essentially the same (neglecting the influence of small amounts of inactivated enzyme and liberated galactose). Hence, measurements on enzymatically modified guars can be seen as a third way to study the influence of solvent quality. In Figures 4 and 9 the relaxation behavior is shown for guar with 43% of its galactose side groups removed. Only a fairly small shift toward higher frequencies is observed, while the magnitudes of the moduli remain unchanged. We take this outcome as another confirmation that interchain bonding does not play a role in the relaxation behavior at high frequencies.

4. Discussion

4.1. Relaxation Behavior of Native Guar. One of the main findings of this study is that new insight into the origins of stress relaxation of guar gum solutions has been obtained after measuring the influence of enzymatic and solvent modifications. Below we shall discuss what are the implications of our experimental results for understanding the relaxation mechanisms at low (<0.03 Hz) and high (>0.03 Hz) frequencies.

Fast Relaxation Mechanism. The insensitivity of the relaxation behavior at high frequencies to the various modifications makes it clear that this behavior is not related to the breakup of weak physical bonds between polymer chains. As already argued in our previous investigation,[10] this leaves only one other conceivable origin for viscoelastic behavior in this frequency range, that is, topological constraints. Considering the possible relaxation mechanisms associated with such constraints, classical reptation by individual chains[21] was already ruled out, in view of the magnitude of the storage modulus and the scalings of several rheological quantities.[10] We therefore think that more complex conformational relaxations play a role involving multichain structures.

One finding which was previously taken as an indication that guar−guar hydrogen bridges might play a role was the quantitative time−temperature superposition at high frequencies that was obtained when using Arrhenius' law, with an activation energy of $10k_BT$ (for $T = 283$ K). Now the H-bonding hypothesis has been rejected, the question arises, what is the physical meaning of this apparent activation energy? We could imagine that the conformational relaxation time of large complex structures would be proportional to the viscosity of the solvent. Making an Arrhenius plot of the viscosity of water between 283 and 333 K, we found a good linearity and an apparent activation energy of $7k_BT$. As an alternative approach, we have also attempted a scaling of the frequency multiplication factors according to the WLF equation. Also in this case we found that the experimental data could be fitted quantitatively (i.e., within their error ranges) by the equation. However, in contrast to the Arrhenius scaling, the WLF scaling is based upon the concept of excluded volume. These findings illustrate that analyzing the temperature dependence of the characteristic relaxation time is not always a suitable tool for assessing the role of weak interchain bonds in the relaxation behavior.

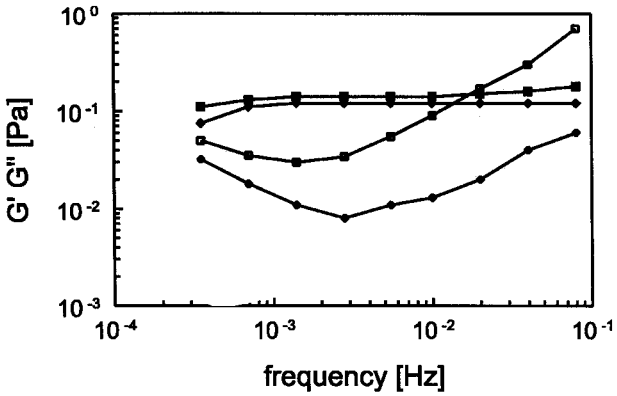

Figure 10. Onset of the relaxation transition at low frequencies for native guar solutions: G'', open symbols; G', filled symbols; □, 1% (w/w) solution of a guar with $M = 910$ kDa; ◇, 4% (w/w) solution of a guar with $M = 270$ kDa.

Perhaps if the accuracy of the TTS scaling factors would be very high and the temperature range sufficiently broad, it might be possible to make a distinction between the Arrhenius and WLF scalings. Unfortunately, for guar gum solutions the accessible temperature range is constrained by the freezing and boiling points of water.

Slow Relaxation Mechanism. It has in this study been corroborated that the relaxation behavior at low frequencies originates from interchain bonds. Both the enzymatic and the solvent modification experiments point in this direction. Decreasing the association tendency of the stickers via the solvent caused a lowering of the storage modulus at low frequencies. In the enzymatic modifications, the number and strength of the bonds are increased, resulting in a strong increase of the G' plateau beyond a certain M/G ratio. Whether the stress relaxation is directly caused by or indirectly and just *triggered* by the breakup of the bonds is a question which remains to be addressed. In any case, the relaxation mechanism must involve time scales of 10^3 s or longer. This can be seen from Figure 10, where the limiting relaxation behavior ($G' \propto \omega^2$ and $G'' \propto \omega$) is not yet reached at the lowest frequency of 3 mHz.

Multichain Structures. Some statements about the structure of native guar gum solutions can also be made, now the relaxation mechanisms have been clarified to a certain extent. Interchain bonding will inherently lead to multichain structures. The presence of such structures was already expected on rheological grounds: a storage modulus at low frequencies generally involves large length scales. Since the guar chains themselves are just linear and not too large (i.e., $R_g < 100$ nm in dilute solutions), the formation of (long-living) multichain structures is needed to obtain these large length

scales. Let us now consider the number of bonds per chain. Obviously, this quantity will be related to the number of stickers (sites available for bonding) per chain, which can be estimated on molecular grounds. Since most of the galactose side groups in guar are arranged in pairs and triplets,[22] a free backbone part will on average consist of two mannose units. Assuming that more than five neighboring free mannose units are needed to form a sticker, as was suggested in the literature,[9,23] a typical chain of 10^6 Da will contain 375 free backbone parts (i.e., mannose groups not bearing a galactose side group) but only a few (O(1−5)) stickers. Still, this is a high enough number to create multichain structures.

Formation of the Structures: Kinetics or Thermodynamic Equilibrium? The presence of large length scales in guar gum solutions raises the question as to what extent kinetics and equilibrium thermodynamics play a role in the structure formation. The answer is likely to depend on the considered length scale. Generally (i.e., following empiric), structures that are not in thermodynamic equilibrium will most probably be found at the largest length scales and the stress relaxations associated with these structures at the lowest frequencies of the measurements. Already in our previous study,[10] it was observed that the storage modulus plateau at low frequencies showed a much weaker dependence on the guar concentration than would be consistent with a thermodynamic equilibrium. Remarkably enough, the dependence of the moduli curves on *temperature* showed to be reversible.

Together these findings point in the direction of a *localized* thermodynamic equilibrium. In this picture, all breakup and bonding events are described with probabilities. In (nondistructive) linear experiments, the breakup chance of a bond depends solely on temperature. The probability for bonding depends not only on the temperature but also on the local environment of the stickers. This environment constrains each sticker to a certain volume (or domain), which makes that bonding can only take place in volumes that are shared by two or more stickers. The typical size of these domains is supposed to be of the order of a few Kuhn segment lengths. More precisely, their size (and hence their probability for overlap) will be determined by the aggregation kinetics in the beginning of the experiment. It is via this kinetics that the concentration dependence of the bonding probability enters the picture and also that the coupling between local and global structure is set. These concepts are also illustrated in Figure 11.

4.2. Tentative Model for Structure and Relaxations in Native Guar Solutions. In section 4.1, statements about the relaxation behavior were made at an abstract level. In our opinion, drawing more specific conclusions about the structure and relaxations in guar gum solutions is not feasible on the basis of the present results and the available literature. To obtain a focus for further research, we will here consider which detailed microscopic picture is the most probable on the basis of our findings.

4.2.1. Proposed Structure and Relaxations. We think that the presence of a few bonds (i.e., O(1−5) on average) per chain will lead to a variety of structures, which can be classified as follows: (i) linear structures involving one or a few bonded chains; (ii) branched (treelike) structures involving several chains; (iii) large aggregated structures involving many chains. These

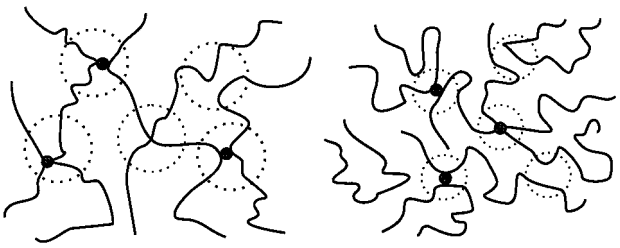

Figure 11. Impression of a localized thermodynamic equilibrium. The small filled circles represent interchain bonds. Large dotted circles represent domains shared by two stickers (i.e., where bonding can occur). The size and number density of these overlapping domains depend on concentration but also on the aggregation kinetics. As a result (and as illustrated by the left and right parts), the concentration dependence of the bond density may not be straightforward. Assuming that the domains remain intact when the temperature is changed, the temperature dependence of the bond density will be simply in accordance with a thermodynamic equilibrium.

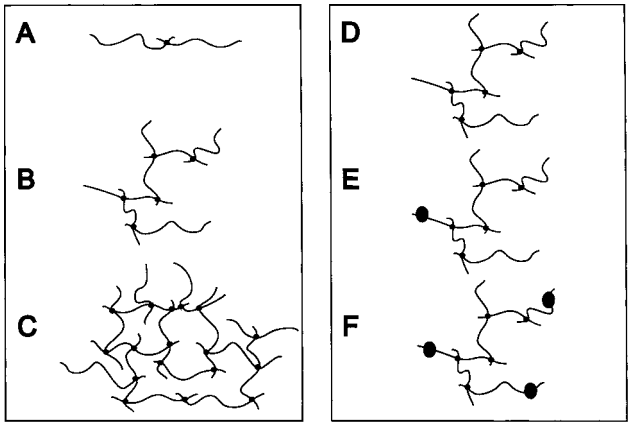

Figure 12. Impression of the different multichain structures that may occur in guar gum solution: A = linear structure, B = branched structure, C = large aggregated structure, D = free structure, E = structure connected at one point to a space-filling network, and F = structure connected at more points to the network.

structures may occur both as free units (Figure 12A−C) and as part of a space-filling network (Figure 12D−F).

Above the critical overlap concentration (where individual chains start to hinder each other), these structures will be intertwined with each other via entanglements. The rheological behavior will depend on the number density distribution of the different structures. Structures that are physically connected via bonds at two or more points to a space-filling network will contribute to this network. For the relaxation of the stress stored in this network, the breakup of bonds is needed. We assume that this will involve a long relaxation time. Structures that are free or only connected at one point to a space-filling network can in principle release their stress via conformational relaxation processes that bear a certain resemblance to the diffusion of polymer arms. The pertaining relaxation times can vary from short to long, depending on the structure size and complexity. For complex structures such as in Figure 12C, it is also possible that they will break up via the bonds before the diffusion process is completed. In that case the relaxation time is not determined by the diffusion time but by the bond breakup time.

This leaves for the low-frequency regime two possible origins for stress relaxation: (1) the breakup of bonds and (2) conformational relaxations of large aggregated

structures. In our opinion the latter process will not likely play a role. If this were to be the dominant relaxation mechanism, then the polydispersity of the aggregates should have given rise to a very broad low-frequency relaxation spectrum that would go over smoothly into the high-frequency spectrum. In contrast, our observations show that the relaxations at high and low frequencies are well separated in the frequency domain.[10] Therefore, we think that the stress relaxations at low frequencies are directly related to the breakup of network bonds.

4.2.2. Proposed Kinetics of Structure Formation. The formation of the complex network structure will be kinetically driven. We imagine that it is formed via (at least) two stages. Right after the guar gum solutions have been subjected to mechanical treatment, distinguishable entities ranging from free chains to small aggregated structures will be present in the solution. A small fraction of these structures will assemble into a space-filling primary (or skeleton) network if the number of potential bonds per chain is small. This will occur on a relatively short time scale, since it involves a more or less free (Brownian) diffusion of small entities. Once the primary network has been formed, the second stage sets in. Now the structures will experience a hindrance in their diffusion from their entanglements with others and from the network. As a consequence, further aggregation will only take place locally between structures that are confined to the same neighborhood. This limited aggregation will lead to a fragile network in which structures of different size (from single to multiple chains) are arrested via connecting bonds and/or topological constraints.

4.2.3. Qualitative Predictions for the Linear Rheological Behavior. *Magnitude of G′ at High Frequencies* (*>0.03 Hz*). In the proposed structure model (discussed above), the polymer solutions will be homogeneous down to the length scale of a few Kuhn segments. The G' associated with the conformational relaxation of multichain structures will be determined mainly by the entanglement concentration, provided that the typical distance between two entanglements is small compared to the size of the diffusing structures. The entanglement concentration is independent of molecular weight and temperature but increases with polymer concentration. In our experiments[10] G' at high frequencies showed qualitatively the same dependences.

Relaxation Time at High Frequencies. The conformational relaxations of the multichain structures will bear a certain resemblance to arm retraction (or starlike reptation) mechanisms. But it will be more complicated in our case, since branched structures are now involved instead of just linear arms. Moreover, there will be distributions for the sizes and shapes of the multichain structures. In any case, the relaxation spectrum can be predicted to be very broad. Indeed, this was observed in our experiments. The temperature dependence of the characteristic relaxation time can be fairly well described by assuming a proportionality to the solvent viscosity. In this approach, thermally induced structural changes are neglected, which might be a simplification at short length scales. The dependences of the typical relaxation time on concentration and molecular weight cannot be predicted. This is because the relaxation time is determined not only by the entanglement concentration but also by the unknown characteristics (size, shape, branching) of the multichain structures.

Magnitude of G′ at Low Frequencies (*<0.03 Hz*). The magnitude of this modulus is determined by the number density of network strands, i.e., polymer strands connected to the space-filling network via at least two points. This number density is hard to predict, since especially at the large length scales probed by the low frequencies, the structure will be determined by kinetics rather than by thermodynamic equilibrium. On changing the concentration or the molecular weight the kinetics will become different but in an unknown way. The temperature dependence of the G' is less difficult to predict, since for this we only need to consider localized equilibria (see section 4.1). Adopting the suggestion that the hydrophobicity of free mannose backbone parts increases with temperature,[13,14] the number of stickers will also increase with temperature, since also a fraction of the smaller free backbone parts (fewer than six mannose units) will start to act as stickers. Thus, a network hardening can occur without the need for Brownian diffusion. The pertaining increase in G' corresponds well to our observations.

Relaxation Time at Low Frequencies. This quantity was outside the range of our measurements. Some qualitative predictions concerning the dependences on experimental variables are possible. In a fragile network, cooperative bonding effects will play a minor role, and therefore the relaxation time will be determined by the breakup time of bonds directly. Obviously, a relaxation time associated with such a highly local process will not depend on the molecular weight or the concentration.

From the above discussion it becomes clear that qualitative predictions for the linear rheological behavior as a function of experimental variables are possible in a number of cases. Also, there are dependences that cannot be predicted due to insufficient knowledge about the structures and kinetics. At least all qualitative statements that can be made on the basis of our tentative picture are consistent with our experimental observations.

4.3. Behavior of Modified Guar Gels. 4.3.1. Tentative Mechanisms of Gelation and Melting. *Gelation*. For gel formation of modified guar gum, many stickers per chain are needed. Then Brownian diffusion of chain segments over appreciable distances (which is hindered by the presence of the initial network) is no longer needed for bond formation, and many bonds per chain can be formed which will result in a strong network. During the gel formation the network will become more compact, thereby causing the observed syneresis.

Influence of Freeze−Thawing on Gelation. In the range between 40 and 50% galactose removal, a gel is only formed after the sample has been frozen. The explanation for this may be provided by the temperature dependence of the bonds: During the freezing step, the temperature decreases, causing fewer and weaker stickers and therefore fewer bonds, in line with our tentative model of section 4.2. Therefore, Brownian diffusion of structures through the solution becomes easier, and the mesoscopic structure evolves more toward a thermodynamic equilibrium which means that the network becomes more dense. Reaching a true equilibrium state is hindered because of excluded volume. In the thawing step, the temperature is increased again and bonds will form again. From the more dense starting state, more interchain bonds are formed, and this can cause the observed gel formation.

Melting Behavior. When the temperature is increased further, microphase separation may occur: Then even more stickers are placed on the chain, causing extra bond formation. This will cause local syneresis, and forces will start to act on the interchain bonds leading to their breakup. This breakup causes the observed decrease of the gel stiffness with increasing temperature (the melting behavior).

From the discussion above it shows that with the same concepts that were used to model the linear viscoelasticity of native guar gum solutions (increase of hydrophobicity with increasing temperature and kinetically determined mesoscopic structures) also the behavior of modified guar gum gels can be rationalized.

4.3.2. Comparison with Locust Bean Gum Gels. The finding that "guar" chains at (average) modification degrees between 40 and 50% can form a gel, but only after a freeze−thawing cycle, is remarkable and not easy to understand. Yet this behavior is comparable with that of LBG, which also forms gels after a freeze−thaw cycle.[2,3] Compared to modified guar gum, LBG (with a M/G ratio of 3.9) lies clearly beyond the M/G limit of 3.27 (50% galactose removal) where guar gum forms a gel without freeze−thawing cycle. So in that sense, modified guar gum forms a gel more easily. This might be caused by the difference in molecular weight (LBG has a molecular weight of 370 kDa, whereas modified guar gum still has a molecular weight of about 730 kDa). The lower molecular weight for LBG will lead to fewer bonds per chain which makes gel formation more difficult. Another reason might be a possible difference in the fine structure of guar gum and locust bean gum (the length distribution of adjacent bare backbone units). This will also influence the number of hydrophobic stickers per chain and therefore the number of bonds per chain.

Besides this difference, also the temperature dependence of the elasticity of locust bean gum gels differs from that of modified guar gum gels: For locust bean gum it has been reported[3] that gels start to melt above 317 K, whereas for modified guar gum we have observed that the melting starts at 288 K. The melting itself might be caused by local phase separation as explained above, but it is not a priori clear what causes the difference in melting temperature. Again, differences in the free backbone length distribution as well as in molecular weight might play a role.

4.4. Recommendations for Further Research. From the previous sections it has become clear that although more light has been shed on the relaxation mechanisms in (modified) guar gum solutions, a further clarification remains desirable. Both theoretical modeling and well-chosen additional experiments could contribute to this. As yet, no microrheological models exist for aggregated structures held together via both long-living bonds and entanglements (i.e., excluded volume). Rubinstein and Semenov[24,25] presented in 1998 a model in which the rheological behavior of (kinetically formed) polymer aggregates is predicted. Bonds were taken into account, but excluded-volume effects were left out for simplicity reasons. They announced that in future work also the latter would be incorporated.

New experiments could also shed more light on the structure and relaxations. By investigating the molecular fine structure of the guar gum[20,22,26] after treatment with α-D-galactosidase, instead of just measuring the overall degree of modification, the nature and number of the stickers could become more clear. This could provide a more solid basis for interpreting the formation, syneresis, and melting behavior of the gels. Studying the structure at the mesoscopic length scales of the chain aggregates could shed more light on the kinetics of aggregation and on the relaxation mechanism at low frequencies. Given the sensitivity of the rheological behavior at low frequencies to mechanical treatment, we think that useful comparisons between samples in the rheological and structural studies will only be possible if the preparation is done following the very same protocol. The utility of small-angle scattering techniques for resolving the mesoscopic structure was shown by Gittings et al.,[27] who performed USALS and SANS experiments on guar gum solutions. Their observations were found to be in accordance with large fractal aggregates (sizes up to 100 μm), with no strong positional correlations between the aggregates. Some chains and aggregates were supposed to exist freely in solution, whereas loosely connected aggregates were also presumed present.

The attribution of the slow relaxation process directly to the breakup of bonds could be corroborated by measuring the concentration and molecular weight dependences of the slow relaxation time. This time could not be measured in the present study, but considering Figure 10 it should be accessible to other instruments like a sensitive creep viscometer. An extension of the frequency range might also allow a comparison with the work of Valentine et al.,[28] who noted that (after transformation of their data over many decades into retardation spectra) close to the gel point very prominent long-time retardation processes were found, which may be attributable to large structures loosely attached to the network.

5. Conclusions

The following can be concluded for the linear rheology of guar gum and enzymatic modifications thereof:

1. Long-living physical bonds are responsible for the rheological behavior at low frequencies. These bonds probably have a hydrophobic origin since the gel stiffness increases with increasing M/G ratio.

2. Weak physical bonds do not cause the rheological behavior at high frequencies. Instead, a conformational relaxation resembling the diffusion of polymer arms plays a role. Multichain structures must be involved in this relaxation, but their precise nature remains unknown.

3. The thermal history dependence of the samples that form gels proves that at least for these samples a thermodynamic equilibrium is not reached.

4. Combining the various observations, a tentative picture for the structure and relaxations could be constructed. This picture is complex but consistent. So far, no quantitative model is able to predict the observed rheological behavior.

5. Via enzymatic modifications, it is possible to induce gel formation. The formed gels show a melting trajectory which starts at 288 K.

Acknowledgment. We thank Prof. Dr. Wim Agterof and Dr. Rob Vreeker of Unilever Research Laboratories Vlaardingen for many stimulating discussions. The work described in this paper is part of the research program of the foundation for Fundamental Research

on Matter (FOM), which is financially supported by the Netherlands Organisation of Scientific Research (NWO).

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MA0103019