Thermoreversible Gels of Hydrophobically Modified Hydroxyethyl Cellulose Cross-Linked by Amylose

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ABSTRACT: Aqueous mixtures of hydrophobically modified hydroxyethyl cellulose (HMHEC) or unmodified hydroxyethyl cellulose (HEC), with amylose (AM) or potato amylopectin (PAP), have been investigated by rheology, turbidity, and ^{1}H NMR measurements. It is concluded that the AM molecules cross-link HMHEC chains by forming helical inclusion complexes with the hydrophobic side chains of HMHEC, leading to thermoreversible, elastic, and thixotropic gels at ambient temperatures. The complexation ability, the setting−melting temperature of the AM−HMHEC complexes, the strength of the association, and the viscoelastic behavior of the gels vary with the polymer concentrations, the pH, and the preparation method. AM−HMHEC complexes were obtained at a pH range of 7−12.5, but a maximum storage modulus was observed at pH ≈ 10. The AM−HMHEC complexation is modified or completely abolished by the addition of surfactant. A weak synergistic interaction was found for mixtures of PAP with HMHEC. No complexation was observed between HEC and either AM or PAP.

Introduction

Amylose (AM) is the essentially unbranched starch fraction consisting almost exclusively of α-D-(1−4) glycosidic bonds. The complex formation between AM and a large number of low-molecular-weight amphiphilic ligands such as surfactants, lipids, and emulsifiers is well documented.[1−4] It is well established that the complexes involve AM helices, with the hydrocarbon chains of the guest molecules inserted in the central cavities of the helices.

The complexation ability varies between different ligands and is related to the hydrophobicity, the steric hindrance, and the length of the hydrocarbon chain of the ligand molecule. It is reported to be highest for saturated monoglycerides, whereas it is very low for lipids with two hydrocarbon chains (like lecithin) and zero for triglycerides.[2,5,6] To some extent, the AM helix can expand or contract as necessary to accommodate the guest molecule, thus forming helices of different diameters.[7−9] Complexes with polar lipids involve single helices with six glucose molecules per turn and an inner diameter of approximately 4.5 Å.[10] When the ligand is bulky or branched, seven or even eight glucosyl residues might be required for one single turn of the helix,[11−13] and the inside diameter of the helix can vary between 4.5 and 6.0 Å.[14] The stoichiometry of the amylose−lipid complex has been shown to vary with the chain lengths of both the AM and the lipid components. In an investigation of complexes of fatty acids with AM samples of different degrees of polymerization, a maximum incorporation, corresponding to nearly 0.5 methylene groups per glucose unit, was observed for the longest fatty acid chain (C_{16}) and the highest degrees of polymerization (DP80 and DP900) of AM of the combinations tested.[15]

Regarding the branched starch fraction, amylopectin, the existence of a lipid complex during starch gelatinization is not fully established. However, a limited amount of complexation, possibly involving the outer branches of the amylopectin molecule, has been suggested from indirect evidence.[16−18]

In contrast to the numerous studies of complexes with low-molecular-weight hydrophobic molecules, little attention has been paid to the possible complexation between AM and so-called hydrophobically modified polymers. The latter are polymers containing short hydrophobic chains attached either as end-caps or as grafted side chains along the water-soluble polymer backbone. Hydrophobically modified polymers have found widespread use as thickening compounds and rheology modifiers in technical formulations.[19] Association phenomena involving hydrophobically modified water-soluble polymers and surfactants are well-known and have been extensively studied over the past decade.[20,21] Surfactant molecules influence the cross-linking between different polymer chains due to the formation of mixed micelles composed of polymer hydrophobic side chains and surfactant molecules.[22] A few previous studies of interactions between hydrophobically modified polymers and starch or starch components have been performed. Okaya et al.[23] studied the viscosity, stability, and transparency of aqueous mixtures of starches with hydrophobically modified poly(vinyl alcohol)s and found effects indicating a complexation. Recently, Gruber and Konish[24] found that combinations of a cationic hydrophobically modified hydroxyethyl cellulose (cat-HMHEC) and AM dissolved together in water at high temperature and carefully cooled, afford increased solution viscosity over either polymer alone. They proposed that the most probable mechanism of interaction was the formation of a helical, cross-linking clathrate between the AM and hydrophobic groups.

In the present investigation we find a similar formation of helical inclusion complexes and a consequent gelation in mixtures of AM with a nonionic hydrophobically modified cellulose derivative. Oscillatory, shear flow, turbidity, and ^{1}H NMR measurements were applied to investigate the molecular events of this com-

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plexation and the viscoelastic properties of the gels. To investigate whether other ligands can compete with the AM−HMHEC complexation, we studied the effects of adding the surfactant sodium dodecyl sulfate (SDS). We also compared the AM−HMHEC mixtures with mixtures between AM and nonmodified HEC and with mixtures of HMHEC or HEC with amylopectin.

Experimental Section

Materials. Potato amylose (AM) with a molecular weight of about 800 000 g/mol was obtained from Sigma Chemical Co. Prior to use, the AM was placed in an oven at 80 °C for 1 h to remove solvent impurities such as butanol.

HMHEC with the commercial name Natrosol Plus grade 331 was obtained from Aqualon. According to the manufacturer, HMHEC has a molar weight of 250 000 g/mol. It carries grafted C_{16} alkyl chains corresponding to a modification degree of 1.7 mol %, based on anhydroglucose units in the cellulose backbone, and the degree of hydroxyethyl group substitution is 3.3 mol %.[25] Prior to use, HMHEC was dissolved in water to a concentration of 1% w/w. Low molecular impurities, such as salt, were removed by dialysis against Millipore water in a Filtron Ultrasette device. The dialysis was performed until the expelled water showed a conductivity of less than 2 μS/cm. After freeze-drying, the polymer was stored in a desiccator. In the present investigation, the HMHEC concentration was 1% w/w or higher, which is well above the critical overlap concentration (ca. 0.2% w/w).

Hydroxyethyl cellulose (HEC) with a molecular weight of about 250 000 g/mol was also obtained from Aqualon. Prior to use, HEC was treated in the same manner as HMHEC.

High amylopectin potato starch (PAP) was provided by Lyckeby Stärkelsen, Sweden. The PAP contains less than 1% AM. It was used as received.

SDS, specially pure, was purchased from BDH Chemicals and used as received. The cmc of SDS is 8.3 mM in pure water.[26]

Sample Preparation. All samples were mixed under conditions where the AM component was dissolved in its denatured (as opposed to helical) conformation. This was accomplished by two different routes of sample preparation. Polymer concentrations are given as weight percent (wt %) throughout.

Alkali Preparation. Mixtures of AM and HMHEC were prepared by adding 0.1 M KOH to dry mixtures of AM and HMHEC. SDS was included in the dry mixtures for the surfactant-containing samples. The samples were stirred overnight. To obtain the desired pH (ca. 7, unless otherwise specified), 0.1 M HCl was added. Finally, the neutral samples were heated until 80 °C for 30 min and cooled. Samples deviating from neutral pH were not heated due to possibilities of bond breakage. All samples were slightly turbid. The turbidity increased slightly with increased levels of AM. All results presented in the paper, including the PAP−HMHEC mixtures, are on samples prepared in this way, unless otherwise specified.

Hot Mixing. Appropriate stock solutions of AM were heated for 1 h at 130 °C (unless otherwise specified), cooled to approximately 90 °C, and finally added to heated (ca.70 °C) solutions of HMHEC. For some sample series the polymers were mixed dry, water was added, and the mixture was then heated to 130 °C.

All samples were centrifuged gently to remove air bubbles. None of the above preparations phase separated macroscopically even after extensive centrifugation.

Rheometry and Viscometry. Rheological measurements under low-amplitude oscillatory shear were performed on a controlled stress Carri-Med CSL100 rheometer (TA Instruments, UK) using a parallel plate geometry (40 mm radius; 800 μm separation). All measurements were performed with 0.5% strain since strain sweeps on selected gels demonstrated that the working deformation was well within the linear viscoelastic region. Each sample was loaded as a hot solution on the platen of the rheometer preset at 85 °C, except for the sample series containing 1% HMHEC and 0.225% amylose at pH 8−13. For the latter series, the samples were quickly heated to 85 °C on the rheometer, to minimize the risk for degradation. The sample periphery was coated with silicone oil to minimize loss of solvent or adsorption of atmospheric moisture. The rheological parameters at a frequency of 1 Hz were monitored on cooling at 2 °C/min to 25 °C. Cooling scans were followed by a 30 min isothermal run and a frequency sweep between 0.01 and 10 Hz (about another 30 min). The rheological routine was completed with a heating run at a frequency of 1 Hz from 25 to 85 °C (2 °C/min). The above sequence of experimental procedures allowed recording of the storage modulus (G'), the loss modulus (G''), tan δ ($= G''/G'$), and the complex viscosity ($\eta^* = (G'^2 + G''^2)^{1/2}/\omega$) as functions of time, temperature, and frequency of oscillation. All samples were then subjected to flow measurements, and the viscosity (η) was recorded as a function of shear rate at 25 °C.

NMR Spectroscopy. Proton NMR measurements were performed on a spectrometer (model ARX500, Bruker Fallanden, Switzerland) operating at 500.25 MHz. Spectra were accumulated at 27 and 80 °C. Integration of the peak areas was performed using Bruker UXNMR software. The mixtures were prepared by hot mixing, but with D_2O as the solvent.

Results

Viscosity of AM−HMHEC Composites. Additions of comparatively small amounts of AM to semidilute solutions of HMHEC resulted in the formation of high-viscosity composites, whereas no viscosity increase was found for mixtures with the nonmodified HEC. These results are consistent with the notion of a complexation between the polymer hydrophobes and AM. Figure 1a,b illustrates some typical flow curves of 1 and 2% HMHEC containing AM at various concentrations. Reference flow curves for an AM−HEC mixture and for a solution of HM−HEC alone are also included.

A region of shear-rate-independent viscosity could not be attained experimentally for AM−HMHEC mixtures containing more than ca. 0.05% AM. The latter mixtures showed a strong shear rate dependence of the viscosity and a pronounced shear thinning behavior (Figure 1). These features suggest that the time scale for the AM−HMHEC association is relatively long. Moreover, the shear rate dependence of the apparent viscosity of the AM−HMHEC composites was reversible only for very low AM concentrations (up to 0.04%). All the other AM−HMHEC composites exhibited thixotropy, that is, a lower apparent viscosity on lowering the shear rate as compared with the results obtained on increasing the shear rate. The original low shear viscosities were attained, however, after the shear force was removed. Consequently, there exist junctions in the AM−HMHEC mixtures that break and re-form reversibly, and relatively slowly, on shearing the mixtures. As expected, the mixtures with 2% HMHEC showed higher viscosities than the 1% mixtures.

Figure 2 shows the concentration dependence of shear viscosity at a shear rate of 0.05 s^{-1} and the magnitude of the shear hysteresis for the 1% AM−HMHEC composites. Addition of AM leads to a progressive increase of η up to a maximum viscosity slightly above 0.2% of AM, followed by a progressive decrease. Similar maxima were found for the elastic moduli (see below). Gruber and Konish also found viscosity maxima for the AM−catHMHEC complexes.[24]

Viscoelastic Behavior. Examination of the rheological response under low deformations was also performed, and representative frequency dependencies for

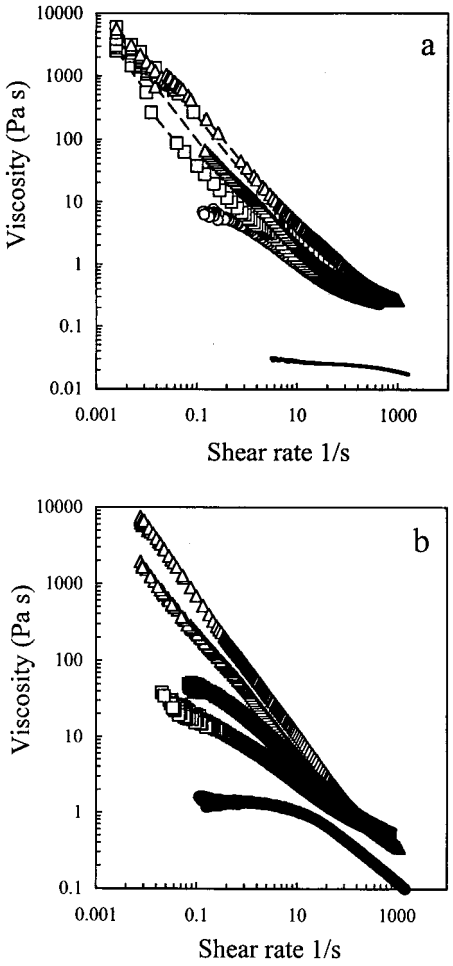

Figure 1. (a) Flow curves (25 °C) for 1% HMHEC with 0.04% (○), 0.15% (□), and 0.225% (△) AM and for 1% HEC with 0.16% AM (—). (b) Flow curves for 2% HMHEC with 0% (○), 0.067% (□), and 0.277% (△) AM.

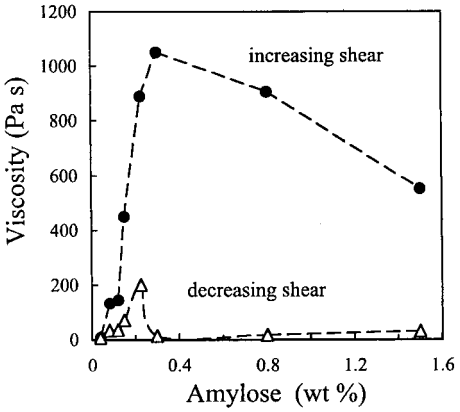

Figure 2. Variation in the viscosities at 0.05 s^{-1} (25 °C) for 1% HMHEC as a function of AM concentration. Increasing shear (●) and decreasing shear (△).

AM−HMHEC composites at 25 °C are presented in Figure 3. The mechanical spectra of the (1 or 2%) AM−HMHEC composites are typical of gels, even at low amounts of added AM: The frequency dependence of G' is quite weak, and G' dominates over G''. The log G' vs log ω plots in Figure 3 give nearly straight lines over the accessible frequency window (3 decades), thus following the commonly observed relation $G' \propto \omega^n$.[27−29] At 25 °C the values of the exponent n are fairly low, in the range 0.049−0.080 for the mixtures of AM with 1%

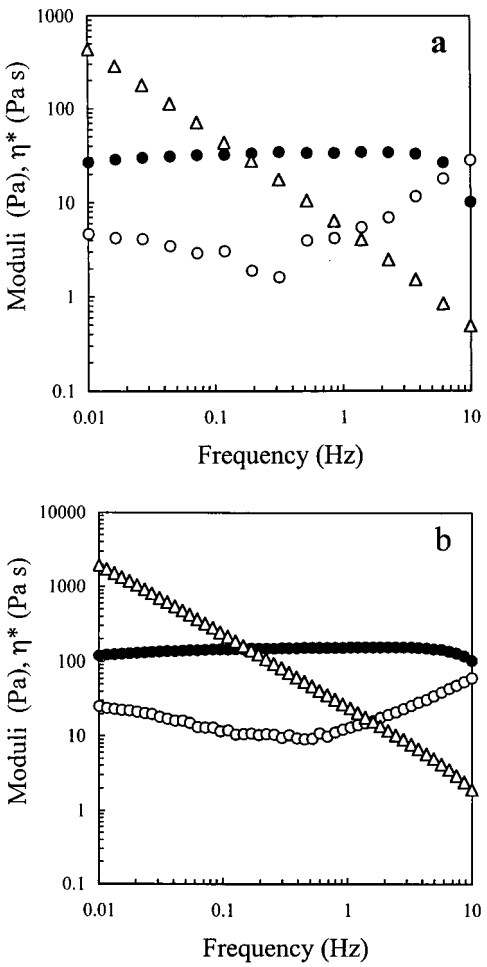

Figure 3. Frequency dependencies of G' (●), G'' (○), and η^* (△) obtained from oscillatory rheological measurements, for (a) 1% HMHEC with 0.225% AM and (b) 2% HMHEC with 0.5% AM (25 °C, 0.5% strain).

HMHEC. As regards the complex dynamic viscosity, the dependence of log η^* on log ω is essentially linear, with no indication of a leveling off to a horizontal plateau at low frequencies and with slopes in the range −0.85 to −0.95 at sufficiently high concentrations of AM (above ca. 0.2 wt %). This is close to the limiting frequency dependence of viscosity for true gels (−1) and steeper than the maximum value of −0.76 observed for random coil polysaccharides interacting only by topological entanglements.[27] Clearly, the AM−HMHEC association results in gels similar to conventional noncovalent polysaccharide gels where networks with strong physical cross-links are formed.

The shear loss modulus of the composites was quite frequency dependent. At low frequencies, G'' decreased or remained almost constant, and then it increased progressively with increasing frequency of oscillation. This behavior was seen for all AM concentrations, but the G'' minimum moved to lower frequencies as the concentration of AM was increased. The minimum in G'' was at much lower frequencies beyond 1.5% of AM, e.g., at ca. 0.03 Hz for 2 and 2.5% AM with 1% HMHEC (data not shown). A similar minimum in the loss modulus is a characteristic of gelatin but is absent in most other thermoreversible biopolymer gels (e.g., agarose).[28] It is assumed to be associated with relaxation processes occurring over much longer time scales and

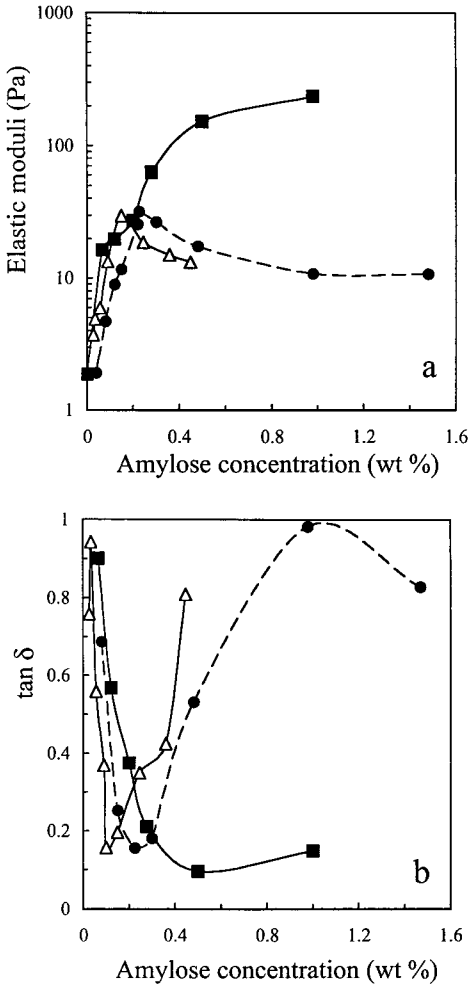

Figure 4. Low deformation rheology of AM−HMHEC mixtures at 25 °C as a function of AM concentration: (a) G' at 1 Hz, 0.5% strain; (b) tan δ at 1.34 Hz. Different symbols represent 1% HMHEC, alkali prepared mixtures (●); 1% HMHEC, hot mixed samples (△); and 2% HMHEC, alkali prepared mixtures (■).

implies is that at longer times the sample must show macroscopic flow.[28,29]

Effects of Varying the Polymer Concentrations. The effect of the composition of the AM−HMHEC mixtures on the elastic modulus, measured at the end of the 30 min cooling routine, is demonstrated in Figure 4a. Measurements were performed for two fixed HMHEC concentrations (1 or 2%) with varying amounts of AM. For samples with 1% HMHEC, the variation of G' with added AM was nonmonotonic, with a maximum at 0.225% AM followed by a minimum at 1.5% AM. These results parallel the nonmonotonic viscosities found in the flow experiments (cf. Figure 2). Further addition of AM resulted in a progressive increase in the elastic modulus (data not shown). The latter increase may most probably be ascribed to an increasing contribution from excess AM, since samples containing AM alone form gels at sufficiently high concentrations.

Also included in Figure 4a are results for mixtures containing 1% HMHEC prepared by hot mixing. Again, a maximum in G' was observed, but at lower AM concentrations (0.15%) than for the corresponding alkali prepared mixtures with 1% HMHEC. The G' value at the maximum was, however, almost the same for the two series of samples. The general appearance of the frequency dependence of the moduli was also the same for the hot mixed samples (not shown) as for the alkali prepared samples (shown in Figure 3). Essentially, the difference between the two sample series was that all data were shifted toward lower AM concentrations for the hot mixed samples. A possible reason for the difference could be that the alkali dissolution procedure results in some degradation of the polymers. To check whether the salt present as a result of the alkali preparation and reneutralization had any effect on the elastic modulus, some samples were prepared by hot mixing in the presence of 0.1 M NaCl. No differences in G' were observed for hot mixed samples with and without added NaCl.

Composites with 2% HMHEC were also investigated as illustrated in Figure 4a. Two observations are noteworthy. First, at low AM concentrations, G' increased more steeply than for systems containing 1% HMHEC. Second, a monotonic increase in G' for the mixtures was found up to the highest concentration (1%) of added AM. At high AM concentrations, the elastic modulus was much higher (by up to more than 1 order of magnitude) for the 2% HMHEC mixtures.

Visual inspection revealed that self-supporting gels developed in all the above mixtures at AM concentrations above 0.08%. However, the turbidity of the gels depended on the AM/HMHEC ratio. To check for possible phase separation, phenomena selected samples were centrifuged extensively and examined for the development of turbidity. None of the above preparations phase separated macroscopically even after extensive centrifugation. Spectrophotometric measurements of the turbidity (not shown) of the samples containing 1% HMHEC showed a smooth increase over the entire range of AM concentration (up to 2%), giving no indication that the drop in modulus might be caused by the onset of a phase separation. It is notable that, although amylose−ligand complexes usually precipitate from solution shortly after their formation,[30−32] this was not observed for the AM−HMHEC composites. No gelation was observed for mixtures of nonmodified HEC and AM.

The effect of the compositional variation on tan δ, measured at a single frequency (of 1.34 Hz) at the end of the 30 min cooling run, is demonstrated in Figure 4b. The graph mirrors the behavior of Figure 4a and reflects the same tendencies: Generally, tan δ was high at low AM contents and decreased progressively. This response again implies the formation of weak gellike networks at low AM and a development of elastic gels at a higher content of AM. For the series containing 1% HMHEC, minima in tan δ appeared at the compositions corresponding to maxima in the elastic modulus, whereas the mixtures with 2% HMHEC showed a leveling off of tan δ above ca. 0.5% AM.

The difference between the 1% and 2% HMHEC series (nonmonotonic vs monotonic dependence on AM concentration) is surprising. One conceivable explanation would be a development of syneresis, causing slippage in the measurements, for the less concentrated systems at AM concentrations above the observed G' maximum. This explanation was considered unlikely, since no signs of an onset of syneresis were seen for any of the samples during cooling experiments (cf. below). Nevertheless, to check for the possibility of slippage, we made additional measurements on hot mixed samples containing 1% HMHEC using a set of crosshatched platens. Moreover, these samples had been mixed at a higher temperature

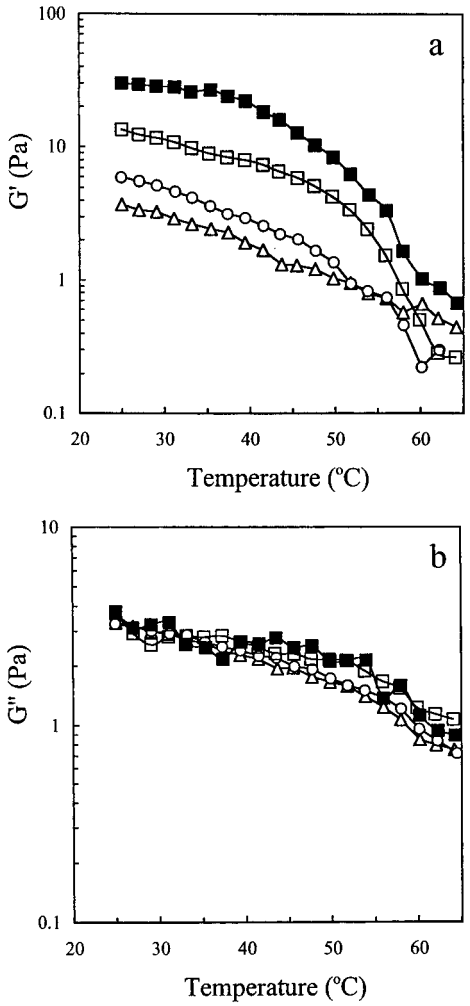

Figure 5. Temperature dependences of the (a) storage and (b) loss moduli for 1% HMHEC with 0.225% (■), 0.15% (□), 0.12% (○), and 0.08% (△) AM. The data displayed were obtained on heating; data from cooling runs were not significantly different.

(155 °C), which was found to yield more homogeneous (less turbid) samples. Since the G' maximum appeared also in these measurements, we conclude that it is a real effect, reflecting bulk properties of the resulting gels.

Thermoreversibility of the AM−HMHEC Gels. Figure 5 shows typical temperature dependencies of the shear storage and loss moduli for the mixtures of 1% HMHEC with different AM concentrations. Within the experimental uncertainty, identical results were obtained in cooling and heating runs. While the loss moduli were fairly insensitive to either temperature or AM content, the storage moduli started to deviate from the baseline at temperatures lower than ∼60 °C, independent of the AM concentration. This temperature was taken as the gel melting or gelation onset temperature, depending on the direction of the temperature change. Composites containing 2% HMHEC (Figure 6) gave results similar to those for the 1% HMHEC mixtures, except that the former samples showed significant thermal hysteresis. The gel melting temperature occurred at 65−70 °C, while the gelation onset temperature remained around 60 °C (or slightly above). For both the 1 and 2% HMHEC gels the moduli traces did not change with time after cooling (data not shown).

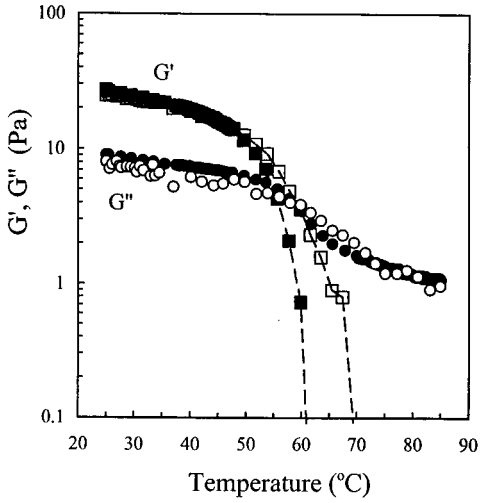

Figure 6. Temperature dependence of the storage and loss moduli of 2% HMHEC with 0.2% of AM; cooling route filled symbols and heating route open symbols (2 deg/min, 1 Hz, 0.5% strain).

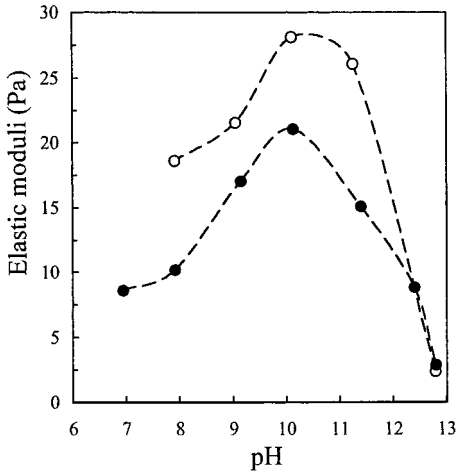

Figure 7. pH dependence of G' for 1% HMHEC with 0.15% (●) or 0.225% (○) AM (25 °C, 1 Hz, 0.5% strain).

No gelation was observed for mixtures of nonmodified HEC and AM.

Effect of pH. A series of samples were taken to different pH values by addition of appropriate amounts of HCl and water after alkali swelling (Figure 7). Acidic mixtures were found to undergo rapid hydrolysis upon heating; hence, only data for neutral and alkaline pH values are included in Figure 7. (Recall that all samples were taken to 85 °C on the rheometer; cf. the Experimental Section.) A maximum in the storage modulus was found around pH 10, followed by a rapid decrease and a final loss of a measurable modulus at very high pH. The latter drop is expected since alkali denatures the helical conformation of AM, which assumes an expanded random coil conformation at high pH.[33] The denaturation of the helix is due to a deprotonation of the hydroxyl groups on AM at high pH; cf. the pK_a of maltose, which is reported to be 11.94 at 25 °C.[34] The reason for the maximum at pH 10 is not clear, but it is possible that residual deprotonated hydroxyl groups on AM and HMHEC serve to make the mixture of the (charged) polysaccharides more homogeneous, which could lead to a more efficient cross-linking. With three hydroxyl groups per sugar unit and a pK_a of 12, one obtains three charged groups per 100 sugar units at pH

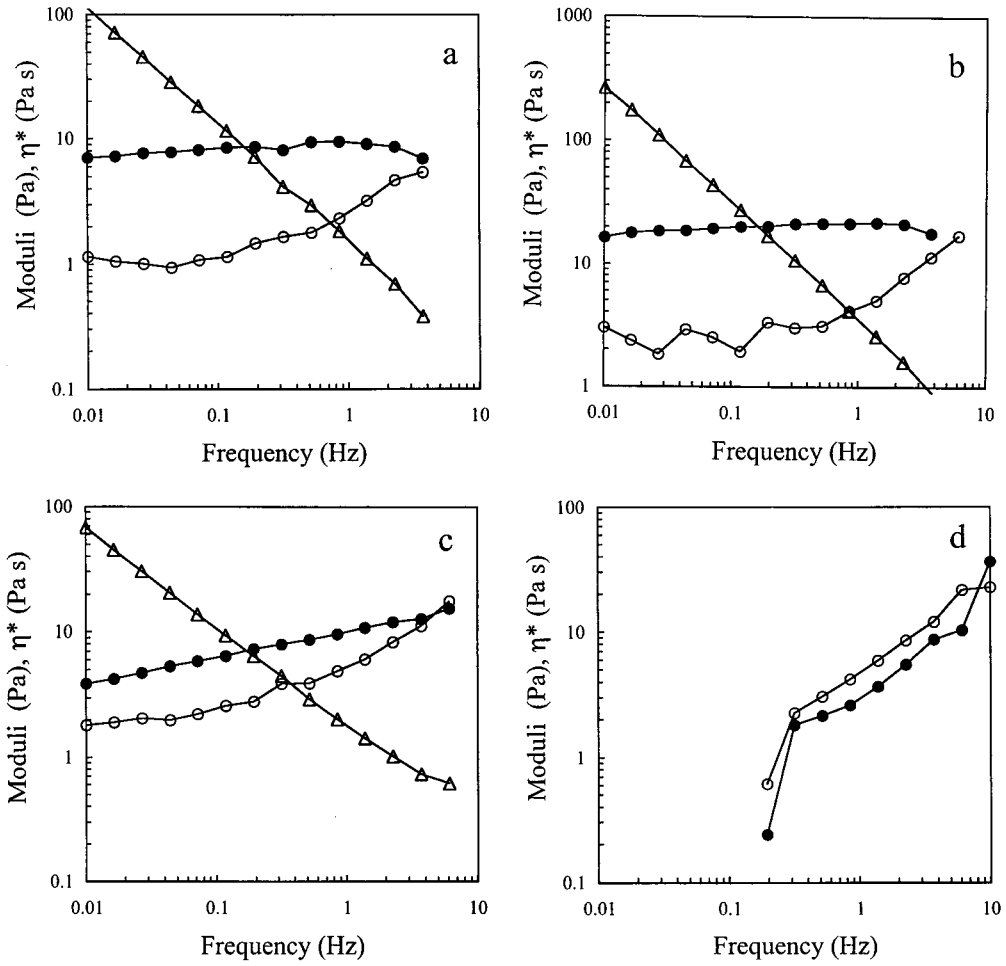

Figure 8. Frequency dependencies of G' (●), G'' (○), and η^* (△) obtained from oscillatory rheological measurements, for 1% HM−HEC with 0.15% AM at different pHs. (a) pH 6.95, (b) pH 10.12, (c) pH 12.39, and (d) 12.7 (25 °C, 0.5% strain).

10. It should also be remembered that the onset of cross-linking occurs at high temperatures (around 60 °C), where the pK_a should be significantly lower than at 25 °C.[34]

Mechanical properties of representative AM−HM-HEC mixtures at different pH values are shown in Figure 8. In the pH range 7−11 a gellike behavior was observed, and the moduli show the frequency dependences reported in Figure 3. At higher pH (e.g., at pH 12.34) a weak gellike behavior appeared, while at pH 12.7 a solution-like response was obtained.

NMR of AM−HMHEC Complexes. In an attempt to gain molecular information on the presence of inclusion complexes, several samples containing 1% HMHEC and varying amounts (0−0.15%) of AM were investigated by proton NMR, both at 80 °C (above the melting temperature of the mixed gels) and at 27 °C. The proton specta of AM−HMHEC mixtures in D_2O were found to contain well-resolved peaks from H-1 of AM, H-1 of HMHEC, and the methylene and methyl protons of the HMHEC hydrophobes (Figure 9). Unfortunately, the AM sample also contained a low-molecular-weight impurity giving overlapping methyl and methylene signals. The NMR spectrum identifies the impurity as butanol, which apparently was not totally removed during the heat treatment of the dry amylose prior to dissolution (cf. Experimental Section).

A previous study by Bulpin et al.[35] has shown that the amylose H-1 signal is broadened, resulting in an intensity loss, when inclusion complexes are formed with fatty acids. Accordingly, we studied the relative intensities of the various peaks from our samples at high and low temperatures and at increasing AM content. The anomeric signal from HMHEC, which is not affected by added AM, was found to be a convenient internal reference for such intensity measurements. For HMHEC alone, the relative intensities of the hydrophobe peaks were independent of temperature. By contrast, in mixtures with AM, both the hydrophobe peaks and the amylose H-1 peak were significantly broadened and had reduced intensities at 27 °C, as compared to 80 °C. This is clearly consistent with an inclusion complexation involving hydrophobes and AM.

Unfortunately, the quantitative information from the spectra was disturbed by the peaks from the butanol impurity. However, their contributions could be subtracted from the measured intensities by the following procedure. The signal from the C-3 methylene protons at 1.58 ppm in butanol did not overlap with any of the HMHEC hydrophobe peaks, and accordingly, its intensity was found to be proportional to the amylose content. Since the intensity of the butanol C-3 methylene signal at 1.58 ppm must be equal to the intensity of the butanol C-2 methylene signal at 1.40 ppm, the contribution from the latter signal to the total methylene peak at 1.4 ppm could be estimated and subtracted. A similar subtraction was done for the methyl peak, taking into account that the intensity of the methyl signal in butanol is $^3/_2$ times higher than the intensity of a methylene signal. The results are shown in Table 1, which clearly shows that

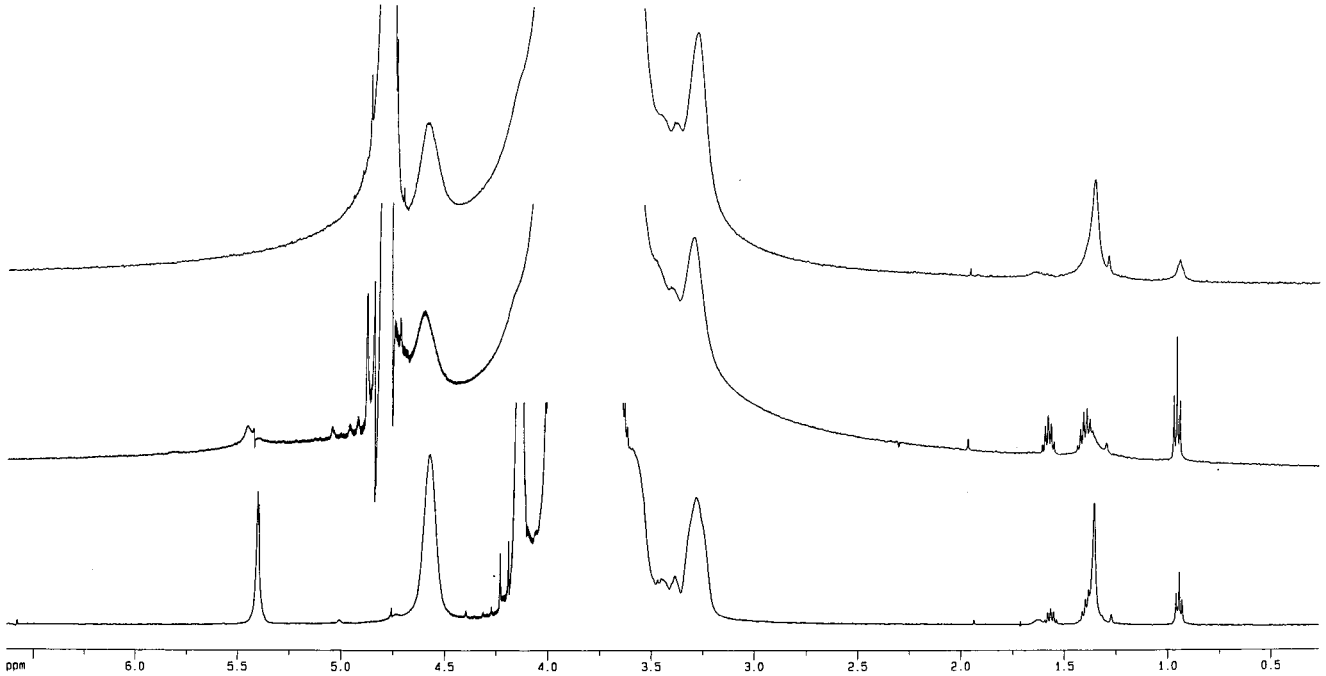

Figure 9. Proton NMR spectra of HMHEC and AM−HMHEC mixtures: top, 1% HMHEC at 27 °C; middle, 1% HMHEC with 0.10% AM at 27 °C; bottom, 1% HMHEC with 0.10% AM at 80 °C. Well-resolved peaks are H-1 (AM) at 5.4 ppm; H-1 (cellulose) at 4.6 ppm; methylene (HMHEC hydrophobes, butanol) at 1.3−1.7 ppm; methyl (HMHEC hydrophobes, butanol) at 0.95 ppm.

Table 1. Measured Intensities (Relative to ^{1}H of Cellulose) of ^{1}H NMR Signals from HMHEC in the Presence of Amylose at 27 °C

c_{AM} (%)	CH_2[a]	CH_3
0.05	0.243	0.022
0.10	0.127	0.008
0.12	0.136	0.011
0.15	0.105	0.005

[a] Main peak at 1.4 ppm.

the intensities of the HMHEC hydrophobe peaks decrease, at ambient temperature, with increasing amylose concentration. A crude linear fit of the data suggests that the signals from the HMHEC hydrophobes would disappear at an AM concentration around 0.2%.

Competition with Surfactant. Surfactants, such as SDS, are expected to compete with the AM−HMHEC complexation, since surfactant complexes are known to form with both the AM and the HMHEC components separately. We performed experiments where SDS was added to mixtures containing 0.15% AM and 1% HMHEC. Interestingly, already low amounts of added SDS (ca.1 mM) resulted in a marked decrease of the elastic modulus of the AM−HMHEC mixtures, as illustrated in Figure 10. Note that the concentration of HMHEC hydrophobes is only 0.5 mM in an 1% HMHEC solution and that, according to Eliasson,[13] only about 1 mM of SDS is needed to saturate 0.15% of AM. A further increase in the surfactant concentration, beyond 1 mM, produces the rheological response that is well-established for AM-free mixtures of HMHEC and SDS: a dramatic increase in G', due to mixed micelle formation between SDS and the HMHEC hydrophobes, followed by a decrease, due to a decreasing number of hydrophobes per mixed micelle (a decreasing functionality of the cross-links) at high surfactant concentrations.[21,22,36] Clearly, SDS destroys the AM−HMHEC complexes very efficiently.

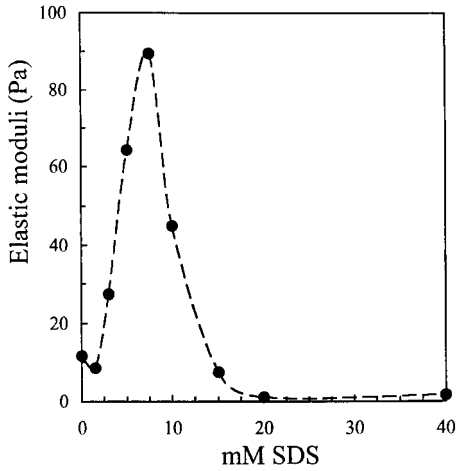

Figure 10. Dependence of G' for 1% HMHEC with 0.15% AM as a function of SDS concentration (25 °C, 1 Hz, 0.5% strain).

As illustrated in Figure 11, the frequency dependencies of the moduli with added SDS are interestingly different than for the surfactant-free AM−HMHEC composites (cf. Figure 8a). The presence of just 1.5 mM SDS is sufficient to give a solution-like behavior. Further surfactant addition increases both moduli and creates a weak gellike composite where both G' and G'' are highly frequent dependent (obviously much more frequent dependent than for the surfactant-free AM−HMHEC composite). Overall, the concentration dependence of the mechanical spectra at SDS concentrations above 1.5 mM is quite similar to that observed previously for the pure SDS−HMHEC system.[25,37,38]

Mixtures with Amylopectin. The rheological response of 1% HMHEC to increasing concentrations of added AP is illustrated in Figure 12. The figure shows G' (Figure 12a) and tan δ values (Figure 12b) from oscillatory measurements at 1 Hz. For comparison, data are included for 1% mixtures of nonmodified HEC with PAP as well as the data from Figure 4 for HMHEC

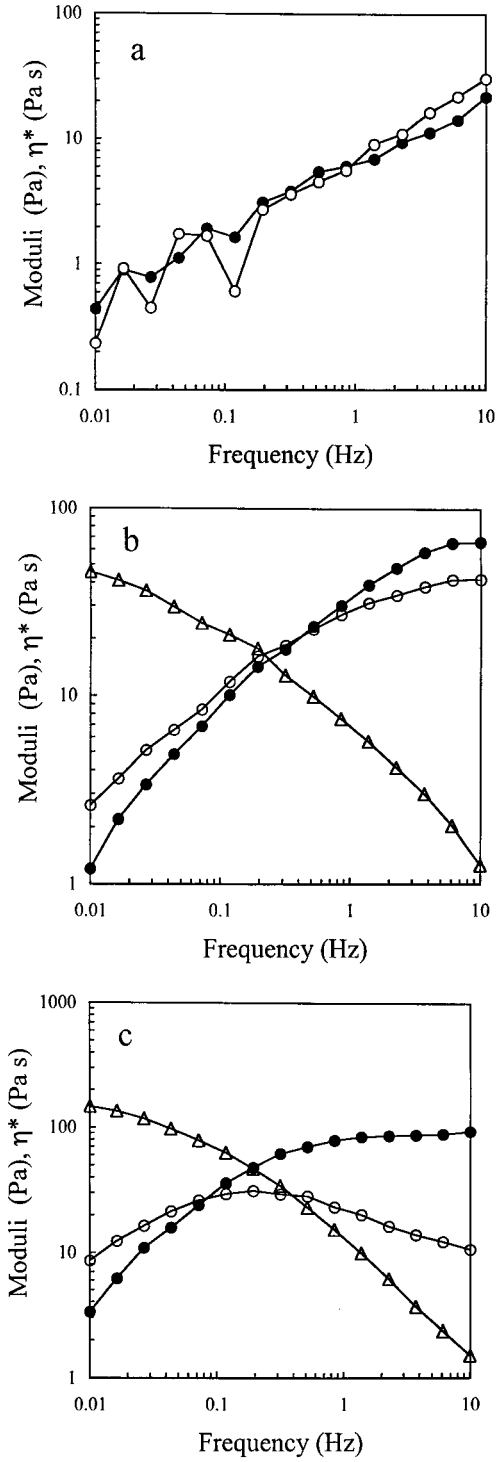

Figure 11. Frequency dependencies of G' (●), G'' (○), and η^* (△) obtained from oscillatory rheological measurements, for 1% HM−HEC with 0.15% AM with (a) 1.5 mM, (b) 3 mM, and (c) 7.5 mM SDS (25 °C, 0.5% strain).

mixtures with AM. Clearly, there is an increase in the storage modulus when PAP is added to HMHEC, while there is very little effect of PAP added to HEC. These results indicate that there is an interaction between PAP and HMHEC that is dependent on the presence of hydrophobes on the latter polymer. On the other hand, the effect of PAP on the G' of 1% HMHEC is much weaker than that of AM, and the tan δ values of the former mixtures remain high at all PAP concentrations, indicating viscous liquidlike, rather than gellike, properties.

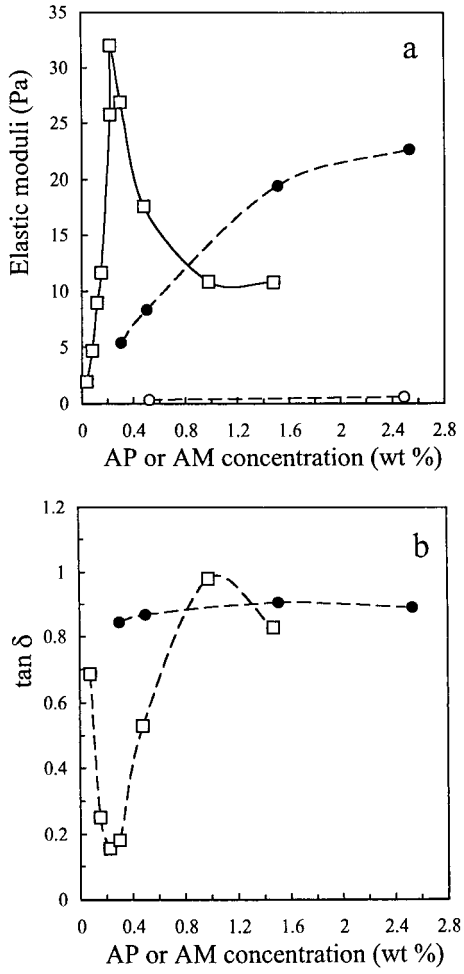

Figure 12. (a) Concentration dependence of G' for 1% PAP−HMHEC mixtures as a function of amylopectin concentration (●) and for 1% PAP−HEC mixtures (○) (25 °C, 1 Hz, 0.5% strain). The G' values of 1% AM−HMHEC mixtures are also included for comparison (□); x axis denoting then the AM concentration. (b) tan δ at 25 °C as a function of amylopectin concentrations for 1% PAP−HMHEC mixtures (●). The tan δ values of 1% AM−HMHEC mixtures are also included for comparison (□).

The response of PAP−HMHEC to increasing small amplitude angular frequency (ω) is also different from that of AM−HMHEC mixtures. Data on G', G'', and η^* for an PAP−HMHEC composite containing 0.3% PAP are shown in Figure 13. Both G' and G'' are found to be highly frequency dependent, with a linear dependence of log G' vs log ω with a slope of 0.324. The plot of log η^* vs log ω is also linear (as for AM−HMHEC mixtures), but the slope is relatively low (−0.65). When the concentration of PAP was increased (data not shown), the frequency dependences of the moduli also increased, while the steepness of the slope of log η^* vs log ω decreased.

Figure 14 shows the changes in small-deformation moduli on cooling−heating observed for 1% HMHEC mixed with PAP at two different concentrations. Both the storage and the loss moduli increased progressively as the temperature was decreased, but their values were similar at each temperature. Flow curves (not shown) were also recorded for mixtures with 1% HMHEC over a wide range of PAP concentrations. A typical shear thinning and thixotropic behavior without a shear-rate-independent plateau was exhibited for all PAP−HMHEC composites.

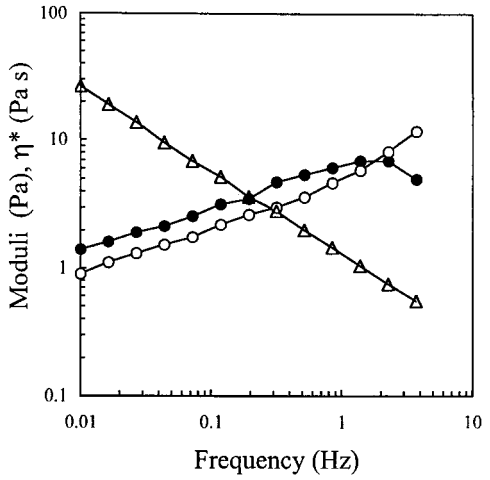

Figure 13. Frequency dependencies of G' (●), G'' (○), and η^* (△) obtained from oscillatory rheological measurements, for 1% HMHEC with 0.3% PAP (25 °C, 0.5% strain).

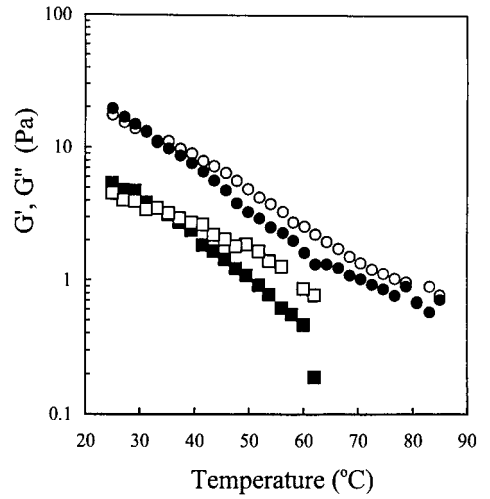

Figure 14. Temperature dependence on cooling and heating of storage (filled symbols) and loss (open symbols) moduli for 1% HMHEC with 1.5% (circles) and 0.3% (squares) PAP (2 deg/min, 1 Hz, 0.5% strain).

Concluding Discussion

Our study confirms the findings by previous investigators[23,24] that an association can develop between starch polysaccharides—AM in particular—and hydrophobically modified polymers. We have clearly established that gellike composites develop in mixtures between HMHEC and AM, if the mixtures are prepared under molecularly dispersed conditions (high pH or temperature) and then brought to ambient temperatures and a neutral or slightly alkaline pH. The associations can be broken by increases in temperature or pH or by subjecting the samples to shear. All these changes were found to be reversible; the gel re-forms when the composite is brought back to its original state.

It is evident that the helical conformation of AM is required for the association and subsequent gelation of the composite, since no association phenomena were observed with denatured AM (high temperatures or high pH). The hydrophobes on HMHEC are similarly essential, since no evidence of an association was found for nonmodified HEC. Moreover, the association is broken when a competing surfactant, SDS, is present at a concentration similar to the concentration of the HMHEC hydrophobes. All this evidence is consistent with the notion that the association is due to the formation of inclusion complexes, where the hydrophobes of HMHEC are included in the central cavities of AM helices. However, our measurements would also be compatible with other modes of association between the HMHEC hydrophobes and helical AM. One could, for instance, imagine an adsorption of the hydrophobes onto small (crystalline) aggregates of AM—although we can find no reason why this type of association would occur. In this context we also note that Gruber and co-workers found little effect of AM on a different type of HMHEC, where the hydrophobes contained more bulky aromatic moieties.[39] It is not clear why a less specific mode of association would be so sensitive to the nature of the hydrophobes. By contrast, it is well established that the development of inclusion complexes between AM and low-molecular-weight amphiphilic molecules is quite sensitive to the shape of the hydrophobic part of the molecule.

One characteristic of the AM—HMHEC complex is the relatively low setting—melting temperature (60—70 °C), as compared with the melting temperature of an amylose—lipid complex. For C_{16} lipid complexes the observed melting temperatures are in the range 90—100 °C.[13] The low transition temperature found in the present study could indicate that only a part of the C_{16} chain on HMHEC can enter into an AM helix for steric reasons. The hydrophobe peaks that are visible in the ^{1}H NMR spectrum at 80 °C, but not at ambient temperatures, are also consistent with the idea that the inclusion complex has "melted" at this temperature.

If the evidence for the inclusion complex is strong, little can be said about the stoichiometry or the higher order structure of the complex at this stage. The low melting temperature indicates a low degree of crystallinity of the AM helices, however, since crystalline amylose—lipid complexes typically have a melting temperature around 110 °C.[13] The fact that the hydrophobes of the inclusion complexes are attached to semiflexible polymer chains would also severely restrict the possibilities for the complexed AM helices to crystallize. Unfortunately, because of the low concentration of inclusion complexes in our composites, it is difficult to study the thermal transition and the molecular nature of the AM—HMHEC complexes with methods conventionally used in the study of amylose—lipid complexes, such as X-ray diffraction or differential scanning calorimetry.

We have observed that the rheological properties of the composites, as well as their turbidity, depend to some extent on the conditions of preparation (alkali preparation or hot mixing and the heating temperature on hot mixing). Moreover, Gruber and co-workers found that the rheology of their composites depended on the rate of cooling after hot mixing.[39] This indicates that the molecular organization of the network that develops on complexation is dependent on sample history. On the other hand, we cannot exclude that part of the differences found in the present study may be due to chemical degradation (depolymerization and/or detachment of hydrophobic side chains), since conditions favoring a molecular dissolution of AM (high temperature or pH) also increase the rate of degradation. It is notable, however, that we have found no effect of the method of preparation on the melting temperature of the complex, as measured by rheology.

The rheology of the AM−HMHEC composite, but not its melting temperature, depends strongly on the concentrations of both polymers. At a fixed HMHEC concentration, the viscosity and the elastic modulus increase steeply with AM concentration at low levels of AM. This initial strong sensitivity on AM concentration may be expected, since the hydrophobe concentration is low. For 1% HMHEC a maximum in elasticity was observed in the vicinity of 0.2% AM, with small variations depending on the method of sample preparation. Previous findings of Gruber and Konish also showed a maximum viscosity enhancement at 0.25% of AM in mixtures with 1% cat-HMHEC. By contrast, we observed no maximum in mixtures with 2% HMHEC, and the latter composites developed a much higher modulus at high AM concentrations. It is interesting to note, however, that both G' and tan δ of the 2% HMHEC composites level off at AM concentations above 0.5%. It is possible that the conditions for the maximum for the 1% HMHEC composites, and the leveling of for 2% HMHEC composites, reflect a saturation of the HMHEC hydrophobes by AM at a concentration ratio of ca. 0.25% AM per 1% HMHEC. This concentration ratio also agrees with the (extrapolated) disappearance of the NMR signals from the HMHEC hydrophobes (cf. above). A weight ratio of 0.25:1 (AM:HMHEC) corresponds to nearly 30 glucose units from AM per HMHEC hydrophobe. On the basis of previous results for amylose−lipid complexes, this seems like a reasonable stoichiometry for a saturated complex.

Several aspects of the complexation between starch components and hydrophobically modified polymers deserve further study. The molecular weight of the AM and the lengths of the hydrophobes on the hydrophobically modified polymer may influence the lifetimes of the complexes and thus the rheology of the composites. The competition with a low-molecular-weight surfactant should also be sensitive to the lengths of the hydrophobes of the surfactant and the HM polymer. Finally, the interaction between HMHEC and PAP, suggested by our results, is interesting. In the present work, we have mainly focused on mixtures dominated by the HM polymer, where we have introduced starch polysaccharides as physical cross-linkers. For PAP in particular, it may be interesting to look at systems at high concentrations of PAP and to study the effects of (low) levels of an added HM polymer. Work along these lines is in progress at our laboratory.

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