# Specific Interactions in Model Charged Polysaccharide Systems†

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Temporary networks were obtained from mixtures of two derivatized chitosans, one with $\beta$-cyclodextrin (CD) cavities and the other with adamantyl (AD) moieties, randomly distributed along the chains. The viscoelastic response of these original systems in which cyclodextrin−adamantane complexes play a role of interchain junctions (sticky points) was investigated as a function of polymer concentration, degree of modification, ionic strength, temperature, and addition of competitive molecules. In the semidilute regime, an increase of the long relaxation times with the density of CD−AD complexes is observed, reflecting the hindrance of local flow processes due to reversible interchain bonds. The strong dependence of the apparent relaxation energy appears as a consequence of the temperature dependence of CD−AD interaction. However, contrary to the long time dynamics, the elastic plateau modulus of the transient network at short times does not seem to be much influenced by the density of stickers. The value of the plateau modulus is mainly dependent on polymer concentration.

## Introduction

It is well-known that some natural polysaccharides such as pectins, with low degrees of esterification, or alginates can lead to the formation of physically cross-linked gels by a cooperative complexation of multivalent counterions (especially $Ca^{2+}$) with galacturonic or guluronic carboxylic groups, respectively.[1] Thermoreversible networks can also be stabilized by cooperative H-bonded zones such as in pectins with a high degree of esterification or in gelatin. Hydrophobic interactions are involved in thermoforming gels such as those with methylcellulose[2] based on the existence of blocks of highly methylated glucose units or hydrophobically modified chitosans[3] or synthetic polymers.[4] Original supramolecular networks were also obtained by inclusion complexation between poly(ethylene glycol) (PEG) grafted dextran and α-cyclodextrins (α-CDs).[5] It was suggested that physical junctions in these systems consist in hydrophobically aggregated cristalline domains formed by PEG−CD inclusion complexes.

More recently, it has been shown that some molecules of biological origin can function as physical cross-links, leading to original properties for the resulting networks. These cross-links include proteins and their ligands such as glucose−concanavalin A,[6] protein−protein interactions leading to coiled-coil domains,[7,8] antibody−antigen complexes,[9] and lactic acid oligomers with complementary D/L conformations.[10]

In the continuing challenge to develop new temporary networks, our approach was to use specific recognition between $\beta$-cyclodextrin and a hydrophobic adamantane derivative, each grafted on a carbohydrate polymer, that is, chitosan. Chitosan is the most important derivative obtained from chitin, chitin being the second most important natural polymer after cellulose. Chitosan is especially interesting due to the presence in the repeating unit of a $-NH_2$ group on the C-2 position allowing specific chemical modification. This polysaccharide becomes water-soluble in acidic conditions (pH < 6), leading to the preparation of biocompatible and often biodegradable polymers with optimized properties, in homogeneous conditions. Thus, the grafting of cyclodextrin molecules, having a hydrophobic cavity and a hydrophilic outer shell, provides a well-defined "host" polymer being able to complex specifically hydrophobic guest molecules, as reported previously.[11]

The junctions in the present system result from the specific complexation between the $\beta$-CD cavity and the hydrophobic adamantyl group which are randomly attached to chitosan. Thus, upon mixing solutions of the two derivatized chitosans, namely cyclodextrin−chitosan and adamantane−chitosan, a large increase of viscosity was observed quasi-instantaneously as a result of the formation of CD−adamantyl (AD) complexes playing a role of labile interchain junctions or stickers. Consequently, the rheological properties of such systems should be closely related to the number of interacting pairs for a finite period of time. The finite lifetime of the cross-links arises from the fluctuations in the relative kinetic energy of the two groups. Such interacting polymers, even at relatively low concentration but in the semidilute regime, show an unusually large thickening behavior (going to a gel-like behavior). In fact, these solutions behave as polymeric solutions with transient cross-links, as described previously in the literature.[12−15] The viscoelastic properties look like those of very high molecular weight and/or concentrated solutions. The solutions behave as a highly viscous fluid, indicating weak and nonpermanent bonds that fluctuate with time. These temporary polymer networks can be defined as three-dimensional infinite arrangements of macromolecules in which interchain junctions are reversibly broken. In this paper, we intend to characterize the mixing of the two polymers (AD−chitosan/CD−chitosan) and examine the influence of various external variables such as concentration and degree of modification of the polymers, temperature, and salt concentration on their rheological behavior.

## Experimental Section

**1. Materials.** The chitosan used has a weight-average molecular weight $M_w$ of 195 000; it is a commercial sample

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from Pronova (Norway) with a degree of *N*-acetylation equal to 0.12. It was purified by solubilization in aqueous $CH_3COOH$ and reprecipitation by NaOH at neutral pH. The polysaccharide was finally washed with deionized water and ethanol and then dried. The $\beta$-cyclodextrin was kindly supplied by Roquette Frères (Lestrem, France). Cyclodextrin grafted chitosan and adamantane grafted chitosan were synthesized in our laboratory as described in detail elsewhere.[11] The CD−chitosan sample has a degree of modification of 0.1 (i.e. on average one CD every 10 glucosamine units). It was mixed with different samples of AD−chitosan having degrees of substitution of 0.05, 0.07, and 0.08. All the polymer concentrations are expressed in monomol/L (number of moles of modified and/or nonmodified glucosamine units/L). The concentrations of the functional groups grafted on the polymers (cyclodextrin or adamantane) are expressed in mol/L.

**2. Rheological Experiments.** Dynamic experiments were performed with a cone plate rheometer (AR1000 from TA Instruments). The cone used has a diameter of 4 cm and an angle of 3°59′, and it was equipped with a cap to avoid vaporization. All the dynamic rheological data were checked as a function of strain amplitude to ensure that the measurements were performed in the linear viscoelastic region. Flow experiments were carried out with the same rheometer as above or with a Contraves LS30 Low-Shear rheometer, depending on the sample viscosity. CD−chitosan and adamantane grafted chitosan solutions were prepared separately at different concentrations in 0.3 mol/L $CH_3COOH$/0.03 mol/L $CH_3COONa$. Salt was added in order to screen the long range electrostatic repulsions between positively charged chains. The dissolution time was at least 1 day at room temperature. Cyclodextrin−chitosan and adamantane−chitosan solutions were then mixed. Gel-like samples were vigorously stirred and allowed to rest for at least 1 h at controlled temperature. We checked that the rheological properties of the samples did not change with time (from 1 to 24 h).

**3. Titration Calorimetry.** Isothermal titration microcalorimetry (ITC) was performed using a Model 4200 microcalorimeter from Calorimetry Sciences Corporation (Utah, USA). In individual titrations, injections of 10 $\mu$L of natural $\beta$-cyclodextrin were added from the computer-controlled 250-$\mu$L microsyringe at an interval of 5 min into the adamantane acetate or AD−chitosan solution (cell volume = 1.3 mL) containing the same solvent as that for $\beta$-CD (pure water or 0.3 mol/L $CH_3COOH$/0.03 mol/L $CH_3COONa$), while stirring at 297 rpm at 25 °C. The observed heat effects under identical injections of $\beta$-CD into a cell containing only the solvent were identical to the heat signals at the end of titration, after the saturation is reached. The raw experimental data were presented as the amount of heat produced per second following each injection of $\beta$-CD as a function of time. The amount of heat produced per injection was calculated by integration of the area under individual peaks by the instrument software, after taking into account heat of dilution. The experimental data were fitted to a theoretical titration curve using the instrument software, with $\Delta H°$ (the enthalpy change in kJ/mol), $K_a$ (the association constant in L/mol), and $n$ (complex stoichiometry) as adjustable parameters.

## Results and Discussion

**1. Solution Properties of Host and Guest Chitosans Alone.** *(a) Cyclodextrin Grafted Chitosan.* Previous studies performed on CD−chitosan solutions in 0.3 mol/L $CH_3COOH$/0.03 mol/L $CH_3COONa$ have shown that the aqueous behavior of this modified chitosan is close to that of the initial chitosan.[16] Nevertheless, at a given concentration, the viscosity of CD−chitosan is slightly larger than that of the initial chitosan, indicating the presence of a few additional interchain interactions induced by the grafted cyclodextrin cavities.

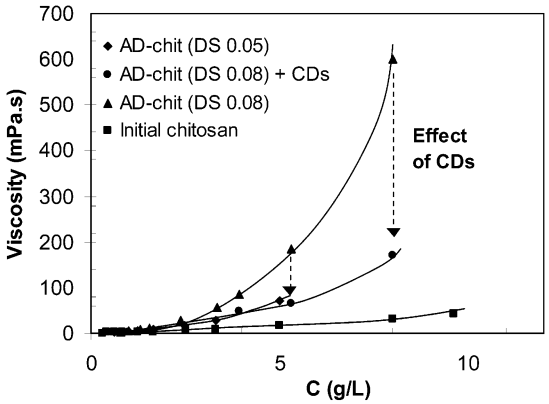


**Figure 1.** Variation of the viscosity with the concentration of solutions of initial chitosan, AD−chitosan with DS = 0.08 alone and in the presence of excess $\beta$-CD, and AD−chitosan with DS = 0.05 in 0.3 mol/L $CH_3COOH$/0.03 mol/L $CH_3COONa$ at 25 °C. ($\dot{\gamma} \rightarrow 0$).

Moreover, it was demonstrated by NMR spectroscopy that the inclusion properties in terms of complex geometry and affinity constant of $\beta$-CD toward small organic guest molecules are not modified by grafting on the polymer.[11]

*(b) Adamantane Grafted Chitosan.* The situation may be different in the case of adamantane grafted chitosan, for which the hydrophobic adamantyl groups promote self-associating behavior in aqueous solution, as reported previously.[16]

The aqueous behavior of AD−chitosan derivatives with different degrees of substitution (0.05 and 0.08) was examined and compared with that of the initial chitosan. Moreover, we addressed the effects of adding natural $\beta$-cyclodextrin to the polymer solutions. The steady shear viscosities of solutions of AD−chitosans were thus measured in the range of concentration from 0.4 to 8 g/L. The results are presented in Figure 1. Since the overlap concentration $C^*$ for the initial chitosan is around 0.9 g/L ($5 \times 10^{-3}$ monomol/L), it can be observed that, in the dilute regime ($C < 1$ g/L), the solution viscosities of the three chitosans are close to each other. Above this concentration, the viscosities become very different. It can be speculated that, around the overlap concentration, intermolecular links form progressively; hydrophobic associations are likely the main cause of the viscosity increase for the AD−chitosan solutions, and the higher the degree of substitution, the higher the viscosity increase. However, owing to the bulky structure of adamantyl groups, these interactions are less strong than those observed for alkylated chitosan derivatives.[3] Upon addition of excess $\beta$-CD, the viscosity of AD−chitosan (DS = 0.08) solutions decreases drastically. This demonstrates the ability of cyclodextrin to cap adamantyl groups, reducing hydrophobic interaction. However, the viscosity of AD−chitosan in the presence of excess $\beta$-CD never reaches the viscosity value of the initial chitosan at the same polymer concentration. This indicates that interchain hydrophobic interaction may compete with CD inclusion complex formation.

Interaction of natural $\beta$-CD with AD−chitosan was further thermodynamically characterized by isothermal titration calorimetry. In particular, we compared complexation between grafted adamantane and $\beta$-CD with that between sodium adamantane acetate and $\beta$-CD. Figure 2 shows the data obtained for the titration of free and grafted adamantane (from AD−

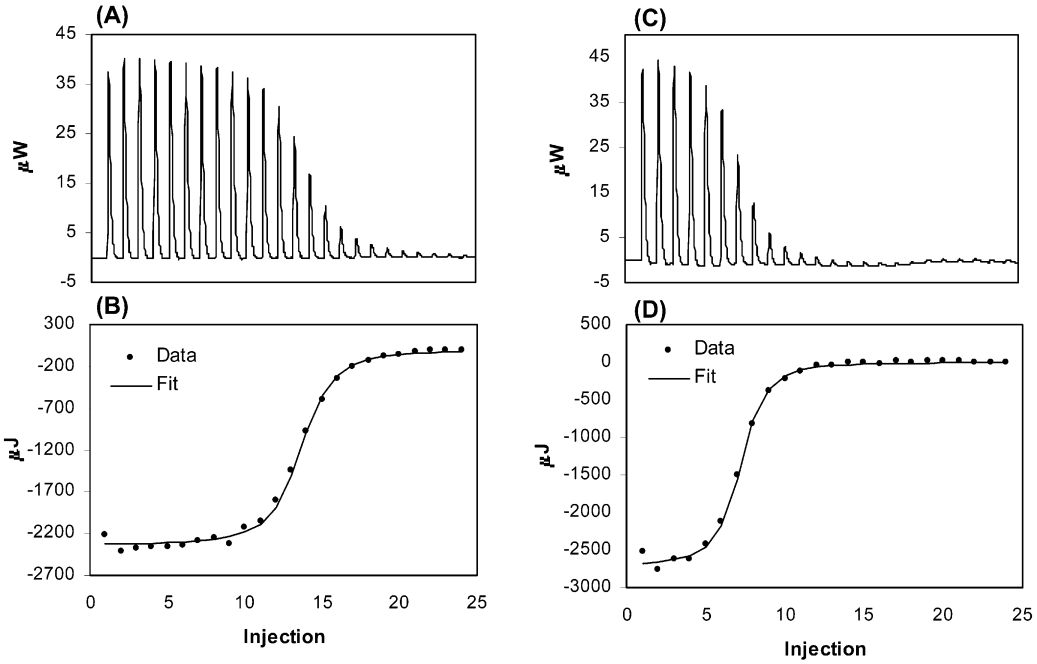


**Figure 2.** Calorimetric titration of sodium adamantane acetate ($10^{-3}$ mol/L in pure water) with $\beta$-CD ($10.73 \times 10^{-3}$ mol/L in pure water) and of grafted adamantane ($1.08 \times 10^{-3}$ mol/L in 0.3 mol/L $CH_3COOH$/0.03 mol/L $CH_3COONa$) with $\beta$-CD ($10.79 \times 10^{-3}$ mol/L in 0.3 mol/L $CH_3COOH$/0.03 mol/L $CH_3COONa$) at 25 °C: (A and C) raw data obtained for 24 automatic injections, each of 10 $\mu$L, of $\beta$-CD to free and grafted adamantane, respectively; (B and D) the integrated curve showing experimental points and the best fit for titration of free and grafted adamantane, respectively.

**TABLE 1: Thermodynamic Parameters for Inclusion Complex Formation of Sodium Adamantane Acetate and Adamantane of AD−Chitosan with Natural $\beta$-CD**

| adamantane molecule | $K_a$ (L/mol) | $\Delta H°$ (kJ/mol) | $T\Delta S°$ (kJ/mol) | $n$ (1AD:$n$CD) |
|---|---|---|---|---|
| sodium adamantane acetate | $1.18 (\pm 0.05) \times 10^5$ | $-22.0 (\pm 0.4)$ | 6.9 | $1.15 (\pm 0.01)$ |
| grafted adamantane | $1.56 (\pm 0.05) \times 10^5$ | $-26.0 (\pm 0.4)$ | 3.6 | $0.48 (\pm 0.01)$ |

chitosan with a DS = 0.08) with $\beta$-CD at 25 °C in water and in 0.3 mol/L $CH_3COOH$/0.03 mol/L $CH_3COONa$, respectively. As shown in Figure 2A and C, exothermic heat is produced after each injection of $\beta$-CD. The magnitude of the released heat decreases progressively with each injection until complete complexation of adamantane is achieved. Parts B and D of Figure 2 display the experimental data and the calculated best fit binding curves which pass very closely through the experimental points. Table 1 shows that free and grafted adamantane have similar $K_a$ and $\Delta H°$ values but different complex stoichiometries. The value of $n$ is 1.15 for free adamantane, indicating that one adamantane molecule is complexed by one CD, which is consistent with the fact that adamantane is deeply included into the CD cavity with no possibility for inclusion of a second adamantane molecule.[17] The value of $n$ of 0.48 for grafted adamantane suggests that one CD interacts with approximately two adamantane molecules, which is unreasonable. The discrepancy between both CD−AD complexes can also be observed on the thermograms. Indeed, the amount of heat evolved for complexation of high affinity free adamantane is essentially constant for the first approximately nine injections, where adamantane is almost completely bound to added $\beta$-CD. Then a steep decrease can be observed, indicating that saturation is almost reached. In the case of grafted adamantane, the drop in amount of heat produced occurs at approximately the fourth injection, as if the concentration of grafted adamantane was about two times lower than that of free adamantane, which is not the case. Moreover, when titration was performed with a two times less concentrated solution of $\beta$-CD, the decrease took place later. Identical effects were observed with the AD−chitosan samples with smaller DS. Thus, these results suggest that only one part of grafted adamantane molecules effectively interacts with $\beta$-CD due to competition with adamantane autoassociation. Nevertheless, the number of complexes formed between grafted adamantane and $\beta$-CD increases with increasing DS. The enthapy value found for the complex formation between free adamantane and $\beta$-CD ($\Delta H° = -22$ kJ/mol, Table 1) is consistent with the values reported in the literature.[18] The binding is largely driven by enthalpy with a small entropy contribution. Moreover, the binding energy is $\sim 10kT$, which should favor the association process between chains substituted by CDs and adamantyl groups, respectively.

**2. Rheological Behavior of CD−Chitosan in the Presence of Adamantane Grafted Chitosan.** Concerning associating polymers leading to temporary networks, different models have been considered to describe their behavior. The first one refers to the dynamics of solutions of unentangled water-soluble telechelic polymers with terminal hydrophobic groups.[19] This model however cannot be applied in the present case, as the associating sites are randomly distributed along the chain. The rheological behavior of unentangled reversible networks formed by polymers with many associating groups per chain was considered theoretically more recently.[20,21] The dynamics of the chain is described by a sticky Rouse model. This means that it resembles that of the Rouse bead−spring chain, but with a slowing down of the motion due to the presence of intermolecular associations (sticky points) which act as dissipative friction centers. Thus, the long time dynamics of such systems is dominated by the number of stickers per chain and their lifetime. In the case of entangled associating polymers, the rheological properties of their solutions are governed by entanglements in addition to the reversible junctions between

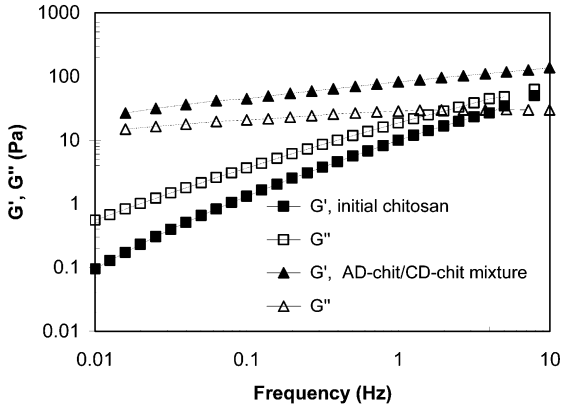


**Figure 3.** Comparison of the storage and loss moduli as a function of frequency for a chitosan solution (30 g/L) and a AD−chitosan (0.0139 monomol/L, 2.46 g/L, DS = 0.07)/CD−chitosan ($6 \times 10^{-3}$ monomol/L, 1.97 g/L, DS = 0.1) mixture in 0.3 mol/L $CH_3COOH$/0.03 mol/L $CH_3COONa$ at 25 °C.

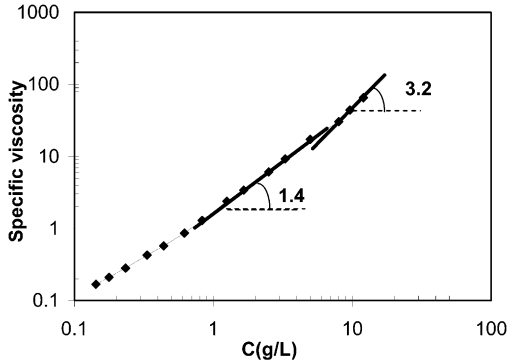


**Figure 4.** Variation of the specific viscosity with the concentration of initial chitosan solutions in 0.3 mol/L $CH_3COOH$/0.03 mol/L $CH_3COONa$ at 25 °C.

different chains due to the presence of stickers. The dynamics of such systems is described by the reptation model, which can be considered as a sticky Rouse motion along the contour of a tube.[21,22]

When a solution of CD−chitosan is added to a solution of AD−chitosan at a total polymer concentration higher than the overlap chain concentration $C^*$ ($\sim 5 \times 10^{-3}$ monomol/L), the rapid formation of a transparent "gel" can be observed macroscopically, resulting from the specific interaction between the grafted CD cavities and adamantane groups. Figure 3 compares the dynamic rheological moduli of a solution of initial chitosan ($C = 0.18$ monomol/L) with those of a AD−chitosan (0.0139 monomol/L, DS = 0.07)/CD−chitosan ($6.6 \times 10^{-3}$ monomol/L, DS = 0.1) mixture. The storage and loss moduli for the mixture of modified chitosans appear to be much larger than those obtained for the initial chitosan although the concentration of the latter is more than 8 times higher than that of the total chitosan chains in the mixture. Moreover, $G'$ is larger than $G''$ within a very large range of frequency for the mixture, reflecting a network-type structure. However, no distinct plateau can be observed for the storage modulus; $G'$ and $G''$ decrease with decreasing frequency, indicating that the network relaxes as a result of the breaking and re-forming of the cross-links (i.e. the CD−adamantane complexes). No maximum for the loss modulus, which yields a good indication of the largest relaxation time, can be observed any more.[14]

**3. Effects of the Concentration and Degree of Modification of Polymers.** Figure 4 reminds us of the main features of the rheological behavior of the initial chitosan. Three different concentration regimes can be distinguished:

(i) The dilute regime $C < C^*$, with $C^* = 5 \times 10^{-3}$ monomol/L.

(ii) The semidilute unentangled regime $C^* < C < C_e$, where $C_e$ is the concentration at which entanglements become elastically effective. Beyond $C^*$, the dependence of the viscosity on concentration is linear in a log−log representation with a slope equal to 1.4. At $C_e \approx 0.06$ monomol/L, corresponding to the appearance of a non-Newtonian behavior, a break in the variation of the viscosity with $C$ is observed, and beyond this value, viscosity scales as $C^{3.2}$. The scaling behavior of viscosity with concentration is in good agreement with the theoretical prediction for polyelectrolytes in saline media[23] and neutral polymers.[24,25] Indeed, the scaling predicted is $\eta \sim C^{5/4}$ for $C^* < C < C_e$ and $\eta \sim C^{15/4}$ for $C_e < C < C^{**}$. In this regime, the viscoelastic properties of the solution are controlled by the Rouse dynamics.

(iii) The semidilute entangled regime with $C_e < C < C^{**}$, where $C^{**}$ corresponds to the transition to the concentrated domain. In this regime the viscoelasticity of the solution is described by the reptation model.

Considering these different regimes, the rheological properties of the AD−chitosan/CD−chitosan system were examined in a range of concentration from $C = 1.8 \times 10^{-3}$ monomol/L to $C = 0.041$ monomol/L where $C$ is the total concentration of monomers with [AD] = [CD]. From $C = 1.8 \times 10^{-3}$ monomol/L to $C = 7.9 \times 10^{-3}$ monomol/L, corresponding to the dilute regime and the beginning of the semidilute one for the initial chitosan, CD−chitosan, and AD−chitosan alone in solution, heterogeneous samples with small pieces of gels dispersed in a dilute polymer solution were obtained. From these samples, no reproducible viscosity measurement was possible. Such a behavior may be attributed to microgel formation. Thus, we can consider that, for these concentrations, we are below the gelation threshold. At $C = 0.010$ monomol/L, which is about two times the overlap concentration of the initial chitosan, a transition in the system behavior takes place; a "homogeneous gel" is observed. We thus examined the viscoelastic properties as a function of total polymer concentration from this critical concentration up to $C = 0.041$ monomol/L for two types of AD−chitosan/CD−chitosan mixtures, one with a AD−chitosan sample having a DS of 0.05 and the other with a AD−chitosan sample having a DS of 0.08 (DS of CD−chitosan = 0.1). Figure 5 shows the effects of polymer concentration on the $G'$ and $G''$ curves as a function of frequency. It can be seen that the storage and loss moduli increase with the polymer concentration for both mixtures. However, the characteristic relaxation time $\tau_c$, obtained from the inverse of the angular frequency $f_c$ obtained from the crossover point of $G'$ and $G''$, which characterizes the slowing down of the dynamics of the system, does not seem to be very affected by the variation of polymer concentration. We tried to form a master curve by shifting the moduli−frequency spectra along the horizontal and vertical directions as for time−temperature superposition. In fact, the $G'$ curves and $G''$ curves could be superimposed on the reference curves, corresponding to the mixture with the higher concentration, only by a vertical shifting in both cases (see Figure 6). The same shift factor was used to obtain master curves for both storage and loss moduli, which is an indication of the validity of the superposition. As mentioned above, the relaxation mechanism does not seem to be affected by a modification of polymer concentration, since only a vertical shifting is necessary. Considering that in this range of concentration entanglements are not yet predominant as for the initial chitosan, this behavior may be explained by the sticky Rouse model.[21] Since intermolecular associations are

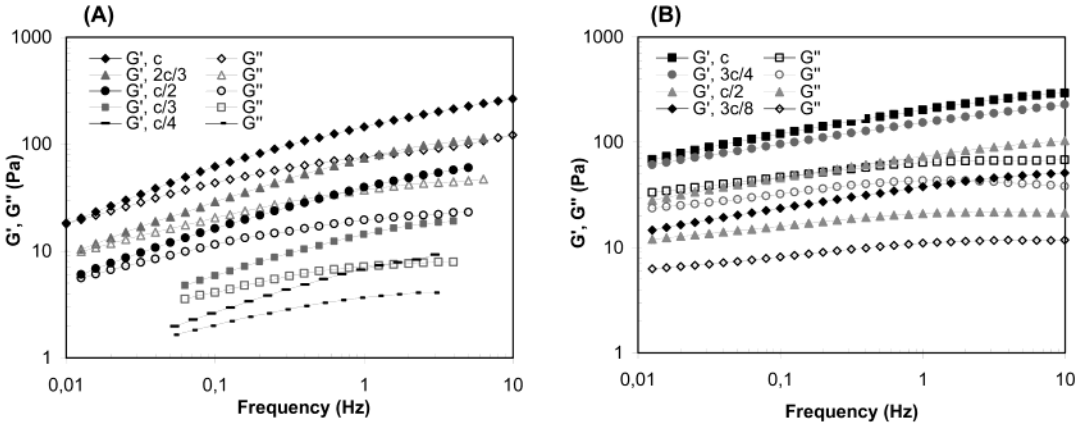


**Figure 5.** Storage and loss moduli dependence on frequency for AD−chitosan/CD−chitosan (DS = 0.1) mixtures ([CD] = [AD]) at various concentrations in 0.3 mol/L $CH_3COOH$/0.03 mol/L $CH_3COONa$ at 25 °C: (A) mixtures performed with AD−chitosan having a DS of 0.05 ($C$ = 0.041 monomol/L, total concentration of monomers); (B) mixtures performed with AD−chitosan having a DS of 0.08 ($C$ = 0.039 monomol/L, total concentration of monomers).

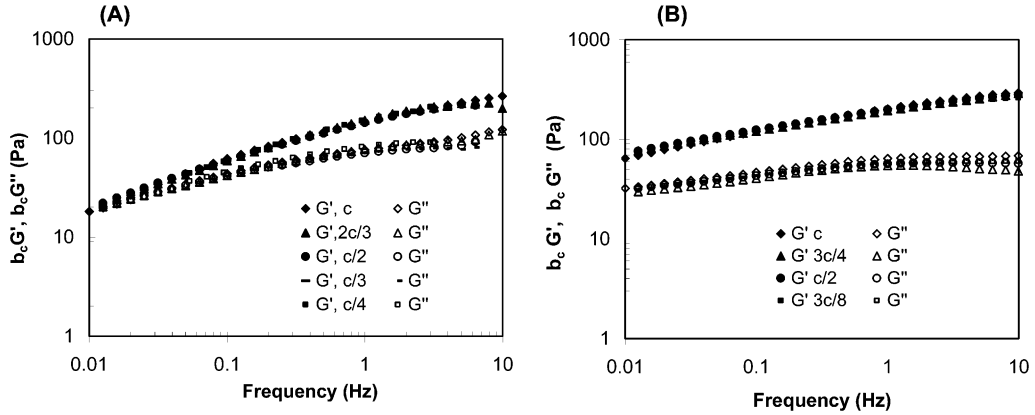


**Figure 6.** Master curves of the $G'$ and $G''$ moduli for the AD−chitosan/CD−chitosan mixtures at different concentrations. For the two AD−chitosan/CD−chitosan systems, the reference curve is the $G'$ curve corresponding to the highest concentration ($C$ = 0.041 monomol/L in part A and $C$ = 0.039 monomol/L in part B).

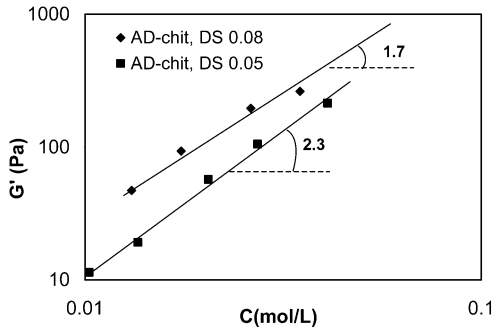


**Figure 7.** Dependence of the $G'$ modulus at $\omega$ = 4 Hz on concentration for the two AD−chitosan/CD−chitosan systems (performed with AD−chitosan samples having a DS of 0.05 and 0.08).

predominant, the concentration dependence of the relaxation time remains weak. Moreover, as only a vertical shifting was used, the dependence of the $G'$ modulus on concentration should be the same whatever the frequency is. Figure 7 shows the variation of $G'$ with total polymer concentration for the two AD−chitosan/CD−chitosan systems. The $G'$ moduli scale as $C^{1.7}$ and $C^{2.3}$. Since the relaxation time appears to be independent of polymer concentration but dependent on the density of stickers, viscosity should follow the same variation with $C$. This was confirmed by the determination of the zero shear viscosities from creep experiments.

As shown by Figure 5, the viscoelastic properties of the AD−chitosan/CD−chitosan system are very sensitive to the content

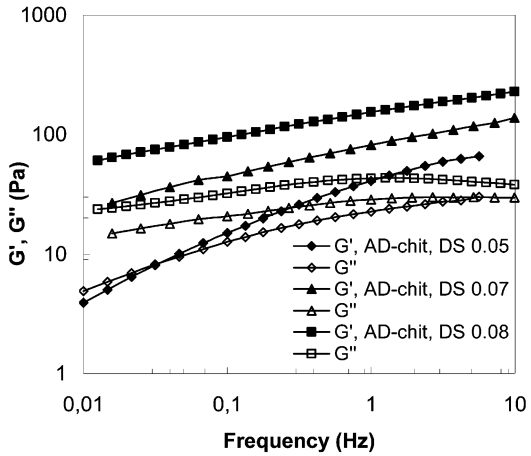


**Figure 8.** Comparison of the storage and loss moduli as a function of frequency for AD−chitosan (0.014 monomol/L)/CD−chitosan ($6.6 \times 10^{-3}$ monomol/L) mixtures with different AD−chitosan samples (DS = 0.05, 0.07, 0.08); solvent, 0.3 mol/L $CH_3COOH$/0.03 mol/L $CH_3COONa$ (25 °C).

in adamantyl groups in AD−chitosan. Figure 8 compares the dynamic rheological moduli of AD−chitosan/CD−chitosan systems prepared from AD−chitosan samples with different DS values. When the DS goes from 0.05 to 0.08, it can be seen that the relaxation time $\tau_c$ largely increases, reflecting a slowing down of the dynamics of the system, which is consistent with the sticky Rouse model ($\tau_{Rouse}$ is proportional to the square of

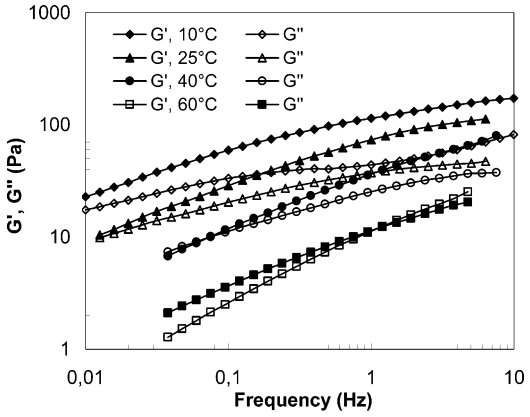


**Figure 9.** Storage and loss moduli dependence on frequency for a AD−chitosan (0.0186 monomol/L, DS = 0.05)/CD−chitosan ($8.8 \times 10^{-3}$ monomol/L, DS = 0.1) mixture in 0.3 mol/L $CH_3COOH$/0.03 mol/L $CH_3COONa$ at different temperatures.

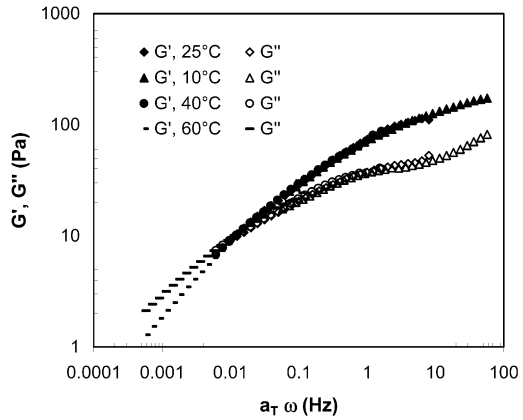


**Figure 10.** Master curves of the $G'$ and $G''$ moduli for the AD−chitosan/CD−chitosan mixture from 10 to 60 °C using the experimental shift factors of Table 2.

the number of interchain bonds per polymer). By shifting the curves horizontally, the storage moduli almost superimpose as well as the loss modulus data (data not shown), which indicates that the long time dynamics is governed by the sticky points.

**4. Effect of Temperature.** The behavior of $G'$ and $G''$ at various temperatures is shown in Figure 9. Owing to the enthalpic nature of the $\beta$-CD−adamantane inclusion complexes, their number decreases as the temperature increases. Also, their exchange rate likely depends on the temperature. From Figure 9, it is clear that raising the temperature leads to a shortening of the longest interaction time scale. Time−temperature superposition[26] allowed us to obtain master curves for the storage and loss moduli (Figure 10). 25 °C was the reference temperature. For both $G'$ and $G''$, the same horizontal shift factors could be applied, whereas no vertical shifting was done. Thus, temperature affects the dynamics of the gel by changing the exchange rate and number of the CD complexes but also chain mobility. The fact that only a horizontal shift is performed indicates that only the dynamics of the chain is modified by temperature. The horizontal shift factors obtained by superimposing the moduli data were compared with those derived from the zero shear viscosities. Indeed, the horizontal shift factor is a measure of the ratio of any specific relaxation time at temperature $T$ to its value at an arbitrary reference temperature $T_0$. According to various molecular theories of viscoelasticity, it has been suggested[26] that, at constant density, the relaxation times are proportional to the quantity $\eta/cT$, where $\eta$ is the zero

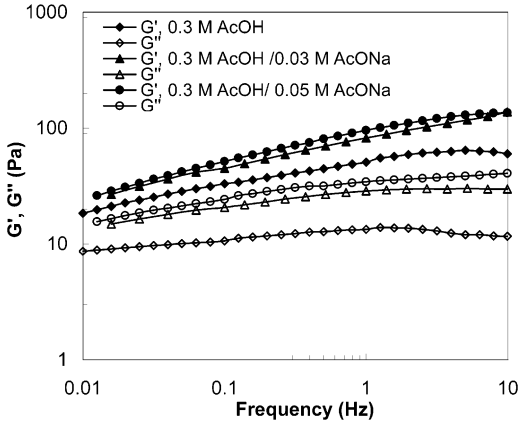


**Figure 11.** Comparison of the storage and loss moduli as a function of frequency for AD−chitosan (0.014 monomol/L)/CD−chitosan ($6.6 \times 10^{-3}$ monomol/L) mixtures with different salt concentrations.

**TABLE 2: Horizontal Shift Factors for the AD−chitosan/CD−chitosan Mixture as a Function of Temperature**

| $T$ (°C) | $a_T$ (exp value) | $a_T$ (calc value) |
|---|---|---|
| 10 | 5.79 | 4.64 |
| 25 | 1 | 1 |
| 40 | 0.16 | 0.23 |
| 60 | 0.015 | 0.013 |

shear viscosity of the polymer solution. The factor $a_T$ can thus be expressed by

$$a_T = \eta T_0/\eta_{T_0} T \quad (1)$$

For calculations of the factors $a_T$ from relation 1, the zero shear viscosities of the AD−chitosan/CD−chitosan mixture at various temperatures were determined from creep experiments. From Table 2, it can be noticed that the calculated values of $a_T$ are in very good agreement with those obtained experimentally.

The apparent relaxation energy $\Delta H_r$ could be estimated from the dependence of the horizontal shift factor on the absolute temperature. The slope of log $a_T$ versus $1/T$ gives $\Delta H_r = 93.1$ kJ/mol for the AD−chitosan (DS = 0.05)/CD−chitosan (DS = 0.1) mixture. This value is much higher than that found for a two times more concentrated solution of initial chitosan ($\Delta H_r = 36.7$ kJ/mol)[27] due to the labile interchain junctions.

**5. Effect of Salt Concentration.** The properties of the network formed by the "host" and "guest" chitosans may be slightly dependent on the balance between attractive interactions due to CD inclusion complexation and repulsive interactions between $NH_3^+$ groups on the chitosan backbone. The balance between attractive and repulsive interactions may be changed by a variation in external salt concentration. It was thus of interest to investigate the influence of $CH_3COO^-Na^+$ concentration on the properties of the network. Effects on dynamic moduli of AD−chitosan/CD−chitosan mixtures are shown in Figure 11. The observed increase of the storage and loss moduli as salt concentration is increased can be explained by the progressive decrease of repulsive interactions between chitosan chains due to electrostatic screening. However, there is a critical concentration beyond which the network collapses. At $CH_3COO^-Na^+$ concentrations higher than $C_s = 0.05$ mol/L, phase separation starts going to complete demixing at $C_s = 0.2$ mol/L.

**6. Competition Experiments.** Since the network based on the CD−chitosan/AD−chitosan mixture results from specific host−guest interactions, we can assume that addition of competitive guest or host molecules should disrupt it. It was

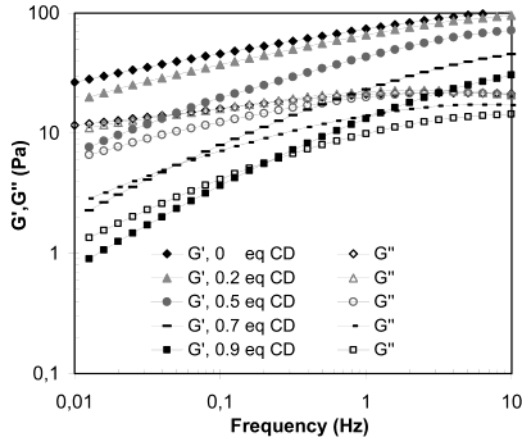


**Figure 12.** Comparison of the storage and loss moduli as a function of frequency for AD−chitosan/CD−chitosan mixtures ($C$ = 0.017 monomol/L, [AD] = [CD]) containing natural $\beta$-CD at different concentrations; solvent, 0.3 mol/L $CH_3COOH$/0.03 mol/L $CH_3COONa$ (25 °C).

thus interesting to examine effects of addition of such molecules on this supramolecular assembly. We selected natural $\beta$-CD as a competitive host molecule. Figure 12 shows the results obtained using different concentrations of free $\beta$-CD. From this figure, it can be seen that the relaxation mechanism is affected by addition of cyclodextrin molecules but the storage moduli seem to tend to a common plateau value. In fact, these competition experiments lead to results similar to those obtained by chemically varying the degree of substitution of AD−chitosan.

In conclusion, the results obtained for the rheological behavior of the mixed AD−chitosan and CD−chitosan, in the semidilute regime of partially overlapping chains, clearly indicate that the mechanism reflects the existence of a temporary multisticker interaction, as previously described in the literature for associating polymers in solution or H-bonded elastomers. The analysis of the data indicates that the relaxation processes involve the chain relaxation but also the stickers which, acting as dissipative friction centers, quench drastically the motions of the chain. The length of the elastic plateau is extended due to the fact that the sticky points dominate the long time dynamics. Consequently, the latter is closely related to the number of stickers per chain and their lifetime. Moreover, due to the temperature dependence of the density and lifetime of stickers, the relaxation mechanism is also influenced by a variation in temperature, as demonstrated by the horizontal shifting. The large apparent relaxation energy also reflects the slowing down of chain motion due to the sticky points.

At the end, contrary to the long time dynamics, the elastic plateau modulus of the transient network at short times seems not to be altered much by the introduction of a few additional cross-link points per chain. The value of the plateau modulus is mainly dependent on polymer concentration.

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