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## Effects of Sodium Chloride, Guanidine Hydrochloride, and Sucrose on the Viscoelastic Properties of Sodium Hyaluronate Solutions

**Abstract:** Effects of sodium chloride (NaCl), guanidine hydrochloride (GuHCl), or sucrose on the viscoelasticity of sodium hyaluronate (NaHA) solutions were studied. NaCl and GuHCl decreased both storage and loss moduli, while sucrose increased both moduli. The critical concentration  $C^*$  was determined as an inflection point in the plot of zero shear specific viscosity vs concentration for NaHA solutions with and without NaCl, GuHCl, or sucrose. It is suggested that sodium ions or guanidinium ions shield the electrostatic repulsion of NaHA molecules, hence reduce the coil dimension, and  $C^*$  shifted to higher concentrations. However, sucrose enhances the entanglement coupling between NaHA molecules and retards the disentanglement of molecular chains or promotes to create hydrogen bonds, and then  $C^*$  for NaHA solutions with sucrose shifts to lower concentrations. This is in agreement with the results of light scattering measurements in the presence of 0.2M NaCl. Both the radius of gyration and hydrodynamic radius of NaHA were reduced in dilute solutions by the addition of sucrose, and added sucrose enhances the interaction between NaHA monomer units. In the case of concentrated NaHA solution, such interactions result to increase the storage and loss moduli because

of the enhancement of temporary network formation. © 1999 John Wiley & Sons, Inc.  
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**Keywords:** hyaluronic acid; dynamic viscoelasticity; steady shear viscosity; light scattering; salt effect; sucrose effect; coil overlap

## INTRODUCTION

Sodium hyaluronate (NaHA) is a linear polysaccharide consisting of disaccharide repeating sequence. The two saccharide-residues are D-glucuronic acid and N-acetyl-D-glucosamine, which are linked by  $\beta(1-3)$  and  $\beta(1-4)$  with each other.<sup>1–3</sup> NaHA is a major macromolecular component of the intercellular matrix of most connective tissues such as cartilage, eye vitreous humor, and synovial fluid. Especially for its prominent viscoelastic properties, hyaluronic acid (HA) acts as a lubricant and shock absorber in synovial fluid.<sup>4–6</sup> Typical molecular weight of HA in normal synovial fluids in human knees was reported as six million.<sup>7</sup> NaHA molecules show various unique solution properties characteristic to the polyelectrolytic nature and stiffness of molecular chains.

Balazs et al.<sup>7</sup> suggested that intraarticular application of highly purified (protein content <0.3%) concentrated (10–20 mg/mL) NaHA with high molecular weight of  $1-3 \times 10^6$  can influence the healing and regeneration of cartilage and soft tissues of joint. It is used as a medicine for arthritis and a surgical aid in ophthalmic surgery.<sup>7–9</sup>

The rheological characteristics of HA molecules that form entanglements and a temporary network in solution have been widely studied.<sup>10–12</sup> The critical concentration above which molecular coils overlap each other was found ca. 1.0 mg/cm<sup>3</sup>,<sup>11</sup> for HA extracted from rooster combs and with the number average molecular weight  $3.9 \times 10^5$ , the weight average molecular weight  $6.8 \times 10^5$ , the z-average molecular weight  $9.6 \times 10^5$ . Welsh et al.<sup>13</sup> reported that both storage shear modulus  $G'$ , which represents the elastic part of the complex modulus, and loss shear modulus  $G''$ , which represents the viscous part, decreased by disruption of molecular network structure on adding of  $\sim 60$  disaccharide units of HA segments, and neither very short chain segments ( $\sim 4$  disaccharides) nor longer segments ( $\sim 400$  disaccharides) did change the viscoelastic properties. They explained the decrease in  $G'$  and  $G''$  by the competitive inhibition. However, Fujii et al.<sup>14</sup> did not find the same result, and indicated that the shorter segments may also enhance the entanglements or promote the association of molecular chains rather than blocking the active sites that build up the transient network structure.

The rheological properties of synovial fluids from normal and diseased joints have been extensively studied by clinical researchers.<sup>7,15</sup> Balazs et al. pointed out that it is very important whether the crossover frequency  $f_c$  at which  $G'$  and  $G''$ , as a function of frequency, cross each other appears or not. They compared normal fluids with pathological fluids and indicated that  $f_c$  shifted to higher frequencies in synovial fluids of aged people and in pathological fluids (osteoarthritis, traumatic arthritis, gout, chondrocalcinosis, and rheumatoid arthritis), which has been caused by the rheological change of synovial fluid from elastic to more viscous behavior.

The flow properties of normal synovial fluid derived from human and cattle joints have been reported.<sup>5,7,16</sup> It is known that normal synovial fluids are shear thinning, showing a power-law behavior at high shear rates, and a constant zero-shear viscosity  $\eta_0$  at lower shear rates. The critical shear rate  $\dot{\gamma}_c$  is the reciprocal of a time constant and marks the onset of shear rate dependent behavior. It could be used as a diagnostic aid for synovial fluids, e.g., in the case of degenerative and chronic arthritis, the reciprocal of  $\dot{\gamma}_c$  becomes lower.<sup>4,16</sup>

The dependence of the polyelectrolyte character of HA on ionic strength has been studied by several groups.<sup>17,18</sup> Morris et al.<sup>10</sup> reported that the coil dimensions reduced with increasing ionic strength in dilute solutions of HA solutions at neutral pH due to the screening of electrostatic repulsion. Kobayashi<sup>19</sup> et al. found that  $G'$  and  $G''$  were decreased by the addition of NaCl, but increased by the addition of sucrose. The effects of the addition of chemical agents on the rheological properties are interesting problems. The hydration of HA is reported as 0.77 cm<sup>3</sup>/g,<sup>20</sup> which is far larger than that of other biopolymers. The rheological properties of biopolymer solutions are strongly influenced by the hydration state. Guanidine hydrochloride (GuHCl) is known as a protein denaturant and a hydrogen-bond breaker, and the addition of GuHCl as well as NaCl or sucrose will change the hydration state of HA. In the present work, dynamic and steady state viscoelastic measurements were performed for NaHA solutions in the presence of sodium chloride, guanidine hydrochloride, or sucrose. Light scattering measurements were also carried out to examine the chain dimension in the dilute solution,

which should be useful for understanding the origin of molecular forces responsible for the rheological properties of NaHA solutions.

## EXPERIMENTAL

### Materials

The samples of powdered HA were extracted from the culture medium of *streptococcus equi*, and lyophilized and then purified in the laboratory of Denki Kagaku Kogyo Co., Ltd. The molecular weights of three samples determined from the intrinsic viscosity using the parameters of the Mark–Houwink–Sakurada equation<sup>21</sup> are  $1.6 \times 10^6$ ,  $1.98 \times 10^6$ , and  $2.02 \times 10^6$ . Molecular weight distribution was estimated by gel permeation chromatography, and the ratio  $M_w/M_n$  was approximately two. Sodium chloride, guanidine hydrochloride, and sucrose of the reagent grade were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and used without further purification. The NaHA solutions of different polymer concentrations were prepared by dissolving in the respective solvents stirring for 20 h at room temperature. The concentration of the solution was determined taking into account of the equilibrium moisture content in the dessicator in the presence of P<sub>2</sub>O<sub>5</sub> (phosphorous pentoxide) at 60°C.

### Measurements

Dynamic and steady state viscoelastic measurements were performed by an RFSII (Rheometrics Fluids Spectrometer; Rheometrics, Inc., NJ, USA) with a cone and plate geometry. The diameter and angle of the cone were 2.5 cm and 0.1 rad, respectively. The strain was 5%, which is within linear viscoelastic regime, and angular frequencies ranged from 0.01 to 100 rad/s in the dynamic measurements. The shear rates ranged from 0.01 to 1000 s<sup>-1</sup> in the steady state measurements. The temperature was fixed at 5 ~ 20°C.

The intrinsic viscosity  $[\eta]$  was determined using an Ubbelohde capillary viscometer at  $25 \pm 0.02^\circ\text{C}$ . The flow time for water was ~ 141 s at 25°C.

Light scattering measurements were carried out using a homemade spectrometer<sup>22</sup> for the NaHA solution in the presence of sucrose in 0.2M NaCl solution. The light source was an Ar ion laser operated at 488.0 nm. The temperature of the sample solution was regulated within  $\pm 0.02^\circ\text{C}$ . The radius of gyration was determined using conventional scattered intensity measurements. Correlation functions of the scattered light intensity were obtained by an ALV-5000/E multiple-tau digital correlator. Since the added sucrose contributes to the correlation functions showing a weak peak with very fast decay rate, which is split clearly from the main peak due to NaHA, such a mode was excluded in the analyses. The hydrodynamic radius of NaHA was determined from the average decay rate of the main peak with slower decay rate and by the Stokes–Einstein equation. Corrections for the refractive index and the solvent viscos-

ity, which depend on the sucrose concentration, were made by use of Abbe's refractometer at 589 nm and capillary viscometer. The effect of the difference of wavelength for refractive index measurements from the light scattering measurements was neglected. Molecular dimensions were determined on the assumption that the molecular weight of NaHA does not change by the addition of sucrose.

## RESULTS

### Mechanical Spectra

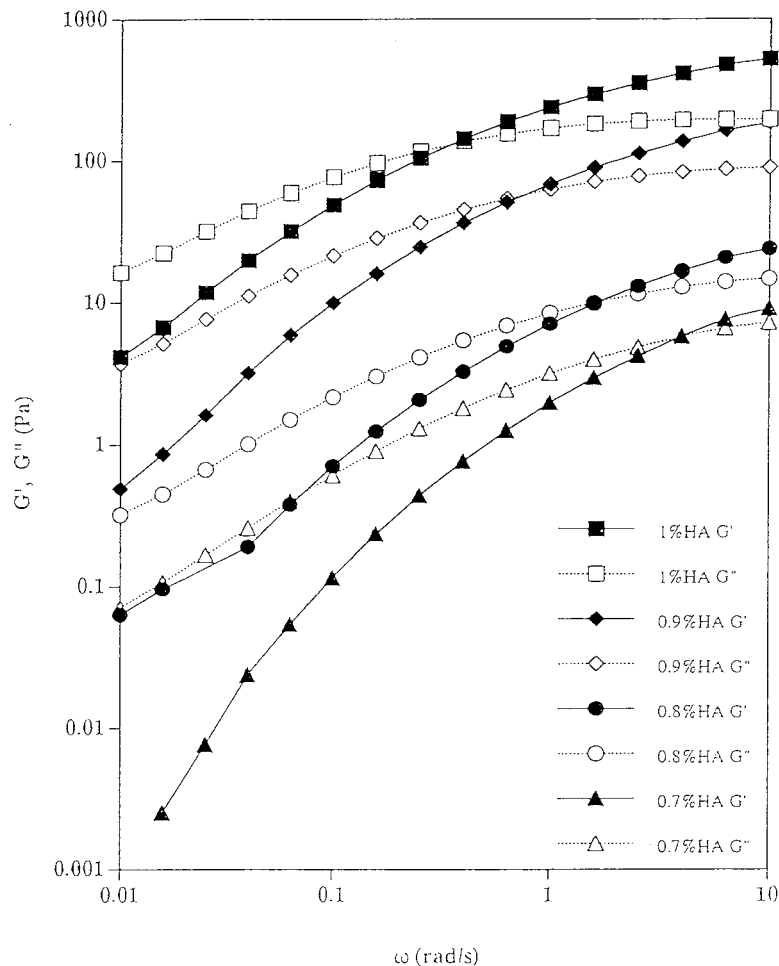
Figure 1 shows the angular frequency dependence of viscoelasticity for NaHA solutions of different concentrations. At lower angular frequencies, the loss modulus  $G''$  is larger than the storage modulus  $G'$ , and both moduli show a pronounced angular frequency dependence, and the solutions behave as viscous liquids. On the other hand, at higher frequencies,  $G'$  is predominant and both moduli show only limited frequency dependence, and the NaHA solutions show an elastic behavior. The rubber-like plateau region was not observed clearly in the accessible frequency range. This is in agreement with previous observations.<sup>10,11,13,19</sup>

Values of  $G'$  and  $G''$  for 1 wt % NaHA solutions with and without NaCl or GuHCl are shown as a function of angular frequency in Figures 2 and 3, respectively. Both storage and loss moduli were decreased by the addition of NaCl or GuHCl, and  $f_c$  shifted to higher angular frequencies with increasing concentration of the added NaCl or GuHCl. The values of  $f_c$  are shown in Table I.

The angular frequency dependence of  $G'$  and  $G''$  for 1 wt % NaHA solutions with and without sucrose in water and in 0.2M NaCl are shown in Figures 4(a) and (b), respectively. Contrary to the situations observed for NaHA solutions in the presence of NaCl or GuHCl, the addition of sucrose increased both moduli and shifted the curves to lower frequencies (shift of  $f_c$  to lower frequencies). The addition of sucrose affected the entanglement structure of NaHA. The plateau region of  $G''$  was observed. The plateau region that appeared only at higher angular frequencies for solutions with low sucrose content tended to appear even at lower frequencies for solutions with high sucrose content.

### Flow Behaviors

The viscosity of NaHA solutions was independent of shear rate at sufficiently low shear rates, i.e., NaHA solutions showed a Newtonian behavior in a restricted range of shear rate. The viscosity that is independent



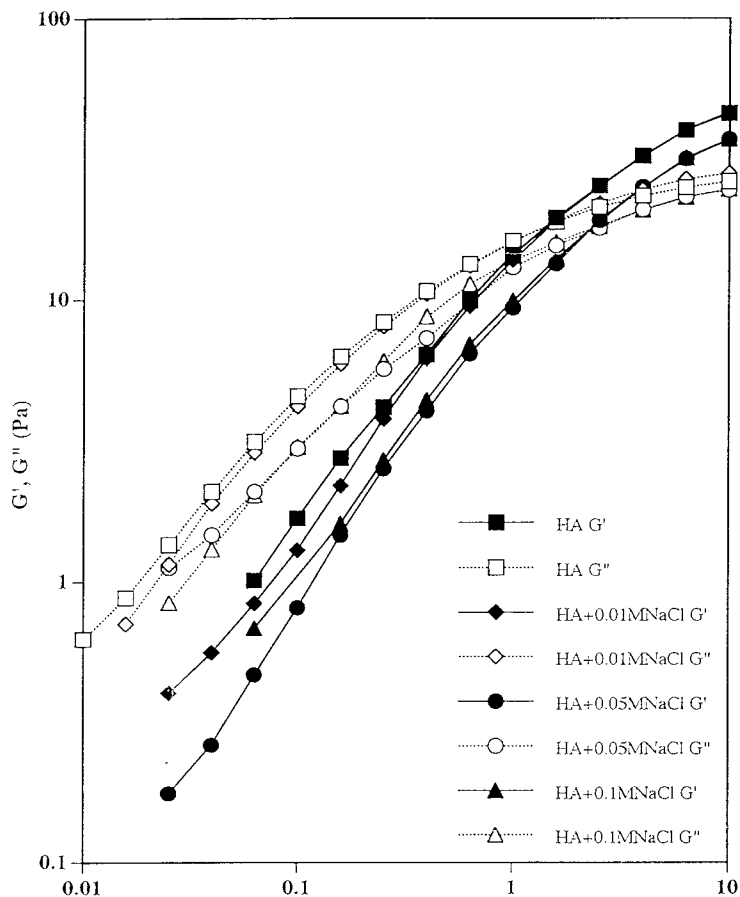
**FIGURE 1** The angular frequency dependence of  $G'$  and  $G''$  for NaHA solutions of various concentrations. Temperature:  $5^{\circ}\text{C}$ ; molecular weight:  $1.98 \times 10^6$ .

of shear rate at very low shear rates is called zero shear viscosity hereafter. The viscosity began to decrease above a certain shear rate, the critical shear rate  $\dot{\gamma}_c$ , i.e., NaHA solutions showed a shear thinning behavior as reported by other workers.<sup>27</sup>

Figures 5 and 6 show the concentration dependence of zero shear specific viscosity for NaHA solutions with and without NaCl or GuHCl, respectively. With increasing concentration of NaCl or GuHCl, the zero shear specific viscosity for NaHA solutions decreased, and the curves shifted downward appreciably. Here, the zero shear specific viscosity was used to normalize the effect of solvent viscosity. Double logarithmic plots of concentration ( $C$ ) and zero shear specific viscosity value ( $\eta_{sp0}$ ) for NaHA solutions showed a pronounced change of slope at a critical concentration ( $C^*$ ) as shown in Figures 5 and 6.

The critical concentration for NaHA solutions was estimated  $C^* \sim 1\text{--}2$  mg/mL ( $0.1 \sim 0.2$  wt %),<sup>7,11</sup> as mentioned in the Introduction, which should depend on the molecular weight, and should shift to lower concentrations with increasing molecular weight. The terms “concentrated” and “dilute” solution will be used hereafter to refer to polymer concentrations above and below  $C^*$ , respectively. NaHA molecules are essentially free to move individually below  $C^*$ , and start to overlap with each other and form a transient network structure above  $C^*$ .

The overlap concentration  $C^*$ , defined in this way, for NaHA solutions with NaCl or GuHCl, was higher than that for NaHA solutions without NaCl or GuHCl, and shifted to higher polymer concentrations with increasing concentration of added NaCl or GuHCl. On adding sodium ions or guanidinium ions, the electrostatic repulsion between NaHA molecules is



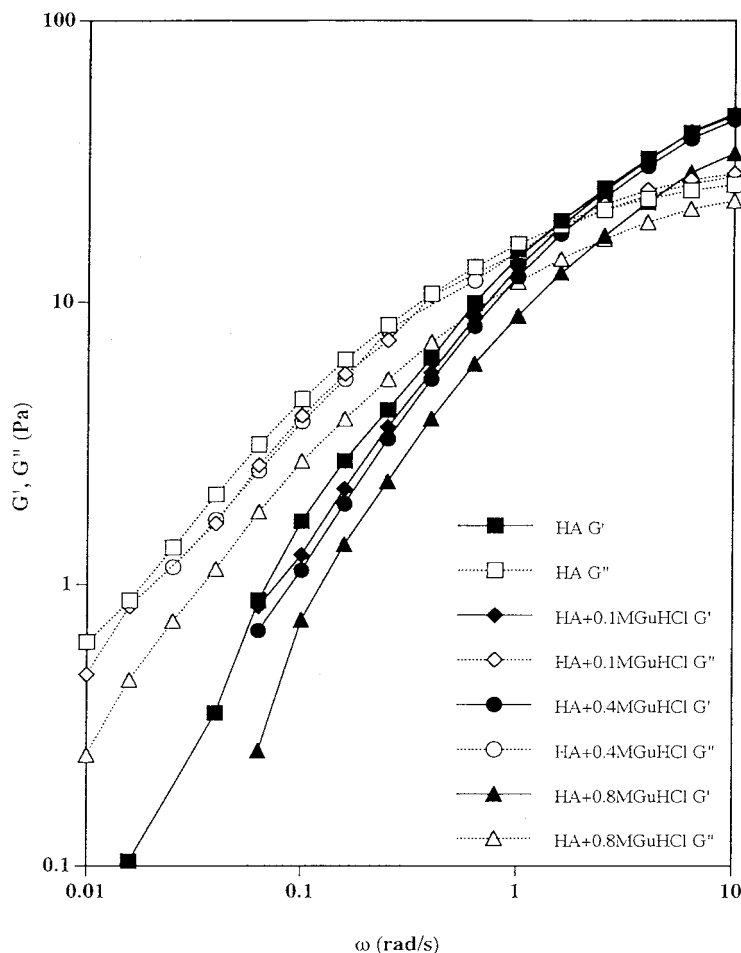
**FIGURE 2** The angular frequency dependence of  $G'$  and  $G''$  for 1 wt% NaHA solutions with and without NaCl of various concentrations. Temperature: 20°C; molecular weight:  $2.02 \times 10^6$ .

shielded and the chain dimension of NaHA molecules is reduced. Therefore, the overlap concentration  $C^*$  shifts to higher concentrations of NaHA.

The behavior of zero shear specific viscosity with the addition of sucrose has been examined both in water and in 0.2M NaCl solution, and has been found to be quite different from that with NaCl or GuHCl. Figure 7 depicts the concentration dependence of zero shear specific viscosity for NaHA with and without sucrose in 0.2M NaCl solution.  $C^*$  for NaHA solution shifted to lower concentrations distinctly with the addition of sucrose both in water (data not shown) and in 0.2M NaCl solution. At very low concentrations, values of  $\eta_{sp0}$  for NaHA solutions with sucrose were smaller than that for NaHA solution without sucrose in 0.2M NaCl solution as shown in Figure 7. A similar tendency was found for the effect of sucrose in the absence of NaCl; values of  $\eta_{sp0}$  for NaHA solutions with sucrose (0.5 and 0.75M) were smaller than that for NaHA solution without sucrose at a very low concentration range of NaHA (data not shown).

In attempting to investigate the effect of the addition of NaCl, GuHCl, or sucrose on NaHA molecular interaction, the zero shear specific viscosity was plotted as a function of the overlap parameter  $C[\eta]$ .<sup>27</sup> According to the Flory–Fox relationship, relatively stiff and extended chains should begin their entanglement behavior at  $C[\eta]$  close to 1.5.<sup>23</sup>

The dimension of polyelectrolytes with random coil conformation is expanded by electrostatic repulsion between chain segments. As is well known,<sup>28</sup> the intrinsic viscosity is related to the volume of the polymer molecule. The reduced viscosity of the NaHA solutions as a function of NaHA concentration without NaCl showed a steep upturn with decreasing concentration of NaHA as has been reported for many polyelectrolyte solutions,<sup>29,30</sup> and has been attributed to the expansion of molecular coils at very dilute region. The intrinsic viscosity could be determined by the conventional extrapolation of the reduced viscosity to zero concentration in the presence of NaCl. The intrinsic viscosity decreased with increasing concen-



**FIGURE 3** The angular frequency dependence of  $G'$  and  $G''$  for 1 wt% NaHA solutions with and without GuHCl of various concentrations. Temperature: 20°C; molecular weight:  $2.02 \times 10^6$ .

tration of added NaCl. Balazs and Laurent<sup>31</sup> have reported a similar experimental result. With increasing concentration of the added sodium chloride, the

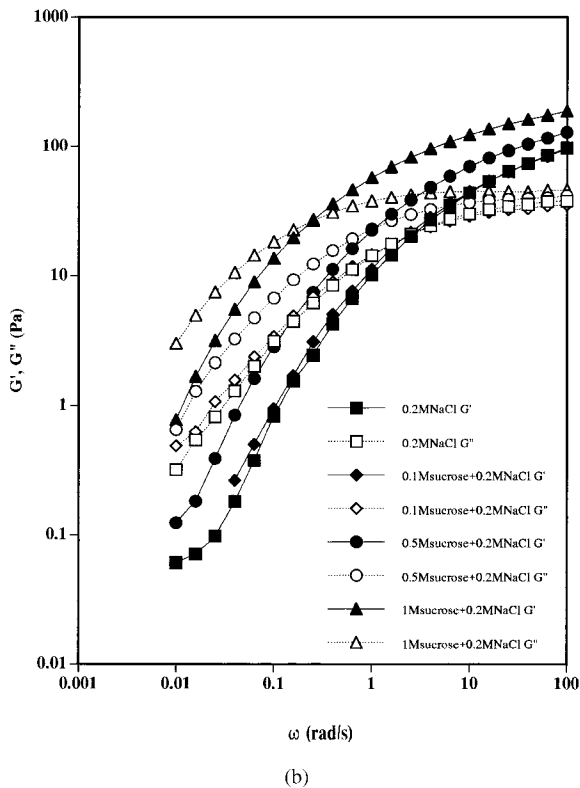
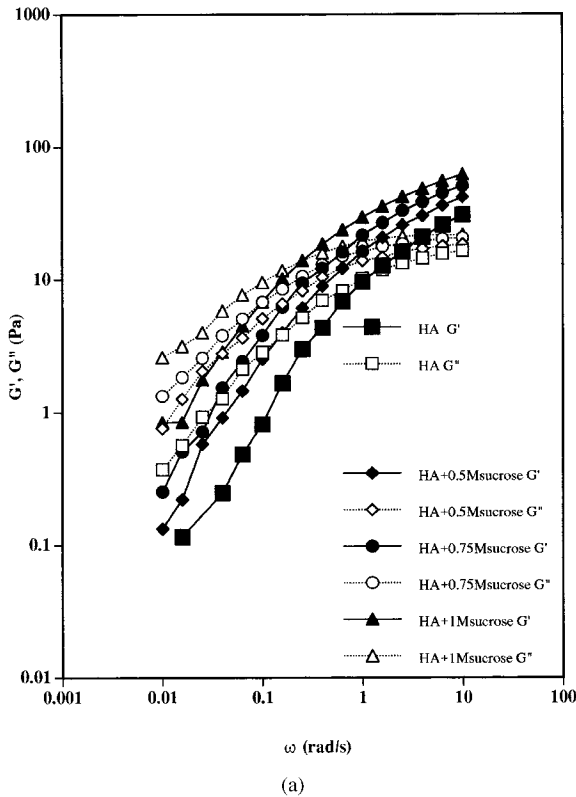
**Table I** The Crossover Frequency  $f_c$  (rad/s) of  $G'$  and  $G''$  for 1 wt % HA with NaCl, GuHCl, Sucrose, and Sucrose in the Presence of 0.2M NaCl

$c$ (M)	NaCl	GuHCl	Sucrose	Sucrose + 0.2M NaCl
0	1.4544			
0.01	1.5292			
0.05	2.2966			
0.1	2.3435	1.4115	1.0652	2.1905
0.2	2.8313			
0.4		1.877		
0.5			0.6528	1.0888
0.75			0.5453	
0.8		2.3796		
1			0.2528	0.2425

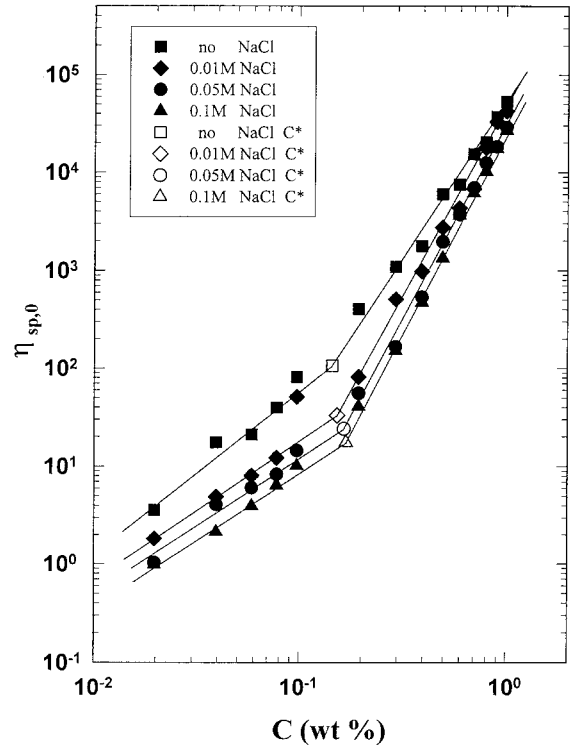
step increase of the reduced viscosity disappeared. Sodium ions may shield the electrostatic repulsion between polyelectrolytic molecular coils. Table II shows the values of intrinsic viscosity  $[\eta]$  and Huggins constant  $k'$  of NaHA solutions with NaCl, GuHCl, or sucrose in the presence of 0.2M NaCl. With increasing concentration of NaCl or GuHCl, the intrinsic viscosity  $[\eta]$  decreased. The addition of a small quantity of sucrose (0.1M) in the presence of 0.2M NaCl decreased the intrinsic viscosity, while the further addition (0.5 and 1.0M) recovered the same value without sucrose.

In all cases of the addition of NaCl, GuHCl, or sucrose, the superposition is well performed and a master curve was obtained. As shown in Figures 8 and 9, the onset of coil overlap of NaHA molecules in the presence of NaCl or GuHCl occurred at a  $C[\eta]$  value of about 4.6 or 2.4, respectively. On adding sucrose in the presence of 0.2M NaCl,  $C^*[\eta]$  was about 3.4 (Figure 10).





**FIGURE 4** (a) The angular frequency dependence of  $G'$  and  $G''$  for 1 wt% NaHA solutions with and without sucrose of various concentrations. Temperature: 20°C; molecular

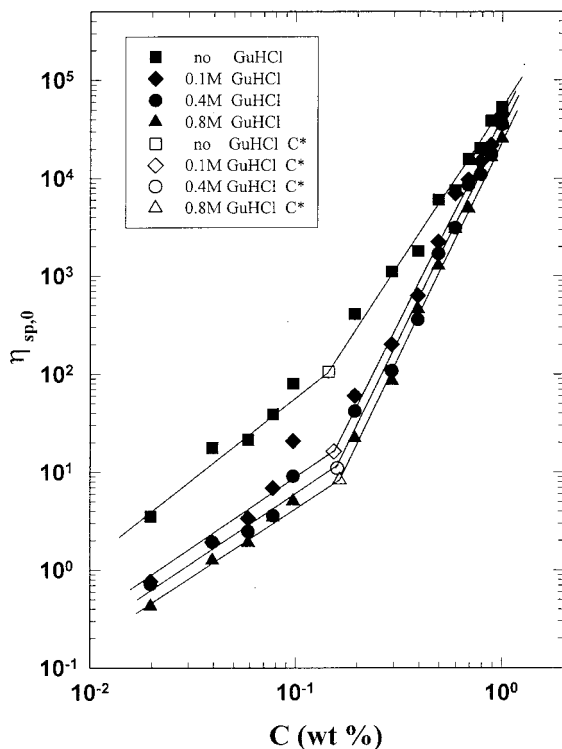


**FIGURE 5** Zero shear specific viscosity for NaHA solutions with and without NaCl as a function of the NaHA concentration. Temperature: 20°C; molecular weight:  $2.02 \times 10^6$ .

## DISCUSSION

The behavior shown in Figure 1 was explained by assuming that the entanglement of molecular chains are transient rather than permanent. At lower angular frequencies, molecular chains disentangle during the long period of oscillation; however, at higher angular frequencies, NaHA molecules cannot disentangle during short period of oscillation and form a temporary network structure. Therefore, the solutions change from predominantly liquid-like behavior at low frequencies to solid-like behavior at higher frequencies. The critical frequency  $f_c$ , at which  $G'$  and  $G''$  cross each other, shifts to lower frequencies with increasing NaHA concentration as expected from the increase of entanglements. The frequency dependence of  $G'$  and  $G''$  of 1% NaHA solution at 5°C (Figure 1) and at 20°C (Figure 2) is quite different:  $f_c$  shifted to a much

weight:  $1.6 \times 10^6$ . Figure 4(b) The angular frequency dependence of  $G'$  and  $G''$  for 1 wt% NaHA solutions with and without sucrose of various concentrations in 0.2M NaCl solutions. Temperature: 20°C; molecular weight:  $1.6 \times 10^6$ .



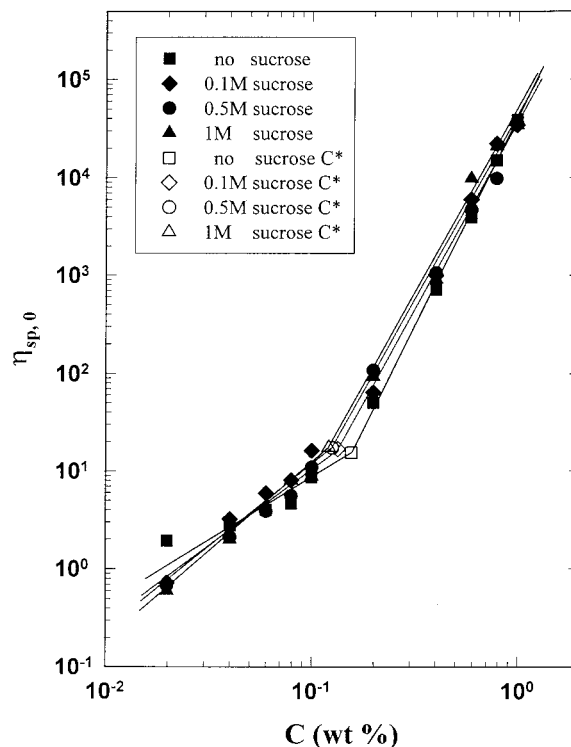
**FIGURE 6** Zero shear specific viscosity for NaHA solutions with and without GuHCl as a function of the NaHA concentration. Temperature: 20°C; molecular weight:  $2.02 \times 10^6$ .

higher frequency with increasing temperature, and both moduli became much more frequency dependent. It is caused by the activation of molecular motion with increasing temperature, and thus the increase in the probability of the disentanglement, which leads to the more liquid-like behavior. Kobayashi et al.<sup>19</sup> compared  $G'$  and  $G''$  as a function of angular frequency for different molecular weights and concentrations. They found that  $G'$  and  $G''$  for NaHA solutions increased and  $f_c$  shifted to lower angular frequencies with increasing molecular weight or concentration of NaHA.

The effect of NaCl is in agreement with the reported results<sup>19</sup> and is explained by the charge screening; electrostatic repulsion between carboxyl groups is suppressed by sodium ions and the coil dimension of NaHA molecules is reduced. The overall behavior of NaHA solutions affected by the addition of GuHCl is the same as that by NaCl, although GuHCl is a disrupting agent of hydrogen bonds and a protein denaturant. It has been reported before for gellan gum solution that a small amount of guanidinium ions shields the electrostatic repulsion between carboxyl groups in gellan gum molecules and increases the number of junction zones; however, an excessive ad-

dition of GuHCl decreases it by the disruption of hydrogen bonds.<sup>24</sup> The effects of GuHCl on the viscoelasticity of  $\kappa$ -carrageenan gels were also explained by the same mechanism: the addition of a small amount of GuHCl increased storage and loss Young's moduli  $E'$  and  $E''$  of  $\kappa$ -carrageenan gels; however, further addition of GuHCl decreased both moduli. The increase was understood by the electrostatic shield of the repulsion between sulfate groups in  $\kappa$ -carrageenan molecules, and the decrease was explained by the disruption of hydrogen bonds.<sup>25</sup> For the GuHCl concentrations used in the present work, the effect of GuHCl on the conformation of NaHA molecules is to shield the electrostatic repulsion and to reduce the chain dimension rather than to disrupt hydrogen bonds. In other words, hydrogen bonding between NaHA molecules should not contribute so much to the viscoelasticity. This should be, however, explored in more detail in the future.

Zero shear specific viscosity expressed by the overlap parameter showed good superposition for various salt concentrations (NaCl or GuHCl), as shown in Figures 8 and 9. The dependence of zero shear



**FIGURE 7** Zero shear specific viscosity for NaHA solutions with and without sucrose in 0.2M NaCl solutions as a function of the NaHA concentration. Temperature: 20°C; molecular weight:  $1.6 \times 10^6$ . The straight line for the data points without sucrose in 0.2M NaCl has a slope of 1.3 in agreement with the results of Figures 5 and 8.

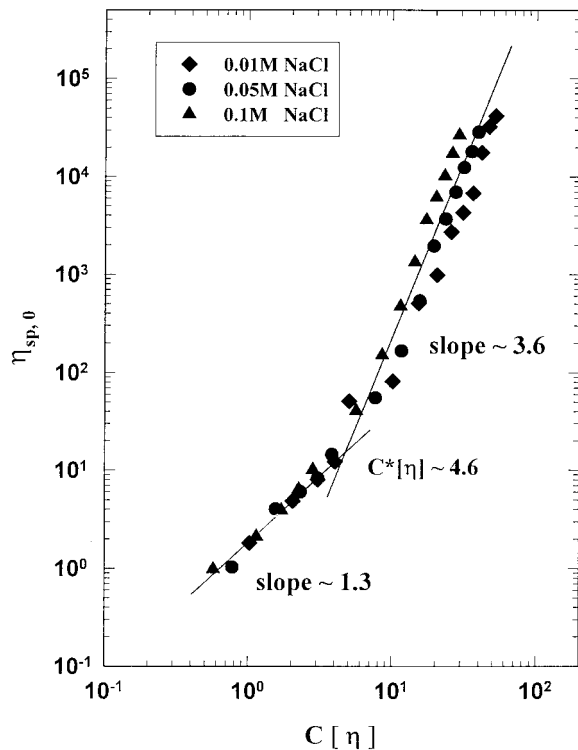
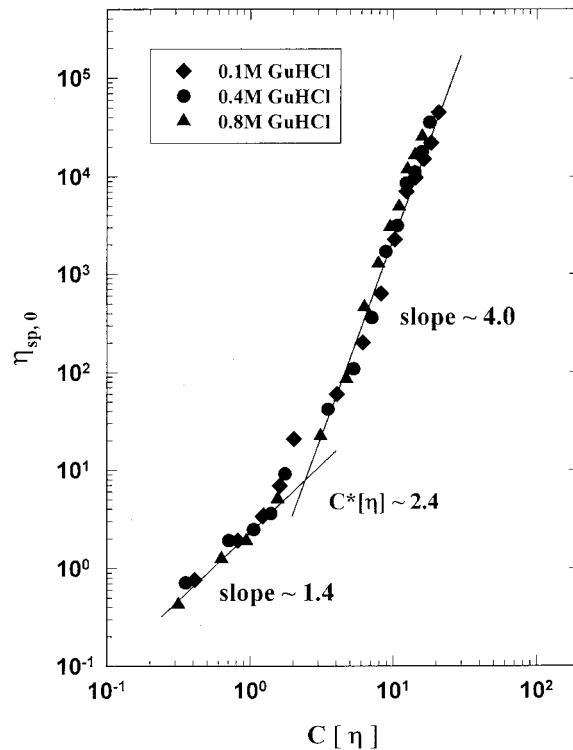


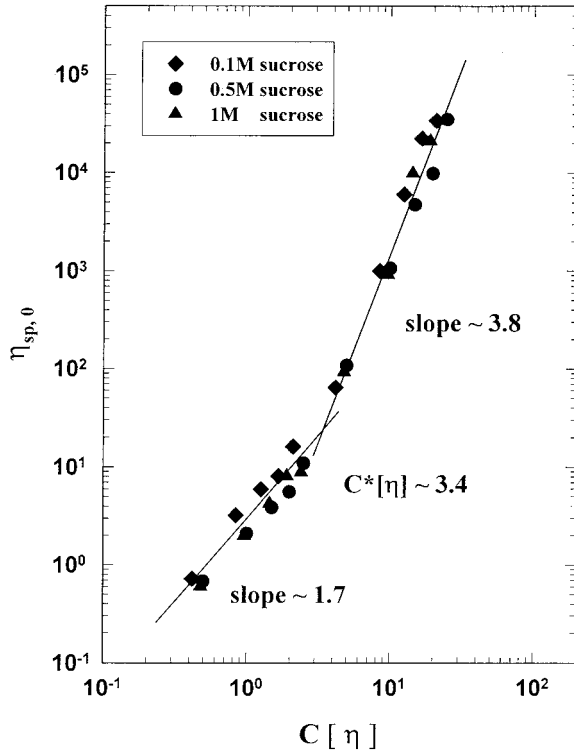
**Table II** Intrinsic Viscosity  $[\eta]$  and Huggins Constant  $k'$  of NaHA Solution with NaCl, GuHCl, and Sucrose of Different Concentrations

$M$	NaCl				GuHCl				Sucrose + 0.2M NaCl		
	0.01	0.05	0.1	0.2	0.01	0.1	0.4	0.8	0.1	0.5	1
$[\eta]$ (dL/g)	52	39.4	29	25	39.5	20.8	18	16	21	25	24
$k'$	1	0.63	0.45	0.37	0.38	0.17	0.18	0.14	0.263	0.655	0.396

specific viscosity on NaHA concentration in the absence of salts deviates significantly from that in the presence of salts. This suggests that the shielding of the electrostatic interaction is an essential factor for the viscoelastic behavior of HaHA in the presence of salts. The slope of the straight line obtained in the double logarithmic plot of the zero shear specific viscosity  $\eta_{sp,0}$  and the polymer concentration  $C$  at a concentration range below  $C^*$  is 1.3 and 1.4 on the addition of NaCl and GuHCl, respectively, which is close to the observed values for many flexible polymer solutions. At concentrations above  $C^*$  ( $\sim 0.1$  wt %), NaHA molecular chains start to overlap with each other and form a transient network structure. The slopes of the straight lines in the concentrated regime

of the double logarithmic plot of  $\eta_{sp,0}$  and  $C$  are 3.6 (NaCl) and 4.0 (GuHCl), which are close to the theoretical prediction  $15/4$ .<sup>26</sup> The value of  $C^*[\eta]$  in the presence of NaCl or GuHCl is  $2.4 \sim 4.6$ , as shown in Figures 8 and 9. These values of exponents  $1.3 \sim 1.4$  and  $3.6 \sim 4.0$ , and  $C^*[\eta]$  in the presence of NaCl or GuHCl determined in dilute and concentrated regimes are in good agreement with those reported by Fouissac et al.<sup>27</sup> Although the present values of  $3.6 \sim 4.0$  are in good agreement with the results of Fouissac et al.,<sup>27</sup> they are a little larger than those found in the melts and concentrated solutions of flexible polymers for which the exponent values of  $3\text{--}3.4$  have been observed experimentally and predicted theoretically, and a definite reason is still open to the future studies.

**FIGURE 8** Zero shear specific viscosity for NaHA solutions with NaCl as a function of coil overlap parameter  $C[\eta]$ . Temperature: 20°C; molecular weight:  $2.02 \times 10^6$ .**FIGURE 9** Zero shear specific viscosity for NaHA solutions with GuHCl as a function of coil overlap parameter  $C[\eta]$ . Temperature: 20°C; molecular weight:  $2.02 \times 10^6$ .



**FIGURE 10** Zero shear specific viscosity for NaHA solutions with sucrose in  $0.2M$  NaCl as a function of coil overlap parameter  $C[\eta]$ . Temperature:  $20^\circ\text{C}$ ; molecular weight:  $1.6 \times 10^6$ .

The addition of sucrose develops the transient network structure of NaHA solution because sucrose increases the viscosity of the solvent (water), and retards the disentanglements of NaHA molecular chains, or the transient network structure is strengthened by the promotion of hydrogen bonding by sucrose via polymer–polymer or polymer–sucrose. The presence of  $0.2M$  NaCl in the solvent seems to enhance the effect of sucrose, e.g., a larger increase of moduli and a shift of  $f_c$  to lower frequencies. The shielding of electrostatic repulsion by sodium ions results in an enhanced effects of sucrose in  $0.2M$  NaCl solution.

The effect of sucrose on the chain dimensions of NaHA molecules in  $0.2M$  NaCl was also examined by

light scattering in the dilute regime. The molecular weight was determined as  $2.26 \times 10^6$  in  $0.2M$  NaCl without sucrose. The NaHA sample for light scattering measurements was a different one from those used for viscoelastic measurements. As is shown in Table III, both the radius of gyration  $R_g$  and hydrodynamic radius  $R_h$  decreased rapidly by the addition of sucrose (even at the addition of  $0.1M$  sucrose). However, the ratio  $R_g/R_h$  increased distinctly at the same time. This ratio relates to the solvent permeability or segment distribution of NaHA molecules. That is, the addition of sucrose might result in a change of segment distribution or conformation of NaHA molecules by the enhancement of interaction between NaHA monomer units. It is possible that hydrogen bonding occurs in the polymer chain via sucrose molecules, and it causes the shrinkage of chain dimension accompanied by the change of segment distribution. When the polymer concentration increases and polymer chains become too entangled, such an interaction takes place also among different NaHA molecular chains. Therefore, the addition of sucrose strengthens the temporary network structure. This picture is not well verified enough at the present stage, and should be explored by the other experiments in the near future.

Zero shear specific viscosity as a function of the overlap parameter again showed a good superposition as shown in Figure 10. The slope of the zero shear specific viscosity on the addition of sucrose in  $0.2M$  NaCl solution in the concentrated regime was 3.8, which is in good agreement with those obtained on the addition of NaCl or GuHCl. The value of  $C^*[\eta]$  3.4 was consistent with the cases in the presence of NaCl and GuHCl. However, the slope in the dilute region was 1.7, showing an appreciable increase than those in the presence of NaCl and GuHCl (Figures 8 and 9). Especially, the values of  $\eta_{sp,0}$  for NaHA solutions with sucrose were smaller than that for NaHA solution without sucrose in  $0.2M$  NaCl at very low concentrations. These facts suggest that the viscoelastic behavior of NaHA in the concentrated regime is quite similar to that in the usual (semi)flexible polymer solutions governed by the entanglement structure, but the situation in the dilute region should be different.

**Table III** The Radius of Gyration  $R_g$  and the Hydrodynamic Radius  $R_h$  of HA Determined by Light Scattering in the Presence of Sucrose and  $0.2M$  NaCl

Sucrose ( $M$ )	0	0.1	0.2	0.3	0.5	1
$R_g$ (nm)	186	170	161	152	147	155
$R_h$ (nm)	113	70.1	66.9	65.6	64.2	67
$R_g/R_h$	1.65	2.43	2.41	2.32	2.29	2.31

This feature of zero shear specific viscosity as well as the dynamic viscoelastic behaviors ( $G'$  and  $G''$ ) is explained by the decrease in coil dimensions of NaHA molecules and by an enhanced interpolymer interaction on the addition of sucrose. The larger exponent value of 1.7 in the dilute region corresponds to the increasing effect of such an interaction. With increasing concentration of NaHA, the interaction between NaHA molecules also becomes more effective simultaneously with the intersegmental interaction in a single polymer chain. In fact, the  $C^*$  values decreased with increasing concentration of the added sucrose. Details of the chain conformation and dimension in water without NaCl is unfortunately unknown, because light scattering measurements without salt was practically difficult. However, we can safely presume that the effect of sucrose without NaCl is essentially similar on the analogy of Figures 4(a) and (b), and an enhanced interpolymer interaction causes the increase of  $\eta_{sp0}$  and the effective decrease of the overlap concentration.

As observed in various polymer solutions, NaHA solutions show a Newtonian behavior only at low shear rates, and they are shear thinning at higher shear rates.<sup>27,31–34</sup> At low shear rates, intermolecular entanglements for concentrated solution disrupted by the imposed deformation are replaced by new interactions between different partners; however, with increasing shear rate, the rate of externally imposed movement becomes greater than the rate of formation of new entanglements and the extent of reentanglement decreases.<sup>35</sup> The viscosity ( $\eta$ ) remains constant at a maximum value (the “zero-shear” viscosity,  $\eta_0$ ) in Newtonian region, but decreases in non-Newtonian region. The non-Newtonian flow behavior (“pseudoplasticity”) of polymer solutions is often fitted to a power law:

$$\eta \propto \dot{\gamma}^{-n}$$

where  $n$  is called the pseudoplasticity index and corresponds to the slope of the curve in the shear-dependent viscosity domain. The critical shear rate ( $\dot{\gamma}_c$ ) corresponds to the onset of the shear-thinning behavior.

Pseudoplasticity index  $n$  as a function of the overlap parameter  $C[\eta]$  for NaHA solutions with different concentration of NaCl, GuHCl, and sucrose were compared. Graessley<sup>35</sup> pointed out that the pseudoplasticity index  $n$  for flexible polymers such as polystyrene and poly( $\alpha$ -methyl styrene) approached 0.8 for  $C[\eta] > 20$ . In the case of weak gels,  $n$  is found to be higher than 0.9.<sup>33</sup> In the present experiment, on

adding sucrose in the presence of 0.2M NaCl,  $n$  for NaHA solutions approached 0.8 for  $C[\eta] > 20$ . This observation is in parallel with the picture that the addition of sucrose enhances the interpolymer interaction and might not be the one like an gelling agent.

## CONCLUSION

NaHA molecular chains are relatively stiff and extended. NaCl and GuHCl shield the electrostatic repulsion of carboxyl groups in NaHA molecules, and reduce the dimension of molecular coils. The effect of sucrose on dilute NaHA solutions is different from that on concentrated solutions. In dilute solution, sucrose reduces the coil dimension and therefore decreases zero shear specific viscosity; however, in concentrated solution, it promotes to form a transient network of NaHA molecules.

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