

Entanglement Network of Chitin and Chitosan in Ionic Liquid Solutions

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ABSTRACT: Concentrated solutions of a chitin from squid pens and of two commercial samples of chitosan were successfully prepared by using an ionic liquid 1-butyl-3-methylimidazolium acetate as a solvent. The dynamic viscoelasticity data for the solutions exhibited rubbery plateaus, indicating the existence of entanglement network of chitin and chitosan in the solutions. To characterize the network, the values of the molecular weight between entanglements (M_e) for chitin and chitosan in the solutions were determined from the plateau moduli. Then the values of M_e in the molten state ($M_{e,melt}$), a material constant reflecting the inherent nature of polymer species, for chitin and chitosan were estimated to be 1.7×10^3 and 3.0×10^3 , respectively. It was found that there was a significant difference in $M_{e,melt}$ between chitin and chitosan. Compared with other polysaccharides such as cellulose and agarose in terms of the number of monosaccharide units between entanglements (N_{unit}), chitin had significantly smaller N_{unit} of 8, while chitosan had equivalent N_{unit} of 19. © 2013 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* 130: 2439–2443, 2013

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INTRODUCTION

Chitin is an amino polysaccharide existing in fungi, plants, and animals,^{1,2} and has been expected as a promising material because of a variety of biomasses.³ Chitosan is also an important polysaccharide easily obtained through deacetylation treatment of chitin. At present chitosan rather than chitin is widely applied to biomedical uses, due to the good solubility to conventional solvents.^{4–7} Improving solubility as well as processability is a key to extend the field of application of the polymers, and the knowledge on rheology is needed to achieve the good processability. Therefore, the object of this study is to find a method for preparing concentrated solutions of chitin and chitosan and to examine their rheological properties. For melts and concentrated solutions, the entanglement coupling originating from the uncrossability that a real polymer chain cannot pass through other chains plays an important role.^{8–11} How many entanglements there are in a system is discussed in terms of the molecular weight between entanglements (M_e). The quantity M_e is a measure of the spacing between two neighboring entanglements and depends on the polymer concentration, but M_e in the molten state ($M_{e,melt}$) becomes a material constant reflecting the inherent nature of polymer species.^{8,9} Both chitin and chitosan are important natural polymers, but the values of $M_{e,melt}$ for the polymers are still unknown.

Some ionic liquids have recently been used as solvents for polysaccharides so far insoluble to conventional solvents.^{12–17}

Actually, we have obtained concentrated solutions of various polysaccharides using an ionic liquid 1-butyl-3-methylimidazolium chloride (BmimCl) as a solvent. The entanglement coupling has been observed as a rubbery plateau on the dynamic viscoelasticity curves and $M_{e,melt}$ has been estimated for the polysaccharides, although actual melts are unavailable.^{18–23} Concerning chitin and chitosan there exists no information on M_e until now. In this study, rheological properties of solutions of chitin and chitosan were investigated. The values of M_e for chitin and chitosan have been estimated, and the difference in $M_{e,melt}$ between chitin and chitosan has also been discussed.

EXPERIMENTAL

Materials

Chitin was obtained from squid pens according to the procedure reported in the literature.²⁴ The squid pens (kindly gifted by Yaegaki Bio-industry, Japan) were first ground into granule with an electric mill. The granular chitin was stirred with 1M hydrochloric acid overnight at room temperature and was washed with water. Then, the crude chitin was treated with 2M sodium hydroxide overnight at room temperature and was washed again with water, followed by the stirs with 2M sodium hydroxide at 100°C for 4 h. The product was washed with water repeatedly until the used water became neutral, and was finally dried in a vacuum oven at 100°C for 3 days. The degree of deacetylation (d_{DA}) for thus obtained chitin was apparently calculated to be -0.08 on the basis of the nitrogen content

determined by elemental analysis, indicating that $d_{DA} \approx 0$. Namely, almost all amino groups in the chitin were considered to take the acetylated form. The molecular weight (MW) of the chitin is not available at present due to the poor solubility to conventional solvents at moderate conditions. Concerning chitosan, two commercial samples of purified grade, purchased from Aldrich (lot STBC6204V, $d_{DA} = 0.68$, Viscosity average MW: 1.4×10^5 – 2.2×10^5 ; chitosan A) and Polysciences (lot 617922, $d_{DA} = 0.95$, MW $\sim 1.5 \times 10^4$; chitosan P), were used without further purification. Despite the nominal MW value, the weight average MW and the number average MW of chitosan P measured by gel permeation chromatography on the basis of pullulan were 3.0×10^5 and 1.0×10^5 , respectively.

To prepare concentrated solutions of chitin, several kinds of ionic liquids were examined as a preliminary experiment. Among the solvents 1-butyl-3-methylimidazolium acetate (BmimAc) gave clear solutions at higher concentrations but at the polymer concentration of 11 wt % the solution became unclear, so that BmimAc was not so good for chitin. BmimAc used here was a product of BASF (Germany); the melting point below -20°C and the density of $1.055 \times 10^3 \text{ kg m}^{-3}$ at room temperature have been reported by the manufacturer.²⁵ Concentrated solutions of chitin and chitosan were prepared in the following way. The powdery polysaccharide was added to BmimAc in a dry glass vessel, and the mixture was quickly stirred with a stainless spatula on a hot plate around 100°C for chitin, and around 80°C for chitosan. Then, the vessels were kept at the temperatures for about 1 day. The concentration of the polysaccharides (c) ranged from 3.2×10^1 to $7.4 \times 10^1 \text{ kg m}^{-3}$ (3–7 wt %) for chitin, and from 3.2×10^1 to $1.1 \times 10^2 \text{ kg m}^{-3}$ (3–11 wt %) for chitosan. In the calculation of c , $1.0 \times 10^3 \text{ kg m}^{-3}$ was assumed for the density of melts of chitin and chitosan, and the reported value of $1.055 \times 10^3 \text{ kg m}^{-3}$ was used for the density of BmimAc.

Measurements

Viscoelasticity measurements were carried out with an ARES rheometer (now TA Instruments) under a nitrogen atmosphere. A cone-plate geometry with a diameter of 25 mm and a cone angle of 0.1 rad. was used. The frequency (ω) dependence of the storage modulus (G') and the loss modulus (G'') for the solutions (including the pure solvent) was measured with a strain amplitude (γ) of 0.1. The temperature (T) ranged from 0 to 100°C . To minimize the effect of moisture, the rheological measurements were made just after finishing the sample preparation. For the pure solvent, BmimAc steady shear flow measurements as well as the dynamic viscoelasticity measurements were performed. The steady shear flow measurements were made at the shear rate ($\dot{\gamma}$) of 0.1 to 10 s^{-1} in the T range of 0 to 60°C . The zero-shear viscosity (η_0) from the dynamic viscoelasticity data was determined by

$$\eta_0 = \lim_{\omega \rightarrow 0} \frac{G''}{\omega} \quad (1)$$

RESULTS AND DISCUSSION

Figure 1 shows $\log \eta_0$ of the neat BmimAc plotted against $1/T$. As is evident, η_0 obtained by the steady flow and dynamic

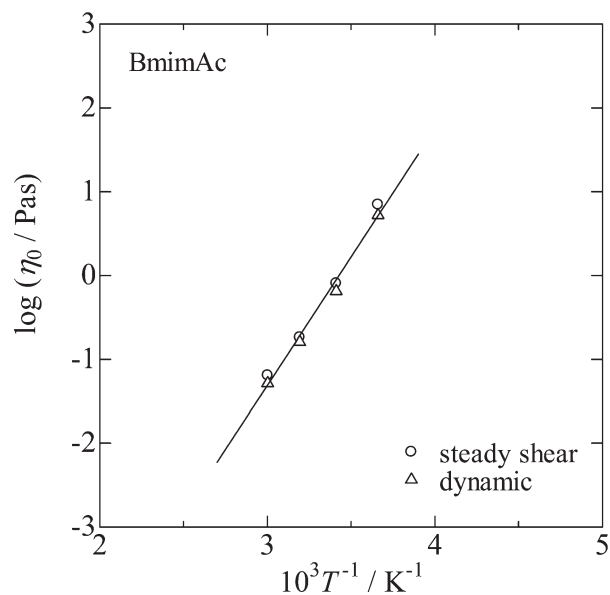


Figure 1. Plot of $\log \eta_0$ for BmimAc obtained from steady shear flow measurements and dynamic viscoelasticity measurement against the reciprocal of T .

viscoelasticity measurements coincides very well at every temperature measured. The line in the figure is the best fit for the data points. All data points appear to fall on the line. If we assume that η_0 obeys

$$\eta_0 = h \exp\left(\frac{\Delta E}{RT}\right) \quad (2)$$

with h and R being a constant and the gas constant, respectively, then the best fit line gives the activation energy of flow (ΔE) of 59 kJ mol^{-1} . We also obtained the value of η_0 at 80°C of $1.4 \times 10^{-2} \text{ Pa s}$ from the best fit line. As will be shown later, this value is much smaller than those of the chitin and chitosan solutions at the same temperature, indicating that the contribution of solvent to G' of the polysaccharide solutions is safely negligible.

Figure 2 shows the ω -dispersion curves of G' and G'' for the chitin solutions. Here, the reference temperature (T_r) is chosen to be 80°C , and a_T stands for the horizontal shift factor. The curves with different c are shifted upwards by a factor of A to avoid overlapping. It is seen that the time–temperature superposition principle holds very well for all G' and G'' curves, indicating that the chitin–BmimAc systems used here are certainly homogeneous solutions. The master curves in Figure 2 have two characteristic domains: the flow zone and the rubbery plateau region (the region of ω where $G' > G''$). The plateau, resulting from the entanglement coupling between polymer chains, becomes wider as c increases. The reason that the plateau of G' is tilted a little is probably due to the polydispersity of the chitin specimen employed. At $c = 3.2 \times 10^1 \text{ kg m}^{-3}$, the lowest c of the chitin solutions in this study, η_0 at 80°C was estimated to be $5.5 \times 10^0 \text{ Pa s}$, which is still much higher than that of the pure solvent ($1.4 \times 10^{-2} \text{ Pa s}$).

Figure 3 shows a_T used to obtain the master curves in Figure 2 plotted against $1/T$ for the chitin solutions. The values of a_T are

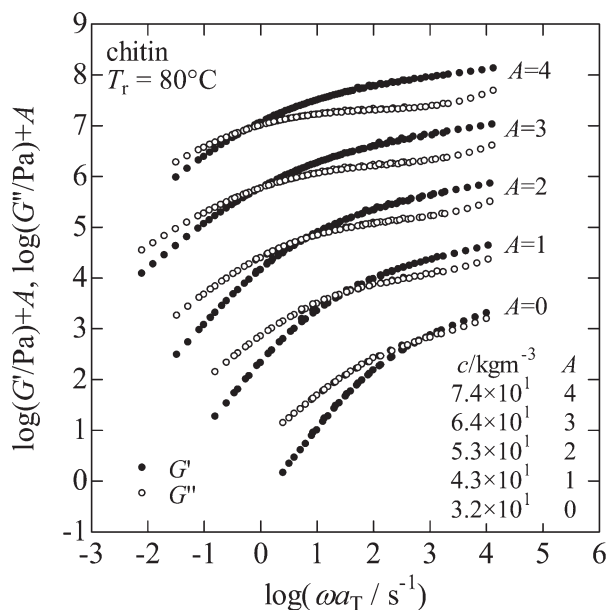
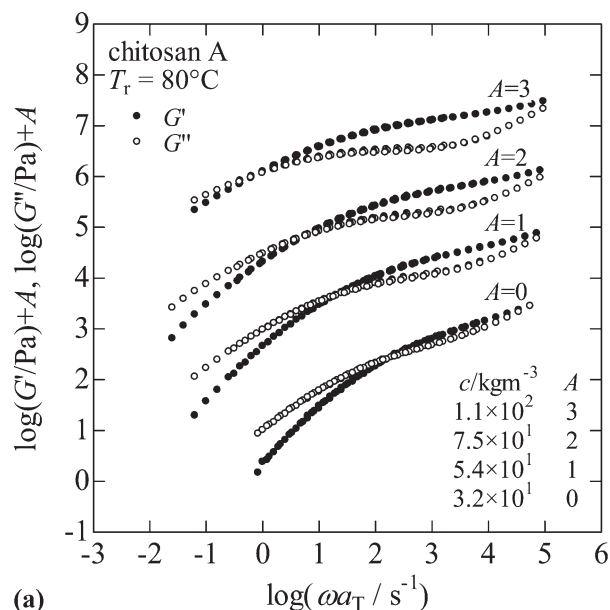


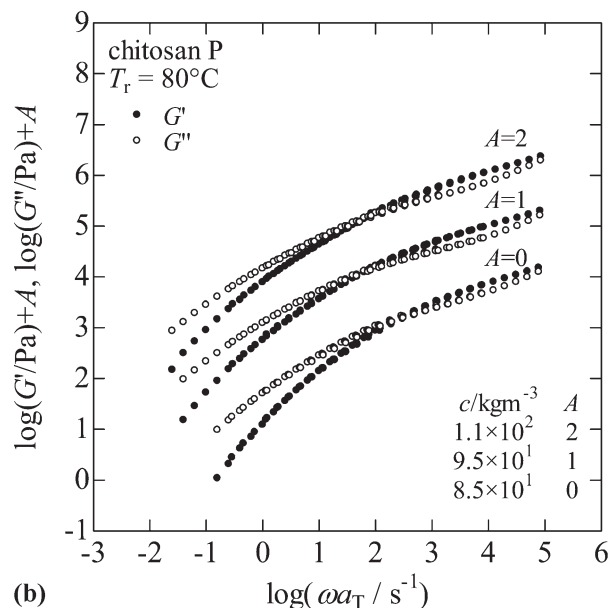
Figure 2. Master curves of ω dependence of G' and G'' for chitin solutions at $T_r = 80^\circ\text{C}$. The curves are shifted upwards by A .

almost independent of c and all data points fall on a single line. This also supports that the solutions are homogeneous. The line in the figure is the best fit one, and gives ΔE (in this case, also the activation energy of flow) of 67 kJ mol^{-1} . This is not far from ΔE for pure BmimAc (59 kJ mol^{-1}), indicating that a_T for these solutions have the similar T dependence as η_0 of BmimAc shown in Figure 1 although the values of η_0 of the solutions are over 10^2 times as high as that of the pure solvent.

Figure 4 illustrates the master curves of G' and G'' for solutions of two types of chitosan samples. The panel (a) corresponds to chitosan A and the panel (b) to chitosan P, and T_r is chosen to be 80°C in both cases. The rubbery plateau is



(a)



(b)

Figure 4. Master curves of ω dependence of G' and G'' for solutions of (a) chitosan A and (b) chitosan P at $T_r = 80^\circ\text{C}$. The curves are shifted upwards by A .

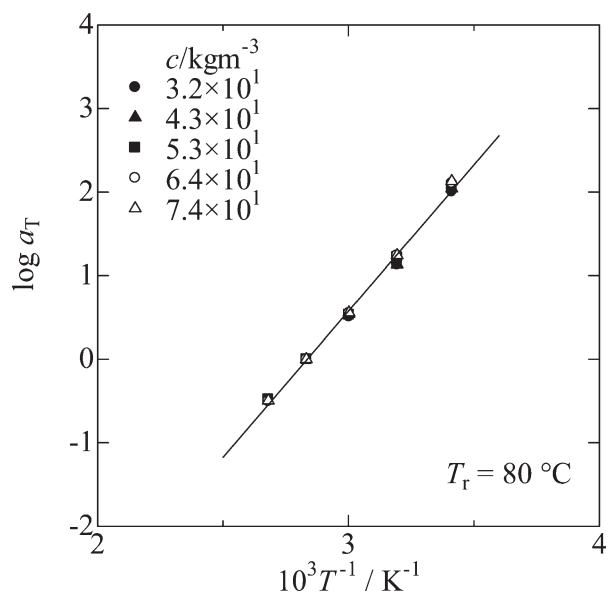


Figure 3. Shift factor for chitin solutions plotted against the reciprocal of T . All data points fall on a single line.

clearly observed on every G' curve in each panel and the plateau becomes long as c increases for each type. If the dispersion curves are compared at the same c , for example at $c = 1.1 \times 10^2\text{ kg m}^{-3}$, it is seen that chitosan A has a wider plateau than chitosan P, indicating that the molecular weight of chitosan A is larger than that of chitosan P. Figure 5 shows the plots of a_T against $1/T$ for the chitosan solutions. The plots contain the a_T data for both types of chitosan, but all data points fall on a single line although there are some clear differences in the molecular characteristics such as molecular weight between two types of chitosan. The line in the figure gives ΔE of 66 kJ mol^{-1} , which is very close to the value for the chitin solutions (67 kJ mol^{-1}).

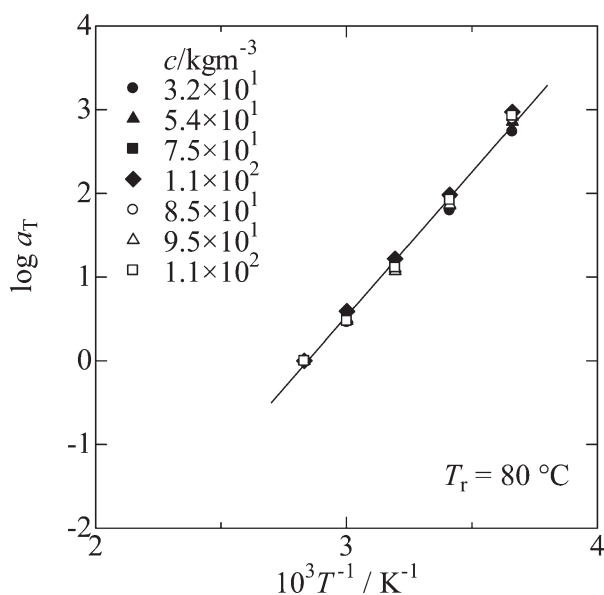


Figure 5. Shift factor for chitosan solutions plotted against the reciprocal of T . Filled and unfilled symbols represent data for chitosan A and P, respectively. All data points fall on a single line.

The plateau modulus (G_N^0) can be determined as the G' value at ωa_T where the loss tangent ($\tan \delta = G''/G'$) curve attains a minimum, if the rubbery plateau is tilted.¹⁹ Once G_N^0 is obtained M_e can be calculated by

$$M_e = \frac{10^3 c RT}{G_N^0} \quad (3)$$

For example, G_N^0 for the chitin solution of $c = 7.4 \times 10^1 \text{ kg m}^{-3}$ (Figure 2) is determined to be $1.1 \times 10^4 \text{ Pa}$, which gives M_e of 2.0×10^4 for chitin at this concentration. The values of M_e for other chitin and chitosan solutions at various concentrations were determined in the similar way and M_e thus obtained are plotted double-logarithmically against c in Figure 6. A straight line with a slope of -1 is also drawn for each polysaccharide in the figure by assuming that the well-known relation $M_e \propto c^{-1}$ can be applicable to these polysaccharides,^{8,10,26} and this line well explains the c dependence of M_e for both polymers. It should be noted that the data points for the two types of chitosan can be fitted by a single (common) line in spite of the different molecular characteristics. If the density of the amorphous chitin and chitosan is commonly assumed to be $1.0 \times 10^3 \text{ kg m}^{-3}$, $M_{e,\text{melt}}$ for the polysaccharides can be estimated as a value of M_e at $c = 1.0 \times 10^3 \text{ kg m}^{-3}$, leading to 1.7×10^3 for chitin and 3.0×10^3 for chitosan. There exists a large difference in $M_{e,\text{melt}}$ between chitin and chitosan. We do not know what generates the difference at present, but to our knowledge the values of $M_{e,\text{melt}}$ for chitin and chitosan are first determined in this study.

The chain properties of chitin and chitosan are compared with those of other polysaccharides examined in our previous studies. For comparison, we use the number of monosaccharide units between entanglements in the molten state (N_{unit}) instead of $M_{e,\text{melt}}$. Here, N_{unit} for chitin and chitosan can be calculated by $N_{\text{unit}} = M_{e,\text{melt}}/M_{\text{unit}}$ with M_{unit} being the molecular weight

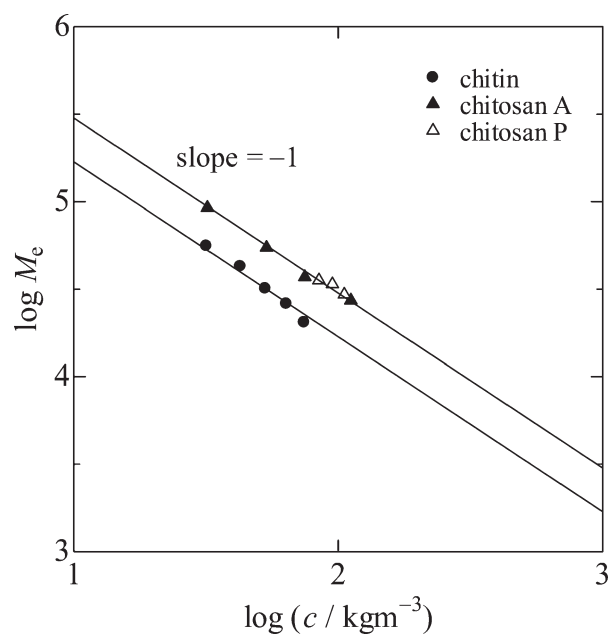


Figure 6. Double-logarithmic plot of M_e against c for chitin and chitosan in solution. Each line is the best fit one with a slope of -1 . $M_{e,\text{melt}}$ is determined as M_e at $c = 10^3 \text{ kg m}^{-3}$.

of the repeating units. Table I lists N_{unit} for chitin and chitosan together with $M_{e,\text{melt}}$ and M_{unit} . The value of N_{unit} of 8 for chitin is significantly smaller than those estimated for several polysaccharides: 20 to 22 for cellulose,^{19,23} 19 for guar gum,²² 15 for agarose,²¹ and 14 for gellan.¹⁸ Concerning chitosan, N_{unit} of 19 is comparable with these reported values. In the previous study, we have considered the relation between $M_{e,\text{melt}}$ and a chain-stiffness parameter (here, we use the characteristic ratio C_∞ as the measure) for D-glucans.²³ Although there exists still controversy, if that N_{unit} is assumed to be a decreasing function of C_∞ then chitin is expected to be stiffer than chitosan and other polysaccharides.

CONCLUSIONS

Solutions of chitin and chitosan were prepared by using Bmi-mAc as a common solvent up to concentrations where there appeared entanglement coupling between the polysaccharide chains. The rubbery plateau zone appearing in the master curves of the ω dependence of G' and G'' obtained for the chitin and chitosan solutions indicated the existence of the entanglement coupling. The values of M_e were determined from the plateau zone and $M_{e,\text{melt}}$ for chitin and chitosan were estimated from the c dependence of M_e to be 1.7×10^3 and 3.0×10^3 , respectively. It was found that the chain properties of chitin and chitosan were significantly different. The value of N_{unit} for chitin was smaller than those of chitosan and other polysaccharides

Table I. Material Constants for Chitin and Chitosan

Sample	M_{unit}	$M_{e,\text{melt}}$	N_{unit}
Chitin	203	1.7×10^3	8
Chitosan	161	3.0×10^3	19

examined in the previous studies, suggesting that chitin has a large stiffness.

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