

Rheological Properties of Concentrated Solutions of Agarose in Ionic Liquid

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ABSTRACT: The rheological properties of agarose solutions were examined under the effect of entanglement coupling between agarose chains. Agarose solutions were prepared by using an ionic liquid 1-butyl-3-methylimidazolium chloride as a solvent. The concentration of agarose was varied from 1.1×10^1 – 2.1×10^2 kg m⁻³. The master curves of the angular frequency (ω) dependence of the storage modulus (G') and the loss modulus (G'') showed a rubbery region in the middle ω region and a flow region at low ω region, respectively. The molecular weight between entanglements (M_e) for agarose was calculated

from the plateau modulus. Moreover, M_e for agarose melt was determined to be 2.3×10^3 from the concentration dependence curve of M_e . By using well-known empirical relations in polymer rheology, information on molecular characteristics of sample agarose was derived. © 2011 Wiley Periodicals, Inc. *J Appl Polym Sci* 123: 3023–3027, 2012

Key words: rheology; agarose; ionic liquid; concentrated solution; entanglement coupling; molecular weight between entanglements

INTRODUCTION

Agarose, a linear chain component of agar,¹ is a popular food additive due to the high gelation ability in aqueous systems.^{2–4} Recently, agarose has also been used as an additive for pharmaceuticals^{5,6} and cosmetics^{7–9} to improve the flow properties as well as the physical properties of the products. Although such applied uses of agarose are increasing, there are hardly any studies on the fundamental chain properties of this polysaccharide. So far the rheological properties of agarose systems have been studied in gels^{10,11} as well as in dilute solutions,¹² but there seem no data on the concentrated solutions. By examining the rheological properties of concentrated polymer solutions (or melts), it is possible to derive the molecular weight between entanglements (M_e), an important chain property reflecting the chain stiffness as well as a measure of how strong the entanglement coupling occurs. To determine M_e for agarose and to compare the value with those of other polysaccharides is interesting and important to highlight the commonality and differentia among the polysaccharides. Since agarose in water form a

gel even at rather low conditions, it is impossible to prepare concentrated solutions of agarose as long as water is used as a solvent.

Actually, we successfully prepared concentrated solutions of another polysaccharide, gellan, by using an ionic liquid 1-butyl-3-methylimidazolium chloride (BmimCl), which is also known to dissolve cellulose,¹³ as a solvent, and successfully investigated the rheological properties in the concentrated region.¹⁴ Concerning agarose, until now there are no reports on the solubility in BmimCl, but BmimCl is expected to be potentially good for agarose and therefore concentrated solutions of agarose must be obtained in the solvent BmimCl.

In this study, concentrated solutions of agarose were prepared by using BmimCl. The values of M_e for several solutions differing in agarose concentration were determined from the dynamic viscoelasticity. The value of M_e for the melt of agarose was estimated from the concentration dependence curve of M_e and was compared with those of gellan and hyaluronic acid.

EXPERIMENTAL

A molecular biology grade of agarose (Research Organics, USA) was used without further purification. Figure 1 shows the chemical structure of agarose: A repeating unit of agarose is composed of (1, 3)- β -D-galactopyranose and (1, 4)-3,6-anhydro- α -L-galactopyranose.² The solvent BmimCl with purity

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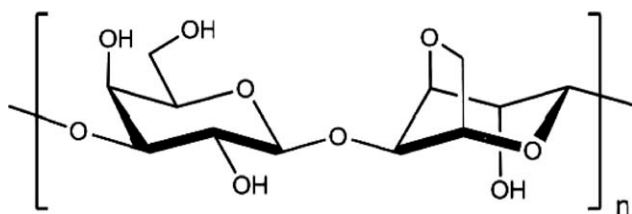


Figure 1 Chemical structure of agarose.

higher than 98.0% was purchased from Aldrich, USA. According to the manufacturer's data sheet, the melting temperature (T_m) of the solvent BmimCl was reported to be 70°C. Agarose solutions were prepared as follows. Agarose was added to BmimCl (preheated to melt) in a dry glass vessel and the mixture was quickly stirred with a stainless spatula on a hot plate at about 80°C. Then, the glass vessel was sealed and was left on the hot plate at about 80°C for complete melting. The concentration of agarose (c) was varied from 1.1×10^1 to 2.1×10^2 kg m^{-3} , i.e., from 1 to 20 wt %. In c calculation, 1.0×10^3 kg m^{-3} was assumed for the density of agarose, and 1.08×10^3 kg m^{-3} (at 50°C) was assumed for BmimCl, which was obtained by pycnometry in the previous study.¹⁴ A fresh bottle of solvent was used to prepare all solution samples and the viscoelasticity measurements were made just after finishing sample preparation, so that no special precaution against moisture absorption was made.

Rheological measurements were carried out with an ARES rheometer (now TA Instruments, USA) with a cone-plate geometry under nitrogen atmosphere. The diameter of the cone and plate was 25 mm, and the cone angle was 0.1 rad. The angular frequency (ω) dependence of the storage and loss moduli (G' and G'' , respectively) for the solutions were measured with a strain amplitude (γ) of 0.1 to 1 at the temperature (T) range of 20–120°C. The value of γ was determined so that the measurement could be performed in the linear viscoelasticity region; for example, γ of 0.1 was employed for the solution of $c = 2.1 \times 10^2$ kg m^{-3} . The strain dependence of G' and G'' for this solution measured at $\omega = 100$ s^{-1} and $T = 120^\circ\text{C}$ was shown in Figure 2. It is seen that G' and G'' are almost independent of γ for $\gamma < 0.2$, which confirms that the measurement was carried out in the linear viscoelasticity region.

Steady shear flow behavior as well as dynamic viscoelasticity was also investigated for the pure solvent BmimCl at a shear rate ($\dot{\gamma}$) of 0.1–100 s^{-1} .

RESULTS AND DISCUSSION

Figure 3 shows $\log \eta_0$ for the pure solvent (BmimCl) plotted against T^{-1} at T of 20–120°C. This T region involves T_m of BmimCl (70°C). Dynamic viscoelastic-

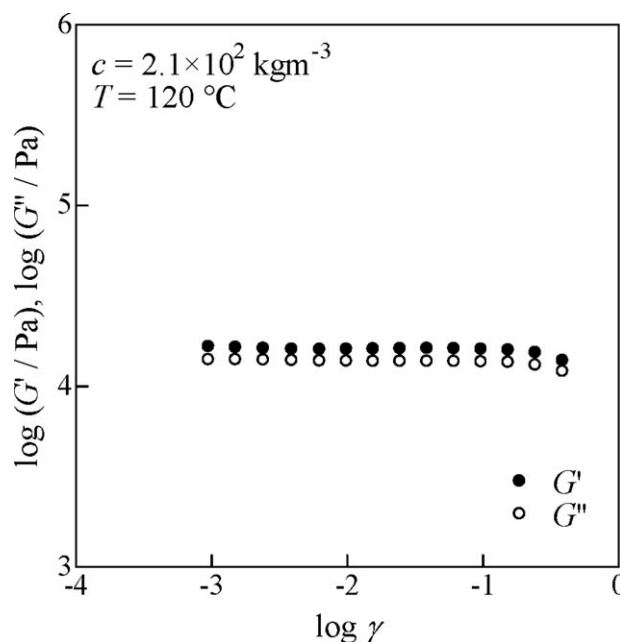


Figure 2 Strain dependence of G' and G'' for the agarose solution of $c = 2.1 \times 10^2$ kg m^{-3} measured at $\omega = 100$ s^{-1} and $T = 120^\circ\text{C}$.

ity measurements were made from high temperature to low temperature side and thus obtained η_0 data are represented by the filled symbols. The open circle in the figure stands for the data point obtained from the steady flow experiment at 80°C, and agrees well with the filled circle at the same T . We were able to make dynamic measurements without problem even at lower temperatures than T_m , and

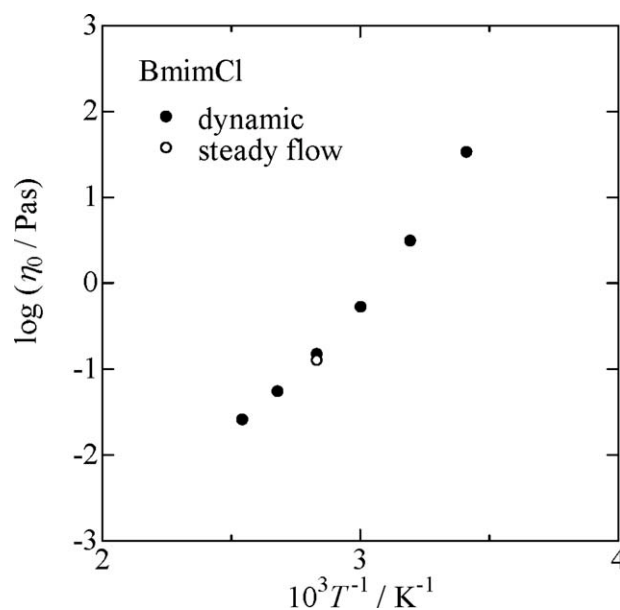


Figure 3 Plot of $\log \eta_0$ for BmimCl obtained from dynamic viscoelasticity measurement against the reciprocal of T . Value of $\log \eta_0$ from steady shear flow measurements at 80°C is also plotted.

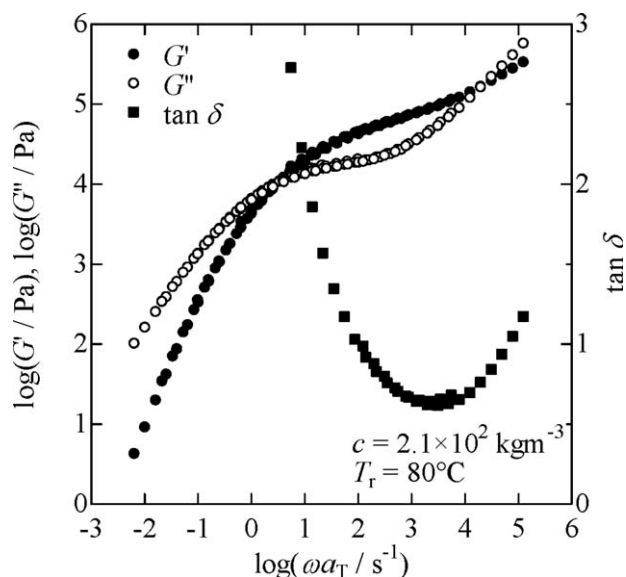


Figure 4 Log-log plot of G' and G'' vs. ωa_T for the agarose solution of $c = 2.1 \times 10^2 \text{ kg m}^{-3}$. Loss tangent $\tan \delta$ is also plotted against ωa_T . Plateau modulus G_N^0 can be determined as G' at ωa_T where $\tan \delta$ curve shows a minimum.

obtained data show that η_0 increase with increasing T^{-1} (i.e., with decreasing T) although the relation between $\log \eta_0$ and T^{-1} becomes nonlinear. This suggests that the supercooling state of BmimCl is “stable” for a rather long time.¹⁴ For the agarose solutions rheological measurements were made without problem, as is the case of pure BmimCl.

In Figure 4 the ω -dependence curves of G' and G'' as well as the loss tangent ($\tan \delta$; $\tan \delta = G''/G'$) for the agarose solution of $c = 2.1 \times 10^2 \text{ kg m}^{-3}$ are shown. These curves correspond to the so-called master curves; here T_r is the reference temperature and is set to be 80°C , and a_T stands for the shift factor. As can be seen from the figure, the time-temperature superposition principle well holds for the solution, and at low ωa_T $G'' \propto \omega$, flow of the system, is observed. In the middle ωa_T region (i.e., approximately at $\log \omega a_T$ of 1 to 4), rubbery region originating from the entanglements of agarose chains emerges. The plateau modulus (G_N^0) is an important quantity characterizing G' in the rubbery region. It is well known that the rubbery region becomes a plateau when the molecular weight distribution of a polymer sample is monodisperse. This is why the rubbery region is often called the rubbery plateau. For the agarose used in this study, however, because of the tilt G' curve in the rubbery region the molecular weight distribution is probably polydisperse rather than monodisperse although we have no information on the molecular characteristics and therefore cannot mention that quantitatively. For the tilt curves, G_N^0 can be defined as the G' value at ω (or, ωa_T) where $\tan \delta$ curve shows a minimum.¹⁴ On the

basis of this definition, the G' curve in Figure 4 gives $G_N^0 = 6.0 \times 10^4 \text{ Pa}$. Because G_N^0 is related to the molecular weight between entanglements (M_e ; in g mol^{-1}) by using the gas constant R as

$$G_N^0 = \frac{10^3 c R T}{M_e} \quad (1)$$

we have $M_e = 1.1 \times 10^4$ for the solution at $c = 2.1 \times 10^2 \text{ kg m}^{-3}$ (i.e., for the solution with $G_N^0 = 6.0 \times 10^4 \text{ Pa}$). The T -dependence curve of a_T is shown in Figure 5. At a given T the values of a_T are almost identical regardless of c , and all data points in the figure appear to fall on a single line. This is interesting because the η_0 curve for the pure solvent (Fig. 3) deviates from the T -dependence of the Arrhenius type.

Figure 6 shows the double-logarithmic plot of M_e against c . The line in Figure 6 is determined by the best fit under the constant value of slope of -1 . The data points fall on the line. This means that the well known relation for melts and concentrated solutions of synthetic polymers,^{16–18} $M_e \propto c^{-1}$, also holds for the agarose solutions examined in this study. By assuming the density of agarose to be $1.0 \times 10^3 \text{ kg m}^{-3}$, M_e for the melts ($M_{e,\text{melt}}$) can be determined from the line in Figure 6, giving $M_{e,\text{melt}}$ of 2.3×10^3 . Taking the molecular weight of the repeating unit (M_{unit}) of 306 into account for agarose (Fig. 1), we have $M_{e,\text{melt}}/M_{\text{unit}}$ of 7.5. This means that the number of monosaccharide units (N_{unit}) between entanglements for melts is about 15 for agarose. Now, we

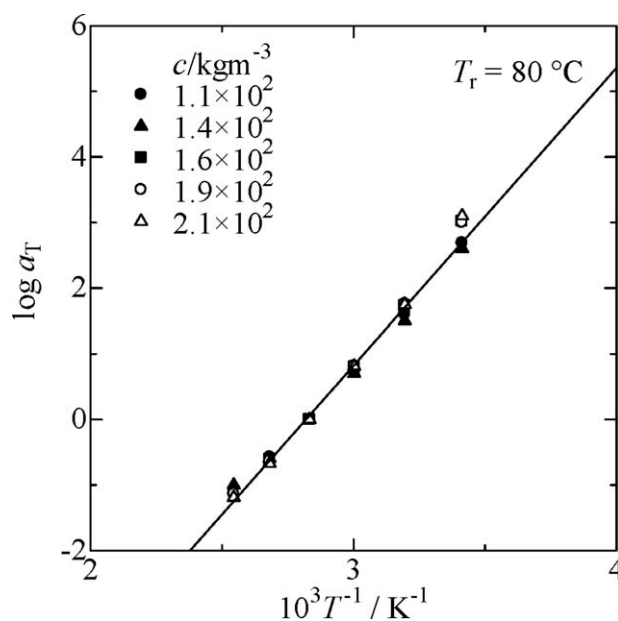


Figure 5 Temperature dependence of shift factor used in plotting master curves of G' and G'' for the agarose solutions of c of 1.1×10^2 to $2.1 \times 10^2 \text{ kg m}^{-3}$. Data are well fitted by a single line.

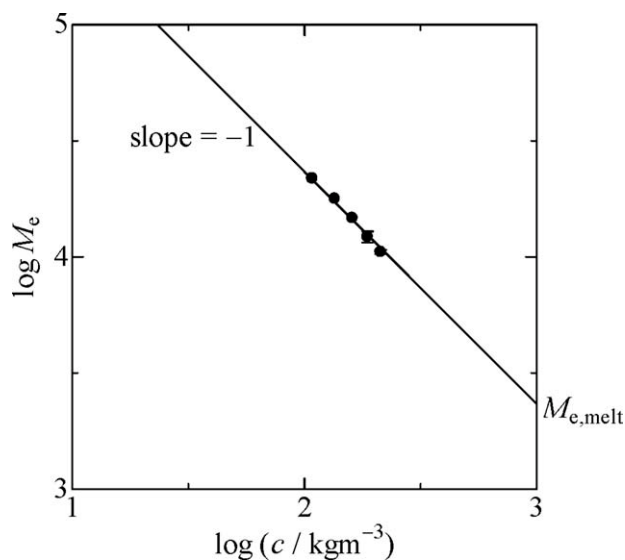


Figure 6 Log-log plot of M_e against c . Straight line is the best fit with a slope of -1 . Intersection at $c = 10^3 \text{ kg m}^{-3}$ gives M_e of agarose melt. Error bars for $c = 1.9 \times 10^2$ and $2.1 \times 10^2 \text{ kg m}^{-3}$ represent the standard deviation.

examine N_{unit} for melts of several polysaccharides, although the data available are actually a few. The values of N_{unit} for gellan¹⁴ and hyaluronic acid¹⁹ are listed in Table I, together with that for agarose. The value of N_{unit} for agarose is very close to those for gellan and hyaluronic acid. It should be noted that these three polysaccharides have the same chemical structure of backbone, i.e., pyranose, but the substituents are different to each other. The backbone structure might be a key factor to determine N_{unit} of polysaccharides. Concerning the relation between $M_{e,\text{melt}}$ and the chemical structure, $M_{e,\text{melt}}$ for agarose is further compared with those of polyethylene and polystyrene. Polyethylene is composed of a linear C—C backbone, whereas polystyrene has a rather bulky substituent, i.e., a phenyl group, in each repeating unit. Consequently, $M_{e,\text{melt}}$ of 1.8×10^4 for polystyrene is much larger than that of 1.3×10^3 for polyethylene.¹⁵ Polysaccharides consisting of pyranose units can be considered a bulky polymer but $M_{e,\text{melt}}$ for agarose is close to that of polyethylene rather than that of polystyrene. This suggests that polysaccharide chains are uniquely flexible in terms of $M_{e,\text{melt}}$ despite the bulky chemical structure, although the reason for the small $M_{e,\text{melt}}$ cannot be stated at the moment.

TABLE I
Comparison of N_{unit} for Polysaccharide Melts

	N_{unit}
Agarose	15
Gellan	14
Hyaluronic acid	~ 10

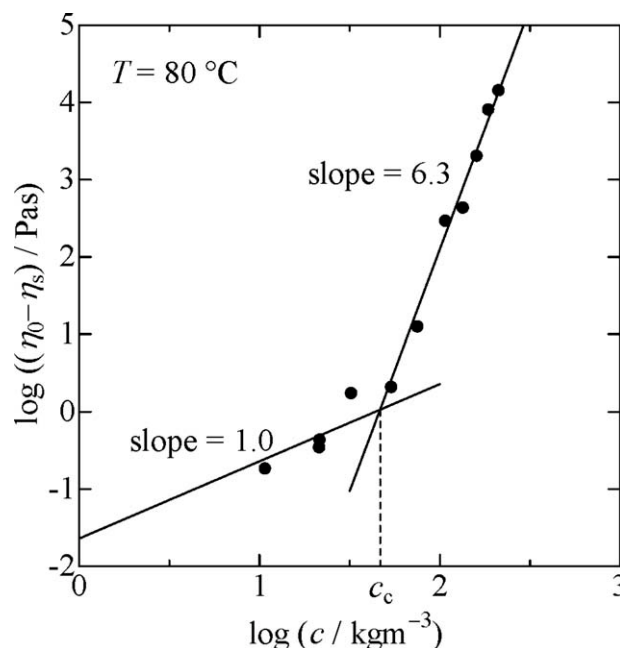


Figure 7 Log-log plot of η_0 against c . Data points at low and high c regions are fitted by straight lines with the slope of 1.0 and 6.3, respectively. Concentration at the intersection corresponds to c_c .

Using well-known empirical relations in polymer rheology, we can derive information on molecular characteristics. Figure 7 shows the double-logarithmic plot of $\eta_0 - \eta_s$ against c for the agarose dilute solutions at 80°C. Here, η_s stands for the viscosity of BmimCl. The data points can be divided into two regions, and the data in each region can be fitted by a straight line. Because the slopes of the lines at low and high c regions are 1.0 and 6.3, respectively, the critical concentration (c_c) for the entanglement formation is determined as a concentration at the intersection, giving c_c of $4.7 \times 10 \text{ kg m}^{-3}$. We can consider that at this critical concentration an average molecular weight of the specimen corresponds to the critical molecular weight for entanglement formation (M_c).^{15,16} The value of M_e for the agarose at c_c was 5.0×10^4 from Figure 6. It is well known that M_c lies between $2M_e$ and $3M_e$. If we assume here $M_c = 2M_e$, then we have $M_c = 1.0 \times 10^5$. This corresponds to an average molecular weight of the agarose used in this study.

CONCLUSION

The value of $M_{e,\text{melt}}$ for agarose was estimated to be 2.3×10^3 from the c -dependence of M_e and was compared with those values for gellan and hyaluronic acid. It was found that N_{unit} for agarose coincides with those for gellan and hyaluronic acid. These three polysaccharides have the same pyranose backbone, suggesting that the backbone structure is a key factor to determine N_{unit} of polysaccharides. It

was also found that polysaccharide chain has a small $M_{e,melt}$ in spite of the bulky chemical structure. The molecular weight of agarose used in this study was also estimated to be 1.0×10^5 from the c -dependence of $\eta_0 - \eta_s$.

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