Optical Rheometry of Silk in Aqueous LiBr Solution

S. K. Siddiquee, P. J. Willcox, J. W. van Egmond,* and S. P. Gido*

Department of Chemical Engineering and Department of Polymer Science and Engineering, University of Massachusetts, Amherst, Massachusetts 01003

Received April 24, 1997; Revised Manuscript Received June 20, 1997[⊗]

ABSTRACT: The rheo-optical response of solutions of silk in aqueous 9 M LiBr of concentrations ranging from 7.5 to 15 wt % was investigated. These samples were found to be birefringent under flow, with the steady state birefringence having a concentration scaling of $c^{3.9}$. This scaling behavior suggests a wormlike conformation of the silk molecules in solution. The relaxation was fast, and Maxwellian behavior was observed at low shear rates. No dichroism was detected suggesting that electrostatic screening suppresses structure formation under shear.

1. Introduction

Silks are the only natural spun fibers. The translucent sheen, dyeing ability, and comfort of silk make it a popular fiber for the clothing industry while its combination of strength and toughness offer potential as a structural fiber. The work to rupture of silk increases with increasing load rate,[1] an unusual trend for most polymers. These favorable properties are attributable to the high degree of molecular orientation within the fibers.

Currently, silk threads are produced by unraveling fibers from *Bombyx mori* silkworm cocoons. These fibers are then spun into thread. It would be desirable to remove some limitations on the production process by finding a method of redissolving the silk molecules and spinning the solution directly into long fibers. The spun-regenerated fibers have poorer mechanical properties than the fibers formed from unraveling silk cocoons. For instance, Matsumoto *et al.*[2] found the tensile strength of their regenerated fiber to be between one-quarter and one-third that of silk fibers taken directly from the cocoon. The reason is uncertain, but it may be partially due to degradation caused by using harsh ionic solvents. However, another reason is that the conformation of the redissolved silk may be very different from that of natural silk taken directly from within the silk glands. How the regenerated silk in solution coagulates is important for success in spinning silk. For stiff fibers of high tensile strength, it is necessary for the molecules to be well extended and oriented along the major axis of the fiber. When the native silk solution is drawn manually from the gland, a well-oriented insoluble filament is easily produced.[3] Why native silk orients so easily in the presence of shear or extensional forces while regenerated silk orients comparably poorly is uncertain but is worthy of investigation.

Silk within the gland is in an aqueous solution with low concentrations of such salts as $CaCl_2$ and KCl.[4] Once the silk fibers have dried, they will only redissolve in water with very concentrated amounts of chaotropic salts.[5] In native silk fibers, the extended fibroin chains are hydrogen bonded together through $-C{=}O$ and $-NH$ groups.[6] This intermolecular hydrogen bonding is sufficiently strong to prevent the separation of the molecules and hence resist their dissolution into pure water. The presence of a high concentration of ions screen intermolecular interactions, thus creating a path for dissolution. This screening also interferes with the reassociation of the molecules in solution that is necessary for successful fiber spinning. The charge distribution along the chain affects the conformation in solution. Thus the presence of small ions is likely to change the structure of the silk molecules. The pH dependence of the silk fibroin conformation and the ion-exchange which occurs in the anterior portion of the silk gland[1] are evidence that the conformation of the silk molecules is dependent on the electrostatic properties of the solution. The concentrations of regenerated silk in solutions that have been dialyzed to remove most of the salts have been limited because of the low solubility of silk in pure water.[2] The removal of the ions may result in the collapse of the silk molecules from their extended state to a spherical state[2] and hence affect their solubility.

Magoshi *et al.*[4] have suggested that conformational changes between random coil and the more ordered β-sheet structures can occur by mechanical shearing and pH variations. On the basis of his experimental results, Iizuka[7] proposed that transitions in silk molecule conformation are shear-induced. There have been few rheological studies of silk solutions. Iizuka's study of dialyzed regenerated silk[5] showed apparent Newtonian behavior at low shear rates for silk concentrations up to 7.3 g/100 mL, with the viscosity independent of shear rate and relaxation abrupt upon the cessation of shear. However, a critical shear rate was found above which a large increase in viscosity was detected and stress relaxation was no longer instantaneous. Above a concentration of 0.5 g/100 mL, Iizuka observed this critical shear rate to increase with silk concentration and to be heavily dependent on the dialysis regime. Iizuka attributed this jump in the viscosity to the onset of crystallization.[5]

In this paper, the shear response of 9 M LiBr solutions is investigated using simultaneous birefringence and dichroism measurements. From the birefringence, the degree of molecular alignment due to flow as well as chain relaxation upon shear cessation can be investigated.

The birefringence anisotropy, $\Delta n'$, is proportional to the conformation tensor, $\langle rr \rangle$.[8] Therefore, the birefringence is a measure of the degree of orientation of the molecules within the sample. With this method, we can study the response to shearing of the degree of alignment of the silk molecules in solution as well as the average angle of orientation, χ', to the flow direction.

[⊗] Abstract published in *Advance ACS Abstracts,* August 15, 1997.

S0024-9297(97)00573-1 CCC: $14.00

If the shear stress is proportional to the conformation tensor, $\sigma_{xy} \propto \langle rr \rangle$, the stress-optical rule holds.[9] This relates the birefringence anisotropy to the shear stress and the first normal stress difference. The stress optical rule has been shown to be true for most semidilute solutions of both rods and flexible coils.[10] However, the rule breaks down at high shear rates when the velocity gradient is sufficient to cause the polymer molecules to approach maximum alignment, resulting in saturation of the refractive index. Failure in the stress-optical rule may also occur due to the presence of form contributions to the birefringence. Form birefringence arises when the solute and solvent refractive indices are sufficiently different and structures with length scales on the order of the wavelength of light are formed so that light is scattered anisotropically. If form contributions to the birefringence are present, we would expect the sample to be dichroic and show evidence of scattering.

Dichroism measures the degree of anisotropy of concentration fluctuations on the length scale of light, as well as the orientation of these fluctuations under flow. Thus flow-induced crystallization can be measured by dichroism. If the silk molecules intrinsically absorb light anisotropically, dichroism will also measure molecular orientation. From the dichroism measurements, we are able to check whether there is any structure formation on the length scale of the probing light.

2. Experimental Section

2.1. Sample. Solutions were prepared from silk obtained by washing and degumming the cocoons of *B. mori* silkworms. The process of degumming involved boiling the cocoons in an aqueous solution of Na_2CO_3 (1% by weight of the cocoons for 2 h) to remove the sericin with which the silk thread is coated. After being rinsed in deionized water, the silk (silk fibroin) was soaked in MeOH for 30 min to extract the wax coating from the fiber. The washed silk is then dissolved in aqueous 9 M LiBr.

The time and temperature required for dissolving the silk varied, depending on the concentration desired. In order to obtain the 15 wt % solution used in these experiments, it was necessary to heat the solution to 80 °C. This solution was used as a stock solution and diluted to 12.5, 10, and 7.5 wt % for the rheo-optical investigation. Previous solutions heated to this temperature have been compared to solutions prepared at ~ 40 °C, and the anisotropy was found to be the same. Therefore, there was insignificant degradation of the polydispersity of the silk fibroin due to heating. Under normal physiological conditions, silk fibroin would be monodisperse. We were concerned that the use of a strong ionic solvent such as 9 M LiBr(aq) would lead to an unacceptable level of degradation of the silk during the period of investigation. Repeating the same shear rate over the duration of the investigation showed no time-dependent reduction of the birefringence which implies that degradation due to solvent action and repeated shearing was insignificant. Values of 300 000[1,8] and 420 000[11] have been reported in the literature for the molecular weight of silk fibroin.

2.2. Rheo-Optics. The optical train (Figure 1) utilized a polarization modulation technique to measure the time-dependent response of birefringence and dichroism during shear and after cessation of shear.[8] The light source was a He−Ne laser of wavelength 632.8 nm. The polarization state generator (PSG) consisted of a polarizer oriented at 0° followed by a half-wave plate rotating at a frequency $\omega = 400$ Hz. After passing through the sample, a beam splitter divides the beam in two. For birefringence measurement, one beam is passed through a polarization state analyzer (PSA) consisting of a left-hand circular polarizer to detector, D1. For dichroism measurement, the other beam is fed directly to the detector, D2.

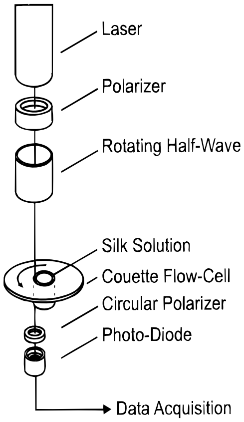

Figure 1. Optical train used in the study. For concurrent birefringence and dichroism measurement, a beam splitter is placed after the flow cell and the second signal is sent directly to an additional photodiode.

With the orientation angles of the birefringence, χ', and the dichroism, χ'' being noncoaxial and for the condition that the retardance for the birefringence and extinction for the dichroism are much less than one (δ', $\delta'' \ll 1$), the intensity at the detector D1, I_1, may be written as:[8,12]

$$\frac{I_1}{I_{10}} = 1 - \delta'' \cos 2\chi'' \cos 4\omega t - \delta'' \sin 2\chi'' \sin 4\omega t \quad (1)$$

where I_{10} is the dc component of the intensity signal of the beam incident on the detector, D1. The intensity at detector D2, I_2, can be written as

$$\frac{I_2}{I_{20}} = 1 + (-\delta' \sin 2\chi - \delta'' \cos 2\chi) \cos 4\omega t + (\delta' \cos 2\chi - \delta'' \sin 2\chi) \sin 4\omega t \quad (2)$$

where I_{20} is the dc component of the intensity signal of the beam incident on the detector, D2.

The intensities may be written in terms of components in-phase with the polarized light exiting the rotating half-wave plate, R'_1 and R''_1 for birefringence and dichroism respectively, and out-of-phase components, R'_2 and R''_2. The retardance and orientation angle of the principle birefringence axis are then calculated to be

$$\delta' = \{(R'_1 - R''_1)^2 + (R'_2 - R''_2)^2\}^{1/2} \quad (3)$$

$$\chi' = \frac{1}{2}\mathrm{atan}\left(\frac{-(R'_1 - R''_1)}{R'_2 - R''_2}\right) \quad (4)$$

Correspondingly, the extinction and orientation angle of the principle dichroism axis may be represented as:

$$\delta'' = \{R''^2_1 + R''^2_2\}^{1/2} \quad (5)$$

$$\chi'' = \frac{1}{2}\mathrm{asin}\left(\frac{R''_2}{R''_1}\right) \quad (6)$$

If the sample is not dichroic, the beam splitter is removed and both R''_1 and R''_2 are set to zero to reduce noise. The birefringence and dichroism are defined respectively as $\Delta n' = \delta'\lambda/2\pi d$ and $\Delta n'' = \delta''\lambda/2\pi d$, where λ is the wavelength of the light passing through the sample, and d is the pathlength through the sample.

The analysis is equivalent to that of Johnson *et al.*[12] for the limit of very small birefringence and dichroism retardance. As can be seen by the above equations, the detector, D1, measures the dichroism directly while the birefringence has to be decoupled from the dichroism for the intensity at detector, D2.

If the stress-optical rule holds, the shear stress, σ_{xy}, and the first normal stress difference, N_1, are related to the birefrin-

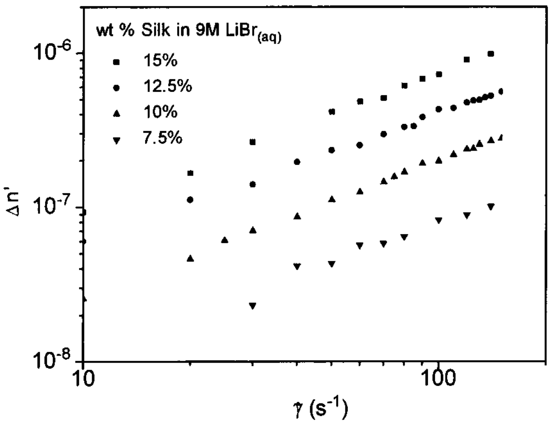

Figure 2. Dependence of the steady state birefringence, $\Delta n'$, versus the shear rate, $\dot{\gamma}$, for the different concentrations of silk in aqueous 9 M LiBr.

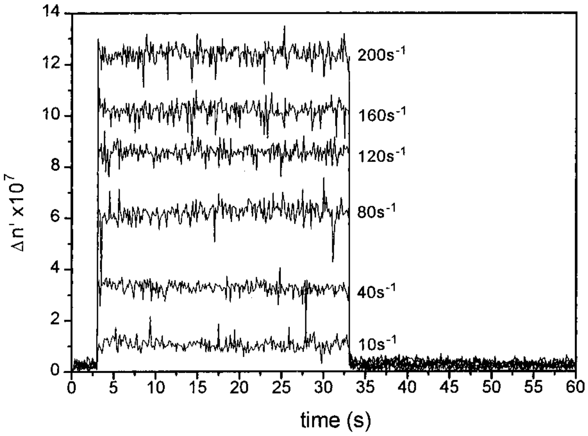

Figure 3. Time-dependent response of the birefringence for 15 wt % silk in aqueous 9 M LiBr for a variety of shear rates.

gence anisotropy by the following expressions:

$$\sigma_{xy} = \frac{n_{xy}}{C} = \frac{\Delta n'}{2C} \sin 2\chi' \tag{7}$$

$$N_1 = \sigma_{xx} - \sigma_{yy} = \frac{\Delta n'}{C} \cos 2\chi' \tag{8}$$

where C is the stress-optical coefficient.[9]

2.3. The Flow Cell. An aluminum couette flow cell with a gap of 2 mm and outer rotating cylinder was used. The laser light propagated along the vorticity axis in the gap between the two cylinders, through two stationary flat quartz windows. An internal diameter for the outer cylinder of 15.9 mm was sufficiently large to ensure a good approximation to homogeneous shear flow. The sample height (path length of the laser beam through the sample, d) was 19.1 mm. Shear rates from 1 to 200 s^{-1} were used, and the data were averaged over up to 20 identical runs to reduce experimental noise. Typically, averaging over 10 runs was sufficient. All experiments were conducted at room temperature (~21 °C).

3. Results and Discussion

Figure 2 shows the variation in the steady state birefringence with shear rate for silk solutions of concentration 7.5, 10, 12.5, and 15 wt %. As expected, the birefringence anisotropy increases with concentration. The birefringence anisotropy for all four solutions show a power law dependence on the shear rate, $\Delta n' = A\dot{\gamma}^n$, with the same exponent, $n = 0.88 \pm 0.03$.

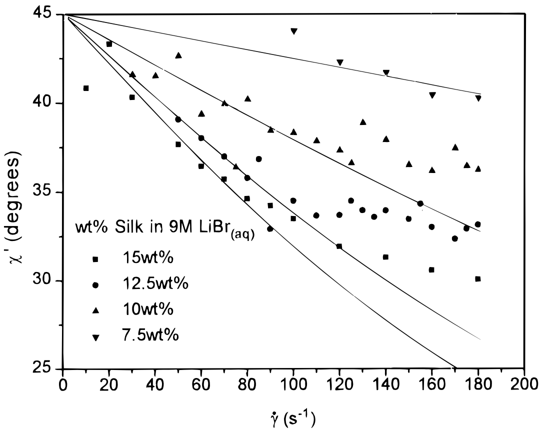

Figure 4. Dependence of the steady state orientation angle, χ', versus the shear rate, $\dot{\gamma}$, for the different concentrations of silk in aqueous 9 M LiBr. The solid lines represent fits of χ' using eq 9.

The time-dependent birefringence behavior for the 15 wt % silk solution is shown in Figure 3. The behavior of the other solutions is qualitatively similar, with the size of the steady state birefringence being a function of shear rate and concentration. With all the solutions, the birefringence rapidly reaches steady state upon start-up of shear and drops rapidly to zero after shear cessation. Since no start-up transients or relaxations are detectable and the fastest sampling rate was 65 ms, the longest relaxation time, τ_r, must be less than this.

The response of the orientation angle follows the behavior of the birefringence anisotropy. As soon as flow starts, the orientation angle reaches the steady state value. The chains assume a random orientation upon flow cessation, indicating full relaxation. Figure 4 shows the steady state birefringence orientation angle, as a function of shear rate. As with more typical polymer solutions, the orientation angle $\chi' \rightarrow 45°$ as the shear rate, $\dot{\gamma} \rightarrow 0$. This is due to the Newtonian limit being reached. The steady state orientation angle tends toward 0° with increasing shear rate. The more concentrated the silk solution, the smaller the orientation angle. Again, this is typical behavior for polymer solutions. There is greater error in the steady state orientation angle at the lower shear rates and lower concentrations due to the smaller signal size and corresponding signal to noise ratio. For the 7.5 wt % solution, the presence of small bubbles had a detrimental effect on the signal to noise ratio.

Assuming compliance with the upper-convected Maxwell model,[10] the longest relaxation time is related to the birefringence orientation angle by the following expression, for lower shear rates $\dot{\gamma} < 1/\tau_r$:

$$\dot{\gamma}\tau_r = \frac{1}{\tan 2\chi'} \tag{9}$$

Using this method, τ_r is estimated as 4.61, 4.12, 2.52, and 0.88 ms for the 15, 12.5, 10, and 7.5 wt % solutions respectively. χ' calculated from these relaxation times is plotted with the experimental values in Figure 4, for all the concentrations. This figure shows the deviation from Maxwellian behavior at shear rates greater than 100 s^{-1} due to shear thinning.

Since the relaxation time constant is small and no dichroism or scattering is detected, the stress-optical rule is assumed to apply. Equation 7 can then be used to relate the rheo-optical data to the shear stress. The steady state shear component, n_{xy}, is seen in this investigation to be similar in behavior to the birefringence. The linear dependence of n_{xy} on $\dot{\gamma}$ at low shear rates coupled with $\chi' \neq 45°$ suggests Maxwellian behavior for $\dot{\gamma} < 50$ s^{-1} (Figure 5). As can be seen from Figure 6, n_{xy} shows a $c^{3.9}$ concentration dependence.

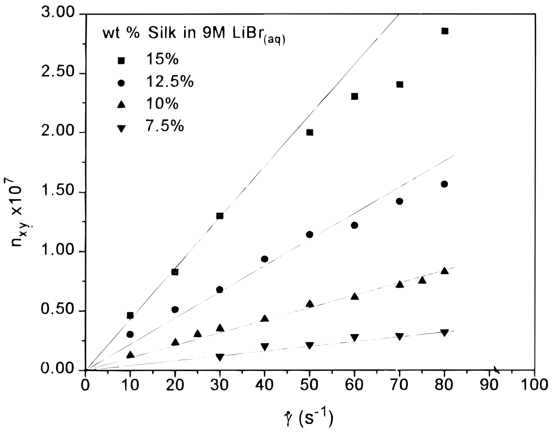

Figure 5. Linear dependence of n_{xy} versus shear rate, $\dot{\gamma}$, for lower shear rates.

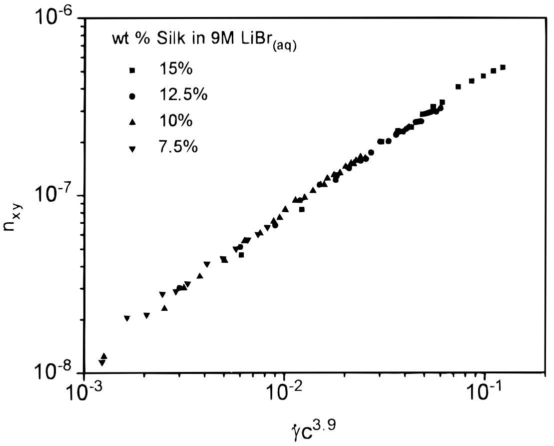

Figure 6. Dependence of the steady state birefringence, n_{xy}, versus $\dot{\gamma}c^{3.9}$, for the different concentrations of silk in aqueous 9 M LiBr.

Little is known about the flexibility of silk molecules. A semiflexible polymer obeying the Kratky–Porod model is usually termed a wormlike chain.[15] The concentration scaling of the viscosity and hence the shear response is different for the limiting behaviors of wormlike chains. In the rodlike limit, where the ratio of contour length to persistence length $L/\alpha \to 0$, the viscosity and shear stress response scale as c^3.[13] As the ratio increases, the polymer chain passes through the wormlike regime, until the limit, $L/\alpha \to \infty$, at which point the polymer follows the same concentration scaling for the stress response as a flexible coil. For this limiting conformation, the specific viscosity has been calculated to scale with $c^{4.25}$.[14] The observed concentration dependence for the silk solutions is very similar to the fourth power dependence, $c^{4.0}$, observed with poly(*n*-hexyl isocyanate) with $L/\alpha \approx 1.8$.[13] For this ratio of contour length to persistence length, poly(*n*-hexyl isocyanate) is a wormlike polymer.[15] This suggests a wormlike conformation for the screened silk molecules in the semidilute concentration regime.

No dichroism is observed with any of the samples, and therefore, there is no evidence of structure formation on the length scale of the probing light due to shear. The form birefringence is most likely negligible. However, the absence of form dichroism cannot normally be used to support this statement. The strong length scale dependence of the form birefringence means that it may not be apparent for particles sufficiently smaller than the length scale of light.[16] Iizuka[5] recorded a critical shear rate at which the shear stress increased suddenly for dialyzed silk solutions of unknown, but small, ionic concentration. For a solution of 7.3 g/100 mL (6.8 wt %), the critical shear rate is approximately 60 s^{-1}. In the range of shear rates studied, no equivalent jump was observed in the birefringence for the silk solution in aqueous 9 M LiBr, as can be seen from Figure 2. If the mechanism of association is electrostatic in nature, the presence of small ions in solution would result in charge screening and the inhibition of the association necessary for structure formation. In the same paper, Iizuka records a steady state birefringence not greater than 3×10^{-8} at a shear rate of 200 s^{-1} for an aqueous silk solution of concentration 6.6 g/100 mL (6.2 wt %). In comparison, at a shear rate of 200 s^{-1}, the steady state birefringence for a 7.5 wt % silk solution in aqueous 9 M LiBr is 1.34×10^{-7}. The implication is that the equivalent degree of molecular alignment is far greater for the solutions in 9 M LiBr than achieved with Iizuka's dialyzed solutions.

4. Conclusions

The rheo-optical investigation of silk in aqueous 9 M LiBr shows a concentration dependence for the birefringence of $\Delta n' \sim c^{3.9}$, and if the stress-optical rule holds, the same concentration dependence is found for the stress response, $\sigma_{xy} \sim c^{3.9}$. This suggests a wormlike conformation for the silk molecules in this solution. Dichroism measurements showed no evidence of crystallization or shear-induced structural transitions in solution.

Acknowledgment. J.W.v.E. would like to acknowledge the partial support from NSF under Grant No. DMR 9400488 (MRSEC). S.P.G. acknowledges the NSF in form of a CAREER Grant, No. DMR 9624306.

References and Notes

(1) Kaplan, D. L.; Lombardi, S. J.; Muller, W. S.; Fossey, S. A. In *Biomaterials: Novel Materials from Biological Sources*; Byron D., Ed.; Stockton Press: New York, 1991.

(2) Matsumoto, K.; Uejima, H.; Iwasaki, T.; Sumino, H. *J. Appl. Polym. Sci.* **1996**, *60*, 503–511.

(3) Magoshi, J.; Magoshi, Y.; Nakamura, S. *Polym. Commun.* **1985**, *26*, 60–61.

(4) Magoshi, J.; Magoshi, Y.; Nakamura, S. In *Silk Polymers: Material Science and Biotechnology*; Kaplan, D., Adams, W. W., Farmer, B., Viney, C., Eds.; ACS Symposium Series 544; American Chemical Society: Washington, DC, 1994; p 292.

(5) Iizuka, E. *J. Appl. Polym. Sci.: Appl. Polym. Symp.* **1985**, *41*, 173–185.

(6) Iizuka, E. *J. Appl. Polym. Sci.: Appl. Polym. Symp.* **1985**, *41*, 163–171.

(7) Iizuka, E. *Biorheology* **1966**, *3*, 141–152.

(8) Fuller, G. G. *Optical Rheometry of Complex Fluids*; Oxford University Press: Oxford, England, 1995.

(9) Fuller, G. G. *Annu. Rev. Fluid Mech.* **1990**, *22*, 387–417.

(10) Larson, R. G. *Constitutive Equations for Polymer Melts and Solutions*; Butterworths: Boston, MA 1988.

(11) Kirkam, K.; Viney, C.; Kaplan, D.; Lombardi, S. *Nature* **1991**, *349*, 596–598.

(12) Johnson, S. J.; Frattini, P. L.; Fuller, G. G. *J. Colloid Interface Sci.* **1985**, *104*, 440–453.

(13) Gatzonis, Y.; Siddiquee, S. K.; van Egmond, J. W. *Macromolecules*, submitted for publication.

(14) Takahashi, T.; Isonu, Y.; Noda, I.; Nagasawa, M. *Macromolecules* **1985**, *18*, 1002–1008.

(15) Yamakawa, H. *Modern Theory of Polymer Solutions*; Harper & Row: New York 1971.

(16) Onuki, A.; Doi, M. *J. Chem. Phys.* **1985**, *85*, 1190–1197.

MA9705735