Excluded-Volume Effects in Sodium Hyaluronate Solutions Revisited

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(Received October 27, 1997)

ABSTRACT: Five sodium salt samples of hyaluronic acid ranging in weight-average molecular weight M_w from 1.9×10^5 to 1.5×10^6 have been studied by light scattering and viscometry in aqueous sodium chloride at 25°C. The intrinsic viscosity ($[\eta]$) data obtained at 10 different NaCl concentrations C_s covering a range from 0.005 to 2.5 M are analyzed along with previous data for lower M_w on the basis of the Yamakawa–Fujii–Yoshizaki theory for unperturbed wormlike chains combined with the quasi-two-parameter (QTP) theory for excluded-volume effects. Fairly satisfactory agreement is observed between theory and experiment at C_s higher than 0.01 M over the range of molecular weight from 3.8×10^3 to 1.5×10^6 , though the degree of agreement tends to lower with decreasing C_s . When the wormlike chain parameters and the excluded-volume strength estimated from $[\eta]$ are used, the QTP theory with the known expression for $\langle S^2 \rangle$ (the mean-square radius of gyration) of the unperturbed wormlike chain is found to describe almost quantitatively the measured $\langle S^2 \rangle$ for Na hyaluronate at $C_s = 0.02$, 0.1, and 0.5 M. Thus, it is concluded that this theory for nonionic chains is applicable, in a fairly good approximation, to $\langle S^2 \rangle$ and $[\eta]$ of the charged polysaccharide in aqueous NaCl unless C_s is lower than 0.02 M.

KEY WORDS: Hyaluronic Acid / Polysaccharide / Polyelectrolyte / Excluded-Volume Effect / Wormlike Chain /

According to recent experimental studies,^{1,2} the quasi-two-parameter (QTP) theory³ based on the wormlike⁴ or helical wormlike³ chain allows an almost quantitative, consistent description of excluded-volume effects on the mean-square radius of gyration $\langle S^2 \rangle$ and the intrinsic viscosity $[\eta]$ for nonionic, linear polymers, both flexible and stiff. In contrast to this situation, our understanding of those effects in ionic chains still remains in a quite unsatisfactory stage.

In our previous work, Hayashi *et al.*^{5,6} examined, as a first step toward solving the electrostatic excluded-volume problem, the applicability of the QTP theory to $[\eta]$ of sodium hyaluronate, a charged linear polysaccharide with intrinsically weak stiffness, in aqueous sodium chloride with the aid of the Yamakawa–Fujii–Yoshizaki theory^{7,8} for unperturbed wormlike cylinders. The general agreement between theory and experiment was not bad over the entire range of weight-average molecular weight M_w ($3.8 \times 10^3 < M_w < 3.5 \times 10^5$) and NaCl concentration C_s ($0.005 \text{ M} \leq C_s \leq 2.5 \text{ M}$) studied, but at a few low C_s the experimental viscosity exponents appeared to exceed the theoretically predictable value in the region of $M_w > 4 \times 10^4$ where excluded-volume effects were significant. Nonetheless, the discrepancies between the theoretical and experimental $[\eta]$ were too subtle to draw a definite conclusion on the applicability of the QTP scheme to Na hyaluronate. Thus it seemed necessary to extend the work to a higher molecular weight region where volume effects are more pronounced.

In the present study, viscosity data were obtained for five hyaluronate samples with M_w of 1.9×10^5 to 1.5×10^6 in aqueous NaCl of different C_s and analyzed along with our previous data^{5,6} to check the applicability of the QTP scheme over a wider range of molecular weight. Radius of gyration data were also obtained from light scattering measurements at some NaCl concentrations. They were used for an independent check of the applicability.

EXPERIMENTAL

Samples

A hyaluronate sample with a purity higher than 99% and an intrinsic viscosity of $25.6 \times 10^2 \text{ cm}^3 \text{ g}^{-1}$ (in 0.2 M aqueous NaCl at 25°C) was supplied by Shiseido Co., Japan. Part of it was degraded to four different (average) molecular weights by heating at 110°C for 25–60 min. The resulting samples (each 5–10 g) and the original one (1.5 g) were then fractionated with 0.2 M aqueous NaCl as the solvent and acetone as the precipitant. The procedure was essentially the same as that employed previously,⁵ but to effect the fractionation, lower and higher molecular weight portions were repeatedly removed from each of the five samples and a small amount of the central portion (0.2–0.5 g) was extracted. The fractions thus obtained were designated as HAT1, HAT2, ..., and HAT5 in order of increasing molecular weight.

Aqueous solutions of these fractions were passed through a mixed-bed ion exchanger (Amberlite IR-120 + IR-400) and then neutralized with 0.1 N aqueous sodium hydroxide (see ref 5). Dry sodium hyaluronate was obtained by lyophilization of the neutralized solutions (for more than 4 days). It was dissolved directly in aqueous NaCl of a given C_s after being further dried *in vacuo* overnight.

Light Scattering

Scattering intensities were measured for the five Na salt samples in 0.02, 0.1, and 0.5 M aqueous NaCl at 25°C on a Fica 50 light-scattering photometer in an angular range from 20 to 150°. Vertically polarized incident light of 436 or 546-nm wavelength was used. The experimental procedures including optical clarification were essentially the same as those described elsewhere.⁹ The intensity data obtained were analyzed by use of the square-root plots of $(Kc/R_\theta)^{1/2}$ vs. c and $(Kc/R_\theta)^{1/2}$ vs. $\sin^2(\theta/2)$ to evaluate M_w , A_2 (the second

virial coefficient), and $\langle S^2 \rangle_z$ (the z -average $\langle S^2 \rangle$). Here, K is the optical constant, R_θ the excess reduced scattering intensity at scattering angle θ , and c the polymer mass concentration.

The measurement was also made at $C_s = 0.005$ M for samples HAT1, HAT2, and HAT3. However, no reliable data for M_w and $\langle S^2 \rangle_z$ could be obtained, and our check of the QTP theory with $\langle S^2 \rangle_z$ data was limited to $C_s \geq 0.02$ M.

The specific refractive index increments $(\partial n/\partial c)_\mu$ of Na hyaluronate in 0.02 and 0.1 M aqueous NaCl at 25°C were determined for dialyzed solutions of a sample with $[\eta]$ (in 0.2 M aqueous NaCl) $= 8.8 \times 10^2 \text{ cm}^3 \text{ g}^{-1}$ using a modified Schulz–Cantow type differential refractometer; the subscript μ attached to $(\partial n/\partial c)$ signifies the condition that the chemical potentials of all diffusible components are held constant. The values of $(\partial n/\partial c)_\mu$ obtained were $0.144 \text{ cm}^3 \text{ g}^{-1}$ for 436 nm and $0.141 \text{ cm}^3 \text{ g}^{-1}$ for 546 nm in both 0.02 and 0.1 M aqueous NaCl. For $(\partial n/\partial c)_\mu$ of 0.5 M NaCl solutions, the previous results⁵ (0.141 and $0.138 \text{ cm}^3 \text{ g}^{-1}$ for 436 and 546 nm, respectively) were used.

Viscometry

Viscosities of aqueous NaCl solutions of Na hyaluronate at 25°C were measured at ten different salt concentrations ranging from 0.005 to 2.5 M using a four-bulb low-shear capillary viscometer (apparent shear rates of 11 – 91 s^{-1} for pure water at 25°C) or a conventional capillary viscometer of the Ubbelohde type (apparent shear rate of about 800 s^{-1}). The apparent values of $[\eta]$ for a given sample, determined by the former viscometer, increased linearly with decreasing shear rate (in the above range) and were extrapolated to zero shear rate without ambiguity; we note that even for the highest molecular weight sample HAT5 in 0.005 M aqueous NaCl, the apparent $[\eta]$ value at the highest shear rate of 91 s^{-1} was only 6% smaller than the value at zero shear rate. No electroviscous effect on the measured $[\eta]$ was considered necessary for the reason mentioned previously.⁶ Huggins' constants were all in the range between 0.32 and 0.41.

RESULTS

Figure 1 illustrates the light scattering envelopes for Na hyaluronate sample HAT5 in 0.1 M aqueous NaCl at 25°C, where the dashed line represents the initial tangent to the curve for $(Kc/R_\theta)^{1/2}$ at infinite-dilution. The numerical data obtained from light scattering measurements are summarized in Table I. Two points are to be noted here. First, the values of M_w for the respective samples at the three different salt concentrations agree within $\pm 2.1\%$. Second, those of A_2 are all on the order $10^{-3} \text{ mol cm}^3 \text{ g}^{-2}$, showing remarkable non-ideality of hyaluronate solutions.

The relations between $\log \langle S^2 \rangle_z$ vs. $\log M_w$ at fixed C_s are shown in Figure 2. They are roughly linear with slopes of 1.18, 1.20, and 1.26 at $C_s = 0.5$, 0.1, and 0.02 M, respectively. The last exponent slightly exceeds the asymptotic value of 1.20 for nonionic, flexible chains in good solvents.¹⁰

The data for zero-shear rate $[\eta]$ are summarized in

Figure 1. Light Scattering envelopes for Na hyaluronate sample HAT5 in 0.1 M aqueous NaCl at 25°C. The wavelength of incident light is 546 nm.

Table I. Results from light scattering measurements on sodium hyaluronate samples in aqueous NaCl at 25°C

Sample	C_s	$10^{-5} M_w$	$10^3 A_2$	$10^{-4} \langle S^2 \rangle_z$
	M		$\text{mol cm}^3 \text{ g}^{-2}$	nm^2
HAT1	0.5	1.92	1.41	0.107
	0.1	1.97	2.51	0.130
	0.02	1.99	5.60	0.173
HAT2	0.5	2.50	1.35	0.144
	0.1	2.50	2.50	0.167
	0.02	2.45	5.41	0.219
HAT3	0.5	4.16	1.16	0.259
	0.1	4.16	2.29	0.328
	0.02	4.33	4.82	0.419
HAT4	0.5	9.25	1.04	0.660
	0.1	9.25	1.95	0.801
	0.02	9.25	3.91	1.2
HAT5	0.5	15.6	0.892	1.2
	0.1	15.2	1.70	1.6
	0.02	15.7	2.2	2.4

Table II, and those at $C_s = 0.005$, 0.02, 0.1, 0.5, and 2.5 M are plotted double-logarithmically against M_w in Figure 3. The data points at each C_s approximately follow a straight line, whose slope is, for example, 0.79 at $C_s = 2.5$ and 1.0 at $C_s = 0.005$ M. These viscosity exponents are somewhat smaller than those (0.84 and 1.14 at 2.5 and 0.005 M, respectively) estimated previously⁶ in the M_w region between 10^4 and 3.5×10^5 , suggesting that the $\log [\eta]$ vs. $\log M_w$ relations for $M_w > 10^4$ have a weak downward curvature in the C_s range studied.

Table II. Intrinsic viscosities for sodium hyaluronate samples in aqueous NaCl with different salt concentrations at 25°C

Sample	$[\eta] \times 10^{-2} / \text{cm}^3 \text{g}^{-1}$									
	0.005	0.0067	0.01	0.02	C_s / M					
					0.05	0.1	0.2	0.5	1.0	2.5
HAT1	11.5	10.5	9.19	7.40	5.92	4.97	4.33	3.96	3.66	3.33
HAT2	14.8	13.3	11.6	9.34	7.18	6.22	5.52	4.95	4.65	4.24
HAT3	26.4	23.7	20.0	15.7	12.8	10.8	9.61	8.05	7.40	6.74
HAT4	59.2	52.8	43.4	33.1	24.8	20.7	17.5	14.7	13.3	11.9
HAT5	93.8	86.3	68.8	52.1	40.1	32.5	27.7	22.9	20.6	18.3

Figure 2. Molecular weight dependence of $\langle S^2 \rangle_z$ for Na hyaluronate in aqueous NaCl with $C_s = 0.02, 0.1$, and 0.5 M at 25°C . **Figure 3.** Molecular weight dependence of $[\eta]$ for Na hyaluronate in aqueous NaCl with indicated C_s at 25°C .

DISCUSSION

Intrinsic Viscosity

In the QTP scheme^{3,11,12} for nonionic wormlike or helical wormlike bead chains, the viscosity expansion factor $\alpha_\eta = ([\eta]/[\eta]_0)^{1/3}$ is a function only of the scaled excluded-volume parameter $\tilde{z}$ defined by

$$\tilde{z} = (3/4)K(\lambda L)z \quad (1)$$

with

$$z = (3/2\pi)^{3/2}(\lambda B)(\lambda L)^{1/2} \quad (2)$$

and

$$K(\lambda L) = (4/3) - 2.711(\lambda L)^{-1/2} + (7/6)(\lambda L)^{-1} \quad \text{for } \lambda L > 6$$

$$= (\lambda L)^{-1/2} \exp[-6.611(\lambda L)^{-1} + 0.9198 + 0.03516\lambda L] \quad \text{for } \lambda L < 6 \quad (3)$$

Here, $[\eta]_0$ is the unperturbed intrinsic viscosity, λ^{-1} is the stiffness parameter, L is the contour length of the chain related to the molecular weight M by $M_L = M/L$, with M_L being the molar mass per unit contour length, z is the conventional excluded-volume parameter, and B is the excluded-volume strength for the interaction between a pair of beads. For the wormlike chain (a special limit of the helical wormlike chain) we have³

$$\lambda^{-1} = 2q \quad (4)$$

$$B = \beta/a^2 \quad (5)$$

where q is the persistence length and β the binary cluster

integral, and a the bead spacing; to be consistent with the notation in our previous work, q is used hereafter instead of λ^{-1} . If the Barrett function¹³ is adopted, α_η^3 in the QTP scheme reads

$$\alpha_\eta^3 = (1 + 3.8\tilde{z} + 1.9\tilde{z}^2)^{0.3} \quad (6)$$

The Yamakawa–Fujii–Yoshizaki theory^{7,8} for $[\eta]_0$ of an unperturbed wormlike chain contains M_L , q , and d (the hydrodynamic diameter in the cylinder model) as the parameters, so that $[\eta]$ for a given M in a perturbed state is determined by M_L , q , d , and B ; note that this theory is essentially equivalent to that¹⁴ based on the touched-bead model if the difference in hydrodynamic diameter is taken into account.

Hayashi *et al.*^{5,6} estimated all the parameters except d as a function of C_s from their $[\eta]$ data for M_w between 3.8×10^3 and 3.5×10^5 ; they determined d to be 1.0 nm at $C_s = 0.2 \text{ M}$ with the aid of Cleland's data¹⁵ for oligomers. Assuming, as in the work of Hayashi *et al.*, that this d value is applicable to any other salt concentrations, we re-estimated q and B at each C_s in such a way that for the previous M_L value,^{5,6} the Yamakawa–Fujii–Yoshizaki theory combined with eq 6 most closely fits the previous and present $[\eta]$ data; we note that Hayashi *et al.* determined M_L using data for low M_w , and also that excluded-volume effects on $[\eta]$ become appreciable almost regardless of C_s when M_w exceeds 1.5×10^4 .⁶ Our analysis showed that only B values at several salt concentrations need to be slightly altered. The results are given in Table III, along with the previous q and M_L data (see ref 6 for discussions on

Table III. Wormlike chain parameters and excluded-volume strength for sodium hyaluronate in aqueous NaCl at 25°C

C_s	M_L^a	q^a	B
M	nm ⁻¹	nm	nm
0.005	400	9.7	19 (16) ^a
0.0067	400	8.5	17 (14) ^a
0.01	400	7.4	13 (11) ^a
0.02	405	6.0	9.0
0.05	410	5.0	7.0 (6.0) ^a
0.1	410	4.8	4.8
0.2	405	4.2	4.0 (3.4) ^a
0.5	400	4.1	2.8
1	400	4.0	2.3
2.5	405	4.0	1.6

^a Previous estimates. **Figure 4.** Comparison between the experimental $[\eta]$ at the indicated C_s (the circles) and the theoretical curves computed from the Yamakawa-Fujii-Yoshizaki theory^{7,8} for unperturbed wormlike chains and eq 6 with $d=1.0$ nm and the parameters in Table III. (○), this work; (⊙), Hayashi *et al.*^{5,6}

the C_s dependence of q or the electrostatic persistence length^{16,17}).

Figure 4 compares the best fit theoretical curves with the $[\eta]$ data at $C_s=0.02, 0.1$, and 0.5 M. The agreement is generally good throughout the entire range of molecular weight. Similar degrees of agreement were found at the other salt concentrations higher than 0.0067 M. However, close examination of Figure 4 shows that the curves deviate slightly upward for M_w between 3×10^4 and 1.5×10^5 and downward for M_w above 3×10^5 . The deviation appears to become larger with lowering C_s .

The comparison at the lowest C_s of 0.005 M is illustrated in Figure 5, where the solid and dashed lines represent the theoretical $[\eta]$ and $[\eta]_0$, respectively. While, as is the case at higher C_s , the experimental relation is essentially linear over a range of M_w from 1×10^4 to 2×10^5 , the solid curve here has a more pronounced downward curvature for $M > 3 \times 10^4$. This difference results in an appreciable upward deviation of the solid curve (for the chosen parameters) from the data points in the M_w range between 3×10^4 and 1.5×10^5 , the deviation amounting to 10–20% (almost twice those at $C_s > 0.01$ M). The theoretical exponent ν_α^3 for α_η^3 in this M range is 0.25, whereas the corresponding *experimen-*

Figure 5. Comparison between the experimental $[\eta]$ at $C_s=0.005$ M (the circles) and the theoretical curve (the solid line) computed from the Yamakawa-Fujii-Yoshizaki theory^{7,8} for unperturbed wormlike chains and eq 6 with $d=1.0$ nm and the parameters in Table III. The dashed line represents the theoretical values in the unperturbed state ($B=0$). The symbols are the same as those used in Figure 4.

tal ν_α^3 value (=the difference between the experimental exponent of 1.14 for $[\eta]$ and the theoretical exponent of 0.79 for $[\eta]_0$) is as large as 0.35, *i.e.*, a value that exceeds the asymptotic exponent 0.30 predicted by eq 6. Note that choice of a smaller B leads to a smaller ν_α^3 and a much larger discrepancy between the theoretical and experimental $[\eta]$ at high M_w .

The QTP theory uses the pseudo (or short-range) potential for the excluded-volume interaction between a pair of beads. This approximation may be considered valid even for polyelectrolytes at low ionic strength if the chains are sufficiently long compared to the range that electrostatic interactions between charged beads extend. In other words, the QTP approximation to α_η^3 for Na hyaluronate should be better for higher M . Interestingly, when M_w exceeds 2×10^5 (Figure 5), such discrepancy in ν_α^3 as mentioned above almost disappears or at least considerably diminishes owing to the decrease in experimental exponent for $[\eta]$. This finding suggests that the observed poor agreement in $[\eta]$, at least, at $C_s < 0.01$ M has something to do with the short-range potential used in the QTP theory. Of course, to ascertain this, we need theoretical work on a chain with both non-electrostatic short-range and electrostatic long-range (Coulomb) potentials.

Although the discrepancies between theory and experiment observed in Figure 4 are somewhat systematic, they are as small as what has been observed for nonionic semiflexible polymers.² A point to note is that for those polymers including Na hyaluronate, small theoretical errors may come from the estimated wormlike chain parameters. In view of these, it is probably fair to say that the QTP theory combined with the Yamakawa-Fujii-Yoshizaki theory has no serious shortcomings at $C_s \geq 0.01$ M.

Radius of Gyration

The radius expansion factor $\alpha_s [=(\langle S^2 \rangle / \langle S^2 \rangle_0)^{1/2}]$ is

Figure 6. Comparison between the experimental $\langle S^2 \rangle_z$ at the indicated C_s and the theoretical curves calculated from eq 7 and 8 with the parameters in Table III. The symbols are the same as those used in Figure 4.

also a function only of $\tilde{z}$ in the QTP scheme, where $\langle S^2 \rangle_0$ (the unperturbed mean-square radius of gyration) for the wormlike chain is given by¹⁸

$$\langle S^2 \rangle_0 = (qL/3) - q^2 + (2q^3/L) - (2q^4/L^2)[1 - \exp(-L/q)] \quad (7)$$

and α_s^2 is expressed by

$$\alpha_s^2 = \left[1 + 10\tilde{z} + \left(\frac{70\pi}{9} + \frac{10}{3} \right) \tilde{z}^2 + 8\pi^{3/2}\tilde{z}^3 \right]^{2/15} \times [0.933 + 0.067 \exp(-0.85\tilde{z} - 1.39\tilde{z}^2)] \quad (8)$$

if the Domb-Barrett function¹⁹ is used. For large L/q with which the present analysis is concerned, $\langle S^2 \rangle_0$ is determined substantially by the first two leading terms of eq 7, so that its z -average ($\langle S^2 \rangle_{0z}$) may be evaluated by use of the z -average L , i.e., M_z/M_L , with M_z being the z -average molecular weight.

In Figure 6, the theoretical curves computed from eq 7 and 8 with the parameters in Table III are compared with our $\langle S^2 \rangle_z$ data at $C_s = 0.02, 0.1$, and 0.5 M and those of Hayashi *et al.*⁵ at 0.5 M, where $\langle S^2 \rangle_0$ has been replaced by $\langle S^2 \rangle_{0z}$ calculated with an M_z/M_w of 1.1, the average obtained previously from sedimentation equilibrium.⁵ All the curves come close to the data points for the respective salt concentrations; though the slope 1.21 of the line for $C_s = 0.02$ M is appreciably smaller than that (1.26) of the corresponding experimental relation, the differences between the theoretical and experimental $\langle S^2 \rangle_z$ values are very small in the molecular weight range studied here. Hence, we may conclude that with the parameters derived from $[\eta]$, the combination of eq 7 and 8 describes the present $\langle S^2 \rangle_z$ data for Na hyaluronate in the three aqueous salts.

As Table III shows, the (total) persistence length (estimated from $[\eta]$) increases remarkably with decreasing C_s . This implies that enhancement of electrostatic repulsion between neighboring charged groups of the hyaluronate chain leads to a considerable increase in $\langle S^2 \rangle_0$. Very recently, Na hyaluronate samples of low

molecular weight ($M_w = 3.8 \times 10^3 - 1.1 \times 10^4$) in 0.02 and 0.1 M aqueous NaCl at 25°C were studied by small-angle X-ray scattering in our laboratory,¹⁹ and it was found that the measured $\langle S^2 \rangle_z$ and scattering functions (in the angular range examined) are almost quantitatively explained by the unperturbed wormlike chain with the q and M_L values in Table III. This confirms the unperturbed dimensions of the polysaccharide in the two aqueous salts estimated indirectly from $[\eta]$. Thus, the agreement observed in Figure 6 can be taken to show that eq 8 is a good approximation to the charged polysaccharide in $0.02, 0.1$, and 0.5 M aqueous NaCl.

CONCLUSIONS

We have examined the applicability of QTP theory³ for nonionic chains to $\langle S^2 \rangle$ and $[\eta]$ of Na hyaluronate, a charged polysaccharide, in aqueous NaCl with the aid of relevant theories^{7,8,18} for unperturbed wormlike chains. The agreement between the theoretical and experimental $[\eta]$ becomes poor as C_s is lowered, but the QTP theory is a fairly satisfactory approximation to both $\langle S^2 \rangle$ and $[\eta]$ of the polysaccharide unless C_s is lower than 0.02 M. Below $C_s = 0.01$ M, this theory combined with the Yamakawa-Fujii-Yoshizaki theory^{7,8} fails to explain the experimental viscosity exponents in a limited range of molecular weight from 3×10^4 to 1.5×10^5 . This failure is tentatively attributed to the short-range potential used in the QTP theory for excluded-volume interactions between beads.

Acknowledgments. We thank Dr. T. Yanaki of Shiseido Co. for providing a hyaluronate sample. This work was supported in part by a Grant-in-Aid (09450363) for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan.

REFERENCES

- See, for flexible polymers, F. Abe, Y. Einaga, T. Yoshizaki, and H. Yamakawa, *Macromolecules*, **26**, 1884 (1993); K. Horita, F. Abe, Y. Einaga, and H. Yamakawa, *Macromolecules*, **26**, 5067 (1993); F. Abe, K. Horita, Y. Einaga, and H. Yamakawa, *Macromolecules*, **27**, 725 (1994); M. Kamijo, F. Abe, Y. Einaga, and H. Yamakawa, *Macromolecules*, **28**, 1095 (1995).
- See, for stiff chains, T. Norisuye, A. Tsuboi, and A. Teramoto, *Polym. J.*, **28**, 357 (1996).
- H. Yamakawa, "Helical Wormlike Chains in Polymer Solutions," Springer, Berlin, 1997.
- O. Kratky and G. Porod, *Rec. Trav. Chim.*, **68**, 1106 (1949).
- K. Hayashi, K. Tsutsumi, F. Nakajima, T. Norisuye, and A. Teramoto, *Macromolecules*, **28**, 3824 (1995).
- K. Hayashi, K. Tsutsumi, T. Norisuye, and A. Teramoto, *Polym. J.*, **28**, 922 (1996).
- H. Yamakawa and M. Fujii, *Macromolecules*, **7**, 128 (1974).
- H. Yamakawa and T. Yoshizaki, *Macromolecules*, **13**, 633 (1980).
- Y. Nakanishi, T. Norisuye, A. Teramoto, and S. Kitamura, *Macromolecules*, **26**, 4220 (1993).
- H. Fujita, "Polymer Solutions," Elsevier, Amsterdam, 1990.
- H. Yamakawa and W. H. Stockmayer, *J. Chem. Phys.*, **57**, 2843 (1972).
- J. Shimada and H. Yamakawa, *J. Chem. Phys.*, **85**, 591 (1986).
- A. J. Barrett, *Macromolecules*, **17**, 1566 (1984).
- T. Yoshizaki, I. Nitta, and H. Yamakawa, *Macromolecules*, **21**, 165 (1988).
- R. L. Cleland, *Biopolymers*, **23**, 647 (1984).
- T. Odijk, *J. Polym. Sci., Polym. Phys. Ed.*, **15**, 477 (1977).
- J. Skolnick and M. Fixman, *Macromolecules*, **10**, 944 (1977).
- H. Benoit and P. Doty, *J. Phys. Chem.*, **57**, 958 (1953).
- C. Domb and A. J. Barrett, *Polymer*, **17**, 179 (1976).
- N. Mizukoshi and T. Norisuye, to be published.