

PH and Ionic Strength Dependences of the Intrinsic Viscosity of Betaine-Type Polyampholyte

Yoshio MUROGA,* Masaki AMANO, Akira KATAGIRI,
Ichiro NODA, and Tadao NAKAYA††

*Department of Applied Chemistry, School of Engineering,
Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464, Japan*

*†† Faculty of Engineering, Osaka City University, Sugimoto-cho,
Sumiyoshi-ku, Osaka 558, Japan*

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ABSTRACT: The intrinsic viscosity, $[\eta]$, was measured for the betaine-type polyampholyte, poly(2-(methacryloyloxy)ethyl 2-(trimethylammonio)ethyl phosphate), in aqueous solution at various pH and ionic strengths, I . The linear relationship between $1/\sqrt{I}$ and $[\eta]$ for ordinary polyelectrolytes was also observed for the polyampholyte at low I near isoionic pH and over a wide range of I at other pH. By comparing I dependence of the electrostatic expansion factor of the polyampholyte with that of poly(sodium acrylate), it is suggested that there is a pronounced intramolecular attraction between the segments due to the oppositely-charged groups even when pH deviates from the isoionic pH.

KEY WORDS Intrinsic Viscosity / Polyampholyte / Potentiometric Titration
/ Polyelectrolyte / Expansion Factor /

As is well known, phospholipids such as phosphatidyl-ethanolamine and -choline play important roles as one of constituents of biological membranes. Focusing on the unique features of phospholipids, some groups have been devoting their efforts to synthesize vinyl polymers containing phospholipid analogues.¹⁻⁵ Recently, Nakaya *et al.*^{6,7} reported the preparation of one of betaine-type polyampholytes, *i.e.*, polymethacrylate containing phosphatidylcholine group, poly(2-(methacryloyloxy)ethyl 2-(trimethylammonio)ethyl phosphate), which has $-\text{PO}_4^-$ group dissociated as a weak acid and $-\text{N}^+(\text{CH}_3)_3$ group dissociated as a strong base. Moreover, they suggested⁶ that the polyampholyte dissolved in water forms a loose hydrophobic area and some uncatalyzed polymerizations are possible in this area. However, the conformation of the polyampholyte chain in aqueous solution remains unclear.

If either acidic or basic group in a polyampholyte is a weak acid or base and the weak acidic or basic groups are more populated than the strong basic or acidic groups in the molecule, one can adjust pH to an isoelectric point where the net charge in the molecule reduces to null. Near the isoelectric point, there is the intramolecular attraction between the oppositely-charged groups on the chain so that the polyampholyte chain is expected to be tightly coiled^{8,9} in salt free solution and to be expanded with increasing ionic strength, I . When one introduces the net charge into the molecule by setting pH apart from the isoelectric point, on the other hand, it is expected that the chain is expanded and the expansion decreases with increasing I . In this work, therefore, we studied the pH and I dependences of the conformation of the polyampholyte chain by measuring intrinsic viscosity, $[\eta]$.

EXPERIMENTAL

Samples

The synthesis of the polyampholyte (Figure 1) was stated in detail in previous papers.^{6,7} In order to reduce the effect of the molecular weight distribution on the experimental result, the fractionation was carried out as follows: The sample was dissolved in a mixture of methanol and acetone (2:7 in volume ratio) so as to have a polymer concentration of ~ 0.29 (w/v %). To the resultant solution was added

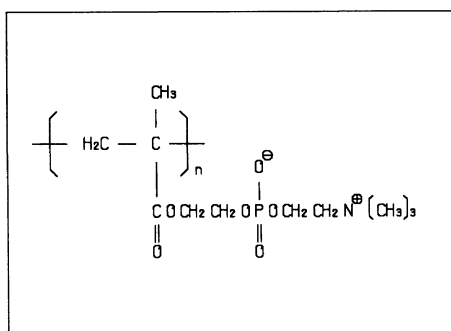


Figure 1. The repeating unit of poly(2-(methacryloyloxy)ethyl 2-(trimethylammonio)ethyl phosphate).

another mixture of the same components (2:6) at room temperature (*ca.* 25°C) until a slight precipitation was effected under stirring. After standing the solution for one day, the concentrated phase was separated from the dilute one. For the polymer recovered from this concentrated phase, the similar fractionation procedure was repeated and the polymer recovered from the final concentrated phase was employed in the present study.

The molecular weight distribution ($M_w/M_n = 3.6$) was evaluated on the basis of pullulan as reference by gel permeation chromatography on Toso GPC HLC-802A (column G5000PW or GMPW) using a mixture of 0.5 M acetic acid and 0.5 M sodium acetate (pH=4.7) as elution solvent. The weight-averaged molecular weight ($M_w = 1.18 \times 10^7$) was determined by light scattering with Automatic Light Scattering, Union Giken LS-601A for 0.1 N NaCl aq. solution of the sample having $\partial n/\partial c = 0.1235$ (mlg^{-1}).

The tacticity of the sample ($rr = 59\%$, $mr = 36\%$, $mm = 5\%$) was characterized from the peak intensity of α -methyl protons in the

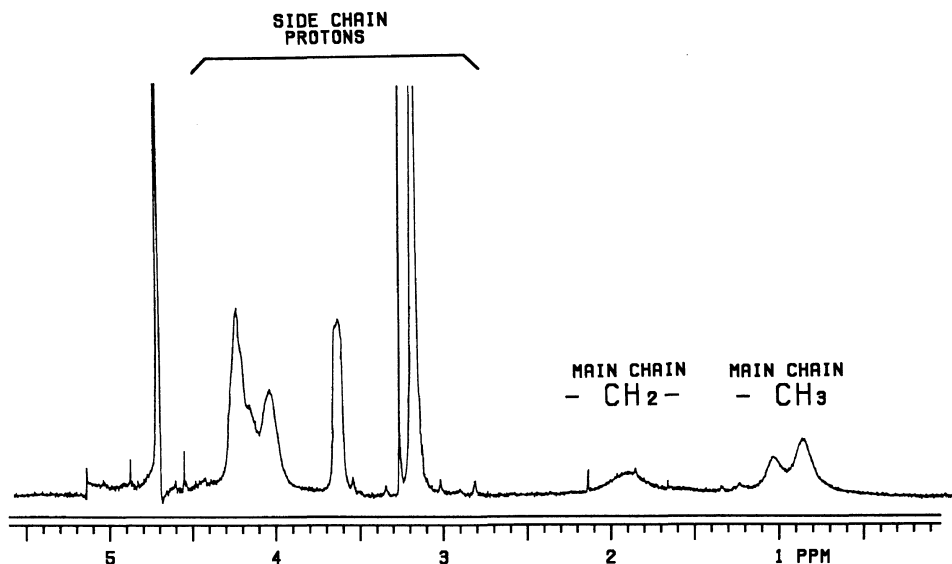


Figure 2. Proton NMR spectrum of the polyampholyte in D_2O solution. Triplet centered at *ca.* 1 ppm, a bump at *ca.* 1.9 ppm, and a sharp peak at *ca.* 4.8 ppm are assigned to α -methyl, methylene protons in the main chain and solvent, respectively. Other signals are assigned to the protons in the side chain.

proton NMR spectrum of its D_2O solution (Figure 2), which was recorded on Varian XL-GEMA 200 spectrometer. Such a tecticity means that the polyampholyte is so-called atactic polymer.

Sample Preparation and Viscosity Measurement

All solutions were prepared with distilled and deionized water. Ionic contaminants in the samples were removed by passing the aqueous solution through a mixed-bed of the ion-exchange resins, Amberlite IRA-400 and IR-120B. The isoionic solution thus prepared showed $pH_{iso} = 3.35$. Since the polyampholyte is extremely hygroscopic in the dry state, the concentration of the solution was determined from the proton NMR spectrum of the solution dissolving a known quantity of a reference sample, phosphoric acid diphenyl ester. Thus, the concentration was determined to the precision of $\pm 3\%$.

The viscosity measurements were carried out at $25 \pm 0.01^\circ C$ by using a Ubbelohde-type capillary viscometer at three pH values, 4.0, 5.0, and 1.0, which were adjusted by the buffer solutions, *i.e.*, a mixture of acetic acid and sodium acetate, a mixture of acetic acid and sodium acetate and a mixture of hydrochloric acid and potassium chloride respectively. At each pH value, I was adjusted to a preassigned one by changing relative amounts of two components dissolved in each buffer solution.

pH Measurement

The measurements were carried out with Corning M255 pH meter at $20 \pm 2^\circ C$, under bubbling of Ar gas over the solution in order to avoid contamination of the solution by CO_2 gas in the air.

RESULTS AND DISCUSSION

The potentiometric titration of the polyampholyte was carried out over both acidic and alkaline pH ranges by adding hydrochloric acid and sodium hydroxide, respectively, to the

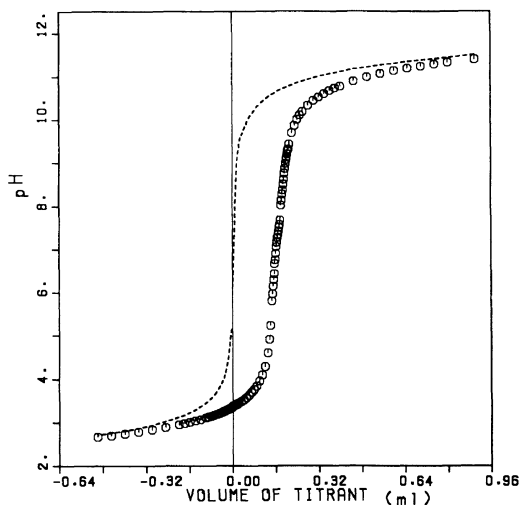


Figure 3. Potentiometric titration curves (circles) of the polyampholyte in aqueous solution and solvent (dotted curve). The isoionic point is found at $pH_{iso} = ca. 3.35$. $C_{p,w} = 0.945$ (g dl $^{-1}$), $C_{NaOH} = 0.05$ (N), $C_{HCl} = 0.05$ (N).

isoionic solution (Figure 3). As is shown in the figure, an inflection point appears in the curve when a small amount of sodium hydroxide is added and this fact implies that the numbers of $-PO_4^-$ groups and $-N^+(CH_3)_3$ groups in the molecule are not equal, but a few bond scissions have occurred somewhere between these ionic groups during the polymerization and/or purification processes.

Here, we assume that the molar weights of the monomer units in the polyampholyte are W_N and W_P , respectively, when the unit has both $-PO_4^-$ and $-N^+(CH_3)_3$ groups and it has $-PO_4^-$ group only. As a result of the dissociation equilibrium (the equilibrium constant = K_{iso}) among $-PO_3OH-$ group and $-PO_4^-$ and H^+ , the polyampholyte aqueous solution having the concentration of $C_{p,w}$ (g dl $^{-1}$) and containing m_0 mole of $-PO_4^-$ groups and n_0 mole of $-N^+(CH_3)_3$ groups in a volume V_0 (ml) exhibits pH_{iso} at the isoionic point. When V_{NaOH} (ml) of C_{NaOH} (N) sodium hydroxide is needed to neutralize the excessive $m_0 - n_0$ mole of $-PO_3OH-$ groups, these quantities are related through the following equations:

$$C_{p,w} = \left\{ \frac{(m_0 - n_0) \cdot W_p + n_0 \cdot W_N}{V_0} \right\} \times 100 \quad (1)$$

$$\text{pH}_{\text{iso}} = -\log[\text{H}^+] \quad (2)$$

$$m_0 - n_0 = \frac{C_{\text{NaOH}} \cdot V_{\text{NaOH}}}{1000} \quad (3)$$

$$K_{\text{iso}} = \frac{\{n_0 \cdot (1000/V_0) + [\text{H}^+]\} [\text{H}^+]}{(m_0 - n_0) \cdot (1000/V_0) - [\text{H}^+]} \quad (4)$$

If a few bond scissions have occurred between $-\text{PO}_4^{\ominus}-\text{CH}_2\text{CH}_2-$ group and $-\text{N}^{\oplus}(\text{CH}_3)_3$ groups, W_p is evaluated to be 238. Thus, substituting $W_p = 238$ and $W_N = 295$ and the observed values of $C_{p,w} = 0.945$ (g dl^{-1}), $\text{pH}_{\text{iso}} = 3.35$, $C_{\text{NaOH}} = 0.05$ (N), $V_{\text{NaOH}} = 0.16$ (ml) and $V_0 = 15$ (ml) into eq 1 to 4, we obtain the ratio $n_0/m_0 = 0.983$. From these values, we evaluate the $\text{p}K_{\text{iso}}$ of $-\text{PO}_3\text{OH}-$ group as 0.78. The magnitude of $\text{p}K_{\text{iso}}$ thus obtained is extraordinary small compared with that for the first step dissociation of phosphoric acid, 1.96. This fact seems to mean that the dissociation of $-\text{PO}_3\text{OH}-$ group was effectively promoted by the presence of $-\text{N}^{\oplus}(\text{CH}_3)_3$ group. Here, it should be noted that W_p cannot unambiguously be known and takes a value between 210 and 238. Such ambiguity of W_p , however, brings about a substantially negligible effect on the resulting $\text{p}K_{\text{iso}}$. From these data, moreover, pH at isoelectric point, where the net charges in the molecule reduces to null, is evaluated as 2.54, assuming that $\text{p}K (= 0.78)$ of $-\text{PO}_3\text{OH}-$ group is not charged between at the isoionic and at the isoelectric points. By using the same assumption, the mole ratios, k , of dissociated basic to acidic groups at $\text{pH} = 4, 5,$ and 1 are estimated as 0.984, 0.983, and 1.54, respectively.

In Figure 4 are shown an example of plots of $\ln(\eta_{\text{rel}})/c$ and η_{sp}/c vs. c at $\text{pH} = 4.0$, where c is the polymer concentration in g dl^{-1} . A good linear relationship between $\ln(\eta_{\text{rel}})/c$ or η_{sp}/c and c is attained irrespectively of I . Such a good linear relationship is also found at $\text{pH} = 5.0$ and 1.0 . To be noted here, it was

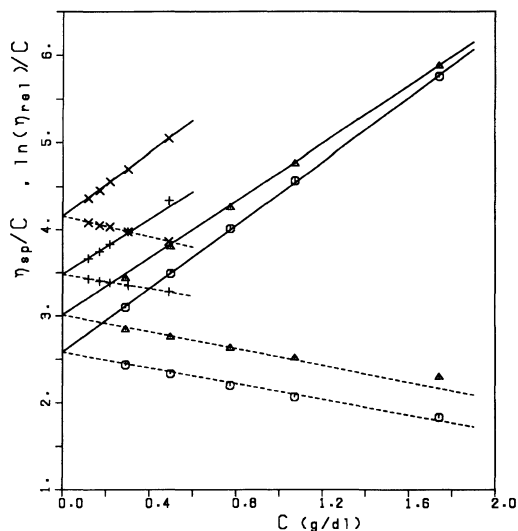


Figure 4. The plot of $\ln(\eta_{\text{rel}})/c$ and η_{sp}/c vs. c at $\text{pH} = 4.0$. The ionic strength is 0.92 (circle), 0.020 (triangle), 0.0021 (plus) and 0.00025 (cross).

Table I. pH and ionic strength (I) dependence of the intrinsic viscosity ($[\eta]$) in (dl g^{-1}).

pH	I	$[\eta]$
1.0	1.0	1.94
	2.3×10^{-1}	2.03
	1.0×10^{-1}	2.16
4.0	9.2×10^{-1}	2.08
	2.0×10^{-2}	2.01
	2.1×10^{-3}	1.98
	2.5×10^{-4}	2.16
5.0	6.7×10^{-2}	1.99
	6.7×10^{-3}	1.95
	6.7×10^{-4}	2.04
	3.4×10^{-4}	2.07

confirmed by proton NMR that the hydrolysis of the polyampholyte was not occurred during the measurement of the viscosity. The $[\eta]$ data obtained by linear extrapolation of $\ln(\eta_{\text{rel}})/c$ and η_{sp}/c to $c = 0$ are summarized in Table I.

The relationship between $[\eta]$ and $1/\sqrt{I}$ for the polyampholyte is shown in Figure 5. In the region of $1/\sqrt{I} > \sim 20$ at $\text{pH} = 4.0$ and 5.0 (Region I) and over a whole region of I at $\text{pH} = 1.0$, $[\eta]$ increases linearly with $1/\sqrt{I}$.

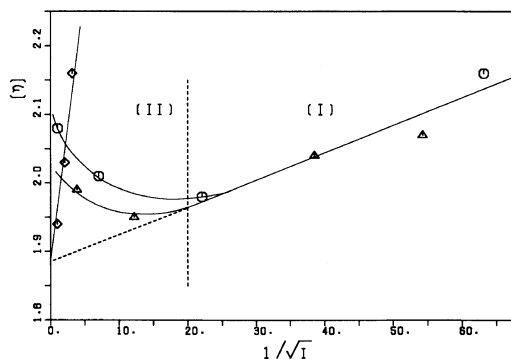


Figure 5. $1/\sqrt{I}$ dependence of $[\eta]$ at pH=1 (diamond), 4 (circle) and 5 (triangle).

Such a linear $1/\sqrt{I}$ dependence of $[\eta]$ is well known in ordinary polyelectrolyte such as poly(sodium acrylate), PNaA.¹⁰ In the regions of $1/\sqrt{I} < \sim 20$ at pH=4.0 and 5.0 (Region II), on the other hand, a reverse tendency is observed. The behavior in Region II at pH=4.0 and 5.0 may suggest the release of attracting interactions between different charges with the increase of I .

Generally speaking in ordinary polyelectrolyte, $[\eta]$ can be linearly extrapolated to infinite I , I_∞ , against $1/\sqrt{I}$, and $[\eta]$ thus obtained, $[\eta]_\infty$, corresponds to a specified and shrank conformation obtained if all charges in the molecule have been completely shielded.¹⁰ As is shown in Figure 5, $[\eta]$ of the polyampholyte showing a linear $1/\sqrt{I}$ dependency also converge to a common value of $[\eta]_\infty$, 1.88, irrespectively of pH. From this observation, it is concluded that the polyampholyte behaves as a polyelectrolyte not only when positive charges are excessively populated in the molecule (pH=1.0), but also even when a slight amount of net charges exists in the molecule (pH=4.0 and 5.0) as long as the magnitude I is sufficiently low.

The cube of expansion factor of a polymer chain for viscosity, α_η^3 , is defined as the ratio of $[\eta]$ of polymer perturbed by the excluded volume effect to that of the unperturbed polymer, $[\eta]_\theta$:

$$\alpha_\eta^3 = \frac{[\eta]}{[\eta]_\theta} \quad (5)$$

and the electrostatic part of α_η^3 for polyelectrolytes, $\alpha_{\eta e}^3$, can be evaluated by¹⁰

$$\alpha_{\eta e}^3 = \alpha_\eta^3 - \left(\lim_{I \rightarrow \infty} \alpha_\eta^3 - 1 \right) \quad (6)$$

where the extrapolation to the infinite ionic strength can be achieved by using the linear relationship between $[\eta]$ or α_η^3 and $1/\sqrt{I}$. As is well established,¹⁰ $\alpha_{\eta e}^3$ of polyelectrolytes depends on $1/\sqrt{I}$ through the factors, $f(i)$, σ , and DP (degree of polymerization):

$$\alpha_{\eta e}^3 - 1 \propto \frac{f(i)}{\sigma^3} \frac{\sqrt{DP}}{\sqrt{I}} \quad (7)$$

where i is a net charge per monomer unit, $f(i)$ is a factor reflecting i dependence of the electrostatic interaction of charges on a polymer chain and σ is a factor reflecting the chain flexibility, which is defined as the ratio of the root-mean squared end-to-end distance of the unperturbed chain, $\langle h^2 \rangle_0^{1/2}$, to that of the ideal chain with free rotation about each carbon-carbon bond, $\langle h^2 \rangle_{0,fr}^{1/2}$.

To clarify the difference in charge interactions at polyampholyte and at ordinary polyelectrolyte, we evaluate the ratio of $f(i)$ for the former to that for the latter. From the data for the polyampholyte in Figure 5, we have

$$\frac{[\eta]}{[\eta]_\infty} = 1 + \frac{A}{\sqrt{I}} \quad (8)$$

where $A=0.0022$ and 0.040 for pH=4 and 5, and 1, respectively. Since eq 6 is rewritten as

$$\alpha_{\eta e}^3 = \frac{[\eta]}{[\eta]_\theta} - \frac{[\eta]_\infty}{[\eta]_\theta} + 1 \quad (9)$$

the electrostatic part of expansion factor for the polyampholyte ($\alpha_{\eta e}^3$)_{AMP} is obtained by substituting eq 8 into eq 9:

$$(\alpha_{\eta e}^3)_{AMP} = 1 + \frac{A}{k \cdot \sqrt{I}} \quad (10)$$

where $k = [\eta]_{\theta}/[\eta]_{\infty}$. When PNaA has the same degree of polymerization ($DP = 4.0 \times 10^4$) as the polyampholyte, $\alpha_{\eta c}^3$ for PNaA is given by:

$$(\alpha_{\eta c}^3)_{\text{PNaA}} = 1 + \frac{B}{\sqrt{I}} \quad (11)$$

where the B values are 1.85 and 3.94 for $i = 0.103$ and 0.400 , respectively.¹⁰ From eq 7, 10, and 11, the ratio of $f(i)$ for the polyampholyte, $f(i)_{\text{AMP}}$, to that for PNaA, $f(i)_{\text{PNaA}}$, is given by:

$$\frac{f(i)_{\text{AMP}}}{f(i)_{\text{PNaA}}} = \frac{A}{k \cdot B} \times \frac{\sigma_{\text{AMP}}^3}{\sigma_{\text{PNaA}}^3} \quad (12)$$

where σ_{PNaA} and σ_{AMP} are the σ values for PNaA and polyampholyte, respectively. The σ value can be evaluated from $\langle h^2 \rangle_0$ determined by $[\eta]_{\theta}$,

$$[\eta]_{\theta} = \Phi \frac{\langle h^2 \rangle_0^{3/2}}{M} \quad (13)$$

where Φ is the well known Flory constant¹⁰ at θ temperature, 2.87×10^{21} . Substituting $[\eta]_{\theta}$ for PNaA¹⁰ and $[\eta]_{\infty}$ for the polyampholyte into eq 13, we have

$$\sigma_{\text{PAA}} = 2.2 \quad (14)$$

$$\sigma_{\text{AMP}}^3 = 27.1[\eta]_{\theta} = 27.1 k[\eta]_{\infty} = 50.9 k \quad (15)$$

Introducing eq 14 and 15 into eq 12, we have

$$\frac{f(i)_{\text{AMP}}}{f(i)_{\text{PNaA}}} = \frac{A}{B} \frac{50.9}{10.6} \quad (16)$$

Here, it is noticed that the net charges in the polyampholyte at pH=4, 5 and 1 are equivalent to that in PNaA having $i = 0.016$, 0.017 , and 0.54 , respectively. Since $\alpha_{\eta c}$ of PNaA at $i = 0.54$ is comparable to that at $i = 0.40$ owing to ion-binding, we have about 0.049 for $f(i)_{\text{AMP}}/f(i)_{\text{PNaA}}$ at pH=1. When i is smaller than 0.1, generally speaking, $f(i)$ is proportional to i^a , where a is ranged from 1 to 2.^{10,11} Therefore, we have $0.047 \sim 0.296$ for B of PNaA at $i = 0.016$ — 0.017 . Using this values in eq 16, we have about 0.036—0.225 for

$f(i)_{\text{AMP}}/f(i)_{\text{PNaA}}$ at pH=4 and 5. Thus, it is clarified that the contribution of $f(i)$ in the $1/\sqrt{I}$ dependence of $\alpha_{\eta c}^3$ is 4.4—28 to 20 times smaller in the polyampholyte than in PNaA.

Thus, the present study shows that a change in I brings about only minor modification in the expansion of the polyampholyte and such a behavior is due to the attractive interactions between oppositely charged groups in the molecule. This conclusion is consistent with our previous observation⁶ that the polyampholyte forms a stable structure in water. When I is higher than 0.0025 at low net-charge (pH=4 and 5), $[\eta]$ is increased with I as is seen in Region II in Figure 5. This may be caused by the release of the attractive interactions between the charges and between hydrophobic groups such as α -methyl groups.

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REFERENCES

1. S. Nakai, T. Nakaya, and M. Imoto, *Makromol. Chem.*, **178**, 2963 (1977).
2. S. Nakai, T. Nakaya, and M. Imoto, *Makromol. Chem.*, **179**, 2349 (1978).
3. T. Umeda, T. Nakaya, and M. Imoto, *Makromol. Chem., Rapid Commun.*, **6**, 285 (1985).
4. A. Furukawa, T. Nakaya, and M. Imoto, *Makromol. Chem.*, **187**, 311 (1986).
5. N. T. Thuong and P. Chabrier, *Bull. Sci. Chim. Fr.*, 667 (1974).
6. T. Ouchi, K. Nomoto, Y. Hosaka, M. Imoto, T. Nakaya, and T. Iwamoto, *J. Macromol. Sci. Chem.*, **A21** (6&7), 859 (1984).
7. T. Nakaya, H. Toyoda, and M. Imoto, *Polym. J.*, **18**, 881 (1986).
8. G. Ehrlich and P. Doty, *J. Am. Chem. Soc.*, **76**, 3764 (1954).
9. M. Nagasawa and I. Noda, *J. Am. Chem. Soc.*, **90**, 7200 (1968).
10. I. Noda, T. Tsuge, and M. Nagasawa, *J. Phys. Chem.*, **74**, 710 (1970).
11. M. Fixman and J. Skolnick, *Macromolecules*, **11**, 863 (1978).