Agarose Gel Electrophoresis of Polyelectrolytes

Kensuke SHIMIZU, Naoki SAKAI, and Akira TAKAHASHI

Department of Industrial Chemistry, Mie University, Tsu, Mie, 514, Japan

ABSTRACT: Agarose gel electrophoresis of synthetic polyelectrolytes, sodium polystyrene sulphonate (NaPSS), poly(acrylic acid) sodium salts (Na-PA) and carboxymethyl cellulose sodium salts (CMC) was studied. Using NaPSSs which have narrow molecular weight distribution, depending on the relative dimensions of radii of gyration of polyions, R_g, and pore size in the gel, $\bar{a}$, it was found that the electrophoretic migration mode is separated into three regimes, namely (1) Ogston ($R_g \leq \bar{a}$), (2) reptation without stretching ($R_g > \bar{a}$) and (3) reptation with stretching, ($R_g \gg \bar{a}$). These findings are quite similar to the agarose gel electrophoresis of DNA first oserved by Slater and Noolandi. Using Na-PSS as standard samples, it was found that, in the regime of reptation without stretching, the friction coefficient f of a monomeric unit is proportional to the cube root of the molar volume of a monomer unit of Na-PA. For measurement of molecular weight of polyions with unknown molecular weight by gel electrophoresis, in the Ogston regime correction for the observed mobility is unnecessary if we use Na-PSS as a standard. However, in the regime (2), mobility should be corrected by evaluating the f of unknown polyions.
KEY WORDS   Agarose / Electrophoresis / Gel / Polyelectrolyte / Poly(acrylic acid) / Polystyrenesulphonate / Carboxy Methyl Cellulose / Reptation /

In order to separate as well as to characterize biological polyelectrolytes such as deoxyribonucleic acid (DNA) and sodium dodecyl sulfate denatured proteins, agarose gel electrophoresis was introduced in the 1960's. It was shown that the method is useful not only for determination of molecular weight but also for separation of DNA fragments depending on their sizes. However, it has often been observed that with increasing molecular size of DNA, electrophoretic separation of DNA depending on chain length becomes difficult. To overcome this shortcoming, pulse field gel electrophoresis has been introduced by Schwartz and Cantor.[17] Then, intensive experimental and theoretical studies on gel electrophoresis of DNA began. However, the extension of this method to characterize synthetic polyelectrolytes has hardly been done.[1-3]

Ferguson[4] studied gel electrophoresis of pituitary proteins and polypeptides based on the investigation of Ogston in 1958.[5] Ferguson derived an equation which correlates the electrophoretic mobility, $\langle \dot{x} \rangle / E$ in a gel in terms of the free mobility, μ_0 in bulk solution, and agarose gel concentration, C_A.[4,6] That is

$$\log \langle \dot{x} \rangle / E = \log \mu_0 - b C_A (R_g + r)^2 \qquad (1)$$

where b is a constant, R_g is the radius of gyration of polyions and r is the radius of rod like fibers which construct the gel structure.[5]

However, this model is only effective for the migration of small polyions whose size is smaller than the average pore size $\bar{a}$ in the gel. Therefore, to explain electrophoretic behavior of larger size DNA, the reptation model was introduced by Zimm, Lumpkin, and Déjardin[7-9] and Slater and Noolandi.[10-14]

Furthermore, to generalize this model covering longer chain length of DNA, Slater, Noolandi, Rousseau, Turmel and Lalande[15] differentiated the migration mode of DNA into three regimes by comparing the radii of gyration of DNA with the average pore size of

agarose gel, $\bar{a}$, namely (1) Ogston regime[4-6] where $R_g < \bar{a}$, (2) reptation without stretching regime[7-14,16] ($R_g > \bar{a}$) and (3) reptation with stretching regime[7-14] ($R_g \gg \bar{a}$).

It is considered that the theory is equally applicable to the gel electrophoresis of synthetic polyelectrolytes since the theory uses only the concept of segments.

The purpose of this work is (1) comparison of the agarose gel electrophoresis experiments of a synthetic polyelectrolytes, sodium polystyrene sulphonate with the theory[1] and clarify the migration mechanism of the polyion, (2) establishment of the method to characterize polyelectrolytes whose molecular size is unknown.

EXPERIMENTAL

Five samples of sodium polystryene sulphonate (Na-PSS) with narrow molecular weight distributions were purchased from Pressure

Table I. Characteristics of sodium polystyrene sulphonates, sodium poly(acrylate)s, and sodium carboxy methyl celluloses

	Code	$M_w \times 10^{-3}$	M_w/M_n	DP
Na-PSS	S1	88	1.05	430
	S2	177	1.05	860
	S3	354	1.05	1720
	S4	690	1.05	3350
	S5	1060	1.05	5150
	S6	2990	—	14510
	S7	5360	—	26020
	S8	8050	—	39080
Na-PA	A1	53.7	—	570
	A2	141	—	1500
	A3	434	—	4600
	A4	495	—	5200
	A5	597	—	6350
	A6	773	—	8200

	Code	$M_w \times 10^{-3}$	M_w/M_n	Degree of substitution	DP
Na-CMC	C1	326	1.41	1.28	1200
	C2	327	1.33	1.54	1150
	C3	393	1.73	2.47	1100

Chemical Co. Three other Na-PSSs of higher molecular weight were fractionated samples and kindly donated from Prof. T. Kato and Mr. S. Usuki. Their characteristics are shown in Table I. Sodium salts of poly(acrylic acid) (Na-PA) (Toa Gosei Chem. Co.) and sodium carboxy methyl celluloses. (Na-CMC) (Daicel Chem. Co.) were fractionated samples and their characteristics are also shown in Table I.

Agarose (type HGT(p)), trishydroxy methyl amino methane and boric acid (all are electrophoresis grade) were purchased from Nacalai Tesque Co., and methylene blue was supplied from Nakarai Chem Co. Twice distilled water was used as the solvent.

METHODS

Preparation of Agarose Gel

Agarose was dissolved in 100 ml of the tris-boric acid buffer solution (pH = 8.1) by boiling. After cooling to approximately 60°C, the agarose solution was poured into 15 cm × 15 cm gel bed (Bio-Rad) equipped with a 15 well comb (Bio-Rad) with 0.55 cm × 0.2 cm teeth. The gel was allowed to solidify for an hour at room temperature.

Gel Electrophoresis

Gel electrophoresis of polyions was performed by a Mitumi Model SJ-1051. The gel was placed in the submarine cell, and the cell was filled with tris–boric acid buffer solution. The 5.0×10^{-2} ml sample solution (50 μg of samples dissolved in the buffer solution) was injected into the well. Electrophoresis was carried out at various agarose gel concentrations under continuous electric fields ($E = 1.2$ V cm^{-1}) during 2—40 h.

After electrophoresis, the gel was immersed in the methylene blue (0.25 wt%) solution for about 30 min and Na-PSS as well as Na-PA and Na-CMC bands were visualized by staining.

902

RESULTS AND DISCUSSION

The radii of gyration of Na-PSS in the tris–boric acid buffer solution were evaluated from the intrinsic viscosity of Na-PSS in the buffer solution by making use of the Flory–Fox equation[23]

$$[\eta] = \Phi \frac{R_g{}^3}{M} \qquad (2)$$

where $\Phi = 1.5 \times 10^{21}$, since in general Φ for polyelectrolytes is somewhat lower than 2.1×10^{21}.[24] The results are shown in Table II.

After gel electrophoresis for the specified time t(h), positions of polyions were visualized by methylene blue staining, and then distances, d(cm) of the center of polyions bands from the comb position of the gel were measured. The average migration velocity of polyions $\langle \dot{x} \rangle$ is calculated as $\langle \dot{x} \rangle = d/t$ and the electrophoretic mobility is given by

$$\langle \dot{x} \rangle / E = d/(t \cdot E) \quad (cm^2 \cdot v^{-1} \cdot h^{-1}) \qquad (3)$$

The experimental reproducibility of $\langle \dot{x} \rangle / E$ was $\pm 10\%$.

Figure 1 shows logarithmic plots of mobility of Na-PSS vs. logarithm of $1/P$, where P is the degree of polymerization (DP) of Na-PSS, for various agarose gel concentration. At 0.5% of agarose gel concentration, the mobilities increase linearly with decreasing DP with the
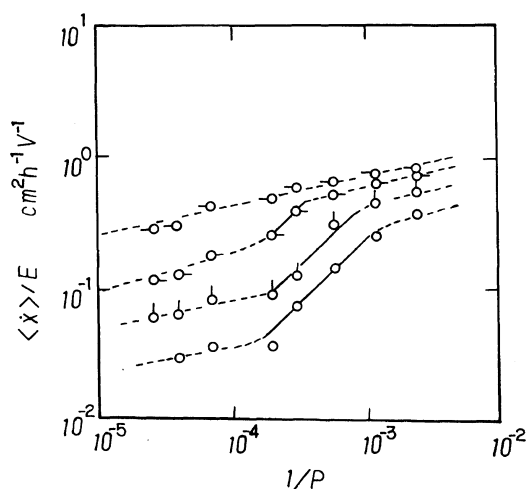
slope of 0.2. However, above 1.0% of agarose gel concentration, the mobility first decreased with increasing DP and then further decreased with the slope of 1 and finally reached a smaller slope line at very high DP.

Our experimental results for the elecrophoresis of Na-PSS are analyzed by the theory of Noolandi et al.[15] in the next section.

I. Comparison with the theory of Slater and Noolandi

(1) *Ogston Regime.* If R_g in eq 1 is fixed, *i.e.*, at a constant molecular weight of polyions, the plot of logarithm of mobility as a function of C_A is expected to give a straight line with a slope of $K_r = b(R_g + r)^2$ and an intercept of $\log \mu_0$, where K_r is known as the retardation coefficient.[15]

In Figure 2, following eq 1 the logarithm of mobility is plotted against agarose gel concentration, which is generally known as the Ferguson plot.[4] For samples of Na-PSS below $M_w = 1.77 \times 10^5$, the plots are straight lines. However, further increase of molecular weight causes departure from linearity. Hence, for determination of K_r, we took initial slopes of

Table II. Radii of gyration of sodium polystyrene sulphonates estimated by eq 4

Code	$M_w \times 10^{-3}$	$[\eta]$ in tris–boric acid buffer solution $(100 \, ml \, g^{-1})$	R_g Å
S1	88	0.20	120
S2	177	0.50	181
S3	354	0.88	277
S4	690	1.05	410
S5	1060	2.10	529
S6	2990	4.90	995
S7	5360	7.80	1413
S8	8050	10.7	1730

Figure 1. The logarithmic plot of mobility, $\langle \dot{x} \rangle / E$ of Na-PSS vs. logarithm of $1/P$. Field strength, $E = 1.2 \, V \, cm^{-1}$ and agarose gel concentration. —◯, 0.5%; ◯—, 1.0%, ◌, 2.0%; ◯, 3.0%. Solid lines have a slope of 1.
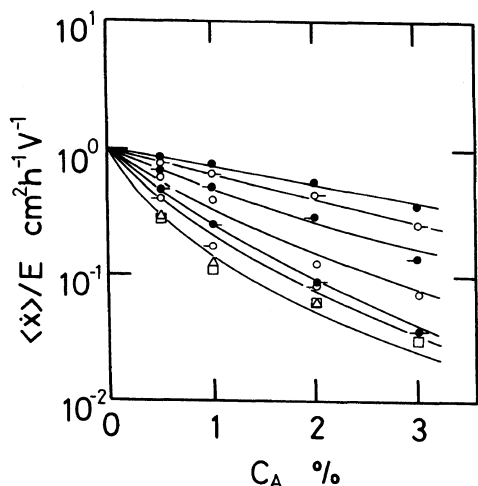
Figure 2. The logarithmic plots of mobility, $\langle \dot{x} \rangle / E$ of Na-PSS $vs.$ agarose gel concentration. Field strength, $E = 1.2\,\mathrm{V\,cm^{-1}}$ and molecular weight of Na-PSS, $M_w \times 10^{-3}$: ●, 88.0; ○—, 177.0; —●, 354.0; ○, 690.0; ●—, 1060; —○, 2990; □, 5360; △, 8050.
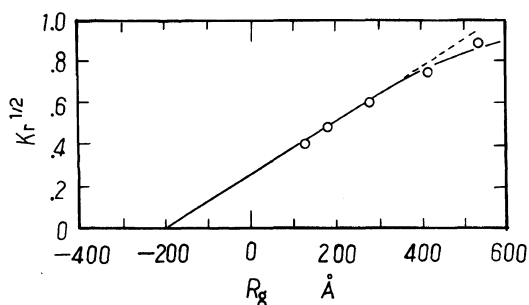

Figure 3. The relationship between the square root of retardation coefficient, $K_r^{1/2}$ and radii of gyration, R_g of Na-PSS. The intercept of this plot at $K_r^{1/2} = 0$ reveals the average fiber radii in agarose gel, $R = 200\,\text{Å}$.

the curves in Figure 2.

Furthermore, the intercepts of the plots almost coincide at one point indicating that the electrophoretic mobility μ_0 in the bulk solution is independent of molecular weight, which has already been confirmed for the electrophoresis of linear polyelectrolytes in bulk salt solution.[18-22]

Since $K_r^{1/2} = b^{1/2}(r + R_g)$ then the plot of $K_r^{1/2}$ against R_g allows us to estimate the average fiber radius, and we obtained $r = 200\,\text{Å}$
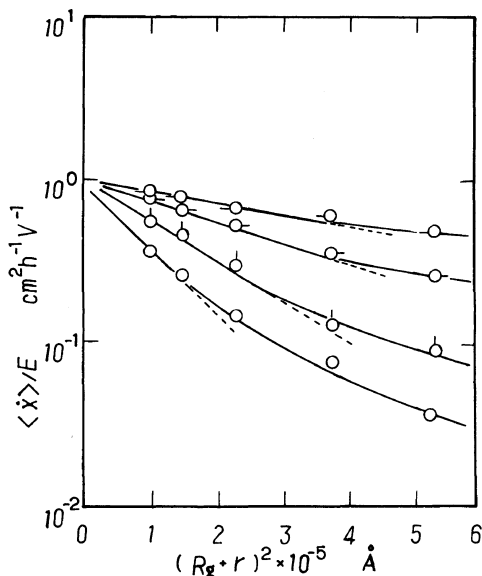
Figure 4. The logarithmic plots of mobility, $\langle \dot{x} \rangle / E$ of Na-PSS $vs.$ $(R_g + r)^2$. Field strength $E = 1.2\,\mathrm{V\,cm^{-1}}$ and agarose gel concentration, C_A: —○, 0.5%; ○—, 1.0%; ○, 2.0% ○, 3.0%.

Table III. Average pore size $\bar{a} \sim R_g$ and gel concentration

$A/\%$	$\bar{a}/\text{Å}$
0.5	500
1.0	290
2.0	150
3.0	80

in the agarose gel at $K_r^{1/2} = 0$ as shown in Figure 3.

Upon evaluated r and R_g, in Figure 4, the mobility is plotted against $(R_g + r)^2$ (eq 1) and the plots are linear for smaller size of Na-PSS, while, for larger size of NaPSS, the plots deviate from straight line.

The average pore diameter $\bar{a}$ may be evaluated by the method of Ogston[4,15] if one knows R_g of polyions. According to the theory, at $R_g \simeq \bar{a}$, the following relationship holds

$$(\langle \dot{x} \rangle / E)/\mu_0 \simeq 1/2 \qquad (4)$$

The results of estimation of $\bar{a}$ are tabulated

in Table III. Thus, it may be concluded that the mobility of sufficiently small size of polyion belongs to Ogston regime.

(2) *Reptation Regime.* The mobility of polyions in reptation regime[7-14] is given by

$$\langle \dot{x} \rangle / E \cong (Q/3\zeta)[(1/P)+(E'^2/3)] \qquad (5)$$

where Q is the total charge on a polyion, ζ is the friction coefficient for translational motion of the whole chain along the tube, and E' is the dimensionless reduced electric field defined by $E' = qEl/2kT$ whre q is the effective charge on a polyion segment and l is the length of a segment of the tube. Since $\zeta = fP$, where f is the friction coefficient of a monomer unit and $Q \propto P$, the term $Q/3\zeta$ is independent of molecular weight of polyions.

According to eq 5, if the electric field strength is sufficiently weak and the first term is much larger than the second term in the square bracket, eq 5 is approximated by

$$\langle \dot{x} \rangle / E \cong (Q/3\zeta) \cdot (1/P) \qquad (6)$$

Therefore, in Figure 1, the regions where the double logarithmic plot of $\langle \dot{x} \rangle / E$ vs. $1/P$ has the slope of 1 belongs to the reptation regime without stretching. For example to 3% gel, the range of molecular weight is from 1.77×10^5 to 1×10^6.

(3) *Reptation Regime with Stretching.* When the polyion size becomes extremely large, then $1/P$ is negligibly small compared with E' and the polyion size-dependence of the mobility will disappear.[7-14]

When $M_w > 10^6$ or $DP > 10^4$, then experimental observation shown in Figure 1 almost coincides with this expectation. However we do not have any direct evidence of the stretching of polyions at the present state. Judging from the comparison between average pore size $\bar{a}$ and radius of gyration of polyions of extremely high molecular weight of Na-PSS, the polyion will make a column of connection of blobs in the tube which restricts the polyion. The end-to-end distance of a polyion in the tube is much larger than that of unrestricted
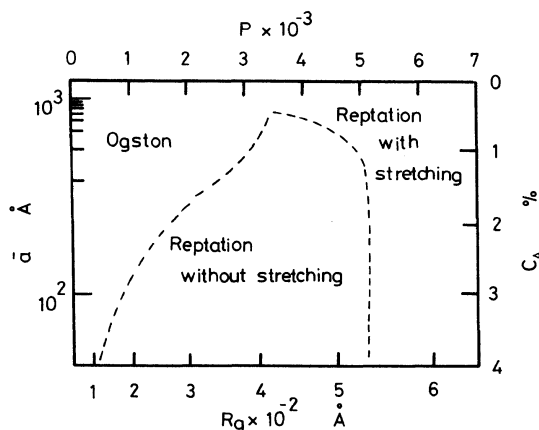

Figure 5. The phase diagram of the agarose gel electrophoresis of Na-PSS at field strength, $E = 1.2 \, \text{V cm}^{-1}$. Degree of polymerization, P and the radii of gyration, R_g (in Å) are given on the upper and lower x axes. Agarose gel concentration, C_A (in %) and the corresponding average pore size $\bar{a}$ (in Å) are given on the left and right y axes.

polyion *i.e.*, a random coil conformation.

II. *Phase Diagram*

The Phase diagram of DNA gel electrophoresis, which indicates three regimes, has been given by Slater and Noolandi *et al.* Following them, the phase diagram for gel electrophoresis of Na-PSS is constructed as Figure 5, which also indicates three regimes, namely Ogston, reptation and stretched reptation.

The Ogston-to-reptation transion point was determined from the onset of nonlinearity on Ferguson plot (Figure 2) and the reptation-to-stretched reptation transition point was determined from the extrapolated intersection of both reptation and streched reptation region (Figure 1).

We can easily find correlations among polyion sizes (molecular weights as well as radii of gyration) and gel concentration with pore size information. The diagram is very useful for molecular characterization of polyions of unknown molecular weight and molecular weight distribution.

III. Calibration Method to Characterize Polyions with unknown Molecular Weight

In the characterization of polyion sizes, the merit of gel electrophoresis experiments is its easiness for comparison with measurements of intrinsic viscosity and/or gel permeation chromatography. However, the gel electrophoresis does not offer absolute mobility which depends on molecular size of polyions. Hence, we must have standard samples of known molecular weight in performing molecular characterization of polyelectrolytes of unknown molecular size as well as molecular weight distribution.[2,3] As standard samples, Na-PSS samples are quite appropriate since they have narrow molecular weight distribution. Using Na-PSS as a standard, the electrophoretic mobilities were measured for Na-PA as well as Na-CMC.[1]

In Table IV, the apparent degree of polymerization P_{app} is compared with the degree of polymerization DP. It is clear that for Na-CMC and low molecular weight Na-PA, which belongs to the Ogston regime, the agreement between P_{app} and DP is good, and therefore we can use Na-PSS as a molecular weight standard without applying any correction.

In the reptation regime, however, P_{app} of Na-PA is smaller than the true DP of Na-PA. This indicates that the careless use of Na-PSS as a standard for molecular weight determination of uncharacterized polyions with different molecular structure leads to erroneous results. In this regime, it should be noted that $\langle \dot{x} \rangle / E$ is a function not only of $1/P$ but also $1/f$,[7-14] i.e.,

$$\langle \dot{x} \rangle / E \propto \frac{1}{fP} \qquad (7)$$

Therefore, we must correct the difference of the friction coefficient of a monomer unit of Na-PSS and Na-PA since the sizes of monomeric unit of both polyions are different. Assuming that the friction coefficient is proportional to the radius of a monomer unit, f is proportional to cube root of molar volume of a monomer unit, i.e.,

$$f \propto r \propto V^{1/3} \qquad (8)$$

and taking the ratio of f for both polyions, we define the specific friction coefficient f_{sp} as

$$f_{sp} = r_{PA}/r_{PSS} = (V_{PA}/V_{PSS})^{1/3} \qquad (9)$$

where V_{PA} and V_{PSS} are the molar volumes of a monomer unit of Na-PA and Na-PSS, respectively. Amoore's method[25] was employed to evaluate V and we obtained $V_{NaPA} = 10.8$ ml (base mol)$^{-1}$, $V_{Na-PSS} = 30.1$ ml (base mol)$^{-1}$, respectively and $f_{sp} = 0.7$. Multiplying f_{sp} to the observed mobility of Na-PA will give corrected DP_c of Na-PA and the results are tabulated in the third column of Table IV. The agreement between the corrected DP_c and DP is fair.

Table IV.

(a) Ogston regime

(1) Na-PA

DP^a	$P_{app} = DP_c$
570	600
1500	1200

(2) Na-CMC

DP	P_{app}
1200	1100
1150	1250
1100	1400

(b) Reptation regime

Na-PA

DP	P_{app}	Corrected $DP_c = P_{app}/f_{sp}$
4600	3200	4600
5200	3000	4200
6350	3800	5400
8200	5000	7250

^{a} DP from Table I.

CONCLUSIONS

The agarose gel electrophoresis of synthetic polyelectrolyte revealed that the migration mechanism is separated into three regimes, namely for a 3% gel (1) Ogston regime ($DP < 10^3$), (2) reptation without stretching regime ($10^3 < DP < 10^4$) and (3) reptation with stretching regime ($DP > 10^4$), which is in conformity with the theory of Slater and Noolandi for DNA electrophoresis.

The phase diagram which offers a very useful guide when one wants to perform the agarose gel electrophoresis of polyions is constructed.

For molecular size determination of polyions of unknown characteristics, Na-PSS having narrow molecular weight distribution is a very good standard. If the sizes of the polyions are in the Ogston regime, no correction is necessary to obtain molecular size. However, if the migration velocity is in the reptation regime, correction for the frictional coefficient of a monomeric unit is necessary.

REFERENCES

1. A. Takahashi and N. Sakai, *Polym. Prepr., Jpn.*, **36**, 1037 (1987).
2. J. L. Chen and H. Morawetz, *Macromolecules*, **15**, 1185 (1982).
3. D. L. Smisek and D. A. Hoagland, *Macromolecules*, **22**, 2270 (1989).
4. K. A. Ferguson, *Metabolism*, **13**, 985 (1964).
5. A. G. Ogston, *Trans. Faraday. Soc.*, **54**, 1754 (1958).
6. D. Rodbard and A. Chrambach, *Proc. Natl. Acad. Sci.*, **65**, 970 (1970).
7. O. J. Lumpkin and B. H. Zimm, *Biopolymers*, **21**, 995 (1982).
8. O. J. Lumpkin, P. Dejardin, and B. H. Zimm, *Biopolymers*, **24**, 1573 (1985).
9. O. J. Lumpkin, and B. H. Zimm, *Biopolymers*, **21**, 2315 (1982).
10. G. W. Slater and J. Noolandi, *Biopolymers*, **24**, 2181 (1985).
11. G. W. Slater and J. Noolandi, *Phys. Rev. Lett.*, **55**, 1579 (1985).
12. G. W. Slater and J. Noolandi, *Biopolymers*, **25**, 431 (1986).
13. G. W. Slater, J. Rousseau, and J. Noolandi, *Biopolymers*, **26**, 863 (1987).
14. G. W. Slater and J. Noolandi, *Polym. Prepr.*, **29**, 416 (1988).
15. G. W. Slater, J. Noolandi, J. Rousseau, C. Turmel, and M. Lalande, *Biopolymers*, **27**, 509 (1988).
16. M. Doi and S. F. Edwards, *J. Chem. Soc., Faraday Trans. II*, **74**, 1789 (1978).
17. D. C. Schwartz and C. R. Cantor, *Cell*, **37**, 67 (1984).
18. M. Nagasawa, A. Soda, and I. Kagawa, *J. Polym. Sci.*, **31**, 439 (1958).
19. I. Noda, M. Nagasawa, and M. Ota, *J. Am. Chem. Soc.*, **86**, 5075 (1964).
20. T. Takahashi, I. Noda, and M. Nagasawa, *J. Phys. Chem.*, **74**, 1280 (1970).
21. M. Nagasawa, I. Noda, T. Takahashi, and N. Shimamoto, *J. Phys. Chem.*, **76**, 2286 (1972).
22. K. Terajima, T. Takahashi, and M. Nagasawa, *Polym. J.*, **8**, 449 (1976).
23. P. J. Flory, "Principles of Polymer Chemistry," Cornell Univesity Press, Ithaca, N.Y., 1953, Chapter 14.
24. A. Takahashi, T. Kato and M. Nagasawa, *J. Phys. Chem.*, **71**, 2001 (1967).
25. J. E. Amoore, *Ann. N.Y. Acad. Sci.*, **116**, 457 (1964).