Electrophoresis of Polyelectrolytes

I. Electrophoretic Study of Poly(vinyl alcohol)

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Abstract

Electrophoretic study of poly(vinyl alcohol) using the Tiselius apparatus showed that the molecule in buffer solutions migrated towards the anode. The number of charges calculated from the mobility was larger than that determined by the conductometric titration: The difference was interpreted as the adsorption of salt-ions inside the polymer coil. The observed mobility was found to be dependent on the polymer concentration: this suggests the existence of inter-macro-ion interaction. The dependence of the mobility on the degree of polymerization was also examined but no appreciable difference was observed.

I. Introduction

A great deal of research has been made on physico-chemical properties of synthetic polyelectrolytes during the last few years. However, only a few authors focused their attention on the electrophoretic behaviors. We intended to study electrophoretic behaviors of a series of polyelectrolytes, which have a fixed number of ionizable groups per molecule and we chose as a sample partially acetalized poly(vinyl alcohol) (PVA) with glyoxylic acid. The present contribution is the first step of the study and is concerned with PVA itself.

The presence of ionizable groups (carboxyl groups) on PVA molecules was pointed out from the polymerization mechanism and Sakurada and Yoshizaki carried out the conductometric titration of PVA to determine the number of acidic groups, which was found to be in agreement with that expected from the polymerization mechanism. This value of carboxyl groups (not more than two per molecule) may be small to detect electrophoretically. On the other hand, there might be a possibility of adsorption of salt-ions as found in the case of proteins by the electrophoresis. Therefore it seems to be interesting to study electrophoretic behaviors of PVA itself.

II. Results

1. Mobility and Polymer Concentration

The effect of polymer concentration on the mobility was observed for PVA. This is illustrated in Fig. 1. The mobility decreases steadily with the polymer concentration at a fixed ionic strength. As will be clearly shown in Part II, the inter-macro-ion interaction seems to be responsible for this dependence.
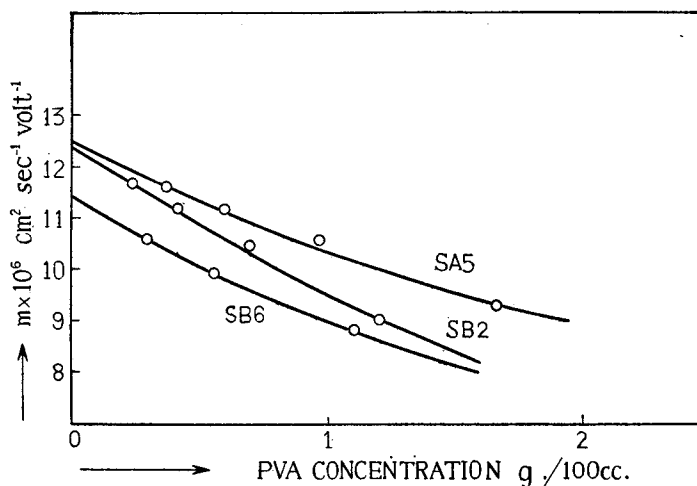

Fig. 1. Concentration dependence of electrophoretic mobility for PVA at 15°C (CH_3COONa–NaOH aqueous solution, ionic strength=0.0927).

2. Mobility and Degree of Polymerization

Table 1 represents the mobility values with PVA's of various degrees of polymerization. R, in this table, is the radius of PVA (assumed to be a sphere) estimated from the viscosity and diffusion measurements[3] and m_0 is the mobility value extrapolated to extreme dilution. Aqueous solution of sodium hydroxyde-sodium acetate (ionic strength 0.0927) was used as the solvent. As is easily seen from this

Table 1. Effect of Degree of Polymerization (P) on Mobility.
Ionic strength $\mu=0.0927$, NaOH–CH_3COONa, pH=12.0

Sample	P	R (A)	c (g./100cc.)	$m \times 10^6$ ($cm^2 \cdot volt^{-1} \cdot sec^{-1}$)	$m_0 \times 10^6$ ($cm^2 \cdot volt^{-1} \cdot sec^{-1}$)
S B 2	1570	115	0.709	10.5	12.5
S B 4	1260	103	0.824	9.9	12.0
S B 6	1010	89	0.558	10.0	11.6
S A 2	460	58	0.673	9.3	12.5
S A 5	350	50	0.993	10.6	12.6
S A 9	160	33	0.682	8.7	11.2

table, the degree of polymerization has no appreciable influence on the mobility. Moreover, despite of the different condition of polymerization, no marked difference in the mobility values between materials *SB* and *SA* has been observed.

3. Mobility and Salt-Concentration

Fig. 2 presents the mobility against salt concentration plots: the mobility unexpectedly increases with increasing salt-concentration. For the charged particle, usually the mobility is expected to decrease due to the growing electrical screening effect as the salt-concentration increases. The results obtained in two solutions of a weak electrolyte (acetic acid) and a strong one (sulphuric acid) are given in Table 2. This indicates that the mobility is determined by the ionic concentration (ionic strength), not by the acid concentration. The ionic strength of the acetic acid solution is calculated from the dissociation constant, $k=1.8\times10^{-5}$, by means of $\alpha^2=k/c$, where α is the degree of ionization and c the molar concentration.
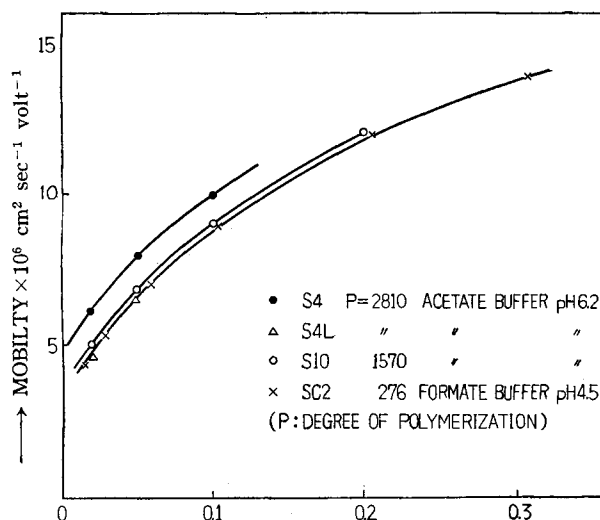

Fig. 2. Electrophoretic mobility vs. ionic strength for PVA in salt solution.

Table 2. Effect of Ionic Strength on Mobility.
Sample S10L. *P*: 1570, PVA concentration=0.80 g./100cc.

Acid	Normality	Ionic strength	$m\times10^6$ $(cm^2\cdot volt^{-1}sec^{-1})$
CH$_3$COOH	0.10	0.0013	0.6
,,	0.25	0.021	1.0
H$_2$SO$_4$	0.095	0.142	8.2

III. Discussion

1. Concentration Dependence of the Mobility

Many theories already proposed on electrophoresis have dealt with a charged particle in the extremely dilute solution and the inter-macro-ion interaction has never been taken into consideration, which would become considerable as the concentration increases. In the high range of concentration, a macro-ion would be shielded electrically

by other macro-ions as well as by salt-ions. It would be to this effect that the concentration dependence of mobility is attributed. We proposed a theoretical treatment of the inter-macro-ion interaction[4] and we will later come back to this problem in Part II.

2. Number of Charges of PVA

Some relations were given earlier on the electric mobility and number of charges of macro-ion. Henry[5] extended the equation proposed by Smoluchowski and derived an expression for the hydrodynamically rigid spherical particles,

$$
m = \frac{E}{6\pi\eta}\left\{\psi_0(1+\lambda)+3\lambda R^3\left[5R^2\int_\infty^R\frac{\psi}{r^6}\,d\dot{r}-2\int_\infty^R\frac{\psi}{r^4}\,dr\right]\right\},
$$

with

$$
\lambda = (\mu_0-\mu')/(2\mu_0+\mu'),
$$

$$(1)$$

where ψ is the electrostatic potential at a distance r from the centre of a rigid sphere (radius, R), ψ_0 the surface potential, $\in$ the dielectric constant, η the viscosity coefficient, μ' the specific conductivity of the sphere, μ_0 the specific conductivity of the solvent.

Using the value of the potential calculated by Debye-Hückel[6], $\psi=\psi_0 R\cdot\exp[-\kappa(r-R)]/r$, we obtain when $\mu_0\gg\mu'$

$$
m = \frac{Q}{6\pi\eta}\frac{f(\kappa R)}{R(1+\kappa R)},
$$

$$(2)$$

where Q is the number of charges of the particle, κ the Debye reciprocal length.

Gorin[7] has proposed a relation for the randomly oriented long cylinder model,

$$
m = \frac{2Q}{\pi Fl\eta}\frac{K_0(\kappa b)}{\kappa b K_1(\kappa b)},
$$

$$(3)$$

where l is the length of long cylinder, b the radius of the section, F a factor dependent on κb[8], and functions K_0 and K_1 are special solutions of the modified Bessel equations of zero and first order respectively.

Recently, Overbeek and Stigter[9] have presented another relation for the spherical model early applied by Debye-Bueche[10]. It reads

$$
m = \frac{Q-Q_{eff}}{Nf}+\frac{Q_{eff}}{F}-\frac{Q_{eff}}{6\pi\eta R'}\frac{\kappa R'}{1+\kappa R'},
$$

$$(4)$$

where Q is the total number of charges, Q_{eff} the effective number of charges, N the Avogadro number, f the friction factor of one bead, F the friction factor of the spherical particle. If $Q\approx Q_{eff}$ and when the shielding ratio $\sigma>5$, Eq. (4) simplifies to

$$
m = \frac{Q}{6\pi\eta R'}\left[\frac{1}{\varphi(\sigma)}-\frac{\kappa R'}{1+\kappa R'}\right],
$$

$$(5)$$

where φ is a function of the shielding ratio. The analogous approach was tried by Hermans and Fujita[11].

According to our study on the viscosity and diffusion of PVA[12], the molecule can be supposed to behave nearly as a rigid shere, and when Debye-Bueche's theory is applied, σ is almost equal to 8. Therefore Eqs. (2) and (5) are applicable to PVA molecules. From the experimental data, the number of charges $n=Q/e_0$ (e_0, the elementary charge) can be estimated by means of these relations and is tabulated in Table 3. For comparative purposes, the number of charges calculated by Eq. (3), with the value $l=2.5 \times P(\text{Å})$, (P the degree of polymerization and $b=2.5\text{Å}$), is also

Table 3. Number of Charges of PVA Estimated from the Mobility.
Ionic strength $\mu=0.0927$, NaOH–CH$_3$COONa

Sample	P	$m_0 \times 10^6$ (e. m. u.) $(\text{cm}^2 \cdot \text{volt}^{-1} \cdot \text{sec}^{-1})$	n		
			Sphere		Cylinder Gorin Eq. (3)
			DH Eq. (2)	OS Eq. (5)	
S B 2	1570	12.5	12.4	8.1	21.8
S B 6	1010	11.6	7.7	5.3	13.0
S A 5	350	12.6	3.3	2.6	4.9
S A 6	160	11.2	1.4	1.4	2.0

DH : Debye-Henry   OS : Overbeek-Stigter

given. As is easily seen from this table, the number of charges increases with P for any case.

The value of $\sigma=8$ was used for Eq. (5). Considering that our experiment was performed in the salt-solution, the value larger than 8 should be assigned to σ. In other words, the number of charges calculated by means of Debye-Henry's relation could be regarded as very close to reality. According to the results by Sakurada and Yoshizaki, however, the number of charges of PVA is independent of the degree of polymerization and cannot be more than 2, but depends on the conditions of polymerization, as inferred from the mechanism of polymerization. The results presented in Table 3 are inconsistent with this information and the number of charges is far from these values. .

3. Effect of Salt-Concentration

Under the experimental conditions mentioned here, the molecular dimension of PVA has heen known to be independent of the salt-concentration[3]. It is possible to estimate the radius by means of Einstein's relation from the results of viscosity and diffusion measurements, and to calculate the number of charges by Eq. (2) from the mobility values at various ionic strengths (presented in Fig. 2). In Fig. 3, the number of charges is given; the dashed curves give those estimated by Eq. (5) with the radius value which was obtained by Debye-Bueche's relation. As is clearly seen, two values are considerably different at high ionic strengths. It is naturally expected that
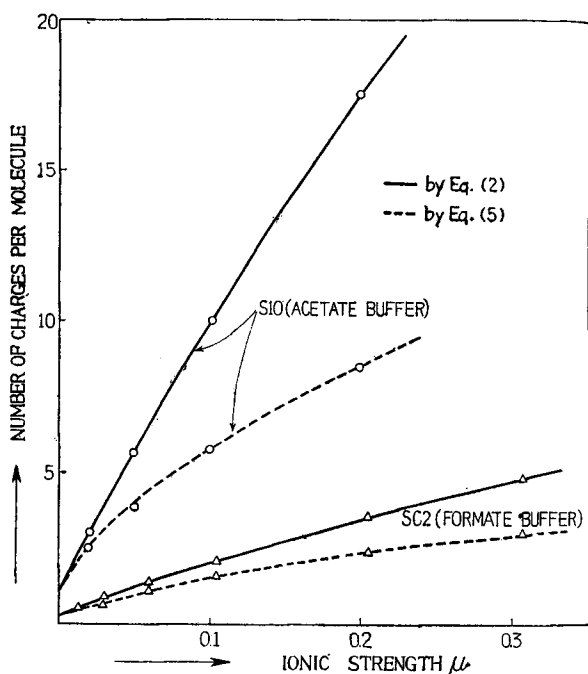
Fig. 3. Number of charges of *PVA* per molecule vs. ionic strength.

σ becomes larger than 8 in high ionic strength range and therefore the number of charges estimated by Eq. (2) would be of more reasonable order. The number of charges does not converge to zero even if extrapolated to zero ionic strength.

a) Charges on *PVA* Molecule

The large number of charges of *PVA* obtained from the mobility connot be explained in terms of present polymerization mechanism. Taking account of the fact that the value increases as the ionic strength increases, one would come to a conclusion: the adsorptive binding of salt-ions inside the sphere of *PVA* molecule. If this reflects the real situation, the number of charges extrapolated to zero ionic strength corresponds to the charges due to ionizations of the ionizable groups on *PVA* molecule, which would be determined by the conductometric titration. Comparison between the number of charges previously reported by Sakurada and Yoshizaki[1] and that obtained by graphical extrapolation of our results to zero ionic strength is shown in Table 4. Agreement is fairly satisfactory.

Table 4. Comparison between the Numbers of Charged of PVA Determined by Conductometric Titration and by Electrophoresis.

Sample	P	Number of charge of a PVA molecule	
		Titration[1]	Electrophoresis
S 4	2810	2.96	2.8
S 4 L	"	0.74	1.1
S 10	1570	0.70	1.0
S C2	276	(0.2-0.4)*	0.3

* Estimated from the results by Sakurada and Yosizaki[1]

b) Equilibrium of Salt-Ion Adsorption

The difference between the number of charges estimated from the mobility at a

finite ionic strength and that due to the ionizable groups of PVA would give the number of ions absorpted by a PVA molecule.

Consider that there exist $[P]$ binding points on PVA molecules to which the salt-ions are bound adsorptively and exists the binding equilibrium

$$[P]+[I] \rightleftarrows [PI],   (6)$$

where $[I]$ is the salt concentration at equilibrium. A binding of salt-ions is assumed to have no influence on the successive bindings and so the equilibrium constant remains unchanged throughout the concentration range studied. Then, if one denotes the number of binding points of a PVA molecule by n, x points of which are assumed to be already occupied, and if one assumes that x is so small that $[I]\cong\mu$, we obtain for 1-1 valent salt,

$$\left. \begin{array}{l} \dfrac{x}{\mu} = \dfrac{n}{K} - \dfrac{x}{K} \\[2mm] \dfrac{\mu}{x} = \dfrac{K}{n_0} + \dfrac{\mu}{n_0} \end{array} \right\}   (7)$$

where K is the equilibrium constant. Therefore x/μ and x, or μ/x and μ should be related in a linear fashion. The results are illustrated in Fig. 4 and Fig. 5, which justify our assumption. From Fig. 5 we obtain $n\cong 6$ per 100 basic molecules of PVA. As to the number of charges, the value obtained by Eq. (2) was used.
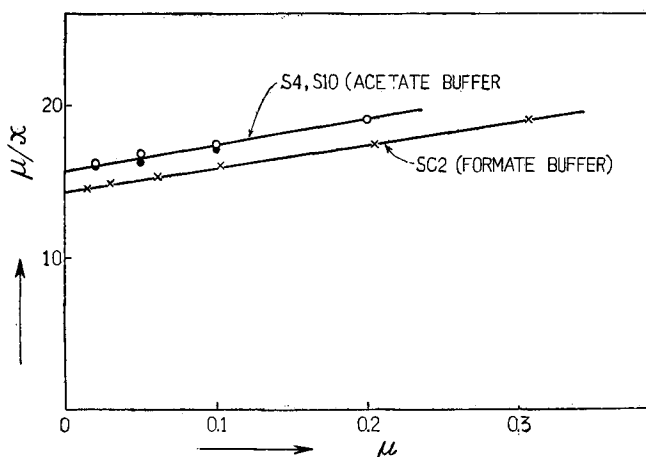
The mechanism of adsorptive binding should

Fig. 4. μ/X vs. μ.

X: Number of binding ions per 100 monomer units of PVA.
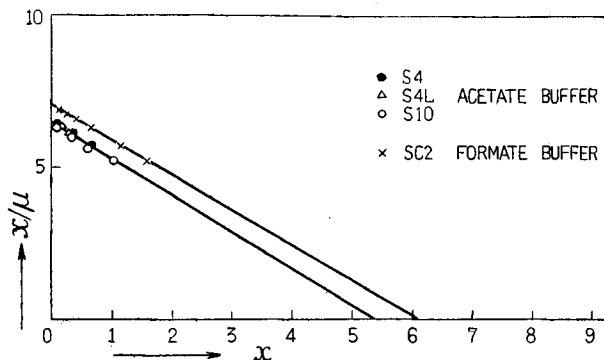
μ: Ionic strength.

Fig. 5. X/μ vs. X.

X: Number of binding ions per 100 monomer units of PVA.

μ: Ionic strength.

be put under further investigation. The treatment which we applied here was already proposed by Klotz[13] for proteins.

IV. Experimental

Apparatus The electrophoretic measurements were performed by the Schlieren diagonal method using HT–A Type (Hitachi Manufacturing Comp.) of the Tiselius apparatus at 15°C ± 0.1°C.

Materials Condition of polymerization of the parent poly(vinyl acetates) was shown in Table 5.

Table 5. Condition of Polymerization of Vinyl Acetate.

Sample No.	Type of polymerization	Catalyst concn. (%, on the wt. of monomer)	Polymeri- zation temp. (°C)	Polymeri- zation time (hrs.)	Conver- sion (%)
S A	Solution (VAc/Ethanol : 70/30)	BPO, 1.0	70	4	86.7
S B	Bulk	BPO, 0.2	60	3	72.0
S C	Solution (VAc/Ethanol : 50/50)	BPO, 1.0	70	4	—
S 4	Bulk	0	80	—	40.0
S 10	Solution (VAc/Ethanol : 62.5/37.5)	AIBN, 0.08	45	—	67.5

VAc: Vinyl acetate, BPO: Benzoyl peroxide, AIBN: Azo-bis-isobutyronitrile.

Parent poly(vinyl acetates) of SA, SC and SB were fractionated by acetone-water mixtures and, after alkali saponification, were washed by methanol for the purification.

S 4 and S 10 were obtained through the courtesy of Mr. Yoshizaki in our laboratory. The subscript L refers to materials, which were treated with HCl to lactonize the carboxyl groups which were supposed to exist at ends of the chain.

The degree of polymerization was calculated from the intrinsic viscosity $[\eta]$ of the aqueous solution by means of $[\eta] = 7.5 \times 10^{-4} \, p^{0.64}$ [2].

Calculation of Electrophoretic Mobility

The electrophoretic mobility m was calculated by

$$m = u/E. \tag{8}$$

where u is the velocity of boundary between buffer and PVA-buffer solution, E the intensity of electric field. u is determined from the displacement of the peak of the descending pattern (Fig. 6) and E from the conductivity of PVA buffer solution, the sectional area of the cell and the electric current.
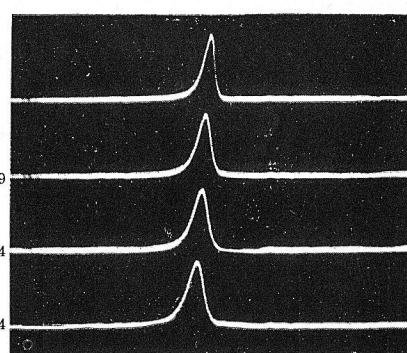
Fig. 6. Electrophoretic patterns of poly(vinyl alcohol).

SB4 Descending patterns   SB4 Ascending Patterns
   Solvent : 0.01N NaOH+CH₃ COONa,
          μ=0.0927, pH=12.0,
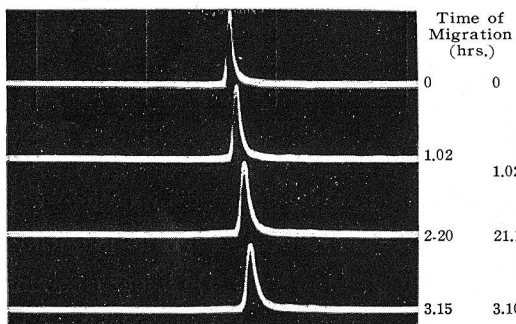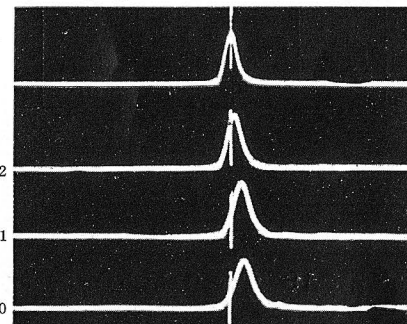   Concn. : 1.648 g./100 cc.
   Voltage gradient : 2.51 v./cm.

Time of Migration (hrs.)

0 — 0
1.37 — 1.39
2.22 — 2.24
3.21 — 3.24
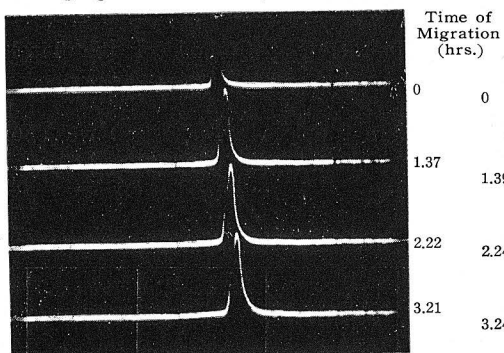
S4 Descending patterns   SC2 Descending patterns
   Acetate buffer ; μ=0.05, pH=6.2   Formate buffer ; μ=0.06, pH=4.5
   Concn. : 0.802 g./100 cc.   Concn. : 1.00 g./100 cc.
   Voltage gradient : 3.65 v./cm.   Voltage gradient : 3.48 v./cm.

Time of Migration (hrs.)

0 — 0
1.02 — 1.02
2-20 — 21.1
3.15 — 3.10

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