Biophysical Chemistry 11 (1980) 425–431

A CONDUCTANCE STOPPED-FLOW STUDY OF POLYELECTROLYTE SYSTEMS. DYNAMIC CONFORMATIONAL CHANGE OF SYNTHETIC MACROIONS AND THEIR COMPLEXATION WITH NEUTRAL POLYMERS

Tsuneo OKUBO
Department of Polymer Chemistry, Kyoto University, Kyoto, Japan

Two dynamic phenomena of polyelectrolyte systems, namely the rapid conformational changes of weak polyelectrolytes (polyacrylic acid (HPAA) and polyethylenimine, induced by pH-jump) and the complexation of HPAA with polyvinylpyrrolidone are first investigated by means of the conductance stopped-flow (κ-SF) technique. The pH-induced relaxations observed by mixing an aqueous solution of HPAA or polyethylenimine with water containing a tiny amount of alkali or acid, are safely ascribed to the conformational transition of the macroions between the compact "coil" and the stretched "rod" forms. The relaxation times are large at high degrees of neutralization and for "rod" conformation. Tentative values for the thermodynamic parameters of the dynamic conformational changes are obtained and discussed. The reaction rates of the polymer complex formation and dissociation of HPAA with polyvinylpyrrolidone are also determined using the κ-SF method. The association constant and the rates of formation and dissociation are 1.84×10^3 M^{-1}, 9.4×10^4 M^{-1} s^{-1} and 51 s^{-1} at 10°C, respectively. We then derive the thermodynamic parameters from the above results.

1. Introduction

Most studies on fast reactions using temperature-jump and stopped-flow techniques have been restricted to spectrophotometric observation of the time change in concentrations of reactants, products and/or intermediates. There exists, however, a variety of association processes and chemical reactions which cannot be followed by spectrophotometric detection. The electrical conductance method is most convenient for dynamic studies of phenomena between ionic species.

The use of conductivity for measuring the rates of fast reactions in solution was first attempted by Saal in 1928 [1]. The technique was basically a continuous flow method, and required large amounts of reagents. In 1955, Sirs [2] and Prince [3] each constructed a conductance stopped-flow (κ-SF) apparatus and studied some fast chemical reactions, e.g., neutralization reaction of carbon dioxide ions in water. However, only a few reports have been published on further applications of the κ-SF technique [4,5,6]. Recently, we constructed a conductance stopped-flow (κ-SF) apparatus and applied it to some ionic reaction systems such as micellar equilibria of ionic surfactants [7], and Ni^{2+}–murexide$^-$ reaction catalyzed by macroions [8].

In this paper, we applied the κ-SF technique to the conformational change of some synthetic macroions (e.g., polyacrylic acid and polyethylenimine). There have been many studies of the conformational changes from compact "coil" to stretched "rod" by neutralization in synthetic weak polyelectrolytes [9,10,11]. Kinetic studies of the transition, however, have been rarely carried out. This is because changes in optical properties are not detected when conformational change occurs, particularly in nonhydrophobic polyelectrolytes such as sodium polyacrylate. Next, we studied the polymer complexation reaction between polyacrylic acid (HPAA) and polyvinylpyrrolidone (PVP) using the κ-SF technique. This reaction system is particularly interesting in relation to the biological phenomena, and the potentiometric, viscometric and IR properties have been studied previously [12–16].

2. Experimental details

2.1. Materials

Sodium polyacrylate (NaPAA, degree of polymerization = 640) was a gift from the Toa Gosei Chemical Co.,

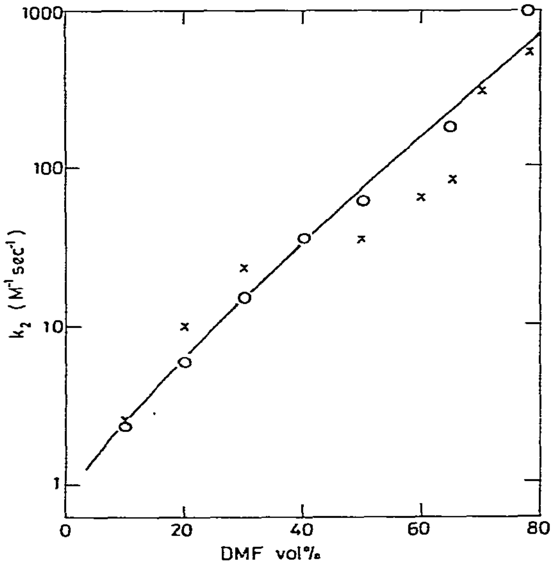


Fig. 1. Rate constants of reactions of malachite green with cyanide ion in H_2O + DMF mixtures at 25°. ○: UV-SF method, X: κ-SF method, [MG] = 1×10^{-5} M, [CN^-] = 1×10^{-2} M.

Nagoya, Japan. An aqueous solution of NaPAA was dialyzed against water for two weeks, and then passed through a column of mixed cation- and anion-exchange resins in their acid forms. Polyethylenimine (PEI) was furnished by the Dow Chemical Co., Michigan (Montrek 12). The molecular weight was 4500 from viscometry. Purification was carried out by passing PEI through columns of ion-exchange resins. Polyvinylpyrrolidone (PVP) was obtained from Tokyo Kasei Chemicals Co., Tokyo (code PVP-K30). The molecular weight was 24500. PVP was further purified by precipitation using acetone. Malachite green of Merck (microscopic and bacteriologic grade) was used without further purification. Potassium cyanide was purchased from Merck (G.R. grade). For the preparation of solutions, deionized water with ion-exchanger resins was further distilled using an auto still.

2.2. Conductance stopped-flow (κ-SF) measurements

The details of the κ-SF apparatus were described in a previous paper [7]. An alternating current of 50 kHz was applied to a Wien bridge. The deviation of the conductance from the equilibrium value was amplified and monitored by a memoriscope, or by a digital memory and X-Y recorder after rectification. The cell consists of a four-jet type mixer (made of Teflon) and an observation cell which has two platinum plates with a 2 mm path length. The distance between the mixer and the center of the platinum plates was 7.5 mm. The temperature of three portions, i.e., mixer, observation cell and solution reserver, was precisely controlled using a thermostatted cell jacket. We can safely neglect the artifact due to the heat of mixing in our apparatus when the heat change is smaller than 37 Jl^{-1} [7]. To check the reliability and versatility of the κ-SF apparatus the rates of some fast reactions from the κ-SF method were compared with those obtained independently from the UV-SF method. The reactions examined are the complexation reaction of Ni^{2+} with murexide anions [7,8], micellar equilibria of ionic surfactants [7], and alkaline hydrolysis of the anionic ester, 4-acetoxy-3-nitrobenzoic acid, in water [17]. Excellent agreement between the two methods were obtained. In this paper, we also measured alkaline fading reaction of malachite green in water-dimethylformamide mixtures. The second-order rate constants, k_2, obtained from the κ-SF method by crosses (see fig. 1) are compared with those from the UV-SF indicated by open circles. A very reasonable agreement is obtained.

2.3. pH, viscosity, and conductance measurements

An F-7SS type pH meter (Horiba-Hitachi, Kyoto) was used for the pH measurements. The specific viscosity was determined using an Ostwald type viscometer. The flow time of water was 256 sec at 25°. The conductivity was measured by a Wayne–Kerr auto balance precision bridge (B-331, Marck II) at a frequency of 10^4 rad/s. The capacitance correction was automatically effected. The precision of the conductivity is approximately ±0.1%. A Jones–Ballinger type cell (platinum plates, cell constant = 4.97 cm^{-1}) was used.

3. Results and discussion

3.1. Dynamic properties of conformational change in polyacrylic acid (HPAA) and polyethylenimine (PEI)

Very clear traces of the conductance change were observed by means of conductance stopped-flow measurements, when an aqueous solution of polyacrylic acid (HPAA) was mixed with a weakly alkaline solution of NaOH. Typical traces of the relaxation effects of HPAA are shown in fig. 2. In most cases, the changes of conductance with time are apparently characterized by two relaxation times: short ones obtained from clear traces as shown in fig. 2 and long ones from very vague traces. Reliable data for longer relaxation times were not obtained. In the present paper, we report the shorter ones only.

In fig. 3, the plots of the reciprocal values of the relaxation times, i.e., τ^{-1}, against the degree of neutralization (α) after mixing are given at constant polymer concentration. Traces of the relaxations of the polymer at $\alpha < 0.2$ were very clear, whereas those at $\alpha > 0.2$ were comparatively vague. Clearly, the reciprocal values decreased in proportion to rising α, and a transition region was observed between -0.1 and 0.3 values of α. This corresponds to the region where PAA changes its conformation from the compact "coil" (for small α) to the extended "rod" forms (for large α) as is seen from the viscosity measurements (see also fig. 3).

In the region of α smaller than -0.1, the conformation of PAA molecules are "coil" or compact "coil". This is because the dissociation of the carboxylic groups of the polymer is almost entirely inhibited in a strongly acidic media, and, furthermore, there is some hydrogen-bonding interactions between the carboxylic groups. On the other hand, when α is larger than 0.3, the conformation of the macroions becomes the stretched "rod" form due to the electrostatic repulsive forces between the carboxylate anions of the polymer. The conformational changes are, therefore, due to transition between the two forms. The transitions of both τ^{-1} and η_{sp} occur at very similar values of α. Thus, we can conclude that the τ^{-1} values of PAA are larger at lower α and for the "coil" form of the polymer.

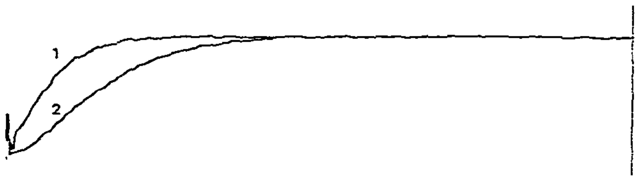


Fig. 2. Typical traces of pH-jump relaxation effect of HPAA by the κ-SF method at 30°. HPAA (5.4 mM) and NaOH (0.188 mM) were mixed. Curve 1: horizontal full scale = 2 s, curve 2: 1 s.

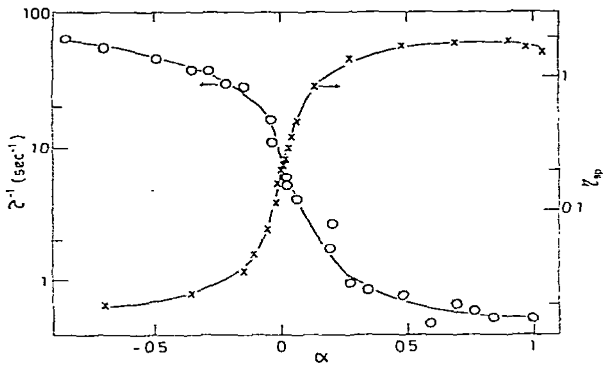


Fig. 3. τ^{-1} (○) and η_{sp} (×) of PAA as a function of α at 15° and 25°, respectively.

We should note here that the τ^{-1} values at small α are extremely large (a limiting value of τ^{-1} is approximately 100 s^{-1}), whereas those at larger α are very small ($\tau^{-1} < 1$ s^{-1}), i.e., the difference between the τ^{-1} values at small and large α is very significant. The radius of gyration, R_G, of HPAA at $\alpha = -0.7$ is roughly estimated to be 70 Å [9]. The small values of R_G indicates that the conformation of HPAA (its degree of polymerization =640) is compact "coil". In this case, the interactions between the chain segments is short-ranged and the fluctuation of such a compact polymer is small and rapid. On the other hand, at $\alpha > 0.5$ the conformation is the extended form, and the maximum length of the polymer is 1600 Å. The fluctuation of such a long chain is not so rapid. Furthermore, the inter-macroion interactions intermediated with their gegenions should not be also neglected, because the existance of ordered structure in NaPAA solutions has been reported. We found that the logarithm of the mean activity coefficient of a polyelectrolyte decreases linearly with the cube-root of polymer concentration. This suggests the existence of a more or less regular

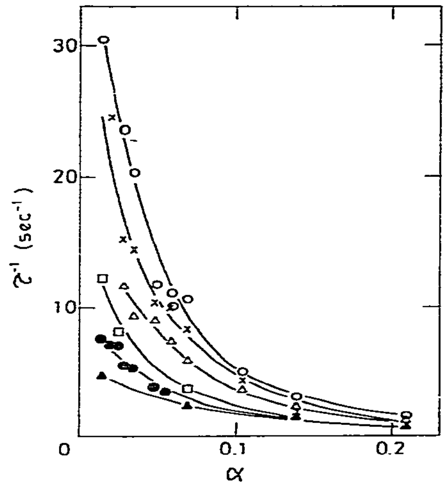


Fig. 4. τ^{-1} of PAA as a function of α between 0 and 0.2 at 10° (▲), 15° (●), 20° (□), 25° (△), 30° (X), and 35° (○). [PAA] = 0.0072 M.

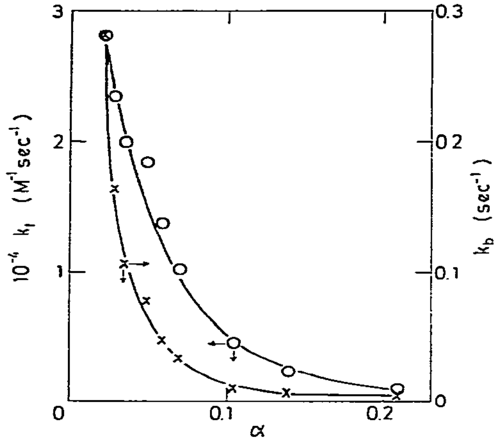


Fig. 5. k_f (○) and k_b (X) as a function of α at 30°. [PAA] = 0.0072 M.

ionic distribution in the solution as a result of intermacroion linkage through the intermediary of gegenions [18,19]. Furthermore, the following experimental supports for such an ordered structure have been documented; the decrease in the single-ion activity of macroions with concentration [20], the low intensity of scattered light from the salt-free polyelectrolyte solutions [21], the catastrophic change of the diffusion coefficient with salt concentration by the dynamic light scattering for poly-L-lysine [22], and the presence of the peak in the scattered intensity of neutron or X-ray beam at small angles for sodium polyacrylate or sodium polymethacrylate [23,24,25]. Thus, we can see that the relaxation period for the conformation change of rod-like macroions is very long. Of course, a clear explanation based on the more detailed experiments is necessary.

The temperature dependence of τ^{-1} values of PAA is measured in the range of α between 0 and 0.2 (see fig. 4). It is clear that τ^{-1} sharply increases with rising temperature and decreases with increasing degree of neutralization.

Now we discuss the apparent reaction rates of the dissociation of protons (k_b), the apparent rates of association (k_f) and the apparent dissociation constant ($K_a = k_b/k_f$) in eq. (1). It is clear that the association

$$\mathrm{HPAA} \rightleftharpoons \mathrm{H^+} + \mathrm{PAA^-} \tag{1}$$

and/or dissociation of protons in eq. (1) are too fast to be followed by the κ-SF method. However, we can assume that the "coil" and "rod" changes in conformation are the rate-determining steps of the fast reactions of protons. The apparent dissociation constant, K_a, is obtained from pH- and conductmetric-methods, and the former gives more reliable data. K_a decreased sharply with increasing α as was observed earlier [1]. The values of k_f and k_b were calculated from K_a and τ values using eq. (2).

$$k_f = \tau^{-1}/(K_a + 2 \times 10^{-\mathrm{pH}} + \alpha C_0)\,, \tag{2}$$

where C_0 denotes the total concentration of PAA. Both k_f and k_b thus obtained were larger at lower α as is clear in fig. 5. The orders of the magnitudes of k_f and k_b seem reasonable because of the large size of the molecule (its molecular weight is 49000). The thermodynamic parameters of the conformational change of PAA at α = 0.02 and at 25° are given in table 1.

Polyethylenimine (PEI, a weakly-basic polymer) also produced clear relaxation curves when we mixed an aqueous PEI solution with water containing a tiny amount of HCl or NaOH. The results of the τ^{-1} values are shown in fig. 6 with the viscosity data. The decreasing tendency of η_{sp} with increasing α above 0.6 is ascribed to the shrinking of the polymer by the elec-

Table 1
Thermodynamic parameters of the "coil"-"rod" conformational change of PAA ($\alpha = 0.02$) at 25° [a]

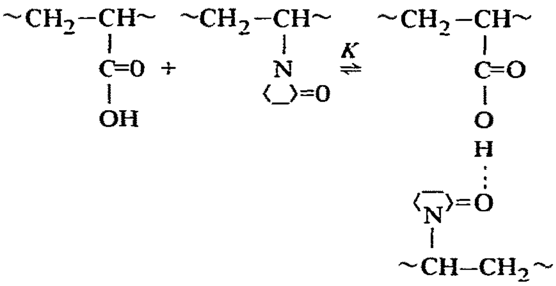
ΔG	-6.8	$\Delta G_f^{\ddagger}$	11.3	$\Delta G_b^{\ddagger}$	18.1
ΔH	~ 0	$\Delta H_f^{\ddagger}$	10.1	$\Delta H_b^{\ddagger}$	11.8
ΔS	17	$\Delta S_f^{\ddagger}$	-4	$\Delta S_b^{\ddagger}$	-21

[a] The units of free energies and enthalpies are in kcal/mole and entropies in e.u.

trostatic shielding effects because the PEI is weakly basic. The conformational transition between "coil" and "rod" forms at $\alpha = -0.2$ and 0.5 did not significantly influence the τ values. However, in the region of α above 0.5, τ^{-1} sharply increased with increasing α. Comparing the data of η_{sp} and τ, we can say that the τ^{-1} values for the "rod" molecules are small and those for the compact "coil" are large. This observation is very similar to that obtained for HPAA.

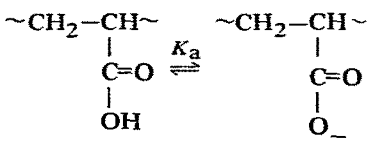
3.2. Kinetics of the polymer complex formation of polyacrylic acid (HPAA) with polyvinylpyrrolidone (PVP)

The proposed rection scheme of the complexation of HPAA with PVP is shown in eq. (3) [16].



$$\begin{array}{c}\sim CH_2-CH\sim\\ |\\ C{=}O\\ |\\ OH\end{array} + \begin{array}{c}\sim CH_2-CH\sim\\ |\\ N\\ \langle\ \rangle{=}O\end{array} \overset{K}{\rightleftharpoons} \begin{array}{c}\sim CH_2-CH\sim\\ |\\ C{=}O\\ |\\ O\\ H\\ \vdots\\ \langle N\rangle{=}O\\ |\\ \sim CH-CH_2\sim\end{array} \qquad (3)$$

When the HPAA-PVP complex is formed, dissociation of the carboxylic groups of HPAA is inhibited. Thus, the concentrations of both protons and carboxylate anions change according to the equilibrium shown in eq. (4).



$$\begin{array}{c}\sim CH_2-CH\sim\\ |\\ C{=}O\\ |\\ OH\end{array} \overset{K_a}{\rightleftharpoons} \begin{array}{c}\sim CH_2-CH\sim\\ |\\ C{=}O + H^+\\ |\\ O_-\end{array} \qquad (4)$$

Since the present reaction system is acidic, the concentration of hydroxide ions is negligible and the following relations hold from eq. (4),

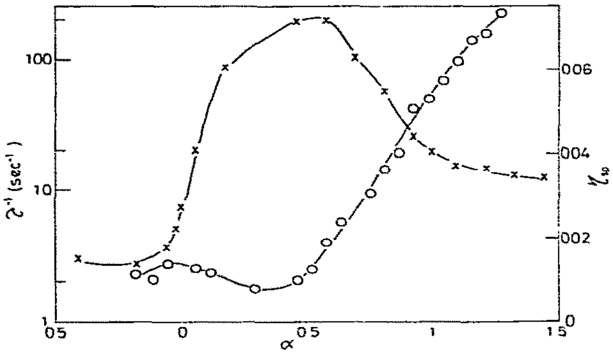


Fig. 6. τ^{-1} (○) and η_{sp} (×) of PEI as a function of α at 25°.

$$K_{a,0} = \frac{[H^+]_0 [PAA^-]_0}{[HPAA]_0} \approx \frac{[H^+]_0^2}{[HPAA]_0}, \qquad (5)$$

$$K_a = \frac{[H^+][PAA^-]}{[HPAA]} \approx \frac{[H^+]^2}{[HPAA]}, \qquad (6)$$

where $[H^+]$, $[PAA^-]$ and $[HPAA]$ denote the concentrations of protons, carboxylate anions and carboxylic acids, respectively. The subscript 0 indicates solutions in the absence of PVP. K_a is the dissociation of HPAA. The ratio of the complexed carboxyl groups to the total concentration of carboxyl groups, θ, is given by the following equations,

$$\log(1-\theta) = \log[HPAA]/[HPAA]_0, \qquad (7)$$

$$= 2(pH_0 - pH) = 2\log(\kappa/\kappa_0), \qquad (8)$$

where, κ_0 and κ are the specific conductance of the reaction solutions in the absence of PVP and in its presence. κ_0 and κ are given by eqs. (9) and (10).

$$\kappa_0 = \kappa_{H^+,0} + \kappa_{PAA^-,0} = 10^{-3}(\Lambda_{H^+} + \Lambda_{PAA^-})[H^+]_0, \qquad (9)$$

$$\kappa = \kappa_{H^+} + \kappa_{PAA^-} = 10^{-3}(\Lambda_{H^+} + \Lambda_{PAA^-})[H^+]. \qquad (10)$$

Thus, θ values are determined from both pH- and conductance-measurements using eq. (8). The changes in the values of pH and κ of the reaction solutions by the addition of PVP are seen in fig. 7 and 8, respectively. There was close agreement between the θ values from pH and conductometric methods.

The association constant between HPAA and PVP,

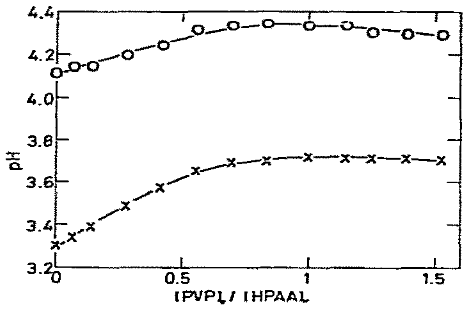


Fig. 7. pH of aqueous HPAA-PVP mixtures at 15°. ○: $[\mathrm{HPAA}]_* = 1.14 \times 10^{-3}$ M, ×: 1.14×10^{-2} M.

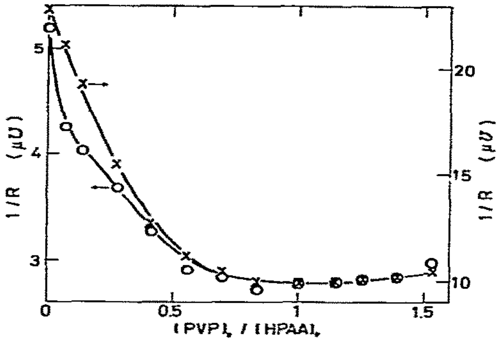


Fig. 8. Conductance of aqueous HPAA-PVP mixtures at 15°. ○: $[\mathrm{HPAA}]_* = 1.14 \times 10^{-3}$ M, ×: 1.14×10^{-2} M.

K, is given as follows:

$$K = \frac{[\mathrm{complex}]}{[\mathrm{HPAA}]\,[\mathrm{PVP}]} = \frac{\theta}{(1-\theta)([\mathrm{PVP}]_* - [\mathrm{HPAA}]_*\theta)} \tag{11}$$

where * indicates the initial total concentrations. The obtained values of K are compiled in table 2. It should be noted that K increases with rising temperature. This may be due to the fact that the driving force of the complexation is, in part, a result of the hydrophobic interactions between the two polymers, because the hydrophobic forces become stronger with rising temperature [26].

Clear traces of relaxation were obtained by mixing aqueous solutions of HPAA and PVP. Typical examples of the traces are seen in fig. 9. The τ^{-1} values obtained at 10 and 15° as a function of $[\mathrm{HPAA}]_*$ ($=[\mathrm{PVP}]_*$) are shown in fig. 10. The reaction at 35° was too rapid to follow by means of the present κ-SF apparatus.

Table 2
Equilibrium and kinetic data of HPAA-PVP complex formation

Temperature (°C)	K (M^{-1})	k_f ($\mathrm{M}^{-1}\,\mathrm{s}^{-1}$)	k_b (s^{-1})
10	$1.8_4 \times 10^3$	9.4×10^4	51
15	$2.3_0 \times 10^3$	$1.2_7 \times 10^5$	55
25	$2.5_3 \times 10^3$	–	–

$[\mathrm{HPAA}]_* = 2.7 \times 10^{-4} \sim 1.1 \times 10^{-2}$ M, $[\mathrm{PVP}]_* = 2.7 \times 10^{-4} \sim 1.7 \times 10^{-2}$ M.

The reaction rates of the complex formation and dissociation, k_f and k_b, were determined from the τ^{-1} and K values using the following equations:

$$\tau^{-1} = k_f\{(K^{-1} + [\mathrm{HPAA}]_* + [\mathrm{PVP}]_*)^2 - 4[\mathrm{HPAA}]_*[\mathrm{PVP}]_*\}^{1/2} . \tag{12}$$

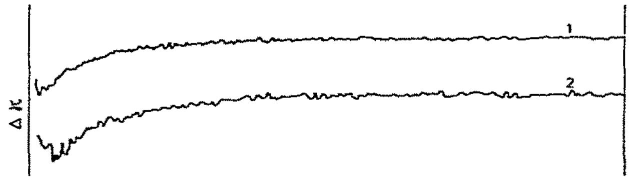


Fig. 9. Typical traces of relaxation effect of HPAA-PVP complexation. Curve 1: $[\mathrm{HPAA}]_* = [\mathrm{PVP}]_* = 3.6 \times 10^{-4}$ M, horizontal full scale: 200 ms, at 15°. Curve 2: $[\mathrm{HPAA}]_* = [\mathrm{PVP}]_* = 5.4 \times 10^{-4}$ M, horizontal full scale: 100 ms, at 10°.

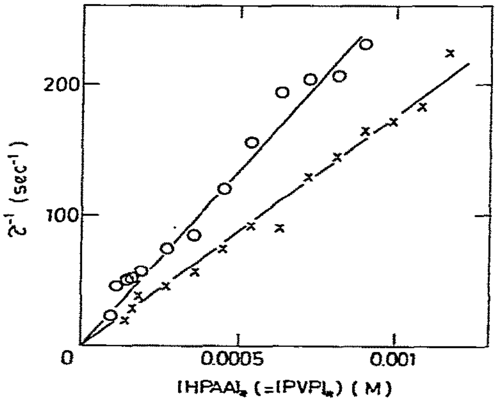


Fig. 10. τ^{-1} of HPAA-PVP complexation at 15° (○) and 10° (×).

Table 3
Thermodynamic parameters of HPAA-PVP complexation at 16.7° [a)]

ΔG	−4.3	$\Delta G_f^{\ddagger}$	10.1	$\Delta G_b^{\ddagger}$	14.4
ΔH	7.2	$\Delta H_f^{\ddagger}$	9.1	$\Delta H_b^{\ddagger}$	1.9
ΔS	40	$\Delta S_f^{\ddagger}$	−3.6	$\Delta S_b^{\ddagger}$	−44

[a)] Calculated from the measurements at 10, 15 and 25°C. The units of free energies and enthalpies are in kcal/mole and entropies in e.u.

$$k_b = k_f / K \,. \tag{13}$$

The obtained kinetic data for the HPAA-PVP system are given in table 2. The complexation rates are very large, in the order of $10^5\,\mathrm{M}^{-1}\,\mathrm{s}^{-1}$. The rates of both forward and backward reactions increases with temperature. The thermodynamic parameters are listed in table 3.

Acknowledgement

The author is greatly indebted to Professor Norio Ise, Kyoto University, for his encouragement and discussion. This paper was presented at the U.S.-Japan Joint Seminar on Electrolytic Aspects of Biopolymers and Biological Systems, Honolulu, Hawaii, June, 1979.

References

[1] R.N.J. Saal, Recl. Trav. Chim. Pays-Bas Belg. 47 (1928) 264, 385.
[2] J.A. Sirs, Trans. Faraday Soc. 54 (1958) 201.
[3] R.H. Prince, Trans. Faraday Soc. 54 (1958) 838.
[4] M.J. Jaycock and R.H. Ottewill, 4th Int. Cong. Surface Act. 2 (1964) 545.
[5] J.M. Corkill, J.F. Goodman, S.P. Harrold and J.R. Tate, Trans. Faraday Soc. 62 (1966) 994.
[6] M.A. Wolff, Chem. Instrum. 5 (1973) 59.
[7] T. Okubo, H. Kitano, T. Ishiwatari and N. Ise, Proc. Roy. Soc. London A 366 (1979) 81.
[8] T. Okubo and N. Ise, Polymer Bulletin 1 (1978) 109.
[9] C. Tanford, Physical chemistry of macromolecules (Wiley, New York, 1961).
[10] S.A. Rice and M. Nagasawa, Polyelectrolyte solutions, 1st Ed. (Academic Press, New York, 1961).
[11] H. Morawetz, Macromolecules in solution, 2nd Ed. (Wiley, New York, 1975).
[12] F.E. Bailey, Jr., R.D. Lindberg and R.W. Callard, J. Polymer Sci. A2 (1964) 845.
[13] V.A. Kabanov, Y. Osada, M. Leskanobrito, J. Reimont and A.N. Grozdetzkii, Vysokomol. Soed. A14 (1972) 2462.
[14] T. Ikawa, K. Abe, H. Honda and E. Tsuchida, J. Polymer Sci. 13 (1975) 1505.
[15] Y. Osada and M. Saito, Makromol. Chem. 176 (1975) 2761.
[16] Y. Osada and M. Saito, J. Polymer Sci., Letter 14 (1976) 129.
[17] H. Kitano, T. Okubo and N. Ise, 10th Symposium on Rapid Reactions, Fukuoka, Oct. (1975).
[18] N. Ise and T. Okubo, J. Phys. Chem. 70 (1966) 1930.
[19] N. Ise and T. Okubo, J. Phys. Chem. 70 (1966) 2400.
[20] N. Ise and T. Okubo, J. Phys. Chem. 70 (1966) 2407.
[21] K.A. Stacey, Light-scattering in physical chemistry (Butterworth, London, 1959).
[22] S.C. Lin, W.I. Lee and J.M. Schurr, Biopolymers 17 (1978) 1041.
[23] J. Cotton and M. Moan, J. Phys., Paris 37 (1976) 75; M. Moan, J. Appl. Crystal 11 (1978) 519.
[24] N. Ise, T. Okubo, Y. Hiragi, T. Hashimoto, M. Fujimura, A. Nakajima and H. Hayashi, J. Amer. Chem. Soc. 101 (1979) 5836.
[25] J. Pleštil, J. Mikeš and K. Dušek, Acta Polymerica 30 (1979) 29.
[26] H.A. Scheraga, G. Nemethy and I.Z. Steinberg, J. Biol. Chem. 237 (1962) 2506.