KINETIC STUDY ON SOME IONIC REACTIONS AND PROCESSES

TSUTOMU ISHIWATARI

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CONTENTS

Chapter 1

Introduction

Recently various types of polyelectrolytes or micelles were synthesized and their catalytic actions were investigated by many workers[1-5]. The catalytic activities of the polyelectrolytes are supposed to be due to various kinds of interactions between the polymer and the reaction substrates. However, studies on the following factors in the catalytic activities of polyelectrolytes, i.e., (1) the influence of the reaction products, (2) the influence of solvation of polyelectrolytes, (3) the influence of the structure of polyelectrolytes and (4) the influence of the charge-transfer type interactions between the substrates and polyelectrolytes, have been insufficient or have not been investigated at all, in spite of their great importance. With such a situation in mind, the author intends to clarify, in this thesis, the roles of these influences both kinetically and thermodynamically, and furthermore, to discuss the construction and versatility tests of conductance stopped-flow apparatus, which is expected to be a useful apparatus for the studies of polyelectrolyte- and micell-catalysed reactions.

The present work is described as follows:

In part I (chapter 2-5), the polyelectrolyte influences on the hydrolysis reactions of various esters or amides and on various interionic reactions will be discussed.

In chapter 2, the alkaline hydrolyses of p-nitrophenyl-3--indoleacetate and N-(indole-3-acryloyl) imidazole are studied in the presence of catalytic polyelectrolytes. The motivation of this study is that, in spite of the importance of the charge--transfer interaction in the hydrolysis reaction systems in vivo, few has been studied on the role of this interaction in the polymer-catalysed hydrolyses of synthesized substrates. The catalytic actions of the polyelectrolytes will be accounted for in terms of the hydrophobic and charge-transfer interactions between the polymers and the substrates. Furthermore in this chapter, the association constants between the polymer and the substrates obtained from the kinetic measurement and those obtained from the spectrophotometric measurement are compared.

In chapter 3, the hydrolysis reactions of p-nitrophenyl caproate, 3-acetoxy-N-trimethylanilinium iodide and 3-nitro-4--acetoxybenzoic acid are investigated in the presence of poly-N--vinylimidazole and the copolymers of N-vinylimidazole and acrylic acid of various composition. The deviation of the first-order plot, i.e., ln[substrate] vs. time plot, from the linearity has often been observed for various combination of reaction substrates and polymer catalysts[6]. It was suspected that this deviation was due to product-inhibition, or the binding of the reaction product onto the catalyst polymer by electrostatic and other interactions. The significance of product inhibition in polymer catalyses will be discussed in detail.

In chapter 4, the role of solvent in polymer catalysis are studied. The solvent effects in polymer "catalysis" are certainly expected to manifest itself clearly for reactions in organic solvents. Some studies have been reported on catalytic influences of polymers in organic solvent-water mixtures[7-15]. However, the hydration or dehydration effect of polymers have not been investigated in detail. In organic solvents, ions are often reported to be dehydrated[16,17]. Furthermore, recent studies of the catalytic effects of reversed micelles and liquid crystals have clearly demonstrated the important role of solvent molecules[18-20]. Thus, it is interesting to examine the catalytic action of polyelectrolytes in organic solvents. Here, the catalytic activities of three kinds of polyethylenimines quaternized with n-octyl bromide, n-lauryl bromide and n-cetyl bromide on the alkaline hydrolysis of p-nitrophenyl acetate, 3-nitro-4-acetoxybenzoic acid and 3-acetoxy-N-trimethylanilinium iodide are studied in n-hexanol-water mixtures. The remarkable role of solvation effect in polymer catalysis will be demonstrated.

In chapter 5, the catalytic effects of ionic latices synthesized by the emulsion copolymerization of acrylic acid and styrene upon interionic reactions between oppositely charged species, i.e., a reaction between $Co(NH_3)_5Br^{2+}$ and OH^- and that between crystal violet and OH^-, are studied. The catalytic polymers used hitherto have been restricted to flexible polymers, and few has been studied on the catalytic effects of polyelectrolytes with fixed molecular conformation. In order to clarify the contribution of structure, ionic latices are used as catalytic polyelectrolytes. The catalytic influences of the latices and a linear polyelectrolyte are also systematically compared.

In part II (chapter 6-8), the polyelectrolyte influences on the association constants of the charge-transfer complexes are studied. The polyelectrolyte influences observed will be accounted theoretically by using Manning theory[21-26].

In chapter 6, the complex formation of analogs of nicotinamide adenine dinucleotide, namely, 3-carbamoylpyridinium compounds quaternized with chloroethylated adenine, thymine and theophylline with indole derivatives are studied in the presence of simple- and polymeric electrolytes and a surfactant, and in their absence. These electrolyte effects are explained theoretically in terms of the "secondary salt effect" using the

Manning theory, suggesting that the electrostatic forces are most important to the stability of the complex. Roles of the hydrophobic and charge-transfer type forces between the substrates and the polyelectrolyte catalyst are also discussed in detail.

In chapter 7, the complex formations of anionic flavinmononucleotide (FMN) with indole derivatives are investigated in the presence of polyelectrolytes. The importance of the electrostatic interactions between the reactant ions and macroions will be discussed, in conformity with the "secondary salt effect".

In chapter 8, the charge-transfer complex formation between poly-4-vinyl-N-alkylpyridinium salts or a polymer containing FMN residues and indole derivatives are investigated. New theoretical equations for the polymer systems are derived from the Manning theory.

In part III (chapter 9-10), the construction of conductance stopped-flow (κ-SF) apparatus and the studies on various ionic reactions by the κ-SF set-up will be reported. Development of apparatus for measuring the fast reaction rates made it possible to study more in detail the catalytic action or the state of polyelectrolytes and micelles in solution. However, as is well known, almost of all studies on fast reactions by using temperature-jump and stopped-flow methods have so far restricted to spectrophotometric observation of the time changes of the concentrations of reactants and/or products. Nevertheless, there exists a variety of association processes and chemical reactions which cannot be followed by the spectrophotometric detection. It is easily understood that the electrical conductance method is convenient for the kinetic studies of phenomena between ionic species such as an association- dissociation equilibrium of micelles.

In chapter 9, the construction of the conductance stopped--flow apparatus and its reliability and versatility tests are discussed. Comparison is made with the established ultraviolet stopped-flow or relaxation methods for a complexation reaction of Ni^{2+} with murexide, a nucleophilic reaction of malachite green with CN^- or OH^- and an alkaline hydrolysis of 4-acetoxy-3-nitrobenzoic acid.

In chapter 10, the rapid ionic equilibria of solutions of cationic and anionic surfactants are studied by the concentration--jump method with the use of the κ-SF technique. The slower relaxation step in micellization kinetics, is measured and discussed for sodium dodecyl sulfate, sodium tetradecyl sulfate, sodium hexadecyl sulfate and hexadecyl trimethylammonium bromide.

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PART I

STUDY OF POLYELECTROLYTE INFLUENCES ON THE HYDROLYSIS REACTIONS

Chapter 2

Catalytic Effects of Poly-4-vinyl-N-alkylpyridinium Salts on the Hydrolyis of Ester and Amide Containing Indolyl Groups

Introduction

Polyelectrolytes are known to have "catalytic" effects on various chemical reactions. This polyelectrolyte catalysis could be attributed to the electrostatic stabilization of activated complex by macroions. Furthermore, it was proposed that the hydrophobic interactions in addition to the electrostatic ones play an important role for the polyelectrolyte catalysis.[1]

In this chapter, the role of the charge-transfer type interactions of macroions in esterolyses is studied. For this purpose, ester- and amide- containing indolyl derivatives as "donor" type substrates and poly-4-vinylpyridinium salts as "acceptor" catalysts are used.

The association constants of the substrate-polymer complex in the "Michaelis-Menten" type reaction scheme are estimated from the kinetic measurements and also from the spectrophotometric measurements of the charge-transfer absorptions.

Experimental

Material

p-Nitrophenyl-3-indoleacetate (p-NPIA) was prepared by mixing 3-indoleacetic acid and p-nitrophenyl trifluoroacetate in dry pyridine by the procedure of Sakakibara and Inukai.[2,3] Yellow crystals were obtained, m.p. 108.2 - 108.5°C (lit.[3] m.p. 108.0 - 108.5°C). Anal. Calcd. for $C_{16}H_{12}N_2O_4$: C, 64.9 ; H, 4.1 ; N, 9.5 ; O, 21.6. Found: C, 65.0 ; H, 4.1 ; N, 9.5 ; O, 21.3.

N-(Indole-3-acryloyl)imidazole (IAI) was obtained by the mixed anhydride method from the recrystallized 3-indoeacrylic acid and imidazole. The product was recrystallized three times from ethyl acetate-n-hexane, m.p. 200 - 200.5°C (lit.[3] m.p. 199.0 - 200.5°C). Anal. Calcd. for $C_{14}H_{11}N_3O$: C, 70.9 ; H, 4.7 ; N, 17.7 ; O, 6.7. Found : C, 71.0 ; H, 4.5 ; N, 17.6 ; O, 7.0.

Polymers used are poly-4-vinyl-N-alkylpyridinium salts (RPVP), i.e., poly-4-vinyl-N-propylpyridinium bromide (C3PVP), poly-4--vinyl-N-benzylpyridinium chloride (BzPVP), and a copolymer of 4-vinyl-N-benzylpyridinium chloride and 4-vinyl-N-cetylpyridinium bromide (19:1) (C16BzPVP). The preparation method of these polymers was described in a previous paper.[4]

Deionized and distilled water was used for the solution preparation.

Kinetic Measurement

The hydrolysis of IAI and p-NPIA were followed by measuring decreases in their absorbances with time at 380 nm and 400 nm, respectively. A Stopped-Flow Spectrophotometer (RA-1100, Union Engineering Co.) and a High Sensitivity Spectrophotometer (SM-401 Union Engineering Co.) equipped with a thermostated cell-holder were used. The kinetic study was always done in a Borax buffer solution of pH = 10.4 at 25°C.

Determination of Association Constants

The association constants, i.e. K* and K, of the donor substrates with the acceptor polymers RPVP were evaluated by the spectrophotometric and kinetic method, respectively. The spectrophotometric measurement of determining K* value was carried out i phosphate buffer solution of pH 7.0 at 25°C in order to suppress the hydrolysis reaction of the substrates. The K* values were obtained from the slope of the $(\varepsilon - \varepsilon_0)/N$ vs. ε plots (Foster-Hammick-Wardley plots$^{5)}$, where ε is the absorbance of the mixture of substrate and catalyst polymer, ε_0 is the sum of absorbance of substrate and that of catalyst polymer, and N denotes the concentration of the polymer. The kinetic determination of K value was done from the plots of $1/(k_{obs} - k_1)$ against $1/[A]$ in equation (1 and (2), where D, A, DA and P

$$\begin{tikzcd} D + A \arrow[r, shift left, "K"] \arrow[d, "k_1"] & D\cdot A \arrow[l, shift left] \arrow[d, "k_2"] \\ P & P \end{tikzcd} \quad (1)$$

$$\frac{1}{k_{obs} - k_1} = \frac{1}{k_2 - k_1} + \frac{1}{K(k_2 - k_1)} \cdot \frac{1}{[A]} \quad (2)$$

denote the substrate, polymer, substrate-polymer complex and reaction product, respectively. k_{obs}, k_1 and k_2 are the observed first-order rate constant, the first-order rate constants of the hydrolysis reaction of the substrate and the complex, respectively.

Results and Discussion

The optical density at 380 nm was plotted against time for hydrolysis reaction of IAI in the presence of BzPVP and in its absence in Figure 1. The linearity was excellent in a wide range of the conversion. The hydrolysis of p-NPIA also showed a linearity as excellent as for IAI. Thus, the kinetic data were treated as the pseudo-first order reactions hereafter, because $[OH^-] \gg [IAI]$ or [p-NPIA].

As is easily seen from Figure 2 and 3, the alkaline hydrolysis reactions of IAI and p-NPIA were enhanced by the addition of the cationic polyelectrolytes, and the magnitudes of

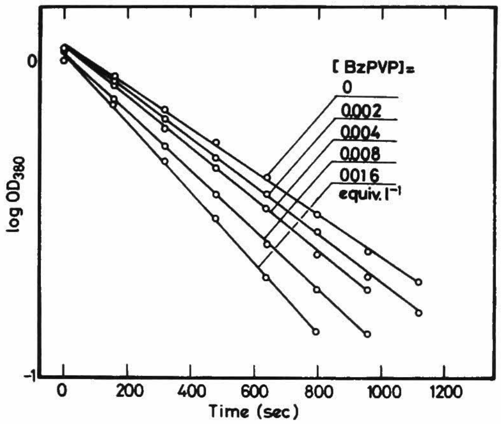

Figure 1 The first order plots for the hydrolysis of IAI with varying concentration of BzPVP

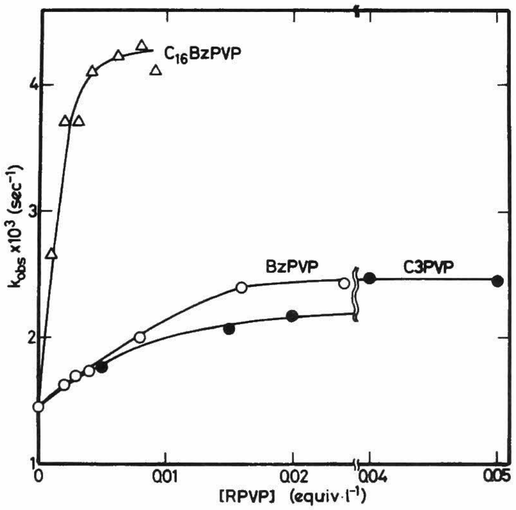

Figure 2 Observed rate constants for the hydrolysis of IAI with varying concentrations of RPVP at 25°C. pH = 10.4, $[IAI] = 3.96 \times 10^{-5}$M

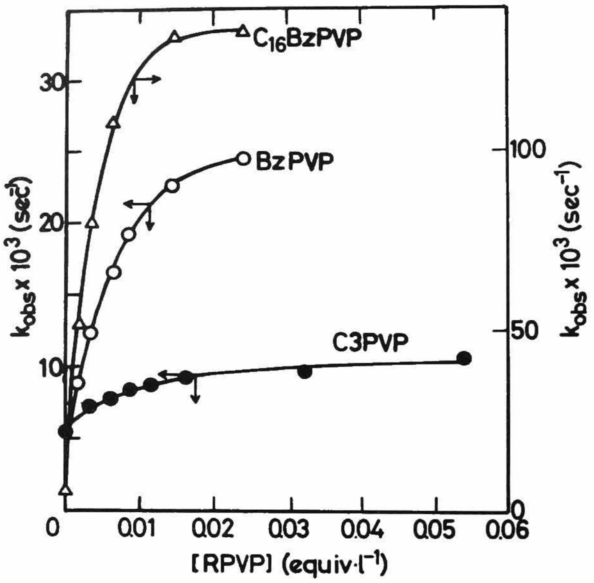

Figure 3 Observed rate constants for the hydrolysis of p-NPIA with varying concentrations of RPVP at 25°C. [p-NPIA] = 3.87×10^{-5}M, pH = 10.4

the enhancement were in the order, C16BzPVP > BzPVP > C3PVP. This order is understandable because the hydroxide ions and the substrates are interacting with the polymer by both the electrostatic forces and the hydrophobic and charge-transfer type interactions.

Another notable feature of the curves in Figure 2 and 3 is the saturation phenomenon. This may imply the validity of the reaction scheme given by equation 1 in the present systems. The $1/(k_{obs} - k_1)$ vs. 1/[BzPVP] plots for the hydrolyses of IAI and p-NPIA are shown in Figure 4. The values of k_2 and K thus

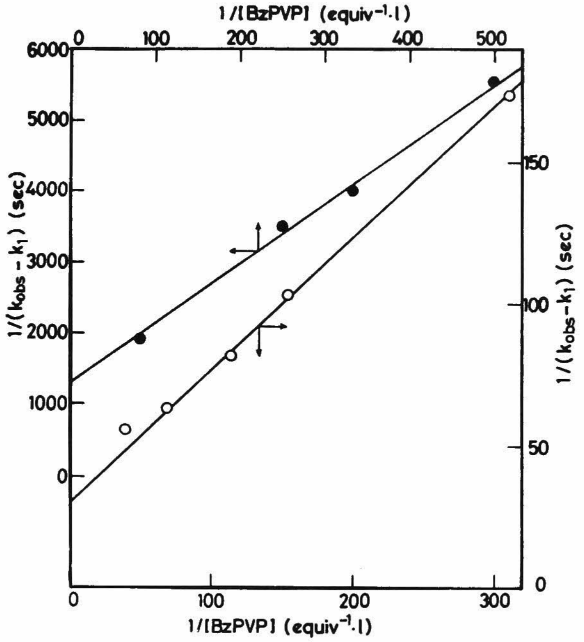

Figure 4 $(k_{obs} - k_1)^{-1}$ vs. $[BzPVP]^{-1}$ plots for the hydrolysis of p-NPIA and IAI with BzPVP at 25°C. [p-NPIA] = 3.87×10^{-5}M (o), [IAI] = 3.96×10^{-5}M (●), pH = 10.4

obtained are compiled in Table I and II with other thermodynamic parameters. The thermodynamic quantities such as $\Delta G^{\neq}$, $\Delta H^{\neq}$ and $\Delta S^{\neq}$ were calculated from the temperature dependencies of k_{obs}. The enthalpy of activation, $\Delta H^{\neq}$, decreased by the addition of the polymers except BzPVP for p-NPIA hydrolysis. Furthermore, k_2 values were larger than k_1 value. This may imply that the esters

Table I Thermodynamic Parameters for the Hydrolysis of

p-Nitrophenyl-3-indoleacetate at 25°C

[p-NPIA]=3.87×10^{-5}M, Borax buffer(pH=10.4)

Polymer	[Polymer] (equiv.l^{-1})	10^3k_{obs} (sec^{-1})	$\Delta G^{\neq}$ ($kcal.mol^{-1}$)	$\Delta H^{\neq}$ ($kcal.mol^{-1}$)	$\Delta S^{\neq}$ (e.u.)	K (M^{-1})	10^3k_2 (sec^{-1})
none	——	7.59(k_1)	22.3	14.4	-27	—	—
C3PVP	0.0165	12.0	20.1	13.2	-23	101	13.2
BzPVP	0.0200	19.5	19.8	14.9	-16	84.6	32.9
C16BzPVP	0.0200	95.2	18.8	8.99	-33	337	87.6

Table II Thermodynamic Parameters for the Hydrolysis of N-(Indole-3-acryloyl)imidazole at 25°C $[IAI]=3.96\times10^{-5}M$, Borax buffer(pH=10.4), K* : the formation constant obtained spectrophotometrically

Polymer	[Polymer] (equiv.l^{-1})	10^3k_{obs} (sec^{-1})	$\Delta G^{\neq}$ (kcal.mol^{-1})	$\Delta H^{\neq}$ (kcal.mol^{-1})	$\Delta S^{\neq}$ (e.u.)	K (M^{-1})	10^3k_2 (sec^{-1})	K* (M^{-1})
none	——	1.45(k_1)	21.3	19.6	-5.7	—	—	—
C3PVP	0.0345	1.81	21.2	16.0	-17	74.3	2.64	270
BzPVP	0.02	2.25	20.9	16.8	-14	44.5	3.62	710
C16BzPVP	0.005	4.17	20.7	13.3	-25	49.2	5.11	980

bound to polymers are more reactive than the esters themselves, though the reason is not clear.

The k_2 values increased in the following order, C3PVP < BzPVP < C16BzPVP. This order indicates that the reactivity of the D·A complex increases as the hydrophobicity of the complex increases, because the order of the strength of the hydrophobicity is reported to be the same as the order of the k_2 values.[6-8] The deactivation of the activated complex of the D·A → P process by the hydrophobic and electrostatic forces of the polymers may be a main reason for the enhancement of the reactivity.

Next, the association constants are discussed. The constants obtained from the kinetic measurements (K) shown in the two tables increased in the order ; BzPVP < C3PVP < C16PVP. This order does not correspond to that of the hydrophobicity, which may be partly due to the strong steric hinderance in the case of BzPVP-substrate binding, and partly to the large contribution of the charge-transfer interactions between the substrates and the catalysts.

The difference absorption spectra of the complexation of IAI with C3PVP are demonstrated in Figure 5. From the changes of the absorption peaks at 410 nm, the association constants, K*, were obtained using the Foster-Hammick-Wardley plots. The plots are shown in Figure 6 for IAI + C3PVP, IAI + BzPVP and IAI + C16BzPVP systems. To prevent the progress of the hydrolysis reactions, the

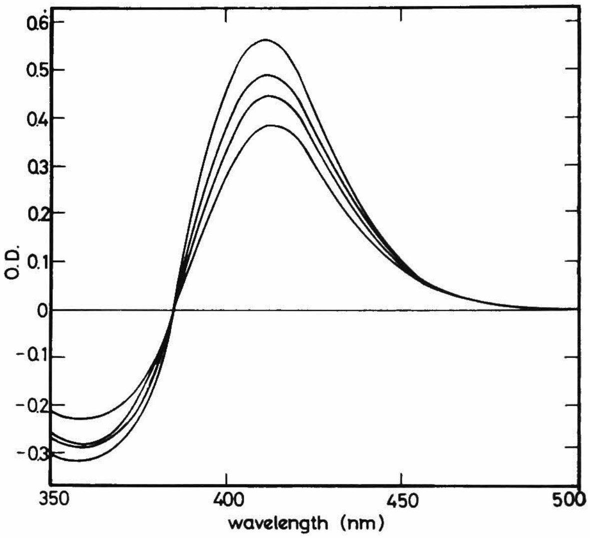

Figure 5 Difference spectra of IAI-C3PVP complex at 25°C. [IAI]=3.96×10^{-5}M, [C3PVP]=0.005-0.04 equiv.1^{-1}

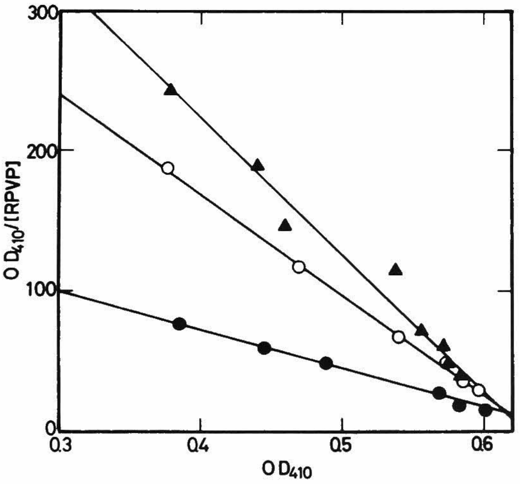

Figure 6 Spectrophotometric determination of the formation constant of the IAI-RPVP complex at 25°C. ▲ : C16BzPVP, ○ : BzPVP, ● : C3PVP [IAI]=3.96×10^{-5}M, [C16BzPVP]=0.001-0.01 equiv.1^{-1}, [BzPVP]=0.002-0.02 equiv.1^{-1}, [C3PVP]=0.005-0.04 equiv.1^{-1}

measurements were carried out at pH = 6.0. The linearities of the plots were excellent for all systems. The spectrophotometric determination of K* was impossible for p-NPIA and polymer systems because the solubility of the ester was very low in water. It is interesting to note that the association constants, K*, for IAI obtained from the spectrophotometric measurements were in the same order as the hydrophobicity, i.e., C3PVP < BzPVP < C16BzPVP (see Table II). Furthermore, K* was clearly larger than K.

The results may suggest that all complexes are not hydrolyzed in these systems. It is highly plausible that some complexes are bound to one another by the strong hydrophobic interactions, and they become inactive for the hydrolysis. This disagreement between the orders of K and that of K* may also suggest the important contribution of the charge-transfer type interactions for the reactivity of the D·A complexes in addition to the electrostatic and hydrophobic interactions.

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Chapter 3

Polyelectrolyte Catalysts Repelling Reaction Products. Hydrolysis Reactions of Esters in the Presence of Copolymers of N-Vinylimidazole and Acrylic Acid.

Introduction

Catalytic influences of synthetic polymers on the hydrolysis of various esters have been extensively studied hitherto[1-5]. However, almost of all studies has been concerned with or restricted to the catalytic effect on the initial rates. In most cases of the systems of polymer catalysts, the reaction order deviates largely from the ideality by the so-called product inhibition[6].

In this chapter, the several systems of hydrolysis reactions are studied, in which the polymer catalysts would repell or attract the products by the electrostatic- and hydrophobic-interactions. Mainly, the curves of the time conversion of the products are examined.

Experimental

Esters used are anionic 3-nitro-4-acetoxybenzoic acid (NABA), cationic 3-acetoxy-N-trimethylanilinium iodide (ANTI) and neutral p-nitrophenyl caproate (PNPC).

NABA and ANTI were synthesized by the methods of Overberger et al.[7,8], and purified by recrystallization from benzene and nitromethane, respectively. PNPC was purchased from Sigma and was used without further purification.

Copolymers of N-vinylimidazole (NVIm) and acrylic acid (AA) of various composition (IV, V, VII and X) and a homopolymer poly-N-vinylimidazole (PNVIm) were obtained by the radical polymerization in methanol at 70°C by using AIBN as an initiater. The monomer fraction (%) in the copolymers shown in Table I were

Table I Composition of the Copolymers

Copolymer	NVIm (%)	AA (%)
IV	12.5	87.5
V	17.5	82.5
VII	25.4	74.6
IX	30.8	69.2
X	46.0	54.0

determined from the measurements of elementary analysis, light absorbances of NVIm residues and conductometric titrations.

Benzyltrimethylammonium iodide, phenol, sodium sulfonate and caproic acid were guaranteed reagents commercially available. Water was deionized and further distilled for solution preparation.

Kinetic measurements were conducted in Clark-Lubs' borate buffer of pH=7.8 at 30°C by using a high sensitive spectrophotometer equipped with a thermostated cell-holder (SM-401, Union Engineering Co. Hirakata, Osaka-fu). The reaction rates were determined from the absorption increase at 400 nm for PNPC, 416 nm for NABA and 293 nm for ANTI, respectively. pH titration curves of the copolymers were drawn by using a automatic-pH-titrator (Radiometer Co.).

Results and Discussion

Figure 1 shows the representative pH titration curves of the copolymers, where the copolymers dissolved in sodium hydroxide solution were titrated with hydrochloric acid solution. In the series of titrations, the sum of the concentrations of the monomer residues were fixed at constant value. The apparent values of the pKa of the copolymers were evaluated to be 10±0.3 and 5.4±0.3

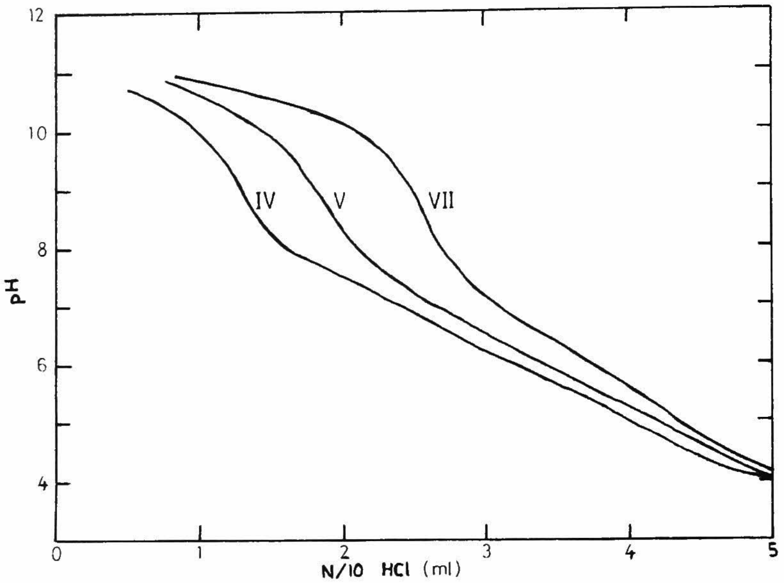

Figure 1 pH titration curves of the copolymers by N/10 HCl solution. [Copolymer]=4.80×10^{-3}equiv·l^{-1}, [NaOH]=5.00×10^{-3}M

respectively from the Figure, and the reliable values of the AA content dependence of the pK values was not obtained from our titration curves. Under the experimental condition of pH=7.8, the imidazole groups dissociate in part and the acrylic acid (AA) groups do almost completely.

The initial reaction rates of hydrolysis of three kinds of esters, i.e., ANTI, NABA and PNPC are shown as a function of imidazole content of the catalysts in Figure 2. In these measurements, the concentration of NVIm residue is fixed at 5.00×10^{-3}

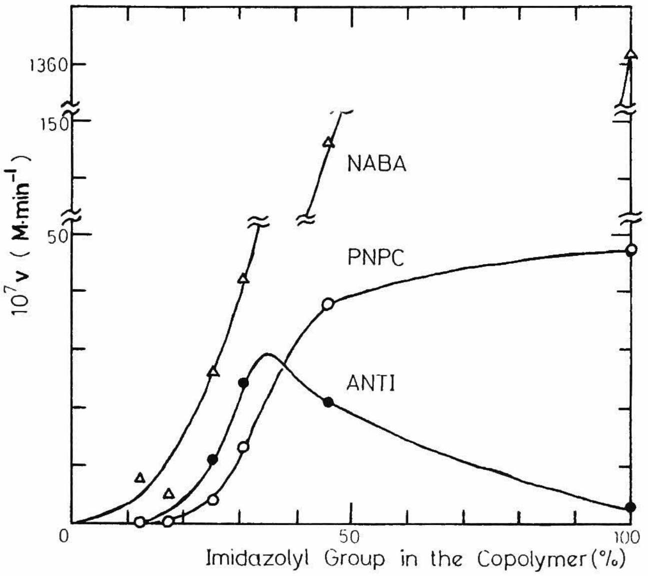

Figure 2 Hydrolysis reaction rates of esters in the presence of copolymers of various composition at constant [NVIm].
$[PNPC]=4.98\times10^{-5}M$, $[NABA]=[ANTI]=5.00\times10^{-4}M$
$[NVIm]_{\text{in polymer}}=5.00\times10^{-3}\text{equiv}\cdot l^{-1}$

equiv·l^{-1}. A common feature of these curves is the sudden increase of the reaction rate at NVIm content of 20-30%. This suggests the existence of the cooperativity between imidazolyl groups and the carboxylic groups, which causes the increase in the reactivity (or nucleophilicity) of the imidazolyl group. The initial reaction rate for anionic NABA increased almost exponentially with NVIm content in the copolymer. This is attributed to the increase of the highly reactive nucleophilic

NVIm residue and also to the decrease of AA concentration which repulse the anionic NABA. The initial rate for ANTI, on the other hand, increased with NVIm content in low content region, but turned to decrease in high NVIm content region passing a maximum. This decrease of the rate is due to the electrostatic repulsive forces between cationic ANTI and cationic NVIm residue and also to decrease of electrostatic attractive force between ANTI and AA residue of the same polymer. The initial rate (V) vs. imidazole content plot for neutral PNPC showed a sigmoid curve, which is understandable by the fact that neutral PNPC does interact neither with anionic AA residues nor cationic NVIm residues by the electrostatic force.

These experimental findings clearly indicate the important contribution of the electrostatic force between esters and catalyst to the catalytic action.

Now, the reaction curves as a function of time are studied. The first order plots for the hydrolysis reactions of PNPC in the presence of the PNVIm and VII are compared in Figure 3. The concentration of PNPC was about 16 times larger than that of the imidazolyl group of the polymers. The plot in the presence of VII gave excellent straight line in all range of conversion whereas that in PNVIm showed a slight deviation from linearity at high conversion of the reaction. The deviation is clearly

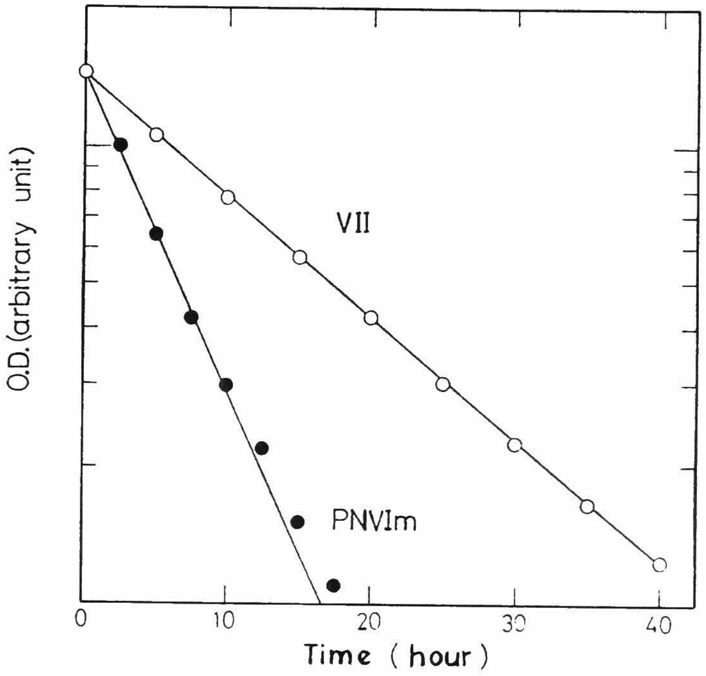

Figure 3 The first-order plots for the hydrolysis of PNPC in the presence of PNVIm and copolymer VII.
$[PNPC]=5.00\times10^{-5}M$, $[NVIm]_{in\ polymer}=5.00\times10^{-3}equiv\cdot l^{-1}$

attributable to the product-inhibition, i.e., anionic products (p-nitrophenol, caproic acid) are adsorped by slightly cationic imidazolyl groups on the polymer. In the presence of the copolymer VII, on the other hand, the anionic product would be electrostatically repelled by the anionic AA groups on the polymer, and therefore the product-inhibition is not expected.

Next, the first order plots for the hydrolysis reactions of

PNPC and ANTI in the presence of VII are shown in Figure 4. The

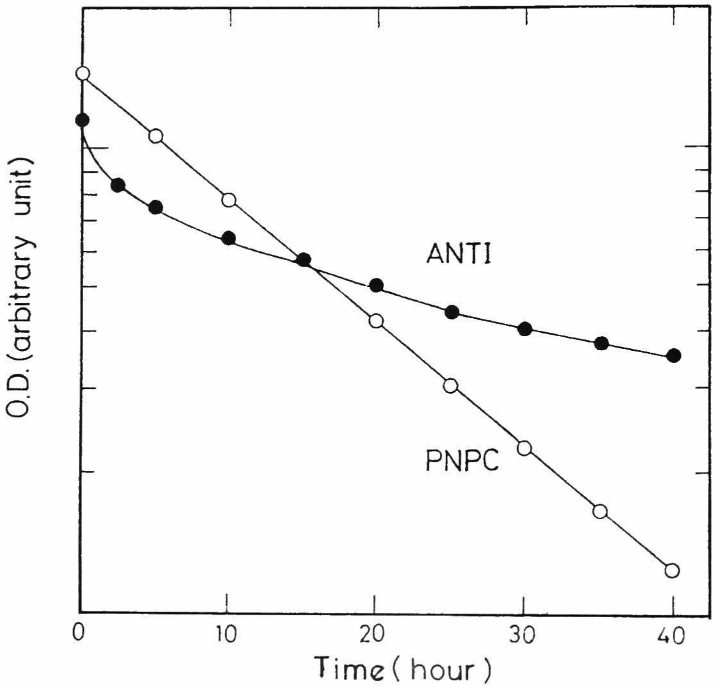

Figure 4 The first-order plots for the hydrolysis of PNPC and ANTI in the presence of copolymer VII.
[PNPC]=5.00×10^{-5}M, [ANTI]=5.00×10^{-4}M
$[\mathrm{NVIm}]_{\text{in polymer}}=5.00\times10^{-3}$equiv·$1^{-1}$

plot for PNPC yielded excellent straight line. On the other hand, the plot for ANTI gave curved line. This implies that the copolymer was most effective in the initial stage only, and the catalytic activity sharply decreased with the reaction progress by the product-inhibition, i.e., the cationic products are strongly adsorped by the anionic polymer. This product binding with the catalyst cause the decrease of the anionic groups of the polymer. Thus, the catalytic action is reduced because the strength of the attractive forces between the cationic substrate and the polymer is weaken.

Figure 5 shows the influence of the model compound benzyl-

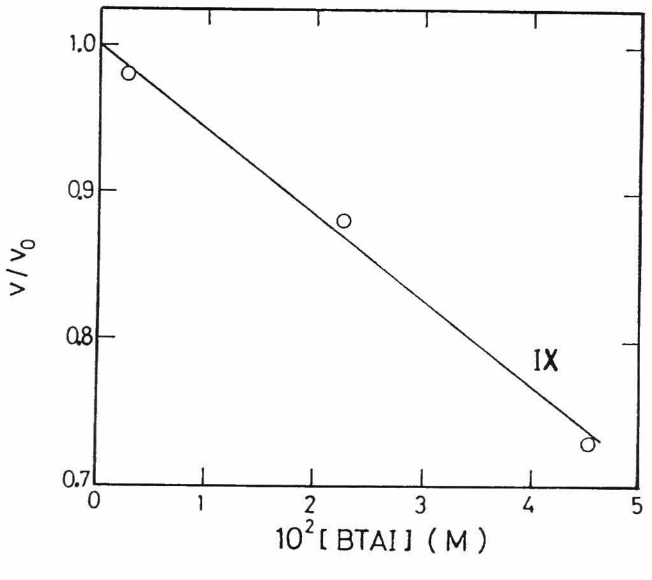

Figure 5 Influence of model compound of reaction product for the hydrolysis of ANTI in the presence of copolymer IX.
[ANTI]=5.00×10^{-4}M, $[\mathrm{NVIm}]_{\mathrm{in\ polymer}}=2.00\times10^{-3}$equiv.$l^{-1}$

-trimethylammonium iodide (BTAI) of the reaction product of ANTI on the hydrolysis of ANTI. The reaction rate decreased with the addition of cationic BTAI in the presence of the copolymer IX. This is attributed to the electrostatic adsorption of BTAI on the catalytic polymer, and therfore to the decrease of the affinity between ANTI and the polymer.

In the case of PNPC hydrolysis, caproic acid and phenol are assumed to be models of the reaction products. The reaction rates of the present system are shown in Figure 6. In the

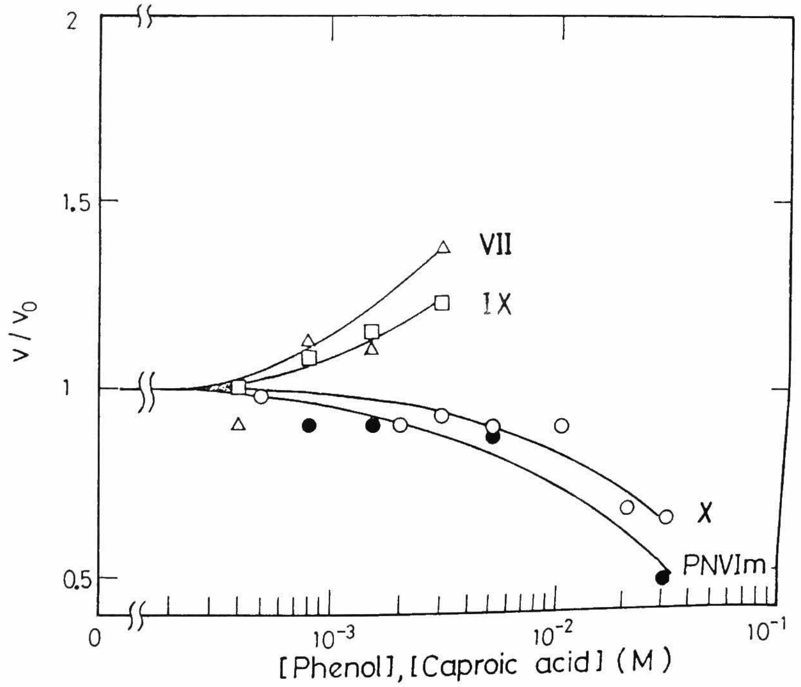

Figure 6 Influence of model compounds of reaction product for the hydrolysis of PNPC in the presence of various copolymers and PNVIm. $[PNPC]=4.98\times10^{-5}M$, $[NVIm]_{in\ polymer}=5.00\times10^{-3}equiv\cdot l^{-1}$

evaluation of the initial reaction rate, small catalytic activity of the added model compound was substracted from the observed reaction rate. Clearly, catalytic activity of the homopolymer PNVIm decreased by the addition of the model compound. This is also ascribed to the adsorption of anionic model compound to PNVIm, which corresponds to the product-inhibition. On the other hand, as the polymer becomes more anionic (X, IX, VII), the product-inhibition becomes weaker. In the case of IX and VII, even a slight enhancing effect was observed. This slight increase of the reaction rate may be due to the slight increase of the apparent pK value of the added phenol because of the anionic environment around the polymer. In any case, it is supposed that product-inhibition does not occur for anionic catalyst polymers.

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Chapter 4

Role of Solvent in Polymer "Catalysis". Polyelectrolyte Catalysis on the Esterolysis of Neutral and Ionic Esters in Hexanol-Water Mixtures

Introduction

The gigantic rate enhancement and retardation effects of macroions on chemical reactions, in particular on interionic reactions, were intensively investigated,[1-5] and the main cause for the catalysis was ascribed to strong electrostatic and hydrophobic interactions between the reactant species and the macroions.

For comparatively simple reaction systems, the theoretical interpretation by the Brönsted-Bjerrum-Manning theory was fairly successful, suggesting that electrostatic stabilization of the activated complex is responsible for the acceleration and that of the reactant is a cause of the deceleration.[6,7,8]

Recent studies of high pressure influence on the polyelectrolyte catalysis revealed that the solvation and desolvation effects of reactant ions and/or activated complex are playing a key role in addition to the electrostatic stabilization mentioned above.[9] These solvent effects in polymer "catalysis" are certainly expected to manifest itself clearly for reactions in organic solvents. Some studies have been reported on catalytic influences of polymers in organic solvent-water mixtures.[10-18] However, the hydration or dehydration effect of polymers were not investigated in detail. In organic solvents, ions are often reported to be dehydrated.[19,20] Furthermore, recent studies of catalytic effects of reversed micelles and liquid crystals have clearly demonstrated the important role of solvent molecules.[21,22,23]

Thus it was thought interesting to examine the catalytic action of polyelectrolytes in organic solvents. In this chapter, the influence of polyelectrolytes on the alkaline hydrolysis of various esters in n-hexanol containing small amounts of water was studied.

Experimental

Materials

Poly-N-octylethylenimine bromide (C8PEI), poly-N-laurylethylenimine bromide (C12PEI) and poly-N-cetylethylenimine bromide

(C16PEI) were prepared by quaternization of polyethylenimine with octyl bromide, lauryl bromide and cetyl bromide, respectively. The polyethylenimine was donated from Nippon Shokubai Co. Tokyo, and its degree of polymerization was 100. The quaternization was carried out in nitromethane and nitroethane mixture (1:1) at 50°C for 4 days. The degree of quaternization of C8PEI, C12PEI and C16PEI were 55, 58 and 59 %, with respect to the total amount of nitrogen atoms in the polymer, respectively. The purification was carried out by reprecipitation with ethylacetate. These polymers were soluble in n-hexanol but insoluble in water. Cetyltrimethylammonium bromide (CTABr) and Brij35 [$C_{12}H_{25}(OCH_2CH_2)_{23}OH$] were commercially available and were used without further purification.

p-Nitrophenyl acetate (PNPA) was commercially available and further purified twice by crystallization from chloroform. 3--Acetoxy-N-trimethylanilinium iodide (ANTI) was synthesized by the method of Overberger et al.[24] and was purified by recrystallization in nitromethane. 3-Nitro-4-acetoxybenzoic acid (NABA) was prepared by the method of Overberger et al.[25] and was crystallized from benzene.

8-Anilino-1-naphthalenesulfonic acid ammonium salt (ANS) was purchased from the Wako Pure Chemical Co. Osaka and purified by repeated crystallization from water.

n-Hexanol was of a spectral grade and further distilled before use. For solution preparation, water was deionized and then distilled.

Kinetic Measurement

The reaction was initiated by adding 20 μl of n-hexanol solution of the esters into 3 ml of the polymer solution. The polymer solution was prepared by mixing desired amounts of the polymers, sodium hydroxide solution and water into n-hexanol. The reaction rates were determined from the absorption changes at 400 nm for PNPA, 416 nm for NABA and 293 nm for ANTI respectively using a High Sensitivity Spectrophotometer equipped with a thermostated cell-holder (SM-401, Union Engineering Co. Hirakata, Osaka-fu).

Fluorescence Measurement

The fluorescence measurements were carried out using ANS as a hydrophobic probe with a Fluorescence Spectrophotometer (FS-401, Union Engineering) at 25°C.

Light Scattering Measurement

The low angle light scattering measurements of the polymer solution were done with KMX-6 Photometer (Chromatix Co. Mountain View, Cal.) at the room temperature. The measurement angle was fixed at 5.9°.

Results and Discussion

The influence of water content on the hydrolysis rate constant, k_{obs}, of PNPA in n-hexanol-water mixtures is shown in Figure 1. Clearly, the k_{obs} sharply increased with decreasing

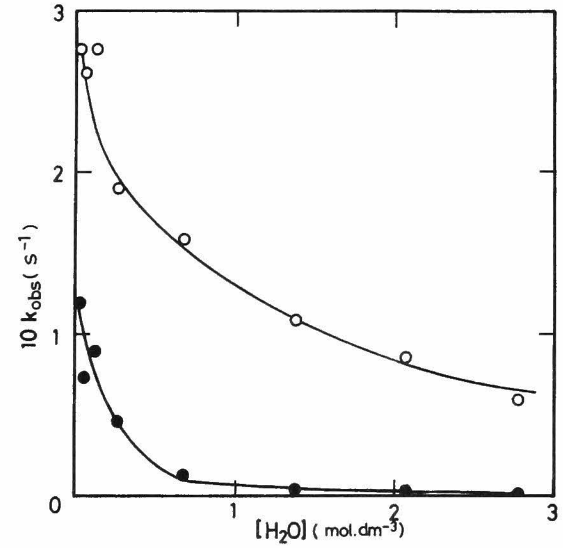

Figure 1. Influence of H_2O on the alkaline hydrolysis of PNPA in n-hexanol at 25°C. [PNPA]=2.5×10^{-5}M, [OH^-]=1.00×10^{-3}M, [C12PEI]= 0(○), 5.51×10^{-3}(●) equiv.l^{-1}

water content. This indicates that the hydroxide ions are dehydrated in n-hexanol and the nucleophilicity of the ions is enhanced as compared with that of the hydrated ions in aqueous solution. The similar observations on the enhanced reactivity of the dehydrated nucleophiles have been reported in organic solvents.[19,20]

Figure 2 demonstrates the influence of C12PEI on the

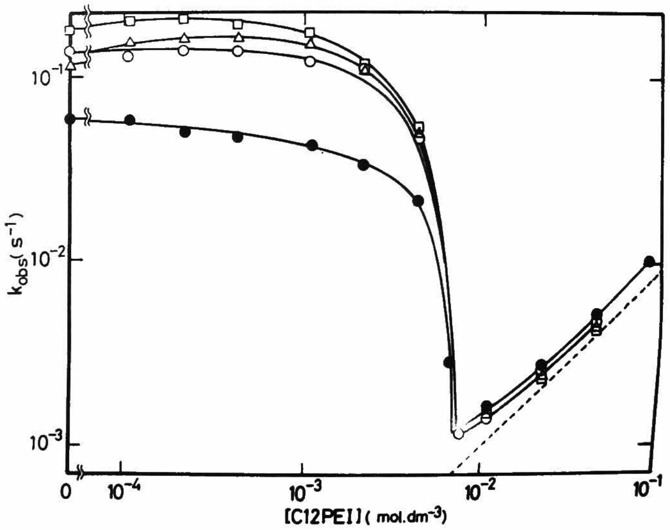

Figure 2 Influence of C12PEI on the alkaline hydrolysis of PNPA in n-hexanol at 25°C. [PNPA]=2.50×10^{-5}M, [OH^-]=1.00×10^{-3}M, [H_2O]=5.56×10^{-2}(□), 2.78×10^{-1}(Δ), 5.56×10^{-1}(○), 2.78 M(●)

hydrolysis of PNPA in n-hexanol. First, the k_{obs} slightly increased by addition of a tiny amount of C12PEI ([C12PEI] < 10^{-3}

(mol dm^{-3})) at low concentrations of water. Then, the rate constants decreased sharply ([C12PEI] = $10^{-3} \sim 10^{-2}$ (mol dm^{-3})), and again turned to increase at high concentrations ([C12PEI] > 10^{-2} (mol dm^{-3})). The dotted line in this Figure shows the rate constant of the hydrolysis carried out without addition of OH^-.

In the absence of the polymer, the weakly hydrated hydroxide ions react with PNPA molecule as is schematically shown in Figure 3-1. The first increase in k_{obs} by the addition of C12PEI may be

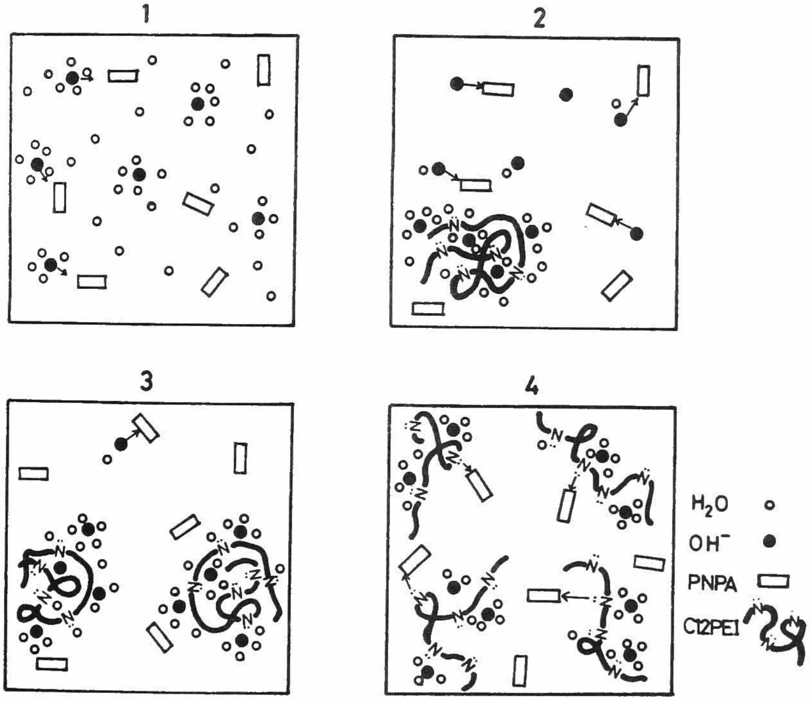

Figure 3 Schematic description of the hydrolysis of PNPA in the presence of C12PEI in n-hexanol containing small amounts of water.

due to the dehydration effects on OH^- by C12PEI (Figure 3-2). Thus, the concentration of water in the bulk phase decreases as a result of preferential absorption by the polymers, which would favor dehydration and enhanced reactivity of OH^-. The enhancement of the nucleophilicity of water molecules by the polymer in organic solvent is improbable because reaction was not observed in the absence of OH^-. It should be mentioned here that OH^- was also accumulated around the cationic polymer by the electrostatic forces. Therefore it will be easily speculated that both OH^- and water molecules are absorbed by C12PEI and the esterolysis in the bulk phase is retarded. This factor will be one of the reasons why k_{obs} decreased in the second region. The other reason is the decrease of the reactivity of OH^- absorbed in the polymer domain, because OH^- in this region is surrounded by a large amount of the absorbed water molecules and subsequently are strongly hydrated (Figure 3-3). The third observation, i.e., the increase in k_{obs} at the higher concentration of C12PEI, is due to the nucleophilic attack of the free imino groups of C12PEI upon the esters (Figure 3-4). This conclusion is derived because the k_{obs} values in the presence of OH^- and in their absence (dotted line) were very close to each other.

Figure 4 shows the influence of a cationic surfactants, CTABr, and a nonionic one, Brij35, upon the alkaline hydrolysis of PNPA.

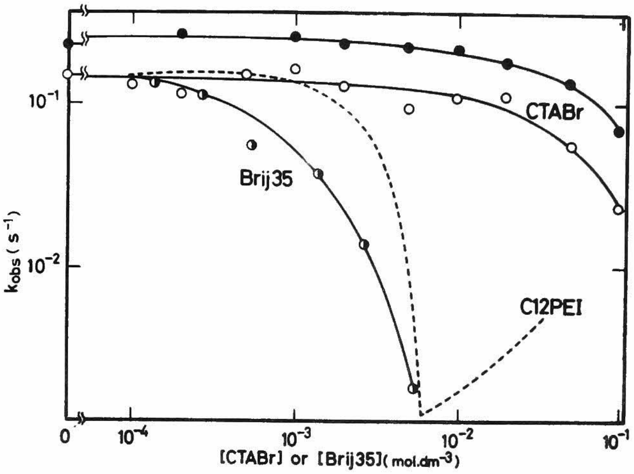

Figure 4 Influence of CTABr of Brij35 on the alkaline hydrolysis of PNPA in n-hexanol at 25°C. ●,○: CTABr, ◑: Brij35, ---: C12PEI, $[PNPA]=2.50\times10^{-5}M$, $[OH^-]=1.00\times10^{-3}M$, $[H_2O]=5.56\times10^{-2}M$(●), $5.56\times10^{-1}M$(○, ◑, ---)

The data of C12PEI was reproduced by the broken line for comparison. The effect of CTABr was small compared with those of C12PEI and Brij35. Under the condition employed, the formation of the reversed micelles is highly plausible. The results show that the so-called "water pools" located in the center of the micelle are not so effective for the rate-retarding effects. This may be partly due to the factor that the reversed micelle of CTABr is not so stable as that of polymer because CTABr is of low molecular weight. The strong inhibition by Brij35 is attributable to the oxyethylene groups of the surfactant, which would absorb water molecules effectively. It is interesting that both CTABr and

Brij35 retarded the reaction, similarly to C12PEI.

The influences of C16PEI and C8PEI on the rate constants for the hydrolysis of PNPA are shown in Figure 5. The rate-retarding effect in the second concentration region of C8PEI was more remarkable than that of C16PEI, and the strength of the inhibition was in the order, C8PEI > C12PEI > C16PEI. This may be due to the higher affinity to water molecules of the less hydrophobic polymer.

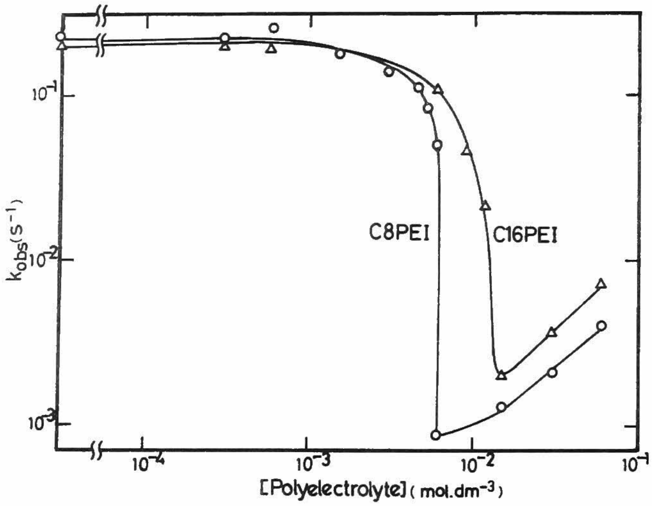

Figure 5 Influences of C8PEI and C16PEI on the alkaline hydrolysis of PNPA in n-hexanol at 25°C. Δ: C16PEI, o: C8PEI; [PNPA]=2.50×10^{-5}M, [OH^-]=1.00×10^{-3}M, [H_2O]=5.56×10^{-2}M

The thermodynamic parameters for the alkaline hydrolysis of of PNPA with and without C12PEI are listed in Table I.[26] The enthalpy of activation, $\Delta H^{\neq}$, in hexanol-water is slightly smaller

Table I Thermodynamic Parameters for the Hydrolysis of PNPA at 25°C

([PNPA] = 2.50×10^{-5}M, [OH^-] = 1.00×10^{-3}M)

	[C12PEI](M)	$10^3k_{obs}(s^{-1})$	$\Delta G^{\neq}(kJmol^{-1})$	$\Delta H^{\neq}(kJmol^{-1})$	$10^{-2}\Delta S^{\neq}(JK^{-1}mol^{-1})$
1	0	8.78	84.5 ± 2.0	23 ± 2	-2.1 ± 0.1
2	0	145	77.8 ± 2.6	20 ± 2	-1.9 ± 0.1
3	9.18×10^{-4}	138	77.8 ± 2.5	10 ± 1	-2.3 ± 0.1
4	9.18×10^{-3}	1.22	89.5 ± 6.9	16 ± 2	-2.4 ± 0.2
5	2.14×10^{-2}	2.32	87.9 ± 5.9	26 ± 3	-2.1 ± 0.3

1 : in aqueous solution

2,3,4,5 : in mixed solvent(hexanol/water), [H_2O] = 5.56×10^{-1}M than that in water (compare 1 and 2). This is consistent with the high reactivity of the dehydrated hydroxide ions in the concentration of 9.18 × 10^{-4} mol dm^{-3} corresponding to the first region above mentioned, in which OH^- was inferred to be dehydrated by the addition of the macroions.

The influence of C12PEI upon the hydrolysis of an anionic ester, i.e. NABA, was examined (Figure 6). The magnitude of k_{obs}

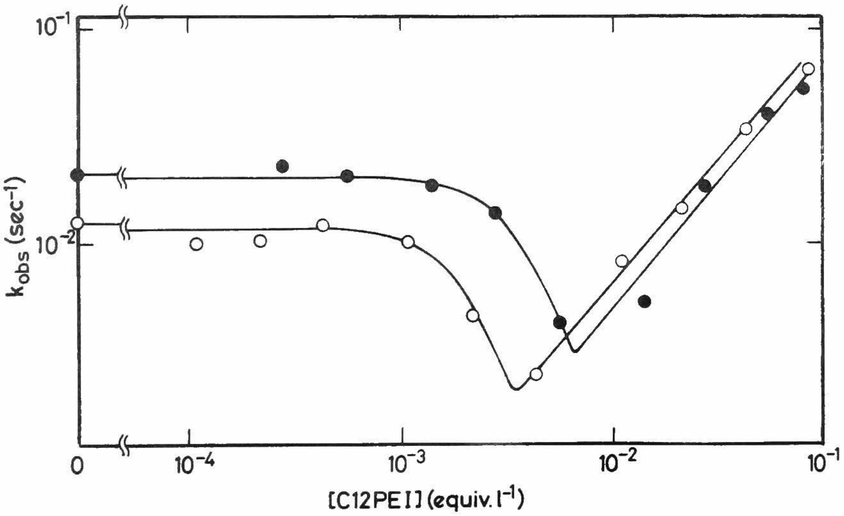

Figure 6 Influence of C12PEI on the alkaline hydrolysis of NABA in n-hexanol at 25°C. [NABA]=2.50×10^{-5}M, [OH^-]=1.00×10^{-3}M, [H_2O]=1.39×10^{-1}M(●), 5.56×10^{-1}M(○)

was about one order of magnitude smaller than that for PNPA. This is in part due to the electrostatic repulsion between similarly charged reactants, namely anionic ester NABA and OH^-. The k_{obs} values in the concentration region of C12PEI higher than 5×10^{-3} mol dm^{-3}, which corresponds to the third region, are much larger than those for PNPA. This is certainly due to an electrostatic interactions between the cationic polymer and the anionic ester. However, the polymer concentration dependence of the reaction rates were similar for PNPA and NABA.

Figure 7 shows the influence of C12PEI upon the hydrolysis rate of

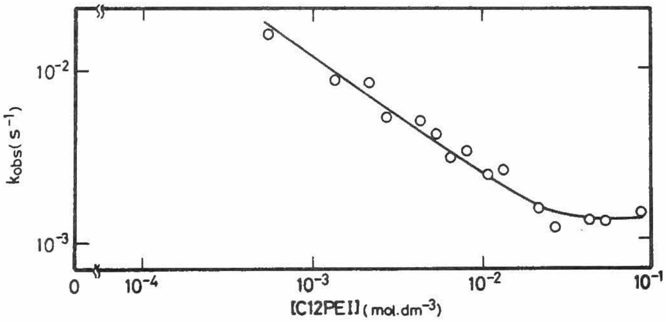

Figure 7 Influence of C12PEI on the alkaline hydrolysis of ANTI in n-hexanol at 25°C. $[ANTI]=2.50\times10^{-5}M$, $[OH^-]=1.00\times10^{-3}M$, $[H_2O]=5.56\times10^{-1}M$

the cationic ester ANTI. The k_{obs} values at low polymer concentrations (in the first region) could not be determined because the reaction was too fast to follow by our spectrophotometer. The very rapid alkaline hydrolysis is due to the strong electrostatic forces between the cationic ester and the hydroxide anions. When the polymer concentration was raised, k_{obs} was decreased, though not so clearly as was the case for PNPA. The sharp increase of k_{obs} at higher polymer concentration was not observed, because the nucleophilic attack of the cationic C12PEI on the cationic ester ANTI is difficult by strong electrostatic repulsion forces.

In order to confirm that the sharp bends of the curves observed in Figure 2 are not resulted from aggregation of C12PEI, light scattering measurements of the polymer solutions were conducted. Figure 8 shows the C/R_θ vs. C plots for C12PEI in n-hexanol containing small amounts of water, wher R is the

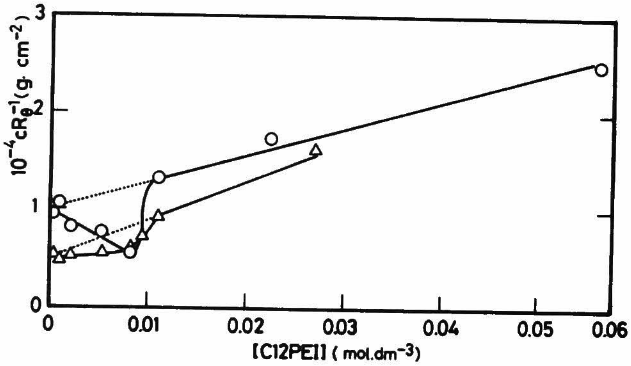

Figure 8 Light Scattering of C12PEI in n-hexanol containing small amounts of water at θ=5.9°. $[OH^-]=1.00\times10^{-3}$M, $[H_2O]=5.56\times10^{-2}$M(o), 1.11×10^{-1}M(Δ)

Rayleigh ratio at a fixed angle ($\theta = 5.9°$) and C the concentration of C12PEI in $g \cdot cm^{-3}$. First, the slopes of the c/R_θ vs. C plots in the low concentration region of C12PEI are small (or even negative). At $[C12PEI] \simeq 10^{-2}$ mol dm^{-3}, the c/R_θ value increased sharply, and then followed by gradual increase at the higher concentration. It is notable that the drastic jump in this Figure and the sharp bends of the curves in Figure 2 were observed at the same concentration. The first small slope of the c/R_θ vs. C plot may be attributed to a compact conformation of C12PEI.because there exists OH^- in excess over the amount equivalent to C12PEI. Thus, it may be suggested that, when [C12PEI] increases conformational change of C12PEI occurs at $[C12PEI] \simeq 10^{-2}$ mol dm^{-3} by shortage of OH^-. However, it must be noted that the intercept of the C/R_θ vs. C plots at the low concentrations of C12PEI coincided well with the extrapolated ones of the data at the higher concentration region, as is shown by dotted lines. This agreement might indicate that the molecular weight of C12PEI in the solution does not change. Thus, it is claimed that aggregation of C12PEI does not occur with increases in its concentration. More quantitative light scattering measurement is now in progress.

In order to confirm the preferential hydration of C12PEI suggested above from the kinetic measurements, the fluorescence spectra of ANS were measured. Figure 9 shows the fluorescence

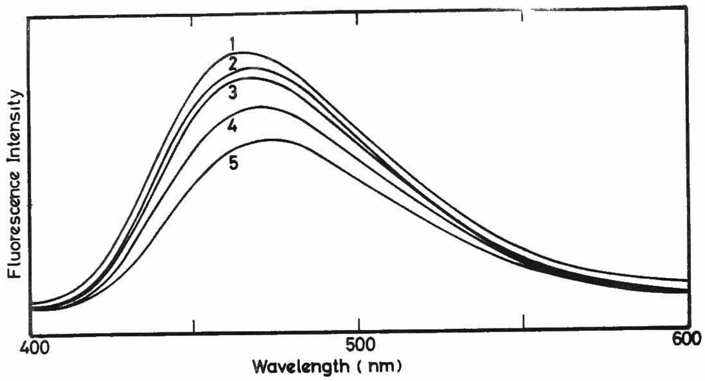

Figure 9 Effect of H_2O on the fluorescence spectrum of ANS in H_2O/n-hexanol solutions. [ANS]=2.50×10^{-5}M, [H_2O]=0 M(1), 6.94×10^{-2}M(2), 2.78×10^{-1}M(3), 6.94×10^{-1}M(4), 1.39M(5) excitation wavelength 380 nm

spectra of ANS in n-hexanol containing various amounts of water. The fluorescence intensity decreased by the addition of water with concomitant red shift of the position of the fluorescence maximum. This shows that ANS is effective as a hydrophobic probe as was often reported.[27,28,29] As is shown in Figure 10, by the addition of C12PEI, first the fluorescence intensity slightly increased (curves 1, 2 and 3) and then turned to decrease drastically (curves 4, 5 and 6). Curve 1 indicates the fluorescence spectrum of ANS without C12PEI. The initial increase may reflect the strong hydrophobicity of the C12PEI polymer and concentration of ANS around the polymer. The subsequent decrease suggests that the water molecules are accumulated around C12PEI (hydration effect),

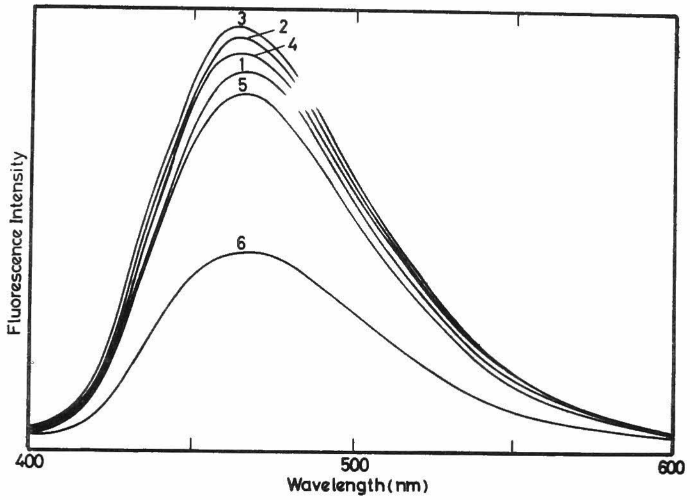

Figure 10 Effect of C12PEI on the ANS fluorescence spectrum in H_2O/n-hexanol/C12PEI solutions. [ANS]=2.50×10^{-5}M, [H_2O]=1.39×10^{-1}M, [C12PEI]=0(1), 1.38×10^{-3}(2), 2.75×10^{-3}(3), 1.38×10^{-2}(4), 2.75×10^{-2}(5), 9.61×10^{-1}(6)M
excitation wavelength 380 nm

and the fluorescence intensity of the hydrophobic probe was rapidly weakened.

Summarizing the above results, it is stated as follows: the hydroxide ions are dehydrated in n-hexanol and the nucleophilicity of the ions become high as compared with that in aqueous solution. By the addition of the hydrophobic cationic polymer, the reactivity is largely lowered, which is due to the "hydration" effect in water-rich region around the polyion chain. The hydration effect is consistent with the measurements of fluorescence and light-scattering.

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Chapter 5

"Catalytic" Effects of Electrically Charged Polymer Latices on Interionic Reactions

Introduction

"Catalytic" effects of polyelectrolytes on interionic reactions have intensively been studied experimentally and theoretically.[1-5] The polymers used in most of these studies have been restricted on linear, flexible polyelectrolytes, and few have been studied on the catalytic effects of polyelectrolytes with fixed molecular conformation.[4]

Electrically charged latices, rigid and spherical polymeric particles, have various interesting properties in common with macroions. First of all, these latex particles and macroions have electric charges in rather restricted spaces. This is expected to cause similar behaviors in solution properties and in their functions. For example, in aqueous solutions of charged latex, ordered distribution of the particles is maintained.[6] According to recent studies,[7-12] there exists also more-or-less regular lattice-like distribution of macroions in polyelectrolyte solutions. Because both the latex particles and the macroions create extremely high electrostatic potential field around themselves, large "catalytic" effects observed for macroion systems can be expected also for the latex systems. As a matter of fact, preliminary results[4,13] were presented from our laboratory, showing that negatively charged latex particles, like anionic macroions, could accelerate Hg^{2+}-induced aquation of $Co(NH_3)_5Br^{2+}$. Recently, Calvin et al. found that the quenching efficiency of rhodamine 101 emission increased in the presence of a cross-linked copolymer of styrene, butadiene and acrylic acid.[14] It was suggested that the latex surface served to bring the electron donor (the quencher) and the acceptor (rhodamine 101) into proximity by hydrophobic forces and enhance the bimolecular reaction.

In this chapter, anionic latices of various sizes and charge densities which contained acrylic acid-and styrene-groups are synthesized, and catalytic effects of these latices and sodium polyacrylate (as a reference) on the reaction between bromopentaamminecobaltic complex $Co(NH_3)_5Br^{2+}$ and OH^- and on the fading reaction of crystal violet by OH^- are studied. These two reactions have been studied in the presence of flexible polyelectrolytes in detail.[15-17]

Experimental

Materials

Eight different kinds of latices were synthesized by copolymerizing of styrene (St) and acrylic acid (AA) in the presence of surfactants in aqueous solution. The surfactant was a 1 : 1 mixture of nonionic Emulgen 920 (Alkylphenolethyleneoxide adduct, Kao Atlas Co., Japan) and Pronon 208 (Polypropyleneglycoleethyleneoxide adduct, Nippon Yushi Co., Japan). Copolymerization was carried out in nitrogen gas flash at 70°C using potassium persulfate as initiator. Water used for the copolymerization was deionized before use. The purification of the latices were carried out by dialysis against distilled water.

Some kinds of latices were reported to decompose in alkaline solution.[18,19] Therefore, the stability of the latices we synthesized was checked. The alkaline solutions of the latices were centrifuged every one hour after the solution preparation, and the supernatant solutions were titrated with a HCl solution. These latices were found to be stable in NaOH solutions of concentrations smaller than 0.1M during the kinetic measurements.

According to electron micrograph, the latex particles were monodisperse and almost perfectly spherical. From the picture we estimated the diameter.

The AA content in the copolymer latices was determined by titration with a NaOH solution (see the fifth column of Table I). The value coincided well with that in the monomer mixtures except S-7 and S-8. Thus, considering that the hydroxide ion cannot easily penetrate into hydrophobic polystyrene domain, it may be presumed that the polyacrylic acid chains exist mainly on or near the surface of the latex particles.[18,20,21] In order words, the latex particle may be visualized as polystyrene (St) core coated with polyacrylic acid layer. The approximate number of the polyacrylic acid layer was calculated from this model on the assumption that a latex particle consists of close-packed microspherical particles representing AA-monomer units and void. S-4 has the thickest ionic layer as is shown in Table I.

Polyacrylic acid (HPAA) was commercially available and purified by precipitation in acetone followed by dialysis against distilled water. HPAA was then neutralized with aqueous NaOH solution to obtain sodium polyacrylate (NaPAA). Bromopentaamminecobaltic perchlorate $[Co(NH_3)_5Br](ClO_4)_2$ was prepared as described before.[22] Crystal violet (CV) of guaranteed grade was purchased from Merck and was used without further purification.

Table I Properties of the latices

Latex	Weight % of surfactant in the reaction mixture	Weight % of monomers in the reaction mixtures	Mole % of AA in the monomer mixture	Mole % of AA in the latex
S-1	0.92	18.4	0.1	0.28
S-2	0.92	18.4	0.5	0.64
S-3	0.92	18.5	1.0	0.93
S-4	0.91	18.7	3.0	2.43
S-5	1.82	18.3	1.0	0.79
S-6	3.57	18.0	1.0	0.89
S-7	0.36	2.0	0.5	2.13
S-8	0.18	1.0	0.5	2.66

Table I Properties of the latices (continued)

Diameter of latex (μ)	Total number of AA monomers in a latex particle (α)	Calculated number of the AA monomer beads coating the St core (β)	Number of AA layers[a] (α/β)
0.48	9.70×10^5	1.92×10^6	0.51
0.68	6.28×10^6	3.80×10^6	1.7
0.54	4.69×10^6	2.42×10^6	1.9
0.59	1.55×10^7	2.86×10^6	5.4
0.38	1.33×10^6	1.17×10^6	1.1
0.097	2.58×10^4	7.79×10^4	0.33
0.047	7.12×10^3	1.85×10^4	0.39
0.051	1.11×10^4	2.14×10^4	0.52

a. The number was obtained from the total number of AA monomers (α) in a latex divided by the number of the beads (3 Å in radius) representing monomer units (β), which were assumed to cover the surface of the St core with the two-dimensional face-centered lattice structure.

Kinetic Measurements

Rate constants of the $Co(NH_3)_5Br^{2+}$ + OH^- and CV + OH^- reactions were determined by the time dependencies of the absorbancies at 255 nm or 300 nm, and of that at 600 nm, respectively, by a high sensitive spectrophotometer (SM-401, Union Engineering Co. Hirakata, Osaka-fu). A high pressure spectrophotometer (Union Engineering)[15] was also used for the determination of the volume of activation.

Results and Discussion

Figure 1 shows the influence of the latices and flexible and linear polymer, i.e., sodium polyacrylate (NaPAA), on the rates of the $Co(NH_3)_5Br^{2+}$ + OH^- reaction. All latices decreased the rate constant, and the order of the retarding effect was S-4 > S-3 > S-5 > S-6 > S-2 > S-1 > S-7 > S-8. This order coincided better with the order of the thickness of the AA layers given in Table I, rather than with that of AA content of the latices. The reaction between $Co(NH_3)_5Br^{2+}$ and OH^- is one of the interionic reactions between oppositely charged species, and is known to be retarded by the addition of polyelectrolytes.[1-5] Thus, the order of the

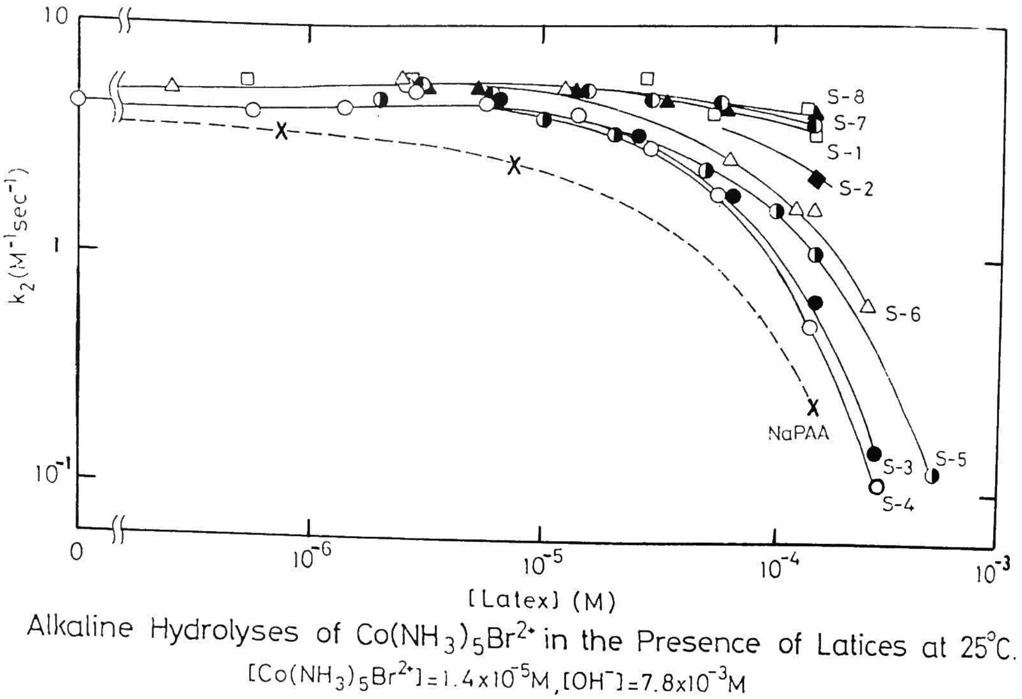

Figure 1 Alkaline hydrolysis of $Co(NH_3)_5Br^{2+}$ in the presence of latices at 25°C.
$[Co(NH_3)_5Br^{2+}]=1.43\times10^{-5}M$,
$[OH^-]=7.79\times10^{-3}M$

inhibitory effect of the latices is understandable by the order of the strength of electrostatic interactions of the AA layers with the reactant ions, i.e., Co-complex ions.

Thermodynamic parameters for the reaction between $Co(NH_3)_5Br^{2+}$ and OH^- are listed in Table II. It must be admitted that the activation parameters are associated with a basic ambiguity in polyelectrolyte-containing systems as was discussed in Reference 15. This ambiguity is unavoidable also in

Table II Thermodynamic Parameters for the Reaction of $Co(NH_3)_5Br^{2+}$ with OH^- at 25°C[a]

Latex	k_2 (1/M·sec)	$\Delta G^{\neq}$ (kcal/mol)	$\Delta H^{\neq}$ (kcal/mol)	$\Delta S^{\neq}$ (e.u.)	$\Delta V^{\neq}$ (ml/mol)
none	5.59	16.4±0.3	25.3±2	30±8	32±2
S-1	3.45	16.7	22.6	20	-
S-2	2.21	17.0	21.9	17	-
S-3	0.669	17.7	26.7	30	-
S-4	0.485	17.9	25.9	27	-
S-5	1.06	17.4	22.3	16	-
S-6	1.61	17.1	25.5	28	-
S-7[b]	4.30	16.6	25.3	29	27
S-8[b]	4.81	16.5	24.1	25	27
NaPAA	0.238	18.3	22.4	14	-

a. $[Co(NH_3)_5Br^{2+}] = 1.43 \times 10^{-5}M$, $[OH^-] = 9.03 \times 10^{-3}M$, $[Latex] = 1.50 \times 10^{-4}M$

b. $[OH^-] = 4.0 \times 10^{-3}M$

electrically charged polymer latex systems. Thus, comparison of the parameters for various latex particles must be only qualitatively significant. Except for S-3, all of the enthalpy of activation ($\Delta H^{\neq}$), entropy of activation ($\Delta S^{\neq}$) and volume of activation ($\Delta V^{\neq}$) decreased by the addition of the latices and NaPAA. This suggests that the inhibitory effects of the latices are due to the desolvation of the reactant ions, mainly, Co--complex ions in the present reaction. Precisely, the reactants are desolvated to a larger extent than the activated complex. This desolvation brings about decrease in $\Delta S^{\neq}$. This tendency has often been observed for the interionic reactions between oppositely charged species in the presence of linear macroions.[16,23] Thus, the present results on the latices suggest that there is no basic difference in the catalyses of flexible and rigid macroions.

Next, the influences of the latices on the alkaline fading reaction of a hydrophobic dye, i.e., crystal violet (CV), were studied (see Figure 2). In this case, also, the reaction rate became smaller in the presence of the latices. However, we could not find significant difference in the retarding effects of various latices. Furthermore, the retarding effect of NaPAA was small. These results indicate that the hydrophobic interaction between the latex and CV is more important than the electrostatic one. The hydrophobicity of the latex is due to the St chains near

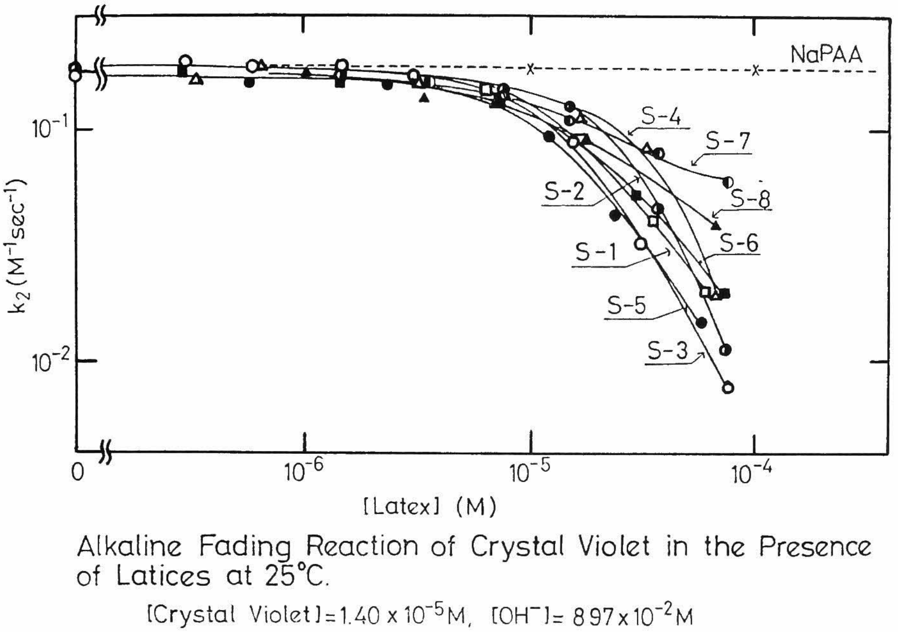

Figure 2 Alkaline fading reaction of crystal violet in the presence of latices at 25°C.
[CV]=1.40×10^{-5}M, [OH^-]=8.97×10^{-2}M

the surface of the latex. It is clear from the table I that the ionic layer of the S-4 is thick and the St chains have a small chance to be exposed to the solution phase. Therefore, the weak hydrophobic interaction of S-4 with CV and hence its relatively weak retarding effect are understandable.

The activation parameters for the alkaline fading reaction in the presence and absence of the latices are compiled in Table III. Both $\Delta S^{\neq}$ and $\Delta V^{\neq}$ in the absence of the latices were negative, and the $\Delta H^{\neq}$ and $\Delta S^{\neq}$ decreased by the addition of the latices except for S-6. Therefore, the inhibitory effect of the latices is

Table III Thermodynamic Parameters for the Alkaline Fading Reaction of Crystal Violet at 25°C[a]

Latex	k_2 (1/M·sec)	$\Delta G^{\neq}$ (kcal/mol)	$\Delta H^{\neq}$ (kcal/mol)	$\Delta S^{\neq}$ (e.u.)	$\Delta V^{\neq}$ (ml/mol)
none	0.157	18.5±0.2	15.1±1	-11±5	-0.9±1
S-1	0.0430	19.3	16.1	-11	-
S-2	0.0609	19.1	6.9	-41	-
S-3	0.105	18.8	7.4	-38	-
S-4	0.0580	19.1	9.0	-34	-
S-5	0.0619	19.1	8.7	-35	-
S-6	0.157	18.5	15.6	-10	-
S-7[b]	0.0918	18.9	10.4	-28	2.9
S-8[b]	0.0809	18.9	10.2	-29	1.3
NaPAA	0.157	18.5	15.6	-10	-

a. [CV] = 1.4 × 10^{-5}M, [OH^-] = 0.0909 M, [Latex] = 3 × 10^{-5}M
b. [CV] = 1.4 × 10^{-5}M, [OH^-] = 0.1 M, [Latex] = 1.5 × 10^{-5}M

caused by the decrease in $\Delta S^{\neq}$, similarly to the $Co(NH_3)_5Br^{2+} + OH^-$ reaction. However, the $\Delta V^{\neq}$ increased by the addition of the latices as is seen from the table, although not much data are available. These results may be interpreted as follows; first, the negative values of $\Delta S^{\neq}$ and $\Delta V^{\neq}$ in the absence of the latices would be due to enhanced formation of the "ice-berg" structure in the course of activation as is demonstrated in Figure 3-a. The

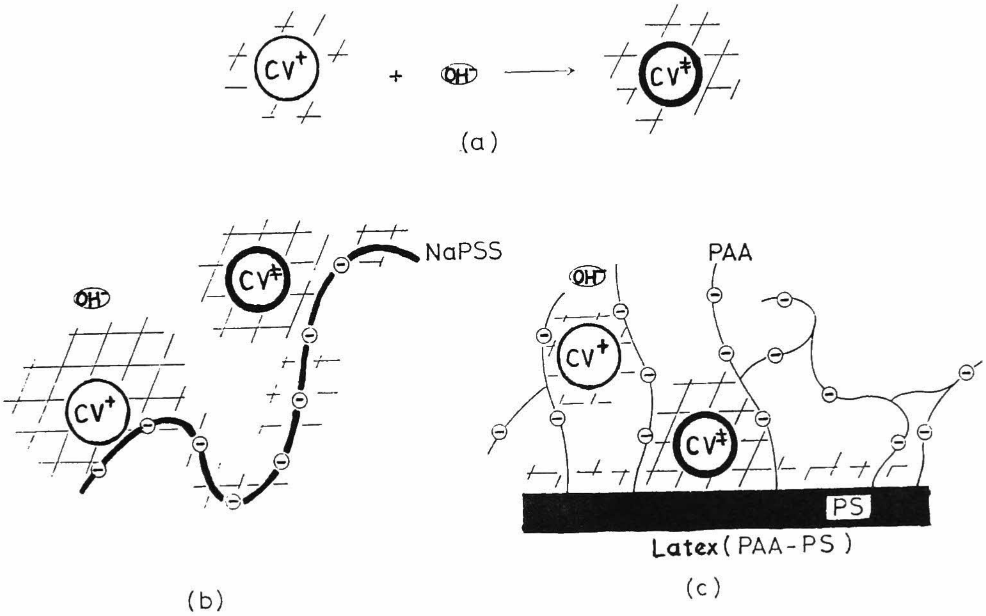

Figure 3 Schematic description of alkaline fading reaction of crystal violet in the absence and presence of NaPSS or latices.
(a) without polymer, (b) with flexible polymer,
(c) with latex particle.

enhancement in the "ice-berg" structure formation causes the decreases in $\Delta S^{\neq}$ and $\Delta V^{\neq}$,[23] when hydrophobic solutes are incorporated into the bulky, low-density ice structure. In this respect, we note, the partial molar volumes of some aliphatic and aromatic hydrocarbons in water are smaller than those in pure liquids by about 20 ml mole^{-1} or 6 ml/mole^{-1}.[24,25] Of course, the electrostatic desolvation effect,[16] which gives rise to positive $\Delta V^{\neq}$ and $\Delta S^{\neq}$ for the interionic reactions, must not completely be neglected.

Next, the influence of the latex particles on $\Delta S^{\neq}$ and $\Delta V^{\neq}$ is discussed. The fact that the latices decreased $\Delta S^{\neq}$ and increased $\Delta V^{\neq}$ would be due to the difference in the interaction of the latex particle with the reactant (CV^+) and the activated complex, ($CV^{\neq}$); the CV^+ ions are affected by the AA groups by the electrostatic interaction, which are in the outer region of the latex particle. On the other hand, $CV^{\neq}$ is neutral and hydrophobic. Thus, $CV^{\neq}$ would be near the St groups of the latex as is shown in Figure 3-c. Considering that the degree of freedom of the $CV^{\neq}$ near the latex core is more strongly restricted than that of the CV^+ near the comparatively flexible AA groups, the decrease of $\Delta S^{\neq}$ by the latex addition is understandable. The reason for the increase of $\Delta V^{\neq}$ by the addition of S-7 and S-8 latices may be in part due to the fact that the hydrophobicity of the St core is already too high to be further enhanced by the $CV^{\neq}$ addition. Another reason for the $\Delta V^{\neq}$ increase would be that the dyes in the activated state ($CV^{\neq}$) locate very near the surface of St groups, and both $CV^{\neq}$ and St groups of the latex surface cannot be incorporated effectively in the "ice-berg" structure of water. This is because the St parts of the latex are strongly restricted from free movement. On the other hand, the reactants (CV^{+}) locate near the AA groups and are free from the binding to the other hydrophobic groups such as St groups of the latices. Then, the CV^{+} molecules will be easily included in the "ice-berg". Thus, the $\Delta V^{\neq}$ increases by the addition of the latices.

Then, the changes in the thermodynamic parameters by the addition of sodium polystyrenesulfonate (NaPSS), i.e., a hydrophobic, anionic, flexible, linear and water-soluble polymer would be discussed. By NaPSS addition, both $\Delta S^{\neq}$ and $\Delta V^{\neq}$ for the fading reaction of CV clearly increased.[23] These increases were interpreted as follows; the cationic dyes, CV^{+}, are very near the styrenesulfonate groups by the electrostatic and hydrophobic interaction, whereas the activated complex ($CV^{\neq}$) is neutral and the attractive force between $CV^{\neq}$ and PSS anions is due to the hydrophobic interaction only. Furthermore the PSS chains are flexible, unlike the latex case. Therefore, we may expect more enhanced incorporation of the PSS + CV^{+} into the ice-berg structure than the PSS + $CV^{\neq}$ system.

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PART II

STUDY OF POLYELECTROLYTE INFLUENCES ON THE CHARGE-TRANSFER COMPLEX FORMATION REACTIONS

Chapter 6

Polyelectrolyte Influences on the Complexation Equilibrium between Model Compounds of Nicotinamide Adenine Dinucleotide and Indole Derivatives

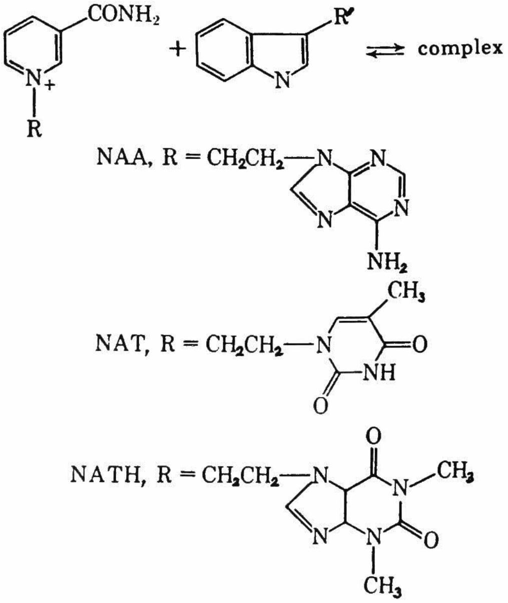
Introduction

In a preceding paper,[1] analogs of nicotinamide adenine dinucleotide, NAD^+, namely, 3-carbamoylpyridinium compounds quaternized with chloroethylated adenine (NAA), thymine (NAT), and theophylline (NATH) were synthesized and their equilibria and reaction rates with cyanide ions were examined. These kinds of analogs which contain neither phosphate nor carbohydrate residues but nucleic acid bases only are very interesting for investigation of the charge-transfer interactions.[2,3]

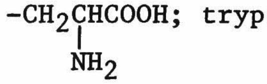
Kosower first suggested that NAD^+ formed charge-transfer complexes with enzyme.[4] The indoles are charge donors and the site of the electron acceptance of the coenzyme is thought to be primarily the nicotinamide moiety of NAD^+. It is now firmly founded that the charge-transfer interactions exist between NAD^+ or its model compounds having pyridinium moiety, i.e., 1-benzyl-3-

-carbamoylpyridinium chloride and tryptophan or other indole derivatives.[5-11] Furthermore, a series of model compounds in which indoles have been directly incorporated into pyridinium chlorides have been synthesized and studied by Shifrin in detail by the spectroscopic method.[8,12]

In the present work, systematic studies were carried out on the charge-transfer interactions of the model compounds with indole derivatives, i.e., L-tryptophan, indole-3-acetic acid, tryptamine, etc. in the presence of polyelectrolytes and in their absence.



(1)

indoleacetic acid (IA), R' = $-CH_2COOH$; indolepropionic acid (IP), R' = $-CH_2CH_2COOH$; indolebutyric acid (IB), R' = $-CH_2CH_2CH_2COOH$; tryptophan (Try), R' =  $-CH_2\underset{NH_2}{CH}COOH$; tryptamine (TA), R' = $-CH_2CH_2NH_2$

The phenomena, that interionic complex formation between similary charged ionic species is facilitated by increasing concentration of simple electrolytes, or ionic strength, whereas that between ionic species of the opposite signs is retarded, are well established as the secondary salt effect.[13] However, the influence of polyelectrolytes on the association phenomena have not been reported. As continuation of the previous work on polyelectrolyte "catalysis" on the reaction rates,[14-18] this point is investigated in the present work.

Experimental Section

Materials

The coenzyme model compounds, NAA, NAT and NATH were prepared by the Menschutkin reaction of nicotinamide with N-chloroethyladenine, thymine and theophylline. The details of the preparation were reported in preceding papers.[1,19] Indole-3-acetic acid (IA) and indole-3-butyric acid (IB) from Merck (for biochemical) were used without further purification. Indole-3-propionic acid (IP), L-tryptophan (Try), tryptamine (TA) hydrochloride, KCl and $CaCl_2$ were guaranteed reagents.

Sodium poly(ethylenesulfonate)(NaPES), degree of polymerization 770, was a gift from the Hercules Powder Co., Wilmington, Del. Sodium Poly(styrenesulfonate)(NaPSt) was kindly donated by the Dow Chemical Co., Midland, Mich. The molecular weight was 6,300,000. The purification of these anionic polyelectrolytes was described in the preceding paper.[1] The copolymer of diethyldiallylammonium chloride and sulfur dioxide (DECS) is a strongly basic polyelectrolyte prepared by Harada et al.[20,21] n--Cetyltrimethylammonium bromide (CTABr) was commercially available. Deionized water was further distilled using a Yamato Auto Still (model WAG-21) for the preparation of aqueous solutions of the model compounds, indole derivatives and polyelectrolytes.

Equilibrium Constants

The apparent association constant, K, in the absence of foreign salts was evaluated using the Foster-Hammick-Wardley method[22] and the Behme-Cordes method[23] from the slope of the $(\varepsilon - \varepsilon_0)/N$ against ε plot, where ε_0 is the sum of absorbances of indole derivative and coenzyme model compound, ε the absorbance of the equilibrium mixture, and N the concentration of the indole derivative. The association constant in the presence of the added salt was derived from the equation, $K^{-1} = N[a\beta/(\varepsilon - \varepsilon_0) - 1]$, where a is the concentration of the coenzyme model compound, and β the molar extinction coefficient of the charge-transfer complex at a given wave length (340 or 350 nm). The reproducibility of the value of K was about 5 % both in the presence and absence of polyelectrolytes. The association constants were determined using a Hitachi spectrophotometer (Model EPS-3T) equipped with a thermostated cell holder.

Results and Discussion

When the coenzyme models were added to a solution of indoleacetate (pH 6.0 in pure water), a faint yellow color appeared instantaneously. As is seen from Figure 1, the difference spectra

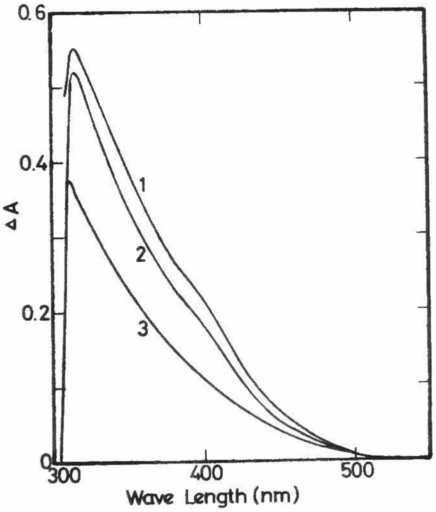


Figure 1 Charge-transfer difference spectra of NAA (curve 1), NAT(2) and NATH(3) with indoleacetate at 25°, [NAA]=[NAT]=[NATH]=2 mM, [IA]=30 mM(curve 1 and 2), 16 mM(3), at 340 nm, in H_2O

indicate the appearance of a quite diffuse band as a long tail to the longer wave length. The spectra are quite similar to those found for NAD^+-indoleacetate and other coenzyme models-indoleacetate mixtures.[6,7,11] It may be quite plausible that indoleacetate is charge-donor and the pyridinium moieties of NAA, NAT and NATH are charge-acceptors.

The representative Foster-Hammick-Wardley plots are given in Figure 2. The $(\varepsilon - \varepsilon_0)/N$ vs. $(\varepsilon - \varepsilon_0)$ plots yielded in all cases

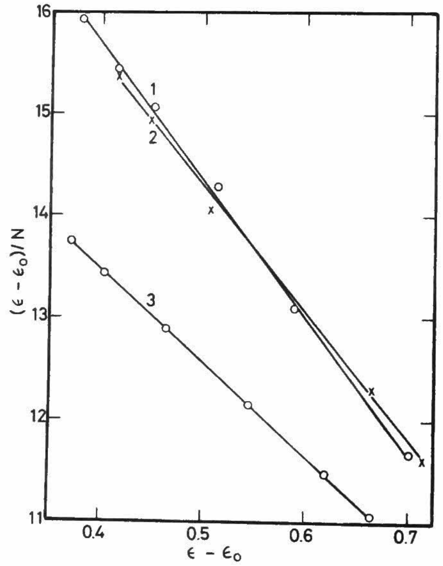


Figure 2 Foster-Hammick-Wardley plots for NAA(curve 1), NAT(2) and NATH(3) Complexes with indoleacetate at 25°; [NAA]=[NAT]=[NATH]=2 mM, [IA]=27 - 60 mM, at 340 nm

straight lines which imply the formation of 1 : 1 reversible complexes. The K values were obtained from the plot (see Table I).

Table I Charge-Transfer Association Constants of Model Compounds of Coenzyme I with Indole Derivatives at 25°

indole derivative	pH	K (M^{-1})		
		NAA	NAT	NATH
indoleacetate[a]	6.0	11.8(860)	9.7(940)	13.3(790)
indolepropionate[b]	6.8	-	-	13.5(700)
indolebutyrate[b]	6.5	-	-	10.1(870)
tryptaminehydrochloride[a]	6.0	-	-	1.08(2000)
l-tryptophan[a]	6.0	-	-	5.63(750)

[NAA] = [NAT] = [NATH] = 2 mM, [indole derivative] = 24 - 96 mM, in H_2O. The values in parentheses are molar extinction coefficients of charge-transfer complexes at 340 or 350 nm.
a. At 340 nm, b. At 350 nm.

The K values of nicotinamide adenine dinucleotide (β-isomer)(βNAD$^+$) with tryptamine and L-tryptophan were reported by Alivisatos et al.[6] to be 14.55 M^{-1} and 13.40 M^{-1}, respectively in water at 27° and ionic strengths of 0.02 - 0.1 M. The K values of the present model compounds with tryptamine are fairly small compared with those of NAD$^+$, because of electrostatic repulsive forces between the donor and acceptor. The K values of the model compounds decreased in the order, indole acetate (anionic) > L-tryptophan (neutral) > tryptamine (cationic). It should be noted here that the K values of the present model compounds with indoleacetate are larger than those of the other model compounds investigated hitherto, i.e., 1-benzyl-3-carbamoyl pyridinium chloride (4.1 M^{-1} at 25° in 0.017 M phosphate buffer, at pH = 6.7),[5] 1-methyl-4-carbamoyl pyridinium chloride (4.0 M^{-1} at 25°, in 1 % ethanol-water),[11] and 1-methyl-3-carbamoyl pyridinium chloride (4.4 M^{-1} at 25°, in 1 % ethanol-water),[11] which contain no nucleic acid bases. This suggests that the nucleic acid bases enhance the susceptibility of the pyridinium ring as an electron-acceptor to the indole attack as an electrondonor. The charge-transfer interactions between tryptophan and nucleic acid bases were recently found by the fluoresence and emission spectra and by the infra-red spectra.[3]

The simple- and poly-electrolyte effects on the association constants will now be discussed. The salt effects on the K value of the NATH-IA complex are shown in Figure 3. As is clearly seen, K decreased by addition of the simple-, poly- and micelle-electrolytes. The effects of poly- and micelle-electrolytes were larger than those of simple electrolytes, which should be ascribed to high electrostatic potential of the micelles and macroions. The retarding actions of KCl and $CaCl_2$ are the so-called "secondary salt effect" on the equilibrium constant.[13] The quantitative discussion will be given later.

In Figure 4 the retarding effects of an anionic polyelectrolyte, NaPES, on the NATH-IA, NATH-IP and NATH-IB

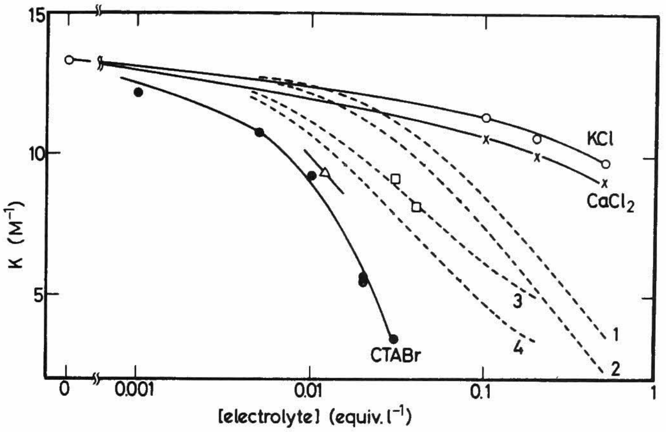


Figure 3 Electrolyte effects on the charge-transfer complex formation between NATH and indoleacetate at 25°; [NATH]=2 mM, [IA]=30 mM, at 340 nm; obsd. ○ : KCl, × : $CaCl_2$, □ : DECS, Δ : NaPSt, ● : CTABr; theory broken curve 1 : KCl, 2 : $CaCl_2$, 3 : DECS(ξ=1.4), 4 : NaPSt(ξ=2.85)

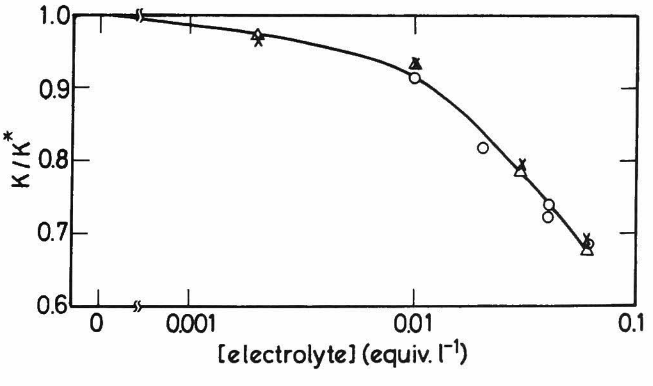


Figure 4 Retarding effect of NaPES on the charge-transfer complex formation between NATH and indole derivatives at 25°; ○ : IA, Δ : IP, × : IB; [NATH]=2 mM, [IA]=[IP]=[IB]=30 mM, at 340 nm

complexations at 25° are shown. The changes of the equilibria are mainly due to the electrostatic interactions between NaPES and NATH, or NaPES and indole derivatives. It should be mentioned that the retarding actions of NaPES or CTABr did not depend on the model compounds as is clearly shown in Figure 5.

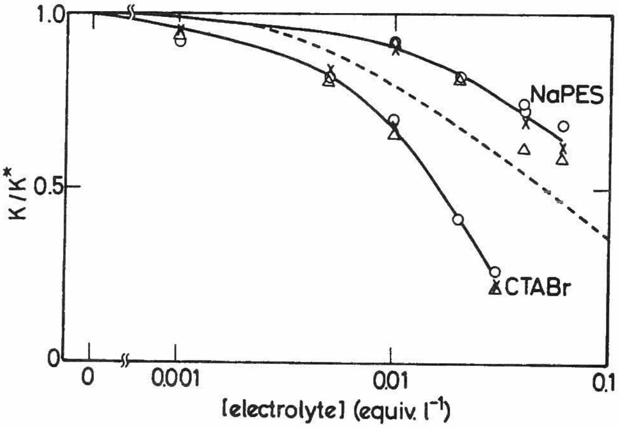


Figure 5 Retarding effect of NaPES and CTABr on the charge-transfer complex formation between coenzyme model compounds and indoleacetate at 25°; [NAA]=[NAT]=[NATH]=2 mM, [IA]=30 mM, at 340 nm; ○ : NATH, Δ : NAA, × : NAT; broken curve : Manning's theory for NaPES(ξ=2.85)

The charge-transfer spectra of the NATH-Try complexation did not show any change by addition of NaPES or DECS, when the concentrations of NATH, Try, NaPES and DECS were 2 mM, 96 mM, 20 mequiv. l^{-1} and 20 mequiv. l^{-1}, respectively, and pH was 6.0. This may be due to the interactions between the polyelectrolytes and neutral tryptophan being very weak under the experimental conditions.

When the anionic polyelectrolyte, NaPES, was added to the NATH-TA·HCl system, the solution turned to yellow and turbid. This shows that NaPES adsorbs both of NATH and TA by the electrostatic attractive forces and strikingly enhances the change--transfer complexation. The determination of the association constants in the presence of NaPES was not possible, of course.

The charge-transfer complexation of NATH-TA system was slightly enhanced by cationic polyelectrolyte, DECS, and KCl, as is seen in Table II. The calculated values were obtained using theories to be mentioned later (eqs. 7 and 16). The reason for the enhancement will be described later.

Now let us discuss the influence of simple- and polyelectrolytes on the association constant in terms of the "secondary salt effect" theoretically. First, the "secondary salt effect" of simple electrolyte is considered. When a charge-transfer complex is formed between an electron-donor D and an acceptor A (eq. 2), the thermodynamic equilibrium association constant, K_o, is defined by eq. 3.

$$D + A \rightleftharpoons D\cdot A \tag{2}$$

$$K_o = \frac{a_{D\cdot A}}{a_D \cdot a_A} \tag{3}$$

Table II Enhancing Effect of KCl and DECS on the Charge-Transfer Complex Formation between NATH and Tryptamine Hydrochloride at 25°

Electrolyte	Concn. of electrolyte (equiv.l^{-1})	K/K* obsd.[a]	K/K* calcd.[b]
KCl	0.02	1.04	1.18
	0.1	1.24	1.84
	0.2	1.41	2.73
DECS	0.0535	1.08	1.46
	0.0764	1.12	1.59
	0.115	1.18	1.76
	0.153	1.23	1.89
	0.191	1.30	1.98

a. [NATH] = 2 mM, [TA·HCl] = 30 mM, at 340 nm. pH = 6.0 The K value in the absence of foreign salt (K*) is 1.08 M^{-1} (see Table I)

b. From eq. 7 for KCl and eq. 16 for DECS.

where a denotes the activity. The activity is the product of the activity coefficient, f, and the concentration. Thus, eqs. 4 and

5 are obtained.

$$K_o = \frac{[D\cdot A]}{[D][A]} \times \frac{f_{D\cdot A}}{f_D \cdot f_A} = K \frac{f_{D\cdot A}}{f_D \cdot f_A} \tag{4}$$

$$K = K_o \frac{f_D \cdot f_A}{f_{D\cdot A}} \tag{5}$$

It is able to observe only the ratio of the concentrations of D, A, and D·A, (not the ratio of the activities) ; the K values (not K_o) can be obtained by experiments. In very dilute aqueous solutions, the activity coefficients, f_D, f_A and $f_{D\cdot A}$, can be safely estimated by the Debye-Hückel theory as follows,

$$\log f_i = -0.509\ z_i^2 I^{1/2} \quad \text{at } 25° \tag{6}$$

where z_i is the valency of the ionic species, i, and I the ionic strength. From eqs. 5 and 6 we get

$$\log K = \log K_o + 1.018\ Z_D Z_A I^{1/2} \tag{7}$$

The comparison of the observed K values for the complex formation between unlikely charged ionic species in the presence of KCl and $CaCl_2$ and values calculated from eq. 7 is demonstrated in Figure 3. It is seen that the observed retarding effect by $CaCl_2$ is larger than that by KCl, though the agreements between the observed and calculated K/K* values are not so good, especially at high concentrations. This deviation may be due to (1) the ionic strength being so high (0.1 - 1) that the application of the Debye-Hückel theory is difficult and (2) both of two reactants, the donor and acceptor ions, being too large to be assumed as point charges.

Next, the "secondary salt effect" of poly-electrolyte on the interionic association constants between unlikely charged ionic species are discussed. In this case, log $f_{D \cdot A}$ can safely be neglected compared with log f_D or log f_A, because the activity coefficients of the electrically neutral complex are not sensitive to changes in ionic concentration. Therefore, the following relation is obtained from eq. 5.

$$\log K = \log K_o + \log(f_D \cdot f_A) \tag{8}$$

In discussing the polyelectrolyte effect, it is convenient to use the association constant without added salt, K*, as a reference. K* is then given as follows.

$$\log K^* = \log K_o + \log(f_D{}^* \cdot f_A{}^*) \tag{9}$$

where the asterisk denotes the reference state. From eqs. 8 and 9, eq. 10 is derived.

$$\log K/K^* = \log(f_D \cdot f_A/f_D{}^* \cdot f_A{}^*) \tag{10}$$

Since the mean activity coefficient, $f_{\pm}$, of the electrolyte D^-A^+

is given by $(f_D \cdot f_A)^{1/2}$, eq. 11 is obtained.

$$\log K/K^* = 2 \log f_{\pm}/f_{\pm}^* \tag{11}$$

Manning developed a theoretical expression for $f_{\pm}$ of added simple salts in dilute polyelectrolyte solutions as follows (eq. 48 of ref. 24)

$$f_{\pm}^2 = \frac{\xi^{-1}n_e+n_s}{n_e+n_s} \exp\left[\frac{-\xi^{-1}n_e}{\xi^{-1}n_e+2n_s}\right], \quad \xi>1 \tag{12}$$

where ξ is the charge density parameter given by the relation,

$$\xi = \frac{e_o^2}{DkTb} \tag{13}$$

where b is the distance between the neighboring charges on an infinite linear line charged macroion and D the dielectric constant of solvent and e_o denotes the electronic charge. n_e and n_s are the equivalent concentrations (number of ions per cm^3) of macroions and the number of added simple salts per cm^3. Equations 11 and 12 are combined and the following relation is obtained.[25]

$$\ln K/K^* = \ln \frac{1 + \xi^{-1}X}{X + 1} - \frac{X}{X + 2\xi} \tag{14}$$

where X is $n_e/(n_s + n_{R_1} + n_{R_2})$, n_{R_1} and n_{R_2} denote the numbers of reactant 1 and reactant 2, respectively.

In Figure 5, a theoretical curve(broken curve) for the retardation by NaPES calculated by eq. 14 is shown. The parameter value of ξ was taken to be 2.85 for NaPES (b was calculated to be 2.5 Å from the molecular structure). The dielectric constant, D, of water was estimated to be 78.54 from Wyman's data.[27] The theory fairly well predicts the observed strong retarding effect of polyelectrolytes. The observed K/K* values were, however, a little larger than the calculated, which may be due to the fact that the complexation reactions occurred between comparatively large and complicated ions for which the theory is not valid. The comparison for the CTABr containing system with theory was not carried out, since the CTABr micelle is spherical,[28] for which theory is not valid, either.

Figure 3 shows the comparison of the K values of the NATH-IA system with the theory in the presence of DECS and NaPSt. In the case of DECS the agreement between the theory (curve 1) and the observed values (open circle) is good. As is seen from the figure, the observed retarding effect of NaPSt is larger than that obtained from the theory (eq. 14). This will be due to the strong hydrophobic nature of the NaPSt[16]; the NATH molecules accumulate around the PSt macroion because the electrostatic and hydrophobic attractive forces between the hydrophobic PSt macro-anion and the hydrophobic NATH univalent cation are extremely strong and are retarded from the complexation with indoleacetate anions, which are far from PSt macro-ion on account of the electrostatic repulsive forces between them.

It must be noted here that eq. 14 is also valid for the cyanide addition reaction of NAA studied earlier in the presence and absence of polyelectrolytes.[1] In Figure 6, the observed equilibrium association constant, K, was compared with the theory (eq. 14). A good agreement between the theory and the experiment was obtained, particularly at low polymer concentration regions.

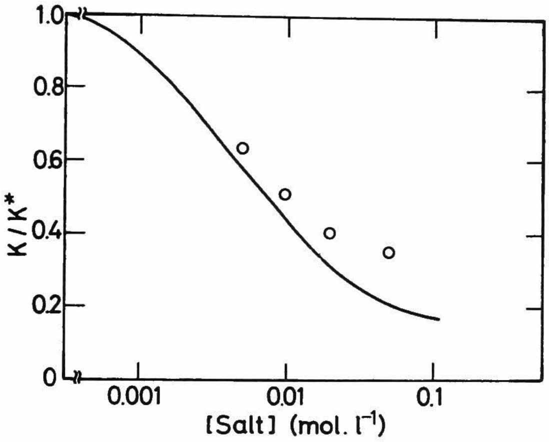


Figure 6 Comparison of observed and calculated K values for NAA-CN^- reaction in the presence of NaPES at 25°: [NAA]=0.198 mM, [KCN]=4 mM, [KOH]=1 mM; o : NaPES added, obsd. (from ref. 1); —— : calculated

Deviations at high polymer concentration regions may be due to the imperfection of Manning's limiting law in such a concentration range.

Now, the "secondary salt effect" of cationic polyelectrolyte on the interionic association constants between cationic charged ionic species, such as between a coenzyme model compound and tryptamine is discussed. In this case, all of the activity coefficients, f_D, f_A and $f_{D\cdot A}$, in eq. 5 are those of co-ions of macroion and given by eq. 15 (see eq. 47 of ref. 24).

$$f_+ = \exp\left[- \frac{\xi^{-1} n_e}{2(\xi^{-1} n_e + 2n_s)} \right], \qquad \xi > 1 \tag{15}$$

Thus, the ln K/K* is expressed as follows :

$$\ln K/K^* = \frac{1}{1 + \xi\left(\frac{2}{X_1} + \frac{2}{X_2}\right)} \tag{16}$$

where $X_1 = n_e/n_{R_1}$ and $X_2 = n_e/n_{R_2}$. n_{R_1} and n_{R_2} denote the numbers of reactant 1 and 2. The calculated values of the NATH-TA complexation in the presence of DECS using eq. 16 were shown in Table II. The calculated ones in the presence of KCl were derived using the Debye-Hückel theory (eq. 7). As is seen from the table, the observed enhancing effect of simple- and poly-electrolyte is supported by the theories, though the agreements between the observed and the calculated K/K* values are not so good for both KCl and DECS. This deviation may be attributed to NATH and TA being too large to be assumed as point charges and the high concentrations of KCl and DECS.[29]

Next, the temperature dependence of the association constants is discussed. The K value for the NAA-IA system decreased with increasing temperature. This reflects the fact that the reaction 1 is exothermic. Table III gives the free energy (ΔG), enthalpy

Table III Thermodynamic Constants of Charge-Transfer Complexation between NAA and Indoleacetate at 25°[a]

Electrolyte	ΔG[b]		ΔH[b]		ΔS[b]	
	Obsd.	Cald.	Obsd.	Cald.	Obsd.	Cald.
none	-1.47	(-1.47)	-4.44	(-4.44)	-10.0	(-10.0)
KCl	-1.34	-1.05	-4.67	-4.68	-11.2	-12.2
$CaCl_2$	-1.30	-0.88	-4.80	-4.75	-11.7	-12.9
DECS	-1.20	-1.24	-4.48	-4.49	-11.0	-10.9
NaPES	-1.19	-1.10	-4.44	-4.47	-10.9	-11.4

a. [NAA] = 2 mM, [IA] = 30 mM, at 340 nm

b. The units of ΔG and ΔH are $kcal.mol^{-1}$, and ΔS in e.u. The experimental uncertainty is believed to be ±0.05 for ΔG, ±0.2 for ΔH and ±2 for ΔS.

(ΔH) and entropy (ΔS) of the NAA-IA complex formation. By the addition of simple- or poly-electrolytes, ΔG increased, which is in agreement with the observed changes of K. Both ΔH and ΔS decreased with added electrolytes, though slightly.

The thermodynamic parameters for the interionic complexation can be computed theoretically from the Debye-Hückel theory and from the Manning theory ; ΔG and ΔH in the presence of polyelectrolyte were obtained from eq. 14 as follows.

$$\Delta G - \Delta G^* = RT\left[-\ln\frac{\xi^{-1}X+1}{X+1} + \frac{X}{X+2\xi}\right] \tag{17}$$

$$\Delta H - \Delta H^* = RT\left[\frac{X}{X+\xi} - \frac{2\xi X}{(X+2\xi)^2}\right]\left(1+\frac{T}{D}\cdot\frac{dD}{dT}\right) \tag{18}$$

where the asterisk denotes the absence of added polyelectrolyte. The changes of ΔG and ΔH of the charge-transfer complex formation by the addition of simple electrolytes (KCl or $CaCl_2$ in the present case) are given by the Debye-Hückel theory.

$$\Delta G - \Delta G^* = -2RT\sqrt{\frac{2\pi N}{1000}}\left(\frac{e_o^2}{DkT}\right)^{3/2}\left(\sqrt{n_{R_1}+n_{R_2}+\frac{z^2+z}{2}n_s} - \sqrt{n_{R_1}+n_{R_2}}\right) \tag{19}$$

$$\Delta H - \Delta H^* = 3RT\sqrt{\frac{2\pi N}{1000}}\left(\frac{e_o^2}{DkT}\right)^{3/2}\left(1+\frac{T}{D}\cdot\frac{dD}{dT}\right)\left(-\sqrt{n_{R_1}+n_{R_2}+\frac{z^2+z}{2}n_s} - \sqrt{n_{R_1}+n_{R_2}}\right) \tag{20}$$

ΔS can be determined by eq. 21

$$\Delta G = \Delta H - T\,\Delta S \tag{21}$$

In Table III, the calculated values are also compiled. The structural parameter, ξ, was taken to be 1.37 for DECS (b was estimated to be 5.2 Å from the molecular structure). As is clear from the table, the agreement between the theory and experiment was excellent ; particularly the observed ΔH values were accurately predicted by the electrostatic interaction theories, that is., eqs. 18 and 20. The larger changes of ΔG, ΔH and ΔS by the addition of $CaCl_2$ compared with those by KCl are also accounted for by eq. 20. From the agreements, it can be stated that the electrostatic interactions between the complex-forming ions and added electrolyte are most significant to the charge-transfer complex formations in the present cases.

From the above theoretical considerations, it is possible to conclude that the polyelectrolyte influence under consideration on the charge-transfer complex formation constant is due to the changes of the activities of the complex forming ions, i.e., the "secondary salt effect". This conclusion implies that the accumulation or condensation effect of ions in the polymer domain in the narrow sense of the words are not basically an appropriate factor. It should be noted that the same conclusion has been reached from a theoretical consideration (on the basis of the Debye-Hückel theory and the Manning theory) on the rate-enhancing effect of a cationic poly-electrolyte on ionic reactions between cationic species.[26,30]

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Chapter 7

Polyelectrolyte Effects on the Charge-Transfer Complex Formation between FMN and Indole Derivatives

Introduction

Flavin mononucleotide (FMN) is a coenzyme of flavoprotein (the old yellow enzyme) found in mitochondria. FMN is also known to take part in the oxidative phosphorilation and form a complex with tryptophan.[1] Charge-transfer forces play important roles in the FMN-indoles complex formation.[1,2] Harbury et al.[3] and Isenberg et al.[4] have measured the apparent association constants of FMN with tryptophan etc.

Wilson[5] investigated the effects of pH, ionic strength and electrostatic forces upon the complexes, and found that the association constants of FMN complexation increased in the following order ; indoleacetate<tryptophan<tryptamine. This order shows the important contribution of the electrostatic forces between the donors and acceptors.

In a preceding paper,[6] the polyelectrolyte influence on the complexation equilibrium between model compounds of nicotinamide adenine dinucleotide (NAD) and some indole derivatives was investigated. In this chapter, the electrostatic forces in the FMN complexes are studied more in detail.

Experimental

Meterial

FMN was purchased from Sigma Chemical Co., St. Louis, and was used without further purification. Indoleacetate from Merck (for biochemical use) was used. L-Tryptophan, tryptamine hydrochloride, KCl and Na_2SO_4 were guaranteed reagents.

Sodium polyethylene sulfonate (NaPES), degree of polymerization 770, was a gift from the Hercules Powder Co., Wilmington, Del. Sodium polystyrene sulfonate (NaPSS) was kindly donated by the Dow Chemical Co., Midland, Mich. The molecular weight was 6,300,000. Dilute solutions of these anionic polyelectrolytes were converted to the acid form by passing them through columns of cation- and anion-exchange resins. Completeness of the conversion was checked by using an atomic absorption spectrophotometer. The corresponding sodium salts were prepared by neutralization of the aqueous solutions with sodium hydroxide. The copolymer of diethyldiallylammonium chloride and sulfur dioxide (DECS) is a strongly basic polyelectrolyte prepared by Harada et al.[7,8]

Measurements of Equilibrium Constants

The apparent association constant, K, in the absence of foreign salts was evaluated using the Foster-Hammick-Wardley plots[9] or the Behme-Cordes method[10] from the slopes of the $(\varepsilon - \varepsilon_0)/N$ vs. $(\varepsilon - \varepsilon_0)$ plot, where ε_0 is the sum of absorbances of the indole derivatives and FMN, ε the absorbance of the mixture, and N the concentration of the indole derivatives. K in the presence of the polymers was derived from the equation, $K^{-1} = N[a\beta/(\varepsilon - \varepsilon_0)]$, where a is the concentration of FMN, and β the molar extinction coefficient of the charge-transfer complex (at 500 nm in the present experiments). The reproducibility of the value of K was estimated to be about 5 % both in the presence and absence of polyelectrolytes. A Hitachi Spectrophotometer (Model EPS-3T) equipped with a thermostated cell holder was used. All these measurements were conducted in 0.2 M aqueous Sörensen Phosphate buffer(Na_2HPO_4 + KH_2PO_4) of pH 6.8 unless described otherwise. The light path of the cell was 1.0 cm. Since FMN is quite photosensitive and decomposes under the exposure of light, the sample solutions are avoided from light exposure as carefully as possible.

Results and Discussion

As is seen in Figure 1, the Foster-Hammick-Wardley plots, i.e. $(\varepsilon - \varepsilon_o)/N$ vs. $(\varepsilon - \varepsilon_o)$ plots yielded in all cases straight lines, which implies the formation of 1 : 1 reversible complexes.

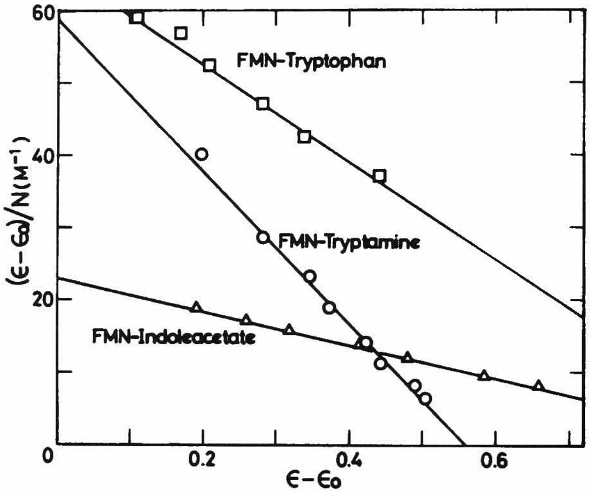

Figure 1 Foster-Hammick-Wardley plots for the Complex Formation of FMN with Tryptamine, Indoleacetate and Tryptophan at 25°C. [FMN] = 3×10^{-4}M, or 6×10^{-4}M, [Tryptamine] = 5×10^{-3} - 8×10^{-2}M, [Indoleacetate] = 5.1×10^{-3} - 8.2×10^{-2}M, [Tryptophan] = 1×10^{-3} - 1.6×10^{-2}M

The association constant values (K) for the complex formation with FMN thus obtained were 109 M^{-1} for tryptamine, 69.3 M^{-1} for L-tryptophan and 23.3 M^{-1} for indoleacetate, respectively at 25°C ([FMN] = 3×10^{-4} or 6×10^{-4} M, [tryptamine] = 10^{-2} M, [L-tryptophan] = 4×10^{-3}M, [indoleacetate] = 2.04×10^{-2} M).

This order suggests that the electrostatic forces between FMN and indole derivatives plays an important role. The thermodynamic parameters obtained for the three complexes are compiled in Table 1.

Table 1 Thermodynamic Parameters for the Complexation of FMN with Tryptamine, Indoleacetate, and Tryptophan at 25°C

Complex	K ($l.mol^{-1}$)	ΔG ($kcal.mol^{-1}$)	ΔH ($kcal.mol^{-1}$)	ΔS (e.u.)
FMN-Tryptamine[a)]	109	-2.78	-4.0	-4
FMN-Indoleacetate[b)]	23.3	-1.87	-3.0	-4
FMN-Tryptophan[c)]	69.3	-2.52	-3.0	-2

a) $[FMN] = 3 \times 10^{-4}M$, $[Tryptamine] = 10^{-2}M$
b) $[FMN] = 6 \times 10^{-4}M$, $[Indoleacetate] = 2.04 \times 10^{-2}M$
c) $[FMN] = 6 \times 10^{-4}M$, $[Tryptophan] = 4 \times 10^{-3}M$

Figure 2 shows the influences of poly- and simple electrolytes on the charge-transfer complex formation between FMN and tryptamine. All electrolytes retarded the complexation. It is interesting to note that the retarding effects of DECS and NaPES were larger than that of simple electrolyte KCl, which should be ascribed to high electrostatic potential of the macroions. As is well known,[6] the

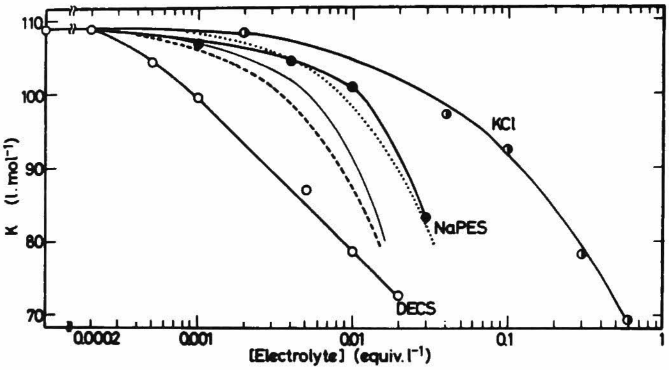

Figure 2 Charge-Transfer Complex Formation of FMN with Tryptamine in the Presence of Polyelectrolytes at 25°C.
[FMN] = 3 × 10^{-4}M, [Tryptamine] = 0.01M, Sörensen-phosphate Buffer (pH = 6.8)
Theoretical Curves (..... : KCl, ----- : NaPES, ——— : DECS)

thermodynamic activity coefficients of the two ionic reactants, i.e., FMN and tryptamine, would be changed by the addition of the polymers and the association constant decreases.

The association constants of FMN with tryptamine in the presence of polyelectrolytes are given by Manning's theory[11] as follows ;

$$\ln(K/K^*) = \ln\frac{\xi^{-1}X+1}{X+1} - \frac{1}{2}\cdot\frac{3X}{X+6\xi} \tag{1}$$

for the case of NaPES

and

$$\ln(K/K^*) = \frac{1}{2}\ln[1 - (1 - \tfrac{1}{2}\xi^{-1})\tfrac{1}{2}X] - \frac{3}{8}\cdot\frac{\xi^{-1}X}{(1+\xi^{-1}-2)X+6} \tag{2}$$

for DECS

where X is given by $n_e/(n_S + n_D + n_A)$, n_e, n_S, n_D and n_A are the concentrations (number of ions per cm^3) of the polymer, foreign salt, tryptamine and FMN, respectively, and ξ is the charge density parameter. The details of the derivation of the equation should be consulted to the preceding paper.[6]

Furthermore, the so-called secondary salt effects of the present complexation systems containing KCl is given by the Debye-Hückel theory ;

$$\ln(K/K^*) = -4.69(I^{1/2} - I^{*1/2}) \qquad (3)$$

where I is the ionic strength and the asterisk implies the reference state. The calculated values of K were also shown in Figure 2 by the dotted, broken and fine solid lines for the cases of KCl, NaPES and DECS, respectively. In the calculations, the valency of FMN was assumed to be -2 under the present experimental conditions. Both the theoretical and experimental K values decreased with addition of electrolytes. The agreement between the observed and calculated values was good at low concentrations. Considering the fact that both FMN and tryptamine are large and complex molecules, the success of the application of the equations derived from the simplified theories is rather astonishing. Furthermore, this agreement suggests that the influence of the added electrolytes is the secondary salt effect as was proposed earlier.[6]

Next, the polyelectrolyte influences on the FMN-indoleacetate complexation are examined. In the present case, the complexation was enhanced by the addition of DECS most efficiently, and by KCl or NaPES slightly. The reason of the enhanced complexation by DECS would be due to the stabilization effects of anionic FMN and indoleacetate by the macrocations. Using the Manning theory in the estimation of the activity coefficients of the reactants in the presence of the cationic polymer, the changes of the K values are given by equation 4.[12]

$$K/K_R = X_A/X_{AR} \tag{4}$$

The theoretical K value increased with electrolyte addition, as was observed. The agreement with observed values is satisfactory particularly at low concentrations of the polymer (see Figure 3), which suggests again that the polymer action is basically the secondary salt effect.

The influence of an anionic polyelectrolyte, i.e., NaPES, on the complexation reaction was theoretically given also by the Manning theory as follows ;[13]

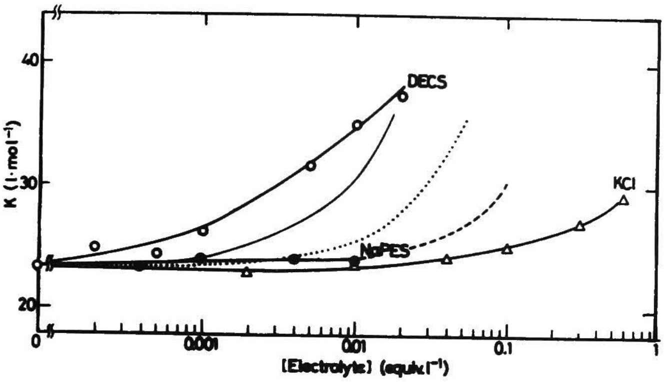

Figure 3 Charge-Transfer Complex Formation of FMN with Indoleacetate in the Presence of Polyelectrolytes at 25°C.
[FMN] = 6 × 10^{-4}M, [Indoleacetate] = 2.04 × 10^{-2}M,
Sörensen-phosphate Buffer (pH = 6.8)
Theoretical Curves (..... : KCl, ----- : NaPES, ——— : DECS)

$$\ln(k/K^*) = \frac{2\xi^{-1} n_e}{\xi^{-1} n_e + 6n_A + 2n_D + 2n_S} \tag{5}$$

where n_A, n_D and n_S are the concentrations of FMN, indoleacetate and 1-1 type salt, respectively. The magnitude of the change of K is comparatively small, but the agreement between the observed and the calculated are excellent.

In Figure 3, the calculated values of K from the Debye-Hückel theory were also given for the FMN + indoleacetate + KCl systems. As is clear in the Figure, the calculated values were larger than the observed. The reason for the discrepancy is not clear yet, but may be partly due to the fact that the valency of the FMN molecule is larger than -2, and it has a large volume in contradiction with the point charge approximation adopted in the theory.

The equilibrium constants of FMN and tryptophan were insensitive to NaPSS and KCl addition as is seen in Figure 4.

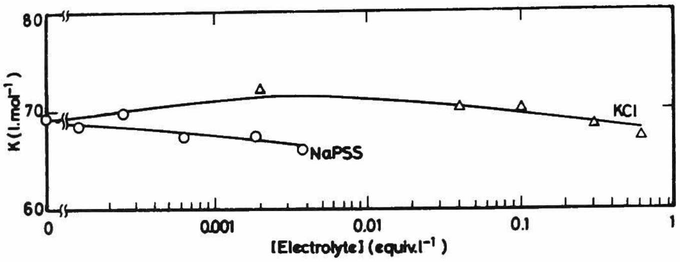

Figure 4 Charge-Transfer Complex Formation of FMN with Tryptophan in the Presence of Electrolytes at 25°C.
[FMN] = 6×10^{-4}M, [Tryptophan] = 4×10^{-3}M,
Sörensen-phosphate Buffer (pH = 6.8)

This is understandable because the tryptophan molecules are neutral in the present experiments and the ion-molecule interactions are so weak compared with the interionic forces.

The conclusions are (1) the strength of the complex formation between FMN and indole derivatives is largely influenced by the electrostatic forces between reactant ions and between reactant ions and macroions, and (2) the changes of the equilibrium constants can be accounted for by the "secondary salt effect".

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Chapter 8

Complex Formation of Poly-4-vinyl-N-alkylpyridinium Salts or Polymer Containing FMN with Indole Derivatives

Introduction

Various polymeric substances are known to accelerate or decelerate chemical reactions[1-6]. The catalytic activity is due to various interactions between the polymer catalysts and reaction substrates, i.e., (1) electrostatic, (2) hydrophobic, (3) hydrogen-bond type, (4) coordination or inclusion type and (5) charge-transfer type interactions. In most cases, these interactions are cooperative.

In the preceding papers[7,8], the influences of polyelectrolytes on the charge-transfer type complex formations between coenzyme model compounds of nicotinamide adenine dinucleotide (NAD^+) and indole derivatives or between flavin mononucleotide (FMN) and indole derivatives were studied. As is well known, pyridinium salts and FMN form charge-transfer complexes with indole derivatives in aqueous solution[9].

In this work, complex formations between poly-4-vinyl-N-

-alkylpyridinium salts (RPVP), i.e., a cationic polymer, and indole derivatives or between anionic polymers containing FMN residues (PFMN) and indole derivatives are investigated in the presence of various salts. These polymers were found to be electron acceptors.

Experimental

Material

Poly-4-vinyl-N-propylpyridinium bromide (C3PVP), poly-4-vinyl-N-butylpyridinium bromide (C4PVP) and poly-4-vinyl-N-benzylpyridinium chloride (BzPVP) were prepared by the Menschutkin reaction of poly-4-vinylpyridine (degree of polymerization ; 3,800) with propyl bromide, butyl bromide and benzyl chloride, respectively. The details of the preparation were reported earlier[10]. The degrees of quaternization of C3PVP, C4PVP and BzPVP were 0.95, 0.95 and 0.92, respectively.

The FMN polymer (PFMN) was prepared by a method described by Kamogawa[11] ; after reaction of methylolated polyacrylamide with FMN sodium salt, excess amounts of methanol was added to the solution to block the unreacted methylol groups of the polymer in order to prevent crosslinking and the solution was kept being stirred at 50°C for 10 hours. Then, the solution was neutralized with aqueous ammonia and precipitated into ethanol. The polymer was dissolved in water and reprecipitated into ethanol. This operation was repeated three times and the polymer was freeze-dried from water to yield yellow material. From the visible light absorbance spectrum of this PFMN, the extent of the reaction with FMN was determined to be 46 %.

Indoleacetate from Merck (for biochemical use) was used without further purification. Tryptamine hydrochloride, L-tryptophan, KCl and $CaCl_2$ were commercially available guaranteed reagents. The copolymer of dimethyldiallylammonium chloride and sulfur dioxide (DMCS) is a strongly basic polyelectrolyte prepared by Harada et al.[12,13] Water was deionized and further distilled before use.

Determination of Equilibrium Constants

The equilibrium association constants, K, were determined using the Foster-Hammick-Wardley method[14] from the absorbances of the charge-transfer band at 330 nm for C3PVP, C4PVP or BzPVP and at 500 nm for PFMN in Sörensen phosphate buffer of pH = 6.8 at 25°C by using a Hitachi Spectrophotometer (EPS-3T).

Thermodynamic parameters, i.e., the free energy (ΔG), enthalpy (ΔH) and entropy (ΔS), for the charge-transfer complex formation were determined by measuring the association constants at 20, 25 and 30°C.

Results and Discussion

Complexation between Polymers and Indole Derivatives

Figure 1 shows the difference spectra of the complex formation

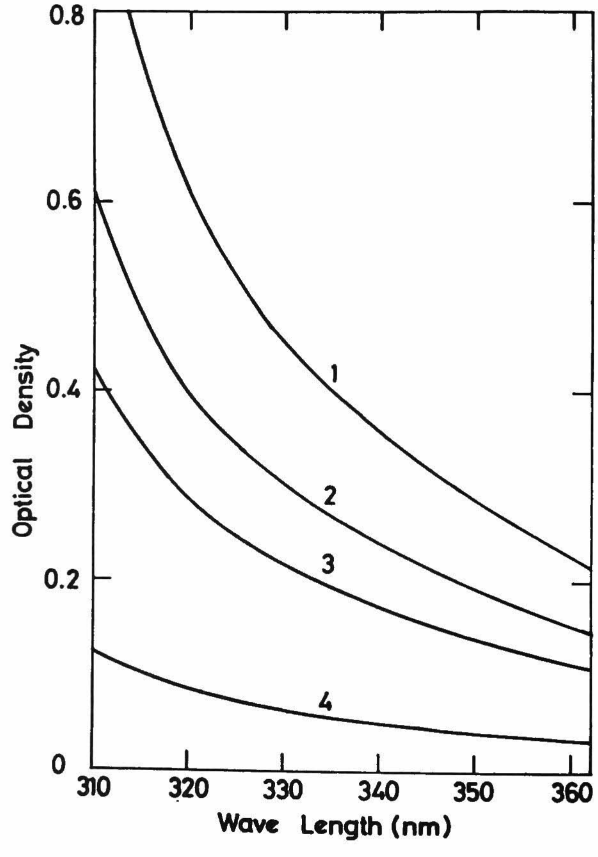

Figure 1 Difference absorbance spectra of complex formation of C4PVP with indoleacetate at 25°C. Curve 1 : [C4PVP]=[Indoleacetate] =0.01 mol.l^{-1}, 2 : 0.00667, 3 : 0.005. 4 : 0.00167

between C4PVP and indoleacetate. The peaks of the spectra were at about 310 nm. These spectra were quite diffuse bands having long tail at longer wavelength. The shapes of these spectra were quite similar to those of corresponding low molecular weight derivatives, i.e., NAD^+ with indoleacetate[15-17]. It is quite understandable that the pyridinium moieties of C4PVP are electron acceptors and indoleacetate is electron donor.

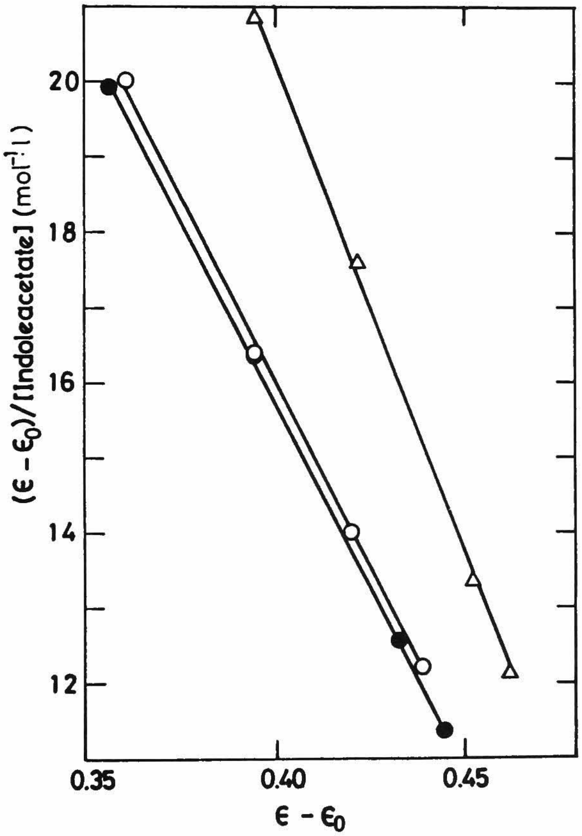

Figure 2 Foster-Hammick-Wardley plots for C3PVP-indoleacetate (●), C4PVP-indoleacetate (o) and BzPVP-indoleacetate (Δ) at 25°C.
[Indoleacetate]=0.018-0.048mol.1^{-1} (o); 0.018-0.042 M (●,Δ)
[C3PVP]=[C4PVP]=[BzPVP]=2×10^{-3} mol.1^{-1}

The representative Foster-Hammick-Wardley plots to calculate the association constants of charge-transfer complexes between C3PVP, C4PVP or BzPVP, and indoleacetate are demonstrated in Figure 2. The $(\varepsilon - \varepsilon_o)$/[indoleacetate] vs. $(\varepsilon - \varepsilon_o)$ plots showed straight lines for all systems, where ε_o was the sum of absorbances of indoleacetate and RPVP, and ε the absorbance of the equilibrium mixture. This linearity suggests the formation of 1 : 1 complexes between indoleacetate and pyridinium moieties of C3PVP, C4PVP and BzPVP.

The association constants, K, determined from the Foster-Hammick-Wardley plots are listed in Table I. As is seen from this

Table I Association Constants (K) of Complexes between RPVP and Indoleacetate at 25°C (pH = 6.0)

Polyelectrolyte[a]	[Indoleacetate] $(\mathrm{mol\ l^{-1}})$	K $(\mathrm{mol^{-1}.l})$
C3PVP	0.018-0.042	97±1
C4PVP	0.018-0.048	98±1
BzPVP	0.018-0.042	123±2

a) Polyelectrolyte concentration : 0.002 $\mathrm{mol.l^{-1}}$

Table, the K values are about 100 for these polymers. The K value for the complexation between a corresponding pyridinium salt of low molecular weight, i.e., 1-benzyl-3-carboxamide pyridinium chloride and indoleacetate is reported to be $4.1\text{mol}^{-1}\text{l}$[9]. Thus, the complex formations between poly-4-vinylpyridinium salts with indoleacetate are about 25 times stronger than that between the low molecular pyridinium salt and indoleacetate. This would be ascribed to very strong electrostatic interactions between the macrocations and indole anions compared with those between monomeric cations and indoles. Furthermore, the K values slightly increased in the order C3PVP < C4PVP < BzPVP. This implies that the complexes became more stable by the hydrophobic interactions in addition to the electrostatic ones.

Table II shows the association constants of C4PVP + L-tryptophan- and C4PVP + tryptamine hydrochloride-complexes. As is clear from the Table, the K value of the C4PVP + L-tryptophan case was larger than the C4PVP + tryptamine pair. The valencies of L-tryptophan and tryptamine hydrochloride are considered to be zero and +1, respectively, under the experimental condition. Thus, the former complexation would not be influenced by electrostatic interaction between the acceptors and donors, whereas the latter would be retarded. It is noted that the strength of the complexation was in the order RPVP + indoleacetate > RPVP +

Table II Association Constants (K) of Complexes between C4PVP and L-Tryptophan or Tryptamine Hydrochloride[a]

Indole Derivative	[Indole Derivative] ($mol\ l^{-1}$)	[C4PVP] ($mol.l^{-1}$)	K ($mol^{-1}l$)	pH	Temp. (°C)
L-Tryptophan	0.024-0.048	0.002	7.4	5.5	25
Tryptamine	0.24-0.48	0.01	1.0±0.3	4.2	35
Tryptamine	0.12-0.35a)	0.01	1.4±0.3	4.0	35

a) Ionic strength was adjusted to 0.35 with KCl

tryptophan > RPVP + tryptamine, which is explainable in terms of the electrostatic interaction between the two reactants of the complexation.

The complexation constants of PFMN with tryptamine hydrochloride, L-tryptophan and indoleacetate were 89.0, 53.9 and 22.9 $mol^{-1}.l$, respectively. This order of K is also understood by the electrostatic interactions. It should be noted that the association constans between these polymers and indole derivatives were of the same order as or a little smaller than those of the complexation of the corresponding monomer, i.e., FMN (109, 69.3 and 23.3 $mol^{-1}l$ for FMN + tryptamine hydrochloride, FMN + tryptophan and FMN + indoleacetate, respectively).

Complexation in the Presence of Foreign Salts

Figure 3 shows the influences of KCl, $CaCl_2$ and DMCS on the K

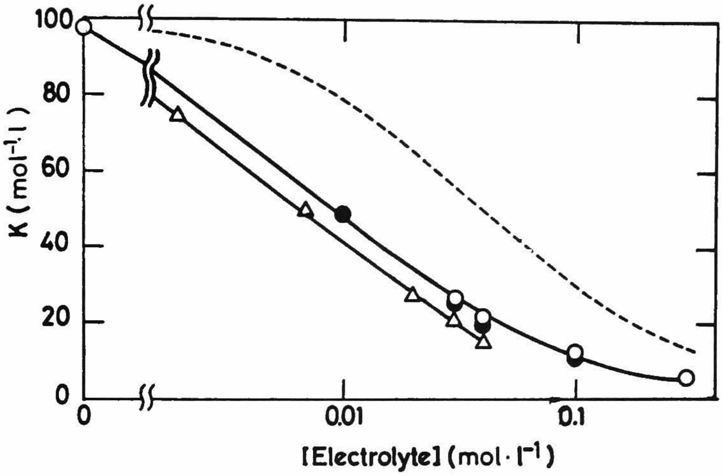

Figure 3 Influences of KCl(o), $CaCl_2$(●) and DMCS(Δ) on the complex formation of C4PVP with indoleacetate at 25°C
[C4PVP]=2×10^{-3}mol.l^{-1}, [Indoleacetate]=0.024mol.l^{-1}
Theory : KCl (---)

values of the complex between C4PVP and indoleacetate. As is clearly seen from this Figure, the added electrolytes retarded the (+z, -1) type complex formation. The K values decreased to about one tenth of that without added electrolytes by addition of salts at a concentration of 0.1 mol.l^{-1}. This is because the complexation is one of the reactions between oppositely charged species.

In the previous work[7,8], the polyelectrolyte action on the complexation reactions was successfully explained theoretically by using Manning theory[18]. The salt influence was found to be the "secondary salt effect". For the present complexation systems, i.e., C4PVP + indoleacetate + KCl, the theory gives the following relation;

$$\ln\frac{K}{K^*} = -\ln\frac{n_e+n_o+n_s}{n_e+n_o} + \xi^{-1}\ln\left(\frac{\xi^{-1}n_e+n_o+n_s}{\xi^{-1}n_e+n_o}\sqrt{\frac{\xi^{-1}n_e+2n_o}{\xi^{-1}n_e+2n_o+2n_s}}\right)$$

$$+\frac{\xi^{-2}n_en_s}{(\xi^{-1}n_e+2n_o+2n_s)(\xi^{-1}n_e+2n_o)} \qquad (1)$$

where K and K* are the association constants with and without KCl, and n_e, n_o and n_s are the concentrations (number of ions per cm^3) of C4PVP, indoleacetate and KCl, respectively. ξ is the charge density parameter given by the relation[18],

$$\xi = e^2/DkTb \qquad (2)$$

where b is the distance between neighboring charges on an infinite linear line charged macroion, D the dielectric constant of solvent, and e_o denotes the electronic charge. The ξ value of C4PVP was taken to be 2.91. The calculated value (broken line in Figure 3) was fairly larger than the observed one. The reason for this discrepancy is not clear at present.

The influences of added electrolytes (KCl, NaPES and DMCS) on the association constants between PFMN and tryptamine hydrochloride, i.e., (-z, +1) type complexation (Figure 4) were also studied. The added electrolytes retarded the charge-transfer complex formation. Theoretical curve for the retarding effects of KCl is also shown in this Figure according to equation (1). The agreement between the calculated and observed values is fairly satisfactory. These

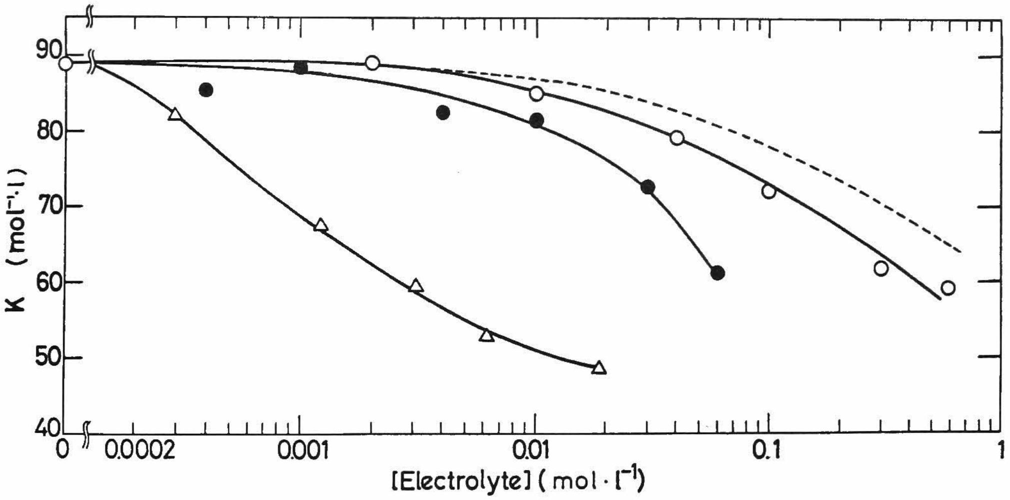

Figure 4 Influences of KCl(o), NaPES(●) and DMCS(Δ) on the complex formation of PFMN with tryptamine hydrochloride at 25°C

[PFMN]=6×10^{-4}mol.1^{-1}, [Tryptamine]=0.01mol.1^{-1}

Theory : KCl (---)

results indicate the important contribution of the electrostatic interaction between the ionic species.

Thermodynamic parameters are listed in Tables III and IV. The theoretical values were computed by the Manning theory. The free energy (ΔG) and the enthalpy (ΔH) of the reaction system between C4PVP and indoleacetate in the presence of simple electrolyte (KCl) were given from equation (1) as :

$$\Delta G - \Delta G^{*} = -RT\left[-\ln\frac{n_e+n_o+n_s}{n_e+n_o} + \xi^{-1}\ln\left(\frac{\xi^{-1}n_e+n_o+n_s}{\xi^{-1}n_e+n_o}\sqrt{\frac{\xi^{-1}n_e+2n_o}{\xi^{-1}n_e+2n_o+2n_s}}\right) + \frac{\xi^{-2}n_e n_s}{(\xi^{-1}n_e+2n_o+2n_s)(\xi^{-1}n_e+2n_o)}\right] \tag{3}$$

$$\begin{aligned}\Delta H - \Delta H^{*} = \xi^{-1}RT\Bigg[&\ln\left(\frac{\xi^{-1}n_e+n_o+n_s}{\xi^{-1}n_e+n_o}\sqrt{\frac{\xi^{-1}n_e+2n_o}{\xi^{-1}n_e+2n_o+2n_s}}\right)\\ &+ \xi^{-1}n_e n_s\Bigg(\frac{2(\xi^{-1}n_e+2n_o)^2+2n_s(\xi^{-1}n_e+4n_o)}{(\xi^{-1}n_e+2n_o+2n_s)^2(\xi^{-1}n_e+2n_o)^2}\\ &+ \frac{1}{(\xi^{-1}n_e+2n_o+2n_s)(\xi^{-1}n_e+2n_o)} - \frac{1}{(\xi^{-1}n_e+n_o+n_s)(\xi^{-1}n_e+n_o)}\Bigg)\Bigg]\\ &\times\left(1+\frac{T}{D}\frac{dD}{dT}\right)\end{aligned} \tag{4}$$

Table III Thermodynamic Parameters for the Complex Formation between C4PVP and Indoleacetate at 20°C[a]

Electrolyte	[Electrolyte] $(mol.l^{-1})$	K $(mol^{-1}l)$	ΔG $(kcal.mol^{-1})$		ΔH $(kcal.mol^{-1})$		ΔS (e.u.)	
			obs.	cal.[b]	obs.	cal.[b]	obs.	cal.[b]
None	0	98.4	-2.7	(-2.7)	-1.7	(-1.7)	3.5	(3.5)
KCl	0.1	11.3	-1.4	-2.0	-1.7	-1.8	-0.7	+0.7
$CaCl_2$	0.1	10.0	-1.4	---	-1.6	---	-0.9	---
DMCS	0.03	21.1	-1.8	---	-1.5	---	1.2	---

a) [C4PVP] = 0.002 $mol.l^{-1}$, [Indoleacetate] = 0.024 $mol.l^{-1}$, pH=6.0
b) The theoretical calculation gives only the relative values of the parameters. Thus, these values were obtained using the observed values in the absence of KCl

Table IV Thermodynamic Parameters for the Complex Formation between PFMN with Tryptamine Hydrochloride at 25°C[a]

Electrolyte	[Electrolyte] ($mol.l^{-1}$)	K ($mol^{-1}.l$)		ΔG ($kcal.mol^{-1}$)		ΔH ($kcal.mol^{-1}$)		ΔS (e.u.)	
		obs.	cal.[b]	obs.	cal.[b]	obs.	cal.[b]	obs.	cal.[b]
None	0	89.0	(89.0)	-2.66	(-2.66)	-4.8	(-4.8)	-7.1	(-7.1)
KCl	0.3	61.8	70.6	-2.44	-2.52	-4.4	-5.1	-6.5	-8.7
NaPES	0.03	74.9	---	-2.56	---	-4.8	---	-7.4	---
DMCS	6.16×10^{-3}	55.4	---	-2.38	---	-3.9	---	-5.2	---

a) [PFMN] = 6×10^{-4} $mol.l^{-1}$, [Tryptamine] = 0.01$mol.l^{-1}$, pH=6.8

b) The theoretical calculation gives only the relative values of the parameters. Thus, these values were obtained using the observed values in the absence of KCl.

As is clear from the Table, the agreement between the experiment and theory was mostly good.

Figure 5 shows that the K values between PFMN and L-tryptophan were almost unaffected by the addition of KCl or NaPES. This is quite reasonable because L-tryptophan is electrically neutral. On the other hand, DMCS retarded the complexation. Some specific interaction besides electrostatic interaction may exist between DMCS and L-tryptophan.

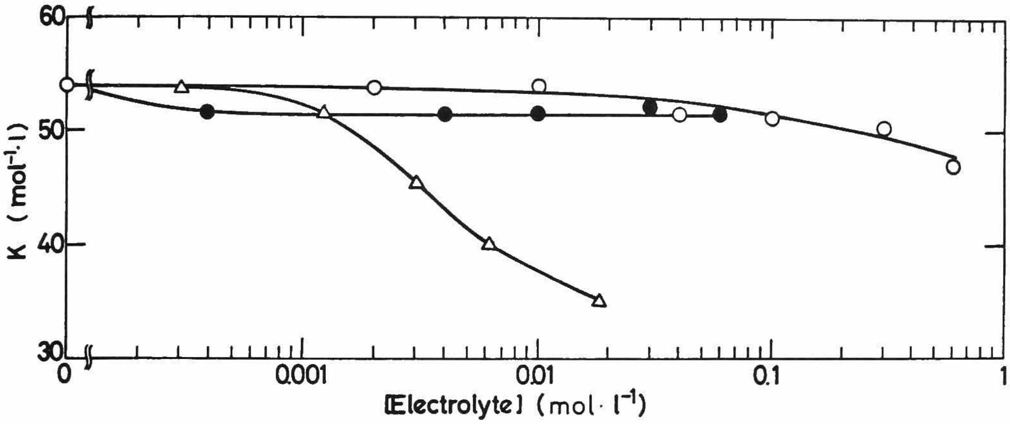

Figure 5 Complex formation of PFMN with tryptophan in the presence of KCl(o), NaPES(●) and DMCS(Δ) at 25°C
[PFMN]=6×10^{-4}mol.1^{-1}, [Tryptophan]=0.01mol.1^{-1}

The K value for the complex formation between PFMN and indoleacetate increased with increasing concentration of KCl, NaPES and DMCS as is shown in Figure 6. These enhanced complexation is

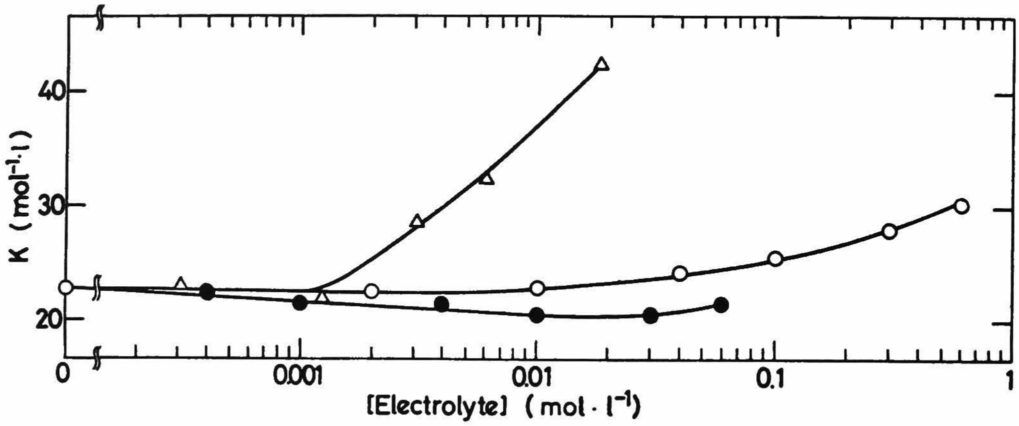

Figure 6 Complex formation of PFMN with indoleacetate in the presence of KCl(o), NaPES(●) and DMCS(Δ) at 25°C
[PFMN]=6×10^{-4}mol.1^{-1}, [Indoleacetate]=0.021mol.1^{-1}

ascribed to electrostatic shielding effect of added electrolytes on the electrostatic repulsive force between similarly charged species, i.e., PFMN macroanions and indoleacetate anions.

On the basis of these results, it is concluded that the charge-transfer complex formations of RPVP or PFMN with various indole derivatives are greatly influenced by electrostatic interactions, and are explained by the "secondary salt effect".

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PART III

CONDUCTANCE-STOPPED FLOW STUDY ON VARIOUS IONIC REACTIONS

Chapter 9

Conductance Stopped-Flow Apparatus and Its Reliability Tests

Introduction

As is well known, almost of all studies on fast reactions by using temperature-jump and stopped-flow methods have been so far restricted to spectrophotometric observation of the time change of concentrations of reactants and/or products. However, there exist a variety of association processes and chemical reactions which cannot be followed by the spectrophotometric detection. It is easily understood that the electrical conductance method is convenient for kinetic study of phenomena between ionic species. The use of the conductance for measuring the rates of fast chemical reactions in solution was first tried by Saal.[1] His technique was basically a flow method and required large amounts of reagents. About twenty years ago, Sirs[2] and Prince[3] constructed a conductance stopped-flow (κ-SF) apparatus and measured the rates of the reaction of carbon dioxide with hydroxide ion in water and of the hydrolysis of triphenylchlorosilane in aqueous acetone. However, no reports have been published on further application of the conductance stopped-flow technique. In this chapter, a conductance stopped-flow apparatus constructed recently is described and some results obtained by this apparatus on several interionic reactions or processes are reported. The main purpose is to compare these results with those independently obtained by UV spectrophotometric methods in order to show the reliability and versatility of the conductance stopped-flow apparatus.

Experimental

Materials

Nickel(II)nitrate (G. R. grade) was purchased from Wako Pure Chemicals, Osaka. A Ni^{2+} solution was prepared by dissolving the $Ni(NO_3)_2$ into deionized and distilled water. The concentration of the solution was determined by the conductometric titration with an EDTA solution. Murexide was a product of Dojindo Co. Ltd., Kumamoto, Japan. The concentration of the dye was determined using a reference value of the molar extinction at 520 nm spectrophotometrically.[4] G.R. grade potassium nitrate was used. The pH of the solution was adjusted by using a dilute HCl solution to 4.0.

Malachite green (MG) of Merck (grade for microscopy and bacteriology) was used without further purification. Potassium cyanide was purchased from Merck (G.R. grade).

4-Acetoxy-3-nitrobenzoic acid (NABA) was prepared by the method of Letsinger et al.[5]

Deionized water obtained with cation- and anion-exchange resins was further distilled for the preparation of solutions. The specific conductance of the purified water was smaller than $5 \times 10^{-7}\ \Omega^{-1}\mathrm{cm}^{-1}$ at 25°.

Spectroscopic grade ethanol was used.

Conductance Stopped-Flow Measurements.

The principle of the κ-SF apparatus was described by Sirs[2] and Prince.[3] Two platinum plates (2 mm × 10 mm) with a 2 mm path length were fixed on inside walls of the observation cell. The observation cell was made of epoxy resin; inside dimension 2 mm × 2 mm × 10 mm. The distance between the mixer and the center of the platinum plate was 7.5 mm. The mixer, which was made of Teflon, was of a four-jet type and its high efficiency of mixing was demonstrated by Hiromi, Nitta and Nagamura[6] for UV-SF measurements. A leading wire from the electrode is shielded by polytrifluorochloroethylene. The dead time of the apparatus was estimated to be as short as that of our spectroscopic stopped--flow apparatus to be mentioned below (namely 1 msec) because the essential structure of the apparatuses (including the mixing device) was the same. For each individual run, 0.2 ml of solution was required at most. A value of 1.30 cm^{-1} was obtained for the cell constant. The block diagram of the instrument is given in Figure 1. An ac current of 50 kHz was given to the Wien bridge.

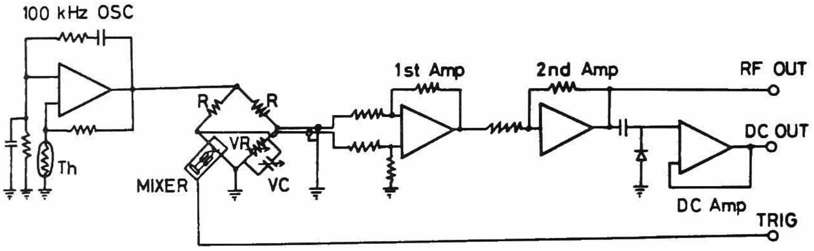


Figure 1 Block diagram of conductance stopped-flow apparatus

The applied voltage across the cell is kept 2 volt r.m.s. The maximum current used was of the order of 1 mA; the average value was 100 μA. The time change of the deviation of the solution conductance from its equilibrium value was amplified in two steps and monitored by using a memoriscope (Hitachi, Model V-018) and/or by a digital memory (Union, Hirakata, Japan) and an X-Y recorder (Riken Co., Tokyo) after rectification. It was ascertained that relaxation times obtained from the conductance deviation were not influenced by the rectification in a range of the relaxation times of 1 msec to 100 sec. Before actual experiments, the electrode system was calibrated with KCl aqueous solution to check the polarization. The polarization effects caused by electrolysis was concluded to be negligible except at high concentrations of electrolyte above 0.1 M, because the equilibrium value of the conductance stayed unchanged for 30 minutes.

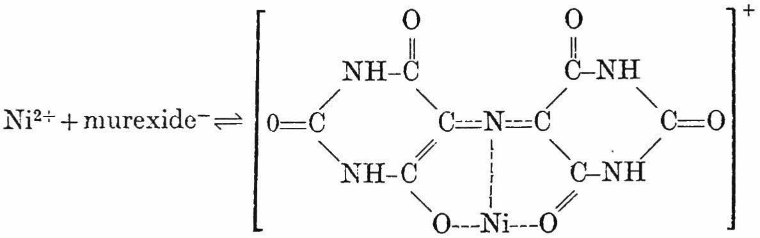
Spectrophotometric Stopped-Flow Measurements

The comparative measurements were carried out using a stopped-flow spectrophotometer (model RA 1100), a product of the Union Engineering, Hirakata, Japan. The details of the apparatus were described elsewhere.[7] The dead time was 1.0 msec by the flow-speed measurements.

Results and Discussion

A. Complexation Reaction of Ni^{2+} with Murexide

The complexation reaction of Ni^{2+} with murexide anion is one of the interionic reactions between oppositely charged ionic species. The conductance of the reacting solution is expected to decrease on complex formation.



The rate constants and the reaction mechanism were thoroughly investigated by some researchers.[8,9] Recently, the rate-retarding influence of polyelectrolytes on this complexation reaction was investigated by using a spectrophotometric stopped-flow method.[10,11]

Clear relaxation traces were obtained after concentration jump by mixing of a $Ni(NO_3)_2$ solution with a murexide solution using the conductance stopped-flow apparatus (see Figure 2). The

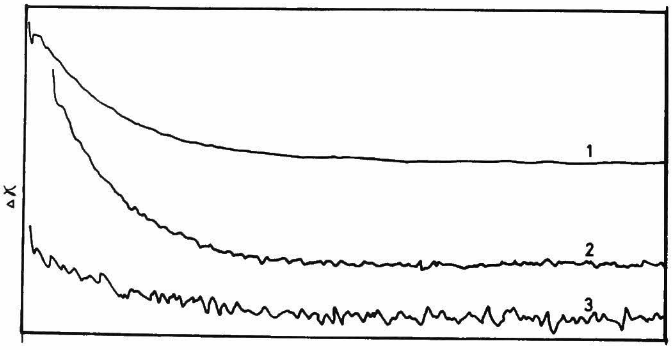


Figure 2 Typical traces of concentration-jump relaxation for Ni^{2+}-murexide reactions at 25°. $[Ni^{2+}]$=3 mM, [murexide]=50 μM, full scale = 500 msec, curve 1: $[KNO_3]$=0 M after mixing, curve 2: $[KNO_3]$=0.01 M, curve 3: $[KNO_3]$=0.1 M

reproducibility of the trace was satisfactory especially for lower concentrations of KNO_3. The total changes of conductance were estimated to be smaller than 2, 0.5 and 0.05 % for curves 1, 2 and 3, respectively.

The relaxation times (τ) obtained for the complexation reaction are listed in Table I, where the literature values of τ determined by the spectrophotometric temperature-jump technique[8c] are also cited. Excellent agreement is noticiable. As is well known, for the temperature-jump measurements, a comparatively large amount of foreign salt (KNO_3 in the present case) must be added to the reaction solutions in order to attain reasonable Joule heat. For the present method, the relaxation data became more reliable at lower concentrations of added salt. It must be noted that the present apparatus is useful even when the concentration of murexide is extremely low (5×10^{-5} M).

Table I Comparison of the Relaxation Times of Ni^{2+}-Murexide Complexation Reaction from the Conductance Stopped-Flow Method with Those from Temperature-Jump Method[a]

$[Ni^{2+}]$	τ (msec)							
	15°C		25°C				36°C	
(M)	κ-SF[d]	T-jump[b]	κ-SF[c]	κ-SF[d]	κ-SF[e]	T-jump[b]	κ-SF[d]	T-jump[b]
0.0005	560	-	220	170	240	-	130	-
0.001	470	420	150	130	140	166	100	83
0.002	230	250	90	78	91	108	58	50
0.003	210	200	65	57	81	83	29	26
0.004	190	160	47	44	58	58	24	-
0.005	130	130	32	40	35	50	22	19
0.007	100	-	26	28	29	-	18	-

a. [Murexide] = 100 μM (15°C. 36°C), 50 μM (25°C)
b. from ref. 8 c, $[KNO_3]$ = 0.1 M, pH = 4
c. $[KNO_3]$ = 0 M, pH = 4
d. $[KNO_3]$ = 0.01 M, pH = 4
e. $[KNO_3]$ = 0.1 M, pH = 4

B. Nucleophilic Reaction of Malachite Green with CN^- or OH^-

The fading reaction is also an interionic reaction between oppositely charged ionic species, and have been investigated in detail by using spectrophotometric method including stopped-flow technique.[12-20]

In aqueous media, the reaction rates of the fading with OH^- or CN^- are slow ($\tau = 10^2$-10^4 sec). Therefore, the rate in the presence of an aprotic solvent such as dimethylformamide (DMF) was tried to measure using the κ-SF apparatus. The second-order rate constants, k_2, from the κ-SF (cross) were compared with those from the UV-SF (open circle) in Figure 3. A reasonable agreement

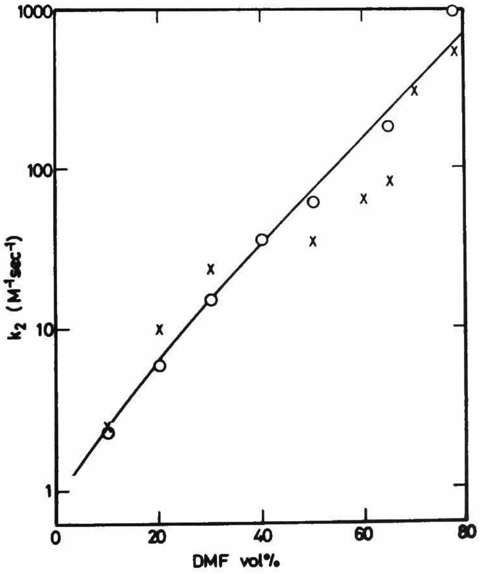


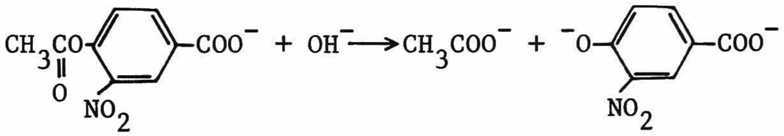
Figure 3 Rate constants of reactions of malachite green with cyanide ion in H_2O + DMF mixtures at 25°. [MG]=10 μM, [CN^-]=10 mM, ○ : UV-SF, × : κ-SF

was obtained. In pure water, the κ-SF apparatus gave less reliable relaxation curves. However, the obtained k_2 was 1.33 M^{-1} sec^{-1} at 25°, which was in close agreement with k_2 = 1.40 $M^{-1}sec^{-1}$ at 25° obtained by Duynstee and Grunwald[13] by the spectrophotometric measurements.

It should be mentioned that no relaxation curve was detected by mixing aqueous alkaline or acid solution (NaOH or HCl < 0.01 M) with pure water, which may indicate that the heat of dilution does not cause serious problems. Richards et al.[21] reported that 1 $mcal \cdot ml^{-1}$ was evolved by dilution of a HCl solution (0.02 M) with pure water to 0.01 M. When equimolar aqueous alkali and acid solutions (0.5 - 10 mM) were mixed, conductance was "observed" to change in the κ-SF measurement. The time order of this trace was 400 msec irrespectively of the concentration (0.5 - 10 mM of HCl and NaOH) and temperatures (10 - 40°C). This may be due to the fact that the solution temperature is raised by exothermic mixing and then heat is released from the solution to the thermostatted cell jacket with that time order, causing temperature lowering and hence conductance change. Such a conductance change was not observed when the concentrations of alkali and acid are smaller than 0.5 mM. According to Richard et al.[22] and Lambert et al.,[23] the heat of neutralization evolved by mixing HCl (0.5 mM) and NaOH (0.5 mM) solutions is 8.8 $mcal \cdot ml^{-1}$. Thus, the artifact caused by the heat of neutralization is safely neglected in our apparatus when the heat evolved or absorbed is smaller than about 9 $\mathrm{mcal \cdot ml^{-1}}$; corresponding to a temperature change of 0.009°C.

C. Alkaline Hydrolysis of an Anionic Ester, 4-Acetoxy-3-nitrobenzoic Acid (NABA)

The alkaline hydrolysis reaction is one of the interionic reactions between similarly charged ionic species.



In connection with the study on polymer catalysis, a variety of hydrolytic reactions have been investigated hitherto.[7,24-34] All of these studies were carried out by spectrophotometric method. For the interionic reactions between similarly charged ionic species, there can be expected only small conductance changes by the reaction, compared with those between oppositely charged species. The alkaline hydrolysis of NABA was tried to follow by the κ-SF method at comparatively high reactant concentrations. The typical traces caused by mixing an NABA solution with an alkali solution are demonstrated in Figure 4. The measurements at 0.1 mM or lower concentrations of NABA were unsuccessful and

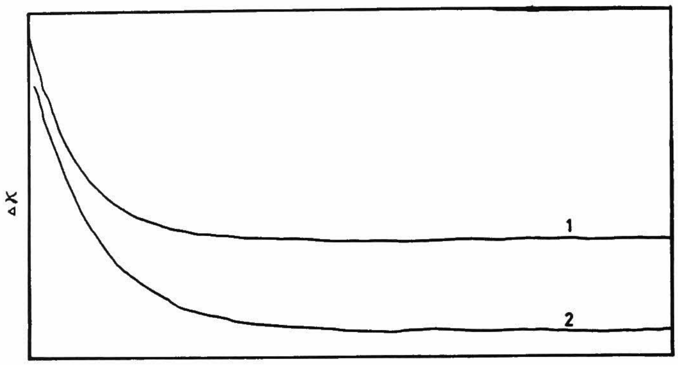


Figure 4 Typical traces of the alkaline hydrolysis of NABA by the κ-SF method. $[OH^-]$=12.5 mM, full scale = 100 sec, curve 1:[NABA]= 1 mM, curve 2: [NABA]=5 mM

direct comparison of the κ-SF method with UV-SF under such a condition was difficult. In Table II, two techniques at high

Table II Comparison of the Relaxation Times, τ, of the Alkaline Hydrolysis of NABA Determined from Conductance Stopped-Flow Method with Those from Spectrophotometric Stopped-Flow Technique at 25°

[NABA]	(mM)	0.1	0.6	1	2	3	5
τ (sec)	κ-SF	-	9.1	8.2	8.2	9.3	10.4
	UV-SF	7.14[a]	8.1	8.5	8.8	9.0	10.2

a. Taken from ref. 31

concentrations are compared. The value of τ at [NABA] = 0.1 mM was taken from our previous work.[31] The agreement between the values from the two methods is gratifying as is seen from the table.

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Chapter 10

Conductance Stopped-Flow Study on the Micellar Equilibria of Ionic Surfactants

Introduction

Recently, two relaxation processes for the micellar equilibria were reported by means of temperature-jump and/or pressure-jump methods around and above the critical micelle concentration (c.m.c).[1,2,3] According to the model proposed, two processes, with relaxation times τ_1 and τ_2 respectively, are shown by the following simplified mechanism, where n is the aggregation number. The fast process (τ_1) has been assigned to the association-dissociation equilibrium of monomeric

$$(n+1)A \underset{k_D}{\overset{k_F}{\rightleftharpoons}} A_n + A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} A_{n+1} \qquad (1)$$

$$\text{(slow)} \qquad\qquad \text{(fast)}$$

ions with micelles, and the slow process (τ_2) to the micellization-dissolution process. The faster process has been measured and discussed by previous authors who used temperature-jump, pressure-

-jump and ultrasonic methods. However, only a few measurements of τ_2 have been reported. This is because the τ_2 process is often too slow to be determined accurately by the methods previously employed. Therefore, application of the stopped-flow method for the measurements of τ_2 seems promising.

When aggregation of ionic surfactants takes place, it is reasonable to suppose that the electrical conductance of the solution would be altered. It is then likely that the kinetics of the micellization-dissolution process could be followed by using the stopped-flow method to measure the changes in conductance.

In this report, we wish to investigate the kinetic aspect of some ionic surfactants by using a conductance stopped-flow apparatus (κ-SF) which was constructed in our laboratory. The use of conductance for measuring the rates of fast chemical reactions in solution was first investigated by Saal.[4] His technique was basically a flow method and required large amounts of reagents. About twenty years ago, Sirs[5] and Prince[6] constructed a conductance stopped-flow apparatus and measured the rates of the reaction of carbon dioxide with hydroxide ion in water and of the hydrolysis of triphenylchlorosilane in aqueous acetone. However, only a few reports have been published on further applications of the conductance stopped-flow technique.[7,8,9]

Experimental

Materials

Sodium dodecyl sulphate (SLS), sodium tetradecyl sulphate (STS) and sodium hexadecyl sulphate (SCS) were purchased from Merck These surfactants were further purified by refluxing with n-hexane for two weeks and recrystallizing three times from a mixture of ethanol and n-butanol. The purity of the surfactants was examined by using gas chromatography to detect the presence of alcohols. The apparatus used was GCG-550T of Yanagimoto Mfg Co., Kyoto. The dimensions of the column were 2.2 m × 3 mm diam. The stationary phase was Chromosorb w with carrier Thermon-1000(0.2 mm diam., products of Wako Pure Chemical Co., Osaka). Helium was used as carrier gas. The column, injection and detector temperatures were 220, 270 and 260°C, respectively. The flow rate and the bridge current were 13 ml min^{-1} and 150 mA. The purities of SLS, STS and SCS were estimated to be 99.8–99.9, 99.8–99.9, and 99.98–99.99 %, respectively. Hexadecyl trimethylammonium bromide (CTABr) was obtained from Nakarai Chemical Co. (Kyoto), and purified by refluxing with n-hexane and recrystallizing from a mixture of water and ethanol.

Phenolphthalein (PP) from Merck (grade for microscopy or bacteriology) was used without further purification. NaCl and

KNO_3 were commercially available guaranteed reagents.

Spectroscopic grade alcohols were used. Deionized water obtained from cation- and anion-exchange resins was further distilled for the preparation of the solutions. The specific conductance of the purified water was smaller than 5×10^{-7} S cm^{-1} at 25°C.

Spectrophotometric stopped-flow measurements

The comparative measurements were carried out with a stopped--flow spectrophotometer (model RA1100), a product of the Union Engineering, Hirakata, Japan. The details of the apparatus are described elsewhere.[10] The dead time was 1.0 ms by the flow-speed measurements.

Surface tension measurements

The surface tensions of aqueous surfactant solutions were measured with a Wilhelmy type surface tensiometer. The details of the apparatus are described by Okubo et al.[11] The values of c.m.c. of SLS, STS, SCS and CTABr were 8.2, 2.1, 0.45 and 1.1 mM at 25°C, respectively.

Results and Discussion

In Figure 1 the typical trace of concentration-jump relaxation

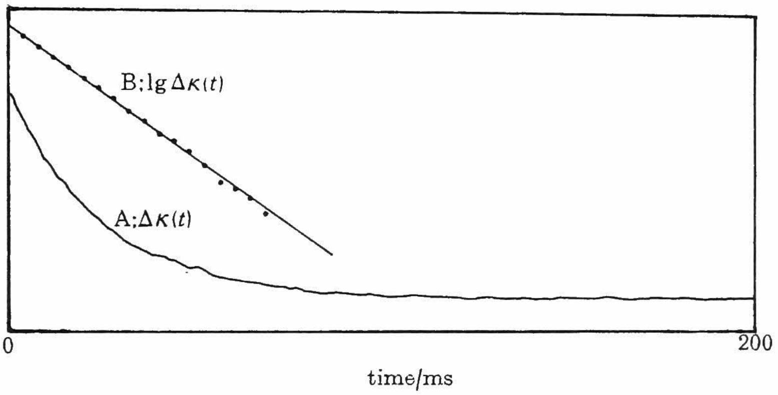

Figure 1 A typical trace of conductance change and plot of lg Δκ against time of SLS in pure water by the κ-SF method. [SLS]=100 mM; T=35°C

effects is shown for sodium dodecyl sulphate, SLS. It was obtained by mixing an aqueous surfactant solution with pure water. The first order plots of lg Δκ against t are also shown in the Figure (curve B). The changes in conductance were roughly estimated to be 10 %. We note that the change of conductance with time was seen to be characterized by a single relaxation time. We further note that this relaxation time was very close to the reported τ_2 values when comparison was possible. Hereafter, therefore, this relaxation time is denoted by τ_2. The other relaxation times ascribable to τ_1, for example, could not be detected. This is reasonable because τ_1 is too small to be observed by the present stopped-flow method.

The plots of τ_2^{-1} against [SLS] for the H_2O + SLS system at various temperatures are given in Figure 2. The literature values

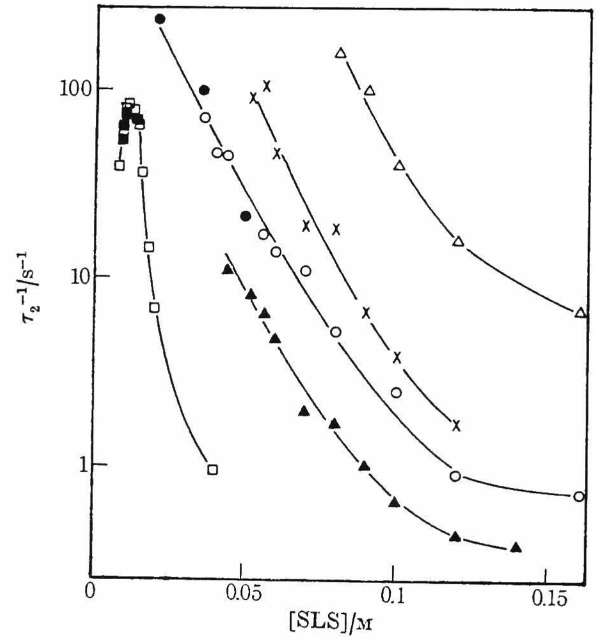

Figure 2 Plots of τ_2^{-1} against [SLS] from the κ-SF measurements on SLS in pure water. Δ, 35°C; ×, 25°C; ●, 20°C; o, 20°C, from ref.3; ▲, 15°C; □, 10°C; ■, 10°C, from ref.1

of τ_2^{-1} of Folger et al.[1] at 10°C and of Aniansson et al.[3] at 20°C are also shown in the Figure. Excellent agreement was obtained. It is clear that the present κ-SF method makes it possible to measure the τ_2 at higher concentrations than was possible with the previous measurements. It was found that τ_2^{-1} decreased sharply with increasing concentration and increased with rising temperature as already reported.[1,2] It should be noted that τ_2^{-1} becomes very small, even smaller than 1 s^{-1}, at high concentrations and at low temperatures. The sharp decrease of τ_2^{-1} with concentration is not inconsistent with the interpretation that the τ_2 process corresponds to the multimode association steps of monomer molecules. Aniansson et al.[3] and Chan et al.[12] tried to interpret the sharp decreases of τ_2^{-1} theoretically. It cannot be denied, however, that the decrease is partly due to the increase of the aggregation number of micelle with increasing concentration, as proposed by Mysels & Princen,[13] and this will be discussed below.

Now, we discuss the artefact which would be caused by the heat evolved on mixing. The time scale for the dissipation of this heat is believed to be around 400 ms as mentioned before. Almost all the τ_2^{-1} values shown in Figure 2 are larger than 1/400 ms^{-1} ($\approx 3s^{-1}$) so that we need not pay much attention to the artefact, except at small values of τ_2^{-1}. The data on the heat of dilution of SLS are not available but we estimated the value of this contribution by using the data for sodium decyl sulphate (SDS)

On mixing a 0.08 M aqueous SDS solution with pure water, 30 Jl^{-1} was reported to be absorbed.[14,15] This value is smaller than the limit which is detectable by the present κ-SF apparatus (namely 37 Jl^{-1}). Thus, we neglect the artefact even when τ_2^{-1} is small.

As is clearly seen in Figure 3, the τ_2^{-1} of SLS was reduced by the addition of NaCl. This may be due to the increase of the aggregation number (n) of the micelle on addition of NaCl.[16-18]

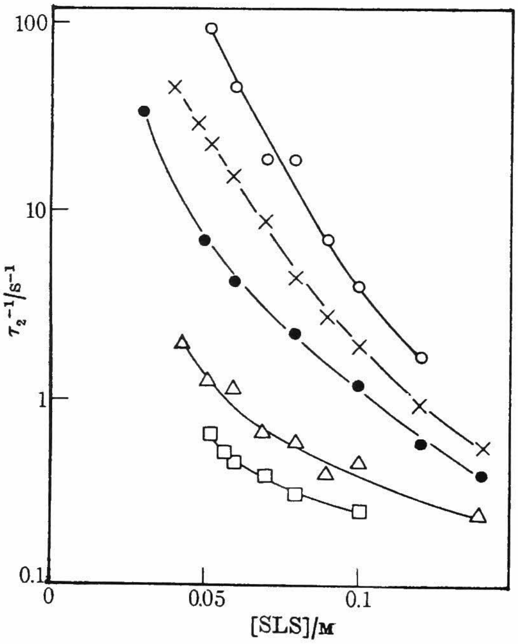

Figure 3 Plots of τ_2^{-1} of SLS against [SLS] in the presence of NaCl at 25°C. ○, [NaCl]=0 M; ×, 2.5 mM; ●, 5 mM; △, 10 mM; □, 15 mM

However, the lowering of the c.m.c. by the salt would also be an important factor.[3]

The τ_2^{-1} values of other anionic surfactants such as sodium tetradecyl sulphate (STS) and sodium hexadecyl sulphate (SCS) were also successfully measured by the κ-SF method in wider ranges of surfactant concentrations than used in the previous studies, and were in good agreement with those obtained earlier.[3,12] The results for STS and SCS are shown in Figures 4 and 5 respectively.

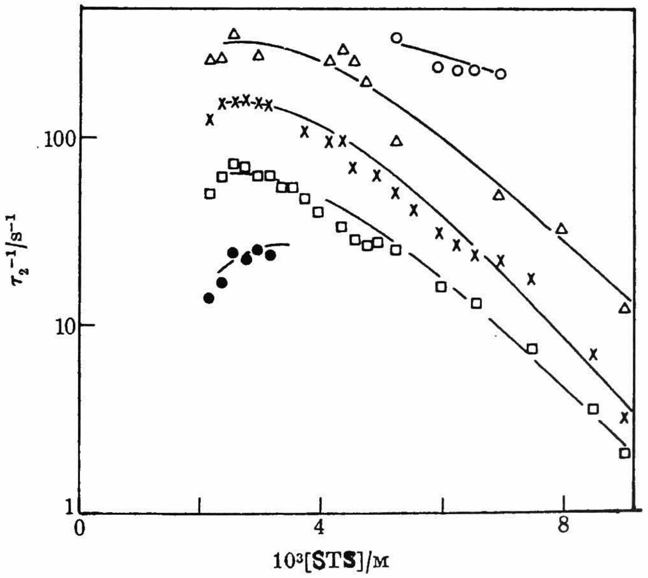

Figure 4 Plots of τ_2^{-1} against [STS] from the κ-SF measurements on STS in pure water. ○, 40°C; △, 35°C; ×, 30°C; □, 25°C; ●, 20°C

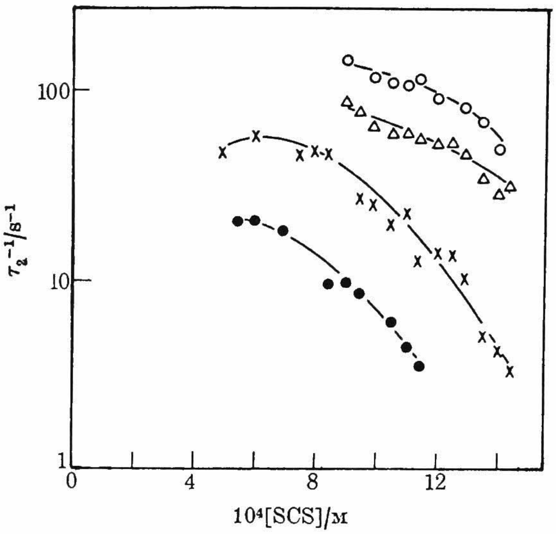

Figure 5 Plots of τ_2^{-1} against [SCS] from the κ-SF measurements on SCS in pure water. ○, 45°C; △, 40°C; ×, 35°C; ●, 30°C

From the comparison of the τ_2 values shown in Figures 2, 4 and 5, τ_2^{-1} was found to decrease in the order SLS > STS > SCS. This may be ascribed to the fact that the aggregation number (n) increases in reverse order, i.e., SLS ($n \approx 64$) < STS ($n \approx 80$) < SCS ($n \approx 100$)

The τ_2^{-1} of a cationic surfactant, hexadecyl trimethylammonium bromide (CTABr), in pure water at various temperatures are given in Figure 6. The values of τ_2^{-1} decreased with concentration, as was the case with SLS. We should note that τ_2^{-1} of CTABr is much smaller than that of SLS. This may be partly due to the aggregation number of the former, n = 79-85[19] being larger than that of the latter, n = 40-70.[16,20,21]

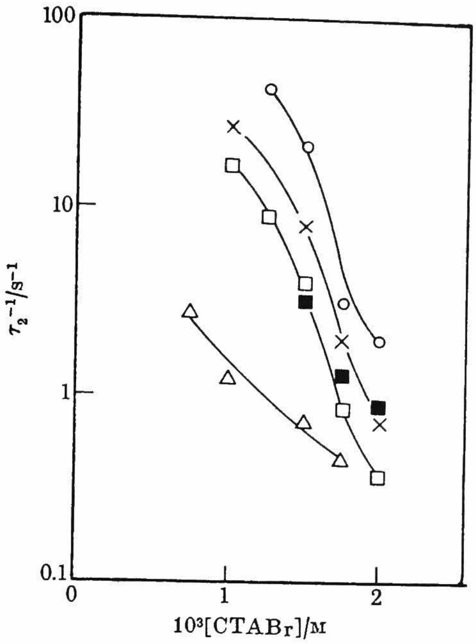

Figure 6 Plots of τ_2^{-1} against [CTABr] from the κ-SF measurements on CTABr in pure water. ○, 40°C; ×, 30°C; □, 25°C; Δ, 20°C

In order to ascertain the relability of the κ-SF data, u.v.-SF measurements were carried out by using phenolphthalein as an indicator in weakly alkaline media. The values of τ_2^{-1} (filled square in Figure 6) obtained from the ultraviolet study agreed satisfactorily with those obtained from the κ-SF method at 25°C.

From the above results the following points may be concluded. (1) The κ-SF method gave clear traces of the micellar relaxation which were ascribed to the τ_2 step. We note that the relaxation data from the κ-SF method can be obtained in a higher concentration range of surfactants than from the temperature-jump and pressure--jump techniques adopted hitherto. (2) The reciprocal relaxation times (τ_2^{-1}) of all ionic surfactants examined, i.e. SLS, STS, SCS and CTABr, decreased sharply with surfactant concentration above c.m.c. Furthermore, τ_2^{-1} increased with increasing temperature. (3) τ_2^{-1} decreased on addition of foreign salts such as NaCl.

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Chapter 11

Summary

In this thesis, the catalytic influences of various polyelectrolytes on various ionic reactions, i.e., hydrolysis reactions charge-transfer complex formation reactions, reactions between $Co(NH_3)_5Br^{2+}$ and OH^- or between crystal violet and OH^- and micelle formation reactions etc., were studied. These studies are summarized here.

In Chapter 1, the outline of these studies on catalytic influences of polyelectrolytes upon various ionic processes was sketched.

In Chapter 2, the alkaline hydrolyses of p-nitrophenyl-3--indoleacetate (p-NPIA) and N-(indole-3-acryloyl)imidazole (IAI) were studied in the presence of poly-4-vinyl-N-propylpyridinium bromide (C3PVP), poly-4-vinyl-N-benzylpyridinium chloride (BzPVP), and copolymer of 4-vinyl-N-benzylpyridinium chloride and 4-vinyl--N-cetylpyridinium bromide (C16BzPVP). The hydrolyses were enhanced by the addition of these cationic polyelectrolytes. The magnitudes of the enhancement were in the order, C16BzPVP > BzPVP > C3PVP, which is explainable in terms of the hydrophobicity of the

polymers. The result and activation parameters obtained indicated that the substrates bound to polymers were more reactive than free substrates. The association constant obtained from the kinetic measurements (K) increased in the order, BzPVP < C3PVP < C16BzPVP. This may suggest important contribution of charge-transfer interaction between the polymers and the substrates besides electrostatic and hydrophobic interactions. The association constant between the polymers and IAI was also obtained independently from the spectrophotometric measurement (K*) using the charge-transfer absorption bands. The agreement between K and K* was not satisfactory, and the reason was discussed.

In Chapter 3, copolymers of N-vinylimidazole (NVIm) and acrylic acid (AA) of various composition and a homopolymer poly-N--vinylimidazole (PNVIm) were synthesized and their catalytic activities on the hydrolysis reactions of neutral p-nitrophenyl caproate (PNPC), cationic 3-acetoxy-N-trimethylanilinium iodide (ANTI) and anionic 3-nitro-4-acetoxybenzoic acid (NABA) were studied. The reaction rate vs. imidazole content in the polymer plots for these esters exhibited different curvatures according to the valency of the esters. This is ascribed to the different affinity between esters and catalytic polymers. The first-order plot for PNPC in the presence of PNVIm gave a slight deviation from linearity at high conversion of the reaction, which is attributed to the product-inhibition. On the other hand, the product-inhibition was not observed for the hydrolysis of PNPC in presence of copolymers, because anionic product was repelled by the the anionic AA residues in the copolymers. The influences of the added model compounds of the reaction products on the reaction rates for PNPC and ANTI were also investigated.

In Chapter 4, the catalytic activities of three kinds of polyethylenimines quaternized with n-octylbromide, n-lauryl bromide and n-cetyl bromide (C8PEI, C12PEI, C16PEI) on the alkaline hydrolysis of p-nitrophenyl acetate (PNPA), 3-nitro-4--acetoxy-benzoic acid (NABA) and 3-acetoxy-N-trimethylanilinium iodide (ANTI) were studied in n-hexanol-water mixtures. The reactivity of OH^- was found to be enhanced in the mixtures with decreasing water content. Furthermore, the remarkable role of the solvation effect of the polymers was shown; the polymers absorbed water molecules strongly and selectively from the mixture of n--hexanol-water. These solvation and desolvation effects were also studied by the fluorescence and light-scattering measurements.

In Chapter 5, anionic latices, copolymers of styrene (St) and acrylic acid (AA), were synthesized, and then "catalytic" influences on interionic reactions between oppositely charged species, i.e., reaction between $Co(NH_3)_5Br^{2+}$ and OH^- and that between crystal violet (CV) and OH^-, were studied. The latices retarded the reactions. For the former reaction, latices of higher AA content had greater retarding effect, indicating that electrostatic interaction is important. For the latter reaction, on the other hand, latices of higher AA content had smaller retarding effect, which implies that the hydrophobic interaction of latices with reactant ions plays a more important role than the electrostatic interaction. The thermodynamic parameters were derived and discussed in terms of these interactions. The "catalytic" influences of latices and a linear polymer (polyacrylic acid) were also compared.

In Chapter 6, the charge-transfer complex formation of analogs of nicotinamide adenine dinucleotide (NAD^+), namely, 3--carbamoylpyridinium compounds quaternized with chloroethylated adenine (NAA), thymine and theophylline, with indole derivatives were studied in the presence of simple- and poly-electrolytes and a surfactant, and in their absence. The indoles are indoleacetate, indolepropionate, indolebutyrate, L-tryptophan and tryptamine. The association constants, K, for the model compounds-indoleacetate systems were 10 - 13 M^{-1} at 25°, which were larger than those of NAD^+-indoleacetate system. The polyelectrolytes were sodium poly(ethylenesulfonate), sodium-poly(styrenesulfonate) and a copolymer of diethyldiallylammonium chloride and sulfur dioxide. The surfactant was cetyltrimethylammonium bromide. All the electrolytes decreased the association constants between the model compounds and indoleacetate, and increased those between the model compounds and tryptamine. The charge-transfer complex formation of the model compounds with L-tryptophan was not influenced with addition of simple- and poly-electrolytes. These effects were discussed theoretically in terms of the secondary salt effect using the Debye-Hückel theory and Manning's theory on polyelectrolytes. The thermodynamic quantities of the complex formation between NAA and indoleacetate, i.e., the free energy (ΔG), enthalpy (ΔH) and entropy (ΔS) were derived. ΔH and ΔS were found to decrease by addition of salts, which was also successfully explained theoretically by using the Deby-Hückel theory and the Manning theory.

In Chapter 7, the complex formations of flavin monomucleotide (FMN) with tryptamine, indoleacetate and tryptophan were investigated in the presence of polyelectrolytes, i.e., sodium polyethylene sulfonate (NaPES), sodium polystyrene sulfonate (NaPSS) and a copolymer of diethyldiallylammonium chloride and sulfur dioxide (DECS).

The complexation of FMN and tryptamine was strongly retarded by the macrocations and macroanions, whereas that of FMN and indoleacetate was enhanced by the macrocations. Furthermore, the equilibrium constants of FMN-tryptophan complex were insensitive to the addition of polyelectrolytes. These results suggest that the complexation of FMN was strongly influenced by the electrostatic interactions between the reactant ions and macroions in addition to those between the reactant ions, in conformity with the secondary salt effect.

In Chapter 8, charge-transfer type complex formations between poly-4-vinyl-N-propylpyridinium bromide (C3PVP), poly-4-vinyl-N--butylpyridinium bromide (C4PVP) or poly-4-vinyl-N-benzylpyridinium chloride (BzPVP) and indole derivatives or between polymer containing flavin mononucleotide residues (PFMN) and indole derivatives were studied in the presence of foreign salts of simple and polyelectrolytes. The association constant (K) of the complex formation of RPVP with indoleacetate increased in the order : BzPVP > C4PVP > C3PVP, which indicated the important contribution of hydrophobic force. The addition of simple and polyelectrolytes caused the decrease of the association constants. This was explained by the "secondary salt effect" of the salts on interionic reactions. The importance of the electrostatic interactions in the complexation systems was demonstrated. The retarding action of simple and polyelectrolytes on the K values was discussed theoretically with the aid of Debye-Hückel and Manning theory, respectively.

In Chapter 9, a conductance stopped-flow apparatus was constructed and used for study or rapid ionic associations and chemical reactions in solution. Comparison was made with established ultraviolet stopped-flow or relaxation methods for a complexation reaction of Ni^{2+} with murexide, a nucleophilic reaction of malachite green with CN^- or OH^-, an alkaline hydrolysis of 4-acetoxy-3-nitrobenzoic acid. Excellent agreement was obtained between the conductance and ultraviolet methods, when comparison is possible. An artifact due to the heat of neutralization by mixing acid and alkaline solutions has been discussed.

In Chapter 10, rapid ionic equilibria of solutions of cationic and anionic surfactants were studied by the concentration-jump method with the use of the conductance stopped-flow technique. The slow relaxation times (τ_2) assigned to the micellization--dissolution process were obtained from the traces of the conductance changes in a much wider concentration range than in the previous methods. The τ_2 of sodium dodecyl sulphate (SLS) observed coincided fairly well with those obtained from the temperature-jump technique by Folger et al. (1974) and by Aniansson et al. (1976), when comparison was possible. In a wide concentration range above c.m.c. τ_2^{-1} of SLS sharply decreased with increasing concentration, increased with temperature, and decreased with addition of foreign salts. The τ_2^{-1} of sodium tetradecyl sulphate (STS) and sodium hexadecyl sulphate (SCS) showed similar trends. The magnitudes of the τ_2^{-1} were in the order SLS > STS > SCS. The τ_2^{-1} of hexadecyl trimethylammonium bromide solution, which was much smaller than those of the anionic micelles studied, also showed similar dependence on concentration (above c.m.c.) and temperature.

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Chapter 5	J. Phys. Chem., in Press
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