### References and Notes

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# Catalytic Activity of Poly(4(5)-vinylimidazole-*co*-acrylic acid) in the Hydrolyses of 3-Acetoxy-*N*-trimethylanilinium Iodide and *p*-Nitrophenyl Acetate in Aqueous Solution

**Takeo Shimidzu,* Akihiro Furuta, and Yukimasa Nakamoto**

*Department of Hydrocarbon Chemistry, Faculty of Engineering, Kyoto University, Kyoto 606, Japan.* *Received May 23, 1973*

ABSTRACT: The catalytic activity of poly(4(5)-vinylimidazole-*co*-acrylic acid) (copoly(VI-AA)) in hydrolyses of 3-acetoxy-*N*-trimethylanilinium iodide (ANTI) and *p*-nitrophenyl acetate (PNPA) in an aqueous solution containing no metal ion has been studied. The copolymer had random sequence. The neutral imidazole fraction as well as the carboxylate fraction in the copolymer were estimated by potentiometric titrations. Both fractions increased with VI content in the copolymer, even in the same pH solution. The hydrolysis of ANTI followed the Michaelis-Menten-type kinetics, and that of PNPA followed the second-order kinetics. Though profiles of total hydrolyis rates for ANTI and PNPA were different, rates of hydrolytic step gave similar profiles; that is, the rates decreased sharply with the VI content in the copolymer in which the VI content was lower than 30 mol %. The effective interaction of ANTI on the copolymer appeared at 30 mol % VI content in the copolymer. The slope in the Brönsted plots with the present reaction was steeper than that obtained in the nucleophilic hydrolysis by Bruice. The molar extinction coefficients of the imidazole moiety in the copolymers also changed decreasingly with VI content in the copolymers. Those results lead to a tentative scheme of the cooperation between imidazole and carboxylic acid moieties. A carboxylic acid moiety not only changes the fraction of the neutral imidazole moiety in the copolymer, but also the nucleophilicity of the imidazole moiety. Such a multiple effect can be considered as the special province of the polymer catalyst.

As a model of catalysis by α-chymotrypsin, many esterolytic catalyses by copolymers containing imidazole or the other nucleophile have been studied.[1-19]

Generally speaking, the role of enzyme consists of the attraction of substrate and the catalysis. On this point, copolymer of 4(5)-vinylimidazole (VI) and acrylic acid (AA) can be thought to be one of the model of the enzyme, because it has an electrostatic attractive moiety and a catalytic moiety. The hydrolysis reaction of a positively charged substrate, of which charge is opposite in sign to the copolymer, will realize a model reaction system. The farseeing study of the catalytic reaction using copoly(VI-AA) has been made by Overberger and Maki.[7] However, the above-mentioned two roles had not been separated. In the present study, to simplify the reaction condition, an aqueous solution containing no metal ion was adopted as a reaction medium. As a result, the above-mentioned two roles in the hydrolysis have been separated. Additionally, an interesting result with respect to an cooperative action between imidazolyl and carboxyl groups in the copoly(VI-AA) is found in the present study.

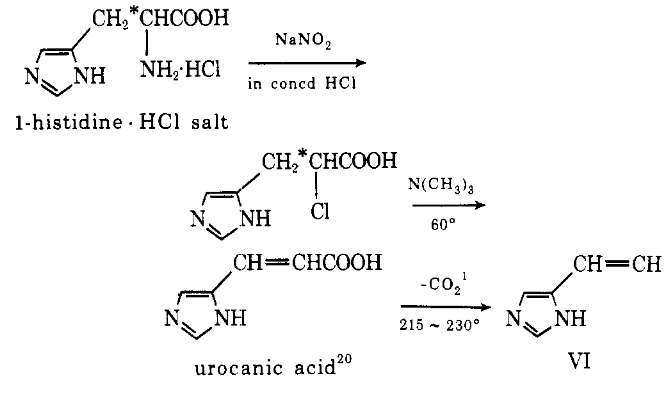
## Experimental Section

**Substrate.** ANTI was synthetized by the method of Overberger *et al.*,[5] and was purified by recrystallization in nitromethane, twice, mp 210.2-212.0° (lit.[5] mp 209-210°). PNPA was purchased from Tokyo Kasei Ltd., and was purified in the usual manner.

**Copoly(VI-AA).** Copoly(VI-AA)'s with various copolymer compositions were obtained by radical copolymerization of 4(5)-vinylimidazole (VI) and acrylic acid (AA).

Compound VI was synthetized *via* decarbonation of urocanic acid as shown in Scheme I. The decarbonation was achieved under the pressure of 2-3 mm, and the product, 4(5)-vinylimidazole, was distilled immediately at the same condition. The copolymerization was achieved as follows. Monomers were placed in an ampoule with methanol, where the concentration of the monomer in the solution was about 17 mol %. AIBN (0.01 mol %) (for monomer) was added to the solution. The ampoule was sealed under nitrogen and the polymerization was carried out at 70° with vigorous shaking agitation. A copolymer was precipitated with

Scheme I



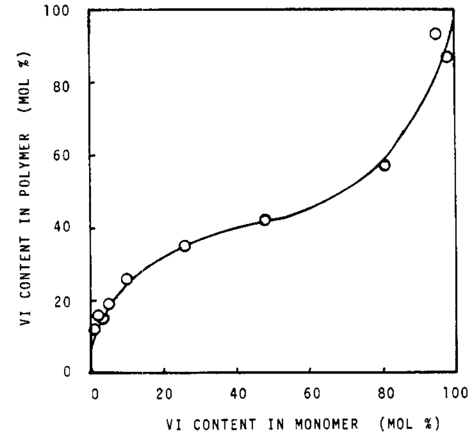


**Figure 1.** Feed and copolymer composition relationship in the copolymerization of 4(5)-vinylimidazole and acrylic acid.

acetone and was washed acetone-methanol, then dried under a reduced pressure. The method of purification of the copolymer by the dialysis with a dilute ammonia solution is not recommended because of the unreliability in its salt form. Composition of the copolymer was determined by the content of carbon and nitrogen in the elemental analysis. Results are summarized in Table I. Figure 1 shows monomer and polymer composition in the copolymerization. From Figure 1, monomer reactivity ratio was estimated as $\gamma_1 = 0.25$ and $\gamma_2 = 0.20$ (suffix 1 shows VI and suffix 2 does AA).

**Esterification of the Carboxylic Acid in the Copolymer and Nmr Measurement of the Esterified Copolymer.** The esterification of the carboxylic acid in the copolymer was accomplished by the treatment of excess of the diazomethane solution with the copolymer, overnight. The diazomethane in ether solution was prepared by the procedure of DeBoer *et al.*[21] An esterified copolymer was washed with ether and dried *in vacuo.*

220-MHz nmr spectrum of the esterified copolymer was measured in $CD_3OD$, using $Me_4Si$ as an internal standard. The copolymer of VI-19 was slightly soluble in $CD_3OD$, so that the measurement of nmr was achieved in suspension.

**Measurement of Hydrolysis Rate.** A polymer solution was prepared by dissolving the copolymer in 0.02 $M$ tris(hydroxymethyl)aminomethane·HCl solution (pH 9.0 ~ 9.1) in which the concentration of carboxylic acid moiety of the copolymer was adjusted to be $(5 \pm 0.3) \times 10^{-4}$ mol/l. Aqueous solution of ANTI was prepared to be $1.02 \times 10^{-2} \sim 5.00 \times 10^{-2}$ mol/l. and alcoholic solution of PNPA was prepared to be $2.06 \times 10^{-3}$ mol/l.

Measurement of the hydrolysis rate was achieved using Beckman DB-G spectrophotometer. The copolymer solution (3 ml) was placed in the cell of 1-cm optical path and the cell was allowed to stand for 30 min, in a temperature-controlled chamber. Then, 0.2 ml of the ANTI or the PNPA solution was added and mixed immediately. The change in optical density was followed. The wavelength of 293 nm for the hydrolysis of ANTI and that of 400 nm for the hydrolysis of PNPA were adopted.

Initial hydrolysis rate, $V_0$, was obtained by an linear extrapolation of the rate at 10% conversion. The rate of the hydrolysis by the copolymer, $V_{cat}$, was defined as $V_{cat} = V_0 - V_{0,blank}$, where $V_{0,blank}$ shows the rate without the copolymer. The hydrolysis of PNPA in the presence of the copolymer obeyed good second-order kinetics. Accordingly, the rate of the hydrolysis was expressed as

**Table I**
**Results of Copolymerization of 4(5)-Vinylimidazole (VI) and Acrylic Acid (AA)**

| Copolymer | Feed VI (mol %) | Polym Time (hr) | Conv (wt %) | Found VI (mol %) |
|---|---|---|---|---|
| VI-12 | 1 | 1.2 | 1 | 12 |
| VI-15 | 3.5 | 4.7 | 2 | 15 |
| VI-16 | 2 | 2.3 | 4 | 16 |
| VI-19 | 5 | 0.8 | 20 | 19 |
| VI-26 | 10 | 0.8 | 19 | 26 |
| VI-35 | 26 | 0.8 | 8 | 35 |
| VI-42 | 48 | 1.6 | 11 | 42 |
| VI-57 | 81 | 4.9 | 15 | 57 |
| VI-87[a] | 98 | 6.6 | 30 | 87 |
| VI-93[a] | 95 | 5.7 | 21 | 93 |

[a] The copolymer was soluble in methanol, so that it was purified by washing with acetone.

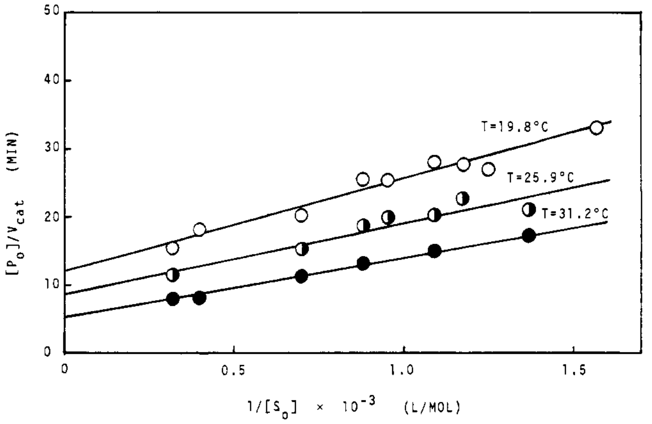


**Figure 2.** Lineweaver-Burk plots for the imidazole-acrylic acid copolymer (VI content, 26 mol %) catalyzed hydrolyses of ANTI.

$$V_{cat} = k[P_0][S_0]$$

where $[P_0]$ is the concentration of the imidazolyl group in the copolymer and $[S_0]$ is the initial concentration of the substrate, PNPA. While, the hydrolysis of ANTI showed a saturation with respect to the substrate. And an approximately linear relationship in Lineweaver-Burk plot was obtained. Then, a Michaelis-Menten kinetics[22] can be applied. The hydrolysis can be presented as

$$P + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} PS \xrightarrow{k_3} P + R$$

where P, S, PS, and R show catalytic moiety in the copolymer, the substrate, complex of the catalytic moiety and the substrate, and hydrolyzed product, respectively. The rate of the hydrolysis is expressed as

$$V_{cat} = k_3[P_0][S_0]/(K_m + [S_0])$$

where $K_m$ is the Michaelis-Menten constant which is identical with $(k_2 + k_3)/k_1$, $[P_0]$ is the concentration of imidazolyl group in the copolymer and $[S_0]$ is the initial concentration of the substrate, ANTI. Figure 2 shows an example of the Lineweaver-Burk plot in the case of the hydrolysis of ANTI by the copolymer VI-26. The rate of the hydrolysis, $k_3$, and Michaelis-Menten constant, $K_m$, were obtained from the intercept and the slope in the Lineweaver-Burk plot, respectively.

**Potentiometric Titration of Copoly(VI-AA) Solution.** The copolymers except VI-93 were dissolved in 0.01373 $N$ NaOH aqueous solution. The copolymer VI-93 was dissolved in 0.01373 $N$ HCl aqueous solution. The concentrations of the solutions were made up $(1.19 \pm 0.10) \times 10^{-2}$ mol/l.[23] The solution was titrated with 0.2 $N$ HCl solution (0.2 $N$ NaOH solution for VI-93) and the pH was measured. Figure 3 shows a typical titration curve. For the copolymer containing less imidazolyl group, the titration curve was separable for the carboxylate group and for the imidazolyl group, as can be seen in curve 1 in Figure 3. While, for that containing more imidazolyl group (over 50 mol %), the titration curve was

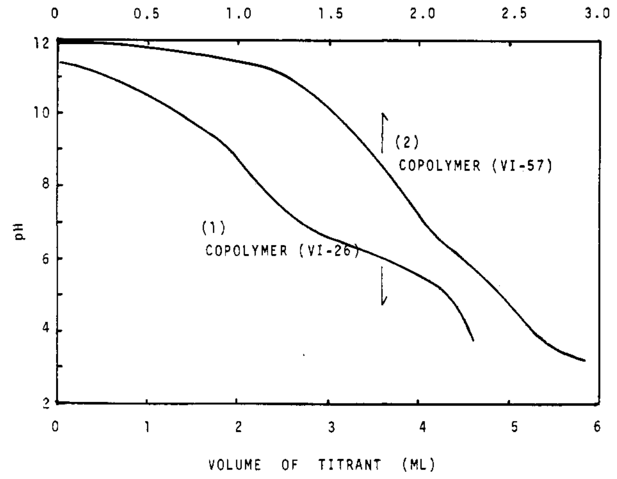


Figure 3. Titration curves of the copolymer.

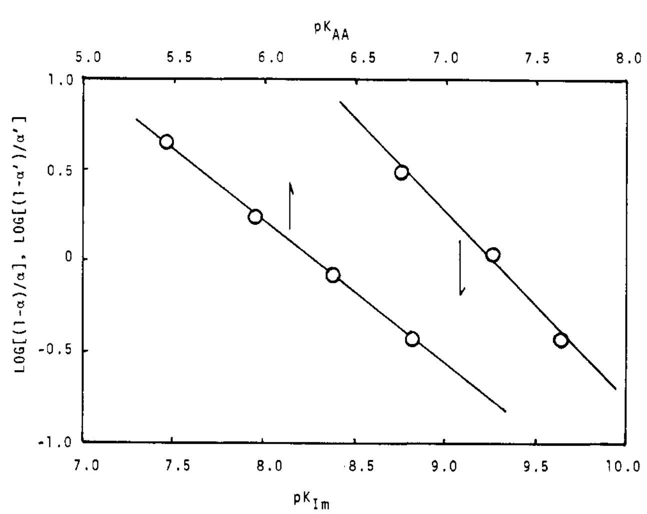


Figure 4. Modified Henderson-Hasselbach plots for carboxyl group and imidazolyl group (the copolymer, VI-26).

not separated for the carboxylate and the imidazolyl groups as is seen in curve 2 in the figure. In the former case, the separation can be made at the inflection point, and we estimated p$K$ and $n$ values of the imidazolyl and carboxyl groups using the modified Henderson-Hasselbach equation such as

$$\mathrm{pH} = \mathrm{p}K - n \log[(1 - \alpha)/\alpha]$$

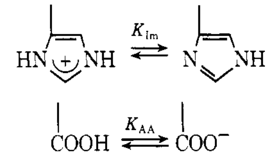
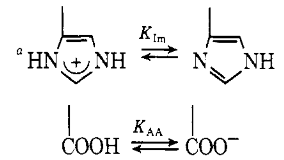


Figure 4 shows the modified Henderson-Hasselbach plots for the imidazolyl and carboxyl groups. And the results of the potentiometric titrations were summarized in Table II.

**Molar Extinction Coefficient of the Imidazole Moiety in the Copolymer.** From the uv spectrum of the copolymer solution in the range of 212-220 nm at 26°, the molar extinction coefficient of the imidazole moiety was estimated. The molar extinction coefficients of a protonated imidazole ($\epsilon_1$) and of a neutral imidazole ($\epsilon_2$) were obtained from their optical densities ($A_1$, $A_2$) at which they reached a constant value by adding the excess of 1 $N$ HCl and by adding the excess of 0.05 $N$ NaOH, respectively, taking into account the volume change in the titration.[24] Absorbancy caused by the carboxylic acid moiety can be neglected. At both conditions of pH values below 1.0 (more than 0.2 ml of 1 $N$ HCl added) and over 10.5 (more than 0.8 ml of 0.05 $N$ NaOH added), the optical densities of copolymer solutions became a constant, respectively. Both molar extinction coefficients ($\epsilon_1$, $\epsilon_2$) decreased with the imidazole content in the copolymer. In addition, the fraction of the neutral imidazolyl group in each copolymer at pH 9.0 was obtained, using $A_1$ and $A_2$ values. Results are tabulated in Table III.

Table II
Result of Potentiometric Titration[a]

| Copolym | VI % | p$K_{\mathrm{Im}}$ | $n_{\mathrm{Im}}$ | p$K_{\mathrm{AA}}$ | $n_{\mathrm{AA}}$ |
|---|---|---|---|---|---|
| VI-12 | 12 | (9.40) | | 6.05 | 1.46 |
| VI-19 | 19 | 9.36 | 1.00 | 6.18 | 1.48 |
| VI-26 | 26 | 9.27 | 1.00 | 6.28 | 1.28 |
| VI-35 | 35 | 9.10 | 1.28 | 6.33 | 1.02 |
| VI-42[b] | 42 | 8.92 | 1.78 | 6.14 | 1.00 |
| VI-93 | 93 | 5.12 | 2.36 | | |



p$K_{\mathrm{Im}}$ and $n_{\mathrm{Im}}$ values of VI-12 and p$K_{\mathrm{AA}}$ and $n_{\mathrm{AA}}$ values of VI-93 were not determined, for their very small amount of titration volume in log $[(1 - \alpha)/\alpha]$ *vs.* pH plot, respectively. [b] The titration curve was not separated for carboxyl and the imidazolyl groups.

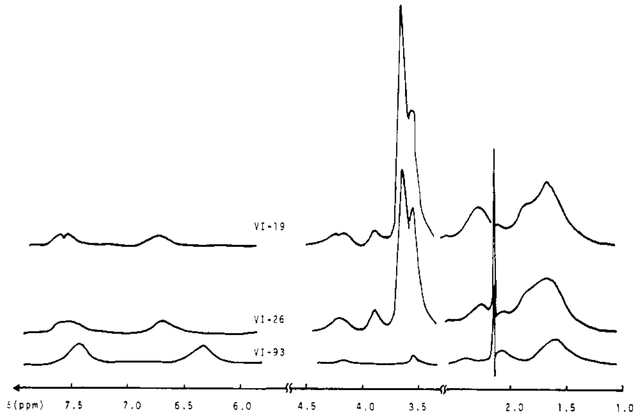


Figure 5. Nmr spectra of esterified copolymers with methyl group.

## Results and Discussion

**Characterization of the Copolymer by 220-MHz Nmr Spectrum.** Figure 5 shows the 220-MHz nmr spectra of the copolymers, VI-19, VI-26, and VI-93. The assignment of signals is tabulated in Table IV.

Signals of two protons of the imidazole showed upfield shift in the spectrum of the copolymer containing higher imidazole moiety. This result can be explained by a consideration that the stacked imidazole moiety is inducing an opposite magnetic field.

Methyl group in the esterified copolymer showed two different peaks (δ in ppm) both in the vicinity of δ 3.64 ~ 3.66 and 3.54 ~3.56. From the result that the copolymer VI-93 (the copolymer containing higher imidazole moiety) did not show the signal at the vicinity of δ 3.54 ~ 3.56, signals in vicinities of δ 3.64 ~ 3.66 and 3.54 ~ 3.56 were assigned to the AA-AA-AA triad and the AA-VI dyad, respectively.

Though two signals of the methyl group were overlapped by each other, the peak ratio was estimated from their half-widths. Their ratios were compared with ratios which were obtained from monomer reactivity ratios by Bovey's method.[25] The result is shown in Table V. As the ratios resulted by two methods agreed roughly, it can be thought that the copolymers were random ones.

**Interaction of ANTI with Copoly(VI-AA).** The rate of hydrolysis of ANTI by copoly(VI-AA) had a tendency of the saturation at a high substrate concentration, and differed from that of PNPA. This suggests that there is an interaction between the substrate, ANTI, and the carbox-

### Table III
### Optical Densities ($A$), Molar Extinction Coefficient ($\epsilon$), and Fraction of the Neutral Imidazolyl Group in the Copolymer at pH 9.0

| Copolymer | $A_1{}^c$ ($\lambda_{max}$), nm | $\epsilon_1$ | $A_2{}^d$ ($\lambda_{max}$), nm | $\epsilon_2$ | pH 9 $A$ | pH 9 $\alpha^e$ |
|---|---|---|---|---|---|---|
| VI-12[a] | 0.417 (214) | 5760 | 0.305 (212) | 4210 | 0.398 | 0.17 |
| VI-19[a] | 0.599 | 5080 | 0.429 | 3640 | 0.522 | 0.45 |
| VI-26[a] | 0.867 (214) | 5040 | 0.648 (215) | 3770 | 0.790 | 0.35 |
| VI-35[b] | 0.790 | 4540 | 0.546 | 3140 | 0.675 | 0.47 |
| VI-42[a] | 1.50 (216) | 4240 | 1.15 (220) | 3250 | 1.27 | 0.66 |
| VI-57[b] | | | 1.81 | 3950 | 1.78 | 1.00 |

[a] Concentrations were (4.9 ~ 5.3) × $10^{-4}$ mol/l. for –COOH in the copolymer. [b] Concentrations were (3.2 ~ 3.5) × $10^{-4}$ mol/l. for –COOH in the copolymer. [c] Optical densities at which all of the imidazole moiety in the copolymer were protonated. [d] Optical densities at which all of the imidazole moiety in the copolymer were neutral. [e] $\alpha$ shows the dissociation of the protonated imidazole moiety.

### Table IV
### Assignment of Signals in Proton Magnetic Resonance of the Copoly(VI-AA)

| $\delta$ (ppm) | Assignment |
|---|---|
| 1.0–2.5 | –CH, –$CH_2$ in polymer chain |
| ~3.55 | $COOCH_3$ adjacent to imidazole moiety |
| ~3.65 | $COOCH_3$ not adjacent to imidazole moiety |
| 6.3–6.8 | 4(5)-H in imidazole moiety |
| 7.4–7.6 | 2-H in imidazole moeity |

### Table V
### Ratio of Sequences of -AA-AA-AA- Residue and -AA-VI- Residue

| Copolymer | (-AA-AA-AA-) : (-AA-VI-) Nmr | (-AA-AA-AA-) : (-AA-VI-) Bovey |
|---|---|---|
| VI-19 | 1.3:1.0 | 1.7:1.0 |
| VI-26 | 1.0:1.2 | 1.0:1.4 |

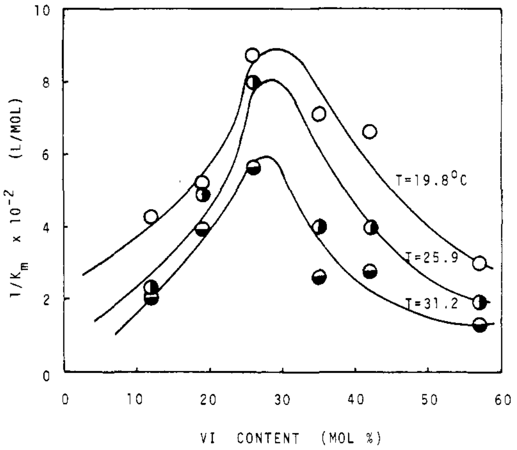


**Figure 6.** Plots of $1/K_m$ against copolymer composition (pH 9.0).

ylate anion in the copolymer. The interaction, which Overberger and Maki had not obviously found in the presence of salt in the reaction solution,[7] was taken into account in the salt free solution. The reciprocal value of the above-mentioned Michaelis-Menten constant, $1/K_m$, is a measure of the magnitude of the effective interaction for the hydrolysis. Figure 6 shows the relation between the copolymer composition and $1/K_m$.

A value of the effective interaction for the hydrolysis, $1/K_m$ was not proportional to the amount of carboxylic acid moiety in the copolymer, and gave the optimum at about 30 mol % of VI content in the copolymer. The most preferable carboxylic acid content in sequence of the copolymer for the hydrolysis reaction is about 70 mol %. This result shows that the effective interaction for the hydrolysis is affected by the AA adjacent to the imidazolyl group in the copolymer.

The effective interaction value decreased with temperature.

**Rates of Hydrolysis of ANTI, $k_3$, and That of PNPA, $k$.** The rates of hydrolysis of ANTI, $k_3$, and that of PNPA, $k$, against the copolymer composition are shown in Figure 7. In both cases of ANTI and PNPA, the rates of hydrolyses decreased monotonously with the imidazole content in the range of VI content up to about 40 mol %, and it increased in the range of that over about 40 mol %.

It is very interesting result that the rate of the hydrolysis increased with AA content in the range of VI content below 40 mol %. It might be suggested that there is a cooperative action between the imidazole and the carboxylic acid moieties. The discussion concerning the cooperation will be made later. The increase in the rate in the range of VI content higher than 40 mol % is explained as increase in the neutral imidazole moiety and a cooperation of contiguous imidazole moieties.[2,4] Formerly, Overberger *et al.* discussed that cooperative action of Im–COOH and that of Im–Im from over-all kinetics.

It is noteworthy result that the dependence of $k$ for the copolymer composition differed from what Overberger and Maki had obtained as a total rate of hydrolysis.[7] This difference might be attributed to the estimation of the copolymer composition or the nature of the reaction solution.

**Cooperative Action between Imidazole and Carboxylic Acid Moieties.** The above-mentioned rate was determined for the total imidazole moiety in the copolymer. Usually, the hydrolysis is achieved by the neutral imidazole predominantly. The neutral imidazole fraction, $\alpha$, which was determined by the titration curve of the copolymer at the present reaction condition (pH 9.0), is plotted against the copolymer composition in Figure 8. The neutral imidazole fraction increased with the imidazole content in the copolymer in spite of the same pH condition.

Figure 9 shows the relationship between the hydrolysis rate for the neutral imidazole group in the copolymer and the imidazole content in the copolymer. Comparing with Figure 7, a steep rise in the rate in the condition of lower neutral imidazole content can be seen.

Figure 10 shows the Brönsted plots, the relationship between the logarithm of the rate and p$K$ value of the imidazole, p$K_{Im}$, which was obtained by the potentiometric titration. In the various copolymers, of which VI contents are less than 40 mol %, p$K_{Im}$ value increased with AA content in the copolymer. In the case of the hydrolysis of PNPA, the neutral substrate, the slope of the curve was steeper than that for the nucleophilic hydrolysis of PNPA that Bruice had obtained formerly (broken line).[26] This

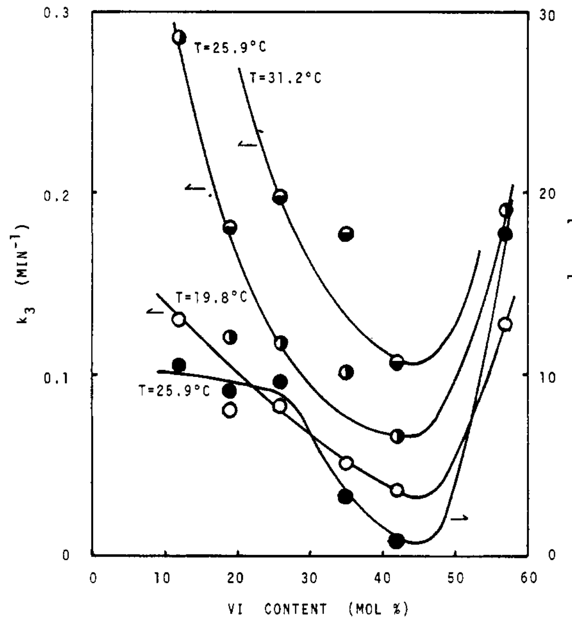


Figure 7. Plots of $k_3$ and $k$ against copolymer composition: (○ ◑ ◒) ANTI (pH 9.0); (○) PNPA (pH 9.1).

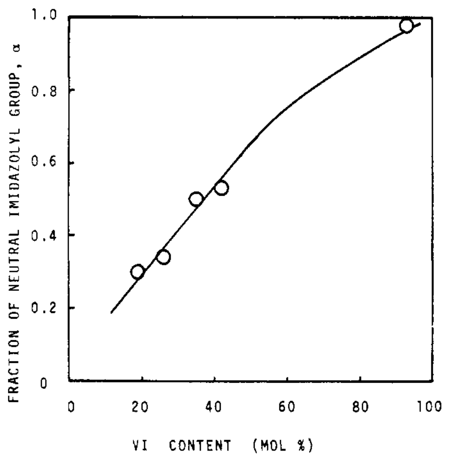


Figure 8. Plots of fraction of neutral imidazolyl group against copolymer composition.

result leads to two interpretations; that the nucleophilic activity of imidazole moiety changes with the copolymer composition and that a cooperative action between imidazole and carboxylic acid moieties takes place. For the cationic substrate, ANTI, a similar tendency can be seen.

Figure 11 shows the molar extinction coefficient for the imidazole moiety in the copolymer at 212–220 nm against the neutral imidazole fraction, $\alpha$. The copolymer containing the lower imidazole fraction gave the higher extinction coefficient, and the extinction coefficient decreased linearly with the fraction of the neutral imidazole moiety, $\alpha$, in each copolymer. From these results, it is concluded that the increase of the extinction coefficient at the same neutral imidazole moiety for various copolymers corresponds to the increase in the activity of nucleophilicity.

From Figures 8 and 11, two important results were obtained. One is that the fraction of the neutral imidazole moiety decreased with AA content in the copolymer, the other is that the molar extinction coefficient of the neutral imidazole moiety increased with AA content in the copolymer. Such phenomena might be realized by the peculiar cooperation of imidazole and carboxylic acid moieties, since they are obliged to be constellated in close vicinity in the copolymer. Such a multiple effect can be considered as the special province of the polymer catalyst.

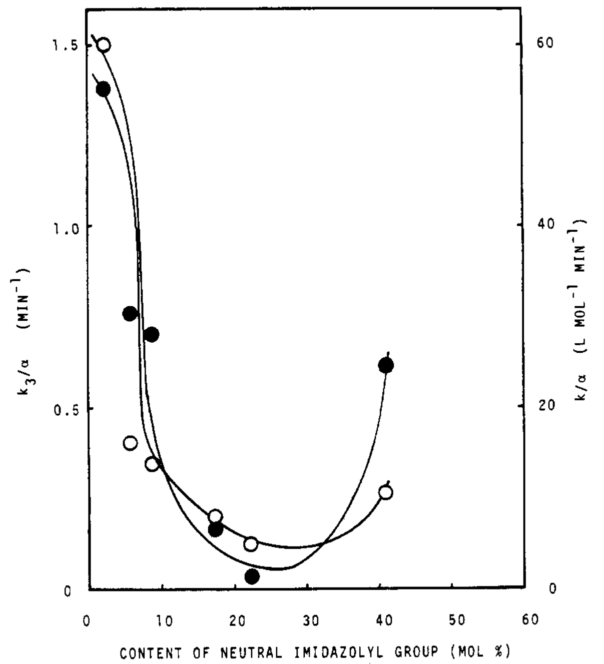


Figure 9. Plots of $k_3/\alpha$ for ANTI and $k/\alpha$ for PNPA against content of neutral imidazolyl group: (○) ANTI, pH 9.0, 25.9°; (●) PNPA, pH 9.1, 25.9°.

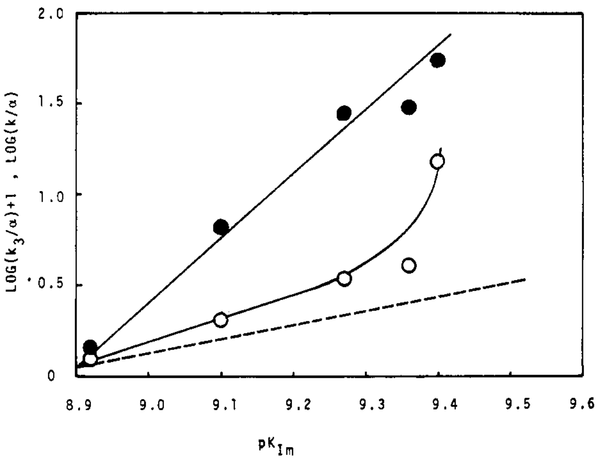


Figure 10. Plots of log $(k_3/\alpha)$ + 1 for ANTI and log $(k/\alpha)$ PNPA against p$K_{\text{Im}}$ obtained from the titration curve: (○) ANTI; (●) PNPA.

The following conclusion can be obtained. Of the copolymer less than 40 mol % in VI content, the carboxyl group increases the nucleophilicity of the imidazole. In the present reaction, both that the increase in AA content decreases p$K_{\text{AA}}$ and that n value in the copolymer containing lower imidazolyl group is larger than unity are good evidences for the existence of nondissociated carboxylic acid. Accordingly, it can be thought that the nondissociated carboxylic acid in the copolymer affects the catalytic activity.

This point differs from the discussion of Overberger and Maki.[7] The nondissociated carboxylic acid might act as a proton donator in the catalysis.

**Overall Hydrolysis Rate.** In the case of ANTI, the total hydrolysis rate can be expressed by the product of the reaction rate, $k_3$, and the magnitude of the effective interaction, $1/K_m$. Figure 12 shows the relation between the copolymer composition and the total hydrolysis rates, *i.e.*, $k_3/\alpha K_m$, $k_3/K_m$ for ANTI, and $k/\alpha$ for PNPA. The inflection point in the curve of $k_3/\alpha K_m$ and optimum point in the curve of $k_3/K_m$, appeared at 30 mol % in VI

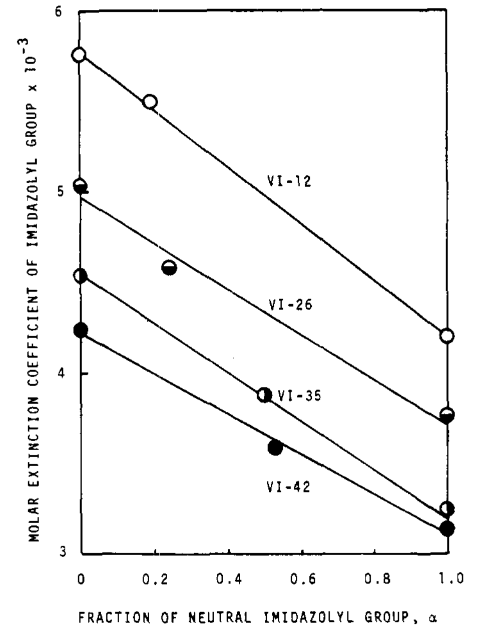


**Figure 11.** Plots of molar extinction coefficient of imidazolyl group in the copolymers against their neutral imidazole fraction.

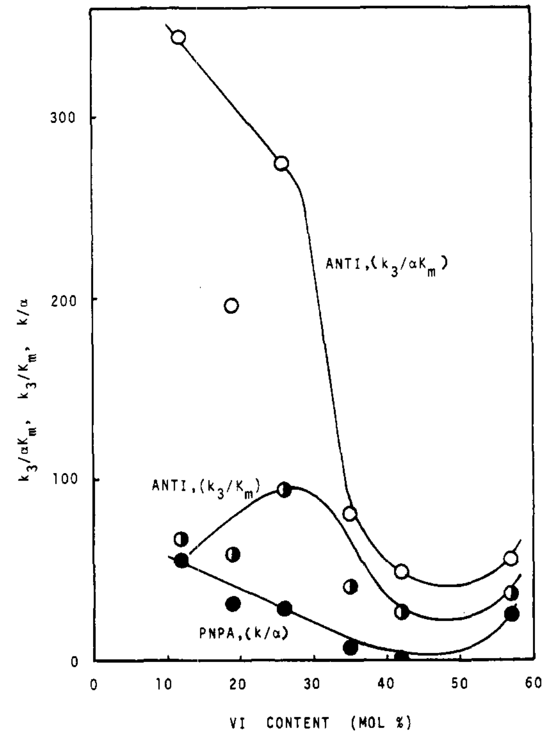


**Figure 12.** Hydrolysis rates, $k_3/\alpha K_m$, $k_3/K_m$, $k/\alpha$, against VI content in the copolymer (pH 9.0 for ANTI and pH 9.1 for PNPA).

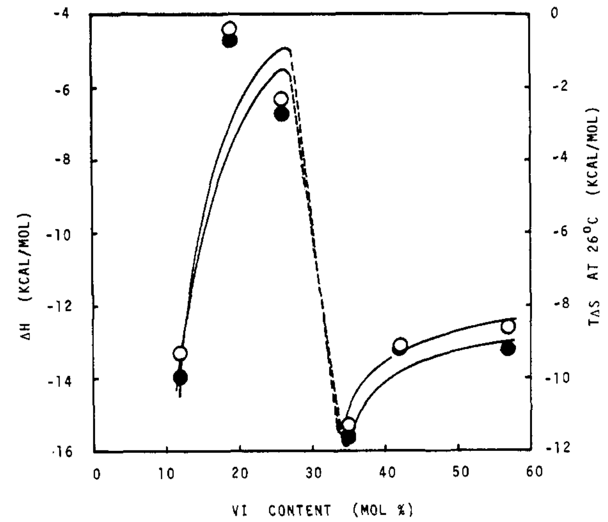


**Figure 13.** Changes in enthalpy and entropy of the complex formation: (○) enthalpy; (●) entropy.

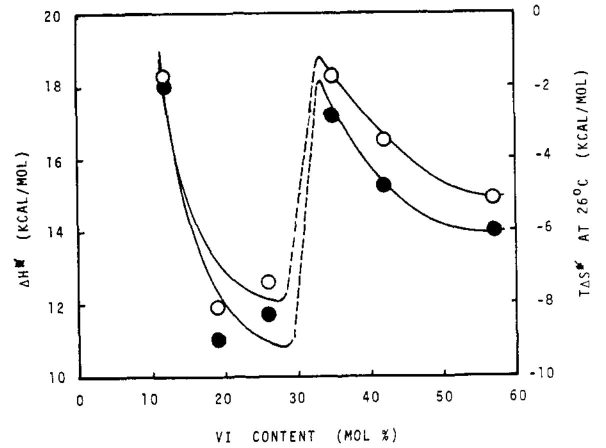


**Figure 14.** Enthalpy and entropy of activation in the hydrolysis: (○) enthalpy; (●) entropy.

content in the copolymer. From this fact, it is considered that the effective interaction influences the overall hydrolysis rate. In the case of PNPA, the overall hydrolysis rate decreases with the imidazole content in the copolymer, since there is no effective interaction between the copolymer and the substrate, PNPA.

**Thermodynamic Quantities of the Hydrolysis.** From the temperature dependency of the effective interaction, $1/K_m$, enthalpy ($\Delta H$), and entropy ($\Delta S$) changes in the effective interaction has been estimated. Figure 13 shows $\Delta H$ and $\Delta S$ with respect to the copolymer composition. Both parameters are discontinuous at 30 mol % of imidazole content in the copolymer. Both parameters are compensated for each other. Referring the result of the above-mentioned potentiometric measurement, the discontinuity can be explained as a configurational change of the copolymer in the reaction solution. That $n$ value which was obtained from the modified Henderson-Hasselbach equation in the potentiometric titration for the imidazole moiety in the copolymer (Table II) became more than unity in the range of imidazole content more than 30 mol % in the copolymer shows the existence of an interaction between the imidazole moieties themselves.

$\Delta H^*$ and $\Delta S^*$ of the hydrolysis reaction are shown in Figure 14. Those parameters are discontinuous at the same VI content as above mentioned. A similar explanation can be given. Both parameters are also compensated by each other. That both parameters in the both ranges are compensated by each other can be considered as due to an organization of water molecule on the copolymer. Addition of ethanol to the reaction system will affect the organization of water and made $\Delta H^*$ and $\Delta S^*$ decrease and did $\Delta H$ and $\Delta S$ increase. Results are tabulated in Table VI.

The hydrolysis in the present study is mainly caused by nucleophilic reaction by imidazole moiety in the copolymer. On the other hand, the imidazole moiety in α-chymotrypsin induced a proton transfer from hydroxy moiety to carboxylic acid moiety. The proton transfer raises the nucleophilicity of the hydroxy moiety. That is, the catalysis of α-chymotripsin proceeds by general acid-base catalysis.

In the nucleophilic hydrolysis by the imidazole moiety

**Table VI**
**Thermodynamic Properties of Hydrolyses**

| Copolymer | | Reaction Solvent (kcal/mol) Water[a] | 30% Ethanol[b] |
|---|---|---|---|
| VI-12 | $\Delta H^*$ | 18.3 | 5.38 |
| | $T\Delta S^{*c}$ | −2.46 | −16.3 |
| | $\Delta H$ | −13.3 | 9.40 |
| | $T\Delta S^c$ | −9.97 | 13.5 |
| VI-26 | $\Delta H^*$ | 12.6 | −5.05 |
| | $T\Delta S^{*c}$ | −8.61 | −26.8 |
| | $\Delta H$ | −6.63 | 23.2 |
| | $T\Delta S^c$ | −2.69 | 27.2 |

[a] pH 9.0. [b] pH 9.1. [c] $T = 26.0°$.

in the present study, the cooperative action between imidazole and carboxylic acid moieties was observed. Moreover, the carboxylic acid moiety acts as an attracting moiety for the substrate, ANTI. The attraction was caused by electrostatic interaction. In this action, $\Delta H$ was estimated to be $-4 \sim -5$ kcal per mol and $\Delta G$ was done to be $-3.3 \sim -3.9$ kcal per mol, respectively.

**Acknowledgment.** The authors are grateful to Professor Kenichi Fukui for his unfailing encouragement.

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# Kinetics and Thermodynamics of the Polymerization of the Cyclic Phosphate Esters. II. Cationic Polymerization of 2-Methoxy-2-oxo-1,3,2-dioxaphosphorinane (1,3-Propylene Methyl Phosphate)[1]

**Grzegorz Lapienis and Stanislaw Penczek***

*Polish Academy of Sciences, Centre of Molecular and Macromolecular Studies, Lodz 40, Poland.*

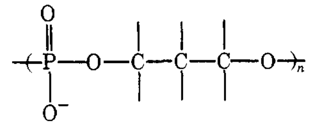
*Received August 15, 1973*

ABSTRACT: Kinetics and thermodynamics of cationic polymerization of 1,3-propylene methyl phosphate, a six-membered cyclic ester of phosphoric acid, have been investigated. The reaction was performed in $(CH_2Cl)_2$ and in bulk, using cationic initiators such as salts of $Ph_3C^+$ or 1,3-dioxolan-2-ylium with $PF_6^-$, $AsF_6^-$, and $SbF_6^-$ counterions. These initiators, in contrast to, *e.g.*, $Ph_3C^+SbCl_6^-$, react quantitatively with the monomer, yielding the propagation cyclic tetraalkoxyphosphonium ion. In the investigated temperature range of 60–155° the polymerization proceeds with marked depropagation. Interaction of the active species with macromolecules leads to chain termination caused by the formation of the nonstrained tetraalkoxyphosphonium ions. The kinetics of the mechanism involving the propagation, depropagation and termination steps was resolved and the respective rate constants determined in the investigated temperature range. Thus, $\Delta H_p^* = 19.5$ kcal/mol, $\Delta S_p^* = -15.6$ eu, $\Delta H_d^* = 20.6$ kcal/mol, $\Delta S_d^* = -10.2$ eu, $\Delta H_t^* = 11.6$ kcal/mol, $\Delta S_t^* = -48.1$ eu, $\Delta H_p = -1.1$ kcal/mol, and $\Delta S_p° = -5.4$ eu. At 25° in bulk $\Delta F_{ls} = -0.78$ kcal/mol.

There are two major methods already explored and used in the preparation of the oligonucleotides. Both are based on the polycondensation principles coupled with chemical activation of the reacting components.[2]

These methods, although limited and demanding the protection of the reactive groups in the nucleotide involved, led to the successful synthesis of various polynucleotides. Reactions of this kind, and further step by step synthesis of the copolynucleotides as well as the polycondensation of blocks of two or more different nucleotides were responsible for the tremendous progress in this field in the last decade.[2b]

In principle, formation of the backbone consisting of a sequence of six atoms



$$-\!\!\left(\begin{array}{c}\mathrm{O}\\ \|\\ \mathrm{P}\\ |\\ \mathrm{O^-}\end{array}-\mathrm{O}-\overset{|}{\underset{|}{\mathrm{C}}}-\overset{|}{\underset{|}{\mathrm{C}}}-\overset{|}{\underset{|}{\mathrm{C}}}-\mathrm{O}\right)_n$$

should also be possible either by a polymerization of six-