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MECHANISM OF ENZYME ACTION

Semiannual Report

by

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1

Foreword

The work described in this report was authorized under Project 1B014501B71A, Life Sciences Basic Research in Support of Materiel (U). This work was started in March and completed in December 1967.

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Digest

Solvolytic rate measurements of esters catalyzed by synthetic, imidazole-
containing polymers have revealed many analogies to those of naturally oc-
curring enzymic processes. In particular, the multifunctional interaction of
catalytic groups at the active site of an enzyme with a substrate can also
exist in synthetic, polymeric imidazoles. Reactions catalyzed by polymers with
pendent imidazole or benzimidazole functions have revealed the existance of
cooperative interactions between two imidazole (or benzimidazole) functions,[1-3]
the utilization of electrostatic attraction of substrate to a charged site on
a polymer chain thereby accumulating the substrate in the vicinity of cata-
lytically active groups,[1,4] and cooperative interactions between imidazole
and hydroxyl or phenol functions.[5]

In a continuation of the study of copolymers of 4(5)-vinylimidazole with
acrylic acid, believed to be synthetic, macromolecular models for acetyl-
cholinesterase,[4] the solvolytic rates of neutral, negatively, and positively
charged phenyl esters were determined as a function of the copolymer composi-
tion and sequence distribution. In this manner, a proper distribution of
charged groups (carboxylate) and catalytically active functions (imidazole)
was obtained which had optimal catalytic activity.

Since the copolymers containing high contents of acrylic acid could be-
have principally as polyelectrolytes, the effect of anionic poly(acrylic acid)
on the solvolytic rates of neutral, negatively, and positively charged sub-
strates was also investigated. In this system a strong inhibition of the

solvolytic rate of the positively charged ester 3-acetoxy-N-trimethylanilinium

iodide (ANTI) was observed. The dissociation constant of this system (poly-

anion-substrate) was found to be of the same order of magnitude as that of

acetylcholinesterase with its substrate.

The preparation of poly(3-vinyl-1,2,4-triazole), which was reported in

the Final Report covering the Period March 4, 1964-March 4, 1967, Contract

No. DA18-035-AMC-121(A), has been improved. Solvotic reactions employing

1,2,4-triazole and poly(3-vinyl-1,2,4-triazole) with neutral and negatively

charged substrates are being investigated. Results appear to indicate that

the nucleophilic reaction of neutral triazole with substrate does not contri-

bute significantly to the reaction rate, while that of either anionic triazole

or general base catalyzed triazole is most important in the solvolytic process.

An investigation of the effects of solvent composition on the catalytic

activities of imidazole-containing polymers is in progress. Alteration in

solvent composition (alcohol-water) has shown marked differences in catalytic

capabilities.[3,5] By varying the concentration of ethanol and water in the

solvent system, it may be possible to correlate the effects of a tight polymer

coil to enhanced catalytic action, since the imidazole groups would have a

greater probability of being in juxtaposition and can act as multifunctional

catalysts. Light scattering measurements will be employed to determine the

end-to-end distance in the polymer. At present, the effect of varying solvent

on apparent pK_1 measurements have been performed, as well as the effect of

solvent composition on polymer solubility (turbidity measurements).

It was recently reported that solvolytic reactions catalyzed by a co-polymer of 4(5)-vinylimidazole and p-vinylphenol indicated the existence of co-operative, multifunctional interactions between pendent, neutral imidazole, and anionic phenol groups.[5]   In order to further elucidate this cooperative interaction a methylene compound with terminal imidazole and phenol groups was synthesized.   Catalytic effects of this compound and of poly-4(5)-vinylimidazole on the solvolytic rates of phosphoric esters are presently under investigation.

Table of Contents

<u>List of Illustrations</u>

List of Illustrations (Concluded)

Results and Discussion

I. **Effects of Copolymer Composition and Monomer Sequence on Catalytic Activity of Copolymers of 4(5)-Vinylimidazole and Acrylic Acid.**

In the last report[6] results of the solvolyses of the neutral ester p-nitrophenyl acetate (PNPA), the negatively charged ester 4-acetoxy-3-nitrobenzoic acid (NABA) and the positively charged ester 3-acetoxy-N-trimethylanilinium iodide (ANTI), each catalyzed by a series of copolymers of 4(5)-vinylimidazole and acrylic acid at one pH value, were given.

When the positively charged ester ANTI was employed, a bell-shaped curve was obtained in a plot of the second-order catalytic rate constant vs. mole % imidazole in the copolymers. This curve showed that maximum catalytic activity occurred in the region of 46 mole % imidazole when the ionic strength was 0.02. However, when the ionic strength was increased to 0.50, no optimal effects were observed. These results and an analysis of the sequence distribution in the copolymers support the belief that the isolated neutral imidazole sequence (I) is primarily responsible for catalytic action. The leveling effect of increased ionic strength
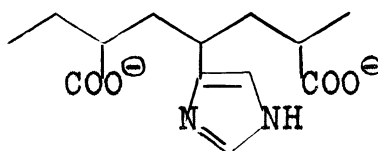
I

strongly suggests that ion-pair formation occurs between the anionic carboxylate sites and the positively charged substrate.

In order to obtain additional information regarding this system, the effect of varying pH on the catalytic action of copolymers of 4(5)-vinylimidazole and acrylic acid with ANTI was studied. In Figure 1 is shown a plot of the second-order catalytic rate constant for solvolysis of ANTI at three pH values vs. mole % neutral imidazole functions. This figure reveals rate maxima in the composition region of 42-50% mole % imidazole for each pH value studied. If this data is compared to a graph of the fraction of isolated neutral imidazole sequences as a function of the mole % imidazole in the copolymers shown in Figure 2 and the preceeding report,[6] it is seen that good agreement exists between the bell-shaped curves with regard to shape and location of maxima. This further substantiates the isolated, neutral imidazole sequence (I) being the most catalytically active component.
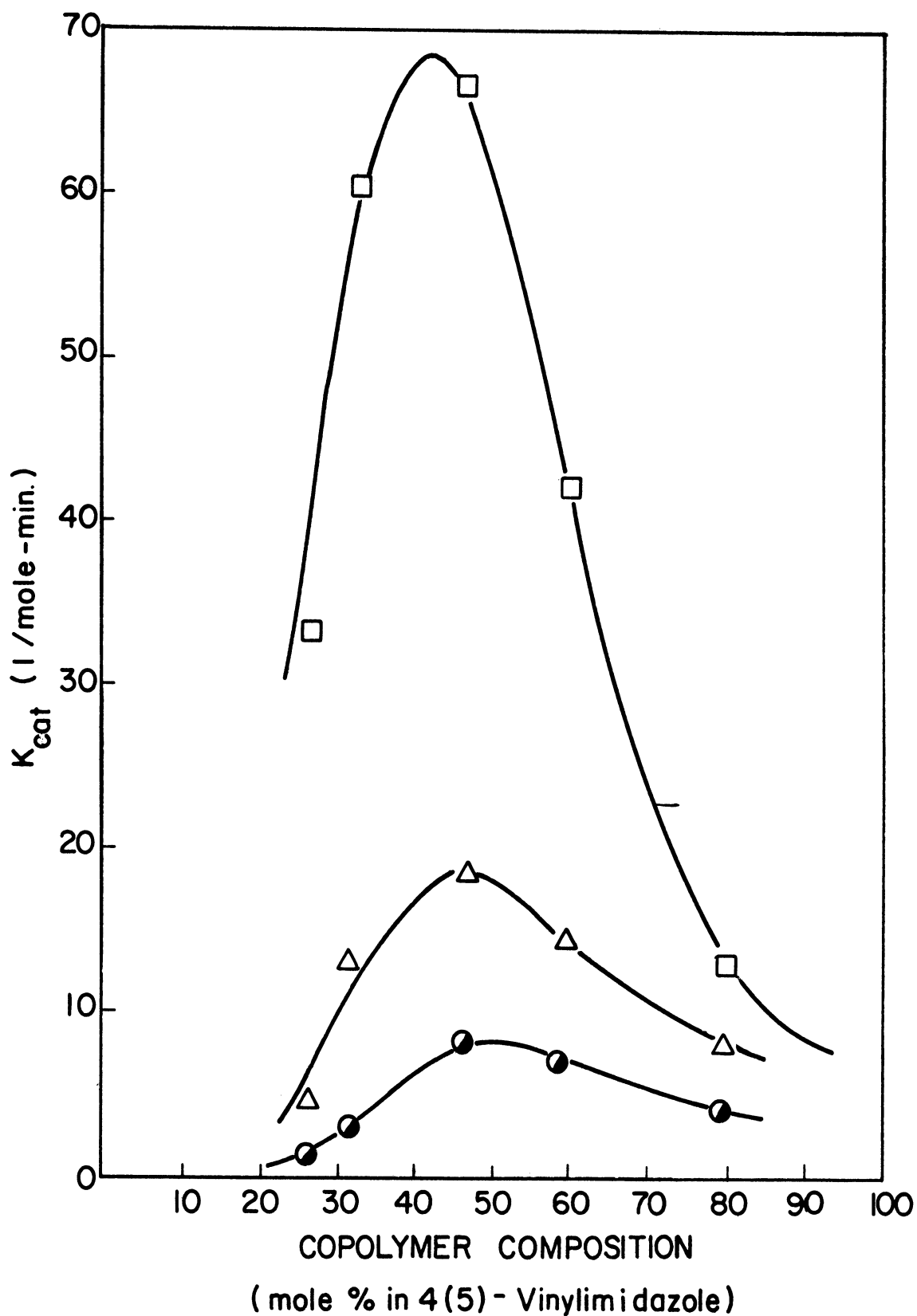
Figure 1. Catalytic rate constants for the solvolysis of ANTI at pH values of 8.38 (●), 8.98 (Δ) and 9.94 (□), catalyzed by copolymers of 4(5)-vinylimidazole and acrylic acid, in 28.5% ethanol-water, ionic strength 0.02, temp. 26°C.
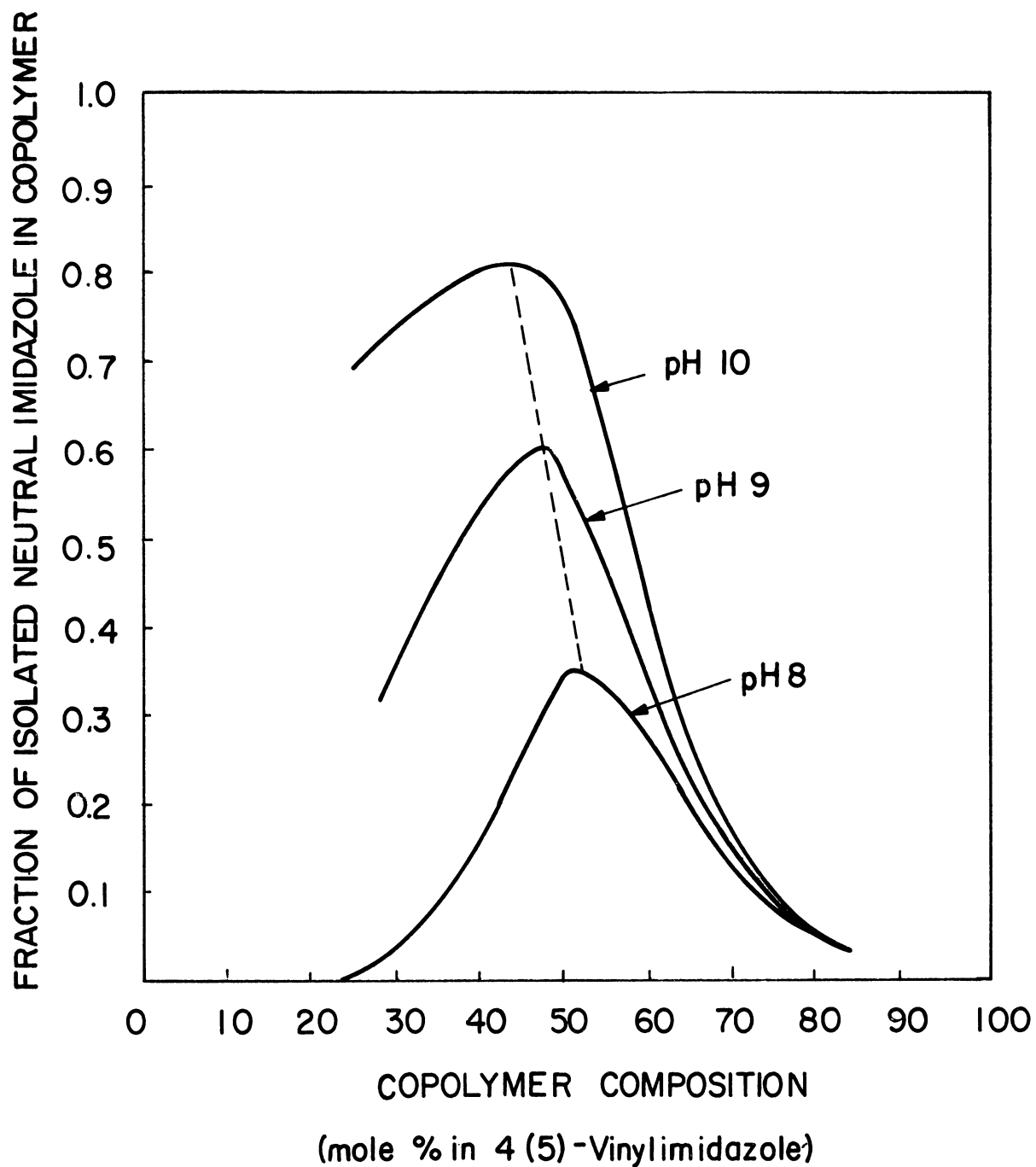
Figure 2. Fraction of isolated, neutral imidazole sequences as a function of the imidazole content in copolymers of 4(5)-vinylimidazole and acrylic acid, at pH values 8, 9, and 10.

If the ratios of the catalytic rate constants (k_{cat}) to the fractions of neutral, isolated imidazole sequences $(a_1 f_1)$ are compared for the different copolymer compositions (Table I), it is noted that the values reported for imidazole contents of 26.1 mole % and 79.4 mole % deviate considerably from the values found for imidazole contents of 31.9, 46.3 and 58.8 mole %.

Table I[(a)]

Ratios of Catalytic Rate Constants to Fractions of Neutral, Isolated Imidazole Sequences as a Function of Copolymer Composition.

$$k_{cat}/a_1 f_1$$

mole % imidazole	pH 8.38	pH 8.95	pH 9.90
26.1	18.7	17.9	48.0
31.9	22.2	33.1	81.0
46.3	19.9	31.3	83.2
58.8	22.2	35.6	91.5
79.4	72.0	130	205

(a) In 28.5% ethanol-water at an ionic strength of 0.02, temp. 26°C.

These data are indicative of two factors involved in the solvolysis of ANTI catalyzed by the copolymers of 4(5)-vinylimidazole and acrylic acid. Firstly, at copolymers compositions greater than 50 mole % imidazole, contributions between imidazole-imidazole sequences could occur in accord to such effects previously observed in reactions catalyzed by poly-4(5)-vinylimidazole.[1,3]

The other factor could be the inhibition of the solvolytic rate of ANTI (presented in section II) caused by an increase in the amount of carboxylate-carboxylate sequences.[7,8]

The effects of pH on the solvolytic rates of PNPA and NABA catalyzed by the above mentioned copolymers and a monomeric analog of the isolated sequences, γ-4-imidazoylbutyric acid, are currently under investigation.

II. Effects of Anionic Poly(acrylic acid) on Solvolytic Rates of Neutral, Negatively, and Positively Charged Esters - An Inhibitory Effect

In general, the effects of polyions (containing no catalytically active functions) on the reaction rates of two small charged reagents can be classified into two categories[7,9]. The large electrostatic potential which characterizes a polyelectrolyte solution can lead to corresponding fluctuations in the local concentration of the charged species. If a reaction involves two ionic species of the same charge, such as hydroxide ions and an anionic ester, a polyion of the opposite sign (polycation) will tend to concentrate the reactive reagents in its vicinity thereby increasing their collision frequency. In such a case the polyion will act as a catalyst. On the other hand, if two oppositely charged species are the reactive reagents, such as a cationic ester and hydroxide ions, a polyion of either charge will tend to concentrate one reagent in its vicinity and repel the other. The polyion will

then act as an inhibitor.

The above effects have been experimentally substantiated[7,8,10,11]. It has also been postulated that if two similarly charged species are the reactive reagents, a polyion of the same charge will accelerate the reaction since both species are repelled from the polymer chain and are in a higher concentration (lower volume) than would be realized in the absence of polyelectrolyte.[7,9]

In order to further understand the behavior of the copolymers of 4(5)-vinylimidazole and acrylic acid towards neutral and charged esters, particularly when the mole % of acrylic acid is high, a study of the solvolytic rates of PNPA, NABA and ANTI was undertaken.

Data for the solvolyses of PNPA, NABA and ANTI solvolysed in the presence and absence of poly(acrylic acid) are listed in Table II. These measurements were obtained in 28.5% ethanol-water at pH 8.98, ionic strength 0.02. In Figure 3, plots of the first-order observed rate constant $(k_{obsd} = k_{measd} - k_{blank})$ $\underline{vs}$. total concentration of poly(acrylic acid) are presented. This figure reveals several surprising effects. It is seen that the reaction with both PNPA and NABA is first-order in both substrate and polyion (hydroxide ion), an effect not previously observed for either a neutral substrate or an anionic substrate whose hydroxide ion catalysis occurs in the presence of a polyanion. Furthermore, the reaction with ANTI clearly reveals an inhibitory effect displaying saturation characteristics, an effect previously reported

by Morawetz and Shafer for the hydrolysis of singly and doubly charged cationic esters in the presence of partially neutralized poly(methacrylic acid).[7]

Table II[a]

First-order Rate Constants for the Solvolyses of PNPA, NABA and ANTI as a Function of Poly(acrylic acid) Concentration, pH 8.98

[Poly(AA)] x 10^3 m	ANTI			10^{-3} min^{-1} NABA			PNPA		
	k_{blank}	k_{measd}	k_{obsd}	k_{blank}	k_{measd}	k_{obsd}	k_{blank}	k_{measd}	k_{obsd}
0.000	7.23	----	0.00	3.03	----	0.00	3.76	----	0.00
0.467	----	6.40	-0.83	----	3.68	0.68	----	4.23	0.47
0.934	----	6.16	-1.07	----	4.33	1.30	----	4.54	0.68
3.000	----	5.56	-1.67	----	7.04	4.01	----	5.72	1.96
4.670	----	5.43	-1.80	----	----	----	----	6.36	2.60

(a) In 28.5% ethanol-water, ionic strength 0.02, temp. 26°c.

Regarding the solvolysis of NABA, the effect of enhanced solvolytic action occurring when two similarly charged species react in the presence of a similarly charged polyion has been theorized[7,9] but never experimentally verified. This effect can be accounted for by the electrostatic repulsion of both reactive species (anionic ester and hydroxide ions) from the polyanion where they are then concentrated in a decreased volume of solution. From this experiment a coefficient of repulsion can be calculated. Consider the rate of solvolysis of substrate in the absence of polyion is given by the expression
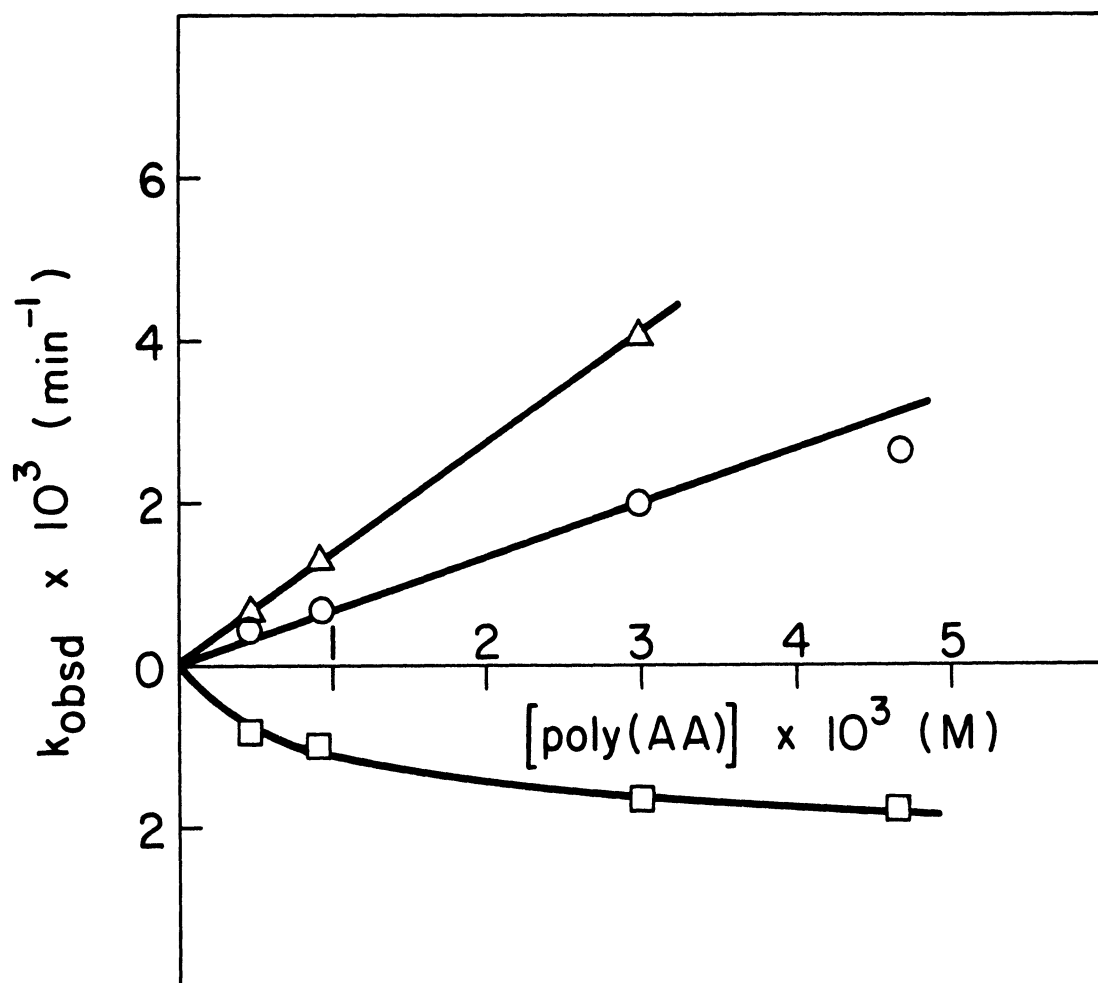
Figure 3. Dependence of first-order observed rate constants for PNPA (O), NABA (△), and ANTI (□) solvolyses on poly(acrylic acid) concentration, 28.5% ethanol-water, ionic strength 0.02, buffer concentration 0.02 M, temp. 26°C.

(1) $R_0 = k_([S][OH^-] + kw + k_b [B][S] = k_0 \dfrac{M_s}{V} \dfrac{M_{OH}}{V} + kw + k_B [B][S]$

where R_0 and k_0 are the rate and rate constant in the absence of polyion, [S] is the substrate concentration, M_s and M_{OH} are the moles of substrate and hydroxide ions, respectively, V is the total volume of solution, k_w is the rate constant for water catalysis, k_B is the rate constant for buffer catalysis and B is the buffer concentration.

In the presence of polyion, the reactive reagents will be ex-cluded from the polyion by a certain factor (according to the Boltzman distribution), and the rate of solvolysis will then be given by

$$R_p = k_0 [S]_p [OH^-]_p + k_w + k_B [B] [S]$$

$$R_p = k_0 \dfrac{M_s}{V-V_p} \dfrac{M_{OH^-}}{V-V_p} + k_w + k_B [B] [S]$$

$$R_p = k_p [S] [OH^-] + k_w + k_B [B] [S] \qquad (2)$$

where R_p and k_p are the rate and rate constant in the presence of polyion, $[S]_p$ and $[OH^-]_p$ are the concentrations of substrate and hydroxide ions resulting from exclusion from the polymer domain, and V_p is the volume from which substrate and hydroxide ions are excluded. This equation assumes that the concentration of reactive species in the vicinity of polyion is negligible.

The observed rate of reaction is then

$$R_p - R_0 = k_0 \, M_s \, M_{OH} \left[\dfrac{1}{\left(V-V_p\right)^2} - \dfrac{1}{V^2} \right]$$

$$R_p - R_0 = k_0 \, M_s \, M_{OH} \left[\frac{2V_p V - V_p^2}{V^2(V-V_p)} \right]$$

$$R_p - R_0 = k_0 \, [S] \, [OH^-] \, \frac{V_p(2V-V_p)}{(V-V_p)^2} \tag{3}$$

Since $V \gg V_p$,

$$R_p - R_0 = k_0 \, [S] \, [OH^-] \, \frac{2V_p}{V} = R_0 \, \frac{2V_p}{V} \tag{4}$$

V_p can be defined as the summation of the excluded volume for each polyion (assuming no interactions of polymer chains),

$$V_p = \sum V_{pi} = Kn_p \tag{5}$$

where K is the effective repulsive volume coefficient associated with one polymer molecule and n_p is the number of polymer molecules. Therefore, since $n_p/V = [P]$

$$\frac{R_p - R_0}{R_0} = \frac{k_p - k_0}{k_0} = 2K[P] \ . \tag{6}$$

Employing equation 6 for the base catalyzed solvolysis of NABA in the presence of anionic poly(acrylic acid) (Figure 4), a straight line is obtained having a slope of 441 M^{-1}. The constant K is then determined to be 220.5 M^{-1}. If the polymer is an extended cylinder in solution, the constant K would then indicate an excluded volume of substrate and hydroxide ions of approximately 240 $\overset{o}{A}$ from a single monomer segment.
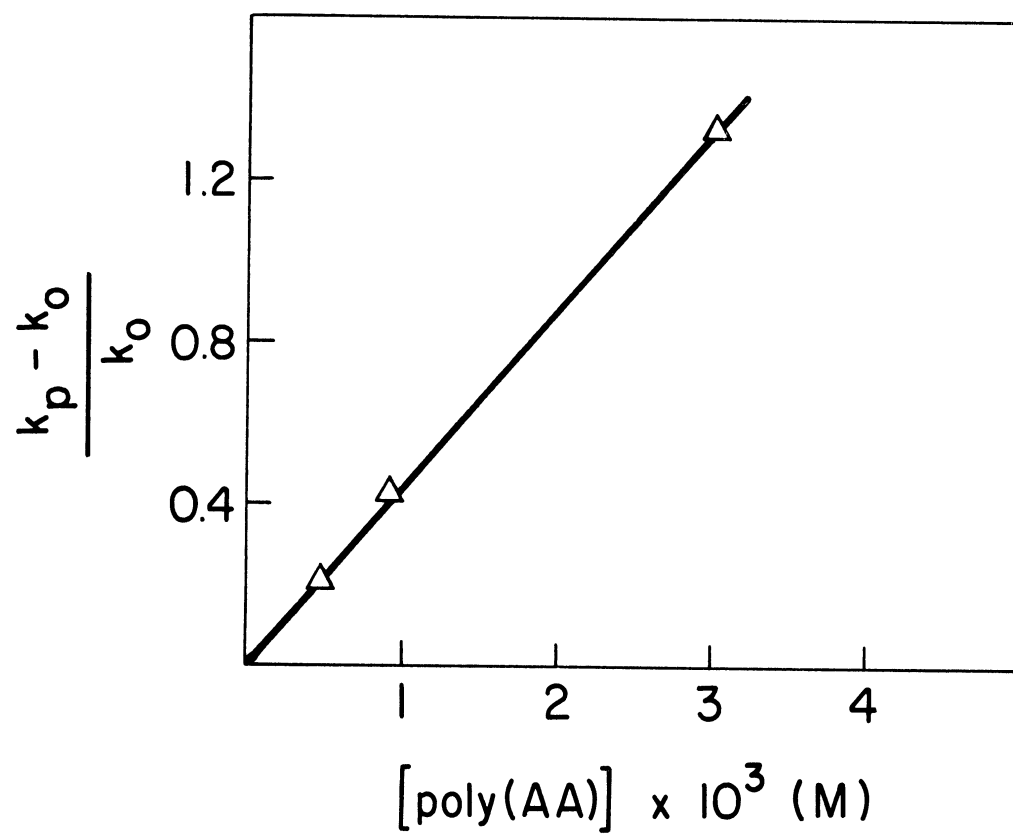
Figure 4. Determination of a coefficient of repulsion.

Previous solvolytic reactions of neutral esters in the presence of polyions have revealed no rate alterations,[7,10] since the ester should neither be concentrated near the polyion nor in the bulk of solution. However, we observed a totally unexpected rate acceleration for the solvolysis of PNPA in the presence of anionic poly(acrylic acid) (Figure 3). In this case it appears that PNPA is excluded from the vicinity of the polyelectrolyte since if it were in the vicinity of the polyanion a rate depression would be expected Another factor involved could be rate acceleration caused by nucleophilic attack of anionic carboxylate groups on the acetyl functions, where PNPA could be attracted to the polyanion by either hydrophobic forces or ion-dipole interactions; however, if this effect were operative, a similar or even greater rate enhancement should occur with the positively charged ester ANTI which would be strongly attracted to the polyanion by electrostatic forces. As mentioned previously, the reaction of hydroxide ions with ANTI in the presence of anionic poly(acrylic acid) revealed a rate depression. This would therefore exclude the possible involvement of carboxylate functions in the solvolysis. At the present time the causes of this effect are not understood and further research is necessary to elucidate this phenomenon.

From Figure 3 it is seen that a strong depression of the solvolytic rate of ANTI is obtained when the reaction takes place in the presence of anionic poly(acrylic acid). Furthermore, at high polymer concentrations the reaction rate approaches a limiting value. This effect is analogous to that previously observed by Morawetz and

Shafer for the hydrolysis of singly and doubly charged cationic esters in the presence of anionic polymeric acids[7,8]. It is accounted for by the electrostatic attraction of the positively charged polyanion, from which the catalyzing hydroxide ions are repelled.

The results of Morawetz and Shafer indicated that a kinetic scheme similar to that obtained for an enzyme-substrate complexation could be obtained from such an inhibitory system. They were able to calculate the dissociation constant K_D from the complex of polymer P with reagent R, i.e.,

$$K_D = \frac{[P][R]}{[PR]} \tag{7}$$

they employed the equation

$$k = \frac{k_0[R] + k_b[PR]}{[R] + [PR]} \tag{8}$$

where k_0 is the rate constant for the uncomplexed substrate and k_b the rate constant for the substrate bound to the polyion, while k is the observed rate constant.

Combining equations 6 and 7 they obtained

$$k = k_b + K_D \frac{(k_0-k)}{[P]} \tag{9}$$

a relationship analogous to that determined by Lineweaver and Burk for enzyme-substrate complexation. They assumed k_0 to be identical to the rate constant resulting from hydrolysis in the absence of polyion. The linear relationship suggested by equation 9 can be used to determine the reactivity of the bound reagent and the dissociation

constant of the polyion-substrate.

In table III data pertaining to the differences in first-order rate constants in the presence and absence of polyion, to be used in plotting equation 9, are presented.

Table III[a]

Differences in First-order Rate Constants for the Solvolysis of ANTI in the Presence and Absence of Poly(acrylic acid).

k_p x 10^3 (min^{-1})	$(k_0 - k_p)/[P]$ (min^{-1} M^{-1})
6.40	1.765
6.16	1.150
5.56	0.551
5.43	0.386

(a) In 28.5% ethanol-water, pH 8.96, ionic strength 0.02. Constants obtained from Table II.

These data are plotted in Figure 5. The value of k_b is determined from the intercept to be 5.25 x 10^{-3} min^{-1}, a value slightly less than the solvolytic rate for the free reagent ($k_0 = 7.23$ x 10^{-3} min^{-1}, see Table II). The dissociation constant K_D for the polyion-substrate complex is determined from the slope to be 7.14 x 10^{-4} M, a value of the same order of magnitude as the Michaelis constant K_m for the reaction of acetylchlinesterase with acetylcholine ($K_m = 2.6$ x 10^{-4}). The latter reaction is in part due to the electrostatic attraction of the positively charged substrate to an anionic site on the enzyme.
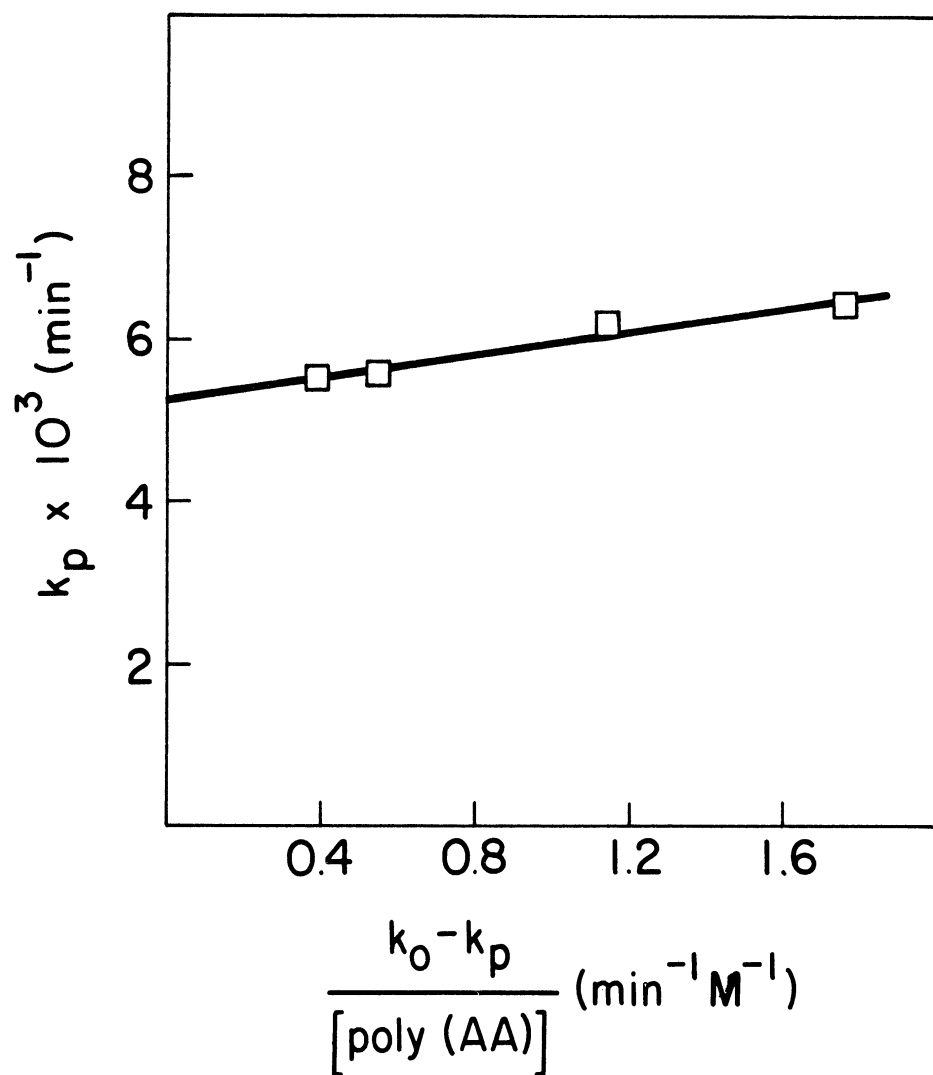
Figure 5. Determination of the polyion-substrate dissociation constant and the reactivity of the bound substrate.

III. Effect of Solvent Composition on Polymeric Imidazole Catalysis

Poly-4(5)-vinylimidazole is soluble in the mixed solvent system of ethanol-water, insoluble in water and difficulty soluble in ethanol Since previous experiments have indicated a marked dependence of solvent composition on the solvolytic rates of esters,[3,5] a study of the varying proportions of ethanol-water on the catalytic cap- abilities of poly-4(5)-vinylimidazole and a copolymer of 4(5)-vinyl- imidazole with styrene (a copolymer containing inert hydrophobic groups) is under investigation.

It can be expected that varying the solvent content will lead to alterations in the polymer conformations, as well as in the ap- parent dissociation constants of the imidazole groups at half neutralization. Since the pendent imidazole functions in poly-4(5)- vinylimidazole are believed to be involved in cooperative, multi- functional interactions, a rate enhancement should occur when the polymer is in a tight coil in solution. In this instance, the catalytic functions could be in juxtaposition for enhanced multifunctional interactions. The dimensions of the polymer will be determined by light scattering measurements. At the present time turbidimetric titrations have been performed on two copolymers of 4(5)-vinylimidazole amd a copolymer of 4(5)-vinylimidazole and styrene. The inert, hydrophobic styrene groups could lead to different conformations than that of poly4(5)-vinylimidazole in ethanol-water solvent systems.

In Figure 6 is shown a plot of the effect of ethanol content on the (apparent) pK_1 values of the imidazole functions for imidazole,

for poly-4(5)-vinylimidazole, and for a copolymer of 4(5)-vinylimidazole
and styrene. These data are tentative and subject to further inves-
tigation. The poly-4(5)-vinylimidazole employed in this investigation
was prepared by free radical polymerization in benzene (sample No. 2,
see Table IV) from which the growing polymer chain precipitates.
This plot reveals that for poly-4(5)-vinylimidazole and (sample No. 5)
copoly[4(5)-vinylimidazole-styrene] the apparent pK_1 values for the
imidazole groups show a minimum in the region of 50% ethanol-water,
whereas the pK_1 values for imidazole decrease with increasing ethanol
content.

In Figures 7 and 8 the turbidity of samples No. 1 and 2
(Table IV) of poly-4(5)-vinylimidazole and sample No. 5 of copolymer
of 4(5)-vinylimidazole and styrene are shown (for two pH values)
vs. varying ethanol content. The conditions employed in polymerization
for a series of homopolymers and are shown in Table IV.

Table IV

Homopolymerization and Copolymerization Conditions of 4(5)-Vinyl-

imidazole with Styrene

Sample No.	[4(5)VIm]	[Styrene]	AIBN	Solvent	Temp.	Time	% Conv.
1	0.011 m	---	0.02 g	methanol	65°	24 h	33.1
2	0.011 m	---	0.02 g	benzene	65°	24 h	88.3
3	0.0079 m	0.0025 m	0.02 g	methanol	65°	40 min	2.9
4	0.0071 m	0.0033 m	0.02 g	methanol	65°	40 min	1.1
5	0.0071 m	0.0033 m	0.02 g	methanol	65°	13.5 h	69.7
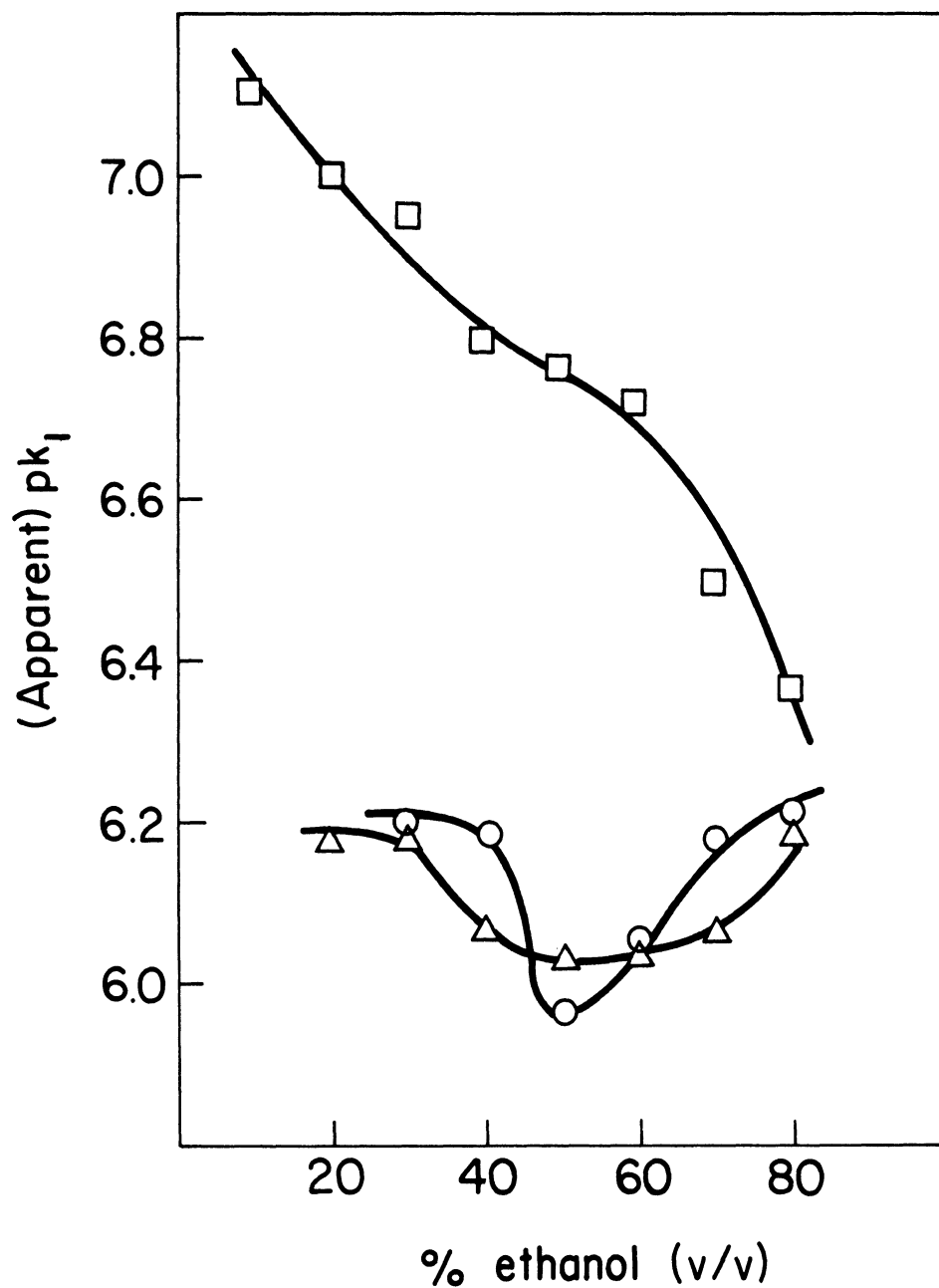
Figure 6. Effect of solvent composition (ethanol-water) or (apparent) pK_1 values for imidazole (□), poly-4(5)-vinylimidazole [sample No. 2, (△)], and copoly [4(5)-vinylimidazole-styrene] [sample No. 5, (0)], ionic strength 0.05, temp. 26°C.
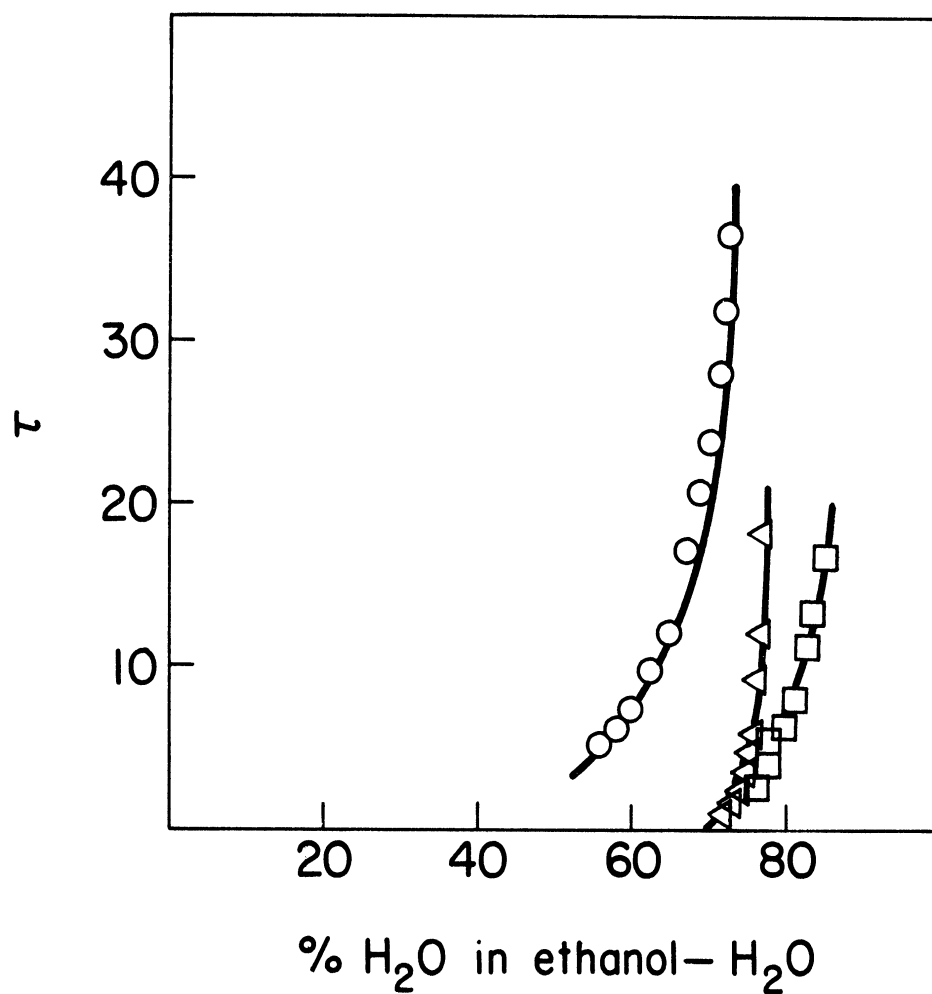
Figure 7. Turbidity of poly-4(5)-vinylimidazole [samples No. 1 (□) and 2 (△)] and copoly [4(5)-vinylimidazole-styrene] [sample No. 5 (O)] at pH 8.0 as a function of increasing water content in ethanol-water solvent systems, buffer (TRIS-HCl) concentration 0.1 M, ionic strength 0.1, temp. 26°C.
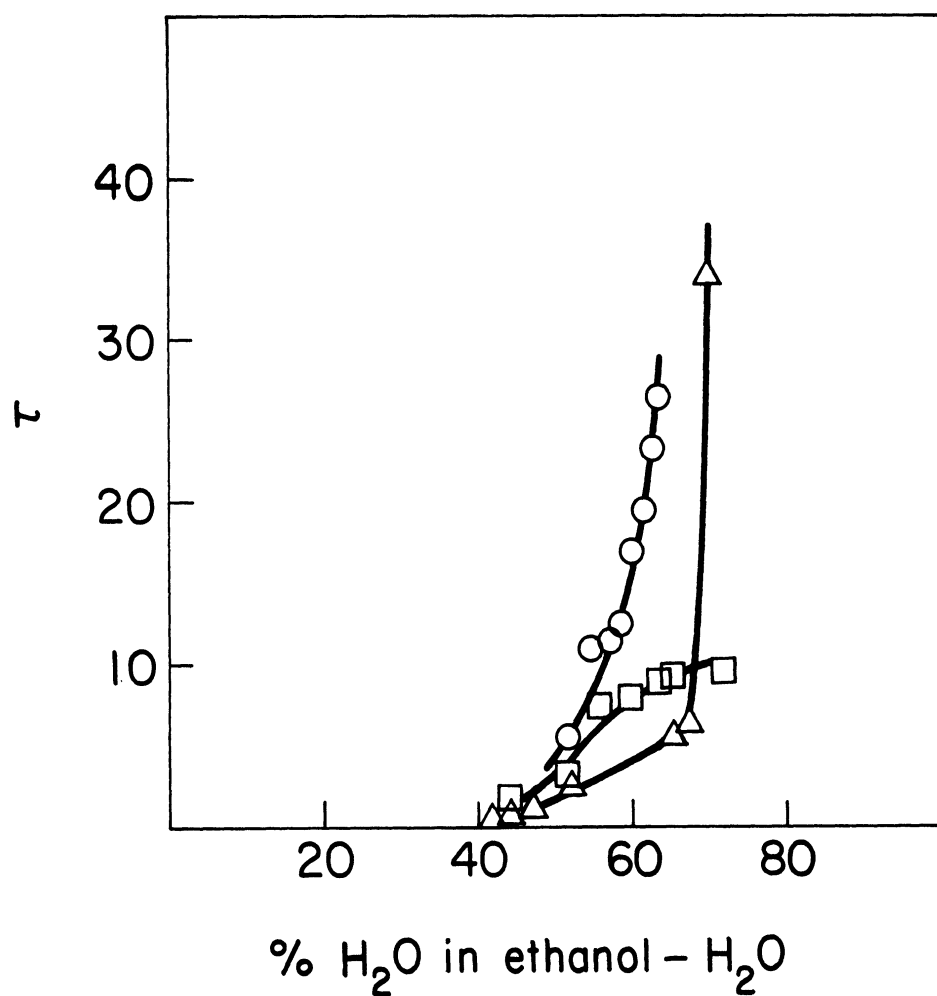
Figure 8.   Turbidity of poly-4(5)-vinylimidazole [samples No. 1 (□) and 2 (Δ)] and copoly[4(5)-vinylimidazole-styrene] [sample No. 5(O)] at pH 9. Δ as a function of increasing water content in ethanol-water solvent systems, buffer (TRIS-HCl) concentration 0.1 M, ionic strength 0.1, temp. 26°C.

From Figures 7 and 8 it is noted that the copolymer (sample No. 5) precipitates more easily from solution than either samples No. 1 or 2 with increasing water content, and that sample No. 2 (having a higher intrinsic viscosity than sample No. 1) precipitates before sample No. 1.

From these results it should be possible to obtain an optimal catalytic activity in the regions where the polymers are approaching their minimum solubility, i.e., when the tightly coiled polymers will have an even higher "effective local concentration" of catalytically active functions.

IV. Catalytic Activities of 1,2,4-Triazole and Poly-3-vinyl-1,2,4-triazole

The preparation of poly-3-vinyl-1,2,4-triazole from the thermal decomposition of 3-β-acetoxyethyl-1,2,4-triazole was previously reported[6]. An improved synthesis has been obtained based on the dehydration of 3-β-hydroxethyl-1,2,4-triazole over molecular sieves 5A. This procedure gives the monomer in 15-20% yield, nmr and infrared spectra consistent with the vinyl structure. 3-Vinyl-1,2,4-triazole polymerizes on standing as well as by free radical and thermal initiation.

Solvolytic rates of PNPA, NABA, NABS and ANTI catalyzed by 1,2,4-triazole and poly-3-vinyl-1,2,4-triazole in several solvent systems are under investigation. At the present time some data have been completed for reactions in 28.5% ethanol-water.

The concentration dependence of the first-order observed rate constant for the solvolyses of PNPA, NABA and NABS, each catalyzed

by 1,2,4-triazole and by poly-3-vinyl-1,2,4-triazole, is shown in Figure 9. This figure reveals that the reaction is an overall second-order process, i.e., first-order in both catalyst and substrate.

In Figure 10 the pH-rate profiles for PNPA, NABA and NABS solvolyses are presented. It is seen that 1,2,4-triazole is a catalyst superior to poly-3-vinyl-1,2,4-triazole in the pH region investigated. Although this is not an unusual occurrence, the marked enhancement of the monomeric triazole catalyzed reaction with increasing pH strongly suggests the involvement of anionic triazole functions which could arise from a general base catalysis.

Potentiometric titrations of 1,2,4-triazole and poly-3-vinyl-1,2,4-triazole in 28.5% ethanol-water gave pK_2 values of 10.1 and 11.0, respectively. The pK_1 value for 1,2,4-triazole was determined to be 2.3. For poly-3-vinyl-1,2,4-triazole, the apparent pK_1 value appears to be lower than its monomeric analog. Therefore, at intermediate pH most of the triazole functions are in the neutral form. In this region both polymeric and monomeric catalysis appear to be equally inefficient, suggesting that the nucleophilic reaction of triazole on substrate does not contribute significantly to the rate of reaction. Indeed, the participation of anionic functions is clearly revealed in Figures 11 and 12 where the second-order catalytic rate constant k_{cat} is plotted vs. α_2 (the fraction of anionic triazole functions) for monomeric and polymeric catalyses, respectively. It is interesting to note that if these figures are compared, the reaction of (anionic) poly-3-vinyl-1,2,4-triazole is
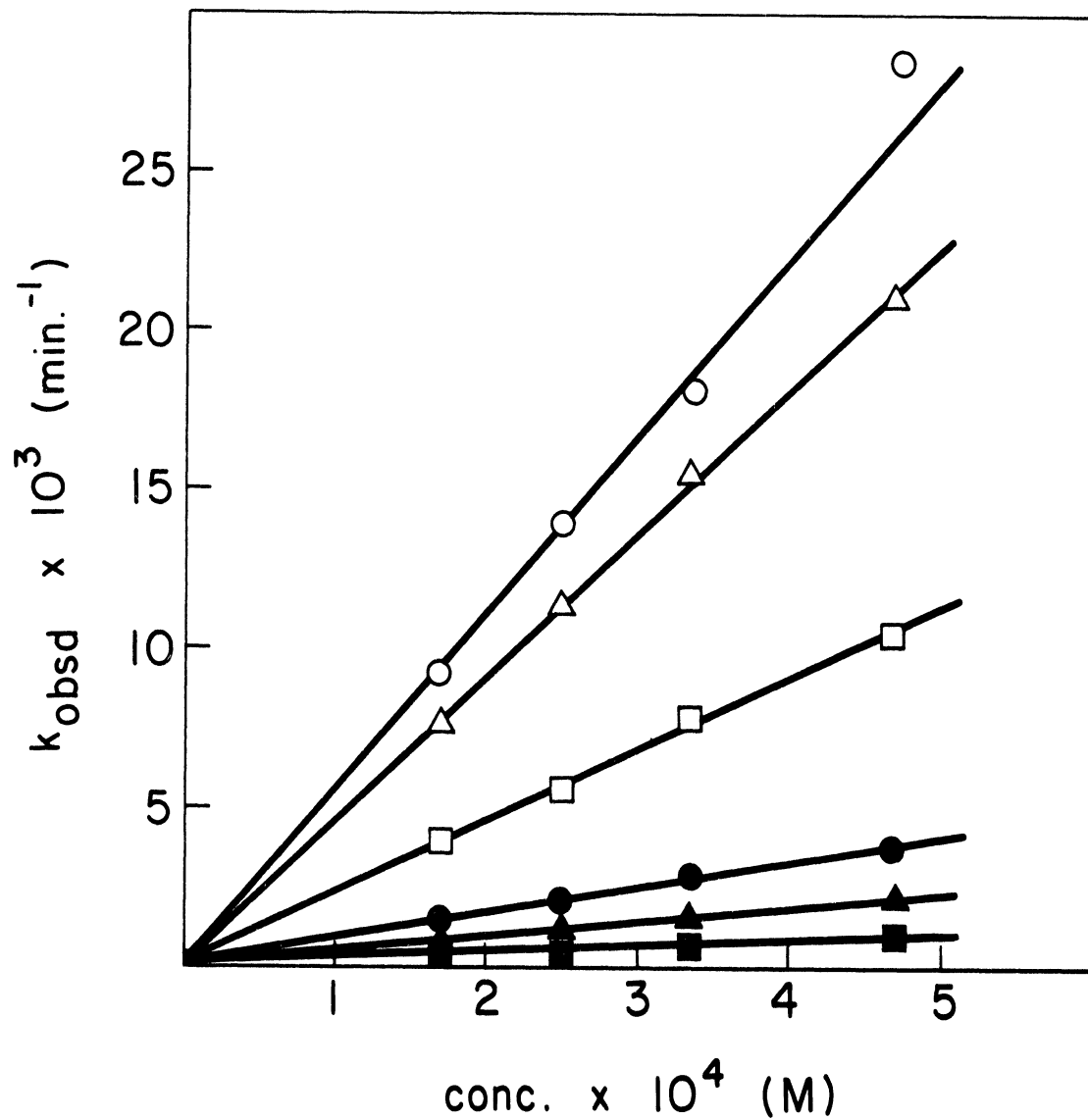
Figure 9. Dependence of the solvolytic rates of PNPA (O,●), NABA (□,■), and NABS (△,▲) on the concentrations of 1,2,4-triazole and poly-3-vinyl-1,2, 4-triazole, respectively; in 28.5% ethanol-water, pH 8.89, buffer concentration (TRIS-HCl) 0.02 M, ionic strength 0.02, temp. 26°C.
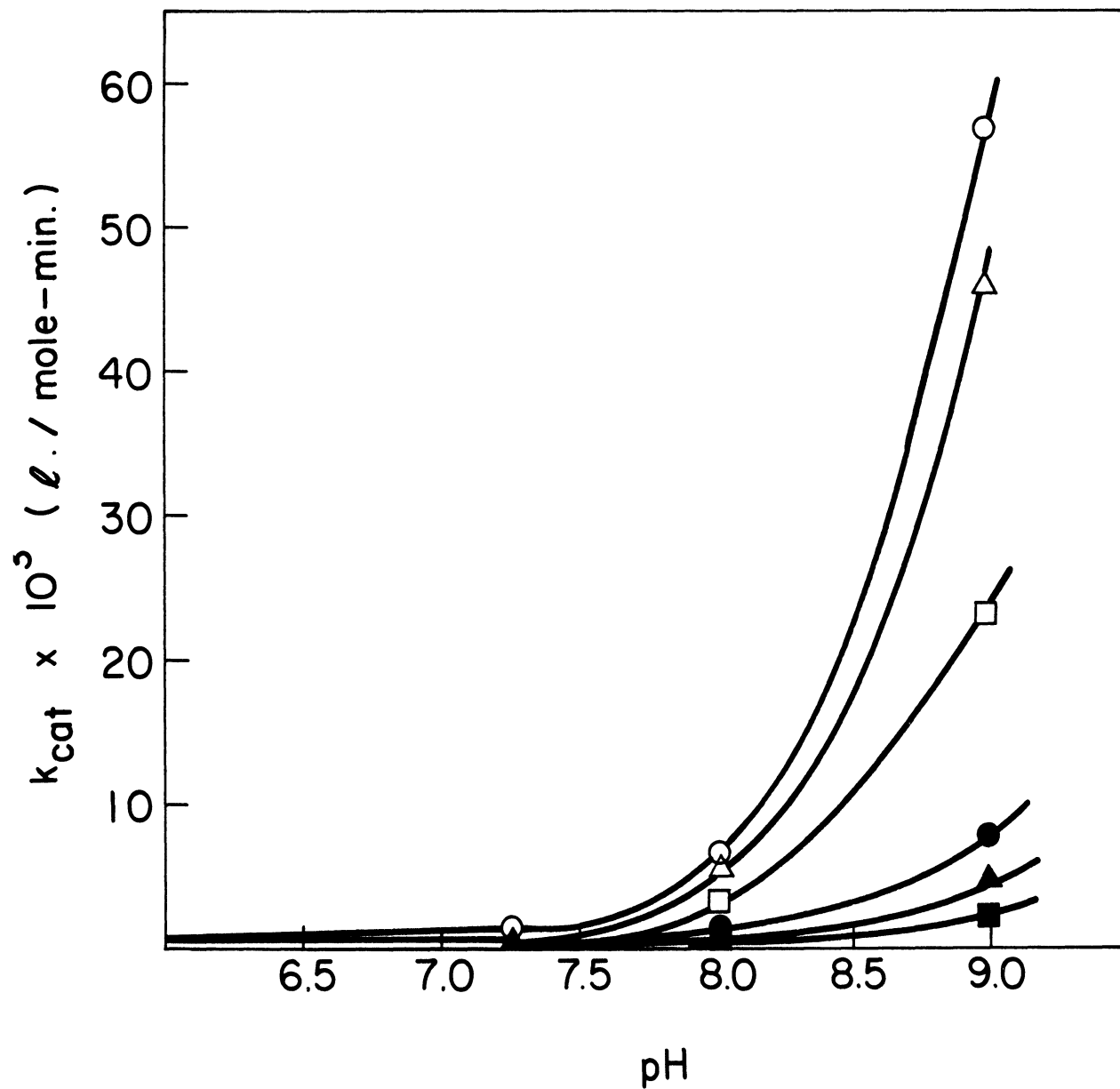
Figure 10.   pH-rate profiles for the solvolyses of PNPA (O,●), NABA (□,■), and NABS (△,▲) catalyzed by 1,2,4-triazole and poly-3-vinyl-1,2,4-triazole, respectively; 28.5% ethanol-water, ionic strength 0.02, buffer (TRIS-HCl) concentration 0.02 M, temp. 26°C.
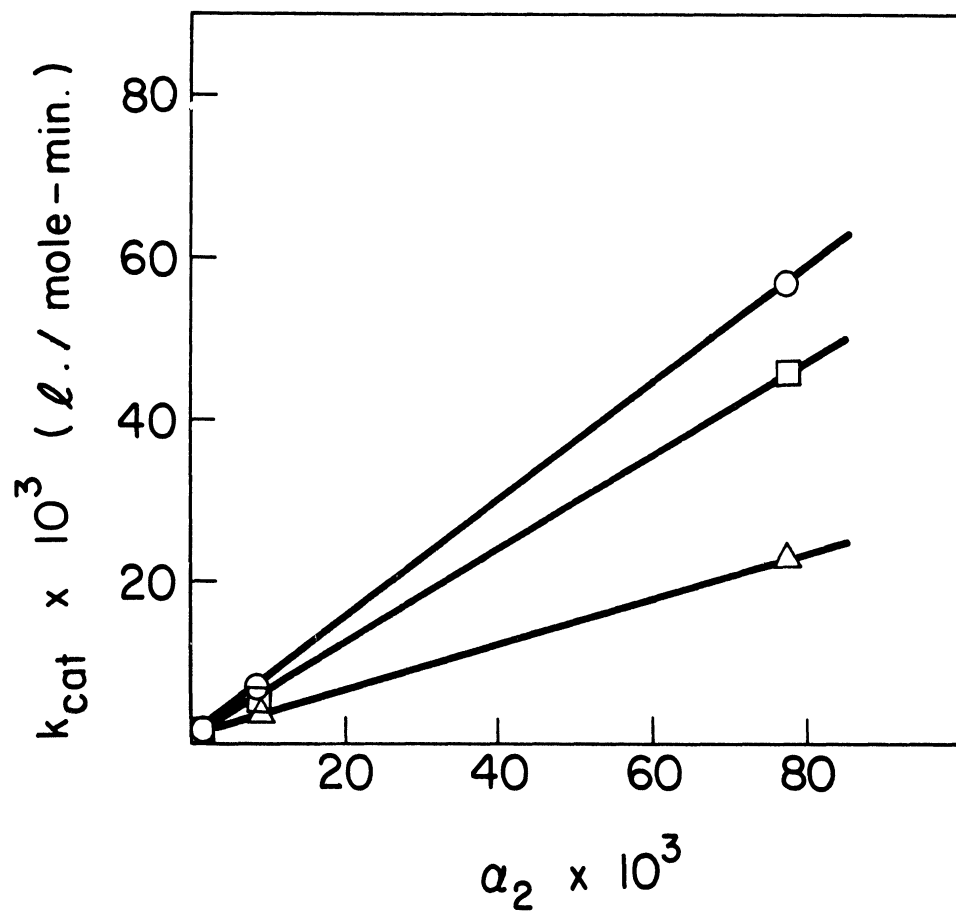
Figure 11. Dependence of the catalytic rate constants for the solvolyses of PNPA (O), NABA (□), and NABS (△) on the fraction of anionic 1,2,4-triazole functions, 28.5% ethanol-water, ionic strength 0.02, buffer (TRIS-HCl) concentration 0.02 M, temp. 26°C.
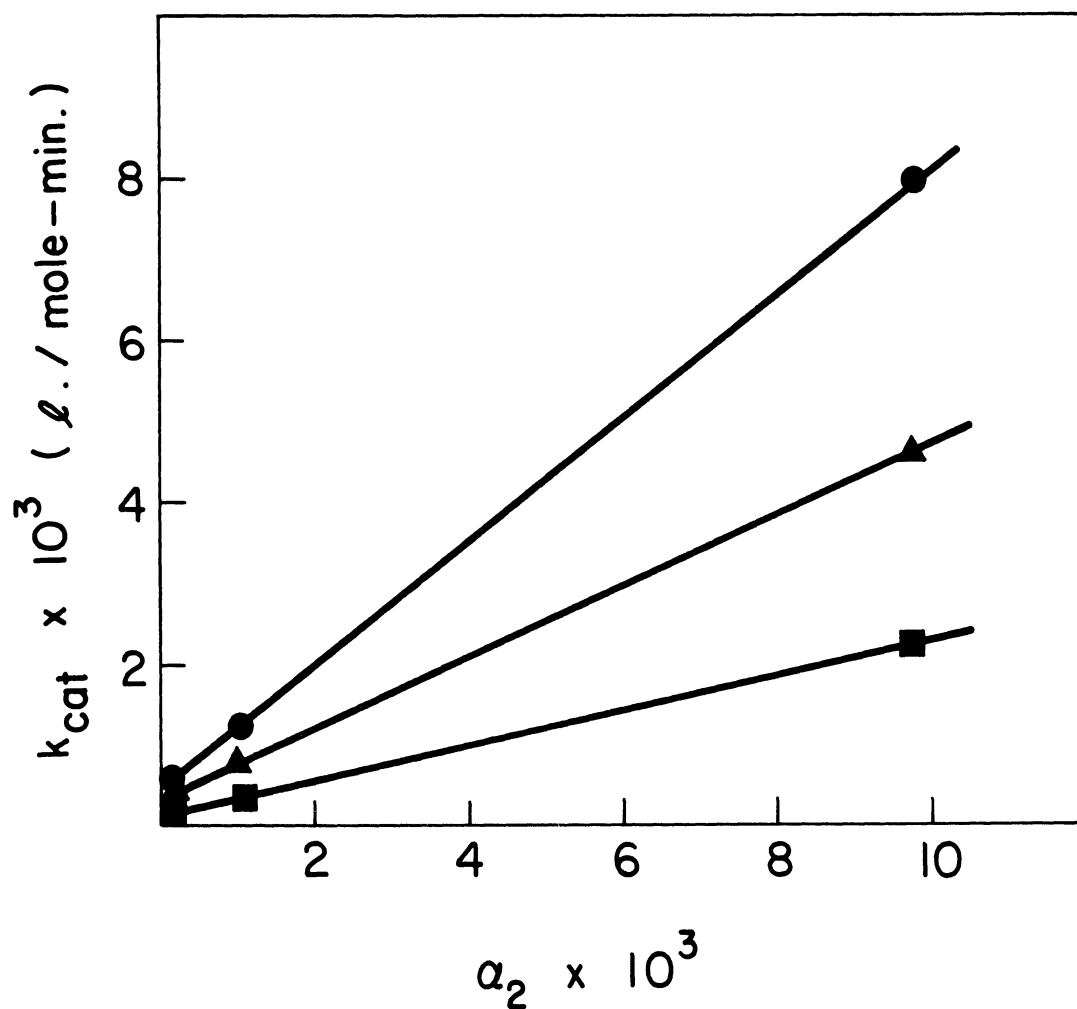
Figure 12. Dependence of the catalytic rate constants for the solvolyses of PNPA (O), NABA (□), and NABS (Δ) on the fraction of anionic 1,2,4-triazole functions in poly-3-vinyl-1,2,4-triazole, 28.5% ethanol-water, ionic strength 0.02, buffer (TRIS-HCl) concentration 0.02 M, temp. 26°C.

approximately as efficient as (anionic) 1,2,4-triazole in catalyzing the solvolysis of PNPA. On the other hand, the reaction of the polymer with NABS is considerably less efficient than that of its monomeric analog. This may indicate that anionic sites exist on the polymer chain which repel the anionic substrate.

These results indicate that the rate expression for the solvolysis of the substrates studied is composed of at least three terms, _viz._

$$\text{Rate} = k_1 \, [\text{Tz}][\text{S}] + k_2 \, [\text{Tz}][\text{OH}^-][\text{S}] + k_3 \, [\text{OH}^-][\text{S}]$$

The existence of bifunctional catalysis (e.g., $k_4 \, [\text{Tz}]^2[\text{S}]$) has not yet been determined.

We are currently investigating the effect of N-methyltriazole on the solvolytic rates of the above montioned substrates, and possibly the deuterium isotope effect. In this manner we hope to substantiate this unexpected general base catalysis of esters containing good leaving groups.

V. __Preparation of 4(5)-[9-(p-hydroxyphenyl)nonyl] imidazole__

A copolymer of 4(5)-vinylimidazole and p-vinylphenol has been found to be a very efficient catalyst for the solvolyses of neutral, negatively and positively charged substrates.[5] The strong enhancement of this copolymer, relative to the solvolytic rates o obtained with imidazole, poly-4(5)-vinylimidazole, phenol, poly-_p_-vinylphenol, and a copolymer of 4(5)-vinylimidazole-_p_-methoxystyrene, was rationalized by the cooperative interaction between imidazole and

anionic phenol participating in a multifunctional attack on substrate.

The synthesis of the model compound 4(5)-[9-p-hydroxyphenyl)nonyl]-imidazole (VIII) was undertaken to determine if such cooperative interactions can occur in small molecules by employing a proper spacing between the catalytic functions.

The following synthetic has been successfully employed.

$$HOOC-(CH_2)_8-COOH \xrightarrow{Ac_2O} -[O-\overset{O}{\overset{\|}{C}}-(CH_2)_8-\overset{O}{\overset{\|}{C}}-]\, O \xrightarrow[AlCl_3]{CH_3O-\bigcirc}$$

II

$$CH_3O-\bigcirc-\overset{O}{\overset{\|}{C}}-(CH_2)_8\ COOH \xrightarrow{H_2NNH_2} CH_3O-\bigcirc-(CH_2)_9\ COOH$$

III   IV

$$\xrightarrow{SOCl_2} CH_3O-\bigcirc-(CH_2)_9-\overset{O}{\overset{\|}{C}}-Cl \xrightarrow[\text{(2) HCl g}]{\text{(1) } CH_2N_2} CH_3O-\bigcirc-(CH_2)_9-$$

V   VI

$$-\overset{O}{\overset{\|}{C}}-CH_2Cl \xrightarrow{H_2N\overset{\oplus}{C}H-NH_2 OAc^{\ominus}} CH_3O-\bigcirc-(CH_2)_9-\left[\begin{smallmatrix}N\\\\N\\H\end{smallmatrix}\right] \xrightarrow{H^+}$$

VII

$$HO-\bigcirc-(CH_2)_9-\left[\begin{smallmatrix}N\\\\N\\H\end{smallmatrix}\right]$$

VIII
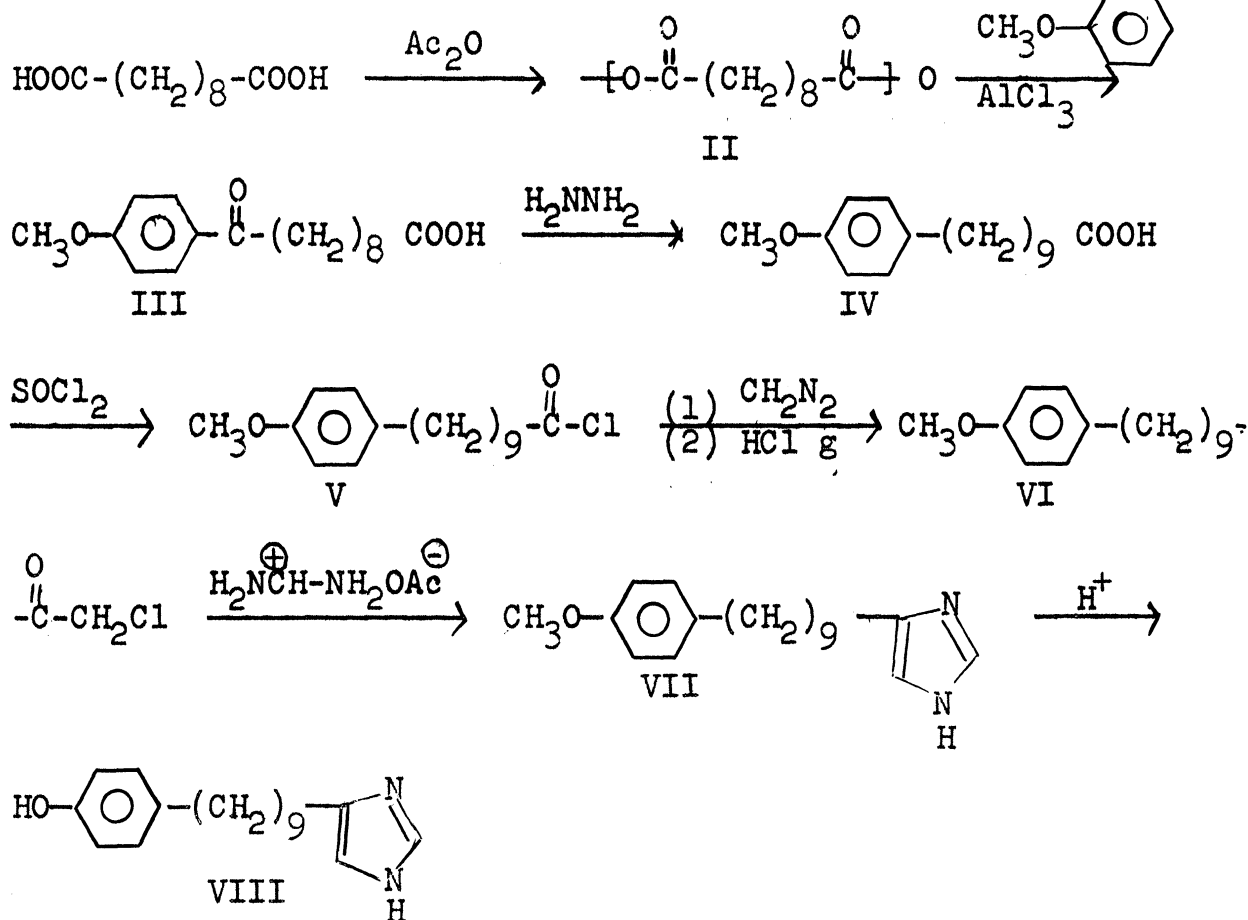
Solvolytic rate measurements are currently in progress.

VI. Experimental

3-Vinyl-1,2,4-triazole. Into an activated 4" column of molecular sieves (dried at 300° for 30 min., 0.4 mm) at room temperature

was placed 0.1 to 0.2 g of 3-β-hydroxyethyl-1,2,4-triazole. Mixing was effected by shaking the stoppered column. The column was then connected to a dry-ice condenser and then immersed into an oil bath pre-heated to 280-300°. After 9-10 min., vacuum (0.4 mm) was applied and both monomer and water condensed on the cold finger. Water was subsequently removed by lyophilization. Yield 15-20%, m.p. 38-40°.

Anal. Calcd. for $C_4H_5N_3$: C, 50.52; H, 5.29; N, 44.17. Found: C, 50.34; H, 5.34; N, 44.24.

Poly (sebacic acid anhydride) (II) (1 mole) was prepared by the procedure of Hill[12].

9-(p-Methoxyphenyl-9-keto) nonanoic Acid III. To 1 mole of poly(sebacic acid anhydride) in a mixture of 250 ml dry nitrobenzene and 1200 ml. dry tetrachloroethane was added 266 g (2 mole) of aluminium chloride with vigorous stirring at 0°. To this mixture was then added 108 g (1 mole) of anisole dropwise, keeping temperature below 5°. The violet colored solution was stirred for a further 4 h and then left to stand at 0-5° for 3 days. The violet complex was destroyed with 250 ml. 37% hydrochloric acid, from which a white solid precipitated. The solvent was removed by steam distillation and a brown solid resulted. The solid was washed several times with benzene (1 l.) and the benzene solution evaporated in vacuo. The solid obtained was dissolved in 1 N sodium hydroxide solution and acidified with hydrochloric acid. The solid obtained was twice ex-

tracted with 500 ml of hot water to remove any unreacted sebacic acid. After decolorization with Norit-A. in 50% ethanol-water, the product was obtained in 51% yield (74-3 g), m.p. 97-99°. Analytical sample recrystallized from cyclohexane yielded colorless needles, m.p. 98-98.5°.

Anal. Calcd. for $C_{17}H_{24}O_4$: C, 69.84; H, 8.27; O, 21.89. Found: C, 69.83; H, 8.34; O, 22.04.

10-(p-Methoxyphenyl) nonanoic Acid (IV). To 10.0 g (0.034 mole) 9-(p-methoxyphenyl-9-keto) nonanoic acid in 80 ml of diethylene glycol was added 5.0 g of sodium hydroxide pellets and 5 ml of 85% hydrazine hydrate. The mixture was refluxed for 1 h. The water formed during the reaction was distilled off slowly until the reaction temperature reached 200°. The mixture was refluxed for 3 h at 200° and then cooled and added to 500 ml water. The pH was adjusted to 6 with hydrochloric acid. A light-yellow solid was obtained (9.75 g, 94% yield) which was recrystallized from heptane. Solution was decolorized with Norit-A. and colorless crystals were obtained, m.p. 64.5-66.5°. Further recrystallization from heptane gave m.p. 66.5-67.5°.

Anal. Clacd. for $C_{17}H_{26}O_3$: C, 73.35; H, 9.41; O, 17.24. Found: C, 73.27; H, 9.42; O, 17.09.

<u>10-(p-Methoxyphenyl) nonanoyl Chloride (V)</u>. To 24.5 g

mole) of 10-(<u>p</u>-methoxyphenyl) nonanoic acid was added 100 ml thionyl

chloride. The mixture was refluxed for 4 h and the excess thionyl

chloride was distilled off at atmospheric pressure. The residual

oil was then distilled at 160-170°/0.05 mm yielding 14.5 g (55%)

of a colorless liquid.

<u>Anal</u>. Calcd. for $C_{17}H_{25}O_2Cl$: C, 68.79; H, 8.49; Cl, 11.95.
Found: C, 68.96; H, 8.53; Cl, 11.84.

<u>α-Chloromethyl-(9-p-methoxyphenyl) nonyl Ketone (VI)</u>. To

14.5 g (0.050 mole) 10-(β-methoxyphenyl) nonanoyl chloride was added

excess diazomethane in ether (prepared from N-nitroso-N-methylurea)

at 0° under a nitrogen atmosphere and anhydrous conditions. The

solution was stirred for 45 min and dry hydrogen chloride was added.

The mixture was left at 0° for an additional 45 min. The ether

solution was then washed with 3-100 ml portions of ice-water and once

with 100 ml 0.1 N sodium bicarbonate solution. The solution was

dried over anhydrous sodium sulfate and the ether evaporated giving

13.2 g (87% yield) of colorless crystals, m.p. 59-61°. Analytical

sample recrystallized from dry heptane, m.p. 60.5-61°.

<u>Anal</u>. Calcd. for $C_{18}H_{27}O_2Cl$: C, 69.55; H, 8.76; Cl, 11.41.
Found: C, 69.39; H, 8.64; Cl, 11.46.

<u>4(5)-[9-(p-hydroxyphenyl) nonyl] imidazole</u>. A solution of 1.2 g

of crude 4(5)-[9-(p-methoxyphenyl) nonyl] imidazole dissolved in
12 ml of glacial acetic acid was saturated with 6 g of dry hydrogen
bromide gas. The reaction mixture was stood at room temperature
for 24 h, at 50° for 2.5 h, and 70° for 1 h. The acetic acid was
distilled off and the residue suspended in anhydrous ether. Ammon-
ia gas was bubbled through the solution until pH 9 was reached. The
ether was then evaporated under reduced pressure yielding a light-
yellow solid. The product was crystallized from 50% ethanol-water,
m.p. 104-106.5°. Further recrystallization from 60% ethanol-water
gave colorless needles, m.p. 106-108.5°.

Anal. Calcd. for $C_{18}H_{26}N_2O$: C, 75.48; H, 9.15; N, 9.78.
Found: C, 75.34; H, 9.03; N, 9.80.

4(5)-[9-(p-Methoxyphenyl) nonyl] imidazole (VII). In 250 ml
of dry diethylene glycol was added 14.0 g (0.045 mole) α-chloromethyl-
(9-p-methoxyphenyl) nonyl ketone and 18.3 g (0.175 mole) of dry
formamidine acetate salt. The mixture was heated for 50 h at 135°
under a nitrogen atmosphere during which time the solution became
brown. The diethylene flycol was removed in vacuo and the residual
oil was added to a Dowex 50 Wx8 ion exchange resin (acidic form).
The desired compound was released by treatment with 0.1 N ammonium
hydroxide in 90% ethanol-water. The brown solution was evaporated
yielding a brown oil which was distilled at 180°/ 0.025 mm. A
light-yellow waxy solid was obtained (5 g, 37% yield), m.p. 58-73°.
Analytical sample recrystallized from diethyl malonate, m.p. 74-76°.

Anal. Calcd. for $C_{19}H_{28}N_2O$: C, 75.95; H, 9.39; N, 9.33.
Found: C, 75.86; H, 9.24; N, 9.31.

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Charged esters Polymeric imidazole catalysis						
1,2,4-Triazole 4(5)-[9-(p-Hydroxyphenyl)nonyl]-						
Catalytic activity imidazole						
Solvolytic rates						
Inhibitory effect						
Monomer sequence						
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