MECHANISM OF ENZYME ACTION

Semiannual Report

by

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Foreword

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Digest

The reactions of imidazole and benzimidazole containing polymers with re-
active esters have revealed several analogies to those of enzyme catalyzed
catalyzed processes. Although the reactions of synthetic macromolecules have
been considerably less efficient than those of natural macromolecules, the
synthetic polymers have revealed a higher reactivity than their monomeric
analogs,[2,21] a specificity in their solvolytic reactions,[2,4,22] a competi-
tive inhibition by substances similar to the reactive substrate,[22,23] a bi-
functional interaction between two catalytic functions and a substrate,[2,3,11]
and a complexation (saturation) by high and low molecular weight esters.[22,24]

Anionic species of 1,2,4- and 1,2,3-triazoles were shown to be the active
species in the catalytic solvolysis of p-nitrophenyl acetate, sodium 3-nitro-
4-acetoxybenzenesulfonate (NABS), and 3-acetoxy-N-trimethylanilinium iodide
(ANTI). The effectiveness of a particular species as a catalyst is related
to its pK_a value. It was expected from the similarity in structure of imidaz-
ole and 1,2,4-triazole, that poly-3-vinyl-1,2,4-triazole would perhaps show
enhanced catalytic effects compared to its monomeric analog 1,2,4-triazole if
similar cooperative interactions proposed for poly-4(5)-vinylimidazole[2] are
also operative in the poly-3-vinyl-1,2,4-triazole system. Furthermore, the
reported catalytic effect of 1,2,4-triazole in peptide syntheses,[25,26] pro-
vided much hope in such an expectation.

The experimental data and the pK_a relationship mentioned above may serve
as a guide for further investigations in the catalytic behavior of other five-
membered nitrogen heterocycles and their corresponding polymers, or to tailor
make a copolymer to demonstrate a certain type of bifunctional interaction.

The reaction between hydroxide and tris(choline chloride) phosphate has
been investigated in the presence and absence of various polymers. The effect
of salt concentration on the reaction in the presence of polyacrylic acid was
investigated. This investigation offers a method for estimating the dissocia-
tion constants for multiplicharged species in solution and the equilibrium
constant for the displacement of $TCPCl_3$ from the polymer by K^+ ions being dis-
placed. The number of polymeric pendent groups required to bind one substrate
molecule was estimated to be 4.0 for polyvinyl sulfonic acid between pH 8.0
and pH 10.0 and this number for polyacrylic acid appears to decrease from 4.0
to 2.7 between pH 8.0 and pH 10.0.

Other polymers had little effect on the rate of the reaction except poly-
p-vinylphenol which enhanced the rate between 2.5 and 1.25 times and copoly-
4(5)-vinylimidazole-acrylic acid which inhibited the reaction as if the vinyl-
imidazole were not present in the polymer.

An investigation of the solvolysis of a paraffinic, anionic ester catalyzed by poly-4(5)-vinylimidazole has revealed rapid solvolytic rates which appear to approach those of enzymic reactions when the solvent system contains a high water content.[12] The results of the effects of temperature and pH on the solvolyses of 3-nitro-4-acetoxybenzoic acid (NABA) and 3-nitro-4-dodecanoyloxy-benzoic acid (NDBA) are reported.

The synthesis of 5(6)-vinylbenzimidazole has been reported,[27] but the last step in the sequence of reactions is not reproducible and gives yields of 0% to 67%. A new synthetic approach is being developed with the purpose of preparing large amounts of 5(6)-vinylbenzimidazole, in a reproducible manner, to be used in the preparation of various copolymers and the subsequent study of their catalytic activities.

The synthesis of stereospecific poly-4(5)-vinylimidazole (both syndiotactic and isotactic) is being investigated. The behavior of such an orderly structured polymer toward esterolysis reactions would be expected to be more pronounced than that of the random poly-4(5)-vinylimidazole utilized in previous studies. It may more closely mirror the activity of naturally occurring enzymes.

The catalytic effect of polyvinylimidazole and various copolymers on the hydrolysis of dinucleoside (3'-5') monophosphates and nucleoside (2'-3') cyclic phosphates is being studied to determine any rate enhancing effects over imidazole monomer. Copolymers may also show a rate enhancing effect due to the presence of two imidazole moieties at the active site of the hydrolytic enzyme ribonuclease.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

6

Results and Discussion

I. ESTEROLYTIC CATALYSES BY TRIAZOLES

Studies of the solvolytic reactions of the neutral ester p-nitrophenyl acetate (PNPA) catalyzed by 1,2,4-triazole[I] and by poly-3 vinyl-1,2,4-triazole[II] were undertaken in 28.5% ethanol-water solution at 26°C and with an ionic strength, μ, of 0.02 M.
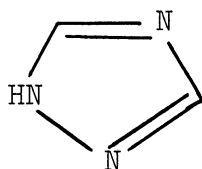
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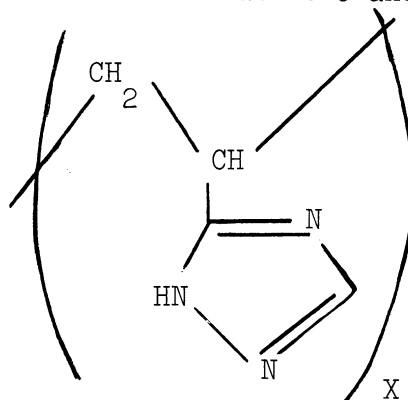
II

A study of the dependence of solvolytic rate on poly-3-vinyl-1,2,4-triazole concentration revealed that the catalytic rates for the solvolyses of PNPA were directly proportional to the polymer concentration. Similarly, it was shown that the reactions catalyzed by 1,2,4-triazole were also linearly related to the catalyst concentration.[1] Although both of these processes are second-order reactions, it appears that at pH 8, the polymer is a less efficient catalyst than its monomeric analog. A similar situation, however, has previously been found for the solvolyses of PNPA catalyzed by poly-4(5)-vinylimidazole and imidazole, where at pH values below 7.5 the polymer was a less efficient catalyst than imidazole.[2]

In order to study in more detail the characteristics of poly-3-vinyl-1,2,4-triazole and 1,2,4-triazole catalyzed reactions, the solvolyses of neutral, anionic (sodium-3-nitro-4-acetoxybenzenesulfonate, NABS) and cationic (3-acetoxy-N-trimethylanilinium iodide, ANTI) substrates were investigated over a range of pH values. In the solvolysis of the neutral ester PNPA in the pH region 7 to 9, instead of observing an enhancement of the polymeric reaction rate as was expected from analogous studies of poly-4(5)-vinylimidazole, a reduced catalytic effect was observed in comparison with its monomeric analog 1,2,4-triazole. Poly-3-vinyl-1,2,4-triazole was found to be a much poorer catalyst than 1,2,4-triazole even at high pH values. In fact, for the

solvolysis of PNPA, 1,2,4-triazole was found to have a second-order rate constant (k_{cat}) approximately 50% greater than that of poly-4(5)-vinylimidazole at pH 9 under similar experimental conditions.[2]

The ineffectiveness of poly-3-vinyl-1,2,4-triazole as a catalyst was also revealed in the solvolyses of both negatively charged substrates NABS and NABA and the positively charged substrate ANTI. At each pH value investigated, it was found that 1,2,4-triazole was a more efficient catalyst than poly-3-vinyl-1,2,4-triazole in the solvolyses of all the substrates studied. Furthermore, no distorted bell-shape pH-rate profile was observed for the poly-3-vinyl-1,2,4-triazole catalyzed solvolysis of NABS in the pH region where the polymer was partially protonated and the substrate was completely anionic, i.e., between pH 1.7 to 4.1, in fact, no measurable rate could be detected in this pH region. Such an effect has been reported for several polymeric systems whereby the partially protonated sites on the polymer chain accumulate the anionic substrate into a high concentration of neutral, catalytically active functions.[2] It is indeed surprising that no selective catalysis, i.e., enhanced reaction rates, was found for any of the polymeric reactions. Although polymeric systems can be less efficient catalysts than their monomeric analogs, particularly with neutral substrates, they usually exhibit marked selectivity towards substrates which carry an opposite charge to that of the charged group on the polymer chain. This type of cationic-neutral interaction was obviously not noted in the poly-3-vinyl-1,2,4-triazole system.

Upon inspection of the pH-rate profile data,[1] it is noted that there is an increased reaction rate for the 1,2,4-triazole and the polymer catalyzed reactions as the pH of the solution is increased, the 1,2,4-triazole reaction being increased to a greater extent. Such phenomena are reminiscent of the pendent benzimidazole catalyzed reaction of PNPA, NABS, and NABA (3-nitro-4-acetoxybenzoic acid) where it was reported that these reactions at high pH values involved the participation of anionic functions in a cooperative interaction with neutral functions.[3]

If there are any cationic-neutral, neutral-neutral, or anionic-neutral interactions, such as proposed for poly-4(5)-vinylimidazole, poly-4-vinylpyridine and poly-5(6)-vinylbenzimidazole,[2-5] operative in the poly-3-vinyl-1,2,4-triazole catalyzed reactions, then it would be expected that poly-3-vinyl-1,2,4-triazole would show some enhanced catalytic effects with one of the four substrates. Since no such bifunctional interactions are indicated with poly-3-vinyl-1,2,4-triazole, the catalytic process may involve either cationic, neutral, or anionic 1,2,4-triazole participation.

At pH 7.25, 1,2,4-triazole, though a better catalyst than poly-3-vinyl-1,2,4-triazole, is not a particularly efficient catalyst. Calculations of the fractions of neutral 1,2,4-triazole (α_1) based on the pK_2 value of 2.28 showed that $\alpha_1 = 1$ at pH 6.3. At pH 7.25, almost all of the 1,2,4-triazole species are still in the neutral form, but on the anionic side of the isoelectric point. As the pH value is increased beyond the isoelectric point, the fraction

8

of neutral 1,2,4-triazole species will obviously decrease, since there will be an increase in the anionic species. Therefore, for all the substrates studied, the neutral 1,2,4-triazole species could not be responsible for the drastic increase in catalytic rates as the pH is increased from 7 to 9.

The observed catalytic effect of 1,2,4-triazole could apparently be accounted for by the anionic 1,2,4-triazole species. If this assumption is true, then a linear relationship between the second-order rate constant, k_{cat}, and the anionic fraction of 1,2,4-triazole species, α_2, should be obtained. Indeed, such a linear relationship was obtained for 1,2,4-triazole catalyzed solvolyses of PNPA, NABS, and ANTI.[1] Since pendent, neutral-neutral, and anionic-neutral interactions are believed to be unlikely for the poly-3-vinyl-1,2,4-triazole catalyzed solvolyses of all the substrates studied, and that the neutral pendent 1,2,4-triazole, it would appear that the mechanisms of catalyses by the polymer are analogous to those of 1,2,4-triazole. Indeed, a linear relationship was found between k_{cat} and α_2 for the poly-3-vinyl-1,2,4-triazole catalyzed solvolyses of all the substrates.[1]

Since the catalytic activity of 1,2,4-triazole ($pK_2 = 10.28$) is apparently due to its anionic species, it would be expected that anionic species participation would also be operating in the 1,2,3-triazole ($pK_2 = 9.50$) system. The pK_1 value of 1,2,3-triazole was found to be too low to be determined by differential potentiometric titration under the same conditions studied for the 1,2,4-triazole system.

The studies of the dependence of the solvolytic rates of PNPA on 1,2,3-triazole concentrations revealed linear relationships, an indication that these are overall second-order reactions. In a study of the solvolyses of PNPA, NABS, and ANTI by 1,2,3-triazole over the pH range 7.25 to 8.80 (Figure 1), drastic increases in the catalytic rates were observed as the pH was increased.

When the second-order catalytic rate constants k_{cat} were plotted against α_2 linear relationships were indeed obtained for each substrate investigated (Figure 2). These results indicate that 1,2,3-triazole is more efficient for the solvolysis of ANTI, less efficient for PNPA, and least efficient for NABS. These observations are consistent with the fact that the anionic 1,2,3-triazole species is responsible for these solvolytic processes, since ANTI would be electrostatically attracted by the anionic species, while NABS would be repelled and PNPA would experience no such electrostatic effect. This trend was also observed with 1,2,4-triazole. A study of 1,2,3-benzotriazole ($pK_2 = 8.65$) showed similar results.

Studies of the catalytic effects of imidazole, 1,2,4-triazoles, 1,2,3-triazoles, and 1,2,3-benzotriazole in the solvolyses of the substrates studied indicated that their catalytic activities are related to their pK_1 or pK_2 values. A relationship of this type has been described by Brønsted where the logarithm of the catalytic rate constant is proportional to the pK_a values of the base.[6]

9
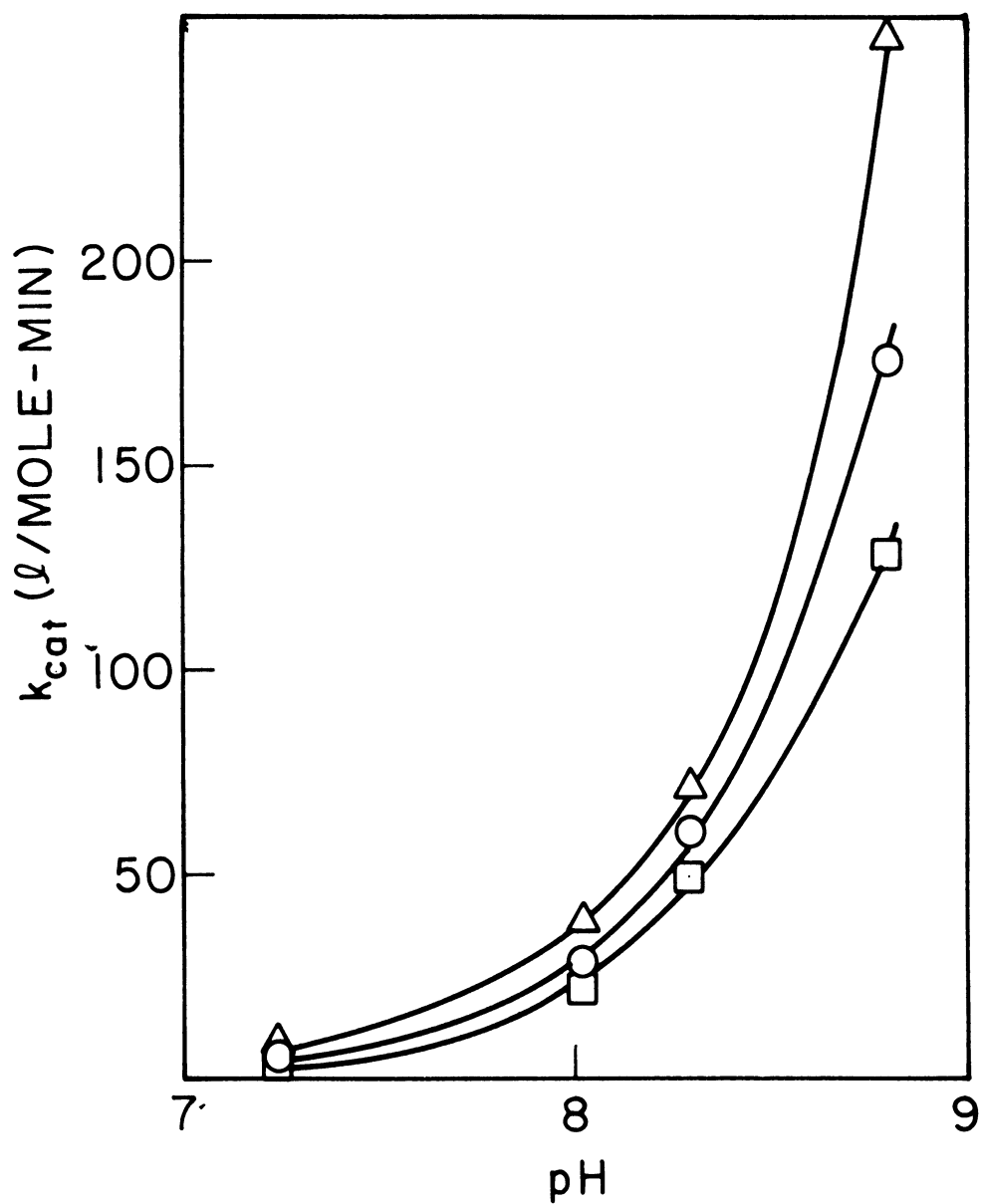
Figure 1. pH-Rate profiles for the solvolyses of PNPA (O), NABS (☐), and ANTI (△) catalyzed by 1,2,3-triazole in 28.5% ethanol-water, ionic strength 0.02, 26°C.
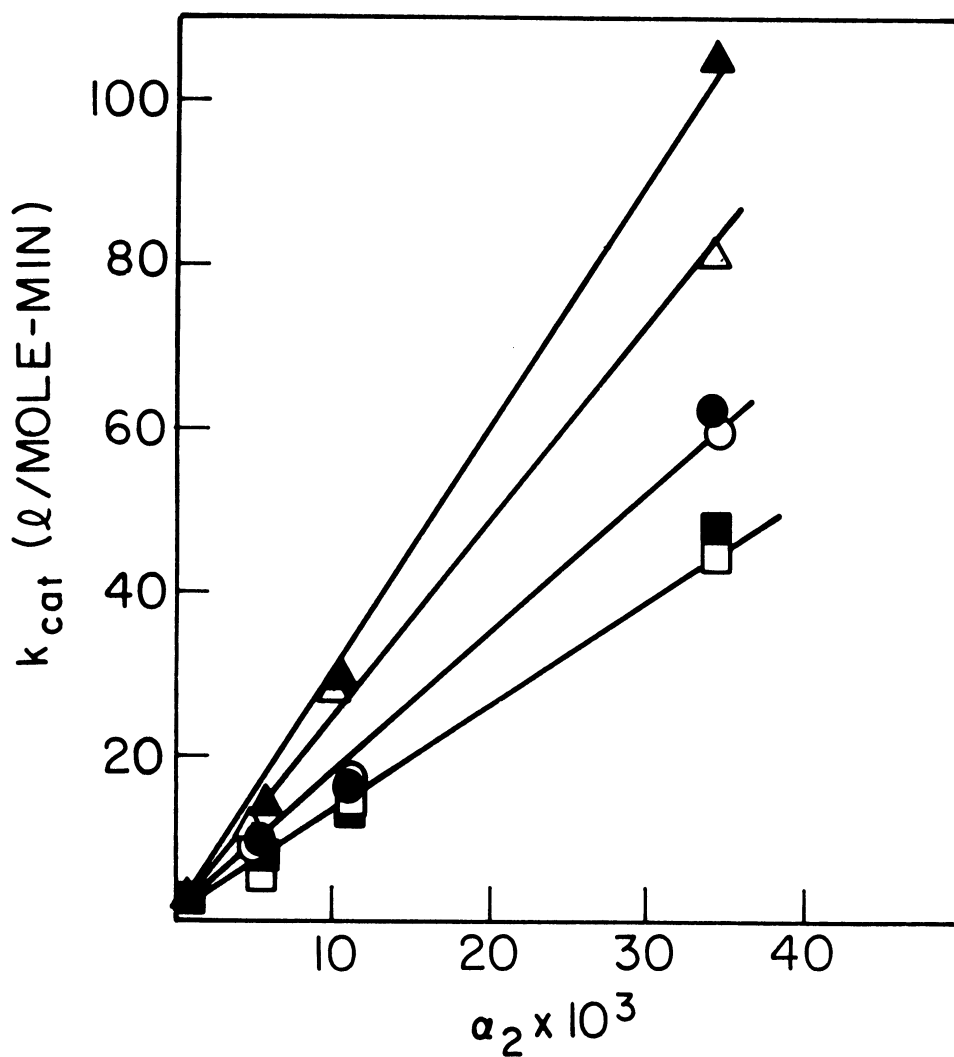
Figure 2. k_{cat}-α_2 profiles for the solvolyses of PNPA (O,●), NABS (□,■), and ANTI (△,▲) catalyzed by 4-methyl-1,2,3-triazole and 4-isopropyl-1,2,3-triazole, respectively; in 28.5% ethanol-water, ionic strength 0.02, 26°C.

The second-order catalytic rate constants (k_{cat}) thus far obtained for the imidazole and triazole systems are related to the total concentration of the catalysts, and not to the concentration of the catalytically active species. Therefore, in order to consider a relationship between the catalytic abilities of the various catalysts, the corrected second-order catalytic rate constants should be used, i.e., k_{cat} has to be corrected by a factor of $1/\alpha_i$ where α_i is the fraction of the catalytically active species present at any pH. We could then define k_i, the corrected second-order catalytic rate constant in the following manner,

$$k_i = k_{cat}/\alpha_i$$

Where i = 1 or 2 for neutral or anionic species, respectively. When i = 2, then k_2 is obtained from the slope of a plot of k_{cat} versus α_2.

The neutral substrate PNPA would be the best system to study for a consideration of the relationships between k_i and pK_i values because electrostatic effects are unlikely. The corrected second-order catalytic rate constants k_i are summarized in Table I. Poly-3-vinyl-1,2,4-triazole, 3-methyl- and 3-isopropyl-1,2,4-triazole are not considered, since steric factors are involved in their solvolytic reactions.

TABLE I

CORRECTED SECOND-ORDER CATALYTIC RATE CONSTANTS FOR THE SOLVOLYSES
OF PNPA IN 28.5% ETHANOL-WATER AND μ = 0.02

Catalyst	i	k_i(1/mole-min) (average)	pK_i	No. of Runs
1,2,4-Triazole	2	825.0	10.28	7
1,2,3-Triazole	2	998.0	9.50	7
4-Methyl-1,2,3-triazole	2	1570.0	10.25	7
4-Isopropyl-1,2,4-triazole	2	1629.0	10.25	7
1,2,3-Benzotriazole	2	247.0	8.65	7
4(5)-Methylimidazole	1	32.0	7.40	4
Imidazole	1	24.3	6.95	*
N-Methylimidazole	1	8.2	6.92	*

*Values obtained from References 2 and 5.

A linear relationship was realized when log k_i was plotted against pK_i. This behavior is analogous to that expected by the Brønsted relationship, except that both pK_1 and pK_2 values are considered together. These findings

indicate that the catalytic activity of a species is solely dependent upon its pK_i value and not on the nature of such species, i.e., if an imidazole compound having a pK_1 of 9.50, then its neutral species would have the same catalytic activity of anionic 1,2,3-triazole species at any pH; conversely, we could predict that anionic tetrazole, $pK_2 = 4.89$[7] would exhibit a k_2 of 1 l/mole-min in the solvolysis of PNPA. Similarly, the neutral species of pyrazole $pK_1 = 2.53$,[8] would also be a poor catalyst for such reactions, a k_1 of less than 1 l/mole-min is predicted. Therefore, the nature and activity of a five-membered nitrogen heterocycle could apparently be predicted to a certain degree in ester solvolyses when no steric factor is involved. This k_i and pK_i relationship could be expressed in the following empirical equation,

$$\log k_i = npK_i + \log C$$

where both C and n are constants, n and log C were determined to be 0.612 and -2.97, respectively.

II. APOLAR BONDING IN MACROMOLECULE-SUBSTRATE COMPLEXATION

The rapid solvolytic reactions of NDBA which appear to follow the Michaelis-Menten mechanism, presumably involve an accumulation of the substrate in the vicinity of the polymer chain by either an electrostatic interaction between a protonated PVIm and negatively charged NDBA or by apolar interactions between the interacting hydrocarbon components. In order to ascertain the main contribution of both interactions, the temperature dependence and the pH dependence of the solvolytic reactions of NDBA were measured and compared with the solvolytic reactions of NABA. Completely different behaviors of NDBA and NABA were observed (Figures 3, 4, 5). Although the PVIm catalyzed solvolytic reaction of NDBA was shown to follow the Michaelis-Menten mechanism, that of NABA is a simple second-order reaction for substrate and catalyst concentration at the different pH values studied. Thus, it is concluded that the rapid solvolytic reaction of NDBA is attributed mainly to the strong apolar interactions between the long aliphatic chain of the substrate (dodecanoyl group) and the long main chain of the catalyst, based on the composition of the reaction solvent.

The solvolytic reaction rates of NDBA catalyzed by PVIm in 20% and 30% (vol) ethanol at pH 7.90 ($\mu = 0.02$) were measured by the technique of stopped-flow spectroscopy. Saturation behavior in 20% and 30% (vol) ethanol as well as that in 43.7% (vol) ethanol was observed. The Michaelis constants, k_m, and the first-order rate constants, k_2, were calculated using the treatment by Lineweaver and Burke. The results are tabulated in Table II.
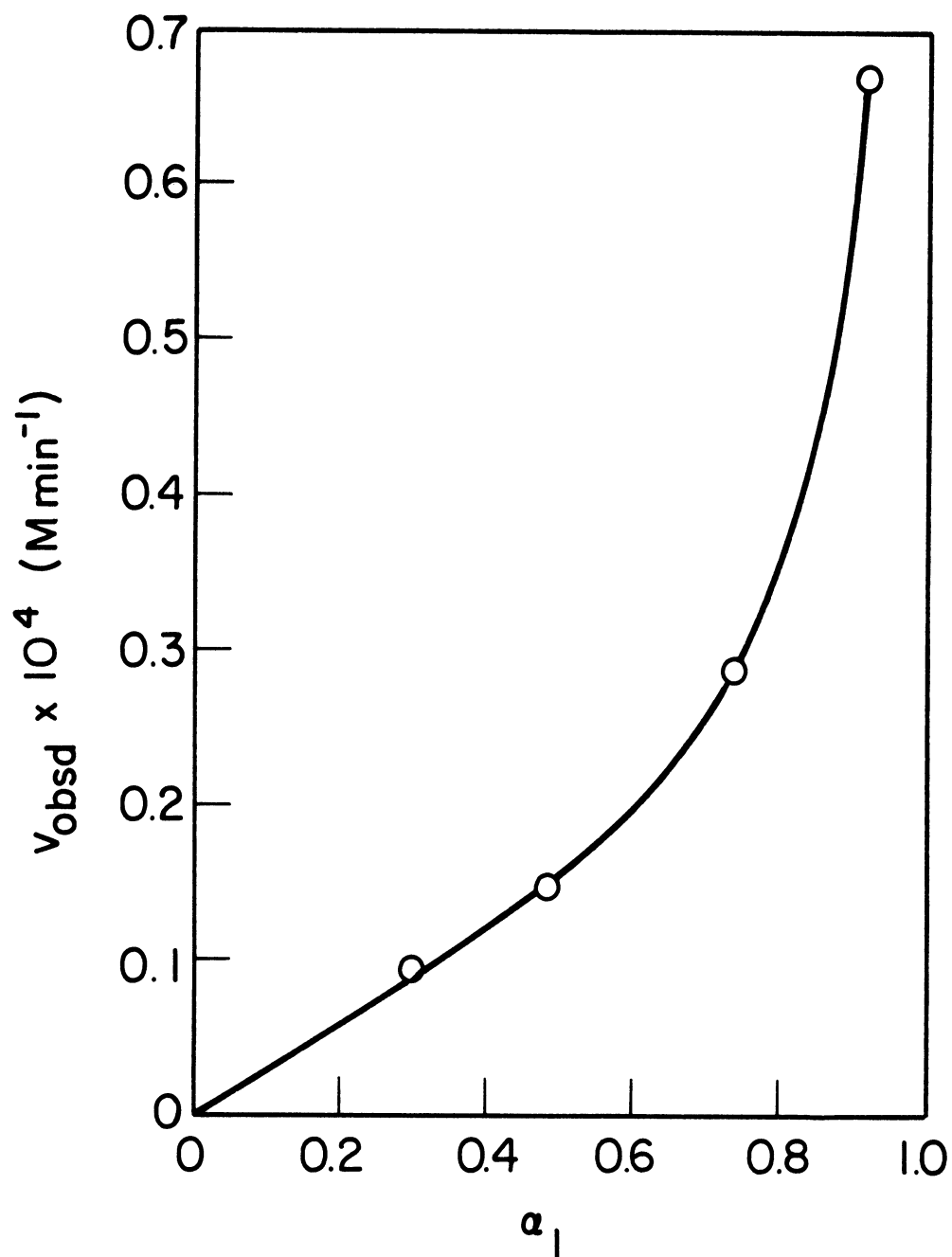
Figure 3. pH-Rate profile for PVIm catalyzed NDBA solvolysis; in 25% ethanol-water, ionic strength 0.02, 25°C; [NDBA] = 5 x 10^{-5}, [PVIm] = 5 x 10^{-4}.

Figure 4. Temperature dependence of PVIm, imidazole catalyzed solvolyses of NABA, NDBA; in 25% ethanol-water, ionic strength 0.02, pH 7.30 ~ 8.11, [PVIm] = 9.38 x 10^{-4}; [NDBA] = 5 x 10^{-5}; [NABA] = 5 x 10^{-5}.
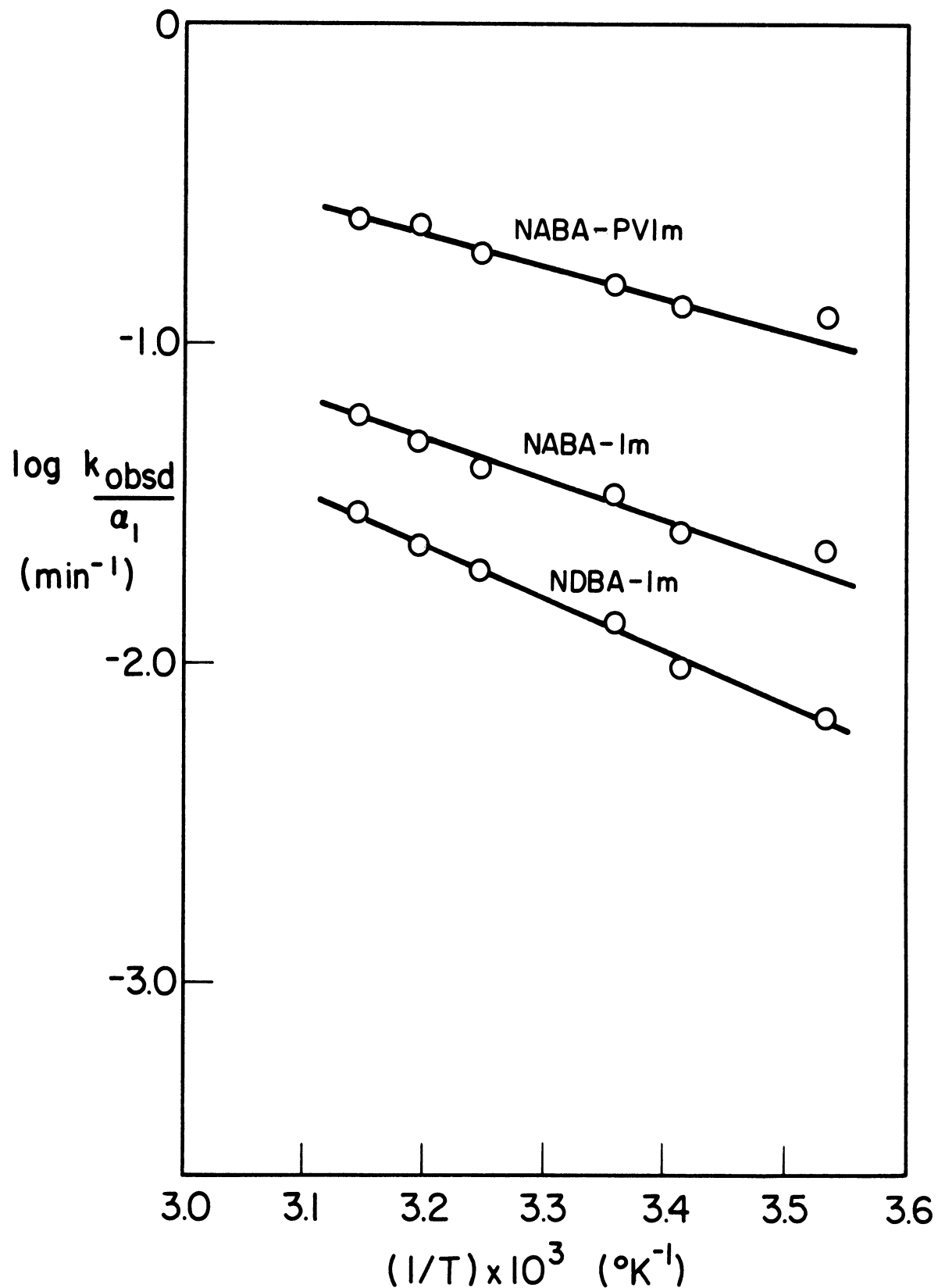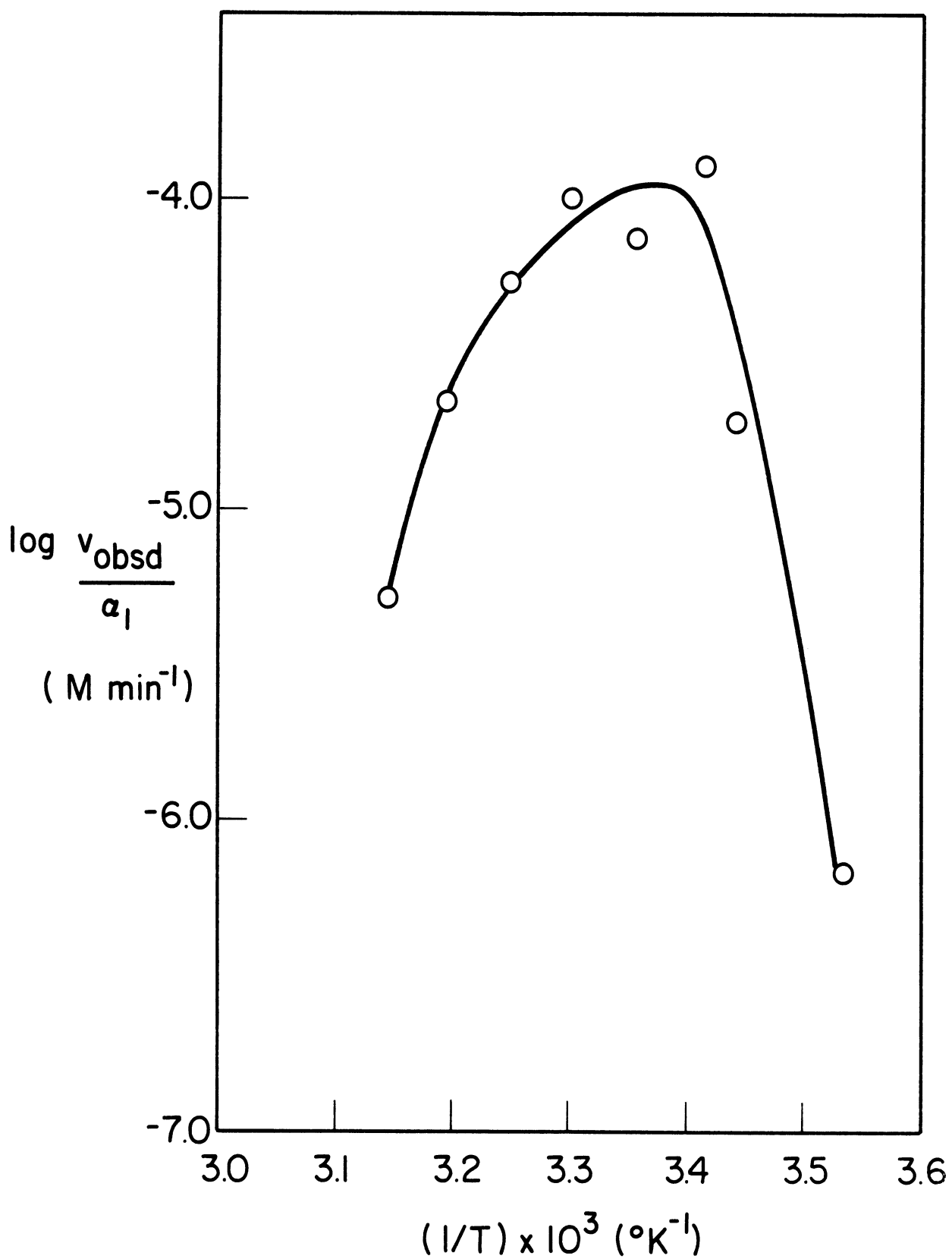
Figure 5. Temperature dependence of PVIm catalyzed solvolysis of NDBA; in 25% ethanol-water, ionic strength 0 02, pH 7.30 ~ 8.11, [NDBA] = 5 x 10^{-5}, [PVIm] = 5 x 10^{-4}.

TABLE II*

MICHAELIS CONSTANTS AND SECOND-ORDER RATE CONSTANTS FOR THE SOLVOLYSIS
OF NDBA CATALYZED BY POLY-4(5)-VINYLIMIDAZOLE

% ETOH (vol)	[NDBA] [M]	K_m[M]	k_2(sec^{-1})
20	8.9×10^{-5}	$(3.82 \pm 1.09) \times 10^{-5}$	(11.4 ± 1.8)
30	1.78×10^{-4}	$(3.11 \pm 0.8) \times 10^{-4}$	(20.7 ± 4.0)

*pH 7.90, $\mu = 0.02$.

It was found that the deacylation rate of dodecanoyl PVIm, known as the intermediate compound, is about 100 times smaller than that of dodecanoyl-imidazole. Therefore, the overall rate determining step of the PVIm-catalyzed solvolytic reaction of NDBA is the deacylation step.

III. THE EFFECT OF POLYELECTROLYTES ON THE HYDROLYSIS OF TRIS-(CHOLINE CHLORIDE) PHOSPHATE

In studies of the effects of polymers on the reactions of small molecules, two conditions may be encountered. First, the reaction rate constant within the polymer region is the same as the intrinsic rate constant in the absence of polymers, and, second, the rate constant within the polymer domain is not the same as that outside the polymer domain. The second condition is observed if the groups on the polymer chain actually enter into the mechanism of the reaction, whereas effects observed when there is no chemical interaction in the rate determining step are due to concentration, solvent, and structure changes and electrostatic field effects.

Polyelectrolytes, which have high charge densities, have dramatic effects on ionic reactions. The present work has involved the investigation of the hydrolysis of the charged phosphate ester, tris(choline chloride) phosphate (TCPCl$_3$) in the presence of polyions. The effect of ionic strength on the reaction between hydroxide ion and TCPCl$_3$ was investigated in the presence and absence of polyions, since ionic strength greatly affects ionic reactions and also affects the properties of highly charged polyions.

It has been observed that counterions in polyelectrolyte solutions have low activity coefficients.[9] Part of this effect may be ascribed to long-range electrostatic forces which would be large in the case of polyions which have high charge densities. Another part of this effect is due to actual ion pair or complex ion formation between counterions and charged groups along the polymer chain.

Tris(choline chloride) phosphate (TCPCl$_3$) has three positive charges,

$$\left\{ \begin{array}{l} O - C_2H_4\ \overset{+}{N}\ (CH_3)_3 \\[2pt] | \\ O = P - O - C_2H_4\ \overset{+}{N}\ (CH_3)_3 \\[2pt] | \\ O - C_2H_4\ \overset{+}{N}\ (CH_3)_3 \end{array} \right\} (Cl^-)_3$$
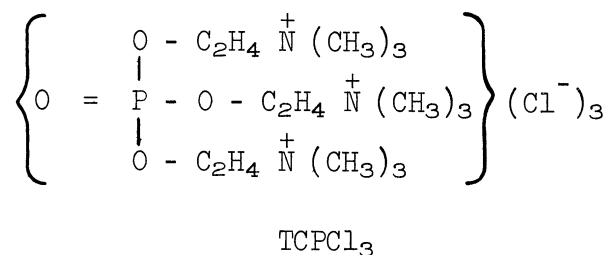
$$TCPCl_3$$

and it would be expected that this molecule would be strongly affected by polyanions.

Unlike the other esters used in our investigations, $TCPCl_3$ has no significant ultraviolet absorption which dictates that spectrophotometric methods could not be used in following the hydrolysis reaction. Since the reaction between $TCPCl_3$ and water gives the strong acid diester and choline, and the products of the reaction with hydroxide ion are entirely neutral, it was decided that the reaction could be followed with a pH-stat. In principle, a pH-stat can follow any reaction that proceeds with a change in pH.

Since $TCPCl_3$ is a bolion, it is not likely to be completely ionized even in dilute aqueous solution. With three ionizable sites there can be three dissociation constants corresponding to:

$$TCPCl_3 \overset{k_1}{\underset{}{\rightleftharpoons}} TCPCl_2^{\oplus} + Cl^{\ominus} \overset{k_2}{\underset{}{\rightleftharpoons}} TCPCl^{2\oplus} + 2Cl^{\ominus} \overset{k_3}{\underset{}{\rightleftharpoons}} TCP^{3\oplus} + 3Cl^{\ominus}$$

An interesting method for evaluating these constants is in studies of the effect of ionic strength on the hydrolysis of this material. The equation for the salt effect in ionic reactions used is[10]:

$$\log r = \log r_o + 1.02\ Z_a Z_b \sqrt{I}$$

where r is the observed rate, r_o is the rate at zero ionic strength, Z_a and Z_b are the charges of species A and B, respectively, and I is the ionic strength. In the case of $TCPCl_3$ (Table 3), if $\log r$ is plotted versus $\sqrt{I}$, a straight line is not obtained which indicates that there is a change in the charged product with change in ionic strength for the reaction between $TCPCl_3$ and OH^-. From the slope of the curve for this reaction it is possible to estimate the number of chloride ions dissociated from $TCPCl_3$ and hence the dissociation constants. These constants were estimated to be 0.5, 1.5, and 2.5, which represent charges of 0.5, 1.5, and 2.5, respectively. The dissociation constant K_x may be written:

$$K_x = \frac{[TCPCl_{4-x}][Cl^-]}{[TCPCl_{3-x}]}$$

18

When the charges are at half-integral values, the ratio $\dfrac{[\text{TCPCl}_{4-x}]}{[\text{TCPCl}_{3-x}]} = 1$, and

the dissociation constant equals the chloride ion concentration at these points. In the case of TCPCl_3 these constants are estimated to be 0.23, 0.07, and 0.009 for k_1, k_2, and k_3, respectively.

TABLE III*

HYDROLYSIS OF TCPCl_3 IN THE PRESENCE AND ABSENCE OF POLYACRYLIC
ACID AT 60°C, pH 9.0, 10% ETHANOL-WATER

I	$r_o \times 10^6$	$r_{PAA} \times 10^6$
0.011	20.0	0.69
0.031	13.5	--
0.051	12.0	2.7
0.071	8.9	4.0
0.111	7.5	5.1
0.161	6.2	5.9
0.211	5.6	5.4
0.411	4.5	4.5

*Initial rates r_o and r in moles
per liter per minute; $[\text{TCPCl}_3] =$
$10^{-3}\underline{M}$ $[\text{PAA}] = 10^{-2}\underline{M}$, 80% ionized.

In spite of the tendency of TCPCl_3 to form ion pairs, it would be expected that it ionizes completely in the field of a polyanion. In the investigation of the effect of ionic strength on the inhibition of the hydrolysis of TCPCl_3 by the potassium salt of polyacrylic acid (PAA), an equilibrium may be assumed between the substrate and polymer-bound potassium ions:

$$\text{TCPCl}_{3f} + x\text{K}_b^{+} \rightleftarrows \text{TCP}_b^{3+} + x\text{K}_f^{+} + 3\text{Cl}^{-}$$
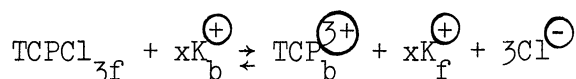
where TCPCl_{3f} represents the substrate not bound to the polymer, K_b^{+} represents potassium ions bound to the polymer, TCP_b^{3+} represents polymer bound substrate, and K_f^{+} represents free potassium ions. Since Cl^{-} is not involved in the interaction with PAA the equilibrium constant may then be written:

$$K = \left[\frac{\text{TCP}_b^{+3}}{\text{TCPCl}_3}\right]\left[\frac{\text{K}_f^{+}}{\text{K}_b^{+}}\right]^{x} \tag{1}$$

19

In logarithmic form the above equation may be written:

$$\log K = \log\left[\frac{TCP_b^{+3}}{TCPCl_3}\right] + x \log\left[\frac{K_f^+}{K_b^+}\right] \qquad (2)$$

It is then assumed that TCP_b does not hydrolyze at all, and that all polymer sites not occupied by substrate are occupied by potassium ions. Under the experimental conditions employed in this investigation, the polymer is 80% ionized.

The following analysis is used to estimate the values of K and x:

Let $j = [TCPCl_3]_f/[TCPCl_3]$, the fraction of $TCPCl_3$ which is free. Since the rate of hydrolysis is assumed to be zero for the bound substrate, and equal to the blank rate for the unbound substrate, then:

$$j = \frac{r}{r_o}$$

where r is the observed rate in the presence of polymer and r_o is the rate in the absence of polymer at any ionic strength, I. Equation (1) now may be written

$$K = \left[\frac{1 - j}{j}\right]\left[\frac{I - 3 - P - x(1 - j)}{P - x(1 - j)}\right]^x \qquad (3)$$

where I is the millimolar ionic strength and P is the millimolar concentration of ionized polymer sites. (I-3) represents all of the potassium ions and P - x(1 - j) represents the potassium ions bound to the polymer. Since [PAA] = 10.0 and it is 80% ionized, P = 8.0 in this investigation. The calculated values for j and (1 - j) are presented in Table IV. Equation (2) may now be expressed

$$\log K = \log\left[\frac{1 - j}{j}\right] + x \log\left[\frac{I - 11 + x(1 - j)}{8 - x(1 - j)}\right] \qquad (4)$$

It is assumed that the value of x is between zero and three. It is also as-sumed that the change in the quantity $\log\left[\dfrac{I - 11 + x(1 - j)}{8 - x(1 - j)}\right]$ is linear with a change in x since $I \gg x(1 - j)$. The approximate equations for log K are pre-

sented in Table V and the values for log K at x = 1, x = 2, and x = 3 are presented in Table VI.

TABLE IV*

CALCULATED FRACTIONS FOR $TCPCl_3$ BOUND TO AND FREE FROM THE POLYMER
DETERMINED FROM THE DATA IN TABLE III

I	$TCPCl_{3f}$	$TCPCl_{3b}$
11	0.04	0.96
51	0.25	0.75
71	0.45	0.55
111	0.71	0.29
161	0.89	0.11
211	0.95	0.05

*Total concentration of $TCPCl_{3f}$
and $TCPCl_{3b}$ equals 1 milli-
mole per liter. I is in milli-
moles per liter.

TABLE V*

APPROXIMATED EQUATIONS USED IN THE CALCULATION OF K AND x DETERMINED
FROM EQUATION (4) AND THE VALUES OF TABLE IV

I	Equation (4); log K =
51	0.477 + x(0.699 + 0.04x)
71	0.087 + x(0.875 + 0.03x)
111	-0.388 + x(1.097 + 0.008x)
161	-0.908 + x(1.279 + 0.004x)
211	-1.278 + x(1.398 + 0.0015x)

*I is in millimoles per liter.

TABLE VI*

VALUES OF LOG K CALCULATED AT x = 1, x = 2, and
x = 3 FROM THE EQUATIONS IN TABLE V

I	x = 1	Log K x = 2	x = 3
51	1.216	2.035	2.935
71	1.022	1.957	2.982
111	0.713	1.818	2.975
161	0.375	1.666	2.965
211	0.122	1.524	2.928

*I is in millimoles per liter.

The values of x is seen to be three since this is the value where the equilibrium constant is actually constant; this is as expected because $TCPCl_3$ has three positive charges. The value for K is $(9.0 \pm 0.5) \times 10^2$. At the lowest value of ionic strength, the rate of hydrolysis of bound $TCPCl_3$ is significant, about 4% of the rate in the absence of polymer; at this ionic strength essentially all of the $TCPCl_3$ would be bound to the polymer.

With polyvinylsulfonic acid, the rate of the hydrolytic reaction of $TCPCl_3$ at pH 8.0, 9.0, and 10.0 was found to linearly decrease with respect to polyvinylsulfonic acid (PVS) concentration. With PVS, it was found that a zero rate occurs at about 4.0 charges per phosphorous atom or about 1.33 eq polymer per charge of $TCPCl_3$ at all pH values. For PAA at pH 8.0 the solvolytic rate is also zero at 4.0 charges per phosphorous atom.

The hydrolysis of $TCPCl_3$ was also carried out with other polymers. Poly-3-vinyl-1,2,4-triazole was used because triazole has a low pK_2 and has strong nucleophilic character. There was, however, no effect on the rate of the reaction at a pH of 9.0 and a temperature of 60°C; the polymer was 6% ionized under these conditions. Copoly-4(5)-vinylimidazole-acrylic acid, which had such a dramatic effect on the positively charged ester 3-acetoxy-N,N,N-trimethylanilinium iodide, was tried and found to have an inhibitory effect as if the imidazole groups were not present; the imidazole groups had no effect on $TCPCl_3$. Poly-p-vinylphenol[11] was tried and a rate enhancement between 1.2 and 2.5 times the blank rate was observed at a pH of 9.0 and a pH of 10.0. The polymer was insoluble at ethanol concentrations less than 30% but the effects observed were much greater when the polymer was precipitated. When the polymer precipitated, however, it clogged the porous pin of the reference electrode which subsequently made the pH sensing very erratic. Polyethyleneimine was tried since it has a high concentration of nucleophilic groups, but no effect was observed at pH of 9.0. Polyacrolein oxime was tried since the oxime function is known to be especially effective in reactivating phosphorylated acetylcholinesterase. There was no change in the rate of hydro-

lysis at pH 9.0, 9.5, or 10.0.

IV. ESTEROLYTIC BEHAVIOR OF DIMERIC ANALOGS

It was found that the solvolysis of p-nitrophenyl acetate could be catalyzed by 4(5)-[2'hydroxyphenyl]imidazole[12] and that there is an exponential increase in rate with increasing pH above pH 9. The rate equation may be expressed as

$$k_{cat} = k_1 \alpha(Im) + k_2 \alpha(Im) \, \alpha(PhO^-) + k_3 \alpha(PhO^-)$$

k_2 is a bifunctional rate constant and k_1 and k_3 are monofunctional rate constants. In order to evaluate the third term of this equation, 1-carbamyl-methyl-4(5)-[2'-hydroxyphenyl]imidazole has been prepared. Kinetic studies of this compound as well as other ortho-substituted phenol derivatives, such as o-phenyl phenol and o-tert-butyl phenol have been completed and the results are presented in Table VII.

TABLE VII

DETERMINATION OF APPARENT DISSOCIATION CONSTANTS AND
SOLVOLYSIS OF p-NITROPHENYL ACETATE IN 30% PROPANOL-WATER; $\mu = 0.02$

Compounds	pK' *	k_2' **
1-carbamylmethyl-4(5)-[2'-hydroxy-phenyl]-imidazole	11.25	44.6
o-phenyl phenol	11.35	78.0
o-tert-butyl phenol	ca. 12.50	233.0
4(5)-[2'-hydroxyphenyl]imidazole	11.04	246.0

*pK[1] obtained from acid-base titrations.
**k_2' is the observed rate divided by the actual phenoxide
  concentration.

A Brønsted type plot is constructed by plotting pK versus log k_2. A straight line is obtained with a similar slope to the p-substituted phenols but with a different intercept. From this plot a value of 14.8 is obtained for k_2' at 10.04, the pK' value of 4(5)-[2'-hydroxyphenyl]imidazole, which is actually the catalysis contribution of the phenol portion of the 4(5)-[2'-hydroxyphenyl]-imidazole.

The k_1 term of the rate equation could be evaluated from the solvolysis data of 4(5)-[2'-methoxyphenyl]imidazole, then the bifunctional catalysis term could be easily evaluated as 229, which is considerably larger in comparison to the values of the monofunctional catalysis rates.

Catalysis shown below (I) might operate for the solvolysis of p-nitrophenyl acetate. Substitution in the para position with an electron withdrawing group such as a quaternized amine should favor this type of operation even in solutions of low pH (II).

Scheme 1

I   II
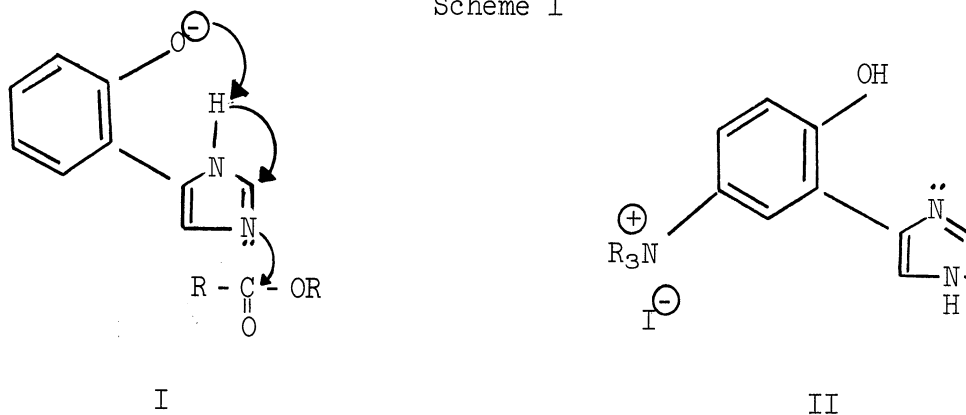
Further studies with these systems are in progress.

V. NEW SYNTHETIC ROUTE FOR 5(6)-VINYLBENZIMIDAZOLE

Poly-5(6)-vinylbenzimidazole has revealed unusual catalytic activity with charged and uncharged substrates.[2,13] At high pH values, the polymer was a better catalyst than benzimidazole with p-nitrophenyl acetate (PNPA) and the negatively charged substrate, 4-acetoxy-3-nitrobenzoic acid (NABA). Benzimidazole anions were found unreactive with the negatively charged substrate. The difference between the monomer and polymer catalyst was explained by the readiness of the benzimidazole functions on the polymer chain for terfunctional interactions, i.e., those involving two benzimidazole groups, one in the neutral and one in the anionic form, in addition to the uncatalyzed nucleophilic reaction. With this in mind, a study of copolymers of benzimidazole seems in order. Scheme 2 is a new synthetic approach to the preparation of monomer 5(6)-vinylbenzimidazole in a reproducible manner and in substantial amounts. The ability of 5(6)-vinylbenzimidazole to participate in copolymerizations seems to be feasible considering its resemblance to many monomers which do.

Preliminary results show that compound V has been prepared as the ammonium salt but is not quite pure. Since purity is essential for the last step of the reaction sequence to succeed without forming polymer, further work in this area is being undertaken.

Scheme 2
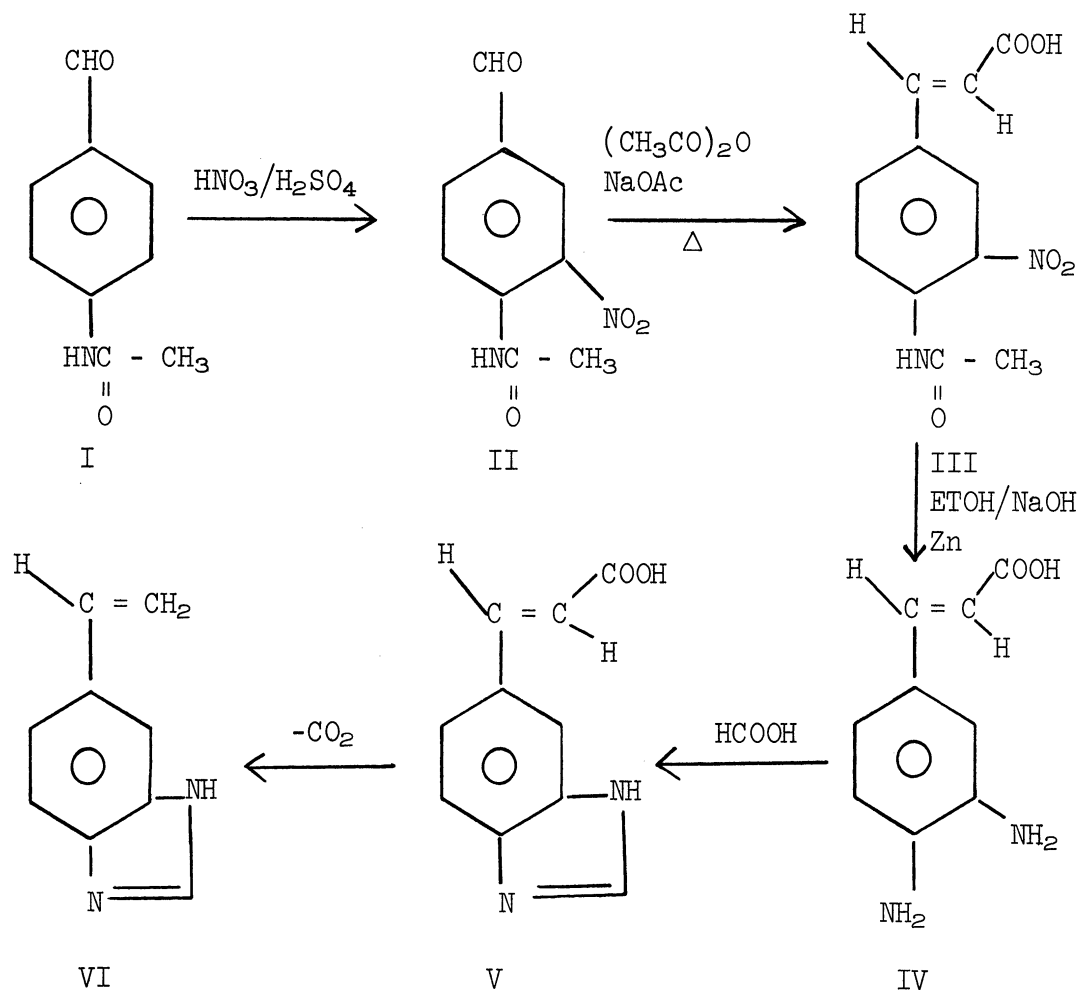
VI. THE STEREOSPECIFIC SYNTHESIS OF POLY-4(5)-VINYLIMIDAZOLE

The preparation of the syndiotactic polymer may be accomplished by low temperature reactions; this form generally being the thermodynamically more stable one. Therefore, photo-initiated free radical polymerization in bulk or solution at -50°C should yield predominantly syndiotactic polymer presuming an appreciable free energy of activation difference $(\Delta(\Delta F^{\ddagger}))$ between the two forms.

The isotactic forms of many vinyl monomers have been prepared via cationic and anionic initiators. These, however, afford special problems in the 4(5)-vinylimidazole case due to the reactive -NH function in the imidazole ring. This could perhaps be overcome by employing a blocking group which will be sufficiently electron releasing to allow polymerization via an anionic mechanism, yet be quantitatively removable from the polymer. The N-trimethylsilyl derivative of 4(5)-vinylimidazole is just such a compound. It can be prepared by addition of hexamethyl disilazane to 4(5)-vinylimidazole.[14]
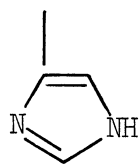
$$H_2C = CH \qquad\qquad\qquad CH_2 = CH$$
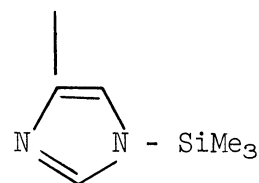
The extent of stereospecificity of the polymers obtained via the above-mentioned routes may be determined by a high resolution NMR technique as developed by Bovey and co-workers.[15] This method utilizes the fact that the β-methylene protons of isotactic polymers will be nonequivalent and appear as an AB pattern, whereas the β-methylene protons of the syndiotactic form will be chemically equivalent and thus have one chemical shift value.

VII. CATALYTIC HYDROLYSIS OF DINUCLEOSIDE (3'-5') MONOPHOSPHATES AND NUCLEO-
SIDE (2'-3') CYCLIC PHOSPHATES WITH POLY-4(5)-VINYLIMIDAZOLE

Pancreatic ribonuclease (RNase) converts ribonucleic acid (RNA) to a complex mixture of uridine 3'-phosphate, cytidine 3'-phosphate and a series of oligonucleotides, each of which terminates with a pyrimidine nucleoside-3'-phosphate.

The active site of pancreatic ribonuclease is said to contain:

(1) Two imidazole (histidine) functions and a lysine function.[16]

(2) Two imidazole functions and one carboxyl function.[17]

(3) Three imidazole functions or two imidazole functions and one lysine function.[18]

Although these reports are somewhat conflicting, there is unanimity in postulating imidazole at the active site; also the presence of another charged group seems to be indicated. Since imidazole has been shown to cleave similar species, Scheme 3, it is possible that poly-4(5)-vinylimidazole would be more effective.

26

Scheme 3[(19,20)]
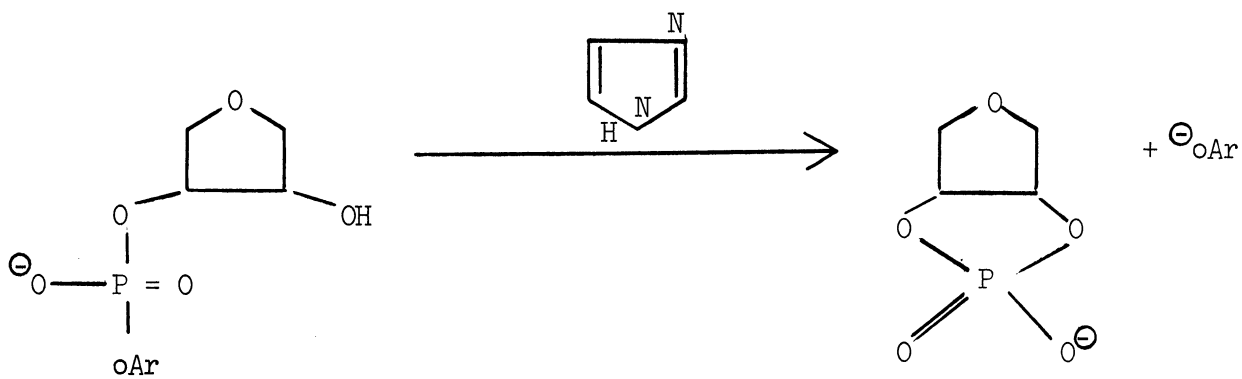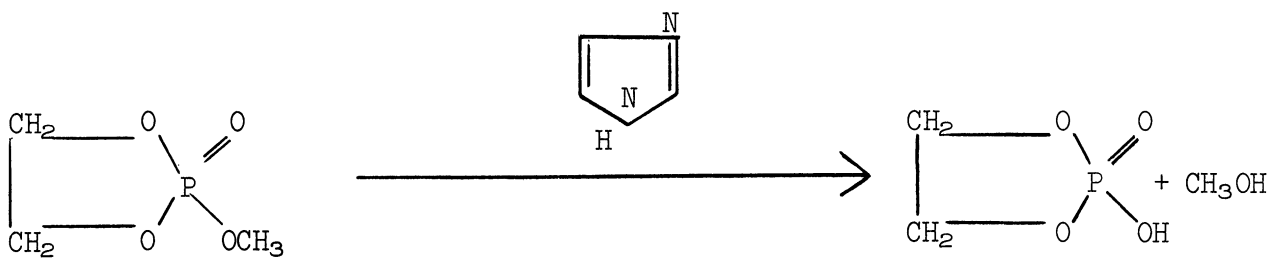
A greatly simplified mechanism for the action of ribonuclease on RNA given here, Scheme 4, emphasizes the similarity to the model reactions in Scheme 3.

Scheme 4[(15)]

$\beta_1 = \beta_2 =$ purine or pyrimidine base
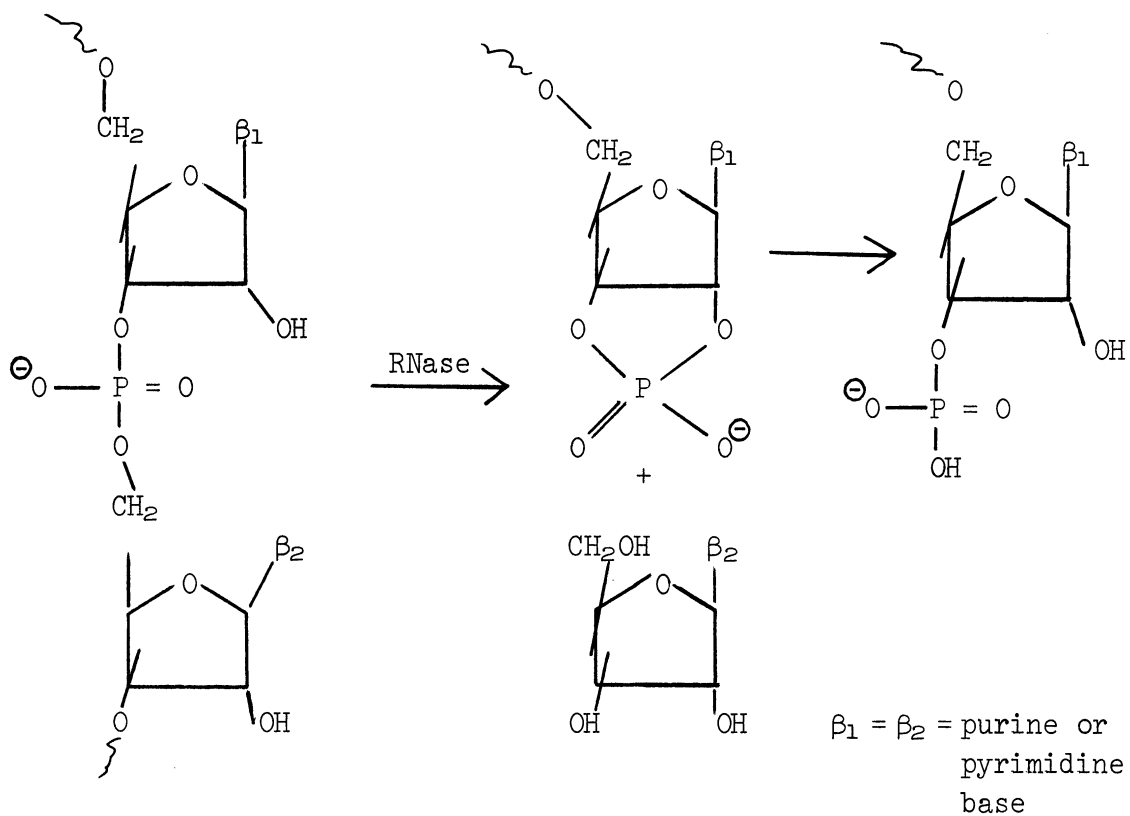
In addition to the study of the effect of poly-4(5)-vinylimidazole, the effect of various copolymers of 4(5)-vinylimidazole and acrylic acid and 4(5)-vinylimidazole and p-vinylphenol may show interesting rate enhancements.

VIII. GLOSSARY

PNPA	p-nitrophenyl acetate
α_1	fraction of neutral functions
α_0	fraction of cationic functions
α_2	fraction of anionic functions
NABS	sodium-3-nitro-4-acetoxybenzenesulfonate
k_{cat}	second-order catalytic rate constant
NABA	3-nitro-4-acetoxybenzoic acid
PVIm	poly-4(5)-vinylimidazole
NDBA	3-nitro-4-dodecanoyloxybenzoic acid
$TCPCl_3$	tris(choline chloride) phosphate
PAA	polyacrylic acid
RNA	ribonucleic acid
PVS	polyvinylsulfonic acid
RNase	ribonuclease

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13. ABSTRACT

The objectives of the research are to synthesize new vinyl polymers and to study their catalytic activities toward either phosphate or carbon esters. Utilizing 1,2,4- and 1,2,3-triazoles in the catalytic solvolyses of p-nitrophenyl acetate, sodium 3-nitro-4-acetoxybenzenesulfonate and 3-acetoxy-N-trimethyl-anilinium iodide, it was found that the effectiveness of a particular species as a catalyst was related to its pK value. The reaction between hydroxide ion and tris (choline chloride) phosphate has been investigated in the presence and absence of various polymers. The effect of salt concentration on the reaction with polyanions was also investigated. The effects of temperature and pH on the solvolyses of 3-nitro-4-acetoxybenzoic acid and 3-nitro-4-dodecanoyl-oxybenzoic acid have been studied to determine the extent of apolar bonding in poly-4(5)-vinylimidazole catalyzed reactions. A new synthetic approach for 5(6)-vinylbenzimidazole as well as the synthesis of stereospecific poly-4(5)-vinylimidazole (both syndiatactic and isotactic) are described. The catalytic effects of poly-4(5)-vinylimidazole and various copolymers on the hydrolysis of nucleoside phosphates are being investigated to determine any rate enhancing effects.

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Catalytic activity						
Solvolytic reactions						
Poly(3-vinyl-1,2-4-triazole)						
Charged esters						
Poly 4(5)-vinylimidazole						
Tris (choline chloride) phosphate						
5(6)-Vinylbenzimidazole						
Stereospecific						
Dinucleoside (3'-5') monophosphate						
Nucleoside (2'-3') cyclic phosphates						
Nonpolar bonding						
Polyelectrolytes						