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MECHANISM OF ENZYME ACTION

Semiannual Report

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## Foreword

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## Digest

The reactions of imidazole and benzimidazole containing polymers with reactive esters have revealed several analogies to those of enzyme 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,<sup>(1-3)</sup> a specificity in their solvolytic reactions,<sup>(2-4)</sup> a competitive inhibition by substances similar to the reactive substrate,<sup>(4,5)</sup> a bifunctional interaction between two catalytic functions and a substrate,<sup>(3,6,7)</sup> and a complexation (saturation) by high and low molecular weight esters.<sup>(4,8)</sup>

In a further investigation of the catalytic interactions between pendent neutral and anionic imidazole groups, mathematical evidence is presented which indicates that this reaction is dependent upon both monomeric and dimeric neutral imidazole interactions. Similarly, for poly-4-vinylpyridine catalyzed reactions, it is possible that a similar neutral-neutral interaction occurs.

An investigation of the solvolysis of a paraffinic, anionic ester catalyzed by poly-4(5)-vinylimidazole has revealed several unusual catalytic effects which appear to be solvent dependent. When the solvent system contains a high water content, very rapid solvolytic rates are obtained. These solvolytic rates appear to approach those of enzymic reactions. Under appropriate conditions it is also possible to obtain saturation phenomena which illustrate the contribution of hydrophobic interactions in polymer-substrate complexation.

Since polymeric catalysts have revealed many analogies to hydrolytic enzymes, we are attempting to prepare a new type of polymeric catalyst which can undergo oxidation-reductions reactions. We are also continuing our investigation of salt effects in the hydrolysis of tri(choline chloride) phosphate and the solvolytic effects of dimeric imidazole-phenol model systems.

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## MECHANISM OF ENZYME ACTION

### Results and Discussion

#### I. DISCUSSION OF RECENT VISIT TO EDGEWOOD ARSENAL

During a recent visit to the Research Laboratories, Physical Research Laboratory of Edgewood Arsenal, C. G. Overberger and J. C. Salamone presented a review of the effects of imidazole containing polymers on the solvolytic rates of carbon and phosphorous esters. Initially, C. G. Overberger discussed the types of cooperative interactions which have been found between two pendent imidazole groups and a substrate. He also discussed the cooperative interactions between imidazole and hydroxyl, imidazole and phenol, as well as imidazole and carboxylate groups. Such cooperative effects are in part the cause of the enhanced catalytic abilities of the polymers in relation to their low molecular weight analogs.

The catalytic ability of 1,2,4-triazole and poly-1,2,4-triazole were also discussed. It was mentioned that this system was quite different from the imidazole system in that the triazole reactions were principally dependent upon the anionic functions.

J. C. Salamone then discussed the effects of hydrophobic bonding which were under investigation using poly-4(5)-vinylimidazole as the catalyst and neutral and anionic, paraffinic esters as substrates. The largest catalytic effect was noted for the system which utilized both hydrophobic and electrostatic forces to effect complexation between substrate and polymer.

The effects of ionic strength on the solvolytic rate of tri(choline chloride) phosphate in the presence of anionic polyacrylic acid were also discussed. The results of this subject are presented in Section IV.

#### II. BIFUNCTIONAL INTERACTIONS IN POLYMERIC IMIDAZOLE AND PYRIDINE SYSTEMS

In the catalytic solvolysis of PNPA by poly-4(5)-vinylimidazole, two types of cooperative mechanisms were proposed for the enhanced catalytic effect in 28.5% ethanol-water solution at pH values above 7.5(3). Interactions of anionic and neutral, pendent imidazole groups with the substrate were considered to be the most likely mechanism for the solvolysis of PNPA. The alternative cooperative interaction of two neutral pendent imidazole groups in the same concerted manner was also considered, particularly for the polymer in 10% methanol-water solution at intermediate pH values.<sup>(9)</sup> The proposed anionic-neutral interaction mechanism was supposedly substantiated by a study of the activation parameters for the solvolysis of PNPA.<sup>(9)</sup>

The enhanced catalytic effect of poly-4(5)-vinylimidazole in the solvolysis of PNPA was observed to begin at  $\text{pH} \approx 7.5$ . Calculations of  $\alpha_0$  (the fraction of cationic functions) and  $\alpha_1$  (the fraction of neutral functions) showed that at  $\text{pH} 8$ ,  $\alpha_0 = 0.1$  and  $\alpha_1 = 0.9$ , while  $\alpha_2$  was estimated to  $10^{-8}$ . Since this system is on the cationic side of the isoelectric point, it would appear to be very unlikely that an anionic-neutral interaction could participate in the catalytic process.

The argument that poly-N-vinylimidazole and poly-2-methyl-N-vinylimidazole are poor catalysts for the solvolysis of PNPA<sup>(3)</sup> because these catalysts contained no anionic sites and could not have any anionic-neutral interaction, supposedly substantiated the proposed anionic neutral imidazole interaction in the poly-4(5)-vinylimidazole catalyzed process. However, a closer examination of the poly-N-vinylimidazole and poly-2-methyl-N-vinylimidazole systems appears to support further either the interaction of two neutral, pendent imidazole groups or individual neutral, pendent imidazole groups in the catalytic solvolysis of PNPA by poly-4(5)-vinylimidazole in 28.5% ethanol-water solution. The investigation of PNPA solvolysis catalyzed by poly-N-vinylimidazole was performed in the  $\text{pH}$  range of 7-9.<sup>(3)</sup> The apparent  $\text{pK}_1$  of the pendent imidazole groups in this polymer was determined to be 4.4. Therefore, under the conditions studied, the polymer was practically neutral. The increase in the fraction of the neutral, pendent groups was very small from  $\text{pH} 7$  ( $\alpha_1 = 0.93$ ) to  $\text{pH} 9$  ( $\alpha_1 = 0.97$ ). It is not surprising to find that the catalytic rates remained almost constant, since there was no significant increase in  $\alpha_1$ . For the N-alkylated polymers, the solvolytic mechanism would seem to imply either an individual pendent, neutral imidazole reaction or a bifunctional pendent, neutral-neutral imidazole reaction.

The lack of an enhanced catalytic effect for poly-2-methyl-N-vinylimidazole in the solvolysis of PNPA cannot be used as a substantiation of the anionic-neutral pendent imidazole group interaction proposed for poly-4(5)-vinylimidazole. In this case the low apparent  $\text{pK}_a$  (4.8) of poly-2-methyl-N-vinylimidazole and the added steric factor of the 2-methyl substitution are also involved. Therefore, whether poly-2-methyl-N-vinylimidazole is a good catalyst or not is irrelevant to the catalytic properties of poly-4(5)-vinylimidazole.

The second order catalytic rate constants for the poly-4(5)-vinylimidazole catalyzed solvolysis of PNPA in 28.5% ethanol-water solution obtained above  $\text{pH} 8.4$  should be considered with reservation, since it was found that the precipitation range of this polymer was in the vicinity of  $\text{pH} 8.5$ . A heterogeneous system would obviously further complicate the process. It was in this region that such anionic-neutral interactions were proposed.

It was previously reported for the monomeric imidazole catalyzed solvolysis of PNPA that there were no interfunctional interactions between two imidazole functions and the substrate.<sup>(10,11)</sup> Studies of the activation parameters of poly-4(5)-vinylimidazole and poly-N-vinylimidazole catalyzed solvolyses of PNPA indicated that multifunctional interactions appeared to be the pathway that



lead to the enhanced effects observed for poly-4(5)-vinylimidazole in 28.5% ethanol-water and 10% methanol-water solutions, while no effects were possible for poly-N-vinylimidazole. A neutral-neutral interaction was proposed for poly-4(5)-vinylimidazole and PNPA in 10% methanol-water, while an anionic neutral interaction was considered to be most likely for the 28.5% ethanol-water solution system. From a consideration of the facts that poly-4(5)-vinylimidazole was investigated in the cationic side of the isoelectric point and that the poly-N-vinylimidazole and poly-2-methyl-N-vinylimidazole reactions do not necessarily support an anionic-neutral reaction in the poly-4(5)-vinylimidazole system, it is possible that the poly-4(5)-vinylimidazole solvolyses of PNPA in 10% methanol-water and 28.5% ethanol-water both involve neutral-neutral interactions.

If the neutral-neutral interaction ( $\alpha_1^2$ ) is the principle factor responsible for the observed enhanced catalytic effect of poly-4(5)-vinylimidazole in the solvolysis of PNPA, and if the neutral, pendent imidazole group ( $\alpha_1$ ) also participates to a lesser extent, then we would express  $k_{cat}$  explicitly by the following equations:

$$k_{cat} = k_{11}\alpha_1^2 + k_1\alpha_1 \quad (1)$$

then

$$\frac{k_{cat}}{\alpha_1} = k_{11}\alpha_1 + k_1 \quad (2)$$

Using equation (2), a linear plot of  $k_{cat}/\alpha_1$  versus  $a_1$  should be obtained if the proposed neutral-neutral pendent group interaction is operative in 28.5% ethanol-water, as well as in 10% methanol-water. The data obtained for the catalyzed solvolyses of PNPA by poly-4(5)-vinylimidazole in both 28.5% ethanol-water and 10% methanol-water solutions<sup>(3,9)</sup> were treated graphically according to equation (2). Indeed, as revealed in Figure 1, a linear relationship was obtained for each solvent system with intercepts at  $\alpha_1 = 0.25$  and values of  $k_{11}$  of 30.50 l/mole-min and 102.70 l/mol-min for 28.5% ethanol-water and 10% methanol-water, respectively. These results strongly indicate that neutral-neutral interactions are very probable for both of these systems.

A close examination of the rate  $\alpha_1$  profiles for the catalytic solvolyses of NABS by poly-4(5)-vinylimidazole<sup>(7)</sup> and by poly-4-vinylpyridine<sup>(2)</sup> reveals that maximal catalytic rates for these polymers are found to be in the vicinity of  $\alpha_1 = 0.75$ . The intercept on the  $\alpha_1$  axis at 0.25 for the catalytic solvolyses of PNPA by poly-4(5)-vinylimidazole seems to be related to this value. This intercept suggests that at  $\alpha_1 = 0.25$  the polymer will have no catalytic activity and that at  $\alpha_1$  values below 0.25, the polymer will either have no catalytic activity or will instead function as an inhibitor. There is no reason, however,

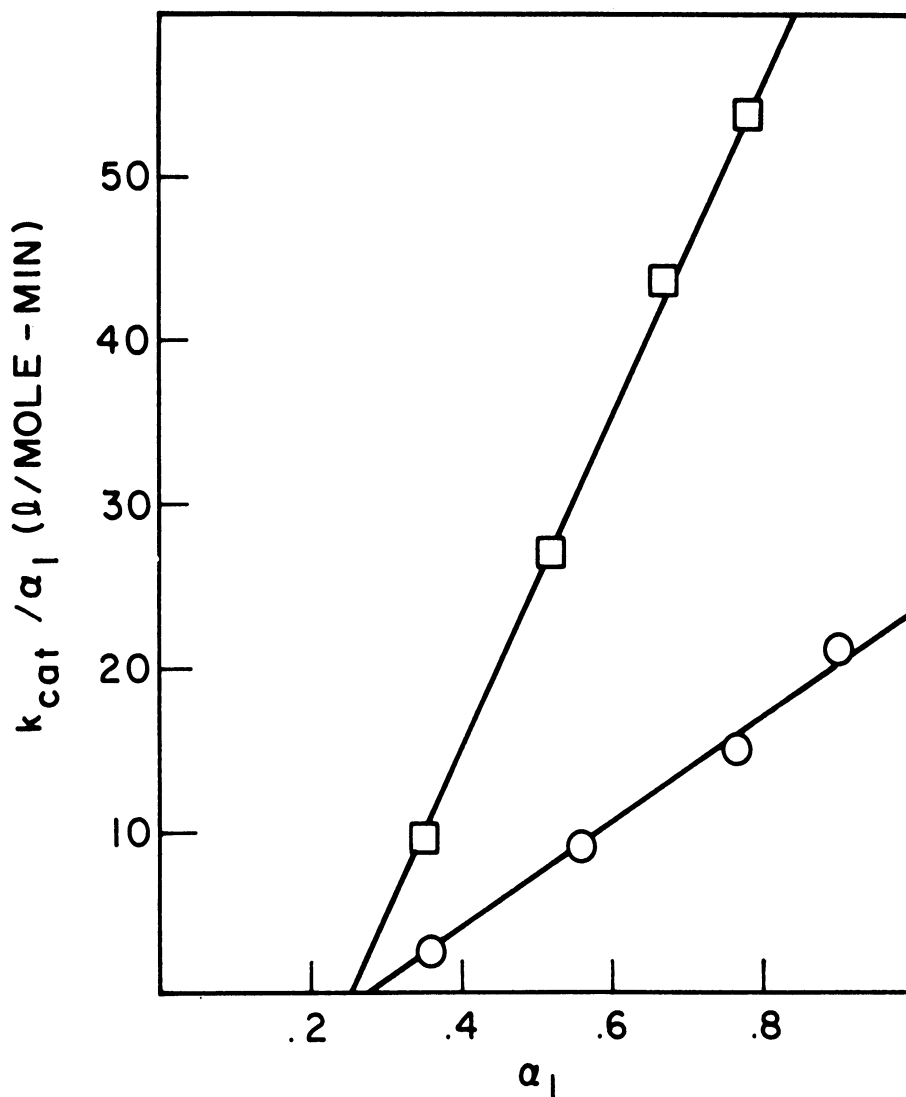


Figure 1.  $k_{cat}/\alpha_1 - \alpha_1$  profiles for the solvolyses of PNPA catalyzed by poly-4(5)-vinylimidazole in 28.5% ethanol-water (O), and 10% methanol-water (□); ionic strength 0.02, 26°C.

why the partially protonated polymer should function as a competitive inhibitor toward a neutral substrate, although such an effect may occur with an oppositely charged ester.<sup>(12)</sup>

At  $\alpha_1 \approx 0.25$ , poly-4(5)-vinylimidazole is ca. 75% protonated. Two neutral, pendent imidazole groups will, on an average, be separated by three protonated, pendent imidazole groups. The polymer chain would be extended because of internal electrostatic repulsion between the protonated functions, and the polymer would be more rigid than a polymer having a protonation of less than 75%. These effects coupled together would render difficult the interactions of neutral-neutral pendent imidazole groups. Consequently, catalytic activity would not be detectable below  $\alpha_1 = 0.25$  if the neutral-neutral interactions are solely responsible for the catalytic effect in these solvolytic processes.

Therefore, we may state that if neutral-neutral interactions are important in a catalyzed solvolytic process, then a separation of two pendent, neutral groups by a certain number of inert pendent groups would be expected to make such neutral-neutral interactions impossible to occur. Indeed, such an effect has previously been noted in solvolytic reactions catalyzed by a copolymer of 4(5)-vinylimidazole and p-methoxystyrene. In this case, the catalytic imidazole functions, when diluted by the inert p-methoxyphenyl groups, were very inefficient catalysts.<sup>(7)</sup> For the case of poly-4(5)-vinylimidazole, this would mean that the neutral-neutral interactions could not extend beyond the fourth unit; therefore,  $\alpha_1 = 0.25$  may be considered as the threshold value for such an interaction to occur.

The above statement is related to the observed maximum rate at  $\alpha_1 = 0.75$  for the catalytic solvolysis of NABS by poly-4(5)-vinylimidazole. At  $\alpha_1 = 0.75$  the polymer is about 25% protonated, that is, every protonated, pendent imidazole group is separated by three neutral, pendent imidazole groups. The observed maximum rate at  $\alpha_1 \simeq 0.75$  would mean that in the case of a cationic-neutral interaction, the three neutral pendent groups contribute to the maximum interaction upon the negative substrate which is bound to a protonated, pendent group by electrostatic force. Therefore, the observed distorted bell-shape rate- $\alpha_1$  profiles for the solvolyses of negatively charged substrates by poly-4(5)-vinylimidazole and by poly-4-vinylpyridine can be interpreted by following the above reasoning. That is, if we assume the fourth neutral pendent group interacts insignificantly with the negative substrate bound to the protonated pendent group by electrostatic force, then the rapid decrease of the catalytic rates at values above 0.75 would be a direct result of the decrease of the cationic sites. The gradual decrease of the catalytic rates at  $\alpha_1$  values less than 0.75 is probably a consequence of the gradual decrease of the neighboring neutral pendent group contribution.

In the case of the poly-4-vinylpyridine catalyzed solvolysis of the neutral substrate 2,4-dinitrophenyl acetate (DNPA), both the nonlinear rate- $\alpha_1$  profile and the inefficiency of the polymer in comparison to its monomeric analog 4-picoline were attributed to the decreased nucleophilicity of the pendent pyridine groups.<sup>(2)</sup> This nonlinear relationship, however, can also be considered by the cooperative interaction of two pendent, neutral pyridine groups. If we assume that neutral-neutral interactions are also operative in the poly-4-vinylpyridine system, then according to equation (2), a linear relationship would be expected to occur between  $k_{\text{cat}}/\alpha_1$  and  $\alpha_1$ . Indeed, such a linear relationship was obtained, and an intercept in the vicinity of  $\alpha_1 = 0.2$  was found (Figure 2). In this case, it is obvious that no anionic-neutral interaction is possible, since the pyridine moiety cannot exist in the anionic form. It is interesting to again note that for a polymeric catalyst and a neutral substrate, there seems to be no catalytic effect at  $\alpha_1$  values below 0.2. Although the poly-4-vinylpyridine system appears to be dependent on neutral-neutral interactions, such as described for poly-4(5)-vinylimidazole, it is unusual that the polymeric catalyst is less efficient than its monomeric analog. This effect may, however, be caused by the high content of ethanol in the solvent system

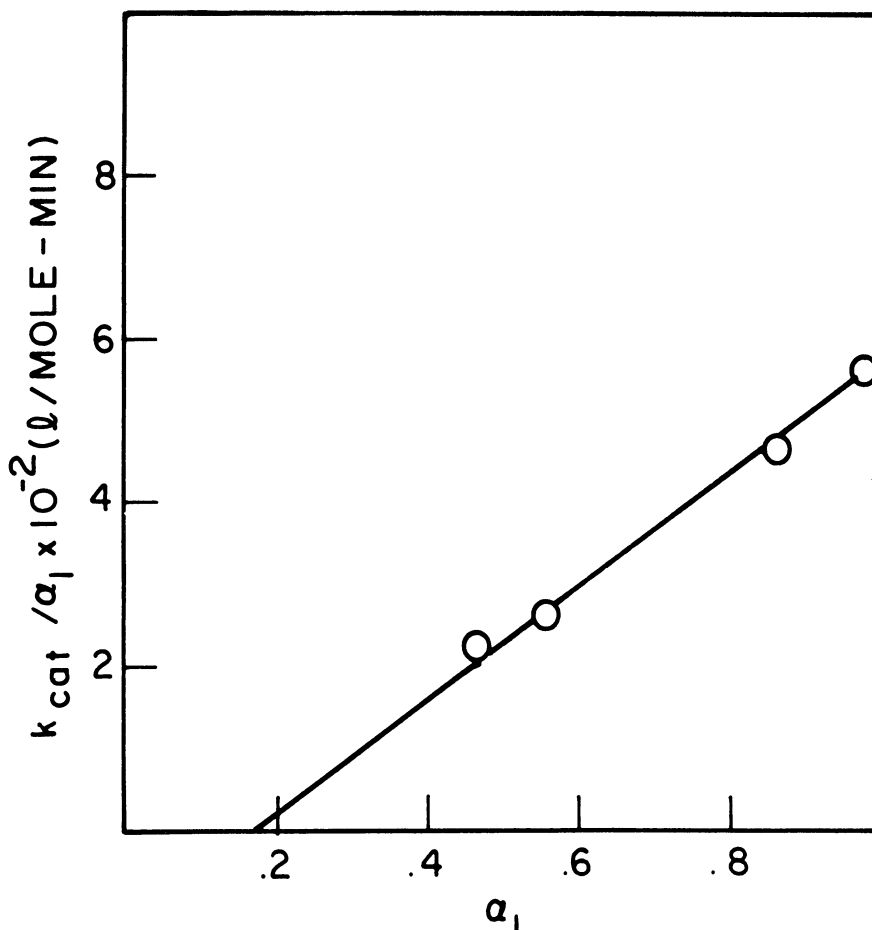


Figure 2.  $k_{cat}/\alpha_1 - \alpha_1$  profile for the solvolyses of DNPA catalyzed by poly-4-vinylpyridine in 50% ethanol-water, 0.04M in KCl, 36.8°C.

(50% ethanol-water). Analogous results have been reported for the decreased catalytic behavior of poly-4(5)-vinylimidazole in solvents of high alcohol contents.<sup>(13)</sup>

Therefore, we may generalize that for any polymer catalyzed solvolysis of an active ester, where neutral-neutral pendent group interactions are important to the solvolytic process, there should be a threshold  $\alpha_1$  value below which the polymer can either have no effect on the reaction rate or can inhibit the solvolytic reaction. For any polymer reactions involving cationic-neutral or anionic-neutral pendent group interactions, there should be a maximum catalytic rate at a certain  $\alpha_1$  value. It would be interesting to investigate the solvolysis of a neutral substrate by a synthetic polymeric catalyst at  $\alpha_1$  values below 0.2, an area which has not been reported.

### III. HYDROPHOBIC BONDING IN MACROMOLECULE-SUBSTRATE COMPLEXATION

The complexation of catalytically active, synthetic macromolecules with low and high molecular weight reagents has been investigated by several workers.<sup>(4,8,14,15)</sup> These association reactions, which are characteristic of

enzyme-substrate complexation, presumably involved an accumulation of the substrate in the vicinity of the polymer chain by either electrostatic or hydrophobic forces. Although the kinetically determined binding constants of these reactions were similar to those of enzymic reactions, the synthetic macromolecule catalyzed processes have been characterized by relatively low reactivities.

It has been recently found that partially protonated poly-4(5)-vinylimidazole was able to complex with the negatively charged ester sodium 3-nitro-4-acetoxybenzenesulfonate (NABS) under conditions in which the substrate concentration was in excess of the polymeric catalyst concentration.<sup>(8)</sup> This saturation effect was not obtained when the neutral ester p-nitrophenyl acetate (PNPA) was employed. These results suggested that the saturation of poly-4(5)-vinylimidazole with NABS was facilitated by electrostatic forces, whereas the lack of saturation with PNPA indicated that hydrophobic forces were insufficient to accumulate the neutral ester in the vicinity of the polymer chain.

Reactions of poly-4(5)-vinylimidazole with neutral and charged substrates in alcohol-water solvent systems have indicated that cooperative, multifunctional interactions among pendent imidazole functions and a substrate were possible.<sup>(13)</sup> Such cooperative interactions appear to be in part responsible for the enhanced catalytic ability of the polymer relative to its monomeric analog, imidazole. In order to further improve the rate of the cooperative imidazole attack on a substrate, it was felt that a very rapid poly-4(5)-vinylimidazole catalyzed reaction could be attained if both hydrophobic and electrostatic forces contributed to the complexation of a substrate with the macromolecule. The substrate chosen for this investigation was 3-nitro-4-dodecanoyloxybenzoic acid (NDBA), a long paraffinic chain analog of the anionic ester 3-nitro-4-acetoxybenzoic acid.<sup>(3)</sup> The solvolysis of NDBA by poly-4(5)-vinylimidazole in ethanol-water solvent systems was expected to involve predominantly hydrophobic interactions in solvents containing a high water content (higher polarity) and electrostatic interactions in solvents containing a high ethanol content (lower polarity). In alcohol-water solvent systems, we have previously observed that a high alcohol content apparently increased the electrostatic interaction between poly-4(5)-vinylimidazole and an anionic ester (NABS) and decreased the apolar interaction with a neutral ester (PNPA).<sup>(7)</sup> These results are in contrast to those of similar reactions conducted at a lower alcohol concentration where the apolar interaction increases relative to the electrostatic interaction.

In Table I are presented the times for half solvolyses of NDBA catalyzed by imidazole and by poly-4(5)-vinylimidazole for several ethanol-water solvent systems. In each case, the catalyst concentrations were greater than the substrate concentration such that a first order disappearance of NDBA could be expected.<sup>(3)</sup> The imidazole catalyzed reactions were found to be second-order processes in all solvent systems, and it is noted that the half-lives for NDBA solvolyses increased considerably with increased ethanol content. The poly-4(5)-vinylimidazole catalyzed reaction was, however, more complex. At ethanol contents of 60, 80, and 90% the polymeric reaction was a second-order process and the polymer was more efficient than its monomeric analog by a factor of 3.5

at 60%, of 4.8 at 80%, and of 13 at 90%. This increase in polymeric reactivity could in part be caused by an increase in electrostatic forces as well as by a possible contribution of hydrophobic forces. Since poly-4(5)-vinylimidazole is insoluble in water or ethanol, it is conceivable that hydrophobic interactions could increase as the limit of solubility of the polymer is approached.

TABLE I\*

EFFECT OF ETHANOL-WATER SOLVENT COMPOSITION ON TIME FOR HALF SOLVOLYSES OF NDBA CATALYZED BY POLY-4(5)-VINYLMIDAZOLE AND IMIDAZOLE

Ethanol (%, volume)	PVIm (min)	Imidazole (min)
15	< 0.5	146
20	< 0.5	197
30	< 0.5	352
40	15	635
60	454	1,610
80	861	4,146
90	826	11,093

\*pH 7.9, ionic strength 0.02; half solvolyses times were determined for catalyst concentrations of  $1.90 \times 10^{-4}m$  and substrate concentration of  $1.78 \times 10^{-4}m$ .<sup>(16)</sup>

From Table I it is also noted that the reactivity of poly-4(5)-vinylimidazole relative to imidazole, increases dramatically at ethanol contents of 15% to 40%. In fact, at low ethanol contents these reactions were too fast to measure by our standard procedure.<sup>(3)</sup> These extremely rapid catalyses, which are currently under investigation by stopped-flow spectroscopy, are indicative of a strong hydrophobic reaction between the paraffinic substrate and the vinyl polymer.

In the region of the 40% ethanol-water the polymer reactions were found to deviate from yielding a first-order solvolysis of NDBA. In Figure 3A the initial rates ( $v_{\text{obsd}}$ )<sup>(8)</sup> for the solvolysis of a fixed concentration of NDBA as a function of increasing concentration of neutral polymer (PVIm) appear to be approaching a limiting value. Such an occurrence is indicative of macromolecule-substrate complexation, although this is unexpected owing to the higher concentration of catalyst to substrate that was employed.<sup>(4)</sup> When the concentration of neutral poly-4(5)-vinylimidazole was fixed below a varying concentration of NDBA (Figure 3B), it was again found that the initial solvolysis rates were approaching a limiting value.

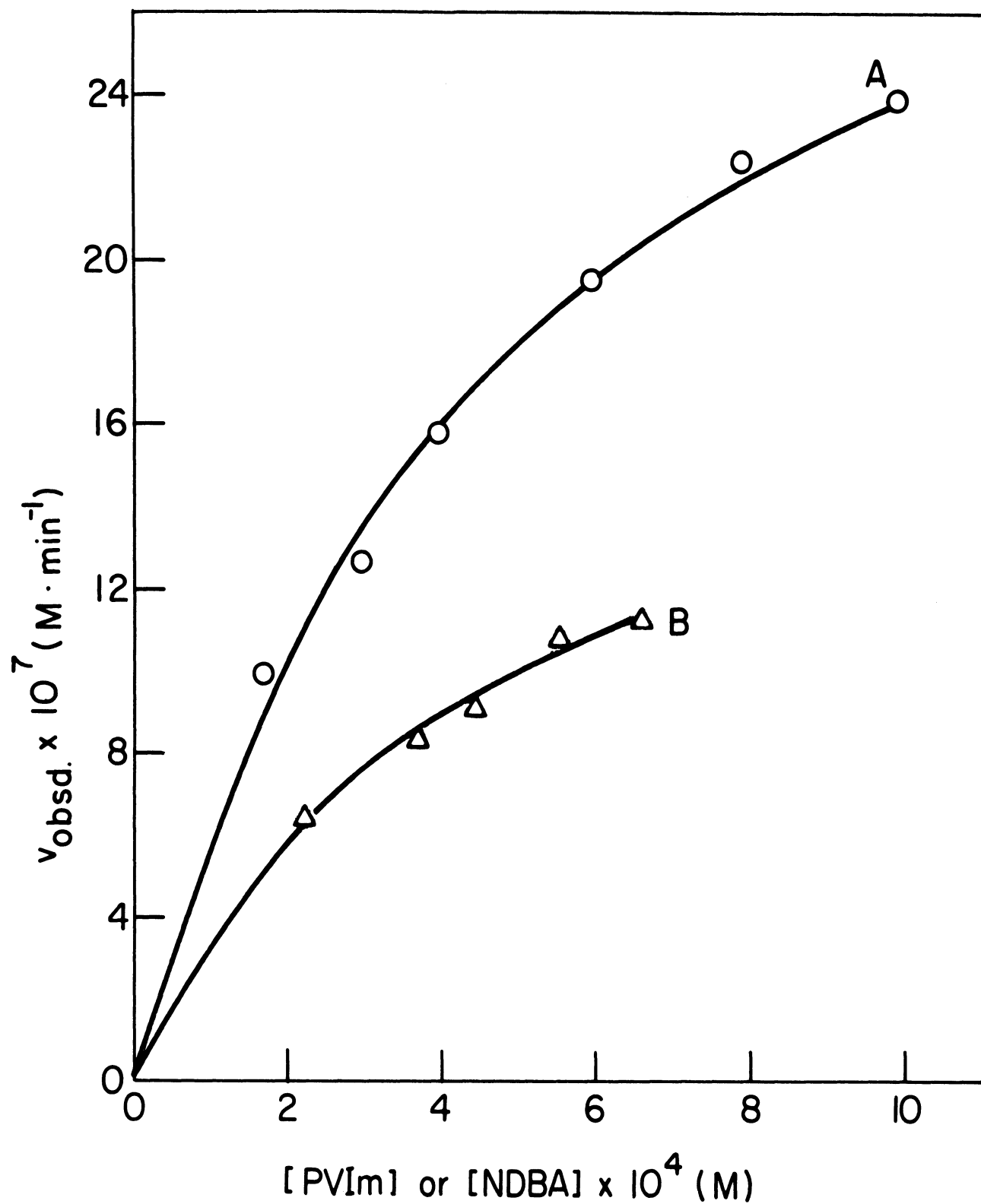


Figure 3. The observed initial rates of the poly-4(5)-vinylimidazole catalyzed solvolysis of NDBA as a function of catalyst and substrate concentration.

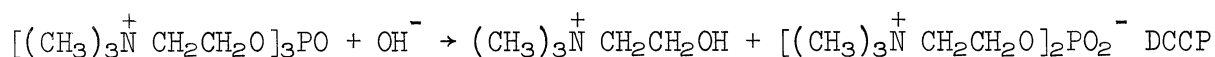
The latter kinetic data are characteristic of the Michaelis-Menten mechanism,<sup>(8,17)</sup> in which the catalyst (an enzyme) reacts with the substrate to form a catalyst-substrate complex, and this complex is decomposed giving back the free catalyst and the products of the reaction. If it is assumed that systems A and B of Figure 3 can be described by the Michaelis-Menten mechanism for complexation, and that there are no interactions between bound materials, which could affect this complexation, it is possible to determine the Michaelis constants ( $K_m$ ) for these reactions; by utilizing a modified Lineweaver-Burk plot,<sup>(8)</sup>  $[P4VIm]/v_{obsd}$  can be plotted versus  $[P4VIm]$  when poly-4(5)-vinylimidazole concentration is in excess (Figure 4A) and  $[NDBA]/v_{obsd}$  can be plotted versus  $[NDBA]$  when substrate concentration is in excess (Figure 4B). From a least squares treatment of these data, values of  $K_m$  were determined to be  $(4.77 \pm 0.57) \times 10^{-4}M$  for  $[P4VIm] > [NDBA]$  (A) and  $(4.53 \pm 0.64) \times 10^{-4}M$  for  $[NDBA] > [P4VIm]$  (B). The first order rate constants for the decomposition of the macromolecule-substrate complex were calculated<sup>(3)</sup> to be  $0.032 \pm 0.004 \text{ min}^{-1}$  for A and  $0.088 \pm 0.013 \text{ min}^{-1}$  for B. The novel similarity of the  $K_m$  values would appear to suggest that the mechanisms of complexation for both systems are similar, regardless of which material is in excess concentration.

Although the determined values of  $K_m$  are the same order of magnitude as those of certain enzyme-substrate reactions, the efficiencies of these polymeric reactions in 43.7% ethanol-water leave much to be desired. We hope to report in the near future the results of the rapid solvolyses of NDBA at low ethanol contents, as well as the effects of temperature, pH and ionic strength on the solvolyses of a variety of neutral and anionic paraffinic substrates.

#### IV. THE EFFECT OF IONIC STRENGTH ON THE INHIBITION OF A PHOSPHATE ESTER

In the last summary progress report<sup>(15)</sup> and at our recent visit to Edgewood Arsenal we discussed briefly the effect of ionic strength on the hydrolysis of tri(choline chloride) phosphate (TCCP) in the presence and absence of anionic polyacrylic acid. We have now completed our investigation of this system.

The kinetics of the reaction



were followed by means of a pH-stat at pH 9.0 in the presence and absence of polyacrylic acid, and at varying ionic strength. The influence of ionic strength ( $\mu$ ) on the rate of the uncatalyzed reactions is as predicted by Laidler<sup>(19)</sup> for reactions which involve charged species; i.e., the logarithm of the initial rate is linearly related to the square root of the ionic strength. This behavior arises from the change in the activity coefficient with a change in  $\mu$  as predicted by the Debye-Hückle theory—the equation is



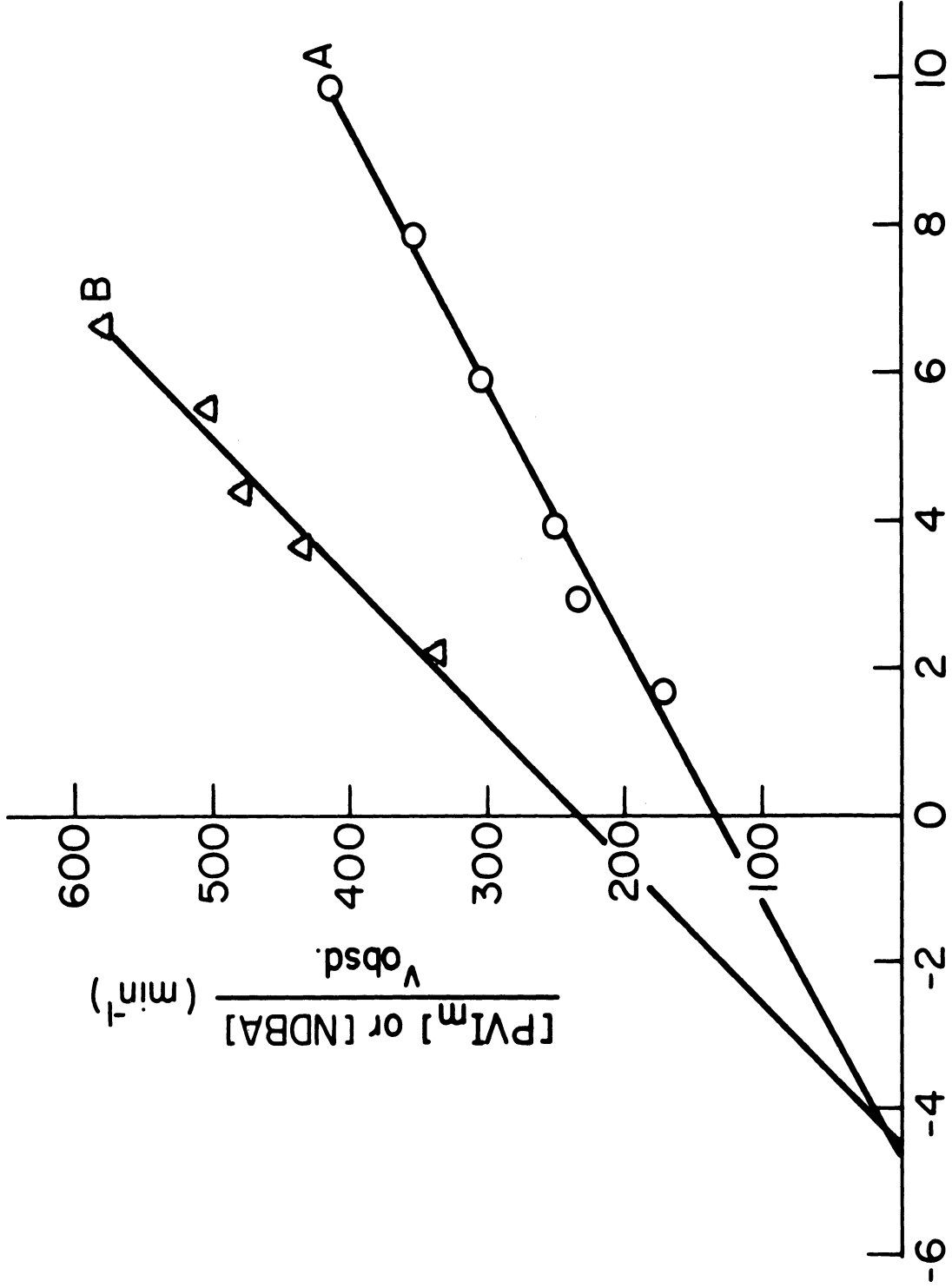


Figure 4. Modified Lineweaver-Burk plots for [PVI<sub>m</sub>]/v<sub>obsd.</sub> vs. [PVI<sub>m</sub>] and [NDBA]/v<sub>obsd.</sub> vs. [NDBA].

$$\log K = \log K^\circ + 1.02 Z_A Z_B \mu$$

where  $K$  is the rate at any  $\mu$ ,  $K^\circ$  is the rate at zero  $\mu$ , and  $Z_A$  and  $Z_B$  are the charges of A and B, respectively. For the blank reaction,  $1.02 Z_A Z_B$  is found to be  $-0.75$ . Since hydroxide ion has a charge of  $-1$ , the charge of phosphate center under attack would then have a charge of  $+0.75$ . This appears to be an unusual method for ascertaining the charge of phosphorous under conditions in which the ester is hydrolyzed.

In the presence of polyacrylic acid the reaction was strongly inhibited, and this effect decreased with increasing ionic strength (Table II). The inhibition factor is defined by  $K^\circ/K$ .

TABLE II\*

EFFECT OF IONIC STRENGTH ON THE INHIBITION OF TCCP

		(M.min <sup>-1</sup> )		
[KCl]	$\mu$	$6 + \log K^\circ$	$6 + \log K$	$K^\circ/K$
0.00	0.011	1.685	0.603	12.10
0.02	0.031	1.580	0.796	6.10
0.04	0.051	1.515	0.940	3.76
0.06	0.071	1.460	1.032	2.68
0.10	0.111	1.370	1.130	1.74
0.15	0.161	1.290	1.130	1.45
0.20	0.211	1.210	1.080	1.35

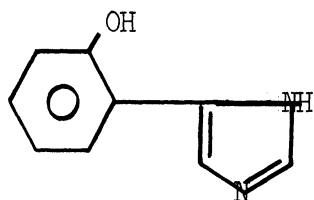
\*pH 9.0, 10% ethanol-water; [polyacrylic acid] = 0.01 M, [TCCP] = 0.001 M.

From Table II it can be seen that as the ionic strength is increased, the inhibition factor is markedly decreased. These data suggest that the increased salt content shields the triply charged TCCP from the anionic carboxylate of the polymer chain.

## V. ESTEROLYTIC BEHAVIOR OF DIMERIC ANALOGS

In our last summary progress report<sup>(18)</sup> the preparation of the dimeric imidazole-phenol dimer, 4(5)-(2-hydroxyphenyl)imidazole (I), was presented.

In Table III are listed the second order catalytic rate constants for the solvolysis of *p*-nitrophenyl acetate (PNPA) catalyzed by imidazole, phenol, and 4(5)-(2-hydroxyphenyl)imidazole. The  $pK_1$  for the imidazole group of I was determined to be 6.0, while the  $pK_a$  of the phenol group of I was determined to



(I)

TABLE III\*

SECOND-ORDER RATE CONSTANTS FOR THE SOLVOLYSIS OF PNPA CATALYZED  
BY IMIDAZOLE, PHENOL, AND IMIDAZOLE-PHENYL DIMER

pH	Imidazole	Phenol	Dimer
7.32	8.4	-	2.2
8.13	11.5	-	2.3
9.22	11.8	-	3.3
9.81	10.1	-	13.6
10.34	11.3	7.25	32.7

\*In 30% n-propanol-water,  $\mu = 0.02$ ,  $26^\circ$ .

be 11.0. Since the  $pK_a$  of monomeric phenol is 10.4, it is conceivable that the enhanced action of I may be caused by a cooperative interaction of imidazole and phenoxide groups. A situation, such as this, has been reported for a copolymer of 4(5)-vinylimidazole and p-vinylphenol.<sup>(7)</sup>

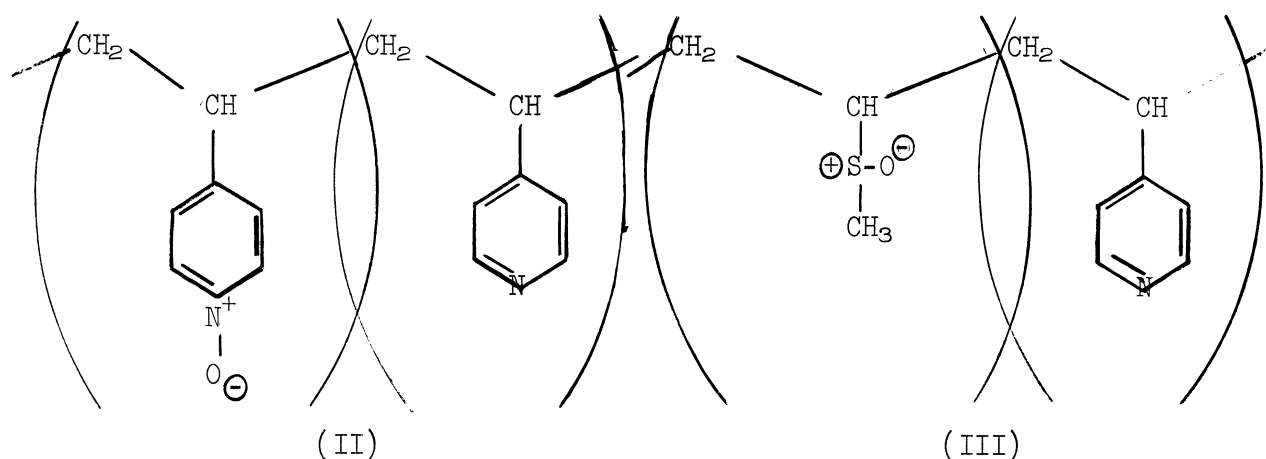
Further studies with I are in progress.

## VI. OXIDATION-REDUCTION POLYMERS

Since polymeric catalysts have revealed several analogies to hydrolytic enzymes, we are attempting to prepare a synthetic, polymeric system which may reveal similarities to the redox enzymes.

A system, which is amenable to an oxidation-reduction reaction and one which is of importance as a preparative organic procedure, is the oxidation of halides or tosylates to carbonyl compounds. Using the concept of bifunctional interactions between two pendent groups on a polymer and substrate, it is possible that copolymers of 4-vinylpyridine and 4-vinylpyridine-N-oxide (II) or 4-vinylpyridine and vinylmethyl sulfoxide (III) would be very efficient oxidative agents. For each of these copolymers the halide or tosylate could be attacked quickly by the oxide. The tetrahedral intermediate thus formed could

then be decomposed by a nucleophilic attack from a neighboring neutral pyridine group, thereby giving the desired aldehyde or ketone.



These systems represent a type of synthetic polymer not yet investigated, since the oxidation-reduction process is dependent upon a cooperative attack between two functional groups and the substrate. The preparations of these copolymers are currently in progress.

#### VII. SIGNIFICANCE OF THIS RESEARCH

The research described in this report has indicated how polymeric reactions have revealed many analogies to the catalytic reactions of enzymes. Although these synthetic, polymeric catalysts have appeared to be considerably less efficient than enzymes in their hydrolytic reactions, recent evidence indicates that such rapid reactions are also possible using the principle of hydrophobic and electrostatic interactions between a substrate and a synthetic macromolecule.

It is hoped that the reactions described above will lead to a more comprehensive understanding of the behaviors of naturally occurring macromolecules, as well as elucidating the behaviors of synthetic macromolecules with low molecular weight materials.

#### VIII. GLOSSARY

- PNPA - p-nitrophenyl acetate
- $\alpha_1$  - fraction of neutral functions
- $\alpha_0$  - fraction of cationic functions
- $\alpha_2$  - fraction of anionic function
- DNPA - 2,4-dinitrophenyl acetate
- $k_{cat}$  - second-order catalytic rate constant
- NABS - sodium 3-nitro-4-acetoxybenzenesulfonate
- PVIm - poly-4(5)-vinylimidazole
- NDBA - 3-nitro-4-dodecanoyloxybenzoic acid
- TCCP - tri(choline chloride) phosphate

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13. ABSTRACT <p>In this report are presented the effects of cooperative interactions between two pendent imidazole functions on a polymer chain and a substrate. Such cooperative interaction lead to enhanced catalytic efficiencies of polymeric catalysts. The effect of hydrophobic and electrostatic binding in synthetic, polymeric catalysis is discussed. Reactions of this type appear to be as efficient as an enzymic process. Inhibition of the hydrolysis rate of the triply charged phosphate ester tri(choline chloride) phosphate in the presence of anionic polyacrylic acid is discussed. A new oxidation reduction system employing macromolecules and low molecular weight materials is presented.</p>			



14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Cooperative interaction						
Bifunctional interaction						
Imidazole						
Poly-4(5)-vinylimidazole						
Hydrophobic bonding						
Electrostatic bonding						
NDBA						
Charged substrated						
Inhibition						
Hydrophobic interactions						
Enzyme-substrate complexation						
Saturation						
Phosphate ester						
Tri(choline chloride) phosphate						
Anionic polyacrylic acid						
Dimeric analogs						



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