

Investigation of a Synthetic Catalytic System Exhibiting Substrate Selectivity and Competitive Inhibition^{1,2}

Robert L. Letsinger and Irvin S. Klaus³

Contribution from the Department of Chemistry, Northwestern University, Evanston, Illinois 60201. Received March 20, 1965

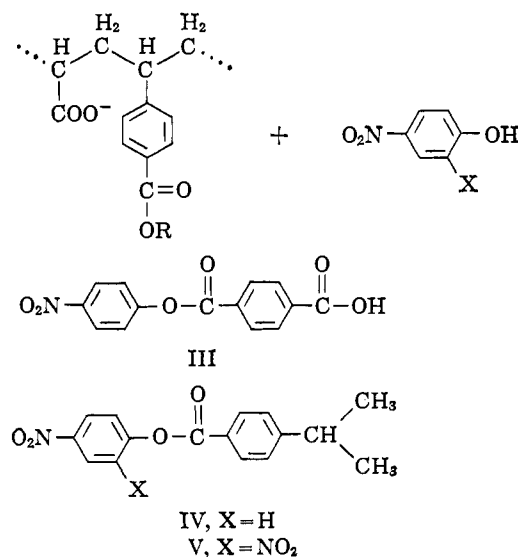
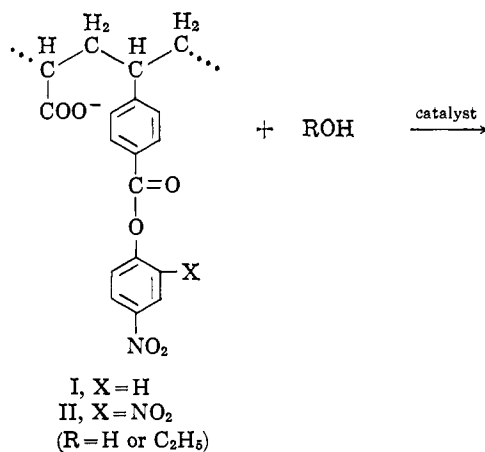
Catalysis by poly(N-vinylimidazole) of the solvolysis of mono- and dinitrophenyl ester groups in polymeric substrates bearing carboxyl groups exhibits several features characteristic of enzymatic reactions, namely, appreciable catalytic activity at low catalyst concentration, kinetics indicative of saturation of the catalyst at high substrate concentration, competitive inhibition by a substance (polyacrylic acid) possessing the same binding groups as the substrate, and catalysis of the reaction of the polymeric substrates (e.g., acrylic acid-2,4-dinitrophenyl p-vinylbenzoate copolymer) possessing binding sites in preference to structurally similar substrates (e.g., 2,4-dinitrophenyl p-isopropylbenzoate), which lack binding sites for the catalyst. These properties are attributed to the formation of a catalyst-substrate complex which increases the probability of encounter between nucleophilic sites on the catalyst and the labile ester groups on the substrate.

In a previous paper we reported that partially protonated poly(4-vinylpyridine) and poly(N-vinylimidazole) are unusually effective catalysts for the solvolysis of nitrophenyl esters, such as potassium 4-acetoxy-3-nitrobenzenesulfonate, which bear a negative charge in the nitrophenyl portion of the molecule.⁴ Recently Overberger, *et al.*, studying 4-acetoxy-3-nitrobenzoic acid and poly-4(5)-vinylimidazole, provided another example of the high activity of a cationic, polymeric catalyst toward an anionic substrate.⁵ The enhanced activity of the partially protonated basic polymers was attributed to electrostatic attraction between the substrates and the polymer, which tends to concentrate the substrate in the region of the active nucleophilic centers.⁴

These systems clearly demonstrate the significant role that electrostatic charge may play in nucleophilic catalysis in polymeric systems. As examples for "selective catalysis"⁴ and as models for enzymatic reactions, however, they suffer several deficiencies. (1) The reaction rates increased linearly with the catalyst concentration; no saturation phenomena characteristic of strong association between catalyst and substrate could be observed. (2) While negatively charged substrates solvolyzed unusually rapidly, no cases were found in which the relative order of solvolysis of two closely related substrates could be reversed by changing from a non-

selective to a selective catalyst. (3) Competitive inhibition was not demonstrated.

For development of selective, synthetic catalytic systems, which in kinetic behavior and specificity of action more closely resemble the enzymatic ones, it appeared that substrates should be sought which would be much more strongly bound to the catalyst than in the cases heretofore examined. With the expectation that a polyanionic substrate would satisfy this condition we undertook a study of polymeric substrates which contained multiple binding sites as well as nitrophenyl ester groups (reaction sites). The substances selected for investigation were copolymers of acrylic acid and p-nitrophenyl p-vinylbenzoate (I) or 2,4-dinitrophenyl p-vinylbenzoate (II). In the range of pH 7 most of the carboxyl groups should be ionized and therefore serve as binding sites. Moreover, the carboxyl groups would be sufficiently far removed from the neighboring ester groups in these substances that intramolecular catalysis of solvolysis by the carboxylate anions should



(1) Paper IV in the series on Selective Catalysis. For a preliminary account see R. L. Letsinger and I. Klaus, *J. Am. Chem. Soc.*, **86**, 3884 (1964). Papers I-III: *ibid.*, **84**, 3122 (1962); **85**, 2230 (1963); **85**, 2223 (1963).

(2) This research was supported by a grant from the National Science Foundation (G25069).

(3) Toni-Gillett Fellow, 1961; Lubrizol Foundation Fellow, 1962.

(4) R. L. Letsinger and T. J. Savereide, *J. Am. Chem. Soc.*, **84**, 114 (1962); **84**, 3122 (1962).

(5) C. G. Overberger, T. St. Pierre, N. Vorchheimer, and S. Yaroslavsky, *ibid.*, **85**, 3513 (1963); **87**, 296 (1965).

be negligible.⁶ The present paper reports the results obtained with the copolymers and also with three low molecular weight substrates, *p*-nitrophenyl hydrogen terephthalate (III), *p*-nitrophenyl *o*-isopropylbenzoate (IV), and 2,4-dinitrophenyl *p*-isopropylbenzoate (V), which served as models for evaluating the reactivity of the copolymers. The stoichiometry is typified by the following equation which represents the solvolysis of one of the ester groups in the polymer chain.

Experimental

p-Nitrophenyl *p*-Vinylbenzoate. To 2.5 g. (0.0165 mole) of *p*-vinylbenzoic acid⁷ and an excess of *p*-nitrophenol (5.3 g., 0.0381 mole) in 50 ml. of dry pyridine at room temperature was added dropwise with stirring 3.5 g. (0.017 mole) of *N,N'*-dicyclohexylcarbodiimide dissolved in 25 ml. of pyridine. Stirring was continued for 24 hr., whereupon the solid which had precipitated was removed by filtration. The filtrate was evaporated and the residue was dissolved in ether and washed successively with several portions of dilute acid, aqueous sodium carbonate, and water. Evaporation of the ether layer afforded *p*-nitrophenyl *p*-vinylbenzoate, which after two recrystallizations from ethanol melted sharply at 103° and weighed 1.4 g. (31.5%). Strong bands in the infrared spectrum (5–9- μ region) were found at 5.85, 6.20, 6.28, 6.59, 6.70, 7.12, 7.40, and 7.86 μ .

*Anal.*⁸ Calcd. for $C_{15}H_{11}NO_4$: C, 66.91; H, 4.12; N, 5.20. Found: C, 66.95; H, 4.27; N, 5.37.

2,4-Dinitrophenyl *p*-Vinylbenzoate. A solution of 3.76 g. (0.0185 mole) of *N,N'*-dicyclohexylcarbodiimide in 40 ml. of pyridine was added to 100 ml. of a solution of 2.75 g. (0.0185 mole) of *p*-vinylbenzoic acid and 3.41 g. (0.0185 mole) of 2,4-dinitrophenol in pyridine. After the mixture had stood overnight at room temperature with stirring, it was filtered and the solvent was removed at reduced pressure. Two recrystallizations of the residue from chloroform–hexane afforded 3.95 g. (66%) of 2,4-dinitrophenyl *p*-vinylbenzoate, m.p. 143–144°. The infrared spectrum contained strong bands at 5.72, 6.20, 6.60, 7.45, and 7.86 μ . The analytical sample, m.p. 145°, was further recrystallized from benzene–hexane. A mixture melting point taken with *p*-vinylbenzoic acid was 123–129°.

Anal. Calcd. for $C_{15}H_{10}N_2O_8$: C, 57.33; H, 3.21; N, 8.92. Found: C, 57.36; H, 3.28; N, 9.00.

p-Nitrophenyl *p*-Isopropylbenzoate. *p*-Isopropylbenzoic acid (9.0 g., 0.055 mole, m.p. 116.5–117.5°) was mixed with excess thionyl chloride and allowed to stand at room temperature for 3 hr.; thereafter excess thionyl chloride was removed under reduced pressure and 9.0 g. (0.065 mole) of *p*-nitrophenol in 60 ml. of dry pyridine was added. After the mixture was warmed on the steam bath for several hours, the pyridine was evaporated under reduced pressure and the residue was dissolved in chloroform. Washing the chloroform solution several times with dilute acid, water, aqueous sodium carbonate, and water, followed by evaporation

of the chloroform in a rotatory evaporator, yielded *p*-nitrophenyl *p*-isopropylbenzoate, which after recrystallization several times from ethanol weighed 9.3 g. (60%) and melted at 104.5–105°, λ_{max} 5.80 μ .

Anal. Calcd. for $C_{16}H_{15}NO_4$: C, 67.36; H, 5.30; N, 4.91. Found: C, 67.46; H, 5.44; N, 4.97.

2,4-Dinitrophenyl *p*-Isopropylbenzoate. A solution containing *p*-isopropylbenzoyl chloride, prepared from 1.64 g. (0.01 mole) of the acid as in the previous experiment, 1.84 g. (0.01 mole) of 2,4-dinitrophenol, and 1 ml. of pyridine in 50 ml. of benzene was heated at reflux for 3 hr. After the mixture had cooled, it was filtered and the filtrate was evaporated to dryness. The residue was extracted with boiling ether. On cooling the extract, 1.22 g. (37%) of 2,4-dinitrophenyl *p*-isopropylbenzoate crystallized, m.p. 116.5–117.5°, λ_{max} 5.71 μ . A mixture with *p*-isopropylbenzoic acid melted at 95–105°.

Anal. Calcd. for $C_{16}H_{14}N_2O_8$: C, 58.18; H, 4.27; N, 8.48. Found: C, 58.41; H, 4.19; N, 8.70.

p-Nitrophenyl Hydrogen Terephthalate. *p*-Nitrophenol (6.58 g., 0.047 mole) in 100 ml. of pyridine was added dropwise to 10.1 g. (0.050 mole) of terephthalyl chloride in 30 ml. of pyridine at about 100°. After standing overnight the mixture was filtered to remove a small amount of di-*p*-nitrophenyl terephthalate, m.p. 245°.

Anal. Calcd. for $C_{20}H_{12}N_2O_8$: C, 58.82; H, 2.96; N, 6.91. Found: C, 58.36; H, 3.28; N, 6.80.

The filtrate was then evaporated and the residue, after washing with ether and dilute hydrochloric acid, was recrystallized from benzene–hexane to give 3.15 g. (22%) of *p*-nitrophenyl hydrogen terephthalate, m.p. 210–211.5°. The analysis correspond to the half ester plus $\frac{1}{6}$ molecule of benzene.

Anal. Calcd. for $C_{14}H_9NO_6 \cdot \frac{1}{6}C_6H_6$: C, 60.00; H, 3.36; N, 4.67. Found: C, 60.31; H, 3.32; N, 4.82.

The infrared spectrum of *p*-nitrophenyl hydrogen terephthalate had bands characteristic for the 2,4-dinitrophenyl ester carbonyl (5.72 μ), carboxyl carbonyl (5.90 μ), carboxyl O–H (3.5, 3.8 and 4.0 μ), and the nitro group (6.59 and 7.40 μ). The compound was completely soluble in aqueous sodium bicarbonate solution and it reprecipitated from the solution on addition of acid.

Acrylic Acid-*p*-Nitrophenyl *p*-Vinylbenzoate Copolymer (I). A mixture of 0.40 g. (5.7 mmoles) of acrylic acid, 0.60 g. (2.23 mmoles) of *p*-nitrophenyl *p*-vinylbenzoate, and 10 mg. of benzoyl peroxide in 20 ml. of dry benzene was warmed at 60° for 72 hr. under a nitrogen atmosphere. Benzene was evaporated, and the solid residue was ground under ether and extracted several times with ether to give 0.7 g. of copolymer. The milliequivalent of ester groups per gram of polymer was determined by hydrolyzing 0.0144 g. of polymer in 10 ml. of 0.1 *M* aqueous sodium hydroxide. From the absorbance (0.598 at 404 $m\mu$) of a solution obtained by diluting 1.0 ml. of this solution to 100 ml. with water, and the extinction coefficient of *p*-nitrophenol in alkali (1.84×10^4), the milliequivalents of ester per gram of polymer was found to be 2.3.

Acrylic Acid-2,4-Dinitrophenyl *p*-Vinylbenzoate Copolymer (II). A vial containing 1.95 g. (6.2 mmoles)

(6) The copolymer of acrylic acid and *p*-nitrophenyl acrylate, though more readily available, was unattractive since it solvolyzes very rapidly in aqueous solutions as a consequence of intramolecular catalysis. See E. Gaetjens and H. Morawetz, *J. Am. Chem. Soc.*, **83**, 1738 (1961).

(7) J. R. Leebrick and H. E. Ramsden, *J. Org. Chem.*, **23**, 935 (1958).

(8) The analyses were made by Miss H. Beck and by the Micro-Tech Laboratories, Skokie, Ill.

of 2,4-dinitrophenyl *p*-vinylbenzoate, 0.81 g. (11.2 mmoles) of acrylic acid, 10 mg. of benzoyl peroxide, and 30 ml. of benzene was flushed with nitrogen, sealed, and suspended in an oven at 65° for 96 hr. The vial was then opened and the liquid was decanted from the precipitate and evaporated at reduced pressure. The combined solids were ground under ether and washed with ether to give 1.0 g. of polymer which softened at approximately 160°. A portion (0.6 g.) of this substance was dissolved in dioxane and partially reprecipitated by addition of a mixture of ether and hexane (3:1). Two-tenths of a gram of white polymer (softening point approximately 255°) was obtained as a precipitate. From the absorbance of a solution obtained by hydrolyzing 33.9 mg. of this copolymer with aqueous sodium hydroxide and from the extinction coefficient of 2,4-dinitrophenol in alkaline solution (1.13×10^4) it was found that the polymer contained 1.9 mequiv. of ester groups per gram.

Poly(acrylic Acid). Freshly distilled acrylic acid was warmed at 120° until polymerization appeared to be complete. The resulting material was stirred with water and the mixture was centrifuged to remove the insoluble portion. Addition of concentrated hydrochloric acid to the solution afforded a precipitate, which was recovered by centrifugation and redissolved in water. On freezing the solution and subliming the water at reduced pressure a porous cake of poly(acrylic acid) was obtained.

Viscosity Determinations. Relative viscosities were determined in a modified Oswald viscometer. Serial dilutions were made and the intrinsic viscosity, $[\eta] = \lim_{C \rightarrow 0} 2.3/C \log t/t_0$, was obtained from the plot of reduced viscosity, $2.3/C \log t/t_0$, vs. C , where C is the concentration of the polymer in grams per deciliter, t is the average flow time for the polymer solution, and t_0 is the average flow time for the solvent. The intrinsic viscosities obtained were: poly(N-vinylimidazole), 0.97; acrylic acid-*p*-nitrophenyl *p*-vinylbenzoate copolymer (I), 1.26; and acrylic acid-2,4-dinitrophenyl *p*-vinylbenzoate copolymer (II), 0.087.

Reagents. 2,4,6-Trimethylpyridine (collidine), b.p. 171–172°, was distilled at atmospheric pressure. The sample of N-methylimidazole was a fraction which distilled at 93° (3 mm.). Reagent grade imidazole, m.p. 90.5–91°, was used directly. Dioxane was refluxed over sodium for 60 hr. and distilled (b.p. $99.9 \pm 0.1^\circ$) prior to use. The N,N-dimethylformamide was first shaken with potassium hydroxide and distilled from calcium oxide. It was then warmed with phthalic anhydride and redistilled at reduced pressure. The fraction collected boiled at 52.5° (22 mm.). Solutions of nitrophenyl esters in N,N-dimethylformamide thus purified were stable and did not turn yellow.

Poly(N-vinylimidazole) was kindly provided by the Badische Anilin und Soda Fabrik, Ludwigshafen, Germany. Prior to use the polymer was washed successively with boiling benzene, hexane, and ethyl ether, and then freed of adhering solvents by evacuation at 1 mm. for 24 hr.

Spectrophotometric Titration. A titration was carried out by adding portions of 10% hydrochloric acid in 50% (weight) aqueous ethanol from a syringe microburet (0.2 μ l. per division) to 80 ml. of 50% aqueous alcohol

which was $8 \times 10^{-4} M$ in poly(N-vinylimidazole) and 0.01 M in potassium chloride. The temperature was $25 \pm 0.2^\circ$. After addition of each increment a sample of the polymer was withdrawn and the absorbance determined at 225 $m\mu$. The absorbance varied from 1.199 at pH 3.70 to 1.325 at pH 7.90. Addition of aqueous sodium hydroxide brought the absorbance to 1.326 at pH 9.30. The results are given in Figure 1.

Kinetics. The reactions were carried out in 50% (weight) ethanol-water solution $1 \times 10^{-2} M$ in potassium chloride and $2 \times 10^{-2} M$ in heterocyclic base units. When the base unit concentration of the catalyst (polyvinylimidazole,⁹ imidazole, or N-methylimidazole) was less than $2 \times 10^{-2} M$, sufficient collidine, which does not function as a nucleophilic catalyst, was added to bring the total concentration to $2 \times 10^{-2} M$. The pH was adjusted by addition of hydrochloric acid. For the reactions of the monomeric esters 0.25 ml. of the substrate solution ($6.14 \times 10^{-3} M$ ester in dioxane) was added to 25 ml. of the catalyst solution, which had previously been brought to the reaction temperature ($30 \pm 0.2^\circ$ or $25 \pm 0.2^\circ$). With the dinitrophenyl esters, a portion of the reaction mixture was transferred to a cuvette and the absorbance change at 412 $m\mu$ followed in a Beckman DU spectrophotometer. A_∞ was obtained after sufficient time had elapsed that no further change in absorbance could be detected. With the *p*-nitrophenyl esters, which hydrolyzed very slowly in 50% aqueous ethanol, the reaction mixture was kept in a thermostated bath. At suitable intervals 2-ml. aliquots were removed and added to 1 ml. of 10% aqueous hydrochloric acid to quench the reaction. The absorbance was read at 344 $m\mu$, where the absorbances of acidic and alkaline solutions of *p*-nitrophenol are the same. A_∞ values were obtained from aliquots of the reaction mixture which were added to aqueous sodium hydroxide to complete the solvolysis.

With the polymeric substrates it was necessary to exercise considerable care in mixing the substrate solution with the solution of poly(N-vinylimidazole) in order to avoid formation of a precipitate or development of turbidity. In the case of copolymer II equal volumes (12.5 ml.) of solvent and of catalyst solution, each 0.01 M in potassium chloride, were brought to 25°. To the solvent portion was added with vigorous stirring 0.25 ml. of a dioxane solution of copolymer II $6.14 \times 10^{-3} M$ in ester units. The catalyst solution was then added, again with vigorous stirring, to the substrate solution thus prepared. A sample of the mixture was transferred quickly to a cuvette for the absorbance reading. The mixing procedure for copolymer I was similar except that N,N-dimethylformamide was used as a solvent for the polymer in place of dioxane. Clear solutions were obtained in all cases except for the experiment involving poly(N-vinylimidazole) and copolymer II at the highest ester concentration ($4.9 \times 10^{-4} M$, see Figure 6), for which a bluish cast, indicative of some colloidal suspension, was apparent. For the reactions involving polyacrylic acid as an inhibitor, experiments were carried out in which polyacrylic acid was added first to the solution

(9) Throughout this paper the concentration of poly(N-vinylimidazole) is expressed as moles/l. of imidazole groups in the polymer. Similarly, the concentration of the polymeric substrate is expressed as moles/l. of ester groups.

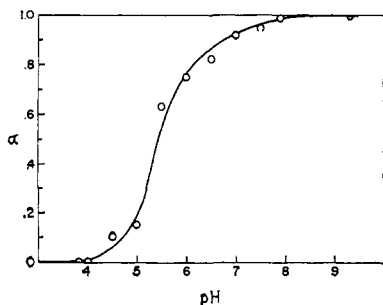


Figure 1. Titration of poly(N-vinylimidazole) in 50% aqueous ethanol at 25°C.

of poly(N-vinylimidazole) and also in which the polyacrylic acid was added first to the substrate solution to see if the order of mixing influenced the inhibitor's action. No effect was found, indicating that equilibrium was achieved before appreciable solvolysis of the substrate.

To see if the spectral changes observed actually reflected the conversion of ester groups to the nitrophenol, a reaction of copolymer II ($6.14 \times 10^{-5} M$) catalyzed by poly(N-vinylimidazole) ($1 \times 10^{-4} M$) was followed by repeatedly scanning the spectrum between 290 and 400 $m\mu$. The final spectrum corresponded to that of 2,4-dinitrophenol and an isosbestic point occurred at 313 $m\mu$. This experiment shows that the absorbance increase was due to formation of 2,4-dinitrophenol, not to development of turbidity that was visually undetectable.

Treatment of Kinetic Data. The rate data for individual experiments with the monomeric substrates (*p*-nitrophenyl hydrogen terephthalate, *p*-nitrophenyl *p*-isopropylbenzoate, and 2,4-dinitrophenyl *p*-isopropylbenzoate) and for the copolymer of acrylic acid and *p*-nitrophenyl *p*-vinylbenzoate were satisfactorily accommodated by a first-order rate equation. Pseudo-first-order rate constants are indicated in the tables as k_{obsd} . For the reactions of acrylic acid-2,4-dinitrophenyl *p*-vinylbenzoate copolymer, curvature in the first-order plots was observed. This feature was a characteristic of the substrate and did not depend upon the catalyst employed. It is not clear why copolymers I and II differ in their kinetic pattern; however, it may be noted that complex kinetics are often encountered in reactions of polymeric substrates.^{6,10} The reactions of copolymer II may be compared by means of initial velocities. The results are essentially the same as for the treatment that follows, which we prefer since it shows that the effect of changing the catalyst or the initial concentrations is a characteristic of the system over a wide range of substrate conversion. It was found that reasonably good straight lines were obtained when $(A - A_0)/(A_\infty - A)$ was plotted against time for reactions of copolymer II catalyzed by imidazole, N-methylimidazole, and poly(N-vinylimidazole) as well as for a reaction conducted in the buffer solution in absence of an imidazole-type catalyst. Typical results are given in Figure 2. A_0 , A_∞ , and A represent the absorbancies at initial time, at completion of the reaction, and at the time of a measurement. As shown

(10) See, for example, J. Moens and G. Smets, *J. Polymer Sci.*, **23**, 931 (1957).

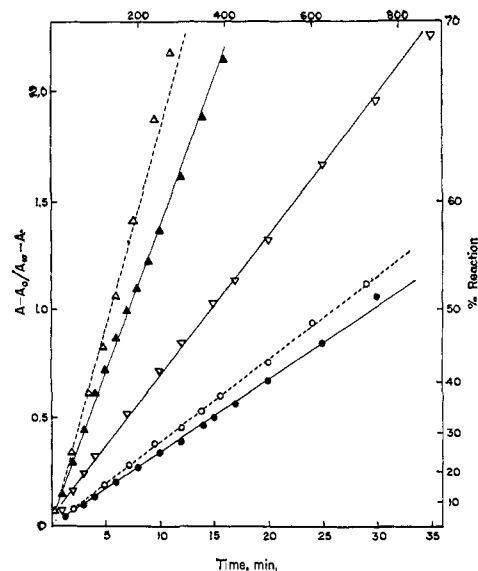


Figure 2. Solvolysis of copolymer II ($6.14 \times 10^{-5} M$ in ester groups): \circ , without catalyst; \bullet , with 0.02 M N-methylimidazole; Δ , with $2.5 \times 10^{-5} M$ poly(N-vinylimidazole); ∇ , with $5 \times 10^{-4} M$ poly(N-vinylimidazole); \blacktriangle , with 0.02 M poly(N-vinylimidazole). Solid lines, read bottom time scale; broken lines, read top time scale.

in Figure 3, the fit was somewhat less satisfactory for the experiments in which the initial substrate concentration was varied, but it was good enough to permit a meaningful comparison of the data. These plots correspond to rate eq. 1. The relatively slow rate of

$$(A - A_0)/(A_\infty - A) = k't \quad (1)$$

solvolysis at high conversions may be ascribed to the low intrinsic reactivity of the ester groups in the partially hydrolyzed polymer. Equation 1 represents the case in which the intrinsic reactivity of the ester groups is proportional to C/C_0 and the instantaneous rate is given by eq. 2, where C and C_0 are the concentration of the ester groups at a given time and at zero time.

$$\frac{-dC}{dt} = k'C(C/C_0) \quad (2)$$

Results

Previous studies^{4,5} of selective catalysis of nitrophenyl esters, $RCOOR'$, involved substrates with a negative charge in the nitrophenyl portion of the molecule, R' . In contrast, the negative charges in copolymers I and II are located in the R portion of the ester. To ascertain whether compounds of this general type also exhibit enhanced catalytic rates in the presence of positively charged catalysts, we first investigated the reaction of a simple monomeric substrate, *p*-nitrophenyl hydrogen terephthalate. Data for solvolysis of this ester in the presence of poly(N-vinylimidazole) are plotted in Figure 4. Throughout most of the pH range investigated the substrate exists largely as a monoanion and the catalyst possesses positive sites as well as nucleophilic centers. A pronounced maximum is apparent in the rate-pH profile, indicative of a rate enhancement stemming from the negative charge on the substrate. It may be noted that *p*-nitrophenyl benzoate, an electrically neutral substrate used as a control,

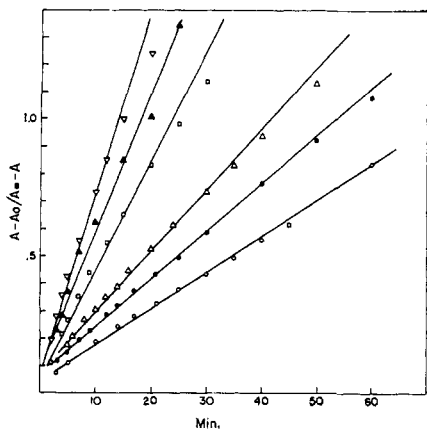


Figure 3. Solvolysis of copolymer II in presence of 5×10^{-4} M poly(N-vinylimidazole) at 25° . Concentration of ester groups in substrate $\times 10^4$, for curves left to right, 0.246, 0.430, 1.23, 3.46, 3.68, and 4.91, respectively.

behaved in a normal manner in the solvolytic reaction (see Figure 4).

Supported by this evidence, we continued with the preparation and study of the polymeric substrates. Results obtained with the acrylic acid-*p*-nitrophenyl *p*-vinylbenzoate copolymer (I) and with *p*-nitrophenyl *p*-isopropylbenzoate, a model for the ester portion of the polymer, are summarized in Table I. These substrates

Table I. Solvolysis of *p*-Nitrophenyl *p*-Vinylbenzoate-Acrylic Acid Copolymer (I)^a and *p*-Nitrophenyl *p*-Isopropylbenzoate (IV)^b at $30 \pm 0.2^\circ$

Expt.	Substrate	Catalyst	Catalyst concn., mole/l.	pH	$k_{\text{obsd}} \times 10^6$, min. ⁻¹
1	I	0	...	7.5	1.4
2	I	Im	5×10^{-4}	7.5	1.8
3	I	Im	2×10^{-2}	7.5	13.0
4	I	PVI	5×10^{-4}	7.5	15.0
5	I	0	0	7.9	2.7
6	I	PVI	5×10^{-4}	7.9	12.0
7	I	PVI	2×10^{-2}	7.9	47.0
8	I	PVI	2×10^{-2}	8.5	14.0
9	I	PVI	2×10^{-2}	4.7	3.3
10	IV	0	...	7.5	1.6
11	IV	Im	5×10^{-4}	7.5	5.3
12	IV	PVI	5×10^{-4}	7.5	1.8
13	IV	0	...	8.5	6.4
14	IV	Im	5×10^{-4}	8.5	14.0
15	IV	PVI	5×10^{-4}	8.5	8.1

^a 1.68×10^{-4} M ester groups. ^b 2.0×10^{-4} M; PVI = poly(N-vinylimidazole); Im = imidazole.

constitute a good pair for study of selective solvolysis since they differ only at positions eight carbon atoms or more removed from the ester function. Several features merit comment. First, the rate of solvolysis of copolymer I in the presence of poly(N-vinylimidazole) is faster at pH 7.9 than at either 4.7 or 8.5 (experiments 7-9), in accord with the findings for *p*-nitrophenyl hydrogen terephthalate. Second, whereas imidazole is more effective than poly(N-vinylimidazole) in catalyzing the solvolysis of *p*-nitrophenyl *p*-isopropylbenzoate (experiments 11 and 12 and 14 and 15), poly(N-vinylimidazole) is considerably more effective than imidazole in catalyzing the solvolysis of copolymer I

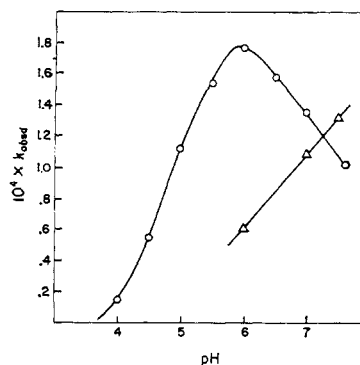


Figure 4. Solvolysis of *p*-nitrophenyl hydrogen terephthalate (O) and *p*-nitrophenyl benzoate (Δ), each 2×10^{-4} M, in presence of 0.02 M poly(N-vinylimidazole) at 30° .

(experiments 2-4). Indeed, the experiments provide a good example of selectivity in catalysis; *p*-nitrophenyl *p*-isopropylbenzoate solvolyzes three times faster than copolymer I in the presence of 5×10^{-4} M imidazole (experiments 2 and 11) whereas copolymer I solvolyzes eight times faster than *p*-nitrophenyl isopropylbenzoate in the presence of 5×10^{-4} M poly(N-vinylimidazole) (experiments 4 and 12). The rate constants, k_{obsd} , represent the actual solvolysis of the substrates and include the contribution from the spontaneous reaction characteristic of the catalyst-free solutions. On the basis of rate constants for the catalyzed portion of the reactions alone, $k_{\text{obsd}} - k_{\text{solvent}}$, the selectivity would appear considerably greater. Finally, it is interesting that $k_{\text{obsd}} - k_{\text{solvent}}$ for copolymer I increases less than fivefold when the poly(N-vinylimidazole) concentration increases 40-fold. This result suggests that the substrate may be saturated with catalyst at the higher catalyst concentration and that catalyst in excess of that needed to complex with the substrate has little effect upon the solvolytic reaction.

The reactions of the *p*-nitrophenyl esters in 50% aqueous alcohol are very slow, requiring several days for completion, even in favorable cases. For a detailed study of the effect of substrate and catalyst concentrations upon the rate of solvolysis it was desirable to work with more reactive esters. Accordingly, the copolymer of acrylic acid and 2,4-dinitrophenyl *p*-vinylbenzoate (II) was prepared and examined. This substrate proved to be relatively labile, particularly in the presence of poly(N-vinylimidazole). Rate data for typical reactions of copolymer II are given in Table II. At pH 7.5 and an imidazole unit concentration of

Table II. Solvolysis of Acrylic Acid-2,4-Dinitrophenyl *p*-Vinylbenzoate Copolymer (6.14×10^{-5} M in Ester)

Catalyst	Catalyst concn., mole/l.	$10^3 \times k'_{\text{obsd}}$, min. ⁻¹
None	...	1.54
N-Methylimidazole	5×10^{-4}	2.0
Poly(N-vinylimidazole)	5×10^{-4}	65
Poly(N-vinylimidazole)	0.25×10^{-4}	7.0

5×10^{-4} M, poly(N-vinylimidazole) is 140 times as effective as N-methylimidazole in catalyzing the solvolysis of copolymer II (measured by $k'_{\text{obsd}} - k'_{\text{solvent}}$).

A striking result was obtained when the reaction of copolymer II was studied as a function of the concentration of poly(N-vinylimidazole). As shown in Figure 5, $k'_{\text{obsd}} - k'_{\text{solvent}}$ increases rapidly with catalyst concentration in the range of 2.5×10^{-5} to 5×10^{-4} M imidazole units. At high concentrations, however, the curve levels off and $k'_{\text{obsd}} - k'_{\text{solvent}}$ is independent of the catalyst concentration. The kinetic picture is markedly different when N-methylimidazole is used as the catalyst. This substance, which is comparable to poly(N-vinylimidazole) with respect to nucleophilic properties but lacks the binding sites of the polymeric catalyst, is ineffective at low concentrations. As the concentration is increased the rate of solvolysis of the substrate increases throughout the range of the experiments (5×10^{-4} M to 1×10^{-2} M).

A similar curve was obtained when the amount of poly(N-vinylimidazole) was held constant (5×10^{-4} M) and the concentration of copolymer II varied over a 20-fold range (2.46×10^{-5} to 4.91×10^{-4} M in initial ester groups). The results are presented in Figure 6 in the form of initial velocity, $v_i = (k'_{\text{obsd}} - k'_{\text{solvent}}) \cdot C_0$, plotted against C_0 , the initial ester concentration. As in many enzymatic reactions the initial velocity is proportional to the substrate concentration at low values of the substrate concentration but it levels off at high substrate concentrations.

If the high activity of poly(N-vinylimidazole) at pH 7.5 in catalyzing solvolysis of copolymer II is due to formation of a substrate-catalyst complex, it would be expected that a substance possessing binding groups similar to the substrate should act as a competitive inhibitor. This expectation was realized in experiments with poly(acrylic acid) as the inhibitor. The data are given in Table III.

Table III. Inhibition by Poly(acrylic Acid)^a

Poly(acrylic acid), mole/l. ^b	$10^3 k'_{\text{obsd}}$, min. ⁻¹
0	65
5×10^{-4}	28 ^c
10×10^{-4}	14 ^c

^a Copolymer I, 6.14×10^{-5} M in ester groups; poly(N-vinylimidazole), 5×10^{-4} M in imidazole units. ^b Of acid groups. ^c k' decreased somewhat as the reaction progressed. These values correspond to the first 30% of the reaction. They are close to values for k'_{obsd} (25×10^{-3} and 16×10^{-3} min.⁻¹, respectively), calculated from the substrate and inhibitor concentrations and from binding and rate data derived from Figure 6. In the calculations it was assumed that the binding constants for carboxylate in copolymer I and in polyacrylic acid are the same and that the ratio of carboxylate to ester in copolymer I is 3:1, as indicated by ultraviolet data.

Finally, it was demonstrated that copolymer II solvolyzes selectively in preference to 2,4-dinitrophenyl *p*-isopropyl benzoate when poly(N-vinylimidazole) is employed as the catalyst. In noncatalyzed solvolyses and with N-methylimidazole as a catalyst, the monomeric substrate reacts faster than copolymer II. Pertinent data are summarized in Table IV. The result is similar to that for copolymer I with poly(N-vinylimidazole) and imidazole as catalysts, and it provides another good example of discrimination by a catalyst on the basis of substrate structural variations distant from the

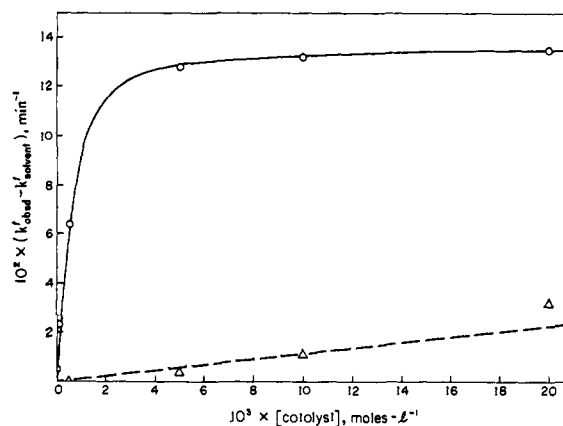


Figure 5. Reaction of copolymer II (6.14×10^{-5} M in ester) catalyzed by poly(N-vinylimidazole (O) and by N-methylimidazole (Δ) at 25°. The solid line is calculated from eq. 6.

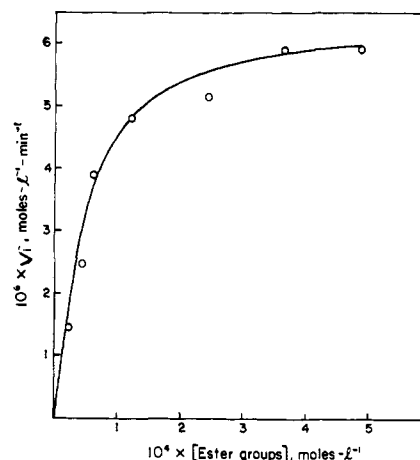


Figure 6. Reaction of copolymer II catalyzed by 5×10^{-4} M poly(N-vinylimidazole) at 25°. The solid line is calculated from eq. 4.

functional groups that undergo covalent change. The reversal in reactivity stems from the unusually high reactivity of a substrate in the presence of a catalyst which possesses complementary binding sites.

Table IV. Selective Catalysis

Catalyst	$10^3 k$, min. ^{-1a}	$10^3 k'$, min. ^{-1b}
None	2.2	1.54
N-Methylimidazole (2×10^{-2} M)	56.5	33
Polyvinylimidazole (5×10^{-4} M in imidazole units)	3.1	65

^a Dinitrophenyl isopropylbenzoate. ^b Copolymer I.

Discussion

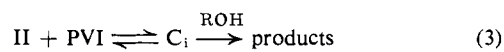
It is clear that poly(N-vinylimidazole) in low concentration at pH 7.5 is a highly effective catalyst for the solvolysis of the copolymers of acrylic acid with *p*-nitrophenyl *p*-vinylbenzoate and 2,4-dinitrophenyl *p*-vinylbenzoate. Neither imidazole nor N-methylimidazole show comparable activity toward these substrates. Furthermore, poly(N-vinylimidazole) does not show unusual activity toward *p*-nitrophenyl *p*-isopropyl benzoate or 2,4-dinitrophenyl *p*-isopropyl benzoate,

substrates which serve as models for the ester portion of copolymers I and II. This fact permits a demonstration of selective catalysis in which the relative solvolytic rates of two similar substrates may be reversed by changing the catalyst (see Results).

The striking reactivity of copolymers I and II in the presence of poly(N-vinylimidazole) seems best explained on the basis of the reversible formation of a complex between the substrate and catalyst, within which nucleophilic nitrogen sites on the catalyst act on the ester groups of the substrate. The complex is held together by attraction between the carboxylate ions in the substrate and protonated nitrogen atoms in the catalyst. Since the binding sites in both the substrate and the catalyst are insulated electronically from the active nucleophilic and reaction sites by a chain of two or more saturated carbon atoms, rate enhancements resulting from inductive or resonance effects of the binding groups may be eliminated from consideration. At pH 7.5 most of the carboxyl groups in the polymeric substrates should be ionized. A spectrophotometric titration of the poly(N-vinylimidazole) revealed that very few, ~5%, of the basic groups in the catalyst are protonated at this pH. That only a relatively low percentage of the nitrogen sites need be protonated to obtain strong binding is reasonable in view of the fact that both components in the complex are polymeric. When *p*-nitrophenyl hydrogen terephthalate served as the substrate, a higher fraction of protonated sites was necessary for effective binding. In this case the maximum effective catalytic activity of poly(N-vinylimidazole) was obtained at pH 6.0, at which one-fourth of the nitrogen sites are protonated.

Strong support for the concept that poly(N-vinylimidazole) and copolymer II form a complex is provided by the competitive inhibition exhibited by poly(acrylic acid) and by the shape of the curves shown in Figures 5 and 6. The solid lines in Figures 5 and 6 are drawn from equations based on the assumption that substrate II and the polymeric catalyst form a complex reversibly (eq. 3), and that the catalytic effect of poly(N-vinylimidazole) outside the complex is negligible compared to that within the complex. The equilibrium constant, K , is defined by eq. 5, and the rate constant

characteristic of the catalyzed reaction, $k'_{\text{obsd}} - k'_{\text{solvent}}$, is given by eq. 6. In these equations $[C_i]$ is



$$v_i = k[C_i] \quad (4)$$

$$\frac{[C_i]}{[E_0 - C_i] \left[\frac{P_0}{n} - C_i \right]} = K \quad (5)$$

$$k'_{\text{obsd}} - k'_{\text{solvent}} = \frac{v_i}{E_0} \quad (6)$$

the initial concentration of the complex expressed as mole/l. of the initial ester groups, v_i is the initial reaction velocity, E_0 and P_0 are the concentrations in mole/l. of the total substrate and catalyst introduced into the solution, and n is the number of imidazole units per ester group in the initial complex. The lines in Figures 5 and 6 were constructed on the assumption that K is 4.2×10^4 l./mole, n is 11, and k is 0.137 min.⁻¹. The agreement between the experimental points and the calculated curves supports the general picture developed for the catalytic reaction and the choice of values for K , n , and k . Since a single set of parameters suffices to correlate the data in both Figures 5 and 6, it appears that the composition of the complex is essentially the same in these solutions whether the polyester or the poly(N-vinylimidazole) is in excess. The value of n seems quite reasonable in view of the composition of the polymers and the low extent of protonation of the poly(N-vinylimidazole) at pH 7.5.

As catalysts, the enzymes are distinguished by their high efficiency and by their selectivity toward structurally similar substrates. The former characteristic appears to depend upon the concerted action of two or more groups in the enzyme on the labile functional group of the substrate. Numerous studies on synthetic model compounds have clearly demonstrated that high reaction rates may indeed result from the concerted action of catalytically active groups. The latter feature appears to depend, in part at least, upon the preferential binding of certain substrates to the enzyme. The present study demonstrates that selective catalysis may also be achieved in synthetic systems in which reversible substrate-catalyst binding occurs.