phosphate was essentially the only product and was isolated in 92% yield. A portion of this material (0.01 mmole) was treated with 80% acetic acid (2 ml.) for 3.25 hr. at room temperature. On chromatography in solvent A, three bands were detected. The main band (73%) corresponded to methyl adenosine-3′ phosphate. In addition there was a small amount (8% of adenosine-3′ phosphate and a band traveling faster than methyl-adenosine-3′ phosphate corresponding, presumably, to methyl-5′-O-tetrahydropyranyl-adenosine-3′ phosphate (19%). The sample of methyl adenosine-3′ phosphate thus obtained when incubated with spleen phosphodiesterase was degraded essentially completely to adenosine-3′ phosphate.

Cytidylyl-(2′ or 3′→5′)-adenosine.—To an anhydrous pyridine solution (1 ml.) of pyridinium $N^6,O^{2'},O^{3'}$-triacetyl-adenosine-5′ phosphate (0.06 mmole) of 5′-O-dimethoxytrityl-$N^6,O^{2'}(O^{3'})$-dibenzoylcytidine, DCC (150 mg.) was added and the sealed reaction mixture was kept in the dark at room temperature for 3 days. Water (0.5 ml.) was then added and after a further 12 hr., the solution was evaporated to a gum which was taken up in 80% acetic acid (5 ml.). After 3 hr. at room temperature, the solution was evaporated to dryness and the residue shaken with 25 ml. of concd. ammonium hydroxide for 12 hr. The solution was then filtered from the insoluble material and after concentration was chromatographed in solvent A on a sheet of Whatman No. 31 paper. The band corresponding to cytidylyl-(2′ or 3′→5′)-adenosine (0.012 mmole, 26%) was eluted with water. Digestion with pancreatic ribonuclease under the standard conditions followed by chromatography in solvent A showed 40% of the synthetic product to be resistant to the action of the enzyme.

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF NORTHWESTERN UNIVERSITY, EVANSTON, ILL.]

Selectivity in Solvolyses Catalyzed by Poly-(4-vinylpyridine)[1]

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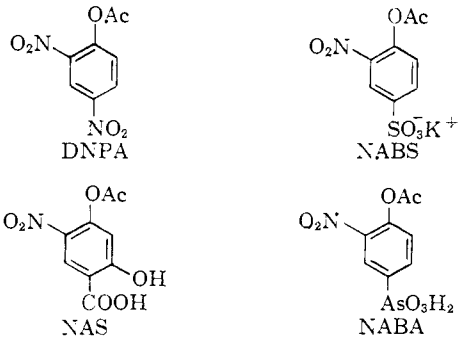
Partially protonated poly-(4-vinylpyridine) in an ethanol water solution serves as a particularly effective catalyst, relative to 4-picoline or to either non-protonated or highly protonated poly-(4-vinylpyridine), in solvolyses of nitrophenyl acetates which bear a negative electric charge. It is a poorer catalyst than 4-picoline for the solvolysis of 2,4-dinitrophenyl acetate, an electrically neutral substance. The selectivity of the partially protonated polymer with respect to charged substrates is attributed to polymer–counter ion electrostatic interaction, which increases the local concentration of an anionic substrate in the region of the polymer coil.

A catalyst is here termed "selective" if it distinguishes between substrates that differ only at positions far removed from the functional group undergoing transformation. Although highly selective catalysts, enzymes, are elaborated in abundance in living systems, little progress has been made in synthesizing agents with comparable properties. The action of an enzyme may be represented schematically by the sequence: E + S → E–S → E + products, where E, S and E–S represent an enzyme, the substrate or substrates, and an enzyme–substrate complex, respectively. Selectivity in these reactions may be achieved by association of the substrate with the catalyst prior to the major covalent change in the substrate.

We report in this paper a study of the catalytic properties of poly-(4-vinylpyridine) in aqueous ethanol solutions. This polymer appeared promising as a selective catalyst since in mildly acidic solution it would possess both cationic sites and basic nitrogen sites. The former should serve to bind anionic substrates[3] and the latter should act as catalytic centers for the hydrolysis of nitrophenyl esters.[4] In addition, the relative number of basic and cationic sites would be subject to control, and, as a result of the flexibility of the polymer chain, both functions would coexist within a given molecule in a great variety of spatial relationships.

2,4-Dinitrophenyl acetate (DNPA), potassium 3-nitro-4-acetoxybenzenesulfonate (NABS), 5-nitro-4-acetoxysalicylic acid (NAS) and 3-nitro-4-acetoxybenzenearsonic acid (NABA) were selected as substrates. The dinitrophenyl acetate was chosen to illustrate the solvolytic behavior of an uncharged ester; the remaining esters, to reveal the effect of charge interaction involving polymer and substrate on the course of a catalyzed solvolysis.



Of the previous attempts to achieve selectivity with synthetic catalysts, the most favorable results were obtained with a cross-linked sulfonated polystyrene resin, Dowex-50.[5] Whitaker and Deathe-

(1) This research was supported in part by a grant from the National Science Foundation, G7414. For a preliminary report, see R. L. Letsinger and T. J. Savereide, *J. Am. Chem. Soc.*, **84**, 114 (1962).

(2) Hercules Powder Co. Fellow, 1958; Public Health Service Research Fellow, 1960.

(3) Numerous studies of counter ion binding by polyelectrolytes have been reported. See, for example: F. T. Wall and W. B. Hill, *J. Am. Chem. Soc.*, **82**, 5599 (1960); F. T. Wall and M. J. Eitel, *ibid.*, **79**, 1550, 1556 (1957); A. M. Liquori, F. Ascoli, C. Botre, V. Cresenzi and A. Mele, *J. Polymer Sci.*, **40**, 169 (1959); M. Nagasawa and I. Kagawa, *ibid.*, **25**, 61 (1957); I. Kagawa and K. Katsuura, *ibid.*, **17**, 365 (1955); H. P. Gregor and D. H. Gold, *J. Phys. Chem.*, **61**, 1347 (1956); P. Doty and G. Ehrlich, *Ann. Rev. Chem.*, (1952); H. Morawetz, A. M. Kotliar and H. Mark, *J. Phys. Chem.*, **58**, 19 (1954). It is noteworthy that H. Ladenheim, E. M. Loebl and H. Morawetz, *J. Am. Chem. Soc.*, **81**, 20 (1959), found that poly-(4-vinylpyridine) functioned selectively in a non-catalytic reaction, quaternization with α-bromoacetamide and bromoacetate ion. See also H. Ladenheim and H. Morawetz, *ibid.*, **81**, 4860 (1959).

(4) The subject of nucleophilic catalysis in the hydrolysis of nitrophenyl esters was reviewed recently by M. L. Bender, *Chem. Rev.*, **60**, 53 (1960).

(5) J. R. Whitaker and F. E. Deatherage, *J. Am. Chem. Soc.*, **77**, 3360, 5298 (1955).

rage found that glycylglycine hydrolyzed 35 times faster than acetylglycine with the resin as catalyst, whereas glycylglycine hydrolyzed at only $^1/_6$th the rate of acetylglycine in hydrochloric acid. The resin system was heterogeneous and it may be assumed that the basic peptide was preferentially held in the acid medium within the resin particles. Comparable selectivity has not been reported for non-enzymatic hydrolyses in homogeneous media; however, good evidence for "electrostatic catalysis" in the hydrolysis of *o*-nitrophenyl hydrogen oxalate catalyzed by 2-aminopyridine was obtained by Bender and Chow,[6] and polystyrenesulfonic and polyethylenesulfonic acid were shown by Kern and Sherhag[7] to be somewhat more effective than hydrochloric acid in hydrolyzing dipeptides. An appreciable effect of certain anions on the course of hydrolysis of proteins in dilute acid solutions was noted by Steinhardt and Fugitt.[8] Thus, in the presence of dodecyl sulfate ions, amide bonds in egg albumin were cleaved in preference to peptide bonds.

Experimental Section

Carbon, hydrogen and nitrogen analyses were performed by Miss Hilda Beck. Melting points were taken on a Fisher–Johns melting point block. The infrared spectra were determined with a Baird double beam recording spectrophotometer with the sample in potassium bromide unless otherwise stated.

5-Nitro-4-acetoxysalicylic acid was best prepared by partial acetylation of 2,4-dihydroxy-5-nitrobenzoic acid by a modification of the method of Hemmelmayr.[9] In our hands the original procedure yielded a product, m.p. 147–149°, which appeared to be the diacetate; λ^{KBr} 5.62, 5.88, 12.1 (absent in the monoacetate) μ.

Anal. Calcd. for $C_{11}H_9NO_8$: C, 46.7; H, 3.20; N, 4.95. Found: C, 47.3; H, 3.12; N, 5.16.

However, by using a molar ratio of sodium acetate to dihydroxynitrobenzoic acid of 1:10 rather than 1:1, we obtained a 30% yield of the monoacetate, m.p. 152–154°, lit.[9] m.p. 150°; λ^{KBr} 5.7, 6.05 7.02 (absent in the diacetate) μ.

Anal. Calcd. for $C_9H_7NO_7$: C, 44.8; H, 2.93; N, 5.81. Found: C, 44.6; H, 2.73; N, 6.12.

5-Nitro-4-acetoxysalicylic acid also was prepared independently by nitration of 4-acetoxysalicylic acid. The acetoxysalicylic acid (9.8 g.)[10] was added to a well stirred mixture of concd. nitric acid (13.5 g.) and acetic anhydride (30 ml.) at −20°. After 90 minutes of stirring at −20° the mixture was poured into water and extracted with ether.[11] Distillation at reduced pressure of the volatile matter from the ether extract left 10.5 g. of yellow powder. After several recrystallizations of a portion (9.5 g.) of this powder, 0.35 g. of pale yellow plates of 5-nitro-4-acetoxysalicylic acid was obtained. This product was identical with that produced by acetylation of 2,4-dihydroxy-5-nitrobenzoic acid. Another portion (0.234 g.) of the crude reaction mixture was treated with diazomethane and the products separated by chromatography on silica gel (10% ether in hexane solvent). **Methyl 2-methoxy-5-nitro-4-acetoxybenzoate** (0.11 g., m.p. 100–101°; λ^{CHCl_3} 5.65, 5.82 μ, no O–H absorption) was isolated. The same ester was also obtained by the action of diazomethane on a pure sample of 5-nitro-4-acetoxysalicylic acid.

Anal. (methyl ester). Calcd. for $C_{11}H_{11}NO_7$: C, 49.3; H, 3.76; N, 5.22. Found: C, 48.9; H, 3.97; N, 5.48.

Alkaline hydrolysis of methyl 2-methoxy-5-nitro-4-acetoxybenzoate afforded a good yield of 2-methoxy-4-hydroxy-5-nitrobenzoic acid, m.p. 191–192°, lit. m.p.[12] 192°; λ^{KBr} 2.90, 5.86 μ; and the alkaline hydrolysis of 4-acetoxy-5-nitrosalicylic acid yielded 2,4-dihydroxy-5-nitrobenzoic acid, m.p. 215°, λ^{KBr} 5.97μ.

The midpoint in the titration curve of 5-nitro-4-acetoxysalicylic acid (titrated in 50% aqueous ethanol 0.04 *M* in potassium chloride at 37.6°) occurred at *p*H 2.69.

Potassium 3-Nitro-4-acetoxybenzenesulfonate.—Potassium 3-nitro-4-hydroxybenzenesulfonate (1.0 g.), prepared by the method of Kolbe and Gauhe,[13] was heated 1 hour at reflux in a solution of acetic acid (6 ml.) and acetic anhydride (4 ml.). After addition of 20 ml. of methanol the solution was refluxed for 2 hours, cooled, filtered, and evaporated to dryness. Recrystallization of the residue from acetic acid–benzene (~10% benzene) gave 0.47 g. (40%) of potassium 3-nitro-4-acetoxybenzenesulfonate as a pale tan powder which did not melt below 300°; λ^{KBr} 5.65 μ.

Anal. Calcd. for $C_8H_6NO_7SK$: C, 32.0; H, 2.02; N, 4.68. Found: C, 31.8; H, 2.20; N, 5.19.

Hydrolysis with a dilute potassium hydroxide solution, followed by neutralization with hydrochloric acid, yielded potassium 2-nitro-4-hydroxybenzenesulfonate, as evidenced by the infrared spectrum.

3-Nitro-4-acetoxybenzenearsonic acid was obtained in 53% yield by addition of acetic anhydride to an alkaline solution of 3-nitro-4-hydroxybenzenearsonic acid (Eastman Kodak Co. white label) according to the procedure used by Chattaway[14] for acetylation of phenols. It was washed well with ice-water, dried, and recrystallized from acetone (alcohol free) to yield a white, crystalline acid melting above 300°; λ^{KBr} 3.6, 4.4, 5.66, 6.2 μ.

Anal. Calcd. for $C_8H_8AsNO_7$: C, 31.5; H, 2.64; N, 4.59. Found: C, 31.6; H, 2.71; N, 4.41.

The apparent pK_a of 3-nitro-4-acetoxybenzenearsonic acid (determined by titration in 50% aqueous ethanol 0.04 *M* in potassium chloride at 36.8°) was 3.85.

2,4-Dinitrophenyl acetate was kindly provided by B Zerner.

Poly-(4-vinylpyridine) was obtained as a transparent, solid rod by bulk polymerization of freshly distilled, deoxygenated 4-vinylpyridine (Reilly Tar and Chemical) by the procedure of Fitzgerald and Fuoss.[15] It was fractionally precipitated from solution in *t*-butyl alcohol by successive additions of benzene. The first portion precipitated was redissolved in *t*-butyl alcohol and recovered as a white, porous cake by sublimation of the butanol at low temperature. This sample, which was used for the subsequent studies, was soluble in alcohol and in aqueous acid and had a softening point about 175°. The intrinsic viscosity in 95% ethanol was 0.377. On the basis of the equation of Boyes and Strauss[16] (for 92% ethanol) the average molecular weight was about 60,000.

Anal. Calcd. for C_7H_8N: N, 13.33. Found: N, 13.37.

Poly-(N-vinylimidazole) was generously donated by the Badische Anilin und Soda Fabrik AG., Ludwigshafen A. Rhein, Germany. It came as a white powder and was used without further treatment.

Anal. Calcd. for $(C_5H_6N_2)_n$: N, 29.78. Found: N, 30.17.

Spectrophotometric Titration of Pyridine Bases.—For analysis of the data from the catalyzed solvolyses it was desirable to know the state of ionization of the catalysts as a function of the *p*H of the solution. This information was obtained by spectrophotometric titrations.

(6) M. L. Bender and Y. L. Chow, *J. Am. Chem. Soc.*, **81**, 3929 (1959).

(7) W. Kern and B. Sherhag, *Makromol. Chem.*, **28**, 209 (1958); W. Kern, W. Herold and B. Sherhag, *ibid.*, **17**, 231 (1955).

(8) J. Steinhardt, *J. Biol. Chem.*, **141**, 995 (1941); J. Steinhardt and C. H. Fugitt, *J. Res. Natl. Bur. Stand.*, **29**, 315 (1942). For related cases see G. Schramm and J. Primosigh, *Z. physiol. Chem.*, **283**, 34 (1948); E. Waldschmitt-Leitz and Fr. Zinnert, *Makromol. Chem.*, **6**, 272 (1951).

(9) F. V. Hemmelmayr, *Monatsh.*, **25**, 21 (1904).

(10) This acid was prepared by the procedure of R. Lesser and G. Gad, *Ber.*, **59**, 234 (1926).

(11) It is important that the ether extraction be carried out promptly. In cases where the mixture was first allowed to stand an hour, an isomeric acetate, probably 4-hydroxy-5-nitro-2-acetoxybenzoic acid, was isolated as well as the desired compound. The isomeric acetate melted at 188–189°; analysis: Found: C, 45.0; H, 2.90; N, 5.94. The infrared spectrum was very similar to that of 5-nitro-4-acetoxysalicylic acid but showed a peak at 10.7 μ absent in the spectrum of the lower melting isomer.

(12) H. Goldstein and A. Jaquet, *Helv. Chim. Acta*, **24**, 30 (1941).

(13) H. Kolbe and F. Gauhe, *Ann.*, **147**, 71 (1868).

(14) F. D. Chattaway, *J. Chem. Soc.*, 2495 (1931).

(15) E. G. Fitzgerald and R. M. Fuoss, *Ind. Eng. Chem.*, **42**, 1603 (1950).

(16) A. G. Boyes and U. P. Strauss, *J. Polymer Sci.*, **22**, 463 (1956).

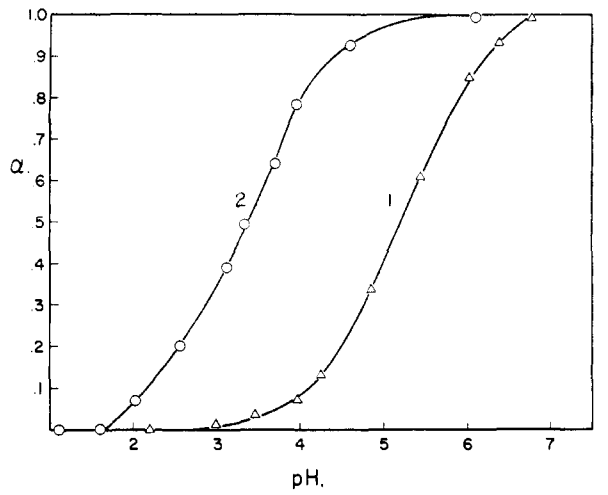


Fig. 1.—Titration curves for 4-picoline (curve 1) and poly-(4-vinylpyridine) (curve 2) in 50% ethanol, 0.04 *M* in KCl.

An aqueous solution of poly-(4-vinylpyridine) (75 ml. of 50% v.v. ethanol–water 5.0×10^{-4} *M* in pyridine units and 0.04 *M* in potassium chloride) was warmed to 37.8° in a water-jacketed beaker. Small portions of 1.2 *M* hydrochloric acid in 50% ethanol–water were added from a syringe microburet, model SB2, Micro-Metric Instrument Co. After each addition, the *p*H of the solution was determined with a Photovolt *p*H meter; a 3-ml. sample then was withdrawn with prewarmed pipets and the absorbance determined at 254 mμ with a Cary model 11 spectrophotometer (temperature in cell compartment, 36.6°). The fraction of non-protonated nitrogen (α) was determined by interpolation from the absorbancies of the polymer in strong alkaline solution (*p*H 11.2, *A* 0.743) and in strong acid solution (*p*H 1.1, *A* 1.750). The titration of 4-picoline was carried out in the same way, with the spectrophotometer set at 255 mμ (at *p*H 2.2, *A* was 1.650; at *p*H 11.0, A was 0.813). The data are presented in Fig. 1.

Plots of $\log((1-\alpha)/\alpha)$ *versus* *p*H were linear for both 4-picoline and poly-(4-vinylpyridine) over the range α 0.06–0.94, in accordance with the modified Henderson–Hasselbach equation[17]: $pK = 5.20 = p\text{H} + 1.10 \log((1-\alpha)/\alpha)$ for 4-picoline and $3.34 = p\text{H} + 1.14 \log((1-\alpha)/\alpha)$ for poly-(4-vinylpyridine).[18]

Kinetic Determinations.—The solvent consisted of equal volumes of ethanol and water, mixed at room temperature. In this system poly-(4-vinylpyridine) remained in solution throughout the *p*H range of interest (1 to 7). Except where noted, the solutions were 0.04 *M* in potassium chloride. The salt was added to minimize salt effects arising from differences in the amounts of hydrochloric acid used to adjust the *p*H of the solutions.

Solvolysis of 5-Nitro-4-acetoxysalicylic Acid.—To an ethanol water solution of poly-(4-vinylpyridine), 0.02 base *M* and 0.04 *M* in potassium chloride, was added concd. hydrochloric acid (vol. less than 1/200 vol. of the polymer solution) to bring the *p*H to 3.50. A. 3.00-ml. portion of this solution was warmed to 39° and added to a quartz cell (1-cm. path) in a thermostatically controlled cell compartment of a Cary spectrophotometer. The polymer solution was allowed to stand 30 minutes in order to attain temperature equilibrium, after which the temperature in the cell remained at 36.8 ± 0.1°. At zero time 0.050 ml. of a freshly prepared ethanol solution of 5-nitro-4-acetoxysalicylic acid (1.2×10^{-2} *M*) was injected into the cell by a microburet and the solution was shaken briefly. The spectrum between 310 and 360 mμ was then scanned repeatedly and the final reading taken after 5 hours (16 half-lives). As the reaction proceeded a broad maximum in the range of 320 mμ (max. for ester) decreased (*A* 1.48 → 1.14) and a new maximum (for 2,4-dihydroxybenzoic acid) developed at 356 mμ (*A* 0.97 → 1.77). A well defined isosbestic point appeared at 331.5 mμ, indicating the absence of any medium-lived intermediates. The changes at both wave lengths corresponded to first-order kinetics through 90% reaction. The rate constant determined from the decrease in absorption at 318 mμ was 0.037 min.$^{-1}$; that determined from the increase in absorption at 356 mμ was 0.038 min.$^{-1}$.

General Procedure A.—The procedure just described was followed except that the spectrophotometer was set to record absorbance as a function of time at a particular wave length. Variations in the quantities of reagents and the *p*H are indicated in the tables and figures. Checks on the initial and final *p*H values showed that the *p*H did not change detectably in the course of the reaction. The solvolyses were followed at 356 mμ for NAS and 360 mμ for the remaining substrates. For the very slow reactions the infinity values of the absorbancies were obtained from solutions containing the appropriate concentrations of the aromatic solvolysis products; otherwise, infinity values were obtained by carrying out the reactions until the spectra no longer changed. In general, good first-order kinetics were obtained; however, at the higher *p*H values (>4) some deviation was noted for the solvolyses of the charged substrates (NAS, NABS, NABA) catalyzed by the polymer. The rate constants tended to decrease as the reaction proceeded. In these cases the first-order rate constants corresponded to about the first 50% of the reaction.

The products of the reactions were identified by the similarity of their ultraviolet spectra with the spectra of the corresponding non-acetylated materials. Furthermore, 2,4-dihydroxy-5-nitrobenzoic acid (68% yield after recrystallization, identified by m.p., mixture m.p. and infrared spectrum) was isolated from a polymer-catalyzed solvolysis carried out with 0.120 g. of NAS. Likewise, 3-nitro-4-hydroxybenzenearsonic acid was isolated from a reaction with NABA. In this case a cellophane dialysis bag was submerged in the reaction solution; it was subsequently removed and the contents evaporated under vacuum to yield 3-nitro-4-hydroxybenzenearsonic acid.

The applicability of Beer's law to polyvinylpyridine solutions of the solvolysis products was tested with standard solutions; Beer's law was obeyed throughout the range investigated (10^{-4} to 4×10^{-4} *M* 3-nitro-4-hydroxybenzenesulfonate and 3-nitro-4-hydroxybenzenearsonic acid, and 5×10^{-5} to 19×10^{-5} *M* 2,4-dihydroxy-5-nitrobenzoic acid, in 0.01 base molar polymer at *p*H 3.6 in 50% ethanol–water, 0.04 *M* in potassium chloride).

Procedure B.—A different technique was used in following the relatively slow reactions of NAS in which catalytic quantities of poly-(4-vinylpyridine) were used. The reaction solvent was 50% (v. v.) aqueous ethanol made 0.04 *M* in potassium acid phthalate and adjusted to the appropriate *p*H by addition of concd. hydrochloric acid. To 22 ml. of this solution was added 1 ml. of the catalyst solution (0.005 *M* poly-(4-vinylpyridine) in ethanol); the mixture then was warmed to 38.3° in a constant temperature bath. One ml. of a standard substrate solution (0.05 *M* 5-nitro-4-acetoxysalicylic acid) was added, the volumetric flask was filled to the 25-ml. mark with the phthalate buffer solution, and the mixture was shaken vigorously for 30 seconds. At periodic intervals 2-ml. aliquots were removed and diluted to 100 ml. with 50% ethanol–water buffered at *p*H 4.9 with 0.02 *M* sodium acetate–acetic acid. The absorbancies were determined at 317 and 357 mμ with a Beckman DU spectrophotometer. The reactions followed first-order kinetics with respect to substrate disappearance and product appearance.

Equilibrium Dialysis Studies.—Since rapid solvolysis precluded direct binding experiments involving the ester substrates and polymer, a substrate model, potassium 4-hydroxy-3-nitrobenzenesulfonate, was used in the binding experiments. This hydroxy derivative should be bound at least as extensively as the acetoxy compound (NABS).

A 10-ml. portion of a poly-(4-vinylpyridine) solution (0.01 *M* in base units, 50% ethanol–water solvent), for which the *p*H had been adjusted to the appropriate value by addition of concd. hydrochloric acid, was placed in a thoroughly washed and soaked 23/100 sausage casing (Visking Corp.). After the ends had been tied, the bag was immersed in 10

(17) (a) W. Kern, *Z. physik. Chem.*, **181**, 249 (1938); (b) A. Katchalsky and P. Spitnik, *J. Polymer Sci.*, **2**, 432 (1947); (c) A. Katchalsky, N. Shavit and H. Eisenberg, *ibid.*, **13**, 69 (1954).

(18) One would expect the coefficient of the log term for 4-picoline to be 1.00. The deviation from unity may therefore reflect an experimental error. The relatively low value of the coefficient (1.14) for the polymer is probably a consequence of the high percentage of alcohol in the solvent. For example, the corresponding coefficient for polymethacrylic acid in aqueous solution is 2; however, the value is markedly reduced in dioxane–water solutions; see ref. 17b.

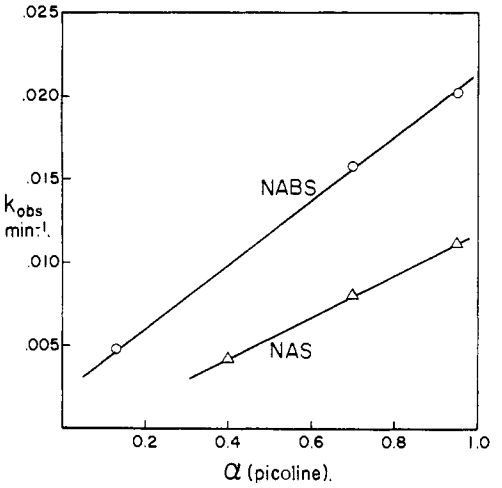


Fig. 2.—Solvolysis of 5-nitro-4-acetoxysalicylic acid (NAS) (2×10^{-4} M) and sodium 3-nitro-4-acetoxybenzenesulfonate (4×10^{-4} M) in 50% ethanol, 0.0157 M in 4-picoline and 0.04 M in KCl at 36.8°.

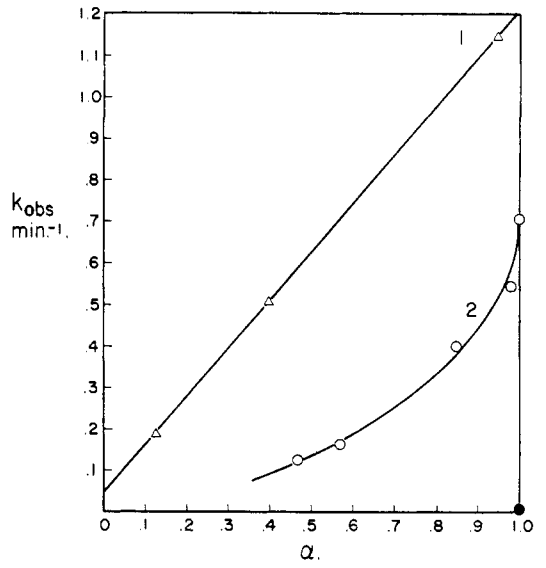


Fig. 3.—Solvolysis of dinitrophenyl acetate (DNPA) (2×10^{-4} M) in 50% ethanol, 0.04 M in KCl at 36.8°: curve 1, 0.0157 M 4-picoline present; curve 2, 0.01 base molar poly-(4-vinylpyridine) present. The pH of the points at $\alpha = 1$ was 6.5; the ● point indicates a reaction carried out in absence of nitrogen bases at pH 6.5.

ml. of ethanol–water (50% v.v.) which was 0.08 M in potassium chloride and 8×10^{-4} M in potassium 3-nitro-4-hydroxybenzenesulfonate. The tube was tightly stoppered and agitated in a wrist action shaker for 24 hours at 25°, whereupon a sample of the solution outside the bag was analyzed

TABLE I

BINDING OF POTASSIUM 4-HYDROXY-3-NITROBENZENESULFONATE TO POLY-(4-VINYLPYRIDINE)

pH	Absorbance "outside" soln.	Sulfonate bound by PVPy. %
5.20[a]	0.900	..
5.00	.860	4
4.47	.830	8
3.52	.805	10
2.93	.820	9

[a] A blank run; no poly-(4-vinylpyridine) in the bag.

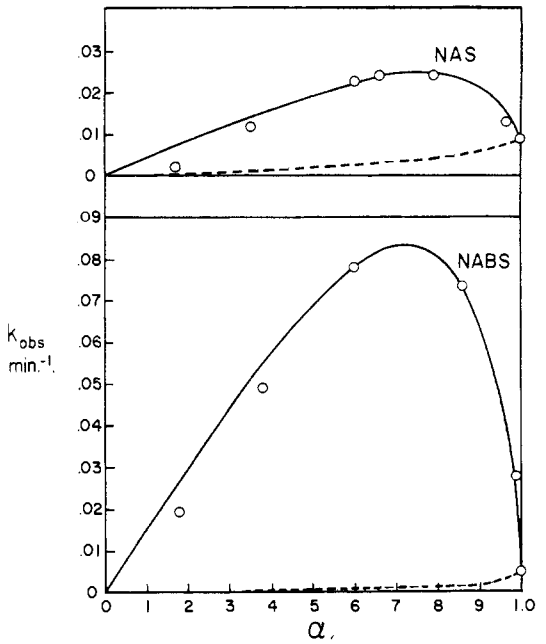


Fig. 4.—Solvolysis of 5-nitro-4-acetoxysalicylic acid (NAS) (2×10^{-4} M) and potassium 3-nitro-4-acetoxybenzenesulfonate (NABS) (2×10^{-4} M) in 50% ethanol, 0.01 base molar in poly-(4-vinylpyridine) and 0.04 M in KCl at 36.8°: experimental points -O-; the solid and dashed lines represent calculated curves (see text).

by its absorption at 343 mμ in a Cary spectrophotometer. At this wave length the absorption did not vary with pH. The absorbance of the "outside solution," which is a measure of the concentration of the sulfonate, varied from 0.900 for a blank run in which no polymer was in the bag to 0.805 for a run with polymer at pH 3.52. In these experiments the polymer nitrogen/substrate mole ratio was 12.5:1; so in the acidic solution sufficient polymer was present to bind all the substrate. The data are presented in Table I as percentage of the substrate which was bound by the polymer.

Results

As controls for the polymer work, the rates of solvolysis of three substrates, 2,4-dinitrophenylacetate (DNPA), 5-nitro-4-acetoxysalicylic acid (NAS) and potassium 3-nitro-4-acetoxybenzenesulfonate (NABS), in aqueous ethanol solutions containing 4-picoline were investigated. The pH of the picoline buffers was varied from 4.3 to 6.5. In Figs. 2 and 3, the pseudo-first-order rate constants for these reactions are plotted as a function of α, the fraction of the picoline present as the free base. The spontaneous solvolysis rates in 50% ethanol–water were neglible compared to the rates for the catalyzed reactions. It was found that the rate constants, k_{obs}, increased linearly with α. This result is in accord with a considerable body of data on catalyzed solvolyses of nitrophenyl esters conducted in aqueous solution.[4]

The shape of the k_{obs}–α plot was quite different when poly-(4-vinylpyridine) was used in place of 4-picoline (Fig. 3). With DNPA, an uncharged substrate, poly-(4-vinylpyridine) was *less* effective as a catalyst than an equivalent amount of 4-picoline and the k–α plot curved upward with increasing α. This behavior of poly-(4-vinylpyridine) may be attributed to a decrease in the nucleo-

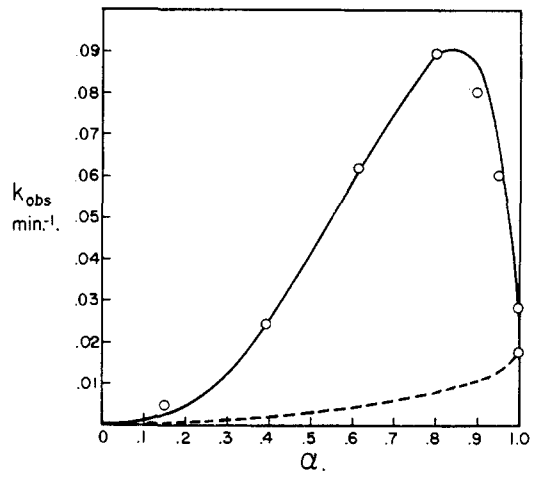


Fig. 5.—Solvolysis of 3-nitro-4-acetoxybenzenearsonic acid (NABA) (4×10^{-4} *M*) in 50% ethanol, 0.01 base molar in poly-(4-vinylpyridine) and 0.04 *M* in KCl at 36.8°; the solid and dashed lines represent calculated curves (see text).

philicity of the free basic groups attendant on an increase in positive charges on the polymer chain.

A striking change in the pattern of solvolysis was observed in the poly-(4-vinylpyridine)-catalyzed reactions of NAS, NABS and NABA, substrates which exist completely or extensively as anions in the *p*H range studied (see Figs. 4 and 5). The reaction rates *increased* as the *p*H was *lowered* below 6.5. A maximum was attained in the range *p*H 3.8–4.0 (α 0.7–0.8), with further decreases in *p*H the values of k_{obs} decreased. For α 0.6 (*p*H 3.6 and 5.45 for the polymer and the picoline solutions, respectively) poly-(4-vinylpyridine) was 9.3 times *more* effective per mole of free amine than picoline in catalyzing the solvolysis of NABS. The corresponding factor was 5.1 for the reaction of NAS.[19] By contrast, under the same conditions the polymer was only two-fifths as active as picoline in the solvolysis of DNPA.

The relation between rate and polymer concentration was explored for NABS at *p*H 3.6. As shown in Fig. 6, the rate of solvolysis was proportional to the polymer concentration in the range 2×10^{-3} to 20×10^{-3} *M* pyridine unit.[20] In these experiments nitrogen base was present in large excess relative to substrate. Significant effects also were produced by relatively small or "catalytic" quantities of polyvinylpyridine. Data are presented in Fig. 6 for experiments in solutions buffered with potassium acid phthalate for which the NAS-polymer nitrogen ratio ranged from 2 to 10. The rates of these reactions were linearly related to the poly-(4-vinylpyridine) concentration. Control reactions in which the polymer was omitted or replaced by 4-picoline were extremely slow.

Potassium chloride had a marked effect upon the rates of the catalyzed solvolyses of the anionic substrates but not upon the *p*H optimum. A comparison of the data in Table II and Fig. 4 reveals that the rates for NABS were approximately twice as great in the salt-free solutions (*i.e.*, those in

(19) The relative superiority of the polymer would, of course, appear much greater if solutions at the same *p*H were compared.

(20) For this relationship to hold it is necessary to keep the salt concentration constant.

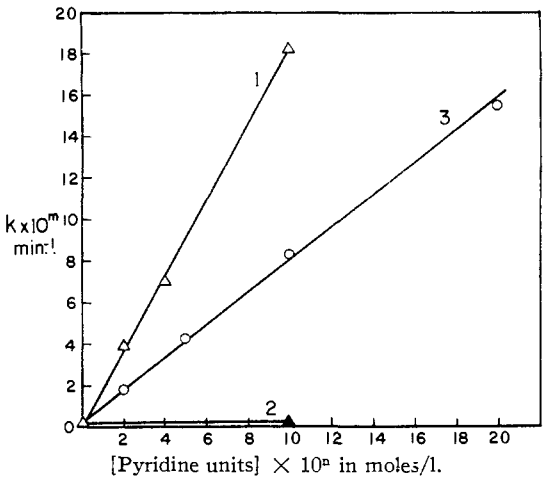


Fig. 6.—Solvolysis of 5-nitro-4-acetoxysalicylic acid (2×10^{-3} *M*) at *p*H 3.6 by experimental method B; $m = n = 4$: curve 1, in presence of poly-(4-vinylpyridine); curve 2, in presence of 4-picoline; curve 3: solvolysis of potassium 3-nitro-4-acetoxybenzenesulfonate (2×10^{-4} *M*) in 50% ethanol in presence of poly-(4-vinylpyridine) at constant salt concentration (adjusted to 0.04 *M* Cl^- by addition of KCl) by exp. method A; $m = 2$, $n = 3$.

which potassium chloride was omitted) as in solutions 0.04 *M* in potassium chloride.

TABLE II

CATALYSIS BY POLY-(4-VINYLPYRIDINE) AND POLY-(N-VINYLIMIDAZOLE); NO SALT ADDED (METHOD A)

Substrate	Vol. % ethanol in solvent	Base (0.01 *M*)	*p*H	$k_{obs.}$, min.$^{-1}$
NABS	50	PVPy	3.50	0.190
			3.60	.220
			4.55	.176
			6.20	.036
			6.80	.010
NABS	50	PVIm	3.10	0.244
			3.85	.438
			4.25	.596
			5.75	.428
			7.70	.133
NABA	10	PVIm	2.50	0.064
			3.20	0.553
			3.80	1.087
			4.60	1.553
			5.60	0.649
			7.50	0.553

Poly-(4-vinylpyridine) is not unique in its catalytic properties. In Table II are also summarized data on solvolyses of NABS and NABA catalyzed by poly-(N-vinylimidazole). A distinct maximum was found in the k_{obs}–*p*H profile in both cases. As expected, the imidazole unit was a more effective catalyst than the pyridine unit. However, for purposes of the present study polyvinylimidazole was less suitable than poly-(4-vinylpyridine) since the change in the ultraviolet spectrum accompanying protonation was so small that an accurate determination of α could not be made.

Discussion

The existence of maxima in the k_{obs}–α profiles for the solvolyses of NAS, NABS and NABA

catalyzed by poly-(4-vinylpyridine) show at least two different factors to be involved in determining the reaction rates; one is favored by a decrease and the other by an increase in the acidity of the reaction medium. It is reasonable that these factors are the amounts of basic nitrogen and protonated nitrogen in the polymer. The solvolytic data may be rationalized qualitatively on the basis that (a) the basic nitrogen catalyzes the actual solvolysis and (b) the protonated nitrogen sites, being positively charged, serve to hold negatively charged substrates within the region of the polymer coils. In the sense that the polymer contains both binding sites and catalytic sites it may be considered to be a model for an enzyme. Substrates such as DNPA which do not carry a negative charge are not subject to the rate enhancement derived from ionic binding.

An estimate of the contribution of binding to the solvolysis rates can be made in the following way. The values of k_{obs} at $\alpha = 1$ should indicate the extent of conventional nucleophilic catalysis by non-protonated polymer. On the assumption that the curve for the DNPA solvolysis (curve 2, Fig. 3) provides a measure of the nucleophilicity of poly-(4-vinylpyridine) in acid solutions, one can derive the dashed curves shown in Figs. 4 and 5, which represent the values of k_{obs} that would be expected if poly-(4-vinylpyridine) functioned only in the conventional sense. The difference between the dashed curves and the experimental points indicates the extent of reaction associated with ionic interaction between the substrate and catalyst. In the most favorable case (NABS) it is estimated that the solvolytic rate at $\alpha = 0.6$ is enhanced by a factor of eighty as a consequence of the electrostatic association.

As a first approximation it could be assumed that the rates of the catalyzed solvolyses, at constant polymer concentration, would be proportional to the product of α, the fraction of catalytic sites, and $1 - \alpha$, the fraction of binding sites. In this case a symmetrical k–α plot with a maximum at α 0.5 would be observed. Actually, the k_{obs}–α curves for the charged substrates do not show this symmetry; so some other factor, or factors, must be operative. One possibility is that the equilibrium constant for binding substrate to polymer is sufficiently large that most of the substrate is in the bound state in the moderately acidic solutions (pH 3.6). This characteristic would account for the shape of the k_{obs}–α profile; however, it would also lead to the prediction that the rates of the catalyzed solvolyses would not be proportional to the polymer concentration, a consequence contrary to fact. Furthermore, equilibrium dialysis studies with poly-(4-vinylpyridine) and potassium 3-nitro-4-hydroxybenzenesulfonate, a model for NABS, indicate that the binding constant would be small (Table I). A second possibility is that asymmetry in the k_{obs}–α curve is produced by the reduction in nucleophilicity of the basic sites which accompanies an increase of positive charges on the polymer chain. However, calculations based on the curvature of the k_{obs}–α profile for an uncharged substrate, DNPA (curve 1, Fig. 3), indicate that this property alone would not account for the bias in the k_{obs}–α plots for the charged substrates. We therefore favor a third possibility, that the polymer undergoes an unfavorable conformational change when the acidity of the medium increases. The change might result either in less binding of substrate or in unfavorable geometrical relationships between the nucleophilic and binding sites. It has been established in other systems that the conformation of a polymer depends upon the charge state.[21]

With substrates which are weak acids or salts of weak acids an additional factor, the state of ionization of the substrate, may influence the k_{obs}–α curve. At high pH the substrate would be ionized and subject to electrostatic binding whereas at low pH it would exist as a neutral molecule with little affinity for the polymer. This factor is probably not significant in the reactions of NABS; however, it may be partially responsible for the decrease in rate observed with NAS at high acidities and is surely involved in the solvolysis of NABA, which is a relatively weak acid. The k_{obs} values for NABA reached a maximum at a somewhat higher α-value and decreased more rapidly on the acid side of the maximum than the k_{obs} values for NAS and NABS. These results support the view that the unusually rapid solvolyses catalyzed by poly-(4-vinylpyridine) in the region of pH 4 involve the substrate in the anionic rather than the acid form.

On the basis of these considerations we suggest that the catalytic rate constant, k_c, for poly-(4-vinylpyridine) may to a good approximation be represented by the equation where k_N is the rate constant for conventional nucleophilic catalysis, α_s is the fraction of substrate in the anion form, α_p is the fraction of the polymeric base in the free base form, and $f(\alpha_p)$ is the function relating the change in the spacing and intrinsic effectiveness of the binding and catalytic sites with the change in acidity of the medium. This equation cannot be tested directly since $f(\alpha_p)$ is unknown; however, it is interesting and perhaps significant that, as shown by the continuous curves in Figs. 4 and 5, a reasonably good fit to the experimental data may be obtained by using this equation and assuming a single expression for $f(\alpha_p)$ for all solvolyses catalyzed by poly-(4-vinylpyridine). The continuous curves in Figs. 3 and 4 were calculated on the basis that $f(\alpha_p) = 1/(1 - 0.86\alpha)$ that $k = 0.04$, 0.156 and 0.221 min.$^{-1}$ for NAS, NABS and NABA, respectively, and that $\alpha_s = 1$ for NAS and NABS. The values for α_s for NABA were calculated on the assumption that the effective pK_a of this acid in the reaction medium was 3.60, and the k_N values were determined from the k_{obs}'s at $\alpha = 1$ and curve 2, Fig. 3, as previously indicated.

$$k_c = k(\alpha_p)(1 - \alpha_p)f(\alpha_p)\alpha_s + k_N$$

Acknowledgment.—Helpful discussions with Professors H. Morawetz and M. Bender are gratefully acknowledged.

(21) For example, A. Oth and F. Doty, *J. Phys. Chem.*, **56**, 45 (1952), found that molecules of polymethacrylic acid in solution extend about sevenfold as the extent of ionization increases.