also gratefully acknowledge the valuable discussions with Visiting Professor Ephraim Katchalski and the financial support by the Research Laboratory, U. S. Army, Edgewood Arsenal (Grant No. DAA-15-67-C-0567) and the National Institutes of Health (Grant No. GM-15256-02).

The Effect of Ethanol–Water Solvent Composition on the Poly[4(5)-vinylimidazole]-Catalyzed Solvolysis of an Anionic, Long-Chain Substrate[1]

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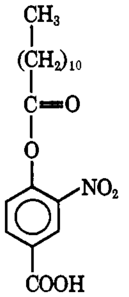
Abstract: The solvolytic reactions of the anionic, long-chain substrate 3-nitro-4-dodecanoyloxybenzoic acid (NDBA) catalyzed by poly[4(5)-vinylimidazole] (PVIm) have been investigated as a function of temperature and pH in aqueous solutions containing varying compositions of ethanol and water. It was found that the polymeric solvolytic reaction of NDBA follows the Michaelis–Menten mechanism in low ethanol composition based on the assumption of a catalyst–substrate complex formation. The maximum catalytic activity of the polymer was observed in *ca.* 30 vol % ethanol–water, relative to that of its monomeric analog imidazole, and this was attributed to the extremely large value of the first-order rate constants, k_2, rather than a favorable Michaelis constant, K_m. The solvolytic reaction of NDBA catalyzed by poly[4(5)-vinylimidazole] in 43.7 vol % ethanol–water presented unusual kinetic features in which a retardation behavior was noted for [NDBA] > [PVIm] and an acceleration behavior for [PVIm] > [NDBA]. These results were related to the formation of the long-lived intermediate compound, dodecanoylpoly[4(5)-vinylimidazole]. This formation also indicates that the deacylation step is an overall rate-determining step in the solvolysis of NDBA catalyzed by poly[4(5)-vinylimidazole]. Nonpolar interactions between the substrate and the catalyst are discussed, based upon the structuredness of the solvent in varying compositions of ethanol.

The enhanced esterolytic action of synthetic, vinyl polymers containing pendent imidazole or benzimidazole groups (in comparison to their monomeric analogs) has been attributed to a variety of cooperative interactions between the pendent catalytic groups and the substrate. Although the efficiencies of the synthetic macromolecular catalysts have been found to be several orders of magnitude less than those of natural enzymes in esterolytic reactions,[2,3] it could be expected that the reactivity of the synthetic macromolecular catalyst could be improved by complex formation with the substrate through either electrostatic or nonpolar interactions followed by a bifunctional interaction from the catalytic functions.

In recent years, contributions of nonpolar interactions on complexation or binding of catalytically active, synthetic macromolecules with low and high molecular weight reagents have been discussed by several workers.[4–6] Recently, we have reported a dramatically large catalytic enhancement for the solvolysis of the long-chain, anionic substrate 3-nitro-4-dodecanoyloxybenzoic acid (NDBA, I) catalyzed by poly[4(5)-vinylimidazole] in an ethanol–water solvent composition of less than *ca.* 40 vol % ethanol in relation to its monomeric analog, imidazole.[1]



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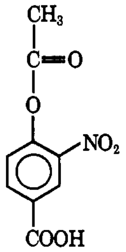

II

Reactivity studies on solvolytic reactions in the presence of detergent micelles have been carried out in order to ascertain the analogies to biological systems.[7–10]

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(1) For a preliminary report of this work, see C. G. Overberger, M. Morimoto, I. Cho, and J. C. Salamone, *Macromolecules*, **2**, 553 (1969); 3, 108 (1970).

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(9) C. Gitter and A. Ochoa-Solano, *ibid.*, **90**, 5004 (1968).

(10) T. C. Bruice, J. Katzhendler, and L. R. Feder, *ibid.*, **90**, 1333 (1968).

It was found that electrostatic attractions are of much more importance for the short-chain esters than for the long-chain esters, while the lyophobic interactions are of much more importance for the long-chain esters than for the short-chain esters, in the reaction of esters with nucleophilic micelles.[10]

In order to investigate further the contribution of nonpolar interactions for macromolecule–substrate complex formation, the solvolytic reactions of NDBA [in comparison to the short-chain substrate 3-nitro-4-acetoxybenzoic acid (NABA, II)] catalyzed by poly[4(5)-vinylimidazole] (relative to the catalytic activity of the monomeric analog, imidazole) were investigated in different compositions of ethanol–water solvent, at different pH values, and at different temperatures. These results would be expected to be helpful in understanding the concept of nonpolar interactions in ethanol–water mixtures as well as facilitating a more detailed understanding of the binding of small molecules with macromolecules.

Experimental Section

N-**Dodecanoylimidazole** was prepared according to the procedure of benzoylimidazole synthesis described by Gerngross.[11] To a solution of imidazole (1.36 g, 0.02 mol) in approximately 25 ml of benzene was added dodecanoyl chloride (2.19 g, 0.01 mol). The mixture was stirred overnight at room temperature. The solution was filtered free of imidazolium chloride and benzene was removed by freeze drying. The yield was 2.43 g (87%), mp 66–67°. It was recrystallized from cyclohexane. *N*-Dodecanoylimidazole in ethanol has an absorption maximum of 245 mμ which is identical with that of acetylimidazole reported by Stadtman:[12] ϵ 2688 cm^{-1} M^{-1} in ethanol at 245 mμ at 26°.

Anal. Calcd for $C_{15}H_{26}N_2O$: C, 71.95; H, 10.47; N, 11.19. Found: C, 71.87; H, 10.59; N, 11.19.

Partially Dodecanoyl-Substituted Poly[4(5)-vinylimidazole] (D-PVIm. Sample No. 1). A mixture of methanol (50 ml) and poly-[4(5)-vinylimidazole] (0.5 g, 0.00531 mol, based on the molecular weight of monomer) was refluxed for 20 min. After cooling and filtration, a solution of dodecanoyl chloride (0.6 g, 0.00274 mol) diluted with 10 ml of methanol was added. The mixture was allowed to stir at room temperature for 18 hr. The mixture was concentrated at reduced pressure, benzene was added, and the product was filtered and washed with excess benzene.

Anal. Found: C, 46.93; H, 5.41; N, 20.28; Cl, 18.71 [(VIm/D-VIm)/(HCl) = 27.8/1/21].

Partially Dodecanoyl-Substituted Poly[4(5)-vinylimidazole] (D-PVIm. Sample No. 2). Trifluoroacetic anhydride (2.1 g, 0.01 mol) and dodecanoic acid (2.0 g, 0.01 mol) were allowed to react at room temperature for 30 min. Poly[4(5)-vinylimidazole] (0.1 g, 0.00106 mol in vinylimidazole units) was added to this mixture and the solution was stirred vigorously for 24 hr at a temperature of 40–50°. The cooled reaction mixture was concentrated to remove excess mixed anhydride. The concentrated solution was precipitated into methanol–benzene with stirring in a high-speed mixer and dried under vacuum at 45°. The D-PVIm was dissolved in methanol, precipitated into benzene, and dried under vacuum at room temperature.

Anal. Found: C, 46.11; H, 4.60; N, 12.89; F, 17.49 [(VIm/D-VIm)/(CF_3COOH) = 6.28/1/4.86].

3-Nitro-4-dodecanoyloxybenzoic acid (NDBA) was prepared according to the procedure of Overberger and Cho[13] and was recrystallized from cyclohexane.

Anal. Calcd for $C_{19}H_{27}NO_6$: C, 62.45; H, 7.45; N, 3.83. Found: C, 62.31; H, 7.45; N, 3.80.

A portion of NDBA used in this investigation was kindly supplied by Dr. I. Cho, mp 82°.

p-Nitrophenyl dodecanoylate (PNPD) was kindly provided by Dr. I. Cho.

Kinetic Measurements. Catalyst solutions were prepared by the previously described procedures,[2] and substrate solutions were also prepared in the same buffer system containing the same composition of ethanol as the catalyst solutions.

1. Slow Reactions. Kinetic measurements and the methods of calculation of the first-order observed rate constants and of the initially observed rate constants were performed as previously described.[2]

2. Rapid Reactions. These kinetic studies were performed with a Durrum-Gibson stopped flow spectrophotometer. Changes of transmittance at a proper wavelength[2] with time were observed on a Tektronix Model 564 oscilloscope, which retains tracings for photography and observation as long as needed. Mixing times in this apparatus are estimated as less than 20 msec.

After transformation of the directly obtained data (transmittance) into the optical density, the rates of solvolysis, v_{measd}, are determined by dividing the molar extinction coefficient, ϵ, into $[A_t - A_0]/t$, *i.e.*, $v_{\text{measd}} = [A_t - A_0]/(t\epsilon)$, where A_t is the absorption of releasing phenolate ion as a function of time (t) at the previously described wavelength,[2] and A_0 is the absorption of the substrate–catalyst mixture at a moment of mixing, *i.e.*, at $t = 0$. The initial observed rate for the catalyzed reaction, v_{obsd}, is obtained by subtraction of v_{blank} for the uncatalyzed reaction from v_{measd}, *i.e.*, $v_{\text{obsd}} = v_{\text{measd}} - v_{\text{blank}}$.

Results and Discussion

Reaction System. It has been reported that the reactivity of poly[4(5)-vinylimidazole] for the solvolysis of the long-chain, anionic substrate NDBA, relative to imidazole, increases dramatically at ethanol contents of 20–40 vol %, and also that the polymeric catalytic efficiencies for the solvolysis of the neutral substrates PNPA (*p*-nitrophenyl acetate) and PNPH (*p*-nitrophenyl heptanoate) increase slightly with increasing ethanol composition in 60, 80, and 90 vol % ethanol–water mixtures.[1,2] In order to properly analyze the data it is important to ascertain whether the reaction system is homogeneous, or in the form of premicelles or micelles of long-chain substrate and poly[4(5)-vinylimidazole]. Since aggregation of substrate would be reflected in the solvolytic rates, the first-order constants for NDBA were measured at constant pH and ionic strength, but while varying the initial substrate concentration. In the approximate concentration range of 10^{-5}–10^{-3} M, no appreciable change in k_{blank} was noted for the solvolysis of NDBA (Table I). In order to determine the critical micelle concentration of dodecanoic acid, the absorbance of a 4 × 10^{-4} M solution of 3-nitro-4-hydroxybenzoic acid (HNBA) at λ 400 mμ and μ 0.02 was measured as a function of dodecanoic acid concentration at pH 8.0 in a 43.7 vol % ethanol–water mixture. The decrease in absorbance of HNBA began in the concentration range of dodecanoic acid of

Table I.[a] First-Order Rate Constants for the Solvolysis of NDBA Catalyzed by Imidazole and Base as a Function of Substrate Concentration

	(First-order rate constant) × 10^3, min^{-1}	
[NDBA] × 10^4 M	k_{blank} for base	k_{obsd} for imidazole
11	0.124	0.497
8.8	0.0989	0.476
6.6	0.122	0.395
4.4	0.115	0.368
2.2	0.092	0.380
1.1	0.0805	0.356
0.88	0.115	0.437

[a] 43.7 vol % ethanol-water, μ = 0.02, pH 7.99, 26°; [Im] = 4.4 × 10^{-4} M.

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[11] O. Gerngross, *Chem. Ber.*, 46, 1908 (1913).

[12] E. R. Stadtman in "The Mechanism of Enzyme Action," John Hopkins Press, Baltimore, Md., 1954, p 581.

[13] C. G. Overberger and I. Cho, *J. Polym. Sci., Part A-1*, 6, 2741 (1968).

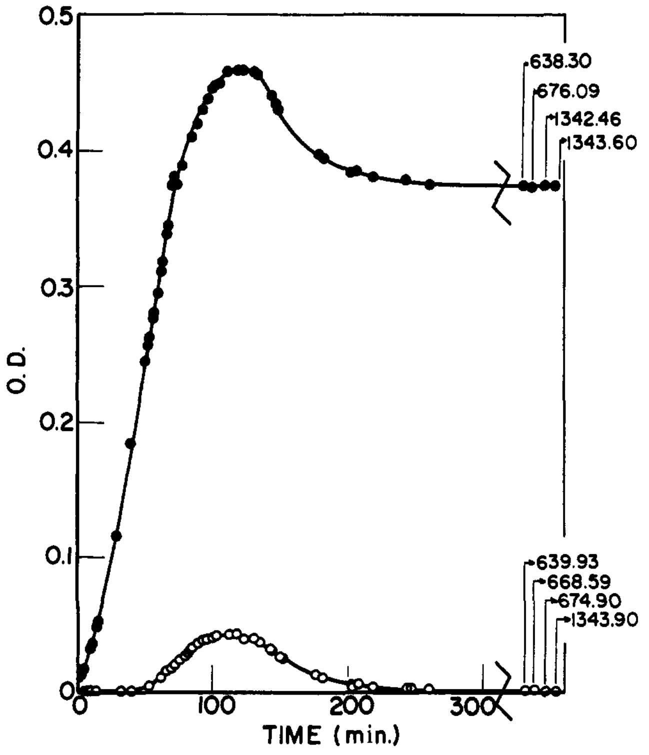

Figure 1. Unusual absorbance behavior at 400 mμ (●) and 570 mμ (○); [PVIm] = 1.9×10^{-4}, [NDBA] = 8.8×10^{-5}, pH 8.00, μ = 0.05, in 43.7 vol % ethanol–water, numbers show time (minutes) after mixing.

ca. 4×10^{-3} *M*. It has been found that the critical micelle concentration (cmc) of surfactants decreases with rising alcohol content up to the alcohol concentration corresponding to the minimum partial molar volume [*ca.* 8 mol % of ethanol at 30°; *ca.* 18.2 wt %, *ca.* 22.3 vol % at 15°], but beyond that concentration it rises sharply;[14] the negatively charged esters involving different aliphatic chain lengths and phenolphthalein induce formation of micelles of the positively charged micelle-forming agent trimethylhexadecylammonium bromide (CTA$^+$) or form salts with CTA$^+$ equally well.[10] Thus, the influence of the partially protonated poly[4(5)-vinylimidazole] (1.88×10^{-3} *M* for 30 vol % ethanol, 2.34×10^{-3} *M* for 20 vol % ethanol) on the absorbance of pinacyanol chloride (0.000261 g/dl, *ca.* 6.71×10^{-3} *M*) as a function of dodecanoic acid concentration was examined at pH 7.9, μ = 0.02 in 20 and 30 vol % ethanol–water mixtures at a wavelength of 610 mμ. It was found that no change in the absorbance was observed in the range of dodecanoic acid concentration less than 1×10^{-3} *M* and that a flocculation (visible to the eye) occurs when negatively charged dodecanoic acid (at a concentration of $2\text{–}4 \times 10^{-3}$ *M* for 30 vol % ethanol) is mixed with a partially protonated poly[4(5)-vinylimidazole]. This demonstrates that a precipitate is formed in the reaction system before stable micelles are formed. Thus, it was concluded that the employed reaction system is homogeneous, *i.e.*, below the cmc.

Unusual Absorbance Behavior. In the usual solvolyses, the absorption of the phenolate ion leaving from the various phenyl esters increases with time and approaches a maximum value in absorption which corresponds to the total concentration of the initially fed substrate.[15] The poly[4(5)-vinylimidazole]-catalyzed NDBA solvolysis in ethanol compositions of less than *ca.* 40 vol %, however, revealed that the absorption of the phenolate ion leaving from NDBA increased with time, but the absorption eventually decreased beyond a certain point in the OD–time plot (Figure 1). This phenomenon is an unusual absorbance behavior. It was not clear whether the unusual absorbance behavior was a kinetically necessary step for the solvolytic reaction or an optical problem which occurred during the reaction. Therefore, a careful examination of this absorbance behavior was performed.

The absorption change was followed against time during the solvolytic reaction in the range of 350–750 mμ by a Perkin–Elmer 202 spectrophotometer, and it was found that there was absorption in the visible light region, not only at 420 mμ (phenolate ion) but also at higher wavelengths. Since the absorption of HNBA disappears beyond λ 550 mμ, the observed absorption in the wavelength region beyond 550 mμ was apparently associated with a light scattering effect along the optical path with regard to the following results.

The total intensity of the integrated scattered light is related to optical density by eq 1 and to the wavelength by eq 2. Equations 1 and 2 predict that a plot of i_s/I_0

$$i_s/I_0 = 1\text{-}10^{-(\mathrm{OD})} \tag{1}$$

$$i_s/I_0 \propto 1/\lambda^4 \tag{2}$$

vs. $1/\lambda^4$ shows a linear relationship,[16] if the size and the concentration of particles are nearly constant during a certain period in the solvolytic reaction. Exact straight lines were indeed obtained in a plot of i_s/I_0 *vs.* $1/\lambda^4$ using the data of absorption change followed with time during a solvolytic reaction in the wavelength range of 350–750 mμ (solvolytic conditions, 39.4 vol % ethanol, pH 7.9, μ = 0.02, *ca.* 25°) (Figure 2). The plots in Figure 2 are made from the data obtained in the nonabsorbance region of HNBA, *i.e.*, at 550–730 mμ. These plots provide strong evidence for the light scattering effect as an explanation for the unusual absorbance behavior during the solvolytic reaction.

Dodecanoyl Substitution. The cause of this unusual absorbance was considered to be the formation of a partially dodecanoyl-substituted poly[4(5)-vinylimidazole] (D-PVIm), which is produced as an intermediate compound and which begins to precipitate during the solvolytic reaction in low alcohol composition because of its low solubility. In order to study this assumption that the long-lived intermediate is the cause of the light scattering effect, dodecanoylimidazole (D-Im) and partially dodecanoylated poly[4(5)-vinylimidazole] (D-PVIm) were synthesized and their deacylation rates were studied.

It is known that imidazole at concentrations much less than that of the ester such as PNPA brings about complete hydrolysis of the ester by a first-order reaction with respect to PNPA and imidazole.[17] These results suggest that imidazole acts as a true catalyst turning

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[14] F. Franks in "Physico-Chemical Processes in Mixed Aqueous Solvents," F. Franks, Ed., Elsevier, New York, N. Y., 1967, pp 50–70; E. M. Arnett, pp 105–128.

[15] C. G. Overberger, T. St. Pierre, N. Vorchheimer, J. Lee, and S. Yaroslavsky, *J. Amer. Chem. Soc.*, 87, 296 (1965).

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[17] T. C. Bruice and G. L. Schmir, *Arch. Biochem. Biophys.*, 63, 484 (1956); *J. Amer. Chem. Soc.*, 79, 1663 (1957).

over substrate many times in the course of the reaction with the formation of the very unstable *N*-acetylimidazole as an intermediate compound.

The solubility tests for polymer were performed with D-PVIm containing different amounts of dodecanoyl groups in a 40 vol % ethanol–water mixture, with μ 0.05 and at pH 8.00. Sample no. 1 (VIm/D-VIm/HCl = 27.8/1/21) was very soluble but sample no. 2 (VIm/D-VIm/CF_3COOH = 6.28/1/4.86) was insoluble at the conditions employed. The solubility tests for D-PVIm, sample no. 2, and D-Im at μ 0.02 with potassium chloride (no buffer) at room temperature showed that D-PVIm is more difficultly soluble in low ethanol composition than D-Im.

Deacylation rates were followed spectrophotometrically by measurement of the change of absorption of the acylimidazole group at 245 mμ. Kinetic experiments and determination of the first-order rate constants, k_{obsd}, were performed in the usual manner.[18] Plotting the extent of reaction, $A_t - A_\infty$, against time on semilogarithmic graph paper gave first-order kinetics.

Imidazole and poly[4(5)-vinylimidazole] catalyses for the solvolyses of the dodecanoyl groups from D-Im were investigated in different catalyst concentrations under the same conditions as the solvolytic reactions catalyzed by unsubstituted imidazole or by unsubstituted poly[4-(5)-vinylimidazole]. In both cases, however, varying the concentration of imidazole and poly[4(5)-vinylimidazole] at pH 8.00, μ 0.02, had no effect on the rate of these reactions. Although imidazole catalysis has been found at a high concentration of imidazole (10^{-2} to 10^{-1} *M*) in the case of acetylimidazole,[18] it is reasonable that no catalysis was observed in this investigation at such a low concentration of catalyst (10^{-4} *M*) as was used in the solvolytic reaction of the dodecanoyl groups.

It was also found that the deacylation rates of dodecanoyl compounds increased with increasing tris(hydroxymethyl)aminomethane (Tris) concentration. Consequently, the rates of deacylation from dodecanoyl compounds were obtained by determining the first-order reaction rates, k_{obsd}, at different concentrations of Tris buffer in 43.7 vol % ethanol–water mixture and extrapolating to zero buffer concentration (Table II).

Table II.[a] First-Order Rate Constants for Deacylation of D-Im and D-PVIm at Zero Buffer Concentration

	$k_{obsd} \times 10^2$, min^{-1}		
pH	D-PVIm[b]	D-Im	Acetylimidazole[c]
7.90		0.92	1.83
8.07	0.0017 ± 0.0006	2.78	2.27

[a] 43.7 vol % ethanol–water. [b] Sample no. 1. [c] Data obtained in pure water.

The k_{obsd} values were calculated by the following rate expression for acetylimidazole hydrolysis using a pK_1'

$$v = k_{obsd}{}^*[\text{acetylimidazole}]_0 = [2.8(1 - \alpha_1) + 0.005\alpha_1 + 19000\alpha_1[\text{OH}^-]][\text{acetylimidazole}]_0 \quad (3)$$

of 3.6 at μ = 0.2.[18] The value of k_{obsd} had the same magnitude (disregarding differences in the solvolytic systems) for the deacylation of D-Im and acetylimidazole, while the value of k_{obsd} for deacylation of D-PVIm was *ca.* 100 times smaller than that of D-Im. Consequently, it was ascertained that the D-PVIm formed as the intermediate in the course of the solvolytic reaction is very stable and has a very low solubility. These results strongly support the above assumption as the origin of light-scattering effect.

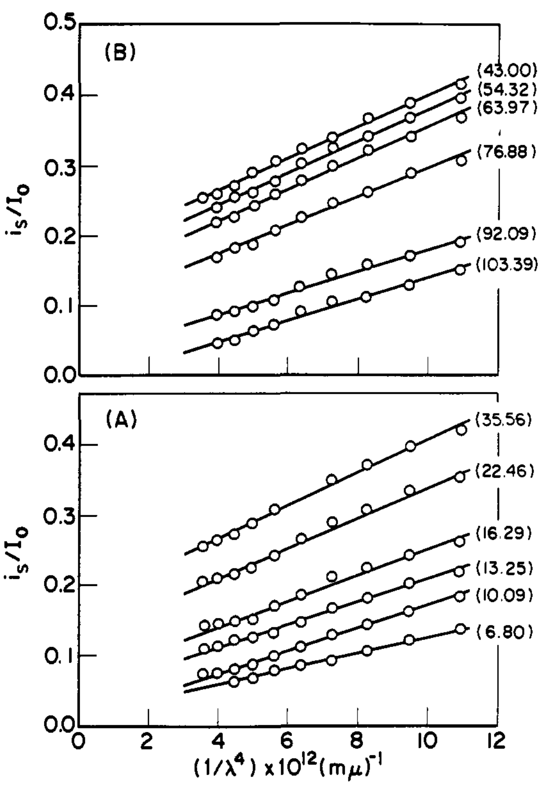

Figure 2. Plots of i_s/I_0 *vs.* $1/\lambda^4$ as a function of time for the solvolytic reaction of NDBA catalyzed by poly[4(5)-vinylimidazole]; μ = 550–730 mμ, pH 7.90, μ = 0.02, *ca.* 25°, in 39.4 vol % ethanol; numbers show time (minutes) after mixing; (A) are plots up to maximum absorption and (B) are plots up to an equilibrium state after passing maximum absorption.

Furthermore, the formation of stable D-PVIm as the intermediate could explain the inhibition effect, *i.e.*, small turnover number, in the course of the solvolytic reaction when [NDBA] > [PVIm] (see section on kinetic parameters). Consequently, the deacylation step might be the overall rate-determining step in the solvolytic reaction of NDBA catalyzed by poly[4(5)-vinylimidazole]. Although the liberation of phenolate ion from NDBA is accelerated by poly[4(5)-vinylimidazole] at the initial stage (in the case of [NDBA] > [PVIm]), the overall solvolytic reaction is almost inhibited. The quaternary salt of poly(4-vinylpyridine) and 2-(2′-chloroethyl)pyridine, which contained a small fraction of unsubstituted 4-vinylpyridine groups, also showed marked retardation and inhibition at degrees of low conversion after the initial acceleration stage in the hydrolysis of PNPA.[4]

It has been reported that the rate of breakdown of the intermediate N^α-myristoylhistidine (Im-acyl) is quite slow in the hydrolysis of straight-chain acyl esters of *p*-nitrophenol in the presence of mixed micelles of N^α-myristoyl-L-histidine and cetyltrimethylammonium bromide, and that the acylated imidazolyl group of the N^α-

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[18] W. P. Jencks and J. Carriuolo, *J. Biol. Chem.*, 234, 1272, 1280 (1959).

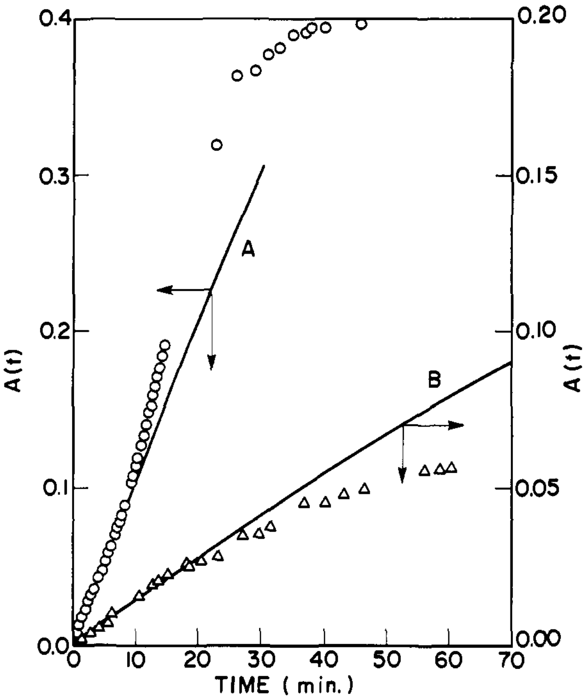

Figure 3. The poly[4(5)-vinylimidazole]-catalyzed solvolysis of NDBA as a function of time for (A) $[E]_0 > [S]_0$ ([NDBA] = 1.1 × 10^{-4} and [PVIm] = 1.1 × 10^{-3}) and (B) $[S]_0 > [E]_0$ ([NDBA] = 1.1 × 10^{-4} and [PVIm] = 2.43 × 10^{-5}); pH 7.99, 43.7 vol % ethanol–water, $\mu = 0.02$, 26°. The solid lines were calculated from $A(t) = 0.4675[1 - \exp(-0.02222t)]$ for (A) and $A(t) = 0.4675[1 - \exp(-0.003803t)]$ for (B).

myristoyl-L-histidine is buried in a hydrophobic region of the micelles.[9] The unusual stability of the resultant intermediate in the course of the solvolytic reaction of NDBA catalyzed by poly[4(5)-vinylimidazole] could be explained in the same manner. Although the solvolytic reaction system has been ascertained not to be composed of micellar aggregates in the absence of D-PVIm, the participation of nonpolar bonding at low solubility and the unusual stability for D-PVIm implies that the nonpolar dodecanoyl side chains are directed away from the water, placing most of the acylimidazole groups within nonpolar regions.

Steady-State Approximation. Since a principal object is to investigate the participation of nonpolar bonding in the solvolytic reactions catalyzed by synthetic polymers, the initial stage during which the solvolytic reaction is not complicated by the resultant intermediate is the most interesting to investigate.[4]

In order to determine the kinetic parameters for this reaction, two sets of conditions may be tested. Under the first set of conditions, [substrate] > [catalyst], low concentrations of catalyst are used in the presence of excess substrate. In the second, [catalyst] > [substrate], low concentrations of substrate are used in the presence of excess catalyst. It is necessary to derive the initial, steady-state rate for the two sets of conditions given above; the Michaelis–Menten mechanism (eq 4) can be

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_2} \mathrm{ES'} + \mathrm{P} \qquad (4)$$

employed for these solvolytic reactions, where E is the catalyst, S is the substrate, ES is the catalyst–substrate complex, ES′ is the intermediate compound (acylated catalyst), and P is the phenolate ion leaving group. The steady-state equations for initial velocity are

$$v = k_2[\mathrm{S}]_0[\mathrm{E}]_0/([\mathrm{S}]_0 + K_\mathrm{m}) \text{ for } [\mathrm{S}]_0 > [\mathrm{E}]_0 \qquad (5)$$

$$v = k_2[\mathrm{E}]_0[\mathrm{S}]_0/([\mathrm{E}]_0 + K_\mathrm{m}) \text{ for } [\mathrm{E}]_0 > [\mathrm{S}]_0 \qquad (6)$$

where $K_\mathrm{m} = [k_{-1} + k_2]/k_1$ and subscript 0 refers to initial concentration. Thus kinetic data for the two sets of conditions were analyzed by initial steady-state kinetic equations (eq 5 and 6) in the solvolytic reaction of NDBA catalyzed by poly[4(5)-vinylimidazole]. The second set of conditions (eq 6), however, is advantageous in that in low concentrations of substrate, the resultant intermediate and products would not greatly interfere with the reaction system studied by either precipitation or retardation.

Kinetic Parameters Obtained in 43.7, 30, and 20 Vol % Ethanol–Water Mixtures. 43.7 Vol % Ethanol–Water. The solvolytic rates of NDBA catalyzed by poly[4(5)-vinylimidazole] in high ethanol composition (60, 70, and 80 vol % ethanol) were satisfactorily described by a simple second-order rate equation, but in low ethanol compositions they completely differ in their kinetic pattern.[1] The solvolysis of NDBA catalyzed by poly-[4(5)-vinylimidazole] was investigated over a wide range of substrate conversion under the two sets of conditions, *viz.*, $[S]_0 > [E]_0$ and $[E]_0 > [S]_0$. It was found that the reaction mechanism is changed in the course of the solvolytic reaction (for both sets of conditions) when the absorbance ($A_t - A_0$) was plotted against time (Figure 3, A_t and A_0 represent the absorbance at any time of a measurement and at the initial time, respectively). The solid lines were calculated by the following equations according to the Hommes' analysis treatment[19] for enzyme-catalyzed reaction for (B), $[S]_0 > [E]_0$, and the modified Hommes' analysis for (A), $[E]_0 > [S]_0$

$$\text{(A)} \qquad P(t) = S_0[1 - e^{-k_2E_0t/(E_0 + K_\mathrm{m})}] \qquad (7)$$

$$\text{(B)} \qquad P(t) + S_0[1 - e^{-k_2E_0t/(S_0 + K_\mathrm{m})}] \qquad (8)$$

The fit was somewhat less satisfactory for the calculated lines, except during the initial stage, *i.e.*, the time interval following the initial steady-state equation was considerably short. It is of interest that the behavior in the solvolytic reaction completely differs between the two sets of conditions. The catalytic rate is accelerated after a certain time interval for $[E_0] > [S]_0$, leading to an acceleration effect (Figure 3A), whereas it is retarded for $[S]_0 > [E]_0$, leading to a retardation effect (Figure 3B). This behavior of retardation was substantiated by the unusual stability of the resultant intermediate, while the acceleration behavior was substantiated by the fact that the solvolytic rate constants of NDBA catalyzed by D-PVIm are larger than that catalyzed by poly[4(5)-vinylimidazole], on the basis of the formation of D-PVIm as the intermediate (Table III). The large catalytic efficiency of D-PVIm is also part of the evidence for the concept that polymer chains which contain pendent long aliphatic chain groups contribute to an increase in the nonpolar interaction for binding with substrate. This is consistent with the results obtained by Klotz, *et al.*[5]

The saturation kinetics, which are strong support for the concept that catalyst and substrate form a complex, were observed when the solvolysis of NDBA was studied as a function of the concentration of neutral imidazole

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[19] F. A. Hommes, *Arch. Biochem. Biophys.*, **96**, 28 (1962).

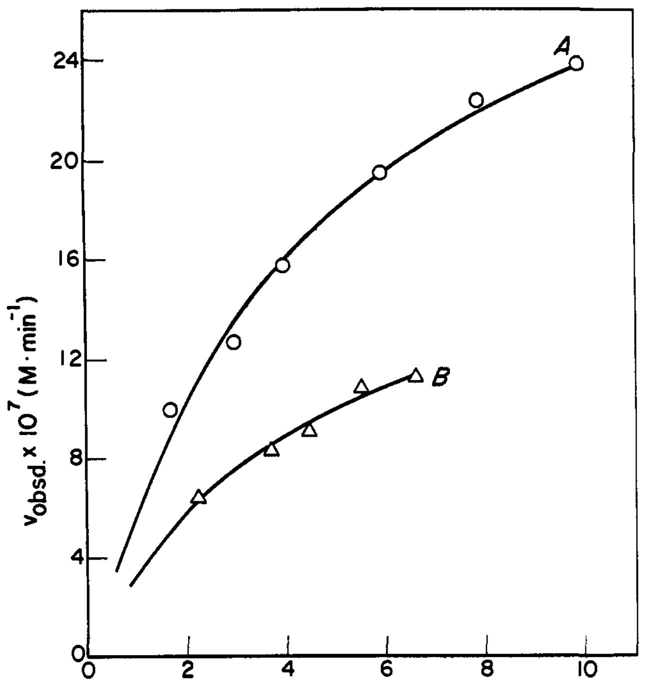

Figure 4. The observed initial rates of the poly[4(5)-vinylimidazole]-catalyzed solvolysis of NDBA as a function of (A) varying concentration of neutral polymer (α_1[PVIm]) and fixed concentration of substrate ([NDBA] = 1.1 × 10^{-4}) and (B) varying concentration of substrate and fixed concentration of neutral polymer ([PVIm] = 2.43 × 10^{-5}); pH 7.99, 43.7 vol % ethanol–water, μ = 0.02, 26°. The solid lines were calculated from the determined kinetic constants obtained from Figure 5.

groups in poly[4(5)-vinylimidazole] for $[E]_0 > [S]_0$ (Figure 4A) and of the concentration of NDBA for $[S]_0 > [E]_0$ (Figure 4B). Such an occurrence is indicative 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 might be held by either a nonpolar interaction between the long aliphatic group involved in substrate and the main carbon backbone of the polymer or by a coulombic attraction between the carboxylate anion in the substrate and the protonated imidazole groups along polymer chain, or by both of these processes. The solid lines B and A in Figure 4 are drawn from eq 5 and 6, respectively, based on the determined kinetic constants obtained in Figure 5.

Table III. Partially Dodecanoyl-Substituted Poly[4(5)-vinylimidazole]- and Unsubstituted Poly[4(5)-vinylimidazole]-Catalyzed Solvolysis of NDBA in 43.7 Vol % Ethanol–Water, μ = 0.05, pH 8.0, [NDBA] = 9.93 × 10^{-5} M

Sample	Polymer concn, g/dl	$v_{obsd} \times 10^6$, M min^{-1}
D-PVIm sample no. 1	3.21	2.86
D-PVIm sample no. 2	3.21	5.54
PVIm	3.21	2.58

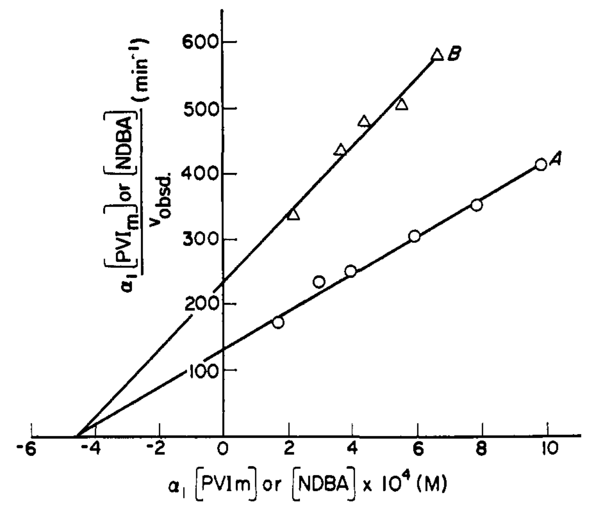

Figure 5. Modified Lineweaver–Burk plots for (A) α_1[PVIm]/v_{obsd} *vs.* α_1[PVIm] and (B) [NDBA]/v_{obsd} *vs.* [NDBA] for the poly-[4(5)-vinylimidazole]-catalyzed solvolysis of NDBA in 43.7% ethanol–water, pH 7.99, μ = 0.02, 26°. The solid lines were computed by a least-squares treatment.

If it is assumed that the solvolytic reaction of NDBA can be treated by the initial steady-state analysis of the mechanism described by eq 4 for the two sets of conditions and that rate enhancements resulting from the interaction of the binding groups or resultant intermediate compounds can be eliminated, it is possible to determine the Michaelis constant K_m for these reactions.

$$\text{(A)}\quad [E]_0 > [S]_0,\ \bar{V}_m = k_2[S]_0$$

$$[E]_0/v_{obsd} = K_m/\bar{V}_m + [E]_0/\bar{V}_m \qquad (9)$$

$$\text{(B)}\quad [S]_0 > [E]_0,\ \bar{V}_m = k_2[E]_0$$

$$[S]_0/v_{obsd} = K_m/\bar{V}_m + [S]_0/\bar{V}_m \qquad (10)$$

By utilizing a modified Lineweaver–Burk plot,[20,21] [PVIm]/v_{obsd} can be plotted *vs.* [PVIm] when the catalyst concentration is in excess (eq 9, Figure 5A) and [NDBA]/v_{obsd} can be plotted *vs.* [NDBA] when the substrate concentration is in excess (eq 10, Figure 5B). From a least-squares treatment of these data, values of K_m were determined to be (4.77 ± 0.57) × 10^{-4} M for [PVIm] > [NDBA] (A) and (4.53 ± 0.64) × 10^{-4} M for [NDBA] > [PVIm] (B). The first-order rate constants, k_2, for release of phenolate ion from the macromolecule–substrate complex were calculated[1] to be 0.032 ± 0.004 min^{-1} for A and 0.088 ± 0.013 min^{-1} for B. It is noted that the novel similarity of K_m values would appear to suggest that the mechanisms of complexation described in eq 4 for the two sets of conditions are similar, regardless of which material is in excess.

20 Vol % and 30 Vol % Ethanol–Water. The kinetic parameters obtained in lower ethanol composition than *ca.* 40 vol % were studied by initial, steady-state kinetic analysis using the data obtained by the technique of stopped flow spectroscopy. Since the long-lived resultant intermediate interferes with the solvolytic reaction, the case of $[E]_0 > [S]_0$ was chosen in this system. A modified Lineweaver–Burk plot (eq 9) showed straight lines for 20 and 30 vol % ethanol–water (Figure 6). From a least-squares treatment of these data, values of K_m and k_2 were determined to be (3.82 ± 1.09) × 10^{-5} M and 11.4 ± 1.8, respectively, for 20 vol % ethanol–water and (3.11 ± 0.8) × 10^{-4} M

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(20) C. G. Overberger, R. Corett, J. C. Salamone, and S. Yaroslavsky, *Macromolecules*, 1, 331 (1968).

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Table IV. Kinetic Parameters for the Poly[4(5)-vinylimidazole]-Catalyzed NDBA Solvolysis with Varying Ethanol Content, μ = 0.02, 26°

Ethanol, vol %	pH	Set of conditions	Fixed, initial concn, M	$K_m \times 10^4$, M	k_2, min^{-1}
43.7	7.99	$[S]_0 > [E]_0$	$[E]_0 = 2.4 \times 10^{-5}$	4.53 ± 0.64	0.088 ± 0.013
		$[E]_0 > [S]_0$	$[S]_0 = 1.1 \times 10^{-4}$	4.77 ± 0.57	0.032 ± 0.004
30	7.90	$[E]_0 > [S]_0$	$[S]_0 = 1.78 \times 10^{-4}$	3.11 ± 0.8	20.7 ± 4.0
20	7.90	$[E]_0 > [S]_0$	$[S]_0 = 8.90 \times 10^{-5}$	0.38 ± 0.11	11.4 ± 1.8

and 20.7 ± 4.0, respectively, for 30 vol % ethanol-water. All of the kinetic parameters obtained by the initial steady-state kinetic analysis are summarized in Table IV.

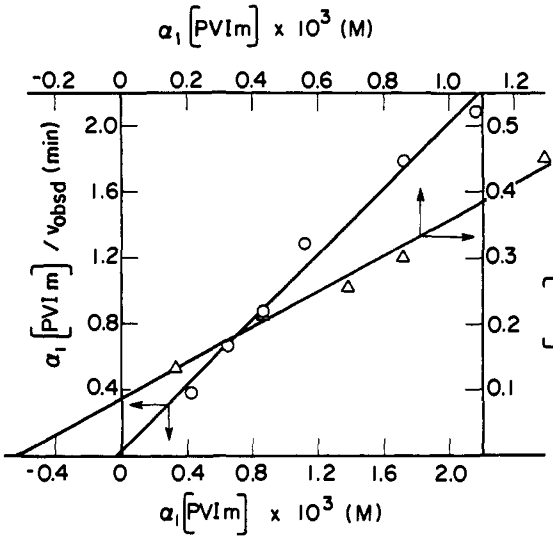

Figure 6. Modified Lineweaver-Burk plots for α_1. [PVIm]/v_{obsd} *vs.* α_1[PVIm], using the kinetics data for poly[4(5)-vinylimidazole]-catalyzed solvolysis of NDBA in 20 vol % (O, [NDBA] = 8.90 × 10^{-5}), 30 vol % (Δ, [NDBA] = 1.78 × 10^{-4}) ethanol-water; pH 7.9, μ = 0.02, 26°.

It was found that the Michaelis constants dramatically decrease with a decrease in ethanol composition, particularly in 20 vol % ethanol under the conditions employed. Furthermore, the first-order rate constants, k_2, for release of phenolate ion from the complex are 10^2 to 10^3 larger in 20 and 30 vol % ethanol-water than in 43.7 vol % ethanol-water. It is apparent from inspection of Table IV and the previously reported results[1] that the relatively low catalytic activity in 20 vol % is substantiated by the relatively small value of k_2, in spite of the very small value of K_m.

The increase in catalytic activity of poly[4(5)-vinylimidazole] for the solvolysis of PNPA (*p*-nitrophenyl acetate), relative to imidazole, with decreasing ethanol content was attributed to an increase in the bifunctional interactions of the pendent, neutral imidazole functions.[2,3] In the solvolysis of NDBA, however, the k_2 value for 30 vol % ethanol-water was larger than that for 20 vol % ethanol-water. This indicates that in 20 vol % ethanol the polymer is a tighter coil than in 30 vol % ethanol, and that the long-chain substrate NDBA is orientated with difficulty in the "active sites" of the polymer, thereby lowering the k_2 value in 20 vol % ethanol. A smaller substrate such as PNPA could presumably fit more easily into these cavities than could NDBA. Further, in 20 vol % ethanol it is reasonable to assume that there are strong nonpolar interactions between the polymer and the substrate. This would lead to a situation in which the imidazole groups are on the exterior of the polymer-substrate complex, which would be on the interior, and this would cause difficulty in proper orientation for most efficient catalysis. These results suggest that the K_m value is associated with the magnitude of the nonpolar interactions as a function of ethanol composition and that the k_2 value is associated with not only the bifunctional catalytic interactions, but also with the magnitude of the nonpolar interactions in the polymer-substrate complex.

Nonpolar Interactions. The composition of a binary mixture of polar and nonpolar solvents could be expected to alter the effects of nonpolar interactions. It was found that the magnitude of nonpolar interactions increases with decreasing ethanol content, according to the Michaelis constant obtained in the poly[4(5)-vinylimidazole]-catalyzed solvolysis of the long aliphatic chain, anionic ester NDBA in 43.7, 30, and 20 vol % ethanol-water mixtures, whereas at 60, 80, and 90 vol % ethanol contents, the polymeric reactions did not follow eq 4 and these reactions were overall second-order processes.

It has been realized that the medium may have an effect on the nonpolar interaction, and that such an interaction is due to the large positive entropy change in water and the small positive enthalpy change, which leads to the favorable negative free energy change, where the entropy factor is related to the structuredness of the solvent.[22-24] Therefore, it is imperative that the role of the medium (*i.e.*, external structure surrounding the molecule) is understood in order to gain an understanding of nonpolar interactions as a function of ethanol composition.

The complexity of ethanol-water mixtures is clearly demonstrated by their thermodynamic and volumetric properties.[24] The addition of ethanol to water has been shown to increase the structuredness of the medium and this reached a maximum at an ethanol mole fraction (x_2) of 0.1 (*ca.* 27 vol % at 25°).[14] Beyond this maximum point the degree of the structuredness decreased until the system became an ordinary binary mixture at $x_2 > 0.25$ (*ca.* 54 vol % at 25°). Apparently, the effect of adding a third solute to an aqueous solution is greatly affected by the structure of ethanol-water mixture. Partial molar heats of solution at infinite dilution were carefully measured for the introduction of a number of salts and nonelectrolytes to ethanol-water mixtures.[25,26] These results indicate

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(26) E. M. Arnett, W. G. Bentrude, J. J. Burke, and P. McDuggleby, *ibid.*, **87**, 1541 (1965).

endothermic maxima in the same solvent region of aqueous ethanol (*ca.* $x_2 \sim 0.1$–0.2), where the degree of structuredness of the system shows a maximum. The magnitude of such a maximum for a given nonelectrolyte molecule is related to the size of the nonelectrolyte molecule, *i.e.*, the volume which the solute occupies and this maximum are greater than that for salts of the same molar volume. The introduction of a third component is, therefore, another means of perturbing the solvent structure. Thus, endothermic maxima for both nonelectrolytes and salts in going from water to highly aqueous alcohol can result either because the solute is a more effective structure maker in water than in the binary solvent, or a more effective structure breaker in the latter.

When these results are extended to the formation of nonpolar bonds in the reaction system, the thermodynamic description could present the best current picture of the formation of nonpolar bonds in ethanol–water mixtures, in view of the striking tendency for enthalpy and entropy changes to mirror each other in highly aqueous media.[27] On the basis of the assumption that salt and Tris buffer do not effectively act as a structure breaker and that enthalpy can be taken as an indicator of net structure change, the formation of the nonpolar interaction in 30 and 43.7 vol % ethanol–water may be due to a negative enthalpy change, $\Delta H^0 < 0$, because the solutes poly[4(5)-vinylimidazole] and NDBA act as the structure breaker in the solution process. In 20 vol % ethanol–water the nonpolar interaction may be due to a positive entropy change, $\Delta S^0 < 0$, because addition of the solutes appears to result in more structure formation than when addition is made to 30 and 43.7 vol % ethanol–water. It is quite reasonable to obtain considerably smaller value of K_m for 20 vol % ethanol, relative to those of 30 and 43.7 vol % ethanol.

Effects of pH. It has been found that the catalytic enhancement for the solvolyses of NABA and NABS (sodium 3-nitro-4-acetoxybenzenesulfonate) are mainly due to the electrostatic accumulation of the anionic substrates in the vicinity of partially protonated poly[4(5)-vinylimidazole].[2,3] Since the NDBA molecule includes a long aliphatic chain which facilitates complex formation by nonpolar interactions and a carboxylate anion which is helpful for electrostatic interactions, it was of interest to investigate the studies of the dependence of the solvolytic rate of NDBA on pH in low ethanol compositions in order to understand the driving force for the complex formation.

If contributions from the electrostatic and nonpolar interactions are necessary for binding, the pH–rate profile may show a bell-shaped portion of a curve and a curve of increasing slope, such as occurs in the solvolysis of NABS by poly[5(6)-vinylbenzimidazole][28] and in the solvolyses of NABA and NABS by a copolymer of 4-(5)-vinylimidazole and *p*-vinylphenol.[3,29] If the electrostatic effect is negligible, we can expect to observe the type of pH–rate profile which occurs for the solvolysis of PNPA by poly[4(5)-vinylimidazole],[3,15] *i.e.*, a curve of increasing slope with increasing α_I.

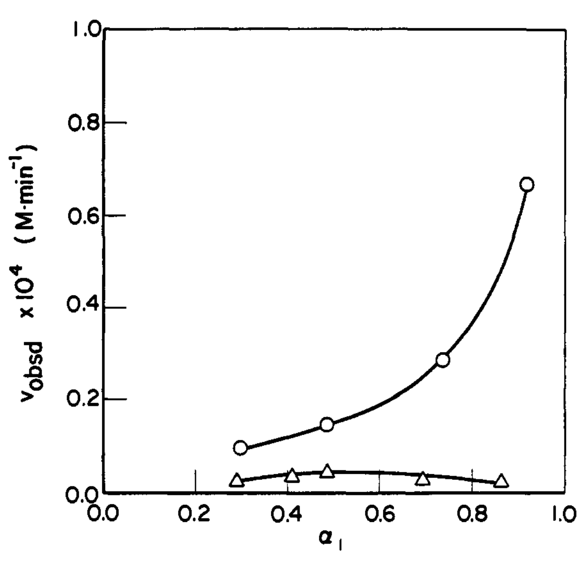

Figure 7. α_I rate profiles for the solvolysis of NDBA and NABA catalyzed by poly[4(5)-vinylimidazole]: ○, Δ[NDBA] = 5×10^{-5}, [PVIm] = 5×10^{-4} in 25 wt % ethanol–water; Δ, [NABA] = 5×10^{-5}, [PVIm] = 5×10^{-4} in 30 vol % ethanol–water; μ = 0.02, 26°.

In *ca.* 30 vol % ethanol, the pH–rate profile for the solvolysis of NDBA catalyzed by poly[4(5)-vinylimidazole] showed that the rate is apparently not dependent on the fraction of protonated imidazole functions as indicated by the lack of enhanced catalysis (bell-shaped rate profile) at low and intermediate pH (Figure 7, upper curve). The lower curve in Figure 7 illustrates the reaction of poly[4(5)-vinylimidazole] with NABA, in which the reaction rate is dependent upon the fraction of neutral and protonated imidazole functions. It was also observed that the rate of imidazole-catalyzed NDBA solvolysis is directly proportional to the fraction of the neutral imidazole.

The reduced action of poly[4(5)-vinylimidazole] (in comparison to imidazole) in the solvolysis of PNPA at low α_I values has been explained by two factors; first, a decrease in nonpolar interactions of the neutral ester with the diminished nonpolar regions of the polyion, and, second, a lack of bifunctional (neutral–neutral) imidazole catalysis.[3] It was found that the catalytic rate for the solvolysis of NDBA is faster by a factor of 4.8 at $\alpha_I = 0.6$ and of 3.8 at $\alpha_I = 0.3$ than that for the solvolysis of NABA at low Ph values (Figure 7). This may indicate that the nonpolar interaction is not strongly affected by an increasing protonation of imidazole residues in the conditions employed. This also agrees with the concept that a neutral ester is not excluded from a catalytically active polyion such as has been reported for the solvolysis of PNPA catalyzed by copolymers of 4(5)-vinylimidazole and acrylic acid.[3,30] Therefore, the reduced activity of poly[4(5)-vinylimidazole] for the solvolysis of PNPA (in comparison to imidazole) at low α_I values can presumably be attributed to a lack of bifunctional (neutral–neutral) imidazole catalysis.

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(30) C. G. Overberger and H. Maki, *Macromolecules*, **3**, 214, 220 (1970).

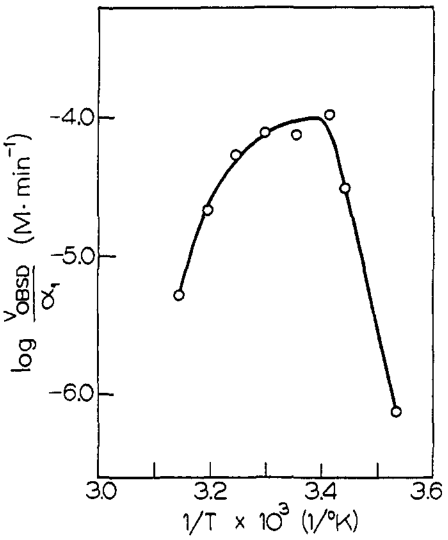

Figure 8. Temperature dependence of the rate of the solvolysis of NDBA catalyzed by poly[4(5)-vinylimidazole]; 25 wt % ethanol–water, μ = 0.02, pH 7.8 (at 25°), [NDBA] = 5×10^{-5} M, [PVIm] = 5×10^{-4} M, (O) experimental points.

The pH–rate profile for the solvolysis of NDBA catalyzed by poly[4(5)-vinylimidazole] shows the same behavior as the pH–rate profile for the solvolysis of PNPA. This result suggests the involvement of bifunctional interactions of two neutral imidazole functions in the solvolytic process and of nonpolar interactions with a negligible electrostatic contribution for complexation. Evidence for the involvement of nonpolar interactions for complex formation in the solvolysis of NDBA was also obtained by the saturation phenomenon for the solvolysis of NDBA and by the fact that no saturation phenomenon was observed for the solvolysis of NABA under similar conditions. Recently, it was reported that the electrostatic interaction between copolymers of 1-vinyl-2-methylimidazole and 1-vinylpyrrolidone and of 1-vinyl-2-methylimidazole and acrylamide and NABA was negligible, relative to hydrophobic interactions for binding.[6]

Effect of Temperature. Frank showed that addition of the first increment of a low molecular weight alcohol to water produces at first an increase in the degree of structuredness of the solvent.[14,26] However, the extreme for the heat of mixing of alcohol, the partial molar volume of alcohol, and the ultrasonic absorption and velocity in the high water region were less pronounced at higher temperature than they were at room temperature.[26] This indicates that the structuredness of solvent in the highly aqueous ethanol solution is collapsed by thermal energy as the temperature increases.

Therefore, the studies of the temperature dependence on the solvolysis of NDBA catalyzed by poly[4(5)-vinylimidazole] could further elucidate the influence of the medium on complex formation and the role of nonpolar interactions.

The slope of the plot of log v_{obsd} *vs.* $1/T$ in the solvolysis of NDBA showed not only a deviation from linearity but also a positive value in the conditions employed as would be expected from the mechanism of eq 4 in which two reaction steps, *viz.*, complex formation and solvolysis, are involved (Figure 8). The slope of the plot of log k_{obsd} *vs.* $1/T$ was negatively linear in the imidazole-catalyzed NDBA solvolysis, and the activation parameters for this system are listed in Table V.

Table V.[a] Activation Parameters for the Imidazole-Catalyzed Solvolysis of NDBA in 25 Wt % Ethanol

	$\Delta E^{\ddagger}$	$\Delta H^{\ddagger}$	$\Delta F^{\ddagger}$	$T\Delta S^{\ddagger}$	$\Delta S^{\ddagger}$
Imidazole	7.65	7.08	18.06	−10.98	−38.1

[a] All values are in kilocalories per mole except ΔS (eu). Measurements were performed at μ = 0.02, pH 7.30 ∼ 8.11, [NDBA] = 5×10^{-5} M, [Im] = 9.38×10^{-4} M.

The temperature dependence for the latter was expected to be normal, since simple first-order reactions with respect to both the substrate and the catalyst have been observed.

Figure 8 reveals that the solvolysis of NDBA catalyzed by poly[4(5)-vinylimidazole] in 25 wt % ethanol–water can be separated into two regions; the first region controlled by the complex formation step at a higher temperature than 20° and the second region controlled by a step for release of phenolate ion at lower temperature than 20°, if the temperature dependence of the bifunctional catalytic interactions is assumed to be negligible in the conditions employed. It should be noted that the catalytic maximum was observed at *ca.* 17°.

The temperature dependence of the poly[4(5)-vinylimidazole]-catalyzed NDBA solvolysis is a different situation than that of the enzymatic reaction which is generally attributed to a combination of two phenomenologically different events. One is related to enzyme stability and the other is related to a kinetic effect of temperature on reaction rates.[31] Although the unusual dependence of temperature on the solvolysis of NDBA by poly[4(5)-vinylimidazole] can be related to a combination of a complex formation step and a releasing step of the phenolate ion, it also seems likely that the effect of temperature on the conformation of the polymer should be considered.

The results of the dependence of temperature in the solvolysis of NDBA catalyzed by poly[4(5)-vinylimidazole] supports the mechanism of eq 4 for the system and furthermore elucidates the contribution of nonpolar interactions for the complex formation on the basis of the "structuredness" of the solvent system.

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