The Esterolytic Activity of Poly(1-methyl-4- and -5-vinylimidazole) in Water

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ABSTRACT: The water solubility of poly(1-Me-5-VIm) has made it possible to achieve phenomenal rate enhancements and to gain even greater insight into the mechanism of catalysis by polymeric imidazoles. The poly(1-Me-5-VIm)-catalyzed hydrolysis of S_{12}^- exhibited saturation in excess catalyst and in excess substrate. Inhibition of the poly(1-Me-5-Vim) catalyzed hydrolysis of S_n^- type substrates by analog inhibitors was also observed. The saturation apparently did not follow a simple Michaelis–Menten mechanism; however, the results could be rationalized by analogy to certain enzymatic systems. Multisite enzymes have long been known to display kinetic patterns different from that exhibited by enzymes with only one active site, i.e., such phenomena as sigmoidal rate vs. [S] plots. These phenomena may arise entirely as a result of the multisite nature of the enzyme. Consequently, a synthetic macromolecular catalyst with multiple sites might also be expected to display such characteristics. The poly(1-Me-5-VIm)-catalyzed hydrolysis of S_{12}^- is apparently the first synthetic system in which such phenomena have been observed. The intermediacy of an apolar polymer substrate complex for the poly(1-Me-5-VIm)-catalyzed hydrolysis of S_{12}^- in water was given support by studies of the effect of temperature on the rate of hydrolysis. Activation parameters were determined for catalysis by 1,5-DMIm and by poly(1-Me-5-VIm). These results showed that the rate enhancement exhibited by the polymer was due entirely to a favorable entropy term.

The most important physical property of poly(1-Me-4-VIm) and poly(1-Me-5-VIm) is water solubility. The methylation of the imidazole ring prevents the intermolecular H bonding which causes the general insolubility of poly[4(5)-VIm], without significantly increasing the apolar character of the macromolecule. This result is very fortuitous, because even though the rate enhancements achieved through hydrophobic interactions in ca. 28.5% ethanol–water are quite dramatic, much greater rate enhancements should be observed in pure water. Hydrophobic interactions are more a property of water than of a particular nonpolar solute. Water molecules surrounding each individual nonpolar solute molecule orient in such a way as to maximize hydrogen bonding. This results in a structuring of the water molecules in the immediate area of the solute.[1-3] Association of any two (or more) nonpolar molecules results in a general decrease in the degree of ordering of the water molecules. this corresponds to a dramatic increase in the entropy of the system as a whole. The enthalpy change resulting from such an association is zero or only slightly positive.[4] Thus, the phenomenon of apolar bonding is favorable only because of an entropy effect derived from the tendency of water to hydrogen bond.

The kinetics in water were carried out both in excess substrate and in excess catalyst. In excess catalyst, the kinetics were most often analyzed by determining the slope of plots of ln $(A_\infty - A_t)$ vs. time, the linearity of which demonstrated the first-order nature of the catalyses. The poly(1-Me-4-VIm)-catalyzed hydrolyses in water were abnormal giving nonlinear plots of ln $(A_\infty - A_t)$ vs. time even in excess catalyst. Consequently, initial rates were determined from the plots of absorbance vs. time. In all cases the kinetics in excess substrate were evaluated from the initial slope of the absorbance vs. time curve. This was done even with the short chain substrate which exhibited simple first-order kinetics.

1. The Critical Micelle Concentration (cmc) of Long-Chain Substrates in Water. Apolar interactions can lead to micellarization of surfactant molecules such as those utilized in this work as substrates. Furthermore, micelles have been shown to influence the rate of hydrolysis of long-chain esters, catalyzed by small molecules.[5] It is not expected, however, that micelles would influence the catalytic activity of a macromolecule (high polymer) in a similar manner. The dimensions of a polymer molecule are such that it would not be adsorbed into a micelle. Consequently, the micelle would only serve as a substrate reservoir in catalysis by a macromolecular species. However, inasmuch as this fact has not been demonstrated unequivocally, we have followed accepted practice carrying out all kinetic studies at substrate concentrations well below their measured cmc values.

Overberger, Glowaky, and Vandewyer have investigated the cmc of the S_n^- series in alcohol–water media;[6] thus it is known from their results that the studies in 28.5% ethanol–water were carried out below the cmc of the substrates employed.

It was necessary, however, to determine the cmc values of these substrates in a totally aqueous medium. The dye method of determining the cmc is fraught with difficulty and inaccuracy.[7] Therefore, the cmc values in water were determined conductometrically.[8] In addition to the substrates, S_7^- and S_{12}^-, cmc values were determined for two substrate analogs; 2-heptanoylbenzoic acid, I_7^-, and 2-undecanoylbenzoic acid, I_{11}^-.

It was desirable to determine the cmc's in pH 7.90, aqueous buffer, μ 0.02 at 26°. The conductivity of the buffer itself was quite high and even though there was only a small difference between the conductivity of buffer and that of buffer plus substrate, it was possible to determine the cmc's of S_7^-, I_7^-, and I_{11}^- in such a medium. The difference between the conductivity of buffer and that of buffer plus S_{12}^-, however, was so small that it was impossible to determine its cmc in buffer conductometrically. Specific conductance, $\bar{L}$, is proportional to the mobility of the ions involved[9]

$$\bar{L} = \frac{\alpha CF}{1000}(U_{A^+} - U_{B^-}) \qquad (1)$$

where the U values are the ionic mobilities. Thus it is apparently a lack of mobility of S_{12}^- that makes it impossible to determine its cmc in $\mu = 0.02$, pH 7.90 buffer. The most suitable alternative was the determination of the cmc of the sodium salt of S_{12}^- in conductivity water. Table I lists the determined cmc values.

2. The Esterolytic Activity of Poly(1-Me-4-VIm) in Aqueous Buffer. As was reported in the first paper of this series,[10] poly(1-Me-4-VIm) is not catalytically active toward PNPA in 28.5% ethanol–water. Similarly, poly(1-Me-4-VIm) exhibits little catalytic activity toward S_2^-,

Table I
The Cmc of Substrates in Water[a]

Substrate	Cmc, 10^3 M
S_7^-	6.25
S_{12}^-	1.15[b]
I_7^-	5.0
I_{11}^-	3.1

[a] Cmc's in H_2O, pH 7.90, μ = 0.02, 26°. [b] Value determined for sodium salt in pure water, 26°.

Table II
The Poly(1-Me-4-VIm)-Catalyzed Hydrolysis of the S_n^- Series[a]

Substrate	Initial rate V_i, 10^7 M min^{-1}	k_{obsd}, 10^3 min^{-1}	r[b]
S_2^-	3.31	4.95	0.45
S_4^-	1.17		
S_7^-	1.36	1.54	0.18
S_{12}^-	44.72[c]	88.67	19.6

[a] Hydrolysis in 96.7% H_2O, 3.3% CH_3CN, pH 7.90 buffer, μ = 0.02, 26°, [poly(1-Me-4-VIm)] = 5×10^{-4} M, [S_n^-] = 5×10^{-5} M. [b] $r = k_{obsd}$[poly(1-Me-4-VIm)]/k_{obsd}[1,4-DMIm]. [c] Average of five kinetic runs.

S_4^-, or S_7^- in pH 7.90 aqueous buffer. Surprisingly, poly(1-Me-4-VIm) shows considerable activity toward S_{12}^-. Table II and Figure 1 present the data, as compared to 1,4-DMIm.

The reactions were carried out at 5×10^{-5} M substrate and 5×10^{-4} M catalyst; however, the kinetics could not be interpreted in a first-order manner. Initial rates were therefore determined from the plots of absorbance vs. time. These curves for the poly(1-Me-4-VIm) (5×10^{-4} M) catalyzed hydrolysis of S_{12}^- (5×10^{-5} M) were quite unusual. The reaction starts out very rapidly and at about 50% conversion it suddenly decelerates. When a fresh aliquot of S_{12}^- (enough to adjust the concentration to 5×10^{-5} M) was added to an old kinetic run of 5×10^{-4} M poly(1-Me-4-VIm) and 5×10^{-5} M S_{12}^-, which had gone to infinity, the rate starts out much more slowly and levels out at a rate similar to that of a virgin kinetic run (see Figure 2). This might indicate some kind of product inhibition; however, when the poly(1-Me-4-VIm) ($0.5 \rightarrow 10 \times 10^{-4}$ M) catalyzed hydrolysis of S_{12}^- (5×10^{-5} M) was studied, it was found that the higher the polymer concentration the more abnormal were the plots of absorbance vs. time (see Figure 3). The higher the catalyst concentration, the greater the inhibition and the lower the conversion at which the abnormal behavior appears.

One would expect that simple product inhibition would become more pronounced as the catalyst concentration decreases. It is possible that the abnormal behavior observed in these kinetic runs results from the phenolate ion-induced formation of multiple-stranded polymer coils. Such multiple-stranded coils would form more readily at higher polymer concentrations. The negative slope of the viscosity vs. concentration curves of the imidazole polymers and the concavity of certain of these curves support such a possibility (see preceding paper).[11]

3. The Poly(1-Me-5-VIm)-Catalyzed Hydrolysis of the S_n^- Series. The poly(1-Me-5-VIm)-catalyzed hydrolysis of the S_n^- series has been evaluated in pH 7.90 aqueous buffer at 26°. A curve similar to that generated in the poly(1-Me-5-VIm)-catalyzed solvolysis of the S_n^- series in etha-

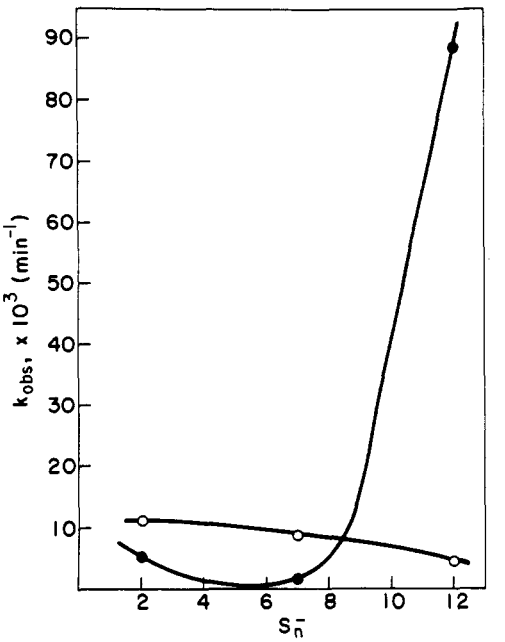


Figure 1. The poly(1-Me-4-VIm)-catalyzed hydrolysis of the S_n^- series. Hydrolysis in 96.7% H_2O, 3.3% CH_3CN, pH 7.90, μ = 0.02, 26°, [catalyst] = 5×10^{-4} M, [S_n^-] = 5×10^{-5} M: ●, poly(1-Me-5VIm); ○, 1,4-DMIm.

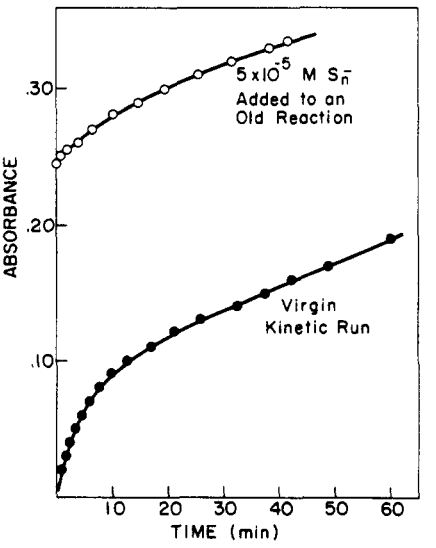


Figure 2. Poly(1-Me-4-VIm)-catalyzed hydrolysis of S_{12}^-. Hydrolysis in 96.7% H_2O, 3.3% CH_3CN, pH 7.90, μ = 0.02, 26°, [catalyst] = 5×10^{-4} M, [S_{12}^-] = 5×10^{-5} M: ●, virgin kinetic run; ○, old kinetic run.

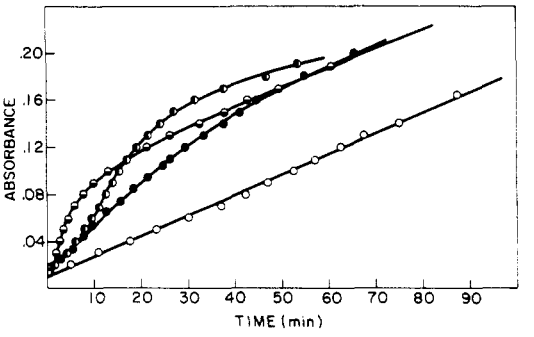


Figure 3. Hydrolysis of S_{12}^- by varying concentrations of poly(1-Me-4-VIm). Hydrolysis in 96.7% H_2O, 3.3% CH_3CN, pH 7.90, 26°, μ = 0.02, [S_{12}^-] = 5×10^{-4} M; [poly(1-Me-4-VIm)] 0.5×10^{-4} M (○), 2.5×10^{-4} M (◐), 5×10^{-4} M (◒), 10×10^{-4} M (●).

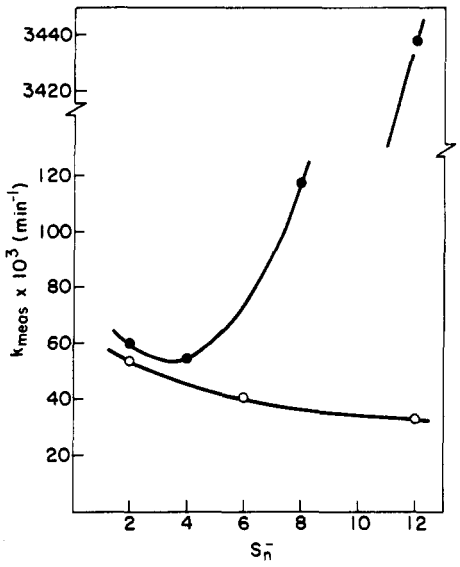


Figure 4. The poly(1-Me-5-VIm)-catalyzed solvolysis of the S_n^- series. Hydrolysis in pH 7.90 aqueous buffer, μ = 0.02, 26°, [catalyst] = 5×10^{-4} *M*, [S_{11}^-] = 5×10^{-5} *M*: ●, poly(1-Me-5-VIm); ○, 1,5-DMIm.

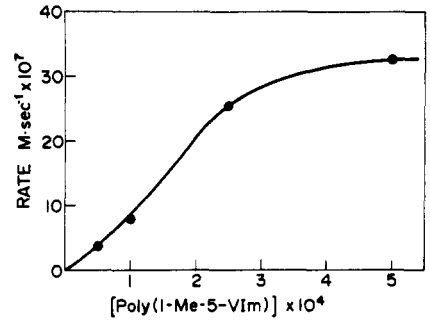


Figure 5. Saturation curve, excess catalyst poly(1-Me-5-VIm)-catalyzed hydrolysis of S_{12}^-. Rate (k_{meas}[S]) of the poly(1-Me-5-VIm)-catalyzed hydrolysis of S_{12}^- in H_2O. [S_{12}^-] = 5×10^{-5} *M*, μ = 0.02, pH 7.90, 26°.

nol–water[11] is obtained with the exception that the rates are much faster and enhancement is realized at shorter chain length. Figure 4 and Table III present the data as compared to 1,5-DMIm. One should notice that the rate of hydrolysis of S_7^- is twice as fast as that of S_4^-. The hydrolysis of S_{12}^- was followed on the stopped flow spectrophotometer, exhibiting a half-life of less than a minute.

4. Saturation Kinetics. The Poly(1-Me-5-VIm)-Catalyzed Hydrolysis of the S_n^- Series. The poly(1-Me-5-VIm)-catalyzed hydrolysis of S_2^-, S_4^-, S_7^-, and S_{12}^- in pH 7.90 aqueous buffer has been studied with respect to both the concentration of substrate and catalyst. The poly(1-Me-5-VIm)-catalyzed hydrolysis of S_2^-, S_4^-, and S_7^- proved to be first order in both substrate and catalyst over the range of concentration studied. The poly(1-Me-5-VIm)-catalyzed hydrolysis of S_{12}^- in pH 7.90 aqueous buffer exhibited saturation phenomena both in excess substrate and in excess catalyst.

A series of stopped flow kinetic runs were carried out at 0.5, 1.0, 2.5, and 5×10^{-4} *M* poly(1-Me-5-VIm) in the presence of 5×10^{-5} *M* S_{12}^-. The plot of the rate of hydrolysis vs. [poly(1-Me-5-VIm)] shown in Figure 5 displays marked saturation. Table IV lists the corresponding data in tabular form. The shape of the curve is sigmoidal rather than hyperbolic. Consequently, a simple Michaelis–Menten mechanism cannot be applied. No such problem was encountered in the poly(1-Me-5-VIm)-catalyzed hydrolysis of S_{18}^- in 28.5% ethanol–water. It is interesting to note that if a mechanism such as the following

Table III
The Poly(1-Me-5-VIm)-Catalyzed Solvolysis of the S_n^- Series[a]

Substrate	k_{meas}, 10^3 min^{-1}	r[b]
S_2^-	60.3	1.13
S_4^-	54.3	
S_7^-	117.3	2.92
S_{12}^-	3438	106

[a] Hydrolysis in pH 7.90 aqueous buffer, μ = 0.02, 26°, [catalyst] = 5×10^{-4} *M*, [S_n^-] = 5×10^{-5} *M*. [b] $r = k_{meas}$[poly(1-Me-5-VIm)]/k_{meas}[1,5-DMIm].

Table IV
The Rate of Hydrolysis of S_{12}^- as a Function of the Concentration of Poly(1-Me-5-VIm)[a]

[Poly(1-Me-5-VIm)], 10^4 *M*	k_{meas}, sec^{-1}	Rate k_{meas}[S_{12}^-], *M* sec^{-1} $\times 10^7$
0.5	7.12	3.51
1.0	15.5	7.98
2.5	50.7	25.5
5.0	57.3	32.5

[a] Hydrolysis in pH 7.90, H_2O; μ = 0.02; 26°; [S_n^-] ≃ 5×10^{-5} *M*.

$$nE + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E_n \cdot S \xrightarrow{k_2} E' + P_1 + (n-1)E \xrightarrow{k_3} E + P_2$$

where n catalytic sites simultaneously bind one substrate molecule were proposed, the overall rate equation would be[12,13]

$$\text{rate} = V = \frac{V_{max} E^n}{K_m + E^n} \quad (2)$$

alternatively, substrate molecules could be bound sequentially in rapid succession.[14] This equation describes a sigmoidal curve. Rearranging to

$$v/V_{max} = E^n/K_m + E^n \quad (3)$$

and taking the reciprocal of eq 3 yields the following relationship.

$$V_{max}/v = (K_m/E^n) + 1 \quad (4)$$

By rearranging and taking the log, eq 5 is obtained. This is analogous to the Hill equation for the binding of O_2 or CO to hemoglobin.[15]

$$\log \left[\frac{v}{(V_{max} - v)}\right] = n \log E - \log K_m \quad (5)$$

From this relationship, it is apparent that a plot of log [v/($V_{max} - v$)] vs. log E will give a straight line of slope n (see Figure 6). Assuming a V_{max} value of 40×10^{-7} *M* sec^{-1} and making such a plot an n value of 1.71 is obtained. This n value would indicate that there is an average of 1.71 imidazole residues per active site.

Isocitrate dehydrogenase is an enzyme which displays a sigmoidal rate vs. [substrate] curve. The plot of log (v/($V_{max} - v$)) vs. log [isocitrate concentration] has a slope, n, of 3.9. This enzyme contains four identical polypeptide chains and four active sites. The sigmoidal curve suggests that the binding of substrate at one site influences the

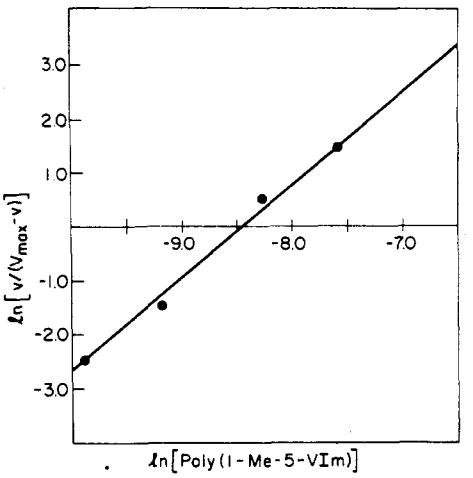


Figure 6. The poly(1-Me-5-VIm)-catalyzed hydrolysis of S_{12}^-. Hydrolysis in water, pH 7.90, μ = 0.02, 26°, $[S_{12}^-] = 5 \times 10^{-5}$ *M*.

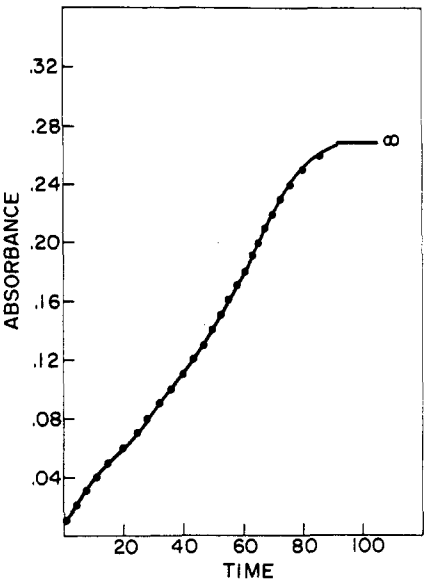


Figure 7. The poly(1-Me-VIm)-catalyzed hydrolysis of S_{12}^-. Hydrolysis in pH 7.90, 96.7% H_2O, 3.3% CH_3CN, μ = 0.02, 26°, [catalyst] = 5×10^{-6} *M*, $[S_{12}^-] = 5 \times 10^{-5}$ *M*.

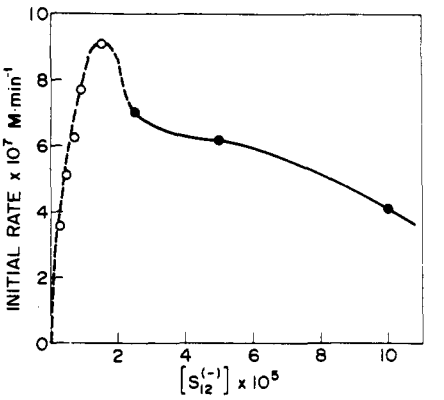


Figure 8. Saturation curve, excess substrate poly(1-Me-5-VIm)-catalyzed hydrolysis of S_{12}^-. (●) Initial rate of the poly(1-Me-5-VIm)-catalyzed hydrolysis of S_{12}^- in 96.7% H_2O/CH_3CN, [poly(1-Me-5-VIm)] = 5×10^{-6} *M*, μ = 0.02, pH 7.90, 26°. (○) Values obtained from the absorbance vs. time plot of a single kinetic run $[S_{12}^-] = 5 \times 10^{-5}$ *M*, [poly(1-Me-5-VIm)] = 5×10^{-6} *M*, μ = 0.02, pH 7.90, 26°.

other active sites so that they now bind much more strongly than did the first site.[12]

Monod, Wyman, and Changeux[16] and Blangy, Buc, and Monod[17] have postulated that the catalytic activity of enzymes may be affected and controlled by interaction with small molecules, not only directly, at or close to the active site, but also indirectly at distant secondary *allosteric sites*. This type of interaction may be recognized and distinguished from the more common direct action of such modifiers (activators or inhibitors) at the catalytic site by certain characteristics, including anomalous kinetic order with respect to substrate (plots of v vs. s yield sigmoidal rather than hyperbolic curves) which suggests multiple interacting binding sites. Allosteric effects resulting from interactions between identical ligands, i.e., a substrate binding to the enzyme at more than one site, have been termed *homotropic* allosteric effects.[18]

Poly(1-Me-5-VIm) is a rather apolar macromolecule with multiple catalytic sites. In an aqueous environment, hydrophobic small molecules should bind, nonspecifically, along the polymer backbone. This binding can change the conformation of the macromolecular catalyst, thus affecting the activity of each catalytic site differentially. S_{12}^- might therefore be termed a homotropic allosteric inhibitor of catalysis by poly(1-Me-5-VIm).

The question at this point is why does the poly(1-Me-5-VIm)-catalyzed solvolysis of S_{18}^- in 28.5% ethanol–water exhibit a hyperbolic plot of rate vs. [poly(1-Me-5-VIm)] (see Figure 10 of the preceding paper)?[11] Before proposing an answer, it would be better to relate the results of the poly(1-Me-5-VIm)-catalyzed hydrolysis of S_{12}^- in water in the presence of excess substrate.

Because the poly(1-Me-5-VIm), in excess, catalyzed hydrolysis of S_{12}^- exhibited saturation, it was felt that saturation should also occur in excess substrate. It was realized that studies in excess substrate could not be carried out under conditions totally analogous to those used in excess catalyst, due to solubility limitations with respect to the substrate. Consequently, the kinetics in excess substrate were followed at 5×10^{-6} *M* catalyst and ($1 \rightarrow 10 \times 10^{-5}$ *M*) substrate. Whereas the kinetics in excess polymer, ca. 10^{-5} *M*, had to be followed using the stopped flow spectrophotometer, in excess substrate ($2.5 \rightarrow 10 \times 10^{-5}$ *M*) and 5×10^{-6} *M* poly(1-Me-5-VIm) it was possible to follow the kinetics manually.

That a saturation phenomenon was occurring was evidenced by the zero-order plots of absorbance vs. time for the hydrolysis of S_{12}^- in excess. There was considerable undulation in the plots of absorbance vs. time, and there was concern that reproducibility might be a problem in systems with polymer concentrations as low as 5×10^{-6} *M*. Therefore, the reaction at 5×10^{-6} *M* poly(1-Me-5-VIm) and 5×10^{-5} *M* S_{12}^- was repeated many times. Undulating curves were obtained in all cases (see Figure 7); however, the initial rates showed an average deviation of only 4.3%.

Studies of the hydrolysis of S_{12}^- at initial concentrations of 2.5, 5.0, and 10×10^{-5} *M* in the presence of 5×10^{-6} *M* poly(1-Me-5-VIm) showed that the initial rate became slower as the initial concentration of substrate was increased (apparent substrate inhibition). Figure 8 and Table V present the data.

The plots of absorbance vs. time were again undulating. Over the entire reaction period, the undulating absorbance vs. time curves exhibited a continuous upward drift, concavity, indicating that the reactions were proceeding faster at the end than in the beginning. This correlates with the observation of faster rates at lower initial substrate concentrations. Another anomally which correlated with inhibi-

Table V
Rate of Hydrolysis of S_{12}^- as a Function of Substrate Concentration[a]

$[S_{12}^-]$, 10^5 M	Initial rate V_i, 10^7 M min^{-1}
10.0	4.06
5.0	6.17
2.5	6.97
1.49[b]	9.16[b]
0.89[b]	7.70[b]
0.67[b]	6.24[b]
0.44[b]	5.12[b]
0.23[b]	3.55[b]

[a] Hydrolysis in pH 7.90, 96.7% H_2O, 3.3% CH_3CN buffer, μ = 0.02, 26°, [poly(1-Me-5-VIm)] = 5×10^{-6} M. [b] Values determined from the absorbance vs. time plot of a single kinetic run.

tion at higher substrate concentrations was the driving of the catalysis to greater than 95% conversion before any slowing in the rate of release of phenolate was observed. In order to ascertain what the substrate concentration might be at which a maximum rate was observed, a single absorbance vs. time curve was analyzed in the region of greater than 80% conversion. The rate values were taken to be the slopes of the various tangent lines to the curve in this region. The maximum occurs at a substrate concentration of less than 2×10^{-5} M (see Figure 8).

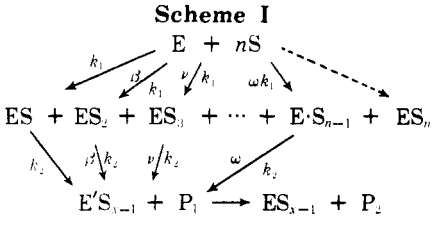
Overberger, Glowaky, and Vandewyer[6] observed maxima in the initial rate vs. substrate concentration curves for the poly[4(5)-VIm]-catalyzed hydrolysis of excess S_{12}^- in 10, 15, and 20 vol % 1-propanol–water. They interpreted these maxima as kinetic verification of the cmc's determined via the pinacyanol chloride dye method.[19] This, in fact, may not be the case; the dye method is known to give abnormally low cmc values.[7]

In this work, the maximum substrate concentration employed is over an order of magnitude less than the cmc value determined conductometrically.

The substrate inhibition can be explained in terms of the multiplicity of catalytic sites on the polymer molecule. Binding of one molecule of substrate and subsequent reaction at one catalytic site can affect either the affinity for a second molecule of substrate (k_1) or the rate of breakdown of E·S, (k_2), or both k_1 and k_2.[20] The mechanism then would not consist in the simple formation and breakdown of a binary E·S complex, but would more closely resemble the mechanism in Scheme I. It is likely that $k_2 \gg \beta k_2 > \nu k_2$, etc. Higher substrate levels displace the reaction pathway toward the E·S_n intermediate, thus, inhibition results. This is a noncompetitive inhibition pattern similar to that described by Hofstee[21] for the oxidation of xanthine by xanthine oxidase.

It would now be appropriate to return to the question of why the shape of the curve for the poly(1-Me-5-VIm)-catalyzed hydrolysis of S_{18}^- in 28.5% ethanol–water is hyperbolic (see Figure 10 of the preceding paper).[11] If one follows the rate vs. substrate concentration plots for an enzyme

Scheme I



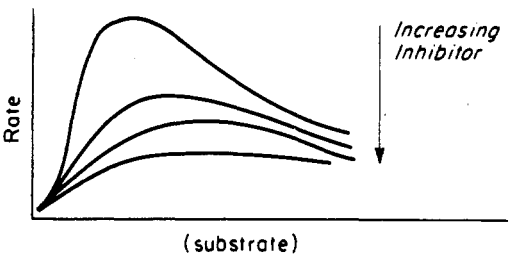
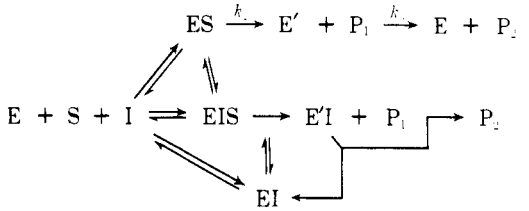


Figure 9. Masking effect of inhibitor.

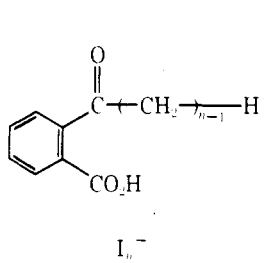
Scheme II



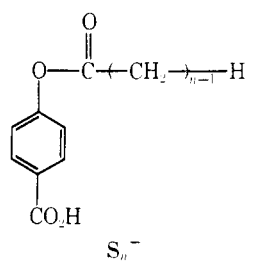
and substrate exhibiting substrate inhibition, in the presence of higher and higher inhibitor concentrations, the inhibition by the substrate itself becomes masked by that of the inhibitor (see Figure 9).[21] One might consider ethanol to be an inhibitor,[22] and thus, in 28.5% ethanol–water the inhibitor concentration would be high enough to mask any substrate inhibition. Similarly, with excess catalyst in 28.5% ethanol–water it is likely that the ethanol masks the differential activity of the various imidazole residues, preventing allosteric behavior.

5. Inhibition Studies. A classical method of demonstrating that a reaction is proceeding through an E·S complex is to carry out the reaction in the presence of a substrate analog which has dimensions similar to those of the substrate but lacks the critical functionality.[23] In the presence of such a species, a catalyst inhibitor complex, E·I, is formed in competition with E·S. Since the initial rate of release of product is equal to k_2[E·S] + k_2'[ESI], the formation of EI competitively suppresses the conversion of substrate to product (Scheme II).

Lane, while working with Overberger, prepared a series of 2-acylbenzoic acids, I_n^-, and he has kindly given permission for these materials to be evaluated as analog inhibitors of the hydrolysis of S_n^- type substrates by polymeric catalysts.



I_n^-



S_n^-

Inhibition studies of the poly(1-Me-5-VIm)-catalyzed hydrolysis of S_n^- were carried out with S_2^-, S_4^-, and S_7^- ($0.5 \rightarrow 5 \times 10^{-4}$ M) in the presence of 5×10^{-4} M I_7^- or I_{11}^-. The concentration of poly(1-Me-5-VIm) employed was 2.5×10^{-5} M. It was possible to inhibit the poly(1-Me-5-VIm)-catalyzed hydrolysis of S_4^- by adding 5×10^{-4} M I_{11}^-, but not I_7^- (see Figure 10). In addition, I_7^- did not even inhibit the poly(1-Me-5-VIm)-catalyzed hydrolysis of S_2^-.

Considering all the data presented in this section on hydrolysis in water, if all catalyses (irregardless of substrate

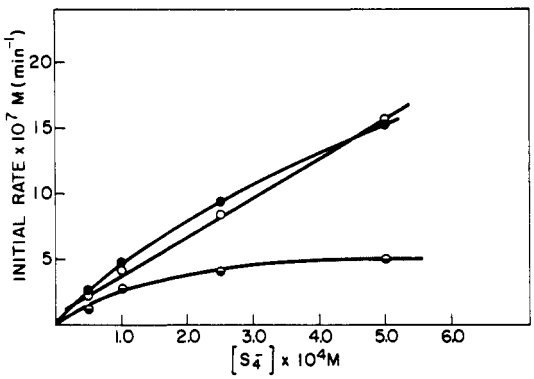


Figure 10. Inhibition of the poly(1-Me-5-VIm)-catalyzed hydrolysis of S_4^-. Hydrolysis in 96.7% H_2O, 3.3% CH_3CN, pH 7.90, μ = 0.02, [catalyst] = 2.5×10^{-5} *M*: [I_n^-] 0 (●); [I_7^-] 5×10^{-4} *M* (○); [I_{11}^-] 5×10^{-4} *M* (◒).

Table VI
Rate of the Poly(1-Me-5-VIm)-Catalyzed Hydrolysis of S_{12}^- as a Function of $[I]_{11}^-$ [a]

$[I_{11}^-]$, 10^4 *M*	Initial rate V_i, 10^7 *M* min^{-1}
1.0	5.82
2.5	7.54
5.0	7.00
9.7	10.66

[a] Hydrolysis in pH 7.90, 96.7% water, 3.3% CH_3CN buffer, μ = 0.02, 26°, $[S_{12}^-]$ = 5×10^{-5} *M*, [catalyst] = 5×10^{-6} *M*.

chain length) are proceeding by a Michaelis–Menten type mechanism

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E \cdot S \xrightarrow{k_2} E' + P_1 \xrightarrow{k_3} E + P_2$$

the following points can be made. (1) In the hydrolysis of S_2^-, S_4^-, and S_7^-, k_1 is less than $k_2 + k_{-1}$. Stated in another way, k_m is large as compared to the substrate concentrations employed and $E \cdot S_{2,4,\text{or}\,7}$ does not accumulate. (2) For the hydrolysis of S_{12}^-, k_1 is greater than $k_2 + k_{-1}$. Alternatively, k_m is small as compared to the substrate concentrations employed and $E \cdot S_{12}^-$ accumulates. (3) I_7^- should behave like S_7^- and I_{11}^- should behave like S_{12}^-; therefore, no appreciable build up of $E \cdot I_7^-$ or $E \cdot I_7^- \cdot S_n^-$ (n = 2, 4, or 7) should occur. On the other hand, $E \cdot I_{11}$ and $E \cdot I^- \cdot S_{12}^-$ should accumulate giving rise to inhibition.

It would, thus, be expected that I_{11}^- would be capable of inhibiting the hydrolysis of S_{12}^-. The poly(1-Me-5-VIm)-catalyzed hydrolysis of S_{12}^- was followed in the presence of (1 → 9.67×10^{-4} *M*) I_{11}^-. The concentrations of catalyst and substrate employed were 5×10^{-6} and 5×10^{-5} *M*, respectively. The data show that no inhibition of the initial rate occurs. In fact, the reaction proceeds faster in the presence of inhibitor. Table VI lists the data.

This trend correlates with the substrate inhibition previously observed. The effect of the inhibitor is to competitively bind to the polymethylene chain, displacing substrate; this effectively decreases the concentration of bound substrate molecules, just as would occur at lower substrate concentrations. Thus, the effect of inhibitor is to enhance the rate.

If one looks closely at the plots of absorbance vs. time shown in Figure 11, one observes that while the curve for the hydrolysis of S_{12}^- in the absence of inhibitor (Figure 7) is similar to that for the hydrolysis of S_{12}^- in 1×10^{-4} *M* I_{11}^-, the curve for the hydrolysis of S_{12}^- in the presence of

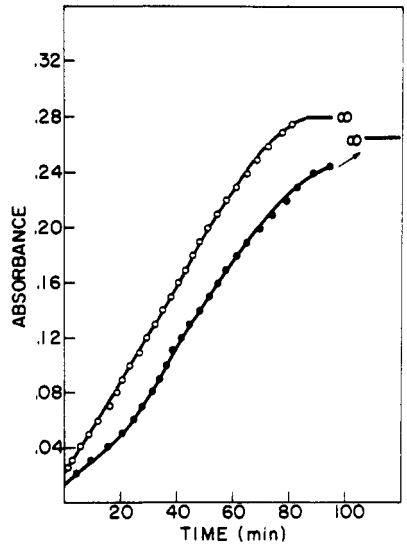


Figure 11. The poly(1-Me-5-VIm)-catalyzed hydrolysis of S_{12}^- in the presence of the inhibitor, I_{11}^-. Hydrolysis in 96.7% H_2O, 3.3% CH_3CN, pH 7.90, μ = 0.02, 26°, [catalyst] = 5×10^{-6} *M*, $[S_{12}^-]$ = 5×10^{-5} *M*: $[I_{11}^-]$ 1×10^{-4} *M*, ●, 2.5×10^{-4} *M*, ○.

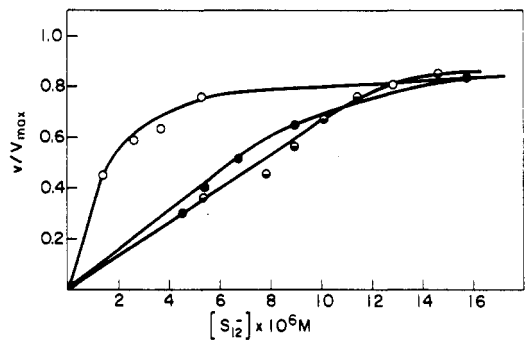


Figure 12. Normalized rate vs. $[S_{12}^-]$ plots: hydrolysis by poly(1-Me-5-VIm) in the presence of I_{11}^-. Hydrolysis in 96.7% H_2O, 3.3% CH_3CN, pH 7.90, μ = 0.02, 26°, [catalyst] = 5×10^{-6} *M*: $[I_{11}^-]$ 2.5×10^{-4} *M* ○, 5×10^{-4} *M* ◒, 9.67×10^{-4} *M* ●.

2.5×10^{-4} *M* I_{11}^- is almost perfectly linear over greater than 80% of the reaction. It should be noticed that the reaction does not exhibit as high a conversion at a constant rate of release of phenolate in the presence of inhibitor as it does in the absence of inhibitor (ca. 95%). Thus, the last 20% of each individual inhibited reaction presents an opportunity to obtain rate vs. substrate concentration plots at a given inhibitor concentration. Further, at 80% conversion, the concentration of S_{12}^- should be low enough that actual inhibition should be observable.

The rate was determined at regular intervals along the last 20% of the curves for the hydrolysis of S_{12}^- in the presence of I_{11}^-. Figure 12 shows the normalized curves, v/V_{max} vs. $[S_{12}^-]$, in the presence of 2.5, 5.0, and 9.67×10^{-4} *M* I_{11}^-. It is apparent from these curves that increasing the concentration of I_{11}^- from 2.5×10^{-4} to 5×10^{-4} *M* in the presence of 1 → 18×10^{-6} *M* S_{12}^- has an inhibitory effect typical of a competitive inhibitor.[23] Increasing the concentration from 5.0 to 9.67×10^{-4} *M* yields no greater inhibition.

The phenomena which have just been described here are not without precedent. Physiologists and enzymologists are well acquainted with substances which play a dual role, sometimes stimulating, sometimes depressing.[24,25] I_{11}^-, thus, might be considered to be an allosteric activator for the poly(1-Me-5-VIm)-catalyzed hydrolysis of S_{12}^- in high

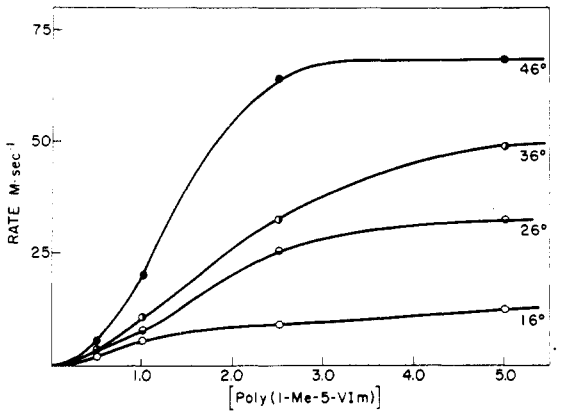


Figure 13. The effect of temperature on the rate of hydrolysis of S_{12}^- by poly(1-Me-5-VIm). Hydrolysis in water, pH 7.90, μ = 0.02, $[S_{12}^-] = 5 \times 10^{-5}$ *M*.

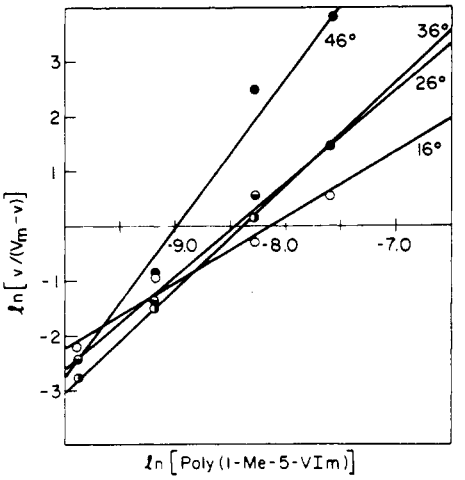


Figure 14. The effect of temperature on the rate of hydrolysis of S_{12}^- by poly(1-Me-5-VIm). Hydrolysis in water, pH 7.90, μ = 0.02, $[S_{12}^-] = 5 \times 10^{-5}$ *M*: temp 16° ○, 26° ◒, 36° ◑, 46° ●.

substrate concentrations and an inhibitor of the hydrolysis of S_{12}^- at low substrate concentrations.

6. The Effect of Temperature on the Rate of the Poly(1-Me-5-VIm)-Catalyzed Hydrolysis of S_{12}^- in Water. The poly(1-Me-5-VIm)-catalyzed hydrolysis of S_{12}^- was carried out at 16, 26, 36, and 46° in the presence of 5×10^{-5} *M* S_{12}. The concentration of catalyst was varied from 0.5 to 5×10^{-4} *M*. In addition, the 1,5-DMIm-catalyzed hydrolysis of S_{12}^- was monitored at 26, 36, and 46° in the presence of 5×10^{-5} *M* S_{12}^- and 5×10^{-5} *M* 1,5-DMIm. Plotting the rate vs. [poly(1-Me-5-VIm)] at the four different temperatures gives a series of sigmoidal curves (see Figure 13). Saturation was most pronounced at 46°; the maximum rate was also greatest at 46°. This observation is additional support for the intermediacy of an apolar polymer–substrate complex.[26] The formation of the hydrophobic bond is accompanied by a marked increase in entropy and may be accompanied by a positive enthalpy change. This positive enthalpy of interaction causes a characteristic increase in the stability of many hydrophobic bonds up to about 60°.

V_{max} was estimated by inspection of the curves in Figure 13. Assuming the allosteric mechanism, n was evaluated according to eq 5 from the slope of plots of ln $[v/(V_{max} - v)]$ vs. ln [E] (Figure 14). Subsequently, K_m was calculated using eq 24. Table VII lists the values of the kinetic parameters at 16, 26, 36, and 46°.

K_m decreases dramatically as the temperature increases; correspondingly the average number of imidazole residues per active site increases as the temperature increases.

Since $V_{max} = k_2[E \cdot S]$, the slope of a plot of ln V_{max} vs. $1/T$(°K) should give the energy of activation, E_a, for the liberation of phenolate anion from the substrate.

$$ES \xrightarrow{k_2} E' + P_1$$

Figures 15 and 16 show the Arrhenius plots and Table VIII lists the thermodynamic parameters, as compared to those for the 1,5-DMIm-catalyzed hydrolysis of S_{12}^-. It is quite apparent from these data that the enhanced activity of the

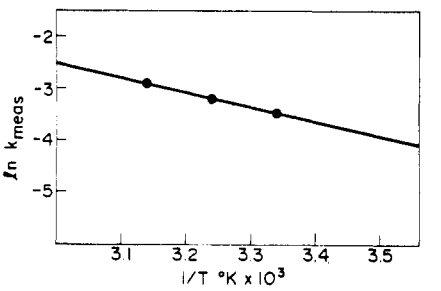


Figure 15. The 1,5-DMIm-catalyzed hydrolysis S_{12}^- arrhenius plot. Hydrolysis in 96.7% H_2O, 3.3% CH_3CN, pH 7.9, μ = 0.02, $[S_{12}^-] = 5 \times 10^{-5}$ *M*, [1,5-DMIm] = 5×10^{-4} *M*.

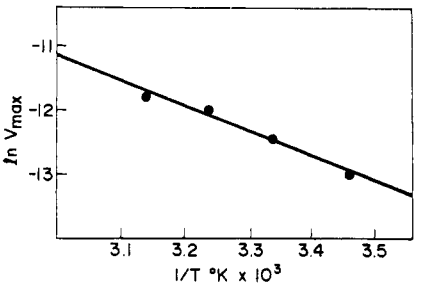


Figure 16. The poly(1-Me-5-VIm)-catalyzed hydrolysis of S_{12}^- arrhenius plot. Hydrolysis in water, pH 7.9, μ = 0.02, $[S_{12}^-] = 5 \times 10^{-5}$ *M*, [poly(1-Me-5-VIm)] = (0.5 → 5×10^{-4} *M*).

Table VII
The Poly(1-Me-5-VIm)-Catalyzed Hydrolysis of S_{12}^- as a Function of Temperature[a]

Temp, °C	V_{max},[b] 10^7 *M* sec^{-1}	n	K_m, *M*[c]
16	20	1.18	1.3×10^{-4}
26	40	1.71	2.2×10^{-6}
36	60	1.88	1.4×10^{-7}
46	75	2.75	1.0×10^{-10}

[a] Hydrolysis in water, pH 7.90, μ = 0.02. [b] Estimated from curves in Figure 13. [c] Calculated from eq 5.

Table VIII[a]
Activation Parameters for the Catalyzed Solvolysis of S_{12}^- (pH 7.90, 26°)

Catalyst	$\Delta E^\ddagger$	$\Delta H^\ddagger$	$\Delta F^\ddagger$	$T\Delta S^\ddagger$	$\Delta S^\ddagger$
Poly(1-Me-5-VIm)	7.67	7.08	8.13	−1.5	−5.02
1,5-DMIm[b]	5.50	4.91	11.55	−6.6	−22.2

[a] All values are in kcal/mol except ΔS (eu). Hydrolysis in water, μ = 0.02, pH 7.90, $[S_{12}^-] = 5 \times 10^{-5}$ *M*. [b] Hydrolysis in 96.7% H_2O, 3.3% CH_3CN, μ = 0.02, pH 7.90, $[S_{12}^-] = 5 \times 10^{-5}$ *M*, [catalyst] = 5×10^{-4} *M*.

polymeric catalyst is due totally to a favorable entropy term.

The increase in entropy realized through the binding of substrate might be reflected as a reduction in the number of translational or rotational degrees of freedom, or through a change in the conformation of the polymer. Whatever the situation might be, this binding evidently assists in the catalysis by producing a state which is closer to the transition state.

The activation parameters for the catalyses were calculated using the following equations

$$\ln k = E_a/RT + \ln S \quad (6)$$

$$\Delta S^{\ddagger} = R \ln \frac{N_0 h[k/([1 - \Delta H^{\ddagger}]/RT)]}{RT} \quad (7)$$

$$\Delta F^{\ddagger} = \Delta H^{\ddagger} = T\Delta S^{\ddagger} \quad (8)$$

the activation energy from the slope of the Arrhenius plots. No corrections were made for the change of pK_a of solvent or catalyst with temperature.

Experimental Section

A. Synthesis. Preparation of Inhibitors, 2-Heptanoylbenzoic Acid (I_7^-) and 2-Undecanoylbenzoic Acid (I_{11}^-). The substrate analogs utilized as inhibitors I_7^- and I_{11}^- were kindly provided by Mr. C. Lane of this laboratory.

The materials were prepared by the reaction of the appropriate organo-cadmium reagent with phthalic anhydride. Thus, the reaction of phthalic anhydride with *n*-hexylcadmium yielded 53% of 2-heptanoylbenzoic acid, I_7^-, as a hydroscopic white crystalline solid. Anal. Calcd for $C_{14}H_{18}O_3$: C, 71.77; H, 7.74. Found: C, 71.65; H, 7.69.

Analogously, the reaction of *n*-decylcadmium with phthalic anhydride gave a 53% yield of 2-undecanoylbenzoic acid, I_{11}^-, mp 68–72°. Anal. Calcd for $C_{18}H_{26}O_3$: C, 74.45; H, 9.03. Found: C, 74.54; H, 8.96.

B. Potentiometric Titrations. About 75×10^{-3} mmol of sample and 0.3 ml of 1 *N* HCl were diluted in a thermostated cell to 15 ml with either 28.5% ethanol–water or water. The respective solutions were subsequently titrated with 1 *N* NaOH or 0.25 *N* NaOH. The concentration of acid was such that at the end point of the titration, the ionic strength (μ) = 0.02. The NaOH was added incrementally from a micropipet (Manostat Digi-Pet). The change of pH was monitored at every addition of NaOH by a Radiometer Type TTTl-titrator. Blank titration curves were obtained in water at 16, 26, 36, and 46° and 28.5% ethanol–water at 26° by titrating 15 ml of 10^{-2} *N* HCl. The differences between the amounts of 1 *N* NaOH added to the blank solution at the same pH (Δ ml) were plotted as a function of pH to give the differential titration curves.[27] The pK_a of the respective samples was determined at half-neutralization via a plot of pH vs. log $\alpha_1/(1 - \alpha_1)$ from the modified Henderson–Hasselbach equation

$$pH = pK_a + n \log \alpha_1/(1 - \alpha_1)$$

C. Determination of Cmc Values. All resistance measurements were made at 25° with a Wheatstone bridge and Wagner ground oscillator (1000 Hz) and an oscilliscope detector. For determining the resistance of S_7^- and I_{11}^- in pH 7.90 buffer, μ = 0.02, a fill-type conductivity cell, k = 4.537, was employed. For determining the resistance of the sodium salt of S_{12}^- in conductivity water, a pipet conductivity cell, k = 0.263 (Beckman Instruments, C 0.10), was used.

Resistance is related to the specific conductance by the following relationship[9]

$$\bar{L} = (1/R)(1/A) = k/R$$

Plots of $\bar{L}$ vs. concentration yielded nonlinear curves. The cmc value was taken as the intersection of lines tangent to the curve at high substrate concentrations and at low substrate concentrations.[8]

D. Viscosity Measurements. All viscosity measurements were carried out at 26° using Cannon-Ubbelohde Semi-Micro dilution viscometers.

E. Kinetic Measurements. 1. Slow Reactions in Excess Catalyst. (a) Preparation of the Solution for Kinetics. To 2.9 ml of a buffered catalyst solution in a 1 cm quartz cell, at 26°, was added 0.10 ml of an acetonitrile solution of substrate. The final concentration of species in the cell was the following: [catalyst] = 5×10^{-4} *M* and variable, [substrate] = 5×10^{-5} *M*, μ = 0.02, [buffer] = 0.02 M, and 26.7% ethanol, 3.3% acetonitrile, 70% water by volume or 96.7% water and 3.3% acetonitrile by volume. The buffer employed above pH 6 was tris(hydroxymethyl)aminomethane–HCl. For pH 6 and below the systems were buffered with sodium acetate–acetic acid. In determining the pH-rate profiles the substrate was added as an ethanol–water solution; thus, the final solvent composition was simply 28.5% ethanol–water by volume.

(b) Treatment of Data. All data obtained under conditions of [catalyst] ≫ [substrate] were, unless otherwise noted, treated as first-order kinetics by plotting ln $(A_\infty - A_t)$ vs. time. The slope of this line was taken as the pseudo-first-order rate constant (k_{meas}). Whenever the blank rate was significant, k_{obsd} was calculated, $k_{obsd} = k_{meas} - k_{blank}$. The second-order rate constant k_{cat} was calculated either from k_{meas} or from k_{obsd}: $k_{cat} = k_{obsd}/[catalyst]$.

2. Fast Reactions in Excess Catalyst. All reactions which had a half-life of less than 2 min were followed on a Durrum-Gibbs stopped flow spectrophotometer. The data were obtained on an oscilloscope trace of % T vs. time; this trace was photographed to supply a permanent record. The % T was read off the photographs at certain time intervals, then converted to absorbance.

With respect to the poly(1-Me-5-VIm)-catalyzed hydrolysis of S_{12}^- in water, the data were treated as pseudo-first-order kinetics by plotting ln $(A_\infty - A_t)$ vs. time. With respect to the 4(5)-VIm-containing copolymer-catalyzed solvolysis of S_{12}^- in 28.5% ethanol–water, initial rates were obtained from plots of absorbance vs. time.

3. Reactions in Excess Substrate and Anomalous Reactions. (a) Preparation of Solutions for Kinetics. Essentially the same procedure was followed as described in section Ela except the catalyst concentration was held constant and the substrate concentration was variable.

(b) Treatment of Data. In all cases in excess substrate, initial rates were obtained from plots of absorbance vs. time. This was done even when the reaction in excess substrate obeyed pseudo-first-order kinetics, as determined by the linearity of plots of ln $(A_\infty - A_t)$ vs. time.

Reactions in excess catalyst which were anomalous, either because of accelerative kinetic behavior or because of other effects, were also analyzed by monitoring the initial rate.

For purposes of comparison the initial rate could be related to k_{obsd} by the following relationship: $k_{obsd} = V_i/[substrate]$.

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Photosensitized Isomerization of Stilbene by Phenyl Vinyl Ketone–2-Vinylnaphthalene Copolymer

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Abstract: Phenyl vinyl ketone–2-vinylnaphthalene copolymers were found to be effective polymer photosensitizers for the isomerization reaction of stilbene, especially at low concentration, compared with poly(phenyl vinyl ketone) or monomeric ketones, such as acetophenone. Phosphorescence spectra of the copolymer and its lifetime measurements revealed that the high efficiency is due to the stabilization of the triplet states of the polymer by energy transfer from the benzoyl group to naphthyl ones. The maximum rate of the photosensitized isomerization is attained by the copolymer containing 16 mol % of the 2-vinylnaphthalene unit, in which the triplet energy is completely transferred to the naphthyl group and its lifetime is rather long. The further increase of the content of the naphthalene unit, however, depressed the rate because of the destabilization of the naphthalene triplet state by dimer or excimer formation. The difference in the mechanism of photosensitization between the copolymer and poly(phenyl vinyl ketone) is also discussed.

Recently much attention has been paid to the photophysical processes in polymer systems and extensive studies concerning excimer formation and energy transfer processes have been reported.[2] One of the most interesting results obtained in these studies is that the photoexcited energy migrates along the polymer chain. The energy migrating along the chain is expected not only to bring about the chemical reaction of the polymer itself, such as bond scission,[3] but also to excite other molecules by intermolecular energy transfer. In this study we elucidate the latter process in detail to obtain an effective polymer photosensitizer.

Although several attempts have been carried out to use polymer sensitizers for isomerization reactions,[4–6] no remarkable advantage of polymers has been obtained yet. The efficiency is similar to that of the corresponding monomer and ultimate conversions are the same.

Necessary properties of an effective polymer photosensitizer are as follows: it should have (1) low singlet excited state energy and rather high triplet energy, (2) high intersystem crossing yield, (3) long lifetime in the triplet state, and (4) a favorable structure in which triplet energy migrates effectively. We used phenyl vinyl ketone–2-vinylnaphthalene copolymer, which is expected to satisfy the above properties, for the sensitization of photoisomerization of *trans*-stilbene.

Experimental Section

Phenyl vinyl ketone monomer was synthesized according to the method of Mannich and Heilner[7] and purified in vacuo by fractional distillation before use. 2-Vinylnaphthalene was recrystalized from cyclohexane twice. Copolymerizations of these two monomers were carried out in benzene at 60° using azobisisobutylonitrile as an initiator.

Zone-refined *trans*-stilbene was sublimed in vacuo before use. Benzene was fractionally distilled twice. All samples were prepared in vacuo at less than 10^{-5} mm.

Photoillumination was carried out with a mercury line of 366 nm by the use of a super high pressure mercury lamp and a monochrometer. Optical absorption and emission spectra were measured with a conventional recording spectrophotometer (Hitachi EPS-3T) and a fluorescence spectrophotometer (Hitachi MPF-2A) with a phosphorescence measurement attachment, respectively. The lifetimes of the triplet state were also determined by the use of the above phosphorescence measurement attachment and a synchroscope.

Results and Discussion

I. Poly(phenyl vinyl ketone). Aromatic ketones, such as acetophenone or benzenophenone, are effective photosensitizers of the photoisomerization of stilbene.[8] Not only monomeric ketones but also polymeric ones are expected to photosensitize the isomerization in high yield. Figure 1 shows the photoisomerization of *trans*-stilbene to the cis form in the presence of poly(phenyl vinyl ketone) (molecular weight = 40,000) as well as a corresponding monomeric ketone, acetophenone, in benzene solution at 10°. The concentrations of these two sensitizers are adjusted so as to have the same absorption at 366 nm. The isomerization was followed by optical absorption at 325 nm due to *trans*-stilbene. Poly(phenyl vinyl ketone) is an effective photosensitizer. However, its efficiency is similar to that of acetophenone. Even when the concentration of *trans*-stilbene is changed from 10^{-4} to 10^{-6} M, significant difference between the polymer and acetophenone is not observed. The result indicates that the advantage of polymer is not enough to bring about higher efficiency to this polymer than acetophenone, though the delocalization of the triplet excited energy may facilitate the energy transfer from the polymer to stilbene.[9]

II. Phenyl Vinyl Ketone–2-Vinylnaphthalene Copolymer. Copolymerization is a convenient way to introduce desirable photophysical and chemical properties in polymer photosensitizers. We used here 2-vinylnaphthalene as a comonomer because of the characteristic nature of the naphthalene unit. The triplet state energy of naphthalene lies between those of acetophenone and stilbene and its singlet state energy is higher than both of them. In addition the lifetime of its triplet state is much longer than