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Kinetics and Mechanism of Hydrolysis of Ruheman's Purple in the Absence and Presence of Micelles in Aqueous Medium

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The mechanism of acid hydrolysis of Ruheman's purple has been investigated and the effect of micelles on the rate has been examined. First order rate constant for the hydrolysis reaction decreases linearly with decreasing hydrogen ion concentration up to pH 3 and levels off thereafter with no reaction occurring above pH 7. Increasing ionic strength decreases the rate in accordance with the Bronsted-Christiansen equation. The salt effect in the dipole-dipole reaction is probably a reflection of the fast equilibrium steps involving ions preceding the rate-determining step. Solvents like ethylene glycol and dioxane have no effect, indicating that the rate-determining step of the reaction does not involve ionic species. A mechanism of the reaction has been proposed which is consistent with our observed results. In the presence of micelles the reaction rate was strongly inhibited by the cationic micelles of hexadecyltrimethylammonium bromide, whereas anionic micelles of sodium dodecyl sulfate showed a slight enhancement. The micellar data have been analyzed on the basis of available models.

The reaction of amines with ninhydrin yields a color product, Ruheman's purple sometimes erroneously referred to as "azine-bis-indandione. This reaction has immense biological and analytical significance as it serves as a model for several biochemical reactions that occur in the metabolism of deamination and transpeptidation[1,2] as well as it is involved in the most common method for the analysis of amino groups.[3,4] The course and mechanism of ninhydrin reaction has been extensively studied by Friedman and Williams[5] who investigated the effect of various parameters on the formation of Ruheman's purple. However, the mechanism of reverse reaction, namely the decolorization reaction, of Ruheman s purple in the presence of acids has not been investigated in detail. We have undertaken a systematic analysis of the kinetics and mechanism of decolorization reaction of Ruheman's purple in the hope that the data on the reverse reaction might provide insight into the ninhydrin reaction.

The reaction responsible for the hydrolytic instability was also studied in the presence of cationic micelles of hexadecyltrimethylammonium bromide (CTAB), sodium dodecyl sulfate (SDS), and poly(oxyethylene) (23) 1-dodecanol (Brij-35, Lauromacrogol). Micelles are known to affect the rates of reactions due to several factors by differential distribution of the substrates inside and outside the micelles and by perturbing the thermodynamic parameters of the reaction.[6] The interpretation of the mechanism of catalysis or inhibition of reaction rates by micelles has received considerable attention in view of the analogies drawn between the micellar and enzyme-catalysis. The rate of the present reaction is considerably inhibited in the presence of cationic micelles of CTAB. The data obtained have been analyzed quantitatively on the basis of available models.

Experimental

Ruheman's purple (RP) was prepared by the method of Ruheman.[11] The product was further purified by employing a method similar to that of Davidson.[12] The final product was obtained as ammonium salt. Sodium dodecyl sulfate obtained from Fisher Scientific Co. was recrystallized twice from 95% ethanol.[13] CTAB (extra pure, Sisco Research Laboratories) was crystallized twice with hot methanol–ether[13] before use. HCl and KCl of analytical grade(B.D.H.) were used without further purification. Organic solvents (analytical grade, B.D.H.) were further purified by the usual methods[14] before use.

The hydrolysis was studied using a Beckman DU spectrophotometer at the absorption maximum of RP (570 nm).

Results and Discussion

Reaction in the Absence of Micelles. First order rate constant for the reaction of RP in acidic medium was found to increase linearly with the increase in the hydrogen ion concentration (Fig. 1). Graphical analysis of the results gave the value of second order rate constant $k'=0.94\ \mathrm{mol^{-1}\,dm^3\,s^{-1}}$, comparable to that obtained earlier by Sattar and Chaturvedi[15] ($k'=0.86\ \mathrm{mol^{-1}\,dm^3\,s^{-1}}$). However, when the reaction was studied over the pH range 2—11, the initial

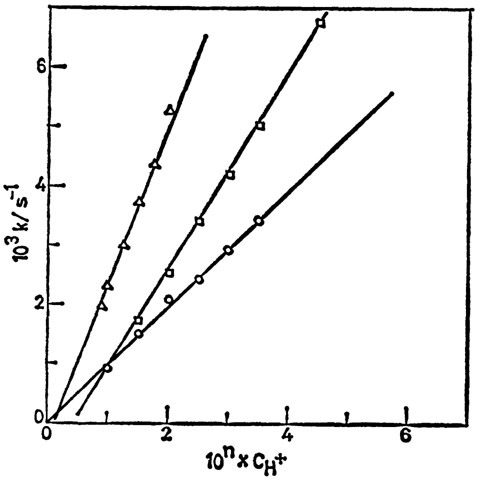


Fig. 1. Variation of first order rate constant with H^+ ion concentration in $\mathrm{mol\,dm^{-3}}$ for the hydrolysis of RP at 25 °C in acid medium in the presence and absence of surfactants. (○) Aqueous medium; (△) in presence of $2\times10^{-3}\ \mathrm{mol\,dm^{-3}}$ CTAB; (□) in presence of 0.01 mol dm^{-3} SDS. Value of n is 3 in presence of CTAB and 1 in the other two cases.

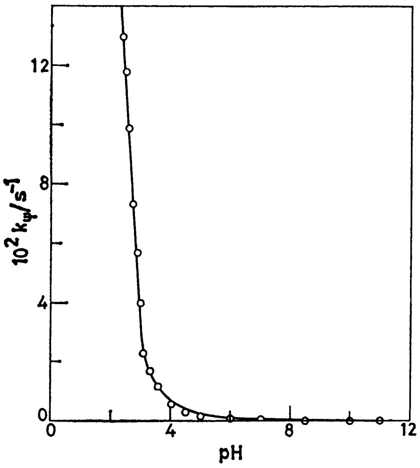


Fig. 2. Variation of first order rate constant with pH for the hydrolysis of RP at 25 °C at constant ionic strength (0.20).

Table 1. Effect of ionic strength on the reaction rate[a)]

μ	$10^3\ k/s^{-1}$	μ	$10^4\ k/s^{-1}$
0.003	3.258	0.523	1.503
0.023	2.802	0.603	1.517
0.043	2.123	0.763	1.401
0.083	2.111	0.843	1.381
0.263	1.746	0.923	1.259
0.363	1.631		
0.443	1.554		

a) Concentration of H^+ ion $= 3\times10^{-3}$ mol dm^{-3}.

linear dependence of rate on pH faded at pH$\approx$3. No hydrolysis of RP was observed at pH>7 (Fig. 2). This pattern of change in the rate with increase in pH differs from that observed by Cordes and Jencks[16)] for the hydrolytic scission of $\rangle$C=N- bond in schiff bases. They observed a rate maximum with the increase in pH followed by a pH region where the rate was unaffected by the increase in pH. In the reaction of RP no change in the reaction rate was observed with the change in RP concentration at fixed H^+ ion concentration. This shows that the reaction rate is independent of the initial RP concentration.

Dependence of the RP reaction on neutral salts added was investigated. Reaction rate was found to decrease with the increase in the ionic strength of the medium (Table 1) at constant H^+ ion concentration. Use of the following equation which is an approximation of Bronsted-Christiansen equation[17,18)] gives the impression as if the reaction is taking place between two oppositely charged ions. However, despite

$$\log k' = \log k'_0 + \frac{1.02 Z_A Z_B \sqrt{\mu}}{1+\sqrt{\mu}} \qquad \text{(i)}$$

the applicability of above equation, observed salt effect seems to be secondary salt effect, a purely thermodynamic effect, arising from the change in activity of the dissociable reactant species. It seems that the salt effect is a reflection of the equilibrium steps involving ions occurring prior to the rate determining step in the overall mechanism (*vide infra*).[19)] The magnitude and direction of secondary salt effect on reaction rate depends on concentration terms that appear in the overall rate expression.

Table 2. Effect of solvents on the hydrolysis of RP in acid medium at 25 °C[a)]

Composition of solvent (v/v)	$k'/\text{mol}^{-1}\ \text{dm}^3\ \text{s}^{-1}$		
	Acetone	Dioxane	Ethylene glycol
5%	0.85	0.84	0.91
10%	0.78	0.84	0.91
15%	0.72	0.84	0.92
20%	0.67	0.82	0.92
25%	0.65	0.84	0.92
30%	0.69	0.88	0.92
40%	—	—	0.90

a) Concentration of H^+ ion $= 4\times10^{-3}$ mol dm^{-3}.

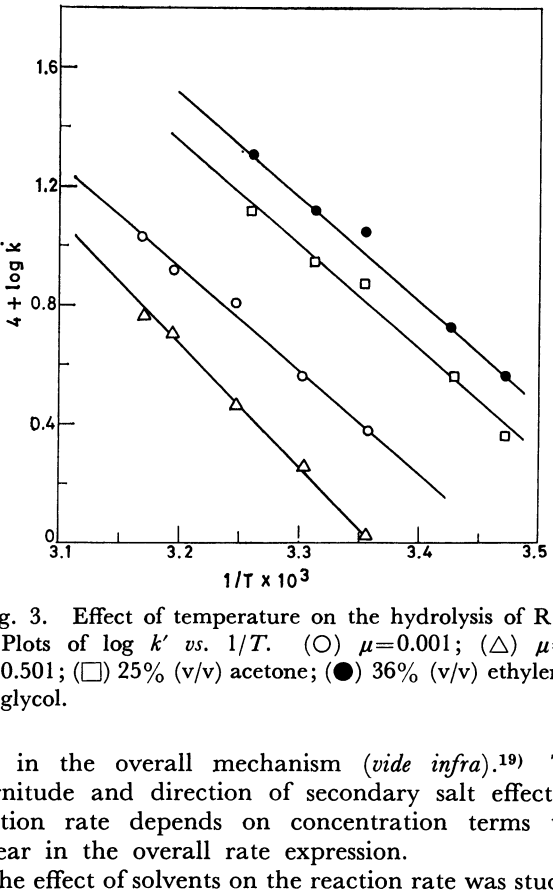


Fig. 3. Effect of temperature on the hydrolysis of RP. Plots of log k' *vs.* $1/T$. (○) μ=0.001; (△) μ=0.501; (□) 25% (v/v) acetone; (●) 36% (v/v) ethylene glycol.

The effect of solvents on the reaction rate was studied in the presence of 5—30% (v/v) of acetone, dioxane, and ethylene glycol in aqueous medium (Table 2). Reaction rate was found to be independent of increasing concentration of ethylene glycol and dioxane but a slight decrease was observed in the presence of acetone. The solvent effects give a positive indication that the rate determining step of the reaction involves no ionic species.

Effect of temperature on the reaction rate was studied in the range 288—320 K at ionic strength of 0.001 and 0.501. In order to examine the influence of temperature on the specific solvent effect, studies were carried out in 25% acetone and 36% (v/v) ethylene glycol, which have the same dielectric constant. The reaction rate was found to follow the Arrhenius equation (Eq. 2) under all the four experimental conditions (Fig. 3). Graphical analysis of

Table 3. Thermodynamic quantities of activation for the hydrolysis of RP at 25 °C

Thermodynamic quantity	Aqueous medium $\mu=0.001$	Aqueous medium $\mu=0.501$	Acetone 25% (v/v)	Ethylene glycol 36% (v/v)
E^*/kJ mol^{-1}	66.9	79.5	54.4	54.4
ΔG^*/kJ mol^{-1}	92.0	92.0	92.0	87.8
Z/mol^{-1} dm^3 s^{-1}	2.8×10^8	1.9×10^{10}	3.1×10^6	5.0×10^6
ΔS^*/J K^{-1} mol^{-1}	−87.8	−54.4	−125.5	−125.5
ΔH^*/kJ mol^{-1}	66.9	75.3	37.6	37.6

log k *versus* $1/T$ plot gave the value of activation energy E^* which was then used to calculate other activation parameters, ΔG^*, ΔS^*, ΔH^*, and Z, using Eqs. 3—5. The values of the parameters at 25 °C are

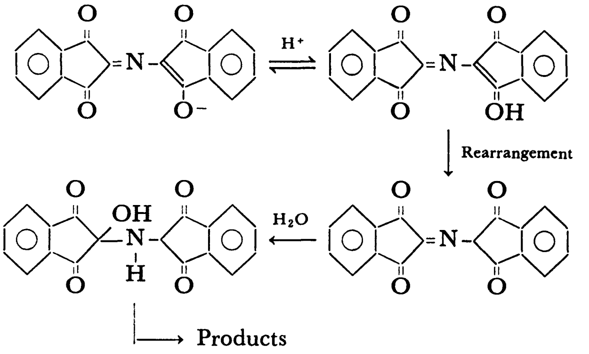
$$\log k' = \log Z - E^*/2.303RT \quad (2)$$

$$\Delta G^* = 2.303RT\left(\log \frac{RT}{Nh} - \log k'\right) \quad (3)$$

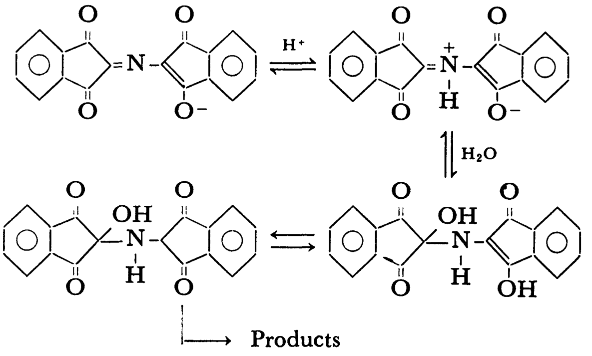
$$\Delta S^* = 2.303R\left(\log Z - \log\left(e\frac{RT}{Nh}\right)\right) \quad (4)$$

$$\Delta H^* = \Delta G^* + T\Delta S^* \quad (5)$$

given in Table 3. High negative entropy values −90.8 J K^{-1} mol^{-1} and −55.2 J K^{-1} mol^{-1} at $\mu=0.001$ and 0.501, respectively, suggest bimolecular nature of the rate determining step.[20] Moreover, activation energies obtained for isodielectric mixture of 25% acetone (55.6 kJ mol^{-1}) and 36% ethylene glycol (54.8 kJ mol^{-1}) are similar, reflecting the fact that the decrease in rate observed in the presence of acetone is due to nonelectrostatic terms generally associated with the effect of solvent on the reaction rate.[21]



Scheme 1.



Scheme 2.

A tentative reaction mechanism for the hydrolysis of RP, proposed by Friedman and Williams,[5] is given in Scheme 1. No justification other than the product analysis has been given by these authors. Moreover, the proposed mechanism can not explain the observed deviation from the linear relationship observed at pH⩾4. Another objection to proposed mechanism is that RP is known to be strongly acidic (pK_a of RP is almost equal to that of H_2SO_4) pK_a⩽0,[5] hence, even in fairly strong acidic solution (pH≈2) RP will remain in ionic form and no protonation can occur at the negatively charged oxygen. We propose a reaction mechanism (Scheme 2) which is consistent with our observed results. At relatively low pH, when concentration of H$^+$ ion is much higher than that of RP, attack of water on the zwitterionic species to give α-hydroxy amine intermediate [x] seems to be the rate determining step. This is supported by the high negative value of entropy (−87.8 J K^{-1} mol^{-1}) and also by the absence of any significant solvent effect. The observed salt effect, though unusual for a dipole-dipole reaction, is not unique for a reaction between two dipoles, where a fast equilibrium step preceeds the rate-limiting step. For example, Sinha and Katiyar[22] and others[23] has also observed a pronounced salt effect on the color-fading reaction of rosaniline. Though this reaction is ion-dipole reaction, it could be cited as an example because theoretically an ion-dipole reaction like a dipole-dipole reaction, should show no pronounced salt effect. Such salt effects might be due to reflection of fast equilibrium steps involving ions, in the overall reaction mechanism as pointed out by Hine.[19] The observed deviation from the linear behavior at pH⩾4 can also be explained by the proposed mechanism. At higher pH, concentration of H$^+$ ion and RP become similar, and the rate of formation of zwitterion progressively decreases with the increase in pH. No reaction takes place at pH⩾7 because of absence of first protonation step.

Reaction in the Presence of Micelles. Change in absorption maximum from 570 nm to 579 nm was observed in the presence of 0.01 M CTAB. However, no such shift was detected in the presence of 0.02 M SDS or 0.01 M Brij-35. This indicates binding between RP and CTAB micelles. The reaction was studied at different surfactant concentrations keeping the hydrogen ion concentration constant in each case. An inhibition of reaction rate was observed in the presence of cationic surfactant (CTAB) whereas the reaction rate was slightly enhanced in the presence of anionic micelles of SDS. However, no definite trend of reaction rate emerged in the presence of nonionic surfactant of Brij-35 (Fig. 4). At fixed SDS or CTAB

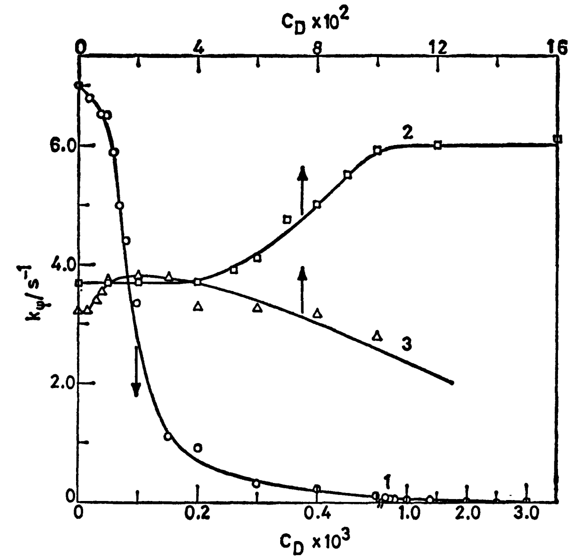
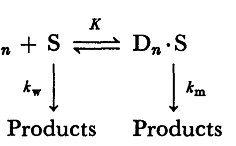


Fig. 4. Effect of surfactant concentration (mol dm^{-3}) on the reaction rate at 25 °C. (○) CTAB; (□) SDS; and (△) Brij-35.

concentration, a linear increase in reaction rate was observed with increase in hydrogen ion concentration (Fig. 1).

Surfactant micelles provide an unusual medium which may affect the reaction rate in more than one way:[7-10,23] (a) a decrease in entropy of the reactants arising due to their binding on the micellar surface, *i.e.*, by virtue of relatively higher concentration of reactants in micellar phase in comparison to bulk phase, (b) a high degree of closeness attained by reactants in the micellar phase, and (c) relative stabilization or destabilization of substrate arising due to electrostatic and hydrophobic interaction between substrates and micelles. Moreover, in the hydrolysis of RP, the micelles may affect the first equilibrium step where proton gets associated with anion to give zwitterion or the subsequent rate determining attack of water molecule to form an α-hydroxy amine. It appears that in the presence of CTAB, because of favorable hydrophobic as well as electrostatic interactions the anionic imine gets bound with the surface of cationic micelle, shifting the protonation equilibrium towards the lefthand side, retarding the reaction rate. However, in the presence of SDS, hydrophobic forces oppose the electrostatic repulsion. However it seems that a fraction of anionic substrate does bind with the anionic micelles, where it is more prone to proton attack, and hence a slight acceleration in the reaction rate is observed. Thus the effect of micelles on the reaction is consistent with the mechanism we proposed.

Quantitative Treatment of the Micellar Data. Simple kinetic model proposed by Menger and Portnoy[24] (Scheme III) was applied to explain quantitatively



Scheme 3.

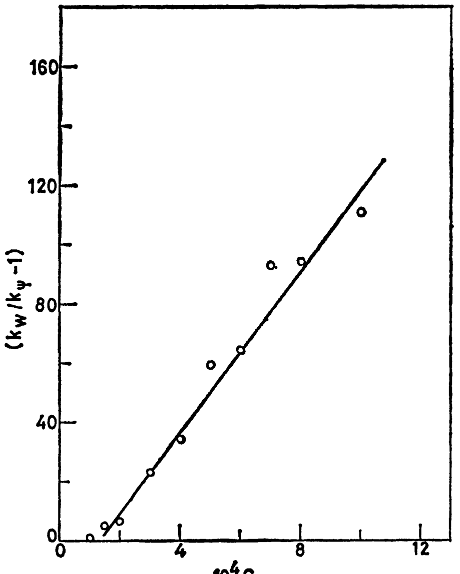


Fig. 5. Variation of $(k_w/k_\psi - 1)$ with the surfactant concentration (mol dm^{-3}) for the hydrolysis of RP at 25 °C in the presence of CTAB.

the effect of cationic and anionic micelles on the reaction rate. This kinetic scheme provides the following rate equation:

$$\frac{k_\psi - k_w}{k_m - k_\psi} = \frac{K}{N}(C_D - \text{cmc}), \qquad (7)$$

where k_ψ is the observed rate constant and k_w and k_m are the rate constants in aqueous and micellar pseudophases, respectively. K is the equilibrium constant for the association of substrate S with micelle D_n, N is the aggregation number and C_D is the stoichiometric surfactant concentration. This equation predicts a linear relationship between $\frac{k_\psi - k_w}{k_m - k_\psi}$ and C_D. However, for micellar inhibited reaction we can further assume that $k_m = 0$.[8] Hence, we obtain

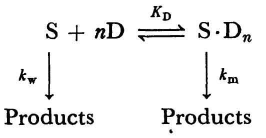
$$\frac{k_w}{k_\psi} - 1 = \frac{K}{N}(C_D - \text{cmc}). \qquad (8)$$

The results obtained in the presence of CTAB were analyzed using Eqs. 7 and 8. Slope of the straight line thus obtained (Fig. 5) gives the value of K/N, which was further utilized to calculate the fraction (α) of RP present in the micellar phase by means of

$$K/N = \frac{\alpha}{1-\alpha} \cdot \frac{1}{C_D - \text{cmc}}. \qquad (9)$$

The values of K/N, cmc, and α for the acidic hydrolysis of RP in the presence of CTAB correspond to 1.36×10^4 mol^{-1} dm^3, 1.32×10^{-5} mol dm^{-3} and 99.27%, respectively. Since the effect on the reaction rate is very small, no quantitative analysis was carried out for the catalysis observed in the presence of SDS.

Recently, Piszkiewicz[25] has proposed a different kinetic scheme based on the observed sigmoidal dependence of rate constant on surfactant concentration. According to his model (Scheme 4) a substrate molecule combines with n detergent molecules to form a catalytic aggregate.



Scheme 4.

Mathematical formulation based on this scheme gives

$$\log\left(\frac{k_\psi - k_w}{k_m - k_\psi}\right) = n \log [D] - \log K_D, \qquad (10)$$

which predicts a linear relationship between $\log (k_\psi - k_w)/(k_m - k_\psi)$ and log [D] with a slope equal to n. In this equation [D] is the total detergent concentration, K_D the dissociation constant of surfactant-substrate complex and n the index of cooperativity. This equation gives a set of parameters which have a different significance from that obtained from Menger and Portnoy's scheme,[24] *e.g.* at $\log (k_\psi - k_w)/(k_m - k_\psi) = 0$, $n \log [D] = \log K_D$. Also at $\log (k_\psi - k_w)/(k_m - k_\psi) = 0$, catalysis by the surfactant shows one-half of its maximum effect, the value of log [D] at this concentration being designated by $\log [D]_{50}$. Another parameter which can be used to compare the effect of micelles is the index of cooperativity 'n'. Double log plot for the hydrolysis of RP in presence of CTAB is shown in Fig. 6. For the CTAB inhibited reaction, when k_m is assumed to be zero, the values of $\log [D]_{50}$ and n obtained from the plot correspond to −4.0 and 2.66 respectively.

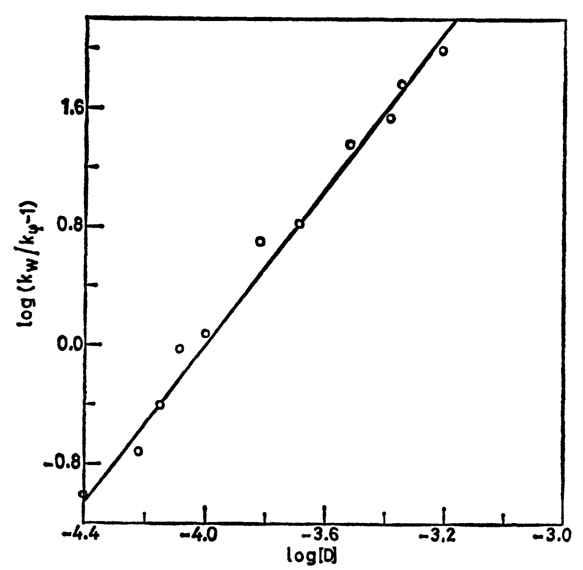


Fig. 6. Plot of $\log (k_w/k_\psi - 1)$ *vs.* log [D] for the hydrolysis of RP in the presence of CTAB.

Successful application of both the kinetic schemes to the hydrolysis of RP in the acid medium is not surprising. Though the two schemes are based on different assumptions, the final mathematical equations based on them are similar (Eqs. 7 and 10). However, the kinetic parameters associated with these equations have quite different significance. Piszkiewicz's model draws its strength from a model used for enzyme catalysed reactions showing positive homotropic interaction (also called positive cooperativity). Positive cooperativity is reflected in the value of n, the index of cooperativity. In the micellar systems, the value of n reflects the average number of surfactant molecules associated with each substrate molecule. Another useful parameter which we obtained by the application of Piszkiewicz's model is $\log [D]_{50}$, the concentration of surfactant where it shows half of its maximum effect. On the other hand the scheme of Menger and Portnoy[24] is based on the distribution of substrate into micellar and aqueous pseudophases and has been extensively applied to micelle catalysed and micelle inhibited reactions. It puts more emphasis on the interaction between the substrate and micellar aggregation, whereas Piszkiewicz's model gives prominence to interaction between substrate and surfactant molecule. Hence the simultaneous application of both models provides a better insight and understanding of micellar effects on the reaction rate.

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