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INFLUENCE OF MICELLES ON TRANSIMINATION REACTIONS IN NON-ENZYMIC SYSTEMS

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Abstract—Cationic micelles of cetyltrimethyl ammonium bromide (CTAB) inhibit the acid catalysed transimination reactions of tetramethylazine-*bis*-barbiturate (TMABB), barbituryl-azine-indandione (BAI) and azine-*bis*-indandione (ABI) with semicarbazide. Whereas a similar reaction with azine-*bis*-barbiturate (ABB) is not affected. The magnitude of inhibition by factors of 2, 14 and 113 for TMABB, BAI and ABI respectively, follows the increasing order of substrate hydrophobicity. Anionic micelles of sodium dodecyl sulphate (SDS) and nonionic micelles of polyoxyethylene (23) dodecanol (Brij 35) have no or little effect on these reactions. Quantitative analysis of the data gave $1.675 \times 10^3\ M^{-1}$ and $3.652 \times 10^4\ M^{-1}$ values for K/N for CTAB inhibited reactions of BAI and ABI respectively. The amount of BAI and ABI present in the micellar phase is found to be 83.2% and 99.1% respectively at 3×10^{-3} M CTAB. Inhibition of the reactions at concentrations well below cmc has been attributed to substrate induction of the micellization and to the presence of functional pre-micellar aggregates.

Usefulness of reactions occurring in presence of micelles as models of certain biological processes occurring at the interfaces prompted many workers to study various organic reactions in the presence of micelle forming substances.[1] This analogy is based on structural similarities between micelles and enzyme proteins and resemblence in properties like substrate specificity, ability to bind the substrate in a non-covalent manner and applicability of Michaelis–Menten type saturation kinetics.[2] Recently Piszkiewicz applied the Hill-model to micelle catalysed reactions and showed that micelle catalyzed reactions could be the models of enzyme catalysed reactions which show positive homotropic interactions.[3]

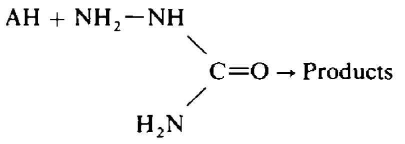
This paper describes the effect of cetyltrimethyl ammonium bromide (CTAB), sodium dodecyl sulphate (SDS) and polyoxyethylene (23) dodecanol (Brij 35) micelles on transimination reactions of murexide or azine-*bis*-barbiturate (ABB), tetramethyl azine-*bis*-barbiturate (TMABB), barbituryl-azine-indandione (BAI) and azine-*bis*-indandione (ABI) also known as Ruheman's purple with semicarbazide.

To our knowledge no data exists in literature on the effect of micelles on these reactions. This study has been undertaken in order to understand the mechanism of micellar catalysis in these reactions and the role of increasing substrate hydrophobicity.

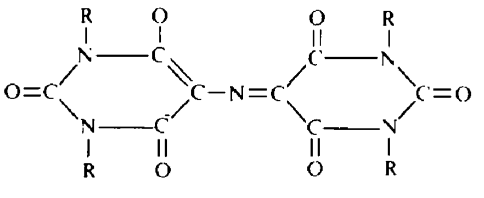
RESULTS AND DISCUSSION

Effect of pH and buffer concentration on transimination reactions in aqueous medium shows the specific acid catalysed nature of these reactions.[10] Catalysis is due to protonation of A^- in a rapid equilibrium step as shown below:

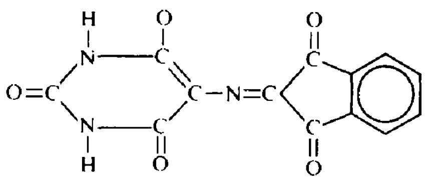
$$A^- + H^+ \rightleftharpoons AH$$



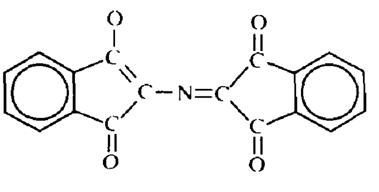
Scheme 1.



R = H, ABB; R = CH$_3$, TMABB



BAI



ABI

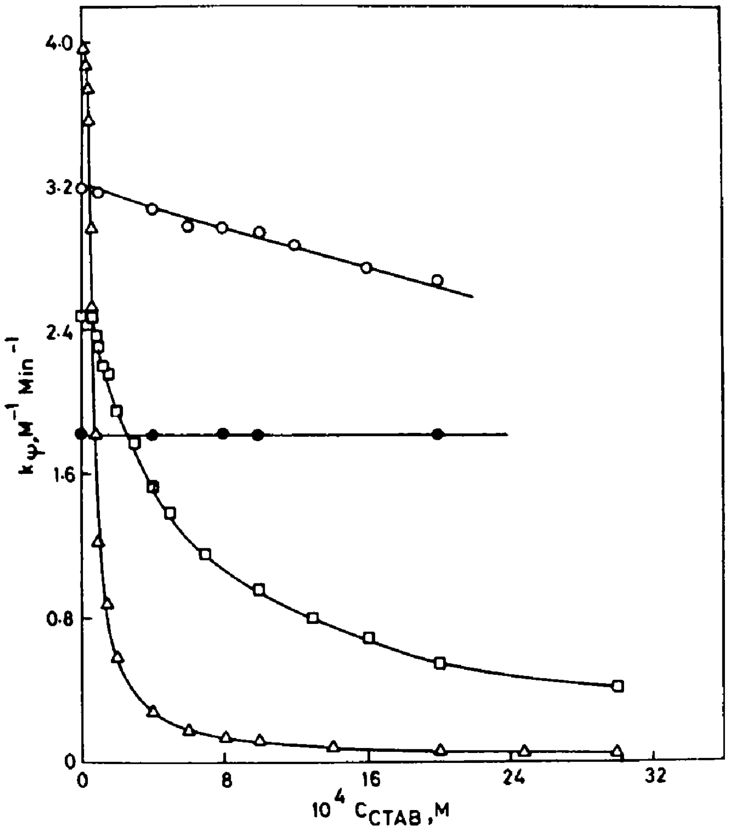


Fig. 1. Bimolecular rate constants plotted against the concentration of CTAB for the reactions of ABB (●), [ABB] = 6.4×10^{-5} M, $\mu = 0.4$; TMABB (○), [TMABB] = 4.7×10^{-5} M, $\mu = 0.3$; BAI (□), [BAI] = 4.5×10^{-5} M, $\mu = 0.26$; ABI (△), and [ABI] = 3.4×10^{-5} M, $\mu = 0.17$; with semicarbazide at 25°C.

It has been proposed that the attack of semicarbazide on protonated azine anion (A^-) leading to the formation of *gem*-diamine intermediate is the rate determining step.

Effect of cationic micelles

CTAB micelles do not affect the position of absorption maxima of ABB (520 nm) and TMABB (530 nm). Whereas these micelles shift the absorption maxima of BAI from 548 nm to 552 nm and of ABI from 570 nm to 580 nm. This shift is indicative of interaction of CTAB micelles with BAI and ABI. It can be seen from Fig. 1 that CTAB has no effect on ABB reaction whereas TMABB, BAI and ABI reactions are inhibited. The overall inhibition is dependent upon the hydrophobicity of the azine and the electrostatic interaction between the micelle and hydronium ion. The extent of inhibition by factors of 2, 14 and 113 at 1×10^{-2} M = CTAB for TMABB, BAI and ABI respectively follows the increasing order of substrate hydrophobicity. In spite of the favourable electrostatic interactions between azine anion and cationic micelle, lack of hydrophobicity in ABB and TMABB appears to be the cause of no or negligible effect of micelles on the rate. On the other hand increased hydrophobicity in BAI and ABI causes strong binding between azine anion and cationic micelle. The micelle bound azine anion is thus shielded from the attack of hydronium ion whose concentration at cationic micellar surface is diminished due to electrostatic repulsion. The observed inhibition (Fig. 1) of BAI and ABI reactions is therefore due to a shift of the equilibrium (Scheme 1) to the left, resulting in the lowering of the concentration of protonated azine molecule which is involved in the rate limiting attack of semicarbazide.

Effect of pH, substrate and reagent concentrations at a fixed concentration of detergent are similar to that in aqueous medium. The results indicate that basic role of these parameters in the course of reaction is not affected in presence of micelles.

The qualitative conclusions drawn in CTAB inhibited reactions of BAI and ABI are substantiated by analysing the data according to a standard scheme.[11,12]

$$\begin{tikzcd}
D_N + S \arrow[r, shift left, "K"] \arrow[d, "k_w"'] & D_NS \arrow[l, shift left] \arrow[d, "k_m"] \\
\text{Products} & \text{Products}
\end{tikzcd}$$

Scheme 2.

This scheme gives the following relationship for the observed rate constant, k_ψ.

$$k_\psi = \frac{k_w + k_m K\left(\frac{C_D - CMC}{N}\right)}{1 + K\left(\frac{C_D - CMC}{N}\right)} \quad (1)$$

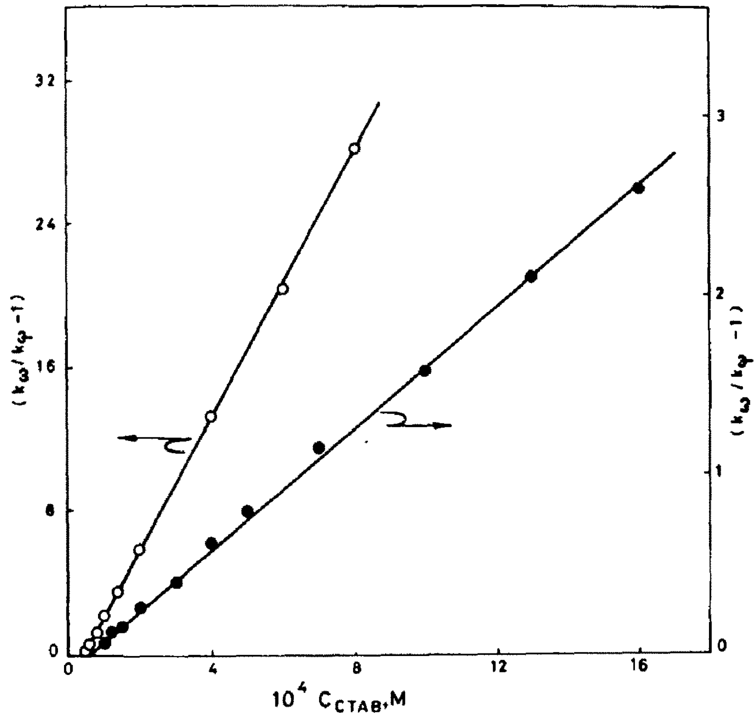


Fig. 2. Quantitative treatment of the kinetic data for the reactions of BAI (●) and ABI (○) with semicarbazide in presence of CTAB micelles.

where k_w and k_m are rate constants in aqueous and micellar phases respectively, K is the association constant between substrate (S) and micelle (D_N), C_D is the concentration of the detergent, CMC is the critical micelle concentration and N is aggregation number of the micelle. In the reactions under investigation k_ψ continued to decrease at very high detergent concentrations also and true saturation was not observed and hence k_m is approximated as equal to zero. Such assumptions have been made earlier for similar strongly inhibited reactions involving hydrophobic substrates which are incorporated into the micelles and the resulting equation (*vide supra*) was successfully employed for the calculation of K.

$$\left(\frac{k_w}{k_\psi} - 1\right) = \frac{K}{N}(C_D - \mathrm{CMC}) \qquad (2)$$

As required by the equation (2) plots of $(k_w/k_\psi - 1)$ against C_D are linear (correlation coefficients 0.9995 and 0.9998 for BAI and ABI respectively). Slopes gave K/N values as $1.675 \times 10^3\ M^{-1}$ and $3.652 \times 10^4\ M^{-1}$ for BAI and ABI respectively. It may be noted that the value of K/N which is a relative measure of binding between substrate and micelle is more for ABI than BAI indicating higher affinity of more hydrophobic molecules to the micelle. From the intercepts of the plots, CMC values (4.38×10^{-5} M and 4.18×10^{-5} M in presence of BAI and ABI respectively) have been obtained. These low values compared to the one in literature[14] (9.2×10^{-4} M) may be due to substrate induced micellisation and presence of counter ions.[1a] The amount of substrate present in the micellar phase (α) can be estimated following the equation (3) which is generally used to find out the substrate incorporated into the micelles from solubility measurements.[12,13]

$$\frac{K}{N} = \frac{\alpha}{(1-\alpha)}\,\frac{1}{C_D - \mathrm{CMC}}. \qquad (3)$$

The amount of BAI and ABI present in the micellar phase at 3×10^{-3} M CTAB was found to be 83.2% and 99.1% respectively. It is of interest to note that most of the substrate is incorporated into micelles where the reaction rate is negligible.

Inhibition at well below the reported CMC values of the detergent can be due to the presence of functional pre-micellar aggregates in addition to the possible lowering of CMC in presence of the substrate and counter ions. The presence of such functional pre-micellar aggregates can be confirmed by using a model recently adopted by Piszkiewicz.[3] According to this model substrate S and n molecules of detergent D combine to form a catalytic micelle D_nS which may then react to yield product. This model gives Equation (4) after approximating k_m as equal to zero.

$$\log\left(\frac{k_w}{k_\psi} - 1\right) = n \log C_D + \log K_D. \qquad (4)$$

Here K_D is the dissociation constant of D_nS back to its free components and n is the average number of detergent molecules per substrate molecule in a functional aggregate. The data of BAI and ABI inhibited reactions fits this equation well (correlation coefficients 0.9985 and 0.9928 for BAI and ABI respectively) and n values from slopes of the plots were found to be 1.08 and 2.84 for BAI and ABI reactions respectively. This low and nonintegral value of n

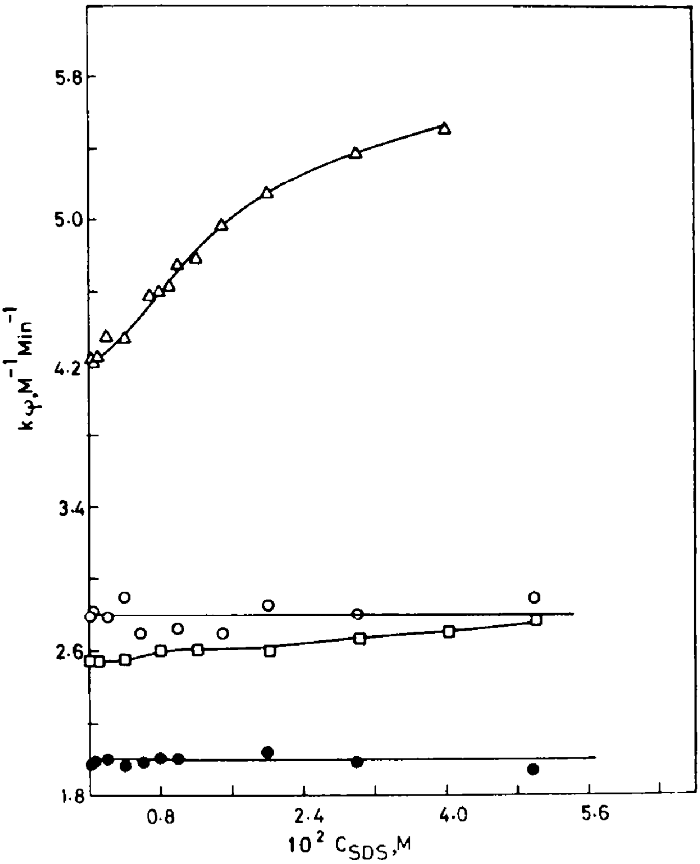


Fig. 3. Bimolecular rate constants plotted against the concentration of SDS for the reactions of ABB (●), [ABB] = 6.4×10^{-5}, $\mu = 0.3$; TMABB (○), [TMABB] = 6.9×10^{-5} M, $\mu = 0.4$; BAI (□), [BAI] = 4×10^{-5} M, $\mu = 0.2$ and ABI (△), [ABI] = 3.2×10^{-5} M; $\mu = 0.3$; with semicarbazide.

suggest that multiple equilibria are operational in the formation of inhibitory premicellar aggregates.

Effect of anionic and nonionic micelles

Unlike in the presence of CTAB micelles, SDS and Brij 35 micelles have no effect on the absorption maxima indicating the absence of any strong interactions between micelles and the substrate. Interestingly SDS micelles have no effect and Brij 35 micelles have negligible effect on ABB and TMABB reactions (Fig. 3 and Table 1). This might again be due to the lack of hydrophobicity in ABB and TMABB. However, a feeble catalysing effect on BAI and ABI reactions was observed (Fig. 3 and Table 1) in the presence of SDS as well as Brij 35. This might be due to the slightly increased concentration of substrates at anionic and non-ionic micellar surfaces due to hydrophobic interactions in spite of the unfavourable electrostatic interactions. Such very weak effects by one charge type of a surfactant and catalysis or inhibition (in this case inhibition by CTAB) by another charge type of surfactants have been observed by earlier workers.[11,15]

Table 1. Effect of Brij 35 on reactions of ABB, TMABB, ABI (all at pH 4.8) and BAI (at pH 6.0) with semicarbazide at 25 C

$C_D \times 10^3$, M	k , M^{-1} min^{-1}			
	ABB[a]	TMABB[b]	BAI[c]	ABI[d]
0	2.627	4.151	2.487	4.440
0.05		4.180	2.472	4.485
0.10	2.542	4.164		4.661
0.12			2.571	
0.50	2.613	4.121	2.641	5.906
1.00	2.721	4.288	2.686	5.587
5.00	2.856		2.630	4.317

a, $\mu = 0.1$ and 5.4×10^{-5} M ABB;
b, $\mu = 0.08$ and 5.1×10^{-5} M TMABB;
c, $\mu = 0.2$ and 4.9×10^{-5} M BAI;
d, $\mu = 0.08$ and 4.0×10^{-5} M ABI.

EXPERIMENTAL

Materials. A BDH sample of azine-*bis*-barbiturate was further purified by salting out in the manner described by Davidson.[4] Tetramethyl azine-*bis*-barbiturate was prepared by the method of Gysling and Schwarzenbach.[5] Barbituryl-azine-indandione was obtained by condensation of uramil with ninhydrin and azine-*bis*-indandione was prepared by the reaction of hydrindantin with ammonium acetate, both following the method of Ruhemann.[6,7] TMABB, BAI and ABI were recrystallised by employing a similar method used for ABB. An extra pure AR sample of cetyltrimethyl ammonium bromide, was used after recrystallising, following the reported method.[8] Sodium dodecyl sulphate was of electrophoresis purity grade of BIO-RAD Labs. Brij 35 obtained from Sigma Chemical Co., (U.S.A.), was used without further purification. Semicarbazide hydrochloride (BDH) was recrystallised from aqueous ethanol before use. Potassium chloride, sodium acetate, sodium hydroxide, potassium dihydrogen ortho-phosphate used in the reactions were analytical grade samples.

Methods. The reaction was studied spectrophotometrically at the visible absorption maxima of the compounds using a Beckman DU spectrophotometer. The temperature of the cell compartment was maintained at 25 ± 0.1°C by using a Beckmann dual thermospacer set in combination with an externally connected thermostat. pH measurements were carried out by a Elico pH meter.

A suitable pH (4.8 for ABB, TMABB, ABI and 6.0 for BAI) was selected such that the rate of hydrolysis of these compounds is negligible compared to the rate of transimination reactions. The desired pH was obtained by neutralising semicarbazide hydrochloride with known amount of sodium acetate. This particular combination under experimental conditions also provided buffering action to the system. The reaction of BAI at pH 6.0 was studied in phosphate buffer. The reactions were studied under pseudo-first order conditions where the concentration of the base was in excess over that of azines. Slopes of the plots were obtained by least square analysis. In calculating the concentrations of free base for obtaining the second order rate constants, a pK_a value[9] of 3.43 at 25°C and Henderson's equation were used.

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