

MICELLAR EFFECTS ON KINETICS AND EQUILIBRIUM OF SYNTHESIS AND HYDROLYSIS OF BENZYLIDENEANILINE

A GENERAL KINETIC CONCEPTION OF MICELLAR CATALYSIS

K. MARTINEK, A. K. YATSIMIRSKI, A. P. OSIPOV and I. V. BEREZIN
 Laboratory of Bio-Organic Chemistry ("A" Building), Lomonosov State University,
 Moscow 117234, USSR

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Abstract—A comparison of effectiveness of micellar catalysis in first-, second- and third order reactions has been made. To this end the effect of sodium dodecylsulphate micelles on the rates of pH-independent and acid-catalysed reactions of hydrolysis and synthesis of benzylideneaniline has been studied. The effect of the above detergent on the equilibrium of interaction between aniline and benzaldehyde has also been investigated. It has been found that

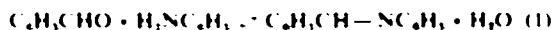
- (1) The apparent equilibrium constant (K_{app} , 1/mole) increases at the optimal concentration of the detergent approximately 10-fold. The shift of the equilibrium caused by the micelles is due to a heightening of reagents concentration in the micellar phase. The true equilibrium constant of the reaction in the micellar phase, K_m , practically equals the equilibrium constant, K_s , determined in the absence of the detergent.
- (2) When the aqueous medium is replaced by the micellar one, pH-independent reactions of hydrolysis and synthesis of benzylideneaniline are retarded, the true rate-constants in the micellar phase being approximately three times lower than those in water. Nevertheless, the fact that the reagents are concentrated in the micelles results in a 3.5-fold increase in the over-all rate of the pH-independent synthesis. The acceleration of the acid-catalyzed hydrolysis and synthesis is much greater, 20 and 125-fold times, respectively.

These results are in good agreement with the kinetic conception proposed in terms of which an increase in the kinetic order of a chemical reaction by unity should result in a P-fold increase in the effectiveness of micellar catalysis, P being the partition coefficient of the reagent for water micelle system.

INTRODUCTION

Catalysis by an enzyme requires the specific binding of substrate molecule to a catalytic site and a chemical interaction with this site, which may directly utilize the binding forces to decrease the free energy of activation of the catalyzed reaction (reviews^{1,2} for example). Micellar catalysis may be viewed as a model of the enzymatic process where the non-covalent binding of the reagents with the micelle ("extraction" of the reagents from water to the micellar "phase") results in a heightening of reagents concentration in the micellar phase and, thus, in an increase in the reaction rate.^{3,4} These views have been now developed into a comprehensive kinetic concept of micellar catalysis which analyzes acceleration of the n-th order reaction in the presence of micelles.

To compare effectivity of micellar catalysis in the reactions of different kinetic order we have studied the effect of sodium dodecylsulphate (SDS) micelles upon the kinetics of synthesis and hydrolysis of benzylideneaniline.



The rate of the forward and backward reactions does not depend upon concentration of H^+ ions in the 9–13 pH-range and at pH = 9 there occurs specific acid catalysis.^{5,6} Earlier the effect of cationic detergent, cetyltrimethylammonium bromide, on acid-catalyzed hydrolysis of benzylideneaniline was studied.⁶

Studying reaction (1) we have elucidated the question, unknown heretofore of the effect of detergent micelles on equilibrium between non-charged molecules

THEORY

Micellar catalysis in the n-th order reaction

The apparent rate constant for the n-th order reaction



running both in the aqueous and micellar "phases" may be represented as follows:

$$k_{app} = \frac{k_m P^n C_D V + k_s (1 - C_D V)}{(1 + C_D V(P - 1))^n} \quad (3)$$

where k_b and k_m are the n -th order rate constants in the aqueous and micellar "phase" respectively, P is partition coefficient of the reagent between the two phases, C_D is concentration of the detergent from which CMC* is subtracted, V is molar volume of the detergent.⁹ How an equation of type (3) is derived was exemplified earlier with a bimolecular reaction.² Briefly, it is assumed the simultaneous course of the reaction in both phases between which there soon establishes an equilibrium partition of reagents; the equilibrium is maintained throughout the reaction. It is also assumed that the reagents produce no effect on formation of micelles and, among other things, they do not shift CMC; that seems to hold only in the case of dilute solutions.

Analyzing Eq. (3) we wish to deal only with the case when the reagent concentrates greatly in the micellar phase, i.e. when $P \gg 1$. Bearing this in mind and assuming also that $C_D V \ll 1$, Eq. (3) may be presented as:

$$k_{app} = \frac{k_m P^n C_D V + k_b}{(1 + P C_D V)^n} \quad (4)$$

With $n = 1$ the dependence of the apparent rate constant, k_{app} , upon the concentration of detergent tends to the limiting value, k_m , in accordance with the hyperbolic law. At other values of n this function has a maximum and the maximal acceleration in the presence of the detergent micelles is equal to:

$$\left\{ \frac{k_{app}}{k_b} \right\}_{max} = \frac{k_m}{k_b} \left(\frac{n-1}{n} \right)^{n-1} P^n \quad (5)$$

This equation holds if the rate of reaction in the aqueous phase is neglected, i.e. if it is assumed that $k_m P^n C_D V \gg k_b$. As is seen from Eq. (5), micellar effect upon the n -th order reaction is determined by two factors: the changed reactivity of the substances on their being transferred from the aqueous to the micellar phase (k_m/k_b) and by the reagents being concentrated. The former factor depends only on the mechanism of the reaction and on the orientation of the reagents in the micelles, whereas the latter includes only the partition constant of the reagent between water and micelles (P) and depends on the order of reaction (n). It is the latter fact that makes it possible to use micelles for effective acceleration of high-order reactions.

Let us see what can be done with Eq. (5). At usual values of P , from 10^2 to 10^3 , sec⁻¹, the maximal acceleration of the reaction amounts to $10 \cdot 10^2$ for a second order and $10^3 \cdot 10^3$ for a third-order

reaction only at the expense of the reagent being concentrated in the micellar phase (i.e. at $k_m = k_b$). In the general case the effectivity of micellar catalysis increases as the kinetic order of the reaction grows. This is clearly seen from Eq. (6) which at $k_m = k_b$ may be derived from Eq. (5):

$$\left\{ \frac{k_{app}/k_b}{(k_{app}/k_b)_{n \text{ th order}}} \right\}_{max} = \frac{n^n P^n}{(n+1)^{n-1} (n-1)^{n-1}} \quad (6)$$

This rate-constants ratio does not depend much on n ; when n changes from 2 to 5 the factor before P in Eq. (6) acquires the values of 0.6 and 0.8 respectively. In other words, when only the concentration effect is at play (i.e. at $k_m = k_b$) the ratio of maximal acceleration observed for the reactions of the $(n+1)$ th and n -th order in the presence of micelles is almost equal to the P value.

Micellar effect upon equilibrium of a chemical reaction

The equilibrium state in a chemical reaction



is described by a usual expression whatever the detergent concentration:

$$K_{app} = \frac{[AB]_t}{[A]_t [B]_t} \quad (8)$$

where $[AB]_t$, $[A]_t$, and $[B]_t$ are total concentrations of the reagents which have material balance relationship with actual concentrations of the reagent in both phases.

$$[A]_t = (1 - C_D V)[A]_b + C_D V[A]_m$$

$$[B]_t = (1 - C_D V)[B]_b + C_D V[B]_m \quad (9)$$

$$[AB]_t = (1 - C_D V)[AB]_b + C_D V[AB]_m$$

Here indices m and b should be referred to the micellar and aqueous phase respectively. In the case of equilibrium partition of the reagents between the aqueous and micellar phases one may derive from Eqs. (8) and (9) that

$$K_{app} = K_b \frac{1 + K_{AB} C_D}{(1 + K_A C_D)(1 + K_B C_D)} \quad (10)$$

where $K_b = [AB]_b/[A]_b[B]_b$ is equilibrium constant when the detergent is absent. Binding constants K_{AB} , K_A and K_B are described¹⁰ by usual expressions:

$$\begin{aligned} K_{AB} &= (P_{AB} - 1)V \\ K_A &= (P_A - 1)V \\ K_B &= (P_B - 1)V \end{aligned} \quad (11)$$

where P_{AB} , P_A and P_B are partition coefficients of

* If the reagent is an ionic or polar compound, the second, non-aqueous phase will be represented only by the surface layer of the micelle which could be assumed to have a certain width and hence, volume. Eq. (3) will then be valid but the volume, V , will be "effective"

the reagents between the two phases:

$$\begin{aligned} P_A &= [A]_m/[A]_a \\ P_B &= [B]_m/[B]_a \\ P_{AB} &= [AB]_m/[AB]_a \end{aligned} \quad (12)$$

The equilibrium constant in the micellar phase, $K_m = [AB]_m/[A]_m[B]_m$ and that in the aqueous phase, K_a , are interrelated in the following way:

$$K_m = K_a \frac{P_{AB}}{P_A P_B} \quad (13)$$

The mode of deriving apparent equilibrium constant expressed as (10) is basically the same as that used to derive kinetic Eq. (4). Therefore, the above equations may be made use of only in the case of dilute solutions.

RESULTS AND DISCUSSION

Effect of SDS micelles on equilibrium of the reaction of benzylideneaniline formation

Figure 1 shows the plot of the apparent equilibrium constant, K_{app} , against detergent concentration. Having analyzed this curve one may find the partition constants of the reagents if Eq. (10) is used (Appendix). These values, K_A , K_B and K_{AB} , are given in the Table. To estimate partition coefficients, P , from experimental constants K (see Eq. (11)), we assumed the specific volume of SDS in micelles to be equal to 0.9 ml/g;⁹ then V is equal to 0.26 l/mole.

Knowing the partition coefficients of the reagents, one may calculate the true equilibrium constant of reaction (1) in the micellar phase. It follows from Eq. (13) that K_m equals 5.1 M^{-1} which practically coincides⁹ with the equilibrium constant $K_a = 6 \text{ M}^{-1}$ measured without the detergent. That means that the observed increase in the apparent equilibrium constant, K_{app} (approximately, by one order), is due to the reagents and product being concentrated in the micelle.

Equation (10) is symmetrical in relation to constants K_A and K_B , therefore one may ascribe one of them to aniline and the other to benzaldehyde only after partition constants of these substances have

⁹It should be noted, however, that estimation of the value of P and, consequently, that of K_m depends upon the assumed value of the effective molar volume, V . Therefore, the coincidence of the values of K_a and K_m should not be overestimated. Besides, it should be taken into account that the equilibrium also involves water, whose concentrations in the micellar and aqueous phase can hardly be thought to coincide. Analysing the results in terms of Eq. (7) we make an assumption that the concentrations of water in the two phases are equal. The corresponding correction will by no means undermine the validity of our conclusions as lower, and not higher, value of K_m will be obtained.

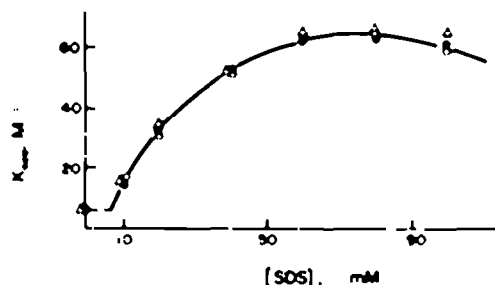


Fig. 1 Effect of sodium dodecylsulphate on the equilibrium constant of formation of benzylideneaniline. The results were obtained as ratios of rate constants of the forward and backward reactions (—○—, pH 8.0 and —●—, pH 10.5, from the data in Figs 3 and 4) and also from equilibrium measurements (△, equilibrium was achieved both in the forward and backward reactions of process (1) at pH 6–11). In the latter case the average values of 4 measurements are given. Each measurement differing from the average value not more than by 10%. The curve is a theoretical one, drawn in accordance with Eq. (10). Experimental conditions: 20°, 5 vol% of methanol, 0.04 M borate buffer, concentrations of reagents aniline from 2.0×10^{-3} to 1.6×10^{-3} M, benzaldehyde from 1.0×10^{-3} to 5.4×10^{-3} M, benzylideneaniline 1×10^{-4} and 2×10^{-4} M.

been determined independently. This was done by us using gel filtration. The experimental data obtained are given in Fig. 2. Constants K_A and K_B found for benzaldehyde and aniline respectively, are listed in the Table. One may see that these values practically coincide with those found in equilibrium measurements in terms of the K_{app} vs C_D plot (Fig. 1). This coincidence should be interpreted to mean that Eq. (10) is correct and, that the above described concept of the effect of micelles on the equilibrium of a chemical reaction is valid.

Effect of SDS micelles on the rates of pH-independent hydrolysis and synthesis of benzylideneaniline

Figure 3A shows the relationship between the

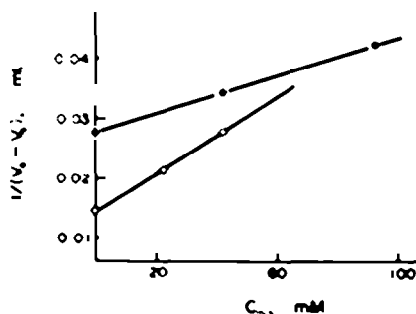


Fig. 2. Gel filtration results for benzaldehyde (—○—) and aniline (—●—), given in the coordinates of Eq. (25). The points at $C_D = 0$ are obtained at SDS concentrations just below the CMC. Experimental conditions: 25°, pH 5.7 (0.006 M phosphate buffer). For details see Methods.

Table 1

K_A, M^{-1}	K_B, M^{-1}	K_A, M^{-1}	K_B, M^{-1}	K_{AB}, M^{-1}	P_A	P_B	P_{AB}
$6 \cdot 0^{*}$	$5 \cdot 1^{\dagger}$	8^{*} 6^{\ddagger}	20^{*} 21^{\ddagger}	550^{*}	$32^{\#}$	$78^{\#}$	$2120^{\#}$

k_{app}, min^{-1}	$k_{app}, \text{min}^{-1} M^{-1}$	k_{app}, min^{-1}	$k_{app}, \text{min}^{-1} M^{-1}$	$k_{app}, \text{min}^{-1} M^{-1}$	$k_{app}, \text{min}^{-1} M^{-1}$
6.3×10^{-4}	38×10^{-4}	2×10^{-4}	10.4×10^{-4}	4×10^{-4}	2×10^{-4}

* Experimental conditions are given in Fig 1

† Calculated according (13) from P_A , P_B , P_{AB} and K_B values

‡ Calculated according Eqs (19) and (20) from reaction equilibrium-detergent concentration profile (see Figs 1, 5)

Determined by gel-filtration method. Experimental conditions are given in Fig 2

• Calculated according (11) from K_A , K_B and K_{AB} values with V taken as 0.26 l/mole

• In absence of SDS. Other experimental conditions are given in Figs 3 and 4, respectively

• Calculated from the data given in Fig 3, assuming $V = 0.26$ l/mole

* $K_A = 9.7 M^{-1}$ (26°, pH 10.5, 30 vol% of methanol) **

† $k_{app} = 0.055 \text{ min}^{-1} \times M^{-1}$ (26°, pH 10.5, 30 vol% of methanol) **

apparent constant of the rate of hydrolysis of benzylideneaniline and detergent concentration. The data obtained indicate that this relationship has a limit at sufficiently high detergent concentration. This agrees with theoretical prediction (14) which follows from Eq. (4) at $n = 1$. In Eq. (14) k_{app} and k_{app} are rate constants of the first order reaction in

$$k_{app} = \frac{k_{app} K_A C_D + k_{app}}{K_A C_D + 1} \quad (14)$$

the micellar and aqueous phases respectively. The value of k_{app} , found as the limiting values of k_{app} at high concentrations of the detergent and also the value of k_{app} , measured without the detergent are presented in the Table

SIDS micelles also produce some effect on the rate of synthesis of benzylideneaniline, which follows from the data in Fig 3B. The theoretical equation describing dependence of apparent rate constant of a second order reaction upon detergent concentration was derived earlier³ and may be presented as follows:

$$k_{app} = \frac{(k_{app}/V) K_A K_B C_D + k_{app}}{(1 + K_A C_D)(1 + K_B C_D)} \quad (15)$$

where k_{app} and k_{app} are rate constants of second order reaction in the micellar and aqueous phases, respectively. Basing on Eq. (15), from the data in Fig 3B one may estimate k_{app} , if the values of K_A and K_B found by equilibrium measurements and listed in the Table are used. The resulting value of $k_{app}/k_{app} = 5.2 M^{-1}$ is practically equal to the value of the equilibrium constant, K_{AB} , found from the equilibrium data (Table).

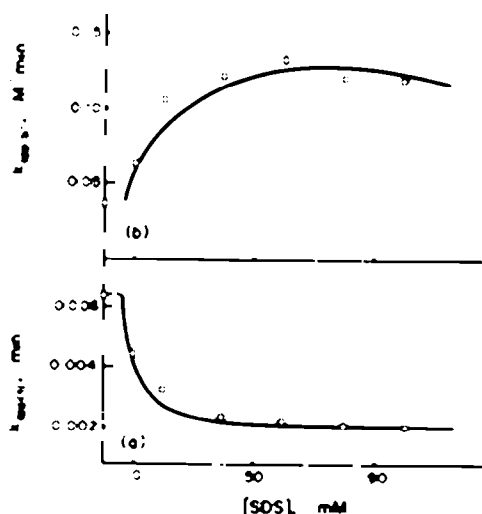


Fig 3 Effect of sodium dodecylsulphate on the kinetics of hydrolysis (A) and synthesis (B) of benzylideneaniline at pH 10.5. In the diagram there are average values of 2-3 measurements, each measurement differing from the average value not more than by 10 per cent. The curves are theoretical ones, drawn in accordance with Eqs (14) and (15). Experimental conditions: 20°, 5 vol% of methanol, 0.04 M borate buffer, concentrations of the reagents: benzylideneaniline $2 \times 10^{-4} M$, aniline $1.6 \times 10^{-3} M$, benzaldehyde $5.4 \times 10^{-3} M$.

As one may see from the Table, when the aqueous medium is replaced by the micellar one, deceleration of both the hydrolysis and synthesis reactions of benzylideneaniline takes place, i.e. $k_{app} < k_{app}$ and $k_{app} < k_{app}$. In both cases (hydrolysis and synthesis) there occurs an almost equal decrease in

the true rate constants (approximately a 3-fold decrease). As a result, the pseudo-monomolecular reaction (hydrolysis), where the fact of the reagent being concentrated in the micellar phase should not influence the overall reaction rate, really retards (Fig 3A). Things are different in the case of second order reaction, such as synthesis, as its overall rate in spite of less favourable rate constant in the micellar phase, not only does not decrease in the presence of micelles, but even increases 3.5-fold (Fig 3B). This is due to the fact that the reagents (aniline and benzaldehyde) are concentrated in the micelles. These data confirm the concept described above that effectivity of micellar catalysis increases as does the kinetic order of the reaction. Still greater acceleration may be attained in a third-order reaction, see below.

Effect of SDS micelles on the rate of acid-catalyzed reactions of hydrolysis and synthesis of benzylideneaniline

Figure 4A shows micellar effect upon the acid-catalyzed hydrolysis of benzylideneaniline. The theoretical dependence of k_{app, H^+} , upon C_D may be derived based on the same considerations as were used for k_{app} (Eq. 4) or K_{app} (Eq. 10), the only difference being that it is not necessary to include the material balance equation (should a buffer be present) to determine the activity of H^+ ions in the surface layer of the micelle. This value may be drawn directly from the activity in the body of solution using partition coefficient P_H . The equation derived in this way looks as follows:

$$k_{app, H^+} = \frac{k_{m, H^+} \cdot K_{AB} P_H \cdot C_D \cdot k_{b, H^+}}{1 + K_{AB} C_D} \quad (16)$$

As is seen in Fig 4A the experimental dependence of the apparent rate constant, k_{app, H^+} , upon C_D has a maximum, which should not be the case in accordance with theoretical expression (16). The reason for this phenomenon seems to be the following. In the simplest case of the Boltzmannian distribution of ions¹⁰ the P_H should be presented as:

$$P_H = e^{-\Psi/RT} \quad (17)$$

where Ψ is surface potential. For an anionic detergent, such as SDS, $\Psi = 0$ is evident. The absolute value of Ψ for this detergent depends, other things being equal, upon concentration of counterions.^{10, 11} As the concentration of counterions increases, the absolute value of Ψ decreases. In our experiments the concentration of counterions increased as that of the detergent went up. This entails a decrease in

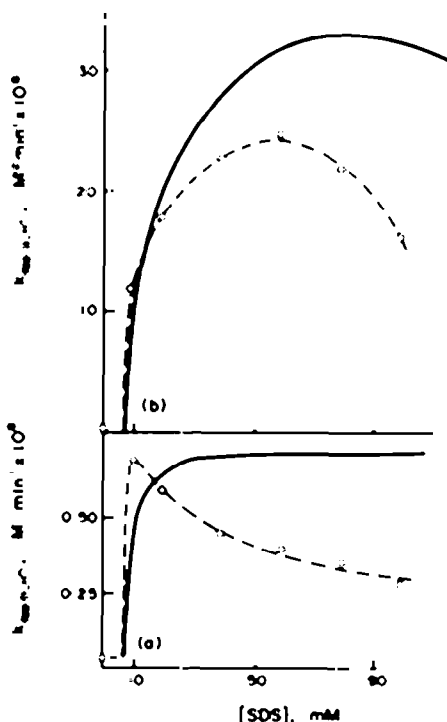


Fig 4. Effect of sodium dodecylsulphate on the kinetics of hydrolysis (A) and synthesis (B) of benzylideneaniline at pH 8.0. The solid curves are theoretical ones, drawn in accordance with Eqs. (16) and (18). Experimental conditions: 20°, 5 vol% of methanol, 0.04 M borate buffer, concentration of reagents benzylideneaniline 2×10^{-4} M, aniline from 2×10^{-2} to 5.4×10^{-2} M, benzaldehyde from 1×10^{-3} to 2×10^{-2} M

Ψ and, hence, in P_H . Therefore the maximum in the curve (Fig 4A) may be accounted for by the variation of the value of P_H as the concentration of the detergent grows. Besides, it should be noted that as the concentration of SDS increases, CMC decreases and the micelles grow in size.^{11, 12} These facts did not allow us to consider SDS effect upon acid-catalyzed reaction on strictly quantitative basis. The following approximation was suggested. Let us assume that $\Psi = 100$ mV, which corresponds to the value of the ζ potential⁹ of SDS micelle at CMC when no electrolytes were added.¹² Then $P_H = 55$. Let us assume, that like in pH-independent reaction, the true rate constant of acid-catalysed hydrolysis in micellar phase is 3 times lower than in the aqueous phase, i.e. let us assume that $k_{m, H^+} = 1.3 \times 10^9$ M⁻¹ min⁻¹. These assumptions were also made when the results pertaining to acid-catalyzed synthesis of benzylideneaniline were analyzed (Fig 4B). The value of k_{m, H^+} , was assumed to be equal to 6.7×10^9 M⁻¹ min⁻¹. The theoretical equation (18) for k_{app, H^+} , will be derived based on the same concept as was made use of for Eq. (16).

* In the general case the value of Ψ in Eq. (17) depends upon positioning the reagent molecule within a micelle.¹¹ The approximation that $\Psi = \zeta$ is correct if the reaction is believed to occur in the aqueous surface layer of the micelle.

$$k_{\text{observed}} = \frac{(k_{\text{ms}} \cdot \mu \cdot \sqrt{V}) K_A K_B P_{\text{II}} \cdot C_D \cdot k_{\text{BEM}}}{(1 + K_A C_D)(1 + K_B C_D)} \quad (18)$$

The theoretical curves drawn basing on these assumptions and Eqs. (16) and (18) are presented in Fig 4A and 4B. As was to be expected, the results of these experiments agree with the theoretical predictions only at low detergent concentrations.

CONCLUSION

In spite of the fact that only semiquantitative analysis of the experimental results was possible, the following conclusion may be drawn:

(a) The effect of SDS micelles on pseudo-monomolecular (pH-independent) hydrolysis of benzylideneaniline is that of a 3-fold deceleration of the reaction, whereas in pseudo-bimolecular reaction (acid-catalysed hydrolysis) there is a ~20-fold acceleration of the reaction (cf Figs 3A and 4A).

(b) The effect of SDS micelles on second order reaction of pH-independent synthesis of benzylideneaniline is that of 3.5-fold acceleration; third order reaction such as acid-catalysed synthesis becomes ~125 more rapid (cf Figs 3B and 4B).

These results agree with the concept described above (Theory), in terms of which the effectivity of micellar catalysis increases with the increasing of kinetic order of a chemical reaction. When the latter increases by unity, the effectivity of micellar catalysis increases 30–60 times in the reaction system studied by us. This confirms the validity of theoretical prediction (6) as partition coefficients P for the reagents have the values between 30 and 80 (Table).

Micellar effect upon the equilibrium state of the chemical reaction between two neutral molecules which we have elucidated allows an analysis of one rather important aspect of micellar catalysis. If micelles had affected only the free energy of the transition state of the reaction, then the equilibrium of a catalysed reaction would not have shifted. But such shift always take place in the presence of micelles—for ionic equilibria this is a well-known fact of pK shifts of acids and bases sorbed by the micelles;¹¹ in this work there is an example of non-ionic equilibrium. This means that micelles produce a marked effect on the ground state of the reaction. Moreover, as was shown in the present work, the fact of the reagents being concentrated in the micelles may be the major reason for acceleration of the reaction in the presence of micelles, whereas the transfer of reaction from water to micelles phase *per se* often causes a retardation of a reaction.² These facts indicate that a detailed kinetic analysis of micellar catalysis should be based on a general concept proposed which deals separately

with both processes: that of partitioning reagents between water and micelles and that of the reaction running in the micellar phase.

APPENDIX

Estimation of partition constants of the reagents between the aqueous and micellar phases using the data on equilibrium measurements

At $K_{AB} C_D \gg 1$ Eq. (10) may be transformed as is shown below (later when partition constants have been found, one may make an accurate estimation of the validity of the above assumption)

$$\frac{K_B C_D - 1}{K_{\text{obs}}} = \frac{K_A + K_B C_D}{K_{AB}} + \frac{K_A K_B C_D}{K_{AB}} \quad (19)$$

The experimental data are given in the co-ordinates of Eq. (19) in Fig 5A. When this dependence is extrapolated to the zero value of C_D , we have $1/K_{AB} = 0.18 \times 10^{-3} \text{ M}^{-1}$.

Using the value of $1/K_{AB}$ Eq. (19) may be now transformed in the following way:

$$\frac{K_B}{K_{\text{obs}}} - \frac{1}{K_{AB} C_D} = \frac{K_A + K_B}{K_{AB}} + \frac{K_A K_B}{K_{AB}} C_D \quad (20)$$

Fig 5B shows the experimental data presented in the co-ordinates of Eq. (20). The slope of the line and the intercept on the Y-axis are equal respectively to $(K_A + K_B)/K_{AB} = 0.05$ and $K_A K_B / K_{AB} = 0.3 \text{ M}^{-1}$. From these expressions one may find K_A and K_B .

EXPERIMENTAL

Materials. Benzylideneaniline was synthesized as described,¹² m.p. 52° (lit. 51–52°).¹³ Aniline and benzaldehyde used were freshly distilled. Sodium dodecylsulphate

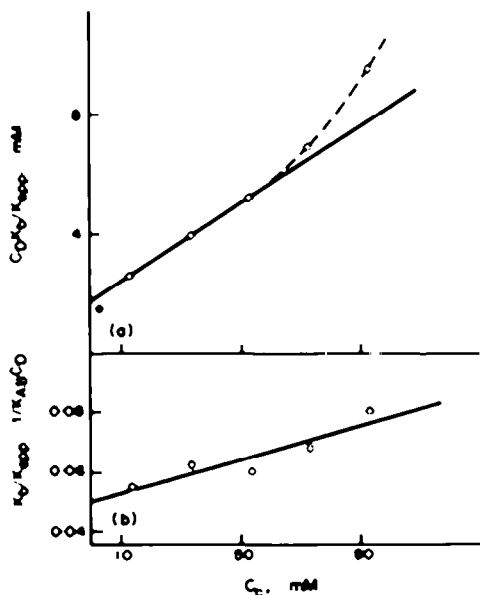


Fig. 5. Determination of partition constants of reagents from the data on the effect of SDS on the equilibrium constant of benzylideneaniline formation (using the data of Fig. 1). The experimental results are presented: (A) in co-ordinates of Eq. (19), (B) in co-ordinates of Eq. (20).

*The black point in Fig 5A does not fit the linear relationship which is described by Eq. (19), because at $C_D = 4 \times 10^{-3} \text{ M}$ condition $K_{AB} C_D \gg 1$ is not observed.

was a commercially available sample (Schuchardt), not additionally purified. The critical micelle concentration (CMC) 6×10^{-3} M at 20°, in water) was estimated by the dye method,¹⁴ using Rodamine 6G.

Components of buffer solutions used were analytical grade reagents of Soyuzkhimreaktiv.

Kinetic and equilibrium measurements. Acid-catalysed reactions of hydrolysis and synthesis of benzylideneaniline were studied at pH 8.0, measuring initial reaction rates which are respectively equal to

$$v_{s, H^+} = k_{s, H^+} [benzylideneaniline]_i a_{H^+} \quad (21)$$

and

$$v_{h, H^+} = k_{h, H^+} [aniline]_i [benzaldehyde]_i a_{H^+} \quad (22)$$

Here a_{H^+} is hydrogen ion activity in bulk solution.

At pH 10.5 initial rates of hydrolysis and synthesis do not depend upon concentration of H^+ ions and can be described by following expressions

$$v_s = k_{s, OH^-} [benzylideneaniline]_i \quad (23)$$

$$v_h = k_{h, OH^-} [aniline]_i [benzaldehyde]_i \quad (24)$$

The initial rate of appearance or disappearance of benzylideneaniline was followed spectrophotometrically.

Equilibrium constant, K_{eq} , was determined by two methods, as a ratio of rate constants of the forward and backward reactions and in accordance with Eq. (8) using the equilibrium concentration of benzylideneaniline measured spectrophotometrically. In the latter case the equilibrium state was attained both in hydrolysis and synthesis of benzylideneaniline. Measurements were performed in the 6–11 pH-range.

Spectrophotometry. Measurements were carried out in a Hitachi EPS-3 recording spectrophotometer supplied with a thermostate cuvette holder. Molar absorbance of benzylideneaniline at 330 nm is 5.3×10^3 M⁻¹ cm⁻¹ (0.02 M borate buffer, pH 6–11, 5 vol% of methanol, 20°), it increases somewhat (not more than by 20%) in the presence of the detergent. Therefore we introduced respective corrections at every concentration of SDS.

pH Measurements. pH was measured by means of a Radiometer instrument.

Gel filtration. Chromatographic assay was carried out in a column 1.6 cm in a diameter with a bed height of about 30 cm. The column was jacketed and operated at 25°. Benzaldehyde experiments were carried out using Sephadex G 15. Since aniline was found to appear as an asymmetrical peak, gel filtration of this substance was performed with Biogel P-4. In gel filtration experiments

recommendations given in¹⁵ were taken into account.

Affluent volume, V_e , corresponding to the maximum concentration in emerging band depends upon the concentration of the detergent equilibrating the column. The following equation was used

$$\frac{1}{V_e} - \frac{1}{V_e^0} = \alpha K_s C_D + \alpha \quad (25)$$

where α is experimental constant.¹⁶ The movement of a band containing a sample of the enzyme, α -chymotrypsin, gave the excluded volume $V_e = 18.0$ ml for Sephadex G 15 and $V_e = 17.7$ ml for Biogel P 4.

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