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ORIGINAL ARTICLE

Catalytic effect of CTAB on the interaction of dipeptide glycyl-tyrosine (Gly-Tyr) with ninhydrin



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Abstract The effect of cationic micelles of cetyltrimethylammonium bromide (CTAB) on the interaction of dipeptide glycyl-tyrosine (Gly-Tyr) with ninhydrin under varying conditions has been studied spectrophotometrically at 70 °C and pH 5.0. The reaction followed first- and fractional-order kinetics with respect to [Gly-Tyr] and [ninhydrin], respectively. Increase in total concentration of CTAB from 0 to $70 \times 10^{-3} \text{ mol dm}^{-3}$ resulted in an increase in the pseudo-first-order rate constant (k_{ψ}) by a factor of ca. 3. Quantitative kinetic analysis of $k_{\psi} - [\text{CTAB}]$ data was performed on the basis of pseudo-phase model of the micelles (proposed by Menger and Portnoy and developed by Bunton) and Piskiewicz model. A possible mechanism has been proposed and the kinetic data have been used to evaluate the micellar binding constants K_S ($268 \text{ mol}^{-1} \text{ dm}^3$ for Gly-Tyr) and K_N ($64 \text{ mol}^{-1} \text{ dm}^3$ for ninhydrin).

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1. Introduction

Surfactants have been widely used in both industrial and domestic fields since the time the first surface-active product was prepared commercially by Schollar in Germany in 1930 (Myers, 2005). They have an impact on almost all aspects of our daily life, either directly in household detergents and personal care products or indirectly in the production and processing of materials which surround us (Lange, 1999; Zana, 2003; Myers, 2005). Surfactants play a vital role in oil industry

too, e.g., in enhanced and tertiary oil recovery (Nasr-El-Din and Taylor, 1998). Such a wide arena of applications has become possible only due to their special amphiphilic nature being soluble in both aqueous and nonpolar media. Surfactants reduce the interfacial tension between oil and water by adsorbing at the liquid–liquid interface. Due to their dual character, the surfactant molecules self-associate (or micellize) when the concentration in a solution reaches a threshold value (the so-called critical micelle concentration (cmc)). The cmc is a key property of a surfactant because a subtle balance exists at cmc which is related to the free energy difference between the surfactant monomers and micelles.

The above mentioned applications are the culmination of a wealth of data available on surfactant related research, both from the academic and industrial R&D research groups. Many more applications are bound to come, like those related to catalysis by surfactant micelles and/or by surfactant-modified adsorbents.

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Nomenclature

Symbols Meanings

k_{obs}	rate constant in aqueous medium
k_{ψ}	rate constant in micellar medium
Gly-Tyr	glycyl-tyrosine
K_N	micellar binding constant for ninhydrin
K_S	micellar binding constant for glycyl-tyrosine
N	ninhydrin
(Gly-Tyr) _w	glycyl-tyrosine in aqueous medium
(Gly-Tyr) _m	glycyl-tyrosine in micellar medium
N_w	ninhydrin in aqueous medium
N_m	ninhydrin in micellar medium
k'_w	pseudo-first-order rate constant for condensation of ninhydrin in non-micellar pseudo-phase
k'_m	pseudo-first-order rate constant for condensation of ninhydrin in micellar pseudo-phase
D_n	micellized surfactant

k_w	second-order rate constant in aqueous medium
k_m	second-order rate constant in micellar medium
M_N^S	molarity of ninhydrin bound to the micellar head-groups
$k_{\psi cal}$	calculated value of rate constant in micellar medium
k_2^m	second-order rate constant for reaction in the Stern layer
K_D	dissociation constant of the micellized surfactant
n	index of cooperativity
E_a	activation energy
ΔH^{\ddagger}	enthalpy of activation
ΔS^{\ddagger}	entropy of activation
k	rate constant
K	equilibrium constant

Ninhydrin has established itself as an analytical tool in the fields of chemistry, biochemistry, and forensic science. Due to the formation of a purple-colored product diketohydrindylidene-diketohydrindamine (DYDA), the use of ninhydrin for the detection and estimation of amino acids/peptides have been the subject of numerous investigations because of its potential ability to reveal fingerprints (Joullie et al., 1991; Friedman, 2004). The method, though useful, still has much room for improvements. Continuous efforts are, therefore, being made to improve the method (Joullie et al., 1991). Our group too has made notable contribution toward the enhancement of DYDA yield (hence increased sensitivity) of ninhydrin-amino acid reaction involving surfactant micelles, solvents and complexation with metal cations (Kabir-ud-Din et al., 2001, 2003; Kabir-ud-Din and Fatma, 2007; Kabir-ud-Din and Siddiqui, 2010; Khan et al., 2010). However, studies on ninhydrin-peptide reaction are scanty (Akram et al., 2006, 2008) and, therefore, in search of enhanced utility, we have studied the Gly-Tyr-ninhydrin reaction in a micelle mediated aqueous medium under varying experimental conditions. For this purpose we used cationic surfactant cetyltrimethylammonium bromide (CTAB) and the results are described in this paper that provide evidence of the catalytic effect of cationic micelles of CTAB on the title reaction.

Peptides and proteins are the most characteristic chemical compounds found in living cells. One example is kyotorphin, found in the brain, where it acts as part of the system used to regulate pain. Dietary proteins are digested to dipeptides and amino acids, and the dipeptides are absorbed more rapidly than the amino acids, because their uptake involves a separate mechanism. Dipeptides activate G-cells found in the stomach to secrete gastrin.

2. Experimental**2.1. Materials**

Gly-Tyr (SRL, 99.0%), ninhydrin (Merck, 99.0%), CTAB (Merck, 99.0%), acetic acid (Merck, 99.0%), and sodium ace-

tate (Merck, 99.0%) were used as received. The stock solutions of Gly-Tyr, ninhydrin, and CTAB were prepared in $\text{CH}_3\text{COONa}-\text{CH}_3\text{COOH}$ buffer solution (pH 5.0), which was prepared by mixing of 30 cm^3 of 0.2 mol dm^{-3} acetic acid and 70 cm^3 of 0.2 mol dm^{-3} sodium acetate (Britton, 1942). Double-distilled and deionized water (specific conductance $(1-2) \times 10^{-6} \text{ ohm}^{-1} \text{ cm}^{-1}$) was used throughout. The pH measurements were made using ELICO LI-122 pH meter in conjunction with a combined electrode (glass-saturated calomel electrode).

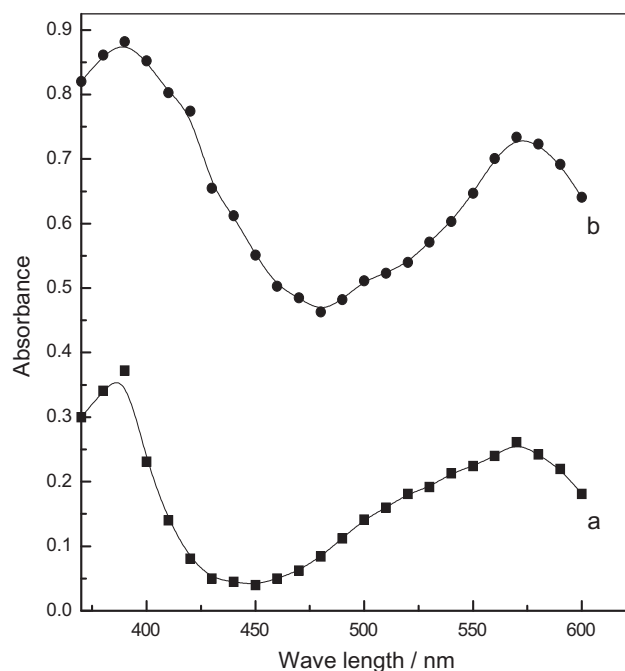


Figure 1 Spectra of the reaction product of ninhydrin with Gly-Tyr in absence (a) and presence of CTAB (b) in buffer solution. Reaction conditions: $[\text{CTAB}] = 30 \times 10^{-3} \text{ mol dm}^{-3}$, $[\text{ninhydrin}] = 6.0 \times 10^{-3} \text{ mol dm}^{-3}$, $[\text{Gly-Tyr}] = 3.0 \times 10^{-4} \text{ mol dm}^{-3}$, temperature = $70 \text{ }^\circ\text{C}$, pH 5.0.

2.2. Spectra of the product

Spectra of the product formed by the reaction between ninhydrin and Gly-Tyr in buffer solution have been taken in aqueous and in micellar media. These results are shown graphically in Fig. 1. The absorbance increases with increase in CTAB concentration. As no shift in λ_{\max} (570 nm) was detected in the presence of CTAB, it is inferred that the same product is formed in both the media. The absorbance maximum ($\lambda_{\max} = 570$ nm) is usually utilized for qualitative and quantitative analyses.

2.3. Kinetic measurements

The reaction was followed spectrophotometrically by observing the appearance of purple color as a function of time at 570 nm (Fig. 1) using a single beam spectrophotometer (SHIMADZU-model UV mini 1240, Japan). A three-necked reaction vessel (fitted with double-walled condenser to prevent evaporation) containing Gly-Tyr solution, buffer, and CTAB (when required) was kept immersed in an oil-bath thermostated at the desired temperature. For stirring and maintaining at an inert atmosphere, pure nitrogen gas (free from CO_2 and O_2) was bubbled through the reaction mixture. The reaction was started by adding a requisite volume of thermally equilibrated ninhydrin solution and the zero-time was taken when half of the ninhydrin solution had been added. The experiments were carried out under pseudo-first-order conditions by taking [ninhydrin] in excess over [Gly-Tyr]. The pseudo-first-order rate constants in the absence (k_{obs} , s^{-1}) and presence of CTAB (k_{ψ} , s^{-1}) were calculated up to 80% completion of the reaction using a computer

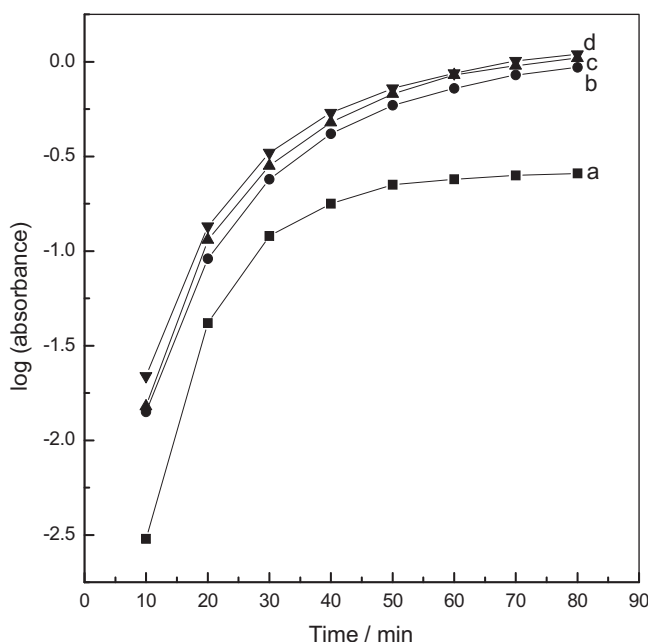


Figure 2 Plots of log (absorbance) vs. time for the reaction of Gly-Tyr and ninhydrin in absence (a) and presence of CTAB; $30 \times 10^{-3} \text{ mol dm}^{-3}$ (b); $40 \times 10^{-3} \text{ mol dm}^{-3}$ (c); $50 \times 10^{-3} \text{ mol dm}^{-3}$ (d). Reaction conditions: [ninhydrin] = $6.0 \times 10^{-3} \text{ mol dm}^{-3}$, [Gly-Tyr] = $3.0 \times 10^{-4} \text{ mol dm}^{-3}$, temperature = 70°C , pH 5.0.

program. Other details regarding pH measurements and kinetic methodology were the same as described elsewhere (Kabir-ud-Din et al., 2000). Plots of log (absorbance) vs. time were made, keeping the fixed concentration of the reactants in aqueous and CTAB micelles ($30, 40, \text{ and } 50 \times 10^{-3} \text{ mol dm}^{-3}$) at temperature 70°C and pH 5.0 (Fig. 2).

2.4. Determination of critical micellar concentration (cmc) by conductivity measurements

The critical micellar concentration (cmc) values of CTAB solutions under different experimental conditions were estimated conductimetrically using ELICO (Type CM 82T, Hyderabad) conductivity bridge. The break points of nearly two straight-line portions of the specific conductivity vs. concentration plots provided the cmc values. Experiments were performed under different conditions, i.e., solvent being water, water + Gly-Tyr, water + ninhydrin, water + Gly-Tyr + ninhydrin; the respective cmc values are ($\times 10^4$) 9.1, 9.0, 8.9, and 9.0 (at 30°C); 12.4, 11.8, 11.9, and 12.3 mol dm^{-3} (at 70°C).

3. Results and discussion

3.1. Effect of pH on the reaction rate

To see the role of pH, the kinetic experiments were performed at pH varying from 4.0 to 6.0 while all other parameters keeping fixed in aqueous as well as in micellar media (CTAB) (Fig. 3). It is observed that the rate constant increases up to pH 5.0 and then becomes almost constant. Every elementary reaction of α -amino acids/peptides and ninhydrin depends upon the $[\text{H}^+]$ because the reaction proceeds through the

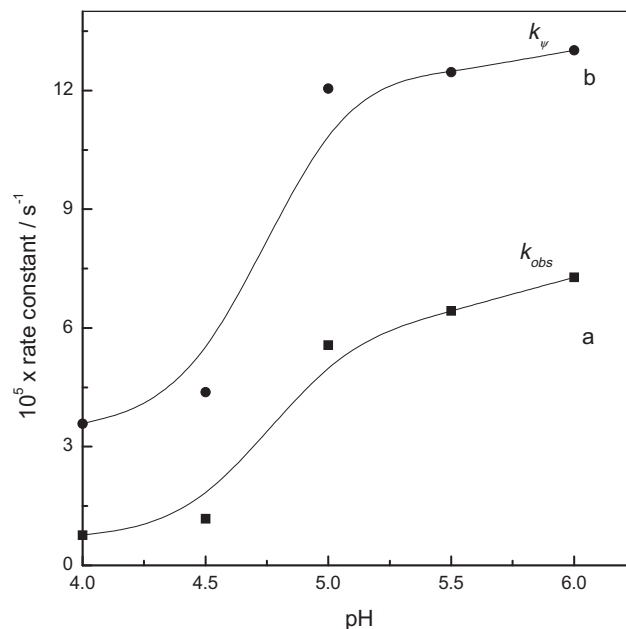


Figure 3 Plots of observed rate constants vs. pH for the reaction of ninhydrin with Gly-Tyr in absence (a) and presence of CTAB (b). Reaction conditions: [CTAB] = $30 \times 10^{-3} \text{ mol dm}^{-3}$, [ninhydrin] = $6.0 \times 10^{-3} \text{ mol dm}^{-3}$, [Gly-Tyr] = $3.0 \times 10^{-4} \text{ mol dm}^{-3}$, temperature = 70°C .

formation of an intermediate which has Schiff base linkage ($>C=N-$). The product of Schiff base formation is acid catalyzed and pH 5.0 is the optimum pH. All subsequent kinetic measurements were, therefore, made at fixed pH (= 5.0).

3.2. Effect of [Gly-Tyr] on the reaction rate

The dependence of the reaction rate on [Gly-Tyr] was determined by carrying out the kinetic runs at different concentrations of Gly-Tyr (keeping [ninhydrin] (6.0×10^{-3} mol dm $^{-3}$), temperature (70 °C), and pH (5.0) constant) in both aqueous and micellar media (Table 1). As the k_{obs} and k_{ψ} values were independent of initial concentration of Gly-Tyr, the order of reaction with respect to [Gly-Tyr] is confirmed to be unity in both the media.

The rate law is, therefore, written as Eq. (1).

$$\text{rate} = d[\text{Product}]/dt = (k_{\psi} \text{ or } k_{obs})[\text{Gly-Tyr}] \quad (1)$$

3.3. Effect of [ninhydrin] on the reaction rate

To investigate the effect of [ninhydrin] on the reaction rate, the kinetic experiments were performed at different concentrations of ninhydrin ranging from 6.0×10^{-3} to 40.0×10^{-3} mol dm $^{-3}$ at constant [Gly-Tyr] (3.0×10^{-4} mol dm $^{-3}$), temperature (70 °C), and pH (5.0). The rate constant values in the two media are recorded in Table 1. The rate constant vs. [ninhydrin] profile is non-linear and pass through the origin (Fig. 4); this indicates the order with respect to [ninhydrin] to be fractional in both the media.

3.4. Effect of temperature on the reaction rate

A series of kinetic runs were carried out at different temperatures (60–80 °C) under identical reaction conditions in absence and presence of CTAB micelles (Table 1). The activation parameters were evaluated using linear least squares regression

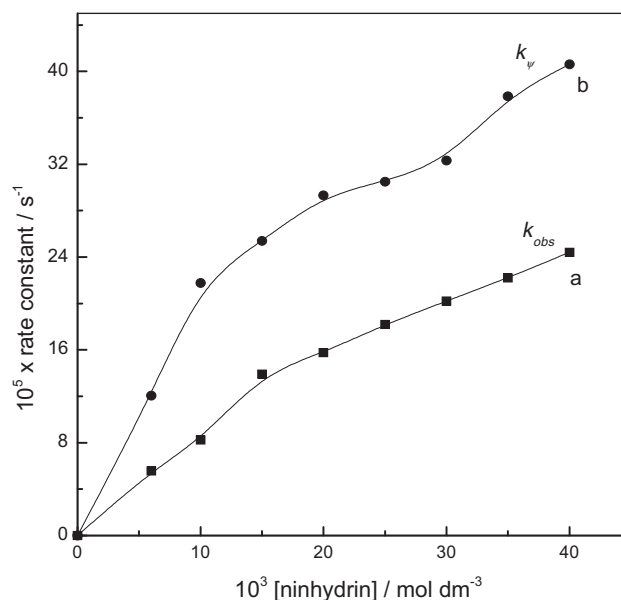


Figure 4 Plots of observed rate constants vs. [ninhydrin] for the reaction of ninhydrin with Gly-Tyr in the absence (a) and presence of CTAB (b). Reaction conditions: [CTAB] = 30×10^{-3} mol dm $^{-3}$, [Gly-Tyr] = 3.0×10^{-4} mol dm $^{-3}$, temperature = 70 °C, pH 5.0.

technique. The observed data were found to fit the Arrhenius and Eyring equations.

4. Reaction in absence of CTAB micelles

Different amino acids/peptides (except proline) react with ninhydrin with different rates but all produce the same final product (Joullie et al., 1991). The amount of the reaction products depends upon temperature, pH and reactant concentrations. As per the well known general mechanism of the reaction

Table 1 Dependence of pseudo-first-order rate constants (k_{obs} or k_{ψ}) on [Gly-Tyr], [ninhydrin] and temperature for the reaction of Gly-Tyr with ninhydrin.

10^4 [Gly-Tyr] (mol dm $^{-3}$)	10^3 [ninhydrin] (mol dm $^{-3}$)	Temperature (°C)	$10^5 k_{obs}$ (s $^{-1}$)	$10^5 k_{\psi}^a$ (s $^{-1}$)
2.0	6	70	5.6	11.9
2.5			6.1	12.2
3.0			5.6	12.1
3.5			6.6	12.4
4.0			6.2	12.5
3.0	6	70	5.6	12.1
	10		8.3	21.8
	15		13.9	25.4
	20		15.8	29.3
	25		18.2	30.5
	30		20.2	32.3
	35		22.2	37.9
	40		24.4	40.6
3.0	6	60	1.9	5.2
		65	2.3	7.8
		70	5.6	12.1
		75	14.9	15.5
		80	20.1	21.6

^a [CTAB] = 30×10^{-3} mol dm $^{-3}$.

(Khan et al., 2010), the condensation between carbonyl group of ninhydrin and amino group of Gly-Tyr takes place. The reaction starts through the attack of lone-pair of electrons of amino nitrogen (of Gly-Tyr) to the carbonyl carbon (of ninhydrin) to give Schiff base. This Schiff base is unstable and hydrolyses to give 2-amino-indanedione, which reacts slowly with another ninhydrin molecule to yield the product (DYDA) (Scheme 1).

On the basis of observed rate law $d[P]/dt = k_{obs} [\text{Gly-Tyr}]$ and the proposed mechanism (Scheme 1), the rate equation is derived as Eq. (2)

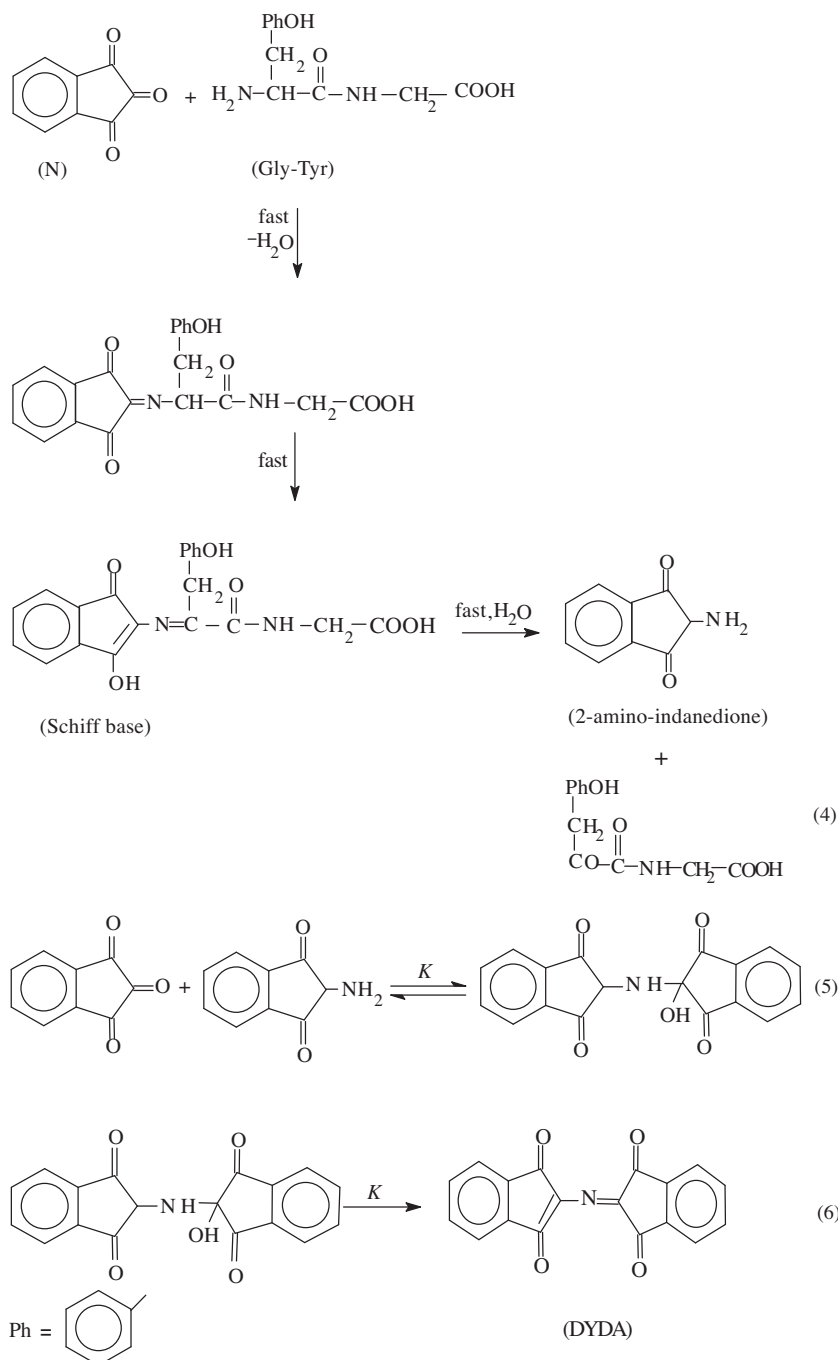
$$k_{obs} = \frac{kK[N]}{1 + K[N]} \quad (2)$$

([N] being the total concentration of ninhydrin).

On rearrangement, Eq. (2) gives

$$\frac{1}{k_{obs}} = \frac{1}{k} + \frac{1}{kK[N]} \quad (3)$$

As envisaged, the double reciprocal profile of $1/k_{obs}$ vs. $1/[N]$ is linear (Fig. 5). The values of K and k (the equilibrium and rate constants of Eqs. (5) and (6)) were evaluated from intercept and slope with respective values being $1.0 \times 10^{-3} \text{ s}^{-1}$ and



Scheme 1 Gly-Tyr-ninhydrin reaction mechanism.

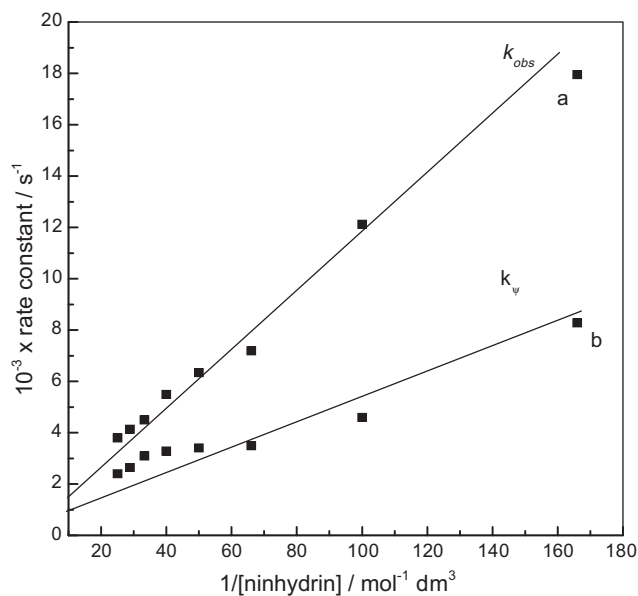


Figure 5 Plots of inverse of observed rate constants vs. $1/[\text{ninhydrin}]$ for the reaction of ninhydrin with Gly-Tyr in the absence (a) and presence of CTAB (b). Reaction conditions: $[\text{CTAB}] = 30 \times 10^{-3} \text{ mol dm}^{-3}$, $[\text{Gly-Tyr}] = 3.0 \times 10^{-4} \text{ mol dm}^{-3}$, temperature = 70°C , pH 5.0.

$10.0 \text{ mol}^{-1} \text{ dm}^3$ in aqueous medium. The calculated values of rate constants (obtained by substituting k and K in Eq. (3)) are in close agreement with k_{obs} , which supports the proposed mechanism, as also confirming the validity of the rate Eq. (3).

5. Reaction in presence of CTAB micelles

As no change in the position of maximum was observed in the presence of CTAB micelles (Fig. 1), it is inferred that the same product (purple in color) is formed both in the aqueous and in micellar media. Furthermore, it is assumed that the pathways outlined in Scheme 1 are being followed in CTAB micelles too (as no change in the behavior was noticed regarding the effect of variables – see Figs. 1–5). In the discussion that follows, quantitative kinetic analyses for the title reaction in CTAB micellar system are presented.

To investigate the effect of CTAB micelles on the reaction rate, the kinetic experiments were performed in the presence of varying amounts of CTAB micelles, keeping other parameters constant. An enhanced reaction rate was observed in the presence of the cationic CTAB micelles. Common to bimolecular reactions catalyzed by micelles, the plot of k_ψ vs. $[\text{CTAB}]$ shows a rate maximum at $[\text{CTAB}] = 70 \times 10^{-3} \text{ mol dm}^{-3}$ (Fig. 6). A further increase in $[\text{CTAB}]$ ($> 70 \times 10^{-3} \text{ mol dm}^{-3}$) results in a slow decrease in the reaction rate.

The behavior in the presence of CTAB micelles may be discussed in terms of pseudo-phase model of the micelles developed by Menger and Portnoy (1967) and Khan (2006) and modified by Bunton (1997) and Vera and Rodenas (1986). Accordingly, the reaction scheme for ninhydrin interaction with Gly-Tyr in the presence of CTAB micelles may be given as shown in Scheme 2.

In Scheme 2, $[\text{D}_n]$ represents the micellized surfactant ($=[\text{CTAB}]_T - \text{cmc}$). The pseudo-first-order rate constants,

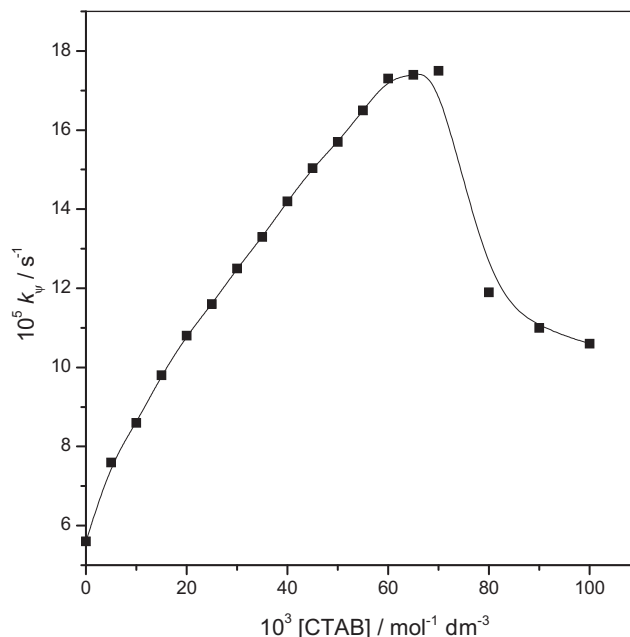
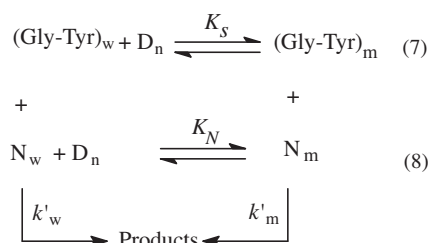


Figure 6 Effect of $[\text{CTAB}]$ on the observed rate constant (k_ψ) for the reaction of ninhydrin with Gly-Tyr. Reaction conditions: $[\text{ninhydrin}] = 6.0 \times 10^{-3} \text{ mol dm}^{-3}$, $[\text{Gly-Tyr}] = 3.0 \times 10^{-4} \text{ mol dm}^{-3}$, temperature = 70°C , pH 5.0.



Scheme 2 Gly-Tyr-ninhydrin reaction in micellar medium.

k'_w and k'_m , stand for condensation of Gly-Tyr in non-micellar pseudo-phase and micellar pseudo-phase, respectively.

Scheme 2, in conjunction with the observed rate being unity in $[\text{Gly-Tyr}]$, leads to Eq. (9)

$$k_\psi = \frac{k'_w + k'_m K_s [\text{D}_n]}{1 + K_s [\text{D}_n]} \quad (9)$$

which, when modified, yields Eq. (10)

$$k_\psi = \frac{k_w [\text{N}] + (K_s k_m - k_w) \text{M}_N^S [\text{D}_n]}{1 + K_s [\text{D}_n]} \quad (10)$$

where $k_w = k'_w / [\text{N}_w]$ and $k_m = k'_m / \text{M}_N^S$ (k_w and k_m are second-order rate constants) and M_N^S , being the molarity of ninhydrin bound to the micellar headgroups, is given as Eq. (11).

$$\text{M}_N^S = \frac{[\text{N}_m]}{[\text{D}_n]} \quad (11)$$

Values of M_N^S were estimated in the following manner. Upon solving $K_N = [\text{N}_m] / [\text{N}_w] ([\text{D}_n] - [\text{N}_m])$ and mass balance $[\text{N}]_T = [\text{N}_w] + [\text{N}_m]$, quadratic Eq. (12) resulted, which was solved for $[\text{N}_m]$ with the help of a computer program with

Table 2 Thermodynamic parameters, rate and binding constant values for the reaction of Gly-Tyr with ninhydrin at pH 5.0.

Parameters and constants	Aqueous	CTAB
E_a (kJ mol ⁻¹)	135.9	69.4
ΔH^\ddagger (kJ mol ⁻¹)	133.0	66.7
$-\Delta S^\ddagger$ (J K ⁻¹ mol ⁻¹)	294.7	307.1
$10^3 k_m$ (s ⁻¹)	–	0.7
$10^5 k_w$ (mol ⁻¹ dm ³ s ⁻¹)	–	5.6
K_S (mol ⁻¹ dm ³)	–	268.0
K_N (mol ⁻¹ dm ³)	–	64.0
$10^4 k_2^m$ (mol ⁻¹ dm ³ s ⁻¹)	–	0.98

K_N as an adjustable parameter. M_N^S was then calculated with the help of Eq. (11).

$$K_N[N_m]^2 - (1 + K_N[D_n] + K_N[N]_T)[N_m] + K_N[D_n][N]_T = 0 \quad (12)$$

In order to determine k_m and K_S kinetically, we need the cmc under kinetic conditions, which were determined conductimetrically. For a given value of cmc, k_m and K_S were calculated from Eq. (10) using non-linear least squares technique. Such calculations were carried out at different presumed values of K_N . The best value of K_N was considered to be one for which the value of $\sum d_i^2$ ($d_i = k_{\psi\text{obsi}} - k_{\psi\text{cali}}$) turned out to be a minimum. These values are recorded in Table 2. The fitting of the calculated data (K_S , k_m and K_N) to Eq. (10) is evident from the calculated values of the rate constants, $k_{\psi\text{cal}}$, shown in Table 3.

An alternative data treatment was also carried out: Eq. (9) can be written as Eq. (13) (Khan, 2006),

$$\frac{1}{(k_w - k_\psi)} = \frac{1}{(k_w - k_m)} + \frac{1}{(k_w - k_m)K_S[D_n]} \quad (13)$$

The equation predicts a linear plot between $1/(k_w - k_\psi)$ and $1/[D_n]$. This treatment assumes that only one substrate is incorporated into the micellar phase (Bunton, 1997). It has been used successfully for a number of reactions because the data treatment with the non-linear Eq. (10) is more difficult than that with the alternative linear Eq. (13).

The rate increment in the presence of positively charged micelles could be attributed to the stabilization of Schiff base intermediate on positively charged micellar surface, thus increasing the concentration of intermediate in Stern layer. The reactant ninhydrin having the Π -electrons enhances the possibility of its partitioning between water and cationic charged micelles. Therefore, both reactants (ninhydrin and Gly-Tyr) get associated/incorporated into the Stern layer (i.e., aqueous surface of micelles, supposed to be the usual site of ionic micelle-mediated organic reactions (Khan, 2006)). Thus, the overall increment of rate constants occurs due to concentrating the reactants in the micellar phase.

The decrease in k_ψ beyond $[CTAB] = 70 \times 10^{-3}$ mol dm⁻³ can be explained as follows. At $[CTAB] > 70 \times 10^{-3}$ mol dm⁻³, practically all the substrate has been incorporated into the micellar phase. When bulk of the substrate is incorporated into the micelles, addition of more CTAB generates more cationic micelles, which simply take up the ninhydrin molecules into the Stern layer, and thereby deactivate them; because a reac-

Table 3 Dependence of the pseudo-first-order rate constant k_ψ on the [CTAB] for the reaction of Gly-Tyr^a with ninhydrin^a and their comparison with $k_{\psi\text{cal}}^b$.

$10^3 [CTAB]$ (mol dm ⁻³)	$10^5 k_\psi$ (s ⁻¹)	$10^5 k_{\psi\text{cal}}^b$ (s ⁻¹)	$(\frac{k_\psi - k_{\psi\text{cal}}}{k_\psi})$
0	5.6	–	–
5	7.6	7.4	+0.03
10	8.6	10.3	-0.12
15	9.8	12.1	-0.23
20	10.8	10.5	+0.03
25	11.6	9.9	+0.15
30	12.5	10.6	+0.15
35	13.3	11.1	+0.15
40	14.2	12.6	+0.17
45	15.0	14.2	+0.05
50	15.7	15.6	+0.01
55	16.5	15.0	+0.09
60	17.3	17.8	-0.03
65	17.4	16.6	+0.05
70	17.5	17.3	+0.01
80	11.9	12.0	-0.01
90	11.0	10.8	+0.02
100	10.6	10.1	+0.05

^a [Gly-Tyr] = 3.0×10^{-4} mol dm⁻³, [ninhydrin] = 6.0×10^{-3} mol dm⁻³, temperature = 70 °C and pH 5.0 (sodium acetate-acetic acid).

^b The calculated values of rate constants ($k_{\psi\text{cal}}$) were obtained by resubstituting the required values in Eq. (10).

tant molecule in one micelle should not react with the other in another micelle. Another reason of decrease in k_ψ could be a result of counter ion inhibition.

The second-order rate constants (k_m , s⁻¹) in the micellar pseudo-phase cannot be compared directly with the rate constants in water (k_w , mol⁻¹ dm³ s⁻¹) because the k_m is calculated by taking concentration as a mole ratio, M_N^S (see Eq. (11)). To compare k_m with k_w , Bunton (1997) and Khan (2006) used the volume element for reaction within the micellar pseudo-phase. Stern layer volume of one mole of CTAB is about 0.14 mol dm⁻³. The second-order rate constant (k_2^m , mol⁻¹ dm³ s⁻¹) for reaction in the Stern layer is then given as $k_2^m = 0.14k_m$.

For bimolecular reactions, in general, $k_w > k_2^m$. In the present case, the two values are comparable. Micellar surfaces are water rich but are less polar than pure water and do not provide a uniform reaction medium because micelle is a porous cluster with a rough surface and deep water filled cavities. On the other hand, the variations in k_w/k_2^m are not related in any obvious way to estimate polarities of micellar surfaces.

In order to calculate the dissociation constant of the micellized surfactant back to its components (K_D) and the index of cooperativity (n), the Piszkievicz model (Piszkievicz, 1977), analogous to the Hill model applied for the enzyme-catalyzed reactions, was used. In micellar systems, the value of n reflects the average number of the surfactant molecules associated with each substrate molecule. The Piszkievicz model relates the n and K_D and its contribution to the rate is given by Eq. (14)

$$k_\psi = \frac{k'_m[D]^n + k'_w K_D}{K_D + [D]^n} \quad (14)$$

where, n describes “index of cooperativity”, $[D]$ is the concentration of surfactant, and K_D is the dissociation constant of micellized detergent back to its component.

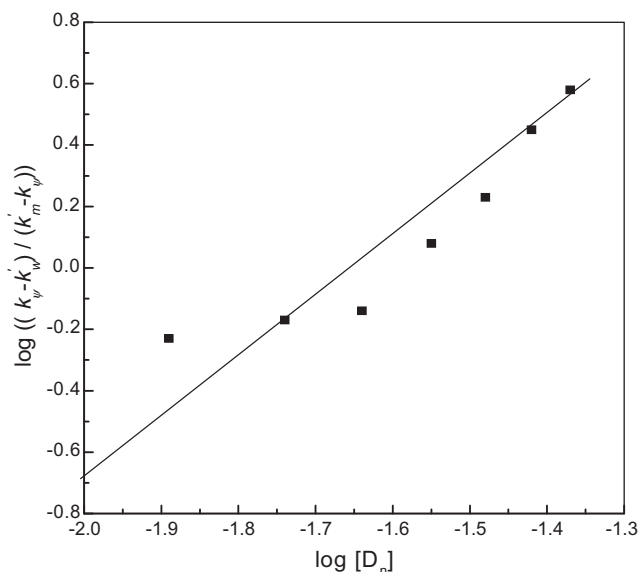


Figure 7 Plot of $\log((k_{\psi} - k'_w)/(k'_m - k_{\psi}))$ vs. $\log[D_n]$ for the reaction of Gly-Tyr with ninhydrin. Reaction conditions were the same as in Fig. 6.

On rearrangement, Eq. (14) gives Eq. (15).

$$\log \left[\frac{k_{\psi} - k'_w}{k'_m - k_{\psi}} \right] = n \log[D] - \log K_D \quad (15)$$

According to Eq. (15), a plot of $\log((k_{\psi} - k'_w)/(k'_m - k_{\psi}))$ vs. $\log[D]$ should be a straight line with a positive slope ($=n$). Such a plot has been realized in the CTAB catalysis of the present study (Fig. 7). K_D and n are 4.67 and 1.8, respectively. A value of n greater than unity indicates positive cooperativity, i.e., the binding of the first molecule of a substrate makes it easier for subsequent molecules to bind. The advantage of Eq. (14) is that it does not require the knowledge of the cmc of surfactant used.

The values of activation parameters such as activation energy (E_a), enthalpy of activation (ΔH^{\ddagger}) and entropy of activation (ΔS^{\ddagger}) are given in Table 2. CTAB lowers the values of activation parameters (ΔH^{\ddagger} and ΔS^{\ddagger}) more than aqueous. This decrement in the parameters occurs not only through the stabilization of transition state but also through adsorption of substrates on the micellar surface. It is not possible to explain the mechanistic feature of apparent values of activation parameters (ΔH^{\ddagger} and ΔS^{\ddagger}) because the values of the rate constant k_{ψ} do not show a single elementary step; it is a complex function of true rate, binding, and ionization constants. The fitting of rate constant k_{ψ} at various temperatures was estimated and was found that Eyring equation is applicable to micellar media, and the sensitivity of the micelle structure to temperature is kinetically unimportant.

6. Conclusion

The reaction between dipeptide Gly-Tyr and ninhydrin has been studied in both aqueous and in micellar media. By comparing the values with those obtained in aqueous medium, we find that the presence of cationic micelles of CTAB catalyze the reaction. The value of activation energy (E_a) clearly indi-

cates that CTAB micelles play the role of catalyst and provides a new reaction path with lower activation energy. This suggests the adsorption/association of both reactants on the micellar surface as well as through stabilization of transition state. Finally, we can conclude that the interaction of Gly-Tyr with ninhydrin in micellar media could successfully be treated using the pseudo-phase and Piszkiwicz models. Quantitative treatment of the kinetic data seems justified as k_{ψ} and $k_{\psi cal}$ are in close agreement within experimental errors.

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