



CATIONIC MICELLAR EFFECT ON THE REACTION BETWEEN DIPEPTIDE GLYCYL-ALANINE (GLY-ALA) AND NINHYDRIN WITH AND WITHOUT SALT ADDITIVES

Mohd. Akram,^{[a]*} Adel A. M. Saeed^[a] and Kabir-ud-Din^[a]

Keywords: surfactant, dipeptide (Gly-Ala), ninhydrin, catalysis, micelles, TTAB, CTAB, CPC, salts

The effect of cationic conventional surfactants myristyltrimethylammonium bromide (TTAB), cetyltrimethylammonium bromide (CTAB), and cetylpyridinium chloride (CPC) on the interaction of dipeptide glycyl-alanine (Gly-Ala) with ninhydrin has been studied spectrophotometrically under different conditions. The reaction rates are higher in the presence of surfactants but the reaction order remains the same in both the media (first- and fractional-order with respect to [Gly-Ala] and [ninhydrin]). Quantitative kinetic analyses of k_p -[surfactant] data were performed on the basis of pseudo-phase model of the micelles (proposed by Menger and Portnoy and developed by Bunton and Romsted) and Piszkiwicz model wherein the micellar binding constants K_S for Gly-Ala and K_N for ninhydrin with surfactant micelles were evaluated. The catalytic efficiency in TTAB increased by added electrolytes which had been discussed in detail.

* Corresponding Authors

Tel: +91 09411040048

E-Mail: drmohdakram@rediffmail.com

[a] Department of Chemistry, Aligarh Muslim University, Aligarh-202002, India

Introduction

Surfactants are amphiphiles that contain polar or ionic head groups and apolar tails. They form association colloids, known as micelles, when they self-associate at concentrations above the critical micelle concentrations. In ionic head micelles, for example, the aqueous solution-micelle interfacial region contains the ionic head groups, the Stern layer of the electrical double layer with the bound counterions, and water. The remaining counterions are contained in the Gouy-Chapman portion of the double layer that extends further into the aqueous phase.¹ A micelle or a micellar aggregate constitutes an inhomogeneous microreaction environment, which is highly dynamic, in the sense that it is in rapid equilibrium with the constituent monomers in aqueous phase. So that, a micelle is not a separate phase, like aqueous phase, although it does provide microreaction medium, which is called pseudophase, in which micellar mediated reactions occur. Micellar catalysis of numerous reactions is an area of current research because of the parallel behaviour of macromolecules and enzymes.²

The ninhydrin (triketohydrindene hydrate)-mediated colour formation is the most widely used method for detection and quantitative estimation of amino acids/peptides.³ The so-called 'ninhydrin reaction' forms a product known as 'Ruhemann's purple' which is attributed to be anion of diketohydrindylidenediketohydrindamine (DYDA), and this product can be quantitatively measured at 570 nm. To improve the sensitivity, however, modifications in the method are continuously being made.^{3,4} In this regard studies by our group had shown success toward increased sensitivity of ninhydrin-amino acid reaction by involving

surfactant micelles, solvents and complexation with metal cations.⁵⁻¹⁰ As studies on ninhydrin-peptide reaction are limited,¹¹⁻¹³ systematic kinetic and mechanistic studies of the Gly-Ala-ninhydrin reaction in absence and presence of cationic micelles of myristyltrimethylammonium bromide (TTAB), cetylpyridinium chloride (CPC), and cetyltrimethylammonium bromide (CTAB) at different temperatures have been performed. Also, it is found that various added salts can affect the overall course of the reaction. Therefore, the investigation concerns the reaction in aqueous and micellar media with and without salts.

Experimental Section

Materials and Methods

The surfactants (TTAB, $\geq 99\%$, Sigma, India; CPC, 99%, Merck, Germany; CTAB, 99%, Merck, Germany), glycyl-L-alanine ($\geq 99\%$ (NT), Aldrich, Switzerland), ninhydrin (99%, Merck, India), sodium acetate anhydrous ($\geq 99\%$, Merck, India), acetic acid glacial (99-100%, Merck, India), sodium nitrate purified (99 %, Merck, India), sodium sulphate ($\geq 98\%$, Merck, India), sodium phosphate (96%, Aldrich, USA), sodium salicylate (99.5%, CDH, India), sodium benzoate (99.5%, Merck, India), sodium tosylate (70-80%, (HPLC), Fluka, Switzerland), and sodium oxalate ($\geq 99.5\%$, S.D. Fine-chem Ltd., India) were used as received. Demineralized double-distilled water was used throughout the work (specific conductivity (Λ): $(0.8 - 2.1) \times 10^{-6} \text{ S}^{-1} \text{ cm}^{-1}$). Stock solutions of the reactants and the surfactants were prepared in acetic acid - sodium acetate buffer which was prepared by mixing acetic acid (0.2 mol dm^{-3}) and sodium acetate (0.2 mol dm^{-3}) up to desired volume.¹⁴ The pH measurements were made using a digital Systronics pH meter model MK-VI (Ahmedabad-India) in conjugation with a combined electrode (glass-saturated calomel electrode) and standardized using WTW buffer solutions

(Germany). A Systronics conductivity meter model 306 (Ahmedabad-India) with platinized electrodes was used for the conductivity measurements.

Kinetic measurements

For each set of kinetic experiments, the requisite volumes of Gly-Ala, buffer and surfactant solutions (when required) were taken in a three-necked reaction vessel (also fitted with a double-surface water condenser), which was then kept in an oil bath at the experimental temperature. The reaction was started by adding a requisite volume of thermally equilibrated ninhydrin solution; zero-time was taken when half of the ninhydrin solution had been added. Pure N₂-gas (free from O₂ and CO₂) was bubbled through the reaction mixture for stirring as well as to maintain an inert atmosphere. Pseudo-first-order conditions were maintained in all the kinetic runs by using excess of ninhydrin over Gly-Ala concentration (≥ 10 times). The absorbance of the product DYDA was measured at 570 nm (λ_{max} -vide infra) at definite time intervals with a Shimadzu UVmini-1240 Spectrophotometer. Other details regarding kinetic methodology were the same as described elsewhere.⁵⁻¹³

Determination of CMC

The critical micellar concentration (CMC) values of the TTAB, CPC, and CTAB solutions under the experimental conditions were determined conductometrically. The values in the presence and absence of reactants have been obtained from the break points of nearly two straight line portions of the specific conductivity vs. concentration plots.¹⁵ Experiments were carried out under different conditions, i.e., solvent being water, water + Gly-Ala, water + ninhydrin or water + Gly-Ala + ninhydrin and the respective CMC values are recorded in Table 1.

Viscosity measurements

Using Ubbelohde viscometer the viscosity measurements were made at 70 ± 0.1 °C. The method of viscosity measurements was the same as reported elsewhere.¹⁶

Results and Discussion

Spectra of the product

The UV-visible spectra of the product formed by the reaction between Gly-Ala and ninhydrin in the buffer solution were recorded in the absence and presence of surfactant micelles (Figure 1). We see that the absorbance is higher in presence of micelles than in aqueous medium with no shift in λ_{max} (570 nm), i.e., the wave length of maximum absorbance remains the same in both aqueous and micellar media. It is, therefore, concluded that the purple-coloured product of Gly-Ala reaction with ninhydrin to be the same in aqueous and micellar systems.

Table 1. The CMC values for CPC, CTAB, and TTAB at 30 °C and 70 °C using electrical conductivity technique.

| System | CMC·10 ³ mol dm ⁻³ 30 °C | CMC·10 ³ mol dm ⁻³ at 70 °C |
|---------------------------|--|---|
| Pure CPC | 1.06 | 1.40 |
| CPC + Ninhydrin | 1.26 | 1.35 |
| CPC + Gly-Ala | 1.05 | 1.29 |
| CPC + Gly-Ala+ Ninhydrin | 1.19 | 1.31 |
| Pure CTAB | 0.98 | 1.27 |
| CTAB + Ninhydrin | 1.29 | 1.41 |
| CTAB + Gly-Ala | 0.93 | 1.09 |
| CTAB + Gly-Ala+ Ninhydrin | 1.07 | 1.38 |
| Pure TTAB | 3.90 | 5.11 |
| TTAB + Ninhydrin | 4.32 | 5.40 |
| TTAB + Gly-Ala | 3.80 | 4.30 |
| TTAB + Gly-Ala+ Ninhydrin | 4.25 | 5.53 |

The kinetics of the reaction of glycyl-alanine and ninhydrin was, therefore studied under varying experimental conditions spectrophotometrically by following the appearance of purple colour at 570 nm. The results are described below.

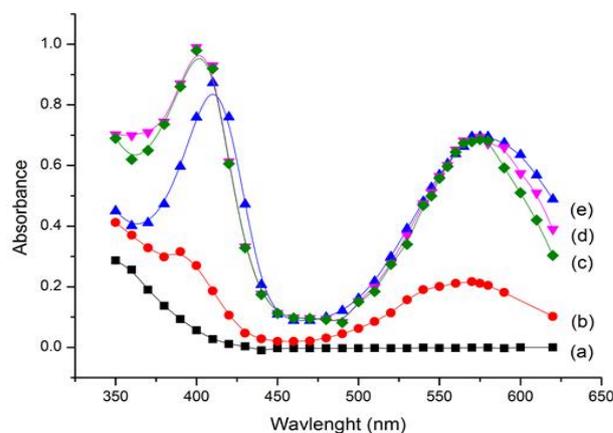


Figure 1. Spectra of reaction product of ninhydrin (6.0×10^{-3} mol dm⁻³) with Gly-Ala (2.0×10^{-4} mol dm⁻³), surfactant (20×10^{-3} mol dm⁻³), pH = 5.0 and temperature = 70 °C in the absence of surfactant immediately after mixing the reactants (a) in the absence of surfactant (b), in the presence of TTAB (c), CTAB (d), and CPC (e), spectra (b) to (e) were recorded after the completion of the reaction

Dependence of Reaction Rate on pH

To find out the sensitivity of the reaction on the pH, the kinetic experiments were performed at pH varying from 4.0 to 6.5 while all other parameters were kept fixed in aqueous as well as in micellar media (Figure 2.).

Table 2. Dependence of pseudo-first order rate constants (k_{obs} or k_{Ψ}) on [Gly-Ala], [ninhydrin] and temperature at pH = 5.0.

| 10^4 [Gly-Ala], mol dm ⁻³ | 10^3 [Ninhydrin], mol dm ⁻³ | Temperature, °C | $10^5 k_{\text{obs}}^a$, s ⁻¹ | $10^5 k_{\Psi}^b$, s ⁻¹ | | | |
|---|---|--------------------|--|-------------------------------------|------|------|------|
| | | | | TTAB | CTAB | CPC | |
| 1.0 | 6.0 | 70 | 13.0 | 60.9 | 61.7 | 30.2 | |
| 1.5 | | | 13.5 | 63.2 | 64.4 | 30.8 | |
| 2.0 | | | 14.1 | 63.9 | 65.1 | 30.2 | |
| 2.5 | | | 14.3 | 61.0 | 62.6 | 29.6 | |
| 3.0 | | | 14.6 | 64.8 | 60.6 | 27.9 | |
| 2.0 | 6.0 | 70 | 14.1 | 63.9 | 65.1 | 30.3 | |
| | | | 10 | 16.5 | 96.8 | 69.1 | 55.2 |
| | | | 15 | 31.5 | 110 | 95.9 | 55.5 |
| | | | 20 | 47.8 | 124 | 96.9 | 81.5 |
| | | | 25 | 45.5 | 122 | 106 | 81.7 |
| | | | 30 | 52.7 | 126 | 123 | 89.0 |
| | | | 35 | 53.5 | 123 | 120 | 85.8 |
| 2.0 | 6.0 | 60 | 3.15 | 18.9 | 14.4 | 7.80 | |
| | | 65 | 8.23 | 33.2 | 49.9 | 17.6 | |
| | | 70 | 14.1 | 63.9 | 65.1 | 30.3 | |
| | | 75 | 20.8 | 101 | 96.9 | 36.7 | |
| | | 80 | 47.9 | 127 | 98.8 | 51.5 | |

^a in the absence of surfactant. ^b in the presence of [surfactant] = 20×10^{-3} mol dm⁻³

It is observed that the optimum pH value is 5.0 and then the reaction rate becomes almost constant. Every elementary reaction of α -amino acids/dipeptides and ninhydrin depends upon the $[H^+]$ because the reaction proceeds through the formation of an intermediate which has Schiff base linkage ($>C=N-$). The product of this reaction also has this type of linkage. Since the Schiff base formation is acid catalysed and pH 5.0 is the optimum pH, all subsequent kinetic runs were made at pH = 5.0 (vide infra).

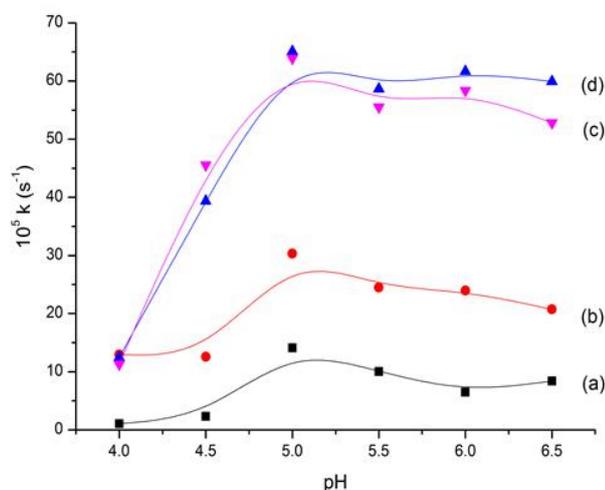


Figure 2. Plots of reaction rate constant vs. pH for the reaction of ninhydrin with Gly-Ala in the absence (a) and presence of [CPC] = 20×10^{-3} mol dm⁻³ (b), [TTAB] = 20×10^{-3} mol dm⁻³ (c), and [CTAB] = 20×10^{-3} mol dm⁻³ (d). Reaction conditions: [Gly-Ala] = 2.0×10^{-4} mol dm⁻³, [ninhydrin] = 6.0×10^{-3} mol dm⁻³, temperature = 70 °C.

Dependence of Reaction Rate on Gly-Ala Concentration

To find the dependence on [Gly-Ala], the reaction was carried out under pseudo-first-order conditions of [ninhydrin] \gg [Gly-Ala] in the range of (1.0×10^{-4} to 3×10^{-4} mol dm⁻³ of [Gly-Ala] at constant [ninhydrin] of 6.0×10^{-3} mol dm⁻³, temperature (70 °C) and pH (5.0). The k_{obs} values are recorded in Table 2. As the values of rate constants (k_{obs} and k_{Ψ}) were found to be independent of the initial concentration of Gly-Ala, the order of reaction with respect to [Gly-Ala] is unity in both the media.

Dependence of Reaction Rate on Ninhydrin Concentration

The effect of ninhydrin concentration was determined by carrying out a series of kinetic experiments at different concentrations of ninhydrin with fixed [Gly-Ala] (2.0×10^{-4} mol dm⁻³), temperature (70 °C) and pH (5.0) constant (Table 2). The plots of rate constants versus [ninhydrin] (Figure 3) give non-linear profile and curved passing through the origin that indicates the order to be fractional with respect to [ninhydrin] in both the media.

Dependence of Reaction Rate on Temperature

A series of kinetic runs were carried out at different temperatures (60 to 80 °C), with fixed reactant concentrations both in the absence and presence of micelles (Table 2). The calculated rate constant values were found to satisfy the Arrhenius and Eyring equations. The activation energy (E_a) resulted from the slope of the lines of Figure 4. The activation enthalpy (ΔH^\ddagger) and activation entropy (ΔS^\ddagger) were calculated using linear least squares regression technique.

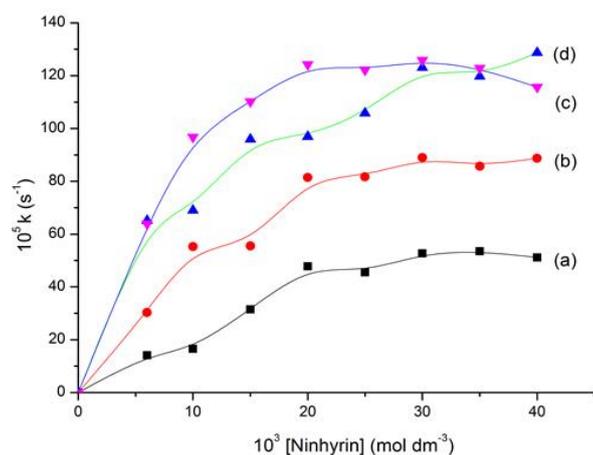


Figure 3. Effect of [ninhydrin] on the reaction rate of ninhydrin with Gly-Ala in the absence (a) and presence of CPC (b), TTAB (c), and CTAB (d). Reaction Conditions: [Gly-Ala] = 2.0×10^{-4} mol dm $^{-3}$, [surfactant] = 20×10^{-3} mol dm $^{-3}$, pH = 5.0, temperature = 70 °C.

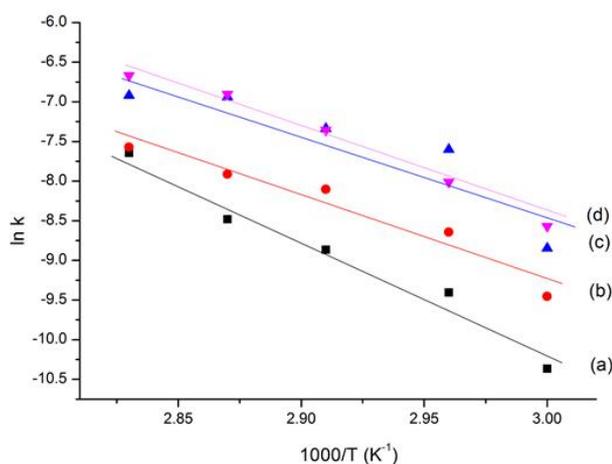
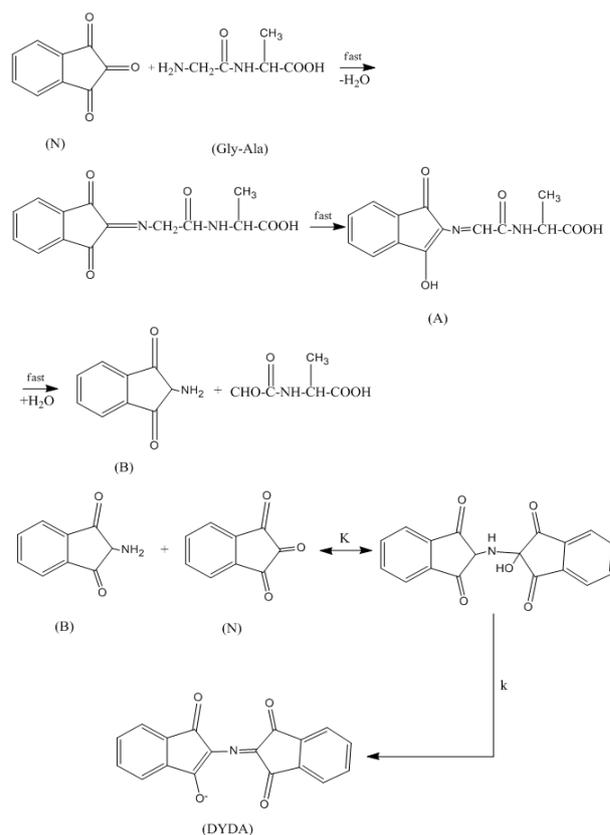


Figure 4. Arrhenius plot for the reaction of ninhydrin with Gly-Ala in the absence (a) and presence of CPC (b), CTAB (c), and TTAB (d). Reaction Conditions: [Gly-Ala] = 2.0×10^{-4} mol dm $^{-3}$, [ninhydrin] = 6.0×10^{-3} mol dm $^{-3}$, [surfactant] = 20×10^{-3} mol dm $^{-3}$, pH = 5.0.

Reaction in Aqueous Medium

On the basis of several studies made on the kinetics of amino acid-ninhydrin (triketohydrindene hydrate) reactions it has been established that the scheme involves oxidation of the amino acid to carbon dioxide and an aldehyde possessing a carbon atom less than the amino acid with the simultaneous reduction of the tri-ketone to hydrindantin and the condensation of the hydrindantin with the ammonia liberated by the oxidation of the amino acid, forming the blue coloured ammonium salt of diketohydrindylidene-diketohydrindamine (DYDA). Further, the amount of the coloured reaction product depends mainly upon temperature, pH and reactant concentrations. In the present case, condensation between carbonyl group of ninhydrin and amino group of Gly-Ala takes place.^{17,18} The reaction starts through the attack of lone-pair of electrons of amino nitrogen (of Gly-Ala) to the carbonyl carbon (of ninhydrin) to give Schiff base A (Scheme 1). This Schiff base is unstable and hydrolyses to give 2-amino indanedione, B, which reacts slowly with another ninhydrin molecule to yield the product P (DYDA).



Scheme 1. Gly-Ala-ninhydrin reaction mechanism

On the basis of the observed rate law $d[P]/dt = k_{\text{obs}} [\text{Gly-Ala}]_{\text{T}}$ and the proposed mechanism (Scheme 1), the following rate equation is derived

$$k_{\text{obs}} = \frac{kK[\text{Nin}]_{\text{T}}}{1+K[\text{Nin}]_{\text{T}}} \quad (1)$$

where $[\text{Nin}]_{\text{T}}$ = total concentration of ninhydrin.

Alternatively,

$$\frac{1}{k_{\text{obs}}} = \frac{1}{k} + \frac{1}{kK[\text{Nin}]_{\text{T}}} \quad (2)$$

which envisages a straight line between $1/k_{\text{obs}}$ and $1/[\text{Nin}]_{\text{T}}$ with a positive slope ($=1/kK$) and an intercept ($=1/k$). Indeed it was found so (Figure 5), and thus confirmed the validity of the proposed mechanism. From the intercept and slope, the respective values of k and K were evaluated, which are: $1.23 \times 10^{-3} \text{ s}^{-1}$ and $6.87 \text{ mol}^{-1} \text{ dm}^3$ respectively, in aqueous medium.

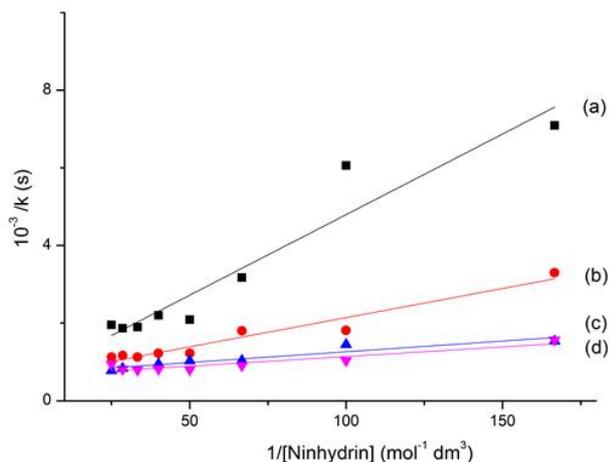
Reaction in the Presence of Surfactant Micelles

To investigate the surfactant concentration effect on the reaction rate, [TTAB], [CTAB], or [CPC] were varied at constant [ninhydrin] (6.0×10^{-3} mol dm $^{-3}$), [Gly-Ala] (2.0×10^{-4} mol dm $^{-3}$) and pH 5.0 at 70 °C (Table 3). The rate constant (k_{ψ}) increased ca.4-5x with increase in [surfactant] from (0 to 30×10^{-3}) mol dm $^{-3}$; then the k_{ψ} decreased noticeably (Figure 6).

Table 3. Effect of [TTAB] on the pseudo-first-order rate constants (k_{Ψ}) for the reaction of ninhydrin with Gly-Ala at pH = 5.0, [Gly-Ala] = 2.0×10^{-4} mol dm $^{-3}$, [ninhydrin] = 6.0×10^{-3} mol dm $^{-3}$ and temperature = 70 °C.

| 10^3 [Surfactant], mol dm $^{-3}$ | $10^5 k_{\Psi}$, s $^{-1}$ | | | $10^5 k_{\Psi cal}^a$, s $^{-1}$ | | | $(k_{\Psi} - k_{\Psi cal})/k_{\Psi}$ | | |
|--|-----------------------------|------|------|-----------------------------------|------|------|--------------------------------------|--------|-------|
| | TTAB | CTAB | CPC | TTAB | CTAB | CPC | TTAB | CTAB | CPC |
| 0 | 14.1 | 14.1 | 14.1 | | | | | | |
| 1.0 | 15.7 | 14.0 | 15.2 | 14.3 | 13.0 | 14.0 | +0.09 | +0.07 | +0.07 |
| 3.0 | 18.7 | 15.3 | 17.6 | 17.6 | 17.9 | 16.4 | +0.06 | +0.07 | +0.06 |
| 5.0 | 26.5 | 34.5 | 18.4 | 23.4 | 31.5 | 17.4 | +0.11 | +0.09 | +0.05 |
| 7.0 | 33.3 | 39.1 | 21.4 | 26.9 | 39.4 | 22.3 | +0.19 | -0.007 | -0.04 |
| 10.0 | 40.5 | 49.9 | 26.8 | 43.9 | 46.8 | 27.1 | -0.08 | +0.06 | -0.01 |
| 12.0 | 43.0 | 52.8 | 27.7 | 50.2 | 50.1 | 29.4 | -0.17 | +0.05 | -0.06 |
| 15.0 | 47.6 | 53.2 | 28.6 | 56.1 | 55.1 | 31.2 | -0.16 | -0.04 | -0.09 |
| 20.0 | 63.9 | 65.1 | 30.3 | 61.1 | 57.7 | 34.9 | +0.04 | +0.11 | -0.15 |
| 30.0 | 59.4 | 63.6 | 32.6 | 62.8 | 62.0 | 38.2 | -0.05 | +0.03 | -0.17 |
| 40.0 | 48.6 | 53.2 | 31.3 | 63.2 | 64.3 | 39.9 | -0.30 | -0.21 | -0.28 |
| 50.0 | 45.3 | 50.3 | 31.2 | 64.3 | 65.3 | 40.2 | -0.42 | -0.29 | -0.29 |
| 60.0 | 36.6 | 49.8 | 30.7 | 64.4 | 68.7 | 42.1 | -0.76 | -0.38 | -0.37 |
| 70.0 | 30.4 | 35.9 | 28.3 | 65.7 | 70.1 | 44.1 | -1.16 | -0.95 | -0.56 |
| 90.0 | 20.9 | -- | -- | 66.3 | -- | -- | -2.17 | -- | -- |
| 100.0 | -- | 32.9 | 28.3 | -- | 72.7 | 46.9 | -- | -1.21 | -0.66 |

^acalculated values using Eq. (3).

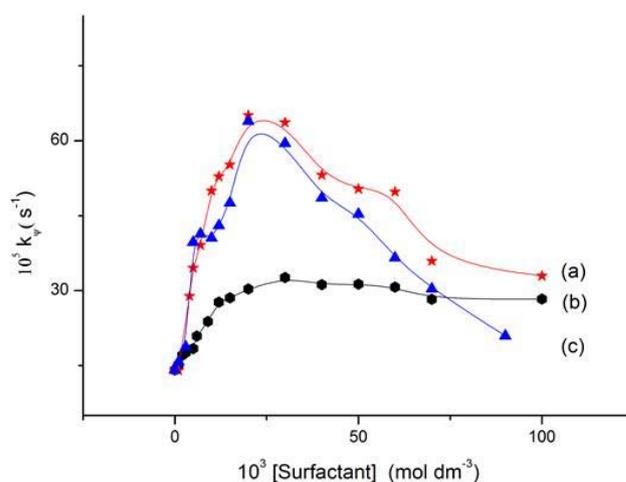
**Figure 5.** Plots of $1/k$ versus $1/[\text{ninhydrin}]$ for the reaction of Gly-Ala with ninhydrin in the absence (a) and presence of CPC (b), CTAB (c), and TTAB (d). Reaction Conditions: same as in Figure 3.

The existence of maximum in the k_{Ψ} – [surfactant] profile shape can be explained by considering that in this case, the reaction takes place in the aqueous as well as in the micellar pseudophases. The increase in rate constant at low surfactant concentrations results in an acceleration of the reaction because the organic substrate incorporates into the micelles and the contribution of the reaction occurring in the small volume of the micellar pseudophase (the so-called Stern layer) increases. However, as [surfactant] increases, a diminution in the Gly-Ala ion concentration in the micellar pseudophase is provoked by the greater number of micellar aggregates present in the reaction media. This effect is the one responsible for the decrease in k_{Ψ} observed at high surfactant concentrations.¹⁹ Another reason for decreasing k_{Ψ} could be a result of counterion inhibition.

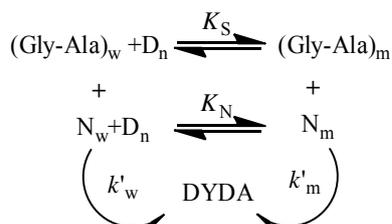
It was mentioned^{20,21} that the head group size of the surfactant is one of the factors that decides the packing of the surfactant monomers into a micelle; if so, we would expect difference of packing of the CPC, CTAB and TTAB

surfactant monomers. Of course, with aromatic pyridinium ring in CPC, there would be delocalization of charge as well as less charge shielding in comparison to CTAB and TTAB. Additionally, there may be an orientational effect. This effect must be taken into account with the effect of side chain (R) of the dipeptide to describe the reaction rate. The nature of k_{Ψ} – [surfactant] profile has been found experimentally similar with rate being CTAB \approx TTAB > CPC (Figure 6).

The same first- and fractional-order kinetics for [Gly-Ala] and [ninhydrin], respectively, was followed in both aqueous and micellar media. Another thing, the absorption band of the product remains unchanged in the presence of TTAB, CTAB, or CPC micelles (Figure 1). Thus, we summarize that the reaction mechanism remains the same in the presence of conventional cationic micelles as that in aqueous medium.

**Figure 6.** Effect of surfactant structure and concentration on the reaction rate for the interaction of ninhydrin with Gly-Ala. Reaction conditions: [Gly-Ala] = 2.0×10^{-4} mol dm $^{-3}$, [ninhydrin] = 6.0×10^{-3} mol dm $^{-3}$, pH = 5.0, temperature = 70 °C, in the presence of CTAB (a), CPC (b) and TTAB (c).

The rate increase for many reactions upon addition of surfactants has been explained on the basis of the following Scheme, proposed by Menger and Portnoy²² and developed by Bunton²³ and Romsted.²⁴



Scheme 2. The pseudo-phase model for the reaction of Gly-Ala with ninhydrin in micellar medium

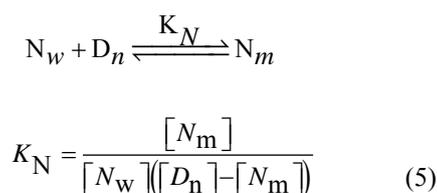
Although several kinetic equations based on this general Scheme 2 have been developed, the most successful appears to be that of Romsted who suggested Equation (3), which takes into account the solubilization of both the reactants into micelles as well as mass action model

$$k_{\Psi} = \frac{k_w [\text{Nin}]_T + (K_S k_m - k_w) M_N^S [D_n]}{1 + K_S [D_n]} \quad (3)$$

where k_w and k_m are the second order rate constants, referring to aqueous and micellar pseudo phases, respectively, K_S is the binding constant of the Gly-Ala to the cationic micelles, and $[D_n] = [\text{surfactant}] - \text{CMC}$. M_N^S is the mole ratio of bound ninhydrin to the micellar head group, given by

$$M_N^S = \frac{[N_m]}{[D_n]} \quad (4)$$

Values of M_N^S were estimated by considering the equilibrium



and the mass balance

$$[\text{Nin}]_T = [N_w] + [N_m] \quad (6)$$

Calculation of k_m and K_S requires CMC under kinetic conditions which has been determined conductimetrically. For a given value of CMC, the k_m and K_S were calculated from Equation (3) using the non-linear least squares technique. Such calculations were carried out at different presumed values of K_N . The best value was considered to be the one for which the value of $\sum d_i^2$ (where $d_i = k_{\Psi \text{ obs}i} - k_{\Psi \text{ cal}i}$) turned out to be a minimum. The fitting of the evaluated data (K_S , k_m and K_N) to Equation 3 is evident from the calculated values of rate constants, $k_{\Psi \text{ cal}}$, recorded in Table 3.

The observed catalysis is due to the increased concentration of both ninhydrin and Gly-Ala in the Stern layer of micelles. Besides this, micelles also exert a medium effect influencing reactivity (the effect arises from a combination of cage, preorientation, microviscosity, polarity and charge effects).²⁵

In order to calculate the dissociation constant of the micellized surfactant back to its components (K_D) and the index of cooperativity (n), the Piszkiwicz model,²⁶ analogous to the Hill model applied for the enzyme-catalysed reactions, was used. In the micellar systems, the value of n reflects the average number of surfactant molecules associated with each substrate molecule. The Piszkiwicz model relates n and K_D and its contribution to the rate is given by

$$k_{\Psi} = \frac{k'_m [D_n]^n + k'_w K_D}{K_D + [D_n]^n} \quad (7)$$

On rearrangement, Equation (7) gives

$$\log \left(\frac{k_{\Psi} - k'_w}{k'_m - k_{\Psi}} \right) = n \log [D] - \log K_D \quad (8)$$

According to Equation (8), a plot of $\log((k_{\Psi} - k'_w)/(k'_m - k_{\Psi}))$ versus $\log[D]$ should be a straight line with a positive slope (n). Such a plot has been realized in the CPC, CTAB, and TTAB catalysis of the present study (Figure 7).

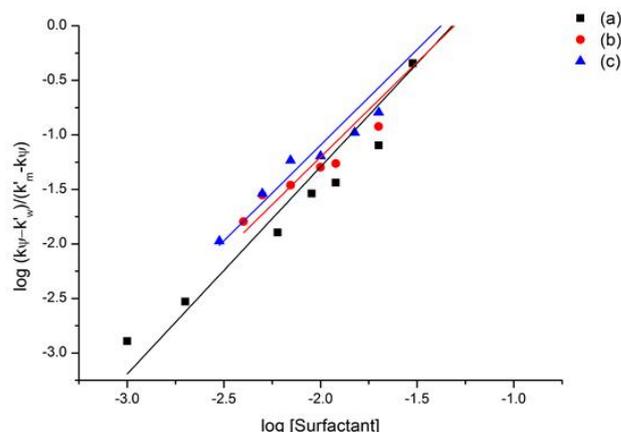


Figure 7. Piszkiwicz plot of $\log(k_{\Psi} - k'_w/k'_m - k_{\Psi})$ vs. $\log[\text{Surfactant}]$. Surfactant = [CPC] (a), [CTAB] (b), and [TTAB] (c). Reaction Conditions: same as in Figure 6.

The K_D and n are: 1.29×10^{-3} , 1.9 (CPC), 3.49×10^{-3} , 1.74 (CTAB), and 1.12×10^{-3} , 1.76 (TTAB), respectively. A value of n greater than unity indicates positive cooperativity, i.e., the binding of the first molecule of the substrate makes it easier for subsequent molecules to bind. The advantage of Equation (8) is that it does not require the knowledge of CMC of surfactant used.

Activation parameters such as activation energy (E_a), enthalpy of activation (ΔH^\ddagger) and entropy of activation (ΔS^\ddagger), are summarized in Table 4. Comparing the values with those obtained in aqueous medium we find that the presence of surfactants lowers the ΔH^\ddagger with a substantial negative ΔS^\ddagger . This lowering occurs not only through the adsorption of both the reactants on the micellar surface but also through stabilization of the transition state. The fitting of the observed k_ψ at different temperatures to the equation was examined and it was found that the Eyring equation is applicable to the micellar media and the sensitivity of micelle structure to temperature is kinetically unimportant. A meaningful mechanistic explanation of the apparent values of ΔH^\ddagger and ΔS^\ddagger is not possible because the k_ψ does not represent single elementary kinetic step; it is a complex function of true rate, binding and ionization constants.

Table 4. Thermodynamic parameters, and k_m , K_S values for the reaction of Gly-Ala and ninhydrin at pH = 5.0 and temperature = 70 °C.

| Parameters and constants | Aqueous | TTAB | CTAB | CPC |
|---|---------|------|------|------|
| E_a (kJ mol ⁻¹) | 127 | 98.1 | 87.9 | 90.0 |
| ΔH^\ddagger (kJ mol ⁻¹) | 124 | 95.3 | 85.1 | 87.2 |
| ΔS^\ddagger (JK ⁻¹ mol ⁻¹) | -306 | -297 | -299 | -305 |
| ΔG^\ddagger (kJ mol ⁻¹) | 216 | 184 | 174 | 178 |
| $10^3 k_m$ (s ⁻¹) | | 0.74 | 8.62 | 5.54 |
| $10^3 k_w$ (mol ⁻¹ dm ³ s ⁻¹) | | 23.5 | 23.5 | 23.5 |
| K_S (mol ⁻¹ dm ³) | | 270 | 214 | 162 |
| K_N (mol ⁻¹ dm ³) | | 45.5 | 59.1 | 61.3 |

Salt Effect

The salt effect on micellar catalysis should be considered in the light of competition between the reactant(s) and counterion for micellar binding sites as well as their effect on the aqueous solubility of substrates. Experimentally, for the title reaction, this effect was explored in the condition of [TTAB] (20×10^{-3} mol dm⁻³), [ninhydrin] (6.0×10^{-3} mol dm⁻³), [Gly-Ala] (2.0×10^{-4} mol dm⁻³), pH (5.0) at 70 °C (Tables 5 and 6). Salts, as additives, in micellar systems acquire a special place due to their ability to modify the systems' properties.²⁷

Table 5. Effect of inorganic salts on pseudo-first-order rate constants (k_ψ) for the reaction of ninhydrin with Gly-Ala at pH = 5.0, [Gly-Ala] = 2.0×10^{-4} mol dm⁻³, [ninhydrin] = 6.0×10^{-3} mol dm⁻³, [TTAB] = 20.0×10^{-3} mol dm⁻³ and temperature = 70 °C.

| [Salt], mol dm ⁻³ | $10^5 k_\psi$, s ⁻¹ | | |
|------------------------------|---------------------------------|---------------------------------|---------------------------------|
| | NaNO ₃ | Na ₂ SO ₄ | Na ₃ PO ₄ |
| 0 | 63.9 | 63.9 | 63.9 |
| 0.05 | 67.9 | 90.4 | 48.2 |
| 0.1 | 98.1 | 82.4 | 23.6 |
| 0.2 | 100 | 62.2 | 13.1 |
| 0.3 | 104 | 48.2 | 11.4 |
| 0.4 | 106 | 45.8 | 8.10 |
| 0.5 | 99.9 | 43.9 | 4.30 |
| 0.6 | 99.4 | 42.9 | 1.42 |
| 0.7 | 105 | 42.3 | 0.08 |
| 0.8 | 98.3 | 41.4 | 0.06 |
| 0.9 | 95.9 | 41.0 | 0.04 |

Table 6. Effect of organic salts on pseudo-first-order rate constants (k_ψ) for the reaction of ninhydrin with Gly-Ala at pH = 5.0, [Gly-Ala] = 2.0×10^{-4} mol dm⁻³, [ninhydrin] = 6.0×10^{-3} mol dm⁻³, [TTAB] = 20.0×10^{-3} mol dm⁻³ and temperature = 70 °C.

| [Salt], mol dm ⁻³ | $10^5 k_\psi$, s ⁻¹ | | | |
|------------------------------|---------------------------------|--------|-------|---|
| | NaSal | NaBenz | NaTos | Na ₂ C ₂ O ₄ |
| 0 | 63.9 | 63.9 | 63.9 | 63.9 |
| 0.5 | 75.5 | 80.2 | 78.9 | 67.4 |
| 1.0 | 95.2 | 112 | 82.3 | 72.6 |
| 3.0 | 82.4 | 106 | 89.2 | 83.5 |
| 5.0 | 76.2 | 97.0 | 95.3 | 76.6 |
| 7.0 | 69.2 | 96.2 | 80.2 | 62.4 |
| 10.0 | 66.3 | 94.6 | 79.8 | 57.5 |
| 20.0 | 61.8 | 90.9 | 81.7 | 48.9 |
| 30.0 | 60.9 | 87.2 | 81.3 | 47.8 |
| 40.0 | 52.1 | 82.1 | 74.8 | 33.1 |
| 50.0 | 50.0 | 62.8 | 74.8 | 30.6 |
| 80.0 | 45.3 | 30.1 | 56.3 | 24.7 |

Figure 8 shows that the rate increases at low concentration of NaNO₃, and then becomes almost constant. However, in Na₂SO₄ a slight increase in the rate is observed, then a decrease which becomes almost constant. At low concentration range, the reactant solubility is affected and they are driven off toward the micellar surface. The increased concentration brings about increase in k_ψ . When the salt concentration is high, the exclusion effect prevails with consequent decrease in k_ψ . As regards Na₃PO₄, it shows a sharp decreasing effect.

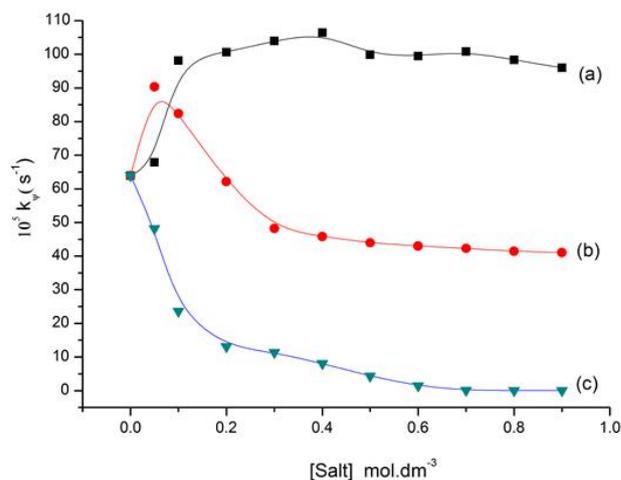


Figure 8. Effect of [inorganic salt] on the reaction rate for the interaction of ninhydrin with Gly-Ala in the presence of surfactant. Reaction Conditions: [Gly-Ala] = 2.0×10^{-4} mol dm⁻³, [ninhydrin] = 6.0×10^{-3} mol dm⁻³, [TTAB] = 20×10^{-3} mol dm⁻³, pH = 5.0, temperature = 70 °C. NaNO₃ (a), Na₂SO₄ (b), Na₃PO₄ (c).

The main reason for this is the change in pH which equals ~ 12 which destroys the buffering effect. On the other hand, the biocompatible hydrophobic salts (the so-called 'hydrotropes') sodium salicylate (NaSal), sodium benzoate (NaBenz), sodium tosylate (NaTos), and sodium oxalate (Na₂C₂O₄) produce marked rate enhancement at low salt concentration, passing through a maximum as the [salt] was increased (Figure 9, Table 6).

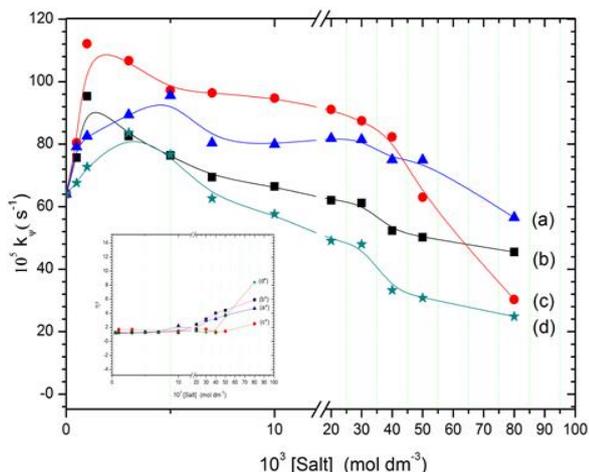


Figure 9. Effect of [organic salt] on the reaction rate (a, b, c, d) and on solution viscosity (a*, b*, c*, d*) (inset) for the reaction of ninhydrin with Gly-Ala in the presence of surfactant. Reaction Conditions: same as in Figure 8. NaSal (a,a*), NaBenz (b,b*), NaTos (c,c*), Na₂C₂O₄ (d,d*).

The addition of these organic hydrophobic salts means that we are adding ionic species having hydrophobic character and, therefore, they can interact with micelles both electrostatically and hydrophobically.²⁸ Therefore, in addition to neutralization of micellar positive charge, they will restrict solubilization sites to hydrophobic substrates. Thus, they catalyse the reaction by virtue of increased concentration of reactants in the Stern layer. The decreased rate observed at relatively higher concentrations of added organic salts is a consequence of the adsorption of hydrophobic anions at the micellar surface and the exclusion of substrate from the micellar surface. The progressive withdrawal of the substrate from the reaction site (micellar surface) would slow down the rate, as was indeed observed. Another factor which could inhibit the rate is the possible micellar growth at higher [salt] as reflected by viscosity data (Figure 9).

In our case the change in morphology from spheroidal micelles to rod-shaped (as inferred by viscosity increase)²⁹ would have certain changes on the characteristics of the micelle. In rod-shaped micelles the counterions bind more tightly and, therefore, suppress the interactions at the micellar surface.

Conclusions

Kinetic experiments between Gly-Ala and ninhydrin have been performed in aqueous and micellar media by studying the reaction spectrophotometrically at 570 nm. We found that the presence of conventional cationic micelles of TTAB, CTAB, and CPC accelerate the reaction and this is supported by comparing the values of activation parameters in both the media. Finally, we can conclude that interaction of Gly-Ala 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 the experimental error.

Acknowledgement

One of the authors (Adel A. M. Saeed) thanks Aden University for providing financial support.

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Received: 22.10.2013
Accepted: 13.12.2013.