Micellar, salt, and organic solvent effects on the rate of [Cu(II)–Gly-L-Ala]$^+$ complex–ninhydrin reaction

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A B S T R A C T

The kinetics of the title reaction has been investigated both in the aqueous and aqueous cationic micelles of tetradecyltrimethylammonium bromide (TTABr) solutions using UV–vis spectrophotometry at 343 K and pH 5.0. Ninhydrin reacts with [Cu(II)–Gly-L-Ala]$^+$ complex in the stoichiometric ratio of 1:1 in both media. To explain the dependence of the reaction rate on [TTABr], pseudo-phase model proposed by Berezin et al. [27] and developed by Bunton [28] and Vera and Rodenas [29] was used. The reaction follows first- and fractional-order kinetics with respect to [Cu(II)–Gly-L-Ala]$_T^+$ and [ninhydrin]$_T$, respectively, in aqueous and TTABr micelles. The values of k_m, k_w, K_A, K_{Nin}, E_a, $\Delta H^\#$, $\Delta S^\#$, and $\Delta G^\#$ have been calculated. The effect of inorganic (NaNO$_3$, Na$_2$SO$_4$, Na$_3$PO$_4$)/organic (NaBenz, NaSal) salts and organic solvents on the reaction rate has also been seen. A mechanism consistent with observed kinetic data has been proposed.

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1. Introduction

Surfactants are an interesting class of materials and it is their dual characteristic (hydrophobic–hydrophilic nature) that makes them so useful and intriguing. Due to this inherent dual character the surfactant molecules self-associate into micelles and other nano-scale structures in aqueous solution. Nowadays, various aspects of human activities and life depend on surfactant applications, such as environmental remediation, human healthcare, and industrial procedures. Such a wide arena of applications has become possible only due to their special amphiphilic nature being soluble in both aqueous and nonpolar media. The addition of a surfactant can accelerate/inhibit a chemical reaction in solution at a concentration higher than its critical micelle concentration so that the reaction can proceed in the environment of surfactant aggregates (micelles) by mimicking the micro-environments of bio-macromolecular ensembles.

Following its discovery by Siegfried Ruhemann in 1910, ninhydrin, or more systematically 2,2-dihydroxy-1,3-indandione, became a great analytical tool used for the identification/quantitative estimation of amino acids, peptides, amino sugars and amines. Comparison with other fingerprint reagents, such as 1,8-diazafluoren-9-one (DFO) [1], fluorescamine and o-phthalaldehyde (OPA) [2,3], ninhydrin is a "universal" tool used in biomedical, bioinorganic, forensic and agricultural fields for detection/estimation of the amine group [4]. However, the chemical reactions involving ninhydrin are complex and their effectiveness depends on the development conditions, such as the reactant concentrations, the nature of medium, and temperature. The reaction of ninhydrin with amino acids/dipeptides forms a purple-colored product known as diketohydrindylidene–diketohydrindamine (DYDA). As the color of DYDA fades at room temperature, many attempts were carried out to stabilize it [5–7]; metal ion coordination being one of such strategies [5,7].

The chemistry of metal ion-coordinated amino acids is of interest in its own right due to applications in metallomics, metalloproteomics [8], pharmacological/biochemistry/forensic/bioinorganic sciences from the viewpoints of (1) evaluating their binding constants and speciation, (2) synthesizing dipeptides and other derivatives [9], (3) models for metalloenzymes and metalloproteins, (4) their role in colorimetric estimation, and (5) increasing stability and sensitivity of detection of amino acids, in general, and latent finger prints, in specific [10]. Copper (as a metal) is essential for all life, but only in small quantities. Copper has received a special attention due to the wide range of complexation with proteins and enzymes which play different roles in biological systems [11,12], such as redox enzymes and hemocyanin.

The effect of solvent on chemical reactions was reported by Oden while the theory to explain solvent effect on the reaction rates was proposed by Hughes and Ingold [13]. It has been suggested that any change in solvent from a polar to non-polar one leads to increased or decreased reaction rates depending on the reaction type [14]. Since the time it was found that addition of acetic acid could improve the sensitivity of ninhydrin reaction [14], many claims, based on changes of composition, solvent and development conditions, to improved ninhydrin reagents have been made.

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Table 1
Critical micelle concentration (CMC) values of TTABr in the absence and presence of reactants [Cu(II)–Gly-L-Ala]$^+$ = 4.0×10^{-4} mol dm^{-3}, [ninhydrin]$_T$ = 10.0×10^{-3} mol dm^{-3}, [TTABr]$_T$ = 20.0×10^{-3} mol dm^{-3} at 303 K and 343 K.

Solution	10^3 CMC (mol dm^{-3})	
	303 (K)	343 (K)
Water	3.900	5.110
Water + 10% DMSO	4.500	8.121
Water + 10% DO	5.648	11.892
Water + 10% AN	6.108	12.561
Ninhydrin	4.320	5.400
Ninhydrin + 10% DMSO	4.692	8.382
Ninhydrin + 10% DO	5.852	12.201
Ninhydrin + 10% AN	6.352	12.987
[Cu(II)–Gly-L-Ala]$^+$	2.770	4.210
[Cu(II)–Gly-L-Ala]$^+$ + 10% DMSO	4.032	7.801
[Cu(II)–Gly-L-Ala]$^+$ + 10% DO	5.468	11.724
[Cu(II)–Gly-L-Ala]$^+$ + 10% AN	6.034	12.473
[Cu(II)–Gly-L-Ala]$^+$ + ninhydrin	3.960	5.240
[Cu(II)–Gly-L-Ala]$^+$ + ninhydrin + 10% DMSO	4.550	8.169
[Cu(II)–Gly-L-Ala]$^+$ + ninhydrin + 10% DO	8.169	12.028
[Cu(II)–Gly-L-Ala]$^+$ + ninhydrin + 10% AN	6.165	12.624

The present studies on [Cu(II)–Gly-L-Ala]$^+$ complex–ninhydrin reaction in surfactant system may find some applications to improve contrast visualization of ninhydrin-developed fingerprints that may prove a step forward from the methods already in use. For this purpose, we have chosen salts, organic solvents and conventional TTABr surfactant to enhance the usefulness of the method.

2. Experimental

2.1. Materials and methods

Copper(II) sulfate pentahydrate (Merck, >99%), glycyl-L-alanine (Gly-L-Ala, Aldrich, >99%), tetradecyltrimethylammonium bromide (TTABr, Sigma, >99%), ninhydrin (Merck, 99%), sodium acetate trihydrate extrapure (s.d. fine, >99%), acetic acid glacial (Merck, >99%), acetonitrile (AN, Merck, >99.0%),1,4-dioxane (DO, Merck, >99.0%), dimethyl sulfoxide (DMSO, Merck, >99.0%), sodium nitrate purified (Merck, 99%), sodium sulphate (Merck, >98%), sodium phosphate (Aldrich, 96%), sodium benzoate (NaBenz, Merck, 99.5%), sodium salicylate (NaSal, CDH, 99.5%), and calcium hydroxide (Sarabhai M. chemicals, >95.0%) were used as supplied.

Stock solutions of Cu(II)–glycyl-L-alanine, ninhydrin and TTABr were prepared in sodium acetate-acetic acid buffer of pH 5.0. Double-distilled and deionized water (specific conductance $(1–2) \times 10^{-6}$ Ω^{-1} cm^{-1}) was used throughout. The pH measurements were made using Systronics MK-VI pH meter in conjugation with a combined electrode (glass-saturated calomel electrode) and standardized using buffer solutions. For the conductivity measurements, a Systronics 306 conductivity meter with platinized electrodes was used. The critical micelle concentration (CMC) values of TTABr surfactant under the reaction conditions were determined at 303 K and 343 K (Table 1). For the viscosity measurements, Ubbelohde viscometer was used at 343 K.

2.2. Job's method of continuous variations

Composition of the product between [Cu(II)–Gly-L-Ala]$^+$ complex and ninhydrin was determined by applying Job's method of continuous variations by heating the reactants at 368 K for 2 h, and measuring their absorbance at λ_{max} (vide infra). The stoichiometry of the complex formed in TTABr micelles/organic solvents seems to be the same as in aqueous medium. It was observed that 1 mol of metal–dipeptide complex associated with 1 mol of ninhydrin in aqueous, micellar and organic solvents to give the reaction product (Fig. 1).

2.3. Kinetic study

The requisite volumes of [Cu(II)–Gly-L-Ala]$^+$ complex and TTABr were taken in a three-necked round-bottomed flask equipped with a double-walled condenser to prevent evaporation. The reaction vessel was then immersed in an oil bath at the desired temperature. Purified N$_2$ gas (free from CO$_2$ and O$_2$) was bubbled through the reaction mixture for stirring as well as to maintain an inert atmosphere. The reaction was initiated by adding standard ninhydrin solution. The formation of the product was monitored periodically by recording absorbance at 340 nm (λ_{max}) (Fig. 2) with the help of a single beam UV–vis spectrophotometer (Shimadzu UV mini-1240, Japan). During all kinetic sets, pseudo-first order conditions were maintained by keeping [ninhydrin]$_T$ in excess. The first-order rate constants in aqueous and micellar systems were calculated up to three half-lives by using the following equation: rate constant = $2.303/t \log (Abs_\infty - Abs_0) / (Abs_\infty - Abs_t)$, where Abs_0, Abs_∞ and Abs_t are the absorbance values initially, at completion of the reaction, and at time t, respectively.

3. Results and discussion

All the UV–vis spectra of the product of ninhydrin and [Cu(II)–Gly-L-Ala]$^+$ were taken after completion of the reaction in the absence and presence of TTABr/TTABr-organic solvents (Fig. 2). Although higher absorbance values were obtained in micellar media/micellar mediated organic solvents, the absorbance maxima of the product remained the same in the presence of micelles/micelles-organic solvents. This indicates that the reaction product is the same in micelles/micelles–organic solvents as that of aqueous solution. The absorbance maximum (λ_{max} = 340 nm) is usually utilized for qualitative and quantitative analyses.

3.1. Rate law

To see the role of copper ion-coordinated glycyl-L-alanine concentration [Cu(II)–Gly-L-Ala]$^+$ (varying from 2.0×10^{-4} to 5.0×10^{-4} mol dm^{-3}) on the reaction rate, the experiments were carried out under pseudo-order conditions by taking [ninhydrin]$_T$ = 10×10^{-3} mol dm^{-3} in excess over [Cu(II)–Gly-L-Ala]$^+$ at 343 K (Table 2, Fig. 3). The kinetics displayed a first order disappearance of [Cu(II)–Gly-L-Ala]$^+$ in the two systems (i.e., aqueous and micellar media) as defined by rate law (Eq. (1)):

$$\text{Rate} = (k_{obs} \text{ or } k_\psi)[\text{Cu(II)-Gly-L-Ala}]_T^+ \tag{1}$$

where k_{obs} or k_ψ is the pseudo-first order rate constant in aqueous and micellar media, respectively, and [Cu(II)–Gly-L-Ala]$_T^+$ is the total concentration of metal–peptide complex. Before proceeding further, the effect of pH on the rate of [Cu(II)–Gly-L-Ala]$^+$–ninhydrin reaction was studied in the pH range 4.0–6.0 at 343 K in the micelle-mediated aqueous medium. It was found that on increasing pH, rate increased up to pH = 5.0 (Fig. 4) and thereafter had no effect on the rate, indicating formation of the Schiff base (>C=N–) [15] in the vicinity of pH 5.0. Consequently, all the subsequent kinetic measurements were made at pH 5.0.

The effects of reactant concentrations (i.e., copper ion-coordinated glycyl-L-alanine and ninhydrin) on the rate constants were also seen. It was found that the rate constants were dependent on [Cu(II)–Gly-L-Ala]$_T^+$ ($2.0–5.0 \times 10^{-4}$ mol dm^{-3}) at constant [ninhydrin]$_T$ (10×10^{-3} mol dm^{-3}), pH 5.0, and temperature 343 K, both in the absence and presence of [TTABr]$_T$ (20×10^{-3} mol dm^{-3}). This confirms the order for [Cu(II)–Gly-L-Ala]$_T^+$ to be unity. However, at different [ninhydrin]$_T$ varying from 6.0 to 40.0×10^{-3} mol dm^{-3} with other experimental conditions constant, the rate of reaction was fractional-order in aqueous and micellar media (Table 2). About three-fold increase in k was observed by increasing [ninhydrin]$_T$ from 6.0×10^{-3}
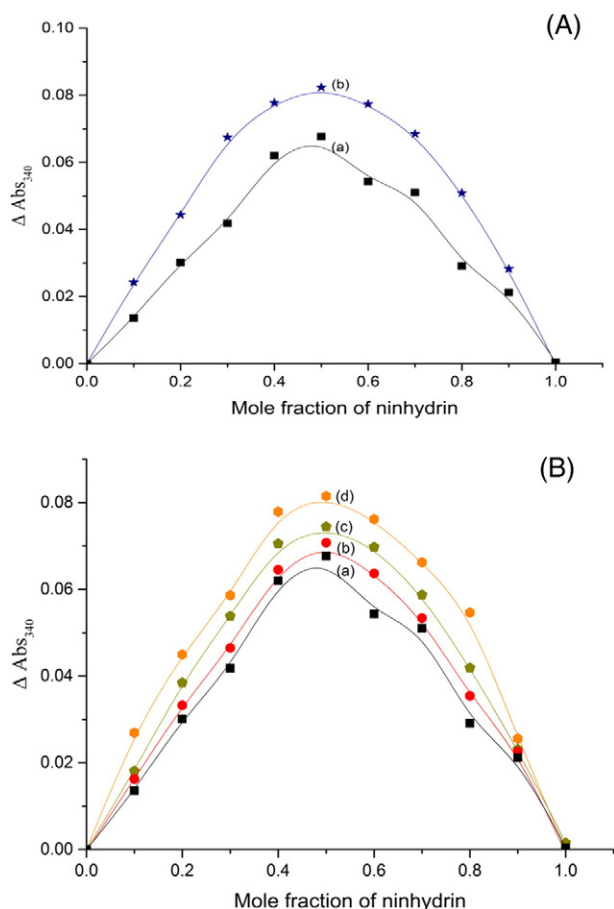
Fig. 1. (A) Plots of ΔAbs_{340} vs. mole fraction of ninhydrin for determination of composition of the product formed by the interaction of [Cu(II)–Gly-L-Ala]$^+$ complex with ninhydrin: in aqueous (a); in the presence of TTABr (b). (B) Plots of ΔAbs_{340} vs. mole fraction of ninhydrin: in aqueous (a); in the presence of DMSO (b); DO (c); AN (d). Reaction conditions: [TTABr]$_T$ = 20.0 × 10^{-3} mol dm^{-3}, 20.0% organic solvent (v/v), pH = 5.0.

to 40 × 10^{-3} mol dm^{-3} at fixed [TTABr]$_T$ (20 × 10^{-3} mol dm^{-3}). A fractional-order in [ninhydrin] is also confirmed by the linearity of log k — log [ninhydrin]$_T$ plot.
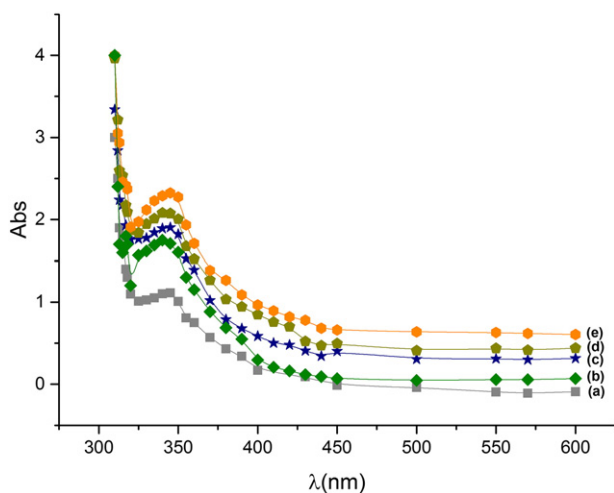

Fig. 2. Absorption spectra of the reaction product of [Cu(II)–Gly-L-Ala]$^+$ complex with ninhydrin in the absence (a) and presence (b–e) of TTABr: (b) in aqueous medium; (c) 10.0% DMSO; (d) 10.0% DO; (e) 10.0% AN. Reaction conditions: [Cu(II)–Gly-L-Ala]$^+$ = 4.0 × 10^{-4} mol dm^{-3}, [ninhydrin]$_T$ = 10.0 × 10^{-3} mol dm^{-3}, [TTABr]$_T$ = 20.0 × 10^{-3} mol dm^{-3}, pH = 5.0, temperature = 343 K.

Table 2
Effect of [Cu(II)–Gly-L-Ala]$^+$, TTABr, and ninhydrin concentrations on the pseudo-first-order rate constants (k_{obs}/k_ψ) for the reaction at 343 K and pH 5.0.

10^4 [Cu(II)–Gly-L-Ala]^+_T (mol dm^{-3})	10^3 [TTABr]$_T$ (mol dm^{-3})	10^3 [ninhydrin]$_T$ (mol dm^{-3})	10^4 k_ψ (s^{-1})
2.0	20.0	10.0	29.8
3.0			30.3
4.0			30.2
4.5			30.2
5.0			30.3
4.0		6.0	20.1
		10.0	30.2
		15.0	35.6
		20.0	44.6
		25.0	52.1
		30.0	63.5
		35.0	62.4
		40.0	65.3
4.0	0	10.0	19.9
	3.0		21.2
	5.0		22.8
	7.0		24.9
	10.0		27.6
	15.0		28.2
	20.0		30.2
	25.0		33.3
	30.0		34.7
	40.0		33.9
	50.0		24.7
	60.0		19.0
	70.0		16.2
	90.0		14.2

3.2. Reaction in the absence of surfactant

It is well known that lone pair electrons of amino group are necessary for nucleophilic attack on the carbonyl group of ninhydrin [16–21]. In the complex ([Cu(II)–Gly-L-Ala]$^+$), this lone pair is not free, and therefore, nucleophilic attack is not possible. The reaction, therefore, proceeds through condensation of coordinated carbonyl group of ninhydrin (Nin) within the coordination sphere of Cu(II). For that reason, the reaction proceeds through the inner sphere complexation on copper(II)–glycyl-L-alanine complex with ninhydrin. The condensation of Gly-L-Ala and ninhydrin with the same copper ion is an example of kinetic template mechanism [22]. Such type of condensation reaction has a feature of a CLAM (Combination-of the-Ligands-Attached-to the same-Metal-ion) reaction and takes place when condensed product acts as a potential tridentate metal binding ONO
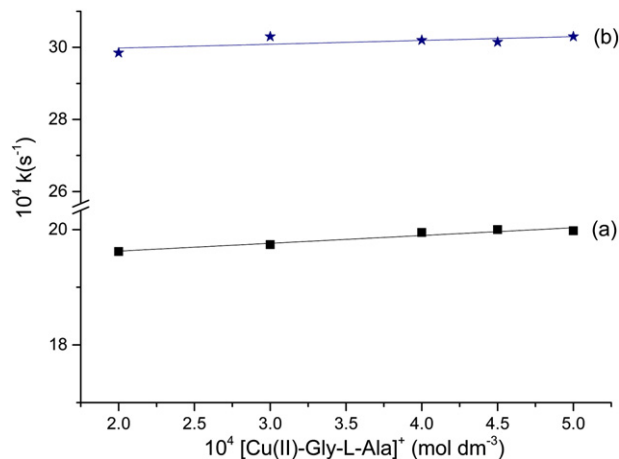

Fig. 3. Plots of rate constants (k) vs. [Cu(II)–Gly-L-Ala]^+_T for the reaction in aqueous (a); in the presence of TTABr (b). Reaction conditions: [TTABr]$_T$ = 20.0 × 10^{-3} mol dm^{-3}, [ninhydrin]$_T$ = 10.0 × 10^{-3} mol dm^{-3}, pH = 5.0, temperature = 343 K.
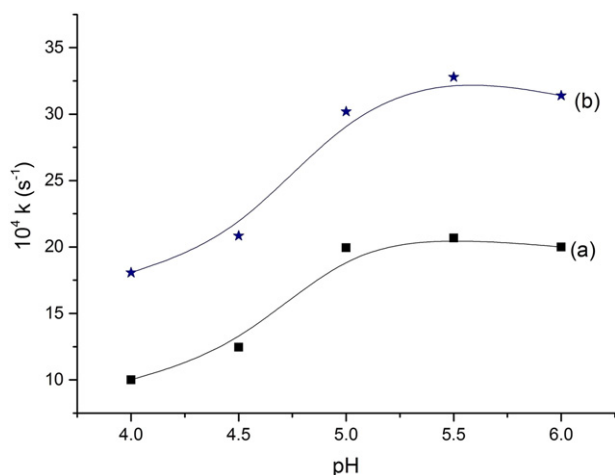
Fig. 4. Plots of rate constants (k) vs. pH variation for the reaction in aqueous (a); in the presence of TTABr (b). Reaction conditions: $[TTABr]_T = 20.0 \times 10^{-3}$ mol dm^{-3}, $[ninhydrin]_T = 10.0 \times 10^{-3}$ mol dm^{-3}, $[Cu(II)-Gly-L-Ala]^+ = 4.0 \times 10^{-4}$ mol dm^{-3}, temperature = 343 K.

donor ligand producing stable-5-membered metal chelates [23]. In order to confirm the cleavage of carboxyl group (–COOH), we have experimentally tested that no carbon dioxide is evolved in the present case. Clearly, Cu(II) inhibits the cleavage of carboxyl group by reducing its escaping tendency and enhancing the electrophilic character of carbonyl group. It seems that the hydrolysis of reaction product is not possible because the lone pair of ninhydrin on carbonyl group is not free during the coordination to the Cu(II). Therefore, the role of Cu(II) in this reaction is to stabilize the end product toward hydrolysis [24].

The mechanism (Scheme 1), proposed on the basis of the above discussion involves two kinetically distinguishable steps: The first step represents the interaction between copper ion-coordinated Gly-L-Ala (A) with anhydrous form of ninhydrin (Nin) to form an inner-sphere complex (B). The second step is the formation of Schiff base (i.e., end product).

With the help of mechanism shown in Scheme 1, the rate equation is given as Eq. (2):

$$d[P]/dt = kK[Nin]_T[Cu(II)-Gly-L-Ala]_T^+ / (1 + K[Nin]_T). \quad (2)$$

On comparing with Eq. (1), Eq. (2) can be written as follows:

$$k_{obs} = kK[Nin]_T / (1 + K[Nin]_T). \quad (3)$$

Eq. (3) can also be written as follows:

$$1/k_{obs} = 1/kK[Nin]_T + 1/k. \quad (4)$$

Accordingly, a plot between $1/k_{obs}$ and $1/[Nin]_T$ yielded straight line with positive intercept (1/k) and slope ($=1/kK$). The respective values of k and K were 9.35×10^{-3} s^{-1} and 21.7 mol^{-1} dm^3. The calculated values of rate constants (k_{cal}), obtained by substituting k and K in Eq. (3), are in close agreement with the k_{obs}, which supports the mechanism and confirms the validity of Eq. (3).

### 3.3. Reaction in the presence of TTABr surfactant

In the presence of TTABr micelles, the reactants may be considered to be distributed in aqueous and in micellar pseudo-phases. Several sets with varying [TTABr] were performed at constant [Cu(II)–Gly-L-Ala]$^+$ (4.0×10^{-4} mol dm^{-3}), [ninhydrin] (10.0×10^{-3} mol dm^{-3}) and pH (5.0) at 343 K (Table 2). The increase in [TTABr] from 0 to 30.0×10^{-3} mol dm^{-3} led to an increase in rate constant from 19.9×10^{-4} s^{-1} to 34.7×10^{-4} s^{-1}. Further increment in [TTABr] had

a decreasing effect on the reaction rate. The [k_ψ–TTABr] profile is in excellent agreement with the results of previous investigations [21,25,26] for monomeric surfactants (Fig. 5). Like aqueous medium, the kinetics showed the first- and fractional-orders in [Cu(II)–Gly-L-Ala]$_T^+$ and [ninhydrin]$_T$, respectively.

The catalytic behavior of TTABr surfactant can be rationalized in terms of pseudo-phase model (Scheme 2) proposed by Berezin et al. [27] and developed by Bunton [28] and Vera and Rodenas [29].

In Scheme 2, S_n ($=$[TTABr] − CMC) is the micellized surfactant, w and m represent the aqueous and micellar pseudo-phases, respectively, and K_A is the binding constant of the [Cu(II)–Gly-L-Ala]$^+$ to the TTABr micelle written in terms of micellized surfactant S_n. The observed rate Eq. (1) and Scheme 2 yield Eq. (5):

$$k_\psi = k'_w + k'_m K_A[S_n]/(1 + K_A[S_n]). \quad (5)$$
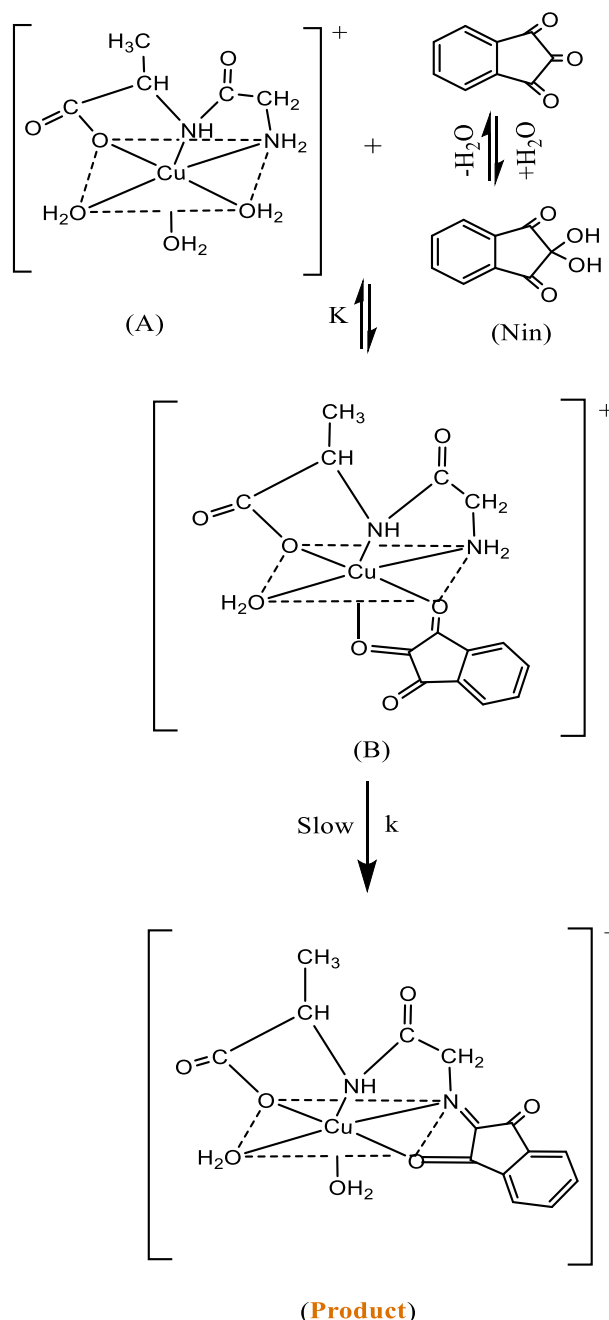
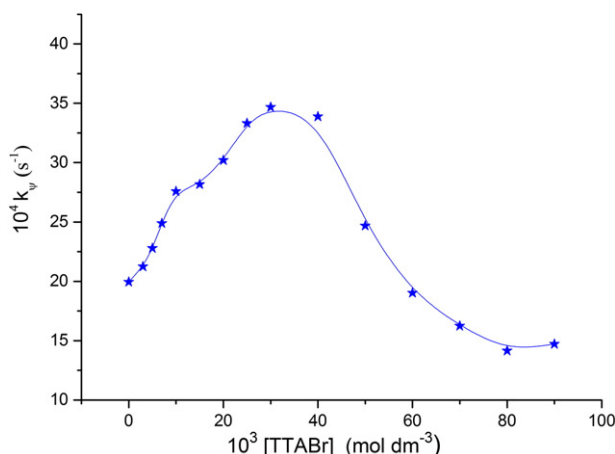**Scheme 1.** Proposed mechanism of the [Cu(II)–Gly-L-Ala]$^+$–ninhydrin reaction.

Fig. 5. Effect of varying TTABr concentration on the rate constant (k_{ψ}) of the reaction. Reaction conditions: $[Cu(II)–Gly\text{-}L\text{-}Ala]_T^+ = 4.0 \times 10^{-4}$ mol dm^{-3}, $[ninhydrin]_T = 10.0 \times 10^{-3}$ mol dm^{-3}, pH = 5.0, temperature = 343 K.

Eq. (5) may be written as follows:

$$k_{\psi} = \left\{ k_w[Nin]_T + (K_A k_m - k_w) M_{Nin}^S [S_n] \right\} / (1 + K_A[S_n]) \quad (6)$$

where $k_w = k'_w/[Nin_w]$ and $k_m = k'_m/M_{Nin}^S$ (k_w and k_m are second-order rate constants) and $M_{Nin}^S = [Nin_m]/S_n$ is the mole ratio of ninhydrin bound to micellar head groups. The best-fit values of k_m and K_A were calculated using a computer based program with the help of non-linear least squares technique. Scheme 2, in conjunction with the mass balance on ninhydrin [30], yield quadratic Eq. (7):

$$K_{Nin}[Nin_m]^2 - (1 + K_{Nin}[S_n] + K_{Nin}[Nin]_T)[Nin_m] + K_{Nin}[S_n][Nin]_T = 0. \quad (7)$$

$[Nin_m]$ was calculated from Eq. (7) with K_{Nin} (the binding constant of the ninhydrin to the TTABr micelle) as adjustable parameter. The CMC values under kinetic conditions are required for the calculation, which were determined by conductivity measurements (Table 1). The evaluated values of rate and binding constants are given in Table 3.

According to the differing properties of micellar pseudo-phase, it is not possible to precisely locate the exact site of the reaction but at least localization of the reactants can be considered. Ionic species can be attracted or repelled by TTABr's micellar surface due to electrostatic force whereas hydrophobic force can bring about the incorporation of the metal complex and ninhydrin into TTABr micelles. The reactant ninhydrin, having π-electrons, enhances the possibility of its partitioning between water and cationic charged micelles [31]. Thus, overall increment of catalytic effect of TTABr micelles is due to the increased concentration of both reactants in the Stern layer. The decrease in rate constant (k_{ψ}) beyond [TTABr] = 30×10^{-3} mol dm^{-3} can be explained as follows. At [TTABr] more than 30×10^{-3} mol dm^{-3}, the reactant has been incorporated into the micellar phase until saturation. When bulk of the substrate is incorporated into the micelles, addition of more TTABr generates more cationic micelles, which simply take up the ninhydrin molecules into the Stern layer, and thereby deactivate them, because a ninhydrin in one micelle should not react with $[Cu(II)–Gly\text{-}L\text{-}Ala]^+$ complex in another [32].

3.4. Thermodynamic parameters

By carrying out kinetic runs at the different temperature (333–353 K) with fixed reactant concentrations, the activation parameters were determined both in the absence and the presence of TTABr micelles. The observed data fitted Arrhenius and Eyring Eqs. (8) and (9):

$$k = A \exp(-E_a/(RT)) \quad (8)$$

$$k = (k_B T/h) \exp(\Delta S^{\neq}/R) \exp(-\Delta H^{\neq}/RT) \quad (9)$$

and

$$\Delta G^{\neq} = \Delta H^{\neq} - T \cdot \Delta S^{\neq} \quad (10)$$

where A = frequency factor, R = gas constant, k_B = Boltzmann constant, and h = Planck constant. The activation energy (E_a) was obtained from the slope of the plot of ln k (y-axis) vs. 1000/T (x-axis). Activation enthalpy ($\Delta H^{\neq}$), activation free energy ($\Delta G^{\neq}$) and activation entropy ($\Delta S^{\neq}$) were calculated using linear least squares regression technique (Table 3). The low activation free energy ($\Delta G^{\neq}$) values in case of TTABr micelles indicate an acceleration of the overall reaction rate by increasing the concentration of transition state, and thus an increase in the reaction rate takes place. Further, a catalytic effect of the micelles through the $[Cu(II)–Gly\text{-}L\text{-}Ala]^+$–ninhydrin interaction is reflected by values of the activation parameters ($\Delta H^{\neq}$, $\Delta S^{\neq}$). The decrease of $\Delta S^{\neq}$ for TTABr shows the formation of well-structured transition state in which reactants are closely oriented in a confined space (i.e., the micellar head group region).
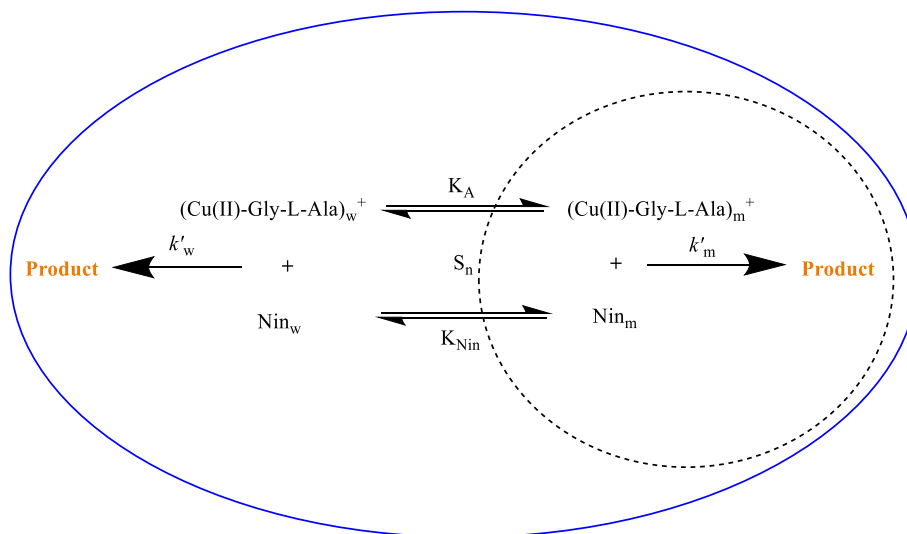
Scheme 2. The pseudo-phase kinetic model for the reaction of $[Cu(II)–Gly\text{-}L\text{-}Ala]^+$ complex with ninhydrin in aqueous and in micellar system.

Table 3
Effect of temperature on the rate for the reaction of $[Cu(II)-Gly-L-Ala]_T^+ = 4.0 \times 10^{-4}$ mol dm^{-3} with $[ninhydrin]_T = 10.0 \times 10^{-3}$ mol dm^{-3} in the absence and presence of $[TTABr]_T = 20.0 \times 10^{-3}$ mol dm^{-3} at pH = 5.0 and values of binding constants and thermodynamic parameters.

Temperature (K)	$10^4 \, k_{obs}$ (s^{-1})	
	Aqueous	TTABr
333	9.29	14.0
338	14.9	21.4
343	19.9	30.2
348	31.8	39.7
353	40.6	48.8
Rate and binding constants		
$10^2 \, k_m$ (s^{-1})[a]	1.9	3.1
$10 \, k_w$ (mol^{-1} dm^3 s^{-1})[a]	2.0	2.0
K_A (mol^{-1} dm^3)[a]	66.0	132.0
K_{Nin} (mol^{-1} dm^3)[a]	64.6	70.6
Parameters		
E_a (kJ mol^{-1})	74.3 ± 0.5	63.1 ± 0.4
ΔH^* (kJ mol^{-1})	71.5 ± 0.5	60.2 ± 0.3
$-\Delta S^*$ (JK^{-1} mol^{-1})	287 ± 5	289 ± 4
ΔG^* (kJ mol^{-1})	158 ± 1	146 ± 2

[a] At 343 K.

3.5. Salt effect

Fig. 6 shows the effect of increasing inorganic/organic salt concentration on the reaction of $[Cu(II)-Gly-L-Ala]^+$ complex and ninhydrin in the presence of TTABr micelles. Salts may modify the substrate–surfactant interaction [33] which can change the rate of the reaction due to change in size and shape [34]. For inorganic salts, the rate increases slightly at low concentration of NaNO$_3$ and then becomes almost constant. However, in Na$_2$SO$_4$ an increase in the rate is observed followed by a slow decrease (Fig. 6A). 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 the rate of reaction. When the salt concentration is high, the exclusion effect prevails with consequent decrease in k_ψ. This view is conceptually related to the different binding models for counter ions [35,36]. However, Na$_3$PO$_4$ does not follow this pattern. This could be due to its acidity effect (pH ≈ 12) which changes the medium to basic and leads to give a red precipitate (i.e., reducing Cu(II) to Cu(I) and destroying the Schiff complex). On the other hand, the hydrophobic salts (the so-called 'hydrotropes'), sodium benzoate (NaBenz) and sodium salicylate (NaSal) produce marked rate enhancement at low salt concentrations, passing through a maximum as the [organic salt] is increased (Fig. 6B). With such hydrophobic salts, penetration of the benzene ring into the micellar palisade layer (a few carbon atoms deep toward core) takes place with the carboxylate group remaining in the outermost region of the micelle (a case of intercalation) [37]. Therefore, in addition to neutralization of micellar surface charge, they restrict interior solubilization of reactants causing an increase in concentration of the latter in the Stern layer; the reaction is thus catalyzed. With further increase in the [organic salt], the above site will be saturated. Once this happens, additional salt will try to get adsorbed at the micellar surface (a case of adsorption) and will thus compete for a site with the reactants (a case of benzoate and salicylate ions' association in the form of adsorption). Consequently, at higher [organic salt] the rate of reaction decreases due to the saturation and adsorption of the Stern layer by hydrophobic anions (i.e., Benz$^-$ and Sal$^-$) which constrain $[Cu(II)-Gly-L-Ala]^+$–ninhydrin reaction in the micellar surface. Another factor which could inhibit the rate is the possible micellar growth at higher [organic salt] as reflected by viscosity data (Fig. 6B, inset).

3.6. Effect of organic solvents

The effect of organic solvents viz., dimethyl sulfoxide (DMSO), 1,4-dioxane (DO), and acetonitrile (AN) on the rate of complex formation was also investigated at fixed $[Cu(II)-Gly-L-Ala]^+$ (4.0×10^{-4} mol dm^{-3}), [ninhydrin] (10.0×10^{-3} mol dm^{-3}), [TTABr] (20.0×10^{-3} mol dm^{-3}), pH (5.0) and temperature (343 K) (Table 4, Fig. 7). Table 1 shows the influence of organic solvents on micellar system as the CMC significantly
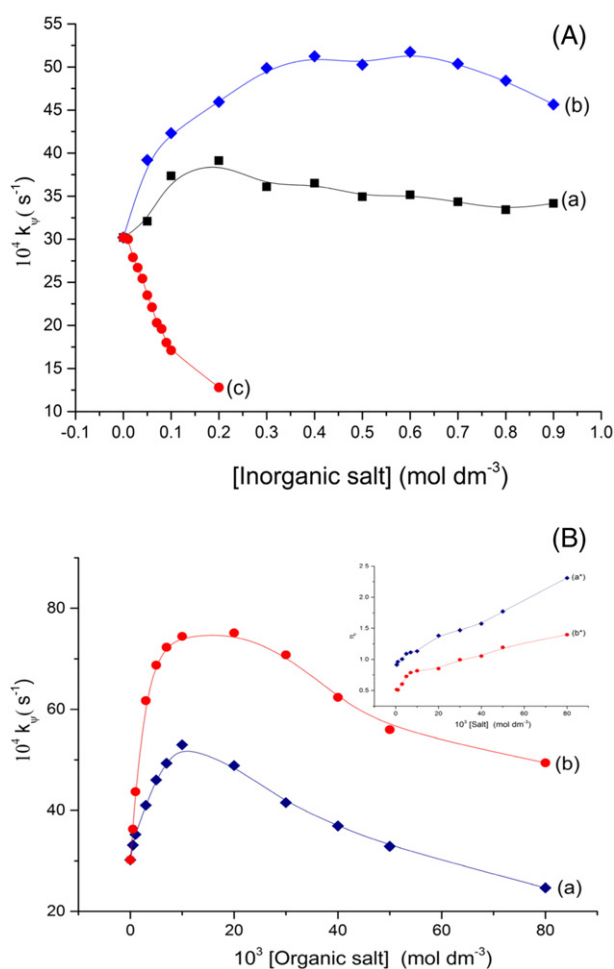
Fig. 6. (A). Effect of [inorganic salt] (NaNO$_3$ (a), Na$_2$SO$_4$ (b), Na$_3$PO$_4$ (c)) on the rate constant (k_ψ) in the presence of TTABr. (B). Effect of [organic salt] (NaSal (a, a*), NaBenz (b, b*)) on the rate constant (a, b) and on solution viscosity (a*, b*) (inset) in the presence of TTABr. Reaction conditions: $[Cu(II)-Gly-L-Ala]^+ = 4.0 \times 10^{-4}$ mol dm^{-3}, $[ninhydrin]_T = 10.0 \times 10^{-3}$ mol dm^{-3}, $[TTABr]_T = 20.0 \times 10^{-3}$ mol dm^{-3}, pH = 5.0, temperature = 343 K.

Table 4
Rate constants (k) for the reaction of $[Cu(II)-Gly-L-Ala]_T^+ = 4.0 \times 10^{-4}$ mol dm^{-3} with $[ninhydrin]_T = 10.0 \times 10^{-3}$ mol dm^{-3} in the absence and presence of $[TTABr]_T = 20.0 \times 10^{-3}$ mol dm^{-3} in aqueous-organic solvent medium at pH = 5.0 and temperature = 343 K.

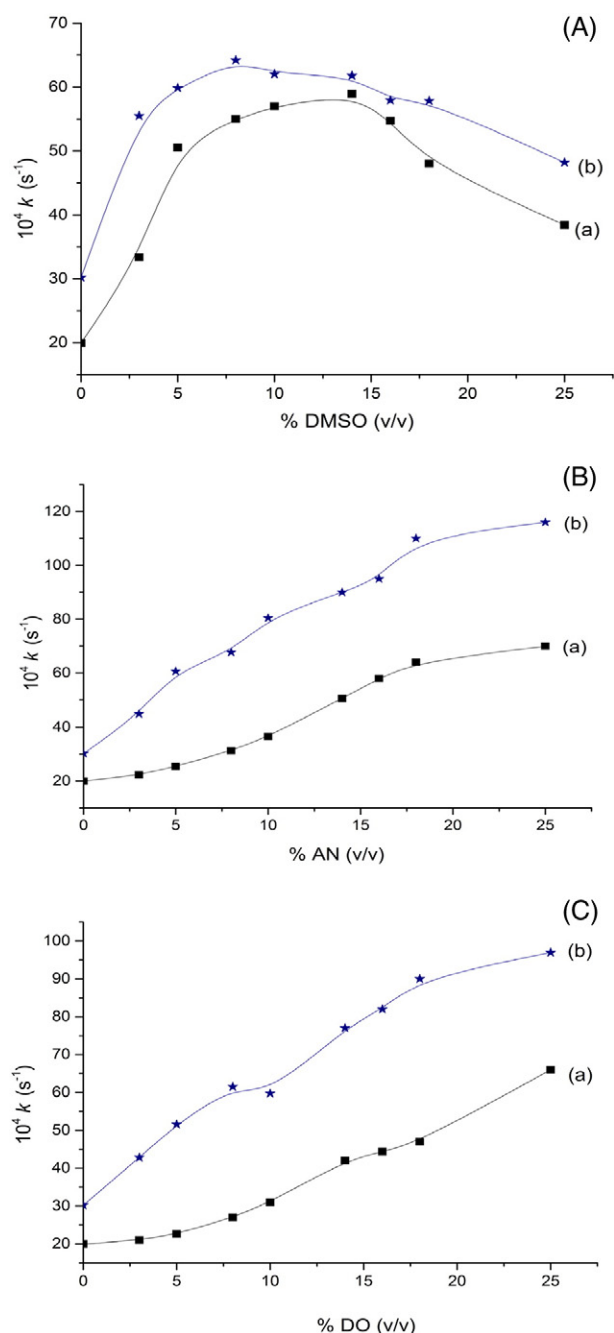| v/v%	(10^4) k (s^{-1})					
	DMSO		AN		DO	
	Aq.	TTABr	Aq.	TTABr	Aq.	TTABr
0	19.9	30.2	19.9	30.2	19.9	30.2
3.0	33.3	55.5	22.2	44.8	21.0	42.8
5.0	50.5	59.8	25.4	60.6	22.6	51.6
8.0	55.0	64.2	31.2	67.7	27.0	61.5
10.0	57.0	62.0	36.5	80.4	31.0	59.7
14.0	58.9	61.8	50.5	90.0	42.0	77.0
16.0	54.7	57.9	58.0	95.0	44.3	82.0
18.0	48.0	57.9	64.0	110.0	46.9	90.0
25.0	38.4	48.2	70.0	116.0	66.0	96.9

Fig. 7. Effect of composition of organic solvents (% v/v): (A) DMSO, (B) AN, (C) DO on the rate constant (k) for the reaction in aqueous (a) and in the presence of TTABr (b). Reaction conditions: $[Cu(II)-Gly-L-Ala]_T^+ = 4.0 \times 10^{-4}$ mol dm^{-3}, $[ninhydrin]_T = 10.0 \times 10^{-3}$ mol dm^{-3}, $[TTABr]_T = 20.0 \times 10^{-3}$ mol dm^{-3}, pH = 5.0 and temperature = 343 K.

increases which means postponement of the micellization. This is due to the tendency of the added organic solvent either to break or make the water structure through solvation of the hydrophobic tail of the surfactant by the hydrocarbon (hydrophobic) part of the organic solvent [38]. However, at low composition, all the three organic solvents have enhanced the rate of the reaction. At high composition, DMSO leads to decrease the rate. This may be regarded to the formation of DMSO–H$_2$O structure which constrains the distribution of the reactants/product in the bulk. For AN and DO no inhibition was recorded due to the relative participation of

water and organic solvents in acid–base equilibria and hydrogen bonding form.

4. Conclusion

The rates of reaction between $[Cu(II)-Gly-L-Ala]_T^+$ (4.0×10^{-4} mol dm^{-3}) and $[ninhydrin]_T$ (10.0×10^{-3} mol dm^{-3}) were determined at 343 K and pH 5.0 in the absence and presence of varying amount of TTABr. By comparing the values with those obtained in aqueous medium, we find that the presence of cationic micelles of TTABr catalyzes the reaction. The value of activation energy (Ea) clearly indicates that TTABr micelles play the role of catalyst and provide 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 the transition state. The effect of salts on micellar catalysis seems to depend on the nature of the salt which could accelerate/inhibit the reaction. Although the presence of TTABr micelles does not bring out any drastic change in the reaction rate, the present study may, however, stimulate and open up a new approach of studying metal ion-coordinated dipeptide–ninhydrin reactions in the presence of surfactants/organic solvents/salts.

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