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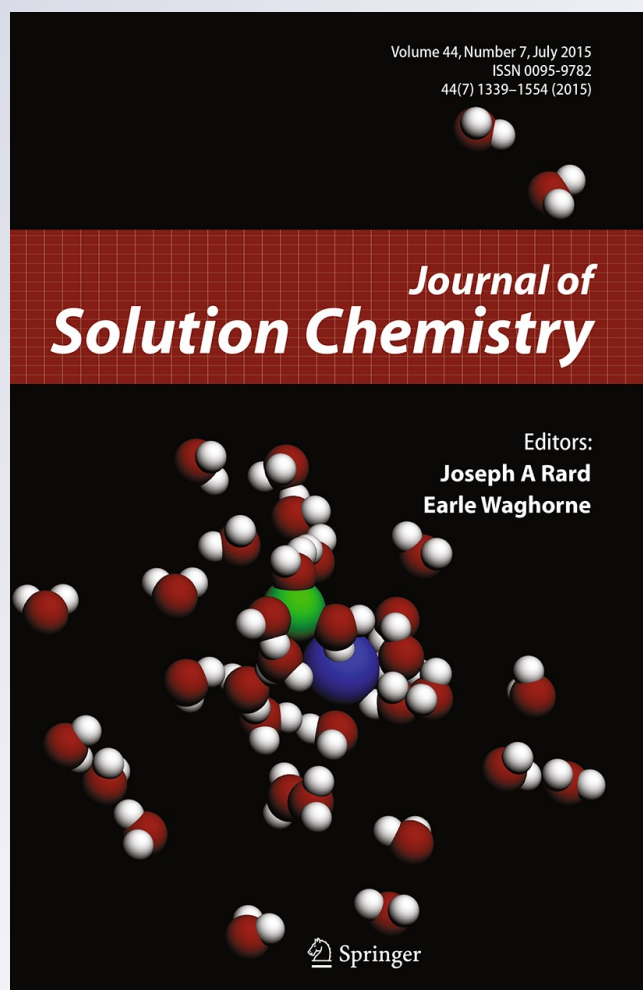
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Catalytic Effect of Cationic Gemini Micelles on the Rate of Condensation between Glycyl-DL-Aspartic Acid and Ninhydrin in the Absence and Presence of Organic Solvents

Mohd. Akram¹ · Adel A. M. Saeed¹ · Kabir-ud-Din^{1,2}

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Abstract The reaction between glycyl-DL-aspartic acid (Gly-DL-Asp) and ninhydrin has been investigated in cationic gemini surfactants [alkanediyl- α,ω -bis (dimethyltetradecylammonium bromide)] (14-*m*-14). The study was carried out as functions of [Gly-DL-Asp], [ninhydrin], solvent (v/v %) and [surfactant] at pH = 5.0 and 70 °C. The reaction followed first-order kinetics in [Gly-DL-Asp] and fractional-order kinetics in [ninhydrin]. The reaction is catalyzed by TTABr/14-*m*-14. Addition of an organic solvent at fixed [surfactant] increases the absorbance as well as the rate of formation of Ruhemann's purple. The results obtained in micellar media are treated quantitatively in terms of pseudophase and Piskiewicz kinetic models. The Eyring equation is valid for the reaction over the range of temperatures used and different activation parameters have been evaluated. The kinetic data have been used to calculate the micellar binding constants K_S for Gly-DL-Asp and K_N for ninhydrin.

Keywords Glycyl-DL-aspartic acid · Ninhydrin · Micellar catalysis · Gemini surfactants · Organic solvents

1 Introduction

The increasing study of chemical kinetics and mechanisms began in the 1930's, but reactions were carried out in moderately concentrated solutions and typically monitored by methods which could not be simply applied to reactions in micelles. However, the situation

✉ Mohd. Akram
drmhakram@gmail.com; drmhakram@rediffmail.com

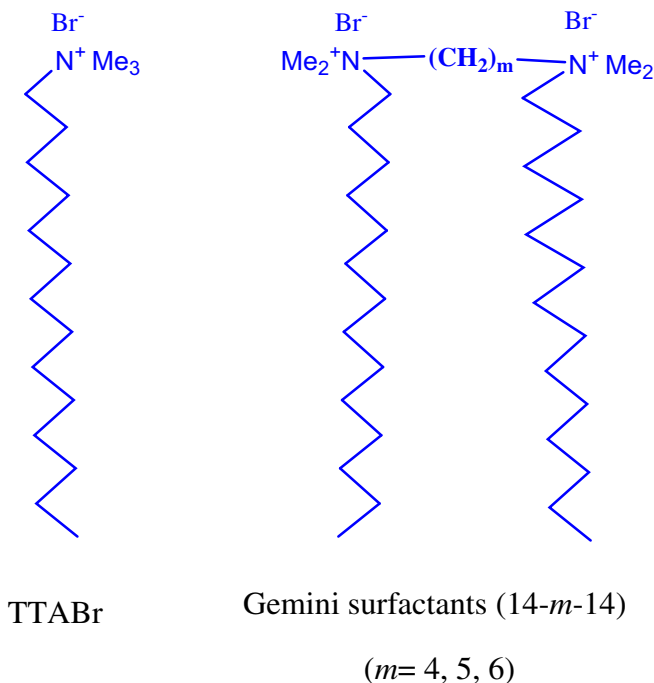
¹ Department of Chemistry, Aligarh Muslim University, Aligarh 202 002, India

² Present Address: Department of Chemistry, Faculty of Natural Sciences, Arba Munch University, Arba Munch, Ethiopia

changed with the introduction of UV–vis spectrophotometers so that the reactions could be followed with very dilute substrate concentration.

Ruhemann [1] suggested the use of ninhydrin for the analysis of amino acids. The reaction of ninhydrin with amino acids/peptides has immense biological significance and serves as a model for several biochemical reactions such as deamination and transpeptidation. Different amino acids/dipeptides (except proline and hydroxyproline) react with ninhydrin with different rates but all produce the same final product, i.e., Ruhemann's purple [2]. The application of ninhydrin for the identification/detection of amino acids/dipeptides has a great importance in disclosing latent fingerprints [3]. The method, though useful, still has much room for improvements. Continuous efforts are, therefore, being made to improve the method [4–6].

Surfactants have uncommon properties and have become an intense subject of investigation in the field of environmental chemistry, biochemistry, industrial chemistry and chemical kinetics [7–9]. The association of many classes of surface-active molecules into micellar aggregates is a well-known phenomenon, and a micellar solution is the dispersion of the aggregates in water/oil media [10]. Micelles are aggregates of surfactant molecules in dynamic equilibrium, constantly disintegrating and reforming [11], and the structure (shape and size) of a micelle depends both on the architecture of the constituent surfactant molecule and the solution conditions such as temperature and presence of additives [12, 13]. Recently, gemini surfactants have attracted considerable attention due to their unique properties [14], such as significantly low cmc, unfamiliar aggregation morphologies, much higher surface activity and high efficiency in decreasing the surface tension of water. A reaction cannot be separated from the medium in which it is performed. A little change can



Scheme 1 The surfactants used in the present study (Me = CH₃)

induce changes in rigidity and surface properties of micelle in surfactant structure, which markedly affect the reactivity of substances [8, 9, 15].

The effect of solvents on chemical reactions is important and it has been suggested that any change in the nature of the solvent, i.e., from a polar solvent to a nonpolar solvent, leads to increase or decrease in reaction rates depending on the type of reaction [16].

Thus, in view of the above, we hereby investigate the interaction of glycyl-DL-aspartic acid with ninhydrin in the absence and presence of TTABr as a conventional surfactant and its related dimeric gemini 14-*m*-14 surfactants (Scheme 1) in aqueous and aqueous–organic solvents.

2 Experimental

2.1 Materials and Methods

2,2-Dihydroxy-1,3-dione (ninhydrin, Merck, 99 %), glycyl-DL-aspartic acid (Sigma, >99 %), sodium acetate anhydrous (Merck, >99 %), acetic acid glacial (Merck, >99 %), 1,4-dibromobutane (Aldrich, >99.0 %), 1,5-dibromopentane (Fluka, >98.0 %), 1,6-

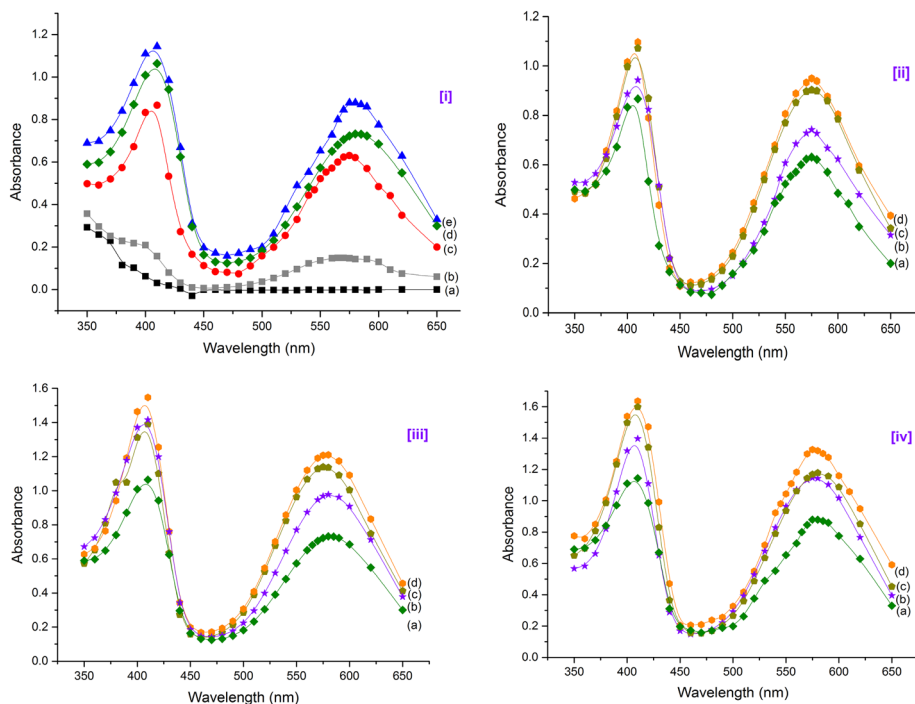


Fig. 1 Spectra of the reaction product obtained by the reaction of ninhydrin ($6.0 \times 10^{-3} \text{ mol} \cdot \text{dm}^{-3}$) with Gly-DL-Asp ($3.0 \times 10^{-4} \text{ mol} \cdot \text{dm}^{-3}$) in buffer ($\text{pH} = 5.0$) at 70°C . **i:** *a, b* in absence of surfactant at zero time (*a*), after completion of the reaction (*b*), (*c–e*) in presence of the surfactant ($50.0 \times 10^{-5} \text{ mol} \cdot \text{dm}^{-3}$) 14-6-14 (*c*), 14-5-14 (*d*), 14-4-14 (*e*). **ii–iv:** *a–d* after completion of the reaction in water (*a*) 20 % DMSO, (*b*) 20 % DO, (*c*) 20 % AN, and (*d*) in the presence of surfactant ($50.0 \times 10^{-5} \text{ mol} \cdot \text{dm}^{-3}$)—**ii** 14-6-14, **iii** 14-5-14, **iv** 14-4-14

dibromohexane (Fluka, >97.0 %), *N,N*-dimethyltetradecylamine (Fluka, >95.0 %), ethanol absolute (Merck, 99.8 %), ethyl acetate (Merck, 99.0 %), hexane (Merck, >95.0 %), acetonitrile (AN, Merck, >99.0 %), 1,4-dioxane (DO, Merck, >99.0 %), and dimethyl sulfoxide (DMSO, Merck, >99.0 %) were used as supplied.

All stock solutions were freshly prepared in acetic acid–sodium acetate buffer of pH = 5.0. The critical micelle concentration (cmc) values of cationic surfactant solutions under different experimental conditions were determined conductimetrically at 70 °C. The measurements were made by a Systronics-306 conductivity meter, using platinized electrodes.

Spectra of the reaction product formed by the reaction between glycyl-DL-aspartic acid and ninhydrin (Nin) were recorded in the absence and presence of surfactants [14-*m*-14] ($m = 4, 5, 6$) ($50.0 \times 10^{-5} \text{ mol} \cdot \text{dm}^{-3}$) with various organic solvents using a SHIMADZU-UV-Vis mini 1240 spectrophotometer (Fig. 1). It is evident that the absorbance of the product is higher in the presence of micelles of 14-*m*-14 surfactants and further increases in the presence of organic solvents with the same absorption maxima ($\lambda_{\text{max}} = 570 \text{ nm}$); this implies that the same final product is formed in both media, i.e., aqueous and micellar. This wavelength of maximum absorption (570 nm) of the product was used to monitor the reaction under different experimental conditions.

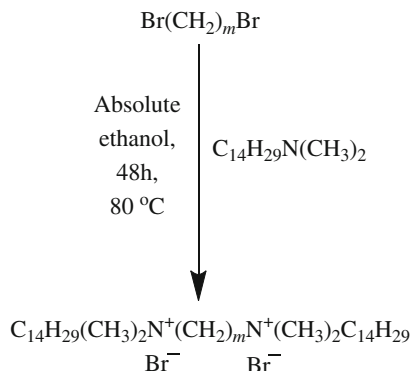
2.2 Synthesis of Gemini Surfactants

The bis(quaternaryammonium) surfactants 14-*m*-14 ($m = 4, 5, 6$) were synthesized as follows. A mixture of *N,N*-dimethylalkylamine and α, ω -dibromoalkane (molar ratio 2.1:1) was refluxed in absolute ethanol with continuous stirring at 80 °C for 48 h to ensure as much as possible a complete bisquaternization (Scheme 2). The progress of the reaction was monitored by using the TLC technique. The observed cmc values as well as the NMR and C, H, N data of the synthesized products were in good agreement to the earlier studies [17, 18].

2.3 Kinetic Measurements

The required solution of dipeptide along with other reagents was taken in a reaction vessel which was then immersed in a thermostated oil bath. The reaction was started by adding the thermally equilibrated ninhydrin solution. A slow stream of pure N₂ gas was passed through the reaction mixture and the progress of the reaction was monitored

Scheme 2 Synthetic route to gemini surfactants 14-*m*-14 ($m = 4, 5, 6$)



spectrophotometrically. The [ninhydrin] was kept in excess (≥ 10 times) in order to maintain pseudo-first-order conditions. Values of pseudo-first-order rate constants (k_{obs} in aqueous and k_{ψ} in micellar media) were obtained from plots of $(\log_{10} [(A_{\infty} - A_0)/(A_{\infty} - A_t)])$ versus time (t) by a least-squares regression analysis of the data.

3 Results and Discussion

At fixed [ninhydrin] (6.0×10^{-3} mol·dm $^{-3}$), temperature (70 °C) and pH (5.0), the kinetics was studied with [Gly-DL-Asp] $_T$ varied from 2.0 to 5.0×10^{-4} mol·dm $^{-3}$ in both media (aqueous, TTABr and 14-*m*-14 micelles). It was found that the order of the reaction with respect to [Gly-DL-Asp] is unity in aqueous and micellar media as the value of the rate constant is independent of the initial [Gly-DL-Asp], (Table 1). Therefore, the kinetics displayed a first order disappearance of [Gly-DL-Asp] as defined by rate law Eq. 1:

$$\text{Rate} = d[P]/dt = -d[\text{Gly} - \text{DL} - \text{Asp}]/dt = k[\text{Gly} - \text{DL} - \text{Asp}]_T \quad (1)$$

where k is the pseudo-first-order rate constant and [Gly-DL-Asp] $_T$ is the total concentration of glycyl-DL-aspartic acid.

To see the role of pH, kinetic runs were performed at different pH varying from 4.0 to 6.5. It is known [19] that the rate at which a Schiff base (see later) is formed is generally high near a pH value of five, and drops at higher and lower pH's. However, for Gly-DL-Asp–ninhydrin reaction at high pH, a slight increase in the rate values is due to the side chain effect of dipeptide which contains carboxylic acid group (H^+ donor) leading to protonation of the OH in the intermediate to allow for its removal as H_2O . At low pH, most of the amine reactant will be tied up as its ammonium conjugate acid and will become non-nucleophilic [20]. Therefore, the detailed kinetic runs were performed at pH = 5.0 keeping other experimental variables constant.

The rate of the reaction, determined at different ninhydrin concentrations $(6.0\text{--}40.0) \times 10^{-3}$ mol·dm $^{-3}$, are summarized in Table 1. The plots of rate constants versus [ninhydrin] $_T$ are non-linear passing through the origin which indicates the order to be fractional with respect to [ninhydrin] $_T$ in both aqueous and micellar media.

To find out dependence of reaction rate on temperature, a series of experiments were carried out at different temperatures with 5 °C interval ranging from 60 to 80 °C in the absence and presence of 14-*m*-14 surfactant micelles (Table 1). The pseudo-first-order rate constants increase with rise in temperature. The values were used to calculate activation parameters (Eyring equation) (Table 2).

3.1 Reaction in Aqueous Media

The reaction has been found to proceed through formation of Ruhemann's purple with the mechanism that follows two main steps: First, the condensation between the carbonyl group of ninhydrin (Nin) and deprotonated amino group of dipeptide (Gly-DL-Asp) takes place. The intermediate Schiff base (A), which hydrolyses fast to give 2-aminoindanedione (B), an aldehyde derivative and carbon dioxide. Second, the reaction of 2-aminoindanedione reacts with another molecule of ninhydrin and gives Ruhemann's purple (Scheme 3).

According to Scheme 3, the rate equation may be given as

$$d[P]/dt = kK[\text{Nin}][\text{Gly} - \text{DL} - \text{Asp}]/(1 + K[\text{Nin}]) \quad (2)$$

Table 1 Dependence of pseudo-first-order rate constants (k_{obs} or k_{ψ}) on [Gly-DL-Asp], [ninhydrin] and temperature

10^4 [Gly-DL-Asp] (mol·dm ⁻³)	10^3 [Ninhydrin] (mol·dm ⁻³)	Temperature (°C)	$10^5 k_{\text{obs}}^{\text{a}}$ (s ⁻¹)	$10^5 k_{\psi}^{\text{b}}$ (s ⁻¹)		
				14-6-14	14-5-14	14-4-14
2.0	6.0	70	4.9	9.8	15.9	28.7
2.5			5.1	10.0	16.0	28.5
3.0			5.3	10.1	16.3	28.6
3.5			5.2	10.3	16.2	28.8
4.0			5.2	10.4	16.3	28.7
3.0	6.0	70	5.3 (5.7)	10.1	16.3	28.6
	10.0		12.1 (12.0)	12.2	17.7	30.1
	15.0		16.2 (15.9)	20.2	24.4	51.4
	20.0		22.7 (22.5)	32.5	40.1	64.4
	25.0		38.6 (38.3)	45.9	51.1	79.2
	30.0		48.3 (49.0)	53.7	60.4	88.7
	35.0		50.9 (51.6)	57.1	65.5	95.5
	40.0		54.4 (55.7)	62.4	73.7	105
3.0	6.0	60	1.3	5.4	6.6	7.81
		65	3.3	7.4	10.9	16.7
		70	5.3	10.1	16.3	28.6
		75	12.0	22.4	28.1	41.1
		80	14.3	31.6	39.2	46.3

Experimental conditions: [14-*m*-14] = 50.0×10^{-5} mol·dm⁻³ (*m* = 4, 5, 6), and pH = 5.0

The values in the parentheses represent the calculated values of k_{obs} using Eq. 3

^a In the absence of surfactant

^b In the presence of [14-*m*-14]

Table 2 Thermodynamic parameter values for the reaction of Gly-DL-Asp and ninhydrin in aqueous and micellar media

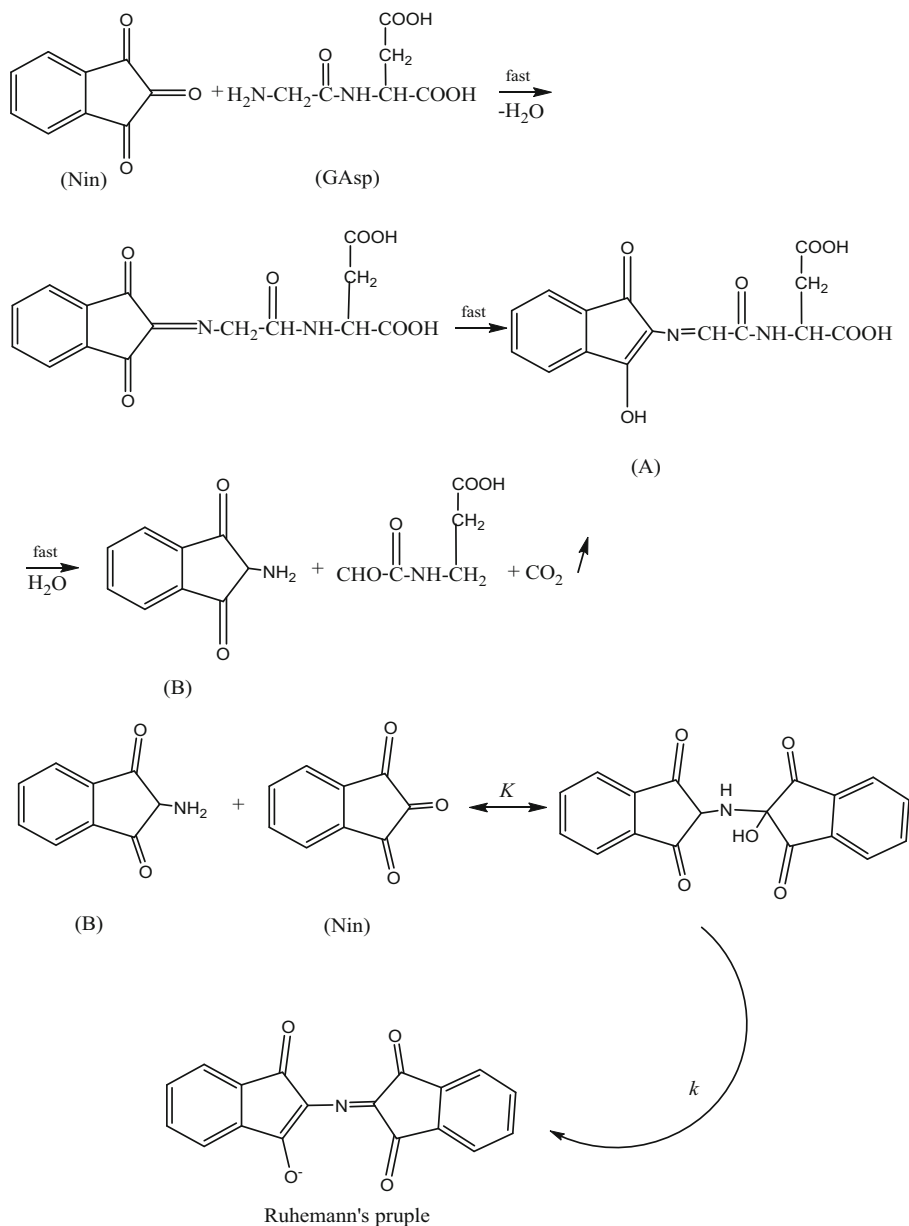
Parameters and constants	Aqueous	14-6-14	14-5-14	14-4-14
ΔS^{\ddagger} (J·K ⁻¹ ·mol ⁻¹)	-256 ± 2	-258 ± 3	-259 ± 3	-60 ± 3
E_{a} (kJ·mol ⁻¹)	117 ± 0.6	89.2 ± 0.7	86.7 ± 0.7	86.5 ± 0.6
ΔH^{\ddagger} (kJ·mol ⁻¹)	117 ± 0.6	89.2 ± 0.7	86.7 ± 0.7	86.5 ± 0.6
ΔG^{\ddagger} (kJ·mol ⁻¹)	211 ± 1	182 ± 0.8	179 ± 0.9	178 ± 0.9

Experimental conditions: [Gly-DL-Asp] = 3.0×10^{-4} mol·dm⁻³, [ninhydrin] = 6.0×10^{-3} mol·dm⁻³, [14-*m*-14] = 50.0×10^{-5} mol·dm⁻³, pH = 5.0, temperature = 70 °C

On comparison of Eq. 2 with Eq. 1, Eq. 3 is obtained:

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

where k_{obs} is the rate constant in aqueous media and [Nin] is the total concentration of ninhydrin.



Scheme 3 Mechanism of the Gly-DL-Asp-ninhydrin reaction

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

Equation 4 envisages linearity between the $1/k_{\text{obs}}$ and $1/[\text{Nin}]$ plots. The values of k ($1.19 \times 10^{-4} \text{ s}^{-1}$) and K (8.45 mol-dm^{-3}) were obtained from the intercept and slope of

such a double reciprocal plot. The values of k and K were resubstituted in Eq. 3 to obtain the calculated values of rate constant (k_{cal}) in various kinetic runs. These values of rate constant in the absence of micellar media (k_{obs}) are parenthesized in Table 1. The close agreement between the observed and calculated values provides supporting evidence for the proposed mechanism.

3.2 Reaction in Micellar Media

The influence of [14-*m*-14] on the reaction rate was investigated by varying surfactant concentrations at constant [Gly-DL-Asp] ($3.0 \times 10^{-4} \text{ mol}\cdot\text{dm}^{-3}$), [ninhydrin] ($6.0 \times 10^{-3} \text{ mol}\cdot\text{dm}^{-3}$) and pH = 5.0 at 70 °C (Fig. 2). It has been found that the pseudo-first-order rate constant (k_{ψ}) increased from $5.3 \times 10^{-5} \text{ s}^{-1}$ to $74.3 \times 10^{-5} \text{ s}^{-1}$ (ca. 14-fold) with increase in [14-4-14] from 0 to $30.0 \times 10^{-3} \text{ mol}\cdot\text{dm}^{-3}$ whereas ca. 4-fold increase occurred with increase in [TTABr] from 0 to $20.0 \times 10^{-3} \text{ mol}\cdot\text{dm}^{-3}$ (Fig. 2 and inset).

At low gemini concentrations (just below their cmc, region I) k_{ψ} increased and then became almost constant; this implies no increase in the reaction rate in region II. The characteristics of regions I and II are the same as with conventional surfactants [21, 22]. The unexpected behavior in region I, as increasing the rate constant values below geminis' cmc, can be understood by considering presence of premicelles in the environment [23]. This result is confirmed by the small cmc values under reaction conditions (Table 3). In region II, the values of the rate constant remain almost constant following the order 14-4-14 > 14-5-14 > 14-6-14 with the same characteristics but much better catalyzing effect than their monomeric counterpart (TTABr).

In the case of region III (Fig. 3) with increasing [gemini], the values of k_{ψ} increase gently and at higher [geminis] a faster increase occurs; this is perhaps due to changes in the micellar structure, which is in conformity with the ^1H NMR studies of the gemini surfactants [24]. Thus, at higher [gemini], an increase in the k_{ψ} value takes place due to

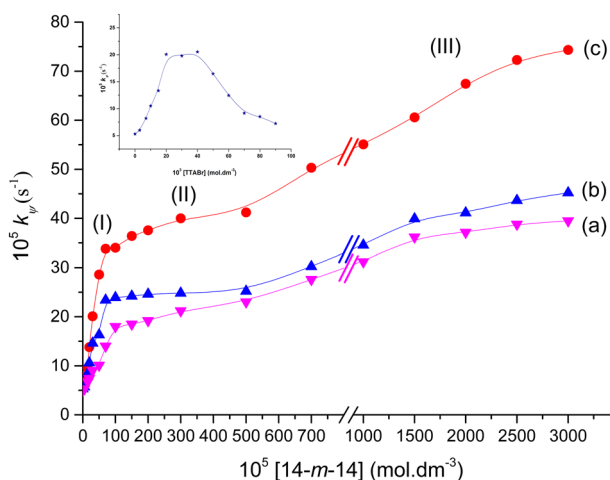


Fig. 2 Influence of [14-6-14] (a), [14-5-14] (b), [14-4-14] (c) and [TTABr] (inset) on the rate of the Gly-DL-Asp-ninhydrin reaction. Experimental conditions: [Gly-DL-Asp] = $3.0 \times 10^{-4} \text{ mol}\cdot\text{dm}^{-3}$, [ninhydrin] = $6.0 \times 10^{-3} \text{ mol}\cdot\text{dm}^{-3}$, pH = 5.0 and temperature = 70 °C

Table 3 The cmc values for 14-*m*-14 systems (*m* = 4, 5, 6) at 70 °C using the electrical conductivity technique in the absence and presence of solvents

Surfactants	10 ³ cmc (mol·dm ⁻³)			
	Absence of solvent	DMSO (20 %)	DO (20 %)	AN (20 %)
14-6-14	0.293	1.3	1.7	2.6
14-5-14	0.278	1.1	1.7	1.9
14-4-14	0.266	1.1	1.1	1.4

Experimental conditions: [Gly-DL-Asp] = 3.0×10^{-4} mol·dm⁻³, [ninhydrin] = 6.0×10^{-3} mol·dm⁻³, pH = 5.0, and temperature = 70 °C

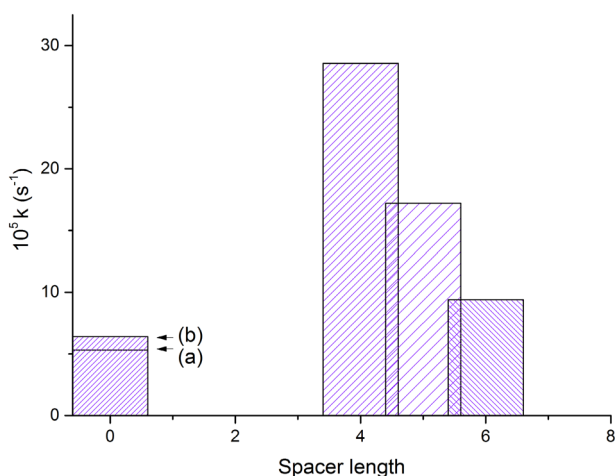


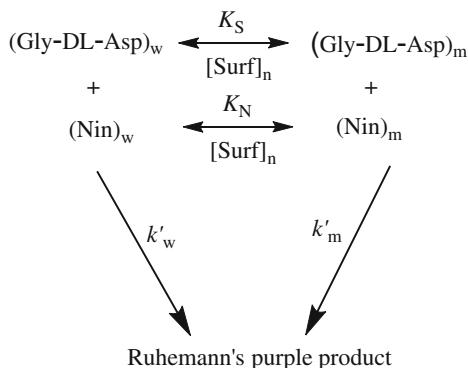
Fig. 3 Spacer length (*m* = 4, 5, 6) effect on the Gly-DL-Asp–ninhydrin reaction in aqueous (a), and in TTABr (b). Experimental conditions: [Gly-DL-Asp] = 3.0×10^{-4} mol·dm⁻³, [ninhydrin] = 6.0×10^{-3} mol·dm⁻³, [TTABr] = 50.0×10^{-5} mol·dm⁻³, [14-*m*-14] = 50.0×10^{-5} mol·dm⁻³, pH = 5.0 and temperature = 70 °C

changes in the aggregate morphology that provides different reaction environments (less polar) [25, 26].

The spacer length (*m*) in 14-*m*-14 series geminis is the key for enhancing the reaction rate by providing different, less polar, microenvironments when decreasing the amount of water in aggregates [24–26]. It is well known that, to minimize its contact with water, a spacer longer than the “equilibrium” distance between two- N⁺Me₂ head groups (the “equilibrium” distance occurs at *m* = 4 in 14-*m*-14 geminis) tends to loop toward the micellar interior [27]. Increased looping will result in a relatively wet Stern layer that will decrease the value of *k*_ψ. Thus, the findings are in agreement with the earlier results that, on increasing the amount of water, the reaction environment produces an inhibiting effect [25].

In order to confirm the proposed mechanism (Scheme 3), the effects of [Gly-DL-Asp], [ninhydrin] and temperature were also studied in the presence of 50.0×10^{-5} mol·dm⁻³ 14-*m*-14 (where *m* = 4, 5, 6). The same first and fractional order kinetics with respect to [Gly-DL-Asp] and [ninhydrin], respectively, were followed. Thus, taking cognizance of the

Scheme 4 The pseudophase model for Gly-DL-Asp–ninhydrin reaction in aqueous/micellar systems



absorption band of the product remaining unchanged in the presence of the surfactant (Fig. 1), we conclude that the reaction mechanism remains the same in the presence of cationic micelles as that in aqueous medium.

The catalytic effect of 14-*m*-14 micelles can be rationalized in terms of the pseudophase kinetic model (Scheme 4) proposed by Menger and Portnoy [28] and modified by Vera and Rodenas [22] and Bunton [29]. According to this model, the reaction occurs in bulk aqueous phase and the micellar pseudophase.

In Scheme 4, k'_w and k'_m are pseudo-first-order rate constants in aqueous and micellar phases, respectively, K_S represents the substrate–micelle binding constant, K_N is the binding constant for ninhydrin, and $[\text{Surf}]_n = \{[\text{Surf}]_T - \text{cmc}\}$. $[\text{Surf}]_T$ stands for the total concentration of surfactant (for the calculation of $[\text{Surf}]_n$, the cmc of surfactant was taken at 70 °C).

The rate law (Eq. 1) and Scheme 3 lead to Eq. 5:

$$k_\psi = (k'_w) + \frac{k'_m K_S [\text{Surf}]_n}{1 + K_S [\text{Surf}]_n} \quad (5)$$

which, when modified, is

$$k_\psi = \left\{ \frac{(k_w [\text{Nin}]_T) + (k_m K_S - k_w) M_N^S [\text{Surf}]_n}{1 + K_S [\text{Surf}]_n} \right\} \quad (6)$$

where k_w and k_m are second-order rate constants in aqueous and micellar phases, respectively, and are equal to $k_w = k'_w / [\text{Nin}_w]$ and $k_m = k'_m / M_N^S$, M_N^S , being the molarity of ninhydrin bound to the micellar head group, given as $M_N^S = [\text{Nin}_m] / [\text{Surf}]_n$, was estimated by considering the equilibrium



$$K_N = [\text{Nin}_m] / \{[\text{Nin}_w] ([\text{Surf}] - [\text{Nin}_m])\} \quad (7)$$

and the mass balance

$$[\text{Nin}]_T = [\text{Nin}_w] + [\text{Nin}_m] \quad (8)$$

Table 4 k_m , k_w , K_S and K_N values for the reaction of Gly-DL-Asp with ninhydrin

Constants	14-6-14	14-5-14	14-4-14
$10^3 k_m \text{ (s}^{-1}\text{)}$	3.5	8.5	13.6
$10^3 k_w \text{ (mol}^{-1}\cdot\text{dm}^3\cdot\text{s}^{-1}\text{)}$	8.8	8.8	8.8
$K_S \text{ (mol}^{-1}\cdot\text{dm}^3\text{)}$	166.0	160.0	150.0
$K_N \text{ (mol}^{-1}\cdot\text{dm}^3\text{)}$	69.8	71.6	75.6

Experimental conditions: $[\text{Gly-DL-Asp}] = 3.0 \times 10^{-4} \text{ mol}\cdot\text{dm}^{-3}$, $[\text{ninhydrin}] = 6.0 \times 10^{-3} \text{ mol}\cdot\text{dm}^{-3}$, $\text{pH} = 5.0$, and temperature = 70°C

For a given value of the cmc, the k_m and K_S values were calculated from Eq. 6 [calculation of k_m (for the micellar phase) and K_S (the substrate–micelle binding constant) requires the cmc under kinetic conditions, which has been determined conductimetrically (Table 3)]. K_N represents the ninhydrin–micelle binding constant. Using the non-linear least-squares technique such calculations were carried out at different presumed values of K_N (Table 4). The larger values of K_S than those of K_N can be explained as due to the presence of the carboxylate group (negatively charged) of Gly-DL-Asp, the substrate strongly associates with the positively charged head group of the micelle. Another reason could be the hydrophobic interaction that can bring the dipeptide into the Stern layer of the micelle.

Due to the various properties of the micellar pseudo-phase, it is not possible to exactly locate the site of the reaction but, at least, the localization of the reactants in the micellar structure can be considered. The micellar pseudophase is regarded as a microenvironment having varying water activity, hydrophobicity and degrees of polarity with distance from the interfacial region to its core [12]. From electrostatic considerations, ninhydrin, due to the presence of π -electrons [3], increases the possibility of partitioning between aqueous and positively charged micelles. On the other hand, hydrophobic interactions can bring about incorporation of Gly-DL-Asp into micelles. Thus, the micelles help in bringing the ninhydrin and the Gly-DL-Asp close together into a small volume, that is, the Stern layer of the micelles (Scheme 5). Therefore, the concentration of the reactants increases, resulting in an increase in the rate constant.

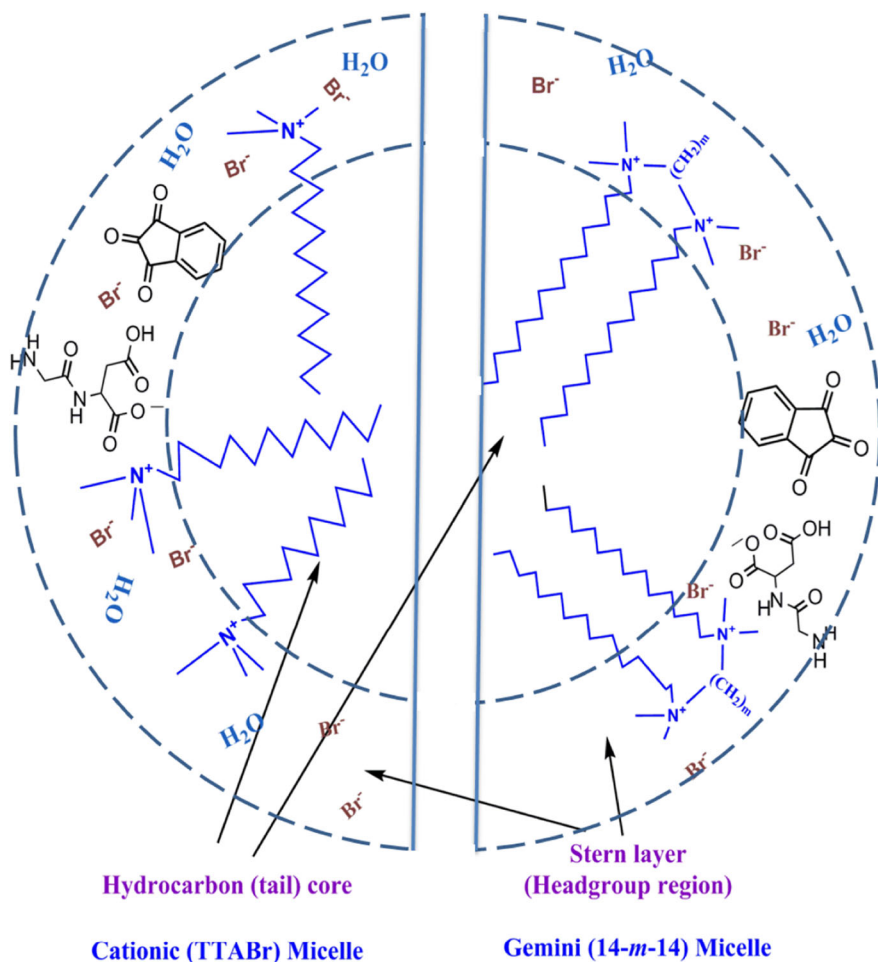
Piszkiwicz's kinetic model [30], which was proposed in analogy to the Hill model [31] for enzyme-catalyzed reactions, was applied to calculate the cooperativity index. The advantage of this model is that it does not require knowledge of the cmc of the surfactant used. Accordingly, the substrate (Gly-DL-Asp) and a number (n) of surfactant molecules (Surf) $_n$, aggregate to form catalytic micelle (Surf) $_n$ –Gly-DL-Asp, which may react to yield Ruhemann's purple product as given by Scheme 6: K_D is the dissociation constant of the catalytic micelle back to its free components. k_m stands for the rate constant for micelle-catalyzed reaction, and k_w is the rate constant for the reaction in the absence of micelle. The rate constant is expressed as a function of concentration of surfactant by:

$$k_\psi = \frac{k_m[\text{Surf}]^n + k_w K_D}{K_D + [\text{Surf}]^n} \quad (9)$$

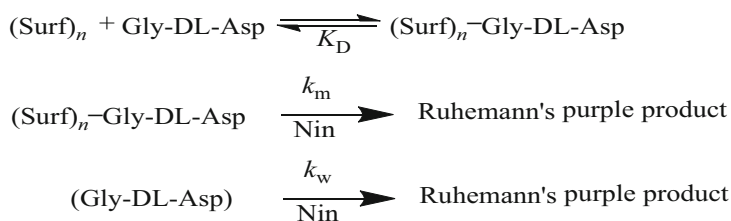
Equation 9 may be rearranged to Eq. 10:

$$\log_{10} \left(\frac{k_\psi - k_w}{k_m - k_\psi} \right) = n \log_{10} [\text{Surf}] - \log_{10} K_D \quad (10)$$

where n describes the stoichiometry of the reaction (known as the cooperativity index). In



Scheme 5 Micellar structure and probable location of reactants in the cationic TTABr/gemini micellar systems



Scheme 6 The Piskiewicz model for the Gly-DL-Asp–ninhydrin reaction

micellar systems, the value of n reflects the average number of surfactant molecules associated with each substrate molecule. From Eq. 10, a plot of $\log_{10}\{(k_\psi - k_w)/(k_m - k_\psi)\}$ against $\log_{10} [\text{Surf}]$ should be a straight line (Fig. 4). The following values of K_D (in

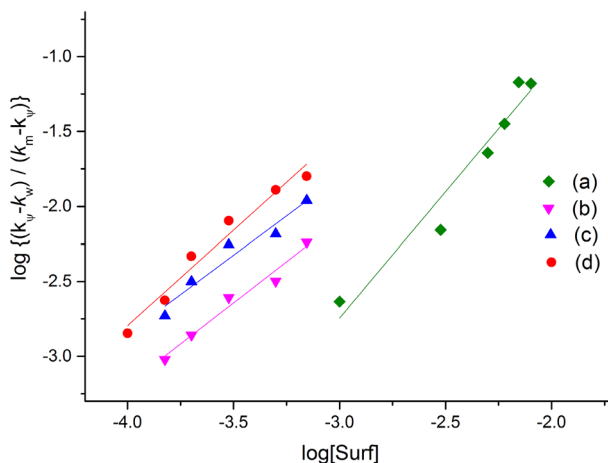


Fig. 4 Piszkwicz plots of $\log_{10} \{(k_{\psi} - k_w)/(k_m - k_{\psi})\}$ against $\log_{10} [\text{Surf}]$ where Surf = TTABr (a), 14-6-14 (b), 14-5-14 (c), and 14-4-14 (d). Experimental conditions: $[\text{Gly-DL-Asp}] = 3.0 \times 10^{-4} \text{ mol-dm}^{-3}$, $[\text{ninhydrin}] = 6.0 \times 10^{-3} \text{ mol-dm}^{-3}$, $[\text{TTABr}] = 20.0 \times 10^{-3} \text{ mol-dm}^{-3}$, $[\text{14-}m\text{-14}] = 50.0 \times 10^{-5} \text{ mol-dm}^{-3}$, pH = 5.0 and temperature = 70 °C

$\text{mol}^{-1}\cdot\text{dm}^3$) and n are: 8.83×10^{-3} , 1.68 (TTABr); 6.20×10^{-3} , 1.10 (14-6-14); 6.31×10^{-3} , 1.05 (14-5-14); and 6.46×10^{-3} , 1.28 (14-4-14), respectively, were determined from the slope and intercept of the line. 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.

3.3 Influence of (v/v %) Organic Solvent

As the absorbance increased with the addition of organic solvents, further investigation was followed on the concentration variation (% solvent (v/v)) with binary solvent systems: water–dimethyl sulfoxide (H_2O –DMSO), water–dioxane (H_2O –DO) and water–acetonitrile (H_2O –AN) to check their effect on the reaction between Gly-DL-Asp and ninhydrin in the absence and presence of 14- m -14 micelles (Fig. 1 [i]–[iv], Fig. 5). Although addition of a non-aqueous solvent suppresses micellization (as reflected in their cmc at 70 °C, Table 3), it has been found that all these solvents accelerate the Gly-DL-Asp–ninhydrin reaction in the absence and presence of surfactants, with the highest enhancement in the presence of 14- m -14 (14-4-14 > 14-5-14 > 14-6-14), while the lowest enhancement was in the absence of surfactant (Fig. 5).

With the increase of solvent volume, the volume of water decreases in a given set of experiments, which results in a decrease of the rate of hydrolysis. This indicates that the side reaction is gradually blocked by the addition of organic solvents. Secondly, Ruhemann's purple is highly soluble in organic solvents thereby resulting in increased intensity. The combined presence of solvents and surfactant micelles show a synergistic effect, which may be due to blockage of side reactions as well as pre-concentration of reactants in the small volume of the micellar surface region.

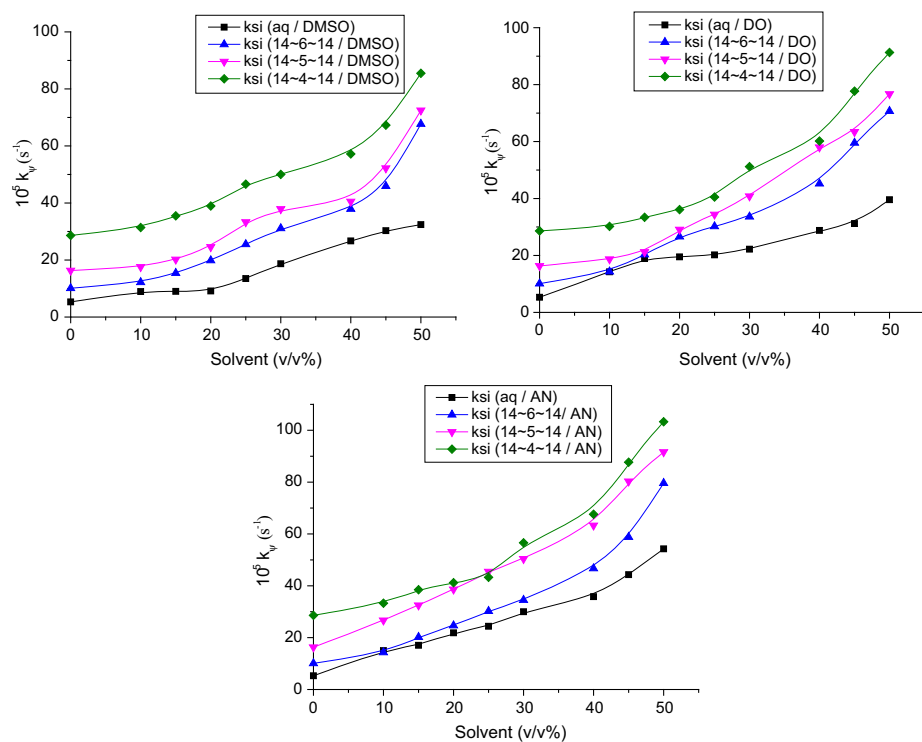


Fig. 5 Influence of organic solvents (v/v %) for the solvents DMSO, DO and AN on the rate of the Gly-DL-Asp-ninhydrin reaction in the absence and presence of 14-*m*-14. Experimental conditions: [Gly-DL-Asp] = 3.0×10^{-4} mol·dm⁻³, [ninhydrin] = 6.0×10^{-3} mol·dm⁻³, [14-*m*-14] = 50.0×10^{-5} mol·dm⁻³, pH = 5.0 and temperature = 70 °C

4 Activation Parameters

The activation energies, evaluated in the case of the gemini surfactants, show the reaction to be catalyzed in the order 14-4-14 > 14-5-14 > 14-6-14. The difference of activation parameters in dicationic 14-*m*-14 micelles as compared to the corresponding aqueous system is as expected (Table 2). This decrease in parameters occurs not only through the stabilization of the transition state but also through adsorption of substrate on the micellar surface. The lower values of E_a suggest that the geminis may act as catalyst and provide a new reaction path with lower activation energy. The decrease in ΔS^\ddagger indicates the formation of a well-structured transition state in which the reactive groups are closely associated with a lesser degree of freedom. The ΔH^\ddagger and ΔS^\ddagger values are associated with the overall rate of reaction. In a complex reaction, each elementary step has its own value of enthalpy and entropy. The observed rate constants are representative of the total reaction rate and are complex functions of true rate, binding and ionization constants. Therefore, for a complex reaction path, a meaningful mechanistic explanation is not possible on the basis of ΔH^\ddagger and ΔS^\ddagger . The fitting of observed k_p at different temperatures to the equation was examined and it was found that Eyring equation is applicable to the micellar media and the sensitivity of micelle structure to temperature is kinetically insignificant.

5 Conclusions

The catalytic effect of 14-*m*-14 gemini surfactants was investigated on the reaction between Gly-DL-Asp and ninhydrin in the absence and presence of organic solvents. The reaction in micellar media was successfully treated using the pseudophase and Piskiewicz models. The binary solvent systems in 14-*m*-14 micelles further increase the rate constant values of the Gly-DL-Asp–ninhydrin reaction. In comparing with monomeric TTABr, a small amount of 14-*m*-14 ($m = 4, 5, 6$) surfactants was sufficient to enhance the reaction rate. This indicates less environmental impact of the smaller surfactant quantity used to carry out the reaction.

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