ZINC DIPEPTIDE COMPLEX ($[ZN(II)\text{-}GLY\text{-}TYR]^+$)–NINHYDRIN REACTION IN PRESENCE OF GEMINI SURFACTANTS: A KINETIC STUDY

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Abstract

Kinetics of the title reaction has been carried out by studying the reaction spectrophotometrically at 80 ºC and pH 5.0 in presence of cationic gemini (alkanediyl-α, ω-bis(dimethylhexadecylammonium bromide)) surfactants. The reaction followed first-order kinetics in $[Zn(II)\text{-}Gly\text{-}Tyr]_T^+$ complex and fractional-order kinetics in $[ninhydrin]_T$. Typical rate constant (k_Ψ) increase and leveling-off regions (like conventional monomeric surfactants, e.g., CTAB) are found with gemini surfactants. Additionally, the gemini surfactants also produced a third region of increasing k_Ψ at higher concentrations. The data are interpreted in terms of *pseudo*-phase model (assuming the association/incorporation of both the reactants at the micellar surface).

Key Words: Micellar catalysis, Zinc-glycyltyrosine complex, Cationic surfactants, Ninhydrin

Introduction

Among natural forces, the hydrophobic-lipophilic effect is one of the most important and necessary forces for the formation of ordered assemblies of amphiphilic molecules, e.g., association colloids, vesicles, biological membranes, etc. [1,2]. Surfactant molecules self associate to form micelles when concentration in a solution reaches a threshold value known as the critical micelle concentration (cmc).

The dimeric (dicationic) gemini surfactants are attracting a lot of interest in several fields (e.g., academic and industrial worlds). They are made up of two amphiphilic moieties connected by a spacer group. Due to their unique properties, they differ from their amphiphilic analogs containing only one head group and one hydrocarbon chain in characteristic features, such as markedly low cmc, high surface activity, low Krafft temperature, unusual rheological properties, multifarious aggregate structures, better wetting ability, and so on.

Protein is an important chemical substance in our life and one of the main targets of all medicines in organism. Confinement of peptides at a surface can induce structure formations and the use of peptidic lipids has been proposed in such a context, aiming at induced structure and functionality of such assemblies that may not be present or may organize differently in free solution [3]. Peptides have been extensively used as a protein model compound recently in the studies of the thermodynamic properties of protein because they contain more complex structures and more components of protein than amino acids. Amino acid side chains of proteins also play essential roles in molecular recognition, information transfer and catalysis.

Ninhydrin is a well known fingerprint-developing agent with interesting forensic, biochemical, pharmacological, and biomedical properties, is extensively used for the identification/detection of amino acids, peptides, amines, and amino sugars in analytical chemistry [4]. Metal-amino acid complexes have been studied in detail from the view points of (1) evaluating their binding constants and speciation, (2) their role in colorimetric estimation, (3) models for metalloproteins, and (4) increasing stability and sensitivity of detection of amino acids, in general, and latent finger prints, in particular [5]. The study of the condensation reactions of the dipeptide-metal complexes with ninhydrin in the presence of micelles may be a better model than studies in water from which to draw conclusions concerning the mechanism of the development of latent fingerprints by ninhydrin. The mechanism of action of an enzyme during the development of latent fingerprints was not ascertained. One possibility is that the enzyme produces amino acids by hydrolysis of proteins present in the fingerprint [6].

The investigation, therefore, concerns the reaction between $[\mathrm{Zn(II)\text{-}Gly\text{-}Tyr}]^+$ complex and ninhydrin in gemini micellar media.

Experimental

N, *N*-dimethylhexadecylamine (Fluka, >95.0%), 1,6-dibromohexane (Fluka, >97.0%), 1,5-dibromopentane (Fluka, >98.0%), 1,4-dibromobutane (Fluka, >98.0%), ethyl acetate (HPLC and spectroscopy grade, 99.0%) and ethanol absolute (Merck, 99.8%) Zinc sulfate heptahydrate (Merck, 99.0%), Gly-Tyr (SRL, 99.0%), ninhydrin (Merck, 99.0%), acetic acid (Merck, 99.0%), sodium acetate (Merck, 99.0%), were used as received. Distilled and deionized water was used throughout. The acetate buffer of pH 5.0 was prepared by mixing 30 $\mathrm{cm^3}$ of 0.20 mol $\mathrm{dm^{-3}}$ acetic acid and 70 $\mathrm{cm^3}$ of 0.20 mol $\mathrm{dm^{-3}}$ sodium acetate [7]. This buffer solution was used as a solvent for preparing all stock solutions. A LI-digital ELICO 122 pH meter in conjunction with a combined electrode was used for pH measurements.

The dimeric gemini surfactants (alkanediyl-α,ω-bis(dimethylhexadecylammonium bromide)) was synthesized by refluxing the corresponding α, ω-dibromoalkanes (s = 4, 5, 6) with *N*, *N*-dimethylhexadecylamine (molar ratio 1:2.1) in dry ethanol with continuous stirring at 80 ºC for 48h. The progress of the reaction was monitored using the TLC technique. Finally, the solvent was removed under vacuum from the reaction mixture and the solid thus obtained was recrystallized several times from ethyl acetate to obtain the compound in a pure form. After recrystallizations, the surfactant was characterized by ^{1}H NMR. All the values obtained were satisfactory, which indicate that the surfactants are well purified [8].

Solutions of the reactants (1:1 molar) were taken in a graduated standard flask, boiled for 2 min, and heated in a controlled manner at 90 ºC for 2h. After completion of the reaction, the flask was brought to room temperature and loss in volume, if any, was maintained by the buffer (pH = 5.0). As a results, a colored complex was formed, which was then stored in dark. This Zn(II)-glycyltyrosine complex was used for further kinetic experiments.

All the UV-visible spectra (Fig. 1) of the solution were recorded after the completion of the reaction between $[\mathrm{Zn(II)\text{-}Gly\text{-}Tyr}]^+$ (3.0×10^{-4} mol $\mathrm{dm^{-3}}$) and ninhydrin (6.0×10^{-3} mol $\mathrm{dm^{-3}}$) in aqueous and gemini micellar media. The absorbance increases with increase in surfactant concentration. No shift in $\lambda_{\max}$ (400 nm) was detected in presence of micelles. The results show that the reaction product is same in presence of micelles as that of aqueous solution. In order to determine the composition of the reaction product formed, Job's method of continuous variations was employed in the presence of gemini micelles. It is found that one mole of ninhydrin reacts with one mole of $[\mathrm{Zn(II)\text{-}Gly\text{-}Tyr}]^+$ complex to give the product.

A 250 ml, three-necked, round-bottomed flask equipped with a double surface water condenser to prevent evaporation and N_2 inlet tube, containing $[\mathrm{Zn(II)\text{-}Gly\text{-}Tyr}]^+$, surfactant and solvent were taken, immersed in an oil-bath thermostated at desired temperature. The reaction was charged with addition of required volume of thermally equilibrated ninhydrin solution. The progress of the reaction was followed spectrophotometrically by pipetting out aliquots at definite time intervals and measuring the absorbance of the reaction product at 400 nm (Fig. 1) using UV-vis spectrophotometer. The *pseudo*-first-order rate constants (k_ψ) were calculated up to 80% completion of the reaction by using a computer program [9].

The cmc values of surfactants in the absence and presence of reactants were obtained from the break points of nearly two straight lines of the specific conductivity *vs.* [surfactant] plots [10]. The experiments were carried out at 30 ºC and 80 ºC under different experimental conditions, i.e., solvent being water, water + ninhydrin, water + $[\mathrm{Zn(II)\text{-}Gly\text{-}Tyr}]^+$, water + ninhydrin + $[\mathrm{Zn(II)\text{-}Gly\text{-}Tyr}]^+$ and respective cmc values are:

(a) 16-6-16 ($\times 10^3$): 0.043, 0.039, 0.046 and 0.048 (at 30 ºC); 0.058, 0.060, 0.057 and 0.054 mol $\mathrm{dm^{-3}}$ (at 80 ºC).

(b) 16-5-16 ($\times 10^3$): 0.034, 0.033, 0.047 and 0.048 (at 30 ºC); 0.055, 0.053, 0.057 and 0.053 mol $\mathrm{dm^{-3}}$ (at 80 ºC).

(c) 16-4-16 (x 10^3): 0.032, 0.031, 0.041 and 0.039 (at 30 ºC); 0.043, 0.044, 0.049 and 0.050 mol dm^{-3} (at 80 ºC).

Results and Discussion

To see the effect of pH on the rate, kinetic runs were carried out under varying condition of pH from 4.0 to 6.0, at fixed [ninhydrin] = 6.0 x 10^{-3} mol dm^{-3}, $[Zn(II)\text{-}Gly\text{-}Tyr]^+$ = 3.0 x 10^{-4} mol dm^{-3}, temperature = 80 ºC and [gemini] = 30 x 10^{-5} mol dm^{-3}. It was found that the value of rate constant increases up to pH = 5.0 and then becomes almost constant. So, all subsequent kinetic measurements were made at pH = 5.0.

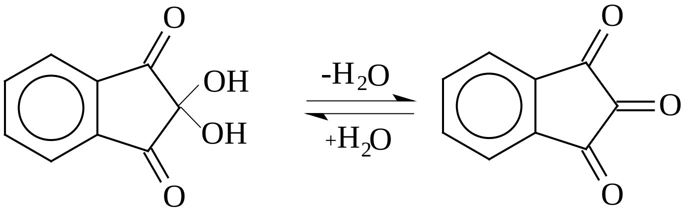
In order to understand the effect of $[Zn(II)\text{-}Gly\text{-}Tyr]^+$ on rate, the experiments were carried out under *pseudo*-first-order conditions of [ninhydrin] >>$[Zn(II)\text{-}Gly\text{-}Tyr]^+$ in the range of 2–4 x 10^{-4} mol dm^{-3} of $[Zn(II)\text{-}Gly\text{-}Tyr]^+$ at constant [ninhydrin] of 6 x 10^{-3} mol dm^{-3}, [surfactant] of 30 x 10^{-5} mol dm^{-3} and an analytical pH 5.0 at 80 ºC (Table 1). The rate constant was invariant with $[Zn(II)\text{-}Gly\text{-}Tyr]^+$ which follows first-order kinetics in $[Zn(II)\text{-}Gly\text{-}Tyr]^+$ complex (Eq. (1)),

$$\frac{d[P]}{dt} = k_{\Psi}[Zn(II)\text{-}Gly\text{-}Tyr]^{+} \qquad (1)$$

The effect of [ninhydrin] upon the rate of formation of product was studied within the [ninhydrin] range of 6 x 10^{-3} mol dm^{-3}–40 x 10^{-3} mol dm^{-3} at 80 °C and in the presence of 30 x 10^{-5} mol dm^{-3} surfactant and 3 x 10^{-4} mol dm^{-3} $[Zn(II)\text{-}Gly\text{-}Tyr]^+$ (Table 1). The rate constants–[ninhydrin] profiles pass through the origin which indicates fractional-order kinetics in [ninhydrin].

On the basis of the above results and previous observations, the mechanism shown in Scheme 1 has been proposed for the reaction of $[Zn(II)\text{-}Gly\text{-}Tyr]^{2+}$ complex with ninhydrin. The lone pair electrons of amino group are necessary for nucleophilic attack on the carbonyl group of ninhydrin [11]. In complex, the lone pair is not free, therefore, nucleophilic attack is not possible. The reaction, therefore, proceeds through condensation of coordinated amino group to the coordinated carbonyl group within the coordination sphere of zinc(II). The coordination of both ninhydrin and Gly-Tyr occurs with the same zinc metal ion (template mechanism [11]) (Scheme 1).

Activation parameters are believed to provide useful information regarding the environment in which chemical reactions take place. In order to learn more about the micro-environments of submicroscopic assemblies, the effect of temperature on reaction rate, a series of kinetic runs were carried out within the temperature range 70 to 90 ºC at fixed [ninhydrin] (6.0 x 10^{-3} mol dm^{-3}), $[Zn(II)\text{-}Gly\text{-}Tyr]^+$ (3.0 x 10^{-4} mol dm^{-3}) and pH (5.0) in the gemini micellar media (16-*s*-16 = 30 x 10^{-5} mol dm^{-3}, *s* = 4, 5, 6). The values of activation parameters were evaluated with the help of linear least squares regression technique (Table 1).



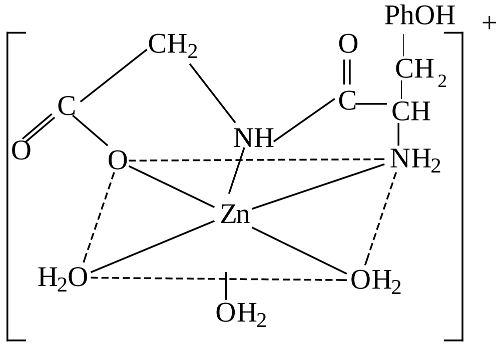
Nin

$$[Zn(II)\text{-}Gly\text{-}Tyr]^{+} + Nin \overset{K}{\rightleftharpoons} [Zn(II)\text{-}Gly\text{-}Tyr]^{+}\text{–}Nin$$

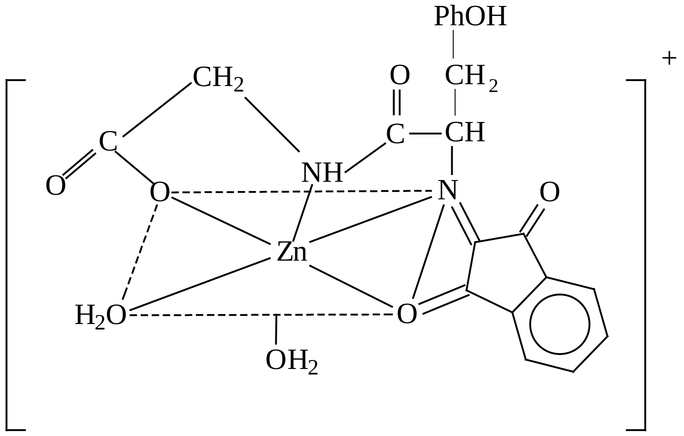
$$[Zn(II)\text{-}Gly\text{-}Tyr]^{+}\text{–}Nin \xrightarrow{k} \text{Product (P)}$$

where,

$[\mathrm{Zn(II)\text{-}Gly\text{-}Tyr}]^+$ =



Product (P) =

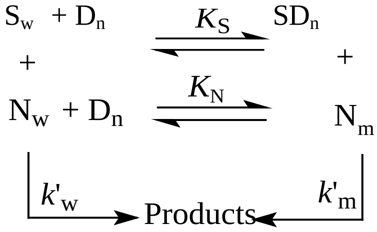


Scheme 1 $[\mathrm{Zn(II)\text{-}Gly\text{-}Tyr}]^+$–ninhydrin reaction mechanism.

Investigations in presence of gemini surfactants disclose that the rate of formation of product is first-order in $[\mathrm{Zn(II)\text{-}Gly\text{-}Tyr}]^+$ and fractional-order in [ninhydrin] (just as in aqueous medium), confirms formation of the same reaction product in both the media. To find out the effect of gemini surfactants on the reaction rate, experiments were performed at varying concentrations of geminis at constant [ninhydrin] (6.0×10^{-3} mol dm^{-3}), $[\mathrm{Zn(II)\text{-}Gly\text{-}Tyr}]^+$ (3.0×10^{-4} mol dm^{-3}), at temperature (80 ºC) and pH (5.0) (Table 2). It has been found that the rate constant increases firstly (part I, [geminis] below their cmc), and then becomes almost constant up to definite concentration (part II, up to 400×10^{-5} mol dm^{-3}) (the characteristics of part I and part II are as conventional surfactant micelles). In the last, k_ψ–values increase sharply (part III) (Fig. 2).

In part I, rate constant (k_ψ) should remain constant because [geminis] are below the cmc but increase in the observed rate constant values may be due to presence of premicelles or preponement of micellization by $[\mathrm{Zn(II)\text{-}Gly\text{-}Tyr}]^+$ and ninhydrin [12]. Regarding part II, the values of rate constant remain almost constant up to 400×10^{-5} mol dm^{-3} of surfactants. The gemini micelles show better environment over the corresponding momomeric single chain surfactants. This could be because of presence of spacer in geminis, which decreases the amount of water in aggregates providing different microenvironment (less polar), thus causing the value of rate constant increases [13]. It has been mentioned that due to proximity of positive charges in gemini surfactants anion binding at the surfaces is increased at the expense of binding of water molecules [14]. The k_ψ–values for all the three geminis at all concentrations follow the order 16-4-16 >16-5-16 >16-6-16 and having same behavior (Fig. 2). It was found several times that the best results were noticed by 16-4-16 gemini surfactant among the series 16-*s*-16 (s = 4, 5, 6) [15]. It is well known that, to minimize its contact with water molecule, a spacer longer than the ‘equilibrium’ distance between two $-\mathrm{NMe_2}$ head groups (the ‘equilibrium’ distance occurs at s = 4 in 16-*s*-16 geminis) tends to loop towards the micellar interior [8]. $s > 4$, increased looping more and will make much more wet to the Stern layer in comparison to s = 4, resulting decrease in the rate constant value (k_ψ). Thus, these findings are in agreement with the earlier results that on increasing amount of water content, the reaction environment behaves as an inhibiting effect [16].

In the range III, with the increasing [geminis]: 400-3000 x 10^{-5} mol dm^{-3} the value of rate constants (k_ψ) increase slowly. Fast increment in rate of reaction occurs at higher [geminis]; this is probably associated with change of micellar structure. This is in conformity with the ^{1}H NMR studies of the surfactants [13,17]. Thus, at higher [geminis], increase in k_ψ–value happens due to changes in the aggregate morphology that provides different reaction environments (less polar).



Scheme 2 Ninhydrin–[Zn(II)-Gly-Tyr]$^+$ reaction in gemini micelles.

The catalytic behavior of geminis surfactants can be rationalized in terms of *pseudo*-phase model (Scheme 2) proposed by Menger and Portnoy [18] for the incorporation/association of reactant into the micellar phase, the most successful appears to be that of Bunton [19] and Romsted [20], who suggested an equation,

$$k_\Psi = \frac{k_w[N]_T + (K_S k_m - k_w)\,M_N^S[D_n]}{1 + K_S[D_n]} \qquad (2)$$

M_N^S being the molarity of ninhydrin bound to the micellar headgroups, [D_n] represents the micellized surfactant (= [surfactant] – cmc), and N_m and SD_n are micellized ninhydrin and [Zn(II)-Gly-Tyr]$^+$ complex, respectively. k_w and k_m are the second-order rate constants, referring to aqueous and micellar pseudo-phases, respectively. To confirm the validity of rate Eq. (2), the rate constants were calculated by substituting the values of k_m and K_S in Eq. (2) and comparing with the observed k_ψ values (Table 2). The close agreement between the observed (k_ψ) and calculated ($k_{\psi cal}$) values supports the validity of the proposed mechanism.

Activation parameters (Table 1), when compared with those obtained in aqueous medium, show that the presence of cationic micelles decreases the enthalpy of activation ($\Delta H^\#$) which indicates that the transition state is highly solvated in the micellar system. The decrease in entropy of activation ($\Delta S^\#$) shows that the transition state is well structured in the case of micelles.

Conclusions

Effect of gemini surfactants was carried out on the kinetics of the title reaction at 80 ºC and pH 5.0. Comparing the values with those obtained in aqueous and CTAB, it was found that gemini micelles catalyze the reaction more. An important point to be noted is that, at present reaction conditions, a smaller amount of surfactants, below the cmc values, was sufficient to accelerate the reaction rate than that of pure water.

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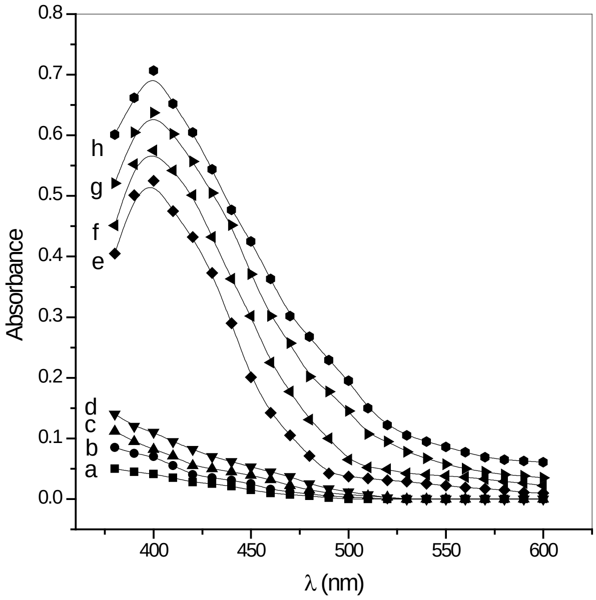

Fig. 1. Absorption spectra of the reaction product of ninhydrin with [Zn(II)-Gly-Tyr]$^+$ in the absence and presence of gemini surfactants: (a) Immediately after mixing the reactants, (b) Same as solution (a) in presence of 16-6-16, (c) Same as solution (a) in presence of 16-5-16, (d) Same as solution (a) in presence of 16-4-16, (e) After heating solution (a) at 80 ºC for 2h, (f) After heating solution (b) at 80 ºC for 2h, (g) After heating solution (c) at 80 ºC for 2h, (h) After heating solution (d) at 80 ºC for 2h. *Reaction conditions*: [16-*s*-16] = 30 x 10^{-5} mol dm^{-3} (*s* = 4, 5, 6), [ninhydrin] = 6.0 x 10^{-3} mol dm^{-3}, [Zn(II)-Gly-Tyr]$^+$ = 3.0 x 10^{-4} mol dm^{-3}, temperature = 80 ºC and pH = 5.0.

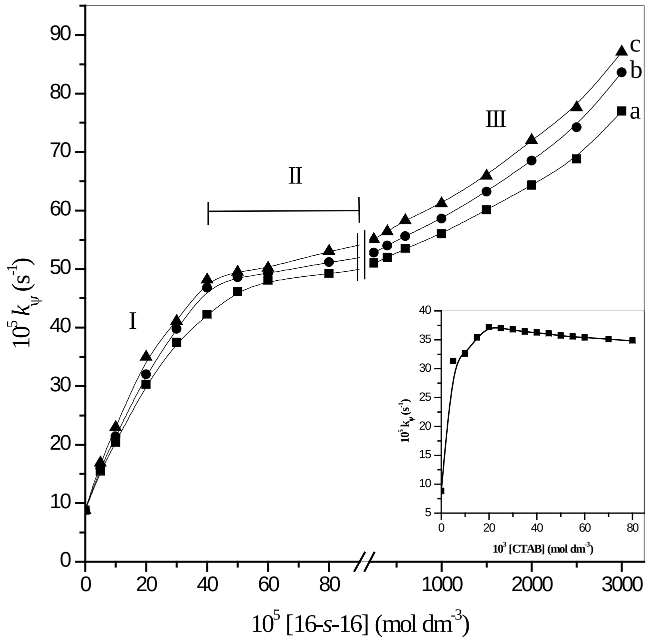

Fig. 2. Effect of varying [geminis] on the reaction rate (k_Ψ) for the reaction of ninhydrin with [Zn(II)-Gly-Tyr]$^+$: (a) 16-6-16, (b) 16-5-16, (c) 16-4-16 and (Inset) for variation of [CTAB]. *Reaction conditions*: [ninhydrin] = 6.0 x 10^{-3} mol dm^{-3}, [Zn(II)-Gly-Tyr]$^+$ = 3.0 x 10^{-4} mol dm^{-3}, temperature = 80 °C and pH = 5.0.

Table 1 Dependence of rate constants (k_Ψ) on [Zn(II)-Gly-Tyr]$^+$, [ninhydrin] and temperature 80 °C at pH 5.0 in presence of gemini surfactants (30 x 10^{-5} mol dm^{-3}).

10^4 [Zn(II)-Gly-Tyr]$^+$ (mol dm^{-3})	10^3 [ninhydrin] (mol dm^{-3})	temp. (°C)	$10^5 k_\psi$ (s^{-1}) 16-6-16	16-5-16	16-4-16
2.0	6	80	37.3	39.8	40.9
2.5			37.6	39.4	41.2
3.0			37.5	39.8	41.1
3.5			37.7	39.3	41.1
4.0			37.3	39.3	40.9
3.0	6	80	37.5	39.8	41.1
	10		40.0	44.7	47.2
	15		44.5	48.1	50.0
	20		47.3	51.5	53.1
	25		49.5	53.7	55.7
	30		51.1	55.2	57.5
	35		52.2	56.6	58.4
	40		52.8	57.3	59.2
3.0	6	70	30.3	31.1	32.7
		75	34.8	35.3	36.7
		80	37.5	39.8	41.1
		85	49.1	50.0	50.9
		90	61.5	63.1	72.1

Activation parameters, rate, and binding constants

	E_a (kJ mol^{-1})	$\Delta H^\#$ (kJ mol^{-1})	$-\Delta S^\#$ (JK^{-1} mol^{-1})	$10^3 k_m{}^a$ (s^{-1})	$K_S{}^a$ (mol^{-1}dm^3)	$K_N{}^a$ (mol^{-1} dm^3)
Aqueous	115.1	112.2	307.0	-	-	-
16-6-16	36.2	33.3	307.7	61.0	110.0	66.2
16-5-16	34.5	31.6	308.1	33.0	104.0	70.9
16-4-16	29.9	27.0	308.6	43.0	96.0	63.6

^{a}At 80 °C

Table 2 Effect of varying [geminis] on the reaction rate for reaction of ninhydrin (6.0 x 10^{-3} mol dm^{-3}) with $[Zn(II)\text{-}Gly\text{-}Tyr]^{+}$ (3.0 x 10^{-4} mol dm^{-3}) at constant pH 5.0 and 80 ºC and their comparison with calculated values ($k_{\Psi cal}$).

10^5 [16-*s*-16] (mol dm^{-3})	16-6-16			16-5-16			16-4-16		
	$10^5 k_\psi$ (s^{-1})	$10^5 k_{\psi cal}$ (s^{-1})	$\frac{k_\Psi - k_{\Psi cal}}{k_\Psi}$	$10^5 k_\psi$ (s^{-1})	$10^5 k_{\psi cal}$ (s^{-1})	$\frac{k_\Psi - k_{\Psi cal}}{k_\Psi}$	$10^5 k_\psi$ (s^{-1})	$10^5 k_{\psi cal}$ (s^{-1})	$\frac{k_\Psi - k_{\Psi cal}}{k_\Psi}$
0	8.8	-	-	8.8	-	-	8.8	-	-
5.0	15.5	-	-	16.0	-	-	16.9	-	-
10.0	20.4	20.5	0.00	21.4	16.2	+0.24	23.0	20.3	+0.12
20.0	30.3	30.1	+0.01	32.0	31.4	+0.02	35.0	34.4	+0.02
30.0	37.5	37.9	-0.01	39.8	40.2	-0.01	41.1	41.2	0.00
40.0	42.2	41.8	+0.01	46.8	46.0	+0.02	48.2	48.8	-0.01
50.0	46.2	45.6	+0.01	48.6	49.3	-0.01	49.5	49.5	0.00
60.0	48.0	48.0	0.00	49.2	49.6	-0.01	50.2	50.3	0.00
80.0	49.2	48.5	+0.01	51.2	51.5	-0.01	53.1	53.4	-0.01
100.0	50.0	49.4	+0.01	52.0	51.8	0.00	54.2	53.8	+0.01
250.0	51.0	50.4	+0.01	52.8	53.5	-0.01	55.1	55.5	-0.01
400.0	52.0	51.7	+0.01	54.0	54.4	-0.01	56.4	56.2	0.00
600.0	53.5	53.0	+0.01	55.6	56.1	-0.01	58.3	58.5	0.00
1000.0	56.0	-	-	58.6	-	-	61.2	-	-
1500.0	60.1	-	-	63.2	-	-	65.9	-	-
2000.0	64.3	-	-	68.5	-	-	72.0	-	-
2500.0	68.8	-	-	74.2	-	-	77.6	-	-
3000.0	77.0	-	-	83.6	-	-	87.1	-	-