

Interaction of Nickel-Dipeptide complex with Ninhydrin in Aqueous and Micellar Media

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Abstract: Ninhydrin reactions using manual and automated techniques as well as ninhydrin spray reagents are widely used to analyze and characterize amino acids, peptides, and proteins as well as numerous other ninhydrin positive compounds in biomedical, clinical, food, forensic, histochemical, microbiological, and nutritional and plant studies. Ninhydrin in aqueous solution reacts with α -amino acids to give a compound known as Ruhemann's purple (diketohydrindylidenediketohydrindamine, DYDA). In order to elucidate the mechanism of the reactions of ninhydrin with $[\text{Ni(II)-Gly-Leu}]^+$ complexes, systematic kinetic studies were performed by following appearance of the respective colored condensation products at different [surfactant], [reactant], [salt] and temperature. The rates of the reaction were determined in both water and surfactant micelles in the absence and presence of various organic and inorganic salts at 70 °C and pH 5.0. The result shows that the reaction follows the same first-order and fractional-order kinetics with respect to $[\text{Ni(II)-Gly-Leu}]^+$ and [ninhydrin], respectively, in both aqueous and micellar medium. Quantitative kinetic analysis of $k_{\text{app}}-[\text{CTAB}]$ data was performed on the basis of the pseudo-phase model of the micelles.

Keywords: Micelle, Kinetics, $[\text{Ni(II)-Gly-Leu}]^+$, CTAB

1. Introduction

Surfactant aggregates in water and in nonpolar solvents have been utilized to mimic the micro-environments of biomacromolecular ensembles. catalysis by micelles resembles the enzymes catalyzed reaction [1-4] and the important similarities are (i) both micelles and enzymes display similar structures by containing hydrophobic core with polar groups on their surfaces; (ii) the micelles and enzymes bind the substrate through non covalent bond (iii) the rate constants of micelle catalyzed reactions, when plotted against detergent concentrations, give sigmoid shaped curves, analogous to positive homotropic interactions (positive cooperativity) in enzymatic reactions. The effect of ionic micelles on the reaction rates of bimolecular reactions is due to the association/incorporation of the two reactants within the small volume of the micellar stern layer [5].

The use of ninhydrin (N) for the detection/ estimation of aminoacids / peptides has great potential in revealing latent finger prints [6]. The use depends on the formation of ruhemann's purple [7]. The method, though useful, still has

much room for improvements. Continuous efforts are, therefore, being made to improve the method [6,8].

Effects of metal ions on ninhydrin reaction were also investigated with this view point, as the presence of metal ions in the system favors the condensation of ninhydrin with the dipeptide by enhancing polarization of the carbonyl group, there by promoting the nucleophilic attack. The present study was aimed to explore the effect of cationic micelles (CTAB) on the reaction of $[\text{Ni(II)-Gly-Leu}]^+$ complex with ninhydrin and to check whether CTAB micelles change the aqueous reaction mechanism. The results and probable explanations are described in this research paper.

2. Experimental

2.1. Materials

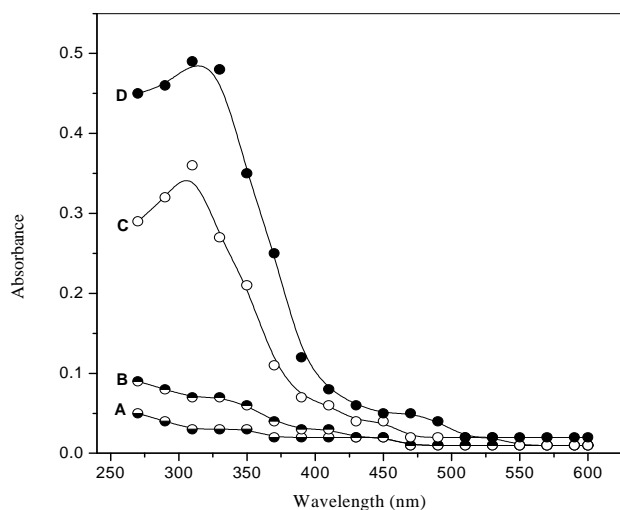
Gly-Leu (LOBA Chemie, 99%), ninhydrin (Merck, 99%), CuSO_4 (Merck, 99%), CTAB (BDH, 99%), sodium benzoate (NaBenz, Merck, 99.5%), sodium salicylate (NaSal, CDH, 99.5%), sodium bromide (LOBA Chemie, 99%), sodium chloride (BDH, 99.9%), sodium sulphate (Qualigens, 99%),

sodium acetate (Merck, 99%) and acetic acid (Merck, 99.9%) were used as received. An acetic acid-sodium acetate buffer of pH 5, which was used as solvent for preparing all stock solutions, was prepared in doubly distilled and deionized water (specific conductance $(1-2) \times 10^{-6} \text{ ohm}^{-1} \text{ cm}^{-1}$).

2.2. Kinetic Measurements

The $[\text{Ni(II)-Gly-Leu}]^+$ complex was made in situ by mixing equimolar i.e. 1:1 solutions of Gly-Leu and nickel sulphate in a three-necked reaction vessel (kept in a thermostatted oil bath). The reaction vessel was fitted with a double surface water condenser to prevent evaporation. The reaction was then started with addition of a thermally equilibrated ninhydrin solution of required volume. Each kinetic run was performed under pseudo-first-order conditions using ≥ 10 -fold excess of [ninhydrin] over $[\text{Ni(II)-Gly-Leu}]^+$. Purified nitrogen gas was bubbled through the reaction mixture for stirring as well as to maintain an inert atmosphere. Aliquots of the reaction mixture were withdrawn at definite time intervals and the absorbance was measured spectrophotometrically at 310nm (λ_{max}) with the help of a Bausch and Lomb spectronic-20 spectrophotometer. The first-order rate constants in the absence (k_{obs} , s^{-1}) and presence of surfactants (k_{Ψ} , s^{-1}) were computed with the help of computer programme, more details about the kinetic methodology, pH measurements, and viscosity measurements can be found elsewhere [9-12].

3. Results and Discussion



Lines are drawn as a guide to the eye.

Fig. 1. Absorption spectra of the reaction product of $[\text{Ni(II)-Gly-Leu}]^+$ and ninhydrin in absence and presence of CTAB micelles at pH 5.0: (A) immediately after mixing the reactants, $[\text{Ni(II)-Gly-Leu}]^+ = 2.0 \times 10^{-4} \text{ mol dm}^{-3}$, $[\text{ninhydrin}] = 6.0 \times 10^{-3} \text{ mol dm}^{-3}$; (B) same as solution(A) in presence of $[\text{CTAB}] = 20 \times 10^{-3} \text{ mol dm}^{-3}$; (C) after heating solution(A) at 70°C for 2h; (D) after heating solution(B) at 70°C for 2h.

Figure 1 shows the UV-Visible spectra of the products formed by the reaction between Ni(II)-Gly-Leu complex (2.0

$\times 10^{-4} \text{ mol dm}^{-3}$) and ninhydrin ($6.0 \times 10^{-3} \text{ mol dm}^{-3}$) in aqueous as well as aqueous CTAB micelles under different conditions. We see that the absorbance increases with CTAB micelles with no shift in λ_{max} , i.e., the wavelength of maximum absorbance ($\lambda_{\text{max}} = 310\text{nm}$) remains the same in both aqueous and micellar media. It is inferred that there is no change of products in the two systems.

To investigate the effect of $[\text{Ni(II)-Gly-Leu}]^+$ complex on the reaction rate, the kinetic experiments were performed at different initial concentrations of Ni(II)-Gly-Leu complex ranging from 1.0×10^{-4} to $3.5 \times 10^{-4} \text{ mol dm}^{-3}$. The concentration of ninhydrin was kept constant ($6.0 \times 10^{-3} \text{ mol dm}^{-3}$) at fixed temperature (70°C) and pH (5.0). Similar studies were made in presence of CTAB micelles. It was found that the value of rate constant was independent of the initial concentration of the $[\text{Ni(II)-Gly-Leu}]^+$ in both the media. The rate law is therefore, as in eq. (1):

$$\text{rate} = \frac{d[\text{Product}]}{dt} = (k_{\text{obs}} \text{ or } k_{\Psi}) [\text{Ni(II)-Gly-Leu}]^+_{\text{T}} \quad (1)$$

The dependence of the rate constant on [ninhydrin] was determined by carrying out the kinetic runs at different concentrations of ninhydrin (keeping $[\text{Ni(II)-Gly-Leu}]^+ (2.0 \times 10^{-4} \text{ mol dm}^{-3})$, temperature (70°C) and pH (5.0) constant) in both aqueous and micellar media the log (rate constants) versus log [ninhydrin] plots are linear with slope (= 0.85) in aqueous and (= 0.87) in micellar media; which indicates the order with respect to [ninhydrin] is fractional in absence and presence of CTAB micelles.

The rate constant values were obtained by conducting kinetic experiments within the temperature range 60 to 80°C in aqueous as well as presence of CTAB micelles (20.0×10^{-3}) with fixed reactants concentration (Table 1).

Table 1. Effect of temperature on pseudo-first-order rate constants (k_{obs}/k_{Ψ}) for the reaction of $[\text{Ni(II)-Gly-Leu}]^+$ with ninhydrin.

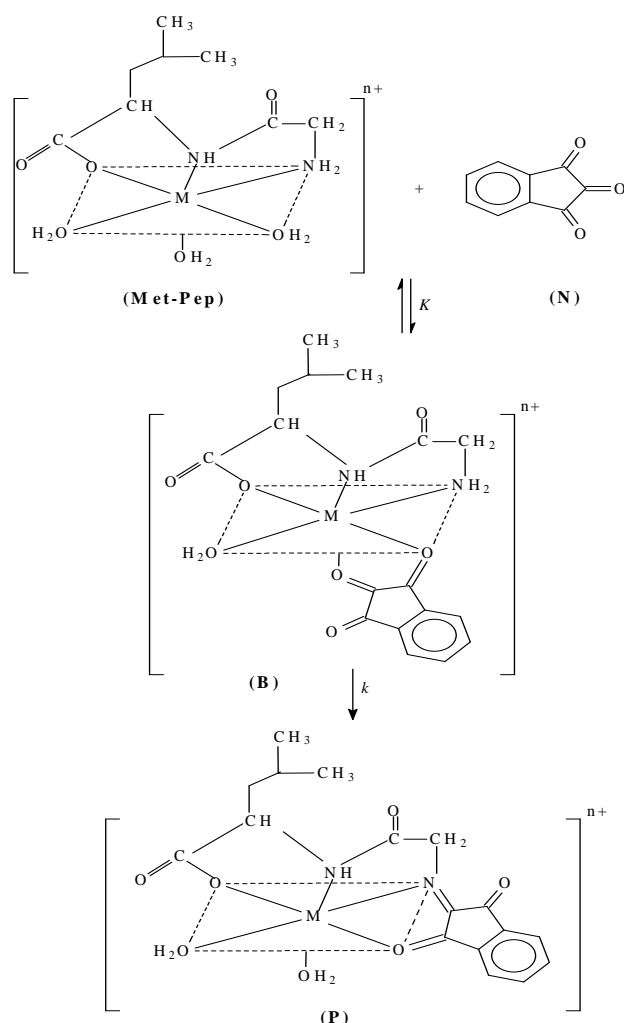
Reaction conditions:		
$[\text{Ni(II)-Gly-Leu}]^+$	=	$2.0 \times 10^{-4} \text{ mol dm}^{-3}$
[ninhydrin]	=	$6.0 \times 10^{-3} \text{ mol dm}^{-3}$
[CTAB]	=	$2.0 \times 10^{-2} \text{ mol dm}^{-3}$
pH	=	5.0

Temperature ($^\circ \text{C}$)	$10^5 k_{\text{obs}} (\text{s}^{-1})$	$10^5 k_{\Psi} (\text{s}^{-1})$
60	1.6	2.6
65	2.5	3.9
70	3.5	6.1
75	5.0	6.8
80	7.6	9.1

Parameters		
$E_a (\text{kJmol}^{-1})$	79.4	65.0
$\Delta H^\ddagger (\text{kJmol}^{-1})$	76.6	62.2
$-\Delta S^\ddagger (\text{JK}^{-1}\text{mol}^{-1})$	151.0	156.0

3.1. Reaction in Aqueous Medium

Detailed investigations reveal that the rate of formation of the product is first-order with respect to Ni(II)-Gly-Leu complex concentration, Equation (1), as confirmed by (i) the initial rate being ($v_0 = k_{\text{obs}} [\text{Ni(II)-Gly-Leu}^+]_{\text{T}}$) directly proportional to the initial concentration of $[\text{Ni(II)-Gly-Leu}^+]_{\text{T}}$ complex, and (ii) constantly of k_{obs} values obtained at different initial concentrations of $[\text{Ni(II)-Gly-Leu}^+]_{\text{T}}$ (Table 1). The plots of k_{obs} versus $[\text{ninhydrin}]_{\text{T}}$ show a fractional-order with respect to $[\text{ninhydrin}]_{\text{T}}$. On the basis of the above results, the following mechanism is proposed for the reaction (scheme 1).



Scheme 1. Ni(II)-Gly-Leu complex ninhydrin complex.

It is well known that lone pair electrons of ammonia group are necessary for nucleophilic attack on the carbonyl group of ninhydrin [6, 13-15].

In complex A this lone pair electron is not free, therefore, nucleophilic attack is not possible. The reaction, therefore, proceeds through, condensation of coordinated amino group of Gly-Leu to the coordinated carbonyl group of ninhydrin with the coordination sphere of Ni (II) (B to P). The coordination of both the reactants (ninhydrin and Gly-Leu) with the same metal ion (nickel (II)) is an example of

template mechanism [16]. The presence of metal ion brings the reaction groups together and provides a better chance for their combination with in its coordination sphere. In order to confirm the cleavage of $-\text{COOH}$ groups, we have experimentally tested that no CO_2 is evolved in the present case. Evidently, nickel (II) inhibits the cleavage of $-\text{COO}$ group by reducing its escaping tendency and by enhancing the electrophilic character of $>\text{C}=\text{O}$ group.

The mechanism (Scheme I), proposed on the basis of above, involves two kinetically distinguishable steps: (i) a rapid ternary labile complex formation between $[\text{Ni(II)-Gly-Leu}^+]_{\text{T}}$ complex and ninhydrin, and (ii) a slower condensation of amino group to carbonyl group. Accordingly, the following rate equation

$$\frac{d[\text{P}]}{dt} = \frac{k K [\text{N}][\text{A}]_{\text{T}}}{(1 + K[\text{N}])} \quad (2)$$

which, on comparison with eq. (1), gives

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

A double-reciprocal plot between k_{obs} and $[\text{N}]$ resulted in a straight line (as envisaged by eq. 2) which allowed us to find the values of k (16.3 s^{-1}) and K ($79.5 \text{ mol}^{-1} \text{ dm}^3$) in the aqueous medium. The calculated values of rate constants (k_{cal} , Table 2, obtained by substituting k and K in eq. 3) are in closed agreement with the k_{obs} which supports the proposed mechanism and confirms the validity of the rate eq. 3.

Table 2. Values of rate parameters (k_m , k_2^m , k_w and k_w/k_2^m) and binding constants (K_S , K_N) for the reaction of $[\text{Ni(II)-Gly-Leu}]^+$ and ninhydrin in micellar media.

Reaction conditions:	
$[\text{Ni(II)-Gly-Leu}^+]$	= $2.0 \times 10^{-4} \text{ mol dm}^{-3}$
$[\text{ninhydrin}]$	= $6.0 \times 10^{-3} \text{ mol dm}^{-3}$
pH	= 5.0
Temperature	= $70 \text{ }^\circ\text{C}$

Parameters and Constants	Values
$10^4 k_m (\text{s}^{-1})$	16.3
$10^5 k_2^m (\text{mol}^{-1} \text{ dm}^3 \text{ s}^{-1})^a$	18.8
$10^5 k_w (\text{mol}^{-1} \text{ dm}^3 \text{ s}^{-1})$	13.5
k_w/k_2^m	10.4
$K_S (\text{mol}^{-1} \text{ dm}^3)$	43.2
$K_N (\text{mol}^{-1} \text{ dm}^3)$	79.5

^asecond-order rate constants (k_2^m) are based on eq. (5).

3.2. Reaction in Presence of CTAB

Preliminary experiments indicate that absorbance of end product increases as the concentration of CTAB micelles increases from 0 to $60.0 \times 10^{-3} \text{ mol dm}^{-3}$ (Fig 2). The absorbance increased from 3.5 to 6.2 (nearly 3a fold) but the wavelength of maximum absorbance (λ_{max}) remained unchanged (vide-supra); this confirms that the product of the

reaction remains the same as in aqueous medium.

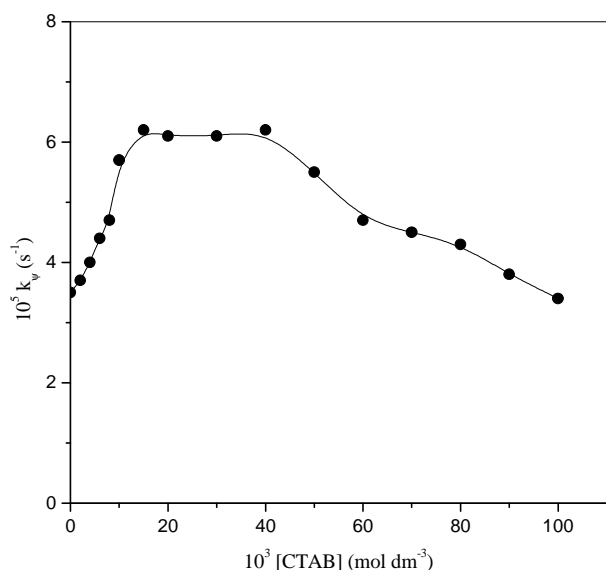
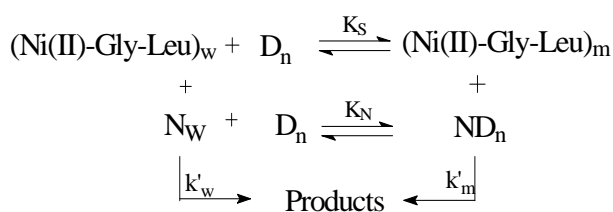


Fig. 2. Effect of [CTAB] on the reaction rate of [Ni(II)-Gly-Leu]⁺ complex with ninhydrin. Reaction conditions: [ninhydrin] = $6.0 \times 10^{-3} \text{ mol dm}^{-3}$, [Ni(II)-Gly-Leu]⁺ = $2.0 \times 10^{-4} \text{ mol dm}^{-3}$, pH = 5.0, temp. = 70 °C.

To find out the behaviour of cationic surfactant on the reaction rate, the effect of varying [CTAB] was studied at constant [Ni(II)-Gly-Leu]⁺ ($2.0 \times 10^{-4} \text{ mol dm}^{-3}$), [ninhydrin] ($6.0 \times 10^{-3} \text{ mol dm}^{-3}$) and pH 5.0 at 80 °C. The observed rate constants are affected by [CTAB] changes and the maximum rate enhancement is *ca* three fold. A plot of k_p versus [CTAB] shows a rate maximum at [CTAB] = $60.0 \times 10^{-3} \text{ mol dm}^{-3}$ (Fig. 2), a very common characteristics of bimolecular reactions catalyzed by micelles [15,16].

It has been established [17] that micelles can cause a change in aqueous reaction mechanism. Therefore, in order to confirm the proposed mechanism (Scheme 1), the effect of reactant concentrations and temperature was studied in presence of CTAB ($20.0 \times 10^{-3} \text{ mol dm}^{-3}$). Just like aqueous medium, the same first-and fractional-order in [Ni(II)-Gly-Leu]⁺ and [ninhydrin], respectively, are being followed. Thus, we can conclude that the reaction mechanism remains the same in presence of cationic CTAB micelles with all possible intermediary situations.

The catalytic behaviour of cationic surfactant (CTAB) can be rationalized in terms of pseudo-phase model (Scheme), proposed by Menger et.al [17] and developed by Bunton [18] and Romsted [19].



Scheme-2

Here *w* and *m* represent the aqueous pseudo-phase and micellar pseudo-phase, respectively, K_S is the binding constant of the [Ni(II)-Gly-Leu]⁺ complex to the cationic micelles, and $[\text{D}_n] = [\text{CTAB}] - \text{CMC}$. k'_w and k'_m are the pseudo-first-order rate constants in aqueous and micellar pseudo-phases. The observed rate law (eq. 1) and scheme II lead to eq. 4.

$$k_p = \frac{k'_w + k'_m K_S [\text{D}_n]}{1 + K_S [\text{D}_n]} \quad (4)$$

Eq. (4) can be modified as Eq. (5)

$$\frac{1}{k_w - k_p} = \frac{1}{k_w - k_m} + \frac{1}{(k_w - k_m) K_S [\text{D}_n]} \quad (5)$$

According to eq. (5), a plot of the left-hand side versus $1/[\text{D}_n]$ should give a straight line with an intercept [= $1/(k_w - k_m)$] and slope $[1/(k_w - k_m) K_S]$. Interestingly, linearity in the plot of $(1/k_w - k_p)$ versus $1/[\text{D}_n]$ was observed, implying that scheme-II model is adequate.

At [CTAB] > $60 \times 10^{-3} \text{ mol dm}^{-3}$ practically all the substrate has been incorporated into the micellar phase. When bulk of the substrate is incorporated into the micelles, addition of more CTAB generates more cationic micelles, which simply take up the ninhydrin molecules in one micelle should not react with the complex in another [20]. Another reason of decreased of decreased in rate constant could be a result of counter ion inhibition.

Micelles mediated reactions occur either inside the stern layer or at the interface between micellar and bulk water solvent [21,22]. However, micellar mediated reactions are also found to occur at the interfacial junction region of stern and Gouy-Chapman layers [23,24]. Micellar surfaces are water-rich [25].

The values of the energy, enthalpy, and entropy of activation, calculated from the Arrhenius and Eyring equations are listed in Table 2. Comparing the values we find that the presence of cationic CTAB micelles decreases the ΔH^\ddagger with a substantial negative ΔS^\ddagger . This decrease in ΔH^\ddagger with CTAB may be related to the slow solubility of [Ni(II)-Gly-Leu]⁺ complex in the micellar phase relative to that in aqueous phase. In addition, a lower value of activation energy clearly suggests the catalytic role of CTAB (catalysts lower the activation energy and provide a new reaction path).

3.3. Salt Effect Results

Rate enhancement or inhibition of micellar catalysis by added electrolytes can be due to the change in shape and size [26] of micelles and/or exclusion of the reactants from the micellar pseudo-phase [27]. However, hydrophobic salts, sodium benzoate (NaBenz), sodium salicylate (NaSal), and sodium tosylate (NaTos) give marked rate enhancement at low salt concentrations, passing through a maximum as the [salt] is increased (Fig. 3).

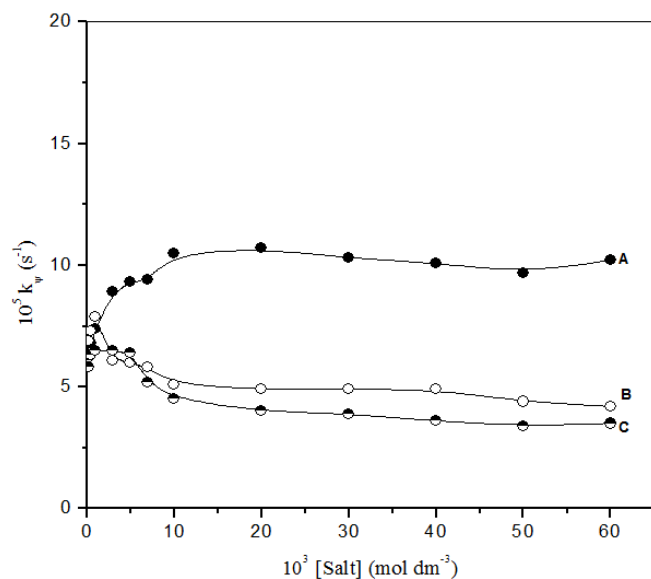


Fig. 3. Effect of NaBenz (A), NaSal (B), and NaTos (C) on the reaction rate of $[\text{Ni}(\text{II})\text{-Gly-Leu}]^+$ complex with ninhydrin. Reaction conditions: $[\text{Ni}(\text{II})\text{-Gly-Leu}^+] = 2.0 \times 10^{-4} \text{ mol dm}^{-3}$, $[\text{ninhydrin}] = 6.0 \times 10^{-3} \text{ mol dm}^{-3}$, $[\text{CTAB}] = 2.0 \times 10^{-2} \text{ mol dm}^{-3}$, $\text{pH} = 5.0$, $\text{temp.} = 70^\circ\text{C}$.

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) [28]. As we increase the [salt], the above site will be saturated. Once this site is fully occupied, additional salt will try to get adsorbed at the micellar surface (a case of adsorption) and will thus compete for a site with reactants (a case of benzoate, salicylate and tosylate ions' association in the form of adsorption). Consequently, [reactant] is decreased at the reaction site by the latter effect (exclusion of substrate). The progressive withdrawal of the substrate from the reaction site would slow down the rate, as was indeed observed.

4. Conclusion

The kinetics of the interaction of $[\text{Ni}(\text{II})\text{-Gly-Leu}]^+$ and ninhydrin has been studied in aqueous and cationic micelles of CTAB. Quantitative treatment of the kinetic data seems justified as observed and calculated rate constant values were in close agreement within experimental errors. Finally, we can conclude that molecule- molecule interactions in micellar media could successfully be treated using the pseudo-phase model.

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