ABSTRACT

There are various chemical and physico-chemical techniques for the qualitative and quantitative estimation of \( \alpha \)-amino acids. Ninhydrin has been widely used for this purpose. Ninhydrin reacts with \( \alpha \)-amino acids in neutral aqueous solution with the evolution of carbon dioxide, the formation of aldehyde and development of deep blue colour of diketohydrindylidene-diketohydrindamine (DYDA). It was observed that the blue colour fades away on keeping and therefore, attempts were made by many investigators\(^1\text{–}^{16}\) to stabilize the colour by adjusting the pH of the reaction media, adding reducing agents, metal ions and organic solvents. The effect of \( \text{Cu(II)} \) and \( \text{Cd(II)} \) has been specifically studied by Ganpathy et al.\(^\text{10, 12}\) and D'Aniello et al.\(^13\) but no work has been reported on the kinetics of the reaction of the metal-amino acid complexes with ninhydrin. In this thesis the work is mainly concerned with the kinetics and mechanism of \( \text{Cu(II)} \) and \( \text{Cd(II)} \) complexes of amino acids (glycine, \( \alpha \)-alanine, aspartic acid, asparagine and serine) with ninhydrin. The amino acids were chosen with different substituents to investigate their effects on the reactivity of their complexes.

In the first chapter a critical review of the work done on the reaction of ninhydrin with \( \alpha \)-amino acids and related
compounds has been given. The work related with the effect of metal ions on this reaction has also been reviewed.

In the second chapter the kinetics and mechanism of the reaction of ninhydrin with copper(II) complexes of amino acids (glycine, α-alanine, aspartic acid, asparagine and serine) have been reported. The composition of the product formed by the interaction of ninhydrin and (copper(II)-amino acid)$^+$ was determined by Job's method of continuous variation and was found to be 1:1. The kinetic studies were performed under different concentrations of ninhydrin, acetate ions and temperatures. The activation parameters calculated on the basis of variation of rate constants with temperature are given in table 1. Ninhydrin reacts with amino acids complexes of copper(II) and gives yellow coloured product (schiff base complex) at $\lambda_{\text{max}} = 375$ nm. The kinetics has been found to follow pseudo first order reaction path with respect to copper(II) complex in presence of excess of ninhydrin. The variation of pseudo first order rate constants with ninhydrin concentrations were found to be in good agreement with the empirical equation (1)

$$\frac{1}{k_{\text{obs}}} = \frac{B_1}{\text{Ninhydrin}} + B_2$$

where $B_1$ and $B_2$ are the unknown empirical parameters. The rate constants were calculated by a computer VAX-11/780. The reaction proceeds through the formation of ternary labile complex
which is a features of template reaction mechanism. On the basis of observed rate data a mechanism for the interaction of ninhydrin with copper(II) complexes of \( \alpha \)-amino acids (except aspartic acid which reacts through different mechanism) has been proposed and given in scheme 1. The mechanism for the reaction of ninhydrin with copper(II) complex of aspartic acid is shown in scheme 2. On the basis of scheme 1 mechanism the following rate equation has been derived.

\[
k_{\text{obs}} = \frac{k_1 K_t \left[ \text{Ninhydrin} \right]}{1 + K_t \left[ \text{Ninhydrin} \right]}
\]

Equation (2) can be rearrange as equation (3)

\[
\frac{1}{k_{\text{obs}}} = \frac{B_1}{\left[ \text{Ninhydrin} \right]} + B_2
\]

with \( B_1 = \frac{1}{k_1 K_t} \) and \( B_2 = \frac{1}{k_1} \). Thus the observed results are compatible with the scheme 1 mechanism. The rate equation for the reaction of aspartic acid-Cu(II) complex with ninhydrin has been derived on the basis of scheme 2 mechanism.

\[
k_{\text{obs}} = \frac{k_1 K_t \left[ \text{Ninhydrin} \right]}{1 + K_t \left[ \text{Ninhydrin} \right] (1 + k_1)}
\]

Equation (4) can be written as

\[
\frac{1}{k_{\text{obs}}} = \frac{C_1}{\left[ \text{Ninhydrin} \right]} + C_2
\]

with \( C_1 = \frac{1}{k_1 K_t} \) and \( C_2 = \frac{(1 + k_1)}{k_1} \).
Equation (5) shows a linear dependence of \(1/k_{\text{obs}}\) on \(1/N\text{ninhydrin}\). Thus the proposed mechanism of scheme 2 follows the observed data. In these studies it was observed that the Hammett-Taft equation is followed by the plot of \(\{\log k_{\text{obs}}(\text{Cu(II)}-\text{amino acid complex}) - \log k_{\text{obs}}(\text{Cu(II)}-\text{alanine complex})\} \text{Vs. Hammett constant (figure 1)}\) and shows the dependence of rate constants on the structure of amino acids-Cu(II) complexes.

In the third chapter the kinetics and mechanism of the reaction of ninhydrin with cadmium(II) complexes of \(\alpha\)-amino acids have been described. The Cd(II) complexes of \(\alpha\)-amino acids react with ninhydrin to give two products with \(\lambda_{\text{max}}\) at 375 nm (yellow coloured product) and 510 nm (red coloured product). These products are formed by two parallel reactions. These reaction paths are found to be pseudo first order with respect to amino acid-cadmium(II) complex. The rate constants \(k_{\text{obs}}\) were calculated by the method described in chapter II. The kinetic studies were performed under different concentrations of ninhydrin, acetate ions and temperatures. Various activation parameters were calculated and are given in table 2. The mechanism for the reaction of ninhydrin with cadmium(II) complexes of \(\alpha\)-amino acids (except aspartic acid) is shown in scheme 3 and that for aspartic acid is shown in scheme 4. In these mechanisms, amino acid-cadmium(II) complexes are labile.
in nature. In one case complex reacts as such with ninhydrin to form a ternary labile complex which gives yellow coloured schiff base complex with $\lambda_{\text{max}}$ at 375 nm. The mechanism is similar to that described for Cu(II) complex. In the other case, the complex is in equilibrium with the zwitter ionic form of amino acid which reacts with ninhydrin to give 2-amino indan dione. Cadmium(II), 2-amino indan dione and ninhydrin react to give a red coloured products at $\lambda_{\text{max}} = 510$ nm. The formation of ternary labile complexes is also a features of template reaction mechanism. For the formation of yellow and red coloured products, following rate equations have been derived (schemes 3 and 4).

\[
(6) \quad k_{1\text{obs}} = \frac{k_{1} K_{t} [\text{Ninhydrin}]}{1 + K_{t} [\text{Ninhydrin}](1 + k_{1})}
\]

and

\[
(7) \quad k_{2\text{obs}} = \frac{k_{2} K_{t} K_{n} [\text{Ninhydrin}]}{1/K_{d} + K_{n} + K_{t} K_{n} [\text{Ninhydrin}](1 + k_{2})}
\]

Equations (6) and (7) can be written as equations (8) and (9) respectively.

\[
(8) \quad \frac{1}{k_{1\text{obs}}} = \frac{B_{1}}{[\text{Ninhydrin}]} + B_{2}
\]

and
Equations (8) and (9) show a linear dependence of $1/k_{1\text{obs}}$ and $1/k_{2\text{obs}}$ on $1/[\text{Ninhydrin}]$. Figure 2 shows that the rate constants of the reaction of ninhydrin with Cd(II) complexes of amino acids depend on the structure of these complexes as required by the Hammett-Taft equation.

In the fourth chapter, the reaction of proline and its copper(II) and cadmium(II) complexes with ninhydrin has been discussed. The reaction of proline with excess of ninhydrin follows an irreversible pseudo first order reaction path for the formation of enol betaine and carbon dioxide. The evolved carbon dioxide was estimated in the same way as for aspartic acid $^{18}$. The kinetic studies were performed under different concentrations of ninhydrin, acetate ions and temperatures. The values of the calculated activation parameters are given in table 3. The rate constants were calculated in the usual way. A mechanism for the evolution of carbon dioxide and for the formation of enol betaine is given in scheme 5 and the following rate equation was derived.

\[
(9) \quad \frac{1}{k_{2\text{obs}}} = \frac{C_1}{[\text{Ninhydrin}]} + C_2
\]

with $B_1 = \frac{1}{k_1 K_t}$, $B_2 = \frac{(1 + k_1)}{k_1}$, $C_1 = \frac{1/k_1 + k_n}{k_2 K_t K_n}$ and $C_2 = \frac{(1 + k_2)}{k_2}$. Equations (8) and (9) show a linear dependence of $1/k_{1\text{obs}}$ and $1/k_{2\text{obs}}$ on $1/[\text{Ninhydrin}]$. Figure 2 shows that the rate constants of the reaction of ninhydrin with Cd(II) complexes of amino acids depend on the structure of these complexes as required by the Hammett-Taft equation.

\[
(10) \quad k_{\text{obs}} = \frac{k_1 K_3 K_{eq, \text{Ninhydrin}}}{(k_2 + k_3)\left\{\frac{L_{\text{H}^+} }{K_{a1}} + K_{eq, \text{Ninhydrin}} + k_1 K_{eq, \text{Ninhydrin}}\right\}}
\]
Equation (10) on rearrangement gives equation (11).

\[
\frac{1}{k_{\text{obs}}} = \frac{B_1}{[\text{Ninhydrin}]} + B_2
\]

with \( B_1 = \frac{(k_2 + k_3)(\frac{[\text{H}^+]}{K_{a1}} + \text{eq.})}{k_1 k_3 \text{eq.}} \) and \( B_2 = \frac{(k_2 + k_3)}{k_3} \)

The reaction of copper(II) and cadmium(II) complexes of proline with excess of ninhydrin also follows an irreversible pseudo first order reaction path. Ninhydrin forms a ternary labile complex with (metal ion - proline)\(^+\) complex, which is a features of template reaction mechanism. The kinetic studies were performed under different concentrations of ninhydrin, acetate ions and temperatures. On the basis of observed data probable mechanisms for the reaction of ninhydrin with proline complexes of copper(II) and cadmium(II) are shown in schemes 6 and 7 respectively and the following rate equation was derived

\[
k_{\text{obs}} = \frac{k_4 K_t [\text{Ninhydrin}]}{1 + K_t [\text{Ninhydrin}]}
\]

on rearrangement equation (12) gives equation (13)

\[
\frac{1}{k_{\text{obs}}} = \frac{C_1}{[\text{Ninhydrin}]} + C_2
\]

with \( C_1 = \frac{1}{k_4 K_t} \) and \( C_2 = \frac{1}{k_4} \) which shows a linear dependence
of $1/k_{\text{obs}}$ Vs. $1/\text{Ninhydrin}$. Cadmium(II) has no significant effect on the rate constants because the cadmium(II) complex of proline is labile in nature but in presence of copper(II) the rate becomes slow due to the stable nature of its complex with proline. The values of activation parameters are given in table 4.

The fifth chapter deals with the studies on the kinetics and mechanism of decarboxylation of aspartic acid with ninhydrin. The kinetic studies were performed under different concentrations of ninhydrin, aspartic acid and temperatures. Various activation parameters were calculated and are given in table 5. The amount of carbon dioxide evolved at different time intervals was estimated using the titration method. Pseudo first order conditions were maintained in all cases by using excess of ninhydrin and the first order rate constants were calculated by the method described in chapter II. A mechanism for the decarboxylation of aspartic acid with ninhydrin for the evolution of two moles of carbon dioxide is shown in scheme 8 and following rate equation has been derived.

\begin{equation}
(14) \quad k_{\text{obs}} = \frac{k_1 k_2 K_d \left[ H^+ \right] \left[ \text{Ninhydrin} \right]}{(k_1 + k_2)(K_II + \left[ H^+ \right] k_{d} + k_1 k_d \left[ H^+ \right] \left[ \text{Ninhydrin} \right])}
\end{equation}

On rearrangement equation (14) gives equation (15).

\begin{equation}
(15) \quad \frac{1}{k_{\text{obs}}} = \frac{B_1}{\left[ \text{Ninhydrin} \right]} + B_2
\end{equation}
with \( B_1 = \frac{(k_{-1} + k_2)(k_{II} + \Sigma H^+ J K_d)}{k_1 k_2 K_d \Sigma H^+ J} \) and \( D_2 = \frac{(k_{-1} + k_2)}{k_2} \)

The proposed mechanism is consistent with the observed rate equation. The evolution of one mole of carbon dioxide takes place through the decarboxylation of \( \alpha \)-COOH group because the second carboxyl group is situated in the \( \beta \) position. The reactivity of the carboxyl group becomes less but may not disappear with the formation of \( \beta \)-keto acid as an intermediate compound which is readily decarboxylated on heating at moderate temperature. Thus we can say that the evolution of second mole of carbon dioxide takes place through the decarboxylation of an unstable intermediate (\( \beta \)-keto acid) which is formed during the course of reaction of aspartic acid and ninhydrin. As the evolution of second mole is very fast therefore, the evolution of two moles of carbon dioxide is a concerted reaction and is a pseudo first order reaction.
\[ R-CH-COO^- + \text{NH}_3 \xrightarrow{K_{Q_2}} R-CH-C-O^- + H^+ \]

\[ A \quad \sim \quad B \]

\[ K_S \xrightarrow{\text{Fast}} + \text{Cu}^{2+} \]

\[ C \]

\[ + \quad \text{N} \]

\[ D \]

\[ E \]

\[ P \sim \]

\((R = -\text{CH}_3, -\text{CH}_2\text{CONH}_2, -\text{CH}_2\text{OH})\)

**Scheme 1**
\[
\begin{align*}
\text{Cd}^{2+} + \text{NH}_2\text{-CH-} \text{COO}^{-} & \quad \text{R} \\
\text{Fast} + & \quad \text{Fast} \\
\text{K}_d \quad & \quad \text{K}_d
\end{align*}
\]

\[
\begin{align*}
\text{R} \quad & \quad \text{R} \\
\text{NH}_2\text{-CH-} \text{COO}^{-} & \quad \text{NH}_2\text{-CH-} \text{COO}^{-}
\end{align*}
\]

\[
\begin{align*}
\text{D} \quad & \quad \text{D} \\
\text{NH}_2 & \quad \text{NH}_2
\end{align*}
\]

\[
\begin{align*}
\text{K}_n \quad & \quad \text{K}_n \\
+ \text{Cd}^{2+} & \quad + \text{Cd}^{2+}
\end{align*}
\]

\[
\begin{align*}
\text{E} \quad & \quad \text{E} \\
\text{Cd-} \text{OH} & \quad \text{Cd-} \text{OH}
\end{align*}
\]

\[
\begin{align*}
\text{K}_t \quad & \quad \text{K}_t \\
+ & \quad +
\end{align*}
\]

\[
\begin{align*}
\text{F} \quad & \quad \text{F} \\
\text{Cd} & \quad \text{Cd}
\end{align*}
\]

\[
\begin{align*}
\text{k}_2 \quad & \quad \text{k}_2
\end{align*}
\]

\[
\begin{align*}
\text{P}_2 \quad & \quad \text{P}_2 \\
\text{(R = -CH}_3\text{,-CH}_2\text{CONH}_2\text{,-CH}_2\text{OH)} & \quad \text{(R = -CH}_3\text{,-CH}_2\text{CONH}_2\text{,-CH}_2\text{OH)}
\end{align*}
\]

\text{SCHEME 3}
\[ \text{Scheme 5} \]
Scheme 6

\[
A \xrightarrow{K_{A2}} B \xrightleftharpoons{Fast} \text{[Cu]^{2+} + } \xrightarrow{K_s} \text{[Cu]^{2+} } \text{[Cu]^{2+} }
\]

\[
\text{N} \xrightarrow{k_1} \text{P}
\]
$\text{Q} - C_0 + Cd^{++} \xrightarrow{K_s \text{ Fast}} \text{B} +$ 

$\text{Cd}^{++} + \text{N} - \text{COO}^{-}$ 

$\text{O}_{\text{Cd}}$ 

$\text{H}_2$ 

$\text{K}_{t}$ 

$\text{CO}_2 + \text{H}_2\text{O}$ 

(E N O L B E T A I N E ) 

SCHEME 7
\[ \text{Scheme 8} \]
\[ \text{Conditions: amino acid - Cu(II) - } \text{Cu(II)} \text{ of amino acids.} \]
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Table - 2

Specific rate constants and activation parameters for  

With cadmium(II) complexes of amino acids.
and temperatures = 393 K, \( n = 1 \times 0 \text{ mol dm}^{-3} \times 10^{-7} \) and

\[
\begin{align*}
N & = 1.0 \times 10^{-7} \text{ mol dm}^{-3} \\
N_{\text{in}} & = 1.0 \times 10^{-7} \text{ mol dm}^{-3}
\end{align*}
\]

Conditions: Princeton program

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<tr>
<td>For decarboxylation</td>
<td>39.01</td>
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\[
\begin{align*}
K_j \text{ mol}^{-1} \\
K_j \text{ mol}^{-1}
\end{align*}
\]

\[
\begin{align*}
t & \text{ for } a_H \text{ or } a_S \uparrow & t & \text{ for } a_S \downarrow & t & \text{ for } a_H \downarrow & E
\end{align*}
\]

Table - 3

Activation parameters for nitrihydrin reaction with
\[
\text{Conditions: } \text{Proline-metallation } \implies \text{Proline-CD(II)-nitrhydrin, 2} = 1
\]

\[
\text{Temperature } = 353 K
\]

\[
\text{Proline-metallation } = \int_{+}^{-} + \int_{-} H_{-} \rightarrow \int_{+}^{-} + \int_{-} H_{-}
\]

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\text{Table - 4: Activation Parameters.}
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\[ T = \text{some equation} \]

\[ \begin{align*}
K_{\text{total}} & = K_{\text{total}} \times \text{some parameter} \\
S & = S \times \text{some parameter} \\
E & = E \times \text{some parameter}
\end{align*} \]

*with naphthazarin*

Table 5: Activation Parameters for Decarbonylation of Aspartic Acid
Figure 1: Variation of rate constant with Hammett constant for the reaction of ninhydrin with Cu(II) - amino acid complexes at $\lambda_{\text{max}} = 375$ nm.
Figure 2: Variation of rate constant with Hammett Constant for the reaction of ninhydrin with Cd(II) - amino acid complexes at $\lambda_{\text{max}} = 375$ nm.

Figure 3: Variation of rate constant with Hammett Constant for the reaction of ninhydrin with Cd(II) - amino acid complexes at $\lambda_{\text{max}} = 510$ nm.
References