ABSTRACT

Ninhydrin has been widely used for the qualitative and quantitative determinations of \(\alpha\)-amino acids. Ninhydrin reacts with \(\alpha\)-amino acids to produce purple coloured product\(^1\)\(^-\)\(^5\) (diketohydrindylidene diketohydrindamine) which is not stable in presence of oxygen and daylight. Many attempts were made to stabilize the coloured product and a number of investigators\(^6\)\(^-\)\(^12\) modified the ninhydrin reagent by adding reducing agents, organic solvents, metal ions etc. In presence of transition metal ions different coloured products have been reported for the amino acid ninhydrin reaction but the mechanism of their formation have not been investigated. The problem related with the kinetic and mechanism of the ninhydrin amino acid reaction is being studied under different experimental conditions in our laboratory for the last decade\(^13\)\(^-\)\(^17\). It was observed that in presence of metal ions the actual reaction takes place between metal-amino acid complex and ninhydrin because the complexation of metal ions is generally a very fast reaction as compared to the reaction of ninhydrin with amino acids. Therefore it was thought worthwhile to study the kinetics and mechanism of the interaction of biologically important metal complexes of histidine with ninhydrin.

Among the naturally occurring amino acids, histidine plays an important role in biological systems and provides potential binding sites in proteins for the coordination of metal ions\(^18\)\(^-\)\(^19\). It has also been reported that some complexes of
transition metal ions with histidine bind molecular oxygen reversibly and thus acts as synthetic oxygen-carriers.\textsuperscript{20-22} Having the importance of histidine in mind the studies on the kinetics and mechanism of its transition metal complexes with ninhydrin were carried out and are reported in this thesis.

In the first chapter a critical review on the importance of histidine and imidazole and its transition metal complexes have been given. The reaction of ninhydrin with amino acids and the role of metal ions on this reaction have been described.

Studies on the kinetics and mechanism of the interaction of copper(II), zinc(II) and nickel(II) complexes of histidine with ninhydrin have been described in II, III and IV chapters respectively. (These complexes react with ninhydrin to give a yellow coloured (\(\lambda_{\text{max}} = 375\, \text{nm}\)) schiff base complexes at pH 5. The composition of the schiff base complex was determined by employing the Job's method of continuous variation and it was found that one mole of metal-histidine complex reacted with one mole of ninhydrin. The pseudo first order rate constants were determined with respect to metal histidine complex by taking ninhydrin in large excess. The studies were carried out under varying conditions of temperature, pH and ionic strength. The reaction was also studied at different concentrations of ninhydrin and it was found that the reaction follows fractional order kinetics with respect to ninhydrin
concentration. The activation parameters were calculated using Arrhenius equation and are summarized in table-1.

The yellow coloured schiff base complex is formed as a result of coordination of ninhydrin to the same metal atom of the histidine complex which is the essential feature of template mechanism (CLAM reaction). Condensation of coordinated amino group of metal-histidine complex and coordinated carbonyl group of ninhydrin takes place within the coordination sphere of the metal ion. Due to the coordination of amino group, the nucleophilic addition-elimination reaction is not possible in these complexes. On the basis of the observed results and discussion the mechanism (Scheme-I) has been proposed and the following rate equation was derived:

\[ k_{obs} = k K_t [Ninhydrin]/(1+K_t[Ninhydrin]) \] .... (1)

where \( k \) is the rate constant and \( K_t \) is the equilibrium constant.

On rearrangement, equation (1) gives equation (2),

\[ 1/k_{obs} = 1/k K_t [Ninhydrin] + 1/k \] .... (2)

A linear plot for the \( 1/k_{obs} \) versus \( 1/[Ninhydrin] \) (Figure-1) provides a satisfactory explanation for the validity of the proposed mechanism and the corresponding rate equation. The values of \( k \) and \( K_t \) have been calculated. These values indicate that the condensation of amino group to carbonyl group is rate
determining step. Substituting the values of \( k, K_t \) and 
\([\text{Ninhydrin}]\) in equation (1), the values of rate constants have
been calculated for the kinetic runs. The good agreement
between the \( k_{\text{obs}} \) and \( k_{\text{calculated}} \) (table- 2-4) further supports
the proposed mechanism.

In fifth chapter the studies related with the kinetics
and mechanism of the interaction of chromium(III) histidine
complex with ninhydrin have been reported. The complexation
of chromium(III) with histidine is very slow process because
the exchange of coordinated water molecules from first
coordination sphere of chromium(III) is very slow. Therefore
the aqueous solution of chromium(III) and histidine was heated
for one hour before adding the ninhydrin solution. Yellow colour
[N-diketohydrindylidene histidinato chromium(III)] was formed
as the product and its composition was determined by Job's
method of continuous variations. It was found that one mole
of chromium(III) histidine complex reacted with one mole of
ninhydrin. The reaction was found to be first order with
respect to chromium(III) histidine complex and fractional order
with respect to ninhydrin. The reaction follows the Arrhenius
equation and calculated values of activation parameters are given
in table-1.

The reaction proceeds through the outer sphere
complexation of the ninhydrin with the coordinated water
molecules of chromium(III) histidine complex, followed by the
rate determining dissociation of coordinated water molecule. Condensation of coordinated amino group and coordinated carbonyl group takes place within the coordination sphere of chromium(III). These are the features of template mechanism. The mechanism of the reaction is given in scheme-II. The following rate equation was derived for the Scheme-II mechanism:

\[ k_{\text{obs}} = \frac{k_1 k_3 [\text{Ninhydrin}]}{(k_2 + k_3 + k_1 [\text{Ninhydrin}])} \quad \ldots \quad (3) \]

On rearranging the equation (3) we get equation (4)

\[ \frac{1}{k_{\text{obs}}} = \frac{(k_2 + k_3)/k_1 k_3 [\text{Ninhydrin}]}{1/k_3} + \frac{1}{k_3} \quad \ldots \quad (4) \]

or

\[ \frac{1}{k_{\text{obs}}} = \frac{1}{K k_3 [\text{Ninhydrin}]} + \frac{1}{k_3} \quad \ldots \quad (5) \]

(Where \( K = \frac{k_1}{k_2} \) and \( k_2 \gg k_3 \))

The observed results were found to be in good agreement with the proposed mechanism and the rate equation.

In the sixth chapter the studies related with the kinetics and mechanism of the interaction of cadmium(II) histidine complex with ninhydrin are described. It was observed that at 30°C only yellow coloured product (\( \lambda_{\text{max}} = 375 \text{ nm} \)) is formed while at temperature > 50°C, two products with \( \lambda_{\text{max}} = 375 \) and 510 nm were formed. The formation of two products yellow Schiff base complex (\( \lambda_{\text{max}} = 375 \text{ nm} \)) and red
coloured complex (\( \lambda_{\text{max}} = 510 \text{ nm} \)) followed first order kinetics with respect to cadmium(II) histidine complex and fractional order with respect to (Ninhydrin). The activation parameters were calculated by carrying out a series of kinetic runs at different temperatures (table-5). On the basis of the observed results the following mechanism (Scheme-III) has been proposed. The reaction proceeds through the coordination of ninhydrin with the cadmium(II) histidine complex which gives a Schiff base complex (\( \lambda_{\text{max}} = 375 \text{ nm} \)). Due to being labile in nature, the decarboxylation and hydrolysis of the Schiff base take place which gives the complex of cadmium(II)-2-aminoindanedione. This intermediate further reacts with another molecule of ninhydrin to give the red coloured complex. The following rate equations have been derived for the formation of yellow and red coloured complexes respectively.

\[
k_{1\text{obs}} = k K_t (\text{Ninhydrin})/(1+K_t(\text{Ninhydrin}) \quad \ldots \quad (6)
\]

On rearrangement, equation (6) gives equation (7),

\[
1/k_{1\text{obs}} = 1/k K_t (\text{Ninhydrin}) + 1/k \quad \ldots \quad (7)
\]

\[
k_{2\text{obs}} = k K_t (\text{Ninhydrin})/(1+K_t(\text{Ninhydrin}) \quad \ldots \quad (8)
\]

On rearrangement, equation (8) gives equation(9),

\[
1/k_{2\text{obs}} = 1/k K_t (\text{Ninhydrin}) + 1/k \quad \ldots \quad (9)
\]

The observed results were found to be in good agreement with the proposed mechanism and confirm the validity of the rate equations.
In the seventh chapter the studies on the interaction of cobalt(II)histidine complex with ninhydrin have been reported. The oxygen gas was bubbled through the reaction mixture containing the cobalt(II)-histidine complex and ninhydrin. The yellow coloured Schlif base complex was formed as the product. The order of the reaction was found to be unity with respect to oxygenated cobalt(II)histidine complex and fractional order with respect to (Ninhydrin). The mechanism is presented in Scheme-IV and the same rate equations was derived as in the case of copper(II), zinc(II) and nickel(II) complexes of histidine which confirms the proposed mechanism.

In absence of oxygen gas (while nitrogen gas was bubbled) yellow and violet coloured products with $\lambda_{\text{max}} = 375$ and 570 nm were obtained having the similar kinetic behaviour as that of oxygenated cobalt(II)histidine complex. The mechanism of the reaction is given in Scheme-V.
### TABLE - 1

Values of the activation parameters for the interaction of metal-histidine complexes with ninhydrin.

<table>
<thead>
<tr>
<th>Metal(II)histidine</th>
<th>$E_a$</th>
<th>$\Delta H^#$</th>
<th>$\Delta G^#$</th>
<th>$-\Delta S^#$</th>
<th>$\ln A$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(kJ mol$^{-1}$)</td>
<td>(kJ mol$^{-1}$)</td>
<td>(kJ mol$^{-1}$)</td>
<td>(JK mol$^{-1}$)</td>
<td>(min$^{-1}$)</td>
</tr>
<tr>
<td>Copper(II) histidine</td>
<td>069.14</td>
<td>066.66</td>
<td>084.53</td>
<td>59.95</td>
<td>19.07</td>
</tr>
<tr>
<td>Zinc(II) histidine</td>
<td>114.74</td>
<td>112.26</td>
<td>081.86</td>
<td>91.95</td>
<td>34.47</td>
</tr>
<tr>
<td>Nickel(II) histidine</td>
<td>058.50</td>
<td>055.57</td>
<td>085.39</td>
<td>71.94</td>
<td>15.11</td>
</tr>
<tr>
<td>Chromium(III) histidine</td>
<td>083.61</td>
<td>081.13</td>
<td>112.19</td>
<td>87.96</td>
<td>22.80</td>
</tr>
</tbody>
</table>

Conditions:

- $[\text{Metal}^{-}\text{histidine}] = 2.0 \times 10^{-4}$ mol dm$^{-3}$
- $[\text{Ninhydrin}] = 6.0 \times 10^{-3}$ mol dm$^{-3}$
- pH = 5.0, $\mu = 1.0$ mol dm$^{-3}$
TABLE - 2

COMPARISON OF THE OBSERVED RATE CONSTANTS AND CALCULATED RATE CONSTANTS.

<table>
<thead>
<tr>
<th>[Ninhydrin] $\times 10^3$ mol dm$^{-3}$</th>
<th>$k_{obs} \times 10^2$ min$^{-1}$</th>
<th>$k_{cal} \times 10^2$ min$^{-1}$</th>
<th>$(k_{obs} - k_{cal}) / k_{obs}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.0</td>
<td>1.15</td>
<td>1.22</td>
<td>-0.074</td>
</tr>
<tr>
<td>10.0</td>
<td>1.86</td>
<td>1.97</td>
<td>-0.059</td>
</tr>
<tr>
<td>14.0</td>
<td>2.64</td>
<td>2.44</td>
<td>+0.081</td>
</tr>
<tr>
<td>18.0</td>
<td>3.65</td>
<td>3.24</td>
<td>+0.088</td>
</tr>
<tr>
<td>22.0</td>
<td>4.09</td>
<td>3.79</td>
<td>+0.070</td>
</tr>
<tr>
<td>26.0</td>
<td>4.49</td>
<td>4.31</td>
<td>+0.060</td>
</tr>
<tr>
<td>30.0</td>
<td>5.22</td>
<td>4.79</td>
<td>+0.077</td>
</tr>
<tr>
<td>34.0</td>
<td>5.60</td>
<td>5.23</td>
<td>+0.064</td>
</tr>
<tr>
<td>38.0</td>
<td>6.17</td>
<td>5.63</td>
<td>+0.068</td>
</tr>
<tr>
<td>42.0</td>
<td>6.36</td>
<td>6.00</td>
<td>+0.047</td>
</tr>
</tbody>
</table>

Conditions:
- $[Cu(II)-\text{histidine}]^+ = 2.0 \times 10^{-4}$ mol dm$^{-3}$
- $[\text{Hydrogen ion}] = 1.0 \times 10^{-5}$ mol dm$^{-3}$
- Ionic Strength = 1.0 mol dm$^{-3}$
- Temperature = 353 K
### TABLE 3
COMPARISON OF THE OBSERVED RATE CONSTANTS AND CALCULATED RATE CONSTANTS.

<table>
<thead>
<tr>
<th>[Ninhydrin] $\times 10^3$ mol dm$^{-3}$</th>
<th>$k_{obs} \times 10^2$ min$^{-1}$</th>
<th>$k_{cal} \times 10^2$ min$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.0</td>
<td>1.037</td>
<td>1.172</td>
</tr>
<tr>
<td>10.0</td>
<td>1.037</td>
<td>1.082</td>
</tr>
<tr>
<td>15.0</td>
<td>2.749</td>
<td>2.712</td>
</tr>
<tr>
<td>20.0</td>
<td>3.485</td>
<td>3.449</td>
</tr>
<tr>
<td>25.0</td>
<td>4.328</td>
<td>4.132</td>
</tr>
<tr>
<td>30.0</td>
<td>5.292</td>
<td>4.979</td>
</tr>
<tr>
<td>35.0</td>
<td>5.901</td>
<td>5.343</td>
</tr>
<tr>
<td>40.0</td>
<td>6.273</td>
<td>6.101</td>
</tr>
</tbody>
</table>

Conditions:

- $[\text{Zn(II)-histidine}] = 2.9 \times 10^{-4}$ mol dm$^{-3}$
- [Hydrogen ion] = 1.0 $\times 10^{-5}$ mol dm$^{-3}$
- Ionic Strength = 1.0 mol dm$^{-3}$
- Temperature = 353 K
TABLE - 4

The comparison of the calculated and observed rate constants.

<table>
<thead>
<tr>
<th>[Ni(nhydrin)] $\times 10^{-3}$ (mol dm$^{-3}$)</th>
<th>$k_{obs} \times 10^2$ (min$^{-1}$)</th>
<th>$k_{cal} \times 10^2$ (min$^{-1}$)</th>
<th>$(k_{obs} - k_{cal}) / k_{obs}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.0</td>
<td>0.760</td>
<td>0.704</td>
<td>+0.071</td>
</tr>
<tr>
<td>10.0</td>
<td>0.888</td>
<td>0.886</td>
<td>+0.002</td>
</tr>
<tr>
<td>15.0</td>
<td>1.124</td>
<td>1.140</td>
<td>-0.014</td>
</tr>
<tr>
<td>20.0</td>
<td>1.309</td>
<td>1.344</td>
<td>-0.026</td>
</tr>
<tr>
<td>25.0</td>
<td>1.395</td>
<td>1.460</td>
<td>-0.046</td>
</tr>
<tr>
<td>30.0</td>
<td>1.644</td>
<td>1.623</td>
<td>+0.013</td>
</tr>
<tr>
<td>35.0</td>
<td>1.654</td>
<td>1.720</td>
<td>-0.039</td>
</tr>
<tr>
<td>40.0</td>
<td>1.701</td>
<td>1.801</td>
<td>-0.057</td>
</tr>
</tbody>
</table>

Conditions:

$[\text{Ni(II)-histidine}]^{+} = 2.0 \times 10^{-4}$ (mol dm$^{-3}$)
$[\text{Hydrogen ion}] = 1.0 \times 10^{-5}$ (mol dm$^{-3}$)
Ionic Strength = 1.0 (mol dm$^{-3}$)
Temperature = 353 K
TABLE - 5

Activation parameters for the interaction of cadmium (II) histidine complex with ninhydrin.

<table>
<thead>
<tr>
<th>Activation parameters</th>
<th>For the yellow Schiff base complex (375 nm)</th>
<th>For the red complex (510 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_a$ (kJ mol$^{-1}$)</td>
<td>77.206</td>
<td>73.282</td>
</tr>
<tr>
<td>$\Delta H^\circ$ (kJ mol$^{-1}$)</td>
<td>74.728</td>
<td>70.804</td>
</tr>
<tr>
<td>$\Delta S^\circ$ (kJ mol$^{-1}$)</td>
<td>81.328</td>
<td>80.926</td>
</tr>
<tr>
<td>$\Delta S$ (JK$^{-1}$mol$^{-1}$)</td>
<td>-22.147</td>
<td>-33.966</td>
</tr>
<tr>
<td>$\ln A$ (min$^{-1}$)</td>
<td>23.108</td>
<td>21.933</td>
</tr>
</tbody>
</table>

Conditions:

\[
[Cd(II)\text{histidine}] = 2 \times 10^{-4} \text{mol dm}^{-3}
\]

\[
[Ninhydrin] = 6 \times 10^{-3} \text{mol dm}^{-3}
\]

$\text{pH} = 5.0, \quad \mu = 1.0 \text{mol dm}^{-3}$
Fig. 1: Plot for the linear dependence of $\frac{1}{K_{obs}}$ on $\frac{1}{[\text{Ninhydrin}]}$ for the interaction of ninhydrin with metal(II) histidine complex.

$[H^+] = 1.0 \times 10^{-5} \text{ mol dm}^{-3}$, $\mu = 1.0 \text{ mol dm}^{-3}$, Temperature = 353 K.

1. Copper(II)histidine
2. Zinc(II)histidine
3. Nickel(III)histidine
N-Diketohydrindylidene histidinato Metal

SCHEME - 1
It

$\text{Scheme - II}$

$\text{N - Diketonohydridylidene histidinato chromium}^{2+}$

$\text{Scheme - III}$
SCHEME IV
REFERENCES

