CHAPTER VII

DISCUSSION & RESULT

KINETIC STUDY OF L.Arg., L.Glu, L. Prolene & GLY. BY NBA IN ALKALINE MEDIA.
In this chapter an attempt has been made to interpret and discuss the experimental results obtained in chapter 3 to 6 and on the basis of results a suitable reaction mechanism for all the oxidation processes investigated in the present thesis has been proposed:

The results obtained in the previous chapters are summarised in the following sections:
7.1 RESULTS IN OXIDATION OF L.Arg., L.Prolene, DL GLU AND GLY. BY NBA IN ALKALINE MEDIA.

In all the four cases, the reaction is of 1st order with respect to NBA. In all cases the various contration of NBA was taken keeping all other reactants concentration as constant.

The graphs were plotted between time and uncosumed NBA. The values of dc/dt was obtained with the help of graphs and rate constants were evaluated ($k_1$).

The values of $k_1$ for each reactants is fairly constant, showing 1st order dependence of NBA.

The reactions showing fractional order with respect to reducing substances (Arg., L. Prolene, DLGLU and GLY.). In order to this, experiments have been carried by changing the concentration of reducing substances keeping all other reagent concentration as constant.

At fixed concentration of NBA and OH⁻ the reaction rate increases with increase in concentration, of Amino acids.

The plot of log $k_{(obs)}$ vs. log [Amino Acid] is linear with slope less than unity. A definite intercept is obtained with $1/k_{(obs)}$ Vs $1/[Amino Acid]$ indicating the formation of complex between oxidant and substrate.
The rate of reaction varies slowly by variation of OH$^-$ concentration.

The rate increases with decrease in OH$^-$. The plot of log $k_{(obs)}$ [OH$^-$] is linear with a slope of less than unity indicating fractional order dependence on OH$^-$. In all cases the rate or reaction was independent to ionic strength of the medium.
7.2 **STOICHIOMETRY AND PRODUCT ANALYSIS:**

Stoichiometric investigation was carried by keeping the excess of N bromoacetamide with fixed concentration of substrate (Amino acids) and the excess of remaining N bromoacetamide was estimated idometrically.

In all the fore cases one mole of N bromoacetamide was found to react with one mole of amino acid in accordance with,

\[
\text{CH}_3\text{CO NHBr} + R\text{.CH (NH}_2\text{)COO}^- + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{CONH}_2 + R\text{CHO} + \text{CO}_2 + \text{NH}_3 + \text{Br}^- \]

\( \text{Where R stands for H (in Glycine).} \)

\( \text{O}^-\text{OC CH}_2\text{CH}^-_2 \) (in Glutamic acid),

\( \text{H}_2\text{NC-NH CH}_2\text{CH}_2\text{CH}_2^- \text{ in Arginine} \)

and

\( \text{Prolene} \)

The experimental analysis of products gives the presence of Acetamide, Aldehydes corresponding to these R values of Amino Acids and the presence of \( \text{Br}^- \). Ammonia and Carbon dioxide is formed along other products.
7.3 DISCUSSION AND INTERPRETATION OF RESULTS.

Oxidation of amino acid by N bromoacetamide under nitrogen atmosphere has failed to induce polymerisation of acrylomitrile ruling out a free radical mechanism.

In alkaline media the proposed oxidising species are anion of NBA and OBr⁻. There is no effect of added acetamide on the rate of reaction in alkaline medium. Hence it is suggested that NBA⁻ is active.

\[
\text{CH}_3\text{CO NHBr}+\text{OH}^- \rightarrow K_1 \text{CH}_3\text{CON}^-\text{Br}+\text{H}_2\text{O} \quad \ldots 2
\]

The zwitterionic and or anionic forms of amino acids can be considered to be the reactive species in alkaline media.

However, the attack of NBA⁻ on the carbon of the zwitterionic forms of amino acid is more feasible than that of the anionic form. Since the farer species has relatively lower electron density than the latter.

The zwitterionic forms of present all the four used amino acids are represented as:

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Zwitterionic form</th>
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<tbody>
<tr>
<td>Glycin(Gly.)</td>
<td>( \text{H} )( \text{N}^- \text{H}_3^+ )( \text{C}^- \text{O} \text{O}^- )</td>
</tr>
<tr>
<td>DL Glutamic Acid</td>
<td>(DLGLU) ( \text{O} )( \text{C} )( \text{H}_2 \text{. C} \text{H}_2 \text{. C} \text{. C} \text{O} \text{O}^- ) ( \text{N}^- \text{H}_3^+ )</td>
</tr>
</tbody>
</table>
In the present work there is formation of complex between anionic form of NBA and zwitterionic forms of Aminoacids. According as:

\[
\text{CH}_3\text{CON}^+\text{Br}^- + \text{RCH(NH}_3^+\text{)COO}^- \xrightarrow{K_2} \left[ \begin{array}{c}
\text{R} -= \text{CH} - \text{COO}^- \\
\text{NH}_3^+ \\
\text{CH}_3 - \text{C} = \text{O}
\end{array} \right] \rightarrow \left[ \begin{array}{c}
\text{R} = \text{CH} = \text{N} - \text{C} - \text{CH}_3 \\
\text{NH}_3^+ + \text{CO}_2^+ + \text{Br}^-
\end{array} \right]
\]

This is followed by the decomposition of the complex in a slow step.

Where \(K_1\) and \(K_2\) are equilibrium constants for equation 2nd and 3rd. \(K_d\) is dissociation constant for complex.
At finally there is a fast reaction as:

\[
R-CH=N-C-CH_3+H_2O \rightarrow RCHO+CH_3CO NH_2
\]

The rate expression derived from the proposed mechanism is given by eq. 6.

\[
\text{Rate} = -\frac{d[NBA]_T}{dT} = K_d [\text{Complex}]
\]

or

\[
\text{Rate} = -\frac{d[NBA]_T}{dT} = K_d K_1 K_2 [NBA] [OH^-][S]
\]

Where \([S]\) stands for substrate (Aminoacids)

The total \([NBA]_T\) can be given as:

\[
[NBA]_T = [NBA]+[NBA^-]+[\text{Complex}]
\]

or

\[
[NBA]_T = [NBA]+K_1 [NBA][OH^-]+K_1 K_2 [NBA][S][OH^-]
\]

Substitution the value of \([NBA]_T\) in equation 9 egstion 10 is obtained as:

\[
\frac{d[NBA]}{dT} = \frac{K_d K_1 K_2 [NBA][S][OH^-]}{1+K_1[OH^-]+K_1 K_2 [S][OH^-]}
\]

By rearranging equation 11 is obtained:

\[
\frac{1}{K_{obs}} = \frac{1}{K_d K_1 K_2 [S][OH^-]} + \frac{1}{K_d K_2 [S]} + \frac{1}{K_d}
\]
The $K$ observed is actual rate constant for over all reaction.

According to equation (11) the plot of $1/K$ obs. Vs. $1/OH^-$ and $1/K$ obs. vs. $1/[\text{Aminoacid}]$ should be linear and with definite intercepts.

This has been found to be the case, supporting the proposed mechanism.

From equation 11 it is evident that $K_d$ can be calculated from the intercept of the plot of $1/K$ obs. Vs. $1/[\text{Aminoacid}]$

Making use of $K_d$, $K_2$ can be evaluated from the intercept of the plot of $1/K$ obs. Vs. $1/OH^-$ substituting the value of $K_d$ and $K_2$, in the slope obtained either from the plot of $1/K$ obs. Vs. $1/[\text{Aminoacid}]$ or from that of $1/K$ obs. Vs. $1/OH^-$, $K_1$ can be calculated.

The values of $K_1$ are 3.3 ±0.06 for these four Aminoacids.

The activation energies for the oxidation of L.Argine, L. Prolene, DL. Glu and Glycine are favourable for the reaction rate.

The $\Delta G$ values as calculated for all these four aminoacids is approximately constant equal to (93±2) indicates that probably the same mechanism prevails in all cases.

The negative values for entropy of activation reflects that the activated complex is more regid.

Thus experimental results and thermodynamic data obtained clearly support the proposed mechanism.