Chapter-IV

Osmium (VIII) catalysed oxidation of L-Proline by alkaline Hexacyanoferrate (III).
1. INTRODUCTION

The chemistry of hexacyanoferrate(III) (HCF) in alkaline medium is well understood\(^1\)\(^-\)\(^8\), in particular its oxidative capacity of oxidation of inorganic and organic compounds. It may be due to its unequivocal stability, solubility, single equivalent change and its moderate reduction potential, \([\text{Fe(CN)}_6]^{3^-} / [\text{Fe(CN)}_6]^{4^-}\) of +0.45V. Thus, this potent oxidant, HCF is used mainly as electron abstracting or proton abstracting agent and also in free radical generation during the oxidation of numerous organic compounds.

Osmium compounds are highly stable in +8 oxidation state. The OsO\(_4\) (Os(VIII)) undergoes reduction up to +2 via +4 and +6 oxidation states in acid medium. However, in alkaline medium it undergoes reduction\(^9\) up to +6 oxidation state only. Together the unique nature and moderate reduction potential\(^10\), Os(VIII) / Os(VI) of +0.85V in acid medium and +0.30V in alkaline medium act as a catalyst with a large number of oxidants in the oxidation of both organic and inorganic substrates\(^11\). The literature survey reveals that the catalytic role\(^12\) of osmium(VIII) is well established. Nevertheless, Os(VIII) has been used as catalyst until today to understand the reaction paths during oxidation of various organic substrates particularly, with HCF wherein HCF regenerates Os(VI)/Os(IV) to Os(VIII).

L-proline (L-Prol) is a non-essential\(^13\) amino acid and it is an important constituent of collagen. It is also an ingredient in energy drinks. A recent report\(^13\) reveals that L-proline is considered to be the world’s smallest natural enzyme and is used in catalyzing the aldol condensation of the acetone to various aldehydes with high stereo-specificity. It is an imino acid and also a unique proteogenic \(\alpha\)-imino acid where the \(\alpha\)-imino group is secondary with pyrrolidine ring. Some of the reports of oxidation of L-proline claim that the ring cleavage takes place between of ‘N’ and ‘C’
of $-\text{NH} - \text{CH} - \text{COOH}$ by retaining $-\text{NH}_2$ group with the main moiety without liberating ammonia and they have proposed\textsuperscript{14} a mechanism for its oxidation as decarboxylation. Nonetheless, the present investigation is undertaken to know whether the reported product results or not. The results in the present study are found to be substantially different and very prominently to establish a mechanism for its oxidation. Hence, the present investigation was undertaken in alkaline medium to setup the oxidative paths of L-proline with a unique oxidant and a catalyst viz., HCF and Osmium(VIII) respectively.

2. EXPERIMENTAL

2.1. Preparation of solutions

Reagent grade chemicals and double distilled water (from KMnO$_4$ in all - glass apparatus) were used. Aqueous solution of HCF was prepared by dissolving K$_3$[Fe(CN)$_6$] (BDH) in water and standardized\textsuperscript{15} iodometrically. L-proline, a colourless crystalline compound (E-Merck) was used without further purification for the preparation of aqueous stock solution. The stock solution of Osmium(VIII) was obtained by dissolving Osmic acid (OsO$_4$) (Johnson-Matthey) in 0.5 mol dm$^{-3}$ sodium hydroxide solution and its concentration was ascertained\textsuperscript{16} against standard Ceric ammonium sulfate solution in acid medium. Aqueous solutions of K$_4$[Fe(CN)$_6$] and L-glutamic acid were used to study the effect of products on rate of reaction. Aqueous solutions of NaOH and NaCl were used to maintain the [OH$^-$] and ionic strength respectively.

2.2. Kinetics

The reaction was initiated by mixing of K$_3$[Fe(CN)$_6$] solution to L-proline which also contained required amounts of Os(VIII), NaOH and NaCl. The reaction at $30 \pm 0.1^\circ\text{C}$
was studied at least 10 times under pseudo-first order condition where [L-Prol] > [HCF]. Progress of the reaction was followed spectrophotometrically by measuring the decrease in the absorbance of [HCF] using Hitachi U-3010 spectrophotometer (Tokyo, Japan) at its $\lambda_{\text{max}} = 420$ nm, as a function of time where other constituents of reaction mixture do not absorb significantly (Figure IV (a) p.126). The obedience to Beer's law of the absorption of HCF at 420 nm in the concentration range of $6.0 \times 10^{-5}$ to $8.0 \times 10^{-4}$ mol dm$^{-3}$ under the reaction conditions had been tested earlier ($e = 1060 \pm 1.5\%$ dm$^3$ mol$^{-1}$ cm$^{-1}$) (Figure IV(b) p.127). An example run is given in the Table IV(a) p.128 and Figure IV(c) p.129. The pseudo-first order rate constants, $k_{\text{obs}}$, were calculated from the slopes of log [HCF] versus time plots which were linear up to 80% completion of the reaction in most of the variation of concentrations of oxidant, reductant and catalyst. Order with respect to each reactant is determined from the slopes of plots of log $k_{\text{obs}}$ versus log(conc.) except in [HCF]. The results were reproducible within $\pm 4\%$.

The moderately higher concentration of NaOH was used to maintain the OH$^-$ concentration in the reaction. Hence, the effect of dissolved CO$_2$ on the rate was examined by carrying out the kinetics in presence of CO$_2$ and N$_2$. It was found that there was no variation of rate constants which indicates that dissolved CO$_2$ had no effect on rate of reaction.

3. RESULTS

3.1. Stoichiometry and Product Analysis

Different sets of various concentrations of reactants and at constant concentration of catalyst in 0.10 mol dm$^{-3}$ NaOH at a constant ionic strength of 0.50 mol dm$^{-3}$ were
Figure IV(a): Spectrum of potassium hexacyanoferrate(III) in aqueous alkaline medium at 30°C.

\[ [\text{HCF}] = 4.0 \times 10^{-4}; \quad [\text{OH}^-] = 0.1; \quad I = 0.5 / \text{mol dm}^{-3} \]
Figure IV(b): Verification of Beer's law at 420 nm with freshly prepared potassium hexacyanoferrate(III) in aqueous alkaline medium at 30°C.

\[ [\text{HCF}] = 4.0 \times 10^{-4}; \quad [\text{OH}^-] = 0.1; \quad I = 0.5 \text{ / mol dm}^{-3} \]
Table IV(a): Example run for the Osmium(VIII) catalyzed oxidation of L-Proline by Hexacyanoferrate(III) in aqueous alkaline medium at 30° C.

\[
\begin{align*}
[HCF] &= 4.0 \times 10^{-4}; \\
[OH^-] &= 0.1; \\
[L\text{-Prol}] &= 4.0 \times 10^{-3}; \\
[Os(VIII)] &= 1.0 \times 10^{-6}; \\
I &= 0.5 \text{ /mol dm}^3
\end{align*}
\]

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<th>Time (min)</th>
<th>Absorbance (420 nm)</th>
<th>[HCF] \times 10^4 (mol dm(^{-3}))</th>
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<tr>
<td>10.0</td>
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</table>
Figure IV(c): Absorbance versus time plot for example run for the oxidation of L-Proline by hexacyanoferrate(III) in aqueous alkaline medium at 30°C.

\[ [\text{HCF}] = 4.0 \times 10^{-4}; \quad [\text{L-Pro}] = 4.0 \times 10^{-3}; \]
\[ [\text{OH}^+] = 0.1; \quad [\text{Os(VIII)}] = 1.0 \times 10^{-6}; \]
\[ I = 0.5 \text{ /mol dm}^{-3} \]
kept for over 24 hours at 30° C. When [HCF] > [L-Prol], the [HCF] was analyzed by measuring its absorbance at 420 nm. It was also estimated iodometrically\textsuperscript{15}. The main oxidative product of L-proline was identified as L-glutamic acid by its spot test in which the intense blue colour was obtained by adding ninhydrin\textsuperscript{17}. It supports the earlier work\textsuperscript{18}. It is also estimated quantitatively as ninhydrin derivative by spectrophotometric method\textsuperscript{19}. It is found that L-proline was oxidized to L-glutamic acid. Other plausible products like glutamic semialdehyde and α-keto acid were not found. Further, the L-glutamic acid was separated from reaction mixture by ether extraction on acidification and subjected to IR scanning. It was observed that the stretching frequencies of –NH\textsubscript{2}, –COOH and carbonyl appeared to be 3430, 3060 and 1684 cm\textsuperscript{-1} respectively, and C-N vibration frequency at 1124 cm\textsuperscript{-1} was also observed. This clearly indicates that the oxidative product of L-proline was found to be L-glutamic acid which is formed by reacting with 4 moles of [Fe(CN)\textsubscript{6}]\textsuperscript{3-} as shown in Equation 1 (Table IV (b) p. 131).

\[
\text{N} - \text{COOH} + 4[\text{Fe(CN)}\textsubscript{6}]^{3-} + 4\text{OH}^- \xrightarrow{\text{Os(VII)}} \text{HOOC - CH}_2\text{- CH}_2\text{- CH - COOH} \\
\text{NH}_2 + 4[\text{Fe(CN)}\textsubscript{6}]^{4-} + 2\text{H}_2\text{O} \quad (1)
\]

### 3.2. Reaction Order

Reaction order and order with respect to each reactant was determined by varying the concentrations of oxidant, reductant, catalyst and alkali in turn, while keeping the others constant.
Table IV(b): Stoichiometry of Osmium(VIII) catalyzed oxidation of L-Proline by Hexacyanoferrate(III) in aqueous alkaline medium at 30°C.

\[ [\text{OH}^-] = 0.1; \quad [\text{Os(VIII)}] = 1.0 \times 10^{-6}; \quad I = 0.5 / \text{mol dm}^{-3} \]

<table>
<thead>
<tr>
<th>Taken</th>
<th>Found</th>
<th>Reacted</th>
</tr>
</thead>
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<td>(mol dm(^{-3}))</td>
<td>(mol dm(^{-3}))</td>
<td>(mol dm(^{-3}))</td>
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3.2.1. Effect of [HCF]

The order in [HCF] in the concentration range, $5.0 \times 10^{-5}$ to $5.0 \times 10^{-4}$ mol dm$^{-3}$ was found to be unity as the plot of log [HCF] versus time was linear with non-variation in slopes for different [HCF] (Table IV(c) p.133). This was also confirmed from the linearity of plots of log absorbance versus time Figure IV(d) p. 134.

3.2.2. Effect of [L-Pro]

The order in L-proline in the concentration range of $1.0 \times 10^{-3}$ to $1.0 \times 10^{-2}$ mol dm$^{-3}$ was found to be less than unity (0.4), which is confirmed from the plot of log $k_{obs}$ versus log (concentration) plot (Table IV(c) p. 133 and Figure IV(e) p.135).

3.2.3. Effect of [OH$^-$]

At a fixed ionic strength of 0.5 mol dm$^{-3}$ and other conditions remaining constant, [OH$^-$] was varied from 0.05 to 0.40 mol dm$^{-3}$. It was noticed that as [OH$^-$] increased the rate of reaction was also increased (Table IV(d) p.136). The plot of log $k_{obs}$ versus log [OH$^-$] was linear (Figure IV(f) p.137) and order was determined as 0.5. Its increasing effect on rate is due to the variation of concentration of hydroxide species of Os(VIII) at different [OH$^-$]. The various forms of hydroxide complexes in alkaline medium such as [OsO$_3$(OH)$_3$]$^-$, [OsO$_4$(OH)$_2$]$^{2-}$ and [OsO$_5$(OH)]$^{3-}$ are in equilibrium with each other and can be shown in Equations (2) and (3).

\[
\text{[OsO}_3(\text{OH})_3]\text{^-} + \text{OH}^- \rightleftharpoons K_1 \text{[OsO}_4(\text{OH})_2]\text{F^-} + \text{H}_2\text{O} \quad (2)
\]

\[
\text{[OsO}_4(\text{OH})_2]\text{F^-} + \text{OH}^- \rightleftharpoons K_2 \text{[OsO}_5(\text{OH})]\text{F^-} + \text{H}_2\text{O} \quad (3)
\]

The total concentration of Osmium(VIII), $[\text{Os(VIII)}]_T$ is sum of different Os(VIII) species concentrations, [OsO$_3$(OH)$_3$]$^-$, [OsO$_4$(OH)$_2$]$^{2-}$ and [OsO$_5$(OH)]$^{3-}$; the
Table IV(c): Effect of [HCF] and [L-Prol] on Osmium(VIII) catalyzed oxidation of L-Proline by Hexacyanoferrate(III) in aqueous alkaline medium at 30\(^0\)C.

\([\text{OH}^-] = 0.1; \quad [\text{Os(VIII)}] = 1.0 \times 10^{-6}; \quad I = 0.5 / \text{mol dm}^3\)

<table>
<thead>
<tr>
<th>[HCF] (\times 10^4) (mol dm(^{-3}))</th>
<th>[L-Prol] (\times 10^3) (mol dm(^{-3}))</th>
<th>(k_{\text{obs}} \times 10^3) (s(^{-1}))</th>
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<th>Calc.</th>
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Figure IV(d): First order plots of Osmium(VIII) catalyzed oxidation of L-Proline by Hexacyanoferrate(III) in aqueous alkaline medium at 30° C.

\[ [HCF] = (1) \ 0.8 \times 10^{-4}, (2) \ 1.0 \times 10^{-4}, (3) \ 2.0 \times 10^{-4}, (4) \ 3.0 \times 10^{-4}, \]

\( (5) \ 4.0 \times 10^{-4}, (6) \ 5.0 \times 10^{-4} \ / \ mol \ dm^{-3}. \)

(Conditions as in Table IV(c) (p.133)
Figure IV(e): Order in [L-Prol] on the Osmium(VIII) catalyzed oxidation of L-Proline by Hexacyanoferrate(III) in aqueous alkaline medium at 30°C

Conditions as in Table IV(c) (p.133)
Table IV(d): Effect of [OH'] and [Os(VIII)] on Osmium(VIII) catalyzed oxidation of L-Proline by Hexacyanoferrate(III) in aqueous alkaline medium at 30°C.

\[ [\text{HC}F] = 4.0 \times 10^{-4}; \quad [\text{L-Pro}l] = 4.0 \times 10^{-3}; \quad I = 0.5 / \text{mol dm}^{-3} \]

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<tr>
<th>[OH'] \text{ (mol dm}^{-3})</th>
<th>[Os(VIII)] \times 10^6 \text{ (mol dm}^{-3})</th>
<th>\text{k}_{\text{obs}} \times 10^3 \text{ (s}^{-1})</th>
<th>\text{k}_{\text{Exp}}</th>
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Figure IV(f): Order in [OH\textsuperscript{−}] on the Osmium(VIII) catalyzed oxidation of L-Proline by Hexacyanoferrate(III) in aqueous alkaline medium at 30\textdegree C.

Conditions as in Table IV(d) (p.136)

![Graph showing the relationship between $4 + \log k_{obs}$ and $2 + \log [\text{OH}^{-}]$.]

Figure IV(g): Order in [Os(VIII)] on the Osmium(VIII) catalyzed oxidation of L-Proline by Hexacyanoferrate(III) in aqueous alkaline medium at 30\textdegree C.

Conditions as in Table IV(d) (p.136)

![Graph showing the relationship between $4 + \log k_{obs}$ and $8 + \log [\text{Os(VIII)}]$.]
complexes having the equilibrium constants $K_1$ and $K_{11}$ as 24 and 6.4 dm$^3$ mol$^{-1}$ respectively$^{20}$, are used to calculate the concentrations of such species (Table IV(e) p.139). The results of such calculations are utilized to draw (Figure IV(h) p.140), and it is seen that, of the concentrations of different species, the variation of only $[\text{OsO}_5(\text{OH})]^{3-}$ with $[\text{OH}^-]$ shows any parallelism with variation of $k_{obs}$ with alkali.

3.2.4. Effect of [Osmium(VIII)]

Effect of varying [Os(VIII)] in the range of $1.0 \times 10^{-7}$ to $4.0 \times 10^{-6}$ mol dm$^{-3}$ in the reaction was studied under the constant concentrations of oxidant and reductant at $4.0 \times 10^{-4}$ and $4.0 \times 10^{-3}$ mol dm$^{-3}$ respectively, when $[\text{OH}^-] = 0.1$ mol dm$^{-3}$ (Table IV(d) p.136). The order in [Os(VIII)] was found to be unity as calculated from log-log plots (Figure IV(g) p. 137).

3.2.5. Effect of Initially Added Products

The effect of initially added products $[\text{Fe(CN)}_6]^{4-}$ and L-glutamic acid were studied in the concentration range of $1.0 \times 10^{-4}$ to $1.0 \times 10^{-3}$ mol dm$^{-3}$ at constant ionic strength and all other conditions being constant. It is found that both of the products did not alter the rate of reaction (Table IV(f) p.141).

3.2.6. Effect of Ionic Strength

The ionic strength of the reaction medium was varied between 0.1 and 0.9 mol dm$^{-3}$ with sodium chloride at constant concentrations of alkali, oxidant, reductant and catalyst at 30°C. The apparent first order rate constants showed a nearly ten fold increase (Table IV(g) p.142). A plot of log $k_{obs}$ versus $\sqrt{I}$ was linear with a unit positive slope (Figure IV(i) p.143).
Table IV(e): Variation of concentration of active species$^\#$ of Osmium(VIII) at different [OH'] with rate constants of oxidation of L-Proline by hexacyanoferrate(III) catalyzed by osmium(VIII) at 30° C.

<table>
<thead>
<tr>
<th>[OH'] (mol dm$^{-3}$)</th>
<th>[OsO$_3$(OH)$_3$]$^+$ x 10$^7$ (mol dm$^{-3}$)</th>
<th>[OsO$_4$(OH)$_2$]$^{2+}$ x 10$^7$ (mol dm$^{-3}$)</th>
<th>[OsO$_5$(OH)]$^{3+}$ x 10$^7$ (mol dm$^{-3}$)</th>
<th>$k_{obs}$ x 10$^3$ (s$^{-1}$)</th>
<th>$k_{cal}$$^#$ x 10$^3$ (s$^{-1}$)</th>
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</thead>
<tbody>
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<td>2.51 s$^{-1}$</td>
</tr>
<tr>
<td>0.50</td>
<td>0.19 x 10$^7$</td>
<td>2.22 x 10$^7$</td>
<td>7.54 x 10$^7$</td>
<td>2.85 s$^{-1}$</td>
<td>2.84 s$^{-1}$</td>
</tr>
</tbody>
</table>

$^\#$ Os(VIII) in various forms at different [OH'] as per equilibria (2) and (3) and $K_1 = 24$ dm$^3$ mol$^{-1}$ and $K_{II} = 6.4$ dm$^3$ mol$^{-1}$ are used to calculate the species (Ref. [17])
Figure IV (h): Variation of concentrations of different Os(VIII) species and rate constants with [OH\(^-\)].

(— • — [Os(VIII)], —— —[OsO\(_4\)(OH)\(_2\)]\(^2^-\), —— —[OsO\(_5\)(OH)]\(^3^-\), —— —log\(k_{obs}\))

(Conditions are as in Table IV(e) p.138)
Table IV(f): Effect of initially added products, \([\text{Fe(CN)}_6]^{4-}\) and L-Glutamic acid on the Osmium(VIII) catalyzed oxidation of L-Proline by Hexacyanoferrate(III) in aqueous alkaline medium at 30\(^0\)C

\[
\begin{align*}
[HCF] &= 4.0 \times 10^{-4}; \\
[L-\text{Prol}] &= 4.0 \times 10^{-3}; \\
[\text{OH}^{-}] &= 0.1; \\
[\text{Os(VIII)}] &= 1.0 \times 10^{-6}; \\
I &= 0.5 \text{ mol dm}^{-3}
\end{align*}
\]

<table>
<thead>
<tr>
<th>[Fe(CN)(_6)](^{4-})/L-glutamic acid</th>
<th>(k_{\text{obs}} \times 10^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(x 10^4) (mol dm(^{-3}))</td>
<td>(s(^{-1}))</td>
</tr>
<tr>
<td>0.8</td>
<td>1.32</td>
</tr>
<tr>
<td>1.0</td>
<td>1.44</td>
</tr>
<tr>
<td>2.0</td>
<td>1.46</td>
</tr>
<tr>
<td>3.0</td>
<td>1.42</td>
</tr>
<tr>
<td>5.0</td>
<td>1.45</td>
</tr>
</tbody>
</table>
Table IV(g): Effect of variation of ionic strength (I) and dielectric constant of the medium on Osmium(VIII) catalyzed oxidation of L-Proline by Hexacyanoferrate(III) in aqueous alkaline medium at 30°C

\[ [\text{HCF}] = 4.0 \times 10^{-4}; \quad [\text{L-Prol}] = 4.0 \times 10^{-3}; \]
\[ [\text{OH}^-] = 0.1; \quad [\text{Os(VIII)}] = 1.0 \times 10^{-5}; \]
\[ I = 0.5 \text{ /mol dm}^{-3} \]

<table>
<thead>
<tr>
<th>Ionic strength (I) ( D = 78.5 )</th>
<th>Dielectric constant (D) ( I = 0.1 \text{ mol dm}^{-3} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>I ( (\text{mol dm}^{-3}) )</td>
<td>( \sqrt{I} )</td>
</tr>
<tr>
<td>------------------------------</td>
<td>------------</td>
</tr>
<tr>
<td>0.1</td>
<td>0.311</td>
</tr>
<tr>
<td>0.2</td>
<td>0.447</td>
</tr>
<tr>
<td>0.3</td>
<td>0.547</td>
</tr>
<tr>
<td>0.5</td>
<td>0.707</td>
</tr>
<tr>
<td>0.7</td>
<td>0.837</td>
</tr>
<tr>
<td>0.9</td>
<td>0.949</td>
</tr>
</tbody>
</table>
Figure IV(i): Effect of variation of ionic strength (I) and dielectric constant of the medium on the Osmium(VIII) catalyzed oxidation of L-Proline by Hexacyanoferrate(III) in aqueous alkaline medium at 30°C

\[
\begin{align*}
[HCF] &= 4.0 \times 10^{-4}; \\
[L\text{-Prol}] &= 4.0 \times 10^{-3}; \\
[OH^-] &= 0.1; \\
[Os\text{(VIII)}] &= 1.0 \times 10^{-6}; \\
I &= 0.5 / \text{mol dm}^{-3}
\end{align*}
\]

(Conditions as in Table IV(g) p.142)
3.2.7. Dielectric Constant of the medium

The dielectric constant of the medium ‘D’ was varied by varying the t-butyl alcohol/\( \text{H}_2\text{O} \) percentage (v/v). Since the ‘D’ for various percentage compositions was not available in literature, they were computed by using their ‘D’ in pure state. In the reaction, as ‘D’ decreases the \( k_{\text{obs}} \) values were increased (Table IV(g) p.142). Earlier the reactions between t-butyl alcohol and oxidant / catalyst were studied. It was observed that there was neither a reaction between solvent and catalyst nor with the oxidant. The graph of log \( k_{\text{obs}} \) versus 1/D was found to be linear with positive slope (Figure IV(i) p.143).

3.2.8. Effect of Temperature

The kinetics were also studied at 30, 35, 40 and 45°C at constant concentrations of all reactants and other conditions being constant. The \( k_{\text{obs}} \) at various temperatures are calculated and tabulated (Table IV(h)A p.145). From the Arrhenius plot of log \( k_{\text{obs}} \) versus 1/T (Figure IV (j) p.146), the energy of activation and other activation parameters were calculated and tabulated (Table IV (h) B p.145).

3.2.9. Polymerization Study

Since the HCF is one equivalent oxidant and oxidation of organic compounds by such an oxidant was expected to intervene the free radical generated by L-proline. Therefore, the intervention of free radical during the reaction was studied by initial addition of monomer, acrylonitrile followed by dilution with methanol, resulting in a copious precipitation. This indicates that the reaction was routed through a free radical path. It is also ascertained by the decrease in rate with initial addition of monomer.
Table IV(h) A. Effect of temperature on Osmium(VIII) catalyzed oxidation of L-Proline by Hexacyanoferrate(III) in aqueous alkaline medium.

\[ \text{[HCF]} = 4.0 \times 10^{-4}; \]
\[ \text{[L-Pro]} = 4.0 \times 10^{-3}; \]
\[ \text{[OH]} = 0.1; \]
\[ \text{[Os(VIII)]} = 1.0 \times 10^{-6}; \]
\[ I = 0.5 \text{ /mol dm}^{-3} \]

<table>
<thead>
<tr>
<th>Temp. (K)</th>
<th>( k_{\text{obs}} \times 10^3 \text{ (s}^{-1}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>303</td>
<td>1.44</td>
</tr>
<tr>
<td>308</td>
<td>1.84</td>
</tr>
<tr>
<td>313</td>
<td>2.29</td>
</tr>
<tr>
<td>318</td>
<td>3.00</td>
</tr>
</tbody>
</table>

B. Activation parameters of Osmium(VIII) catalyzed oxidation of L-Proline by Hexacyanoferrate(III) in aqueous alkali.

<table>
<thead>
<tr>
<th>Activation Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>( E_a ) (kJ mol(^{-1}))</td>
<td>33.5 ± 2</td>
</tr>
<tr>
<td>( \Delta H^# ) (kJ mol(^{-1}))</td>
<td>31 ± 2</td>
</tr>
<tr>
<td>( \Delta S^# ) (J K(^{-1}) mol(^{-1}))</td>
<td>-24 ± 1.5</td>
</tr>
<tr>
<td>( \Delta G^# ) (kJ mol(^{-1}))</td>
<td>38 ± 2</td>
</tr>
<tr>
<td>( \log A )</td>
<td>3.0 ± 0.1</td>
</tr>
</tbody>
</table>
Figure IV(j): Effect of temperature on the Osmium(VIII) catalyzed oxidation of L-Proline by Hexacyanoferrate(III) in aqueous alkaline medium at 30°C

(Conditions as in Table IV(h) A p.145)
4. DISCUSSION

The variation of concentration of Os(VIII) with alkali as shown in Figure IV(h) p.140 indicates that \([\text{OsO}_5(\text{OH})]^2^-\) is the reactive species; its concentration was varied linearly with \([\text{OH}^-]\). The concentrations of the other two species, \([\text{OsO}_4(\text{OH})_2]^2-\) and \([\text{OsO}_5(\text{OH})_3]^+\) are either decreased or increased drastically with various \([\text{OH}^-]\) and are not varied parallel to the variations of \(k_{\text{obs}}\) for different \([\text{OH}^-]\). Hence, they are not considered as reactive species. The formation of \([\text{OsO}_5(\text{OH})]^3^-\) as in equation (3) is important in this study as reported earlier\(^{20}\).

Each fractional order in \([\text{OH}^-]\) and \([\text{L-Prol}]\) is an implicit fact to support the expectation of the pre-equilibrium before rate determining step, and copious precipitate during polymerization study strongly supports the free radical intervention generated from L-Proline in its oxidation by alkaline HCF catalyzed by Os(VIII). First order each in oxidant and catalyst can be accommodated in the mechanism as shown in scheme 1. Hence, the scheme 1 is written in accordance with the above facts and the consideration of active species of Osmium(VIII) in alkali as \([\text{OsO}_5(\text{OH})]^3^-\) in the first equilibrium step.

\[
[\text{OsO}_4(\text{OH})_2]^2^- + \text{OH}^- \overset{K_1}{\longrightarrow} [\text{OsO}_5(\text{OH})]^3^- + \text{H}_2\text{O}
\]

\[
[\text{OsO}_5(\text{OH})]^3^- + \text{\begin{align*}
\text{COOH} \\
\text{H}
\end{align*}} \overset{K_2}{\longrightarrow} \text{Complex}
\]

\[
\text{Complex} + [\text{Fe(CN)}_6]^3^- \overset{k_{\text{slow}}}{\longrightarrow} \text{\begin{align*}
\text{COOH} \\
\text{H}
\end{align*}} + [\text{Fe(CN)}_6]^4^- + [\text{OsO}_5(\text{OH})]^3^-
\]

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L-Proline has two donor atoms, viz., 'N' from imino moiety and 'O' from carboxylic group having a lone pair of electrons. It is a known fact that 'N' is a small potent atom and can donate a pair of electrons to the central metal ion of Os(VIII) to form an adduct. A possible structure of the adduct can be written as

Presence of two -CH₂ groups on either side of N atom favors the positive charge on N atom and makes it easy to form the complex. Thus, formation of complex between Os(VIII) and 'O' atom of carboxylic group can be ruled out. The adduct formed in this way with 'N' might be very reactive and undergoes oxidation easily by HCF. This is evidenced by the fact that in absence of Os(VIII), the reaction between L-proline and HCF was not observed. Therefore, the intermediate as shown in the second step of scheme 1 reacts with HCF in rate-determining step to give a free
radical generated from L-proline. This justifies the unit order each in oxidant and catalyst. Nevertheless, one can predict that the Os(VIII) could oxidize the substrate by yielding Os(VI) and it is re-oxidation to Os(VIII) by HCF. If that is the case the order in substrate would be unity, and no free radical intervention during oxidation could be expected. However, in the present investigation free radical test was positive and order with respect substrate is fraction. Hence, such a possibility is ruled out.

The rate law for the mechanism as in scheme 1 could be derived as

\[
\frac{d[\text{Fe(CN)}_6^{3-}]}{dt} = \text{rate} = k \left[\text{Fe(CN)}_6^{3-}\right] [\text{Complex}]
\]

\[
= k \left[\text{Fe(CN)}_6^{3-}\right] [\text{L-Prol}]_f [\text{OsO}_5(\text{OH})]^{3-}
\]

\[
= k K_2 \left[\text{Fe(CN)}_6^{3-}\right] [\text{L-Prol}]_f [\text{OsO}_4(\text{OH})]^{2-} [\text{OH}]_f
\]

\[\text{(4)}\]

[Os(VIII)]_T is equal to the sum of concentration of [OsO_4(\text{OH})_2]^{2-}, [OsO_5(\text{OH})]^{3-} and [Complex].

\[\text{[Os(VIII)]}_T = [\text{OsO}_4(\text{OH})_2]^{2-}_f + [\text{OsO}_5(\text{OH})]^{3-}_f + [\text{Complex}]\]

\[= [\text{OsO}_4(\text{OH})_2]^{2-}_f + K_1 [\text{OsO}_4(\text{OH})_2]^{2-}_f [\text{OH}]_f + K_1 K_2 [\text{OsO}_4(\text{OH})_2]^{2-}_f [\text{L-Prol}]_f [\text{OH}]_f\]

\[= [\text{OsO}_4(\text{OH})_2]^{2-}_f \{ + K_1 [\text{OH}]_f + K_1 K_2 [\text{L-Prol}]_f [\text{OH}]_f \}\]

\[\therefore \quad \text{[OsO}_4(\text{OH})_2]^{2-}_f = \frac{[\text{Os(VIII)}]_T}{1 + K_1 [\text{OH}]_f + K_1 K_2 [\text{OH}]_f [\text{L-Prol}]_f}\]

\[\text{(5)}\]

Similarly,

\[\text{[(OH)]}_f = \frac{[\text{OH}]_T}{1 + K_1 [\text{OsO}_4(\text{OH})_2]^{2-}} \]

\[\text{and} \quad \text{[L-Prol]}_f = \frac{[\text{L-Prol}]_T}{1 + K_2 [\text{OsO}_5(\text{OH})]^{3-}} \]

\[\text{(6)}\]

\[\text{and} \quad \text{(7)}\]

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In view of low concentration of Os(VIII) used in the present investigation, its concentration in the denominators of Equations (6) and (7) in the parenthesis are negligible. Hence, denominators tend to unity.

Thus, \([\text{OH}]_r = [\text{OH}]_t^-\) and \([\text{L-Prol}]_r = [\text{L-Prol}]_t\) and on incorporating all these and by substituting equation (5) in (4), equation (8) results.

\[
\frac{d[\text{Fe(CN)}_6]^{3-}}{dt} = \text{rate} = \frac{kK_1K_2[\text{Fe(CN)}_6]^{3-}[\text{L-Prol}]_t[\text{Os(VIII)}]_t[\text{OH}]_t}{1 + K_1[\text{OH}]_t + K_1K_2[\text{L-Prol}]_t[\text{OH}]_t}
\]

Or

\[
k_{\text{obs}} = \frac{kK_1K_2[\text{L-Prol}]_t[\text{Os(VIII)}]_t[\text{OH}]_t}{1 + K_1[\text{OH}]_t + K_1K_2[\text{OH}^-][\text{L-Prol}]_t}
\]

For the verification of the rate law, the subscripts 'T' and 'f' are omitted and hence equation (8) becomes,

\[
k_{\text{obs}} = \frac{kK_1K_2[\text{L-Prol}][\text{Os(VIII)}][\text{OH}^-]}{1 + K_1[\text{OH}^-] + K_1K_2[\text{OH}^-][\text{L-Prol}]}
\]

Equation (9) is re-arranged into equation (10).

\[
\frac{1}{k_{\text{obs}}} = \frac{1}{kK_1K_2[L-Prol][Os(VIII)][OH^-]} + \frac{1}{kK_2[L-Prol][Os(VIII)]} + \frac{1}{[Os(VIII)]}
\]

The mechanism as in Scheme 1 and rate law (9) are verified in the form of equation (10) by plotting the graphs of \(1/k_{\text{obs}}\) versus \(1/[\text{OH}^-]\) and \(1/[\text{L-Prol}]\) which should be linear (Figure IV (k) p.151). From the slopes and intercepts of such plots, the values of \(k, K_1\) and \(K_2\) are calculated as \(3.79 \times 10^3 \text{ dm}^3\text{ mol}^{-1}\text{ s}^{-1}\), \(0.52 \text{ dm}^3\text{ mol}^{-1}\) (Literature value: \(6.4 \text{ dm}^3\text{ mol}^{-1}\)), and \(3.78 \times 10^3 \text{ dm}^3\text{ mol}^{-1}\) respectively. The \(K_1\) found in this study is close agreement with reported value\(^{20}\). This justifies the formation of \([\text{OsO}_5(\text{OH})]^{3+}\). Using these values, rate constants under different experimental
Figure IV(k): Verification of rate law (9) of osmium(VIII) catalyzed oxidation of L-Proline by Hexacyanoferrate(III) in aqueous alkaline medium at 30°C.
conditions were calculated. It is found that such calculated rate constants agree with the experimental values (Table IV(c) p.133, Table IV(d) p.136, Table IV(e) p.139).

An increase in rate with increasing ionic strength is in the expected direction as a negatively charged complex, resulted by interacting Os(VIII) species with L-proline, reacts with another negatively charged [Fe(CN)₆]³⁻ in the rate determining step. Ion pairing¹⁰,²² between K⁺ and [Fe(CN)₆]³⁻ is understood. Thus, the ion pairing between Na⁺ and [Fe(CN)₆]³⁻ may also be expected as NaOH and NaCl were used to maintain required concentrations of alkali and ionic strength respectively. Therefore, unit positive slope in the graph of log $k_{obs}$ versus $\sqrt{I}$ explains qualitatively the interaction between the species of similar charges in rate-determining step.

An increase in rate with decreasing the dielectric constant of reaction media is contradictory to the expected direction as ionic species are involved in the slow step as shown in scheme 1. This may be due to the solvation of activated complex at low dielectric constant media rather than that of a higher one where the reactants are more solvated²³.

A negative value of $\Delta S^\#$ (-24 ± 1.5 JK⁻¹ mol⁻¹) suggests that the two ionic species combine in rate determining step to give a single intermediate complex which is more ordered than the reactants²⁴,²⁵. The smaller rate constant of the slow step of the mechanism indicates that the oxidation presumably occurs through an inner-sphere mechanism. This conclusion was supported by earlier reports²⁶-²⁸. The unexpected low value of frequency factor ($\log A = 3.0$) clearly indicates the intervention of free radical and similarly charged ions that are interacting rather than direct interaction between unionized L-proline with Os(VIII) / HCF in a slow step that fortifies the mechanism as shown in scheme 1.
5. CONCLUSION

Oxidation of L-Proline was set up to mimic the biological path. The reaction product was found to be L-glutamic acid. However, the earlier studies reveal that the products were 4-amino butyric acid\textsuperscript{14}, 4-amino buteraldehyde\textsuperscript{29} and keto acids\textsuperscript{30}. The 4-amino buteraldehyde is most unpredictable product, as L-Proline oxidizes through a pyrrolidine ring cleavage without decarboxylation. If aldehyde is formed at all, it would be glutamic semialdehyde.

In the absence of Os(VIII), the reaction between L-Proline and hexacyanoferrate(III) is almost imperceptible, where as the addition of small amount of Os(VIII) favors the spontaneity of the reaction. This might be the reactive species of adduct which is formed by interacting L-Proline with Osmium(VIII). Though the Osmium(VIII) is used as catalyst it did not undergo reduction to Os(VI), but it catalyses through the formation of active adduct and regenerates in the rate determining step by reacting with HCF to give free radical generated by L-Proline.
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