KINETICS AND MECHANISM OF OSMIUM (VIII) CATALYZED OXIDATION OF VALINE BY HEXACYANOFERRATE (III) IN ALKALINE MEDIUM

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ABSTRACT
The osmium(VIII) catalysed reaction between L-valine and hexacyanoferrate (III) [HCF(III)] in alkaline medium has a stoichiometry of 1: 2 with zero order dependence on [HCF(III)]. The reaction is first order with respect to [catalyst] and [alkali] and fractional with [substrate]. No effect of added product was observed. A mechanism involving the formation of a complex between L-Valine and osmium(VIII) were proposed. The main product of the oxidation is the corresponding aldehyde. Effect of temperature was also studied for calculating different activation parameters.

Keywords: Oxidation of L-Valine, hexacyanoferrate, osmium(VIII)

INTRODUCTION
Amino acids act not only as the building blocks in protein synthesis but they also play a significant role in metabolism. Amino acids find a number of applications in biochemical research, metabolism, microbiology, nutrition, pharmaceuticals and fortification of foods and feeds. The kinetics and mechanism of oxidation of amino acids have been studied previously by some researchers. However, the mechanism is different in the different reaction systems. The oxidation of amino acids is also of interest as the oxidation products differ from different oxidants. L-Valine is an essential, non-polar, aliphatic amino acid used to hold proteins together. It is needed for muscle metabolism and coordination, tissue repair and for the maintenance of proper nitrogen balance in the body.

Literature survey reveals that kinetic studies on the oxidation of L-valine using oxidants like N-Bromo Acetamide (NBA), N-Chloro Saccharin (NCSA), KMnO₄, Ce(IV), N-Bromo Succinimide etc were reported earlier.

Kinetic studies on oxidation of a variety of substances, both organic and inorganic, by hexacyanoferrate (III) in alkaline medium have been undertaken by many workers. The oxidation of organic substrates proceeds through outer sphere mechanism, with the electron transfer occurring from the reductant to the central atom via a cyanide ligand. The cyano groups constitute bridging ligands, as they allow simultaneous C- bridging to the metal ion and n- bridging to the other nucleophile. Unless a catalyst is employed these reactions are either extremely slow or do not proceed at all. The most widely investigated catalyst is Os(VIII). The mechanism of the catalysis is quite complicated due to the formation of different intermediate complexes, free radicals and different oxidation states of osmium. Thus in order to explore the mechanism of oxidation by hexacyanoferrate (III) in presence of Os(VIII) catalyst in alkaline medium and to check the selectivity of L-valine towards HCF(III), we have carried out kinetic investigations on the oxidation of L-Valine by HCF(III) in alkaline medium.

EXPERIMENTAL
The standard solution of L-Valine (0.05 mol dm⁻³) (Himedia) was prepared by using double distilled water. The other chemicals used were Hexacyanoferrate [HCF(III)], sodium hydroxide, osmium(VIII) and sodium perchlorate. All chemicals were of AR grade. A 0.01 mol dm⁻³ solution of osmium(VIII) was
prepared in 0.25 sodium hydroxide from osmium tetroxide (Johnson Mathey, London). Its strength was determined by taking aliquot volume of the solution into 0.5 mol dm$^{-3}$ hydrochloric acid adding 10 ml of 10% KI and titrating the liberated iodine with sodium thiosulphate using starch as indicator. Solution of desired concentration is prepared from this stock by suitable dilution. Hexacyanoferrate (II) solution was prepared by dissolving a known amount of K$_4$[Fe(CN)$_6$] in water. Sodium hydroxide (E. Merck) and sodium perchlorate (E. Merck) were used to maintain [OH$^-$] and ionic strength respectively.

**Kinetic Procedure**

A spectrophotometer UV-2000+ (Lab India) model fitted with a thermostatic compartment (Peltier model) and a recorder was used to follow the disappearance of Fe(CN)$_6^{3-}$ at 420 nm. The temperature was maintained at the desired value to within ± 0.1°C. Reaction mixtures having [amino acid]>> Fe(CN)$_6^{3-}$ were used for the kinetics. The reaction was initiated by the quick addition of Fe(CN)$_6^{3-}$ to a solution containing appropriate concentrations of L-valine, NaOH, NaClO$_4$ and Os$^{VIII}$ (after the solutions had reached thermal equilibrium) at a constant temperature of 30°C±0.1°C.

The absorbance was recorded until it reached a value which was approximately one-eighth of the initial absorbance. Plots of absorbance against time were linear indicating a zero order dependence in Fe(CN)$_6^{3-}$. The quotient was the value of $k_o$, the observed pseudo-zero order rate constant. Duplicate kinetic runs showed that the data were reproducible within ± 5%. Beer’s law was obeyed at 420nm over the concentration range employed. The ionic strength was kept constant at 0.5 mol dm$^{-3}$.

**Test for free radicals**

Reaction solutions were degassed with N$_2$ before the initiation of the reaction and the addition of acrylonitrile to the partially oxidized reaction mixtures. No polymerisation of the monomer was noted over a considerable period of time. This experiment, however does not prove that a free radical is not formed. It is possible that the monomer might not be polymerised if the free radical reacts more quickly with one of the species present in the solution than with the monomer. However, in the knowledge that [Os(VIII)] undergoes a two electron change in the oxidation of organic compounds, it might not be erroneous to assume the absence of free-radicals.

**Stoichiometry**

Known amounts of L-valine were allowed to react completely with a known excess of HCF(III) in presence of constant amount of Os(VIII) at 30°C in 0.2 mol dm$^{-3}$ OH$^-$ at an ionic strength of 0.5 mol dm$^{-3}$ (by adding NaClO$_4$) in a closed vessel. The concentration of remaining HCF(III) was then analyzed spectrophotometrically at 420 nm. As per these results the stoichiometry was found to correspond to the equation:

$$\text{RCH(NH}_2\text{)}\text{COO}^- + 2 \text{HCF(III)} + \text{OH}^- \rightarrow \text{RCHO} + 2 \text{HCF(II)} + \text{CO}_2 + \text{NH}_3$$

(1)

Where, R = (CH$_3$)$_2$CH-

The main oxidation products were identified as aldehyde. It was confirmed by adding 2,4-DNP to the product solution. Similarly, ammonia by Nesseler’s reagent and CO$_2$ was qualitatively detected by bubbling nitrogen gas through the acidified reaction mixture and passing the liberated gas through tube containing lime water.

**RESULTS AND DISCUSSION**

**Dependence on Valine**

The concentration of valine was varied from 1.0 × 10$^{-3}$ to 1.0 × 10$^{-2}$ mol dm$^{-3}$ keeping the concentration of all other species constant in the reaction mixture. The pseudo zero order rate constants ($k_o$) were found to increase with increase in the concentration of valine (Table 1) and the plot of log $k_o$ versus log[Val] (Fig.1) was found to be linear with a slope of 0.5 at 30°C. The plots of 1/ $k_o$ versus 1/[val] were found to be straight lines with positive intercepts on the rate axes indicating that the reaction obeys Michaelis- Menten behaviour (Fig.3).

**Dependence on Osmium (VIII)**

The concentration of [Os(VIII)] was varied from 2.0 × 10$^{-6}$ to 10.0 × 10$^{-6}$ mol dm$^{-3}$ at fixed concentration of [Val] = 2.0 × 10$^{-3}$ mol dm$^{-3}$, [OH$^-$] = 0.2 mol dm$^{-3}$, I = 0.5 mol dm$^{-3}$ at temperature 30°C.
The plot of $k_o$ versus $[\text{Os(VIII)}]$ was found to be linear passing through the origin. Passage through the origin implied that alkaline Fe(CN)$_6^{3-}$ did not oxidize the amino acid to any appreciable extent in the absence of osmium(VIII). The plot of $\log k_o$ versus $\log[\text{Os(VIII)}]$ was found to be linear with a slope of 1.02 at 30°C.

**Table-1: Effect of [Os(VIII)], [L-Val], [OH$^-$] and ionic strength, $\mu$, on the pseudo-zero order rate constant ($K_0$) at 30°C ± 0.1°C**

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<tr>
<th>$10^4[HCF]$ (mol dm$^{-3}$)</th>
<th>$10^3[\text{Val}]$ (mol dm$^{-3}$)</th>
<th>$10^4[\text{Os(VIII)}]$ (mol dm$^{-3}$)</th>
<th>$10^2[O H^-]$ (mol dm$^{-3}$)</th>
<th>$\mu$ (mol dm$^{-3}$)</th>
<th>$10^4(k_0)$ mol dm$^{-3}$s$^{-1}$</th>
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**Dependence on Hydroxide Ion**
The concentration of [OH$^-$] was varied from 0.2 to 0.6 mol dm$^{-3}$ at fixed concentration of [Os(VIII)] = 2.0 $\times$ 10$^{-6}$ mol dm$^{-3}$, [Val] = 2.0 $\times$ 10$^{-3}$ mol dm$^{-3}$, $I$ = 0.5 mol dm$^{-3}$ at temperature 30°C. $k_o$ increases proportionately with increasing [NaOH]. The plot of $\log k_o$ versus $\log[OH^-]$ was linear with zero intercept. (Fig. 2). The zero intercept signified the fact that aqueous osmium tetraoxide solution did not act as a catalyst for the reaction.

**Effect of product**
The effect of added product on the rate of reaction was studied by varying the concentration of hexacyanoferrate(II) from 2.0 $\times$ 10$^{-4}$ to 6.0 $\times$ 10$^{-4}$ mol dm$^{-3}$ by keeping the concentrations of all other reactants constant. The pseudo-zero order rate constants thus obtained were found to remain practically constant indicating that HCF(II) did not have any effect on the rate of the reaction.

**Effect of Ionic Strength**
Ionic Strength was varied from 0.5 to 1.5 mol dm$^{-3}$ using NaClO$_4$ solution keeping the concentrations of all other reactants constant. The rate slightly increases with increase in ionic strength. No significant effect of ionic strength ($\mu$) on the rate of reaction was observed.
Effect of temperature

The reaction was studied at different temperature (25-35°C) at fixed concentration of [Os(VIII) ] = 2.0x10^6 mol dm^{-3}, [Val] = 2.0x10^3 mol dm^{-3}, [OH⁻] = 0.2 mol dm^{-3}, I = 0.5 mol dm^{-3}. From the linear Arrhenius plot of \( \log k' \) vs \( 1/T \), values of activation parameters, energy of activation (\( E_a \)), enthalpy of activation(\( \Delta H^\circ \)), entropy of activation (\( \Delta S^\circ \)) and free energy of activation (\( \Delta G^\circ \)) for the composite reaction were calculated.

\[
\text{Fig.-1: Plot of } \log k_0 \text{ versus } \log[\text{Val}]
\]

\[
[HCF] = 2.0 \times 10^{-4} \text{ mol dm}^{-3}; [\text{Os(VIII)}] = 2.0 \times 10^{-6} \text{ mol dm}^{-3}; [\text{OH}^{-}] = 0.2 \text{ mol dm}^{-3}; (I) = 0.5 \text{ mol dm}^{-3}
\]

This study has shown that the reaction has a zero order dependence in \([\text{Fe(CN)}_6^{3-}]\), a first order dependence both in \([\text{Os(VIII)}]\) and \([\text{OH}^{-}]\) and a fractional order in [amino acid].

Under the present experimental conditions, at a \([\text{OH}^{-}]\) of 0.2 mol dm^{-3}, valine exists in the form of anionic species \(RCH(\text{NH}_2)\text{COO}^-\). HCF(III) is a low spin octahedral complex.

\[
\text{RCHNH}_2\text{COOH} \rightleftharpoons \text{CHNH}_2\text{COO}^- + \text{H}^+ \rightleftharpoons \text{RCHN}^+\text{H}_3\text{COO}^-
\]

\[
\text{RCHN}^+\text{H}_3\text{COOH} \rightleftharpoons \text{H}^+ \rightarrow \text{RCHN}^+\text{H}_2\text{COO}^- \rightleftharpoons \text{H}^+ \rightarrow \text{RCHNH}_2\text{COO}^-
\]

Where, \( R = (\text{CH}_3)_2\text{CH}-\)
A first order dependence in [Os(VIII)] and the linearity of the plot between (k')^{-1} and [amino acid]^{-1} (Fig. 3) is considered to suggest the possible formation of transient complex between Os(VIII) and the amino acid similar to that assumed to form between an enzyme and substrate in the well known Michaelis and Menten mechanism for enzyme-substrate reactions. This assumption might not be erroneous because the intermediacy of Os(VIII) - amino acid complexes has been proposed earlier although their existence has not been demonstrated.

Osium (VIII) is known to form different complexes with OH⁻ and species such as OsO₄, [OsO₄(OH)(H₂O)]⁻, [OsO₄(OH)₂]⁻ and [OsO₄(OH)₃]⁻ coexist in fast equilibria with each other in basic medium.

\[
\text{OsO}_4 + 2\text{OH}^{-} \rightleftharpoons [\text{OsO}_4(\text{OH})_2]^{2-} \quad (4)
\]

Where, K= 24 at 308K and \( \mu = 0.5 \text{ mol dm}^{-3} \). Since one electron transfer is involved in the system, the possible existence of free radical intermediate in the oxidation of organic compounds by alkaline hexacyanoferrate (III) is expected, but experimental evidence does not indicate presence of free radicals, hence mechanism based on the formation of soluble complex between amino acid and [Os(VIII)] is suggested. This complex then rapidly decomposes to give aldehyde and osmate (VI) ion, subsequently Os (VIII) ion is then regenerated by oxidation of osmate (VI). Since uncatalysed reaction does not proceed at all and hexacyanoferrate (III) is consumed without taking part in the rate law, it is assumed that the hexacyanoferrate (III) does not react directly until after the rate determining step. Based on these arguments, the following mechanism is proposed

Val⁺ + [OsO₄(OH)₂]²⁻ \( \rightleftharpoons \) Complex (C) \( (5) \)

C + OH⁻ \( \xrightarrow{k_{\text{slow}}} [\text{OsO}_4(\text{OH})_2]^{4+} \) + valine aldehyde + CO₂ + NH₃ \( (6) \)

[OsO₄(OH)₂]^{2+} + 2 [Fe(CN)₆]^{3+} \( \xrightarrow{\text{fast}} [\text{OsO}_4(\text{OH})_2]^{2-} + 2 [\text{Fe(CN)}_6]^{4+} \) \( (7) \)

\[
\frac{-d[\text{Fe(CN)}_6]^{3+}}{dt} = k[C][\text{OH}^-]
\]

(8)

\[
kK_1[V\text{al}^+]_e[\text{OsO}_4(\text{OH})_2]^{2-}_e[\text{OH}^-]_e
\]

(9)

Since \([\text{Os}^{\text{VIII}}]_e = [\text{OsO}_4(\text{OH})_2]^{2-}_e + [C]_e\)
Therefore, $[\text{OsO}_4(\text{OH})^2_2]_e = \frac{[\text{Os}^\text{VIII}]}{1 + K_1[\text{Val}]}$ (10)

Rate $= \frac{kK_1[\text{Val}][\text{Os}^\text{VIII}][\text{OH}]}{1 + K_1[\text{Val}]}$ (11)

Where $[\text{Val}]_e = [\text{Val}]$, the above equation may be written as

Rate $= \frac{kK_1[\text{Val}][\text{Os}^\text{VIII}][\text{OH}]}{1 + K_1[\text{Val}]}$ (12)

This rate equation explains first order dependence each on [osmium(VIII)] and [OH], zero order on [HCF(III)] and fractional order with respect to [Valine].

The activation parameters $E_a$, $\Delta H^\parallel$, $\Delta S^\parallel$, $\Delta G^\parallel$ were evaluated from the plot of log $k'$ vs 1/T and found to be 59.74 KJ mole$^{-1}$, 57.26 KJ mole$^{-1}$, -102.73 J K$^{-1}$mol$^{-1}$ and 94.99 kJ mole$^{-1}$ respectively. The experimental values of $\Delta S^\parallel$ and $\Delta G^\parallel$ were both favourable for electron transfer processes. The high negative values of $\Delta S^\parallel$ indicates that the intermediate complex is more ordered than the reactants$^{30}$ due to loss of degree of freedom.

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