

# Iridium(III) Catalysed Oxidation of Threonine by Hexacyanoferrate(III) in aqueous alkaline medium

Anjali Goel, Ruchi Sharma, Rajni Lasyal

## ABSTRACT

The kinetics of the oxidation of threonine by hexacyanoferrate (abbreviated as HCF) (III) ions catalyzed by Iridium trichloride has been studied spectrophotometrically in aqueous alkaline medium at constant ionic strength of  $0.5 \text{ mol dm}^{-3}$  & temperature  $35^\circ\text{C} \pm 1^\circ\text{C}$ . The stoichiometry of the reaction corresponds to a reaction in which one mole of the amino acid reacts with two moles of hexacyanoferrate(III) ion. The reaction rate shows first order kinetics with respect to  $[\text{HCF(III)}]$ ,  $[\text{OH}^-]$  and  $[\text{IrCl}_3]$ . The order of reaction is also one with respect to [substrate] at its lower concentration tending towards zero at higher concentrations. A plausible reaction mechanism through a transient complex formation between  $\text{IrCl}_3$  and amino acid has been suggested. Activation parameters have also been evaluated using Arrhenius equation. A keto acid,  $\alpha$ -keto hydroxy butyric acid, has been identified as the final product of oxidation by chromatographic and spectroscopic techniques.

## Keywords

Kinetics, Mechanism, Oxidation, Iridium trichloride, threonine, hexacyanoferrate(III) ions.

## INTRODUCTION

Oxidation reactions of  $\alpha$ -amino acids are one of the most relevant biochemical reactions because; such reactions serve as models for protein oxidations [1-3]. Also, uncatalyzed oxidation reactions of  $\alpha$ -amino acids involving a wide range of oxidants are of particular concern in biotechnology and medicine. Simple amino acids present in municipal waste waters cause serious eutrophication in water bodies. Pharma industries dealing with biochemicals and tanneries are some of the major sources of waste waters containing amino acids [4]. It is essential to remove or oxidatively degrade the dissolved amino acids from waste waters. Non enzymatic model oxidation reactions involving  $\alpha$  - amino acids and a wide variety of oxidants are reported plenty in literature [5-10]. Some selective oxidation reactions are reported involving metal catalysts. Transition metal ions of Ag, V, Fe, Ru, Mn etc. are reported to act as catalyst for some of the  $\alpha$  - amino acid oxidations [11-16].

Hexacyanoferrate(III) [17], a potential one electron oxidant is reported to oxidise  $\alpha$  - amino acids to corresponding keto acids. Hexacyanoferrate(III) as an

it abstracts only one electron from the substrate yielding a simple step in any reaction mechanism; and being a one electron oxidant with a redox potential of  $+0.45\text{V}$  of  $\text{Fe(CN)}_6^{3-}/\text{Fe(CN)}_6^{4-}$  couple in alkaline medium, the oxidant exhibits appreciable stability in solution and yields a stable reduction product hexacyanoferrate(II). Interest in the oxidation of inorganic [19] and organic substances [20-21] by hexacyanoferrate(III) has been growing.

The literature on the oxidation of threonine, by HCF(III) catalyzed by Ir(III) is scanty. To understand more about the catalysis of Ir(III) in oxidation by HCF(III) and in order to explore the mechanism of oxidation in presence of Ir(III) ion in the aqueous alkaline medium, here threonine as a substrate for oxidation has been selected.

## EXPERIMENTAL

All chemicals & reagents used were of AR grade. Hexacyanoferrate(III) was used after recrystallization. All the solutions and reaction mixtures were prepared by using double distilled water. Absorbance was recorded on Sys. UV-vis spectrophotometer-117.  $\lambda_{\text{max}}$  for the reaction mixture was 420 nm at which the absorbance was noted only in the

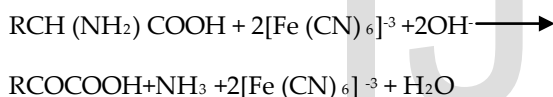
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oxidant has more advantages [18] as, being the mild oxidant, attacks the organic compound at a particular stage,

period in which the  $\lambda_{\text{max}}$  did not change and no precipitate / turbidity appeared.  $\text{IrCl}_3 \cdot 10\text{H}_2\text{O}$  (SRL) was prepared by dissolving the sample in dil HCl. The final strength of iridium trichloride was kept  $3.35 \times 10^{-5} \text{ M}$ .

NaOH, KCl and HCF(III) (AR) grade were used. All the solutions were made in doubled distilled water. The kinetic experiments were carried out by mixing the required quantity of amino acid solution maintained at constant temperature with solution of HCF(III), NaOH, KCl and iridium trichloride kept at the same temperature. The mixture and stock solution of amino acid was then clamped in a thermostat at  $35^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$ . After about 0.5 hour, a required amount of amino acid solution was added to the mixture and stirred to start the reaction. Aliquots were withdrawn from the reaction mixture after repeated intervals of 5 min and the absorbance was recorded. Absorbance vs time plots were made for all the sets. Initial rates  $(dA/dt)_i$  were evaluated after 5 min from the start of the reaction by using plane mirror method and pseudo first order rate constant  $(k_1)$  were calculated by Guggenheim's method.

The stoichiometry of the reaction was studied by estimating the amount of HCF(II) ions produced after definite interval of time with standard solution of ceric (IV) sulphate using ferroin as redox indicator. Estimation of the residual oxidant showed that 1 mole of amino acid consumed 2 moles of hexacyanoferrate(III), corresponding to the following stoichiometry.



Where R represents  $\text{CH}_3\text{-CH-}$

Using the same experimental conditions that were used for the kinetic determinations, solution of substrate and oxidant, in NaOH (ionic strength adjusted by the addition of the requisite amount of KCl), were mixed and kept at atmospheric conditions for 24 hours. The main reaction products were identified as keto acid and ammonia. Ammonia was identified by Nessler's reagent [22] and keto acid by the following methods.

The reaction mixture was extracted with diethyl ether and then concentrated. The concentrated extract was subject to TLC which shows the presence of single white product, keto acid [23-24]. The concentrated extract was evaporated at room temperature. A solid residue was left. It was analyzed by melting point determination, spot test analysis and I.R. spectroscopy. The melting point of the keto acid,  $\alpha$ -keto hydroxy butyric acid is  $98^{\circ}\text{C}$  (Literature value -  $98^{\circ}\text{C}$ ) [17, 25].

## RESULTS AND DISCUSSION

Kinetic experiments were made at different concentration of one reactant keeping the concentration of other constant.

To study the effect of substrate (threonine) concentration on reaction rate, its concentration was varied in the range of  $1 \times 10^{-3}$  -  $10 \times 10^{-3}$  mol  $\text{dm}^{-3}$  at  $35^{\circ}\text{C}$  keeping all other reactants concentration constant (Table-1). The data presented in table-1 shows first order dependence of rate on lower concentration of substrate which tends to be zero order at its higher concentration. The plot of  $[\text{rate}]^{-1}$  vs.  $[\text{S}]^{-1}$  were found linear with small intercept on  $[\text{rate}]^{-1}$  axis satisfying the Michaelis - Menten type relationship. This also suggests the formation of a short lived complex and amino acids prior to the rate determining step.

Secondly, the  $[\text{Fe}(\text{CN})_6]^{3-}$  was varied in the range  $2 \times 10^{-4}$  -  $7 \times 10^{-4}$  mol  $\text{dm}^{-3}$  at fixed [substrate],  $[\text{OH}^-]$  and ionic strength. It was observed that rate depends linearly on the concentration of HCF(III) ion. The straight line plots plotted between initial rates in terms of  $[\text{dA/dt}]_i$  vs concentration of HCF(III) indicates the order in  $[\text{Fe}(\text{CN})_6]^{3-}$  is unity (table 1).

The effect of  $[\text{OH}^-]$  on the rate of reaction was studied at constant [substrate],  $[\text{Fe}(\text{CN})_6]^{3-}$  and ionic strength at  $0.5$  mol  $\text{dm}^{-3}$  at  $35^{\circ}\text{C}$ . A gradual increase in rate with hydroxide concentration reveals first order kinetics with respect to  $[\text{OH}^-]$ .

The effect of ionic strength was studied by varying the [KCl] in the reaction medium. The ionic strength of the reaction medium were varied from  $0.5$  -  $0.7$  mol  $\text{dm}^{-3}$  at constant  $[\text{Fe}(\text{CN})_6]^{3-}$ , [Substrate], and  $[\text{OH}^-]$  as presented in table - 2. It was found that as ionic strength increases, the rate of the reaction increases. It indicates a positive salt effect i.e. both the reacting species (organic substrate and reactant) involved in the oxidation are of similar charges.

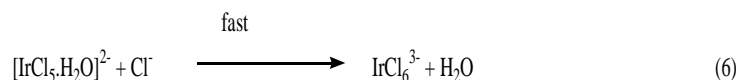
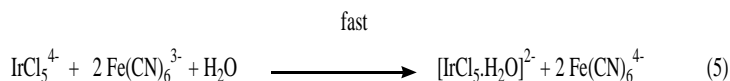
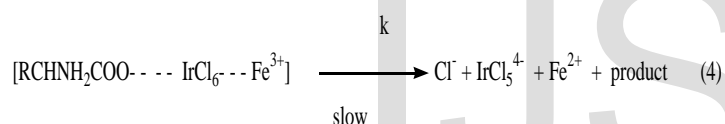
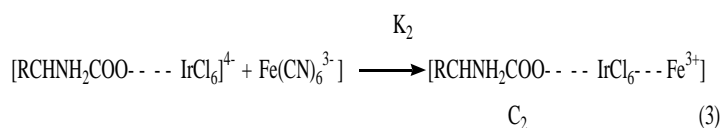
The effect of temperature on the rate of the reaction was studied by carrying out the reaction at four temperatures  $25$ ,  $30$ ,  $35$  and  $40^{\circ}\text{C}$ . The thermodynamic parameters calculated for the oxidation of threonine by alkaline HCF(III) are presented in table 3. A perusal of data shows that the reaction rate are characterised by large negative entropy of activation and a low value of energy of activation. The latter is characteristic of a bimolecular reaction in solution while the former is mainly observed in polar solvents and also suggest the formation of a charged and rigid transition state. Further a high value of the frequency factor observed indicates the involvement of a reactive species which are large in size.

## Mechanism

Keeping in view of the above experimental results, the following mechanistic path has been suggested for the oxidation of threonine by hexacyanoferrate (III) in aqueous alkaline medium in presence of Ir(III) as catalyst.



$\text{C}_1$

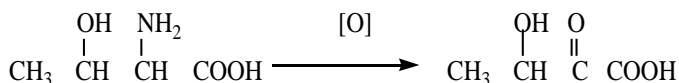


Where R represents  $\text{CH}_3\text{-CH(OH)-}$

It is known that two moles of HCF(III) give one mole of oxygen in alkaline medium.



The final equation in terms of organic substrate may be written as



It is reported that Ir(I) and Ir(II) are the stable species of iridium, but in alkaline medium  $[\text{IrCl}_6]^{3-}$  is the only reacting species of iridium[25-27]. Srivastava et al have reported that the oxidation of amino acids involves the cleavage of N-H and C-H band in the rate determining step [28]. Based on the above facts it is assumed in the present study that the anion forms a loose bonded complex ' $\text{C}_1$ ' with iridium trichloride. The carbonyl oxygen of acid is most likely involved in the formation of complex  $\text{C}_1$ . In the next step the complex ' $\text{C}_1$ ' then combines with HCF(III) through electron abstraction to form complex ( $\text{C}_2$ ). The complex ( $\text{C}_2$ ) then slowly disproportionates into  $\text{Ir}^{1+}$  and HCF(II) along with final product.  $\text{Ir}^{1+}$  is reoxidized to  $\text{Ir}^{3+}$  by two moles of HCF(III) via one electron transfer process. The metal ion complexes with organic substrate make the electron transfer easier [29]. The formation of the complex was proved kinetically by Michaelis – Menton plot i.e. a non - zero intercept of the plot of  $1/\text{rate}$  vs.  $1/[\text{S}]$  (fig. 2). The complex formation between oxidant and substrate was also reported in literature [30].

The reaction rate( $r$ ) is measured in terms of rate of disappearance of HCF(III). According to step(III) the rate of disappearance of HCF(III) would be

$$\text{rate} = \frac{-d[\text{HCF(III)}]}{dt} = k [\text{C}_2]$$

Now the total concentration of  $\text{Ir}^{3+}$  will be  
 $[\text{Ir}^{3+}]_t = [\text{Ir}^{3+}] + [\text{C}_1] + [\text{C}_2] \quad \dots\dots (2)$

**Table 1** Effect of variation of [threonine],  $[\text{OH}^-]$ ,  $[\text{HCF(III)}]$  and Ir(III) on reaction rate at  $\lambda_{\text{max}} = 420 \text{ nm}$ , Temp =  $35^\circ\text{C} \pm 0.1^\circ\text{C}$ ,  $\mu = 0.50 \text{ mol dm}^{-3}$

[Threonine] $\times 10^3$ (mol $\text{dm}^{-3}$ )	[NaOH] (mol $\text{dm}^{-3}$ )	[HCF(III)] $\times 10^4$ (mol $\text{dm}^{-3}$ )	[IrCl <sub>3</sub> ] $\times 10^5$ (mol $\text{dm}^{-3}$ )	[dA/dt] $\times 10^3$ (min <sup>-1</sup> )
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1.0	0.40	3.00	3.35	0.8
2.0	0.40	3.00	3.35	1.4
3.0	0.40	3.00	3.35	1.8
4.0	0.40	3.00	3.35	2.2
5.0	0.40	3.00	3.35	2.6
6.0	0.40	3.00	3.35	3.0
7.0	0.40	3.00	3.35	3.2
8.0	0.40	3.00	3.35	3.2
9.0	0.40	3.00	3.35	3.2
10.0	0.40	3.00	3.35	3.2
3.0	0.10	3.00	3.35	1.0
3.0	0.20	3.00	3.35	2.0
3.0	0.30	3.00	3.35	3.2
3.0	0.40	3.00	3.35	4.2
3.0	0.40	2.00	3.35	0.8
3.0	0.40	3.00	3.35	1.2
3.0	0.40	4.00	3.35	1.8
3.0	0.40	5.00	3.35	2.0
3.0	0.40	6.00	3.35	2.6
3.0	0.40	7.00	3.35	3.0
3.0	0.40	3.00	2.34	2.4
3.0	0.40	3.00	2.68	2.8
3.0	0.40	3.00	3.01	3.2
3.0	0.40	3.00	3.35	3.6
3.0	0.40	3.00	3.68	3.9

**Table-2** Effect of ionic strength on reaction rate,  $[HCF(III)] = 3.00 \times 10^{-4} \text{ mol dm}^{-3}$ ,  $[Threonine] = 3.00 \times 10^{-3} \text{ mol dm}^{-3}$ ,  $[IrCl_3] = 3.35 \times 10^{-5} \text{ mol dm}^{-3}$ ,  $[NaOH] = 0.40 \text{ mol dm}^{-3}$ , Temp. =  $35 \pm 0.1^\circ\text{C}$ ,  $\lambda_{\text{max}} = 420 \text{ nm}$

$[\mu]$ (mol dm <sup>-3</sup> )	0.50	0.55	0.60	0.65	0.70
$(dA/dt)_i \times 10^3$ (min <sup>-1</sup> )	1.1	1.3	1.5	1.8	2.0

**Table -3** Effect of temperature on reaction rate,  $[HCF(III)] = 3.00 \times 10^{-4}$  mol dm<sup>-3</sup>,  $[Threonine] = 3.00 \times 10^{-3}$  mol dm<sup>-3</sup>,  $[IrCl_3] = 3.35 \times 10^{-5}$  mol dm<sup>-3</sup>,  $[NaOH] = 0.40$  mol dm<sup>-3</sup>,  $\mu = 0.50$  mol dm<sup>-3</sup>,  $\lambda_{max} = 420$  nm

PARAMETER	VALUES
Ea ( kcal-mol <sup>-1</sup> )	12.68 ± 0.048
$\Delta H^\ddagger$ ( kcal-mol <sup>-1</sup> )	12.06 ± 0.111
$\Delta S^\ddagger$ e.u.	23.97 ± 0.041
$\Delta F^\ddagger$ ( kcal-mol <sup>-1</sup> )	19.61 ± 0.121
A x10 <sup>7</sup> ( l mol <sup>-1</sup> sec <sup>-1</sup> )	1.03 ± 0.009

Graphical value of Ea = 12.68 kcal mol<sup>-1</sup>

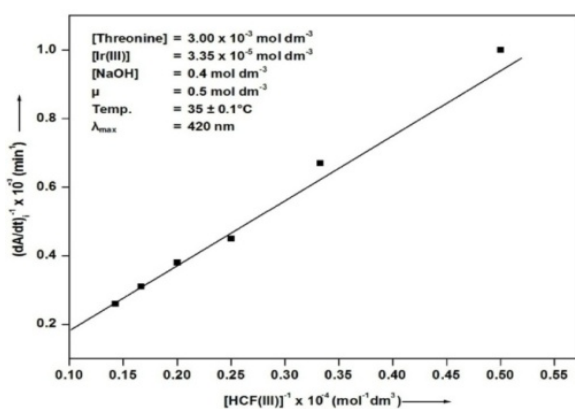


Figure 1. Plot between rate<sup>-1</sup> Vs  $[HCF(III)]^{-1}$

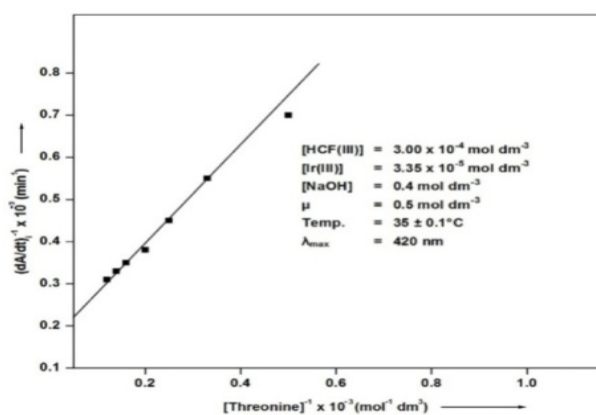


Figure 2. Plot between rate<sup>-1</sup> Vs  $[threonine]^{-1}$

$$rate = \frac{kK_1'K_2[HCF(III)][Ir^{3+}]_t[S][OH^-]}{1 + K_1'[S][OH^-] + K_1'K_2[S][OH^-][HCF(III)]} \quad \dots\dots (3)$$

Substituting the values of  $[Ir^{3+}]$ , (C<sub>1</sub>) and (C<sub>2</sub>) in Equation

(i)

Where  $K_1' = KK_1$

At low concentration of HCF(III), S and OH-Eq. (3) reduces to

$$r = k K_1' K_2 [HCF(III)] [Ir^{3+}]_t [S] [OH^-]$$

The rate law (4) clearly accounts for the first order kinetics with respect to HCF(III), organic substrate, hydroxide ion and catalyst at their lower concentration.

In order to verify this law (3) at higher concentration of above said reactants it could be re-written as-

$$\frac{1}{r} = \frac{1}{k K_1' K_2 [HCF(III)] [Ir^{3+}]_t [S] [OH^-]} + \frac{1}{k K_2 [HCF(III)] [Ir^{3+}]_t} + \frac{1}{k [Ir^{3+}]_t} \quad \text{..... (5)}$$

This equation (5) indicates that the plot of 1/rate vs. 1/[HCF(III)] & 1/rate vs. 1/[S] should give a straight line with positive intercept at 1/rate axis (figure 1 and 2). A close examination of these figures clearly indicate that these are evidently straight line with positive intercept at 1/rate axis. The reaction constant  $k$ ,  $K_1'$  and  $K_2$  calculated from intercept and slope of the straight line plots between rate<sup>-1</sup> vs. [HCF(III)]<sup>-1</sup> and rate<sup>-1</sup> vs. [S]<sup>-1</sup> for organic substrate (threonine) are 1.63, 1.46, 1.28 respectively. The constancy in  $k$ ,  $K_1'$  values clearly shows the validity of derived rate law equation on the basis of proposed mechanism.

## CONCLUSION

Thus from the present study it can be concluded that the proposed method can be used for the removal of dissolved amino acids from waste water into less toxic keto acids. In this study threonine is oxidized to a keto acid,  $\alpha$ -keto  $\beta$  hydroxy butyric acid using iridium (III) as the catalyst and HCF(III) as the oxidant in aqueous alkaline medium. Thus, Ir(III) acts as an effective catalyst in the oxidation of threonine by HCF(III) ions and the reaction proceeds through an electron transfer process. Kinetic study of this amino acids also reveals that Ir(I) is reoxidized to Ir(III) by two molecules of HCF(III) via. one electron transfer process. The mechanism was followed by derived rate law. The mechanism involves the formation of a complex between anion of organic substrate and Ir(III). This complex further forms another complex with HCF(III) which slowly disproportionate into Ir(I), HCF(II).

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