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KINETIC AND MECHANISTIC STUDY OF CATALYZED OXIDATION OF AMINO ACID BY HEXACYANOFERRATE(III) IN AQUEOUS ALKALINE MEDIUM

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ABSTRACT

The kinetics and mechanism of ruthenium(III) catalyzed oxidation of L-serine by hexacyanoferrate(III) were investigated in an aqueous alkaline medium at 35±0.1°C. The reaction rate exhibits first-order dependence with respect to hexacyanoferrate(III) and L-serine at its lower concentrations, tending towards zero-order kinetics at higher concentrations of both hexacyanoferrate(III) and L-serine. The reaction rate exhibits first-order kinetics with respect to ruthenium(III) chloride. The effect of ionic strength of the medium on the reaction rate and the presence of initially added product was also studied. Arrhenius and thermodynamic activation parameters, Ea*, ΔS* and ΔF* have been calculated from the Arrhenius plot by studying the reaction at different temperatures. The proposed and derived rate laws were consistent with the experimental observations, thereby supporting the plausibility of the reaction mechanism suggested.

KEYWORDS: Oxidation, Amino Acid, Hexacyanoferrate(III), Kinetics, Ruthenium Trichloride

Transition metal catalyzed redox processes are an important research area for the mechanistic and synthetic chemists because of the ability of these metals to mediate electron transfer under mild conditions. The redox reactions involving homogeneous catalysts such as transition group metals particularly osmium(VIII), iridium(III) and palladium(II) have extensively been used. Extensive research has been shown on osmium(VIII) (Singh,1986), used as an oxidant (Singh *et al.*,1998; Singh *et al.*, 1999) and a catalyst (Singh *et al.*, 1969) in the oxidation of organic compounds. Osmium forms toxic 'osmates' in acidic medium, thereby restricting its use in alkaline medium. Ruthenium (III)(Mavalangi, 2001, Rathour *et al.*, 2026) and iridium(III) (Gupta *et al.*,2007; Gupta *et al.*,2011) are also known to act as an efficient homogeneous catalyst in the oxidation of several organic compounds in acidic as well as in alkaline medium. In aqueous alkaline media, ruthenium complexes facilitate controlled electron transfer, kinetic analysis to identify rate-determining steps and the nature of reactive species. Among these metals, ruthenium functions as a comparatively more effective catalyst for a wide range of substrates owing to its versatile oxidation states and stability under basic conditions. The redox potential of Ru(IV)/Ru(III) couple (+1.3V) exceeds that of osmium couples, Os(VIII)/Os(VI) is (+0.80–0.85 V), showing effective catalytic activity of ruthenium in +3 state. (Tandon and Gupta, 2011; Singh *et al.*,1990; Singh *et al.*,1990; Nandibewoor *et al.*, 2000).

Various metal complexes are good oxidants in acid or alkaline media under suitable reaction conditions. However, their redox potential determines their oxidation capacity. Several transition metal ions Ce(IV), Cu(III), Ni(IV), Fe(III) in their complex form act as good oxidants in neutral, acidic or basic medium depending upon their redox potential. Hexacyanoferrate (III) ions, $[Fe(CN)_6]^{3-}$, are well-known outer-sphere oxidants that have been used extensively in kinetic investigations because of their distinct electrochemical reactions and stable redox properties. Hexacyanoferrate(III) is widely used as an efficient one-electron oxidant. Its stability, aqueous solubility and moderate reduction potential make it a suitable probe for investigating transition metal-catalyzed oxidation mechanisms. Various oxidation states of iron from +3 to +6 have gained much attention as environmentally friendly oxidants. The coagulating power of hypervalent iron species viz. Fe(IV), Fe(V) and Fe(VI)) have recently been used for removing various impurities from contaminated water (Jiang and Lloyd, 2002; Sharma, 2002). Complex mechanistic pathways involving several electron transfers are frequently revealed by their interaction with organic substrates, especially when transition metal catalysts are present. For instance, in acidic and alkaline media, the redox potential of $[Fe(CN)_6]^{3-}$/ $[Fe(CN)_6]^{4-}$ is + 0.36 V and + 0.40 V, respectively (Day and Selbin, 1964). This implies that hexacyanoferrate(III) works effectively as an oxidant in alkaline media. Hexacyanoferrate(III) is also a single, equivalent and stable oxidant, which reduces experimental error and enables careful data analysis to determine the reaction process. A non-essential amino

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acid, L-serine, has been shown to have numerous beneficial effects by serving as a precursor to important molecules required for protein synthesis, cell division and development. Neural differentiation and survival depend on its function in the central nervous system's synthesis of sphingolipids (Eagle, 1959). The mammalian brain uses the phosphorylated method to synthesize L-serine, which is derived from glucose through glycolysis instead of phosphoenolpyruvate from gluconeogenesis (Maugard *et al.*, 2021). L-serine is an amino acid characterized by its polar hydroxyl group and aliphatic side chain and plays a significant role in biological processes such as protein synthesis and metabolic regulation. Investigating the oxidative degradation of L-serine is essential for elucidating the broader reactivity profiles of amino acids in catalytic environments. However, the application of ruthenium to L-serine oxidation kinetics in alkaline media remains unexamined. In the present study, the kinetics of ruthenium(III) chloride catalysed oxidation of L-serine by hexacyanoferrate(III) in an alkaline medium was investigated to elucidate the reaction pathway and to identify the active species of ruthenium(III) in aqueous alkaline conditions. The findings are presented herein and a plausible reaction mechanism is proposed.

EXPERIMENTAL

MATERIALS AND METHODOLOGY

All chemicals used in this study were of analytical reagent (AR) grade. Double distilled water was used to prepare all the solutions for kinetic experiments and purified to ensure minimal interference from trace contaminants. A stock solution of ruthenium trichloride was prepared by dissolving $RuCl_3$ (Johnson- Matthay Chemical Ltd.) in a minimum amount of hydrochloric acid. The concentration was determined by EDTA titration (Reddy and Kumar, 1995). Mercury was added to ruthenium(III) solution to reduce any ruthenium (IV) formed during the preparation of ruthenium(III). This stock solution was kept overnight. The final strengths of HCl and Ruthenium trichloride were 1.02×10^{-2} M and 4.76×10^{-3} M, respectively. A solution of serine was prepared daily to ensure optimal reactivity and prevent degradation by dissolving a measured amount of recrystallised sample in double distilled water. The solutions of sodium hydroxide and potassium chloride were used to maintain the alkalinity and ionic strength of the reaction solution respectively. The product effect, Ag(I), was investigated using an aqueous solution of $AgNO_3$. The pH meter was used to measure the pH of the medium in the solution. Purified nitrogen gas was passed through distilled water to eliminate any dissolved oxygen to determine how dissolved oxygen affected the rate of reaction and it was observed that no significant distinction was seen between the results obtained in the presence of air and a nitrogen atmosphere.

KINETIC STUDIES

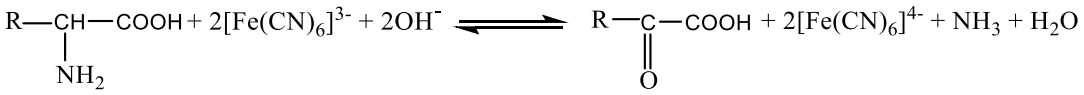
The kinetic measurements were performed on a Systronics 2203 double beam UV-Vis spectrophotometer. The reaction was carried out under pseudo-first order conditions where the concentration of substrate was kept higher than the concentration of the oxidant. Measured amounts of the solutions of potassium ferricyanide, ruthenium (III) chloride, sodium hydroxide and potassium chloride thermostated at 35^oC. A measured amount of serine solution, also thermostated at the same temperature, was rapidly added to the reaction mixture to initiate the reaction. The temperature of the reaction mixture was kept constant at 35 ± 1^0C with an electrically operated thermostat. The progress of the reaction was followed spectrophotometrically at 420 nm to hexacyanoferrate(III). Kinetic measurements were performed monitoring the decrease in oxidant concentration over time at a fixed wavelength. It was clear that there was negligible interference from other species present in the reaction mixture at this wavelength. Preliminary experiments established pseudo-first-order conditions by maintaining a significant excess of L-serine relative to the oxidant, thereby simplifying the kinetic analysis. The initial rate method was employed to determine the reaction orders with respect to each reactant. This approach allowed for the elucidation of the rate law, which is important for proposing a plausible reaction mechanism. The –dc/dt values were determined from the plots of absorbance versus time. The reaction orders for various reactants were determined from the slopes of plots of –dc/dt versus respective concentration of that reactant. Additionally, activation parameters such as activation energy, enthalpy, and entropy were calculated from the temperature dependence of the rate constants, providing further insights into the energy and transition state characteristics of the reaction.

RESULTS AND DISCUSSION

Stoichiometry and Product Analysis

Stoichiometry of the reaction was determined by calculating the number of moles of oxidant required to completely oxidize one mole of organic substrate. For this purpose ratio of the oxidant and organic substrate was changed and complete oxidation of organic substrate was ensured. To determine the stoichiometry of this reaction, varying ratios of hexacyanoferrate(III) to L-serine were equilibrated at 308 K for 24 hours in the presence of sodium hydroxide and potassium chloride.

The ionic strength of the medium was maintained by adding potassium chloride. The concentration of the remaining hexacyanoferrate was subsequently determined spectrophotometrically. This analysis revealed that one mole of L-serine consumes two moles of hexacyanoferrate, establishing a [L-serine]:[HCF(III)] stoichiometric ratio of 1:2, as described by the following equation:



where R= $-CH_2-OH$

The reaction mixture was basified, concentrated, then extracted with ether once the reaction was completed. Thin layer chromatography (TLC) was used to separate the oxidation products, and physicochemical spectrum studies were then used to characterize them. The reaction products were identified as hexacyanoferrate(II), keto acid and ammonia. The development of a reddish-brown precipitate with neutral $FeCl_3$ indicated the synthesis of the L-serine derived keto acid, which was further verified by IR spectral analysis. Nessler's reagent was used to confirm the presence of ammonia.

Reaction Orders

The oxidation of L-serine by hexacyanoferrate(III), catalysed by ruthenium(III) chloride, were examined at various initial concentrations of the reactants in an alkaline medium. The concentration of hexacyanoferrate(III) was varied over a wide range while maintaining constant concentrations of L-serine, sodium hydroxide, ruthenium(III) chloride and the ionic strength of the medium at a constant temperature 35°C. The reaction rates (–dc/dt) were determined from the initial slopes of the individual absorbance versus time plots. The data obtained from the variation of the hexacyanoferrate(III) concentration show that the rate values increased with increasing concentration of the oxidant in the beginning, but at higher concentrations of the oxidant, the increase was not prominent. This trend becomes clear on plotting -dc/dt values versus [oxidant] (Figure 1), where the straight line passing through the origin tends to become parallel to the x-axis at higher concentrations. This shows that the reaction follows first order kinetics at low concentrations, which tends to become zeroth order at higher concentrations of oxidant (Figure 1). The concentration of L-serine was varied while keeping the concentrations of other reactants constant at the same reaction conditions. The reaction rate increases with increasing the concentration of L-serine at its low concentrations but approaches zero-order kinetics at higher concentrations of L-serine, indicating a shift from first-order kinetics to zero-order kinetics with respect to the substrate (Figure 2). The effect of alkali on the reaction rate was also studied, revealing that the rate of the reaction increases with the increase in the concentration of alkali indicates first order kinetics with respect to alkali for the entire range of variation (Table 1). At a fixed temperature of 35^0C, the concentrations of the other reactants were maintained constant while the concentration of ruthenium (III) chloride was changed. The reaction exhibits first-order kinetics with respect to ruthenium (III) chloride throughout its concentration range, as indicated by the observed increase in –dc/dt values with increasing catalyst concentration (Table 1). The order with respect to ruthenium (III) chloride is further confirmed by a straight line passing through the origin for catalyst variation (Figure 3). By varying the potassium chloride concentration while keeping the concentrations of the other reactants constant, the impact of the ionic strength of the medium on the reaction rate was investigated. The findings show that the reaction rate is positively impacted by the ionic strength of the medium (Table 2). The effect of variation of externally added hexacyanoferrate(II) was also studied by keeping the concentrations of the other reactants constant at 35^0C. The results reveal that the rate of the reaction decreases on increasing the concentration of ferrocyanide ions (Figure 4). On the basis of common ion effect decrease in reaction rate on increasing the concentration of externally added hexacyanoferrate(II) shows that prior presence of hexacyanoferrate(II) ions in the reaction mixture slows down the rate of the reaction and thus the rate of reaction is decreased. A known quantity of the scavenger acrylonitrile monomer was first added to the reaction mixture, which was then kept in an inert atmosphere for two hours in order to examine the potential role of free radicals in the process. No precipitate was seen when the mixture was diluted with methanol, suggesting that free radicals are not involved in the process. By changing the t butyl alcohol–water volume fractions in the reaction mixture from zero to thirty times while maintaining all other parameters constant, the impact of the dielectric constant was investigated. Under the experimental conditions, it was discovered that the solvent did not react with the oxidant.

Table 1: Effect of Variation of [L-Serine], [NaOH] and [$RuCl_3$] on the reaction rate at 35 ^{0}C. $K_3[Fe(CN)_6]$ = 1.67 x 10^{-3}M; μ= 0.7M

[L-Serine] x 10^{-2} M	[NaOH] x 10^{-1} M	[$RuCl_3$] x 10^{-5} M	-dc/dt x 10^{-1} M.min^{-1}
1.00	2.00	2.38	0.47
1.10	2.00	2.38	0.53
1.25	2.00	2.38	0.52
1.43	2.00	2.38	0.84
2.00	2.00	2.38	0.96
2.50	2.00	2.38	1.00
3.30	2.00	2.38	1.10
5.00	2.00	2.38	1.25
6.00	2.00	2.38	1.20
8.00	2.00	2.38	1.33
1.25	0.20	2.38	0.13
1.25	0.25	2.38	0.21
1.25	0.50	2.38	0.24
1.25	0.75	2.38	0.33
1.25	1.00	2.38	0.70
1.25	1.50	2.38	0.75
1.25	2.00	2.38	1.75
1.25	2.50	2.38	2.10
1.25	2.00	0.48	0.57
1.25	2.00	0.95	1.60
1.25	2.00	1.43	2.00
1.25	2.00	1.90	2.67
1.25	2.00	2.38	3.33
1.25	2.00	2.86	3.87
1.25	2.00	3.33	4.40
1.25	2.00	3.81	4.60
1.25	2.00	4.28	5.80
1.25	2.00	4.76	6.20

Table 2: Effect of variation of μ on the reaction rate at 35 ^{0}C. $K_3[Fe(CN)_6]$ = 1.67 x 10^{-3}M; [L-Serine] =1.25 x 10^{-2}M; [$RuCl_3$] = 2.38 x 10^{-5}M [OH^-]= 2.0 x 10^{-1} M

μ x 10^{-1} M	0.30	0.40	0.50	0.60	0.70	0.80	0.90	1.00
-dc/dt x 10^{-1} M.min^{-1}	0.23	0.62	0.78	1.00	1.12	1.15	1.45	3.00

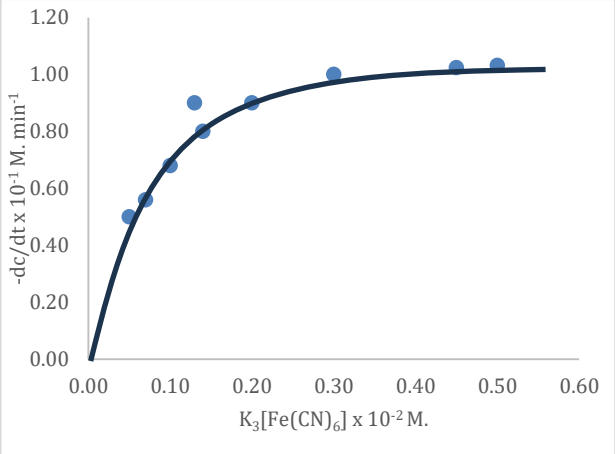


Figure 1: Effect of variation of $K_3[Fe(CN)_6]$ on the reaction rate at 35 ^{0}C. [L-Serine] =1.25 x 10^{-2}M; [OH^-]= 2.0 x 10^{-1}M; [$RuCl_3$] = 2.38 x 10^{-5}M; μ= 0.7M.

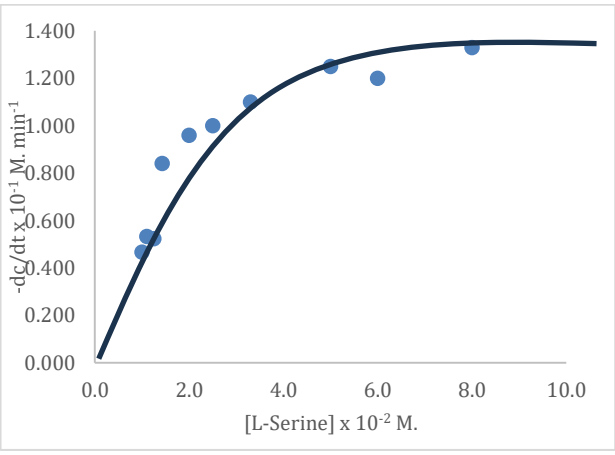


Figure 2: Effect of substrate variation on the reaction rate at 35 ^{0}C. $K_3[Fe(CN)_6]$ =1.67 x 10^{-3}M; [OH^-]= 2.0 x 10^{-1}M; [$RuCl_3$]= 2.38 x 10^{-5}M; μ= 0.7M.

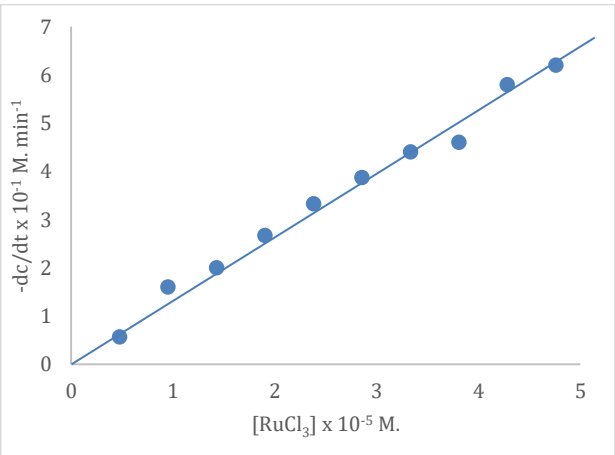


Figure 3: Effect of catalyst variation on the reaction rate at 35 ^{0}C.
$K_3[Fe(CN)_6]$ = 1.67 x 10^{-3}M; [L-Serine] =1.25 x 10^{-2}M; [OH^-]= 2.0 x 10^{-1}M; μ= 0.7M.

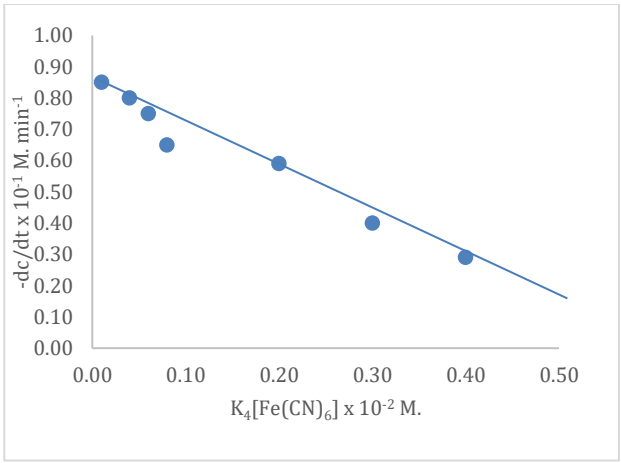


Figure 4: Effect of variation of $K_4[Fe(CN)_6]$ on the reaction rate at 35^{0}C. $K_3[Fe(CN)_6]$ = 1.67 x 10^{-3}M; [L-Serine] =1.25 x 10^{-2}M; [$RuCl_3$] = 2.38 x 10^{-5}M; [OH^-]= 0.2 M; μ= 0.7M.

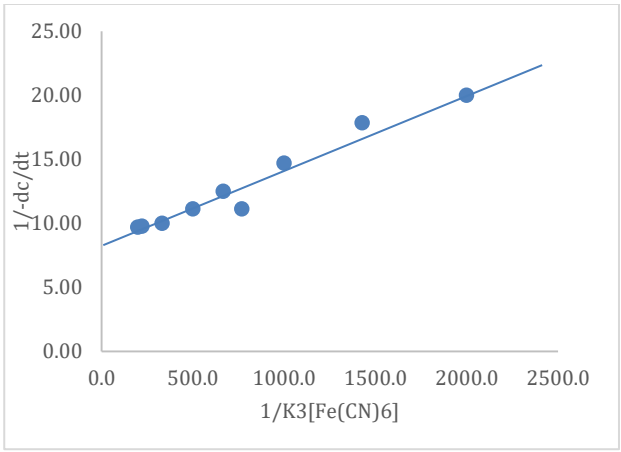


Figure 5: Plot of 1/-dc/dt vs 1/$K_3[Fe(CN)_6]$

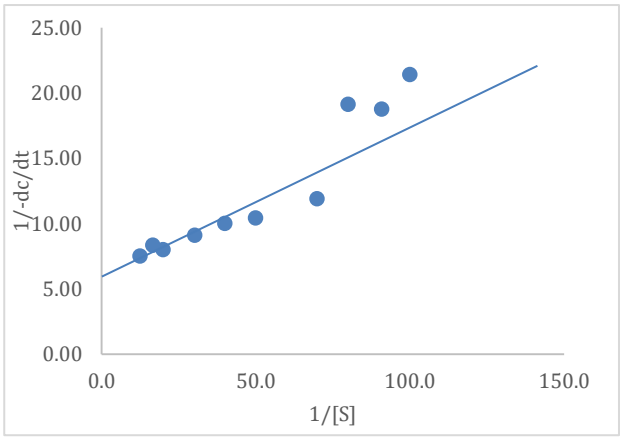


Figure 6: Plot of 1/-dc/dt vs 1/[S]

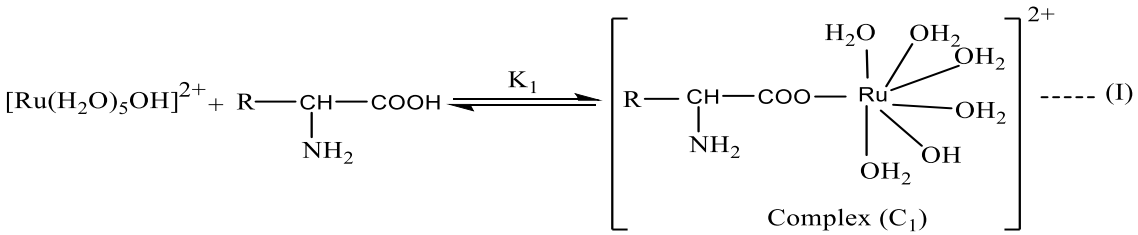
Reactive species of ruthenium(III)chloride in alkaline medium

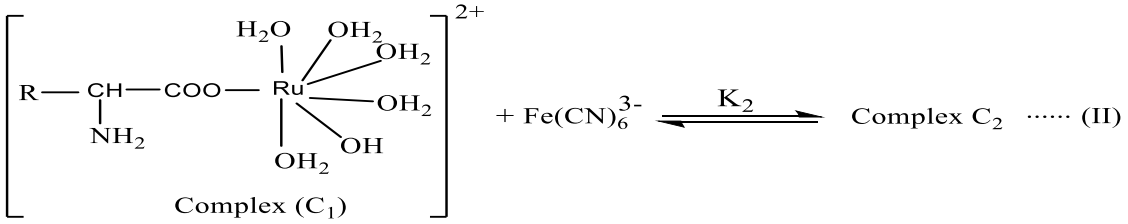
Electronic spectral studies (Cotton and Wilkinson, 1996; Singh, 1977) have confirmed that ruthenium(III) chloride exists in the hydrated form as

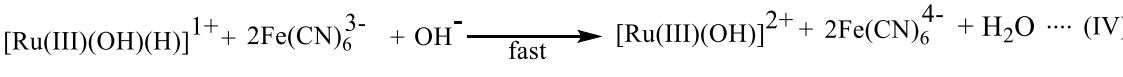
$[Ru(H_2O)_6]^{3+}$. In the present study, it is quite probable that the $[Ru(H_2O)_5OH]^{2+}$ species might assume the general form $[Ru(III)(OH)_X]^{3-X}$. The value of x would always be less than six because there are no definite reports of any hexahydroxy ruthenium species. Water molecules will fill the remainder of the coordination sphere. Hence, under the experimental conditions $[OH^-]$ >> [Ru(III)], ruthenium (III) is mostly present (Kamble and Nandibewoor, 1998; Hiremath, 1998) as the hydrated species, $[Ru(H_2O)_5OH]^{2+}$. The mechanism involves the formation of the active species, $[Ru(H_2O)_5OH]^{2+}$, in an equilibrium step, which reacts with the active form of aspartic acid to give a complex. Spectral evidence for complex formation between catalyst and substrate was obtained from the UV-Vis spectra of the ruthenium (III) species and a mixture of ruthenium (III) and aspartic acid. The evidence for complex formation is also obtained by kinetic studies, from the Michaelis-Menten plot. The complex then reacts with hexacyanoferrate(III) in a slow step to form the product and the hexacyanoferrate(II) and the catalyst is regenerated.

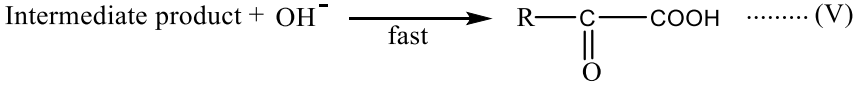
In an alkaline medium, ruthenium (III) exists as,

Since the rate of the reaction rises as $[OH^-]$ increases. $[Ru(H_2O)_5OH]^{2+}$ is regarded as the active form of ruthenium (III). The following mechanism is suggested in light of these data.









where R = $-CH_2-OH$

The concentration of total ruthenium trichloride is given as eq. (1)

$$[Ru(III)]_T = [Ru(III)] + [C_1] + [C_2] \quad .....(1)$$

$$[C_2] = \frac{K_1K_2[S][Ru(III)]_T[Fe(CN)_6]^{3-}}{1+K_1K_2[S][Fe(CN)_6]^{3-}} \quad .....(2)$$

$$\text{rate of reaction} = -\frac{d[Fe(CN)_6]^{3-}}{dt} = k[C_2]\,[OH^-] \quad \cdots\cdots\cdots (3)$$

On substituting the values and rearranging the equation, the final rate law may be given as,

$$-\frac{d[Fe(CN)_6]^{3-}}{dt} = \frac{kK_1K_2[S]\,[Ru(III)]_T[Fe(CN)_6]^{3-}[OH^-]}{1+K_1K_2[S]\,[Fe(CN)_6]^{3-}} \quad \cdots\cdots (4)$$

As per the stoichiometric ratio, eq. (4) is written as eq. (5)

$$-\frac{d[Fe(CN)_6]^{3-}}{dt} = \frac{2kK_1K_2[S]\,[Ru(III)]_T[Fe(CN)_6]^{3-}[OH^-]}{1+K_1K_2[S]\,[Fe(CN)_6]^{3-}} \quad \cdots\cdots\cdots (5)$$

$$k' = \frac{\text{rate}}{[Fe(CN)_6]^{3-}}$$

$$\frac{1}{k'} = \frac{1}{2kK_1K_2[S]\,[Ru(III)]_T[Fe(CN)_6]^{3-}[OH^-]} + \frac{1}{2k\,[Ru(III)]_T\,[OH^-]} \quad \cdots\cdots (6)$$

According to the equation, the rate of the reaction shows first-order kinetics at lower oxidant and substrate concentrations and tends towards zero-order kinetics at higher concentrations of both the L-serine and hexacyanoferrate (III). For the whole concentration range of ruthenium (III) chloride and hydroxide ions, the reaction exhibits first-order kinetics. Equation (6) is in the form of a straight line equation and a plot of 1/rate versus $1/[Fe(CN)_6]^{3-}$ should give a straight line with positive intercept at y-axis which is obtained experimentally also and supports the rate law (5) and the proposed mechanism. The graphs 1/k′ vs. 1/[S] and 1/k′ vs. $1/[Fe(CN)_6]^{3-}$graphs display straight lines with positive intercepts on the Y-axis. The k K_1K_2 and k values calculated from the slope and intercept of the graphs and the values come out to be 68.90 x 10^4 and 25.85 x 10^2 respectively. The suggested mechanism is supported by these graphs.

To gain deeper insights into the kinetic behaviour, the reaction was examined across a range of temperatures at 25°C, 30°C, 35°C, 40°C and 45°C, from which activation parameters including energy of activation (Ea^*), enthalpy of activation (ΔH^*) and entropy of activation (ΔS^*) were calculated using the Arrhenius and Eyring equations. The influence of temperature on the reaction rate was assessed while keeping [L-serine], [hexacyanoferrate], $[OH^-]$ and $[RuCl_3]$ constant, and maintaining ionic strength of the reaction medium constant. The activation energy for the rate-determining step was determined from the slope of the Arrhenius plot of 3+log k versus 1/T (Figure.7) and was found to be 3.993 kcal/mol. The entropy of activation (ΔS^*) and free energy of activation (ΔF^*) for the rate-determining step were also calculated. Using the Eyring equation, the values obtained were $\Delta S^* = -70.343$ cal/mol.K and $\Delta F^* = 25.6587$ kcal/mol. This reflects an efficient catalytic route mediated by the Ru–substrate complex, while the negative indicates a structured transition state involving ion-pairing and electron transfer in the complex consistent with the outer-sphere mechanism and absence of radical polymerization. These values align well with the observed Michaelis–Menten saturation kinetics, first-order dependences on $[OH^-]$ and $[RuCl_3]$, and micellar rate enhancements. The derived rate law accords quantitatively with the kinetic observations under all conditions. These results validate the proposed mechanism involving deprotonation, complexation and rate-limiting electron transfer, thereby emphasizing the pivotal role of ruthenium in facilitating oxidation in alkaline media.

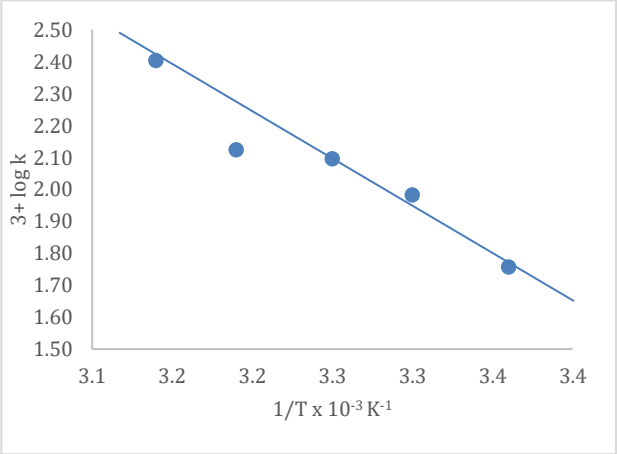


Figure 7: Effect of temperature on the reaction rate. $K_3[Fe(CN)_6]$ = 1.67 x 10^{-3}M; [L-Serine] =1.25 x 10^{-2}M; $[RuCl_3]$ = 2.38 x 10^{-5}M; $[OH^-]$= 2.0 x 10^{-1}M; μ= 0.7M.

CONCLUSION

The kinetics of the ruthenium(III) chloride catalysed oxidation of L-serine by hexacyanoferrate(III) in aqueous acid medium at 35°C was discussed. The reaction proceeds at a measurable rate in alkaline media. Kinetic studies were used to elucidate the reaction mechanism. A negative value entropy of activation and free energy of activation indicates the formation of transition state fair and rapid with lower degree of freedom. Further, the high value of free energy of activation suggests a solvated activated complex. The mechanistic steps of the redox reaction have been analysed, mechanisms proposed and the rate laws derived. The rate laws are consistent with the observed kinetic data, thus justifying the rate laws and hence the proposed mechanism.

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