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Indian J.Sci.Res. 16 (2): 11-19, 2026 **Original Research Article**

KINETIC STUDY OF THE CATALYSED OXIDATION OF ASPARTIC ACID IN AQUEOUS ALKALINE MEDIUM

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ABSTRACT

The kinetic study of ruthenium (III) catalysed oxidation of aspartic acid by hexacyanoferrate (III) was investigated in an aqueous alkaline medium at 35 °C. The reaction rate exhibits a first-order dependence with respect to hexacyanoferrate (III) and the amino acid substrate at its lower concentrations, tending towards zero-order kinetics at higher concentrations of both hexacyanoferrate (III) and amino acid substrate. The reaction rate exhibits first-order kinetics with respect to ruthenium (III) chloride. An increase in the ionic strength of the medium shows a positive effect on the reaction rate, whereas the presence of the initially added product produces a retarding effect on the progression of the reaction. Kinetic investigations conducted at varying temperatures and various activation parameters have been computed. The proposed and derived rate laws were consistent with the experimental observations, thereby supporting the plausibility of the reaction mechanism suggested.

KEYWORDS: Amino Acid, Hexacyanoferrate, Oxidation, Kinetics, Ruthenium Trichloride

Transition metal ions and their complexes are able to catalyse and oxidise a wide variety of reactions and are thus important for the chemical industry. Transition metals play an important role in catalysing numerous redox reactions due to their ability to adopt multiple oxidation states. The kinetics of redox processes involving homogeneous catalysts, particularly platinum group metals such as osmium(VIII), iridium(III) and palladium(II), have been extensively studied. Metal complexes are good oxidants in acid or alkaline media under appropriate reaction conditions. Extensive research has been conducted on osmium(VIII) (Singh, 1986), which has been employed both as an oxidant (Singh *et al.*, 1999; Singh *et al.*, 1998) and as a catalyst (Singh *et al.*, 1969) in the oxidation of various organic compounds. However, the toxic nature of osmium, particularly the formation of hazardous 'osmates' in acidic media, has limited its practical application, making its use more favourable under alkaline conditions. Ruthenium(III) (Mavalangi, 2001; Gupta *et al.*, 2015; Gupta *et al.*, 2017) and iridium(III) (Gupta *et al.*, 2007; Gupta *et al.*, 2011) have also been demonstrated to act as efficient homogeneous catalysts for the oxidation of various organic compounds, functioning effectively in both acidic and alkaline media. It has been observed that, among these metals, Ru(III) functions as a comparatively more effective catalyst for the oxidation of a wide range of substrates. The redox potential of the Ru(IV)/Ru(III) couple is +1.3 V, whereas that of the Os(VIII)/Os(VI) couple is +0.85 V. This significant difference highlights the catalytic efficiency of ruthenium, particularly in its +3 oxidation state (Tandon and Gupta, 2011; Singh *et al.*, 1990; Singh *et al.*, 1990; Nandibewoor *et al.*, 2000).

Transition metals in higher oxidation states can be stabilised through chelation with suitable complexing agents. Such metal complexes often serve as effective oxidising agents in both acidic and alkaline media when used under appropriate reaction conditions. Several transition metal ions, such as Ce(IV), Cu(III), Ni(IV) and Fe(III), in their complexed forms, function as effective oxidising agents in neutral, acidic or basic media, depending on their respective redox potentials. It has been reported that the redox potential of the $[Fe(CN)_6]^{3-}$/$[Fe(CN)_6]^{4-}$ couple is +0.40V in alkaline medium and +0.36V in acidic medium, indicating that hexacyanoferrate(III) functions as a very effective oxidising agent under alkaline conditions (Day and Selbin,1964; Singh *et.al.*, 1999) and thus ferricyanide has extensively been used as a moderate oxidant. Electrode potentials are normally pH dependent for anions whose protons are involved in the oxidation/reduction equation, so the reduction potential of $[Fe(CN)_6]^{3-}$/$[Fe(CN)_6]^{4-}$ couple does not depend on pH of the medium. Hexacyanoferrate(III) is a single-equivalent and stable oxidant (Singh *et al.*,1999) and ideally suited to probe the reaction pathway of aspartic acid with ruthenium(III) chloride used as a homogeneous catalyst.

Amino acids have numerous applications in biochemical research, metabolic studies, microbiology,

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pharmaceuticals and nutrition. The oxidation of amino acids (Gupta, 2015) represents a key class of biochemical reactions, as these processes serve as valuable models for understanding protein oxidation (Hawkins *et al.*, 2003; Stadtman, 1993; Byadagi *et al.*, 2010). The study of amino acid oxidation is of significant interest both from a chemical perspective and for its implications in understanding the mechanisms of amino acid metabolism. Generally, the amino and carboxyl functional groups in $RCH(NH_2)COOH$ undergo chemical transformations, while the hydrocarbon side chain generally remains unaltered. This behaviour is attributed to the higher reactivity of the functional groups compared to the hydrocarbon side chain. Aspartic acid, the ionic form is known as aspartate, is an α-amino acid that is used in the biosynthesis of proteins (Voet *et.al.*,2016) and has vital roles in the body, including protein synthesis, hormone release and nervous system function. It is produced by the body and is also found in foods. Aspartic acid is involved in metabolic pathways such as the citric acid cycle and helps the body to produce energy. It is a non-essential amino acid in humans, meaning the body can synthesise it as needed. The L-isomer of aspartic acid is one of the twenty two proteinogenic amino acids, i.e. the building blocks of proteins. D-aspartic acid is one of two D-amino acids commonly found in mammals. In the present study, the kinetics of aspartic acid oxidation by hexacyanoferrate(III), catalysed by ruthenium(III) chloride in an alkaline medium, were 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.

MATERIALS AND METHODS

All chemicals employed were of analytical reagent (AR) grade and double-distilled water was used for the preparation of all solutions utilised in the kinetic experiments. The stock solution of ruthenium trichloride was prepared by dissolving $RuCl_3$ (Johnson- Matthay Chemical Ltd.) in a minimum amount of hydrochloric acid. The concentration of the solution was determined by EDTA titration (Reddy and Kumar,1995). Mercury was added to the ruthenium(III) solution to reduce any ruthenium(IV) formed during its preparation and the stock solution was allowed to stand overnight to ensure stability. A solution of aspartic acid was prepared by dissolving an appropriate amount of the recrystallized sample in double-distilled water. Sodium hydroxide and potassium chloride solutions were prepared to maintain the alkalinity and ionic strength of the reaction mixture, respectively. To assess the effect of dissolved oxygen on the reaction rate, purified nitrogen gas was passed through the distilled water to remove dissolved oxygen. No significant difference was observed between the results obtained under a nitrogen atmosphere and those conducted in the presence of air.

Kinetic Studies

The kinetic studies were conducted using a Systronics 2203 double-beam UV-Vis spectrophotometer. The reaction was performed under pseudo first order conditions, maintaining the substrate concentration in excess as compared to that of the oxidant. Appropriate quantities of potassium ferricyanide containing the required amount of ruthenium(III) chloride, sodium hydroxide and potassium chloride, were thermostated at 35°C. A known volume of aspartic acid solution, pre-thermostated at the same temperature, was quickly introduced into the reaction mixture to initiate the reaction. The temperature of the reaction mixture was maintained at 35 ± 1°C using an electrically operated thermostat. The progress of the reaction was monitored spectrophotometrically at 420nm, corresponding to the absorption of hexacyanoferrate(III). It was confirmed that interference from other species present in the reaction mixture at this wavelength was negligible. The –dc/dt values were calculated from the plots of absorbance versus time. The reaction orders for the various reactants were determined from the slopes of the plots of –dc/dt versus the respective reactant concentrations.

RESULTS AND DISCUSSION

Stoichiometry and Product Analysis

Different ratios of hexacyanoferrate(III) to aspartic acid were equilibrated at 308 K for 24 hours in the presence of sodium hydroxide and potassium chloride. The ionic strength of the medium was adjusted by adding potassium chloride. The remaining hexacyanoferrate(III) was analysed spectrophotometrically. The estimation of the residual oxidant indicated that one mole of aspartic acid reacted with two moles of hexacyanoferrate(III), establishing the stoichiometric ratio [aspartic acid] : [HCF(III)], 1:2. The stoichiometry for aspartic acid was found to correspond to the following equation:

$$RCH(NH_2)COOH + 2[Fe(CN)_6]3^- + 2OH^- \rightarrow RCOCOOH + 2[Fe(CN)_6]4^- + NH_3 + H_2O$$

where R = $-CH_2-COOH$

After the reaction was completed, the reaction mixture was basified, concentrated, and extracted with ether. The oxidation products were isolated using thin-layer chromatography (TLC) and subsequently characterised through physicochemical spectral analyses. The reaction products were identified as hexacyanoferrate(II), keto acid and ammonia. The formation of the aspartic-derived keto acid was evidenced by the appearance of a reddish-brown precipitate with neutral $FeCl_3$ and further confirmed through IR spectral analysis. The presence of ammonia was verified using Nessler's reagent.

The oxidation of aspartic acid 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 aspartic acid, 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 aspartic acid was varied while keeping the concentrations of other reactants constant at the same reaction conditions. The reaction rate increases with increasing the concentration of aspartic acid at its low concentrations but approaches zero-order kinetics at higher concentrations of aspartic acid, 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 investigated, 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).

The concentration of ruthenium(III) chloride was varied while keeping the concentrations of other reactants constant at a constant temperature. The observed increase in –dc/dt values with increasing catalyst concentration indicates that the reaction follows first-order kinetics with respect to ruthenium(III) chloride over its concentration range (Table 1). A straight line passing through the origin for catalyst variation further confirms that the order with respect to ruthenium(III) chloride is unity (Figure 3). Additionally, the plot of 1/k′ versus 1/[Fey] for oxidant variation show straight lines with positive intercepts at Y-axis (Figure 4). The graph is plotted between 1/k′ versus 1/[S] for substrate variation yielded straight lines with positive intercepts at the Y-axis (Figure 5). The influence of the ionic strength of the medium on the reaction rate was examined by manifold variation of the concentration of potassium chloride while keeping the concentrations of other reactants constant. The results indicate that the ionic strength of the medium has a positive effect on the reaction rate (Table 2). To investigate the effect of the initially added product, hexacyanoferrate(II), the reaction rate was measured over a range of hexacyanoferrate(II) concentrations while maintaining other reactant concentrations constant. The results showed no significant impact of hexacyanoferrate(II) on the reaction rate. The possible involvement of free radicals in the reaction was investigated by initially adding a known amount of acrylonitrile monomer (a scavenger) to the reaction mixture, which was then maintained for two hours under an inert atmosphere. Upon diluting the mixture with methanol, no precipitate was observed, indicating that free radicals do not participate in the reaction. The effect of the dielectric constant was examined by varying the t-butyl alcohol–water volume fractions from zero to thirty times in the reaction mixture, while keeping all other conditions constant. The solvent was found to be non-reactive with the oxidant under the experimental conditions. Zero to thirty times, in the reaction mixture with all other conditions being maintained constant. The solvent did not react with the oxidant under the experimental conditions.

Table 1: Effect of variation of [L-Aspartic Acid], [NaOH] and [$RuCl_3$] on the reaction Rate At 35 °C. $K_3[Fe(CN)_6]$ = 2.0×10^{-3}M.; μ = 0.5M

[L-Aspartic acid] x 10^{-2} M.	[NaOH] x 10^{-2} M	[$RuCl_3$] x 10^{-5} M.	-dc/dt x 10^{-2} M.min^{-1}
0.67	3.33	2.38	4.00
1.00	3.33	2.38	5.67
1.11	3.33	2.38	7.40
2.00	3.33	2.38	7.70
2.50	3.33	2.38	7.30
3.33	3.33	2.38	9.00
5.00	3.33	2.38	10.00
7.00	3.33	2.38	10.50
2.00	1.25	2.38	0.40
2.00	1.43	2.38	0.58
2.00	1.67	2.38	2.00
2.00	2.00	2.38	1.39
2.00	3.33	2.38	2.12
2.00	5.00	2.38	3.67
2.00	10.00	2.38	6.50
2.00	3.33	0.95	2.81
2.00	3.33	2.38	5.20
2.00	3.33	2.86	7.67
2.00	3.33	3.80	12.67
2.00	3.33	4.76	14.50
2.00	3.33	5.712	17.33

Table 2: Effect of variation of μ on the reaction rate at 35 °C. $K_3[Fe(CN)_6]$ = 2.0×10^{-3} M; [L-Aspartic Acid] = 2.0×10^{-2} M; [OH^-]= 3.33×10^{-2} M; [$RuCl_3$] = 2.38×10^{-5} M

μ M.	0.08	0.1	0.4	0.5	0.6	0.7	0.8
-dc/dt x 10^{-2} M. min^{-1}	0.90	1.00	1.50	1.40	1.71	2.00	2.46

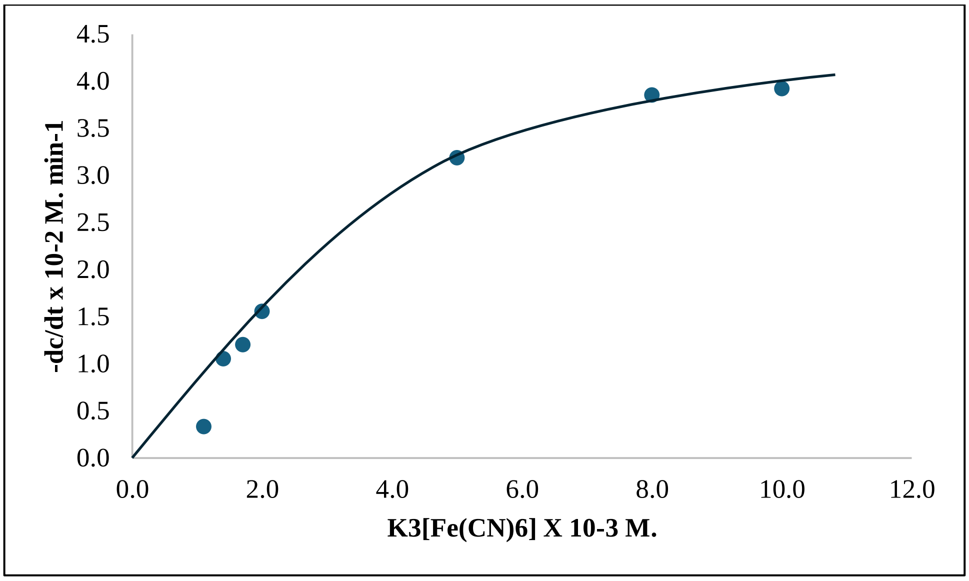

Figure 1: Effect of variation of $K_3[Fe(CN)_6]$ on the reaction rate at 35 °C. [L-Aspartic Acid] = 2.0×10^{-2} M; [OH^-]= 3.33×10^{-2} M ; [$RuCl_3$] = 2.38×10^{-5} M; μ=0.3M

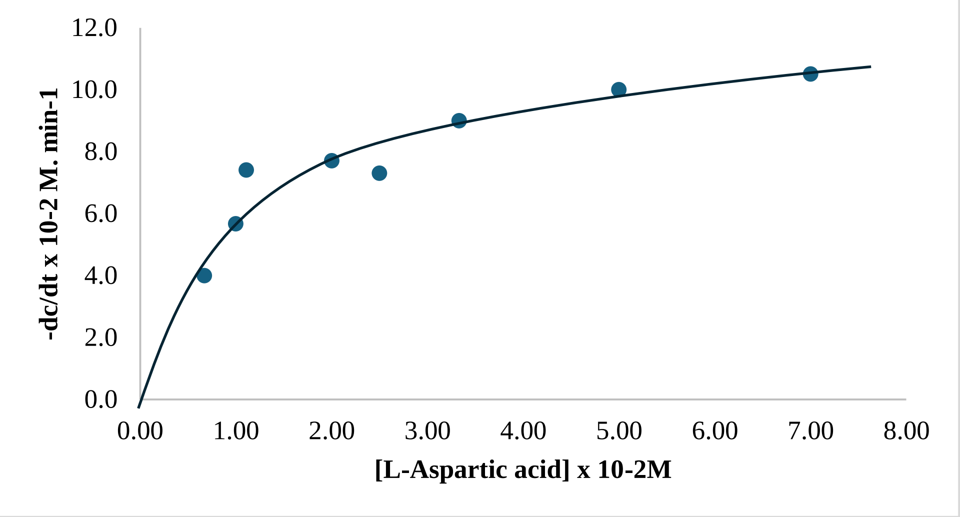

Figure 2: Effect of variation of substrate on the reaction rate at 35 °C. $K_3[Fe(CN)_6] = 2.0 \times 10^{-3}$ M; $[OH^-]= 3.33 \times 10^{-2}$ M; $[RuCl_3]= 2.38 \times 10^{-5}$ M; μ=0.3M

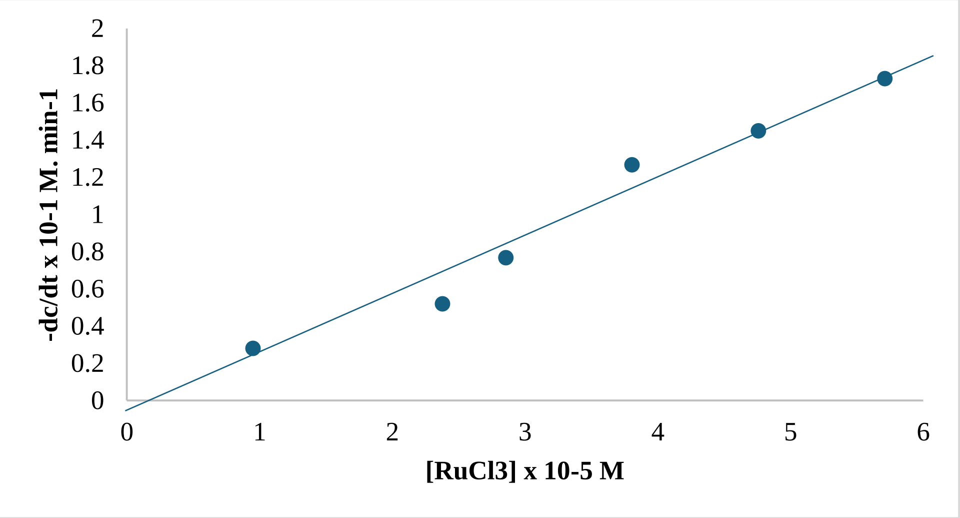

Figure 3: Effect of variation of catalyst on the reaction rate at 35 ^{0}C. $K_3[Fe(CN)_6] = 2.0 \times 10^{-3}$M; [L-Aspartic Acid] = 2.0×10^{-2} M; $[OH^-]= 3.33 \times 10^{-2}$ M; μ= 0.3 M

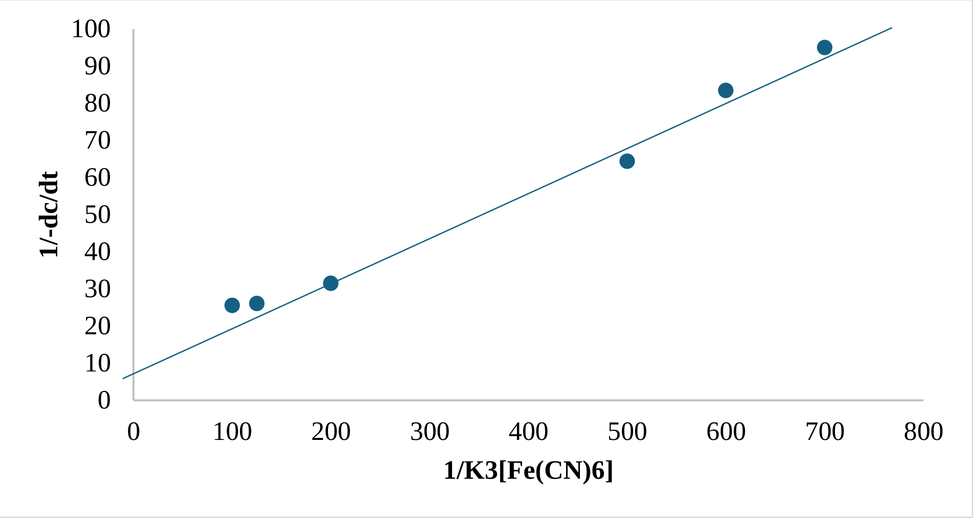

Figure 4: Plot of 1/-dc/dt vs $1/K_3[Fe(CN)_6]$

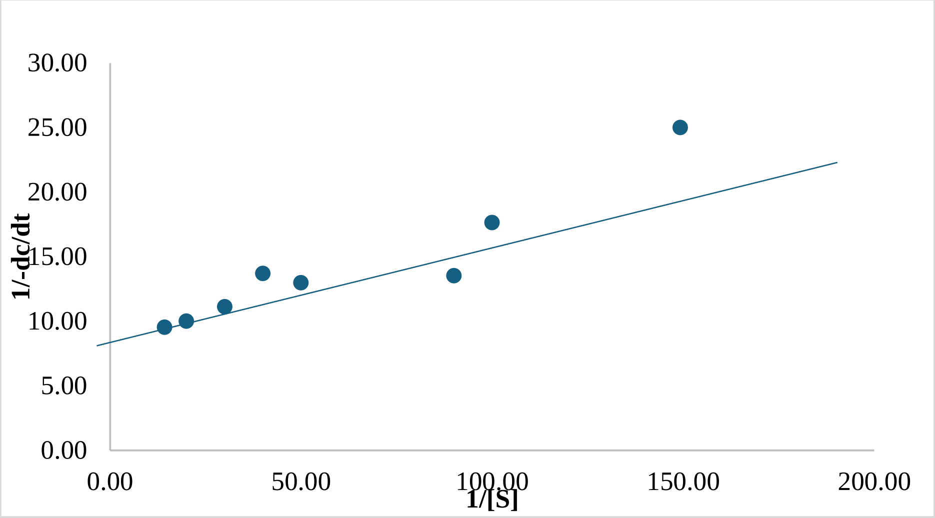

Figure 5: Plot of 1/-dc/dt vs 1/[S]

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 alkaline medium, ruthenium (III) exists as,

$$[Ru(H_2O)_6]^{3+} + OH^- \rightleftharpoons [Ru(H_2O)_5OH]^{2+} + H_2O$$

Since the rate of the reaction increases with an increase in $[OH^-]$. The active form of ruthenium(III) is considered to be $[Ru(H_2O)_5OH]^{2+}$. Considering these observations the following mechanism is proposed.

$$[Ru(H_2O)_5OH]^{2+} + AA \underset{}{\overset{K_1}{\rightleftharpoons}} C_1 \quad \text{....................(I)}$$

$$C_1 + [Fe(CN)_6]^{3-} \overset{K_2}{\rightleftharpoons} C_2 \quad \text{.............(II)}$$

$$C_2 + OH^- \xrightarrow[\text{slow}]{k} [Ru(III)(OH)(H)]^{1+} + \text{Intermediate product} \quad \text{..........(III)}$$

$$[Ru(III)(OH)(H)]^{1+} + 2Fe(CN)_6^{3-} + OH^- \xrightarrow[\text{fast}]{} [Ru(III)(OH)]^{2+} + 2Fe(CN)_6^{4-} + H_2O \quad \text{......(IV)}$$

$$\text{Intermediate product} + OH^- \xrightarrow[\text{fast}]{} \text{Final oxidation product} \quad \text{........(v)}$$

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

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

$$[C_1] = \frac{K_1[S]\,[Ru(III)]_T}{1 + K_1[S] + K_1K_2[S]\,[Fe(CN)_6]^{3-}} \quad \ldots\ldots\ldots\ldots\ldots\ldots(2)$$

On substituting the value of C_1 from eq (2) to (4) and rearranging the equation, the final rate law may be given as,

$$-\frac{d[Fe(CN)_6]^{3-}}{dt} = \frac{k\,K_1\,K_2\,[S]\,[Ru(III)]_T[Fe(CN)_6]^{3-}[OH^-]}{1 + K_1K_2\,[S]\,[Fe(CN)_6]^{3-}} \quad \ldots\ldots\ldots.(4)$$

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

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

The equation shows that the rate of the reaction exhibits first order kinetics at lower concentrations of oxidant and substrate, tending towards zero-order kinetics at higher concentrations of both hexacyanoferrate(III) and aspartic acid substrate. The reaction follows first-order kinetics with respect to ruthenium(III) chloride and hydroxide ion for its entire concentration range. The graphs plotted for 1/k′ vs 1/[OH$^-$] and 1/k′ vs 1/[S] show straight lines with positive intercepts on the Y-axis. These graphs support the proposed mechanism.

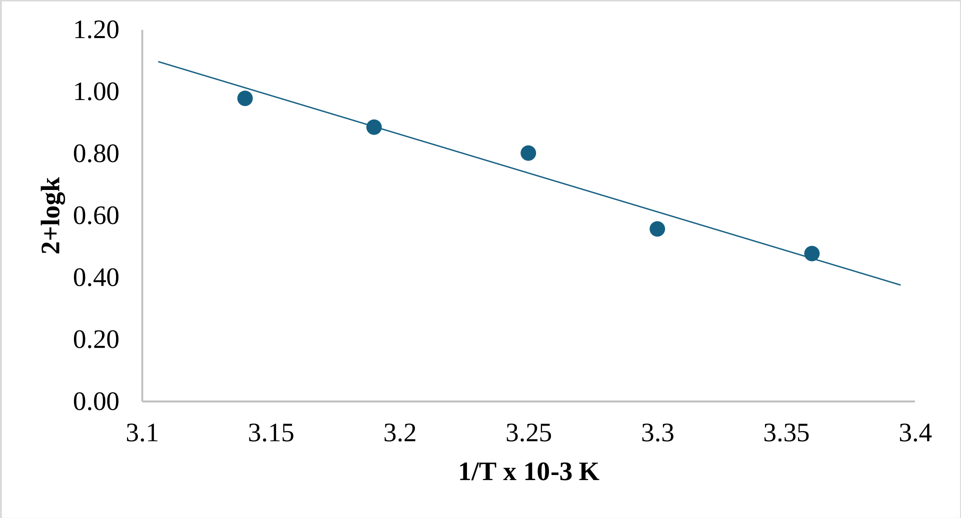

Figure 6: Effect of temperature on the reaction rate. $K_3[Fe(CN)_6]$ = 2.0 x 10^{-3} M; [L-Aspartic Acid] = 2.0 x 10^{-2} M; [OH$^-$]= 3.33 x 10^{-2} M; [$RuCl_3$] = 2.38 x 10^{-5} M; μ=0.3M

To further elucidate the kinetics, the reaction was systematically investigated at different temperatures and the corresponding activation parameters; the activation energy (Ea*), enthalpy of activation (ΔH^*) and entropy of activation (ΔS^*) were computed from the Arrhenius and Eyring equations. The effect of temperature on the reaction was investigated at 25°C, 30°C, 35°C, 40°C and 45°C. The activation energy for the rate-determining step was determined from the plots of log k versus 1/T (Figure 6). The activation energy (Ea) was determined from the slope of the Arrhenius plot and was found to be 3.399 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 ΔS^* = –68.288 cal/mol·K and ΔF^* = 24.432 kcal/mol. The values obtained suggest that the transition state is consistent with the proposed mechanistic path. The experimentally derived rate laws were found to agree with the observed kinetic data. On the basis of all the findings, a plausible mechanistic scheme has been proposed that satisfactorily accounts for the experimental results.

CONCLUSION

The oxidation of aspartic acid by hexacyanoferrate(III), catalysed by ruthenium(III) chloride, proceeds at a measurable rate in alkaline media. Kinetic studies were employed to elucidate the reaction mechanism. The mechanistic aspects of this redox reaction have been analysed, proposed mechanisms documented and the resulting rate laws derived. These rate laws are in excellent agreement with the experimentally observed kinetics, thereby supporting both the validity of the rate laws and the proposed mechanistic reactions.

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