

CATALYSED OXIDATION OF AMINO ACID IN AQUEOUS ALKALINE MEDIUM

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ABSTRACT

Kinetics of ruthenium (III) catalysed oxidation of essential amino acid, arginine by hexacyanoferrate (III) has been carried out in aqueous alkaline medium at 35°C. The rate of the reaction shows first order dependence on hexacyanoferrate (III). The reaction rate is first order at low concentrations of amino acid and alkali both and tends towards zero order at higher concentrations of substrate and alkali. The rate of reaction shows first order kinetics with respect to ruthenium (III) chloride. The variation of ionic strength of the medium and the addition of the initially added product show no effect on the rate of the reaction. There is no effect of dielectric constant on the reaction rate. The reaction was studied at different temperatures and activation parameters have been computed. The rate laws proposed and derived are in agreement with experimental results and a plausible mechanism is proposed.

KEYWORDS: Amino Acid, Hexacyanoferrate, Oxidation, Kinetics, Alkaline Medium.

Transition metals are involved to catalyse many redox reaction as they show multiple oxidation states. The kinetics of redox reactions involving homogeneous catalyst such as platinum group metals particularly osmium(VIII), iridium(III) and palladium (II) have extensively been used from the mechanistic point of view. There are numerous researches 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. But the injurious nature of osmium as the formation of toxic 'osmates' in acidic medium restricted its use in alkaline medium. Ruthenium (III)(Mavalangi,2001) and iridium(III)(Gupta et al.,2007; Gupta et al.,2011)are also known to act as a efficient homogeneous catalyst in the oxidation of several organic compounds in acidic as well as in alkaline medium.

It has been observed that among these metals Ru(III) acts as a comparatively more effective catalyst towards the oxidation of several substrates. The redox potentials of Ru(IV)/Ru(III) is +1.3V and that of Os(VIII)/Os(VI) is +0.85V, which is very low as compared to Ru(IV)/Ru(III). This also shows that ruthenium is an efficient catalyst (Tandon and Gupta, 2011; Singh et al.,1990; Singh et al.,1990; Nandibewoor et. al., 2000) in +3 state.

Transition metal in the higher oxidation state can be stabilized by chelation with suitable complex agent. Metal complexes are good oxidants in acid or alkaline media under appropriate reaction conditions. 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. It is reported that the redox potential of $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ in alkaline medium is +0.40V and in acidic medium is +0.36V indicating that

hexacyanoferrate (III) is a good oxidizing agent in alkaline medium(Day and Selbin,1964). It is a single equivalent and stable oxidant. Hence to explore the reaction path of arginine, a single equivalent oxidant, hexacyanoferrate (III) is used as an oxidant and ruthenium(III) chloride as a catalyst.

Amino acids find a number of applications in biochemical research, metabolism, microbiology, pharmaceuticals and nutrition. Oxidation reactions of amino acids (Gupta, 2015) are one of the most relevant biochemical reactions because such reactions serve as models for protein oxidations (Hawkins et al., 2003; Stadtman, 1993; Byadagi et al., 2010)). It is of great importance both from a chemical point of view and its bearing on the mechanism of amino acids metabolism. Generally the amino and carboxyl functional groups in $\text{RCH}(\text{NH}_2)\text{COOH}$ undergo chemical transformations while the hydrocarbon moiety remains intact. This property is attributed to the higher reactivity of the former compared to R. Arginine, an essential amino acid, is important to life especially to the growth of children. It finds application in medicine and pharmaceuticals. It is also needed to remove toxic ammonia from the body and plays an important role in cell division, immune function and in the release of hormone. In the present study, the kinetics of the oxidation of arginine by hexacyanoferrate (III) catalysed by ruthenium (III) chloride in alkaline medium has been taken to explain the reaction and also to understand the active species of ruthenium (III) in aqueous alkaline medium. The results are discussed in this communication and a plausible mechanism is proposed.

EXPERIMENTAL

Chemicals used were of AR grade. Double distilled water was used to prepare all the solutions for

kinetic experiments. A stock solution of ruthenium trichloride was prepared by dissolving RuCl₃ (Johnson-Matthay Chemical Ltd.) in 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. A solution of arginine was prepared by dissolving an appropriate 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 dissolved oxygen in distilled water was removed by passing purified nitrogen gas to check the effect of dissolved oxygen on the rate of reaction. It was observed that there is no significant difference between the result obtained under nitrogen atmosphere and in the presence of air.

KINETIC STUDIES

The kinetic measurements were performed on a systronics 2203 double beam UV-Vis spectrophotometer. The action was carried out under pseudo-first order condition where the concentration of substrate was kept higher than the concentration of the oxidant. Appropriate amounts of potassium ferricyanide which also contained required amount of ruthenium (III) chloride, solutions of sodium hydroxide and potassium chloride thermostated at 35°C. A measured

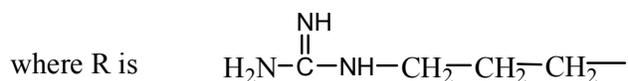
amount of arginine solution, also thermostated at the same temperature, was rapidly added to the reaction mixture to initiate the reaction.

The temperature of the reaction mixture was kept constant at 35 ± 1°C with an electrically operated thermostat. The progress of the reaction was followed spectrophotometrically at 420 nm to hexacyanoferrate (III). It was clear that there is negligible interference from other species present in the reaction mixture at this wavelength. The -dc/dt values were determined from the absorbance versus time plots. The orders for various reactants were determined from the slopes of plots of -dc/dt versus respective concentration of that reactant except oxidant.

RESULTS AND DISCUSSION

Stoichiometry and Product Analysis

Varying ratios of hexacyanoferrate (III) to arginine, in the presence sodium hydroxide and potassium chloride (ionic strength adjusted by the addition of potassium chloride) were equilibrated at 308 K for 24 h. The remaining hexacyanoferrate (III) was then analysed spectrophotometrically. Estimation of the residual oxidant showed that one mole of arginine consumed two moles of hexacyanoferrate (III), corresponding to the stoichiometry, [arginine] : [HCF] = 1 : 2. Stoichiometry for arginine was found to correspond to the equation:



After completion of the reaction, the reaction mixture was basified, concentrated and extracted with ether. The oxidation products were isolated using TLC separation techniques and characterized by physicochemical spectral studies. The reaction products were identified as hexacyanoferrate (II), keto acid and ammonia. The presence of arginine keto acid was shown by the presence of reddish brown precipitate with neutral FeCl₃ and confirmed by IR spectrum. The presence of ammonia is confirmed by Nessler's reagent.

The kinetics of oxidation of arginine by hexacyanoferrate (III) catalysed by ruthenium (III) chloride was investigated at several initial concentrations of the reactants in alkaline medium. The concentrations of hexacyanoferrate (III) was varied for its manifold variation at constant concentration of

arginine, sodium hydroxide, ruthenium (III) chloride and ionic strength of the medium. The rate of the reaction values (-dc/dt) were calculated from the initial slopes of individual graphs between absorbance versus time. The values obtained by the variation of hexacyanoferrate (III) shows that the rate of the reaction increases with the increase in the concentration of hexacyanoferrate (III) indicates first order kinetics with respect to hexacyanoferrate (III) for the entire range of variation (Table 1). The concentration of arginine was varied keeping the concentrations of other reactants and condition constants. The rate of the reaction increases with the increase in the concentration of arginine at their low concentrations and tends to become zero order at higher concentrations of arginine (Fig. 1). The effect of alkali on the rate of reaction was also studied and it

was found that the rate constant increases with the increase in alkali at low concentrations and tends to become zero order at higher concentrations of alkali showing first order tending towards zero order kinetics with respect to alkali (Fig.2). The ruthenium (III) chloride concentrations was varied at constant concentration of other reactants. The increase in $-dc/dt$ values with increasing concentrations of the catalyst and fair constancy in the rate values obtained for molar concentration of the catalyst indicate that the reaction follows first order kinetics with respect to ruthenium(III) chloride for its manifold variation in concentration (Table 2). A straight line passing through the origin is obtained for the catalyst variation confirming the order with respect to ruthenium (III) is unity. Further, the plot of $1/k'$ versus $1/[S]$ for substrate variation (Fig.3) and the plot of $1/k'$ versus $1/[OH^-]$ for alkali variation (Fig. 4) were shown straight lines with positive intercepts on Y-axis. The effect of ionic strength of the medium on the rate of the reaction was also studied by manifold variation of potassium chloride keeping the concentration of other reactants constant and the results show that there is no effect of the ionic strength of the medium on the reaction rate (Table 3). To study the effect of initially added product, hexacyanoferrate (II) on the reaction rate, the rate of the reaction is varied for manifold variation of hexacyanoferrate (II) keeping the concentration of other reactants constant. The results did not show any significant effect on the rate of reaction (Table 3). The intervention of free radicals in the reaction was examined by adding the known quantity of acrylonitrile monomer (scavenger) initially to the reaction mixture and was kept for two hours in an inert atmosphere. On

diluting the reaction mixture with methanol, there is no indication of the precipitate formed, indicating that there is no intervention of free radicals in the reactions. The effect of dielectric constant was studied by varying the t-butyl alcohol-water volume fractions from zero to thirty times, in the reaction mixture with all other conditions being maintain constant. The solvent did not react with the oxidant under the experimental conditions.

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. The remainder of the coordination sphere will be filled by water molecules. Hence under the experimental conditions $[OH^-] \gg [Ru(III)]$, 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 arginine 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 mixture of ruthenium (III) and arginine. The evidence for complex formation is also obtained by kinetic studies, from the Michaelis-Menton 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.

Table 1: Effect of variation of [HCF(III)] on the reaction rate at 35^oC.

([HCF(III)] = 1.66 X 10⁻³ M; [Arginine] = 1.25 x 10⁻² M; [OH⁻] = 1.66 x 10⁻² M; [RuCl₃] = 15.23 x 10⁻⁶ M; μ = 0.1)

K ₃ [Fe(CN) ₆] x 10 ³ M	0.50	0.80	1.00	3.00	3.50	5.00
-dc/dt x 10 ⁵ M.min ⁻¹	3.5	9.0	10.0	26.0	27.5	37.5

Table 2: Effect of variation of [Ru(III)] on the reaction rate at 35^oC.

([HCF(III)] = 1.66 X 10⁻³ M; [Arginine] = 1.25 x 10⁻² M; [OH⁻] = 1.66 x 10⁻² M; μ = 0.1)

[Ru(III)] x 10 ⁶ (mol dm ⁻³)	1.904	3.808	7.616	11.40	15.23	19.04
-dc/dt x 10 ⁵ M.min ⁻¹	0.5	1.0	2.0	3.5	4.2	6.1

Table 3: Effect of variation of [K₄Fe(CN)₆] and μ on the reaction rate at 35^oC.

([HCF(III)] = 1.66 X 10⁻³ M; [Arginine] = 1.25 x 10⁻² M; [OH⁻] = 1.66 x 10⁻² M; [RuCl₃] = 15.23 x 10⁻⁶ M; μ = 0.1)

[K ₄ Fe(CN) ₆] x 10 ³ M	-dc/dt x 10 ⁶ M. min ⁻¹	μ	-dc/dt x 10 ⁵ M. min ⁻¹
0.3	2.40	0.1	5.1
0.5	2.40	0.25	5.0
1.0	2.52	0.4	4.0
1.6	2.46	0.6	3.8
2.0	2.54	0.8	4.2

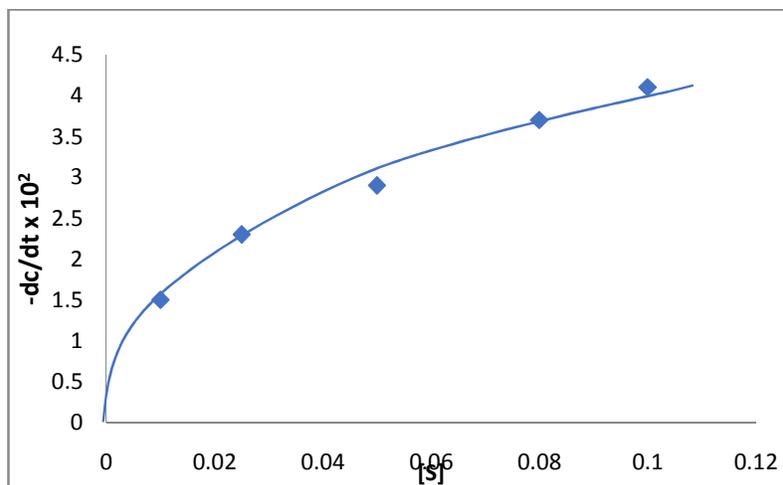


Figure 1: Plot of $-\frac{dc}{dt}$ vs [Arg]

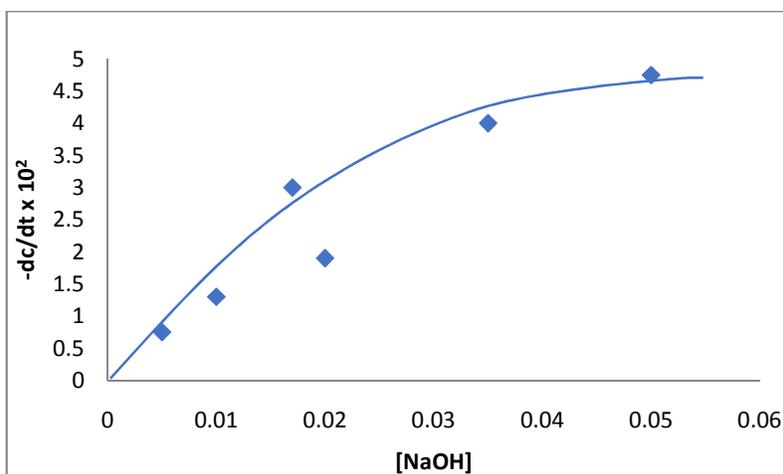


Figure 2: Plot of $-\frac{dc}{dt}$ vs $[OH^-]$

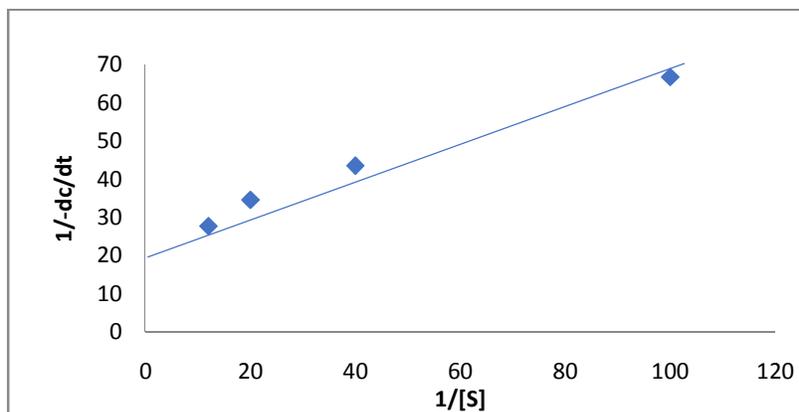


Figure 3: Plot of $1/(-\frac{dc}{dt})$ vs $1/[S]$

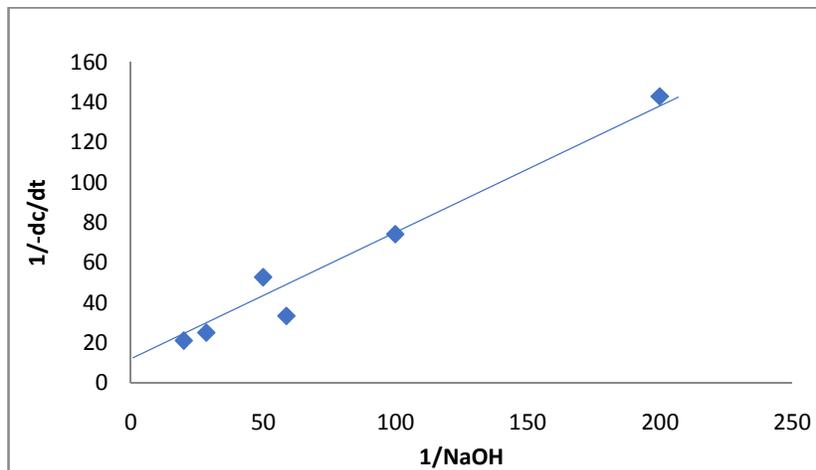


Figure 4: Plot of 1/-dc/dt vs 1/NaOH

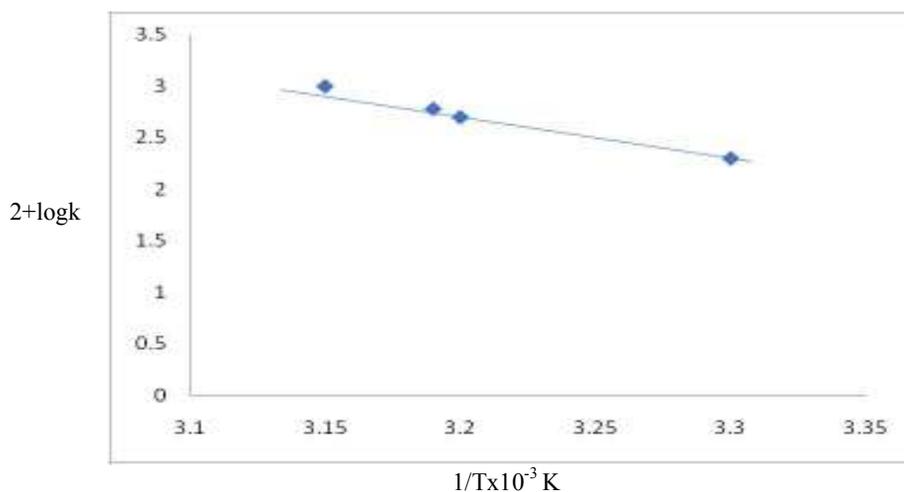
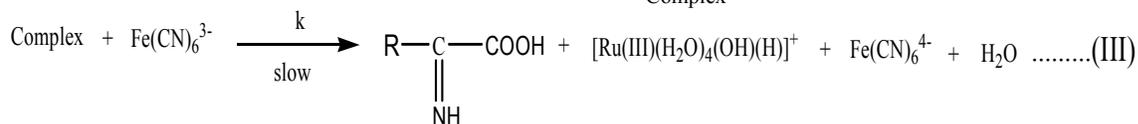
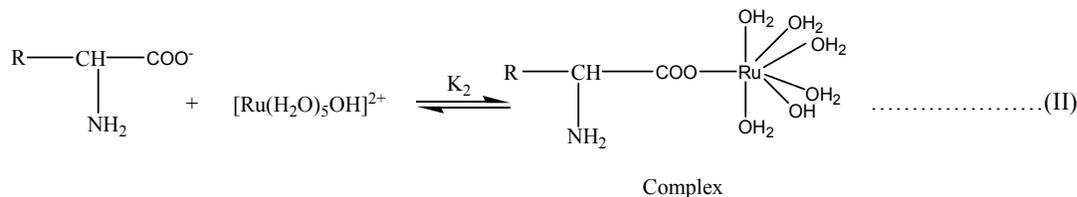


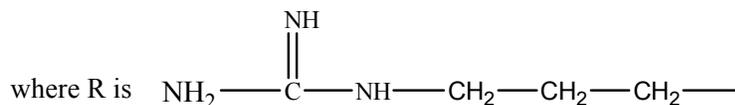
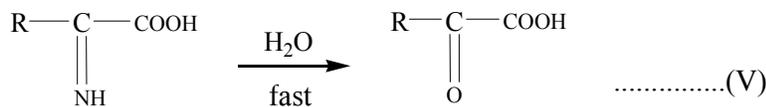
Figure 5: Effect of temperature on the reaction rate

In alkaline medium, ruthenium (III) exists as,



Since the rate of the reaction increases with increase in $[\text{OH}^-]$. The active form of ruthenium(III) is considered to be $[\text{Ru}(\text{H}_2\text{O})_5\text{OH}]^{2+}$. Considering these observations the following mechanism is proposed.





$$\text{Rate of reaction} = -\frac{d[\text{Fe}(\text{CN})_6]^{3-}}{dt} = k[\text{C}][\text{Fe}(\text{CN})_6]^{3-} = kK_2[\text{S}][\text{Ru}(\text{H}_2\text{O})_5\text{OH}]^{2+} [\text{Fe}(\text{CN})_6]^{3-} \dots(1)$$

$$[\text{Ru}(\text{H}_2\text{O})_5\text{OH}]^{2+} = \frac{K_1[\text{OH}^-][\text{Ru}(\text{III})]}{1+K_1[\text{OH}^-] + K_1K_2[\text{S}][\text{OH}^-]} \dots\dots\dots(2)$$

On substituting the value of $[\text{Ru}(\text{H}_2\text{O})_5\text{OH}]^{2+}$ from eq (2) to (1),

The final rate law may be given as,

$$\text{Rate} = \frac{kK_1K_2[\text{S}][\text{OH}^-][\text{Ru}(\text{III})][\text{Fe}(\text{CN})_6]^{3-}}{1+K_1[\text{OH}^-]+K_1K_2[\text{S}][\text{OH}^-]}$$

The equation shows that the rate of the reaction is first order kinetics at low concentrations of oxidant and substrate.

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

$$\frac{1}{k'} = \frac{1}{kK_1K_2[\text{S}][\text{OH}^-][\text{Ru}(\text{III})]} + \frac{1}{kK_2[\text{S}][\text{Ru}(\text{III})]} + \frac{1}{k[\text{Ru}(\text{III})]}$$

The graphs plotted for $1/k'$ vs $1/[\text{OH}^-]$ and $1/k'$ vs $1/[\text{S}]$ show straight lines with positive intercepts on Y- axis. These graphs support the proposed mechanism.

Effect of temperature was studied at four different temperatures 30°C , 35°C , 40°C , 45°C . The energy of activation for the rate determining step was obtained by plotting the graph between $\log k$ vs. $1/T$. Further the energy of activation (ΔE^\ddagger), entropy of activation (ΔS^\ddagger) and free energy of activation (ΔF^\ddagger) were determined by plotting the Arrhenius graph between $\log k$ and $1/T$ at four different temperatures (30°C , 35°C , 40°C , 45°C). The value of energy of activation has been calculated from the slope of the Arrhenius plot i.e. $E_a = 11.440 \text{ Kcal/mol}$. The values of entropy of activation and free energy of activation has also been calculated with respect to slow step. The entropy of activation, $\Delta S^\ddagger = -54.1331 \text{ Kcal/mol}$ and free energy of activation, $\Delta F^\ddagger = 16.68 \text{ Kcal/mol}$ were obtained by Eyring equation.

CONCLUSION

The reaction between hexacyanoferrate (III) and arginine catalysed by ruthenium (III) chloride occurs with measurable rate in alkaline media. Kinetic studies have been used as a tool to know the mechanism of a reaction. Mechanistic aspects of the redox reaction have been documented, mechanisms proposed and the derived rate laws are consistent with the observed kinetics. The derived rate laws are in excellent agreement with the observed rate of the reaction, thus justifying these rate laws and hence the proposed mechanistic scheme.

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