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Research Article

ROLE OF ELLMAN'S REAGENT FOR THE OXIDATION OF PYRIDINE N-OXIDE BY A SUPEROXIDE LIGAND IN A Co^{III}- COMPLEX, IN AQUEOUS ACETATE BUFFER MEDIUM: A KINETICS AND MECHANISTIC STUDIES

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ABSTRACT

In aqueous acetate buffer media (pH=4.0-5.2), reaction between the superoxo complex, $[(dien)(en)Co^{III}(O_2)Co^{III}(en)(dien)]^{5+}$ (**1**) and pyridine N-oxide (C_5H_5NO) proceeds through a complex kinetic pathway and the change of the absorbance of **1** with time also follows a complex pattern. Only in the presence of Ellman's reagent (5,5′-dithio-bis-(2-nitrobenzoic acid), DTNB), the reaction becomes quantitative and each mole of **1** is reduced by one mole of pyridine N-oxide (C_5H_5NO) to produce the corresponding hydroperoxo complex, $[(dien)(en)Co^{III}(HO_2)Co^{III}(en)(dien)]^{5+}$ (**2**), following proton coupled electron transfer (PCET) path. In presence of DTNB, with large excess of pyridine N-oxide (C_5H_5NO) over [**1**], the reaction follows first-order kinetics and the observed rate constant, k_o increase linearly with increase in $[C_5H_5NO]$ but decreases with increase in $[H^+]$. It is also noticed that the reaction rate significantly decreases with increasing proportion of D_2O replacing H_2O in the solvent. Therefore, an H-atom transfer (HAT) from the reducing species to the bridging superoxide in **1** seems reasonable at the rate determining step. Indeed the present work confesses the forthcoming involvement of Ellman's reagent as a radical scavenger.

Keywords: Kinetics, Superoxide, Redox, DTNB, Mechanism, Radical.

1. INTRODUCTION

Pyridine N-oxides and their functionalized derivatives are biologically active and medicinally important [1]. In the recent time the chemistry and appliation of pyridine N-oxide have received very important attention due to its usefulness as synthetic intermediates and their biological importance [2]. In the last few years, there has been an increasing interest in the synthesis and use of related optically active pyridine N-oxides as chiral controllers for asymmetric reactions and also as chiral catalyst [3]. Again the chiral non-racemic pyridine N-oxides have a great utility in various enantioselective processes. The N-O moiety of pyridine N-oxides possesses a unique functionality which can act effectively as a push electron donor and as a pull electron acceptor group [4]. This strong push-pull property has an essential chemical consequence; it accounts for the equally easy synthesis of 4- substituted derivatives of pyridine N-oxides with donor as well as acceptor groups [5, 6]. The pyridine N-oxide derivatives represent a peculiar class of antiviral compound that qualify as promising novel drugs for exploration as potential anti-HIV agents [7]. They have an entirely new mechanism of antiviral action and the capacity to retain antiviral activity against virus strains that have gained resistance to clinically used drugs. At the same time a wide variety of pyridine N-oxide derivatives have been found to be inhibitory against feline corona-virus (FIPV strain) and human SARS-CoV (Frankfurt strain-1) in CRFK and simian kidney (Vero) cell cultures, respectively [8-11]. The oxide part on the pyridine moiety proved indispensable for anti-coronavirus activity. Another important application of pyridine N-oxide (PNO) as dye transfer inhibitor polymers. One of the major problems in the household machine laundry process is dyes bleeding from coloured garments and then the re-depositing on lighter-coloured garments in the same wash load. Improper loading of high quantity of dye transfer inhibitor (DTI) polymer also had severe negative effect on coloured garment in terms of colour striping, patchy and unsightly appearance. In this game, pyridine N-oxide (PNO) polymers were optimized for better dye transfer inhibition property in the 'wash' liquor against reactive dye [12]. The polymer pyridine N-oxide (PNO) also overcomes the shortfalls of commercially available DTI polymers like polyvinyl-pyrrolidone (PVP) [13].

On the other hand, in the biological systems, Superoxide ion ($O_2^{\bullet-}$) is of great significance as a radical and chemical species [14]. It is generated by the immune system to kill invading microorganisms. In phagocytes, superoxide is produced in large quantities by the enzyme NADPH oxidase for use in oxygen-dependent killing mechanisms of invading pathogens [15]. Indeed, all the living cell contain Superoxide ion ($O_2^{\bullet-}$) [16] but the chemistry knowledge of $O_2^{\bullet-}$, especially the bi-nuclear metal bound superoxide is rather scarce. So the chemistry and reaction of -N-O centre and metal bound superoxide is of great significance. The present work represent the role of Ellman's reagent (DTNB) in the reaction between pyridine N-oxide and bi-nuclear Co^{III}- bound superoxide in aqueous acetate buffer medium.

2. EXPERIMENTAL

2.1. Material and instrumentation

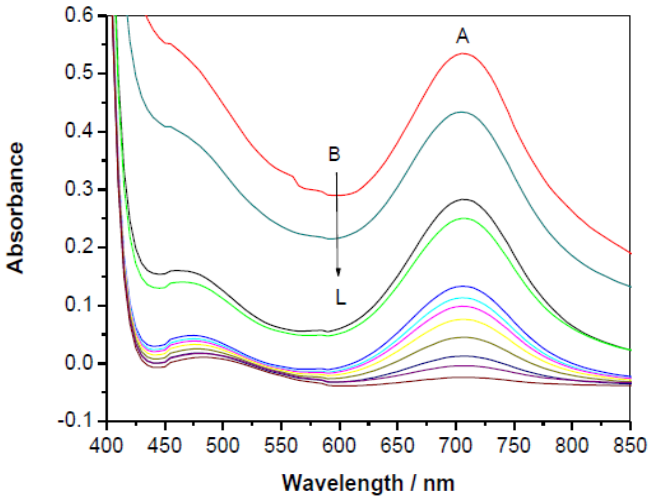
Superoxo complex, μ-superoxo[bis(ethylenediamine) bis (diethylenetriamime) cobalt(III)]$^{5+}$, viz., [(dien)(en) Co^{III} $(O_2)Co^{III}$(en)(dien)]$(ClO_4)_5$ (**1**) was synthesized following the literature method [17, 18]. $NaClO_4$ was prepared by neutralizing $HClO_4$ with $NaHCO_3$ in the usual way. All other materials including dipicolinic acid (dpa, Aldrich), pyridine N-oxide (Aldrich) and 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB; Aldrich) were used as received. All the solutions required for the experiments were prepared in freshly boiled, double distilled water.

Absorbance, UV-VIS spectra and changes in absorbance of the reaction mixture during the kinetic experiments were recorded with a Shimadzu 1800 spectrophotometer equipped with electrically controlled thermostat (±0.1°C) and 1.00 cm quartz cells. A pH meter (Gold-533) with electrodes calibrated with standard buffer solutions was used for pH measurements. C, H, N analyses were made using a 2400 series-II CHN/O analyzer (Perkin ELMER).

2.2. Kinetics

The superoxo complex **1** shows its characteristic absorption maxima at 708 nm where no other reactants absorbs. In aqueous acetate buffer media the reaction of **1** with large excess of C_5H_5NO (C_5H_5NO >> [**1**]) results in the decrease of absorbance of **1** (at 708 nm) (fig. 1) but the reactions kinetics follows a complex pattern.

Only in presence of DTNB, under the same experimental conditions, the absorbance of **1** decrease regularly and such a time resolved UV-VIS spectral change obeyed first order kinetics (fig. 2) at least up to 95% completion of reaction and the first order rate constants (k_o) were evaluated by non-linear least squares fitting of the decay of the absorbance (A_t) with time (t) data to standard first-order exponential decay equation.



pH = 4.8 in acetate buffer (T_{OAc} = 0.2 M), I = 0.5 M, T = 25.0 °C . (A) Spectrum of the pure complex , (B)-(L) spectra of reaction mixture at time intervals 60, 120, 240, 360, 600, 820, 960, 1200, 1440, 16780 and 9720 seconds, respectively.

Fig. 1: Time resolved spectra of 0.50 mM of 1 reacting with. 2M Pyridine N-oxide in presence of 2mM Ellman reagent

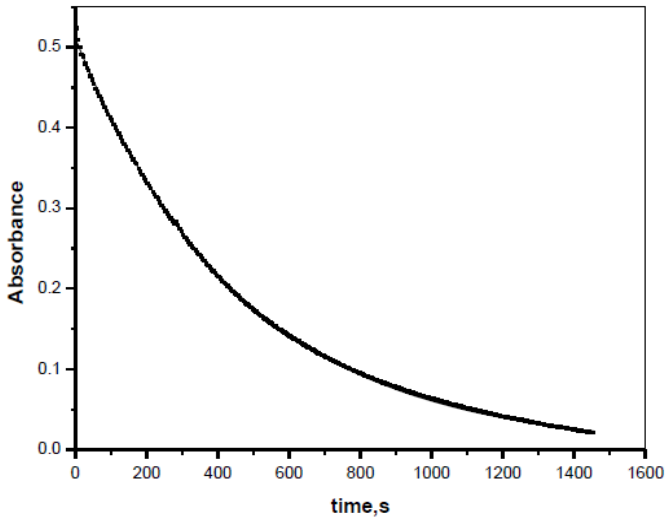


Fig. 2: Decrease in absorbance (points shown in black circles) of 1 with time at 708 nm in its reaction with Pyridine N oxide in presence of 2mM Ellman's reagent gives an excellent fit (solid line) to the first-order exponential decay equation. [1] = 0.50 mM, [C_5H_5NO] = 0.30 M, pH =4.8, *I* = 0.5M ($NaClO_4$), T_{OAc} = 0.2 M, T = 25.0 °C.

Here, it is important to note that from independent experiments, it has been confirmed that under the experimental pH range (4.0-5.2), DTNB neither reacts with **1** nor with C_5H_5NO. On addition of DTNB to the solution of **1** or to the solution of C_5H_5NO, no change in absorption was noticed for at least 2 hours. Since, complex **1** is only stable in acidic media, all the kinetic experiments were conducted in acetic acid-sodium acetate buffer media (pH=4.0-5.2) at room temperature (25.0±0.1°C) under constant ionic strength, *I* of 0.5 M (maintained with $NaClO_4$), mentioned otherwise. Furthermore, dipicolinic acid (dpa, $C_7H_5NO_4$) was added to sequester the ubiquitous metal ions (*vide infra*) present in the reaction media. When the solvent was enriched with D_2O, the pH of the reaction media was estimated using the relation, pD = pH + 0.4 [19, 20].

2.3. Stoichiometry

The stoichiometry of the title reaction was determined spectrophotometrically by measuring the equilibrium absorbance at 708 nm where none other reactants but [**1**] absorbs. For such stoichiometric determination, in the presence of DTNB, excess [**1**] was allowed to react with deficit amounts of [C_5H_5NO] in different ratio. From the difference of the initial (A_0) and equilibrium absorbance (A_e) of the reaction mixtures at 708 nm the concentration of remaining **1** was calculated.

3. RESULTS

3.1. Stoichiometry and reaction product

The results from stoichiometry (table 1), show that in presence of DTNB, each mole of C_5H_5NO exhaust one mole of the superoxo complex **1**. Thus, in presence of DTNB and excess C_5H_5NO, complex **1** is reduced to its corresponding one-electron reduced hydroperoxo complex, $[(en)(dien)Co^{III}(HO_2)Co^{III}(en)(dien)]^{5+}$ (**2**) following PCET pathway. The final UV-VIS spectrum shown in (Fig. 1) match closely to the reported spectrum of hydroperoxo complexes of Co(III) with amine ligands under similar kinetic conditions [21,22].

Table 1: Stoichiometric results for the oxidation of C_5H_5NO by 1 in presence of 2mM DTNB at T_{OAc} = 0.2M, *I* = 0.5 M ($NaClO_4$)

[**1**] /Mm	[C_5H_5NO] / mM	[**1**]left / mM	Δ[**1**] / Δ[C_5H_5NO]
10.5	3.4	7.2	0.97[a]
15.0	4.5	10.6	0.98[b]
20	5.0	15.1	0.98[c]

T = 25.0 °C: [a] pH 4.2, [b] pH 4.0, [c] pH 4.4.

In the experimental conditions the reductantant pyridine N-oxide mainly exist as $C_5H_5NOH^+$ (pKa = 0.79) [23]. The redox reaction between **1** and $C_5H_5NOH^+$ consists of the reduction of the bridging superoxo group and $C_5H_5NOH^+$ does not reduce the Co(III) centers of the complex. This is evident from the fact that simple dinuclear Co(III) complex like, μ-amido-bis [pentaam-minecobalt(III)]$^{5+}$ cannot oxidize $C_5H_5NOH^+$. Thus in absence of DTNB the title reaction can be represented by equation 1.

$$[(en)(dien)Co^{III}(O_2)Co^{III}(en)(dien)]^{5+}\ (\mathbf{1}) + C_5H_5NOH^+ \longrightarrow [(en)(dien)Co^{III}(HO_2)Co^{III}(en)(dien)]^{5+}\ (\mathbf{2}) + C_5H_5NO^{\bullet}\ (\mathbf{1})$$

In presence of DTNB, on reaction with $C_5H_5NOH^+$ the absorbance of **1** at 708 nm decreases regularly and follows first-order rate kinetics (fig. 2). Under the condition of large excess of $C_5H_5NOH^+$ over [**1**], the observed rate, k_o linearly increase with the increase in [**1**] and thus the observed reaction rate is dependent on the first power of [**1**]. On the other hand, at constant [**1**], k_o also increase linearly with increase in C_5H_5NO (fig. 3, table 3), and the linearity confirm dependence of the observed reaction rate on the first power of $C_5H_5NOH^+$ concentration. Thus, the reaction rate is first-order to each of [**1**] and $C_5H_5NOH^+$.

Table 2: Variation of k_o with [C_5H_5NO], [1] = (0.50 mM), [DTNB] = 2mM at pH = 4.01, T_{OAc} = 0.2 M, [dipicolinic acid] = 2.0 mM, *I*=0.5M ($NaClO_4$), T = 25.0°C.

[C_5H_5NO] /M	$10^3 k_o$ / s^{-1}
0.1	0.51
0.2	1.18
0.3	1.68
0.4	2.13
0.5	2.40
0.6	2.93
0.7	**3.2**

The title redox reaction was performed over a pH range of 4.0-5.2 (table 3) and the plot of $1/k_o$ against [H^+] was found to be linearly decreasing (fig. 4). Evidently, the

reactant is undergoing protonation with increasing [H^+] of the reaction media to form the kinetically robust species. We consider the case of complex **1** if it can participate in any protic equilibrium. Earlier works by Hoffman *et al.* [24] confirms that the superoxo bridge of complex **1** cannot abstract any proton as in such case the structure of the linear O-O^- bridge will be destroyed. The structural change is similar to the change of linear peroxo moiety (O-O^{2-}) to the hydroperoxo moiety (>O-OH) [25]. Overall, thus we can assume that complex **1** take part in protic equilibria by abstracting a proton from reaction media and the inverse-proton dependence of the observed rate should arise due to the protonation of the complex **1**.

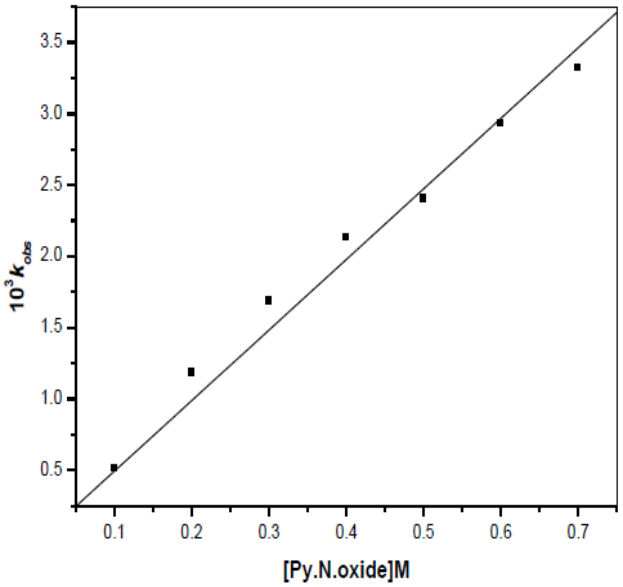


Fig. 3: Linear variation of k_o for the reaction of [C_5H_5NO] and 1 (0.50 mM), [DTNB] = 2mM at pH = 4.8, T_{OAc} = 0.2 M, [dipicolinic acid] = 2.0 mM, *I* = 0.5M ($NaClO_4$), T = 25.0°C.

Table 3: Variation of $1/k_o$ with pH, [1] = 0.50 mM, [C_5H_5NO]=0.3M, T_{OAc} = 0.2 M, [dipicolinic acid] = 2.0 mM, *I* = 0.5M ($NaClO_4$), T = 25.0°C.

Ph	$1/k_o$ / s
4.0	828.17
4.2	695.67
4.4	650.2
4.6	592.25
4.8	567.94
5.0	545.33
5.2	**520.24**

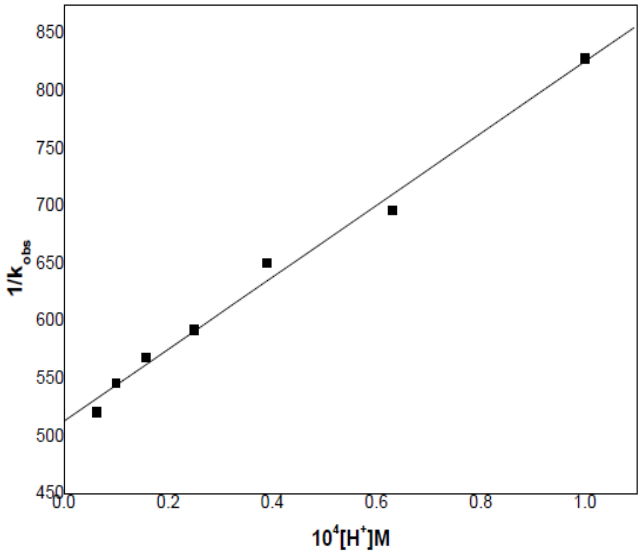


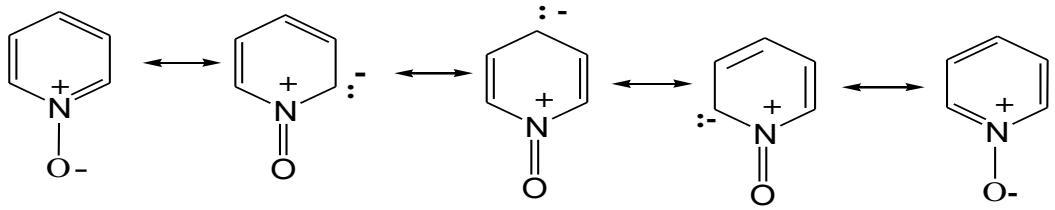
Fig. 4: Plot of $1/k_o$ *vs* [H^+], [1] = 0.50 mM, [C_5H_5NO] = 0.30 M, [DTNB] = 2mM, T_{OAc} = 0.2 M, *I* = 0.5 M ($NaClO_4$), [dipicolinic acid] = 2.0 mM, T = 25.0 °C.

In aqueous solution, pyridine N-oxide (C_5H_5NO) is believed to exist in protonation equilibrium, $C_5H_5NOH^+ \overset{K_a}{\rightleftharpoons} C_5H_5NO + H^+$ and the equilibrium constant for proton donation by the conjugate acid in aqueous solution has been reported to be 0.79 (pKa) [23]. Considering the proton-dependence of the reaction rate, we can assume that $C_5H_5NOH^+$ acts as the primary form of the reductant against **1**. As the proton concentration increases, stability of the species $C_5H_5NOH^+$ also increases. However, it is also noteworthy that the plot shown in fig. 4 yields a substantial intercept value at zero [H^+] and thus we should also consider the role of the C_5H_5NO as the reductant, though the concentration of the species C_5H_5NO is very low in the experimental reaction conditions.

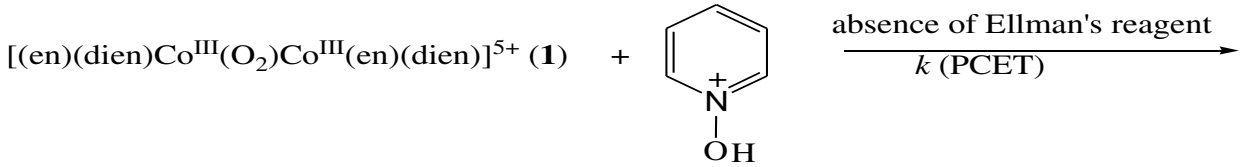
For the title reaction, we also have studied the effect of change of ionic strength on the values of observed rate constant, on increasing the media ionic strength, the reaction rate also increases which also indicates a reaction between two reactive species with similar charges. Since, the oxidant superoxo complex **1** carries positive charges; the other reactive species from C_5H_5NO must also bear positive charge, *i.e.*, $C_5H_5NOH^+$. This strengthens our assumption that the protonated form of C_5H_5NO i.e., $C_5H_5NOH^+$ is the primary reactive species.

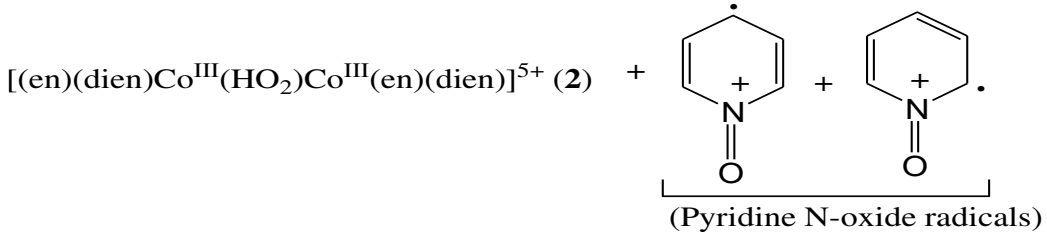
4. DISCUSSION

From the above discussions we can lay the primary mechanistic idea for the redox reaction as that in presence of Ellman's reagent, $C_5H_5NOH^+$ reduces **1** to **2** following PCET pathway and itself gets oxidized to pyridine N-oxide radical species ($C_5H_5NO^{\bullet+}$), which is immediately arrested by DTNB and the corresponding reaction kinetics become simple and first order. The reaction scheme can be represented as-



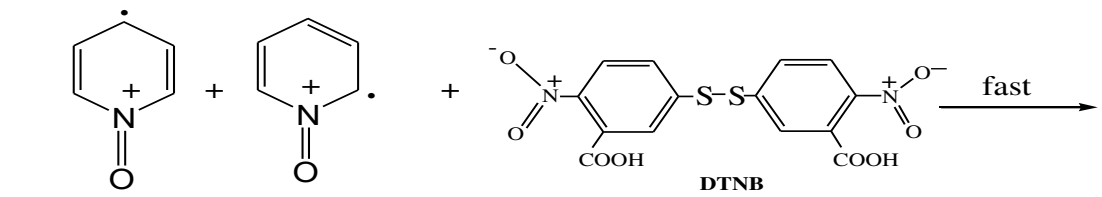
In the experimental conditions, $C_5H_5NOH^+ \underset{}{\overset{K_a}{\rightleftharpoons}} C_5H_5NO$

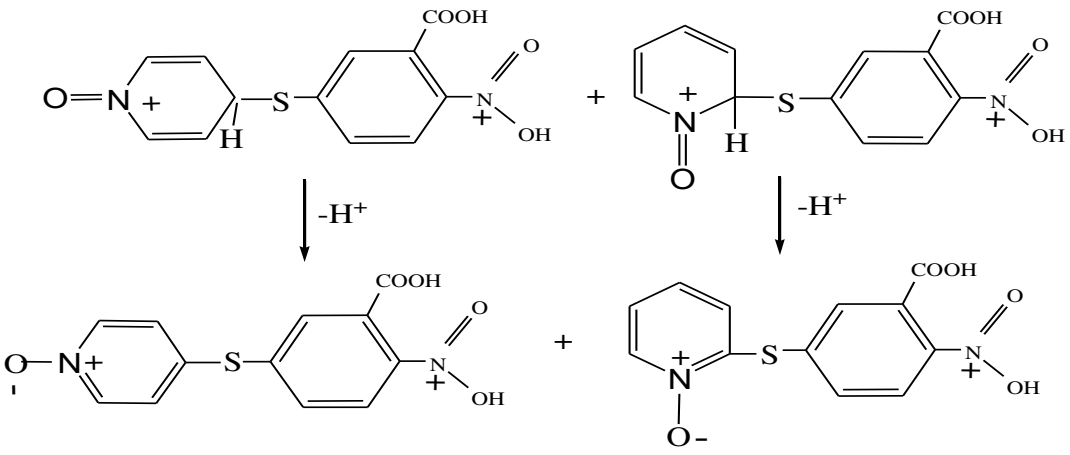




Pyridine N-oxide radicals + **1** $\xrightarrow{\text{fast}}$ products

But in presence of Ellman's reagdents these pyride N-oxide radicals gets arrested, which gives simple and first order reaction kinetics





The formation of cationic pyridine radical species ($C_5H_5NO^{\bullet+}$) from the oxidation of pyridine N-oxide have also been reported by Y. V. Geleti et al. for reactions with photo-excited triplet state of chloanil and in aqueous solution it is also generated by the reaction of pyridine and $OH_2^{\bullet}$ [26]. Since the enrichment of aqueous media with D_2O (from 0 to 93.3 % v/v) yields significant retardation of rate, we can assume that the electron and proton transfer process are synchronous (PCET) [27, 28]. The the cationic pyridine radical species ($C_5H_5NO^{\cdot+}$) is highly reactive in aqueous solution and in absence of DTNB, immediately react with second mole of the superoxo complex (1) and gives the complex kinetics. Only in the presence of Ellman's reagent (DTNB) which removes the cationic pyridine radical species ($C_5H_5NO^{\cdot+}$) immediately through the formation of 2 and 4 -substituted derivative of pyridine N-oxide, the redox interaction between **1** and C_5H_5NO follows a pure first-order reaction path and it can be systematically studied. Hence the proposed reaction scheme in abbreviated form is thus:

$$\mathbf{1} + H^+ \overset{K}{\rightleftharpoons} \mathbf{1}H \qquad (2)$$

$$\mathbf{1} + C_5H_5NOH^+ \xrightarrow{k} \mathbf{2} + C_5H_5NO^{+\cdot} \qquad (3)$$

Equations (2) and (3) lead to the rate equation (4).

$$k_0 = k[C_5H_5NOH^+]/(1 + K[H^+]) \qquad (4)$$

Equation (4) may be rearranged to equation (5).

$$1/k_0 = 1/(k[C_5H_5NOH^+] + K[H^+]/(k[C_5H_5NOH^+]) \qquad (5)$$

A plot of $1/k_o$ *versus* $[H^+]$ was found to be excellent straight line (fig. 4) as expected from equation (5) and yielded $k = 6.51 (\pm 0.4) \times 10^{-3}$ s^{-1} and $K = 2.03 (\pm 0.3) \times 10^4$ M^{-1}. DTNB is generally used as a reagent for quantitative estimation of free sulfhydryl group [29, 30] but in this present work, it seems that DTNB also functions as the scavenger of $C_5H_5NO^{\cdot+}$ generated there from and stop further reactions.

5. CONCLUSION

In aqueous acetate buffer media (pH=4.0-5.2), reaction between the superoxo complex, $[(dien)(en)Co^{III}(O_2)Co^{III}(en)(dien)]^{5+}$ (**1**) and pyridine N-oxide (C_5H_5NO), generates the corresponding hydroperoxo complex, $[(dien)(en)Co^{III}(HO_2)Co^{III}(en)(dien)]^{5+}$ (**2**) and pyridine N-oxide radical ($C_5H_5NO^{\cdot+}$) respectively. The pyridine N-oxide radical ($C_5H_5NO^{\cdot+}$) can further react with complex **1,** the reaction kinetics proceeds through a complex pathway and the change of the absorbance of **1** with time also follows a complex pattern. Ellman's reagent (DTNB) plays an important role in removing the pyridine N-oxide radical ($C_5H_5NO^{\cdot+}$). Hence only in the presence of Ellman's reagent (5,5′-dithio-bis-(2-nitrobenzoic acid), DTNB) the reaction becomes quantitative and each mole of **1** is reduced by one mole of pyridine N-oxide (C_5H_5NO) to produce the corresponding hydroperoxo complex, $[(dien)(en)Co^{III}(HO_2)Co^{III}(en)(dien)]^{5+}$ (**2**) and the reaction follows a first-order kinetics path. Indeed the present work confesses the forthcoming involvement of Ellman's reagent as a radical scavenger.

6. ACKNOWLEDGEMENT

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Conflict of Interest

None declared.

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