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Oxidation of hydroxylamine by Co^{III}-bound superoxo complex containing chelating ancillary ligands: A kinetics and mechanistic study

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In aqueous acetate buffer medium (pH, 3.6 to 4.8) hydroxylamine reduces the one electron oxidant, superoxo ligand in title superoxo complex $[(dien)(en)Co^{III}(O_2)Co^{III}(en)(dien)](ClO_4)_5$ (**A**) to its hydroperoxo complex, $[(en)(dien)Co^{III}(HO_2)$-$Co^{III}(en)(dien)]^{5+}$ (**B**) and itself gets oxidised to N_2O gas following both proton coupled electron transfer (PCET) path and an electron-transfer reaction. The kinetics, stoichiometry and reaction mechanism clearly indicate that oxidation of NH_2OH occurs through the formation of an intermediate aminoxyl ($NH_2O^{\bullet}$) radical, a weak acid (pKa = 12.6±0.3). Addition of excess NH_2OH over **A**, the reaction obeys good first-order kinetics and the reaction rate increases linearly with the increase of $[NH_2OH]$. The reaction rate, however, decreases with increase in $[H^+]$. The plot of $1/k_0$ with $[H^+]$ is linear with a small but noteworthy intercept. The decrease of reaction rate with $[H^+]$ is attributed to the protonation of the superoxo complex **A**, which leads to a kinetic inert product. It is also noticed that the reaction rate significantly decreases with increasing percentage of D_2O replacing H_2O in the solvent. Therefore, transfer of an H-atom (HAT) from the hydroxylamine to the superoxide ligand in **A** assumes plausible at the rate determining step.

Keywords: Hydroxylamine, superoxo, kinetics, reaction mechanisms, oxidation-reduction.

Introduction

Hydroxylamine is an intermediate between nitrous oxide and ammonia as it can be oxidised to nitrous oxide and reduced to ammonia[1]. It is produced by the interaction of SO_2 and NO_x in aqueous solution[2–4]. So study of reactions of hydroxylamine may help us understanding the cooperative transition of NO_x and SO_2 into aerosols that impacts air quality and threatens the health of humans and other terrestrial animals[2]. This may also give us insight about the development of flue gas and the interaction in atmospheric aqueous droplets that leads to acid rain[4,5]. Moreover, hydroxylamine is a commonly used reagent for the synthesis of oximes that act as intermediates in numerous chemical synthesis and are known to produce radical intermediates in various types of reactions[6,7]. Hydroxylamine is a well known antioxidant and its derivative such as hydroxylamine sulfate (HAS) has wide application in agriculture, e.g. for insecticides, acaricidies, herbicides, and germicides hydroxylamine sulfate is widely used as a raw material[8–11]. The various derivative of hydroxyl amine, such as hydroxamic acid, hydroxyl isoxazole, uric acid, oxadiazole, amide, and oxime are also used as raw material for the production of range of important chemicals including CNS sedatives, tear contamination obstructors, incitement, diuretics, blood coagulants, anti-malaria drugs, and diabetes drugs[12]. Again for the treatment of metal surface, extraction of metal ions and their separation hydroxylamine sulfate is also widely used[13]. NH_2OH has also a significant contribution in rubber industry as a vulcanizer[14].

Besides industrial applications, hydroxylamine has a key role to play in combating another great threat to humanity-antibiotic resistance that is emerging and spreading globally at an alarming rate. Misuse and overuse of antibiotic both for human and animal has caused to evolve some bacteria to become anti-biotic resistant which made some common bacterial infection harder to treat. In this regards the hydroxyl amine and its N-substituted derivative is very commonly used to treat the bacterial infection due to its high radical scavenger properties[15]. Hydroxylamine is also a mutagen[16] and in the biological system the enzyme Cytochromes P-460, can effectively convert hydroxylamine to nitrous oxide, a potent

greenhouse gas[17]. Expectedly, its environmental, biological importance as well as its synthetic applications has driven numerous mechanistic investigations of various type of reaction[7,8–10].

On the other hand the chemistry and reactivity of the superoxide ion ($O_2^{\bullet-}$) is very important both for chemists and biochemists as it can be generated chemically and biologically. Superoxide ($O_2^{\bullet-}$) is a respiratory intermediate[18], and is one of the primary links between biology and chemistry for the study of various chemical and biological reactions. But at high concentration of superoxide is extremely toxic[19] due to its capacity to generate the highly reactive hydroxyl radical. Therefore the kinetics and reaction mechanism of metal bound superoxo complexes have falicitate[20]. According to Sykes *et al*.[21] a given metal bound superoxo complex can play important variations of reaction mechanism with seemingly alike type of reducing agents. So far the studies mainly worried with redox conduct of monodentate[22] and bridging superoxide[23]. But the effect of assisting ligands on the redox conduct of metal bound superoxide ligand is not well known. So the reaction mechanism between hydroxyl amine and superoxide is worthy to be investigated and is the focus of this article. In this study I present a metal bound superoxide ligand that reacts with hydroxyl amine in aqueous acetate buffer medium to produce nitrous oxide and a hydroperoxo complex and probe how it happens and what affects its kinetics.

Experimental

Materials and reagents:

The perchlorate salt of μ_2-superoxo[bis(ethylenediamine)bis(diethylenetriamime)cobalt(III)], [(en)(dien)Co^{III} $(O_2)Co^{III}$(en)(dien)]$(ClO_4)_5$ (**A**) was prepared by the literature procedure[24] and recrystallised from 0.3 *M* $HClO_4$. The purity of the complex was checked by measuring absorbance at 708 nm [ε^{708} mol^{-1} dm^3 cm^{-1}: found 1186; reported[24]: 1210±5%], EPR study [*g* = 2.01(found); reported: 2.0304[24]] and elemental analyses (observed; N, 14.91%, C, 16.20%; reported[24]: 14.94% and 16.24% respectively). Hydroxylamine hydrochloride, $NH_2OH.HCl$ (E. Merck) was standardized spectrophotometrically[25]. The catalytic path suppressing agent, dipicolinic acid (dpa, Aldrich)) was reagent grade and used without further purification. Sodium perchlorate was prepared by reacting perchloric acid with $NaHCO_3$ and gradually concentrating the neutral solution. Other solutions and reagent so used are very pure (reagent grade) and therefore no need to further purification.

Instruments and kinetics:

A Shimadzu 1800 spectrophotometer having 1.00 cm quartz cell were used for recording absorbances and UV-Vis spectra. Reactions were conducted *in situ*, in acetate buffer (pH, 3.6–4.8; T_{OAc} = 0.20 *M*) in presence of excess NH_2OH in comparison to [**A**], at an ionic strength 0.5 *M* ($NaClO_4$) in a thermostatic (25±0.1°C) cell located inside the spectrophotometer. The kinetics was studied at 708 nm, the maximum absorption of the superoxo complex (**A**). Reactions followed good first order kinetics up to 95% completion of reaction. The rate constants (k_0) of the first order reaction were assigned 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. Furthermore, to arrest the omnipresent metal ions (*vide infra*), present in the reaction media, $C_7H_5NO_4$ was added. For the measurements of pH a pH meter (Gold-533) was used and the electrode of the pH meter was calibrated using standard buffer solutions. During reporting pH values in D_2O medium a relation pD = pH + 0.4 was used[26,27]. A 2400 series-II CHN/O analyzer (Perkin-Elmer) was used for the analysis of elements C, H and N. EPR spectra were registered with JES-FA Series EPR spectrometer using field strength 250.000 mT and at a frequency of 9114.872000 MHz. Solutions and reagent used for the reaction were prepared in freshly boiled doubly distilled water.

Stoichiometry:

For the determination of stoichiometry of the reaction, excess superoxo complex (**A**) was allowed to react with deficit amount of hydroxylammine in different ratio and at the equilibrium; absorbance of the reaction mixture was measured spectrophotomertrically at 708 nm. From the difference between the initial absorbance (a_0) and equilibrium absorbance (a_e) the concentration of unreacted superoxo complex (**A**) was calculated (Table S1).

Results and discussion

Stoichiometry and reaction products:

Each mole of NH_2OH consumed (ESI Table S1) 2 moles of the superoxo complex (**A**). Moreover, it was observed that

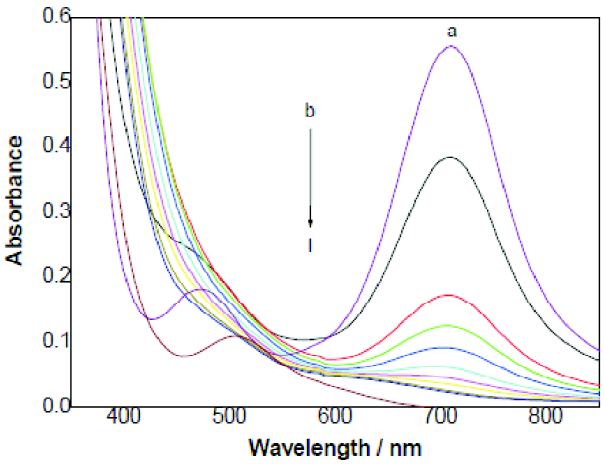


Fig. 1. Time set on spectra between the reaction of 0.50 mM of (**A**) and 20 mM NH_2OH at 25.0°C. pH = 4.0 in aqueous acetate buffer, total acetate = 0.2 *M*, ionic strength = 0.5 *M*, [dpa] = 2.0 mM: (a) Represents the spectrum of original complex and (b)-(l) are the spectra of reaction mixture at different time gaps 60, 120, 365, 450, 610, 710, 900, 1000, 1200, 1800 and 86400 s respectively.

the final spectrum is very closely identical with corresponding hydroperoxo complex (Fig. 1) of (**A**)[28,29]. Therefore it is clear that the superoxo complex (**A**) was converted to the hydroperoxo complex **(B)** (eq. (1)). Again from the stoichiometric calculations it is clear that N_2O is the oxidation product for oxidations of NH_2OH.

$$2[(en)(dien)Co^{III}(O_2)Co^{III}(en)(dien)]^{5+} + NH_2OH \rightarrow 2[(en)(dien)Co^{III}(O_2H)Co^{III}(en)(dien)]^{5+} + N_2O + H_2O \quad (1)$$

Hydroxylamine (NH_2OH) is a familiar reductant and depending on the accurate condtions of reation its oxidation products may vary, such as NO_3^-, NO_2^-, N_2O, or N_2[1]. If the reaction is set up with a one electron oxidant or a hydrogen atom abstractor, an intermediate produts $H_2NO^\bullet$ or its isomer $^\bullet NHOH$ is formed, in whih the oxidation number nitrogen is zero. Several quantum mechanical calculation and EPR spectra predict that $NH_2O^\bullet$ is thermodynamically more stable than $^\bullet NHOH$. Therefore, we presumed that the product of one electron oxidation (or H-atom abstraction) of NH_2OH, was the aminoxyl radical ($H_2NO^\bullet$). $H_2NO^\bullet$ is a weak acid (pKa = 12.6±0.330) which immediately reacted with the second mole of superoxo complex (**A**) in an electron transfer reaction to yield nitroxyl (HNO) (following an electron transfer path). After end of kinetic study, with the reation mixture the chemical test (Griess reagent test) for N_2, NO_2^- and NO_3^- were performed but none of the species give the positive responce[31]. Again from the literature survay it was already stablished that nitroxyl (HNO) in acid medium readly disproportionate to N_2O[32]. Moreovere concurrent decrease of intensity of the EPR spectra of (**A**) (Fig. 2) authenticate the attack of NH_2OH on the metal bound superoxide ligand but not on Co(III) centre. In this connection it is worthy to mention that oxidation of hydroxyl amine in acid medium by one electron oxidant is already reported and the dehydrative dimerisation rate constant of HNO is reported to be very high, $(1.8–7.2)\times10^9$ M^{-1} [32]. After one day the spectra of the reaction mixture gives the identical spectra with that of cobalt(II) chloride hexahydrate at the same conditions (ESI, Fig. S1).Therefore, the hydroperoxo complex of (**A)** is not stable under experimental conditions for a long periods and in the long run, auto-decomposes to Co(II) and other products. Nonetheless, under actual experimental conditions this auto-decomposition of (**B**) is insignificant and does not influence the kinetics and stoichiometric studies.

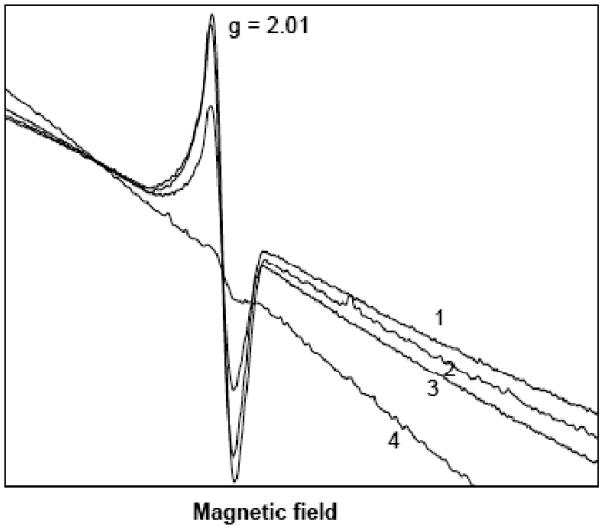


Fig. 2. EPR spectra at liquid nitrogen temperature; Curve (1) is the EPR spectrum of 0.5 mM pure superoxo complex (**A**). Curve (2) and (3) represents the spectra of the reaction mixture at time gaps of 60 and 120 s respectively and the curve (4, almost disappears) is the spectrum of the same reaction mixture after termination of the reaction. [NH_2OH] = 20 mM, pH = 4.0.

Kinetics:

In the experimental condition (aqueous acetate buffer media) the title superoxo complex **(A)** is sufficiently stable with respect to auto-decomposition as the change of absorbance over a long period of time is negligibly small. However in presence of excess hydroxylamine, the peak absorbance of the superoxo complex at 708 nm decreased gradually substantially to zero. Which indicate that the superoxo complex (**A**) is gradually exhausted by the hydroxylamine? The decay action followed very good first-order reaction kinetics (Fig. 3) and the rate constants (k_0) of the reaction increased continuously with [NH_2OH] (Fig. 4, Table 1). The rates of the reaction were energetically influenced by the acidity of reaction media but go on uninterrupted with ionic strength. The plot of $1/k_0$ vs [H^+] was found to linear having a small but notable intercept (Fig. 5, Table 2).

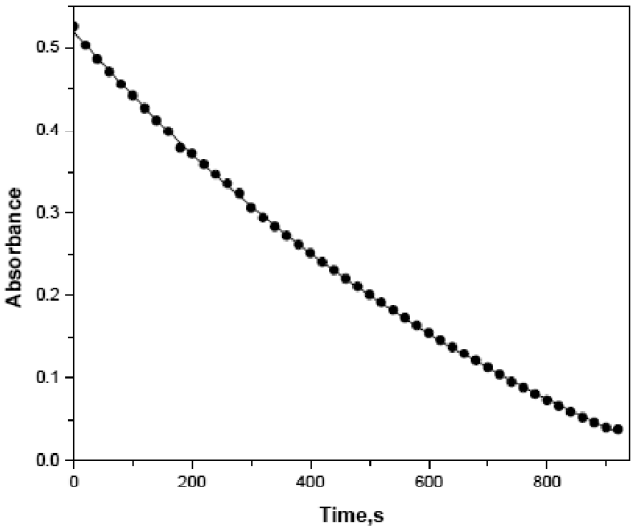


Fig. 3. Fall-off absorbance at 708 nm shown by the solid circles of (**A**) with time in its reaction with NH_2OH gives a very good fit (solid line) to the first-order rate equation, at 25.0°C. [**A**] = 0.50 mM, [NH_2OH] = 20 mM, pH = 4.0, total acetate = 0.2 *M*, ionic strength = 0.5 *M*, [dpa] = 2.0 mM.

Table 1. Variation of k_0 with [NH_2OH], [**A**] = 0.50 mM, pH = 4.0, [dpa] = 2.0 mM, total acetate = 0.2 *M*, ionic strength = 0.5 *M*

[NH_2OH] mM	20	25	30	35	40	45	50
$10^4 \times k_{obs}$ (s)	5.07	6.62	7.48	8.57	9.52	10.63	11.62

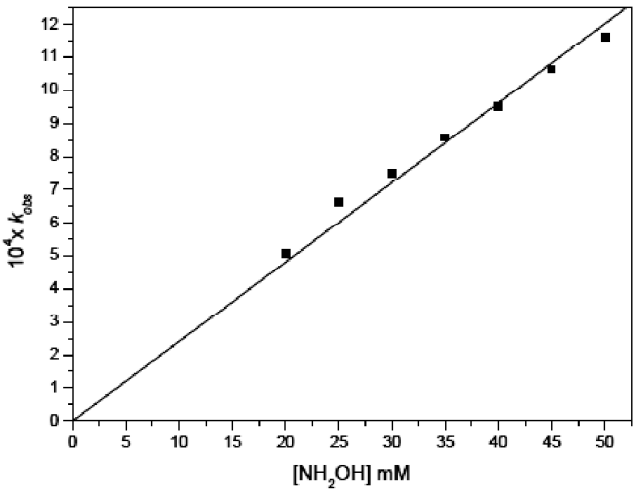


Fig. 4. Plot of k_0 vs [NH_2OH] for the reaction of excess NH_2OH with 0.50 mM of complex (**A**) at 25.0°C. pH = 4.0, [dpa] = 2.0 mM, total acetate = 0.2 *M*, ionic strength = 0.5 *M*.

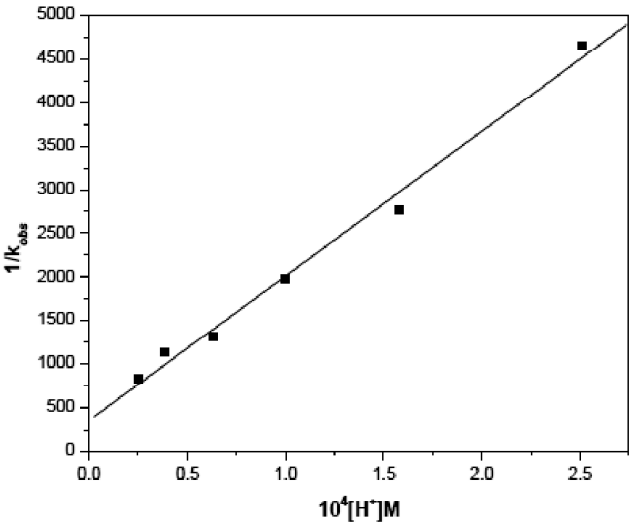


Fig. 5. Plot of $1/k_0$ vs [H^+] at 25.0°C. [**A**] = 0.50 mM, [NH_2OH] = 20 mM, total acetate = 0.2 *M*, ionic strength = 0.5 *M*, [dpa] = 2.0 mM.

Table 2. Variation of k_0 with pH of the medium at 25.0°C, [**A**] = 0.50 mM, [NH_2OH] = 20 mM, [dpa] = 2.0, total acetate = 0.2 *M*, ionic strength = 0.5 *M*

Ph	3.6	3.8	4.0	4.2	4.4	4.6
$1/k_{obs}$ (s)	4657.35	2772.85	1967.91	1309.26	1127.29	825.1

The increase of reaction rate with pH seemed not comprehensible from deprotonation of NH_2OH because hydroxy-

lamine is a weak acid (pKa = 6.01)[32] and our experimental pH range was 3.4–4.6. Rather, a mechanism transferring H-atom (or H^+ + e) to the Co^{III}-bound superoxide ligand (HAT) seemed logical as superoxide was well-known to be a fairly strong base[29]. The perceived pH-dependence on reaction rate undoubtedly demonstrates **A-**H as a kinetically inert species. Therefore as the acidity of the reaction medium increased more and more **A-**H is formed and consequently reaction rate dropped. The protonated form of the superoxo complex (**A-**H) is redox inert because it has no more house to accommodate a additional H^+ following HAT mechanism from hydroxylamine or it reaction intermediate. Therefore at the rate controlling step, **A** is reduced to its analogous hydroperoxo complex (**B**). The plan of action **P_1** below constitutes a caricature of the reaction mechanism.

an electroprotic hydrogen atom transfer (HAT) mechanism (H^+ + e).

Proposed reaction scheme in shortened form is thus:

$$\mathbf{A} + H^+ \underset{}{\overset{K}{\rightleftharpoons}} \mathbf{A\text{-}}H \qquad (2)$$

$$\mathbf{A} + H_2NOH \xrightarrow{k} \mathbf{B} + H_2NO^{\bullet} \qquad (3)$$

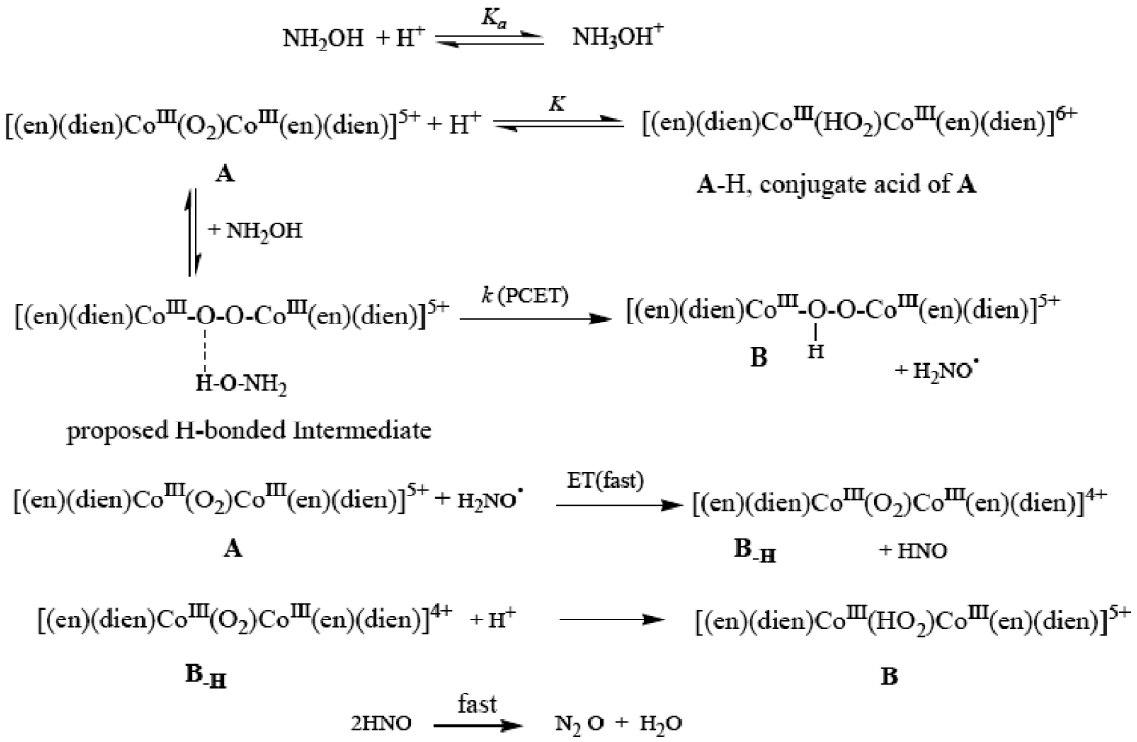
Eqs. (2) and (3) lead to the rate eq. (4).

$$k_0 = k[H_2NOH]/(1 + K[H^+]) \qquad (4)$$

Eq. (4) may be rearranged to eq. (5).

$$1/k_0 = 1/(k[H_2NOH] + K[H^+]/(k[H_2NOH]) \qquad (5)$$

A plot of $1/k_0$ vs $[H^+]$ was found to follow excellent straight-line trajectory (Fig. 5) as expected from eq. (5) and yielded *k*



Plan of action (**P_1**)

The values of k_0 decreased remarkably when solvent H_2O was augmented with D_2O (k_{H_2O}/k_{D_2O} ~ 2). In addition, in the D_2O solvent media the plots of *k*o versus mole % of D_2O was found to be linear (Fig. 6) suggesting convey of a solitary proton at the rate determining step[33]. This subsistence = 14.32±0.4)×10^{-2}/s and *K* = 2.5±0.3)×10^2 M^{-1}. Free superoxide is a strong base (pKa = 4.88)[34] and the presence of a residual basicity in a coordinated superoxide ligand is not unexpected but the basicity of the superoxide ligand due to coordination to two Co(III) centers in is expectedly somewhat

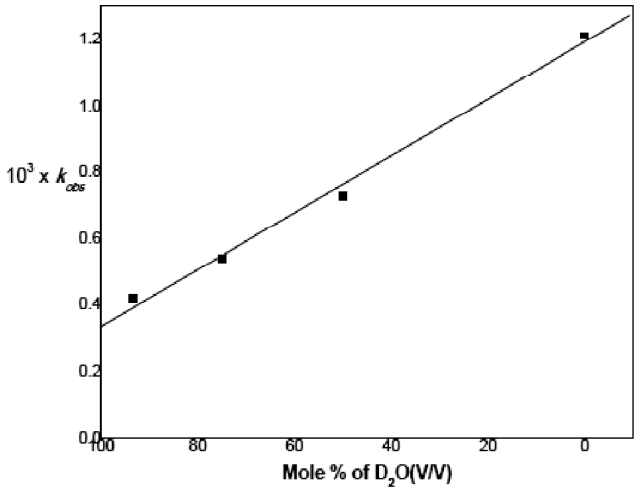


Fig. 6. Effect of D_2O on k_0, [**A**] = 0.50 mM, [NH_2OH] = 0.20 m M, pH/pD = 4.6, total acetate = 0.2 *M*, ionic strength = 0.5 *M*, [dpa] = 2.0 mM, T = 25.0°C.

reduced. Again for the reducing agent, phenol, H-atom transfer mechanism is already an accepted phenomenon[35].

To substantiate the suggested mechanism, **A** was reacted with phenol and N,N-dimethylhydroxylamine. Both the reducing agents reacted with **A** but none of the reactants like phenyl methyl ether and O-methyl hydroxylamine reacted under similar reaction conditions. Therefore the essential condition for the proposed reactions mechanism to proceed is that there must be at least one O-H bond in the reducing agent.

Conclusion

In aqueous acetate buffer medium (pH, 3.6–4.8) hydroxylamine reduces the one electron oxidant, superoxo ligand in $[(dien)(en)Co^{III}(O_2)Co^{III}(en)(dien)](ClO_4)_5$ (**A**) to the corresponding hydroperoxo complex, $[(en)(dien)Co^{III}(HO_2)\text{-}Co^{III}(en)(dien)]^{5+}$ (**B**) and itself gets oxidised to N_2O gas following both proton coupled electron-transfer (PCET) path and an electron-transfer reaction. The kinetics, stoichiometry and reaction mechanism clearly indicate that oxidation of NH_2OH occurs through the formation of an intermediate aminoxyl ($NH_2O^{\bullet}$) radical, a weak acid (pKa, 12.6±0.3).

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