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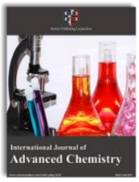

Non enzymatic NADH-dependent reduction of Cis-$[Co(en)_2(H_2O)_2]^{3+}$ in aqueous medium

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Abstract

The kinetics of the electron transfer reaction of NADH with Cis-$[Co(en)_2(H_2O)_2]^{3+}$ has been studied over the range $1.0 \leq 10^2\,[NADH] \leq 3.0$ mol dm^{-3}, $7.0 \leq pH \leq 8.0$ and $20^0C \leq t \leq 35^0C$ in aqueous medium. The rate of electron transfer reaction was found to be first-order dependence each in $[NADH]_T$ and Cis-$[Co(en)_2(H_2O)_2]^{3+}{}_T$. The products of the reaction were found to be NAD^+ and Co(II). The corresponding activation parameters of the electron transfer reactions were found to be as $\Delta H^{\#}=27.55$ kJ mol^{-1} and $\Delta S^{\#}=-189.35$ JK^{-1}mol^{-1}.

Keywords: *Diaquabisethylenediamine Cobalt(III); Electron Transfer; Kinetics; NADH; Nonenzymatic.*

1. Introduction

NADH (Nicotinamide Adenine Dinucleotide) is an important pyridine nucleotide that functions as an oxidative cofactor in eukaryotic cells. NADH plays a key role in the production of energy through redox reactions. It donates electrons by providing a hydrogen molecule to the oxygen molecule to create water during the electron transport chain. During the electron transport chain, NADH become NAD^+ and the H^+ get transported across the membrane. As a result, electrons reduced from NAD^+ are used in the transport chain. NAD serves as a cofactor for dehydrogenases, reductases and hydroxylases, making it a major carrier of H^+ and e^- in major metabolic pathways such as glycolysis, the tricarboxylic acid cycle, fatty acid synthesis and sterold synthesis. The conversion of NAD from its oxidized form (NAD^+) to its reduced form (NADH), and back, provides the cell with a mechanism for accepting and donating electrons. NAD^+/NADH plays a significant role in the reactions associated with glycolysis, oxidative phosphorylation, and fermentation. NADH increases cell energy production. The high reducing power of NADH is used in creating energy. It is used as an antioxidantto neutralize free radicals. The potential value of NAD^+/NADH redox pair is −0.32 volts, which makes NADH a strong reducing agent [Unden et al. 1997]. The reaction is easily reversible, when NADH reduces another molecule and is re-oxidized to NAD^+. This means the coenzyme can continuously cycle between the NAD^+ and NADH forms without being consumed [Pollak et. al. 2007]. NADH absorbs at higher wavelengths, with a peak in UV absorption at 339 nm with an extinction coefficient of 6,220 $M^{-1}cm^{-1}$ [Dawson et. al. 1985]. Solutions of NAD^+ are colorless, stable at neutral pH, but decompose rapidly in acids or alkalis. Upon decomposition, they form products that are enzyme inhibitors [Biellmann et. al. 1979].

Generally, Co(III) Coordination compounds are more priority because their complexes kinetically inert undergo ligand exchange very slowly compared to other transition metals. There are various transition metal complexes which are used for studying electron transfer reactions. Compared to all, octahedral cobalt(III) complexes being substitutionally inert are the more priority. Therefore, recently redox reactions involving octahedral cobalt(III) complexes and NADH have attaracted considerable attentation [Kumari et. al. 2011]. The kinetics of electron transfer reactions of metal ion is strongly influenced by its ligating behavior. NADH makes special attention due to its reductant capability in biological system as well as in fundamental research. Electron transfer reaction of Co(III) complexes with drugs [Bhagwat et. al. 2016] and vitamins [Makote et. al. 1999; Senapati et. al. 2014], have been investigated. Co(III) complexes show higher stability than Co(II) complexes which have more potent antibacterial and antifungal activity [Chylewska et. al. 2013]. Reduction of tungstovanadophosphates [Sami et. al. 2009], bovine ceruloplasmin [Lovstad et. al. 2006] andferric siderophores [Adjimani et. al. 1997] by NADH have been reported. In order to determine the redox behavior of Cis-$[Co(en)_2(H_2O)_2]^{3+}{}_T$, its reaction with NADH has been investigated.

1.1. Materials and methods

All the chemicals used were of A.R grade and double distilled water was used throughout the experiment. The electron transfer reaction was studied at pH= 7.0±0.01 using a pre-standardized Electronics India pH-meter model-101 equipped with glass electrode. The pH of the solution was maintained using phosphate buffer. The kinetic runs were followed by means of a Agilent Cary Win UV-Vis spectrophotometer. Temperature control was maintained within 0.1^0C by a thermostated circulating bath. FT-IR spectrum of the compound was recorded on a Perkin-Elmer Series 2000 spectrophotometer using KBr pellets in the range 400-4000 cm^{-1}. Metal contents were estimated spectrophotometrically using Atomic Absorption Spectrophotometer(AAS).

2. Results and discussion

2.1. Synthesis and conformational analysis

Trans-$[Co(en)_2Cl_2]Cl$ was prepared by the method of Krishnamurty [Krishnmurty et. al. 1972]. It was converted to Cis-$[Co(en)_2(CO_3)]ClO_4$ by treatment with Na_2CO_3, heating the mixture till a deep red solution developed and then precipitated with $NaClO_4$. The carbonate compound was formed as Cis-$[Co(en)_2(CO_3)]ClO_4$. The sample formed was then recrystallised from water and was characterized spectrophotometrically (λ_{max} value =503nm, ϵ= 148.5). The FT-IR spectra of the substrate $[Co(en)_2(CO_3)]ClO_4$ was shown in (Fig. 1). The broad band at 3467 cm^{-1} is due to N-H stretching and at 3077cm^{-1} is due to C-H stretching frequency. The band at 1585cm^{-1} is due to C=O stretching, 1461cm^{-1} corresponds to C-C stretching and 1270cm^{-1} corresponds to C-O stretching. Peaks at 587cm^{-1} and 511cm^{-1} correspond to Co-N and Co-O stretching. All the above peaks suggest the substrate to be $[Co(en)_2(CO_3)]ClO_4$. It was further supported by ^1H- NMR spectra (Fig. 2) of the same product. The peak at δ= 7.26 is due to the twelve hydrogen atoms which shows a longer height as all the protonated hydrogen atoms are symmetrical. As the solvent used was water and the substrate $[Co(en)_2(CO_3)]ClO_4$ was formed using perchloric acid, the lower height peak between the value at δ= (1-2) may be arises. The CHNO analysis was predicted as, Anal. Calc. for Co C_5 H_{16} N_4 O_7 Cl; Co, 17.43; C, 17.73; H, 4.73; N, 16.54; O, 33.09; Cl, 10.49. Found: Co, 17.12; C, 17.25; H, 4.60; N, 16.23; O, 32.62.

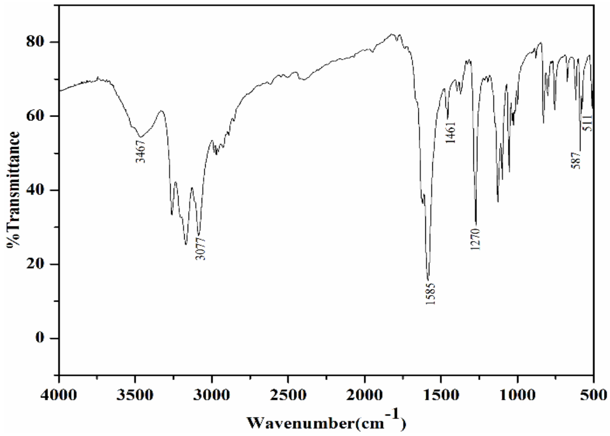

Fig. 1: FT-IR Spectra of the Substrate $[Co(en)_2(CO_3)]ClO_4$.

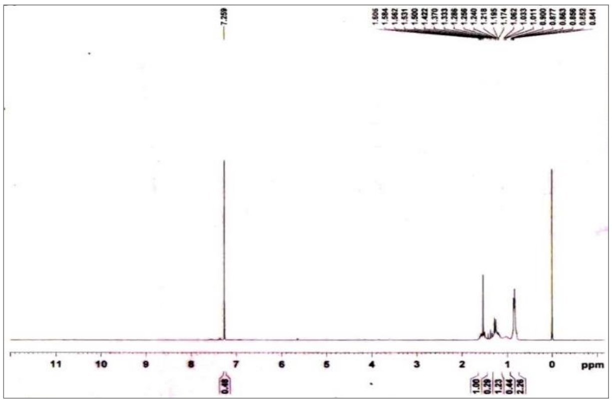

Fig. 2: ^1H-NMR Spectra of the Substrate $[Co(en)_2(CO_3)]ClO_4$.

From all of the above characterization the product was established as $[Co(en)_2(CO_3)]ClO_4$. The structure was shown in (Fig. 3).

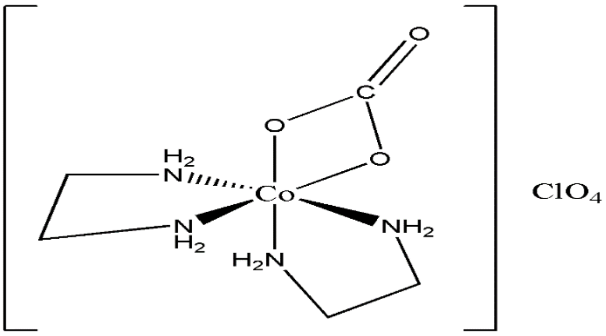

Fig. 3: Structure of Cis- $[Co(en)_2(CO_3)]ClO_4$.

The reduction of Co(III) to Co(II) was determined spectrophotometrically by Kitson's method [Kitson et. al. 1950]. The stable ion was generated in solution form as Cis-$[Co(en)_2(H_2O)_2]^{3+}$ by treating the carbonato complex with standard $HClO_4$ followed by slight warming to remove any dissolved CO_2. This sample was used for reaction studies.

2.2. Kinetic measurements

Free radical reaction was monitored using the absorbance decrease at 503 nm and the k_{obs} values were obtained from the slope of –ln $(A_t - A_\infty)$ versus 't' (min) plots (Fig. 4) using the equation $\ln(A_t - A_\infty) = k_{obs}\, t + C$ where A_t and A_∞ are the absorbance of the reaction mixture at time 't' and at equilibrium respectively. Pseudo first order conditions were maintained throughout the runs by using large ($\geq$ five fold) excess of [NADH]. The molar absorptivity of the product Cis-$[Co(en)_2(H_2O)_2]$ was determined experimentally. The correlation coefficient of the plots used to determine k_{obs}were found to be 0.99 in most cases.

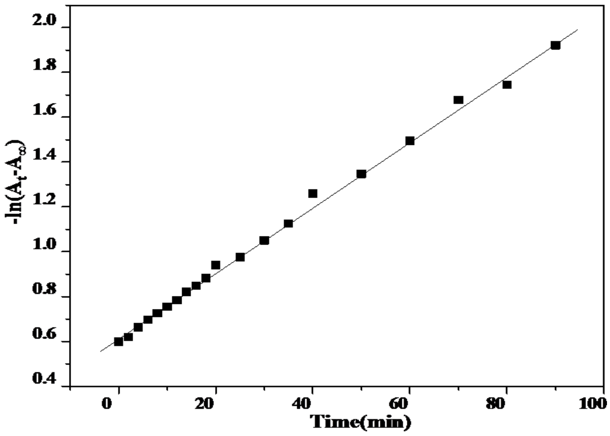

Fig. 4: Determination of K_{obs} Value, $[Co(III)]_T$ = 2.0 $\times 10^{-3}$ mol dm^{-3}, [NADH] = 3.0×10^{-3} mol dm^{-3}, pH=7.0, Temp = 25^{0}C.

2.3. UV- spectral analysis

UV-Vis spectral scan of the reaction mixture was studied spectrophotometrically at different time intervals (Fig. 5). The reactant Cis-$[Co(en)_2(H_2O)_2]^{3+}$ shows two peaks at λ_{max}= 503 nm and 368nm. On mixing with NADH, absorbance decreases at λ_{max}= 503nm. The redox reaction was monitored at λ_{max}= 503nm under pseudo-first order condition keeping [Co(III)] = 2.0×10^{-3} mol dm^{-3} and varying [NADH] = 1.0×10^{-2} - 3.0×10^{-2} mol dm^{-3} having pH= 7.0 which indicates Co[II] formation. The pseudo-first order rate constants were found to increase with increase in concentration of reductant showing first order dependence of rate with [NADH].

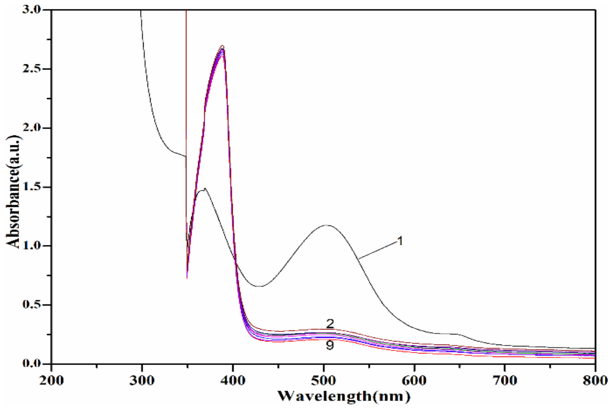

Fig. 5: UV-Vis Spectral Scan of the Reaction Mixture of Co(III) and [NADH] = 1.0 $\times 10^{-2}$ mol dm^{-3}, pH=7.0, Temp = 25^0C. (1) $[Co^{III}]_T$= 2.0×10^{-3} mol dm^{-3} (2) Immediate after mixing (3) ΔT= 10minutes (Curves 2-9).

2.3.1. Effect of [NADH] on reaction rate

The effect of varying [NADH] on the reaction rate was studied by observing the kinetics at constant pH=7.0 between the temperatures (293K-308K). The concentration of Co^{III} was kept constant and 10^4[NADH] was changed from 1.0 to 3.0 mol dm^{-3}. The reaction rates are found in (Table-1).

The plot of k_{obs}/s^{-1} vs. $[NADH]_T$ is linear at four different temperatures indicates that the reaction is first order with respect to $[NADH]_T$. Hence, the order of reaction with respect to $[Co^{III}]$ must be one.

Table 1: Values of K_{obs} at Different Temperatures, Cis-$[Co(en)_2(H_2O)_2]^{3+}$ = 2.0×10^{-3} mol dm^{-3} and pH=7.0

10^2[NADH] mol dm^{-3}	10^4k_{obs} / s^{-1}			
	293K	298K	303K	308K
1.0	1.66	2.16	2.5	3.33
1.5	2.33	2.66	3.16	4.17
2.0	3.0	3.5	4.0	5.50
2.5	3.66	4.16	4.66	6.33
3.0	4.33	4.66	5.5	6.83

2.3.2. Effect of temperature on reaction rate

The effect of temperature on the rate was studied by observing the kinetics at various temperatures (293K-308K). The oxidant concentration was kept constant at 3.0 mol dm^{-3} and the reductant NADH concentration was varied from 1.0×10^{-2} to 3.0×10^{-2} mol dm^{-3}. The k_{obs} was found to increase with increase in temperature (Fig. 6). Activation parameters are calculated using Eyring equation and were found to be as $\Delta H^\# = 27.55$ kJ mol^{-1} and $\Delta S^\# = -189.35$ $JK^{-1}mol^{-1}$. The activation entropy has a negative value which indicates an ordered transition state for the electron transfer reaction.

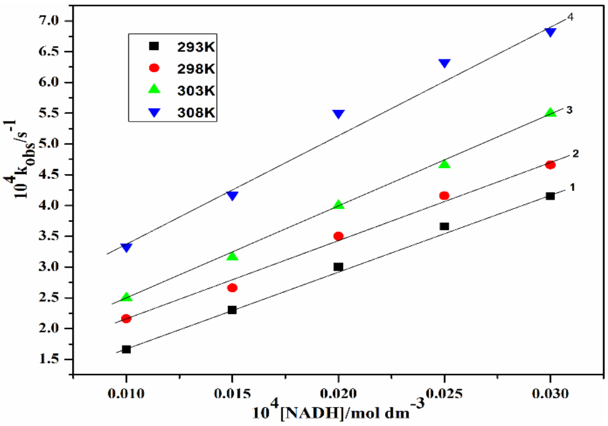

Fig. 6: K_{obs} versus NADH at Different Temperatures (1) 293K, (2) 298K, (3) 303K, (4) 308K.

2.3.3. Rate dependence on pH

The pH was varied from 6.5 to 7.5 using phosphate buffer (KH_2PO_4+Na_2HPO_4) at 298K with [Co(III)]= 2×10^{-3} mol dm^{-3} and [NADH]= 1×10^{-2} mol dm^{-3}, and the calculated value of $10^4k_{obs}(s^{-1})$ was found to be constant (±0.26). In the above pH range, there is no reaction of electron transfer. The electron transfer reaction could not be studied at higher pH because the oxidant Co(III) complex will undergo base hydrolysis.

3. Polymerization study

The participation of free radicals was tested using polymerization study. Initially, a known quantity of acrylonitrile scavenger was added in the reaction mixture, kept in an inert atmosphere for 5 h. On diluting the reaction mixture with methanol, a white precipitate of polymer was formed indicating the formation of free radicals in the reaction. Using this same condition blank experiments either with $[Co^{III}]$ or NADH with acrylonitrile did not give polymerization. The initially added acrylonitrile decrease the rate of reaction indicating the free radical intervenation [Kolthoff et. al. 1953].

4. Stoichiometry and Identification of product

The stoichiometry of the reaction was studied at 30^0C where $[Co^{III}]$ was kept constant at 2.0×10^{-3}mol dm^{-3} and $[NADH]_T$ at 3.0×10^{-2}mol dm^{-3}. A peak was formed at 251nm after three half lives (Fig. 7) indicates the formation of NAD^+. The formation of a peak at 503nm indicates the formation of Co(II)(Fig. 5). The stoichiometry of the reaction can be written as

$$2[Co^{III}]+NADH \longrightarrow 2[Co^{II}]+NAD^{+}+H^{+}$$

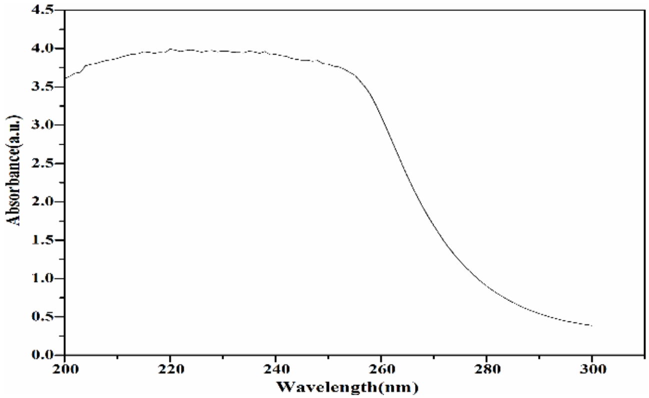

Fig. 7: Scan of reaction mixture with respect to time showing $[Co^{II}]$ formation.
$[Co^{III}] = 2.0\times10^{-3}$ mol dm^{-3}, [NADH]= 1.0×10^{-2} mol dm^{-3}, pH=7.0, Temp = 25^0C.

$$NADH + [Co^{III}] \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} NADH^{\cdot+} + [Co^{II}] \qquad (1)$$

$$NADH^{\cdot+} + H_2O \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} NAD^{\cdot} + H_3O^{+} \qquad (2)$$

$$NAD^{\cdot} + [Co^{III}] \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} NAD^{+} + [Co^{II}] \qquad (3)$$

5. Conclusion

The above mechanism describes the reaction between Co(III) and NADH, which shows electron-proton-electron transfer steps where an electron is transferred from NADH to $[Co^{III}]$ producing $NADH^{\cdot+}$ and $[Co^{II}]$. The solvent water molecule abstracts the proton from $NADH^{\cdot+}$ to produce $NAD^{\cdot}$. For such a mechanism, either electron transfer from NADH to [Co^{III}] or proton transfer from $NADH^{\cdot +}$ to water may be rate-limiting step.

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