

Studies on electron transfer reactions of Keggin-type mixed addenda heteropolytungstovanadophosphates with NADH

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Abstract. The coenzyme nicotinamide adenine dinucleotide (NADH) undergoes facile electron transfer reaction with vanadium (V) substituted Keggin-type heteropolyanions (HPA) $[\text{PV}^{\text{V}}\text{W}_{11}\text{O}_{40}]^{4-}$ (PV_1) and $[\text{PV}_2^{\text{V}}\text{W}_{10}\text{O}_{40}]^{5-}$ (PV_2) in aqueous phosphate buffer of pH 6 at ambient temperature. Electrochemical and optical studies show that the stoichiometry of the reaction is 1 : 2 (NADH : HPA). EPR and optical studies show that HPA act as one electron acceptor and the products of electron transfer reactions are one electron reduced heteropoly blues (HPB), viz. $[\text{PV}^{\text{IV}}\text{W}_{11}\text{O}_{40}]^{5-}$ and $[\text{PV}^{\text{IV}}\text{V}^{\text{V}}\text{W}_{10}\text{O}_{40}]^{6-}$. Oxygraph measurements show that there is no uptake of molecular oxygen during the course of reaction. The reaction proceeds through multi-step electron–proton–electron transfer mechanism, with rate limiting initial one electron transfer from NADH to HPA by outer sphere electron transfer process. Bimolecular rate constant for electron transfer reaction between NADH and PV_2 in phosphate buffer of pH = 6 has been determined spectrophotometrically.

Keywords. Nicotinamide adenine dinucleotide (NADH); heteropoly-tungstovanadophosphate; outer sphere electron transfer reactions.

1. Introduction

Nicotinamide adenine dinucleotide (NADH) is a coenzyme found in all living cells. It is easily oxidized to NAD^+ by the enzymes present in biological system.

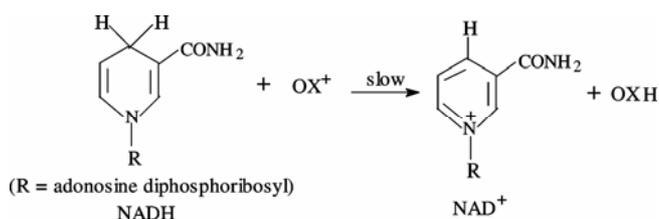


The redox couple NADH/NAD^+ plays an important role in biological reactions involving electron transfer.^{1–3} Studies on the electron transfer reaction of NADH and its synthetic analogue were reported by several workers.^{4–10} The mechanism of reaction of NADH largely depends on the nature of oxidants. When the oxidants are iminium ions of activated carbonyl compounds¹¹ and quinone,¹² there is a transfer of hydride ion from the 4th position of the dihydropyridine ring to the oxidizing agent in the rate limiting step (scheme 1).

Oxidation of NADH by ferrocenium³ and ferricyanide¹¹ proceeds through multi-step electron–proton–electron transfer mechanism in which the

initial electron transfer from NADH to the oxidant is the rate limiting step.

Vanadium plays an important role in a number of biological process.^{13–16} The metal vanadium exists in several oxidation states viz. +1, +2, +3, +4 and +5. Vanadium in +5 oxidation state can act as one electron or two electron acceptor in electron transfer reactions. In the Keggin-type heteropolytungstophosphate, W^{VI} can be replaced by V^{V} without affecting structure of the HPA.^{17,18} Vanadium substituted HPA are ideal candidates for the study of electron transfer reactions.^{19–21} By changing the composition, the redox potential of HPA can be varied to span a wide range. They can formally act as one electron, two electron or even several electron acceptors. Therefore, it was thought of interest to



Scheme 1.

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study the electron transfer reactions of NADH with vanadium substituted HPA. The present investigation may give valuable information on the biologically important reactions involving NADH.

2. Experimental

Disodium salt of NADH (Boehringer Mannheim GmbH) was used as such. Potassium salt of vanadium (V) substituted heteropolytungstovanadophosphates, viz. $K_4[PV^V W_{11} O_{40}] \cdot 2H_2O$ and $K_5[PV_2^V W_{10} O_{40}] \cdot 3H_2O$ were prepared and characterized by the method described by Domaille.²² The pH of the solution was maintained with KH_2PO_4 buffer.²³ All the pH measurements were made with Philips pH meter.

Electrochemical studies were carried out using EG&G Princeton Applied Research Potentiostat/Galvanostat Model 273 A, controlled by M-270 software. Cyclic voltammetric measurements were performed using three electrode assembly of glassy carbon electrode (working), platinum electrode (auxiliary) and Ag/AgCl electrode (reference). Glassy carbon electrode was resurfaced with alumina. The solution was taken in the cell, purged with nitrogen for 5 min. and the cyclic voltammograms (CV) were recorded.

Oxygen consumption studies were carried out using an oxygraph; Model 18172 fitted with Clark electrode (Yellow Springs Co., Yellow Springs, OH). First the electrode was calibrated to 100% air saturation using a solution containing HPA (0.2 mM) in phosphate buffer with constant stirring to obtain a steady base line. Then NADH (0.1 mM) was added and oxygen consumption was measured for about 8 min.

Shimadzu Model UV-1601 UV-Visible spectrophotometer was used to record the optical spectra. EPR spectra were recorded on a Varian E-109 X-band spectrometer at room temperature. The following parameters were set for the measurements: microwave power, 20 mW; modulation amplitude, 0.05 mT; modulation frequency, 100 kHz. For the EPR measurements, a reaction mixture containing PV_1/PV_2 (0.2 mM) and NADH (0.1 mM) in buffer solution (pH = 6) was prepared. The sample solutions after the completion of the reaction were drawn into aqueous flat cell and then placed in the EPR cavity.

The kinetics of oxidation of NADH by PV_2 was investigated under second order condition with equal and unequal concentration of the reactants

(NADH and PV_2). The progress of the reaction was monitored by following the decrease in the absorbance at 340 nm. Typical concentrations of NADH were 0.10 to 0.20 mM while that of PV_2 were 0.15 to 0.20 mM. The reaction was initiated by adding required volume of NADH into the PV_2 in aqueous pH 6.0 phosphate buffer. The quartz cuvette containing the reaction mixture was thermostated to $30.0 \pm 0.2^\circ C$ in the cell holder of a Hitachi Model 200-20 UV-Visible spectrophotometer. Time vs absorbance readings were then recorded and bimolecular rate constants were evaluated using the integrated rate equations.

3. Results and discussion

In the present work, electron transfer reaction of vanadium substituted Keggin-type heteropolyanions (HPA) $[PV^V W_{11} O_{40}]^{4-}$ (PV_1) and $[PV_2^V W_{10} O_{40}]^{5-}$ (PV_2) with NADH has been examined under non enzymic condition.

3.1 Stoichiometry of the reaction

CV of 0.5 mM of NADH in phosphate buffer of pH 6 shows an irreversible anodic peak potential at 0.805 V for the electrochemical oxidation of NADH to NAD^+ (figure 1a). The CV of PV_1 in phosphate buffer (figure 1b) shows a cathodic peak at 0.626 V and that of PV_2 (figure 1c) shows a cathodic peak at 0.473 V. When HPA is added to the aqueous buffered medium (pH = 6) containing NADH, the height of the anodic peak of NADH gradually decreases. For the reaction mixture containing $(NADH + PV_1)/(NADH + PV_2)$ in the ratio 1 : 2 the anodic peak corresponding to the oxidation of NADH and cathodic peak of PV_1/PV_2 completely disappeared (figures 1d and e). This shows that two moles of HPA are needed for the oxidation of one mole of NADH.

UV-Visible spectrum of NADH shows a characteristic peak at 340 nm ($\epsilon = 6300 \text{ mol}^{-1} \text{ cm}^{-1}$) which was employed to monitor the $[NADH]$. To an aqueous buffered medium (pH = 6) containing NADH (0.1 mM) on adding PV_1/PV_2 , the optical density (OD) at 340 nm decreases (figures 2 and 3). By measuring the decrease in OD at 340 nm, the amount of $[NADH]$ consumed and $[NADH]/[HPA]$ was calculated. The optical measurement studies also show that two moles of HPA are needed for the oxidation of one mole of NADH.

3.2 Products of the reaction

Reaction mixtures containing (NADH + PV₁) and (NADH + PV₂) in the ratio 1 : 2 were prepared and kept aside for few hours for the completion of the reaction and then UV-Visible spectrum was recorded (figure 4). The optical spectral features are comparable to those of the respective one electron reduced heteropoly blue (HPB) of the HPA, i.e. [PV^{IV}W₁₁O₄₀]⁵⁻ and [PV^{IV}V^VW₁₁O₄₀]⁶⁻. The characteristic peaks were assigned as per previous investigations^{24,25} where the HPBs were produced by controlled potential electrolysis (table 1). One electron reduced heteropoly blue of the corresponding HPA is the one of the products of NADH oxidation.

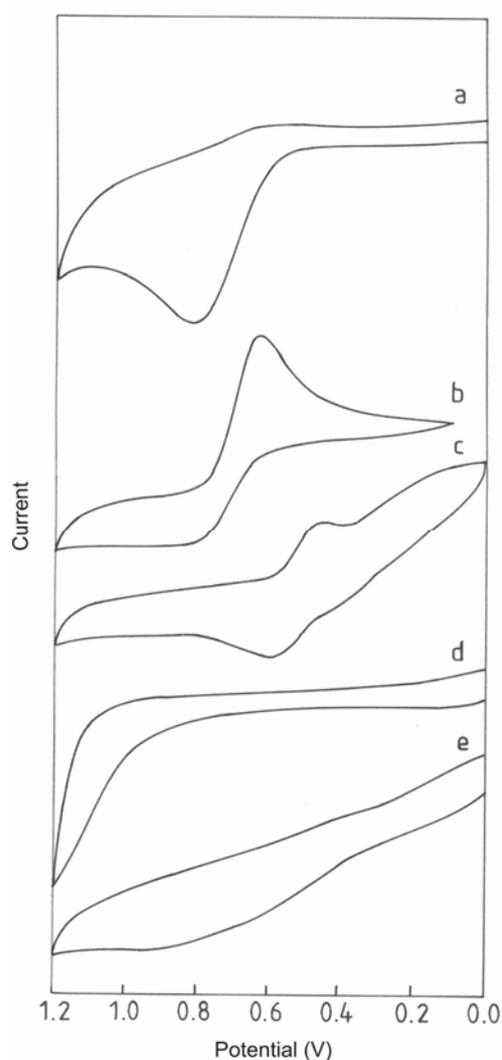
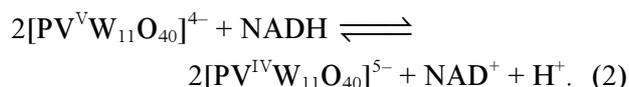


Figure 1. Cyclic voltammograms of (a) 0.1 mM NADH; (b) 0.2 mM PV₁; (c) 0.2 mM PV₂; (d) 0.1 M NADH + 0.2 mM PV₁; (e) 0.1 M NADH + 0.2 mM PV₂ (in phosphate buffer of pH 6; scan rate: 100 mV s⁻¹).

Figure 5a is the EPR spectrum of reaction mixture containing NADH and PV₁ in the ratio 1 : 2 in phosphate buffer of pH 6 after completion of the reaction at room temperature. It shows a typical 8 line pattern expected for vanadium (IV) with *d*¹ configuration (⁵¹V, I = 7/2, 100%). In PV₁, vanadium is in +5 oxidation state and EPR silent. The 8 line EPR spectrum ascertains that during the electron transfer reaction, PV₁ has accepted one electron from NADH to form the HPB, viz. [PV^{IV}W₁₁O₄₀]⁵⁻ and the electron transfer reaction can be represented as follows.



A complication inherent in the study of multi-electron transfer processes by polyoxovanadium containing anions is the capacity of these anions to be reduced by one or more electrons, i.e. in the two electron

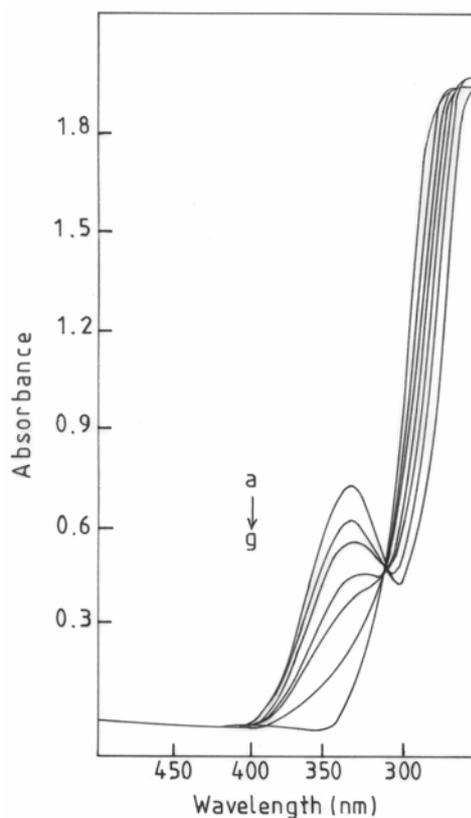
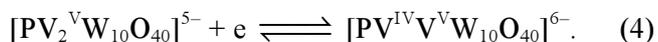
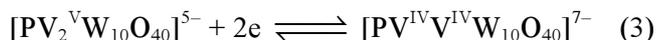


Figure 2. UV-Visible spectra of (a) 0.1 mM NADH; (b) 0.1 mM NADH + 0.03 mM PV₁; (c) 0.1 mM NADH + 0.05 mM PV₁; (d) 0.1 mM NADH + 0.08 mM PV₁; (e) 0.1 mM NADH + 0.1 mM PV₁; (f) 0.1 mM NADH + 0.15 mM PV₁; (g) 0.1 mM NADH + 0.2 mM PV₁ (in phosphate buffer of pH 6; after completion of reaction).

oxidation of NADH (1), PV_2 can accept either two electrons or one electron (3 and 4).



If one mole of HPA accepts two electrons (3), both vanadium (V) will be reduced to vanadium (IV) to give two electron reduced HPB $[PV^{IV} V^{IV} W_{10} O_{40}]^{7-}$. In such case EPR spectrum will give 8 line pattern. If one mole of HPA accepts one electron (4), it will give one electron reduced HPB, $[PV^{IV} V^V W_{10} O_{40}]^{6-}$ i.e. only one of the vanadium (V) will be reduced to vanadium (IV). In order to ascertain the reduction product, EPR spectrum of the reaction mixture containing NADH and PV_2 in the ratio 1:2 in buffered medium (pH = 6) was prepared. After the

completion of reaction, EPR spectrum was recorded at room temperature. The spectrum (figure 5b) shows 15 line pattern.

PV_2 is a 1,2-isomer with the two VO_6 octahedra sharing their corners (VOV bond angle is 150°). The two vanadium nuclei are in +5 oxidation state under same environment. During NADH oxidation, one electron reduced HPB is formed. The resulting one electron reduced HPB $[PV^{IV} V^V W_{10} O_{40}]^{6-}$ is a mixed-valence V^{IV}/V^V species. At room temperature the unpaired electron will be interacting equally with the two adjacent vanadium nuclei (^{51}V , $I = 7/2$, 100%) by a thermally activated hopping process²⁶ and this leads to 15 line pattern in the EPR spectrum at room temperature. The EPR spectrum of the reduction product obtained in the present study is similar to the EPR spectrum of one electron reduced HPB prepared by controlled potential electrolysis²⁶⁻²⁸ of $[PV_2 W_{10} O_{40}]^{5-}$. The EPR parameters are summarized in table 2. The stoichiometric equation for the reduction of PV_2 by NADH may be represented as follows:

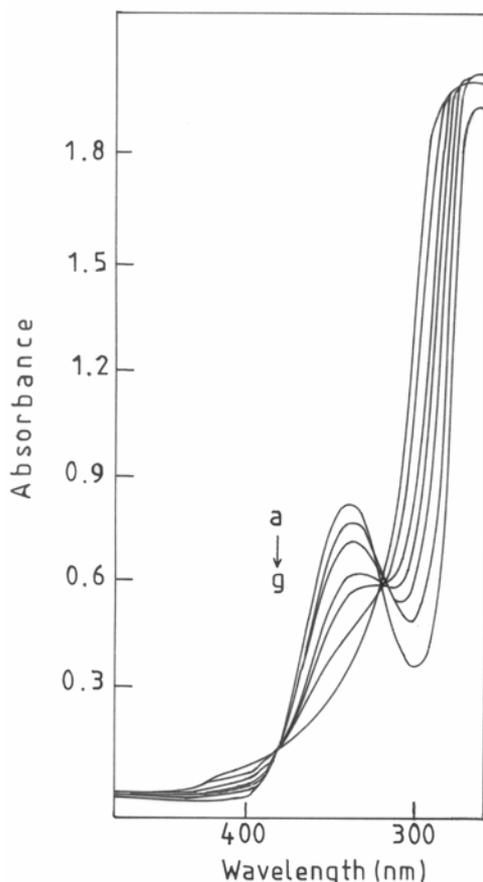
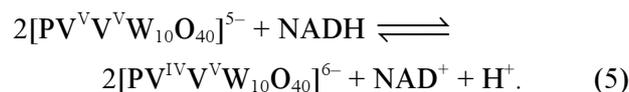


Figure 3. UV-Visible spectra of (a) 0.1 mM NADH; (b) 0.1 mM NADH + 0.03 mM PV_2 ; (c) 0.1 mM NADH + 0.05 mM PV_2 ; (d) 0.1 mM NADH + 0.08 mM PV_2 ; (e) 0.1 mM NADH + 0.1 mM PV_2 ; (f) 0.1 mM NADH + 0.15 mM PV_2 ; (g) 0.1 mM NADH + 0.2 mM PV_2 (in phosphate buffer of pH 6; after completion of reaction).

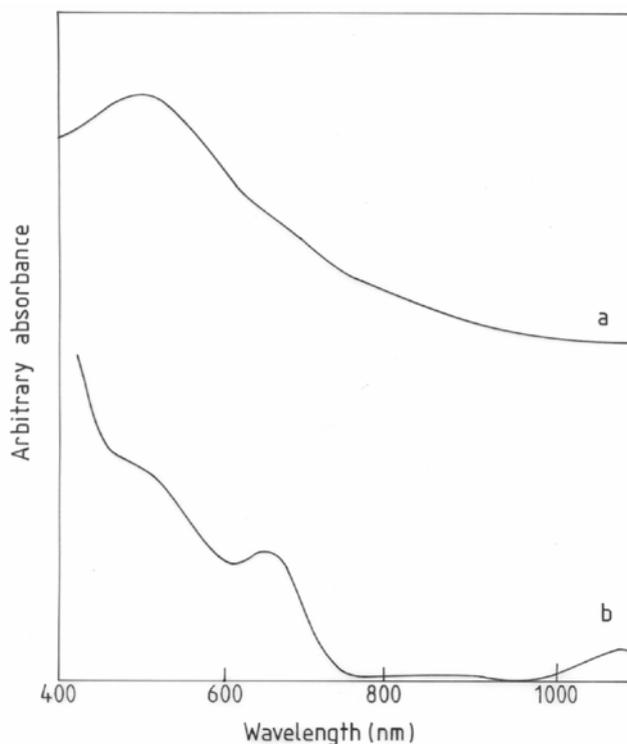


Figure 4. UV-Visible spectra of one electron reduced heteropoly blue (a) $[PV^{IV} W_{11} O_{40}]^{5-}$; (b) $[PV^{IV} V^V W_{10} O_{40}]^{6-}$ (obtained by NADH reduction of PV_1/PV_2).

Table 1. Optical spectral data characteristics of heteropoly blues.

Mode of reduction	$[\text{PV}^{\text{IV}}\text{W}_{11}\text{O}_{40}]^{5-}$ λ (nm)	$[\text{PV}^{\text{IV}}\text{V}^{\text{V}}\text{W}_{10}\text{O}_{40}]^{6-}$ λ (nm)	Assignment	Reference
By NADH	–	1060 (Sh)	$\text{V}^{\text{IV}} \rightarrow \text{V}^{\text{V}}$ (IVCT)	This work
	845 (Sh-b)	–	$d-d$	
	685 (Sh)	651	$d-d$	
	503	500 (Sh)	$\text{V}^{\text{IV}} \rightarrow \text{W}^{\text{VI}}$ (IVCT)	
Electrochemical	–	1130 (Sh)	$\text{V}^{\text{IV}} \rightarrow \text{V}^{\text{V}}$ (IVCT)	24, 25
	835 (Sh-b)	830 (Sh-b)	$d-d$	
	690 (Sh)	645	$d-d$	
	500	526 (Sh)	$\text{V}^{\text{IV}} \rightarrow \text{W}^{\text{VI}}$ (IVCT)	

Sh: Shoulder; Sh-b: Shoulder broad; IVCT: Intervalence charge transfer

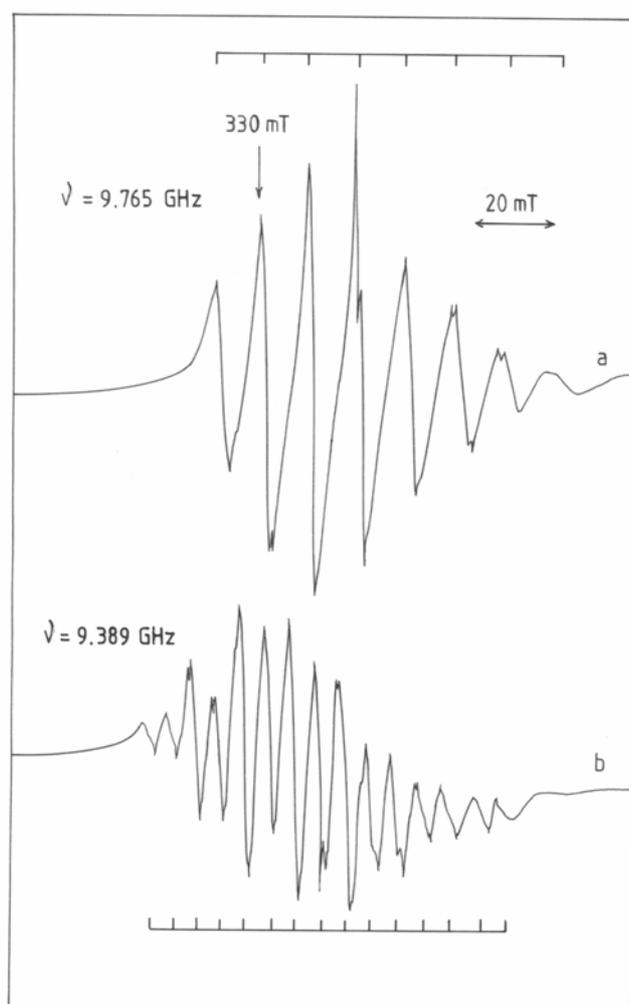


Figure 5. X-band ESR spectra of one electron reduced heteropoly blue (a) $[\text{PV}^{\text{IV}}\text{W}_{11}\text{O}_{40}]^{5-}$; (b) $[\text{PV}^{\text{IV}}\text{V}^{\text{V}}\text{W}_{10}\text{O}_{40}]^{6-}$ (obtained by NADH reduction of PV_1/PV_2) recorded at room temperature.

Optical, electrochemical and EPR studies show that

- (i) 1 mole of NADH reduces two moles of HPA.
- (ii) PV_1 and PV_2 act as one electron acceptor.

- (iii) The products are NAD^+ and one electron reduced HPB of the corresponding HPA.

3.3 Rate-law

The rate of the electron transfer reaction between NADH and PV_1 is very fast and we were unable to follow the kinetics by conventional technique. The rate of the electron transfer reaction between NADH and PV_2 was measured spectrophotometrically by monitoring the decrease in $[\text{NADH}]$ at 340 nm under second order conditions. The reaction rates were measured with equal and unequal concentration the reactants in phosphate buffer of pH 6 at 303 K. The results are summarized in table 3. The reaction follows simple second order kinetics, i.e. order with respect to $[\text{NADH}]$ is one and order with respect to $[\text{PV}_2]$ is also one. The rate law for the electron transfer reaction is

$$-\frac{d[\text{NADH}]}{dt} = k_2[\text{NADH}][\text{HPA}] \quad (6)$$

3.4 Mechanism

The rate of oxidation of NADH by enzymes present in several biological membranes, viz. cat ventricles, human heart erythrocytes, mouse liver plasma membranes and rat liver microsomes was stimulated by vanadates.⁶⁻⁸ Vanadates are capable of oxidizing NADH under non-enzymic conditions. Isopoly-decavanadate in presence of phosphate buffer was found to be an effective oxidant of NADH compared to other forms of vanadates viz. orthovanadate and metavanadate.^{9,10} Based on the experimental results, a mechanism involving superoxide intermediate for the oxidation of NADH by decavanadate was proposed by Vijaya and Ramasarma¹⁰ (scheme 2).

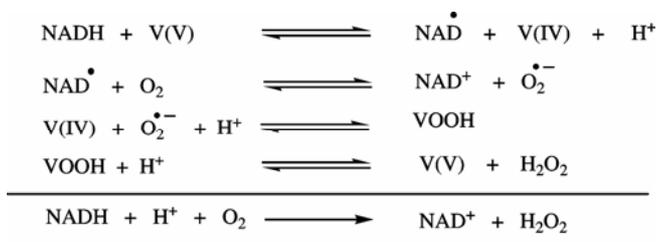
Table 2. ESR spectral data characteristics of heteropoly blues.

Mode of reduction	[PV ^{IV} W ₁₁ O ₄₀] ⁵⁻		PV ^{IV} V ^V W ₁₀ O ₄₀] ⁶⁻		Reference
	<i>g</i> _{iso}	<i>A</i> _{iso} (10 ⁻⁴ cm ⁻¹)	<i>g</i> _{iso}	<i>A</i> _{iso} (10 ⁻⁴ cm ⁻¹)	
By NADH	1.963	91.1	1.969	47.6	This work
Electrochemical	1.952	90.7	1.963	51.5	26–28

Table 3. Bimolecular rate constant for the oxidation of NADH by PV₂ in phosphate buffer of pH 6 at 303 K.

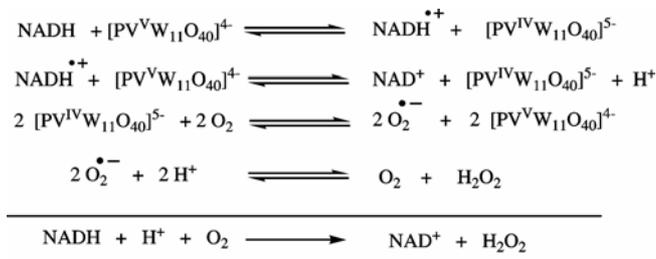
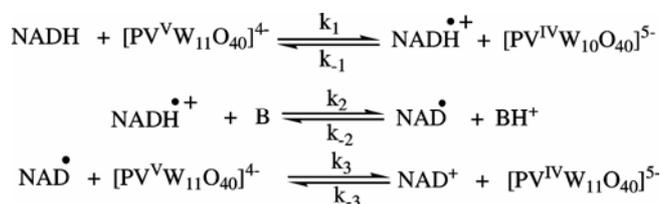
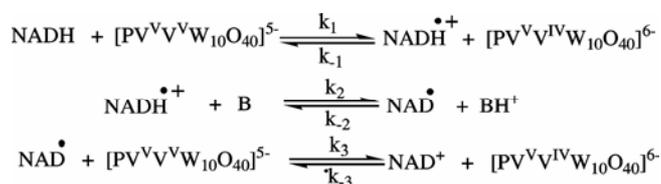
NADH (mM)	PV ₂ (mM)	<i>k</i> ₂ (M ⁻¹ s ⁻¹)
0.10	0.20	25.3
0.12	0.20	22.4
0.15	0.20	21.7
0.20	0.15	21.6
0.20	0.20	24.6

Average *k*₂ = 23.1 ± 1.4 M⁻¹ s⁻¹

**Scheme 2.**

The oxidants employed in the present studies are also polymeric anions containing vanadium (V). The reduced form of the above polyanions, namely heteropolyblues can react with molecular oxygen to produce superoxide radicals.^{29,30} Therefore, there is a possibility that NADH oxidation by PV₁/PV₂ may proceed through superoxide radical intermediate as shown in scheme 3.

If the reaction proceeds with the formation of superoxide radical intermediate, there will be uptake of molecular oxygen, i.e. one mole of oxygen will be consumed for the oxidation of one mole of NADH (scheme 3). In order to ascertain whether there is uptake of molecular oxygen or not, oxygraph measurements were performed. The results clearly show that there is no uptake of molecular oxygen during the course of the oxidation of NADH by PV₁/PV₂. Further for scheme 3, to be operative, HPB will be only an intermediate. But we have found that the end product of NADH oxidation by PV₁/PV₂ is HPB (Optical and ESR data). This excludes the possibility of reaction proceeding through superoxide intermediate in the present study. Based

**Scheme 3.****Scheme 4.****Scheme 5.**

on the results of present studies, the following mechanism can be envisaged for the electron transfer reaction of NADH with PV₁ and PV₂ (schemes 4 and 5).

The above mechanism involves multiple electron-proton-electron transfer steps. Initially an electron is transferred from NADH to HPA to give NADH^{•+}. The solvent water molecule (B) abstracts the proton from NADH^{•+} to give NAD[•] which subsequently reacts with another molecule of HPA to give NAD⁺. For such a mechanism either electron transfer from NADH to HPA or proton transfer from NADH^{•+} to B may be rate-limiting step. For electron-transfer step to be rate-limiting the following condition should be satisfied.

$$k_{-1}[\text{HPB}] < k_2[\text{B}] \quad (7)$$

Assuming equal radii for the reactants, the diffusion controlled rate constant *k*₋₁ can be calculated by the modified Debye equation¹¹

$$k_{-1} = \frac{8RT}{3\eta} \quad (8)$$

At 303 K, with $\eta = 0.01$ P, $k_{-1} = 6.6 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$. If we assume $[\text{HPB}] = 0.2 \times 10^{-3} \text{ M}$ (maximum concentration of HPB formed during oxidation of NADH by HPA), then $k_{-1} [\text{HPB}] = 1.3 \times 10^6 \text{ s}^{-1}$. A laser flash photolysis³¹ shows that $k_2 [\text{B}] = 12 \times 10^6 \text{ s}^{-1}$. The inequality $k_{-1} [\text{HPB}] < k_2 [\text{B}]$ exists even at the end of the reaction. Therefore, the initial electron transfer step appears to be the rate-limiting step in the oxidation of NADH by vanadium substituted Keggin-type heteropolyanions. This mechanism (schemes 4 and 5) is in accordance with the mechanism proposed for the oxidation of NADH by vanadium substituted Dawson-type heteropolyanions³²⁻³⁴ and oxidation of NADH by ferrocenium ions.³

4. Conclusion

Reduction of PV_1/PV_2 by NADH proceeds smoothly in phosphate buffer of pH 6 at ambient temperature. For the reduction of 2 moles of HPA 1 mole of NADH is consumed. The products are NAD^+ and one electron reduced heteropoly blues. There is no uptake of molecular oxygen during the reaction. The reaction is second order. Order with respect to $[\text{NADH}]$ is one and order with respect to $[\text{HPA}]$ also one. The reaction proceeds through multiple electron-proton-electron transfer steps in which the initial one electron transfer is the rate-limiting step.

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