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Kinetics and mechanism of oxidation of pyridoxine by enneamolybdomanganate(IV)

S.V. Nipane, V.M. Gurame, G.S. Gokavi *

Kinetics and Catalysis Laboratory, Department of Chemistry, Shivaji University, Kolhapur 416004, MS, India

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ABSTRACT

Oxidation of pyridoxine (vitamin B6) by enneamolybdomanganate (IV) in aqueous perchloric acid medium has been studied spectrophotometrically at 25 °C under pseudo-first-order conditions. The mechanism involves formation of a precursor complex between the reactants which decomposes in a subsequent slow step to give pyridoxal as the intermediate product. The pyridoxal is further oxidized to a final product, 4-pyridoxic acid, by another oxidant molecule in a fast step. The precursor complex formation is supported both kinetically and spectrophotometrically. The accelerating effect of hydrogen ions on the reaction is due to the formation of active hexaprotonated oxidant species. The protonated enneamolybdomanganate(IV) and the pyridoxine cation are found to be the active species of the oxidant and the substrate respectively. The reaction involves direct two-electron transfer step without any free radical intervention. The effects of ionic strength, solvent polarity and the activation parameters were also in support of the mechanism proposed.

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Polyoxometalates(PMO's) have been utilized in the field of material science, chemistry and biological applications [1] due to their variable redox and acidic properties. The modification of the redox properties of the central metal ion by changing the oxometalate moiety leads to the stability of otherwise unstable metal ions while their acidic properties can be altered by changing the hetero atom at the center. Therefore, because of such variation in almost all physical properties of PMO's it makes them very good acid [2–5] as well as redox [6–8] catalysts. They are also used as analytical reagents, staining agents, precipitating reagent, electron acceptors, antiviral and antitumoral reagents [1] in biochemical applications. The coulombic interaction between polyanions with the cationic sites is the basic mechanism of interaction of biomolecules with PMO. In recent years pyridoxine, vitamin B6, has become a focus of research describing the critical functions of compounds in its cellular metabolism. Pyridoxine is known to be involved in biological activities through its conversion into pyridoxal phosphate, a coenzyme for amino acid decarboxylase [9]. The vitamin B6 exists in six interconvertible forms; pyridoxine, pyridoxal, and pyridoxamine each of which has a corresponding 5-phosphate [9]. The metabolic pathway of degradation of vitamin B6 is its enzymatic oxidative [10] conversion into pyridoxal, 4-pyridoxo-lactone and 4-pyridoxic acid. The major excreted catabolite is 4-pyridoxic acid which is found in urine. Vitamin B6 is widely distributed in food in both free and bound forms and it has wide applications in pharmaceutical industry. Therefore, the degradation pathway of vitamin B6 in presence of an oxidant in aqueous medium is helpful in understanding its biological metabolism. Oxidation of vitamin B6 was reported by using Mn(III) [11] and chloramine-T [12] in acidic as well as by using $KMnO_4$ in alkaline medium [13] and pyridoxal was found to be the major product of reaction. The oxidant used in the present study, enneamolybdomanganate(IV) contains one MnO_6 unit surrounded by nine MoO_6 octahedra and the redox potential of the Mn^{IV}/Mn^{II} couple also decreases from 1.51 V to 1.035 V at pH 3.94 in presence of molybdate ions [14]. Molybdenum, the major content of the oxidant used, is found in several tissues of the human body and is required for the activity of xanthine oxidase which is involved in the catabolism of purines [15]. In continuation of our earlier studies [6,7,16,17], oxidation of vitamin B6 by enneamolybdomanganate(IV) was carried

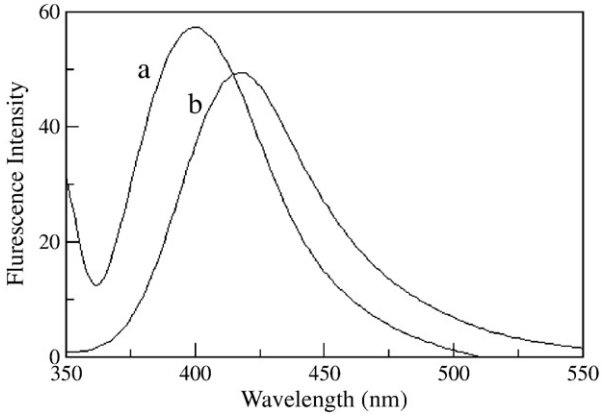

Fig. 1. Fluorescence spectra of (a) Pyridoxine and (b) the product 4-pyridoxic acid.

* Corresponding author. Tel.: +91 2312909391; fax: +91 231 2692333.
E-mail address: gsgokavi@hotmail.com (G.S. Gokavi).

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Table 1
Effect of concentration of oxidant and perchloric acid on the oxidation of pyridoxine by enneamolybdomanganate(IV) at 25 °C. [Pyridoxine] = 0.01 mol dm^{-3} and I = 0.1 mol dm^{-3}.

10^3 $[Mn^{IV}Mo_9O_{32}]^{6-}$ mol dm^{-3}	$10[HClO_4]$ mol dm^{-3}	10^2 k_{obs} s^{-1}
0.2	0.3	2.5
0.6	0.3	2.6
0.8	0.3	2.6
1.0	0.3	2.6
1.2	0.3	2.6
1.4	0.3	2.5
1.6	0.3	2.5
1.2	0.1	1.1
1.2	0.3	2.6
1.2	0.5	3.9
1.2	0.7	5.0
1.2	1.0	7.1

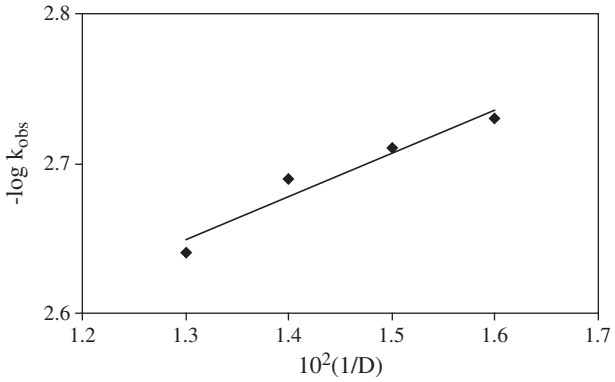


Fig. 2. Plot of log k_{obs} against (1/D) the oxidation of pyridoxine by enneamolybdomanganate(IV) at 25 °C. 10^3 $[Mn^{IV}Mo_9O_{32}]^{6-}$ = 1.2 mol dm^{-3}, [Pyridoxine] = 0.01 mol dm^{-3}, 10 $[HClO_4]$ = 0.3 mol dm^{-3} and I = 0.1 mol dm^{-3}.

out in order to understand the mechanism of oxidative transformation of pyridoxine, the nature of interaction and the probable intermediates.

All chemicals were of reagent grade and double-distilled water was used throughout the work. A solution of pyridoxine hydrochloride (vitamin B6)(Hi Media) was freshly prepared by dissolving an appropriate amount of sample in double-distilled water. Standard solution of perchloric acid was prepared in double distilled water. The ammonium salt of Mn^{IV} complex, $(NH_4)_6[Mn^{IV}Mo_9O_{32}]$ was prepared by reported method [18]. The oxidant was characterized by FTIR and AAS analysis as reported earlier [16,17].

Kinetic measurements were performed on Elico SL-177 spectrophotometer. The kinetics was followed under pseudo-first order conditions keeping large excess of [vitaminB6] over [oxidant] at constant temperature 25 ± 0.1 °C. The reaction was initiated by mixing the previously thermostated solutions of vitaminB6 and $(NH_4)_6$ $[Mn^{IV}Mo_9O_{32}]$ which also contained the required amount of perchloric acid and doubly distilled water. The progress of reaction was followed spectrophotometrically at 468 nm ($\varepsilon = 360 \pm 2$ dm^3 mol^{-1} cm^{-1}) by monitoring the decrease in absorbance of oxidant. The pseudo-first order rate constants were determined from the log [oxidant] versus time plots and the rate constants were reproducible within ±5% and the reaction was studied up to 80% completion.

The stoichiometry was studied by keeping concentration of $[Mn^{IV}Mo_9O_{32}]^{6-}$ constant at 1.0×10^{-3} mol dm^{-3} and varying concentration of vitamin B6 from 1.0×10^{-3} to 2.0×10^{-4} mol dm^{-3} these reaction mixtures also contained required amount of perchloric acid. The concentration of unreacted $[Mn^{IV}Mo_9O_{32}]^{6-}$ was determined after 24 h spectrophotometrically. The stoichiometry was found to be 2 mol of $[Mn^{IV}Mo_9O_{32}]^{6-}$ per mole of vitamin B6 indicating 4-pyridoxic acid as the product. Further, the fluorescence spectra of reaction mixture after 24 h was examined which shows emission at 418 nm (Fig. 1b) characteristic of 4-pyridoxic acid, thus confirming 4-pyridoxic acid as product of reaction [19] as shown in Eq. (1).

$$2\left[Mn^{IV}Mo_9O_{32}\right]^{6-} + C_8H_{11}NO_3 + H_2O \rightarrow 2\left[Mn^{II}Mo_9O_{32}\right]^{8-} + C_8H_9NO_4 + 2H^+ \quad (1)$$

Table 2
Effect of concentration of Pyridoxine on the oxidation of pyridoxine by enneamolybdomanganate(IV). 10^3 $[Mn^{IV}Mo_9O_{32}]^{6-}$ = 1.2 mol dm^{-3}, $10[HClO_4]$ = 0.3 mol dm^{-3} and I = 0.1 mol dm^{-3}.

10^2 [Pyridoxine] mol dm^{-3}	10^3 k_{obs} s^{-1}				
	288 K	293 K	298 K	303 K	308 K
0.5	1.3	1.5	1.8	2.1	2.4
0.8	1.5	1.8	2.1	2.5	3.0
1.0	1.7	2.0	2.5	3.0	3.3
3.0	2.1	2.4	3.0	3.5	4.0
5.0	2.4	2.9	3.5	3.9	4.5

The reaction was also studied in presence of added acrylonitrile to understand the intervention of free radicals. There was no effect of added acrylonitrile on the reaction and also no precipitate due to the polymerization of the added acrylonitrile was observed thus confirming the absence of any free radical formation in the reaction.

The reaction was carried out under pseudo-first-order conditions and the plots of log [oxidant] against time were found to be linear in all the runs up to three half lives of the reaction. The values of pseudo-first-order rate constants were constant between the range of [oxidant] of 2.0×10^{-4} to 1.6×10^{-3} mol dm^{-3} (Table 1) therefore, the order in [oxidant] is unity. The pseudo-first-order rate constants were found to increase (Table 2) as [vitamin B6] increases from 5.0×10^{-3} to 5.0×10^{-2} mol dm^{-3} at a constant [oxidant] of 1.2×10^{-3} mol dm^{-3}. The order in [vitamin B6] was found to be 0.28 as determined from the log k_{obs} against log [vitamin B6]. Since, the order in [vitamin B6] was fractional which indicates the formation of a complex therefore, the kinetic data were used to obtain Michaelis–Menten plot of $(1/k_{obs})$ against (1/[vitamin B6]). Such a plot was found to be linear with an intercept supporting the formation of a complex between the reactants. In order to evaluate thermodynamic parameters the effect of [vitamin B6] was studied at five different temperatures (Table 2). The effect of $[H^+]$ on the reaction was studied by varying the perchloric acid concentration between 0.01 and 0.1 mol dm^{-3} at a constant ionic strength of 0.1 mol dm^{-3}. The rate of reaction is accelerated (Table 1) by increase in $[H^+]$ and the order in $[H^+]$ was found to be 0.8.

The effects of ionic strength and solvent polarity were studied keeping concentration of $[Mn^{IV}Mo_9O_{32}]^{6-}$, vitamin B6 and perchloric acid constant at 1.2×10^{-3} mol dm^{-3}, 0.01 mol dm^{-3} and 0.03 mol dm^{-3} respectively at 25 °C. Sodium perchlorate was used to vary the ionic strength. The rate of the reaction was unaffected with varying ionic strength and the rate of reaction decreases as percentage of acetonitrile increases from 0 to 40% v/v. The plot of log k_{obs} vs. (1/D) is linear with a negative slope (Fig. 2).

Enneamolybdomanganate(IV) is one of the stable heteropolymolybdate containing Mn(IV) as a hetero atom and is noncentrosymmetric. The central Mn(IV) is surrounded octahedrally by six oxygen atoms with a structure of D_3 symmetry. It shows a characteristic charge transfer band at 468 nm due to $A_{2g} \rightarrow {}^4T_{2g}$ transition of Mn^{IV}

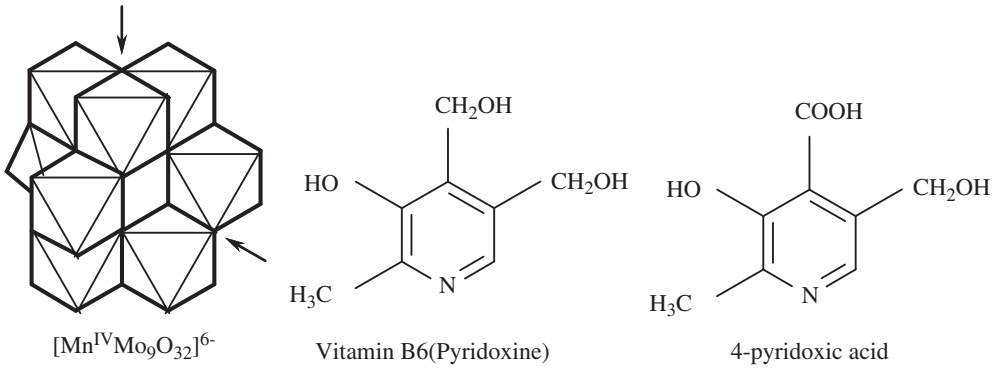


Fig. 3. Structures of enneamolybdomanganate(IV)(Polyhedral representation), pyridoxine and 4-pyridoxic acid.

ion. The oxygen atoms of $[MnMo_9O_{32}]^{6-}$ ion are of two types (Fig. 3) [20], namely those which are directly bound to the central Mn(IV) ion and the rest of the atoms are involved in formation of polyoxometalate moiety around it with Mo atoms. Any perturbation in the environment of the oxygen atoms directly bound to Mn(IV) ion in the enneamolybdomanganate(IV) leads to the change or shift in the peak at 468 nm which has been noticed during the study [20] of the interaction between cations and enneamolybdomanganate(IV). Further, in aqueous solution the UV–VIS spectrum of the anion, $[MnMo_9O_{32}]^{6-}$, shows two isosbestic points in presence of acid at 438 and 522 nm due to its protonation (Fig. 4). In order to understand the interaction between the vitamin B6 and $[MnMo_9O_{32}]^{6-}$, the UV–VIS spectra of the reaction mixture was examined. The anion shows a characteristic peak at 468 nm in aqueous solution and addition of 0.01 mol dm^{-3} perchloric acid the spectrum of protonated anion is obtained with two isosbestic points at 435 and 522 nm as shown in Fig. 4. To an acidic solution of the anion when vitamin B6 is added the reappearance of peak at 468 nm was not observed instead there was a considerable increase in the intensity of absorption (Fig. 4). The reappearance of peak at 468 nm in presence of vitamin B6 would have been expected [20] if its interaction with the oxidant occurs through oxygen atoms of MnO_6 octahedra. Since, the peak at 486 nm was not observed in presence of vitamin B6 (Fig. 4), the interaction between the reactants does not occur through the oxygen atoms of MnO_6 octahedra. On the other hand, there was an increase in the intensity of spectra of $[Mn^{IV}Mo_9O_{32}]^{6-}$ ion (Fig. 4) in the presence of vitamin B6 which indicates formation of a complex between the two. The substrate, pyridoxine contains pyridine nitrogen, a methyl, two alcoholic groups and a phenolic –OH group. The complex formation of pyridoxine with metal ions like V(IV) involves phenolic –OH and the adjacent alcoholic group [21]. Since, the product of the present study is 4-pyridoxic acid, the interaction between $[Mn^{IV}Mo_9O_{32}]^{6-}$ ion and pyridoxine is occurring through the alcoholic group at the 4th position and oxygen atoms of the oxidant which are not directly bound to the central Mn(IV). Further, the polyoxometalates are outer-sphere oxidants [2], therefore, the oxidation of pyridoxine proceeds through formation of a precursor complex (Fig. 5) between the reactants rather than a isolable complex as noticed [21] in case of vanadyl ion. The formation of such precursor complex between

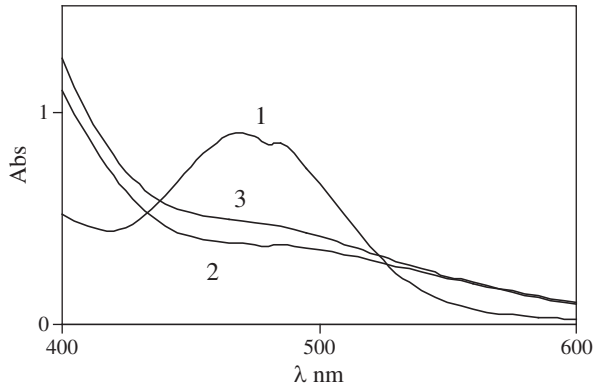


Fig. 4. UV–VIS spectra of enneamolybdomanganate(IV) in (1) water (2) in acidic medium and (3) the reaction mixture. 10^3 $[Mn^{IV}Mo_9O_{32}]^{6-} = 1.2$ mol dm^{-3}, [Pyridoxine] = 0.01 mol dm^{-3}, $10[HClO_4] = 0.1$ mol dm^{-3} and I = 0.1 mol dm^{-3}.

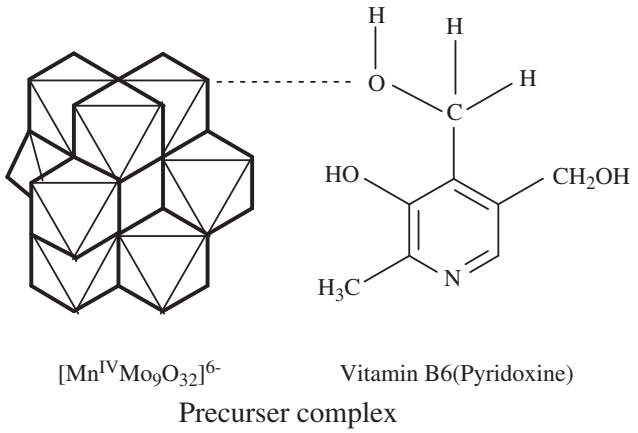


Fig. 5. Structure of the probable transition state of the reaction between pyridoxine and enneamolybdomanganate(IV).

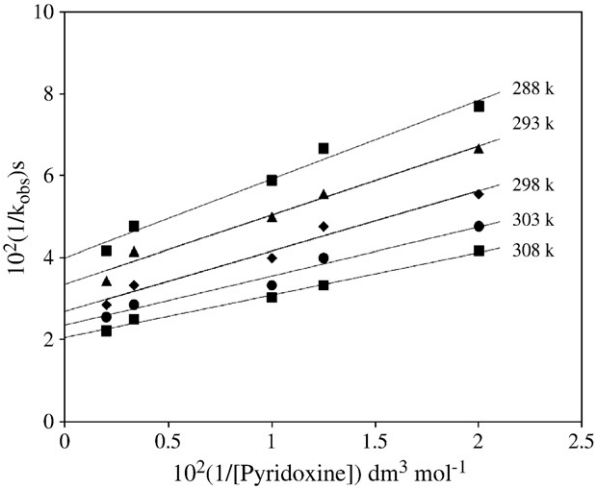
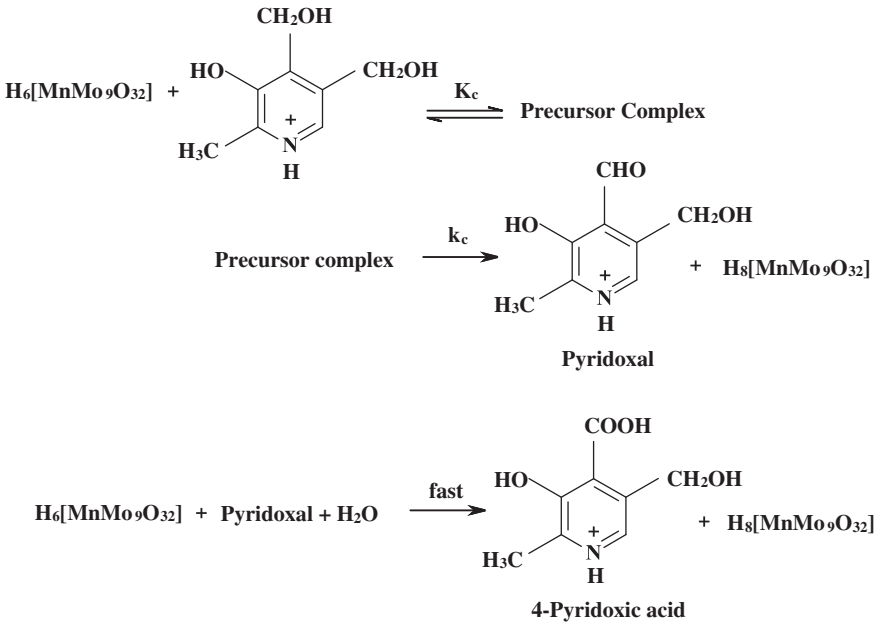


Fig. 6. Michaelis–Menten plot for the oxidation of pyridoxine by enneamolybdomanganate(IV). (conditions as in Table 2).



Scheme 1. Mechanism of Oxidation of vitaminB6 by enneamolybdomanganate(IV).

$[MnMo_9O_{32}]^{6-}$ ion and pyridoxine is supported spectrophotometrically by the increase in the intensity of absorption of the oxidant in presence of substrate (Fig. 4) and kinetically by the Michaelis–Menten plot of $1/k_{obs}$ against 1/[Pyridoxine] (Fig. 6).

The pK of protonation of nitrogen of pyridine of pyridoxine is 5.0 and the phenolic –OH is basic. Therefore, in the acidic medium used in the present study pyridoxine is predominantly exists as monoprotonated form with formation of pyridinium cation. Polyoxometalates are known to undergo protonation and in acidic medium and enneamolybdomanganate(IV) ion is in the form of hexaprotonated species [17]. Therefore, under the reaction conditions, the oxidant exists as $H_6[MnMo_9O_{32}]$ and it is in equilibrium with $H_5[MnMo_9O_{32}]^-$ ions as shown in equilibrium (2). Since, the reaction is catalyzed by hydrogen ions with an order of 0.8 the reactive species of the oxidant is $H_6[MnMo_9O_{32}]$.

$$H_6\left[Mn^{IV}Mo_9O_{32}\right] \overset{K_1}{\rightleftharpoons} H_5\left[Mn^{IV}Mo_9O_{32}\right]^- + H^+ \quad (2)$$

The oxidation of pyridoxine to 4-pyridoxic acid requires four electrons and the oxidant, $[Mn^{IV}Mo_9O_{32}]^{6-}$, is a two electron reagent. Therefore, the reaction involves two two-electron transfer steps with the formation of pyridoxal as an intermediate. The pyridoxine is oxidized to pyridoxal by first oxidant molecule and this pyridoxal is further oxidized to 4-pyridoxic acid by second oxidant molecule. Product 4-pyridoxic acid is confirmed by its characteristic [22] fluorescence spectra (Fig. 1b). The fluorescence spectra of the pure form of pyridoxine under acidic conditions have an emission (Fig. 1a) at 400 nm, whereas the product of the reaction shows an emission at 418 nm at excitation wavelengths of 332 and 320 nm respectively.

The mechanism of the reaction, based on the kinetic results and spectrophotometric examination of the reactants and the reaction mixture can be represented as in Scheme 1. According to Scheme 1 the reaction proceeds with the formation of a precursor complex between the active forms of reactants $H_6[MnMo_9O_{32}]$ and the cation of pyridoxine to form a precursor complex in a prior equilibrium with a constant K_c. The precursor complex thus formed will decompose in a slow second step with rate constant k_c to form pyridoxal. The pyridoxal thus formed reacts in the subsequent fast step with another oxidant molecule to give final product 4-pyridoxic acid. The rate law according to Scheme 1 and protonation equilibria (2) of the oxidant is given by Eq. (3). The rate law 3 explains that the fractional orders with respect to the [Pyridoxine] and $[H^+]$ and the plots of $1/k_{obs}$ against 1/[Pyridoxine] were found to be linear for five different temperatures ranging from 15 to 35 °C (Fig. 6). From the slope and intercept

$$k_{obs} = \frac{k_c K_c [H^+]\ [\text{Pyridoxine}]}{([H^+] + K_1)\ (1 + K_c[\text{Pyridoxine}])} \quad (3)$$

of Fig. 6 the values of rate constant for the slow step, k_c and formation constant of precursor complex, K_c, were calculated and are given in Table 3 along with the activation parameters for the slow step of the reaction. The moderate values of $\Delta H^{\#}$ and $\Delta G^{\#}$ of 22.45 ± 0.6 and 88.17 ± 0.5 kJ mol^{-1} respectively were favorable for electron transfer processes. The negative value of $\Delta S^{\#}$ can be ascribed to the nature of electron pairing and unpairing processes and to the loss of degrees of freedom formerly available to the reactants upon the formation of transition state. The effect of ionic strength indicates that one of the reactant is neutral and decrease in the rate with decrease in the

Table 3
Rate constant for the slow step, k_c, formation constant of precursor complex,K_c and activation parameters for the oxidation of pyridoxine by enneamolybdomanganate(IV). (Conditions as in Table 2).

Temp, K	288	293	298	303	308
10^3 k_c dm^3 mol^{-1} s^{-1}	2.5	3.0	3.7	4.2	4.8
K_c dm^3 mol^{-1}	207	199	190	196	198
$\Delta H^{\#}$	22.45 ± 0.6 kJ mol^{-1}				
$\Delta G^{\#}$	88.17 ± 0.5 kJ mol^{-1}				
$\Delta S^{\#}$	-220 ± 3 J K^{-1} mol^{-1}				

dielectric constant is in conformity with Amis concept for ion–dipole interactions [23].

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