A comparative study of the electrochemical properties of vitamin B-6 related compounds at physiological pH

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Abstract

A comparative study of vitamin B6 group and related compounds in buffered solutions using electrochemical techniques has been performed at neutral pH. Irreversible bi- or tetra-electronic processes are observed for these substances, and the electron transfer coefficient (αn) calculated. It was concluded that either the first or second electron transfer were the rate determining step of the electrode process. The diffusion coefficient of these substances was calculated and the values given follow an inverse tendency to the molecular size. For aldehydes the values obtained were corrected of the hydration reaction.

It is important to remark that catalytic waves were reported for the first time for these compounds. Using a model involving the nitrogen of the basic structure the kinetic constants were calculated for most of them.

Keywords: Vitamin B6; electrochemistry; pyridoxine; diffusion coefficient; catalytic wave

1. Introduction

The chemical characterisation of vitamin B6 group was started more than three decades ago, but still today new aspects have been revised and new information added in an attempt to understand their catalytic activity.

The study of the different chemical species through their electronic absorption and emission bands has been very important in order to elucidate their chemistry in solution. These species present a complex chemical behaviour due to acid-base reactions coupled to tautomeric equilibria and in some cases hydration of aldehyde groups. Figure 1 shows their structure and functional groups, necessary to explain and understand the chemical transformations between them.

Figure 1

Pyridoxal-5'-phosphate (PLP) and pyridoxal (PL) are able to catalyse a great number of biological reactions. Their role in these reactions has been studied using different models involving themselves or different derivatives of vitamin B6.

For a long time, the term "vitamin B6" has been defining a series of compounds with biological activity derived from 3-hydroxi-2-methyl pyridine (1-2). The most important compounds of this group are: pyridoxine (PN), pyridoxal (PL), pyridoxamine (PM), pyridoxal-5'-phosphate (PLP), pyridoxamine-5'-phosphate (PMP) and pyridoxine-5'-phosphate (PNP). From the later, the phosphorylated esters, PLP and PMP, are the most important in enzymatic catalysis. Derivatives of this family of compounds are 4-pyridoxic acid (PA) or its lactone (PAL), obtained as organism degradation by-products (3,4). It is well known that vitamin B6 catabolism consists in the formation of PM, PL and PN by oxidation of the substrate present in position 4 or 5 and finally PA will be produced through oxidation of PL. Related products are their oximes, pyridoxal oxime (PLO) and pyridoxal-5'-phosphate

oxime (PLPO), obtained by a condensation reaction of hydroxylamine with PL and PLP, respectively (5-7). These oximes are particularly interesting as they present fluorescence and phosphorescence and have been used for protein structure studies (8). Other group of compounds is related to vitamin B6 family and can be considered the simplest compounds with a pyridine heterocyclic as are 4-pyridin aldehyde (P), 4-pyridin carboxylic acid (PC), 4-pyridin aldehyde oxime (PO) and 4-aminomethylpyridine (AP).

Most of studies on vitamin B6 have been based on natural derivatives of PN. However, some synthetic derivatives have been also studied in an attempt to simplify or explain the behaviour of these compounds due to the presence of different functional groups on the pyridine ring. The existence of multiple equilibria complicates the understanding of the properties in solution for these compounds that strongly depend on the acid-base characteristics, the polarity of the solvent and the surrounding environment. Studies by UV-Visible spectroscopy (9-11) support the use of a simplified model in which only the acid-base equilibria from the N and the o-hydroxyl group present in the pyridine ring is considered.

From an electrochemistry point of view, the study of vitamin B6 group is also interesting. Most of these studies tried to relate the different electrochemical signals to the different functional groups present in the molecules. It is worth mentioning the first studies from Manousek (12) and Zuman (13,14), both describing the polarographic behaviour for PN, PM, PMP, PL, PLP, PLPO, PLO and other derivatives in a buffered medium. Other studies performed by Llor et al (15,16) compared the different electrochemical response of PLP in aqueous and organic solvents and mixtures of water/organic solvent.

Our interest in recent years has been the study of vitamin B6 compounds by electrochemical techniques. The electrode processes are pH-dependent due to the complex distribution of species in solution provided by acid-base properties, tautomeric equilibria and, in aldehyde compounds, hydration equilibrium (17-25). Electrochemical responses indicate

the exchange of two or four electrons as well as a distribution of characteristic potentials depending of electroactive group. This behaviour is interesting from an analytical point of view.

In this work we show a comparative study of electrochemical of vitamin B6 related compounds under fixed experimental conditions using buffered solutions at neutral pH where zwitterionic species are predominant for the 3-hydroxy pyridine derivatives. This study allows obtaining and collecting of different electrochemical parameters useful for analytical purposes and development of other applications as the design of modified electrodes. Moreover, we analyze the catalytic response observed at more cathodic potential that is explained by the existence of the ring structure base of vitamin B6 compound.

The electronic mobility of the different molecules, regulated by proton transfers on different sites of the molecule, is a very important factor for the design of new interfaces. In this sense, the formation of thiol-derivatized monolayers of the compounds under study on appropriate substrates (habitually gold, platinum or mercury) is being researched in our laboratory at present. The study of these compounds has provided valuable information to be extrapolated to biological environments, where these compounds develop their catalytic activity.

2. Experimental

2.1. Apparatus and instruments

A computer assisted Inelecsa PDC1212 potentiotast was used for classic polarography (CP), differential pulse polarography (DPP) and cyclic voltammetry (CV). For additional voltammetry studies, an AMEL electrochemistry instrument model 433-A fitted with homemade software was also used. The experiments were conducted in a double-walled Metrohm E-505 thermostatic cell fitted with a three electrode system: a saturated calomel

reference electrode (SCE) Ingold 303-NY, a platinum auxiliary electrode from Metrohm and, as working electrodes, a mercury capillary electrode and a dropping mercury electrode (DME) EA290 both also from Metrohm.

The concentration of some compounds, such as PLO and PLPO, were monitored by UV-Vis spectrophotometry using a Perkin Elmer Lambda 3B spectrophotometer.

Temperature for both electrochemical and spectrophotometric experiments was kept constant at 25±0.1ºC by using an thermostatic bath Selecta Frigiterm, model S-382. The pH was monitored using a Crison 2001 pH-meter.

2.2 Reagents

Pyridoxine (PN), 4-pyridine aldehyde (P), pyridoxal (PL), pyridoxal-5'-phosphate (PLP), 4-pyridine aldehyde oxime (PO), 4-pyridin carboxylic acid (PC), 4-pyridoxic acid (PA), amino methyl pyridine (AP), pyridoxamine (PM) and pyridoxamine-5'-phosphate (PMP) were purchased from Sigma.

4-pyridoxic acid lactone (PAL) was synthesised by acidic hydrolysis of PA. A sample of PA in 1 mM HCl was incubated at 37ºC and after 24h the conversion into lactone was complete. The reaction was followed by monitoring absorption spectra at pH 6, the initial band centered at 315 nm changes to 356 nm as the lactone is formed [23].

Pyridoxal oxime (PLO) and pyridoxal-5'-phosphate (PLPO) were synthesised using a method used by Pocker et al (26).

Other reagents purchased from Merck were phosphoric acid, acetic acid, hydrochloric acid, sodium hydroxide and potassium chloride. Acetic/acetate and hydrogen phosphate/di-hydrogen phosphate buffers were used for the experiments where pH needed to be kept constant.

3. Results and discussion

3.1. General behaviour

Electrochemical studies of vitamin B6 compounds are one of the subjects of our investigation as we have indicated in the Introduction section. Aldehydic compounds as pyridoxal and pyridoxal 5'-phosphate attract our attention due to their biological role. Electrochemical reduction of the aldehyde group is complex, as expected because of the hydration reaction or hemiacetal formation in homogenous aqueous medium as well as other electrode reactions as the dimerization of radical intermediate. Dimerisation explains why two waves are obtained (the case of pyridin 4'-aldehyde (17,18)) or combined waves (the case of PL and PLP (19-21)). In all cases, under different pH conditions, the bi-electronic reduction generates the alcohol derivative as principal product. For PLP is also worth mentioning the intramolecular catalysis in the hydration-dehydration reaction due to the presence of a very fast three-centre intramolecular proton transfer between the protonable groups of the molecule (20-22). The oxime reduction on the electrode produces the amine derivative in all the compounds studied (24,25) (Table 1). In aqueous medium, under different pH conditions, the dehydration reaction of de hydroxylamine intermediate is fast and lead to a tetra-electronic wave. In general, the reduction of monocarboxylic acids happens to be a tetra-electronic process to produce the correspondent alcohol through the related aldehyde or the hydrated form, which can be transformed into the respective alcohol at the same potential (Table 1). PA reduction is also a tetra-electronic process (23) and it does not have the correspondent alcohol (PN) as a final product, as observed by NMR and mass spectrometry. PAL should follow a similar reduction process. The most feasible explanation considers that the reduction process involves a hydrated reaction intermediate of pyridoxal, or hemiacetal, adsorbed onto the electrode yielding a product different from the alcohol. The current intensity observed in

our case, as typical profile intensity vs potential, demonstrates that the process is diffusion-controlled. Amine reduction, again under different pHs, is an irreversible bi-electronic process yielding ammonia (24). The characteristic reduction potential close to the proton reduction complicates their study, but the main electrochemical parameters could be compared and linked to the second reduction process on the oximes at more cathodic potentials (24,25) (Table 1).

In this work the electrochemical properties of vitamin B6 family compounds are studied in buffered solutions (phosphate / dihydrogen phosphate buffer 0.1 M) at neutral pH. In these conditions, the neutral species is predominant for the pyridine derivatives whereas the 3-hydroxy pyridine derivatives are in their zwiterionic form. The results obtained by CP, DPP (based on theoretical treatment for signal analysis (27-29)) and CV electrochemical techniques are summarized in Tables 1 to 3.

Tables 1, 2 and 3

The first group of compounds studied is aldehydes, which give a faradaic response in the potential range in between -0.5 to -0.8 V. For 4-pyridin aldehyde a two-step reduction process is obtained, both showing the same intensity, whereas a single two-electron process is observed in the case of pyridoxal and pyridoxal 5'-phosphate. For aldehydes, the reduction product is the correspondent alcohol. The structure of the molecules and the dimerisation of the intermediate radical obtained in the first proton and electron transfer reaction, favours the observation of this two-step process. Another important factor influencing aldehydes reduction is the hydration reaction of the functional group in bulk solution. The rate of dehydration to produce the electroactive species is paramount as greatly influences the limiting current. This is the case for P and PL as limiting current, i_L, is controlled by the

aldehyde dehydration, whereas in the case of PLP is controlled mainly by diffusion. We present the current intensity correction to the hydration reaction in the following section (Table 1).

The oximes, PO, PLO and PLPO, show two reduction processes in the range of potential in between -0.75V and -1.5V. The first one corresponds to the tetra-electronic reduction process of the oxime group to amine and the second one is a bi-electronic process driving to the elimination of ammonia and forming the related saturated compound.

The next group of this study comprises the acids PC and PA, and the lactone derived from PA (PAL). The reduction of this group shows one electronic process and it is observed at potentials close to -1.2V. The reduction of PAL shows a main electronic process followed by a second minor process, probably due to residues of PA coming from the hydrolysis of PAL.

The last group shows the reduction of amines, AP, PM and PMP. These compounds show a bi-electronic reduction process around -1.4V. It can be observed that there is a greater superficial contribution for this process in the case of PM. It can be noted that the obtained response is very similar to that obtained in the second reduction process for oximes. For PMP the reduction occurs close to the depolarisation of the inert electrolyte at pH=7 and the signal is buried under it.

All results agree with the number of electrons obtained by different authors in the literature [30]: bielectronic (aldehydes and amines) and tetraelectronic (oximes and acids) processes. In some cases we obtain peaks due to high adsorption of the electroactive substance or any of the reaction intermediates. It has been checked that the amount of inert electrolyte is not affecting the reduction potential, the limiting current or the shape of the signal obtained.

In general, each group of molecules is electroactive in a range of potential as we can see in Figures 2 and 3 for the pyridine derivatives.

Figure 2

Figure 3

The reduction of the four families of compounds studied in this work shows to be irreversible as it is inferred from the slope of logarithmic analysis of the polarographic signal (greater than expected), from the theoretical adjustment of the DPP peaks (26) and the characteristic shape obtained by voltammetry [31] (Figure 3).

Aldehydes and amines are reduced through bi-electronic transfers (Scheme 1). The results obtained from the slope of the logarithmic analysis in classic polarography showed the transfer to be in between a reversible mono-electronic process and a reversible bi-electronic one. This suggests that this is driven through a single irreversible process involving two electrons with the transfer of the second electron as the controlling step in the reduction process. This is supported by the value obtained for $\alpha \geq 1$ (n_a=1). The exception to this behaviour is AP that shows a slope only slightly greater than that for a mono-electronic reversible process and $\alpha < 1$ (n_a=1), which indicates that the first electronic transfer is the responsible for the kinetic control of the process.

Acids and oximes yield greater slopes than those obtained in a reversible tetra-electronic process (Scheme 1). For PO and PLPO the logarithmic analysis suggests that the electrode process is controlled by the first electronic transfer, while PLO shows control by the second electronic transfer. This is supported by values of $\alpha < 1$ (n_a=1) for PO and PLPO and $\alpha > 1$ (n_a=1) for PLO. In the case of acids, slopes greater than those associated to a reversible bi-electronic process are obtained, which suggest kinetic control by a second electronic transfer

reaction. These models must be considered as an approximation to the overall behaviour of the electrode processes, as more than one elemental step or coupling of chemical reaction might be involved.

Aldehydes	R-CHO $\xrightarrow{2e^-+2H^+}$ R-CH_2OH	[19-21]
Oximes	R-CHNOH $\xrightarrow{4e^-+4H^+}$ RCH_2NH_2	[24,25]
Acids	R-COOH $\xrightarrow{4e^-+4H^+}$ R-CH_2OH (for P)	[17,18]
	Other derivatives (for PA)	[23]
Amines	R-CH_2NH_2 $\xrightarrow{2e^-+2H^+}$ R-CH_3 + NH_3	[24]

Scheme 1

Electrochemical reduction of the vitamin B6 derivatives

(Right: references where the overall process is described)

3.2. Hydration reaction in aldehydes

The electroreduction of free aldehydes is a two-electron transfer coupled with protonation reactions to yield the correspondent alcohol. This process is complicated by the hydration equilibrium as both hydrated and non-hydrated forms (see figure 4) of the studied aldehydes (for PL, hemiacetal form) give a CE process of the type:

$$A{\cdot}H_2O \underset{k_b}{\overset{k_f}{\longrightarrow}} A \xrightarrow{ne^-} B \qquad (1)$$

Using equilibria and rate constants on aldehydes hydration from the literature (32) and the experimental data obtained by polarography, we can calculate the limiting current, the diffusion constant (I) and the diffusion coefficient (D) by using a modification of Koutecky and Brdicka equation (33):

$$\frac{i_K}{i_L - i_K} = 1.386(K^2kt)^{0.545} \qquad (2)$$

where i_L is the intensity of the limiting current, i_K the intensity of kinetic current, K the equilibrium constant expressed as $[A \cdot H_2O]/[A]$, $k=k_f+k_b$ where k_f is the rate constant of formation for the electroactive substance and k_b the rate constant of formation for the non-electroactive form, and t the dropping time. Table 4 shows the results obtained.

Figure 4

Table 4

Using the data of the corrected limiting current for the different aldehydes we can calculate the diffusion coefficient (D) for each one.

Comparing the results for the vitamin B6 compounds studied, the sequence obtained for the diffusion coefficient is: aldehyde > acid > amine > oxime. Within each group we can observe a tendency linked to the molecular size, except in the case of PMP, which cannot be used for comparisons as it was recorded at pH=5 to avoid overlapping with the signal coming from the inert electrolyte.

3.3. Catalytic signal

A common feature for all compounds of this family is the presence of a reduction peak below -1.5V close or partially overlapped to the background response of the buffer (Fig. 3). This behaviour has been observed using all three techniques used in this study and the signal obtained is greater than expected for a bi- or tetra-electronic process. Differences in the intensity of these peaks have been observed, which are not related to the concentration of the electroactive substances (identical in all cases). This process, first time reported in the literature of these compounds, shows characteristic features of an associated catalytic process with independence of the functional groups studied and related with a common structure and involving easy-to-dissociate protons. The compounds of vitamin B6 group contain the same basic structure, pyridine and 3-hydroxi-pyridine rings, with different functional groups in position 4'. A similar behaviour has been described previously for the simpler of these compounds, pyridine (34), as well as other organic compounds containing nitrogen, phosphorus, arsenic or sulphur that are capable of binding a proton to the free electron-pair of these atoms (35-39). Table 5 shows potential and currents of the catalytic process in polarography (CP and DPP) and voltammetry together with the ratio between the catalytic current and the functional group current. Corrections taking into account the number of electrons transferred in the electroactive group are also shown.

Table 5

We observe a single catalytic wave for 4-pyridin derivatives (Figure 3). The 3-hydroxypyridine derivatives show two overlapped waves indicating a catalytic process in two steps (see example of PLP in the inset of Fig 3).

A catalytic process differs from those discussed previously in the fact that the catalytic current is much higher than the limiting current obtained for a process not involving a

chemical reaction (by a factor 50-150 as it could see in the last column of Table 5). We write the process in the case of pyridine derivatives as:

$$\rangle NH^+ + e^- \rightleftharpoons \rangle N\mathbf{:} + 1/2H_2$$

$$\rangle N\mathbf{:} + AH \rightleftharpoons \rangle NH^+ + A^-$$

Scheme 2

where AH is a substance inactive in the electrode with a high concentration which does not modify the process on the electrode (typically the buffer component as proton donor). We are assuming the H^+ will be reduced through recombination with a characterized donor present in solution, although other mechanisms could be possible depending on each electroactive substance and the different intermediates formed on the electrode surface and in the solution. By using the information obtained in the different polarographic experiments we can also calculate the kinetic constants for this process, as shown in table 6.

Table 6

4. Conclusions

Vitamin B-6 related compounds showed irreversible bi- or tetraelectronic reduction at pH=7 on mercury electrodes as it is inferred from the logarithmic analysis of the polarographic waves and the electronic transfer coefficient (αn). This behaviour has also been confirmed by the parameters obtained using DPP and CV. Using the irreversible model it was concluded that either the first or second electronic transfer were the controlling stages of the electrode process.

Using the parameters calculated in this work the different diffusion coefficients of these substances were calculated and corrected values given for the case of aldehydes, in which hydration reactions are present. In general, the obtained values follow an inverse tendency to the molecular size.

It is important to remark that catalytic waves were reported for the first time for these compounds. Using a model involving the pyridine nitrogen and 3-hydroxi groups of the basic structure the kinetic constants were calculated for most of them.

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Figures and legends

Figure 1. Chemical structures of the main compounds for vitamin B6 family. Zwitterions are shown for PN, PL, PLP, PLO, PLPO, PA, PAL, PM, PMP.

Figure 2. Differential Pulse Polarography. Experimental results compared to theoretical curves calculated by using the equation: $I=4I_pL/(1+L)^2$, with $L=\exp[-(E-E_p)/b]$ [26], for: P, PL, and PLO. (●●●) experimental, (….) individual and (——) overall theoretical calculations.

Figure 3. Cyclic Voltammetry. Some compounds and catalytic waves. 0.05 M acetate-phosphate buffer (pH=7). Electroactive substance concentration= 0.1mM . Scan rate: 0.1 V/s. Inset: catalytic wave for PLP.

Figure 4. Hydration equilibria for aldehydes

Table 1. Polarographic results in buffered aqueous solution (pH 7.0) [a],[b]

		c (mM)	$-E_{1/2}$ (mV)	i_L (μA)	n	I ($cm/s^{1/2}$)	$Dx10^6$ (cm^2/s)	$\delta E/\delta\log(i/i_L-i)$ (mV)	αn [c]
Aldehydes	P	0.1	604/757	0.09/0.07	2	2.1	2.4	44/45 [d]	1.4
		1	590/745	1/1	2	2.6	3.2	50/50 [d]	1.2
	PL	0.1	750	0.08 [d]	2	1.1	0.6	64	0.9
		1	765	1.02 [d]	2	1.4	1.0	58	1.0
	PLP	0.1	718	0.20	2	2.7	3.7	50	1.2
		1	715	2.30	2	2.8	4.0	42	1.4
Oximes	PO	0.1	730/1420	0.62/0.30	4/2	8.4/4.0	8.9/8.3	69/95	0.9
		1	766/1460	7.02/3.20	4/2	9.1/4.3	10.3/9.0	71/85	0.8
	PLO	0.1	731/1440	0.54/0.26	4/2	7.3/3.8	7.6/7.8	43/52	1.4
		1	768/1566	5.8/3.1	4/2	7.7/4.2	7.3/8.6	43/44	1.4
	PLPO	1	785/1566	4.8/2.6	4/2	6.8/3.7	5.7/7.0	68/60	0.9
Acids	PC	0.1	1214	0.39	4	5.2	3.5	35	1.7
		1	1208	4.35	4	5.8	4.2	35	1.7
	PA	0.1	1264	0.38	4	5.2	3.4	36	1.6
		1	1264	4.50	4	5.8	4.2	36	1.6
Lactone	PAL	0.1	1170 [e]	0.43	4	5.8	4.3	34	1.7
		1	1200 [e]	5	4	6.2	5.1	34	1.7
Amines	AP	1	1460	3.45	2	11.0	4.8	77	0.8
	PM	0.1	1420	0.25	2	3.4	5.8	44	1.4
		1	1450	3.04	2	4.0	8.2	49	1.2
	PMP	0.1	1359 [f]	0.26	2	3.5	6.3	56	1.0

Where, c= concentration, $-E_{1/2}$= reduction potential, i_L= limiting current, n= number of electrons involved in the electronic process, I=diffusion constant , D= coefficient of diffusion, αn_a= coefficient of electronic transfer

[a] Experimental conditions: mass flow rate of mercury through the capillary, m=0.48 mg/s; dropping time, t=3 s; $I=i_d/c\ m^{2/3}t^{1/6} = 708nD^{1/2}$
[b] '/' separates information relative to the first and second reduction processes.
[c] Calculated from $\delta E/\delta\log(i/i_d-i)=-59/\alpha n_a$
[d] Kinetic control in the limiting zone
[e] Shoulder
[f] pH=5

Table 2. Differential Pulse Polarography results for c = 1 mM at pH=7 [a],[b]

Compound	$-E_p$ (mV)	i_p (μA)	n	$W_{1/2}$ [f] (mV)	b [f] (mV)
P	600/700	0.09/ 0.04	2	78	50/ 55
PL	754	0.03 [c]	2	82	57
PLP	709	0.17	2	45	35
PO	743/1400	0.27/ 0.14	4 / 2	107/ 121	70/ 70
PLO	758/1450	0.49/ 0.17	4 / 2	75/ 75	40/ 50
PC	1214	0.25	4	36	35
PA	1272	0.23	4	48	36
PAL	1172 [d]	0.39	4	44	35
PM	1448	0.19	2	73	45
PMP	1470 [e]	0.11	2		65

Where, $-E_p$= reduction potential, i_p= peak intensity, n= number of electrons involved in the electronic process, $W_{1/2}$= peak width at half height , b=RT/αnF

[a] Experimental conditions: m=0.48 mg/s, t=3 s
[b] '/'separates information relative to the first and second reduction processes
[c] Kinetic control
[d] Shoulder
[e] pH 5
[f] $W_{1/2}$, width of the peak at peak height/2; b=RT/αnF calculated by comparing the experimental results to a theoretical curve [27] for a reduction process where the electronic transfers are coupled to first or second order chemical reactions.

Table 3. Results for Cyclic Voltammetry for c=1 mM at pH=7 [a], [b]

Compound and concentration		$-E_p$(mV)	i_p(μA)	J(μA/cm^2)	$\lvert E_pE_{p/2}\rvert$ (mV)	αn [c]
P	(0.1mM)	624/781	0.38/0.10	27/7	36/ 42	1.3
	(1mM)	630/768	1.60/0.43	75/86	45/40	1.1
PL	(0.1mM)	826	0.15	11	53	0.9
	(1mM)	825	1.45	104	64	0.7
PLP	(0.1mM)	745	0.63	45	36	1.3
PO	(0.1mM)	800/ 1458	1.1/0.73	79/53	70/ 53	0.7
	(1mM)	814/1503	13.0/8.0	1007	67/70	0.7
PLO	(0.1mM)	800/ 1475	2.1/ 0.9	151/65	33/ 42	1.4
	(1mM)	810/1570	18.3/8.1	1316	34/20	1.4
PLPO	(1mM)	859/1623	10.2/7.0	734	71/53	0.7
PC	(0.1mM)	1233	0.8	58	17	2.9
	(1mM)	1240	9.6	712	30	1.6
PA	(0.1mM)	1264	0.8	58	36	1.3
	(1mM)	1270	10	719	29	1.6
PAL	(0.1mM)	1200	1.26	91	29	1.6
	(1mM)	1200	10.8	777	34	1.4
PM	(0.1mM)	1480	0.73	53	32	1.5
	(1mM)	1495	6.9	496	39	1.2
PMP	(0.1mM) [d]	1436	0.89	64	54	0.9
	(1mM)	1502	14.7	1057	68	0.7

Where, $-E_p$= peak potential, i_p= peak intensity, J= electronic current, αn= coefficient of electronic transfer

[a] Experimental conditions: m=0.48 mg/s, t=3 s,
[b] '/' separates information relative to the first and second reduction processes
[c] $|E_p-E_{p/2}|= 47.7/\alpha n_a$
[d] pH 5

Table 4. Corrected values for the reduction of aldehydes without the contribution of the hydration reaction.

Compound	C (mM)	$(i_L)_{exp}$ (μA)	i_L/i_K [a]	$(i_L)_{cal}$ (μA)	I (μA)	$Dx10^6$ (cm^2/s)	$Dx10^6$ [c] (cm^2/s)
P	0.1	0.18	1.70	0.306	3.74	7.0	8.4
	1	2.0		3.40	4.42	9.8	
PL	0.1	0.09	3.09	0.25	3.09	4.8	6.3
	1	1.02	(2.81) [b]	2.87	3.90	7.7	
PLP	0.1	0.27	1.03	0.28	2.88	4.2	4.2
	1	2.3		2.4	2.89	4.2	

Where, c= concentration, i_L= limiting current, i_K = intensity of the kinetic current, I= diffusion constant, D= coefficient of diffusion

[a] calculated from the modified Koutecky and Brdicka equation
[b] calculated from comparing limiting current at different pH values
[c] average value

Table 5. Catalytic signals for c=0.1 mM at pH=7 [a]

	CP		DPP		CV		
Compound	$-E_{1/2}$ (mV)	$-i_L$ (μA)	$-E_p$ (mV)	$-i_p$ (μA)	$-E_p$ (mV)	$-i_p$ (μA)	$(i_{cat}/i_f)n$ [c]
P	1739	31.0	1776	5.8	1883	36.8	154
PL	1738	5.8	1740	1.0	1773/1850	3.8/5.3	122
PLP	1805	9.6	1848	1.1	1725/1895	10.4/5.7	52
PO	1785	30.5	1800	6.2	1824	42.5	156
PLO	1837	6.7	1789	5.9	1779/1841	4.2/3.2	140
PC	1750	8.8	1782	1.1	1895	15.9	80
PA	-	-	-	-	1737/1883	4.4/5	48
PAL	-	-	-	-	1715/1850	10/7.6	56
PM	1832	14	1850	2.5	1832	16 [b]	44
PMP	1830	35	1875	4.3	1800	28.5	62

Where, $-E_{1/2}$= reduction potential, i_L= limiting current, $-E_p$= peak potential, i_p= peak intensity, i_{cat}= intensity of the catalytic current, i_f=limiting functional group current

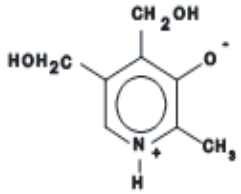
[a] **'/'** separates information relative to the first and second processes
[b] Two overlapped waves. Value of the total current observed.
[c] Ratio of catalytic current (i_{cat}) to limiting functional group current (i_f) corrected by the number of electron transferred in the electroactive group (n).

Table 6. Catalytic rate constants

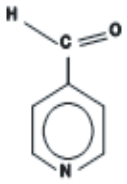
Compound	i_k (μA)	m (mg/s)	$Dx10^6$ (cm^2/s)	i_D [c] (μA)	i_k/i_D	$10^{-3}k_1$ [d] ($M^{-1}s^{-1}$)
P	31.0	0.55	0.4 [a]	0.16	193.8	199.6
PL	5.8	0.55	6.3 [a]	0.14	41.1	9.0
PLP	9.6	0.73	4.2 [a]	0.14	68.6	25.0
PO	30.5	0.48	9.6 [a]	0.16	189.8	191.5
PLO	6.7	0.48	7.5 [b]	0.14	46.9	11.7
PC	8.8	0.55	4.0 [b]	0.11	77.5	31.9
PM	14.0	0.55	7.9 [b]	0.16	87.7	40.9
PMP	35.0	0.73	7.0 [b]	0.18	192.9	197.7

(a) Based on values from table 1
(b) Average values from table 1
(c) Monoelectronic process (reaction: $Ox + 1e^- \rightarrow Red$; $Red + AH \rightarrow Ox + A^-$) ; $i_D=705nD^{1/2}cm^{2/3}t^{1/6}$
(d) $i_k/i_D=1\cdot12\cdot(k_1ct_1)^{1/2} \therefore k_1=(i_k/i_D\cdot12)^2\ 1/c\ t_1$. For maximum current [ref.31]

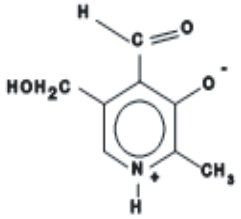
Figure 1.



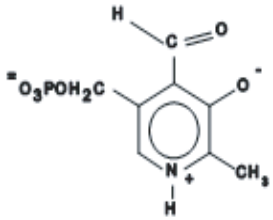
pyridoxine (PN)



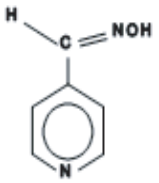
4-pyridine aldehyde (P)



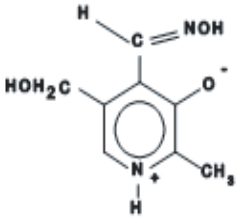
pyridoxal (PL)



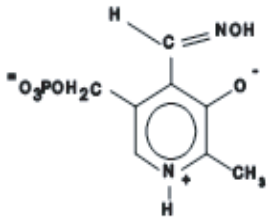
pyridoxal-5'-phosphate (PLP)



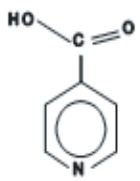
pyridin oxime (PO)



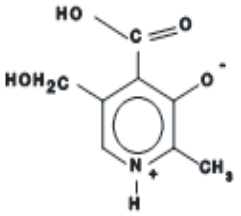
pyridoxal oxime (PLO)



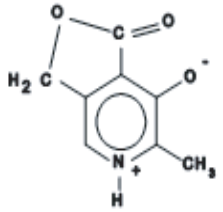
pyridoxal-5'-phosphate oxime (PLPO)



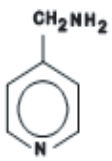
4-pyridin carboxilic acid (PC)



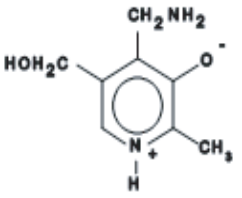
4-pyridoxic acid (PA)



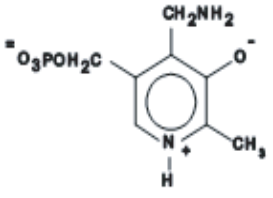
4-pyridoxic acid lactone (PAL)



aminomethyl pyridine (AP)



pyridoxamine (PM)



pyridoxamine-5'-phosphate (PMP)

Figure 2.

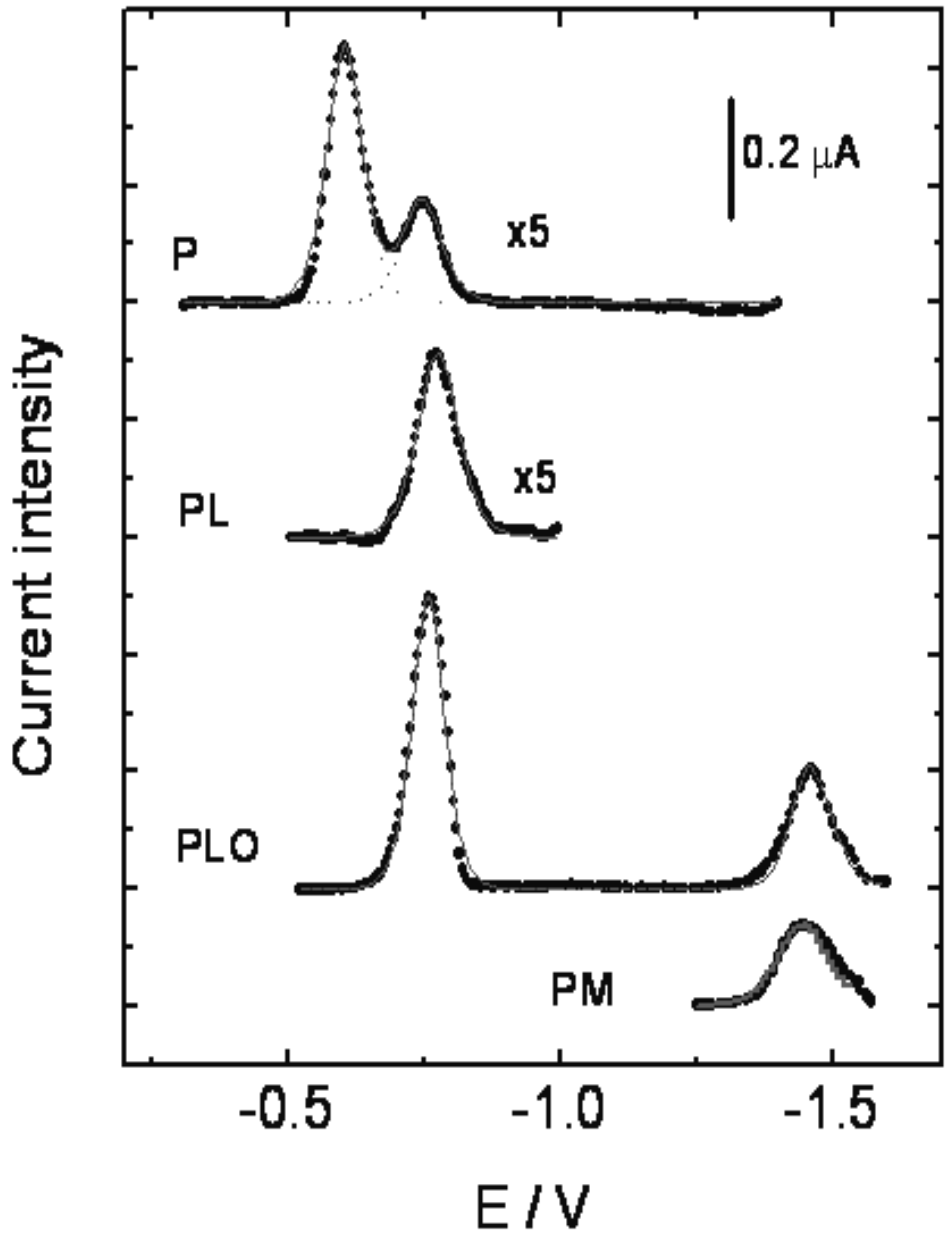


Figure 3

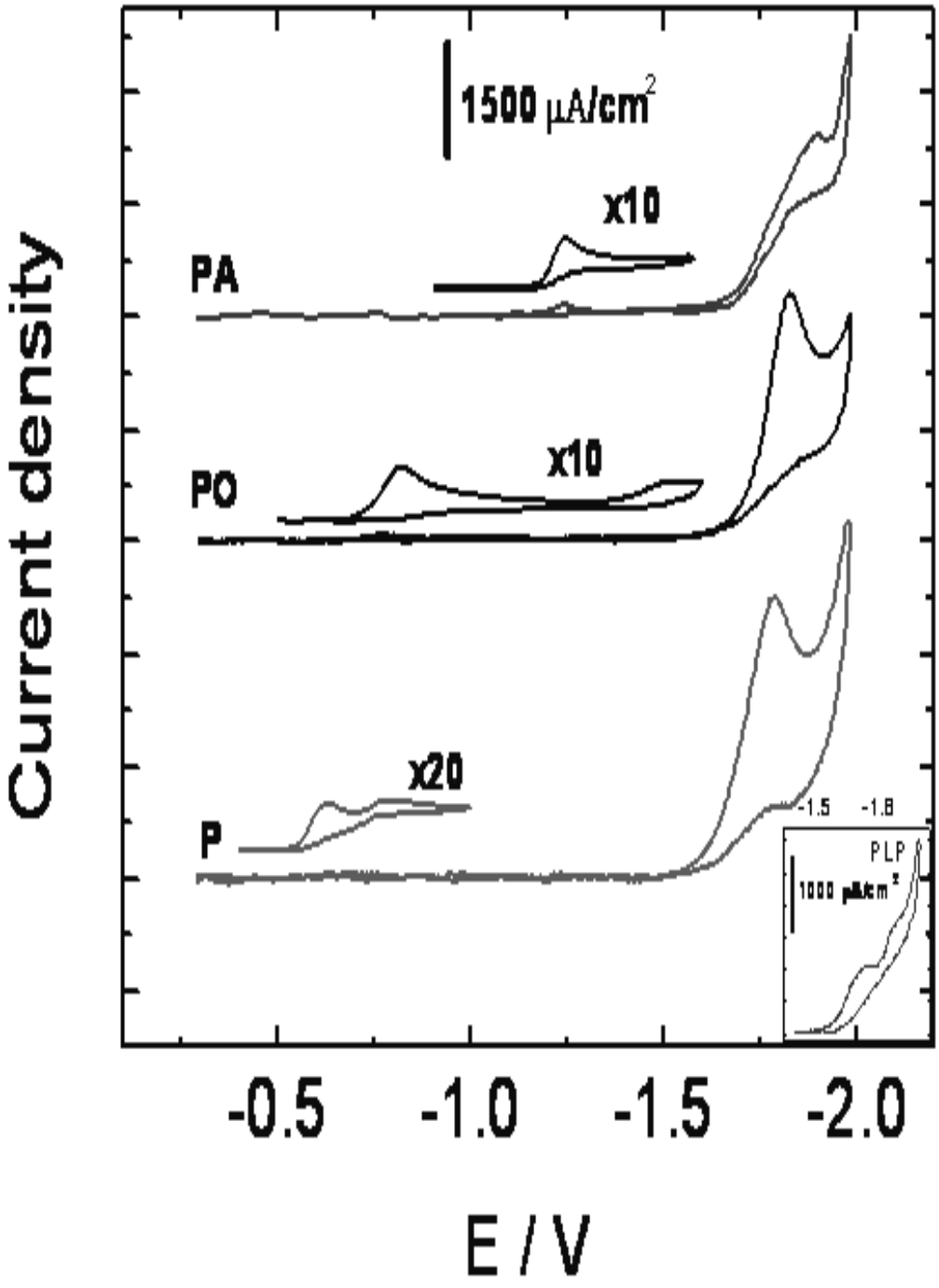

1500 μA/cm²
x10
PA
x10
PO
x20
P
-1.5
-1.8
PLP
Current density
-0.5
-1.0
-1.5
-2.0
E / V

Figure 4