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Electrocatalytic oxidation of NADH at graphite electrodes modified with osmium phenanthroline dione

Ionel Catalin Popescu^{a,*}, Elena Domínguez^a, Arántzazu Narváez^a, Valeri Pavlov^b, Ioanis Katakis^b

^a *Departamento de Química Analítica, Universidad de Alcalá, E-28871 Alcalá de Henares, Madrid, Spain*

^b *Departament d'Enginyeria Química, Escola Tècnica Superior d'Enginyeria Química, Universitat Rovira i Virgili, E-43006 Tarragona, Catalonia, Spain*

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Abstract

We report here a detailed study concerning the electrochemical behavior of Os(4,4'-dimethyl, 2,2'-bipyridine)₂(1,10-phenanthroline 5,6-dione) complex, adsorbed on spectrographic graphite, and about its electrocatalytic activity for NADH oxidation. Cyclic voltammetric measurements, performed in aqueous phosphate buffer solutions, at different scan rates and pH values, allowed us: (i) to relate the redox response of the *o*-quinone ligand (phendione) to that of the Os(II) central ion; (ii) to confirm that, in aqueous solutions, the phendione based redox process globally involves two electrons and two protons; (iii) to estimate the rate constant for the heterogeneous electron transfer corresponding to the phendione redox couple ($k_s \approx 20.1 \text{ s}^{-1}$). The second order rate constant for electrocatalytic oxidation of NADH ($k_{1,[\text{NADH}] = 0} = 1.9 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$, at pH 6.1) as well as its pH dependence (from pH 5.5 to 8.1) were evaluated from RDE experiments, using both Koutecky–Levich and Lineweaver–Burk data interpretations. © 1999 Elsevier Science S.A. All rights reserved.

Keywords: NADH; Electrocatalytic oxidation; Os-phendione; Modified electrode

1. Introduction

The mediated electro-oxidation of β -nicotinamide adenine dinucleotide (NADH) is interesting both from the theoretical and practical point of view. Thus, as it was summarized recently [1,2], it appears that depending on the mediator nature this reaction may: (i) involve the formation of an intermolecular complex between NADH and mediator, as in the case of redox dyes [3–9]; (ii) proceed through a net hydride transfer, as for *o*- and *p*-quinones and some aromatic diamines [10–18]; (iii) take place by two one-electron steps with

a proton transfer to the solvent, as for some polyoxometallates [19,20]. The efficient and reversible recycling of NADH is of particular interest in the construction of dehydrogenase based amperometric biosensors because more than 300 enzymes require nicotinamide co-enzymes as cofactors, and in the fine chemicals industry using NAD^+ -dependent biocatalysts.

Since the direct electro-oxidation of NADH on different conventional electrode materials requires high overpotentials [13,21,22], it is desirable to discover efficient electrocatalysts. The major electrochemical configurations already proposed for such catalytic recycling of NADH were examined critically in comprehensive works [1,2,5,23]. Among these mediating schemes, those based on tetracyanoquinodimethane (TCNQ) [24,25] and a phenoxazinium salt (Meldola blue) [3] gave the highest sensitivities and second order rate

* Corresponding author. Fax: +34-91-8854646.

E-mail address: prof.visit@alcala.es (I. Catalin Popescu)

¹ Permanent address. Department of Physical Chemistry, University 'Babes-Bolyai', RO-3400 Cluj-Napoca, Romania.

constants for the NADH mediated electro-oxidation. However, the TCNQ-modified electrode has as a major drawback the high value of applied potential (about +0.4 V vs. Ag | AgCl | KCl_(sat)), while the Meldola blue scheme has a low chemical stability, especially at high pH values.

In this work, based on some early results [26–28] showing that 1,10-phenanthroline-5,6-dione complexes of Os(II) exhibit electrocatalytic activity towards NADH oxidation, we report on a detailed study concerning the electrochemical behavior of Os(4,4'-dimethyl,2,2'-bipyridine)₂(1,10-phenanthroline-5,6-dione) complex (Os-phendione), adsorbed on spectrographic graphite. Thus, cyclic voltammetry, performed under different experimental conditions, was used to characterize the intrinsic electrochemical behavior of the adsorbed mediator. Then, the kinetics of the mediated electro-oxidation of NADH at an Os-phendione modified graphite electrode were investigated under defined mass transport conditions, using rotating disk electrode experiments.

2. Experimental

2.1. Chemicals

β -Nicotinamide adenine dinucleotide (NADH) was purchased from Sigma (St. Louis, MO, USA) as the disodium salt. Analytical grade, anhydrous acetonitrile was obtained from SDS (Peypin, France). All other reagents were of analytical grade and used as received. Water was obtained by means of a Milli-Q system (Millipore, Milford, MA, USA).

The Os(4,4'-dimethyl, 2,2'-bipyridine)₂(1,10-phenanthroline 5,6-dione) (Os-phendione) complex was synthesized according to procedures published earlier [26,29] and isolated finally as the PF₆⁻ salt form, which is insoluble in water.

2.2. Preparation of Os-phendione modified electrodes

A spectrographic graphite rod (Ringsdorff-Werke, GmbH, Bonn-Bad Godesberg, Germany), of ca. 3 mm diameter, was wet polished on fine (grit 400 and 600) emery paper (Buehler, Lake Bluff, IL, USA). Then, a graphite piece of suitable length was washed carefully with deionized water, dried, and finally press-fitted into a Teflon holder in order to obtain a graphite electrode having a flat circular surface of ca. 0.071 cm², in contact with the solution.

The modified graphite electrodes were obtained by spreading onto the electrode surface 1 μ l of 2 mM Os-phendione solution in acetonitrile, and leaving them for 20 min at room temperature to evaporate the solvent. Before immersion in the test solution the

modified electrodes were washed carefully with deionised water. For each electrode, the surface coverage was estimated from the under-peak areas (corresponding to the phendione ligand), recorded during the cyclic voltammetry measurements, and considering as 2 the number of exchanged electrons [30].

2.3. Electrochemical measurements

Cyclic voltammetry (CV) measurements were carried out in a conventional three electrode electrochemical cell. Ag | AgCl | KCl_(sat) and a coiled Pt wire served as reference and counter electrodes, respectively. The cell was connected to a computer-controlled BAS CV-50W voltammetric analyzer (Bioanalytical Systems, West Lafayette, IN, USA). As noticed, by changing the distance between the working and reference electrode, the uncompensated resistance in solution was negligible.

Steady state amperometric measurements at different rotating speeds of the working electrode were performed using a modulated speed rotator (model AMS-FRX, Pine, Grove City, PA, USA) and the same spectrographic graphite as the disk material. The current intensity-time data were collected using the already mentioned voltammetric analyzer. After the RDE experiments the surface coverage was checked by CV and found to be within $\pm 10\%$ of the initial value.

The supporting electrolyte was a 0.1 M phosphate buffer containing 0.15 M NaCl. The pH was adjusted in the interval 3 to 8 using the appropriate NaOH or H₃PO₄ solutions. For all measurements the solutions were deaerated by nitrogen bubbling for 15 min.

3. Results and discussion

3.1. Electrochemical behavior of the Os-phendione complex adsorbed on spectrographic graphite

The electrochemical behavior of free 1,10-phenanthroline 5,6-dione (phenidone) [26,30–32,35–37] as well as complexed with Ni(II), Co(II), Zn(II) [31–33] or with Cr(III), Fe(III), Ni(II), Co(II), Cu(I), Cu(II), Os(II) and Ru(II) [26,27,34–37] has retained the interest of many researchers. Globally, it has been established that the phendione voltammetric response presents one or two distinct, quasi-reversible and pH dependent waves, in protic or aprotic solvents, respectively [26,27,30–32,35–37], shows a positive shift of the half wave potential due to the divalent metal's complexation [26,31,33,37], exhibits a loss of ligand electroactivity due to the proton or metal cations binding to the pyridine nitrogen sites [35,36] and is affected strongly by the solubility and adsorption differences existing between oxidized and reduced forms of the free ligand and the complex [37].

In a protic solvent the two-electron two-proton reduction, characteristic of the quinone moiety, is evidenced on the corresponding voltammogram by a single wave [26,27,30–32]. In accordance with this, the cyclic voltammogram recorded in an aqueous phosphate buffer solution for the Os-phendione complex adsorbed on the surface of a graphite electrode (Fig. 1) presents also one wave for the phendione ligand, with a formal standard potential of +0.08 V versus $\text{Ag}|\text{AgCl}|\text{KCl}_{(\text{sat})}$ at pH 6.0. At a scan rate of 50 mV s^{-1} , the peak separation ($\Delta E_p = 54 \text{ mV}$), the ratio of the peak current intensities ($I_{\text{pa}}/I_{\text{pc}} = 1.02$) and the full width at half maximum ($E_{\text{FWHM}} = 100 \text{ mV}$ for the anodic peak and 120 mV for the cathodic one) indicate that it is a quasi-reversible voltammetric wave, and reveal the presence of some repulsive interactions between the adsorbed species [38,39]. Moreover, as expected for surface confined redox active species [40] the cyclic voltammograms recorded at low potential scan rates ($2\text{--}25 \text{ mV s}^{-1}$) showed a linear dependence of the peak currents, corresponding to the phendione wave, on the electrode potential scan rate.

The ratio between the phendione and Os(III/II) integrated peaks, corrected for the background current, offers an indirect way to estimate the number of electrons exchanged in the phendione oxido-reduction process. For example, at pH 7, these ratios, and consequently the number of electrons, were found 2.0 ± 0.2 and 2.1 ± 0.1 for the anodic and cathodic peaks, respectively (the results are the average of four independent measurements for the phendione wave, $E^{\circ'} = +0.032 \text{ V}$ vs. $\text{Ag}|\text{AgCl}|\text{KCl}_{(\text{sat})}$, and for the Os(III/II) wave, $E^{\circ'} = +0.703 \text{ V}$ vs. $\text{Ag}|\text{AgCl}|\text{KCl}_{(\text{sat})}$).

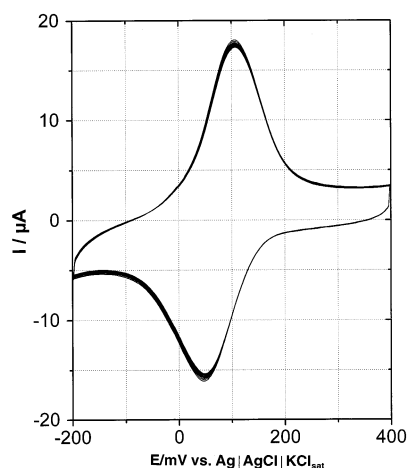


Fig. 1. Consecutive cyclic voltammograms for phendione of the Os-phendione complex, adsorbed on spectrographic graphite. Experimental conditions: starting potential, +400 mV vs. $\text{Ag}|\text{AgCl}|\text{KCl}_{(\text{sat})}$; scan rate, 50 mV s^{-1} ; deoxygenated, 0.1 M phosphate buffer, pH 6.0; number of cycles = 10.

In order to check the stability of the system, the potential of the modified electrode was cycled continuously over the range of -0.2 to $+0.4 \text{ V}$ and the resulting voltammograms were recorded (for convenience, in Fig. 1 only the first ten cycles are shown). A progressive decrease of the anodic and cathodic peak intensity was observed with a tendency towards a lower limit, while the voltammogram shape remained invariant. This behavior proves the good electrochemical stability of the Os-phendione complex, in the potential range used for the NADH recycling, and a relatively strong adsorption on the graphite surface.

As expected for the redox behavior of a compound containing a quinone moiety, the redox potential of the complexed phendione (estimated as the average of cathodic and anodic peak potentials) was pH-dependent, according to the reaction:



where OsQ, OsQH₂ denote the Os complexed phendione and its reduced form, respectively. The pH range explored was restricted deliberately to between 3 and 8 because of the phendione chemical instability [33] at pH values higher than 8, and of NADH hydrolysis in acid solutions [28,41]. The slope of the regression line, $E^{\circ'}/\text{V} = 0.383 - 0.049 \times \text{pH}$, ($r = 0.998$; $n = 11$), estimated from the experimental data in the pH range investigated, does not agree with the theoretical value for a redox process involving an equal number of electrons and protons. However, taking into account the acid–base behavior of the hydroquinone/quinone functionality of the phendione ligand, the best fit between the calculated and the experimental data was obtained when the corresponding $\text{p}K_a$ value was taken as 6.3. Thus, the regression equation becomes: $E^{\circ'}/\text{V} = 0.429 - 0.0591 \times \text{pH} + 0.029 \times \log[1 + 10^{-(6.3 - \text{pH})}]$. In this context it is worth mentioning that from the pH dependence of the formal standard potential for the free phendione, dissolved in a partial aqueous solvent (50% dimethylsulfoxide), it was established [30] that the quinone form is unprotonated, while the hydroquinone one exhibits a $\text{p}K_a$ of 9.0. This acid shift observed for the $\text{p}K_a$ of complexed phendione is consistent with the positive shift (+185 mV, at pH 7) of the formal redox potential of the phendione induced by Os complexation, and with the similar behavior reported previously for divalent metals [26,31,33,37]. This phenomenon possibly also explains the higher stability of the phendione ligand when complexed, compared to the free ligand oxidation.

Using the treatment proposed by Laviron [42], from the variation of the peak potentials with the potential scan rate, the heterogeneous electron transfer rate constant (k_s) for the phendione redox process was estimated. Within experimental error, this rate constant appeared to be independent of pH, in the pH range

(6.5–8) used for NADH mediated electro-oxidation. At pH 6.5, the average value for k_s (20.1 s^{-1}) was higher than that reported for Meldola blue (10 s^{-1}) [3] and of the same order of magnitude as those measured recently for dihydroxybenzaldehyde redox active films [43,44]. Based on the electrochemical behavior of the graphite adsorbed Os-phendione complex, it is concluded that this is electrochemically a quasi-reversible redox couple, involving two electrons and two protons (at $\text{pH} < 6.3$) and having good chemical reversibility in aqueous solutions, a fact that makes it suitable for use as an NADH electro-oxidation catalyst.

3.2. Electrocatalytic oxidation of NADH at an Os-phendione modified graphite electrode

Many compounds containing quinone moieties have been shown previously to be active electrocatalysts for NADH oxidation (for a detailed discussion see [1,2,23,45]). However, it was stressed [1] that these mediators, due to their intrinsic reactivity, can be a source of side reactions that might eventually limit the efficiency and life time of the mediating system. Recently, a new strategy was demonstrated [26–28] that consists of the complexation of a quinoid structure with a transition metal. Due to the supposed higher stability of the radicals involved in the NADH oxidation with such complexes, an improved electrocatalytic activity and stability are expected to be achieved.

Fig. 2(A) shows the phendione cyclic voltammograms obtained for a graphite electrode modified with Os-phendione, in the absence and in the presence of NADH. A dramatic enhancement of the anodic peak currents, associated with the progressive diminishing of the cathodic one, when the NADH concentration was increased, proves the strong electrocatalytic effect of the Os-phendione complex. At pH 7, the catalytic peak was found at +60 mV versus $\text{Ag}|\text{AgCl}|\text{KCl}_{(\text{sat})}$, a significantly lower value compared to the uncatalyzed electro-oxidation of NADH, reported for bare graphite electrodes: +410 mV versus SCE [14] or +550 mV versus SCE [21,46]. Consequently, the decrease of the NADH electro-oxidation overvoltage, due to the electrocatalytic activity of the Os-phendione complex, is greater than 400 mV.

A qualitative estimation of the response rate and signal stability, due to the mediating configuration investigated, is furnished by the rotating disk electrode (RDE) results presented in Fig. 2(B). It can be noticed that, after a change of the electrode rotation speed, the new steady state is reached in less than 10 s.

The sensitivity of the Os-phendione-modified NADH sensor, for stationary ($13.2 \text{ mA M}^{-1} \text{ cm}^{-2}$) as well as for rotating disk electrode ($55.6 \text{ mA M}^{-1} \text{ cm}^{-2}$), estimated from the slope of the linear part of the current intensity versus NADH concentration plot, indicates

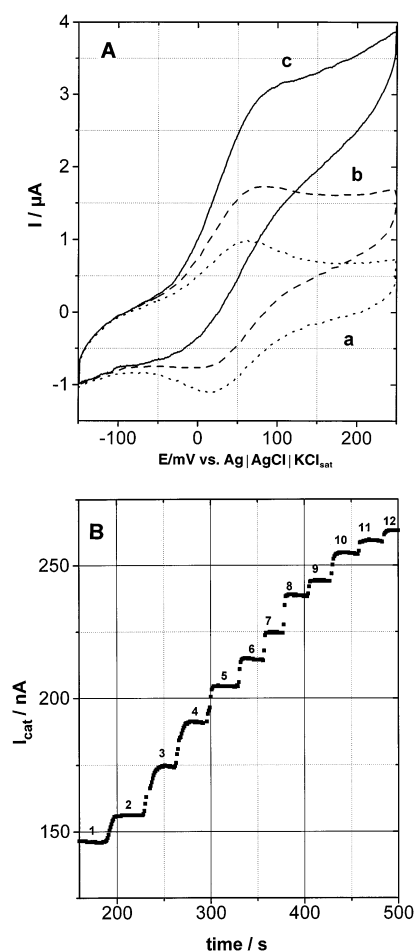


Fig. 2. Electrocatalytic oxidation of NADH on Os-phendione modified graphite electrode: (A) Cyclic voltammetry of modified electrodes: (a) in buffer; (b) in 0.5 mM of NADH; (c) in 2.0 mM of NADH. (B) Steady state current intensity response at increasing rotation speeds for an Os-phendione modified graphite rotating disk electrode. Experimental conditions: (A) surface coverage, $2.2 \times 10^{-10} \text{ mol cm}^{-2}$; scan rate, 10 mV s^{-1} ; starting potential, +250 mV vs. $\text{Ag}|\text{AgCl}|\text{KCl}_{(\text{sat})}$; deoxygenated, 0.1 M phosphate buffer, pH 7.0; (B) rotation speed; (1) 10 rpm; (2) 15 rpm; (3) 25 rpm; (4) 45 rpm; (5) 75 rpm; (6) 115 rpm; (7) 215 rpm; (8) 415 rpm; (9) 815 rpm; (10) 1615 rpm; (11) 2615 rpm; (12) 3615 rpm; surface coverage, $2.3 \times 10^{-10} \text{ mol cm}^{-2}$; applied potential, +200 mV vs. $\text{Ag}|\text{AgCl}|\text{KCl}_{(\text{sat})}$; 0.1 mM NADH; deoxygenated, 0.1 M phosphate buffer, pH 7.0.

that the Os-phendione/graphite configuration is among the most sensitive configurations for NADH detection (for comparison see table 1 from Ref. [1] and table 5 from Ref. [2]).

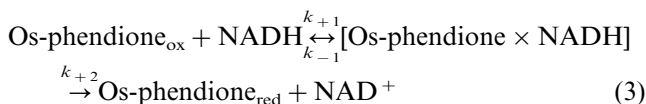
Kinetic measurements of NADH electrocatalytic oxidation at Os-phendione modified graphite electrodes were performed at different NADH concentrations and pH values, using the RDE technique. In all cases, in order to have a global electrode process controlled by the chemical reaction between NADH and the mediator, the applied potential was taken more positive by 120 mV [4] than the actual value of the formal standard potential of the Os-phendione mediator. The well behaved mixed control (diffusional–kinetic), characteris-

tic for the system investigated, is exemplified by the Levich plot (Fig. 3), obtained at pH 6.1, in the NADH concentration range from 0.04 to 2.28 mM, and for rotation speeds from 10 to 3615 rpm.

Based on the hypothesis that a substrate-mediator complex is involved in the mechanism of NADH electrocatalytic oxidation (Michaelis–Menten kinetic model), a general equation describing the dependence of the catalytic current (I_{cat}) on the experimental parameters was proposed [3,4]:

$$\frac{1}{I_{\text{cat}}} = \frac{1}{(nF\Gamma k_{+2})} + \left[\frac{K_M}{(nF\Gamma k_{+2})} + \frac{1}{(0.62nFAD^{2/3}\nu^{-1/6}\omega^{1/2})} \right] \frac{1}{(c_{\text{NADH}})} \quad (2)$$

where $K_M = (k_{-1} + k_{+2})/k_{+1}$, D ($\text{cm}^2 \text{s}^{-1}$) represents the diffusion coefficient of NADH, ν ($\text{cm}^2 \text{s}^{-1}$) the hydrodynamic viscosity, ω (rad s^{-1}) the rotational speed, and c_{NADH} (mol cm^{-3}) the NADH concentration. It is worth mentioning, that it is more convenient to replace the product $nF\Gamma$ by the coulometric charge, obtained experimentally as an average value from the integration of the anodic and cathodic peaks corresponding to the mediator voltammetric wave [44]. The rate constants, k_{-1} , k_{+1} and k_{+2} , appear in the following reaction scheme



In Table 1 are shown the results of the RDE measurements, treated according to both variants proposed [3–5] for the estimation of the relevant kinetic param-

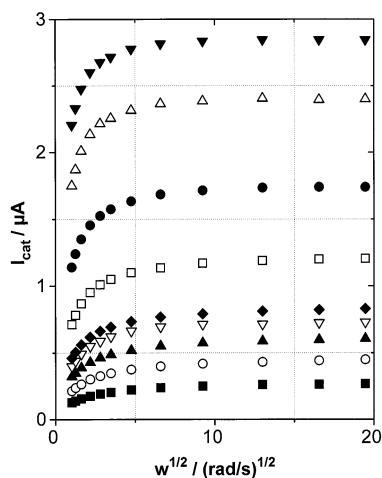


Fig. 3. Levich plot of the steady state electrocatalytic response for an Os-phendione modified graphite RDE at different NADH concentrations (0.04 mM (■), 0.08 mM (○), 0.12 mM (▲), 0.16 mM (▽), 0.20 mM (◆), 0.36 mM (□), 0.68 mM (●), 1.30 mM (△), 2.28 mM (▼). Experimental conditions: surface coverage, 2.6×10^{-10} mol cm^{-2} ; applied potential, +200 mV vs. Ag|AgCl|KCl_{sat.}; deoxygenated, 0.1 M phosphate buffer, pH 6.1.

eters K_M and k_{+2} : (i) the so called ‘Koutecky–Levich’ (KL) interpretation, in fact a $1/I_{\text{cat}}$ versus $\omega^{-1/2}$ plot at a constant NADH concentration; (ii) the so called ‘Lineweaver–Burk’ (LB) interpretation, which is a $1/I_{\text{cat}}$ versus $1/c_{\text{NADH}}$ plot at constant rotational speed.

In order to compare the values provided by the two variants of data treatment, the same experimental data set was used. It was not surprising to notice that both procedures gave practically the same result for k_{+2} , but different values for K_M (Table 1). The discrepancy can be explained if it is observed that, in the K_M calculation according to the KL treatment only the linear regression intercepts are involved, while in the LB procedure both linear regression parameters are used. Moreover, examining the partial results for both data treatments, shown in Table 1, it can be stated that due to the better precision, reflected by the smaller standard deviations of the linear regression parameters, the KL method of data treatment is preferable.

Because of the strong dependence of the second order reaction constant (k_1 , $\text{M}^{-1} \text{s}^{-1}$) for the NADH mediated electro-oxidation on the NADH concentration, defined for the above mentioned reaction scheme, it is unsuitable for a comparison of the catalytic efficiency for different mediating schemes. That is why it was proposed [3] to use its extrapolated value to zero NADH concentration ($k_{1,[\text{NADH}]=0}$), estimated as the k_{+2}/K_M ratio. In Table 2 are presented the $k_{1,[\text{NADH}]=0}$ calculated values for the Os-phendione based mediating configuration, measured at different pH values (from 5.5 to 7.0). As was reported previously for phenoxazine derivatives [5] and 3,4-dihydroxybenzaldehyde (3,4-DHB) electropolymerized films [44], the $k_{1,[\text{NADH}]=0}$ values decrease significantly as the pH increases. In our case the estimated value for $k_{1,[\text{NADH}]=0}$ at pH 7.0 is roughly 30 times lower than the corresponding value for Meldola blue ($2.7 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$, at pH 7.0) [3] and a quarter of the value ($4.3 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$, at pH 7.0) reported for a glassy carbon electrode covered by a 3,4-DHB film [44].

In this context, is it interesting to remark that, within experimental error and in spite of the inherent differences between the systems compared (for example the surface coverage of the electrodes), a surprisingly good agreement is observed between the heterogeneous electron transfer rate constants for the two mediators compared (Os-phendione $k_s = 20.1 \text{ s}^{-1}$, pH 6.5–8.0; 3,4-DHB $k_s = 25 \text{ s}^{-1}$, at pH 7.0). Moreover, it was observed that the linear correlation between E^0 and $\log k_{1,[\text{NADH}]=0}$, found by Gorton [5] for some phenoxazine derivatives, extends to the 3,4-DHB and Os-phendione mediators too (Fig. 4) [The corresponding linear regression equation is: $\log k_{1,[\text{NADH}]=0} = 2.97 + 0.0059 \times E^0$ with the correlation coefficient $r = 0.994$]. Once more, it appears that the electrocatalytic efficiency of different systems used for NADH oxidation is con-

Table 1
Results of data interpretation for electrocatalytic NADH oxidation at an Os-phendione modified RDE ($\Gamma = 2.6 \times 10^{-10}$ mol cm $^{-2}$; pH 6.1)

[NADH]/mM	Linear regression parameters			k_{+2}/s^{-1}	K_M/mM	$10^6 D_{NADH}/cm^2 s^{-1}$
	A/s_A	B/s_B	r/n^a			
<i>'Koutecky–Levich' interpretation</i>						
0.04	$0.00353/3 \times 10^{-5}$	$0.00457/6 \times 10^{-5}$	0.9992/12			
0.08	$0.002101/7 \times 10^{-6}$	$0.00264/2 \times 10^{-5}$	0.9998/12			
0.12	$0.001582/7 \times 10^{-6}$	$0.00159/1 \times 10^{-5}$	0.9996/12			
0.16	$0.00128/1 \times 10^{-5}$	$0.00123/3 \times 10^{-5}$	0.9980/12			
0.20	$0.001153/3 \times 10^{-6}$	$0.001042/6 \times 10^{-6}$	0.9998/12	0.80 ^b	0.43 ^b	4.2 ^c
0.36	$0.000792/5 \times 10^{-6}$	$0.00061/1 \times 10^{-5}$	0.9982/12			
0.68	$0.000554/3 \times 10^{-6}$	$0.000322/8 \times 10^{-6}$	0.9972/12			
1.30	$0.000403/3 \times 10^{-6}$	$0.000171/6 \times 10^{-6}$	0.9930/12			
2.28	$0.000342/2 \times 10^{-6}$	$0.000110/4 \times 10^{-6}$	0.9918/12			
<i>'Lineweaver–Burk' interpretation</i>						
$\omega/rad s^{-1}$						
1.05	$0.00041/7 \times 10^{-5}$	$0.254/6 \times 10^{-3}$	0.9982/9			
1.57	$0.00038/6 \times 10^{-5}$	$0.232/4 \times 10^{-3}$	0.9987/9			
2.62	$0.00036/5 \times 10^{-5}$	$0.206/4 \times 10^{-3}$	0.9985/9			
4.71	$0.00035/4 \times 10^{-5}$	$0.181/4 \times 10^{-3}$	0.9987/9			
7.85	$0.00035/4 \times 10^{-5}$	$0.165/3 \times 10^{-3}$	0.9986/9			
12.0	$0.00035/4 \times 10^{-5}$	$0.155/3 \times 10^{-3}$	0.9986/9			
22.5	$0.00036/4 \times 10^{-5}$	$0.141/3 \times 10^{-3}$	0.9982/9	0.75 ^d	0.39 ^e	6.1 ^e
45.5	$0.00036/4 \times 10^{-5}$	$0.131/3 \times 10^{-3}$	0.9979/9			
85.3	$0.00036/4 \times 10^{-5}$	$0.124/3 \times 10^{-3}$	0.9979/9			
169.1	$0.00038/4 \times 10^{-5}$	$0.118/4 \times 10^{-3}$	0.9979/9			
273.8	$0.00038/4 \times 10^{-5}$	$0.116/3 \times 10^{-3}$	0.9970/9			
378.6	$0.00038/4 \times 10^{-5}$	$0.115/3 \times 10^{-3}$	0.9975/9			

^a r , Correlation coefficient; n , number of experiments.

^b Estimated from dependence of 'K–L' plot intercepts on $1/[NADH]$ $Y = 0.00034 + 0.146 \times [NADH]^{-1}$ ($r = 0.9961$; $n = 8$).

^c Estimated from dependence of 'K–L' plot slopes on $1/[NADH]$ $Y = 8.3 \times 10^{-6} + 0.204 \times [NADH]^{-1}$ ($r = 0.9976$; $n = 8$).

^d Estimated from the average (36.5 ± 1.2 nA $^{-1}$) of 'L–B' intercepts.

^e Estimated from the 'L–B.' slope dependence on $\omega^{-1/2}$. $Y = 0.1071 + 0.159 \times \omega^{-1/2}$ ($r = 0.9997$; $n = 11$).

trolled strongly by the thermodynamic driving force for electron exchange. This observation would point to the direction that the limits of molecular engineering for synthesis of more efficient NADH oxidation electrocatalysts have not been explored fully.

4. Conclusions

The Os(4,4'-dimethyl, 2,2'-bipyridine) $_2$ (1,10-phenanthroline 5,6-dione) complex is adsorbed strongly on graphite surfaces. Its quasi-reversible electrochemical behavior in aqueous solutions involves two electrons and two protons, with an apparent heterogeneous rate constant ($k_s \approx 20.1$ s $^{-1}$)

The adsorbed Os-phendione complex oxidises NADH reversibly and the electrochemical analysis provides evidence that a charge transfer complex is involved in the NADH oxidation mechanism that is apparently controlled by the thermodynamic driving force for electron exchange [5].

The electrocatalytic activity, a good electrochemical

stability and the high responses observed show that this is an advantageous strategy for NADH electrocatalytic oxidation. Taking into account that in our previous study [28] it was also shown that the resulting NAD $^+$ is enzymatically active, it can be stated that the Os-phendione based configuration fulfils well the expected conditions for a successful mediating scheme for the construction of NAD $^+$ -dependent dehydrogenase electrodes. For such electrodes to be reagentless, the coimmobilization of the enzymes, the Osphendione mediator and NAD $^+$ is now being attempted.

Table 2
pH dependence of the rate constant for the NADH electrocatalytic oxidation at an Os-phendione modified graphite electrode (Koutecky–Levich interpretation)

10^{10} Surface coverage/ mol cm $^{-2}$	pH	$10^{-3}k_{1,[NADH]=0}/$ M $^{-1}$ s $^{-1a}$
2.3	5.5	3.1
2.6	6.1	1.9
2.4	7.0	0.9

^a Extrapolated value for zero NADH concentration.

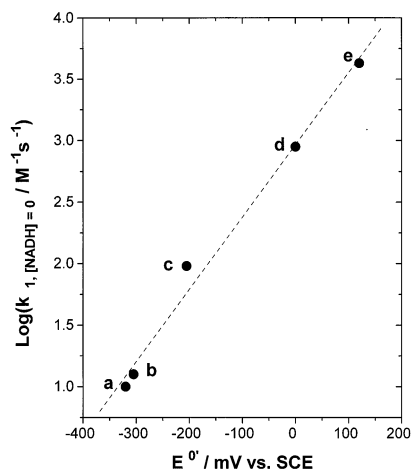


Fig. 4. Dependence of $\text{Log } k_{1,[\text{NADH}]=0}$ on the formal standard potential (E^0) for some phenoxazine dyes: ethyl capri blue, methyl capri blue and galloycyanine (a–c) [5], Os-phendione (d) and 3,4-dihydroxybenzaldehyde electropolymerized film (e) [44]. The mediators were adsorbed on graphite (a–d), or deposited on glassy carbon (e). All rate constants were measured at pH 7. Dashed line represents the least-squares fit.

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