Dedicated to Professor Ionel Haiduc,
President of The Romanian Academy at his 70th anniversary

GRAPHITE ELECTRODES MODIFIED WITH
3,7-di(*m*-AMINOPHENYL)-10-ETHYL-PHENOTHIAZINE

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ABSTRACT. The electrochemical behavior of a new functionalized phenothiazine derivative 3,7-di(*m*-aminophenyl)-10-ethyl-phenothiazine, adsorbed on spectrographic graphite, has been investigated. From cyclic voltammetric measurements, performed in aqueous buffer solutions of different pH values and at different potential scan rates, the rate constant of the heterogeneous electron transfer and the transfer coefficient were estimated. The electrochemical parameters of the voltammetric response and the slope of the linear regressions between the formal standard potentials and pH suggest a quasi-reversible $1e^-/1H^+$ redox process, involving a surface confined redox couple. From the dependence of peak current on the cycling time, the electrochemical stability of the modified electrode was estimated. From cyclic voltammetry measurements performed at +150 mV *vs.* SCE, the electrocatalytic efficiency toward NADH oxidation was evaluated and it was found ~ 67 %, recommending the new synthesized compound as a promising mediator for NADH electrocatalytic oxidation.

Keywords: phenothiazine derivatives, NADH oxidation, modified electrodes.

Introduction

The detection of reduced β-nicotinamide adenin dinucleotide (β-NADH) is very important in enzyme assays, due to its participation in the enzymatic catalysis of more than 300 dehydrogenases, useful both in bioprocesses and in analytical applications [1,2]. The electrochemical oxidation of NADH has been studied intensively during the last two decades [3,4]. Unfortunately, the direct electrochemistry of this cofactor suffers from a high overvoltage at most solid electrodes which leads to the formation of an enzymatic inactive form of NAD^+ and of other side products, causing important interferences [5,6].

All these observations point to different solutions to overcome the problems of electrode fouling and overvoltage. One of the most

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encountered solutions is the usage of chemically modified electrodes with surface adsorbed mediators.

It has been reported that several types of quinone derivatives, phenylenediamines, alkylphenazines and redox dyes, such as phenoxazine and phenothiazine derivatives, present catalytic activity towards NADH oxidation [7,8,9]. However, for various reasons, concerning the stability of the adsorbed mediator, fast heterogeneous electron transfer between the electrode and mediator, fast chemical redox reaction between adsorbed mediator and dissolved NADH etc., phenoxazine and phenothiazine mediators are often considered as being the most suitable in obtaining chemically modified electrodes for electrocatalytic oxidation of NADH [10]. A wide variety of these mediators was studied, including Nile Blue [11], Toluidine Blue [12], Methylene Green [13], Meldola Blue [14] and various derivatives of this parent structures.

In this paper, we report a detailed study on the electrochemical behavior of a new synthesized phenothiazine derivative: 3,7-di(meta-aminophenyl)-phenothiazine adsorbed on spectrographic graphite. Using cyclic voltammetry (CV) measurements, performed in different experimental conditions (scan rates and pH values), the rate constant (k_s) of the heterogeneous electron transfer and the transfer coefficient (α) were estimated as well as the electrocatalytic efficiency for NADH oxidation.

Experimental

Materials

The new functionalized phenothiazine namely 3,7-di(meta-aminophenyl)-10-ethyl-phenothiazine (DAPht) (Figure 1) was synthesized within the Organic Chemistry Department [15] and it was kindly given to us for research purpose.

β-Nicotinamide adenine dinucleotide, reduced form (NADH), was purchased from Sigma (St. Louis, MO, USA) as disodium salt. Phosphate buffer solutions were prepared using $K_2HPO_4 \cdot 3H_2O$ and KH_2PO_4 from Merck (Darmstadt, Germany). All other reagents were of analytical grade and used as received.
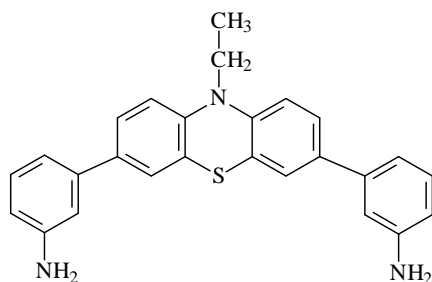
The supporting electrolyte was a 0.1 M phosphate buffer solution and the pH value was adjusted using appropriate H_3PO_4 or NaOH solutions.

Figure 1. Structure of DAPht

Electrode preparation

A spectrographic graphite rod (Ringsdorff-Werke, GmbH, Bonn-Bad Godesberg, Germany), of ~3 mm diameter, was wet polished on emery paper. Then, a graphite piece of suitable length was carefully press-fitted into a PTFE holder in order to obtain a graphite electrode, contacting the solution by means of flat circular surface of ~ 0.071 cm^2.

The modified graphite electrodes were obtained by spreading onto the electrode surface 2 µl of 10 mM solution of DAPht in dimethylsulfoxide and keeping it over night at room temperature in order to evaporate the solvent.

Electrochemical measurements

CV measurements were carried out in a conventional three-electrode electrochemical cell. A saturated calomel electrode (SCE) and a coiled Pt wire served as reference and counter electrode, respectively. The cell was connected to a computer-controlled voltammetric analyzer (Autolab-PGSTAT10, Eco Chemie, Utrecht, The Netherlands).
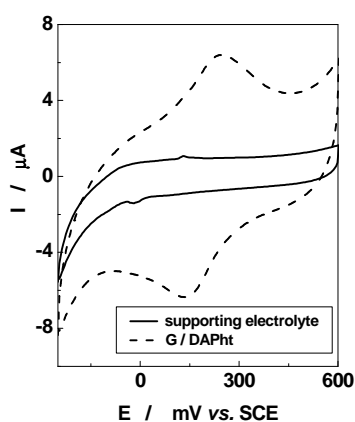
Results and discussions

Figure 2. Cyclic voltammograms for DAPht adsorbed on spectrographic graphite. Experimental conditions: starting potential, −250 mV *vs.* SCE; scan rate, 10 mV s^{-1}; supporting electrolyte, 0.1 M phosphate buffer (pH 7); surface coverage, see Table 1.

I.   Electrochemical behavior of the modified electrodes

The electrochemical behavior of DAPht adsorbed on spectrographic graphite was investigated in different working conditions using CV measurements. Figure 2 shows the electrochemical behavior of this compound. A well-defined peak pair with the formal potential around 200 mV *vs.* SCE (pH7) is observed (Table 1). The electrochemical parameters indicate a quasi-reversible redox process, taking into consideration the value of ΔE as criterion for the reversibility of the process. As the pH decreases the reversibility of the process decreases as well, suggesting that the reaction site is pH sensitive.

Table 1.

Electrochemical parameters of voltammetric response for DAPht adsorbed on the spectrographic graphite. Experimental conditions: see Figure 2.

pH	$E^{0'}$ (mV) vs. SCE	ΔE_{peak} (mV)	I_{pa}/I_{pc}	Γ (10^8 mol cm^{-2})
2	394	148	1.12	1.18
3	343	140	0.98	1.20
5	212	107	1.04	1.18
6	200	107	1.09	1.15
7	195	90	1.05	0.86

Cyclic voltammograms performed in a wide range of scan rates (5-1280 mV s^{-1}), showed a linear dependence of the current intensity on the scan rate (results not shown). Table 2 presents the parameters of the log-log linear regressions corresponding to the peak current dependence on the potential scan rate. The values of the slopes are dispersed around 1 for the reduction process suggesting that the reduced species are well adsorbed on the graphite surface. For the oxidation process, the values are smaller which leads to the assumption that the oxidized species are less adsorbed on the graphite surfaces. This can be explained by the repulsion existing between the positive charged species, generated on graphite electrode during the oxidation step.

Table 2.

Parameters of the log-log linear regressions corresponding to the peak current dependencies on the potential scan rate (5 to 1280 mV s^{-1}) for DAPht adsorbed on graphite. Experimental conditions: supporting electrolyte, 0.1 M phosphate buffer.

pH	Slope (A / V s^{-1})		R / no. of experimental points	
	Oxidation	Reduction	Oxidation	Reduction
3	0.49 ± 0.03	0.73 ± 0.04	0.996 / 4	0.993 / 8
5	0.69 ± 0.03	0.69 ± 0.01	0.993 / 10	0.999 / 10
7	0.54 ± 0.02	0.67 ± 0.02	0.990 / 12	0.996 / 8
9	0.57 ± 0.02	0.89 ± 0.03	0.991 / 15	0.996 / 8

Cyclic voltammograms were recorded also in a pH range from 1 to 8 with the scan rate of 5 mV s^{-1}. Figure 3 shows the linear dependence of the formal potential with the pH and the slope was close to 50.9 mV, suggesting a H$^+$/e$^-$ ratio of 1. The influence of the pH on the electrochemical behavior of the compound is explained by the presence of the free primary amino groups in the compound structure (a similar behavior was reported for thionine [16]) which have the ability to bind a proton. The presence of a positive charge deactivates the phenothiazinic nucleus and, hence, the redox process is more difficult to realize.

The kinetic parameters of the redox process (the heterogeneous electron transfer rate constant, k_s and the transfer coefficient, α) were determined using
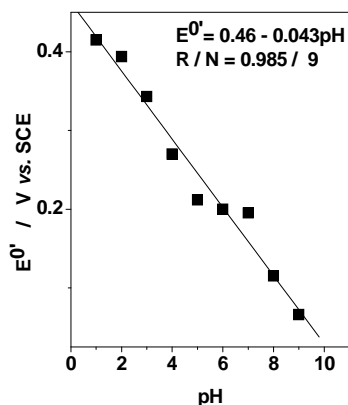
Figure 3. pH dependence of the formal potential of DAPht adsorbed on graphite. Experimental conditions: scan rate, 5 mV s^{-1}; supporting electrolyte, 0.1 M phosphate buffer.

Laviron's treatment [17] and they are shown in Table 3 for different pH values. It can be seen that the redox process is influenced by pH, which can be explained by the fact that the presence of a positive charge in the system makes the electron transfer at the electrode surface more difficult and hence, as the concentration of the hydrogen ions increases, the value of k_s decreases.

As compared to the value of k_s reported for phenothiazine (1,70 s^{-1} at pH 7 [18]) a much lower value of k_s (0.73 s^{-1}, at pH 7) was observed for DAPht. This is due probably both to the presence of ethyl group on the phenothiazinic nitrogen atom and to the mesomeric effect of the two meta-aminophenyl substituents on the phenothiazinic nucleus.

Table 3.

Kinetic parameters of the redox process for DAPht adsorbed on graphite.

pH	k_s (s^{-1})	α	R / no. of experimental points		Γ (10^8 mol cm^{-2})
			Oxidation	**Reduction**	
3	0.23	0.58	0.992 / 5	0.993 / 6	1.20
5	0.37	0.64	0.995 / 8	0.993 / 8	1.18
7	0.73	0.54	0.995 / 6	0.998 / 6	0.86
9	0.98	0.47	0.990 / 4	0.997 / 4	0.83

II. Electrochemical stability of the electrodes

The stability of the G/DAPht modified electrode was studied using repetitive CV measurements, by performing 25 consecutive scans at 50 mV s^{-1}. The dependence of peak current intensity on cycling time is shown in figure 4. It can be observed that the G/DAPht modified electrode presents a stable electrochemical response in time as the variation of peak current intensity on cycling time is almost constant both for cathodic and for anodic process.

III. Electrocatalytic activity for NADH oxidation

Taking into consideration the good electrochemical behavior of DAPht, its electrocatalytic activity for NADH oxidation was studied by cyclic voltammetry. Figure 5 presents the electrochemical behavior of DAPht

adsorbed on graphite, in the absence and in the presence of NADH dissolved in the buffer. The electrocatalytic efficiency for NADH oxidation estimated as $\dfrac{I_{[NADH]\neq0} - I_{[NADH]=0}}{I_{[NADH]=0}}$ ratio, measured at a potential of 150 mV *vs.* SCE, was found equal to 67 %. The electrocatalytic activity of the mediator is proved also by the decrease of the NADH electro-oxidation over potential with 400 mV, since at G/DAPht the catalytic peak was found at 150 mV *vs.* SCE while the uncatalyzed electro-oxidation of NADH at bare graphite electrodes was reported at 550 mV *vs.* SCE [5].
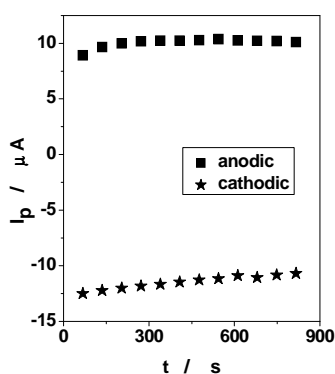
Figure 4. Time dependence of the peak current intensity for G/DAPht modified electrodes. Experimental conditions: scan rate, 50 mV s^{-1}; potential range, -250 to 600 mV *vs.* SCE; supporting electrolyte, 0.1M phosphate buffer (pH 7).
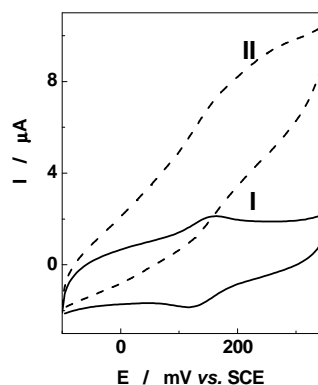
Figure 5. Electrocatalytic oxidation of NADH at G/DAPht modified electrode: (I) 0.1 M phosphate buffer (pH 7); (II) 0.1 M phosphate buffer containing 5 mM NADH. Experimental conditions: scan rate, 5 mV s^{-1}; surface coverage, ~3.5*10^{-9} mol cm^{-2}; starting potential, -100 mV *vs.* SCE.

Conclusions

New modified electrodes were obtained by the adsorption of 3,7-di(*m*-aminophenyl)-10-ethyl-phenothiazine on spectrographic graphite.

The electrochemical studies indicate that the electron transfer process at the new modified electrodes is a quasi-reversible one and its reversibility decreases with the pH decrease.

The slope of the standard formal potential (E$^{0'}$) linear regression *vs.* pH indicates a 1e$^-$/1H$^+$ transfer during the redox process. The redox process is strongly influenced by pH, due to the presence in the mediator molecule of two primary amino groups.

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The kinetic parameters, meaning the heterogeneous electron transfer rate constant (k_s) and the transfer coefficient (α), were determined. It was observed that k_s is affected by pH and by the presence of the ethyl group at the nitrogen atom, which is involved probably in the way in which the mediator binds to graphite.

The new modified electrodes present a good electrocatalytic activity for the NADH oxidation, with an electrocatalytic efficiency of 67%, hence recommending the new synthesized compound as a promising mediator for NADH electrocatalytic oxidation.

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