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Dedicated to Professor Liviu Literat, at his 80th anniversary

ELECTROCHEMICAL BEHAVIOR AND APPLICATIONS OF PHENOTHIAZINE DERIVATIVES BASED ON *BIS*-(10*H*-PHENOTHIAZIN-3-YL)-METHANE

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ABSTRACT. The electrochemical behavior of two phenothiazine derivatives, *bis*-(10*H*-phenothiazin-3-yl)-methane (I) and 1,1-*bis*-(10*H*-phenothiazin-3-yl)-ethane (II), was studied comparatively by cyclic voltammetry in dimethylsulfoxide solution and adsorbed on graphite electrodes. Compound I presents a more favorable electrochemical behavior than compound II, both in solution or adsorbed. The obtained modified electrodes present electrocatalytic activity towards NADH oxidation. Their electrocatalytic efficiency, evaluated from cyclic voltammetry, revealed the following decreasing sequence: I > II.

Keywords: *Phenothiazine derivatives, Modified electrodes, NADH electrocatalytic oxidation.*

INTRODUCTION

The employment of various organic compounds as mediators in facilitating electron transfer from enzyme molecules to electrode surface including phenothiazine derivatives has been reported in the literature [1]. It is known that phenothiazine derivatives, a pharmaceutically important class of heterocycles, with electron-rich sulfur and nitrogen heteroatoms, have been used as electron donor components. [2].

The cyclic voltammetric responses for phenothiazine derivatives adsorbed onto graphite electrodes recommend them for the low potential, sensitive and stable determination of dihydronicotinamide adenine dinucleotide (NADH) [3-7].

The determination of NADH is very important because NADH and its oxidized form, NAD^+, are the coenzymes for a large number of dehydrogenase

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enzymes (more than 300) and components of biomarker systems. However, the direct electrochemical oxidation of NADH at a bare electrode takes place at high overpotential. So, much effort has been devoted to reduce the overpotential of the oxidation of NADH by using redox mediators, including phenothiazine derivatives [8].

In this context, the aim of this work is the study of the electrochemical properties of two new phenothiazine derivatives, *bis*-(10*H*-phenothiazin-3-yl)-methane (**I**) and 1,1-*bis*-(10*H*-phenothiazin-3-yl)-ethane (**II**), in solution and adsorbed on graphite electrodes as possible mediators for NADH oxidation. Cyclic voltammetry measurements (CV) were used to investigate the electrochemical behavior of compounds **I** and **II** under various experimental conditions (different potential scan rates). The modified electrodes obtained by adsorption of these new phenothiazine derivatives on graphite electrodes were tested for electrocatalytic oxidation of NADH.

RESULTS AND DISCUSSION

Electrochemical behavior of compounds in solution

Variable experimental conditions (pH, and scan rates) were employed for the investigation of the electrochemical behavior of compounds **I** and **II** by CV in DMSO solution. Figure 1 presents the cyclic voltammograms obtained for compounds **I** and **II** in DMSO solution, on graphite electrodes. The cyclic voltammograms are characterized by two peak pairs, with formal standard potential *vs.* Ag/AgCl/KCl$_{sat}$ in the positive domain (Table 1).

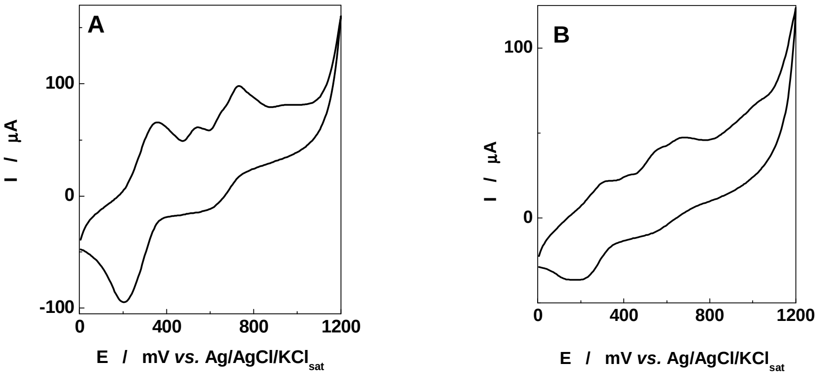


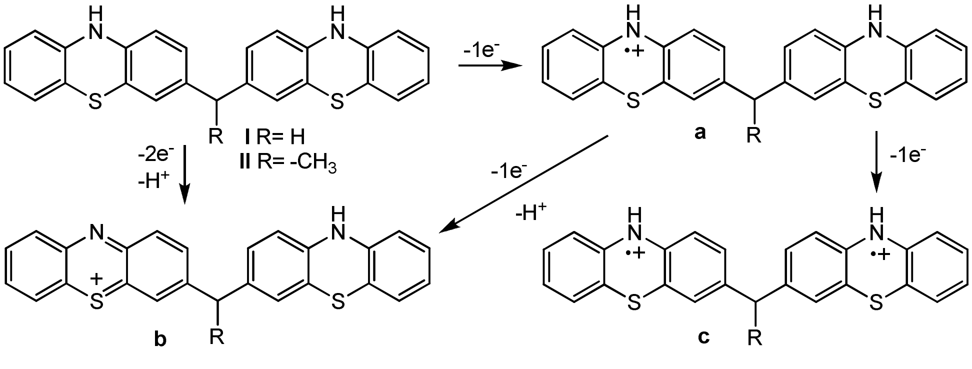
Figure 1. Cyclic voltammograms recorded on graphite electrode for compounds **I** (A) and **II** (B), dissolved in DMSO. Experimental conditions: starting potential, 0 mV *vs.* SCE; potential scan rate, 10 mV s^{-1}; supporting electrolyte, dimethylsulfoxide + 10^{-1} M KCl, pH 7.

Table 1. Electrochemical parameters of the voltammetric response of compounds **I-II** in solution. Experimental conditions: as in Figure 1

Compound	Peak	E_{pa} (mV)*	E_{pc} (mV)*	ΔE_{peak} (mV)
I	**1**	349	208	141
	2	722	602	120
II	**1**	290	212	78
	2	667	543	124

* mV *vs.* Ag|AgCl/KCl$_{sat}$

The first oxidation peak, may be assigned to one-electron oxidation leading to the formation of a stable radical cation (labeled **a** in Scheme 1) generated by one phenothiazine structural unit present in the molecular structure ($E_I^{0/+1}$= 349 mV and $E_{II}^{0/+1}$= 290 mV). The second oxidation peak appears shifted anodically ($E_I^{0/+2}$= 722 mV and $E_{II}^{0/+2}$= 667 mV) and may be assigned to two-electron oxidation leading the formation of phenazathionium cation (labeled **b** in Scheme 1). These oxidation potentials values are in agreement with the reported electrochemical data for unsubstituted phenothiazine ($E^{0/+1}$= 270 mV and $E^{0/+2}$= 750 mV *vs*. Ag/AgCl/KCl$_{sat}$ [9]. In the CV of compound I, presented in figure 1, a peak characterized by lower current intensities appears at intermediate values (aprox. 500 mV) and could be assigned to the formation of a *bis*-radical-cation (labeled **c** in Scheme 1).



Scheme 1

The values of ΔE_{peak} showed that compounds **I** and **II** presents irreversible electrochemical behavior ($\Delta E_p > 59$ mV) on graphite.

A small positive shift of the E_{pa} values for compound **I** was observed (see Table 1) as compared with compound **II**, suggesting that the latter is oxidized easier taking benefit of a weak inductive electron donor effect of the alkyl substituent.

Electrochemical behavior of adsorbed compounds

Figure 2 shows the voltammetric response corresponding to compounds **I** and **II**, adsorbed on the surface of graphite electrode, in a phosphate buffer solution (pH 7). The voltammogram of compound **I** (Figure 2A) shows one well-defined peak pair and one oxidation shoulder. The oxidation waves can be assigned to the formation of stable radical cations generated by the two phenothiazine structural units present in the molecular structure. The first oxidation wave ($E_{pa}^{0/+1}$= 89 mV *vs.* SCE) can be assigned to the radical cation formation of one phenothiazine unit in the molecular structure. The shoulder ($E_{pa}^{+1/+2}$= 400 mV *vs.* SCE), involving the oxidation of the phenothiazinium radical cation to the *bis*-phenothiazinium dication appears shifted anodically and it is characterized by a much lower current intensity, may be due to the electron withdrawing effect induced by the positive charge in the radical cation.

Figure 2B shows the cyclic voltammograms of compound **II** containing a principal oxidation peak $E_{pa}^{0/+1}$ situated close to 5 mV *vs.* SCE. Thus, compound **II** appear to undergo oxidation processes slightly easier as compared to the compound **I**, presenting the same behavior as in solution.

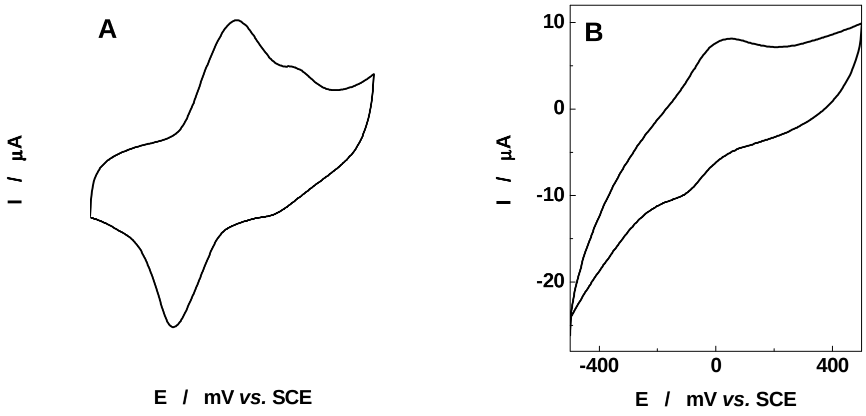


Figure 2. Cyclic voltammograms of compounds **I** (A) and **II** (B) adsorbed on graphite. Experimental conditions: starting potential, -500 mV *vs.* SCE; potential scan rate, 50 mV s^{-1} (A) and 10 mV s^{-1} (B); supporting electrolyte, 0.1 M phosphate buffer, pH 7.

The voltammetric behavior of compounds **I** and **II**, characterized by the electrochemical parameters summarized in Table 2, points out to an irreversible process (ΔE_p > 59 mV), for each investigated compound. The values of the standard formal potentials $E^{0'}$ show greater values for compounds **I** and **II** in comparison with pure phenothiazine, which is characterized by standard formal potential value $E^{0'}$= -107 mV *vs.* SCE, in the same experimental conditions. This fact suggests the more difficult oxidation of both studied compounds as compared to the parent unsubstituted phenothiazine. This behavior can be explained by the bigger size of compound **I** and its different steric orientation towards the electrode surface and reflects the increased stability of compound **I.** It can be observed also from Table 2 that the supplementary substitution with methyl groups of compound **II** leads to a decrease of ΔE_{peak} although compound **I** exhibits the relative current ratio I_{pa}/I_{pc} close to one, specific to adsorbed conditions [10].

Table 2. Electrochemical parameters of the voltammetric response for graphite electrodes modified with compounds **I-II**. Experimental conditions: scan rate, 10 mV s^{-1}; supporting electrolyte 0.1 M phosphate buffer, pH 7

Compound	**E_{pa} (mV)***	**E_{pc} (mV)***	**ΔE_{peak} (mV)**	**$E^{0'}$ (mV)**	**E_{FWHM} (mV)**		**I_{pa}/I_{pc}**
					anodic	**cathodic**	
I	89	-59	148	15	206	169	1.00
II	5	-95	100	-45	200	107	3.82

* mV *vs.* SCE

As expected for surface confined redox species [11], the cyclic voltammograms recorded in a wide range of potential scan rates (10-400 mV s^{-1}) showed a linear dependence of the peak currents (I_p) on the electrode potential scan rate (v) (results not shown), for compounds **I** and **II** adsorbed on graphite electrodes. The slopes of the log I_p *vs.* log v plots for the compounds **I** and **II** were close to the theoretical value (1), in phosphate buffer pH 7.

For both studied compounds, the width at half peak height (E_{FWHM}) was different to that corresponding to the ideal case (E_{FWHM} = 90.6/n mV, where n is the number of electrons). The observed discrepancies prove the existence of repulsive interactions between the adsorbed redox species (radical cations generated in the anodic process) [11,12]. Additionally, as expected for lateral interaction [10], it was noticed that the peak parameters were affected by the surface coverage.

In conclusion, compound **I** presents a more favorable electrochemical behavior than compound **II**, both in solution and adsorbed on graphite electrodes.

Electrocatalytic activity for NADH oxidation

Starting from the favorable electrochemical behavior of the compounds **I** and **II** adsorbed on graphite, their electrocatalytic activity towards NADH oxidation was tested by CV. As can be seen from Figure 3, an enhancement of the anodic peak current, observed in the NADH presence, associated with the progressive diminishing of the cathodic one, proved the electrocatalytic effect for compounds **I** and **II**. The electrocatalytic efficiency, estimated as the ratio $(I_{cat})_{NADH}/(I_{cat})$ was found decreasing in the following sequence: **I** (1.70, measured at -38 mV *vs.* SCE) > **II** (1.12, measured at +28 mV *vs.* SCE).

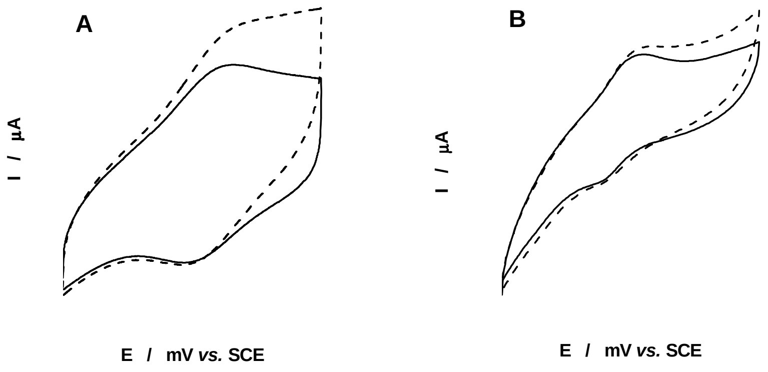


Figure 3. Cyclic voltammograms of compounds **I** (A) and **II** (B) adsorbed on graphite electrodes, in absence and in presence of NADH 5 M. Experimental conditions: starting potential, –200 mV *vs.* SCE (A) and –500 mV *vs.* SCE (B); scan rate, 10 mV s^{-1}; supporting electrolyte, 0.1 M phosphate buffer, pH 7.0.

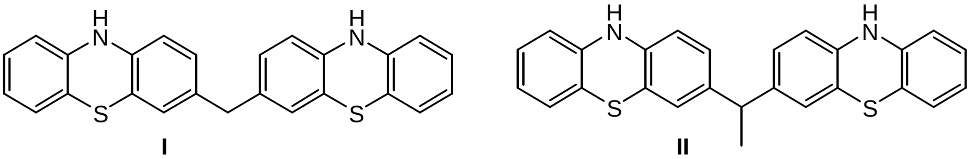
CONCLUSIONS

By comparing the electrochemical behavior of two new phenothiazine derivatives, *bis*-(10H-phenothiazin-3-yl)-methane (**I**) and 1,1-*bis*-(10*H*-phenothiazin-3-yl)-ethane (**II**), it was put on evidence that compound **I** is more difficult to oxidize than compound **II**, on graphite electrodes. in the same experimental conditions (in solution and in adsorbed state). However, the catalytic efficiency of compound I adsorbed on graphite toward NADH oxidation was better than that of compound II. Both graphite electrodes modified with compounds I and II present electrocatalytic effect towards NADH oxidation.

EXPERIMENTAL SECTION

Materials

Bis-(10*H*-phenothiazin-3-yl)-methane (**I**) and 1,1-*bis*-(10*H*-phenothiazin-3-yl)-ethane (**II**) (Scheme 2) are the two phenothiazine derivatives used for electrochemical studies.



I **II**

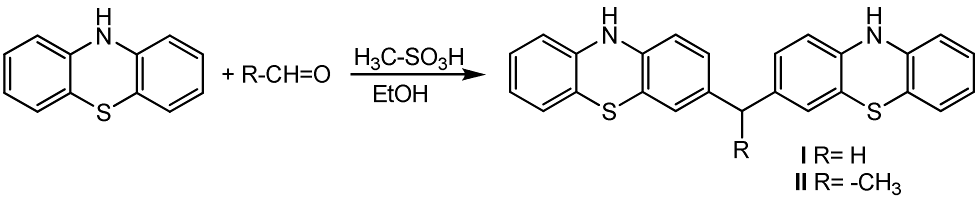
Scheme 2

The supporting electrolyte for voltammetric measurements in solution was dimethylsulfoxide (DMSO, Merck, Darmstadt, Germany) containing 0.1 M KCl and for adsorbed system, phosphate buffer solutions. Phosphate buffer solutions were prepared using $Na_2HPO_4 \cdot 2H_2O$ and $NaH_2PO_4 \cdot H_2O$ from Merck (Darmstadt, Germany).

β-Nicotinamide adenine dinucleotide, reduced form (NADH) was purchased from Sigma (St. Louis, MO, USA) as disodium salt. All other reagents were of analytical grade and used as received.

Synthesis and characterization

Bis-(10*H*-phenothiazin-3-yl)-methane (**I**) was synthesized by the condensation of phenothiazine with formaldehyde according to previously reported procedures [13]. 1,1-*bis*-(10*H*-phenothiazin-3-yl)-ethane (**II**) was synthesized according to similar procedures by the condensation of phenothiazine with acetaldehyde in the presence of catalytic amounts of methanesulfonic acid (Scheme 3).



Scheme 3

The structure assignment of **I** and **II** is supported by spectroscopic data. FT-IR spectroscopy indicates the stretching vibration of N-H bonds by the absorption band situated at about 3330 cm^{-1}. In particular, **II** can be clearly identified by the appearance of the aliphatic protons in NMR spectra. 300 MHz ^1H-NMR spectrum shows a quartet signal situated at 4.5 ppm and a doublet signal situated 1.22 ppm for the four protons of the ethylidene group. For the NH groups in the two equivalent phenothiazine units the signal of the protons appears situated at 8.5 ppm. The substitution in position 3 of the phenothiazine units was assigned according to the aromatic protons coupling pattern observed for the signals situated in the 6.5-7 ppm range in the homocorrelation COSY–45 spectrum.

NMR spectra were recorded using a 300 MHz Brucker NMR spectrometer.

FT-IR spectra were recorded using a Brucker Vector 22 FT-IR spectrometer.

***1,1-Bis*-(10*H*-phenothiazin-3yl)-ethane (II)**

10*H*-phenothiazine (2.5 mmol) solved in ethanol (20 mL), acetaldehyde (1 mmol) and methanesulfonic acid (1 mL) were heated to reflux for 12 hours and generated a green precipitate which was filtered. The precipitate was thoroughly washed with cold ethanol and then it was solubilized in tetrahidrofurane. The insoluble part was removed by filtration. After removing the solvent, 0.25 g green precipitate was obtained (yield 60%).

^1H-NMR (300MHz, DMSO-d_6): δ=1.22 ppm (d, 3H), 4.50 ppm (q, 1H), 6.66 ppm (d, J=6.20 Hz, 2H), 6.76 ppm (d, J=6 Hz, 2H), 6.79 (m, 4H), 6.82 (d, J=6.2 Hz, 2H), 6.96 ppm (d, J=5.8 Hz, 2H), 7.05 (t, 2H), 8.52ppm (s, 2H).

Electrodes preparation

A spectrographic graphite rod (Ringsdorff-Werke, GmbH, Bonn-Bad Godesberg, Germany), of ~ 3 mm diameter, was wet polished on fine (grit 400 and 600) emery paper (Buehler, Lake Bluff, Ill., USA). Then, a graphite piece of suitable length was carefully washed with deionized water, dried, and finally press-fitted into a PTFE holder in order to obtain a graphite electrode having, in contact with the solution, a flat circular surface of ~ 0.071 cm^2.

The modified graphite electrodes were obtained by spreading onto the electrode surface 2 µl of 1 mM phenothiazine derivative solution in dimethylsulfoxide and leaving them for 20 minutes at room temperature to evaporate the solvent. Before immersion in the test solution the modified electrodes were carefully washed with deionized water.

Electrochemical measurements

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

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