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Dedicated to Professor Valer Fărcăşan
at his 85th anniversary

GRAPHITE ELECTRODE MODIFIED BY CHARGE TRANSFER COMPLEX BETWEEN TETRACYANOQUINODIMETHANE AND 16*H,*18*H*-DIBENZO[c,1]-7,9-DITHIA-16,18-DIAZAPENTACENE USED FOR NADH ELECTRO-OXIDATION

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ABSTRACT. The electrochemical behavior of a new charge transfer complex between 7,7,8,8-tetracyanoquinodimethane and 16*H,*18*H*-dibenzo[c,1]-7,9-dithia-16,18-diazapentacene adsorbed on spectrographic graphite has been investigated. Cyclic voltammetry measurements, performed in aqueous buffer solutions at different potential scan rates and pH values, pointed out to a quasi-reversible, surface confined redox process. The voltammetric response involves the transfer of $1e^-/1H^+$, with a heterogeneous rate constant of 26.8 s^{-1} (pH 7.0). The modified electrodes showed a good electrochemical stability as well as moderate electrocatalytic activity toward NADH electro–oxidation.

Keywords: NADH oxidation, charge transfer complex, modified electrodes, TCNQ, phenothiazine derivatives.

INTRODUCTION

The electrochemical oxidation of β-nicotinamide adenine dinucleotide (NADH) has been extensively investigated, its efficient and reversible recycling being of particular interest for the construction of NADH-dependent dehydrogenase based amperometric biosensors and for energy conversion *via* biofuel cell [1-3].

It is known that the direct electro-oxidation of NADH at many bare electrodes is complicated and requires a large overvoltage [4-6], which results in the interference from more easily oxidizable species. Additionally, the adsorbed molecules of NAD^+ could cause electrode fouling at NADH concentration above 0.1 mM [1]. The most efficient way to realize the NADH electro-oxidation at low overvoltages is the use of mediators immobilized on the electrode surface.

Many redox couples could be used as mediators for NADH oxidation, because of the low value of the formal potential ($E^{0'}$) of NAD^+/NADH redox couple (–315 mV *vs.* NHE, at pH 7 and 25^0 C) [7]. In spite of this, only a restricted number of compounds have been selected in order to obtain modified electrodes for electrocatalytic oxidation of NADH [1,2,8,9]. Among these, organic dyes, i.e. phenazines, phenoxazines and phenothiazines derivatives, were used frequently to design efficient electrocatalytic schemes for NADH recycling [10-14]. However, these mediators suffer because of their chemical/electrochemical instability, especially at high pH values [8].

In order to improve the operational stability of NADH sensors, two different approaches have been proposed: (i) more efficient immobilization of the mediator by its electropolymerization [15], by using a polymeric film as an entrapment matrix [8,16] or as a diffusion barrier [1,17]; (ii) improvement of the mediator behavior, as for example by decreasing the mediator reactivity *via* enlarging the intramolecular conjugation system [18,19], by introduction of withdrawing (-I) substituents [20,21], or using charge-transfer complexes between a component, acting as electron donor and an electronegative component acting as an electron acceptor [8].

In this context, based on the fact that the phenothiazine and its derivatives are good electron donors, suitable for forming numerous charge transfer complexes with different electron acceptors, in this work a charge transfer complex (CTC) between 7,7,8,8-tetracyanoquinodimethane (TCNQ) and 16*H,*18*H*-dibenzo[c,1]-7,9-dithia-16,18-diaza-pentacene (DDDP) was investigated as a redox mediator for NADH electro-oxidation. The electrochemical behavior and stability of the CTC adsorbed on spectrographic graphite were investigated by cyclic voltammetry (CV) in different experimental conditions (various potential scan rates and pH values). Finally, using CV measurements, the CTC modified graphite electrodes were examined for their electrocatalytic activity toward NADH oxidation.

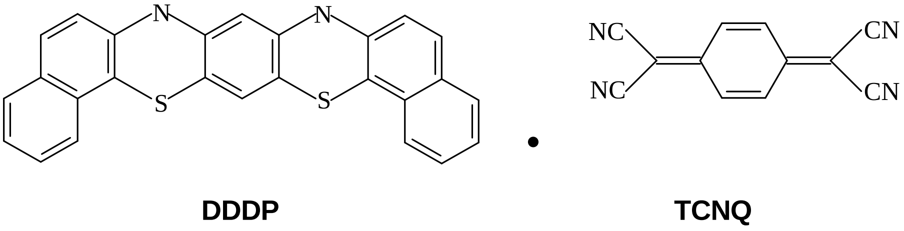
EXPERIMENTAL SECTION

Materials

The charge transfer complex (CTC) between 7,7,8,8-tetracyanoquinodimethane (TCNQ) and 16*H,*18*H*-dibenzo[c,1]-7,9-dithia-16,18-diaza-pentacene (DDDP) (see Scheme 1) was synthesized according to a previously published procedure [22].

β-Nicotinamide adenine dinucleotide, reduced form (NADH), was purchased from Sigma (St. Louis, MO, USA) as disodium salt. Phosphate buffer solutions were prepared using $Na_2HPO_4 \cdot 2H_2O$ and $NaH_2PO_4 \cdot H_2O$ 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. The pH value was adjusted using the appropriate H_3PO_4 or NaOH solutions.



Scheme 1. CTC of DDDP-TCNQ formula.

Electrode 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 doubly distilled 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 5 µl of 10 mM CTC solution in dimethylformamide, 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 doubly distilled water.

For each electrode, the surface coverage (Γ, mol cm^{-2}) was estimated from the under peak areas, recorded during the CV measurements at low scan rate (v < 10 mV s^{-1}), and considering the surface redox valence equal to unity [23]. All presented results are the average of at least 3 identically prepared electrodes, if not otherwise mentioned.

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 DISCUSSION

1. Electrochemical behavior of the CTC-modified graphite electrode

The electrochemical behavior of CTC adsorbed on spectrographic graphite (G/CTC) was investigated using CV measurements, performed in different experimental conditions. As can be seen from Figure 1, the cyclic voltammogram recorded for G/CTC electrode is similar with that recorded for DDDP, presenting one wave with the formal standard potential placed at ~ 265 mV *vs.* SCE (pH 7.0). This similarity proves a reduced intermolecular interaction between DDDP and TCNQ in CTC.

The electrochemical parameters for different pH values, summarized in Table 1, point to a quasi-reversible, one-electron process. The discrepancies

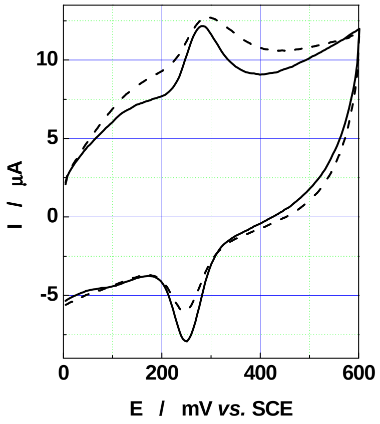


Fig. 1. Voltammetric response of CTC (—) and DDDP (---) adsorbed on spectrographic graphite. Experimental conditions: starting potential, 0 mV *vs.* SCE; scan rate, 50 mV s^{-1}; supporting electrolyte, 0.1 M phosphate buffer, pH 7.0; surface coverage, see Table 1.

between the theoretical value of the peak width at half peak height (E_{FHWM} = 90.6/n mV) and the corresponding experimental values as well as $\Delta E_p \neq 0$ (Table 1) prove the existence of some interactions between the surface-confined redox species [24,25].

Table 1.

Electrochemical parameters of the voltammetric response of CTC, adsorbed on spectrographic graphite. Experimental conditions: as in fig. 1.

pH	ΔE_{peak} (mV)	E_{FWHM} (mV)		I_{pa}/I_{pc}	Surface coverage (nmol cm^{-2})
		anodic	cathodic		
3	38	95	91	1.09	0.9
5	53	124	87	0.69	0.7
7	37	95	70	0.68	0.7
9	57	124	111	1.01	1.3

As expected for surface confined redox active species [25], the cyclic voltammograms recorded for a wide range of potential scan rates (0.01 – 3.2 V s^{-1}) showed a linear dependence of the peak currents (I_p) on the electrode potential scan rate (v) (Figure 2, Table 2). Also, the slope of log I_p *vs.* log v dependence was close to one, confirming once more the existence of adsorbed species. The number of electrons involved in the redox process, estimated from the I_p *vs.* v dependence [26], was found close to 1 (within ± 10 %), in accordance with the predicted value for the cation radical formation.

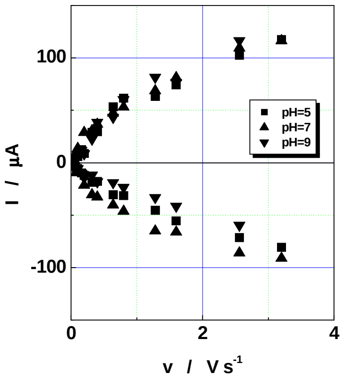


Fig. 2. Peak current dependence on the potential scan rate for the voltammetric response of CTC-modified graphite electrodes. Experimental conditions: starting potential, –1000 mV *vs.* SCE; scan rate, 50 mV s^{-1}; supporting electrolyte, 0.1 M phosphate buffer, pH 7.0; surface coverage, see Table 2.

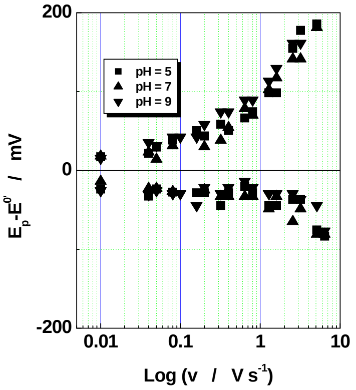


Fig. 3. (E_p - $E^{o'}$) *vs.* logarithm of the scan rate dependence for CTC adsorbed on spectrographic graphite. Experimental conditions: surface coverage, see Table 3; other conditions, see Fig. 1.

Table 2.

Linear regression parameters for the dependence of the peak current on the potential scan rate (0.01 to 0.64 V s^{-1}), observed for CTC-modified graphite electrodes. Experimental conditions: as in fig. 2.

pH	Slope (10^6 A V s^{-1})		R / no. of exp. points		Surface coverage (nmol cm^{-2})
	oxidation	reduction	oxidation	reduction	
3	29.9 ± 2.2	-46.6± 2.8	0.971 / 13	0.981 / 13	0.7 ± 0.2
5	81.1 ± 3.4	-39.8 ± 3.0	0.992 / 11	0.981 / 11	2.2 ± 0.3
7	64.9 ± 6.0	-49.4 ± 6.0	0.967 / 10	0.945 /10	1.1 ± 0.3
9	67.2 ± 3.6	-24.4 ± 2.3	0.985 / 12	0.959 / 12	1.6 ± 0.5

Table 3.

Kinetic parameters for the heterogeneous electron transfer at CTC-modified graphite electrodes. Experimental conditions: as in fig. 3.

pH	k_s (s^{-1})	α	R / no. of exp. points		Surface coverage (nmol cm^{-2})
			oxidation	reduction	
3	38.6	0.44	0.918 / 4	0.966 / 4	1.3 ± 0.4
5	37.5	0.65	0.956 / 5	0.972 / 4	0.5 ± 0.02
7	26.8	0.43	0.979/ 5	0.999 / 5	0.5 ± 0.1
9	42.3	0.65	0.982 / 6	0.986 / 3	1.3 ± 0.4

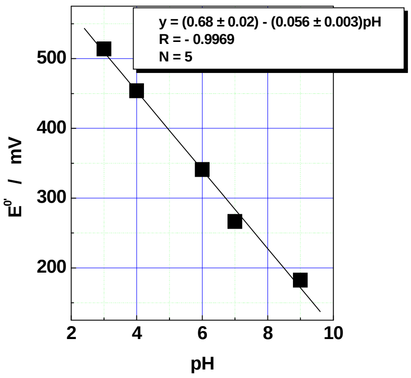


Fig. 4. pH dependence of the standard formal potential for CTC-modified electrodes. Experimental conditions: starting potential, -1000 mV *vs.* SCE; scan rate, 50 mV s^{-1}; supporting electrolyte, 0.1 M phosphate buffer.

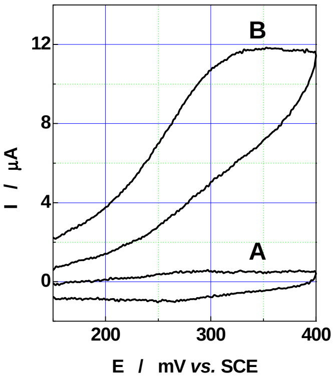


Fig. 5. Electrocatalytic oxidation of NADH at CTC-modified graphite electrode: (A) 0.1 M phosphate buffer, pH 7.0; (B) 0.1 M phosphate buffer containing 5 mM NADH. Experimental conditions: starting potential, +150 mV *vs.* SCE; scan rate, 5 mV s^{-1}; surface coverage, ~ 2.2·nmol cm^{-2}.

The heterogeneous electron transfer rate constant (k_s, s^{-1}) was estimated at different pH values (Table 3) using the treatment proposed by Laviron [27] (Figure 3). Within the experimental error, the heterogeneous electron transfer rate constant (k_s) and the transfer coefficient (α) are not significantly affected by the pH, being equal to 26.8 s^{-1} and 0.43, respectively (pH 7). The k_s value is close to that obtained for DDDP (30.1 s^{-1}) [19], indicating the weak influence of TCNQ presence on the redox behavior of DDDP.

As expected for the redox behavior of an N-unsubstituted phenothiazine moiety-containing compound [28], the CTC formal redox potential ($E^{0'}$, estimated as the average of cathodic and anodic peak potentials) was found to be pH-dependent (Figure 4). The slope (~ 56 mV/ΔpH) of the regression line, corresponding to the $E^{0'}$ – pH dependence, obtained from the experimental data within a wide pH range (3 – 9), proved that in the redox process is involved an equal number of electrons and protons. Therefore, the voltammetric response corresponding to the formation of the CTC cation radical, involves a $1e^-/1H^+$ transfer.

2. Electrocatalytic activity of the CTC-modified graphite electrode

Starting from the favorable electrochemical behavior of CTC adsorbed on graphite, its electrocatalytic activity was tested by cyclic voltammetry. Figure 5 presents the cyclic voltammograms (v = 5 mV s^{-1}) obtained for G/CTC electrodes in the absence and in the presence of 5 mM NADH. In the presence of NADH it can be observed a remarkable enhancement of the anodic peak current, associated with the progressive diminishing of the cathodic one, proving good CTC electrocatalytic effect for NADH oxidation. The electrocatalytic efficiency, estimated as the $(I_{cat})_{NADH}/(I_{cat})$ ratio, at an applied potential of +300 mV *vs.* SCE, was found equal to 28. This value is comparable with that obtained for DDDP (24.3, measured at + 320 mV *vs.* SCE), reflecting again the reduced influence of TCNQ in CTC complex. Taking as reference the potential for NADH electro-oxidation at bare graphite electrodes [5,29,30], an overpotential decrease higher than 100 mV was noticed.

3. Chemical and electrochemical stability of the CTC-modified graphite electrode

The stability of modified electrodes was tested by measuring the variation of mediator electrochemical signal in a defined time range. It is known that the immobilization stability of a mediator on graphite electrodes is decided by the number of conjugated aromatic rings from molecule, therefore the mediator which contains more aromatic rings will lead to the obtaining of a more stable modified electrode. Additionally, the strong interaction between the graphite surface and phenothiazine derivatives (the superposition between π electrons of graphite and the mediator) determines a fast charge transfer between the electrode and mediator [10,31]. The stability of G/CTC electrodes was tested chemically and electrochemically.

Chemical stability of G/CTC electrodes was studied by keeping the electrode at room temperature, and during the time the electrodes were periodically tested. The CTC-modified electrodes presented a voltammetric response after 7 days, but a weak decrease of the surface coverage (~ 85 %) could be observed.

This result proves the utility of CTC strategy in order to increase the stability of DDDP-modified electrodes [32].

Several reasons could be responsible for the electrochemical instability of the G/CTC-modified electrode: (i) the CTC intrinsic electrochemical instability enhanced by the pH increase and applied potential and (ii) the mediator desorption.

The electrochemical stability tests of the G/CTC were realized in potentiodynamic conditions: the electrode potential was continuously cycled within the potential range covering the domain of the mediator redox activity, at different pH values of the contact solution. From the recorded voltammograms a progressive decrease of the electrode surface coverage was observed, while the voltammogram shape remains invariant (results not shown). This behavior proves both the good CTC electrochemical stability in the potential range used for the NADH recycling, and its relatively strong adsorption on the graphite surface.

The kinetic interpretation of the deactivation process showed that it obeys a first-order kinetics. The slopes of kinetic plots were used to determine the values of the deactivation rate constants, as an average of the anodic and cathodic processes (Table 4). Surprisingly, as can be seen from Table 4, at higher pH values the deactivation process is slower, recommending CTC modified electrodes as very useful for the development of NADH amperometric sensors. However, the electrochemical stability of CTC was inferior to that observed for DDDP ($1.8 \cdot 10^{-14}$ mol cm^{-2} s^{-1}; phosphate buffer pH 7). This higher instability of the CTC-modified graphite electrodes could be due to a weaker adsorption of CTC on graphite surface in comparison with DDDP [32].

Table 4.

Deactivation rate constants for CTC-modified graphite electrodes. Experimental conditions: scan rate, 50 mV s^{-1}; supporting electrolyte, 0.1 M phosphate buffer, pH 7.0; time range, 0 - 500 s.

pH	**$\Gamma_{(t=0)}$ (10^{10} mol cm^{-2})**		**k_{deact} (10^{14} mol cm^{-2} s^{-1})**	**R / no. of exp. points**	
	anodic	**cathodic**		**anodic**	**cathodic**
4	9.1 ± 0.5	3.9 ± 0.1	75.1	0.9740 / 5	0.9331 / 5
7	9.9 ± 0.1	8.01 ± 0.05	12.3	0.9053 / 5	0.9799 / 5
8	10.9 ± 0.04	9.07 ± 0.01	4.5	0.8509 / 4	0.9970 / 4

CONCLUSIONS

The adsorption on graphite of a charge transfer complex between 7,7,8,8-tetracyanoquinodimethane and 16*H*,18*H* dibenzo[c,1]-7,9-dithia-16,18-diazapentacene resulted in modified electrodes for NADH oxidation.

The linear dependence between peak current (I_p) and the potential scan rate (v), corroborated with the slope of standard formal potential linear regression ($E^{0'}$) *vs.* pH, proves the existence of a redox couple adsorbed on electrode surface involving $1e^-/1H^+$.

The study of chemical and electrochemical stability of CTC adsorbed on graphite evidences a higher chemical stability of CTC in comparison with DDDP, confirming the proposed strategy to increase the stability of modified electrodes.

The CTC-modified graphite electrodes present a moderate electrocatalytic activity towards NADH oxidation, the electrocatalytic efficiency suggesting the possibility of using them as amperometric sensors for NADH.

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