NADH sensor with electrochemically modified TCNQ electrode

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Abstract

7,7,8,8-Tetracyanoquinodimethane (TCNQ) has been immobilized electrochemically on an edge plane pyrolytic graphite electrode. The electrode was immersed in a solution of TCNQ in acetonitrile and a voltage of 1.0 V vs. Ag/AgCl was applied for 25 min. The surface coverage was $5.3 \times 10^{-10}$ mol cm$^{-2}$. The modified electrode catalyses the electron transfer from dihydronicotinamide adenine dinucleotide (NADH) and is potentially useful as a sensor for substrates enzymatically coupled to NAD$^+/\text{NADH}$.

Key words: Cyclic voltammetry; Sensors; NADH; TCNQ

1. Introduction

The oxidation of NADH is of special interest because of its practical application in the development of amperometric biosensors for NAD$^+$-dependent dehydrogenases [1]. Dehydrogenases depend on the soluble cofactor NAD$^+/\text{NADH}$. In order to lower the overpotential in the electrochemical oxidation of NADH, the use of several electron transfer mediators has been examined either in homogeneous solution or with mediator-modified electrodes. The subject has been reviewed recently [2]. A carbon paste electrode chemically modified with a polymer containing a covalently bound phenothiazene dye, toluidine blue [3] and poly(thionine) modified electrodes [4] have recently been suggested for electrocatalytic oxidation of NADH.

7,7,8,8-Tetracyanoquinodimethane (TCNQ) has been found to facilitate electron transfer from glucose oxidase to pyrolytic graphite electrode and the modified electrode (carbodiimide procedure) responded to glucose in the range up to 70 mM [5]:

This paper reports on a voltage-facilitated immobilization procedure for TCNQ on an edge plane pyrolytic graphite electrode and the electrocatalytic activity of the modified electrode for NADH oxidation.

2. Experimental

2.1. Reagents

7,7,8,8-Tetracyanoquinodimethane (TCNQ, Aldrich) and NADH (Extra Pure for Biochemistry, SRL, India) were used as received. Acetonitrile was distilled over P$_2$O$_5$ before use. The standard buffer was 0.1 M phosphate buffer (pH
7.0) containing sodium perchlorate. All solutions were prepared in doubly distilled water and oxygen free conditions.

2.2. Apparatus

Cyclic voltammetry (CV) was carried out with a three electrode cell (BAS C-1B) with a working volume of 5 cm$^3$. The reference and counter electrodes were a sintered Ag/AgCl electrode and a platinum wire electrode, respectively. The working electrode was fabricated from 3 mm diameter edge plane pyrolytic graphite (EPG) supplied by Le Carbone (Lorraine, France). The EPG electrode was polished with 600 grit silicon carbide paper followed by 1 μm diamond paste. A BAS CV-27 voltammmograph with a BAS X-Y recorder (Model No. MF 8050F) was used for the measurements. All experiments were carried out at 25 ± 0.1°C.

2.3. Immobilization procedure

TCNQ was immobilized on a clean EPG electrode by dipping it in a 10 mM solution of TCNQ in acetonitrile containing 0.1 M sodium perchlorate and applying a positive potential of 1.0 V vs. Ag/AgCl for 25 min. The electrode was washed with distilled water and stored in the phosphate buffer pH 7.0.

3. Results and discussion

The CV of TCNQ in acetonitrile solution is shown in Fig. 1a. The two anodic peaks $E_{pa1}$ and $E_{pa2}$ (+0.250 and $-0.313$ V vs. Ag/AgCl) and the two cathodic peaks $E_{pc1}$ and $E_{pc2}$ (+0.325 and $-0.200$ V) correspond to the electrochemical processes TCNQ $\rightleftharpoons$ TCNQ$^{+}$ and TCNQ$^{+}$ $\rightleftharpoons$ TCNQ$^{2+}$, respectively. These values are in fair agreement with those reported by Hendry and Turner [5] and Sharp [6]. The formal potentials are $0.288$ V and $-0.256$ V, respectively. The CV of the EPG electrode modified with TCNQ in phosphate buffer is shown in Fig. 1b. $E_{pa1}$ and $E_{pa2}$ values are $+0.250$ and $-0.225$ V and $E_{pc1}$ and $E_{pc2}$ values are $+0.100$ and $-0.325$ V, respectively. The observed changes in the shape and position of the peaks are due to surface modifications. The peak positions are also slightly different from those observed for a TCNQ-modified electrode prepared by the dip coating method [7] possibly due to the presence of the sodium perchlorate supporting electrolyte. The surface coverage, as calculated from the voltammograms of the TCNQ-modified electrode at various scan rates using the equation [8]:

$$i_p = n^2F^2AΓν/4RT$$

(where the parameters have their usual significance) is found to be $5.3 \times 10^{-10}$ mol cm$^{-2}$ and corresponds to a monolayer.
The CVs of an TCNQ-modified EPG electrode in the presence of varying concentrations of NADH at pH 7.0 are shown in Fig. 2. NADH is not stable below pH 7.0 [9]. While the peaks corresponding to $\text{TCNQ}^- \rightarrow \text{TCNQ}^{2-}$ are unaffected, those due to $\text{TCNQ} = \text{TCNQ}^-$ change. The oxidation of NADH is accompanied by a shift of the 0.250 V peak to ca. 0.40 V and an increase in current (corresponding to $\text{TCNQ}^-$ oxidation) because the NADH in the solution diffuses towards the electrode and reduces the TCNQ produced electrochemically. As $\text{TCNQ}^-$ is regenerated by NADH during the scan, there is a resultant increase in the anodic current. Simultaneously, the cathodic current decreases. The overall reaction scheme may be depicted as follows:

$$\text{TCNQ} + \text{NADH} \rightarrow \text{TCNQ}^- + \text{NAD}^+ + \text{H}^+ + e^- \quad (1)$$

$$\text{TCNQ}^- \rightarrow \text{TCNQ} + e^- \quad (2)$$

Net: $\text{NADH} \rightarrow \text{NAD}^+ + \text{H}^+ + 2e^- \quad (3)$

The amperometric response of a TCNQ-modified electrode to NADH in solution has been examined. A calibration graph was obtained by measuring the peak current at ca. 0.40 V upon addition of increasing amounts of NADH. The graph is linear over the range 1–10 mM with the equation $i_p (\mu A) = 19.1[\text{NADH}]\text{mM} - 2.8$ (correlation coefficient = 0.999, $n = 7$).

The kinetic aspects of mediator modified electrodes are of interest [10]. An attempt has been made to determine an approximate rate of reaction 1 from analysis of the CVs. Andrieux and Saveant [11] have derived an expression relating the peak current and the concentration for the case when reaction 1 is extremely fast:

$$i_p = 0.496nFAC (DnFv/RT)^{1/2}c \quad (4)$$

where the symbols have their usual significance. From our data on TCNQ modified electrode with a coverage of $5.3 \times 10^{-10}$ mol cm$^{-2}$, the constant was found to be 0.326. Using this, and Fig. 1 of Ref. 11, the rate constant $k$ for reaction 1 was calculated to be $4.8 \times 10^6$ M$^{-1}$ s$^{-1}$. A comparison of this value with other modified electrodes for NADH oxidation has shown that it is similar to that for a 4-[2-(2-naphthyl)vinyl]-catechol-coated electrode (ca. $2.0 \times 10^6$ M$^{-1}$ s$^{-1}$) [12]. Mention must be made that the values for rate constants obtained by CV or rotating disc experiments are about the same [12].

The results reported here demonstrate that a TCNQ-modified electrode can be effectively used for NADH sensing, up to at least 10 mM. The electrode is quite stable, and experiments concerning its use in for amperometric enzyme NAD$^+$/NADH systems are in progress.
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5. References