Tetracyanoquinodimethane (TCNQ) modified electrode for NADH oxidation

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Abstract

A TCNQ-modified edge-plane pyrolytic graphite electrode prepared by a dip-coating procedure shows electrocatalytic activity for NADH oxidation in phosphate buffer solutions (pH 7.0). The modified electrode is stable and shows a linear relation for NADH in the concentration range 1–10 mM. The rate constant between adsorbed TCNQ and NADH in solution has been estimated to be \(1.46 \times 10^7 \text{M}^{-1}\text{s}^{-1}\) at 25°C. The modified electrode has the potential use as a sensor for dehydrogenase-enzyme-based substrates.

1. Introduction

The electrochemical oxidation of NADH to NAD\(^+\) is of considerable interest in the development of amperometric biosensors for NAD\(^+\)-dependent dehydrogenases [1]. Direct oxidation of NADH at solid electrodes occurs at substantial overpotentials, and various redox mediators have been found which exhibit electrocatalytic activity for NADH oxidation and reduce the overpotential. The mediated electrochemistry has been reviewed by Cardosi and Turner [2] and by Schuhmann and Schmidt [3]. More recently, it has been found that electrodes modified with horseradish peroxidase covalently bound to polyvinyl pyridine complexed

[Os(bpy)\(_3\)Cl\(^{2+/2+}\)] [4], poly(3-methylthiophene) [5] and poly(thionine) [6] also show electrocatalytic activity to NADH oxidation.

NADH oxidation at n-methylphenazinemethosulphate(NMP) + tetracyanoquinodimethane (NMP + TCNQ) conducting salt electrodes [7,8] has received considerable attention. The behaviour at NMP + TCNQ or tetrathiafulvalene (TTF) + TCNQ electrodes is rather controversial as electron transfer is likely to occur at the individual mediators formed near the electrode surface as a result of slight dissolution of the conducting salt. In view of this, it was considered interesting to examine the mediating ability of TCNQ in the electrocatalytic oxidation of NADH. Indeed, TCNQ [9], TTF [10] and NMP [11] have been found to mediate the glucose–glucose oxidase reaction. An NMP-modified electrode also catalysed electron transfer from NADH [12]. The electrocatalytic oxidation of NADH at a TCNQ-modified electrode is described in this paper.

2. Experimental

7,7,8,8-tetracyanoquinodimethane (TCNQ) (Aldrich) and NADH (Extrapure for Biochemistry, SRL, India) were used as received. The standard buffer was 0.1 M phosphate buffer (pH 7.0) containing 0.1 M sodium perchlorate. The buffers used for the pH profile study were 0.1 M citric acid + 0.2 M Na\(_2\)HPO\(_4\) (pH 5.0–6.0) 0.2 M NaH\(_2\)PO\(_4\) + 0.2 M Na\(_2\)HPO\(_4\) (pH 6.3–8.0). All the solutions were prepared using double-distilled water and oxygen-free conditions. Cyclic voltammetry experiments were performed using a two-compartment cell with a working volume of 4 ml. The reference electrode and counter-electrode were a saturated calomel electrode (SCE) and a platinum foil (area 1 cm\(^2\)) respectively, A BAS CV-27 voltamnograph was used with a BAS x–y recorder (model MF 8050F). Experiments were carried out at 25 ± 0.1°C.

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The pyrolytic graphite (PG) working electrodes (Le Carbone Lorraine, France), of diameter 3 mm, were either oriented with the disc face parallel to the basal plane (a-b) (BPG) or the edge plane (b-c) (EPG). The PG electrodes were polished with 600 grit SiC paper and 1 µm diamond paste.

The TCNQ-modified electrode was prepared by placing a well-polished EPG electrode in filtered TCNQ solution (10 mg per millilitre of toluene) for 1 h. The electrode was then removed and left to dry in air for 1 h at room temperature. The electrodes were rinsed with phosphate buffer and stored in the same solution.

3. Results and discussion

Since TCNQ is more soluble in CH$_3$CN than in an aqueous medium, cyclic voltammograms (CVs) were recorded in CH$_3$CN (Fig. 1(a)). It can be seen that it exhibits reversible heterogeneous kinetics. The peaks at 0.25 V correspond to the process $\text{TCNQ}^- + e^- = \text{TCNQ}^{2-}$ and are chosen for further investigation. The CV of the TCNQ-modified EPG electrode (prepared as described in Section 2) in phosphate buffer is shown in Fig. 1(b). The peaks, which are characteristic of a surface-modified electrode, show a correspondence with those in Fig. 1(a). The mediator seems to be firmly anchored to the surface of EPG as indicated by stable CVs obtained for a period of several hours. The surface coverage of the electrode was calculated from the voltammograms of the TCNQ-modified electrode at various scan rates using the equation [13]

$$I_p = n^2 F^2 A \Gamma v / 4RT$$

where the parameters have their usual significance, and was found to be $1.2 \times 10^{-8}$ mol cm$^{-2}$ which corresponds to multilayer formation.

Electro-oxidation of NADH at conventional metal electrodes requires an overpotential of 1.1 V and proceeds through the formation of a radical intermediate [2,3]. However, on a PG electrode covered with adsorbed NAD$^+$, oxidation occurs at 0.550 V [14]. We have examined NADH oxidation at unmodified EPG and BPG electrodes. Oxidation at the EPG electrode occurred at 0.38 V and the anodic peak is quite well defined. However, oxidation at the BPG electrode occurred at a higher potential (0.45 V), in agreement with the result reported by Ohsaka et al. [6]. Therefore further investigations were confined to the EPG electrode.

The CVs of the TCNQ-modified EPG electrode in the presence of varying concentrations of NADH are shown in Fig. 2. The oxidation of NADH is accompanied by an increase in the anodic current at ca. 0.38 V/SCE (corresponding to TCNQ$^{2-}$ oxidation). The observed increase in current is due to the fact that the NADH present in solution diffuses towards the electrode and reduces the TCNQ produced electrochemically. As TCNQ$^{2-}$ 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 can be represented as follows:

$$\text{TCNQ} + \text{NADH} \rightarrow \text{TCNQ}^{2-} + \text{NAD}^+ + \text{H}^+ + e^- \quad (1)$$

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

The amperometric response of the TCNQ-modified electrode to NADH in solution has been examined. The calibration curve was obtained by measuring the peak current at a potential of ca. 0.38 V upon addition of increasing amounts of NADH. The calibration plot was linear in the range 1–10 mM.

An attempt has been made to determine the approximate rate of the reaction in eqn. (1) from an analysis of CVs. Andrieux and Saveant [15] have derived an
electrodes has shown that it is close to that for an electrode coated with 4-[2-(2-naphthylvinyl)] catechol [16] (ca. $2 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$) and comparable with that for the TTF + TCNQ electrode [8] ($1.3 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$). It should be noted that the rate constants evaluated by CV and rotating-disc experiments are about the same [16].

The effect of pH on the anodic current of the electrode (with 1 mM NADH) was investigated over the range 5.0–8.0 (Fig. 3). The optimum pH was found to be 7.0.

The results reported here demonstrate that TCNQ-modified electrodes can be effectively used for NADH sensing over an extended concentration range. The results are significant for the development of amperometric enzyme sensors for substrates which are enzymatically coupled to NAD$^+$/NADH. Preliminary investigations have shown that ethanol can be quantitatively oxidized at a TCNQ-modified electrode in the presence of alcohol dehydrogenase and diaphorase. Further work is in progress.

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