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Catalytic oxidation of reduced nicotinamide adenine dinucleotide at a microband gold electrode modified with nickel hexacyanoferrate

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Abstract

Nickel hexacyanoferrate films have been deposited on the surface of a microband gold electrode by cyclic voltammetry. Cyclic voltammograms of the resulting nickel hexacyanoferrate films indicate the presence of a couple of redox peaks which correspond to the hexacyanoferrate(II/III) redox couple. The nickel hexacyanoferrate modified microband gold electrode shows an excellent electrocatalytic activity for oxidation of reduced nicotinamide adenine dinucleotide (NADH) in phosphate buffer solution (pH 7.0), with an overpotential ca. 340 mV lower than that of the bare electrode. The steady-state current increases linearly with NADH concentration in the range of 0.5–8.0 mmol/l. The catalytic rate constant of the modified electrode for the oxidation of NADH is determined using a rotating disk electrode. The further experimental results show that the rate constant decreases with increasing NADH concentration. The mechanism of the oxidation of NADH catalyzed by the nickel hexacyanoferrate modified gold electrode is discussed.

Keywords: Catalytic methods; Microelectrodes; Electrocatalytic oxidation; NADH; Nickel hexacyanoferrate

1. Introduction

Recently, the electrochemical oxidation of the coenzyme nicotinamide adenine dinucleotide (NADH) in aqueous solution has received a great deal of interest, due to its significance both as a substrate for dehydrogenase enzymes [1] and its role in the NAD^+/NADH redox couple of the electron transfer chain [2], and also to develop amperometric biosensors for NAD^+ dependent dehydrogenases. However, the oxidation of NADH at a conventional solid electrode surface is highly irreversible and takes place at considerable overpotentials. The overpotential at pH 7.0 is about 1.1 V at carbon [3] and 1.3 V at platinum [4] electrodes. Furthermore, it has been reported that the reaction involves radical intermediates which cause electrode fouling [5].

The high overpotential for the oxidation of NADH can be considerably reduced when the electrode was modified with some compounds, called mediators, which can undergo fairly fast redox reactions with NADH and which react directly with the electrode or with a variety of other redox couples. Many com-

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pounds were found to reduce the overpotential of the oxidation of NADH. Jaegfeldt et al. [6,7] and Gorton et al. [8–11] have reported the electrocatalytic oxidation of NADH by adsorbed aromatic compounds containing catechol functionalities [6,7] and adsorbed phenoxazines [8–11]. Albery and Bartlett [12] have reported the oxidation of NADH on electrodes made of a conducting organic salt. Bernadette and Christopher [13] reported the oxidation of NADH at a hexacyanoferrate modified nickel electrode. Other mediators, such as 1,2-quinones [14–18], 1,4-quinones [19], and alkylphenazinium ions [20], have been immobilized on the surface of the carbon electrode through covalent binding, direct adsorption, or through adsorption of a polymer containing the mediating functional group, and were used to catalyze the oxidation of NADH. Electrocatalytic oxidation of NADH at the conducting polymer (such as poly(3-methylthiophene) [21], poly(mercaptoquione) [22] and poly(thionine) [23]) modified electrode was recently reported.

Microelectrodes have received considerable attention in some applications due to their many advantages in comparison with conventionally sized electrodes. Microelectrodes have attracted great interest for the determination of biomolecules in small volumes. Although there are many studies on the electrochemical oxidation of NADH using various electrodes [6–23], to our knowledge there are no reports on the electrochemical oxidation of NADH at a gold microelectrode. In order to obtain more redox-related information on NADH, the mechanism of the electrocatalytic oxidation of NADH at chemically modified electrodes and the development of a microsensor which depends on NADH for in vivo analysis and biotechnology were investigated. It is also necessary to study the electrochemical oxidation of NADH at microelectrodes. The aim of this paper is to describe the voltammetric behaviour of NADH in aqueous media at a nickel hexacyanoferrate modified microband gold electrode and the electrochemical performance of the electrode. The inorganic films are stable to electrochemical processes. A noticeable reduction of the overpotential for NADH oxidation and a remarkably stable voltammetric response at the microband gold electrode modified with the films were observed. Nickel hexacyanoferrate appears to be ideal for application in amperometric sensors.

2. Experimental

NADH was obtained from Sigma and used as received without further purification. All other chemicals were of analytical grade. The phosphate buffer solution was made up from Na_2HPO_4 and KH_2PO_4. All solutions were prepared with deionized water. A PAR M270 electrochemical system (EG&G) was used for electrochemical experiments. Rotating disk electrode measurements were carried out with a PAR M636 ring-disk electrode system (EG&G) using a gold disk electrode (4.5 mm diameter).

A three-electrode system with a platinum wire as the counter electrode, a saturated calomel electrode (SCE) as a reference electrode and a modified microband gold electrode (ca. 0.1 μm $\times$ 1.0 cm) as the working electrode were employed. The working electrode was polished with sandpaper and 0.05 μm alumina slurry and then cleaned by sonicating in doubly distilled water. Prior to use, the electrode was pretreated by a continuous cyclic potential sweep from 0 V to -2.0 V at 50 mV/s in phosphate buffer solution (pH 7.0) until a constant background was observed. The nickel hexacyanoferrate film was electrochemically grown on the microband gold electrode in 5×10^{-4} mol/l $Ni(NO_3)_2$ and 5×10^{-4} mol/l $K_3Fe(CN)_6$ aqueous solution containing 0.1 mol/l $NaNO_3$ as supporting electrolyte by a continuous potential sweep between -0.1 V and $+1.0$ V at a scan rate of 50 mV/s. The film thickness was dependent on the number of cycles. The voltammetric experiments of the modified electrode were carried out in the potential range of 0 to $+0.8$ V at 25 ± 0.1° C. All tested solutions were deaerated by passing highly pure nitrogen for 30 min before the electrochemical experiments, and a continuous flow of nitrogen was maintained over the sample solution during experiments. All the electrochemical experiments were performed inside a Faraday cage.

3. Results and discussion

3.1. Nickel hexacyanoferrate modified microband gold electrode

When cycling the potential of the microband gold electrode in a solution containing $Ni(NO_3)_2$ and

$K_3Fe(CN)_6$, the nickel hexacyanoferrate film can grow on the electrode surface, as indicated by the peak currents that increased with increasing numbers of scanning. A series of typical cyclic voltammograms obtained during the deposition process is shown in Fig. 1. The film is doped with alkali-metal cations to achieve charge neutrality in the film and a net diffusion of ions in and out of film takes place when the redox state of the film changes [24]. The cyclic voltammograms of the nickel hexacyanoferrate modified gold electrode at various scan rates, which were obtained in phosphate buffer solution, are shown in Fig. 2. A couple of well-defined redox peaks were observed. Because the nickel cannot be reduced in this potential range, the redox peaks in Fig. 2 correspond to the hexacyanoferrate(II/III) redox couple. The peak width at half-height is about 96 mV, which approaches the theoretical value of a one-electron reaction mechanism (90.6/n mV according to the literature [25]).

As shown in Fig. 2, the ratio of anodic to cathodic peak currents obtained at various scan rates is almost unity, furthermore, the anodic and cathodic currents increase linearly with scan rates up to 200 mV/s, as predicted for a diffusionless system. The separation of peaks at a low scan rate (20 mV/s) is about 12 mV, a further increase of scan rate resulted in a larger separation of peaks, for example, a separation

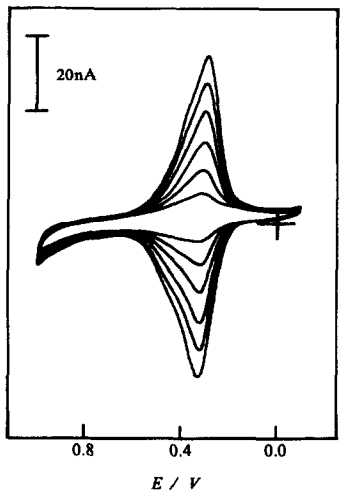

Fig. 1. Cyclic voltammograms for a microband gold electrode in 0.1 mol/l $NaNO_3$ solution containing 5×10^{-4} mol/l $K_3Fe(CN)_6$ and 5×10^{-4} mol/l $Ni(NO_3)_2$ at a scan rate of 50 mV/s.

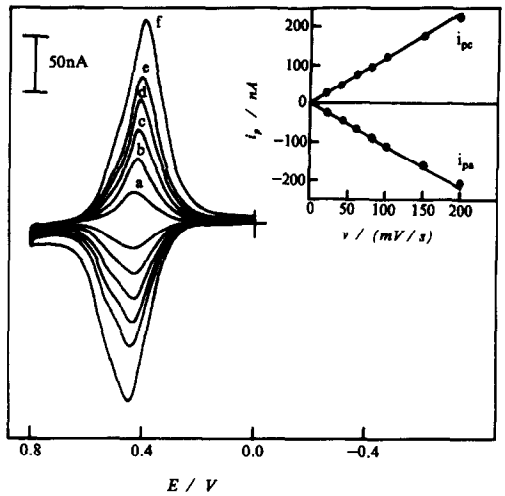

Fig. 2. Cyclic voltammograms of a nickel hexacyanoferrate modified microband gold electrode in 0.1 mol/l phosphate buffer solution (pH 7.0) for a scan rate of (a) 20, (b) 40, (c) 60, (d) 80, (e) 100 and (f) 150 mV/s. The inset shows the dependence of peak currents on the scan rate.

of ca. 52 mV occurs at 150 mV/s. For a fully reversible reaction in which no reactants from the solution take place, the separation of peaks should be zero. The relatively small peak separation indicates fairly fast electrochemical reaction rates [26]. The apparent rate constant obtained from the separation of the peak as a function of scan rate is about 2 s^{-1} [26].

The surface coverage, Γ, can be evaluated from the following equation:

$$\Gamma = Q/nFA \quad (1)$$

where Q is the amount of coulombs obtained from integrating the anodic peak under the background correction, the other symbols have their usual meaning. In the present case, Γ is 3.1×10^{-9} mol/cm^2. The formal potential, $E^{0\prime}$, for the nickel hexacyanoferrate redox couple, which is taken as the mid-point of the anodic and cathodic potential, is 0.422 V. This value is independent of pH in the range of 5.0–8.0.

3.2. Stability of the modified electrode

The stability of the nickel hexacyanoferrate modified electrode was studied by exposing it to air or storing it in buffer solution for a period of time, then recording the cyclic voltammograms. The experi-

mental results indicate that the nickel hexacyanoferrate modified microband gold electrode is not affected by air. Furthermore, there is no loss of redox activity after storing it in the buffer solution for about one week. In addition, the reproducibility of the modified electrode was also examined by repetitive scans in buffer solution. No changes in height and separation of cyclic voltammetric peaks were observed after 100 cycles of repetitive scanning. The results are shown in Fig. 3.

3.3. Catalytic oxidation of NADH at the modified electrode

Fig. 4b shows a cyclic voltammogram of the nickel hexacyanoferrate modified microelectrode in a buffer solution containing 2.0 mmol/l NADH. It can be seen that there is a great increase in the anodic current, corresponding to the oxidation of hexacyanoferrate(II) to hexacyanoferrate(III), compared to the voltammogram in a buffer solution without NADH (Fig. 4a). The reason is that the NADH present in the solution diffuses to the electrode surface and reduces the hexacyanoferrate(III) to hexacyanoferrate(II). As hexacyanoferrate(II) is regenerated by NADH during the sweep, there will be an increase in the anodic current, while the cathodic current is smaller than in the absence of NADH. It can be concluded that overall reactions proceed according to the electrochemical catalytic mechanism as shown below:

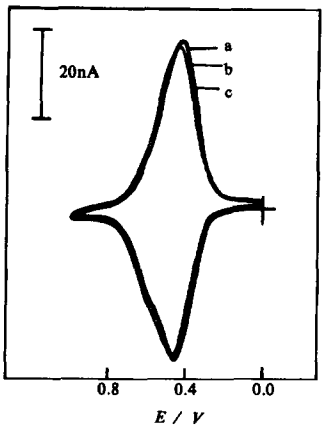

Fig. 3. Cyclic voltammograms of a nickel hexacyanoferrate modified microband gold electrode in 0.1 mol/l phosphate buffer solution (pH 7.0) at the 10th (a), 100th (b) and 150th (c) cycle.

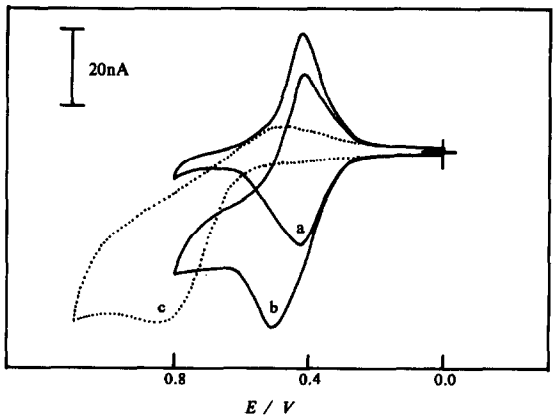

Fig. 4. Cyclic voltammograms of a nickel hexacyanoferrate modified microband gold electrode in 0.1 mol/l phosphate buffer solution (pH 7.0) in (a) the absence and (b) presence of 1.0×10^{-3} mol/l NADH. Curve c shows the oxidation of NADH at a bare gold electrode in the same solution as used for curve b.

$$2Na_2NiFe^{II}(CN)_6 \rightarrow 2NaNiFe^{III}(CN)_6 + 2Na^+ + 2e \quad (2)$$

$$2NaNiFe^{III}(CN)_6 + 2Na^+ + NADH \rightarrow 2Na_2NiFe^{II}(CN)_6 + NAD^+ + H^+ \quad (3)$$

net reaction:

$$NADH \rightarrow NAD^+ + H^+ + 2e \quad (4)$$

The overall oxidation of NADH by the modified electrode is given in Eq. 4.

The catalytic efficiency can be seen directly when Fig. 4b is compared with Fig. 4c. The latter shows a voltammogram of NADH at an unmodified microband gold electrode. The anodic peak potential for the oxidation of NADH at the nickel hexacyanoferrate modified gold electrode is about 0.51 V. At the bare gold electrode, the anodic peak potential is located at ca. 0.85 V. A decrease in overpotential of approximately 340 mV is achieved. This result is much better than the value of 150 mV, obtained by a hexacyanoferrate covered nickel electrode reported in the literature [13]. It probably indicates that the gold-based electrode facilitates much better the electron transfer across the electrode interface.

Successive scanning with stirring in between results in only minor decrease of the peak currents for the oxidation of NADH. This is in accordance with Fig. 3 which indicates that repeated cycling does not result in a loss of redox activity of the nickel hexacyanoferrate film. Further experimental results show that the steady state currents measured at +0.52 V are proportional to the concentration of NADH in the range of 0.5–8.0 mmol/l using an electrode with a surface coverage of 3.1×10^{-9} mol/cm^2. The linear relationship mentioned above establishes the basis for the determination of NADH in the given sample.

Fig. 5 shows the cyclic voltammograms of the nickel hexacyanoferrate modified electrode at various scan rates obtained in 2.0 mmol/l NADH solution. As indicated by Fig. 5, the catalytic oxidation peak potential gradually shifts towards positive potentials with increasing the scan rate; the oxidation currents increase linearly with the square root of the scan rate. These results show that the overall electrochemical oxidation of NADH under consideration might be controlled by the diffusion of NADH in solution and the cross-exchange process between NADH and the redox site of the nickel hexacyanoferrate film.

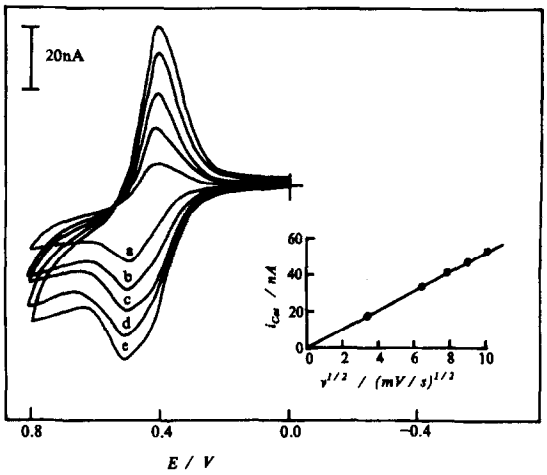

Fig. 5. Cyclic voltammograms of a nickel hexacyanoferrate modified microband gold electrode in 0.1 mol/l phosphate buffer solution (pH 7.0) containing 1.0×10^{-3} mol/l NADH at a scan rate of (a) 20, (b) 40, (c) 60, (d) 80 and (e) 100 mV/s. The inset shows the plot of i_{cat} vs. $v^{1/2}$.

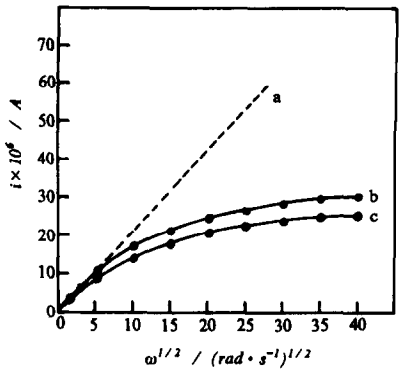

Fig. 6. Levich plot for the oxidation of NADH at a nickel hexacyanoferrate modified microband gold electrode in 0.1 mol/l phosphate buffer solution (pH 7.0). The broken line (curve a) shows the theoretical line for a very fast reaction. An experimentally obtained curve in shown for a $\Gamma = 3.1 \times 10^{-9}$ mol/cm^2, the NADH concentration is (b) 1.6×10^{-3} mol/l and (c) 1.2×10^{-3} mol/l.

3.4. Kinetics of the electrocatalytic oxidation of NADH

It is impossible to obtain quantitative data on electrocatalytic processes only by cyclic voltammetry in the present case, because the rate controlled process is complicated. With rotating disk electrode measurements, however, the electrode can be kept at a stationary potential and a steady state is attained. In addition, the rates of mass transfer at the surface of the rotating disk electrode are much larger than the rates of diffusion, so that the relative contribution of the effect of mass transfer to electron transfer kinetics is smaller.

In our experiments, the disk electrode was kept at +0.52 V just in the potential range the plateau obtained when the potential was scanned slowly during rotation. The anodic current of NADH at the modified electrode increases with increasing rotational speed, although non-linearly in a square root plot (Fig. 6). The broken line is calculated theoretically from the Levich equation. The non-linear behaviour of the modified electrode for NADH proves that a chemical reaction (e.g., reaction (3)) is involved in the rate limiting step. This conclusion is consistent with that obtained for cyclic voltammetry. The catalytic current (i_{cat}) corresponding to the mediated reaction, obtained with the rotating disk elec-

trode, is a function of the currents (i_K) corresponding to the electron cross-exchange between NADH and the nickel hexacyanoferrate site, and the Levich current (i_L) representing the mass transfer of NADH in the solution. Inversion of the Levich equation gives

$$\frac{1}{i_{Cat}} = \frac{1}{i_K} + \frac{1}{i_L} = \frac{1}{nFAk\Gamma c_{NADH}} + \frac{1}{0.620nFA\nu^{-1/6}D^{2/3}c_{NADH}} \cdot \frac{1}{\omega^{1/2}} \quad (5)$$

Where c_{NADH} is the bulk concentration (mol/ml), ω the rotational speed (rad/s), D the diffusion coefficient (cm^2/s), ν the hydrodynamic viscosity (cm^2/s), Γ the surface coverage of nickel hexacyanoferrate (mol/cm^2), k the rate constant for Eq. 3 ($l\ mol^{-1}\ s^{-1}$) and all other parameters have their usual meaning. A Koutecky-Levich plot gives a straight line as shown in Fig. 7. Both the slopes and the intercepts of the lines in Fig. 7 are inversely proportional to the bulk concentration of NADH according to Eq. 5. The rate constant, k, can be calculated from the intercept of the Koutecky-Levich plot and it is found to be 1.3×10^3 l mol^{-1} s^{-1} and 1.1×10^3 l mol^{-1} s^{-1} for NADH concentrations of 1.2 mmol/l and 1.6 mmol/l, respectively. These values are almost comparable with those previously reported for the electrocatalytic oxidation of NADH at the electrodes modified with mediators such as 1,2-benzophenoxazine-7-one [9,11], 5-methylphenazinium- or tetrathiafulvalene-tetracyanoquinodimethane radical salts [12], 4-methyl catechol [15,16] and poly(thionine) [23]. Rate constant, k, values evaluated from the intercepts of plots such as those in Fig. 8 decreased as the NADH concentration increased (as shown in Fig. 8).

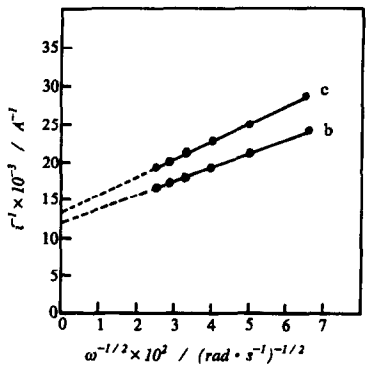

Fig. 7. Koutecky-Levich plot of the same data as in Fig. 6.

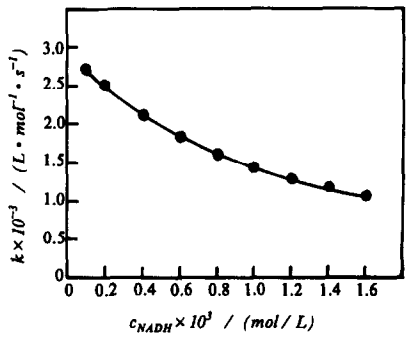

Fig. 8. Variation of the rate constant (k) for the catalytic reaction with the NADH concentration, calculated with Eq. 5.

4. Conclusions

The present study demonstrates that an electroactive, stable, thin film of nickel hexacyanoferrate can be electrodeposited on the surface of a microband gold electrode, and the resulting modified microband gold electrode exhibits an excellent electrocatalytic activity for NADH oxidation in neutral aqueous solution with an overpotential ca. 340 mV lower than that at the bare, unmediated electrode. A quantitative analysis of the mechanism of the electrocatalytic reaction based on rotating disk voltammetry gives a rate constant, k, in the order of 10^3 l mol^{-1} s^{-1}.

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