

ELECTROCATALYSIS OF NICKEL HEXACYANOFERRATE MODIFIED MICROBAND GOLD ELECTRODE FOR OXIDATION OF REDUCED NICOTINAMIDE ADENINE DINUCLEOTIDE

Chen Xin CAI Huang-Xian JU Hong Yuan CHEN*

Department of Chemistry, Nanjing University, Nanjing 210093

Abstract A nickel hexacyanoferrate modified microband gold electrode, which can be prepared by cyclic voltammetry, exhibits excellent electrocatalytic activity for NADH oxidation with an overpotential ca.340mV lower than that at the bare electrode. Quantitative analysis of the electrocatalytic reaction based on rotating disk voltammetry gives a rate constant k of the order of $10^3 \text{ L}\cdot\text{mol}^{-1}\cdot\text{s}^{-1}$.

The electrochemical oxidation of reduced nicotinamide adenine dinucleotide (NADH) in aqueous solution has received a great deal of interest for developing amperometric biosensors for NAD^+ -dependent dehydrogenases. Redox mediators for accelerating the highly irreversible oxidation of NADH at the conventional electrodes have been extensively studied [1-5]. However, up to now there is no report of electrocatalytic oxidation of NADH at a microelectrode. In order to obtain more redox information of NADH and fabricate microsensor which depends on NADH for *in vivo* analysis and biotechnology, it is necessary to study the electrochemical oxidation of NADH at microelectrodes. The aim of this paper is to describe the voltammetric behaviours of NADH at nickel hexacyanoferrate modified microband gold electrode. A noticeable reduction of overpotential for NADH oxidation at microband gold electrode modified with the films was observed. Such work would open up a new area for studying the electrochemical oxidation of NADH by microelectrodes.

The nickel hexacyanoferrate film was electrochemically grown on a microband gold electrode ($0.1\mu\text{m} \times 1.0\text{cm}$) in phosphate buffer (pH 7.0) containing $5 \times 10^{-4} \text{ mol/L}$ $\text{Ni}(\text{NO}_3)_2$ and $5 \times 10^{-4} \text{ mol/L}$ $\text{K}_3\text{Fe}(\text{CN})_6$ by continuous sweep between -0.1V and $+1.0\text{V}$ (vs. SCE) at a scan rate of 50mV/s . The peak currents increasing with increasing numbers of scanning indicates that the nickel hexacyanoferrate film has grown on the electrode surface. The cyclic voltammograms of nickel hexacyanoferrate modified microband gold electrode (defined as NHCF/Au) in phosphate buffer at various scan rates are shown in Figure 1. A

couple of well-defined redox peaks which correspond to hexacyanoferrate(II/III) redox couple were observed. The ratio of anodic to cathodic peak currents obtained at various scan rates is almost unity. The anodic and cathodic currents increase linearly with the scan rate up to 200mV/s . The separation of peaks at low scan rate (20mV/s) is about 12mV . Further increase of scan rate resulted in wider separation of peaks. For example, a separation of ca. 52mV occurs at 150mV/s . The formal potential E^0 for the redox couple is 0.422V . This value is independent of pH in the range of $5.0\sim 8.0$. Stability studies conducted for exposure of NHCF/Au electrode in air as well as stored it in a buffer solution for a period of one week showed that there is no loss of redox activity. In addition, the electrode is stable to electrochemical cycling (> 100 cycles).

Figure 2b shows a cyclic voltammogram of NHCF/Au electrode in a buffer solution containing 2.0mmol/L NADH. It can be seen that there is a great increase in the anodic current compared to that without NADH (Fig.2a). 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 that in the absence of NADH.

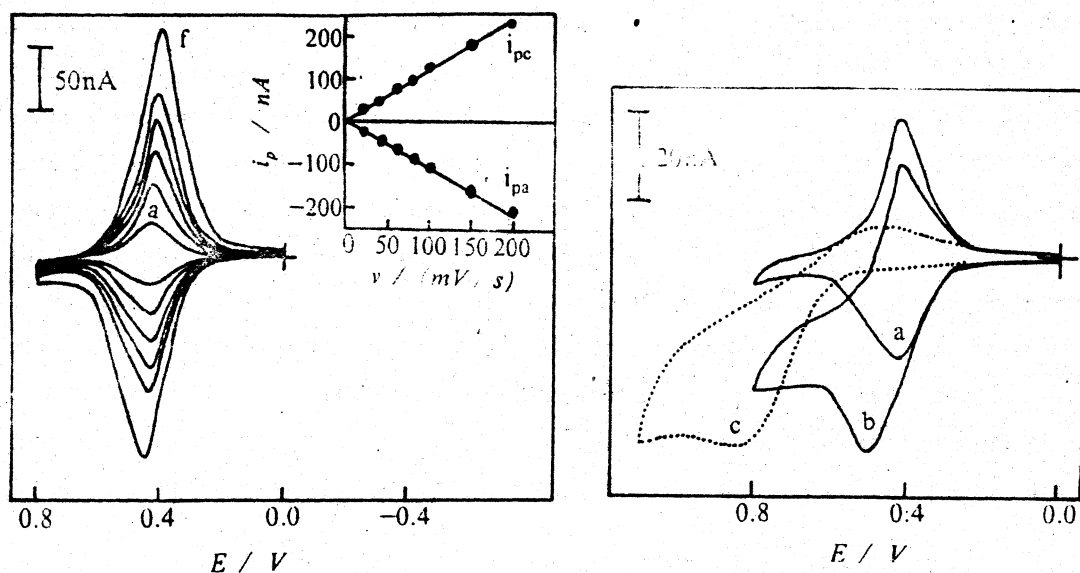


Fig. 1 Cyclic voltammograms of a NHCF/Au electrode in phosphate buffer solution (pH 7.0) for scan rate (a~f): 20, 40, 60, 80, 100 and 150mV/s respectively. The inset shows the plot of i_p vs. v .

Fig. 2 Cyclic voltammograms of a NHCF/Au electrode in phosphate buffer solution (pH 7.0) in absence (a) and in presence (b) of $1.0 \times 10^{-3}\text{mol/L}$ NADH. Curve c shows the oxidation of NADH at a bare gold electrode in the same solution as used for curve b.

The steady state currents measured at +0.52V are proportional to the concentration of NADH in the range of 0.5~5.0 mmol / L. The linear relationship establishes the basis for the determination of NADH.

The catalytic efficiency can be seen directly when Fig.2b is compared with Fig.2c. The anodic peak potential for oxidation of NADH at NHCF / Au electrode is about 0.51V. At the bare gold electrode, the anodic peak potential locates at ca.0.85V. A decrease in overpotential of approximate 340mV is achieved. Further experiments indicate that the catalytic oxidation peak potential (Fig.2b) shifts slightly toward positive position with increasing scan rate and the oxidation currents increase linearly with the square root of scan rate. These results show that the overall electrocatalytic oxidation of NADH under consideration might be controlled by the diffusion of NADH in solution and the cross-exchange process between NADH and redox site of nickel hexacyanoferrate film.

It is impossible to get quantitative data of electrocatalytic process only by cyclic voltammetry in the present case, because the rate controlled process is complicated. But with a rotating-disk electrode, the electrode will 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 the electron transfer kinetics is smaller. A Koutecky-Levich plot for the catalytic oxidation of NADH at NHCF / Au electrode gives a straight line as shown in

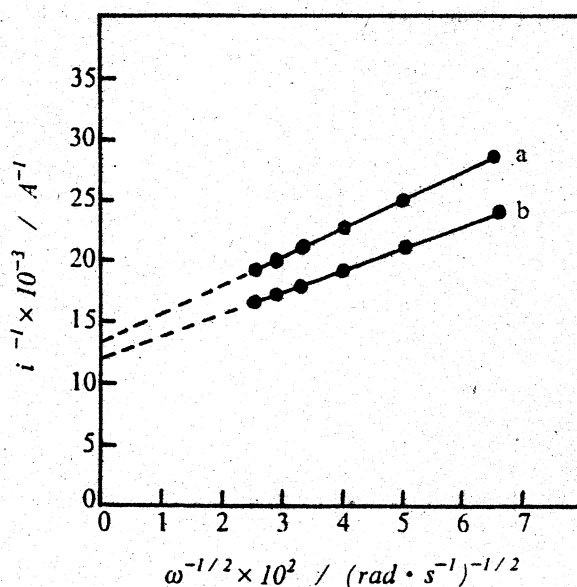


Fig. 3 Koutecky-Levich plot for oxidation of NADH at NHCF / Au electrode with $\Gamma = 3.1 \times 10^{-9}$ mol / cm², the NADH concentration is (a) 1.2×10^{-3} mol / L and (b) 1.6×10^{-3} mol / L.

Figure 3. The catalytic rate constant, k , can be calculated from the intercept of the Koutecky-Levich plot and it is found to be $1.3 \times 10^3 \text{ L.mol}^{-1}.\text{s}^{-1}$ and $1.1 \times 10^3 \text{ L.mol}^{-1}.\text{s}^{-1}$ for the NADH concentrations of 1.2 mmol/L and 1.6 mmol/L with $\Gamma = 3.1 \times 10^{-9} \text{ mol/cm}^2$, respectively. These values are almost comparable with those previously reported for the electrocatalytic oxidation of NADH at the electrodes modified with the mediators such as 1,2-benzophenoxazine-7-one ($8 \times 10^2 \text{ L.mol}^{-1}.\text{s}^{-1}$) [2], 5-methylphenazinium- or tetrathiafulvalene-tetracyanoquinodimethane radical salts ($1.5 \times 10^3 \text{ L.mol}^{-1}.\text{s}^{-1}$) [3], and poly(thionine) ($2.1 \times 10^3 \text{ L.mol}^{-1}.\text{s}^{-1}$) [4].

Acknowledgement

The project was supported by the National Natural Science Foundation of China.

References

- 1 B. Persson, *J. Electroanal. Chem.*, 1990, 287, 61
- 2 L. Gorton, *J. Electroanal. Chem.*, 1990, 292, 115.
- 3 W.J. Albery and P.N. Bartlett, *J. Chem. Soc., Chem. Commun.*, 1984, 234
- 4 T. Ohsaka, K. Tanaka and K. Tokuda, *J. Chem. Soc., Chem. Commun.*, 1991, 122.
- 5 N.F. Atta, A. Galal, A.E. Karagozler, H. Zimmer, J.F. Rubinson and H.B. Mark Jr., *J. Chem. Soc., Chem. Commun.*, 1990, 1347.

(Received 18 October 1994)