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Mediatorless catalytic oxidation of NADH at a disposable electrochemical sensor

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Abstract

We report here a simple, selective and sensitive method for the determination of NADH in neutral aqueous solution by using an electrochemically preanodized screen printed carbon electrode (SPCE*) without the addition of any redox mediator. The preanodization procedure makes the SPCE more electroactive towards NADH oxidation with a decrease of ∼300 mV in the overpotential without electrode fouling. It is proposed that surface reorientation to generate more edge plane sites during the preanodization procedure, as confirmed by a distinctive Raman band at ∼1360 cm^{-1}, is the main cause for the facilitated electron transfer for the oxidation of NADH. Presence of large excess of ascorbic acid does not interfere the detection of NADH and the SPCE* shows individual voltammetric peaks for ascorbic acid and NADH. Under optimized conditions, the obtained calibration plot shows a linear range up to 100 μM with a detection limit (S/N = 3) of 157 nM by flow injection analysis.

Keywords: NADH oxidation; Screen printed carbon electrode; Preanodization; Flow injection analysis

1. Introduction

The electrochemical oxidation of nicotinamide adenine dinucleotide (NADH), a cofactor required in the enzymatic reaction of dehydrogenase, is essential in the development of dehydrogenase-based biosensors. Direct electrochemical oxidation of NADH often occurs with high overpotential leading to the interference from other easily oxidizable species in the sample of interest [1]. Electrode fouling is another concern since NADH gets oxidized to form NAD$^+$ through a (2e$^-$ + 1H$^+$) process with the cleavage of C–H bond to form dimers at the electrode surface [2]. This can then attribute sluggish heterogeneous electron transfer and thereby reduces the sensitivity of the electrode [1,3]. Ceans et al [4] reported the electrocatalytic oxidation of NADH and ascorbic acid on electrochemically pretreated glassy carbon electrodes however the oxidation of NADH in presence of ascorbic acid was not demonstrated. Various chemically modified electrodes have been reported to decrease the high overpotential and to eliminate the electrode fouling effect [2,5–10]. Meanwhile use of mediators with fast electron transfer to catalyze NADH has also been intensively studied [11–18]. Nevertheless the development of a rapid, cheap, disposable and sensitive electrochemical sensor for NADH determination is still a matter of interest.

Recently, Banks and Compton [19] observed an interesting behavior of NADH at an edge plane pyrolytic graphite electrode and proposed that it can conveniently replace carbon nanotube modified electrode [5] for the routine sensing of NADH due to its simplicity of preparation and cost. Edge plane pyrolytic graphite electrodes are advantageous for use as electrode substrates in electroanalysis owing to the highly reactive edge plane sites which allow low detection limits, high sensitivities, improved signal to noise characteristics and low overpotentials. Similarities to the above mentioned principle, in this report another approach for the electrochemical oxidation of NADH by using a disposable preanodized screen printed carbon electrode (designated as SPCE*) was demonstrated. Surface reorientation to generate more edge plane sites during the preanodization procedure is proposed as the main cause for the facilitated electron transfer for the oxidation of NADH. The generation of more edge plane sites during the preanodization procedure was characterized by a distinctive Raman band at ∼1360 cm^{-1} [20,21].

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The stability of the treated electrode was then checked and the response of NADH was finally evaluated by cyclic voltammetry (CV) and flow injection analysis (FIA). The preanodization procedure can not only make the SPCE more electroactive towards NADH oxidation but also provide low susceptibility to electrode fouling. We believe that the SPCE* is a good alternative for the routine sensing of NADH due to its simplicity of preparation, low susceptibility to electrode fouling, low detection limit and insensitivity to interference from ascorbic acid.

2. Experimental

2.1. Chemicals and reagents

β-Dihydronicotinamide adenine dinucleotide reduced disodium salt (MP Biomedicals) and ascorbic acid (Sigma) were used as received without further purification. All other chemicals used were of ACS certified reagent grade. Aqueous solutions were prepared with doubly distilled and deionized water. Unless otherwise mentioned, a pH 7.4, 0.1 M phosphate buffer solution (PBS) was used as supporting electrolyte in all experiments.

2.2. Apparatus

Electrochemical experiments were carried out with a CHI 400 electrochemical workstation (CH Instruments, Austin, TX, USA). The three electrode system consists of an SPCE* as working electrode, an Ag/AgCl reference electrode and a platinum auxiliary electrode. The disposable SPCEs (geometrical area = 0.2 cm^2) were purchased from Zensor R&D (Taichung, Taiwan). The FIA system is consisted of a Cole-Parmer microprocessor pump drive, a Rehodyne model 7125-sample injection valve (20 μl loop) with interconnecting Teflon tube and a Zensor SF-100 thin-layer detecting electrochemical cell specifically designed for SPCE (Zensor R&D, Taichung, Taiwan). A carrier solution of pH 7.4 PBS was used throughout the FIA experiments.

Room temperature Raman spectra were recorded with a 3D Nanometer Scale Raman PL Microscopy system by using a Nanofinder® 30 (Tokyo Instruments, INC) that used a He/Ne laser beam with an excitation wavelength of 633 nm and a CCD detector (Andor DU401-BV) with a readout speed of 1–32 μs/pixel at −70 °C to record the Raman scattered light intensity.

2.3. Procedure

For all experiments, a bare SPCE was electrochemically cleaned by cycling the potential between −0.8 and 1.2 V. The applied potential and time for preanodization was then optimized carefully to get good electrochemical behavior for NADH. In most cases, electrode was electrochemically oxidized (i.e., preanodization) by applying a potential at 2.0 V versus Ag/AgCl for 90 s in pH 7.4 PBS under stirred condition. The as-prepared electrode was further used for the voltammetric studies of NADH.

3. Results and discussion

3.1. Electrochemical oxidation of NADH

Fig. 1 compares the cyclic voltammograms obtained for the oxidation of NADH at a bare SPCE and the SPCE* in pH 7.4 PBS. As can be seen, anodic oxidation of NADH takes place at a much higher potential (ca. 0.73 V) on a bare SPCE. However, a gradually decrease of peak current in subsequent cycles indicates the fouling of the electrode surface (data not shown). Obviously dimerization of the oxidized product NAD$^+$ occurs easily at higher potentials and hence it gets adsorbed on the electrode surface. Compared to the CV behavior observed on a bare SPCE, the SPCE* exhibited a significant shift in peak potential to a less positive value (ca. 0.40 V, Fig. 1B). Furthermore, the peak current was unaltered in the subsequent cycles (Fig. 1C) indicating low susceptibility to electrode fouling of the SPCE*. The negative shift in the oxidation potential also reflects a faster electron transfer reaction at the preanodized electrode. It

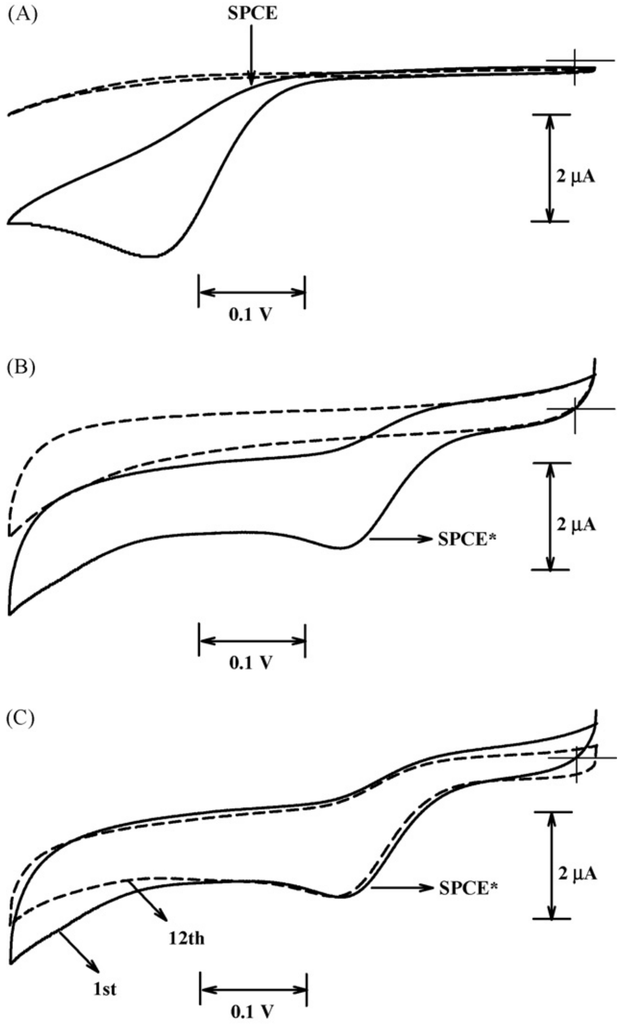

Fig. 1. Cyclic voltammetric responses of a SPCE (A) and the SPCE* (B) in 0.1 M PBS (pH 7.4) without (dotted line) and with (dark line) 1 mM NADH at $\upsilon = 50$ mV/s. (C) Cyclic voltammetric responses of 1 mM NADH at the SPCE* for 24 continuous cycles.