



Iron oxide/carbon black (Fe₂O₃/CB) composite electrode for the detection of reduced nicotinamide cofactors using an amperometric method under a low overpotential

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ARTICLE INFO

Article history:

Received 24 June 2009

Received in revised form 1 October 2009

Accepted 5 October 2009

Available online 14 November 2009

Keywords:

Amperometric detection

Dehydrogenase

Direct oxidation

Iron oxide/carbon black composite electrode

Nicotinamide cofactor

ABSTRACT

An amperometric biosensor for the detection of the reduced nicotinamide cofactors NADH and NADPH was designed, based on the electrochemical oxidation of NAD(P)H with an iron oxide/carbon black composite (Fe₂O₃/CB) electrode. The electrode exhibited excellent performances in that it led to a substantial decrease in the overpotential of electrochemical NADH oxidation. Iron oxide plays a significant role as a catalyst for NADH oxidation and the reaction occurs at +0.00 V (vs. Ag/AgCl). The method of the sensor construction is very simple and the sensor performed well, giving high sensitivity, high stability, and a broad detection range. The sensitivity of this system is 2.54 μA mM⁻¹ and the limit of detection (S/N = 3) is 10 μM. A linear range was observed between 10 μM and 1000 μM of NADH (R² = 0.993), which is preferable to that of the previous studies. The Fe₂O₃/CB electrode also oxidizes NADPH under the same condition and can be applied as an NADPH sensor. Moreover, when the sensor system was integrated into a dehydrogenase-based sensor system, it also showed a good sensing performance.

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1. Introduction

In the recent decades, nicotinamide cofactors such as reduced nicotinamide adenine dinucleotide (NADH) or reduced nicotinamide adenine dinucleotide phosphate (NADPH) have received a considerable interest because these cofactors are essential in hundreds of dehydrogenase reactions, including the biological electron transfer system used by all living organisms. Particularly, measuring NADH is very important because NAD(P)⁺ is used as a cofactor for about 250 NAD⁺-dependent and 150 NADP⁺-dependent dehydrogenases. It can be applied to analytical detection, fermentation, clinical practices, food industry, and dairy industry. Therefore, the development of a detection system for NAD(P)H is attractive (Lobo et al., 1997).

Since the detection of many important biological analytes is carried out using an electrochemical method, a highly sensitive amperometric sensor is desirable. The direct oxidation of NADH on bare electrode surfaces, however, usually requires a high overpotential and suffers from a poor sensitivity. In fact, conventional electrodes such as carbon, gold, and platinum require an overpotential higher than +1.00 V (Jaegfeldt, 1980; Moiroux and Elving, 1978; Samec and Elving, 1983), which causes undesirable side reactions,

remarkably increased background currents, hence, difficulties in obtaining accurate signals. In addition, the direct oxidation of NADH brings about fouling on the electrode surface due to undesirable dimer formations from the intermediates. Therefore, various mediators such as organic dyes (Kumar and Chen, 2007; Maroneze et al., 2008; Zhang and Gorski, 2005; Zhu et al., 2007), thio-substituted nucleobases (Behera and Raj, 2007), transitional metal complexes (Wu et al., 1996), and conducting polymers (Bartlett and Simon, 2003) have been employed for the purpose of decreasing the overpotential and the electrode fouling. However, although the use of such mediators seems promising, they still cause problems such as leakages from the electrode surface, lack of a long-term stability, and toxicity, which have a negative influence on their analytical applications.

Today, with the development of material science, there have been various trials to employ new materials, such as modified carbon electrodes (Campbell and Rishpon, 2001; Prasad et al., 2007) and nanomaterials (Deng et al., 2008; Liu et al., 2008; Zhang et al., 2004). The use of carbon nanomaterial electrodes is of great interest due to their unique structural and electrical properties. Carbon nanotubes (CNT) have wholly activated surfaces and oxygen-containing activated sites that are ideal for the immobilization of enzymes. Carbon nanofibers (CNF) are also used for biosensor electrodes because of their structural advantages for the electrocatalysis of biological molecules. Additionally, various materials such as gold nanoparticle multilayers and Ti-MCM (Dai et

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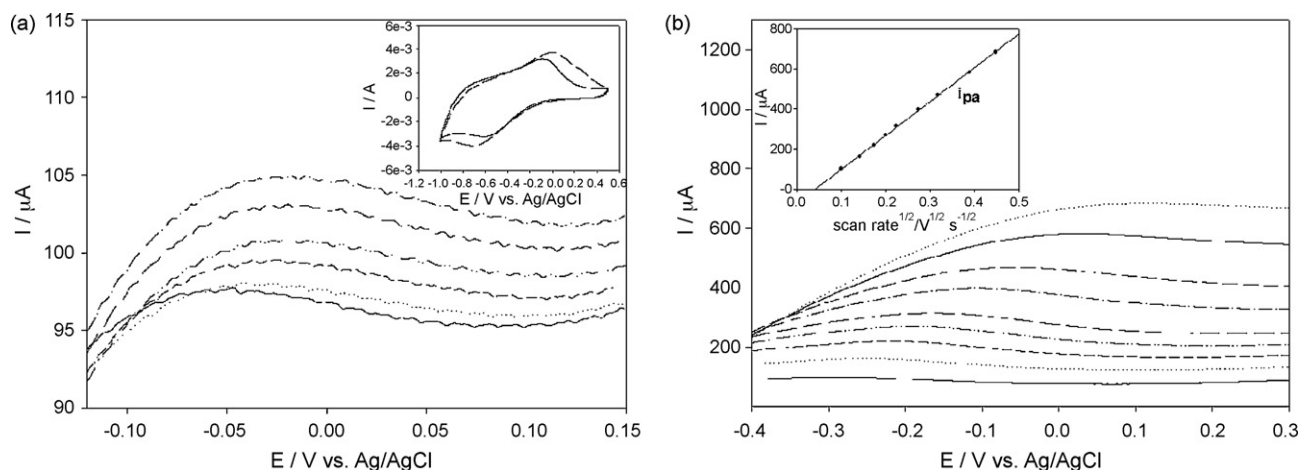


Fig. 1. (a) Linear sweep voltammograms of the $\text{Fe}_2\text{O}_3/\text{CB}$ electrode with different NADH concentrations (0 mM, 1 mM, 2 mM, 3 mM, 4 mM, and 5 mM; arranged from bottom to top, respectively) (insert: cyclic voltammograms in the absence and presence of 1 mM NADH) at a scan rate of 50 mV s^{-1} ; (b) linear sweep voltammograms of the $\text{Fe}_2\text{O}_3/\text{CB}$ electrode at a scan rate of 10 mV s^{-1} , 20 mV s^{-1} , 30 mV s^{-1} , 40 mV s^{-1} , 50 mV s^{-1} , 75 mV s^{-1} , 100 mV s^{-1} , 150 mV s^{-1} , and 200 mV s^{-1} ; arranged from bottom to top, respectively (insert: dependence of the peak current on scan rate) in pH 7.5 potassium phosphate buffer (100 mM).

al., 2007) were employed to decrease the high overpotential and minimize the effect of the electrode surface fouling.

Here, we report a new sensor for the nicotinamide cofactor detection based on the amperometric method. To overcome the limitations of previous studies, an iron oxide/carbon composite ($\text{Fe}_2\text{O}_3/\text{CB}$) electrode was introduced for NAD(P)H oxidation under a very low overpotential without the employment of the mediators or chemical treatment. In our previous study, we found that direct electrochemical NAD(P)H oxidation is possible using a metal oxide electrode (Kim and Yoo, 2009). A metal oxide electrode including iron oxide has a role of an electrocatalyst for the NAD(P)H oxidation. Based on the previous results, we are able to detect NADPH as well as NADH. In addition, the proposed system can be applied to the ethanol detection by using NAD^+ -dependent alcohol dehydrogenase.

2. Experimental

2.1. Reagents

Super P[®] conductive carbon black (CB) was obtained from TIMCAL graphite & carbon (Bodio, Switzerland) and polytetrafluoroethylene (PTFE) was kindly offered from the electrochemical energy conversion and storage laboratory in SNU. Iron oxide (Fe_2O_3), NAD^+ , NADH, NADPH, and alcohol dehydrogenase from *Saccharomyces cerevisiae* (EC 1.1.1.1) were obtained from Sigma (St. Louis, USA). The reagents for buffer preparation were purchased from Junsei (Tokyo, Japan). Isopropanol and ethanol were obtained from Merck (Darmstadt, Germany). All reagents were used without further purification and aqueous solutions were prepared using deionized water from a Milli-Q water purification system (Millipore, France).

2.2. Apparatus

The surface of the prepared electrode was analyzed by SUPRA 55VP field emission scanning electron microscope (FE-SEM; Carl Zeiss, Oberkochen, Germany) with an accelerating voltage of 2 kV.

Electroanalytical measurements such as cyclic voltammetry, linear sweep voltammetry, and chronoamperometry were taken by using an Autolab PGSTAT 302N potentiostat/galvanostat (Eco Chemie, Utrecht, The Netherlands). This instrument was operated using the GPES program (Eco Chemie). Electrochemical impedance spectroscopy (EIS) was carried out using a CHI 660A electrochem-

ical workstation (CH Instruments, Inc., Austin, USA). The EIS data were fit by Zview software. All electrochemical experiments were performed with a 5 mL reaction volume in a 20 mL electrochemical cell (Bioanalytical Systems, Inc., West Lafayette, USA). The electrode body (3 mm dia; BAS) filled with $\text{Fe}_2\text{O}_3/\text{CB}$ or CB and a glassy carbon electrode (GC, 3 mm dia; BAS) was used as the working electrode. Platinum coil was used as the counter electrode and a RE-5B Ag/AgCl electrode (BAS) was used as the reference electrode.

The activity of alcohol dehydrogenase was determined by the change in absorbance of NADH at 340 nm using a Cary 50Conc UV-vis spectrophotometer (Varian, Victoria, Australia), the enzyme was put in 1 mM NAD^+ and 10 mM ethanol at 25°C .

2.3. Preparation of the iron oxide/carbon black composite electrode

The $\text{Fe}_2\text{O}_3/\text{CB}$ electrode was prepared for the NADH determination. The Teflon electrode body was previously cleaned by sonication with deionized water for 15 min. Iron oxide powder was mixed with carbon black at a given ratio (5:1, 10:1, and 15:1). For binding each component, a 0.1 mL PTFE dispersion in water (10 mg mL^{-1}) was used. The colloid was desiccated for 20 min, and carbon black and iron oxide were added on the dried colloidal PTFE. Using 2-propanol, the three components became mingled and kneaded until they were equally distributed. Then, the electrode body was filled with the mixed composite. Prior to every experiment performed with the composite electrode, the electrode was rinsed with deionized water.

3. Results and discussion

3.1. Characterization of the iron oxide/carbon black composite electrode

The iron oxide/carbon black composite ($\text{Fe}_2\text{O}_3/\text{CB}$) electrode was prepared for the amperometric detection of NAD(P)H. The surface distribution of Fe_2O_3 and CB on the surface of the prepared electrode was observed by FE-SEM (Supplementary data). It showed that the prepared electrode had a porous structure and that the composition materials for the electrode were well dispersed. The particle size of iron oxide was hundreds of nanometers and the average particle size of carbon black was approximately 30 nm.

The basic electrochemical properties of the $\text{Fe}_2\text{O}_3/\text{CB}$ electrode were investigated by cyclic voltammetry and linear sweep voltam-

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