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Amperometric determination of NADH with Co_3O_4 nanosheet modified electrode

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ABSTRACT

In this work, we have developed a simple and reliable cobalt oxide (Co_3O_4) based amperometric sensor for the determination of NADH. A sheet shape Co_3O_4 nanooxide was synthesized by the CTAB assisted hydrothermal technique and was characterized by SEM and XPS. Owing to the redox property of Co_3O_4, the operating potential of NADH can be significantly reduced from 0.7 down to 0.1 V. Compared to a commercial Co_3O_4 nanoparticle modified electrode, this nanosheet form cobalt oxide possesses a rapid background subsiding characteristic and a low residual current. This scheme was conducted on a flow injection system with a constant operating potential of 0.1 V (vs. Ag/AgCl, 3 M) in a 0.2 M phosphate buffer at pH 6.0. A suitable linear range from 10 to 100 μM (R=0.999) with a detection limit of 4.25 μM (S/N=3) was obtained. The RSD for 20 successive measurements of 75 μM NADH is only 1.4%, which indicates a high stability and no contamination during NADH oxidation. This scheme did not suffer from conventional antioxidants, including dopamine, uric acid, epinephrine, serotonin, histamine, and 4-acetaminophen, except ascorbic acid. Thus, an ascorbate oxidase was introduced to remove the ascorbic acid before the sample was injected into the flow injection analysis system. After this simple pretreatment, the influence of ascorbic acid was eliminated, successfully.

1. Introduction

NAD^+ and its reduced form, NADH, are important cofactors in the electron transport chain. More than 300 biological enzymatic reactions require NAD^+ as a cofactor to receive the electron after a particular dehydrogenated reaction. Thus, the merits of a NAD^+/NADH sensor are critical due to its possible application in the development of dehydrogenase based biosensors as well as their applications in food processing, environmental analysis, and clinical diagnosis. Several optical approaches have been reported in the determination of the NADH; generally, measuring the absorption of NADH at 340 nm is widely used in identification of enzymatic activity (McComb et al., 1976). Subsequently, because the excited NADH is a suitable fluorophore which has a strong emission at 450 nm, a further fluorescence method provides a high sensitivity in the NADH determination (Podrazky and Kuncova, 2005). In addition, NADH is a suitable co-reactant for the Ru $(bpy)_3^{2+}$ that enhances the electrochemical luminescence (ECL); several ECL based approaches have been reported to provide a highly sensitive scheme to monitor the NADH at nanomolar level (Martin and Nieman, 1997; Deng et al., 2009).

Actually, NADH is a famous reductant in biological systems with a formal reduction potential of −0.32 V vs. NHE (Nelson and Cox, 2005), and electrochemical determination of NADH is a critical issue. However, direct oxidation of NADH required a relatively high overpotential at bare electrodes (Eisenberg and Cundy, 1991; Wang et al., 2001) and usually causes a passive layer to pollute the surface of the electrode (Radoi et al., 2008). Keita and Sampath indicated that this problem is attributed to its electrochemical product which would adsorb onto the surface after self dimerization (Keita et al., 1996; Sampath and Lev, 1998). Besides, the crucial overpotential limits its application in real biological samples from most co-existed antioxidants. In order to overcome this problem, several carbon based materials such as carbon nanotube (Pumera et al., 2006), carbon fiber (Zhao et al., 2010), pyrolytic glassy carbon (Banks and Compton, 2005), and graphene (Keeley et al., 2011) have been proposed to show its facilitation in electrochemical oxidation of NADH; however, the catalytic potential of these carbon based sensors can be shifted down to only around 0.4 V, and analytical result would be still affected by most biological antioxidants.

To solve these dilemmas, several redox mediators were employed to provide another pathway in the catalytic oxidation of NADH. The most popular organic modifiers can be roughly divided into fluorenone (Mano and Kuhn, 1999), phenothiazine (Boguslavsky et al., 1995), phenoxazine (Kubota et al., 1996), and quinone (Jaegfeldt et al., 1981) based modifiers according to their

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basic chemical structure. These mediators could be easily adsorbed on the electrode surface through physical or electrostatic interaction. Generally, the operating potential used in a physically adsorbed modified sensor could be down to less than 0 V; however, these sensors can be operated only in a static condition due to the weak interaction between the surface and the modifier. Electrochemical polymerization provides a more secure scheme to immobilize these mediators on the electrode surface which can be easily held on high convective situation such as a flow injection system and a rotating analysis; however, the catalytic potential of the most polymerized electrode shows a positive shift compared to that of a free molecule. For example, the catalytic potential of a methylene blue modified electrode is −0.036 V (Borgo et al., 2002), but a polymeric electrode required 0.15 V to achieve this approach (Dilgina et al., 2011). Toluidine blue/CNT electrode possesses a catalytic potential at −0.2 V (Lawrence and Wang, 2006), but shifted toward 0.2 V after polymerization (Hasebe et al., 2011).

The application of nanometal or metal oxide particles has emerged as an attractive investigation in recent years. Since the chemical and physical properties of nanomaterials are different from bulk ones, a number of research fields are successful in obtaining a remarkable progress after working with nano materials. Several fabricated techniques, including sol–gel (Laberty-Robert et al., 2006), co-precipitation (Quin and Shi, 1998), and hydrothermal method (Hagrman et al., 2001), have been widely demonstrated. Among these schemes, the hydrothermal method is considered as an attractive method to fabricate nanometal oxides. By controlling the temperature and pressure, a metastable material has been produced (Zhang et al., 2003). In addition, the morphology of the nanostructure is varied according to the conformation of the micelle (Wang et al., 2011). Generally, ethylene diamine tetraacetic acid, sodium dodecyl sulfate, polyethyleneglycol and hexadecyltrimethylammonium bromide (CTAB) have been frequently used as templates in hydrothermal techniques.

Recently, metal oxide based modified electrodes have intensively investigated in the electrochemical determination of several clinical markers due to their interesting catalytic properties (Chen and Lin, 2012; Shih et al., 2009; Zen et al., 2002). However, their application in NADH is still rare. To the best of our knowledge, only Fe_3O_4/CNT modified electrode has been reported to show its catalytic oxidation of NADH at 0 V (Teymourian and Salimi, 2012); however, that report used an acid pre-treated carbon nanotube as its basic matrix, and this matrix has been proven to possess a similar catalytic behavior to that of the NADH at 0 V (Wooten and Gorski, 2010); hence, the role of Fe_3O_4 in the catalytic determination of NADH still needs to be investigated. In this work, the feasibility of catalytic oxidation of NADH by using the redox property of Co_3O_4 is reported. In order to overcome the drawbacks of highly residual current and sluggish response of Co_3O_4 modified electrode, a nanosheet Co_3O_4 synthesized by the CTAB assisted hydrothermal technique was used in this study, which provides a simple and sensitive method with limited interference. The possible mechanism and the analytical performance are also described in this report.

2. Material and methods

2.1. Apparatus

A CHI 832B electrochemical workstation (CH Instruments Austin, TX, USA) was used in the whole electrochemical studies. The flow rate of the FIA system was controlled with a syringe pump (74900 series, Cole-Parmer Instrument Company, Illinois, USA). A steel reactor containing a Teflon vessel was used to synthesize the Co_3O_4 nanosheet. The surface morphology of nanosheet was taken by FE-SEM (LEO1530, Germany) and X-ray Photoelectron Spectroscopy (XPS) was performed by ESCALAB 250 (Thermal VG science, UK). A Ag/AgCl (3 M NaCl) electrode was used as the reference electrode in this scheme.

2.2. Reagents

Cobalt (II) nitrate hexahydrate, and NADH (98%) were purchased from Acros Organics. Urea (99.5%) was obtained from Riedel-dehaën (Seelze, Germany). Serotonin hydrochloride (99%) was purchased from Alfa-Aesar. Hexadecyltrimethylammonium bromide (CTAB, 99%), L (+) -ascorbic acid (99.7%), uric acid, dopamine, histamine (99%), (±)-epinephrine (95%), 4-acetaminophenol, Co_3O_4 nano particle, and CoO were obtained from Sigma-Aldrich (St. Louis, USA). All other chemicals and solvents were of analytical grade and were used as received without further purification. A conductive carbon ink (C10903D14) obtained from Gwent Electronic Materials Ltd. (Pontypool, UK) was used for immobilization of CoO, and Co_3O_4 on the electrode. Ascrobate oxidase (EC 1.10.3.3) was purchased from TOYOBO (Osaka, Japan).

2.3. Fabrication of Co_3O_4 nanosheet

The Co_3O_4 nanosheet was fabricated according to the previous report with certain modification (Liu et al., 2007). Briefly, 6 mmole Co $(NO_3)_2$, 45 mmole urea, and 3 g CTAB were dissolved in a Teflon container containing 100 mL water. Subsequently the container was sealed in a stainless steel reactor. A purple-red cobalt complex would be obtained after sequentially heating at 70 °C and 120 °C for 24 h and 12 h, respectively. This complex was dried in an oven at 70°C overnight and calcined in a tube furnace (F-21100, Thermolyne, USA) at 600 °C for 2 h to remove the organic template and convert their oxidation state into Co_3O_4.

2.4. Procedure of electrode modification and mechanism study

A homemade rotating disc carbon electrode with a diameter of 5 mm was used in the hydrodynamic voltammotry and potentiometric study. This Co_3O_4 modified electrode was constructed by weighting appropriate Co_3O_4 nanosheet, conducting ink and cyclohexanone. The final weight ratio of this suspension mixture is 7:3:20. Subsequently, 2.5 μL of above mixture was placed on the electrode surface and allowed to dry in an oven at 70 °C for 1 h. When not in use, it was kept in a dry container full of nitrogen at room temperature. A dual rotating ring–disk platinum electrode (BAS, IN, USA) immobilized with 70% CoO powder on the central disk electrode was used to investigate the final product of oxygen reduction. A simple potentiometric study by measuring the potential difference between modified electrodes and the reference electrode (Ag/AgCl, 3 M NaCl) was used to evaluate the oxidation state of cobalt oxide.

3. Results and discussion

3.1. Characterization of Co_3O_4 nanosheet

The morphology of the prepared cobalt oxide was characterized by scanning electron microscopy (SEM). Fig. 1A shows a typical SEM image of the Co_3O_4 nanosheet with magnification of 10,000 folds. This image shows that this microscale particle is constructed from several nano rectangular sheets. Further magnification with 100,000 folds as shown in Fig. 1B indicates that