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Amperometric determination of NADH based on poly Ni(II)-curcumin composite film modified glassy carbon electrode

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Abstract: A poly Ni(II)-curcumin (curcumin: 1,7-bis[4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-dione) composite film modified electrode was prepared for amperometric determination of β -nicotinamide adenine dinucleotide (NADH). Scanning electron microscopy and Energy Dispersive X-ray spectroscopy were used to examine the morphology and interface property of the Ni(II)-curcumin film. Under optimum conditions, the amperometric detection of NADH provided wide linear detection range (0.3–300 μM), high sensitivity (230.02 $\mu\text{A mM}^{-1} \text{cm}^{-2}$) and low limit of detection (LOD=0.18 μM , S/N=3). Moreover, the modified electrode showed good stability and reproducibility, indicating that it can improve the analysis signal of NADH with a very convenient way.

Keywords: Amperometric sensing; Electrocatalysis; NADH; Ni(II)-curcumin

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1. Introduction

Nicotinamide adenine dinucleotide (NAD⁺) and its reduced form (NADH) are the most important charge carriers in the biological cells [1,2]. NAD (H) is an important part of the respiratory chain existing in the organs or tissues of prokaryotic and eukaryotic organisms, which participates in cell metabolism and stimulates the production of energy as a coenzyme involved in more than 300 kinds of dehydrogenase enzyme reactions [3,4]. In recent years, studies have shown that NADH also plays an important role in gene expression, immune function and cellular senescence, etc [5–7]. Through the analysis of its concentration and related parameters of detection, analysts can measure the cell metabolic activity and health status [8]. Therefore, sensitive and selective methods for the determination of NADH are in great demand.

Nowadays, there have been kinds of analysis methods to detect NADH, such as spectroscopy [10], high performance liquid chromatography method [11], fluorescence method [12], capillary electrophoresis method [13] and electrochemical analysis [14], etc. Among them, electrochemical analysis method is attractive because of high sensitivity, simple operation and low cost. However, due to NADH has a high overpotential [15], the effect of direct electrochemical oxidation of NADH at bare electrode is not ideal. Liu et al. reported the application of Fe₃O₄ nanoparticles with poly-2,6-pyridinedicarboxylic acid modified glassy carbon electrodes to detect NADH [16]. Hong et al. prepared poly methyl methacrylate and graphene modified electrodes applied to the direct electrochemical behavior research to NADH [17]. Teymourian designed the ionic liquids and multi-walled carbon nanotube nanocomposites, improving the catalytic oxidation activity of NADH on the electrode [18]. It is very necessary to develop novel NADH biosensors to reduce the overpotential and improve the catalytic performance of NADH.

In recent years, because of the stability, efficient catalytic performance and fast electron transfer rate, electrodes coated with electropolymerized metal macrocyclic complexes have caused extensive concerns of the researchers [19,20]. The electrochemical and electrocatalytic properties of macrocyclic complexes of some transition metals have been well studied in various solvents [21,22]. Especially, Nickel macrocyclic complexes have been reported that it can easily be electropolymerized onto electrode surface in alkaline solutions to form stable film that catalyze the oxidation of several substrates, and have been used for many applications, such as the amperometric detection of carbohydrates [20], amines and amino acids [23]. However, it is significant to investigate further application of Nickel macrocyclic complexes.

The polyphenolic flavonoid curcumin is a yellow pigment of turmeric as an interesting eco-friendly material and obtained from the rhizome of the plant *Curcuma longa* with a long history of use in traditional Indian diets and herbal medicine [24]. Under alkaline conditions, curcumin can form stable film with transition metal chelating complex [25]. And it acts mainly as an electron donor and good coordination sites for the formation of inert and stable transition metal complexes such as Ni. In this paper, we prepared Ni(II) and curcumin polymer metal composite modified glassy carbon electrodes (Ni-Cur/GCE) through electrochemical polymerization method. Due to Ni(II)/Ni(III) redox couple in alkaline solution is reminiscent of a nickel oxyhydride species, it has great electro catalytic performance for organics compounds containing hydroxy and amino [26,27], and can be used as a redox reaction medium to accelerate the electro oxidation process between electrode and solution [28]. The electrode preparation method was simple and applied to the analysis and research of NADH successfully.

2. Experimental

2.1 Chemicals and apparatus

Electrochemical measurements were carried out on a CHI 660C electrochemical workstation (Shanghai Chenhua Co., Ltd., China) with a conventional three-electrode system. The modified GCE was used as the working electrode, while the platinum wire served as the auxiliary electrode and standard calomel electrode acted as the reference electrode. Scanning electron micrographs measurements was carried out on a scanning electron microscope (JSM-6700F, 15.0 kV). The EDS spectra were measured during the SEM measurements.

Nickel chloride, sodium hydroxide and curcumin were purchased from Aladdin Chemical Reagent Co., Ltd. (Shanghai, China). All chemicals are commercially available as analytical grade and used without further purification. Ni(II)-ammonia complex was prepared by dissolving 4 mM nickel chloride in 25% ammonia solution, in which NH₃ acted as a complex agent of the Ni(II) ion. The double distilled water was used for all experiments and sodium hydroxide buffer solution was used as the supporting electrolyte.

2.2 Preparation of modified glassy carbon electrode

A glassy carbon electrode (GCE) was polished successively on chamois leather with 0.3 and 0.05 μm Al₂O₃ slurry. Then it was washed with HNO₃ (1: 1, v/v), ethanol and doubly distilled water in an ultrasonic bath. After all the above, the electrode was immersed in 10 mL mixture solution of 10 mM curcumin dissolved in 0.1 M NaOH and 4 mM Ni(II)-ammonia compounds (7:3, v/v) by cyclic voltammetry from 0.2 to 0.8 V for 30 cycles. The obtained Ni-Cur/GCE was preserved in a refrigerator at 4 °C after washing with double distilled water.

3. Results and discussion

3.1 Preparation and Characterization of the Ni-Cur/GCE

The formation and growth of an electropolymerized film of Ni-Cur on the glassy carbon electrode in sodium hydroxide solution containing 10 mM curcumin and 4 mM Ni(II)-ammonia compounds was depicted in Fig. 1A. In the early stages of potential cycles, an anodic current at about 420 mV was observed which might be caused by irreversible oxidation of the curcumin (Fig. 1B). With the current scanning, a pair of redox peaks appeared, indicating that Ni-Cur polymer was fixed on

the electrode surface. The polymerization product of curcumin can be used as a substrate to fixed nickel ion, and the nickel ions infiltrate the polymer membrane with the diffusion in the solution, thus ionic conducting complex film is formed [23]. As curcumin contains two methoxy phenol and a second enol ketone structure, in alkaline conditions, phenol and methoxyl respectively generates free radicals, which transform into adjacent benzene quinone structure adsorbed on the electrode surface (Scheme. 1). With the electrochemical oxidation process, it forms the oxygen structure of O-Ni-O [27].

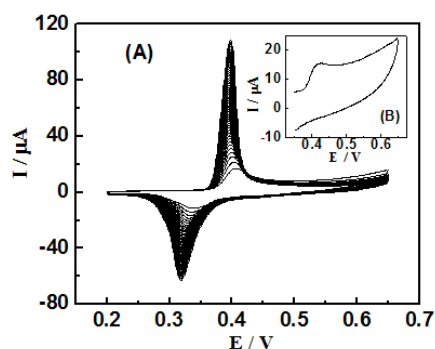
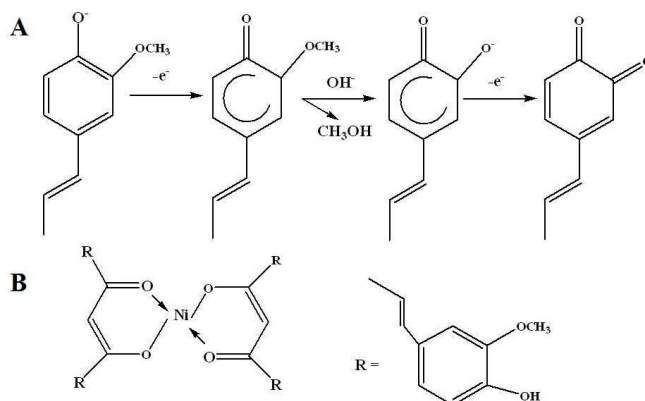


Fig. 1 (A) Consecutive cyclic voltammograms of 0.1 M NaOH solution containing 10 mM curcumin and 40 mM Ni(II)-ammonia complex using a GC. Potential sweep rate was 100 mV s⁻¹. (B) The first cycle in main panel.



Scheme 1. Possible reaction routes involved into electropolymerization of curcumin ligand (A) and the chemical structure of Ni(II)-curcumin complex (B).

To investigate the surface structure and morphology of the Ni-Cur modified film, we performed scanning electron microscopy (SEM). As contrast, Fig. 2A displays a typical SEM image of bare GCE. Fig. 2B shows the Ni-Cur polymer film assembled on the GCE. It can be seen that the modified film becomes rough with some granules decorated on the GCE. Furthermore, the existence of Ni and Carbon was proved by the peaks of Ni and Carbon in the EDS data (Fig. 2C). These results indicated that Ni-Cur composite film has been successfully fabricated.

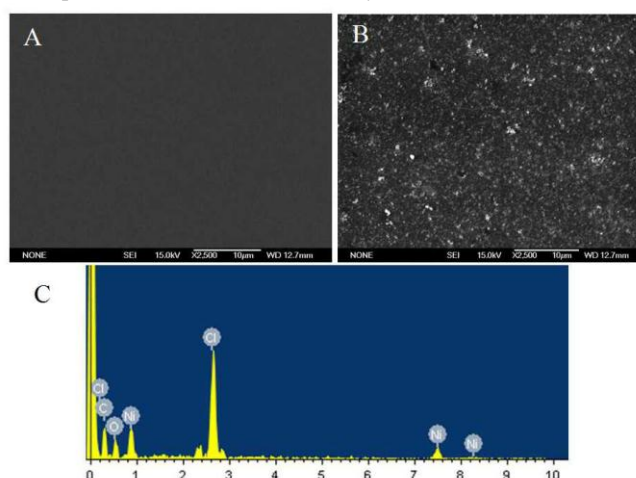


Fig. 2 Scanning electron micrographs of bare GCE (A) and Ni-Cur/GCE (B), EDS of Ni-Cur/GCE (C).

The electrochemical impedance spectra of bare GCE and Ni-Cur/GCE was measured in 0.1 M KCl containing 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ (Fig. 3). Compared with bare GCE, the Ret of Ni-Cur/GCE was larger. This may be caused by the thickness of the modified film and the electrostatic repulsion force between the negatively charged $[\text{Fe}(\text{CN})_6]^{3-/4-}$ and Ni-Cur film, indicating the successful modification of Ni-Cur on the surface of GCE.

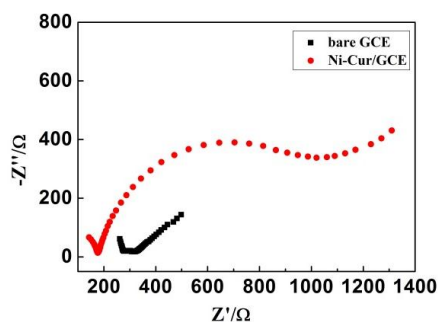


Fig. 3 Electrochemical impedance spectra of GCE and Ni-Cur/GCE in 0.1 M KCl containing 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ at the scan rate of 100 mV s^{-1}

Fig. 4 is the cyclic voltammograms under different scanning speed of Ni-Cur/GCE in 0.1 M NaOH solution. It can be observed that the redox peak potential differs was increasing with scanning speed. According to the Fig. 3, redox peak currents have a linear relationship with the square root of scan rate, indicating that it is mainly controlled by the diffusion process.

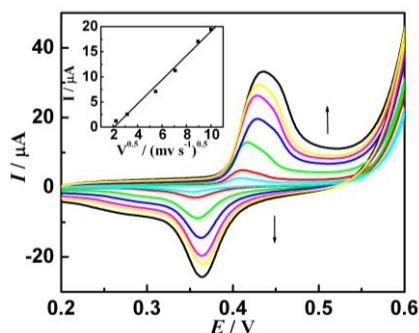


Fig. 4 (A) Cyclic Voltammograms of Ni-Cur/GCE in 0.1 M NaOH solution. Potential sweep rates from inner to outer are: 5, 10, 30, 50, 70, 80, 100 mV s^{-1} . (B) The proportionality of anodic peak currents on the square roots of sweep rate.

3.2 Electrochemical behavior of NADH at Ni-Cur/GCE

The current-time curve of Ni-Cur/GCE in 0.1 M NaOH solution for the determination of NADH was shown in Fig. 5. There is an obvious current step of the modified electrodes at the potential 0.45 V in 0.1 M NaOH solution with adding $100 \mu\text{M}$ NADH continuously. Compared with bare electrode, the current of Ni-Cur/GCE increased about 5 times, showing that the modified electrode has good electrocatalytic ability for NADH. Based on previous reports [26], we can infer reaction mechanism on the modified electrodes:

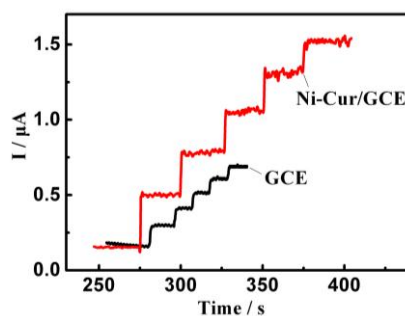
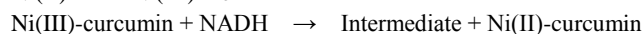
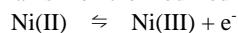


Fig. 5 Amperometric responses of different electrodes with successive addition of $100 \mu\text{M}$ NADH in 0.1 M NaOH solution. Applied potential: 0.45 V.

3.3 Effect of operational parameters

Fig. 6A is current-time curves of the detection of NADH at different volume ratio between curcumin and Ni(II)-amino modified electrodes (a 9:1, b 8:2, c 7:3, d 6:4, e 5:5) with applied potential at 0.45 V. From curve of a to c, it can be seen that NADH current steps increase in turn when the volume ratio (v/v) of curcumin solution and Ni(II)-ammonia solution change from 9:1 to 7:3. Reversely, current steps reduced gradually when the volume ratio varies from 7:3 to 5:5 (curve d-e). The maximum current was obtained when the volume ratio was 7:3 (v/v). Thus we choose 7:3 as the volume ratio (v/v) of curcumin solution and Ni(II)-ammonia solution.

The effect of polymerize cycles on the current response of 50 μM NADH was displayed in Fig. 6B. The volume ratio (v/v) of curcumin solution and Ni(II)-ammonia solution was fixed for 7:3. Results show that, with the aggregate circle number increasing from 10 to 30 laps, the oxidation current of NADH reaches the maximum when polymerization turns to 30 laps, and then decreases when over 30 laps. The reason may be that the mass transfer resistance increased as the polymer film becoming thick, resulting in the decrease of response signal. Herein, 30 laps was selected.

The sensitivity of electrochemical sensor is strongly dependent on the applied potential. Depending on sensitivity and selectivity, the impact of applied potentials from 0.35 to 0.55 V was systematically investigated on the Ni-Cur/GCE. Fig. 6C shows the current responses at different applied potentials after successive addition of 25 μM NADH into 0.1 M NaOH solution containing some potential interferences such as NaCl (100 μM), KNO₃ (100 μM), UA (50 μM), leucine (50 μM) and alanine (50 μM). It can be seen that the current increases slightly with the increasing applied potential when the potential is less than 0.45 V. However, when the potential increased to 0.50 and 0.55 V, the current almost keep constant and the noise of current increased obviously. Thus, 0.45 V was chosen as the working potential for this electrode.

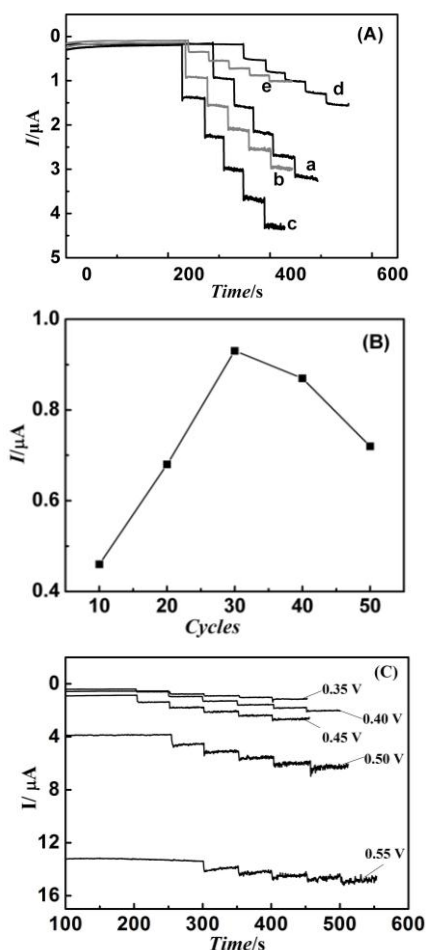


Fig. 6 (A) Current-time curves of the detection of NADH at different volume ratio between curcumin and Ni(II)-amino modified electrodes (a 9:1, b 8:2, c 7:3, d 6:4, e 5:5) with applied potential at 0.45 V. (B) Effect of polymerize cycles on the current response of 50 μM NADH in 0.1 M NaOH solution. (C) Current-time curves obtained at Ni-Cur/GCE upon successive addition of 50 μM NADH with different applied potential.

3.4 Amperometric determination of NADH at Ni-Cur/GCE

Under the optimum conditions, the electrochemical behaviors of different concentrations of NADH were investigated. Fig. 7 shows typical amperometric responses to successive additions of NADH in a stirring 0.1 M NaOH solution at an

applied potential of 0.45 V. Response current (I_p) has a linear relationship with the concentration (c) of NADH. In the range from 0.3 to 300 μM , linear regression equations I_p (μA) = 0.4858 + 0.01623 c (μM) was obtained with the correlation coefficients of 0.997. The detection limit ($S/N=3$) and the sensitivity was calculated to be 0.18 μM and 230.02 $\mu\text{A}\cdot\text{mM}^{-1}\cdot\text{cm}^{-2}$, respectively. The analytical characteristics of Ni-Cur/GCE toward the oxidation of NADH was compared with some other sensors previously reported in Table 1.

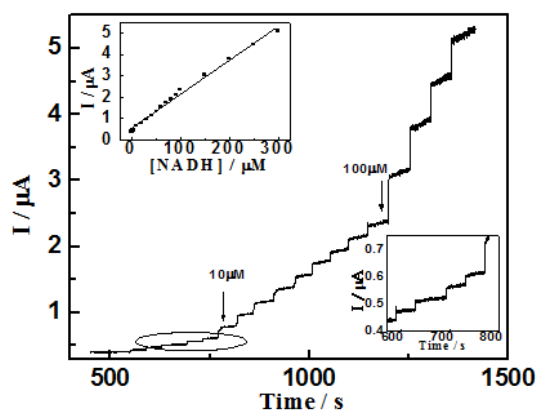


Fig. 7 Current-time curves obtained at Ni-Cur/GCE upon successive addition of different concentrations of NADH into 0.1 M NaOH with applied potential at 0.45 V. Inset (Left) is the calibration curve for NADH at the modified electrode. Inset (Right) is amplification of small concentration curves.

Table 1 Comparison of the analytical performance of the NADH sensor based on the amperometric schemes.

Different NADH sensors	Linear range (μM)	Detection limit (μM)	Sensitivity ($\mu\text{A mM}^{-1} \text{cm}^{-2}$)	Reference
PEDOP/MWCNTs-Pd/GCE	1-13,000	0.18	68	[15]
graphite/poly(methylmethacrylate) composite electrode	4-5,600	3.5	12.91	[17]
Co_3O_4 nanosheet modified electrode	1-30	4.25	2.76	[29]
Graphene-Au nanorods hybrid nanosheets	5-377	1.5	27.07	[30]
Graphene oxide/polythionine/GC	10-3,900	0.1	143	[31]
PEDOT-PSS- Au_{nano} electrode	1-80	0.1	88 \pm 2	[32]
<i>o</i> -aminophenol/GC	1-100	0.15	0.446	[33]
poly Ni(II)-curcumin composite film	0.3-300	0.18	230.02	This work

*GCE: glassy carbon electrode PEDOT: Poly(3,4-ethylenedioxythiophene)

PSS: Poly(styrene sulfonic acid)

3.5 Interference, reproducibility and stability

To investigate possible interferences for the detection of NADH, various compounds were selected. It was found that no significant interference (signal change <5%) for determination of NADH in the presence of NaCl (500 μM), KNO_3 (500 μM), UA (50 μM), leucine (100 μM), alanine (100 μM) (Fig. 8).

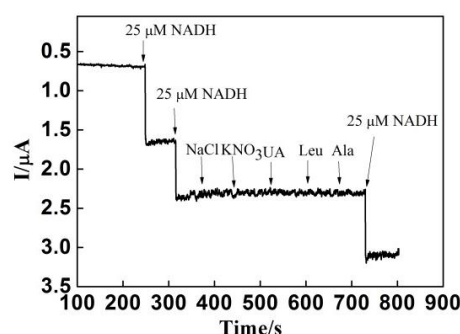


Fig. 8 Current-time curves obtained at Ni-Cur/GCE upon successive addition of 25 μM NADH and NaCl (500 μM), KNO_3 (500 μM), UA (50 μM), leucine (100 μM), alanine (100 μM).

The relative standard deviations (R.S.D.) for six successive determination of 25 μM NADH were achieved as 4.35%, indicating a good reproducibility. Moreover, the storage stability of the Ni-Cur/GCE was investigated. After the modified electrode storing in the refrigerator for 3 weeks, the result shows that the catalytic current response maintains 92.8%.

3.6 Real sample analysis

Human serum samples were selected for analysis by proposed method. The serum samples (15 μL) were added to 10 mL of 0.1 M NaOH solution. All the solutions were analyzed by the standard addition method. The results were listed in Table 2. The good recoveries of the samples indicate that the proposed method could be effectively used for the determination of NADH in commercial samples.

Table 2 Determination of NADH in serum samples

Samples	Detected (μM)	Added (μM)	Found (μM)	Recovery
Serum 1	NADH ND	5	4.94	98.8%
Serum 2	NADH ND	35	36.2	103.4%

4. Conclusions

In summary, a nickel-based chemically modified electrode (Ni(II)-Cur/GCE) has been prepared by electropolymerization of Ni-curcumin complex on GCE. The modified electrode exhibited an excellent electrocatalytic activity on the oxidation of NADH. By using the Ni-Cur modified electrode, NADH can be determined with a wide linear range, low detection limit and high sensitivity. The simple fabrication procedure, high stability and good reproducibility suggest that Ni(II)-curcumin/GCE is an attractive candidate as an electrochemical sensor for the determination of NADH.

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