

A NADH Sensor Based on 1,2-Naphthoquinone Electropolymerized on Multi-walled Carbon Nanotubes Modified Glassy Carbon Electrode

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(Received: Feb. 2, 2012; Accepted: Apr. 16, 2012; Published Online: Jun. 12, 2012; DOI: 10.1002/jccs.201200049)

A new carbon nanotubes modified electrode (poly-Nq-MWCNTs/GCE) was fabricated by electropolymerization of 1,2-naphthoquinone to the surface of multi-walled carbon nanotubes modified electrode by casting method. The morphology of the nanocomposite was characterized by scanning electron microscopy. Cyclic voltammetry and chronoamperometry were applied to investigate the electrochemical properties of the poly-Nq-MWCNTs nanocomposite modified electrode. The result of electrochemical experiments showed that such modified electrode had a favorable catalytic ability to oxidation of β -nicotinamide adenine dinucleotide (NADH). The resulted sensor was sensitiveness to NADH and achieved 95% of the steady-state current within 5s. Furthermore, the anodic peak current was linear to the concentration of NADH for the range from 1.0 μM to 0.14 mM. The linear equation was: $I(\mu\text{A}) = 0.3987 + 0.1035c$ ($\mu\text{mol/L}$), the correlation coefficient $r = 0.9962$, the detect limit is down to $1 \times 10^{-7} \text{ M}$ ($S/N = 3$) and the sensitivity is 0.1035 $\mu\text{A}/\mu\text{mol}$. The well catalytic activity of the sensor was ascribed to the synergistic effect role played by MWCNTs and poly-Nq. Moreover, the based sensor possesses good stability and reproducibility.

Keywords: Multi-wall carbon nanotubes; 1,2-Naphthoquinone; NADH; Modified electrode; Synergistic effect.

INTRODUCTION

NADH is a cofactor of more than 500 enzymes and its regeneration is the most important,¹ which has been widely used in food control, environmental, and clinical analysis.^{2,3} Therefore, there is a need to design and fabrication detection method for this molecule. As reported, the electrochemical detection by redox turnover of NAD^+/NADH couple was the most. Owing to the large overvoltage encountered in NADH oxidation at ordinary electrodes⁴ and strong surface passivation associated with the accumulation of reaction products,⁵ chemically modified electrodes have been regularly used to solve the problems for their electrocatalytic oxidation.⁶ One approach is to immobilize new mediators on the electrode surface to ameliorate analytical performance of the based electrode in virtue of promotion of electron shift between electrode interfaces.⁷⁻¹⁰ Another method is to introduce advanced nanomaterials to the based electrode to enhance the analytical response to NADH.¹¹⁻¹⁶

Carbon nanotubes (CNTs) have attracted great interest since they were discovered in 1991 by Iijima¹⁷ because of their high aspect ratio, electrical conductivity, chemical stability and high mechanical strength. They have enormous

potentials as component of nanoscale electric devices and biosensors to promote electron transfer reactions. As a result of that, CNTs have been considered to be excellent candidates for electrode materials with electrochemical catalysis and supercapacity. As reported quinone derivatives were widely applied as mediators, which enable the electrocatalysis of NADH oxidation. In some cases, these quinones were immobilized^{18,19} or electropolymerized^{20,21} directly on the electrode surface. Recently, efforts have also been focused on the modification of CNTs based composites. Combination of compounds with CNTs will lead to new composite materials possessing the properties of each component, or even with a synergistic effect, which would be useful in the fields of electroanalysis.

In present study, poly-1,2-naphthoquinone and MWCNTs nanocomposite modified glassy carbon electrode (poly-Nq-MWCNTs/GCE) was constructed and used for determination of NADH. The electrochemical behaviors of NADH at the poly-Nq-MWCNTs/GCE were investigated using cyclic voltammetry (CV) and chronoamperometry. Based on the electrocatalytic activities of the modified electrode towards NADH, a sensitive and selective method for NADH determination was established.

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EXPERIMENTAL

Reagents and Apparatus

1,2-Nipthoquinone was purchased from Shanghai Chemical Reagent Company. MWCNTs and NADH obtained from Sigma-Aldrich (USA) were used as received. All other used chemicals were analytical grade. Phosphate buffer solution (PBS) was prepared from 0.1 M Na_2HPO_4 and 0.1 M NaH_2PO_4 aqueous solutions. The preparation of aqueous solution was done with twice distilled deionized water.

Electrochemical experiments were performed on a CHI820B electrochemical workstation (Shanghai Chenhua, China). A three electrode configuration was employed with the poly-Nq-MWCNTs/GC electrode as a working electrode, and SCE electrode and a platinum wire as the reference and counter electrodes, respectively. All of the measurements were carried out at room temperature.

Preparation of the Poly-Nq-MWCNTs/GC Electrode

With the help of ultrasonic agitation, 0.1 mg pretreated MWCNTs were well dispersed into 1 mL deionized water. Before modification, the GCE was carefully polished with 0.05 μm alumina slurry, then ultrasonicated in ethanol solution (1:1, v/v), HNO_3 solution and double-distilled water and dried in the open air. The pretreated GCE was coated with a 5 μL suspension of MWCNTs and allowed to be dried at room temperature. Thus the MWCNTs/GCE was completed.

The electropolymerization of the 1,2-Nq onto the MWCNTs/GCE was carried out as follows. Firstly, put the MWCNTs/GCE into the solution containing of 5 mg/L 1,2-Nq in PBS solution by cycling between -0.5 V and 0.5 V (vs. SCE) at a scan rate of 40 mV/s for 10 cycles. Secondly the modified electrode was rinsed thoroughly with double-distilled water and dried in air for approximately 5 h and noted as poly-Nq-MWCNTs/GCE.

Compared with electrode MWCNTs/GCE and poly-Nq-MWCNTs/GCE, the poly-Nq/GCE was fabricated as stated above.

Chronoamperometric Detection of NADH Using the Poly-Nq-MWCNTs/GCE

The chronoamperometric measurements were done in a total time interval of 15 min, with the solution under constant magnetic stirring and work potentials of 0 V. Calibration curves were built from the current intensities values obtained after stabilization for each addition of NADH.

RESULTS AND DISCUSSION

Preparation and Characterization of Poly-Nq-MWCNTs/GCE

The electrochemical polymerization of 1,2-Nq (5 mg/L) on bare and MWCNTs modified GCE present in PBS (pH 7) were performed by consecutive cyclic voltammogram. The suitable potential range for 1,2-Nq deposition is -0.5 V to 0.5 V. On subsequent cycles, the cyclic voltammograms show redox peaks corresponding to 1,2-Nq growing at the electrode (data not shown). This above result indicates that during the cycle, polymerizations of 1,2-Nq take place on the electrode surface.

SEM was used to characterize the morphologies of the modified electrodes, MWCNTs/GCE and poly-Nq-MWCNTs/GCE (Fig. 1). As shown in Fig. 1A, the MWCNTs with large surface area well distributed on the surface in the form of small bundles and single tubes which is believed to be very beneficial for the performance of the modified electrode. After the polymerization of 1,2-Nq on MWCNTs/GCE (Fig. 1B), the majority of MWCNTs has been entrapped in the poly-Nq film, and informed into the composites with the diameter nanoscaled.

Cyclic voltametric behaviors of the four electrodes: bare GCE, MWCNTs/GCE, poly-Nq/GCE and poly-Nq-MWCNTs/GCE

Fig. 2 show the cyclic voltammograms for the four electrodes obtained at the scan rate of 40 mV/s. Curve a represents the cyclic voltammograms with bare GC electrode for 0.1 M of PBS (pH 7). As shown in curve a, the bare GC electrode did not show discernible redox peaks in

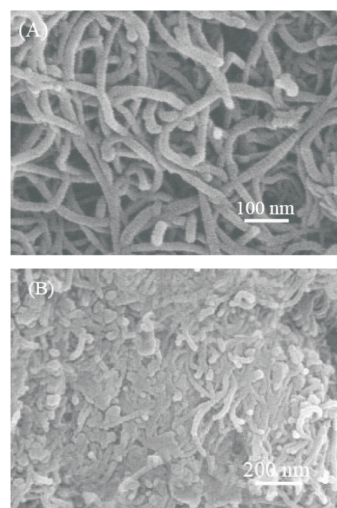


Fig. 1. SEM images of the modified GCEs: MWCNTs/GCE (A) and poly-Nq-MWCNTs/GCE (B).

PBS solution, indicating that electron transfer was usually limited at the bare electrode surface. Curve b represents the cyclic voltammograms with MWCNTs/GCE in PBS solution. There was a pair of redox peaks appeared. Moreover, the MWCNTs/GCE encircled larger area and exhibited larger capacitance than the bare electrode, which demonstrate the MWCNTs is an ideal material for supercapacitors. The electrochemical properties of the poly-Nq modified GC electrode in PBS solution were evaluated in curve c. Two pairs of redox peaks were observed in the curve. These were attributed to particular electrochemical behavior of poly-Nq. Compared with poly-Nq/GCE, the poly-Nq-MWCNTs/GCE was quite different as shown in curve d. The poly-Nq-MWCNTs/GCE shows a 2-fold larger current than MWCNTs/GCE and 4-fold larger than poly-Nq/GCE. It is demonstrated that MWCNTs and poly-Nq play a synergistic effect role in enhancing the sensitivity of GC electrode.

Effect of scanning rate

CV curves of poly-Nq-MWCNTs/GCE were obtained at different scanning rate (see Fig. 3). With the increase of scan rate, the oxidation and reduction peak currents increased, but the peak potentials almost kept unity. The inset of Fig. 3 showed the slope of the peak currents vs. scan rate. The relationship between them is linear in the range of 10–100 mV/s, which illustrated that the process of the electrode reaction is controlled by the adsorption.

Effect of solution pH and stability of the modified electrode

One of the most important parameters in modified

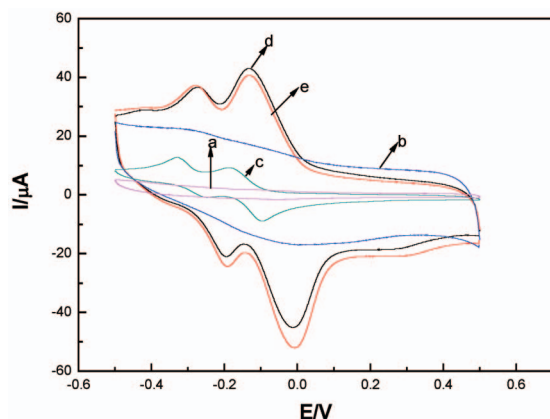


Fig. 2 Cyclic voltammograms of (a) bare GC electrode, (b) MWCNTs, (c) poly-Nq, poly-Nq-MWCNTs modified GC electrodes in PBS (0.1 M, pH 7) (d) and in 0.1 M PBS containing 0.1 mM NADH (e). Scan rates: 40 mV.s⁻¹.

electrode performance is solution pH effect. The effect of solution pH on the peak potential and current was investigated by cyclic voltammetry in the pH range 3–10 and the results are shown in Fig. 4. pH adjustments in solutions were made with 0.1 M phosphoric acid and sodium hydroxide solutions. It was found that two pairs of redox peaks were maintained at acidic, neutral and basic solutions, peak potentials shifted negatively with the increase of solution pH. The formal potential $E^{0'}$ for peak pair I (the left) and peak pair II (the right) were proportional with the solution pH in the range of 3–10 (Fig. 5). The linear regression equations were $E_1^{0'} \text{ (V)} = 0.1727 - 0.057\text{pH}$ for peak pair I and $E_2^{0'} \text{ (V)} = 0.3173 - 0.054\text{pH}$ for peak pair II, with the correlation coefficient 0.9989 and 0.9970, respectively. For electrochemical reaction proton involved: $\text{Ox} + \text{ne}^- + \text{mH}^+$

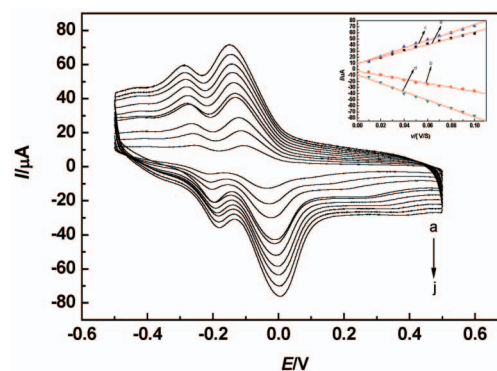


Fig. 3. Cyclic voltammograms of poly-Nq-MWCNTs modified GC electrode at different scan rate: 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 mV.s⁻¹ (a–j) in 0.1 M PBS (pH 7). Inset: the independence of the current response versus the scan rate. a, b for the redox peak on the left and c, d on the right.

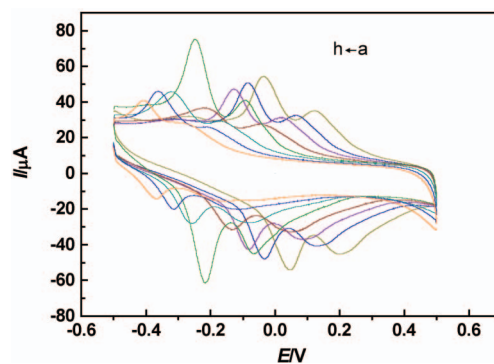


Fig. 4. Cyclic voltammograms of poly-Nq-MWCNTs/GCE in PBS with pH values of 3(a), 4(b), 5(c), 6(d), 7(e), 8(f), 9(g) and 10(h). Scan rates: 40 mV.s⁻¹.

→ Red, the Nerst equation at 25 °C was $E = E^0 + (0.059/n)\lg([Ox]/[Red]) - (0.059m/n)\text{pH}$. Here -0.059 m/n was the slope for the linear regression curve stated above. Therefore, it can easily get that m/n equals to 1, demonstrating that the probable electrochemical reaction of the electrode process is one-proton coupled one-electron transfer for each pair of redox peaks. In addition, the relationship between peak currents and pH value were also explored. The larger anodic and cathodic peak currents were obtained at pH 7. On the other hand, the peak currents reached to the highest at pH 7 which were relative to the gathering efficiency for NADH on the surface of electrode. Along with the change of pH value, the charge for NADH carried also changed. This exist tremendous influence on the interaction between NADH and poly-Nq-MWCNTs membrane. It was easy to see, the gathering efficiency achieved under neutral condition.²² So the pH 7 was chosen in the electrochemical detection of NADH.

The stability of poly-Nq-MWCNTs/GCE was also explored. A good operational stability of the modified electrode can be accessed with less than 0.7% relative deviation for 100 successive scanning by CV methods in 0.1 M PBS solution. The storage stability of the modified electrode was recorded every 3 days. It was found that the modified electrode retained about 93% of its original response after 1 month.

Electrochemical response of poly-Nq-MWCNTs/GCE to NADH

The cyclic voltammograms for the oxidation of 0.1 mM NADH at bare and those modified GC electrode was also studied. With bare GC electrode, there was no obvious response toward the oxidation of NADH, also for the MWCNTs/GCE. As poly-Nq/GCE, although two couples

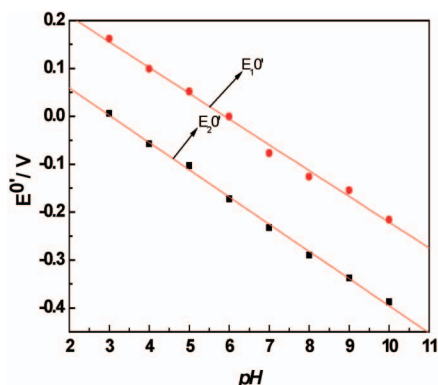


Fig. 5. The plot of formal potential $E^{\circ'}$ vs. pH, where $E_1^{\circ'}$ for the redox pair I on the left and $E_2^{\circ'}$ pair II on the right.

of redox peaks appeared, there was no distinct difference absence and presence of NADH. When NADH was added into the PBS solution for poly-Nq-MWCNTs/GCE, there is a large increase in the oxidation peak current responses while the reduction peak currents decreased (see curve e in Fig. 2). This indicated that poly-Nq-MWCNTs/GCE had electrocatalytic activity for oxidation of NADH, the well catalytic activity was ascribed to that poly-Nq and MWCNTs play a crucial role in enhancing the sensitivity for detection of NADH.

As we known that NADH oxidation has a high overpotential at the surface of electrode and facilitates electrochemical oxidation of NADH. The poly-Nq-MWCNTs/GCE based sensor shows a quite low peak potential around 0 V and high current signal compared to other sensors. Comparing to some other modification on the NADH oxidation, such as CNTs,²³ graphene²⁴ and so on, it had a lower oxidation potential, thus was the most important.

Amperometric determination of NADH

Inset A of Fig. 6 shows the electrocatalytic properties of the modified electrode toward the oxidation of NADH by CV method. Upon addition of NADH into the 0.1 M PBS, the oxidation peak currents increased with NADH concentration.

Chronoamperometric methodology can be used to determination of biomolecule and free radicals in vivo.²⁵ The performance of the poly-Nq-MWCNTs/GCE toward the oxidation of NADH was also studied by the amperometric current-time method. Fig. 6 illustrates a typical current-

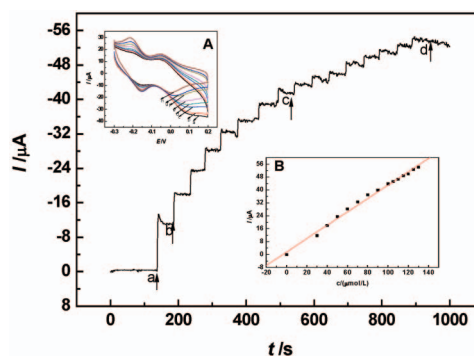


Fig. 6. Amperometric responses of the poly-Nq-MWCNTs/GCE at 0 V to successive addition of NADH (a→b, 30 μM steps; b→c, 10 μM steps; c→d, 5 μM steps) in a stirred 0.1 M PBS (pH 7). The inset (A) is the cyclic voltammograms for NADH at different concentration at poly-Nq-MWCNTs modified GC electrode, (B) is plot of chronoamperometric current vs. NADH concentration.

time curve at 0 V upon successive addition of NADH in the concentration range from 1.0 μM to 0.14 mM. When NADH was added to the stirring 10 mL PBS solution, the oxidation current increased steeply to a stable value. The time to reach 95% of the maximum current was less than 5s. Such a short response time can be mainly attributed to the fast adsorption process of NADH on the film electroactive sites. The modified electrode displayed increasing amperometric responses to NADH with a good linear relationship as stated inset B of Fig. 6. The linear regression equation was $I (\mu\text{A}) = 0.3987 + 0.1035 C (\mu\text{M})$ with a correlation coefficient $r = 0.9962$ and the detection limit is down to 1×10^{-7} M ($S/N = 3$). When the NADH concentration was higher than 0.15 mM, the electrochemical response deviated from linearity and reached a plateau at much higher concentration of NADH.

Table 1 shows the efficiency of the poly-Nq-MWCNTs/GCE electrode for NADH determination compared with other modified electrodes. The present electrode showed a lower LOD wider linear range for some other modified materials.

Sample analysis

In order to estimate the applicability of the methodology, the proposed NADH biosensor was tested by applying it to the measurement of NADH concentration in artificial samples by CV technique. The standard addition method was performed to demonstrate the possibility of NADH detection in artificial samples and results were shown in Table 2.

Interference study and reproducibility

In real sample, the coexisting electroactive species might affect the biosensors response. In order to access the anti-interference capability of the present sensor, the electrochemical response of some common interfering substances were studied. In the determination of NADH, the interference from ascorbic acid (AA) is a major problem due to the fact that these two biomolecules are oxidized at almost the same potential at the traditional electrodes, resulting in the overlap of voltammetric response.²³ Experimental results in our study demonstrated that the poly-Nq-MWCNTs/GCE could be used to detect NADH in the presence of AA without interference. And no significant interference was observed upon addition of physiological levels of uric acid (UA), dopamine (DA), as stated in Fig. 7. This experiment was carried by chronoamperometry at a fixed potential of 0 V. Four successive additions of NADH, ascorbic acid (AA), dopamine (DA) and UA into the electrochemical cell are shown in Fig. 7 (i vs. t). On the chronoamperogram shown, at first step when NADH is added, a sharp increases of current, and the second addition of AA is not followed by a current intensity change up. Also for the third and fourth addition of DA and UA. Therefore, the interferences from the substances coexisting in real samples can actually eliminate and a high selectivity for NADH was obtained. The poly-Nq-MWCNTs/GCE electrode exhibited good reproducibility in the detection of NADH with a relative standard deviation (R.S.D.) of ca. 1.64% found over 10 repeated measurements of 50 μM NADH.

Table 1. Comparison of some properties of the present work with others for NADH detection

Type of modification	Technique	Linear range ($\text{mol}\cdot\text{L}^{-1}$)	Detection limit ($\text{mol}\cdot\text{L}^{-1}$)	Refs
Poly-p-quinone	DPV	$1 \times 10^{-5} \sim 1 \times 10^{-4}$	1×10^{-6}	10
ER-GNO/IL-SPE	CV	$5 \times 10^{-6} \sim 1.2 \times 10^{-2}$	1×10^{-6}	15
PP-MWCNT	CV	$3 \times 10^{-5} \sim 7 \times 10^{-4}$	1.2×10^{-5}	16
IL-graphene/chitosan	CV	$2.5 \times 10^{-4} \sim 2.0 \times 10^{-3}$	no	24
CNT	CV	$2 \times 10^{-4} \sim 5 \times 10^{-3}$	no	23
poly-Nq-MWCNTs/GCE	CV	$1 \times 10^{-6} \sim 1.4 \times 10^{-4}$	1×10^{-7}	This work

Table 2. Determination of NADH in artificial samples and statistical treatment of the experimental data ($n = 5$)

Samples	Amount of L-cysteine (μM)			RSD (%)	Recovery (%)
	Testing value	Added	Found		
Sample I	51.90	50.00	51.02	2.32	102.0
Sample II	51.87	50.00	49.87	1.87	99.74
Sample III	51.83	50.00	50.23	2.04	100.5

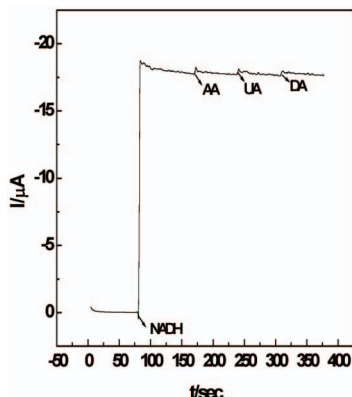


Fig. 7. Current vs. time for the addition of NADH, AA, UA and DA into the electrochemical cell in sequence at 80, 170, 270 and 310 s in 0.1 M PBS (pH 7) at the same concentration 50 μ M.

CONCLUSIONS

This study describes the efficient electrocatalytic detection of NADH using electropolymerized 1,2-naphthoquinone (poly-Nq) nanoparticles catalyst supported on a MWCNTs modified glassy carbon electrode (poly-Nq-MWCNTs/GCE), and experimental parameters were optimized. Other electrodes investigated are: bare GCE, MWCNTs/GCE and poly-Nq/GCE. The poly-Nq and MWCNTs nanocomposites play a synergistic effect role in enhancing the electrocatalytic response towards the detection of NADH in terms of peak current compared to other electrodes investigated. The anodic peak current was linear to the concentration of NADH for the range from 1.0 μ M to 0.14 mM. The linear equation was: $I(\mu\text{A}) = 0.3987 + 0.1035c$ ($\mu\text{mol/L}$), the correlation coefficient $r = 0.9962$, the detect limit is down to 1×10^{-7} M ($S/N = 3$) and the sensitivity is 0.1035 $\mu\text{A}/\mu\text{mol}$. The modified electrode clearly eliminated signal from the interfering effect of coexisting biomolecules such as AA, DA and UA. This study was expected to be useful for providing a sensitive and stable sensor for NADH detection.

ACKNOWLEDGEMENTS

This work was financially supported by the Research Program of Hubei Provincial Department of Education

(No: Q20122507).

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