Chapter 17

Carbon Nanotube Biosensors Based on Electrochemical Detection

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Abstract

The advantages of carbon nanotubes, such as high surface area, favorable electronic properties, and electrocatalytic effect, attracted considerable attention very recently for the construction of electrochemical biosensors. We describe here the construction and application of carbon nanotube/epoxy rigid polymer composite electrochemical biosensor for the detection of important biomarkers, such as NADH and hydrogen peroxide.

Key words: Carbon nanotubes, Biosensing, Electrochemistry, Composite

1. Introduction

Distinctive properties of carbon nanotubes (CNT), such as a high surface area, ability to accumulate analyte, minimization of surface fouling, and electrocatalytic activity, are very attractive for electrochemical sensing (1, 2). Recent studies demonstrated that CNT exhibits strong electrocatalytic activity for a wide-range of compounds, such as NADH (3), hydrogen peroxide (4), neurotransmitters (5), cytochrome *c* (6), amino acids (7), and DNA (8).

Most of the CNT-based electrodes for electroanalytical applications are based on physical adsorption of CNT onto electrode surfaces, usually glassy carbon. However, it is important to note that CNT dispersed in mineral oil (8) or incorporated into Teflon (9) have been used.

The method described in this chapter deals with the outstanding properties of CNT incorporated into an epoxy polymer, forming an epoxy composite hybrid material as a new electrode with

K. Balasubramanian and M. Burghard (eds.), *Carbon Nanotubes: Methods and Protocols*, Methods in Molecular Biology, vol. 625,
DOI 10.1007/978-1-60761-579-8_17,

improved electrochemical sensing properties (10). This CNT–epoxy composite is simple, cheap, and results in interesting electrode material. The preparation and characterization of this multifunctional material is discussed, and electrochemical responses to important biomarkers such as NADH (β-nicotinamide adenine dinucleotide reduced form) and hydrogen peroxide are studied.

2. Materials

2.1. Construction of Carbon Nanotube/Epoxy Composite Electrodes

1. Epoxy resin Epotek H77A and hardener Epotek H77B (Epoxy Technology, Billerica, MA, USA).
2. Multi-wall carbon nanotubes (CNT-200: length, 0.5–200 μm; O.D., 30–50 nm, wall thickness, 12–18 nm; and CNT-2: length, 0.5–2 μm; O.D., 20–30 nm; wall thickness, 1–2 nm; both produced by chemical vapor deposition method) with ~95% purity (Sigma-Aldrich).
3. Graphite powder (particle size 50 μm, BDH, U.K).
4. Nitric acid, diluted to 2 M concentration (Sigma-Aldrich).
5. Cylindrical PVC sleeve (6 mm i.d., 8 mm o.d., and 16 mm long).
6. Copper disk (6 mm o.d. and 0.5 mm thickness).
7. Abrasive paper and alumina paper (polishing strips) (Orion, Spain).
8. Spatula and standard laboratory glassware.

2.2. Analytes and Electrolytes

1. β-Nicotinamide adenine dinucleotide reduced form (NADH) (Sigma-Aldrich).
2. Potassium dihydrogen phosphate and potassium hydrogen phosphate (Sigma-Aldrich).
3. Nitric acid (Sigma-Aldrich).
4. Hydrogen peroxide (30%) (Merck, Germany).

2.3. Electrochemical Biosensing

1. Platinum auxiliary electrode (BAS).
2. Double-junction Ag/AgCl reference electrode (BAS).
3. Carbon nanotube/epoxy composite electrode as working electrode.
4. Autolab PGSTAT 20 (Eco Chemie, Netherlands) connected to a personal computer for electrochemical measurements.
5. Magnetic stirrer.

3. Methods

3.1. Construction of Carbon Nanotube/Epoxy Composite Electrodes

1. Purify the carbon nanotubes by stirring them in 2 M nitric acid at 25°C for 24 h.
2. Clean the copper disk by dipping it in HNO_3/water solution (1:1) in order to remove copper oxide on the surface and rinse it well with bidistilled water.
3. Connect female of 2 mm of diameter and place a metallic thread and then solder this connection to the center of the copper disk.
4. Introduce this connection into the cylindrical PVC sleeve (6 mm i.d., 8 mm o.d., and 16 mm long). The metallic thread allows the connection to remain well-fixed at the end of the cylindrical PVC sleeve (Fig. 1).
5. Mix epoxy resin and hardener manually with spatula in the ratio 20:3 (w/w).
6. Carbon nanotubes/epoxy electrodes have been produced by loading the epoxy resin, before curing, with 20% w/w of carbon nanotubes.
7. When the resin and hardener are well-mixed, the carbon nanotubes should be added and mixed for 30 min. Place the resulting paste into a cylindrical PVC sleeve (6 mm i.d.). Electrical contact should be completed using copper disk and wire.
8. Cure conducting composite is to be cured at 40°C for 1 week.
9. Prior to use, the surface of the electrode should be polished with abrasive paper and then with alumina paper (see Note 1).
10. In the following text, the carbon nanotube/epoxy composite electrodes based on CNT-200 nanotubes will be marked as CNT-200-EC and the ones based on CNT-2 nanotubes will be marked as CNT-2-EC.

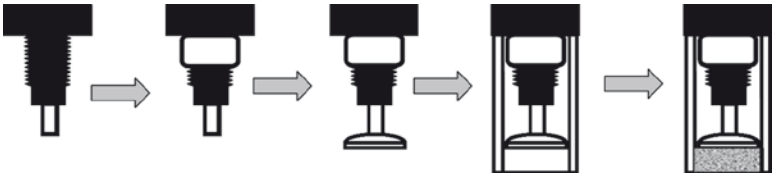

Fig. 1. Details on preparation of carbon nanotube/epoxy composite electrodes

11. In the same way as described above (steps 1–9), prepare graphite/epoxy composite electrode (GEC) for comparative purposes.

3.2. Electrochemical Biosensing Using Carbon Nanotube/ Epoxy Composite Electrodes

1. Prepare supporting electrolyte from phosphate salts. The resulting solution should have a concentration of 50 mM and pH of 7.4 (see Note 2).
2. Add biomarker in the solution and test its electrochemical behavior using cyclic voltammetry. The optimal concentration of NADH and hydrogen peroxide is about 5 mM.
3. Scan voltage from –200 mV to 1,100 mV with sweep rate of 100 mV/s (see Note 3).
4. Determine electrochemical activation potential from cyclic voltammograms (for an example of cyclic voltammogram of NADH, see Fig. 2).
5. Figure 2 compares cyclic voltammograms for 1 mM NADH at carbon nanotube CNT-200-EC (a) and CNT-2-EC (b) electrodes with graphite epoxy composite electrode (c). A broad NADH oxidation peak is observed at +0.72 V (vs Ag/AgCl) for GEC electrode. For CNT-2-EC electrode, well-developed oxidation peak is observed at the very similar potential +0.74 V. Substantial shift to +0.45 V in the oxidation potential was observed at CNT-200-EC. It is interesting to note that the large potential shift to the lower potentials (similar to those observed by Musameh et al. (3)) is observed only at carbon nanotube CNT-200-EC electrode, while NADH oxidation potentials at CNT-2-EC and graphite powder epoxy electrodes are similar and they remain relatively high.

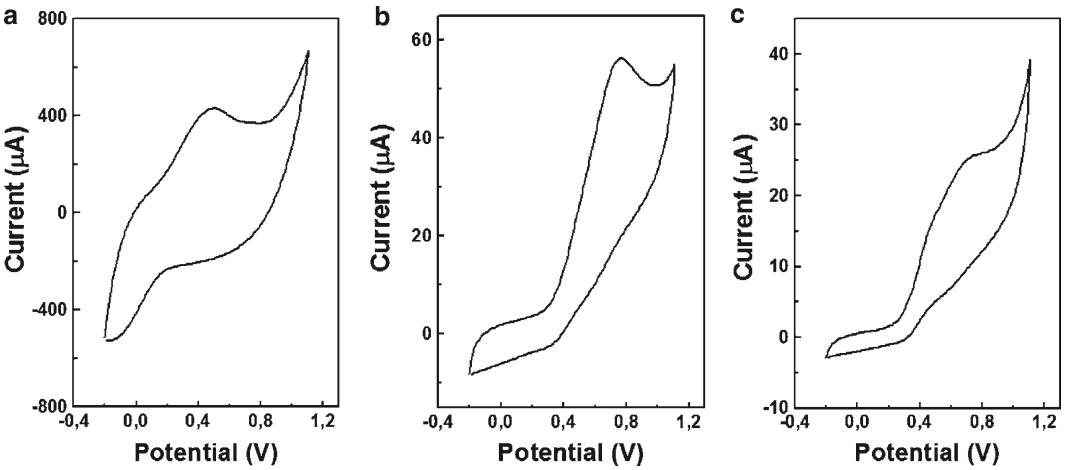

Fig. 2. Cyclic voltammograms for 1 mM NADH of the (**a**, **b**) carbon nanotube/epoxy composite electrodes and (**c**) graphite electrodes. Reprinted with permission from (10)

6. In the same way add hydrogen peroxide to supporting electrolyte in the new electrochemical cell. Optimum concentration of hydrogen peroxide biomarker is 5 mM.
7. Scan voltage from –200 mV to 1,100 mV with sweep rate 100 mV/s.
8. Determine electrochemical activation potential from cyclic voltammograms (for an example of cyclic voltammogram of hydrogen peroxide, see Fig. 3).
9. Figure 3 displays cyclic voltammograms for 1 mM hydrogen peroxide at carbon nanotube CNT-200-EC (a), CNT-2-EC (b), and GEC (c) electrodes. The long-nanotube CNT-200-EC electrode displays oxidation signal around +0.60 V (a), while the short-nanotube CNT-2-EC and graphite epoxy composite electrode (b and c, respectively) shows oxidation around +0.70 V. CNT-200-EC electrode also shows higher sensitivity towards oxidation of hydrogen peroxide (note the different scales).
10. Important characteristic of carbon nanotube-based electrochemical biosensor is the fact that it is prone to passivation. The oxidation of NADH at conventional solid electrodes is prone to the problems with passivation of the surface (3), the stability of response of the electrodes should be evaluated.
11. Figure 4 compares stability of oxidation of NADH at +0.55 V for CNT-200-EC (a), CNT-2-EC (b) and GEC (c) electrodes.
12. The results should indicate that carbon nanotube epoxy composite electrodes provide a good stability towards oxidation of NADH. See an example in Fig. 4.

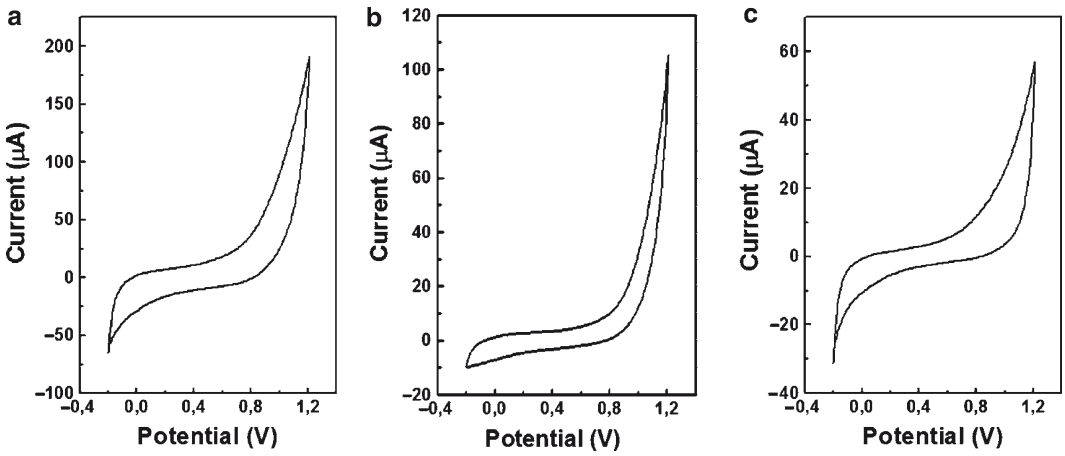

Fig. 3. Cyclic voltammograms for 1 mM hydrogen peroxide of the (**a**, **b**) carbon nanotube/epoxy composite electrodes and (**c**) graphite electrodes. Reprinted with permission from (10)

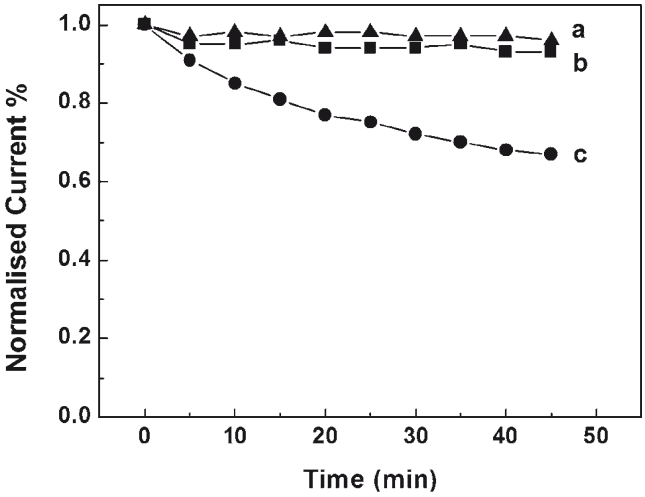

Fig. 4. Stability of 1 mM NADH response using the (*a*) CNT-200-EC, (*b*) CNT-2-EC, and (*c*) GEC electrodes. Operation potential, +0.55 V. Solution stirring ca. 400 rpm. 50 mM phosphate buffer, pH 7.4. Reprinted with permission from (10)

13. Figure 4 shows that carbon nanotube/epoxy composites hold about 96% (CNT-200-EC) and 93% (CNT-2-EC) of original response even after 45 min of immersion in the solution of NADH, while response of GEC electrode decreases by 33% in the same timescale. Similar passivation layer as on the surface of GEC electrode also shows glassy carbon electrode (3, 11).
14. The amperometric response of CNTEC and GEC electrodes towards NADH should be assessed from cyclic voltammograms. In our example in Fig. 2, it was assessed at +0.55 V (see Note 4).
15. Calibration curve should be constructed for both NADH and hydrogen peroxide by adding 100 µM increments of these analytes to electrochemical cell. Carbon nanotube-based electrodes will displays a well-defined concentration dependence over concentration range 0.0–1.0 mM (0.1 mM increments), with sensitivities in tens µA/mM with high correlation coefficients (about 0.995) for NADH.
16. For hydrogen peroxide, the concentration range 0.0–2.0 mM (0.2 mM increments) should provide linear calibration range with of tens µA/mM with correlation coefficients about 0.995.
17. The hydrogen peroxide response was not affected by regenerating (polishing) the surface.
18. Reproducibility of biosensor should be tested. Series of eight successive measurements, each recorded on a newly polished surface will yield to RSD = 5%.

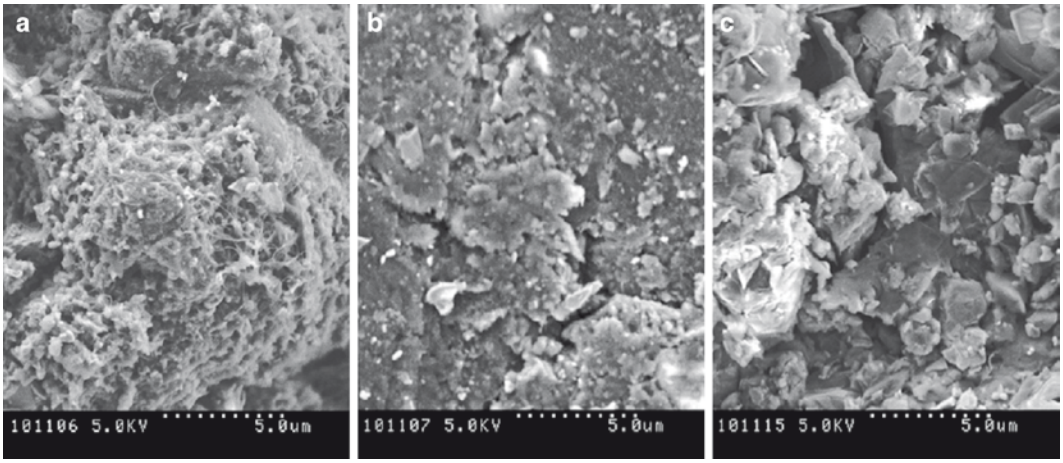

Fig. 5. SEM images for long-carbon nanotube-based epoxy electrode (**a**), short-carbon nanotube-based epoxy electrode (**b**) and conventional GEC electrode (**c**). All electrode surfaces have been polished in the same way as explained in the text. The same acceleration voltage (5 kV) and resolution are used. Reprinted with permission from (10)

4. Notes

1. It is important to characterize carbon nanotube/epoxy composites with scanning electrochemical microscopy (SEM) in order make sure that electrode was fabricated successfully. For an example, see Fig. 5.
2. It is important to note that all electrochemical experiments used bidistilled water.
3. As a standard, solutions during acquiring cyclic voltammogram are static, not stirred.
4. On other hand, amperometric sensing at fixed potential (i.e., observing stability of electrode) should be carried out in steered solution with magnetic stirrer (approximately 400 rpm).

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