NADH Sensing Using a Carbon Nanotube Electrode Reinforced with a Plasma-polymerized Thin Film

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We report on an electrochemical nicotin adenine dinucleotide (NADH) sensor that is based on carbon nanotubes (CNT) and plasma-polymerized film (PPF). The configuration of sensing electrode was CNTs sandwiched between two 6 nm thick PPFs made from acetonitrile on sputtered gold thin film. We optimized the CNT concentration for casting formation onto the under PPF layer. The sensor showed high sensitivity (a sensitivity of 240 μA mM⁻¹ cm⁻², a detection limit of 3.9 μM at S/N = 3, +0.4 V vs. Ag/AgCl), wide dynamic range (a linear response range of 0.009-2.3 mM, a correlation coefficient of 0.993), and rapid response (<7 s in reaching 95% of maximum response). This high performance is attributed that CNTs offer excellent electrocatalytic activity and enhance electron transfer, therefore, PPF and/or plasma process are the electrochemistry-friendly platform for CNT applications.

Key Words : Electrochemistry, Plasma-polymerized Film, Carbon Nanotube, Nicotin Adenine Dinucleotide

1 Introduction
The electrochemical detection of β-nicotinamide adenine dinucleotide (NADH) is of is important because the NAD⁺/NADH couple is the cofactor system for a large number of dehydrogenase enzymes and a component of biomarker systems. However, the direct electrochemical oxidation of NADH to its corresponding oxidized form NAD⁺ at bare electrode is highly irreversible and needs a considerable overpotential (~1 V). Recently, the reports of NADH sensing with carbon nanotubes (CNTs), which are considered to be formed by the folding of graphene layers into carbon cylinders, are increasing because of their excellent electron transfer properties and catalytic activities. However, one problem is that the hydrophobic surfaces of CNTs need to be changed to be hydrophilic ones in order to use in aqueous media. One solution is the dispersion of CNTs with a binder such as other polymers.

Our solution is a method of CNTs embedded into plasma-polymerized nano-thin films (PPFs). The advantage of this strategy is that the surface of CNTs can be easily modified under clean conditions. With making best of this advantage, we have already reported that the PPF is a enzyme-friendly platform in CNT-enzyme biosensors. Our motivation is extension of the potential PPF-base electrochemical sensor. Here, the proposed method not only simplifies the fabrication of CNT electrodes but also enables ultrasensitive NADH sensing.

2 Experimental
2.1 Chemicals
Distilled water, potassium dihydrogenphosphate, disodium hydrogenphosphate, acetonitrile, ammonia, and hydrogen peroxide were purchased from Kanto Chemical Inc., (Tokyo, Japan). NADH (reduced form) was purchased from Sigma (St. Louis, MO, USA). Single-walled CNTs were purchased from Aldrich (Milwaukee, WI, USA). All reagents used in this work were used without further purification.

2.2 Device fabrication
An amperometric NADH sensing device based on CNTs was fabricated on the basis of the semiconductor layer-by-layer processes. The device was formed on a 150 μm thick glass substrate. Its planar dimensions were approximately 50 × 50 mm². All metal layers were sputter-deposited and patterned by a mask process. Glass slides used to fabricate thin-film electrodes were boiled in hydrogen peroxide/ammonia/water (1:1:8 in volume) for 1 h and then rinsed with water and acetone. Gold (Au) thin films were sputtered with an apparatus manufactured by Ulvac (VEP-1000, Tokyo, Japan). Their thicknesses (200 nm) were determined from a surface profiler and a quartz crystal microbalance. A 40 nm thick chromium intermediate layer was used to promote the adhesion of the gold layer. The dimensions of the openings for the working electrode were 5 × 5 mm².

A plasma generator was obtained from Ulvac (VEP-1000, Tokyo, Japan). The first PPF layer made from acetonitrile was deposited onto the sputtered Au electrode of the following parameters: power, 200 W; pressure, 0.6 Pa; deposition rate, 6 nm min⁻¹. The CNTs were dispersed in the solution of 1:1 mixture of ethanol and phosphate buffer solution (20 mM, pH 7.4) in order to prevent the aggregation of the CNTs. The suspended solution was dropped onto the PPF surface and was dried in a vacuum oven. Subsequently, the CNT-adsorbed surface was overcoated with PPF as a second layer. The deposition parameters were mentioned the above.

2.3 Measurements
Cyclic voltammetry was performed with an electro-
chemical analyzer (ALS Instruments, Model 701A, West Lafayette, IN). The three-electrode configuration was used. A reference electrode (Ag/AgCl, RE-1C) and a counter electrode (Pt) were purchased from Bioanalytical Systems Inc. The working electrode was the fabricated device. Electrochemical measurements were carried out in a 10 mL vessel at ambient temperature (20 ± 1°C). As the supporting electrolyte, a phosphate buffer (20 mM, pH 7.4) was used. To prepare samples at designated concentrations, stock solutions of NADH were sequentially added, and the procedure was repeated.

3 Results and Discussion

3.1 Optimization of fabrication process

The fabricated amperometric NADH sensor has a sandwich-like structure denoted as the basic structure of PPF/CNT/PPF/Au. The PPFs synthesized from acetonitrile was made of highly branched and incompletely cross-linked aliphatic hydrocarbon backbone chains containing nitrogen atoms that form a primary amine group. First, the CNT concentrations of solution for casting formation were optimized. Figure 1 shows cyclic voltammograms (CVs) obtained in the absence and presence of NADH as a function of the CNT concentrations. The higher the CNT concentration is, the larger the current increment due to NADH addition is. In CVs with CNT concentrations of 7.5, 15, and 45 mg/mL (Figs. 1c, d, and e), the distinct oxidation peak around +0.4 V due to the oxidation of NADH was observed. This indicates that CNTs reduce the oxidation potential of NADH. However, in CV with the largest concentration of 75 mg/mL (Fig. 1f), both of the oxidation and background currents increased. Therefore, the optimized concentration is determined for 15 mg/mL.

Second, the thicknesses of the PPFs on Au (the first layer) and on CNT (the second layer) were optimized. Figure 2 shows CVs obtained in the absence and presence of NADH as a function of thicknesses of the PPFs. The current in Fig. 2a (no first layer, PPF/CNT/Au) is much larger than that in Fig. 2c (PPF/CNT/PPF/Au), indicating that the CNT layer is well formed on PPF layer than bare Au surface. Therefore, the PPF on Au electrode play a role as an interface between CNT and Au. However, in Fig. 2b (3 nm thickness of the first PPF, PPF/CNT/3 nm PPF/Au), both of the oxidation and background currents increased probably because the PPF was too thin to be covered with Au electrode. Therefore, we considered that the optimum thickness of first PPF layer may be 6 nm.

The current in Fig. 2d (9 nm thickness of the second PPF, 9 nm PPF/CNT/PPF/Au) was smaller than that in Fig. 2c (6 nm PPF/CNT/PPF/Au) because the second

![](image1.png)

Fig. 1 Cyclic voltammograms of the fabricated amperometric NADH sensor using CNTs and PPF (PPF/CNT/PPF/Au) as a function of CNT concentration for casting formation. CNT concentration: (a) 0.75, (b) 1.5, (c) 7.5, (d) 15, (e) 45, and (f) 75 mg/mL. Arrow direction represents the NADH concentration increments at 0, 1.7, 4.2, and 5.8 mM. Conditions: 20 mM phosphate buffer; pH = 7.4; 20°C; scan rate, 50 mV s⁻¹; electrode geometrical area, 25 mm².

![](image2.png)

Fig. 2 Cyclic voltammograms of the fabricated amperometric NADH sensor using CNTs and PPF (PPF/CNT/PPF/Au) as a function of thickness of PPF layers: (a) No first PPF (6-nm-PPF/CNT/Au), (b) 6-nm-PPF/CNT/3-nm-PPF/Au, (c) 6-nm-PPF/CNT/6-nm-PPF/Au, and (d) 9-nm-PPF/CNT/6-nm-PPF/Au. Arrow direction represents the NADH concentration increments at 0, 1.7, 4.2, and 5.8 mM. Conditions: 20 mM phosphate buffer; pH = 7.4; 20°C; scan rate, 50 mV s⁻¹; electrode geometrical area, 25 mm².
PPF layer blocked access of NADH to CNT. The effectiveness of upper PPF layer lied in changing the hydrophobic surface of CNTs to stable hydrophilic environment. The method of formation and modification for the CNT layer is very simple and a clean process compared with previously reported methods that require acid treatment and dissolution in a binder solution.\(^2\)\(^3\)\(^1\)

We considered that the optimum thickness of second PPF layer may be 6 nm.

3.2 Sensor performance

Figure 3 shows the steady-state amperometric response at +0.4 V vs. Ag/AgCl of the electrode. This potential was selected on the basis of CV. The sequential wise increase in NADH addition is observed. This tendency of current increment is similar to CV data shown in Fig. 2. Detection mechanism can be considered for the catalytic ability of the CNTs toward NADH.\(^2\)\(^3\)\(^1\) CNTs catalyze the oxidation of NADH to NAD\(^+\) (oxidation form of NADH), as a result, the Au electrode can detect two electrons as following equation.

$$\text{NADH} \rightarrow \text{NAD}^+ + \text{H}^+ + 2e^- \quad (1)$$

Since CNTs reduces the activation energy of electrochemical reaction on the surfaces, CNTs reduces the oxidation potential (\(< 0.4 \text{ V})\)\(^2\)\(^3\)\(^1\) compared with the carbon electrode (\(\sim 1 \text{ V})\).\(^1\) The CNTs also play a role as an electron transfer conductor. Then, the generated electron was transfer via CNTs to the Au electrode detects the electron, as a result, current increases.

One remarkable characteristic is the small background current (1.5 ± 0.1 μA) compared with the NADH response, showing no need to the baseline calibration for NADH measurement. This is because effective electro-

![Fig. 3 Time-current response to sequential NADH addition at concentrations of 0.30, 0.59, 0.88, 1.2, 17, 23, 32, 42, and 5.0 mM. Polarized potential: +0.4 V vs. Ag/AgCl. Inset: Enlargement of region of low NADH concentration. Response time: 7 s (95% to maximum level). Detection limit 39 μM (S/N = 3).](image)

![Fig. 4 Calibration plot for NADH response using the data in Fig. 3 (n = 3). Correlation line at NADH concentration range (0.009-2.3 mM); sensitivity of 240 μA mM\(^{-1}\) cm\(^{-2}\) (r = 0.993). Inset: Enlargement of the low concentration range.](image)

chemical communication due to nano sandwich structure and permselective coating of the first PPF on Au electrode. The other remarkable characteristic is ultra-sensitivity; the detection limit (signal/noise = 3) was 3.9 μM. Because of the nanometer sandwiched structure, a short response time (< 7 s, this is 95% to maximum response) was obtained.

Figure 4 shows the current vs. NADH concentration based on data in Fig. 3. The characteristics of this devices is good linearity (r = 0.993) at wide concentration region (0.009-2.3 mM) and the sensitivity was 240 μA mM\(^{-1}\) cm\(^{-2}\). Since the devices were fabricated in the same batch, reasonable reproducibility (relative standard deviation: 5% at linear region, n = 3) from sample-to-sample was obtained.

Figure 5 shows the storage stability under polarization at +0.4 V in the presence of 0.3 mM NADH. The electro-

![Fig. 5 Storage stability of the PPF/CNT/PPF/Au electrode. Amperometric detection of 0.3 mM NADH at 0.4 V was carried out in an electrolyte of pH 7.4, 20 mM phosphate buffer solution.](image)
chemical response of the optimized device retained a current response of more than 95% for 7 days though the second PPF-free device (CNT/PPF/Au) showed a significant decrease of current response for same days. This suggests that a second PPF layer made from acetoneitrile prevent leaching CNTs.

4 Conclusions

We demonstrated a new simple and reliable method for fabricating a CNT-based amperometric NADH sensor. The ultrasensitive NADH detection was demonstrated. The plasma-polymeried coating method, that is, a semiconductor-technology-compatible layer-by-layer process, provided a well-defined design of CNT-electrode. In this experiment, PPF modification for CNTs is an effective strategy for an electrochemical communication-friendly platform in amperometric sensors. The method presented here can be easily extended to other electrochemical devices.

References