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Detection of NADH and Ethanol Based on Catalytic Activity of Soluble Carbon Nanofiber with Low Overpotential

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The excellent catalytic activity of a novel carbon nanomaterial, soluble carbon nanofiber (CNF) with good dispersion and wettability, to the oxidation of dihydronicotinamide adenine dinucleotide (NADH) was described for biosensing application. The edge plane sites and oxygen-rich groups presented on the CNF surface could be partially responsible for its electrocatalytic behavior, which induced a substantial decrease by 573 mV in the overpotential of NADH oxidation reaction (compared to a bare electrode). The CNF-coated electrode thus allowed highly sensitive amperometric detection of NADH with a low limit of detection (0.11 μM), low applied potential (+0.06 V), and minimization of surface fouling. Such ability of CNF to promote the electron transfer between NADH and the electrode suggested a new, promising biocompatible platform for development of dehydrogenase-based amperometric biosensors. With alcohol dehydrogenase (ADH) as a model, the ADH/CNF-modified electrode could be constructed by a simple casting process. The proposed biosensor showed rapid and highly sensitive amperometric response to ethanol with acceptable preparation reproducibility and excellent stability.

Dihydronicotinamide adenine dinucleotide (NADH) and its oxidized form, nicotinamide adenine dinucleotide (NAD^+), are the key central charge carriers in living cells. The NADH/NAD^+ couple is the cofactor taking part in more than 300 dehydrogenase enzymatic reactions.[1] The electrochemical oxidation of NADH at the electrode surface has received considerable interest due to its significance both as a cofactor for dehydrogenase enzymes and its role in the electron-transfer chain in biological system, and also due to the need to develop amperometric biosensors for substrates of NAD^+ dependent dehydrogenases. The diversity of these commercially available enzymes offers the possibility to develop many analytical devices based on dehydrogenases, which can be applied in different fields like food control, environmental, and clinical analysis.[2,3] However, the oxidation of NADH at a conventional solid electrode surface is highly irreversible and takes place at considerable overpotentials, which limits the selectivity of the determination in a real sample.[4] Furthermore, the reaction at a high overpotential involves radical intermediates that cause electrode fouling[4,5] and the loss of analytical sensitivity, reproducibility, and operational lifetime.[6]

To solve these problems, many compounds, such as aromatic compounds containing catechol group,[7] phenoxazine dyes,[8] organic salt,[9] metal complexes,[10] and conducting polymer,[11] have been immobilized on the electrode surface as electron-transfer mediators to catalyze the oxidation of NADH and reduce the overpotential. These immobilized mediators produce a series of electrochemical biosensors for NADH and substrates of NAD^+-dependent dehydrogenases. However, most of these biosensors show relatively low sensitivity due to slow diffusion of NADH within these immobilized matrixes or redox self-exchange restrictions within the film. In recent years, with the great progress made in nanoscience and nanotechnology, interest is increasing in exploring the unique properties and potential technological applications of various nanostructures.[12,13] Many nanomaterials, such as peptide nanotubes,[12] poly(1,2-diaminobenzene) nanotubules,[14] and TiO_2 nanostructured films,[15] particularly carbon nanotubes (CNT),[16−21] have been devoted to decreasing the high

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overpotential for NADH oxidation, minimizing surface fouling, and improving electron-transfer kinetics. Depending on the size, shape, and internal structure of these nanomaterials, nanoparticles frequently display unique physical and chemical properties.[22] Owing to the remarkable electronic and mechanical properties of CNT,[23,24] they show promising application in NADH sensing by combining with carbon paste electrodes,[25,26] biopolymers,[27,28] and microelectrodes.[24] By integrating the hydrophilic ion-conducting matrix of chitosan with electron mediator toluidine blue O and CNT, the produced NADH sensor shows very low oxidation overpotential and good analytical performance.[28] This work suggests one new carbon nanostructure, carbon nanofiber (CNF), to decrease the oxidation overpotential of NADH for preparation of highly sensitive NADH and dehydrogenase-based amperometric biosensors.

CNF is a kind of carbon fiber with a nanometer-size diameter and no hollow core.[29] It has been recognized as one of very promising materials based on its nanostructure and particular properties[30] and is expected to be used in various applications such as catalysts or catalyst supports,[31] probe tips,[32] and fuel cells.[33] The oxidation treatment of CNF with nitric acid can produce a range of oxygen-containing groups without degradation of the structural integrity of its backbone.[34] These formed oxygen-rich groups can be expected to act toward facilitating electrocatalytic behavior of NADH as that of CNT.[18] Compared with CNT that have gained considerable attention, CNF possesses less order and more edge sites on the outer wall[29,35] along with better dispersion and wettability.[36] The higher proportion of edge plane defects may lead to more facile electron transfer.[21] The solubility of CNF facilitates its manipulation, including the coating on electrode surfaces for electrochemical biosensing applications, because the insolubility of carbon materials in most solvents is a major obstacle in implementing their widespread use.[37] This work proposes the first biosensing application of CNF with excellent catalytic activity for convenient preparation of highly sensitive sensors for NADH and substrates of dehydrogenase. Compared to peptide nanotube-based[12] and CNT-based[21,27] NADH sensors, the oxidation overpotential and applied potential are further decreased by more than 340 and 240 mV, respectively. Using alcohol dehydrogenase (ADH) as a model enzyme that can be simply immobilized on an electrode surface in the CNF coating process, a sensitive amperometric sensor for ethanol with a low applied potential is proposed. CNF provides a new, biocompatible platform for sensitive biosensors and biomolecular diagnostics.

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EXPERIMENTAL SECTION

Reagents. CNF was a gift from WPI (Sarasota, FA). ADH from bakers yeast and β-NAD^+ from yeast were purchased from Sigma. β-NADH was obtained from Fluka. Multiwall carbon nanotubes (MWCNTs, >95%, 10−20 nm diameter) were purchased from Shenzhen Nanotech Port Ltd. Co. (Shenzhen, China). Other reagents were of analytical reagent grade. All solutions were prepared with doubly distilled water.

Apparatus. All electrochemical measurements were performed on a CHI 660 electrochemical analyzer with a conventional three-electrode system composed of platinum wire as auxiliary electrode, saturated calomel electrode (SCE) as reference electrode, and glassy carbon electrode (GCE) as working electrode. Electrochemical impedance spectroscopy (EIS) was performed with the CHI 660 electrochemical analyzer in a 5.0 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1) mixture with 0.10 M KNO_3 as supporting electrolyte, using an alternating current voltage of 5 mV, within a frequency range of $0.01-10^5$ Hz.

For morphological analysis, the sample films were prepared in the same way as those for voltammetric measurements on different slides treated with nitric acid and a mixture of H_2SO_4 and H_2O_2 (1:1). After Au film was coated to improve the conductivity, these films were examined under a scanning electron microscope (SEM, LEO 1530 VP). X-ray photoelectron spectra (XPS) were recorded on an Escalab MKII X-ray photoelectron spectrometer.

The static water contact angles were measured at 25 °C by a contact angle meter (Rame-Hart-100) employing drops of pure deionized water. The readings were stabilized and taken within 120 s after addition. Fourier transform infrared (FT-IR) spectra were recorded on a Vector 22 FT-IR spectrometer (Bruker). A pH glass electrode (868, Thermo Orion) was used for pH titration.

Preparation of Soluble CNF. Twenty mmilligrams of CNF was dispersed in 30 mL of 30% HNO_3, and the resultant mixture was then refluxed for 24 h at 140 °C. The resulting suspension was centrifuged and the precipitate was washed with water to obtain carboxylic group functionalized CNF. The obtained CNF could be completely dissolved in water at pH 1.0 for preparation of a 5 mg mL^{-1} CNF solution, which was then neutralized to pH 7.0 with 0.1 M NaOH. The homogeneous solution was stable for a long time (at least 3 months). When the CNF concentration was more than 5 mg mL^{-1}, the CNF particles precipitated in the solution. So the solubility of the CNF was 5 mg mL^{-1}. MWCNTs were treated by the same procedure. The treated MWCNTs could not disperse as well as CNF, and only a 5 mg mL^{-1} suspension was prepared.

Electrode Preparation. The GCE was successively polished to a mirror finish using 0.3- and 0.05-μm alumina slurry (Beuhler) followed by rinsing thoroughly with doubly distilled water. After successive sonication in 1:1 nitric acid, acetone, and doubly

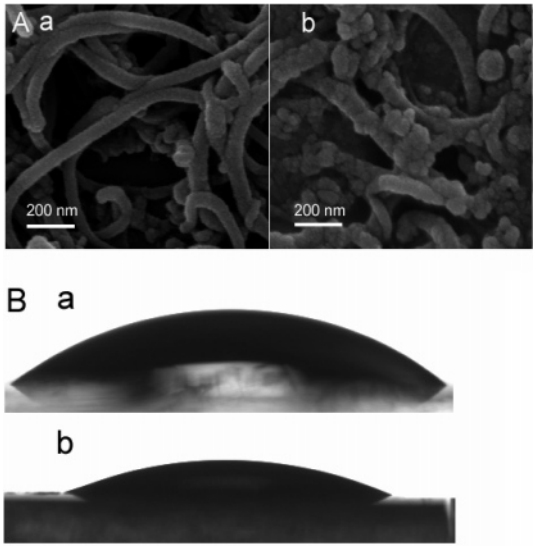

Figure 1. (A) Scanning electron micrographs of glassy carbon electrodes coated with (a) CNF and (b) ADH/CNF films, and (B) contact angle of substrate (a) and CNF-modified substrate (b).

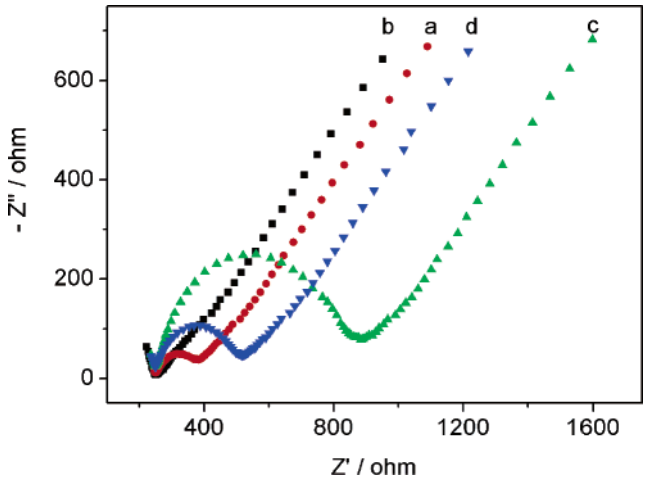

Figure 2. EIS of bare (a), CNF- (b), ADH- (c), and ADH/CNF- (d) modified electrode in 0.10 mM KNO_3 containing 5 mM $K_3[Fe(CN)_6]$/$K_4[Fe(CN)_6]$.

distilled water, the electrode was rinsed with doubly distilled water and allowed to dry at room temperature. A 3.0-μL sample of the 5 mg mL^{-1} CNF solution was dropped on the pretreated GCE and dried in a silica gel desiccator. After 30 min, this coating process was repeated again to obtain a uniform CNF film. Two coating steps ensured the amount of CNF was cast stably on the electrode surface.

Enzyme solution was prepared by dissolving 2 mg of ADH in 1.0 mL of 0.2 M pH 7.0 phosphate buffer solution (PBS). A 3.0-μL aliquot of the ADH solution was dropped on a pretreated GCE and dried in a silica gel desiccator for 30 min. Then 3.0 μL of 5 mg mL^{-1} CNF solution was dropped twice on the membrane at an interval of 30 min to obtain the amperometric biosensor for ethanol. The biosensor was stored in 0.2 M pH 7.0 PBS at 4 °C.

RESULTS AND DISCUSSION

Characterization of CNF and ADH/CNF Films. Figure 1A shows the morphologies of CNF and ADH/CNF films characterized by SEM. The SEM of the CNF film displayed a chemically clean, three-dimensional homogeneous incompact structure (image a). The diameter of the CNF was in the range from 30 to 50 nm. This uniform nanostructure provided a significant increase of effective electrode surface for loading of biomolecules and accelerating electron transfer. When CNF film was cast on the ADH-coated GCE (b), CNF was entrapped in the enzyme matrix. The aggregates of the trapped ADH molecules were distributed regularly and showed an islandlike structure. This excellent electric property and incompact open structure of CNF film facilitated substrate and cofactor NAD^+ access to the ADH and resulted in good amperometric response to both NADH and the substrate.

The biocompatibility of an electrode surface for loading of biomolecules and preserving their bioactivity could be characterized by the hydrophilicity, which could be measured with the contact angle of the substrate. As shown in Figure 1B, the bare and CNF-coated glass slides gave the contact angles of 38° and 24°, respectively. The CNF film showed a lower contact angle, indicating better hydrophilicity. Thus, the casting of CNF on the electrode surface improved its biocompatibility for retaining bioactivity.

The FT-IR spectrum of CNF treated with nitric acid showed two absorption peaks at 1732.2 and 1586.6 cm^{-1}, indicating the formation of the carboxylic and carboxylate groups in the pretreatment process. After loading of ADH on the CNF film, the FT-IR spectrum showed two other absorption bands centered at 1650.6 and 1541.4 cm^{-1}. These shapes of these absorption bands were attributed to the the amide I and amide II infrared absorption bands of ADH. They can provide detailed information on the secondary structure of the polypeptide chain. The complete denaturation of enzyme will eliminate the distinctive absorption bands of amide I and amide II.[38] The absorption bands of ADH in the CNF film were nearly the same as those at 1650.8 and 1541.4 cm^{-1} obtained for the protein itself. This demonstrated that ADH was successfully immobilized in the CNF film and its structure did not alter in the immobilization process. Therefore, CNF film retained the native structure of the coimmobilized enzyme.

XPS analysis of the CNF showed that the acidic treatment introduced the oxygen-rich groups. The oxygen/carbon atomic ratio of CNF surface increased from 1.4 to 14.9 atom % oxygen. Similar to other carbon materials, these oxygen-containing groups should be carboxyl, phenol, and carbonyl groups, which could be verified with pH titration. The titration curve of the obtained soluble CNF (no shown) showed two equivalence points, indicating the presence of different oxygen-containing groups.

Electron-Transfer Kinetics at CNF Membrane. The electron-transfer kinetics of a redox probe at the CNF-modified electrode was investigated with EIS. At a bare GCE, the redox process of the probe, $[Fe(CN)_6^{3-/4-}]$, showed a low electron-transfer resistance (Figure 2, curve a), while the CNF-modified electrode showed a lower resistance for the redox probe (curve b), implying that CNF was an excellent electric conducting material and accelerated electron transfer. When ADH was assembled on the bare electrode, the semicircle increased dramatically (curve c), suggesting that ADH film blocked the electron exchange between

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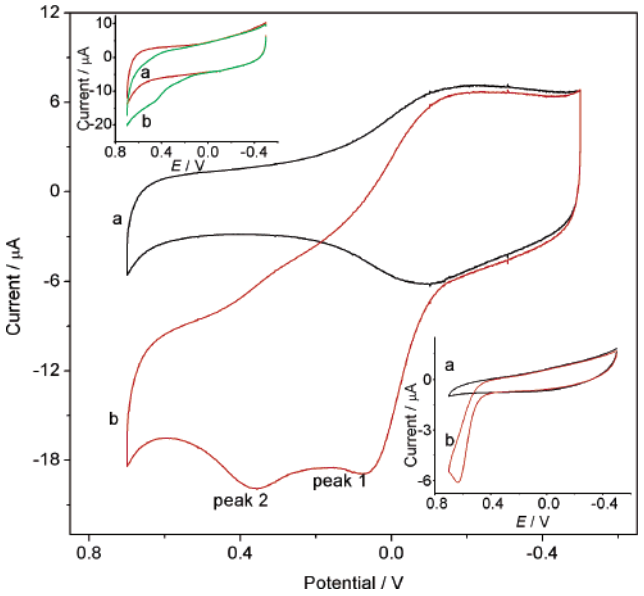

Figure 3. Cyclic voltammograms of CNF-modified electrode in (a) 0.2 M pH 7.0 PBS and (b) (a) + 2.0 mM NADH. Inset: cyclic voltammograms of bare (lower) and untreated CNF-modified (upper) electrodes in (a) 0.2 M pH 7.0 PBS and (b) (a) + 2.0 mM NADH. Scan rate, 10 mV s^{-1}.

the redox probe and electrode surface. However, upon the casting of CNF on the ADH film, the semicircle or electron-transfer resistance decreased obviously (curve d), suggesting that the presence of CNF made the electron transfer easier.

Electrochemical Response of CNF-Modified Electrode to NADH. The fast and reliable detection of NADH at low potential is particularly important. Figure 3 shows the cyclic voltammograms of bare and CNF-modified electrodes in PBS and PBS containing 2.0 mM NADH recorded at 10 mV s^{-1}. The CNF-modified electrode in pH 7.0 PBS showed two redox waves with the peak potentials of −0.13 and −0.10 V, similar to those obtained from CNT.[39] These peaks could be ascribed to the reduction and oxidation of the oxygen-containing groups on the CNF surface.

It is well known that quinone functionalities can mediate electron transfer from NADH.[40,41] Upon addition of 2.0 mM NADH to PBS, the cyclic voltammogram showed two oxidation peaks at +0.062 and +0.361 V, while the reduction peak of the CNF at −0.13 V decreased. At the uncoated electrode, the peak potential of the NADH oxidation is +0.635 V (lower inset in Figure 3). Compared to the oxidation peak at +0.062 V, the presence of CNF resulted in a substantial negative shift of the anodic peak potential for 573 mV. The peak current also increased from 5.11 to 11.39 μA, showing excellent electrocatalytic behavior to the oxidation of NADH. At the untreated CNF-modified electrode, the peak potential for NADH oxidation was at +0.450 V (upper inset in Figure 3), which was 388 mV higher than that for treated CNF. So the electrocatalytic behavior of CNF film partially resulted from the oxygen-rich groups such as phenolic hydroxides[39,42] and quinone,[43] present on the CNF surface (introduced during the acid oxidation). Generally, the oxidation of the basal plane of highly ordered pyrolytic graphite can result in fragmentation of the surface to expose edge plane defects, and electron transfer may be more facile at samples containing a higher proportion of edge plane defects,[21] producing a faster electron-transfer rate.[44,45] The two anodic responses observed could be ascribed to the oxidation of NADH at the edge plane sites on the CNF and those of the underlying GCE.[21] The catalytic activity is generally evident from the defined peak at +0.062 V. Thus, CNF decreased the oxidation overpotential of NADH by ~573 mV, which was larger than 490 mV at a MWCNT film modified GCE.[18] The electrocatalytic oxidation process at the CNF-based NADH sensor occurred at potentials of 340 and 240 mV more negative than those at peptide nanotube-based[12] and CNT-based[21,27] NADH sensors. The greater negative shift of peak potential or lower overpotential for NADH oxidation resulted from the nanostructures and more oxygen-rich groups of the CNF film. CNF possessed more edge sites on the outer wall than CNT,[29] thus showing faster kinetics. XPS analyses showed the oxygen/carbon atomic ratios of treated MWCNTs and CNF surfaces were 11.0 and 14.9 atom % oxygen, respectively, while the untreated MWCNTs and CNF contained 1.8 and 1.4 atom % oxygen, respectively, indicating the acidic treatment produced more oxygen-rich groups on the CNF surface. The titration curves of the obtained soluble CNF and MWCNTs (no shown) also showed the former possessed higher hydrogen (acid) and hydroxide (base) ion capacity. Under the same conditions, the CNF solution needed 179 μL of 0.01 M HCl to reach the first equivalence point and 254 μL of 0.01 M NaOH to reach the second equivalence point, while the MWCNT suspension needed 116 μL of HCl and 130 μL of NaOH to reach the two equivalence points. The higher amount of oxygen-rich groups made the electrocatalytic oxidation of NADH easier at the CNF electrode.

Condition Optimization. The performance of the CNF electrode mainly depended on the amounts of both CNF immobilized on electrode surface and the oxygen-containing groups on the CNF surface, which was related to the oxidation treatment time of CNF. This work optimized the oxidation treatment time. As shown in Figure 4A, the catalytic current increased with increasing oxidation treatment time and reached a maximum value at 24 h due to the increase in oxygen-containing groups. When the treatment time was longer than 36 h, the response decreased because of the structural degradation of CNF backbone.

At the optimized treatment time, with the increasing CNF concentration used for preparation of the modified electrode by the two-coating process, the electrocatalytic current increased and reached a constant value at 5 mg mL^{-1} (Figure 4B), at which the electrocatalytic current increased with increasing layer number (3.0 μL of solution for each layer). However, the obtained electrode gave the best performance for NADH at a CNF layer number of

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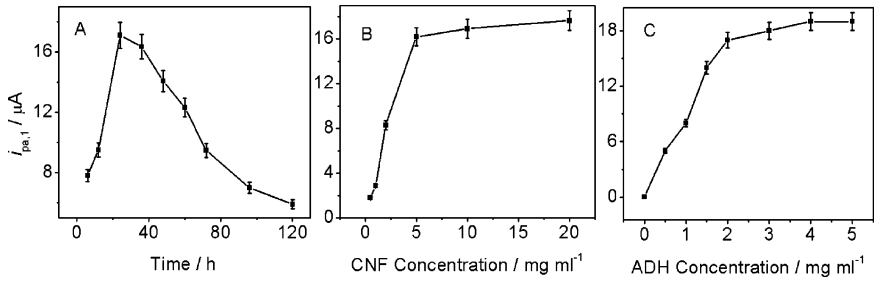

Figure 4. Effects of oxidation treatment time (A) and CNF concentration used for preparation of CNF/GCE (B) on peak current in 0.2 M pH 7.0 PBS containing 2.0 mM NADH, and ADH concentration used for preparation of ADH/CNF/GCE on peak current in 0.2 M pH 7.0 PBS containing 2.0 mM NAD^+ and 2.0 mM ethanol (C).

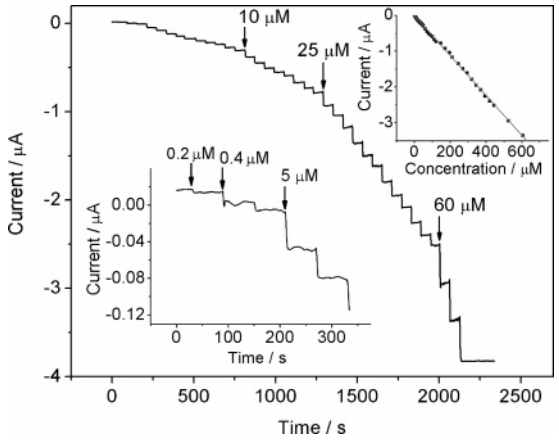

Figure 5. Successive amperometric response of the CNF-modified electrode to NADH in pH 7.0 PBS at +0.06 V. The NADH addition each time is from 0.2 to 60 μM as indicated. Upper inset, linear calibration curve; lower inset, amplified response curve.

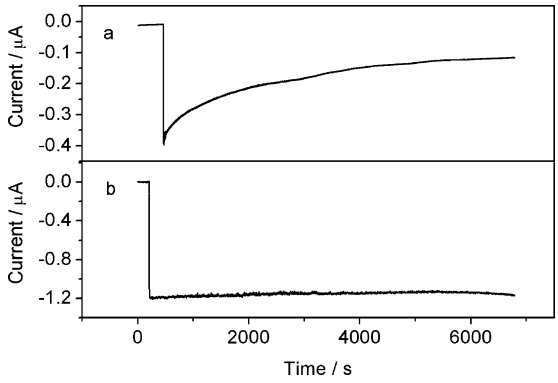

Figure 6. Responses of unmodified electrode at +0.6 V (a) and CNF-modified electrodes at +0.06 V to 210 μM NADH in 0.2 M pH 7.0 PBS.

2. When the layer number was more than 2, the response did not increase. If 6.0 μL of the 5 mg mL^{-1} CNF solution used for the two coating processes was dropped once on the pretreated GCE, the film formed was unstable. Thus, the NADH sensor was prepared by casting two layers of CNF.

The concentration of enzyme immobilized on the electrode surface is another important parameter for substrate detection. Figure 4C shows the effect of the concentration of enzyme dropped on the electrode surface on the voltammetric response of the enzyme electrode. With the increasing concentration of 3.0 μL of enzyme solution, the catalytic peak current increased and then tended toward a constant value. At an enzyme concentration of 2 mg mL^{-1}, the current reached a maximum value, indicating a saturation of enzyme in the CNF film. Thus, 3.0 μL of 2 mg mL^{-1} ADH solution was used for preparation of the substrate biosensor.

Detection of NADH. Figure 5 shows the amperometric response of the CNF-modified electrode at +0.06 V to the successive addition of NADH in pH 7.0 PBS. Immediately after the addition of NADH, the anodic current increased and reached a steady state within 5 s. The response displayed a linear range from 0.2 to 686 μM with a correlation coefficient of 0.999 and a slope of 5.6 nA μM^{-1}. The linear response range was wider than that of 5−300 μM reported previously with a CNT−chitosan-based NADH amperometric sensor at +0.4 V.[27] The sensitivity was also higher than that at a CNT-modified GCE measured at an applied potential of +0.3 V.[18]

The electrocatalytic behavior was highly reproducible, as reflected by a relative standard deviation of 3.5% estimated from the slopes of the calibration plots at six freshly prepared CNF-modified electrodes. At an NADH concentration of 1.0 and 100 μM, the sensor showed relative standard deviations of 4.5 and 4.9% examined for six determinations, respectively. The batch-to-batch reproducibility was estimated with the slopes of calibration plots obtained from six sensors made at the same electrode independently. The relative standard deviation of these slopes was 5.9%. When the sensor was not in use, it was stored in pH 7.0 PBS at 4 °C. A decrease of 4.2% amperometric response was observed after 3 months. When it was stored in a desiccator at room temperature for 3 months, the decrease of amperometric response was 4.5%. When the cyclic voltammogram was recorded once each day, the response of NADH remained 90.1% of its initial response after 30 days. If the sensor was immersed in 0.2 M pH 7.0 PBS with the temperatures from 20 to 70 °C for 20 min, and then used to detect the response of NADH at room temperature, the results showed only slight change. Thus, the stability of this sensor was acceptable, and it was suitable for the determination of NADH.

Another attractive feature of the CNF-modified electrode was its highly stable amperometric response to NADH. Figure 6 compares the amperometric responses of an unmodified and a CNF-modified electrode to 210 μM NADH recorded over a continuous period of 100 min. At the unmodified electrode, the applied potential was held at +0.60 V for NADH oxidation. The bare GCE displayed a rapid decay of the signal with up to 70% current depressions after 100 min, indicating a nearly complete inhibition of the oxidation process. In contrast, the response of the CNF-coated GCE remained stable throughout the entire

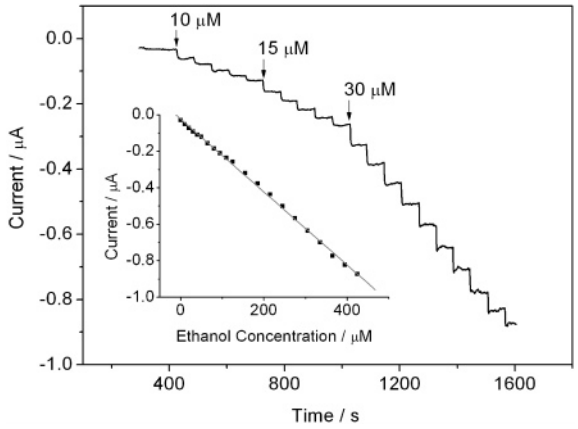

Figure 7. Successive amperometric response of the ADH/CNF-modified electrode to ethanol in pH 7.0 PBS at +0.06 V. The ethanol addition each time is from 10, 15, and 30 μM as indicated. Inset, linear calibration curve.

experiment, with only 3% current diminutions at 100 min. Obviously the oxidation of NADH at the bare GCE led to surface passivation that resulted from the radical intermediates, the oxidation products formed at a high overpotential.[4,5] The surface fouling caused the loss of analytical sensitivity, reproducibility, and operational lifetime.[6] However, the CNF-coated GCE overcame this drawback due to the improved kinetics of electron transfer and more negative potential applied for the oxidation of NADH, which limited the amount of radical intermediates and their dimerization. Thus, the soluble CNF was a potential excellent electrocatalytic material for preparation of sensitive, reproducible, and stable amperometric biosensors for dehydrogenase substrates.

Amperometric Biosensing of Ethanol. Figure 7 shows the steady-state response at an applied potential of +0.06 V for different additions of ethanol in a magnetically stirred 0.2 M pH 7.0 PBS. The response of the sensor was rather fast, and the response time was ~10 s. The anodic current increased linearly with ethanol concentration over the range from 10 to 425 μM. The limit of detection was estimated at a signal-to-noise ratio of 3 to be 3.0 μM, which was much lower than those of 0.1 mM and 49 μM reported for sensors based on injection of the recognition element[46] and Au nanoparticles,[47] respectively.

The relative standard deviation, estimated from the slopes of the calibration plots of six different and freshly prepared ethanol sensors, was 4.6%, indicating good fabrication reproducibility. The sensors could be repeatedly used for ethanol monitoring. The assay precision of the sensors was examined at an ethanol concentration of 100 μM. The relative standard deviation for six determinations was 5.1%. The examination of the batch-to-batch reproducibility showed a relative standard deviation of 7.2% for the slopes of calibration plots of six sensors made at the same electrode independently. The stability of the ADH/CNF-modified electrode was investigated when stored in pH 7.0 PBS at 4 °C. After 30 days, the response current was still retained at 95% value of the initial response. Response current for continuous usage for 30 days remained at 88.3% of the initial response. This implies that the three-dimensional structure of the ADH/CNF film was very efficient for retaining the bioactivity of ADH. After the modified electrode was stored in a desiccator at room temperature for one week, only 87.5% of the initial response remained. Immersion of the ethanol sensor in 0.2 M pH 7.0 PBS at temperatures of more than 40 °C for 20 min would lead to a decrease in amperometric response. These decreases were due to the loss of ADH enzyme activity.

CONCLUSIONS

The new application of soluble carbon nanofiber, an attractive electrocatalytic nanomaterial, for preparation of an amperometric biosensor is proposed. The solubility and biocompatibility of CNF facilitates its manipulation for sensor preparation and biosensing applications. The nanostructures of the CNF film increase the loading of biomolecules. The resulting CNF-modified electrode shows a very efficient electrocatalytic behavior toward the oxidation of NADH at a low overpotential (+60 mV) due to the formation of a high amount of oxygen-rich groups. The accelerated electron-transfer kinetics limits the formation of electrode surface fouling and improves the operational stability, fabrication reproducibility, and sensitivity of CNF-based sensors. The new application of the catalyst described to biosensor development has been demonstrated by the construction of a very simple ethanol biosensor. The sensors for both NADH and dehydrogenase substrates exhibit very good analytical performance with low cost, convenient preparation, and sensitive, rapid, and reproducible detection. Thus, the soluble carbon nanofiber is an attractive amperometric transducer in biosensors for substrates of dehydrogenase enzymes and other practical applications.

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