INTRODUCTION

Carbon nanotube (CNT) is an attractive material for the development of biosensors because of its capability to provide strong electrocatalytic activity and minimize surface fouling of the sensors. This article reviews the recent successful development of biosensors based on CNT materials. Specifically, biosensors from two fabrication regimes have been investigated: 1) the coimmobilization of CNTs and enzymes on electrode surfaces and 2) the growth of controlled-density aligned CNTs for the fabrication of nanoelectrode arrays. In the first regime, the CNTs are either dispersed in solvents [e.g., sulfuric acid, dimethylformamide (DMF)], dissolved in Nafion solution for electrode coating, or mixed with Teflon as an electrode material for reagentless biosensors. In the second regime, the nanoelectrode arrays consisting of millions of vertically aligned CNTs, each acting as an individual electrode, have been fabricated through a nonlithographic method. We also demonstrate the capability of CNTs to promote the oxidation/reduction (redox) reactions of hydrogen peroxide and nicotinamide adenine dinucleotide (NADH), which are involved in a wide range of amperometric biosensors associated with oxidase and dehydrogenase enzymes, respectively. With these electrocatalytic properties of CNTs, the applications of CNT-based biosensors examined in our laboratories include the low-potential detections of glucoses, organophosphorous compounds, and alcohol.

BACKGROUND

There has been enormous interest in exploiting CNTs in electrochemical and biological sensors since they were first introduced in 1991. CNTs are distinguished according to their structural properties: a single-wall CNT (SWCNT) consists of a single graphitic sheet rolled into a cylinder (with 1 to 2 nm o.d. and several microns in length), and multiwall CNT (MWCNT) consists of graphitic sheets rolled into closed concentric tubes (with 50 nm o.d. and microns in length), each separated by van der Waals forces to have a gap of 3.4 Å. Carbon nanotubes have been known to promote electron-transfer reactions of cytochrome c, NADH, catecholamine neurotransmitters, and ascorbic acid. This is attributed to their electronic structure, high electrical conductivity, and redox active sites. Carbon is also a versatile electrode material that can undergo various chemical and electrochemical modifications to produce suitable surfaces for high electrode responses. Carbon electrodes have a wide useful potential range, especially in the positive direction, because of the slow kinetics of carbon oxidation. These excellent properties of CNTs have been successfully exploited in our laboratories to develop either amperometric biosensors based on the immobilization of CNTs, coimmobilization of CNTs and enzymes, or the growth of controlled-density aligned CNTs into the nanoelectrode arrays for the detection of glucoses, organophosphorous compounds, and alcohol, as summarized in the following sections.

BIOSENSORS BASED ON IMMOBILIZATION OF CNTs

Solubilization and Immobilization of CNTs

CNTs have shown potential for applications in chemical/biological sensors and nanoscale electronic devices. A
major barrier for developing such CNT-based devices is the insolubility of CNTs in most solvents. This challenge has been addressed through covalent modification\textsuperscript{[24,25]} or noncovalent functionalization\textsuperscript{[26,27]} of the CNTs. A ‘‘wrapping’’ of CNT in polymeric chains (i.e., poly(p-phenyl-enevinylene)\textsuperscript{[26]} or poly\{[(m-phenylenevinylene)-co-[2,5-dioctyloxy-(p-phenylene)-vinylene]]\}\textsuperscript{[27]} has improved the solubility of CNTs without impairing their physical properties.\textsuperscript{[28]} In our work,\textsuperscript{[5]} a well-known perfluorosulfonated polymer, Nafion, has been used to solubilize single-wall and multiwall CNTs. Because of their unique ion-exchange, discriminative, and biocompatibility properties, Nafion films have been used extensively to modify electrode surfaces and to construct amperometric biosensors.\textsuperscript{[29,30]} Similar to other polymers used to wrap and solubilize CNTs, Nafion bears a polar side chain. We have found that CNTs can be suspended in solutions of Nafion in phosphate buffer or alcohol. Increasing the Nafion content from 0.1 to 5 weight percent (wt.%) results in a dramatic enhancement of the solubility of both single-wall and multiwall CNTs, which can be observed by the naked eye. A homogeneous solution of the Nafion/CNT complex is observed in Nafion solution, but no such solubilization is observed in ethanol or phosphate-buffer solutions containing no Nafion. The CNT/Nafion association does not impair the electrocatalytic properties of CNTs with respect to the redox reaction of hydrogen peroxide. The Nafion-induced solubilization of CNT thus permits a variety of applications, including the modification of electrode surfaces for preparing amperometric biosensors.

Because CNTs are insoluble in most solvents, previously reported CNT-modified electrodes have relied on casting a CNT/sulfuric acid solution onto a surface of electrodes,\textsuperscript{[1,31]} a procedure that is not compatible with the immobilization of biocomponents. Therefore we have developed a new and simple method for preparing effective CNT-based biosensors from CNT/Teflon composite material.\textsuperscript{[6]} Carbon composites, based on the dispersion of graphite powder within an insulator, offer convenient bulk modification for the preparation of reagentless and renewable biosensors. Teflon has been used as a binder for graphite particles for various electrochemical-sensing applications.\textsuperscript{[32,33]} Our approach relies on CNTs as the sole conductive component rather than as the modifier cast on other electrode surfaces. The bulk of CNT/Teflon composites hence serve as a reservoir for the enzymes, in the same manner as their graphite-based counterparts. The preparation is very simple: a certain amount of CNTs is hand-mixed in the dry-state with granular Teflon to obtain a desired composition of CNT/Teflon. The CNT content of the new composites has a large effect upon their electrochemical behavior. Fig. 1a compares the calibration plots for potassium ferricyanide obtained at electrodes containing different MWCNT loadings. All electrode compositions yield highly linear calibration plots over the entire concentration range. The sensitivity increases with the CNT loading between 10 and 60 wt.% and decreases thereafter. The influence of the CNT content was also examined using cyclic voltammetry (CV) experiments. The CV ferricyanide cathodic and anodic currents increased linearly with the CNT content between 30 and 70 wt.% (Fig. 1b). Composites containing more than 70 wt.% CNTs are too dry and porous and have poor mechanical stability. Electrode resistance leading to a poorly defined and negligible CV is observed at the electrodes containing low CNT content. Fig. 1b shows the sensitivity-loading (B) and resistance-loading (A) profiles. Too high Teflon (<30 wt.% CNTs) leads to high resistance to a nearly insulating matrix and low sensitivity, while too high CNT (>75 wt.% CNT) leads to an operation beyond the mass-limiting plateau associated with the electrodes.

![Fig. 1](image-url)
with the shift of the voltammetric signal. Therefore a CNT content of 40 to 60 wt.% is suggested.

The CNT/Teflon composites have the combined advantages of CNTs and bulk composite electrodes that permit a wide range of applications without the need for a graphite surface. Certain amounts of enzymes [e.g., glucose oxidase (GOx) and alcohol dehydrogenase (ADH)] and cofactor (e.g., NAD+) can be mixed with the CNT/Teflon composite and used as electrode materials, depending upon specific needs. The CNT/Teflon coating was later investigated in our laboratory and displayed a marked electrocatalytic action toward hydrogen peroxide and NADH and hence is promising for the development of biosensors for glucose (in connection with oxidase enzymes) and ethanol (in connection with dehydrogenase enzymes), respectively.

**Electrocatalytic Activity of CNTs to Redox Reactions of Hydrogen Peroxide**

Hydrogen peroxide is involved in a wide range of biosensing applications associated with oxidase enzymes. In our laboratories, the enhancement of the redox activity of hydrogen peroxide by CNTs is investigated using the previously described CNT/Nafion\(^5\) and CNT/Teflon\(^6\) electrode materials.

To study the electrocatalytic activity of CNTs for hydrogen peroxide oxidation/reduction, the CNT/Nafion (from a 0.5 wt.% Nafion solution containing 2 mg/mL of CNTs) is coated on a glassy carbon (GC) electrode surface. Fig. 2 displays the cyclic voltammograms for \(5 \times 10^{-3}\) M hydrogen peroxide recorded at a bare GC electrode (A) and the CNT/Nafion-modified GC electrode (B). Significant oxidation and reduction currents starting around +0.20 V are observed on the CNT/Nafion-coated electrode, while none is observed at the bare GC electrode. The CNT/Nafion-coated electrode offers a marked decrease in the overvoltage for hydrogen peroxide reaction and hence allows low-potential amperometric detection. The inset of Fig. 2 shows the amperometric response at 0.0 V to successive additions of hydrogen peroxide. While the modified electrode (B) responds very rapidly and favorably to the changes in hydrogen peroxide concentration, no response is observed at the bare GC electrode (A). A similar decrease in hydrogen peroxide overvoltage is observed at other CNT-modified electrodes (not shown), indicating that Nafion does not impair the electrocatalytic properties of CNT. The CNT/Nafion-coated electrode is reliable and not affected by regenerating the surface; six successive measurements of hydrogen peroxide, each recorded on a freshly polished surface, show reproducible results with %RSD of less than 4.

Similar substantial lowering of the detection potential and significantly improved current signals for hydrogen peroxide by CNTs have also been found at CNT/Teflon electrodes, obtained by packing 60/40 wt.% of CNT/Teflon into the electrode cavity of a glass sleeve with a copper wire as the electrical contact. Control experiments, using graphite-based Teflon composites, were performed in parallel. Fig. 3 compares the hydrodynamic voltammograms (HDVs) for 1 mM hydrogen peroxide at the 60:40 wt.% graphite/Teflon electrode (a) and the 60:40 wt.% MWCNT/Teflon electrode (b). Other conditions are as in Fig. 1a. (From Ref. 6.)
Fig. 4  (a) Cyclic voltammograms for $5 \times 10^{-3}$ M NADH at unmodified (A), MWCNT-modified (B), and SWCNT-modified (C) GC electrodes. Operating conditions: scan rate, 50 mV/sec; electrolyte, phosphate buffer (0.05 M, pH 7.4). Dotted lines represent the background response. (b) Hydrodynamic voltammograms for $1 \times 10^{-4}$ M NADH at the unmodified (A) and the MWCNT-modified (B) GC electrodes. Operating conditions: stirring rate, 500 rpm; electrolyte, phosphate buffer (0.05 M, pH 7.4). (c) Current–time recordings obtained after increasing the NADH concentration of $1 \times 10^{-4}$ M (each step) at unmodified (A) and MWCNT-modified (B) GC electrodes. Inset shows the corresponding calibration curve. Operating conditions: potential, +0.3 V; others as in (b). (From Ref. 1.)
perte doctorate at the graphite/Teflon (a) and CNT/Teflon (b) electrodes. Compared to the graphite/Teflon electrode, the CNT/Teflon electrode responds more favorably to hydrogen peroxide over the entire potential range (0.0 to 1.0 V) with significant response starting at +0.20 V. The Teflon binder is proven to not impair the electrocatalytic properties of CNTs.

Electrocatalytic Activity of CNTs to Redox Reactions of NADH

β-Nicotinamide adenine dinucleotide (NADH) is a cofactor in several hundred enzymatic reactions of NAD+/NADH-dependent dehydrogenases. The electrochemical oxidation of NADH has thus been the subject of numerous studies related to the development of amperometric biosensors. Problems inherent to such anodic detection are the large overvoltage encountered for NADH oxidation at ordinary electrodes and surface fouling associated with the accumulation of reaction products. CNTs have thus been used in our recent work as the new electrode material to reduce the overpotential for NADH oxidation and to alleviate surface fouling problems.

In the previous study, single-wall and multiwall CNTs were dispersed in concentrated sulfuric acid, and each was subsequently cast on a glassy carbon electrode. Fig. 4a shows the cyclic voltammograms of NADH measured at unmodified (A), MWCNT-modified (B), and SWCNT-modified (C) glassy carbon electrodes. Both modified electrodes yield an approximately twofold larger NADH peak, compared to the unmodified electrode. The oxygen-rich groups on the CNT surface, introduced during the acid dispersion, are perhaps responsible for such electrocatalytic behavior for the oxidation of NADH. Fig. 4b shows a HDV of 1 × 10⁻⁴ M NADH, which reflects the electrocatalytic behavior of the CNT coating with varying potentials. The MWCNT-coated electrode (B) responds to NADH over the entire 0.0- to 1.0-V range, while the bare electrode (A) responds only at potentials higher than +0.6 V. Fig. 4c shows that successive additions of 1 × 10⁻⁴ M NADH result in increasing response detected at the CNT-modified electrode (B) but no response at the unmodified electrode (A) when the detection potential was kept low (i.e., 0.3 V). Evidently, the electrocatalytic action of CNT enables the fast response (i.e., 10 sec to reach the steady state) to the change of NADH concentrations at the low-detection potential. The amperometric response of 5 × 10⁻³ M NADH appears to be very stable; the decay of the signal is less than 10% and 25% after a 60-min period at the MWCNT-modified and SWCNT-modified electrodes, compared with 75% and 53% at the graphite-coated and acid-treated electrodes, respectively. This shows the capability of CNTs in resisting the fouling effects and in preventing the diminishing of signals in successive cyclic voltammetric detections. The resistance to fouling of CNT-based electrodes has yet to be understood.

In our more recent study, the electrocatalytic effect of CNTs to facilitate low-potential amperometric measurements of NADH has been investigated using CNT/Teflon electrodes. Fig. 5 compares the amperometric response (at +0.40 V) of the graphite/Teflon (a) and CNT/Teflon (b) electrodes to successive additions of 0.1 mM NADH. Only the CNT/Teflon electrode responds very rapidly to the changes in the level of NADH, producing steady-state signals within 8 to 10 sec. The favorable signals are accompanied by a low noise level.

The CNT coating offers remarkably decreased overvoltage for the NADH oxidation as well as reduced surface fouling effects of the electrodes. These characteristics indicate the great promise of CNTs for developing highly sensitive, low-potential, and stable amperometric biosensors based on dehydrogenase enzymes.

Applications of CNT-Immobile Biosensors

Our laboratories have exploited the capability of CNTs to promote redox activity of hydrogen peroxide to develop oxidase-based amperometric biosensors, including those for detecting glucose and organophosphorous compounds. Similarly, the electrocatalytic properties of CNTs in reducing the overpotential for the redox reaction of NADH suggest their potential use in dehydrogenase-based amperometric biosensors such as those for alcohol detection. These applications of CNT-based biosensors are summarized as follows.
Glucose detection

The CNT/Nafion/GOₓ-modified GC electrode was used in a flow-injection system to measure glucose.[⁵] Fig. 6 compares the amperometric responses for relevant physiological levels of glucose, ascorbic acid, acetaminophen, and uric acid at the CNT/Nafion/GOₓ-modified GC electrode (B) and Nafion/GOₓ-modified GC electrode (A). In Fig. 6, the accelerated electron-transfer reaction of hydrogen peroxide at the CNT/Nafion/GOₓ-modified GC electrode allows for glucose measurements at very low potentials (i.e., −0.05 V) where interfering reactions are minimized. As a result, a well-defined glucose signal (d) is observed, while the signals of acetaminophen (a), uric acid (b), and ascorbic acid (c) are negligible. No such discrimination is obtained at the Nafion/GOₓ biosensor (without the CNT) (A) held at +0.80 V, where large oxidation peaks are observed for all interferences, indicating that the permselective (charge-exclusion) properties of Nafion are not adequate to fully eliminate anionic interferences. In short, the coupling of the permselective properties of Nafion with the electrocatalytic action of CNT allows for glucose detection with effective discrimination against both neutral and anionic redox constituents. Similarly, the CNT/Nafion-coated electrodes have also been demonstrated to dramatically improve the signal of dopamine in the presence of the common ascorbic acid interference.

![Fig. 6](image)

**Fig. 6** Flow-injection signals for $2 \times 10^{-4}$ M acetaminophen (a), $2 \times 10^{-4}$ M ascorbic acid (b), $2 \times 10^{-4}$ M uric acid (c), and $1 \times 10^{-2}$ M glucose (d), at the Nafion/GOₓ-modified GC electrode (A) at +0.8 V, and the MWCNT/Nafion/GOₓ-modified GC electrode (B) at −0.05 V, and flow rate of 1.25 mL/min. (From Ref. 5.)

The detection of glucose has also been performed on CNT/Teflon-based electrodes, which are immobilized with GOₓ enzyme.[⁶] Fig. 7 compares the amperometric response to successive additions of 2 mM glucose at the graphite/Teflon/GOₓ (a) and the MWCNT/Teflon/GOₓ (b) electrodes using operating potentials of +0.6 V (A) and +0.1 V (B). The CNT-based bioelectrode offers substantially larger signals, especially at low potential, reflecting the electrocatalytic activity of CNT. Such low-potential operation of the CNT-based biosensor results in a highly linear response (over the entire 2- to 20-mM range) and a slower response time (~1 min vs. 25 sec at +0.6 V). The glucose biocomposite based on single-wall CNTs results in a more sensitive but slower response than that based on multwall CNTs. The low-potential detection also leads to high selectivity (i.e., effective discrimination against coexisting electroactive species). Despite the absence of external (permselective) coating, the glucose response at +0.1 V was not affected by adding the common acetaminophen and uric acid interferences at 0.2 mM. A similar addition of ascorbic acid resulted in a large...
interference, reflecting the accelerated oxidation of this compound at the CNT surface. Reproducible activity was observed after 2 weeks of dry storage at 4°C, which is in good agreement with the high stability of enzymes in Teflon-based carbon composites.

Organophosphorus compound detection

Organophosphorous (OP) compounds are very toxic and are thus widely used as pesticides and chemical-warfare agents (CWAs). Recently, we have successfully used MWCNTs in developing an amperometric biosensor for OP compounds. Specifically, the MWCNT is used to modify screen-printed carbon electrodes, which are subsequently coimmobilized with acetylcholinesterase (ACHE) and choline oxidase (CHO) enzymes. The MWCNT-modified electrode has demonstrated a significant catalytic effect for the redox reaction of hydrogen peroxide, leading to the development of a novel biosensor for the assay of OP compounds with enhanced sensitivity.

The ACHE enzyme is known to play an important role in cholinergic transmission as a catalyst for the rapid hydrolysis of the neurotransmitter acetylcholine to acetate and choline as follows:

\[
\text{Acetylcholine} + \text{H}_2\text{O} \xrightarrow{\text{ACHE}} \text{Acetate} + \text{Choline} \quad (i)
\]

However, in the presence of OP compounds, the rate of choline production is reduced. The capability of OP compounds to inhibit ACHE activity is well known and thus is being exploited in developing biosensors for OP compound detection. In our work, the amperometric biosensor for OP compounds is based on coimmobilization of ACHE and CHO on a printed CNT electrode. In the biosensor based on ACHE/CHO enzymes, choline that is produced in reaction (i) serves as a substrate for the CHO enzyme in the presence of oxygen to produce hydrogen peroxide as follows:

\[
\text{Choline} + \text{O}_2 \xrightarrow{\text{CHO}} \text{Betaine Aldehyde} + \text{H}_2\text{O}_2 \quad (ii)
\]

The hydrogen peroxide that is produced in reaction (ii) can be detected amperometrically, and the amperometric response is negatively proportional to the amount of an OP compound that is introduced into the system.

The inhibition of ACHE activity by OP compounds is an irreversible process; once exposed to the OP compounds, the enzyme is inactivated, and the sensor can be reused only after an appropriate enzyme reactivation. Therefore we have attempted to develop biosensors that are low-cost and disposable using screen-printed carbon electrodes. To make the sensor, the suspension of MWCNTs in N,N-DMF was cast on the surface of screen-printed carbon electrodes to form a thin-film of CNTs. The screen-printed carbon only serves as a conducting base for the CNT electrode. The electrochemical method was used to oxidize CNTs to create a carboxylic acid group. Both enzymes, ACHE and CHO, were then coimmobilized on the CNTs via carbodiimide chemistry by forming amide linkages between their amine residues and carboxylic acid groups on the CNT surface. The optimum biosensor was found to contain a loading of 0.8 nM ACHE and 1.5 U CHO on the electrode, with a 2-mM ACh as the substrate.

An amperometric method was employed to study the sensor response time of the ACHE inhibition after the spike of methyl parathion, as a representative OP compound. In Fig. 8a, the amperometric response was rapid (i.e., within 30 sec) after the ACh addition, reflecting the fast diffusion of enzyme substrates and the products (as the CNTs are membrane-free porous). When methyl parathion was successively added to the test area of the

Figure 8

(a) Amperometric response of methyl parathion at the CNT/ACHE/CHO-immobilized screen-printed biosensor. Operating conditions: 0.1 M phosphate buffer/0.1 M NaCl (pH 7.4), potential of +0.50 V. (b) Inhibition effects by three organophosphates, chlorpyrifos (A), fenitrothion (B), and methyl parathion (C) on the enzyme activity, measured with the CNT/ACHE/CHO-immobilized screen-printed biosensor. Other conditions are as in (a). (From Ref. 8.)
biosensor, the response decreased significantly and rapidly in the first 10 min and more slowly thereafter. The significant inhibition effect of methyl parathion to the catalytic activity of ACHE reduces the production of hydrogen peroxide, leading to low signals.

In addition to methyl parathion, the inhibition effects of the other two OP compounds were investigated using the CNT-modified, ACHE/CHO-immobilized electrode. Fig. 8b compares the enzyme activities with a function of time after the spike of each OP compound, including (A) chlorpyrifos, (B) fenitrothion, and (C) methyl parathion.

The enzyme activity is the ratio of $I_i$ (a steady-state current obtained in the presence of a given OP compound) to $I_o$ (that obtained in the absence of the OP compound). After spiking with OP compounds, the enzyme activity decreases with time. The high inhibition effect of OP compounds can be correlated to low enzyme activity of ACHE. Their inhibition effects are in the following ascending order: methyl parathion $>$ fenitrothion $>$ chlorpyrifos.

The relative inhibition of methyl parathion at the CNT-modified, ACHE/CHO-immobilized biosensor as a function of methyl parathion concentration was investigated using a preincubation method in which the biosensor was exposed to the incubation solution containing methyl parathion for 10 min before the change in enzyme activity was measured. Successive incubation measurements were performed with varied methyl parathion concentrations. The CNT/ACHE/CHO biosensor has good analytical characteristics for methyl parathion, including a broad dynamic linear range (up to 200 μM, $r^2 = 0.96$), high sensitivity (0.48% inhibition/μM), and low detection limit (LDL = 0.05 μM). These improved characteristics reflect the catalytic activity of CNTs that promotes the redox reaction of hydrogen peroxide produced during ACHE/CHO enzymatic reactions with their substrate, as well as the large surface area of CNT materials. The hand-held electrochemical detector (i.e., CHI1232 from CHI Instrument, Inc.) coupled with the disposable biosensor developed in this work will potentially facilitate the field screening of OP pesticides and nerve agents with fast speed, high efficiency, low cost, and small sample size needed.

**Alcohol detection**

The attractive low-potential detection of NADH, along with minimal surface fouling, makes CNT extremely attractive for amperometric biosensors of ethanol through the incorporation of ADH/NAD$^+$ within the three-dimensional electrode matrix. Specifically, the reagentless biocomposite was prepared by mixing the desired amounts of the ADH enzyme and the NAD$^+$ cofactor with the CNT/Teflon composite to obtain the final composition of 28.5:65:1.5:5 wt.% CNT/Teflon/ADH/NAD$^+$. The mixture was then packed firmly into the electrode cavity of a glass sleeve with a copper wire as the electrical contact. Fig. 9 compares the performance at a low detection potential (i.e., +0.20 V) of the CNT/Teflon-based electrode (b) to that of graphite/Teflon-based electrode (a). Only the CNT/Teflon-based electrode (b) responds favorably to successive additions of 1 mM ethanol. The response is relatively fast (∼60 sec to reach steady state) and nonlinear. The greatly enhanced biosensing of ethanol at the CNT-based electrode suggests the accelerated oxidation of NADH at low-potential detection. In addition to being reagentless, the biosensor does not require a redox mediator (to shuttle the electrons from the NADH product to the surface), which is commonly used for such low-potential detection of ethanol.

**BIOSENSORS BASED ON CONTROLLED-DENSITY ALIGNED CNTs**

**Fabrication**

Although vertically aligned CNTs have good material properties (e.g., good electrical conductivity, ability to...
promote electron transfer reactions) and are of the right size (20 to 200 nm) for nanoelectrode arrays (NEAs), they lack the right spacing. To make each nanotube work as an individual nanoelectrode, the spacing needs to be sufficiently larger than the diameter of the nanotubes to prevent the diffusion layer overlap from the neighboring electrodes.\[45\]

Recently, a nonlithography method that allows the fabrication of low-site-density aligned CNT arrays with an interspacing of more than several micrometers has been developed.\[46\] From these low site density CNTs, the NEAs consisting of millions of nanoelectrodes with each electrode being less than 100 nm in diameter were successfully fabricated.\[7,9,10\] As the total current of the loosely packed electrode arrays is proportional to the total number of individual electrodes, having the number of the electrodes up to millions is highly desirable. The size reduction of each individual electrode and the increased total number of the electrodes result in improved signal-to-noise ratio ($S/N$) and detection limits.\[47,48\]

In growing the low-site-density aligned CNT arrays, Ni nanoparticles were randomly deposited on a 1-cm$^2$ Cr-coated silicon substrate (Fig. 10a) by applying a pulse current to the substrate in NiSO$_4$ electrolyte solution. The size and the site density of the Ni nanoparticles were controlled by the amplitude and the duration of the pulse current. On these Ni particles, the CNTs were grown [Fig. 10b] in the plasma-enhanced chemical vapor deposition (PECVD) system at 650°C for 8 min with 160 sccm NH$_3$ and 40 sccm C$_2$H$_2$ gases with a total pressure of 15 Torr and a plasma intensity of 170 W. The aligned CNT arrays had a site density of $1 \times 10^6$ to $3 \times 10^6$ cm$^{-2}$, a length of 10 to 12 μm, and a diameter of 50 to 80 nm. In the early days of fabricating the electrode arrays,\[7,46\] a thin layer of SiO$_2$ was coated on the surface by magnetron sputtering to insulate the Cr layer. This was followed by applying M-Bond 610 coating (epoxy-phenolic adhesive from Vishay Intertechnology, Inc., Shelton, CT) to further insulate the Cr and provide mechanical support to the CNTs. This insulation is sometimes not sufficient to prevent current leakage, which results in the distortion of the cyclic voltammetry curve. Recently,\[9,10\] we improved the fabrication method using better insulating materials and packing procedures that solve the leakage problem, coupled with a pretreatment of the electrode to reduce the peak separation. We replaced the SiO$_2$ and M-bond coatings used as the passivation layer in our previous attempts with Epon epoxy resin 828 (Miller-Stephenson Chemical Co., Inc., Sylmar, CA) and $m$-phenylenediamine (MPDA) as a hardener. After these steps, the CNTs were half-embedded in the polymer resin, and the protruding part of the CNTs beyond the polymer resin was mechanically removed by polishing with a lens, followed by ultrasonication in water. Then the electronic connection was made on the CNT-Si substrate to make the CNT nanoelectrode arrays (Fig. 10c). Finally, the electrode arrays were pretreated by electrochemical etching in 1.0 M NaOH at 1.5 V for 90 sec prior to the electrochemical characterizations. The lifetime of the NEAs employing new epoxy is significantly improved: there is no degradation for several weeks because of the excellent stability of the epoxy layer.

Applications of Controlled-Density Aligned CNT Electrodes

The nanoelectrode array, consisting of millions of CNT electrodes, yields an excellent sigmoidal voltammogram for K$_3$Fe(CN)$_6$ and a low $S/N$.\[9\] The sigmoidal voltammogram is a characteristic of microelectrodes having radial diffusion. The steady-state current arises because the rate of electrolysis approximates the rate of diffusion of an analyte to the electrode surface.\[49\] The scan-rate-independent limiting current behavior was observed up...
to 500 mV/sec at the nanoelectrode array. This indicates that there is no diffusion layer overlapping between the electrodes because most of the CNTs are separated from their nearest neighbors by at least 5 \( \mu \)m, much larger than the diameter of each nanotube (50 to 80 nm).

The CNT-NEAs have a wide range of applications. They have been investigated in our laboratory as electrochemical sensors without further modification for the detection of drugs, such as 4-acetamidophenol, and metal ions, such as lead.[9]

For use as a glucose biosensor,[10] the GO\(_x\) enzyme molecules were attached to the broken tips of the CNTs via carbodiimide chemistry by forming amide linkages between their amine residues and carboxylic acid groups on the CNT tips. Fig. 11a and b compares the amperometric responses for 5 mM glucose (G), 0.5 mM ascorbic acid (AA), 0.5 mM acetaminophen (AC), and 0.5 mM uric acid (UA) at the GO\(_x\)-modified, CNT-nanoelectrode array and the potentials of +0.4 V (a) and −0.2 V (b). Electrolyte: 0.1 M phosphate buffer/0.1 M NaCl (pH 7.4). (c) Amperometric response at the GO\(_x\)-modified, CNT-nanoelectrode array for each successive addition of \( 2 \times 10^{-3} \) M glucose. Inset shows the corresponding calibration curve. Potential: −0.2 V, other conditions are as in (a–b). (From Ref. 10.)

![Fig. 11](a–b) Amperometric responses for 5 mM glucose (G), 0.5 mM ascorbic acid (AA), 0.5 mM acetaminophen (AC), and 0.5 mM uric acid (UA) at the GO\(_x\)-modified, CNT-nanoelectrode array and the potentials of +0.4 V (a) and −0.2 V (b). Electrolyte: 0.1 M phosphate buffer/0.1 M NaCl (pH 7.4). (c) Amperometric response at the GO\(_x\)-modified, CNT-nanoelectrode array for each successive addition of \( 2 \times 10^{-3} \) M glucose. Inset shows the corresponding calibration curve. Potential: −0.2 V, other conditions are as in (a–b). (From Ref. 10.)
For many years, numerous researches have emphasized the use of anti-interference layers or artificial electron mediators for improving the selectivity of amperometric biosensors. CNTs eliminate potential interferences through the preferential detection of hydrogen peroxide at the CNT-based electrodes. Such development of interference-free transducers will significantly simplify the design and fabrication of biosensors. The biosensors based on low-site-density aligned CNTs are also suitable for the highly selective detection of glucose in a variety of biological fluids (e.g., saliva, sweat, urine, and serum).

CONCLUSION

The electrocatalytic properties of CNTs in promoting the redox reaction of hydrogen peroxide and NADH are being exploited in our work in developing biosensors based on oxidase and dehydrogenase enzymes, respectively. With our CNT-based biosensors, low-potential detections of alcohol, organophosphorous compounds, and glucose are possible with many advantages over conventional devices. The capability of CNTs to reduce the overvoltage for the oxidation of hydrogen peroxide and NADH allows the detection of these species at low potential. At such low potential, most interfering species in the test samples do not undergo oxidation, thus eliminating potential interference. CNTs also minimize the surface fouling of biosensors, thus imparting higher stability onto these devices. While the CNT/Nafion-based biosensors use the electrocatalytic activity of CNTs and the permeselectivity of Nafion to detect glucose with effective discrimination against most neutral and anionic redox constituents, the CNT/Teflon composite allows the reagentless approach to fabricate biosensors with flexible coimmobilization of enzymes and cofactors for specific biosensing needs. Owing to the CNTs’ capability to promote electron-transfer activity, the artificial mediators that shuttle electrons between the enzymes and the electrodes are not required at the CNT-based biosensors, thereby eliminating the dependence upon the electroactive species and enhancing the reproducibility.

For future work, we will investigate the capability of CNTs to promote electron-transfer reactions of other biologically and environmentally important compounds. The biosensor fabrication technology demonstrated in this work holds a great future for developing routine, onsite amperometric biosensors based on both oxidase and dehydrogenase enzymes, such as those for cholesterol, alcohol, lactate, acetylcholine, choline, hypoxanthine, and xanthine. Although this article focuses on biosensors based on CNTs, other oriented conducting nanowires, e.g., oriented conducting polymer nanowires recently synthesized in our laboratory, should also provide an alternative ideal platform for biosensing applications.

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REFERENCES


