

# NANO LETTERS

## Glucose Biosensors Based on Carbon Nanotube Nanoelectrode Ensembles

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### ABSTRACT

This paper describes the development of glucose biosensors based on carbon nanotube (CNT) nanoelectrode ensembles (NEEs) for the selective detection of glucose. Glucose oxidase was covalently immobilized on CNT NEEs via carbodiimide chemistry by forming amide linkages between their amine residues and carboxylic acid groups on the CNT tips. The catalytic reduction of hydrogen peroxide liberated from the enzymatic reaction of glucose oxidase upon the glucose and oxygen on CNT NEEs leads to the selective detection of glucose. The biosensor effectively performs a selective electrochemical analysis of glucose in the presence of common interferents (e.g., acetaminophen, uric and ascorbic acids), avoiding the generation of an overlapping signal from such interferers. Such an operation eliminates the need for permselective membrane barriers or artificial electron mediators, thus greatly simplifying the sensor design and fabrication.

Because of the high demand for blood glucose monitoring, significant research and development efforts have been devoted to producing reliable glucose sensors for *in vitro* or *in vivo* applications.<sup>1–2</sup> The measurement principle of oxidase-based amperometric biosensors previously relied upon the immobilization of oxidase enzymes on the surface of various electrodes and the detection of the current associated with the redox product in the biological reaction. To increase the selectivity and sensitivity of amperometric biosensors, artificial mediators and permselective coatings are often used in biosensor fabrication. Artificial mediators are used to shuttle electrons between the enzyme and the

electrode to allow operation at low potentials.<sup>3–5</sup> This approach can minimize interference with coexisting electroactive species, but the stability and toxicity of some mediators limit their *in vivo* applications. Permselective membranes are also used to eliminate interference.<sup>6–7</sup> Effective, but incomplete, rejection has been reported in most cases. A mediator-free and membrane-free biosensor was described by Wang et al.<sup>8–9</sup> Wang's method provides a means for measuring the cathodic current of enzymatically liberated hydrogen peroxide in metal-dispersed carbon paste biosensors. The idea of a mediator-free and membrane-free biosensor based on the reduction of hydrogen peroxide has provided a new approach for biosensor development.

Recently, electrochemical properties of carbon nanotubes (CNTs) have been unveiled, and their application toward electrochemical sensors and biosensors has gained interest.<sup>10–22</sup>

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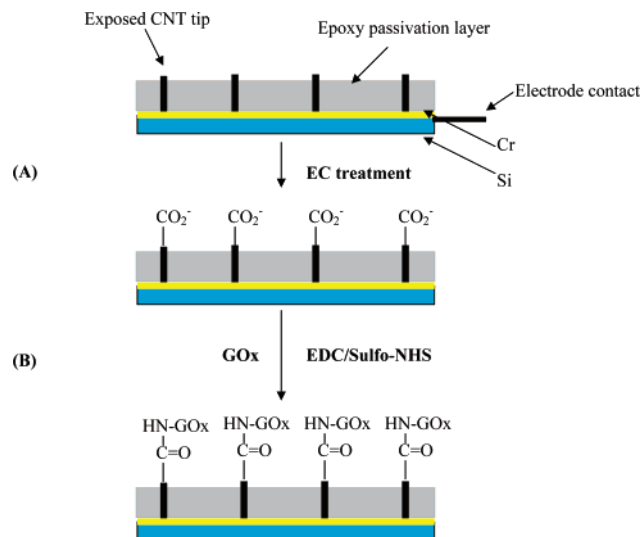
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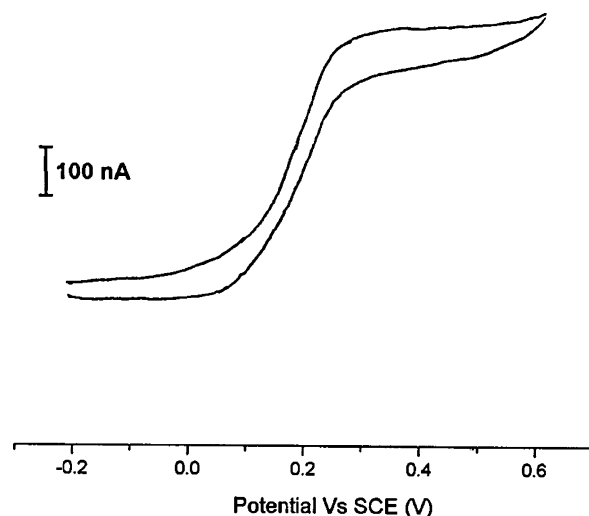
It was found that CNTs have a high electrocatalytic effect and a fast electron-transfer rate.<sup>10–13,19</sup> Wang et al. reported a mediator-free glucose sensor based on a Nafion-coated CNT-modified glassy carbon electrode.<sup>12</sup> The earlier work discussed above takes advantage of the bulk properties of CNTs. Our recent work explored another important feature of CNTs, which is that its ultrasmall size can be very useful in making nanoelectrode. Nanoelectrode ensembles based on low-site density, aligned CNTs were fabricated, and the electrochemical characteristics were investigated.<sup>14</sup> To make each nanotube work as an individual nanoelectrode, the spacing needs to be sufficiently larger than the diameter of the nanotubes to prevent diffusion layer overlap with the neighboring electrodes.<sup>14,23,28</sup> From these low site density CNTs, NEEs consisting of millions of nanoelectrodes (with each electrode being less than 100 nm in diameter) were successfully fabricated. Because the total current of the loosely packed electrode ensembles is proportional to the total number of individual electrodes, the number of electrodes totaling in the millions is highly desirable. The size reduction of each individual electrode and the increased total number of electrodes result in improvements in both the signal-to-noise ratio (S/N) and detection limits.<sup>24,25,27,28</sup> The CNTs were directly grown on the conductive substrate to ensure good electric conductivity. This approach is based on the advantages of CNT materials over conventional macroelectrodes such as increased mass transport and the decreased influence of the solution resistance, which will provide an excellent electrochemical transducer in biosensor applications. The NEEs have more practical value as ultrasensitive electrochemical sensors for chemical and biological sensing. In this paper, we will continue our preliminary report on NEEs based on aligned CNTs and describe their application in the development of a mediator-free and membrane-free glucose biosensor.

The fabrication of CNT nanoelectrode ensembles has been described in previous reports.<sup>14,26</sup> Briefly, Ni nanoparticles were electrodeposited on a Cr-coated Si substrate of 1 cm<sup>2</sup> area; low site density aligned CNT arrays were then grown from those Ni nanoparticles by plasma-enhanced chemical vapor deposition. An Epon 828 epoxy-based polymer with an MPDA curing agent was spin-coated on the substrate and covered half of the CNTs. The protruding parts of the CNTs were removed by polishing.

The enzymes were attached to the broken tips of the CNTs using standard water-soluble coupling agents 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and *N*-hydroxysulfo-succinimide (sulfo-NHS) by forming amide linkages between their amine residues and carboxylic acid groups on the CNT tips<sup>19,29</sup> (Figure 1). The CNT electrodes were pretreated in 1.0 M NaOH at 1.5 V for 90 s. After electrochemical treatment, some functional groups (e.g., carboxylic acid) were created at the CNT tips. The activated CNT NEEs were then immersed in a freshly prepared 10-mL aqueous solution of EDC (10 mg/mL). With stirring, 300 mg of sulfo-NHS was added to the solution. The pH of the solution was adjusted to 7. The reaction was allowed to occur at room temperature for 2 h. Then, the NEE electrode



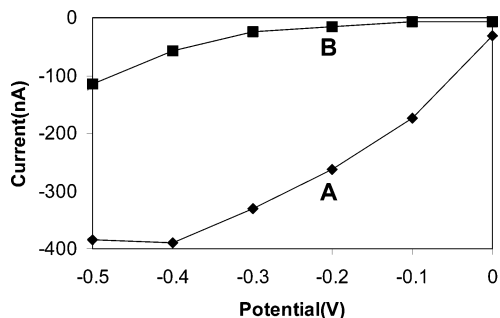
**Figure 1.** Fabrication of a glucose biosensor based on CNT nanoelectrode ensembles: (A) Electrochemical treatment of the CNT NEEs for functionalization (B) Coupling of the enzyme (GOx) to the functionalized CNT NEEs.



**Figure 2.** Cyclic voltammogram of the CNT nanoelectrode ensemble. The curves were taken in a solution of 4 mM K<sub>3</sub>Fe(CN)<sub>6</sub> in 0.5 M KNO<sub>3</sub> at a 100 mV/s scan rate.

was washed quickly with cold water and immediately immersed into a degassed solution (10 mL) with the desired amount of glucose oxidase (GOx) (2 mg/mL) in a 0.1 M, pH 7.4 phosphate buffer solution (PBS) with stirring. The enzyme immobilization reaction was allowed to occur at room temperature for 2 h. The resultant NEE biosensor was washed with a 0.1 M phosphate buffer solution (pH 7.4) containing 0.5% BSA and stored at 4 °C before use.

All experiments were performed using a hand-held electrochemical detector (CHI Instruments, Inc., CHI 1232) connected to a portable computer. The amperometric response of the glucose biosensor based on CNT NEEs to glucose was recorded under steady-state conditions in a 0.1 M phosphate buffer (pH 7.4) by applying a desired potential (for interference experiments, +0.4 and -0.2 V; for the calibration experiment, -0.2 V) to the biosensor. The amperometric experiment was performed in a standard single-



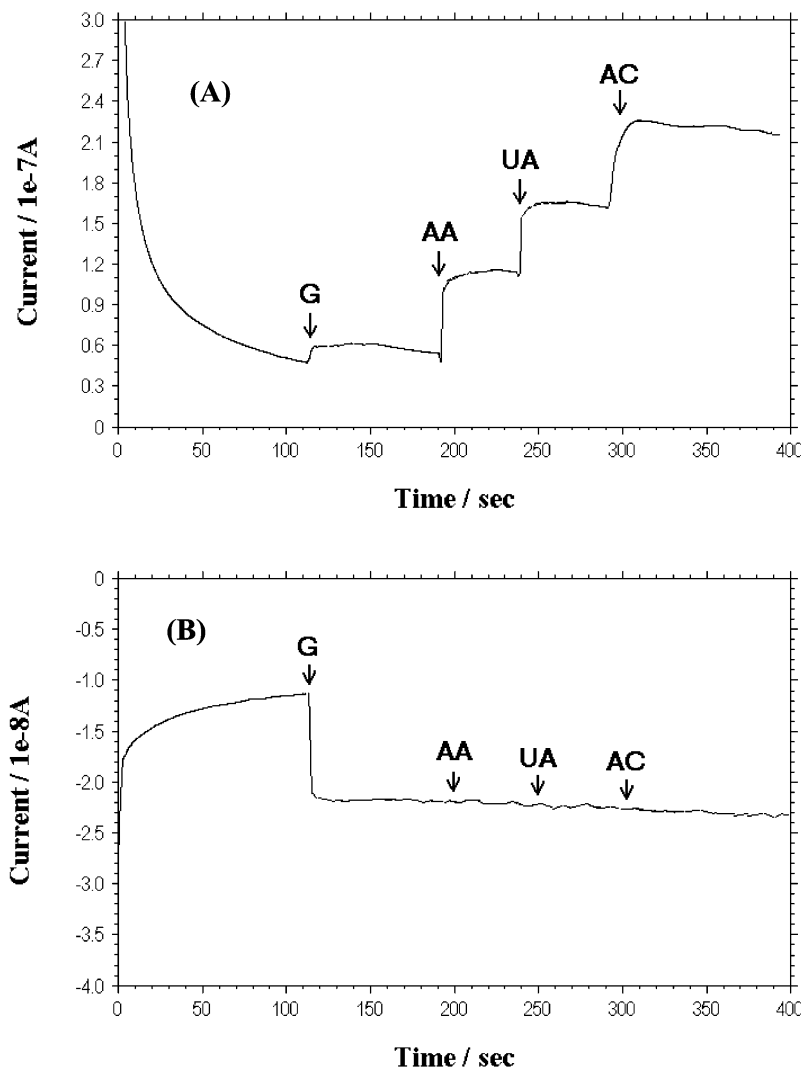
**Figure 3.** Hydrodynamic voltammograms for 1 mM hydrogen peroxide at a CNT NEE (A) and at a glassy carbon electrode (B). The electrolyte and 0.1 M phosphate buffer (pH 7.4) were stirred at 300 rpm.

compartment electrochemical cell that contained an NEE electrode, an Ag/AgCl reference electrode, and a platinum wire auxiliary electrode. The background response of the NEE biosensor was allowed to decay to a steady state with stirring. When the background current became stable, a solution of glucose was injected into the electrolytic cell, and its response was measured.

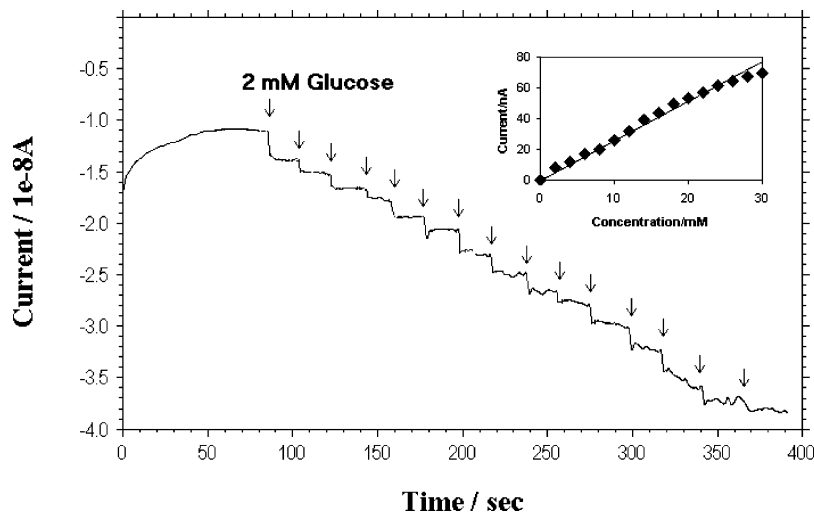
Figure 2 shows the cyclic voltammogram measured with an NEE electrode, with the sigmoidal shape indicating nanoelectrode behavior. This indicates that there is no diffusion layer overlapping between the nanoelectrodes because most of the CNTs are separated from their nearest neighbors by at least 5  $\mu\text{m}$ , which is much larger than the diameter of each nanotube (50 to 80 nm). Very low background current and leakage current are the result of the excellent sealing provided by the spin-coated Epon epoxy resin, which enables the sensitive analysis.

The CNT nanoelectrodes have a strong catalytic effect on reduction of hydrogen peroxide. To compare and verify the selectivity of the NEE, a series of experiments were performed to determine the electrochemical response of the NEE and normal glassy carbon electrode. Amperometric responses of the NEE and glassy carbon electrode were measured in a 1 mM hydrogen peroxide solution over a potential range of 0 to  $-0.5$  V.

Figure 3 illustrates a comparison of hydrodynamic voltammograms for 1 mM hydrogen peroxide at the NEE (A) and at a normal glassy carbon electrode (B). The normal glassy carbon electrode generated only small cathodic responses,



**Figure 4.** Amperometric responses of the NEE glucose biosensor to glucose (G), ascorbic acid (AA), uric acid (UA), and acetaminophen (AC) at potentials of  $+0.4$  V (A) and  $-0.2$  V (B). Other conditions are the same as those in Figure 3.



**Figure 5.** Amperometric responses of a NEE biosensor to successive additions of 2 mM glucose. Other conditions are the same as those in Figure 3.

whereas the CNT NEE generated a significant catalytic reduction current. Figure 3 shows that the range of operation potential for the NEE is relatively broad. In this study, an optimal potential region (0 to  $-0.20$  V) was chosen. At such a low applied potential, the responses of common interference species can be minimized, and the oxygen reduction current can be limited.

The selectivity advantage accrued from the hydrodynamic voltammograms is demonstrated in Figure 4, which compares amperometric responses for relevant physiological levels of glucose, ascorbic acid, acetaminophen, and uric acid at the GOx-modified NEE at potentials of  $+0.4$  and  $-0.2$  V, respectively. Amperometric responses were obtained by a batch addition of interfering species (0.5 mM ascorbic acid (AA), 0.5 mM uric acid (UA), and 0.5 mM acetaminophen (AC)) after the 5 mM glucose addition (G) at two different potentials ( $+0.40$  (A) and  $-0.2$  (B) V). Well-defined cathodic and anodic glucose responses were obtained at the NEE biosensor at potentials of  $+0.4$  and  $-0.2$  V. At an operating potential of  $+0.40$  V, the glucose response is overlapped by large anodic contributions from ascorbic acid, uric acid, and acetaminophen. The use of a lower operating potential greatly reduces these contributions. No interference was observed at a potential of  $-0.20$  V for the interference species, indicating high selectivity toward the glucose substrate. We emphasize that such a highly selective response to glucose is obtained at the NEE biosensor without the use of mediators and permselective membranes.

Cyclic voltammetric experiments indicate that the oxidation of the interfering species at the NEE starts at about  $+0.20$  V (ascorbic acid) and  $+0.30$  V (acetaminophen, uric acid), with no reduction up to  $-0.2$  V (not shown).

The amperometric responses at the NEE glucose biosensor for each successive addition of  $2 \times 10^{-3}$  M glucose are presented in Figure 5; the inset is the calibration curve. Well-defined current responses for glucose were obtained at the NEE biosensor. The reaction occurring at the biosensor is very fast in reaching a dynamic equilibrium upon each addition of the sample solution, generating a steady-state

current signal within 20 to 30 s. The linear response of the glucose biosensor to glucose is up to about 30 mM of glucose, which is higher than the 15 mM required for practical use in the detection of blood glucose. The signal response curve is effective at low detection limits for glucose because of favorable signal-to-noise ratio characteristics at  $-0.2$  V. The limit of detection, based on a signal-to-noise ratio of 3, was 0.08 mM.

In summary, we have demonstrated a new approach for the fabrication of glucose biosensors based on CNT NEEs for the selective and sensitive detection of glucose. CNT NEEs eliminate potential interference through the preferential detection of hydrogen peroxide. Such development of interference-free transducers should simplify the design and fabrication of conventional and miniaturized sensing probes. The glucose biosensor based on an aligned CNT NEE is thus suitable for the highly selective detection of glucose in a variety of biological fluids (e.g., saliva, sweat, urine, and serum). The biosensor fabrication technology demonstrated in this work is readily applicable to the fabrication of other biosensors based on oxidases, such as biosensors for cholesterol, alcohol, lactate, acetylcholine, choline, hypoxanthine, and xanthine.

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