A Glucose Biosensor Based on Deposition of Glucose Oxidase onto Crystalline Gold Nanoparticle Modified Carbon Nanotube Electrode

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A new amperometric biosensor, based on deposition of glucose oxidase (GOD) onto crystalline gold (Au) nanoparticle modified multiwalled carbon nanotube (MWNT) electrode, is presented. MWNTs have been synthesized by catalytic chemical vapor decomposition of acetylene over rare-earth-based AB_2 ($DyNi_2$) alloy hydride catalyst. Purified MWNTs have been decorated with nanocrystalline Au metal clusters using a simple chemical reduction method. The characterization of metal-decorated CNTs has been done using X-ray diffraction analysis, scanning electron microscopy, transmission electron microscopy (TEM), high-resolution TEM, and energy-dispersive X-ray analysis. Amperometric biosensor fabricated by depositing GOD over Nafion-solubilized Au−MWNT electrode retains its biocatalytic activity and offers fast and sensitive glucose quantification. The performance of the biosensor has been studied using cyclic voltammetry, amperometry, and hydrodynamic voltammetry, and the results have been discussed. The fabricated glucose biosensor exhibits a linear response up to 22 mM glucose and a detection limit of 20 μM.

1. Introduction

The physical and chemical properties of the materials used in the construction of biosensors have got significant influence on their performance. Highly sensitive and selective enzyme-based biosensors are used for the detection and quantification of various components present in the biological systems.[1] Highly sensitive enzyme-based biosensors can be fabricated by the incorporation of enzymes with a suitable electrochemical transducer. For retaining their bioactivities and for obtaining their direct electrochemical reactions, these enzymes should be immobilized on the electrode surface.[2] Enzyme immobilization can be attained by a variety of processes such as entrapment techniques,[3] electrochemical copolymerization,[4] covalent or cross-linking,[5] and adsorption.[6]

There are a variety of materials that can be used as electrochemical transducers. Commonly employed electrochemical transducers are either inert metals, such as platinum or gold, or carbonaceous materials.[1,2] The carbonaceous materials include graphite, carbon fibers, porous carbon, carbon spheres, glassy carbon, and carbon nanotubes (CNTs). All these materials help for easy enzyme immobilization, and they possess reproducible electrochemical behavior and useful physical properties.

Glassy carbon electrode (GCE) has been well-established as a biosensor immobilization matrix.[7] Because of its high porosity and low background current over a wide potential range, it can be used as an electrochemical transducer. It is an ideal electrode for the adsorption of large molecules. This electrode material is a mechanically stable compact solid that is impermeable to gases and fluids,[8] but one major disadvantage of the GCE when used in biosensor design is its low sensitivity to peroxide, as well as to other mediators. This can be overcome by introducing oxygen surface groups into the GCE.

Ever since their discovery by Iijima,[9] CNTs have attracted considerable research interest owing to their unique physical and chemical properties. CNTs possess an excellent electron transfer rate, much better than conventional carbon electrodes, and also allows surface chemistry for tethering foreign biomaterials such as enzymes and nucleic acids.[2] Composite materials can be prepared by attaching foreign molecules to the functional groups present in CNTs. We can fabricate efficient biosensors by attaching specific enzymes with CNTs.

For the treatment and control of diabetes, the amount of blood glucose has to be monitored. For this reason, the glucose biosensor is the most extensively studied among the different types of enzyme-based biosensors.[2] In most of the glucose biosensors, glucose oxidase (GOD) is employed as the enzyme as it is of practical use, stable, and inexpensive. GOD from *Aspergillus* is a homodimer containing two tightly bound flavine adenine dinucleotide (FAD) cofactors.[10] It catalyzes the electron transfer from glucose to oxygen accompanying the production of gluconolactone and hydrogen peroxide. This can be represented as follows

$$\text{Glucose} + O_2 \xrightarrow{\text{GOD}} \text{gluconolactone} + H_2O_2$$

From the electrochemical detection of the enzymatically liberated H_2O_2, the quantification of glucose can be achieved. However, the overvoltage necessary for the oxidation or reduction of H_2O_2 at solid electrodes is rather high. Therefore, the modification of the electrode surface is carried out such that the H_2O_2 oxidation/reduction overvoltage is considerably lowered. This can be achieved by the deposition of nanocrystalline metal clusters of noble metals such as palladium, platinum, gold (Au), copper, and iridium on to the surface of the electrochemical transducer—the MWNT.[11−13] Enhancement of mass trans-

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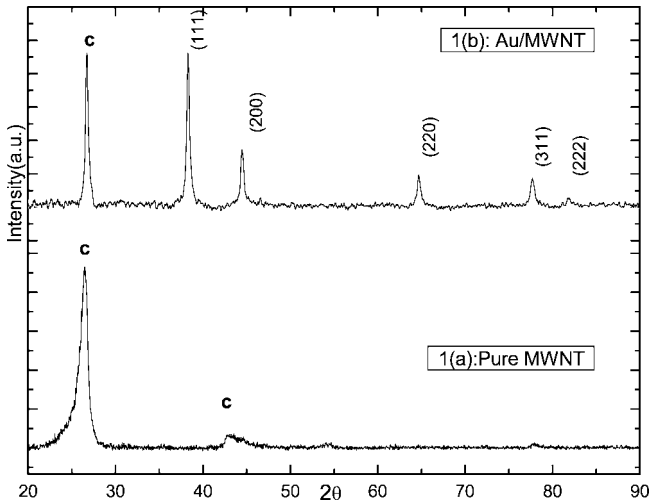

Figure 1. Powder X-ray diffractogram of (a) purified CNTs obtained from $DyNi_2$ alloy hydride and (b) Au−MWNT.

port, catalysis, high effective surface area, and control over electrode micro environment are some advantages displayed by the metal nanoparticles when used for electroanalysis. Lim et al., reported the fabrication of a glucose biosensor based on electrodeposition of palladium nanoparticles and glucose oxidase onto Nafion-solubilized CNT electrode and the fabricated Pd-GOD-Nafion CNT glucose biosensor, which exhibited a linear response up to 12 mM glucose with 1000 U of GOD and a detection limit of 0.15 mM[2].

Among the different noble metals, Au nanoparticles have attracted significant attention in biosensors research over the past few years as they allow proteins to retain their biological activity upon adsorption.[14] Moreover, Au nanoparticles are able to reduce the insulating effect of the protein shell for direct electron transfer.[15] Therefore, Au−CNTs hybrids will constitute biocompatible materials with important electroanalytical features. Various research efforts have been made to attach gold nanoparticles to carbon nanotubes. Even then, there is a lack on reports regarding the performance and applications of such hybrid materials. Very few biosensors using gold nanoparticles−CNT materials can be found in the literature.[16] Wu et al. in 2007 fabricated an amperometric glucose biosensor based on multilayer films prepared via layer-by-layer self-assembly of mutiwalled nanotubes (MWNT), Au nanoparticles, and GOD on the Pt electrode with a wide linear range of 0.1−10 mM glucose and a sensitivity of 2.527 μA/mM,[17] but in that case, the method of fabrication is highly complicated, and due to layer-by-layer deposition, adhesion between MWNT and Au particles is minimal. This can be overcome by directly attaching the Au nanoparticles on MWNT. Therefore, it will be interesting to investigate the performance of a glucose biosensor based on Au−MWNT composite with a lower amount of GOD.

In this paper, we report for the first time the fabrication, characterization, and analytical performance of a glucose biosensor based on the deposition of only 32 units of GOD onto crystalline Au nanoparticle modified MWNT electrode by a very simple technique. MWNTs have been synthesized by catalytic chemical vapor decomposition (CCVD) of acetylene over rare earth (RE)-based AB_2 ($DyNi_2$) alloy hydride catalyst. Purified MWNT have been decorated with nanocrystalline Au metal clusters using a simple chemical reduction method. An amperometric biosensor has been fabricated by the deposition of 32 units of glucose oxidase (GOD) over a Nafion-solubilized Au−MWNT electrode. The performance of the biosensor has been studied using cyclic voltammetry, amperometry, and hydrodynamic voltammetry, and the results have been discussed.

2. Experimental Methods

2.1. Reagents. GOD (EC 1.1.3.4, *Aspergillus niger*, >100 U/mg) was purchased from Alfa Aesar and used as received. MWNT were synthesized in our laboratory by chemical vapor deposition technique as described below. $HAuCl_4 \cdot 3H_2O$ was also obtained from Alfa Aesar. D-(+)-Glucose was purchased from Sigma, and the glucose stock solution was allowed to mutarotate for 24 h at room temperature prior to use and subsequently store at 4 °C. The supporting electrolyte was 0.1 M phosphate buffer at pH 7, unless otherwise stated. One hundred millimolar phosphate buffer was prepared by mixing stock standard solution of K_2HPO_4 and KH_2PO_4, and the pH was adjusted with KOH. The common chemicals used for preparation of buffers, etc., were of analytical reagent grade. All of the solutions were prepared with deionized distilled (DD) water.

2.2. Instruments. The electrochemical measurements were performed with CH Instruments CHI 608C Electrochemical Analyzer/Workstation. A Pt wire counterelectrode, Ag/AgCl (3 M KCl) reference electrode, and glassy carbon electrode (GCE, diameter 3 mm) were inserted into a modified 5−10 mL glass cell (Model CHI-222) for the measurement. All potentials are referred to the Ag/AgCl reference electrode. A magnetic stirrer provided the convective transport at 300 rpm during the amperometric measurements, and the background current was allowed to decay to a steady-state value before spiking the equilibrated β-D-glucose. The powder X-ray diffraction (XRD) patterns were obtained using an X'pert PRO, PANalytical diffractometer with nickel-filtered Cu Kα radiation under ambient air and scanning in the 2θ range of 15−90°, in steps of 0.05°. The TEM images were obtained on a transmission electron microscope (TEM, JEOL JEM-2010F), and the SEM images were taken with a scanning electron microscope (FEI; QUANTA scanning electron microscope).

2.3. Synthesis of MWNT. MWNTs were synthesized by the decomposition of acetylene over RE-based AB_2 ($DyNi_2$) alloy hydride powders using a fixed-bed catalytic reactor as discussed in previous work.[18] The as-prepared MWNT were purified by air oxidation followed by acid treatment. The crystallinity and purity of the samples were verified by XRD (Cu Kα radiation) and thermogravimetric measurements (20 °C/min). These techniques have been explained in detail in our previous work.[18] The samples were characterized using SEM and TEM.

2.4. Preparation of Au−MWNT Composite. In order to decorate the purified MWNT with nanocrystalline Au clusters, a chemical reduction method was used. Nearly 0.02 g of purified MWNTs were treated with 0.075 M $HAuCl_4 \cdot 3H_2O$ followed by magnetic stirring for 12 h. Au salt is reduced by adding a reducing solution, which is a mixture of 0.1 M $NaBH_4$ and 1 M NaOH, during stirring. After the reaction is over, the solution is washed three times with deionized water and filtered using cellulose membrane filters having a pore size of 0.1 μm. The material left out is collected and dried in a vacuum oven at 80 °C for 2 h. The sample was then characterized using XRD, SEM, and energy dispersive analysis of X-rays (EDAX).

2.5. Fabrication of GCE/GOD/Au-MWNT/Nafion Electrode. Before electrode modification, the bare GCE was polished with 0.05 μm alumina slurry, sonicated in deionized water, and dried with a high-purity nitrogen stream to obtain a mirror surface. The Au−MWNTs were sonicated in 0.5% Nafion solution to give a concentration of ~1 mg/ml. Four microliters of the CNT suspension was film-cast onto the surface of the

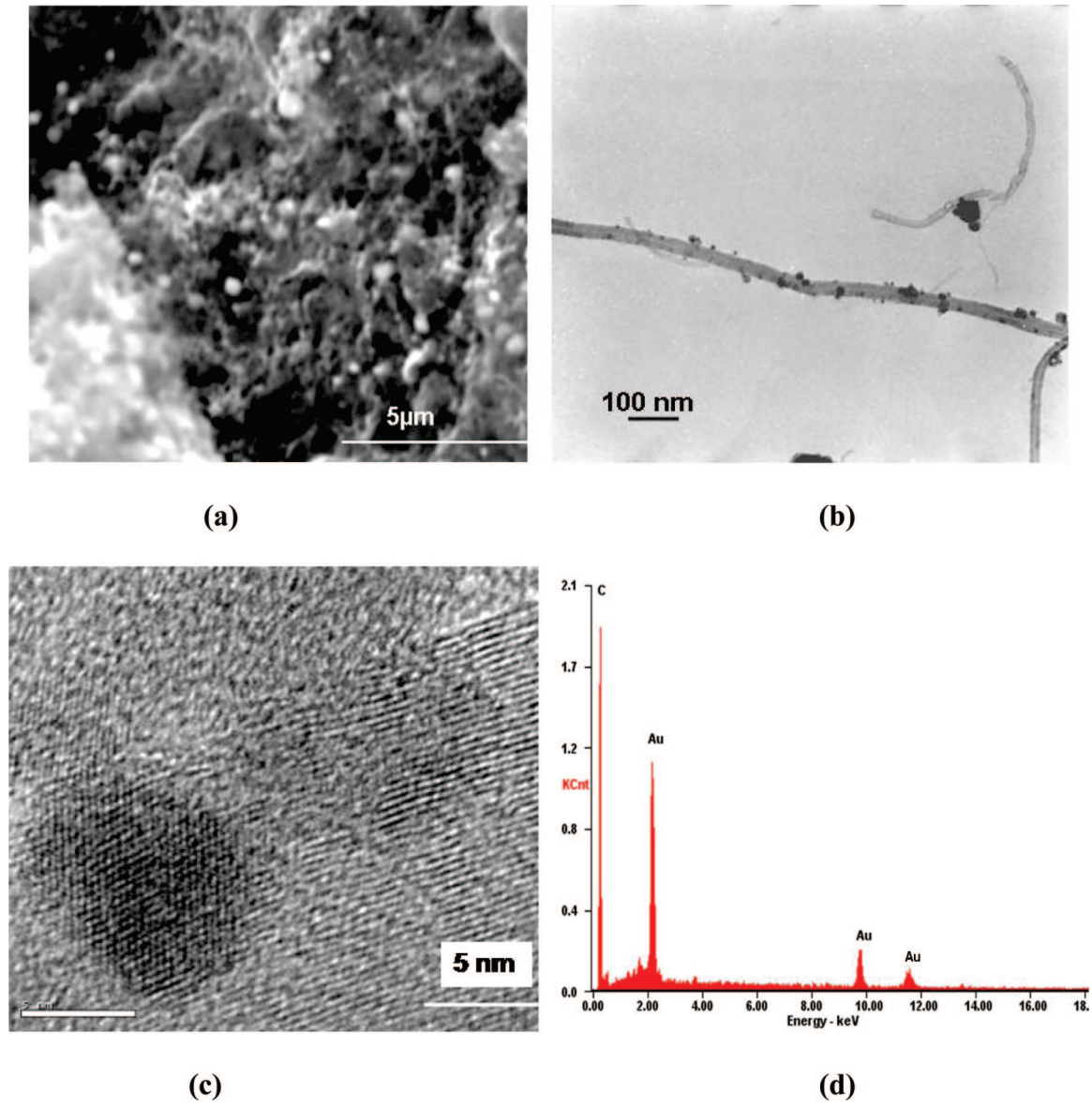

Figure 2. (a) SEM, (b) TEM, (c) HRTEM images, and (d) EDAX pattern of Au−MWNT.

GCE and allowed to dry slowly. Films formed from Nafion-solubilized MWNT are more uniform and stable than those cast from organic solvents. Nafion assists the dispersion of MWNT, whereby the MWNT remain well-dispersed on prolonged standing. A 4 μL portion of 32 U GOD solution was film-cast onto the surface of the GCE/GOD/Au−MWNT/Nafion electrode and allowed to dry slowly at 4 °C. The obtained GOD/Au−MWNT/Nafion electrode was washed carefully with double-distilled water and dried at less than 4 °C. These GCE/Au−MWNT/GOD/Nafion bioelectrodes were coated with an extra 2.5 μL layer of 0.5% Nafion. The electrodes were rinsed with pH 7 buffer and stored in the buffer at 4 °C prior to use.

3. Results and Discussions

3.1. Morphology and Characterization of Au-MWNT used in the Fabrication of Bioelectrode. Figure 1a shows the XRD pattern of purified MWNT using alloy hydrides as catalysts. The peaks are indexed to the reflections of hexagonal graphite. The absence of additional peaks corresponding to the catalytic impurities shows that the impurities have been removed by the acid treatment. The XRD pattern of Au−MWNT nanocomposite material (Figure 1b) shows the reflections of Au along with that for graphitic carbon. The broad peaks reveal the presence of nanostructured crystalline gold particles.

SEM, TEM, and HRTEM images of Au/MWNT are shown in Figure 2(a)−2(c), respectively. SEM (FEI; QUANTA scanning electron microscope) and TEM (JEOL, JEM-3010 electron microscope with an acceleration voltage of 200 KV) images of Au/MWNT reveal uniform distribution of Au nanoparticles throughout the surface of MWNT having an outer diameter of about 30 nm and an inner diameter of about 10 nm. The crystalline nature of the metal particles having average particle size in the range of 5−8 nm dispersed on the MWNT surface is clearly seen from the HRTEM image. The EDAX pattern shown in Figure 2d indicates nearly 20 wt % loading of gold nanoparticles on the surface of MWNTs, and for that the carbon peak has been used as the reference. The EDAX measurement helps only in ensuring the presence of the particular metal on the surface of the MWNT and it gives only its local concentration. The value of electrical conductivity and the inherent purity of MWNT make them excellent candidates for electrochemical application.

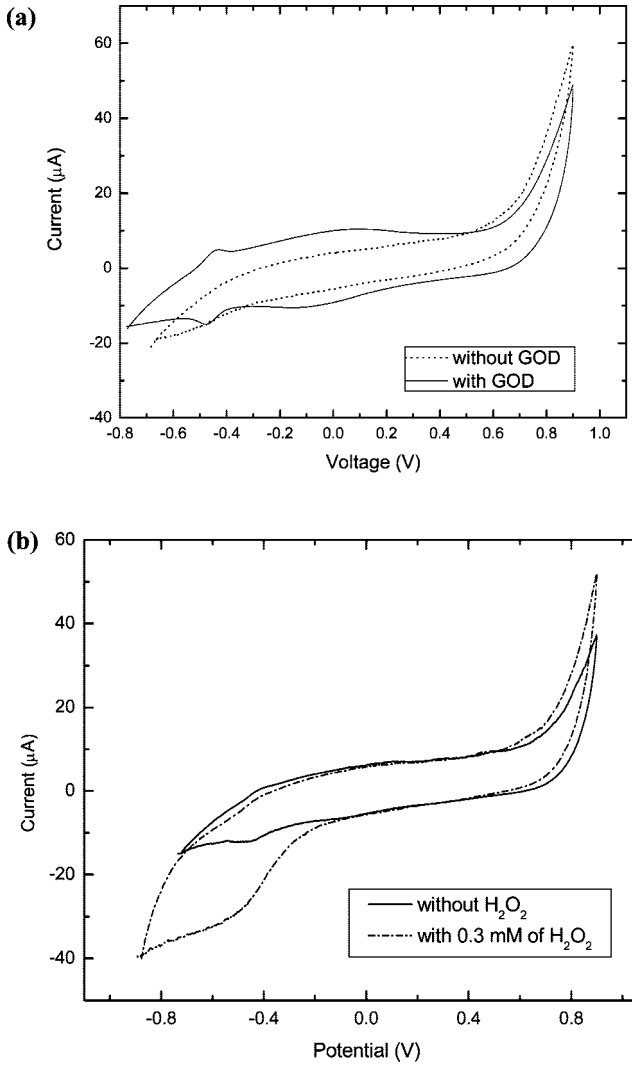

Figure 3. Cyclic voltammographs of (a) GOD/Au−MWNT/Nafion bioelectrode (solid line) and Au−MWNT/Nafion electrode (dotted line) in phosphate buffer solution on the addition of 15 mM of glucose and (b) GOD/Au−MWNT/Nafion bioelectrode before (solid line) and after (dotted line) adding 0.3 mM of H_2O_2 at a sweep rate of 25mV s^{-1}.

3.2. Voltammetric Characterization of GOD/Au-MWNT/Nafion Modified Glassy Carbon Electrode. The application of Nafion-solubilized MWNTs for electrochemical biosensor was exploited by immobilizing GOD in a Nafion-solubilized MWNT film. Cyclic voltammetry is a useful tool for electrochemical evaluation of the transducers. A 0.1 M phosphate buffer at pH 7 was used as a probe to investigate performance of the fabricated glucose bioelectrode. The cyclic voltammetric (CV) responses of the Au−MWNT/Nafion electrode and GOD/Au−MWNT/Nafion electrode toward the enzymatically liberated H_2O_2 were simulated by adding 15 mM glucose at pH 7 buffer, and their performances are illustrated in Figure 3a. The Au−MWNT/Nafion electrode did not exhibit any oxidation or reduction peaks upon the addition of 15 mM glucose. The electrode oxidation and reduction signals were better defined in the presence of GOD. This catalytic effect is attributed to the presence of GOD in the fabricated bioelectrode.

The cyclic voltammetric (CV) response of the fabricated GOD/Au−MWNT/Nafion electrode toward the addition of H_2O_2 to pH 7 buffer is illustrated as Figure 3b. A minor reduction peak was observed at −0.5V at the GOD/Au−MWNT/Nafion modified glassy carbon electrode in the absence of H_2O_2. There is a significant change in the reduction peak due to the addition of H_2O_2. In the presence of H_2O_2, the reduction current obtained at the fabricated bioelectrode was over 5-fold greater than in the first case, but in both cases oxidation peaks are not prominent. This catalytic effect is attributed to the presence of Au nanoparticles, and one can not exclude the possibility that the Nafion-solubilized MWNT itself contributes to the enhanced detection toward H_2O_2. The large distinguishable response toward the detection of H_2O_2 indicates that the charge transport within the composite films is relatively fast, which can be attributed to remarkable electronic properties of Au/MWNT.

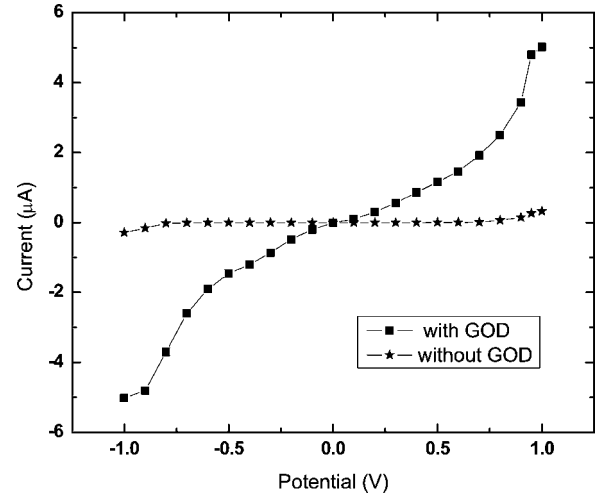

Figure 4. Hydrodynamics voltammographs of Au-MWNT/Nafion electrode in 15 mM glucose in the presence or absence of GOD.

The hydrodynamic voltammograms (HDV) for 15 mM glucose at GOD/Au-MWNT/Nafion electrode is displayed in Figure 4. For the fabricated bioelectrode, the oxidation of the enzymatically formed H_2O_2 starts at potentials more positive than +0.2V and attains saturation above +0.8V. This indicates that the fabricated biosensor has a flexible operating potential range for the monitoring of the oxidation/reduction of H_2O_2. In the absence of GOD, the electrodeposited GOD/Au−MWNT/Nafion electrode is almost amperometrically insensitive to the presence of glucose, except at higher potentials (> +0.7 V) at which direct oxidation of glucose might take place. Hence, the response of the GOD/Au−MWNT/Nafion electrode is due to the entrapped GOD enzymes.

3.3. Determination of Glucose Using Fabricated GOD/Au−MWNT/Nafion Electrode. The typical amperometric $i-t$ curves for the fabricated GOD/Au−MWNT/Nafion electrode at a constant voltage of +0.3 V is shown in Figure 5. A 0.1 M phosphate buffer at pH 7 has been used as the supporting electrolyte, and the amperometric response of the fabricated bioelectrode toward the detection of glucose has been carefully investigated by increasing the concentration of glucose in phosphate buffer solution systematically. An increase in current with the increase in the concentration of glucose has been observed, which indicates the highly sensitive nature of the fabricated bioelectrode to the concentration of glucose in the solution. In each case for a particular glucose concentration, current value stabilized after a certain period of time. The average steady state time has been found to be 25 s, indicating the fast response nature of the fabricated bioelectrode toward the detection of glucose. The steady-state calibration curve (Figure 6) has been drawn using the steady-state current values corresponding to different glucose concentrations. It exhibits a

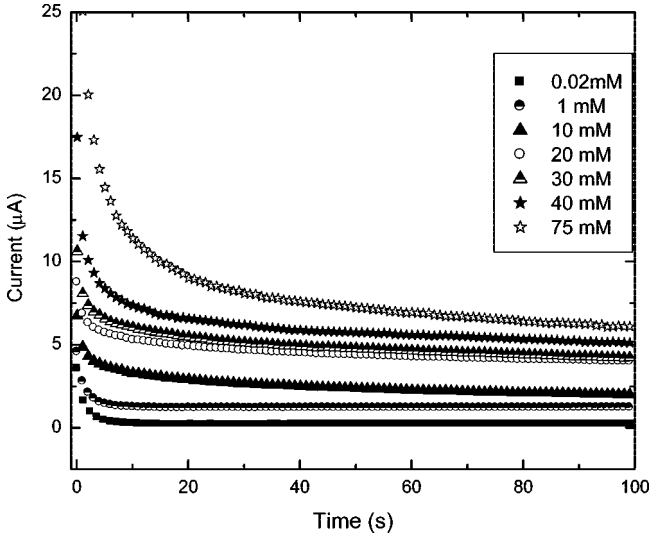

Figure 5. Amperometric $i-t$ curves of the fabricated GOD/Au−MWNT/Nafion electrode for different glucose concentrations.

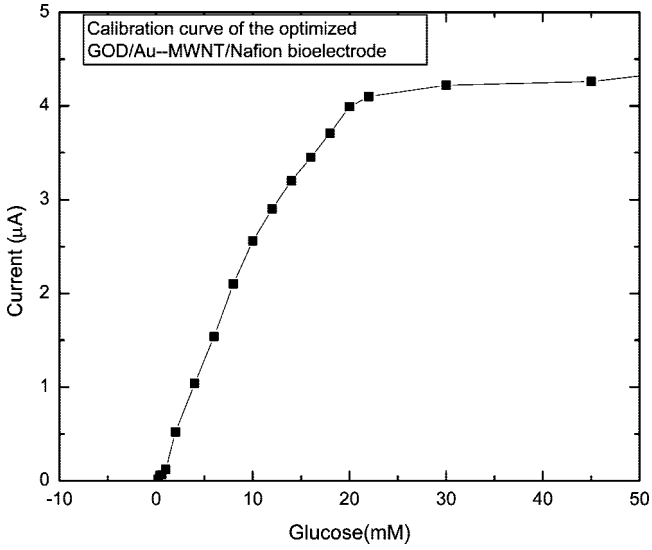

Figure 6. Calibration curve of the fabricated GOD/Au−MWNT/Nafion bioelectrode.

linear range from 0.05 to 22 mM with the detection limit being 20 μM. The fabricated biosensor exhibited a sensitivity of 0.4 μA/mM.

Therefore, the fabricated biosensors exhibits better performance than the Pd-GOD-Nafion CNT glucose biosensor electrode of Lim et al.[2] with a lower amount of GOD loading. The fabrication method is much simpler and equally effective as compared to that of Wu et al.[17]

4. Conclusions

Chemical reduction method is a simple and effective technique to deposit nanocrystalline Au metal particles on the surface of MWNT. With the immobilization of 32 U of GOD on the Au-MWNT/Nafion film, a novel glucose biosensor was fabricated by a simple deposition technique and exhibited many advantages at a low applied potential, such as high sensitivity, low detection limit, good reproducibility, long-term stability, and fast current response. A more controllable, stable, and reproducible deposition of Au−MWNT film onto GCE can be achieved using a homogeneous solution of (0.5%) Nafion/Au−MWNT. The fabricated GOD/Au−MWNT/Nafion electrode has a good glucose-biosensing capability, and it exhibits a linear response up to 22 mM glucose and a detection limit of 20 μM.

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