BIOLOGICAL SENSORS USING DNA FUNCTIONALIZED MULTIWALLED CARBON NANOTUBES

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ABSTRACT

In the present work, we report the electrochemical detecting behavior of MWNT functionalized with single strand DNA. MWNT have been synthesized by catalytic chemical vapour deposition of acetylene over Mm based AB_3 alloy hydride catalyst prepared by hydrogen decripitation technique. Purified MWNT have further been functionalized with aqua regia for better dispersion and to improve its combining capacity with the DNA. Special machine grade DNA [AC] 15 was used for functionalization of MWNT by a sonication process. Structural and morphological characterizations have been carried out using SEM, TEM, HRTEM and FT-IR spectroscopy. Electrochemical performance studies have been carried out using potassium ferro cyanide as the redox electrolyte. Cyclic voltammetry gave well-defined quasi-reversible voltammetric responses, flat and wide potential window, and quick electron transfer showing that DNA-MWNT film is a good electrochemical sensor. Further, we demonstrate highly selective and sensitive detection of a low concentration of dopamine in the presence of excess ascorbic acid.

Keywords: Multi Walled Carbon Nanotubes; DNA, Biological sensor

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14. ABSTRACT

In the first part of this work, electrochemical detecting behavior of MWNT functionalized with single strand DNA was studied. The MWNT was synthesized as described in the report. Purified MWNT was functionalized with aqua regia for dispersion improve combining capacity with the DNA. Special machine grade DNA [AC] 15 was used for functional- ization of MWNT by sonication. Characterizations have been carried out using various techniques. Cyclic voltammetry gave well-defined quasi-reversible voltametric responses, showing that DNA-MWNT film is a good electrochemical sensor. Further, we demonstrate highly selective and sensitive detection of a low concentration of dopamine. In the second part of this work, a new amperometric biosensor, based on deposition of glucose oxidase (GOD) on to crystalline gold (Au) nanoparticle modified multi walled carbon nanotube (MWNT) electrode, is presented.. Purified MWNTs were decorated with nanocrystalline Au metal clusters using a simple chemical reduction method. Amperometric biosensor fabricated by depositing glucose oxidase (GOD) over Nafion-solubilized Au/MWNT electrode retains its biocatalytic activity and offers fast and sensitive glucose quantification.

15. SUBJECT TERMS
Carbon nano tubes, Nanocomposites, Biosensors

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INTRODUCTION

Carbon nanotubes (CNTs) have come among one of the most major discoveries in the last twenty years [1]. The immense range of properties displayed by CNTs has resulted in it being the most heavily researched material in the world. Their unique physical and chemical properties allow them for uses in varied fields. Functionalization and solubilization of these CNTs have imparted them remarkable qualities which allow them to be used effectively in the bio-medical field [2-6]. DNA is one of the one dimensional fundamental molecules in biology. It is a naturally occurring polymer consisting of bases, sugar and phosphates. Fascinating properties of both the DNA molecule and the multi walled nanotubes (MWNT) have inspired scientists over the last few years to combine these two materials and study their properties. Various non-biological applications like covalent conjugation of DNA to oxidizing open ends of single walled nanotubes (SWNT) for self-assembled molecular scale electronic systems, non-covalent binding of DNA to side-walls of SWNT for dispersion [7] and separation of SWNT and DNA-templated CNTs field effect transistors [8]. Recently, there have been several attempts to use CNTs functionalized with DNA as electrode materials for enhanced electrochemical detection resulting in extra-sensitive bio-sensors which can detect the presence of minute concentrations of critical body fluids. Here, we present a novel, cost effective and easy method for the synthesis of MWNT in large quantities using Mischmetal (Bharat Rare Earths Metals, India; composition:- Ce 50%, La 35%, Pr 8 %, Nd 5%, Fe 0.5% and other rare earth elements 1.5%) based AB_3 alloy hydride catalyst, obtained through hydrogen decrepitation technique. Catalytic chemical vapour deposition (CCVD) technique using a single-stage furnace facility has been used to grow these nanostructures in the temperature range 500ºC to 700ºC. The as-grown and purified samples have been characterized using SEM, TEM, HRTEM and FT-IR spectroscopy. MWNT, after functionalization in acid solution, have been attached to the DNA molecules, which were found to be was found to be dispersed very well in solution. Electrochemical performance studies have been carried out using potassium ferro cyanide as the redox electrolyte and DNA-MWNT film electrode and results have been discussed. Further, we report the electrochemical voltammetric properties for DNA-MWNT film electrode, as a detector of low-concentration dopamine in the presence of excess ascorbic acid.

MATERIALS AND METHODS

Catalyst synthesis, Carbon nanotube synthesis and characterization

Mm based AB_3 alloys were prepared by arc melting the constituent elements in a stoichiometric ratio under argon atmosphere. The alloy buttons were re-melted six times by turning them upside down after each solidification to ensure homogeneity. Single-phase formation of alloys was confirmed by powder X-ray diffraction. Each of these alloys was then hydrogenated to their maximum storage capacity of about 1.5 wt % using a high pressure Seivert’s apparatus. Fine powders of alloys, with fresh surfaces and free from oxidation were obtained by several cycles of hydrogen absorption/desorption. The growth of carbon nanostructures has been carried out using a single-stage furnace with precisely controlled temperatures in the range 500 to 700°C. Fine powders of alloy obtained after several cycles of hydrogen absorption/desorption, were directly placed in a quartz boat and kept at the center of a quartz tube, which was placed inside a tubular furnace. Hydrogen (50 sccm) was introduced into the quartz tube for 1 h at 500°C, in order to remove the presence of any oxygen on the surface of the alloy hydride catalysts. Hydrogen flow was then stopped and furnace heated up to the desired growth temperature followed by the introduction of methane at a flow rate of 100 sccm. The pyrolysis was carried out for a duration of 30 min and there after the furnace was cooled to room temperature. Argon flow was maintained through out the experiment. The carbon soot obtained in the quartz boat was purified by air oxidation and acid treatment [9].

Preparation of ssDNA-MWNT

After obtaining the purified samples of MWNT, they were functionalized in an acid solution. 2 mg of MWNT were added to an aqua regia solution (3:1 ratio of sulphuric acid and nitric acid respectively) and functionalization process has been carried out in an ultra sonicator. The beaker was kept in an ice bath to maintain a temperature of about 25^{o}C. The sonication is carried out for around 10 h. This ensures proper dispersion of the MWNT and it also cuts down the length of the individual nanotubes. After the functionalization, the dispersed MWNT were filtered and dried. Single strand DNA was obtained from Bioserve Ltd. The sequence obtained was 5-ACACACACACACACACACACACACACACAC-3, since this disperses CNTs most efficiently [7]. The DNA solution is prepared by the addition of TE buffer to the lyophilized DNA obtained from Bioserve. The TE buffer composition is 10 mM Tris with 1 mM EDTA, pH 7.6. 1 ml of the solution consists of 1 mg of DNA. 1mg of the MWNT was taken and mixed it with 1ml of the (AC) 15 solution and the mixture was sonicated 1 h in a sonicator. The mixture was then centrifuged to remove any insoluble materials and the supernatant was filtered through a disk membrane. The pellet of DNA-MWNT on the membrane was washed with copious amounts of water to remove buffer components and unbound DNA. The Purified and DNA functionalized samples were then characterized by FT-IR, scanning electron microscopy (SEM), transmission electron microscopy (TEM) and high resolution transmission electron microscopy (HRTEM).

Electrode Preparation

A piranha solution (conc sulphuric acid/30 % hydrogen peroxide, volume ratio 7:3) was prepared for cleaning the glass plate. The glass plate was treated with this solution at 95 ^{o}C for 20 min. Then an aqueous solution of the DNA-MWNT was prepared and the solution was applied on the prepared glass plate. It was dried on a heat block and the process has been repeated several times. The electrode was prepared by affixing a copper wire to the glass plate by silver paste and then it was partly mounted by epoxy resin. A small portion in the middle was left bare to measure the voltammetric properties of electrode and to detect dopamine. Cyclic voltammetry was performed in the standard three-electrode system including the DNA-MWNT working electrode, a platinum counter electrode and an SCE reference electrode. The data was recorded with a potentiostat/galvanostat interfaced with a computer. Al the experiments were conducted at room temperature.

RESULTS AND DISCUSSION

Figure 1 (a-c) show the SEM, TEM and HRTEM images of purified MWNT respectively. From these figures it is clear that good quality MWNT have been obtained by CCVD technique using Mm based AB_3 alloy hydride catalyst. Further, HRTEM image reveals the multiwalled nature of carbon nanotubes with each graphene layer being clearly distinguishable since the graphene sheets with a spacing is ~0.34 nm are stacked parallel to the growth axis of carbon nanotubes. The purity of MWNT is found to be of 95 % from thermo gravimetric analysis (TGA).

The FTIR spectrum of acid treated MWNT in the range 700 to 4000 cm^{-1} is shown in Figure 2 (a). A broad absorption band at 3437 cm^{-1} is attributed due to the hydroxyl group (νOH) [10, 11]. This band might have resulted due to water νOH and δH_2O [12] and also the –OH functional groups resulting due to the chemical treatment during the purification process and functionalization process respectively [13]. Bands at 2927 and 2853 cm^{-1} are due to asymmetric and symmetric stretching of CH stretching. A small peak at 1734 cm^{-1} is associated with the C=O stretching of the carboxylic acid (–COOH) group [14]. The peak at 1639 cm^{-1} is due to C=C stretching of the CNTs [15]. The peak at 1384 cm^{-1} is due to O–H bending deformation in –COOH.

A small peak at 1086 cm^{-1} (Figure 2 (b)) is assigned to C=O bond stretching [14]. For the DNA-wrapped MWNT sample, the peak at 1260 cm^{-1} refers to the CH_2-O-P-O phosphate group. The DNA-MWNT complex results in the intensity increase or the appearance of H-bonded O-H and N-H stretching vibration as well as an asymmetrical PO_2^- vibration at 1228 cm^{-1}, a sugar vibration at 880 cm^{-1}, the C=C vibration at 1651 cm^{-1}, and the C=O vibration at 1720 cm^{-1}, which indicate the helix interaction of DNA with MWNT. The EDAX data clearly confirms the presence of nitrogen, phosphorous and sulphur [16]. The origin of these elements can be attributed to the attachment of DNA molecule to the CNT. The FT-IR data clearly confirms the attachment of the DNA molecule to the MWNT.

DNA-MWNT molecules formed are extremely unique due to the fact that they are water dispersible and do not agglomerate, a property completely absent in MWNT (inset in Figure 3). The accompanying SEM photograph clearly shows how the MWNT is thoroughly dispersed and exists in individual nanotubes. The SEM and HRTEM images (Figure 3) show the wrapping of DNA around the individual tubes. We found that the DNA-MWNT hybrid could firmly attach onto a glass substrate and form a uniform film. This is due to the high dispersity of DNA-MWNT. After dispersion, the individual MWNT have a high surface to volume ratio and surface free energy, so they tend to interact with each other as well as the glass substrate to release some of the free energy while the water evaporates. The strains among any of the nanotubes to form the film are equivalent in all directions, since they are individually suspended in water. If the nanotubes are not individually suspended in water, the asymmetric strain from nanotubes will result in an uneven film, which cannot adhere tightly to the glass and will easily fall off when put into solution.

The potential window is one of the most important factors for an electroanalytic electrode, and well-performing electrodes can give a good signal-to-noise ratio with a flat and wide potential window. Redox pairs are usually used to probe the quality of the electrode by cyclic voltammetry [17, 18]. We chose $Fe(CN)_6{}^{4-}$/ $Fe(CN)_6{}^{3-}$ as redox pairs to investigate the electronic transfer properties of the DNA-MWNT electrode, for their voltammetric responses. In Figure 4, the cyclic voltammograms exhibit well-defined redox reaction at potentials of 0.290 and 0.205 V. The electron transfer speed at the interface between the electrode and the solution is faster than that of diffusion of the reactant. From all of the analyses above, we can conclude that the redox reaction of

$Fe(CN)_6^{4-}$/ $Fe(CN)_6^{3-}$ in 1 M KCl is a quasi-reversible reaction [19]. Another advantage we found is that there is no foul on the surface of the electrode, because voltammograms are reproducible without any special pretreatment before each experiment, except rinse with water. It is a very important property for a DNA-MWNT electrode to be used in electrochemical analysis, because the surface of bulk carbon electrodes can usually become deactivated as a function of time when they are exposed to the laboratory atmosphere or working solution. The problem will become more severe when a biological sample is used.

To explore the potential applications of the DNA-MWNT electrode in electrochemical detection, we used low-concentration dopamine (DA) to test its selectivity and sensitivity. DA is an important neurotransmitter and it coexists in the interstitial fluid in the brain with ascorbic acid (AA). The physiological concentration of AA is much higher than that of DA, and the oxidation potential of the AA is close to that of DA, making selective detection of DA a challenging task. Various modified traditional electrodes have been attempted [20, 21] to address this issue, such as glassy carbon electrode, graphite electrode, and noble metal electrode. However, these electrodes usually need complicated pretreatment and modification of the electrode surface chemistry to enhance selectivity and sensitivity. In addition, some of them tend to lose stability and selectivity during measurement, and some of them do not produce well resolved voltammograms [22]. Figure 5 shows results of voltammetric detection of DA using our DNA-MWNT electrode in 0.1 M phosphate buffer solution (PBS) of pH 7.0. Peaks for DA and AA can be clearly identified at 0.304 and 0.055 V respectively, with peak separation of 0.249 V from Figure 5. DNA is rich in active functional groups, such as amino groups, carbonyl groups, and carboxyl groups; these groups can act as media for electron transfer in redox reaction and catalyze the oxidation of DA and AA [23]. As a result, the two anodic peak potentials of DA and AA are well-separated, indicating high selectivity and sensitivity of the DNA-MWNT electrode.

The electron transfer of reactants during electro-oxidation is mainly determined by the conductivity of electrode material as well as the active functional groups on its surface. Owing to their excellent conductivity, metals (such as platinum and gold), glass carbon, and graphite are the materials usually used for electrodes. However, the number of active functional groups on surfaces of these materials is limited, as a result of limited exposed area on electrode surface.

For the DNA-MWNT electrode, though its conductivity is not as good as that of those materials mentioned above, it has much higher surface area, which can accommodate much more active functional groups in a given region. This will significantly enhance the rate of surface catalyzed reaction, which is proportional to surface coverage of functional groups [24]. For a DNA-MWNT electrode, the length of MWNT will also play a role in conductivity. Shorter MWNT will form more contacts and hence producing a higher contact resistively.

CONCLUSION

Wrapping ssDNA on MWNT yields a well-dispersed sample, which can be made into a uniform thin film when attached to a glass substrate. Hence we can fabricate a DNA-MWNT electrode and use it in solution phase. Electrochemical experiments using DNA-MWNT indicates that DNA-MWNT electrode possesses a flat and wide potential window, well defined quasi-reversible voltammograms, and quick electron transfer for $Fe(CN)_6{}^{4-}$/ $Fe(CN)_6{}^{3-}$, suggesting the potential of DNA-MWNT electrode for electrochemical sensor. Further demonstration of high selectivity and sensitivity of this DNA-MWNT electrode is the detection of a low concentration of dopamine.

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Figure Captions

1. (a) SEM, (b) TEM and (c) HRTEM images of purified MWNT
2. FT-IR spectra of (a) carboxyl group functionalized MWNT and (b) DNA functionalized MWNT
3. (a) SEM and (b) HRTEM images of DNA/MWNT
4. Cyclic voltammogram of 10 mM $Fe(CN)_6$ in 1 M KCl
5. Cyclic voltammetric detection at DNA-MWNT electrode in 0.1 M PBS, pH 7.0 for 2 mM DA + 20 mM AA

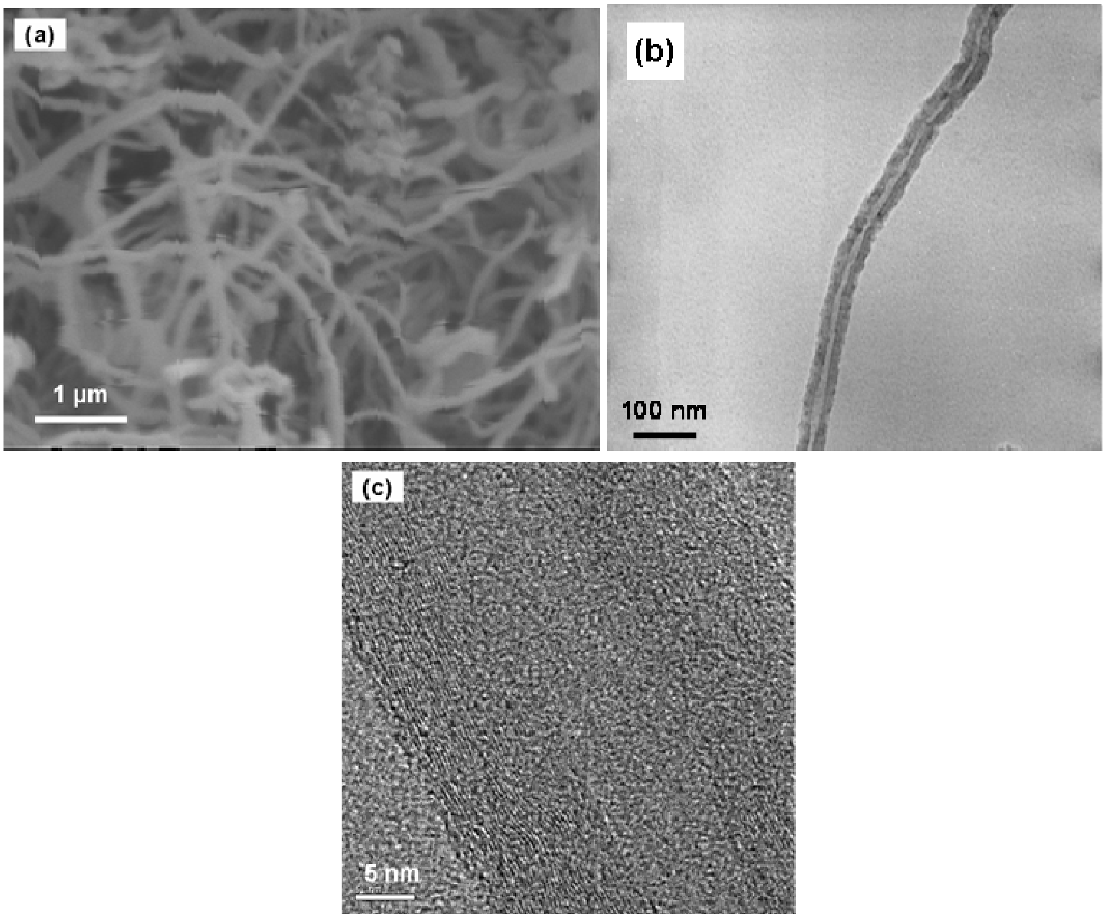

Figure 1

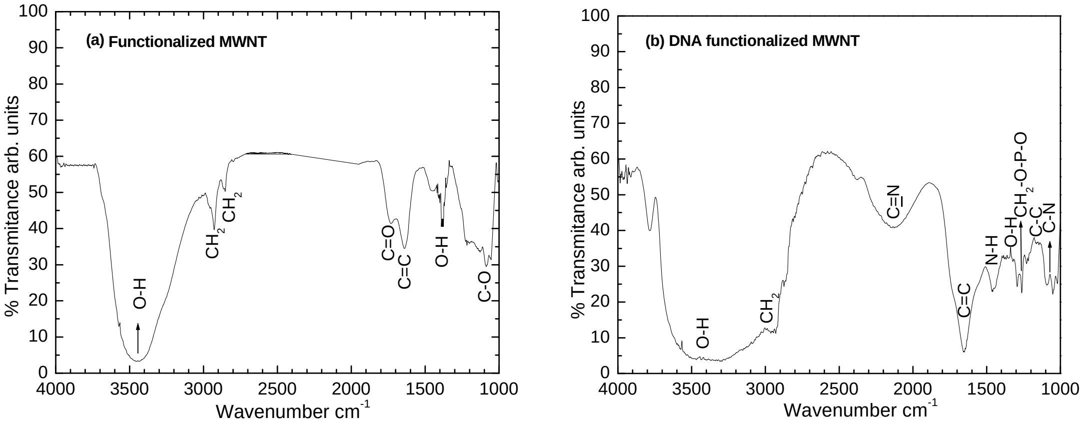

Figure 2

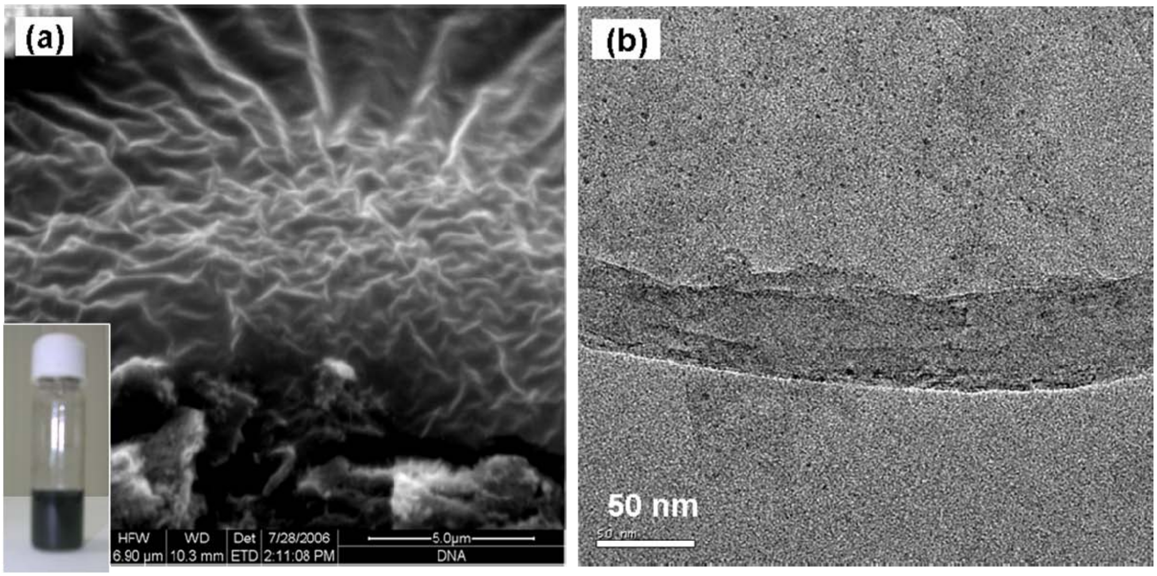

Figure 3

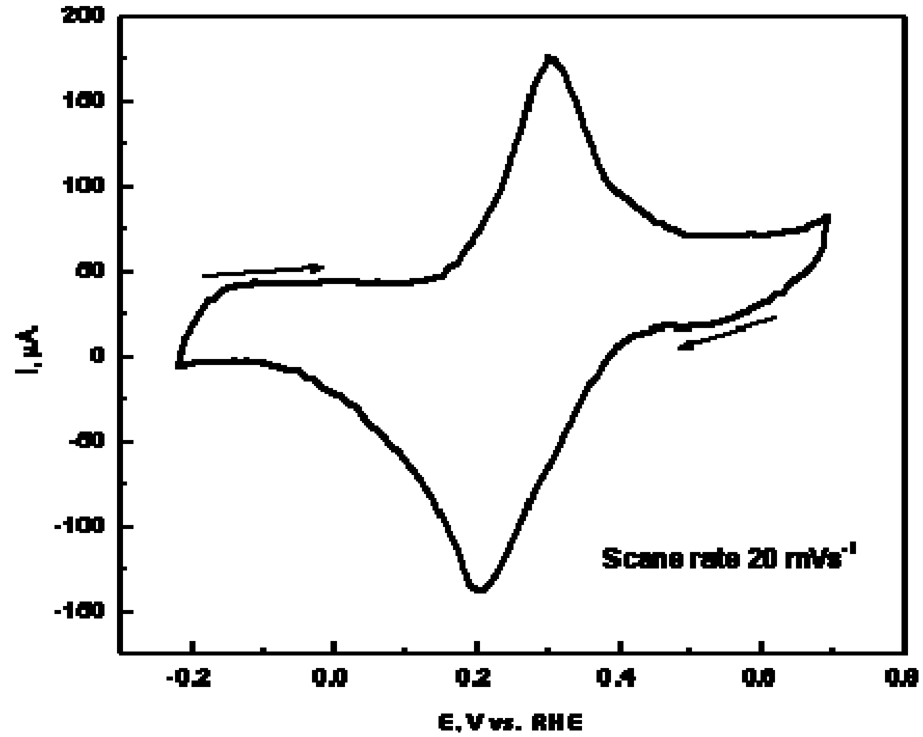

Figure 4

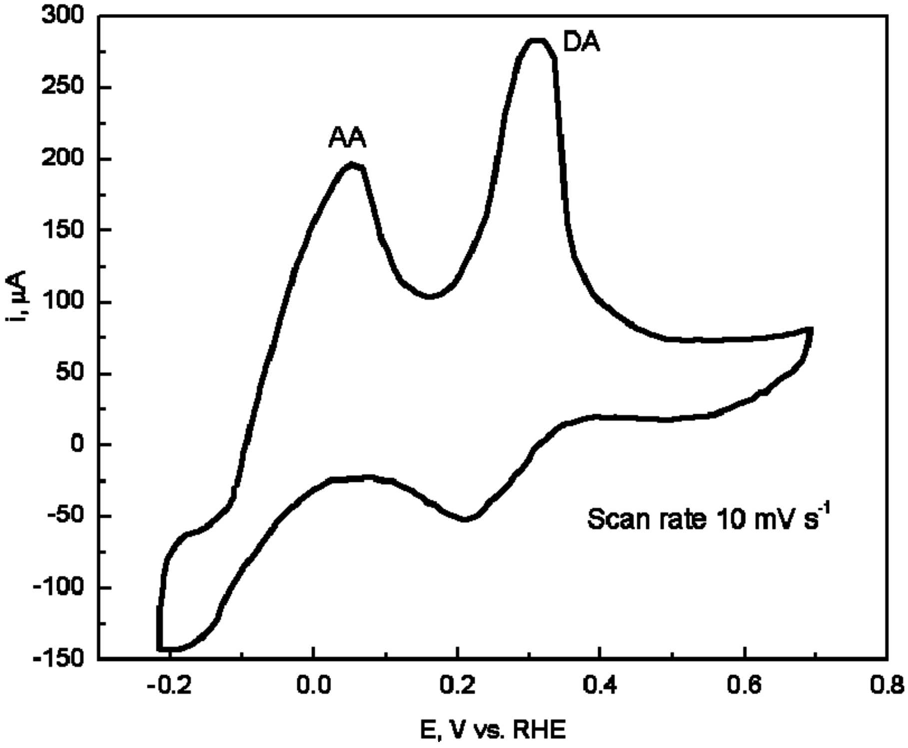

Figure 5

A glucose biosensor based on deposition of glucose oxidase on to crystalline gold nanoparticle modified carbon nanotube electrode

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Abstract

A new amperometric biosensor, based on deposition of glucose oxidase (GOD) on to crystalline gold (Au) nanoparticle modified multi walled carbon nanotube (MWNT) electrode, is presented. MWNT have been synthesized by catalytic chemical vapour 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. The characterization of metal decorated MWNT have been done by using XRD, SEM, TEM, HRTEM and EDX. Amperometric biosensor fabricated by depositing glucose oxidase (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.

Key words: amperometric biosensor, glucose oxidase, multi walled carbon nanotube, Nafion-solubilized

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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 like entrapment techniques[3] electrochemical copolymerization[4] covalent or cross-linking[5] and adsorption[6].

There are a variety of materials, which can be used as electrochemical transducers. Commonly employed electrochemical transducers are either inert metals like platinum or gold, carbonaceous materials[1, 2]. The carbonaceous materials include graphite, carbon fibres, porous carbon, carbon spheres, glassy carbon and carbon nanotubes (CNTs). All these materials help for the 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, which is impermeable to gases and fluids[8]. But one major disadvantage of 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 excellent electron transfer rate, which is 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, 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 over-voltage 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 over-voltage is considerably lowered. This can be achieved by the deposition of nano crystalline 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 transport, 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 attract significant attention in biosensors research over the last few years as they allow proteins to retain their biological activity upon adsorption[14]. More over, 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–carbon nanotube materials can be found in the literature[16]. Wu et al. in 2007 have fabricated an amperometric glucose biosensor based on multilayer films prepared via layer-by-layer self-assembly of muti walled 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, 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 on to crystalline Au nanoparticle modified MWNT electrode by a very simple technique. MWNT have been synthesized by catalytic chemical vapour 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. Amperometric biosensor has been fabricated by the deposition of 32 units of glucose oxidase (GOD) over 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

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 vapour deposition technique as described below. $HAuCl_3.3H_2O$ 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 0.1M 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 adjust the pH 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 (D–D) water.

Instruments

The electrochemical measurements were performed with CH Instruments CHI 608C Electrochemical Analyzer/Workstation. A Pt wire counter electrode, Ag/AgCl (3M 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-K_α 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).

Synthesis of MWNT

MWNT 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-K_α radiation) and thermo gravimetric measurements (20°C/min). These techniques have been explained in detail in our previous work[18]. The samples were characterized using SEM and TEM.

Preparation of Au/MWNT composite

In order to decorate the purified MWNT with nano crystalline Au clusters, chemical reduction method was used. Nearly 0.02 gm of purified MWNT were treated with 0.075M $HAuCl_4.3H_2O$ followed by magnetic stirring for 12 h. Au salt is reduced by adding reducing solution which is a mixture of 0.1 M $NaBH_4$ and 1M NaOH during stirring. After the reaction is over the solution is washed three times with deionised water and filtered using cellulose membrane filters having pore size of 0.1 µm. The 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 (EDX).

Fabrication of GCE/Au-MWNT/GOD/Nafion electrode

Before electrode modification, the bare GCE was polished with 0.05µm alumina slurry, sonicated in deionized water, and dried with high purify nitrogen stream to obtain a mirror surface. The Au/MWNT 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 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. 4µl of 32U GOD solution was film-cast onto the surface of the GCE/ Au-MWNT electrode and allowed to dry slowly at 4°C. The obtained GOD/Au-MWNT 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 2.5µl 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

Morphology and characterization of Au-MWNT used in the fabrication of bioelectrode

Figure 1 (a) 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 (Fig. 1 (b)) shows the reflections of Au along with that for graphitic carbon. The broad peaks reveal the presence of nanostructured crystalline gold particles.

TEM image (JEOL, JEM-3010 Electron Microscope with an acceleration voltage of 200 KV) of Au/MWNT (Fig. 2) reveals a 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. Inset of the figure shows the HRTEM image of the Au nanoparticle and the corresponding EDX pattern. 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 HRTEM image. EDX patterns show about 20 wt% loading of gold on the surface of MWNT and for that the carbon peak has been used as the reference. EDX 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.

Voltammetric characterization of Au-MWNT/GOD/Nafion modified glassy carbon electrode

The application of Nafion-solubilized MWNT for electrochemical biosensor was exploited by immobilizing GOD in Nafion-solubilized MWNT film. Cyclic voltammetry is a useful tool for electrochemical evaluation of the transducers. 0.1M phosphate buffer at pH 7 was used as a probe to investigate performance of the fabricated glucose bioelectrode. The cyclic voltammetric (CV) responses of Au-MWNT/ Nafion electrode and Au-MWNT/GOD/Nafion electrode towards the enzymatically liberated H_2O_2 were simulated by adding 15mM glucose at pH 7 buffer and their performances are illustrated in Fig. 3 (a). Au-MWNT/ Nafion electrode did not exhibit any oxidation or reduction peaks upon the addition of 15mM 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 Au-MWNT/GOD/Nafion electrode towards the addition of H_2O_2 to pH 7 buffer is illustrated as Fig. 3 (b). A minor reduction peak was observed at -0.5V at the Au-MWNT/GOD/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 fivefold greater than in the first case. But in both the 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 towards H_2O_2. The large distinguishable response towards 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.

The hydrodynamic voltammograms (HDV) for 15mM glucose at GCE/Au-MWNT/GOD/Nafion bioelectrode is displayed in Fig.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 GCE/Au-MWNT/GOD/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 GCE/MWNT/Au/GOD/Nafion electrode is due to the entrapped GOD enzymes.

Determination of glucose using fabricated GCE/Au-MWNT/GOD/Nafion bioelectrode

The typical amperometric i - t curves for the fabricated GCE/Au-MWNT/GOD/Nafion bioelectrode at a constant voltage of +0.3 V is shown in Fig. 5. 0.1M phosphate buffer at pH 7 has been used as the supporting electrolyte and the amperometric response of the fabricated bioelectrode towards 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 towards the detection of glucose. The steady-state calibration curve (Fig. 6) has been drawn using the steady state current values corresponding to different glucose concentrations. It exhibits a linear range from 0.05 mM to 22 mM and 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. Conclusion

Chemical reduction method is a simple and effective technique to deposit nanocrystalline Au metal particles on the surface of MWNT. With the immobilization 32 U of GOD on the Au–MWNT/Nafion film, a novel glucose biosensor was fabricated by a simple deposition technique which exhibits high sensitivity, low detection limit, good reproducibility, long-term stability and fast current response at a low applied potential. A more controllable, stable and reproducible deposition of Au-MWNT film onto GCE can be achieved using homogeneous solution of (0.5%) Nafion/Au-MWNT. The fabricated Au-MWNT/GOD/Nafion bioelectrode 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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Figure captions

1. Powder X- ray diffractogram of (a) purified CNTs obtained from $DyNi_2$ alloy hydride, (b) Au/MWNT.
2. TEM images of Au/MWNT with corresponding HRTEM image and EDAX pattern in the inset.
3. Cyclic voltammographs of (a) GOD/Au—MWNT/Nafion bioelectrode (solid line) and Au—MWNT/Nafion electrode (doted line) in phosphate buffer solution on the addition of 15mM of glucose and (b) GOD/Au—MWNT/Nafion bioelectrode before (solid line) and after (doted line) adding 0.3mM of H_2O_2 at a sweep rate of $25mVs^{-1}$
4. Hydrodynamics voltammograph of GOD/Au—MWNT/Nafion electrode in 15mM glucose.
5. Amperometric i-t curves of the fabricated GOD/Au—MWNT/Nafion electrode for different glucose concentrations.
6. Calibration curve of the fabricated GOD/Au—MWNT/Nafion bioelectrode.

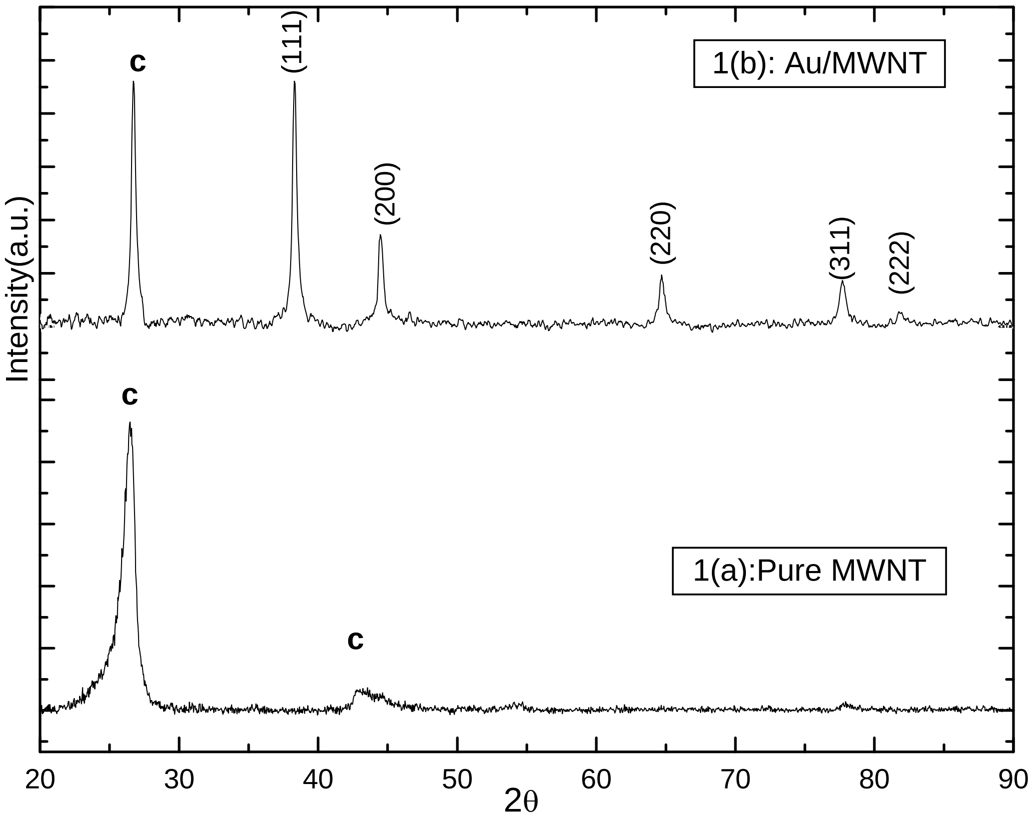

Intensity(a.u.)
2θ
20
30
40
50
60
70
80
90
1(b): Au/MWNT
c
(111)
(200)
(220)
(311)
(222)
c
c
1(a):Pure MWNT

Figure 1

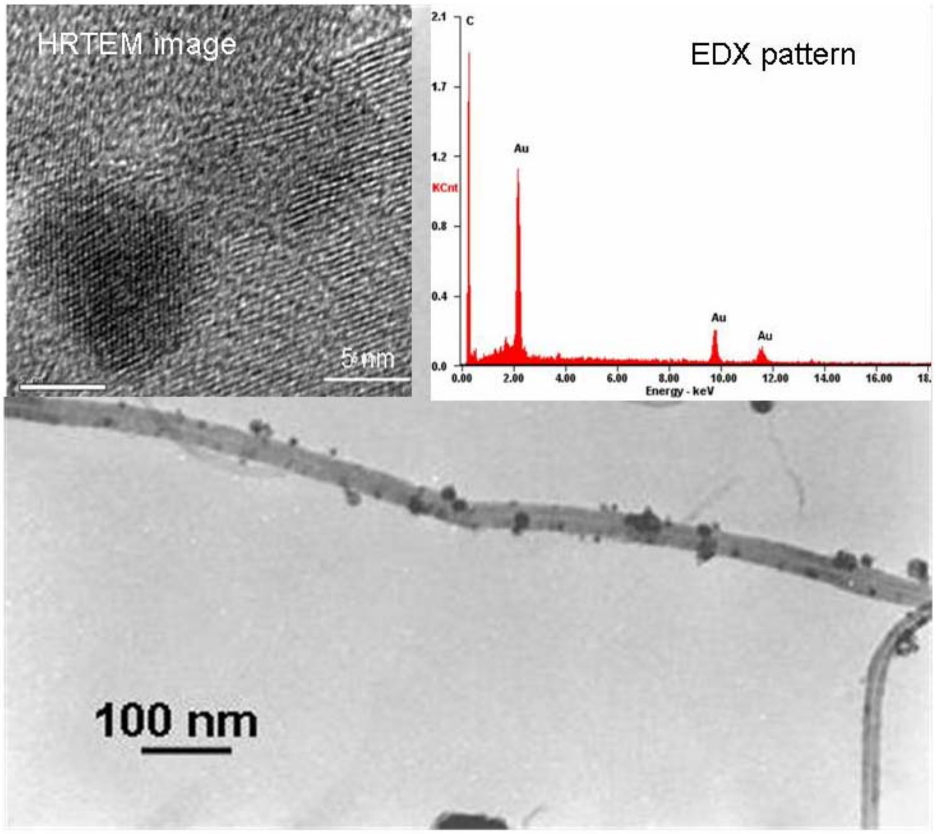
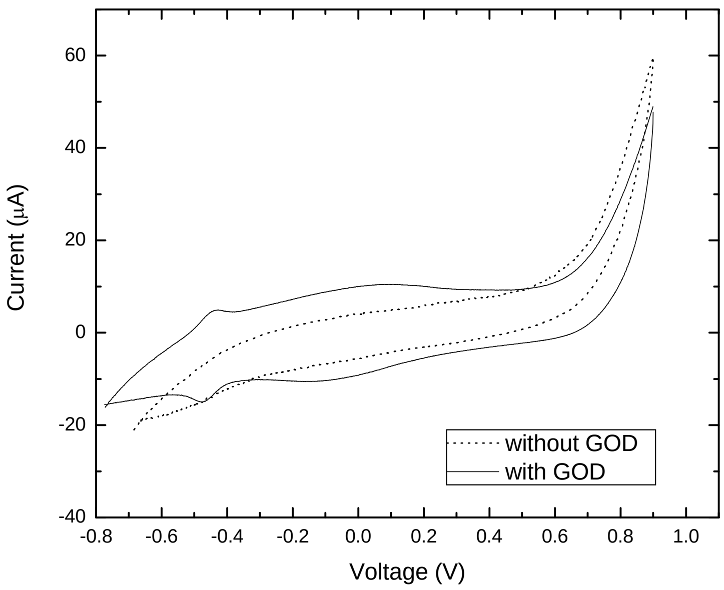
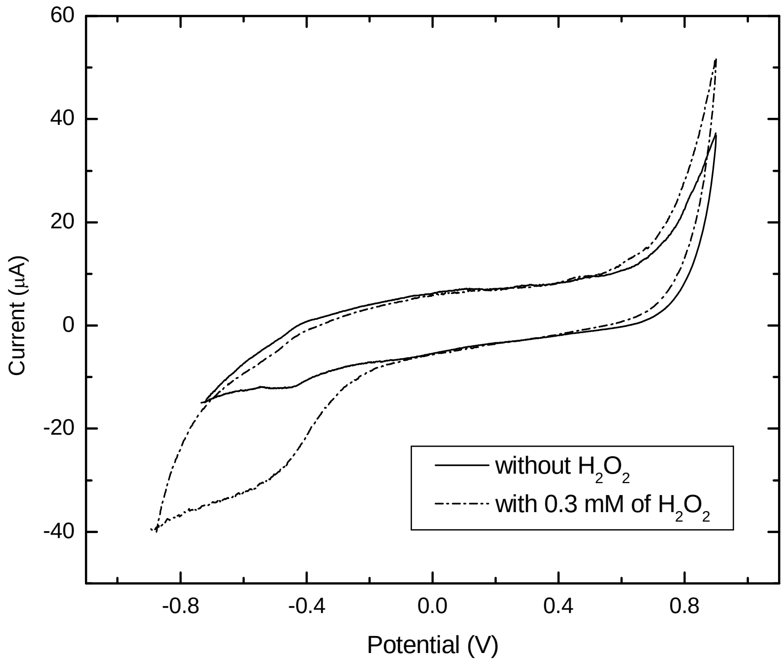

HRTEM image
5 nm
EDX pattern
2.1
1.7
1.2
KCnt
0.8
0.4
0.0
C
Au
Au
Au
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.
Energy - keV
100 nm

Figure 2



3 (a)



3 (b)

Figure 3

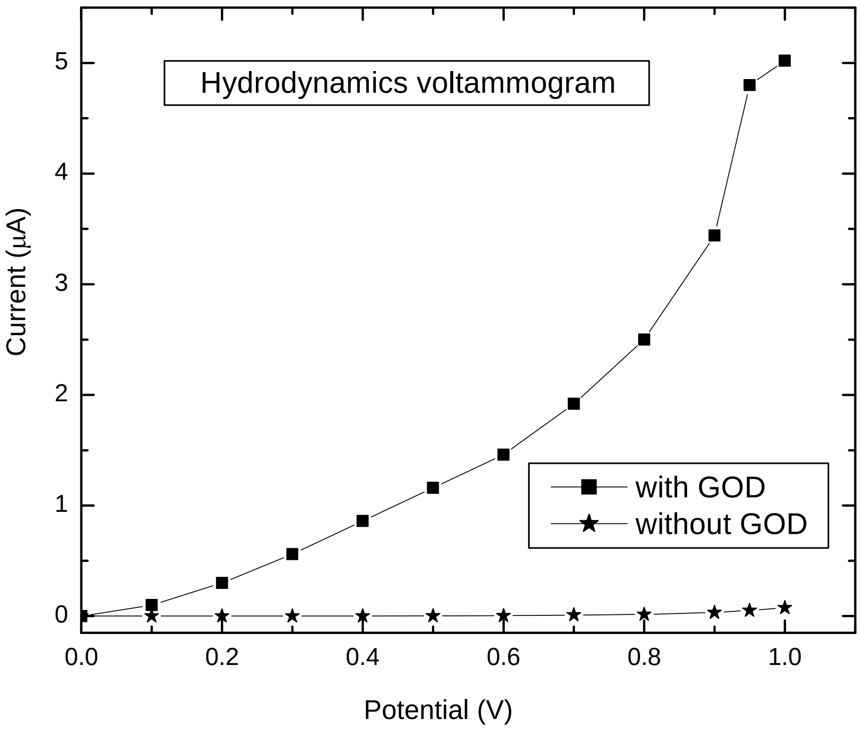

Hydrodynamics voltammogram
with GOD
without GOD
Current (μA)
Potential (V)
0
1
2
3
4
5
0.0
0.2
0.4
0.6
0.8
1.0

Figure 4

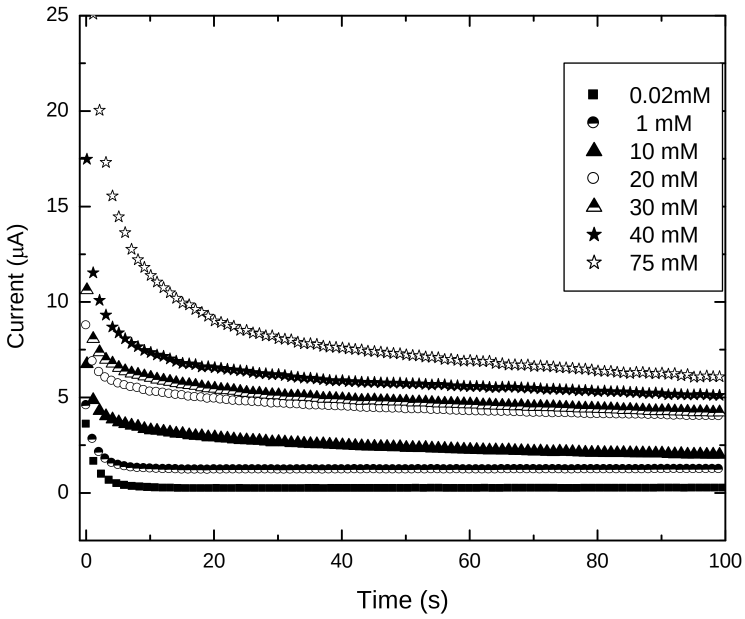


Figure 5

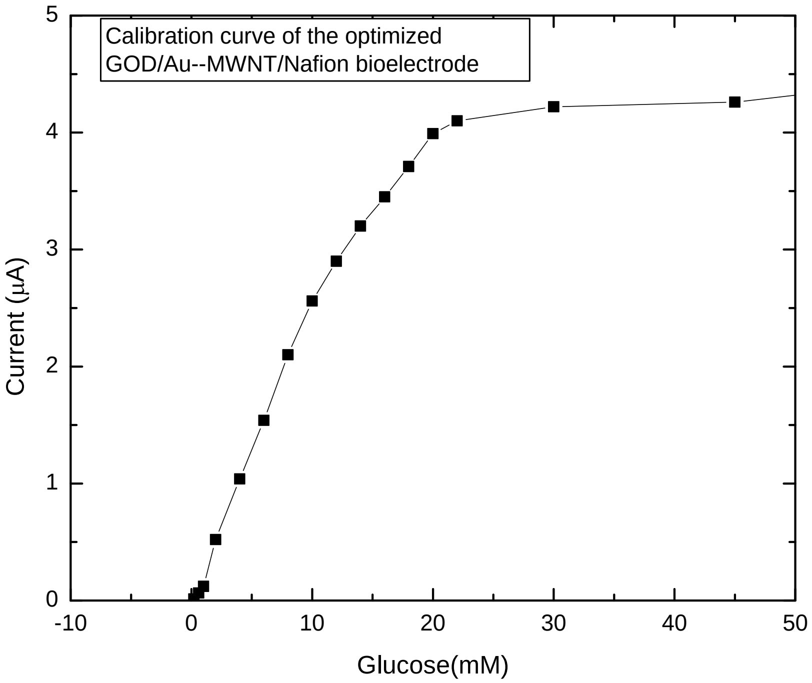


Figure 6