Fabrication of a Gold Nanoparticles Decorated Carbon Nanotubes Based Novel Modified Electrode for the Electrochemical Detection of Glucose

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A modified electrode based on gold nanoparticles decorated multiwall carbon nanotubes (MWNTs), MWNT-Au nano-ME is fabricated. MWNTs are functionalized with 4-aminothiophenol and coated over the glassy carbon electrode. Further, Au nanoparticles are deposited into MWNTs coated GC electrode by electrochemical reduction of HAuCl4. Field emission transmission electron microscope (FETEM) image shows the formation of ∼5 nm sized Au+ nanoparticles without any agglomeration on the MWNTs surface. Further, the presence of Au+ nanoparticles is confirmed through X-ray photoelectron spectroscopic (XPS) studies. The electrocatalytic activity of the MWNT-Au nano-ME towards the detection of glucose is investigated. MWNT-Au nano-ME shows enhanced current response than pristine MWNT-ME over the entire (+0.05 to +0.80 V) potential range. The modified electrode shows linear response to current with the concentration of glucose between 1 and 20 mM. Larger current responses to glucose oxidation are witnessed at +0.60 V than at +0.05 V. However, a large interference signal, reflecting the accelerated oxidation of electroactive interference is observed at +0.60 V. No overlapping signal from the interferents such as ascorbic acid, acetaminophen, and dopamine are observed at the MWNT-Au nano-ME at +0.05 V. Further, the MWNT-Au nano-ME shows high resistance to the toxicity of chloride ions.

Keywords: Multiwall Carbon Nanotubes, Gold Nanoparticles, Glucose, Sensor.

1. INTRODUCTION

Blood glucose monitor is an important tool that helps people with diabetes to assess their physiological status and allow them to properly medicate themselves to have modulated food. The most investigated technology for in vivo glucose monitoring is based on implanted amperometric enzyme electrodes and devices are commercially available and used in clinical practice.1–3

Most of the recent works related to the development of amperometric glucose sensors are based on immobilization of the enzyme such as glucose oxidase,4 or glucose dehydrogenase5 which catalyzes the oxidation of glucose to gluconolactone. The enzyme immobilized electrode surfaces are fabricated to achieve rapid and direct electron transfer.6 Today most commercial amperometric biosensors rely on reactions catalyzed by oxidase enzyme and subsequent detection of H2O2 on platinum electrodes. There is a limitation in selectivity of glucose between the bio-recognition event and the amperometric detection of H2O2. High oxidizing potential (∼700 mV vs. Ag/AgCl) is necessary for the oxidation of H2O2, that results in substantial interference from the oxidation of other compounds in the complex matrices. Further, the byproduct, H2O2, produced by enzymatic reaction denatures the enzymes.

In most of the cases, the enzyme based sensors require a redox mediator7 for shuttling the electron between electrode and analyte. Such mediators can be used as replacement for O2 to increase the selectivity and sensitivity of the biosensors. Ferrocene, quinones, quinoid like dyes, organic conducting salts and viologens have been used as mediators.8 Use of mediators allows the exploitation of other oxidoreductase enzymes, including peroxidases and dehydrogenases.9 However, the stability and toxicity of...
these mediators limit their in vivo applications. In general, the amperometric enzyme electrodes have several inherent problems such as the relatively low output current density and the gradual deterioration of the enzyme activity. A possible solution of these problems is to search for efficient non-enzymatic electrocatalysts for glucose detection.

Carbon nanotubes (CNTs) are one of the most promising nanomaterials for a wide range of technological innovations. Applications of CNTs as electrocatalysts have been investigated widely due to its unique combinational properties. Owing to its high surface area and good electrical conductivity, CNTs have also been regarded as perfect supporting materials for catalysts. Recently, investigations demonstrated that metallic nanoparticles can be decorated on the external walls or encapsulated in the interior of the CNTs. For example, Pt, Au, and Pt-Ru were deposited on CNTs by different methodologies and the electrodes show good electrocatalytic properties for many electrochemical reactions.

In the present investigation, a robust and non-enzymatic glucose sensor electrode based on gold nanoparticles decorated MWNTs (MWNT-Au nano-ME) is developed. The present investigation shows that the direct oxidation of glucose in phosphate buffer is achieved by the novel electrocatalysts without loading any enzyme or mediators. The MWNT-Au nano-ME shows high sensitivity and selectivity towards the detection of glucose. Further, the MWNT-Au nano-ME shows high resistance to the toxicity of chloride ions. The results are presented herein and discussed.

2. EXPERIMENTAL DETAILS

2.1. Chemicals

Multiwall carbon nanotubes, MWNTs (10–50 nm in diameter) obtained from CNT Co., Ltd., Incheon, Korea were rinsed with double-distilled water and dried. 4-Aminothiophenol, nitric acid, thionyl chloride, tetrahydrofuran, auric acid, ascorbic acid, acetaminophen, and dopamine of analytical grade from Aldrich were used as received. Double-distilled water was used throughout the experiments. Aqueous solutions of glucose were prepared in phosphate buffer (pH 7) fresh at the time of experiments.

Surface of glassy carbon (GC) was modified and used (details are given below) as working electrode. GC electrode was polished with a suspension of alumina powder (1/μm) and then rinsed thoroughly with deionized water to remove any alumina residue. Electrochemical pretreatments were done in a 50 mM (NH₄)₂SO₄ solution (pH 3, being adjusted with sulfuric acid). The pretreated electrodes were allowed to stay for 10 min in deionized water and used.

2.2. Functionalization of MWNTs

MWNTs were functionalized with 4-aminothiophenol (ATP) (Scheme 1(A)) as detailed in our previous work. In a typical method, 50 mg of MWNTs were refluxed in 4 M HNO₃ for 24 h and filtered through polycarbonate membrane. The residue, MWNT-COOH, was washed with deionized water and dried under vacuum at 60 °C for

![Scheme 1. Functionalization of MWCNTs and fabrication of MWCNT-Au nano-ME.](image-url)
12 h. 50 mg of MWNT-COOH was refluxed in 100 ml of thionyl chloride at 65 °C for 24 h to obtain MWNT-COCI. MWNT-COCI was filtered, washed with THF and dried under vacuum at room temperature. ATP functionalized MWNTs were prepared by refluxing MWNT-COCI with ATP in THF at 60 °C for 24 h. The ATP functionalized MWNTs (MWNT-ATP) were separated by filtration and dried.

2.3. Fabrication of MWNT-Au nano-ME

A black suspension (MWNT-ATP) was prepared by dispersing 50 mg of MWNT-ATP in DMF with the aid of ultrasonication. About 1.0 μl of the black suspension was dropped on the surface of the pretreated GC electrode and kept at 60 °C for 12 h to evaporate the solvent. The GC electrode coated with MWNT-ATP was washed with water and stored under nitrogen atmosphere.

Au⁺ particles were electrochemically deposited onto GC/MWNT-ATP layer from 0.5 M H₂SO₄ solution of HAuCl₄ by employing a repetitive potential scan from 1.1 V to 0.0 V (vs. SCE) and thus, the modified electrode, MWNT-Au nano-ME was fabricated.

2.4. Characterization

The morphology of MWNT-Au nano was examined by field emission transmission electron microscope (FETEM)—JOEL JEM-2000EX with a field emission gun operated at 200 kV. X-ray photoelectron spectroscopy (XPS) was recorded on VG Microlab and MT 500/L with a Mo Ka X-rays radiation as the X-ray source for excitation. The data were obtained at room temperature and the operating pressure in the analysis chamber was below 10⁻⁹ Torr with analyzer pass energy of 50 eV. The resolution was 0.2 eV.

2.5. Instrumentation

Electrochemical measurements were carried using EG & G PAR Electrochemical Analyzer. The electrocatalytic activity of the modified electrodes was evaluated by using cyclic voltammetry in phosphate buffer solution containing 1 mM K₃[Fe(CN)₆]₃. The amperometric response of the sensor electrode was recorded under steady-state conditions in the phosphate buffer (pH 7) by applying a constant potential (+0.05 or +0.60 V) to the working electrode. The amperometric experiment was performed in an electrochemical cell that contains the modified electrode as working electrode, a SCE reference electrode, and a platinum wire auxiliary electrode. The background response of the modified electrode 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. In the case of flow analysis, current vs. time for variable glucose concentration injections were recorded for injection of a series of concentrated solutions of glucose to the phosphate buffer solution (pH 7).

3. RESULTS AND DISCUSSION

3.1. Fabrication of MWNT-Au nano-ME

Modification of the surface of working electrode (GC) was performed through sequential steps. At first instant, MWNTs were functionalized with ATP (as described in the Experimental Details Section) and MWNT-ATP was coated on the GC electrode surface. In the next step, Au⁺ nanoparticles were deposited onto MWNT-ATP from 0.5 M H₂SO₄ solution containing HAuCl₄ by employing a repetitive potential scan from 1.1 V to 0.0 V (vs. SCE) as described in the literature. Cyclic voltammograms (CVs) show that there exits two cathodic peaks at ~0.78 V and ~0.50 V (vs. SCE). No anodic peak is observed during positive scan of potential. The peak at 0.50 V represents the reduction of solution bound AuIII to Au⁺ and the wave at 0.78 V is attributed to the reduction of adsorbed AuCl₄⁻ ions to Au⁺. This, Au⁺ loaded MWNT modified electrode, MWNT-Au nano-ME was fabricated.

Figure 1 shows the FETEM image of MWNT-Au nano. Spherically shaped Au⁺ particles having average size of about 5–7 nm can be seen on the surface of MWNTs with relative uniformity. The loading of Au⁺ nanoparticles on the surface of MWNTs was 18%, which was estimated by energy-dispersive X-ray spectroscopy. Generally, most metals particles would not adhere to MWNTs due to its hydrophobicity. Further, metal particles tend to aggregate to form bigger metallic particles on the surface of MWNTs. Surface modification and sensitization activation...
have been attempted to improve metal deposition onto the surface of MWNTs. The presence of functional groups on MWNTs increases the metal nucleation. By keeping this in view, in the present instigation, MWNTs were specifically functionalized with 4-aminophenol. During electrolysis of HAuCl₄, Au⁺ nanoparticles are expected to preferentially anchor into the –SH sites of MWNTs (Scheme 1(B)). The interactions between –SH moieties in MWNTs with Au⁺ nanoparticles provide uniform distribution for Au⁺ nanoparticles and further prevent aggregation. Attachment of Au⁺ nanoparticles into –SH groups is well known.²⁰–²³

The oxidation state of Au in the nanoparticles was confirmed by X-ray photoelectron spectroscopy (XPS). Figure 2 presents the XPS spectrum of MWNT-Auₙ₂oₜ showing the characteristic Au 4f/2 and 4f5/2 doublets. The clear peaks centered at 83.1 eV and 87.3 eV are the signature of Au⁺ nanoparticles that exist in MWNT-Auₙ₂oₜ.

3.2. Electro-Catalytic Behavior of MWNT-Auₙ₂oₜ-ME

The electrochemical performance of the MWNT-Auₙ₂oₜ-ME and MWNT-ME was investigated for the ferriyanide (Fe³⁺/Fe²⁺) system. Figure 3 shows the CVs of 1 mM K₃Fe(CN)₆ in the phosphate buffer (pH 7) electrolyte solution at MWNT-Auₙ₂oₜ-ME and MWNT-ME. The excellent electrochemical performance of the MWNT-Auₙ₂oₜ-ME is evident by the reversibility of the Fe³⁺/Fe²⁺ electrochemistry with an ideal peak separation (∆Eₚ) of 61 mV with a half-wave potential of (Eₕw) of 208 mV (vs. SCE) and augmented current response. However, at MWNT-ME, the ∆Eₚ was about 92 mV exhibiting pseudo-reversibility, similar to the one observed at bare GC electrode. A ∆Eₚ value of ∼100 mV was reported for Fe³⁺/Fe²⁺ system at CNT paste electrodes.²⁴ A quasi-reversible electrochemistry (∆Eₚ = 130 mV) was observed for Fe³⁺/Fe²⁺ system at Auₙ₂oₜ/dithiol modified Au electrode.²⁵

The enhanced electro-catalytic activity of MWNT-Auₙ₂oₜ-ME is expected to arise from the smaller (∼5 nm) crystallite sizes of Au⁺ nanoparticles and fine distribution of Au⁺ nanoparticles on the surface of MWNTs via formation of Au⁺ nanoparticles through self-assembly configuration (Scheme 1). Further, Au⁺ nanoparticles provide conduction pathways to accelerate electron transfer through mediation influence of MWNTs. Smaller size (∼5 nm) and uniform distribution of Au⁺ nanoparticles in addition to the existence of MWNTs provide a high surface area for the electrocatalytic sites.

The excellent electrochemical behavior of MWNT-Auₙ₂oₜ-ME encourages us to develop a non-enzymatic electrode for the detection of glucose. MWNT-Auₙ₂oₜ-ME showed augmented current response towards detection of glucose in the phosphate buffer (pH 7). The details are presented below.

3.3. Hydrodynamic Response of MWNT-Auₙ₂oₜ-ME

Hydrodynamic voltammograms were recorded for a solution of 1 mM glucose in phosphate buffer (pH 7) at MWNT-Auₙ₂oₜ-ME and compared with the electrochemical response at MWNT-ME (Fig. 4). Both electrodes show current response over the entire (+0.05 to +0.80 V) potential range, however, a significant increase in peak currents for the oxidation of glucose was noticed with MWNT-Auₙ₂oₜ-ME in comparison to MWNT-ME (Fig. 4). Typically, at the anodic potential of +0.60 V, MWNT-Auₙ₂oₜ-ME shows current response of about 18 μA, which is nearly three
times higher than the current response at MWNT-ME (6.6 µA). The anodic current response at MWNT-Aunano-ME increases rapidly between +0.05 and +0.60 V and levels off at higher potential (>0.60 V). The excellent sensitivity for glucose oxidation at MWNT-Aunano-ME is in a wider potential range arises from the additional surface area provided by Au nanoparticles for the oxidation process. This indicates that electron transfer at MWNT-Aunano-ME is more efficient than at MWNT-ME. Further, MWNT-Aunano-ME has a flexible operating potential window (+0.05 to +0.60 V) for monitoring the glucose oxidation process. And, this wider potential range of operation provides scope for using MWNT-Aunano-ME for glucose detection in potential avoiding signals from the electroactive interferents.

3.4. Amperometric Response for Glucose at Low and High Potentials

We have assessed the electrocatalytic behavior of MWNT-Aunano-ME for glucose (Fig. 5) at a low (+0.05 V) and a relatively higher potential (+0.60 V). Figure 5 compares the amperometric current response to successive additions of 1 mM glucose at MWNT-Aunano-ME (a) and MWNT-ME (b) at the operating potentials of +0.05 V (i) and +0.60 V (ii), respectively. It can be seen from Figure 5 that both electrodes respond to glucose oxidation at +0.05 and +0.60 V. MWNT-Aunano-ME offers larger current signals (Fig. 5(i) and (ii); line (a) than MWNT-ME (Fig. 5(i) and (ii); line (b) reflecting the augmented electrocatalytic activity of MWNT-Aunano-ME. Further, the current noticed at +0.60 V is comparatively higher than noticed at +0.05 V.

It is reported that the amperometric response of glucose is insensitive at Pd-nafion-CNT electrode when the potential was held at +0.70 V, however in another report, Au nanoparticles-flavin adenine dinucleotide/glucose oxidase (Au-FAD/GOx) shows high sensitive for glucose at 0.60 V. In the present investigation, MWNT-Aunano-ME shows higher response to glucose detection at 0.60 V. Further, higher sensitivity (increased current response) is observed at MWNT-Aunano-ME when the applied potential is held at +0.60 V than at +0.05 V (vs. SCE). The steady-state calibration exhibits linearity upto 20 mM with the detection limit being 0.01 mM. MWNT-Aunano-ME reaches 95% of the steady-state current within 6 s. Also, with a low-potential of operation (+0.05 V), MWNT-Aunano-ME shows linearity for the current response in the concentration range of 1–20 mM. MWNT-Aunano-ME exhibits a rapid and sensitive response to the changes of glucose concentration, indicating excellent electrocatalytic behavior.

3.5. Flow Injection Analysis

Flow injection analysis (FIA) is widely used as an analytical tool due to its versatility such as low sample consumption, repeatability of results and high throughput. Further, it is anticipated that FIA can eliminate the diffusion...
restriction of glucose at high concentrations. By considering the ease of FIA in the analytical application, FIA measurements were made using MWNT-Au nano-ME as working electrode. The operating parameters such as flow rate and applied potential were optimized to have better sensitivity at MWNT-Au nano-ME for detection. Figure 6(A) shows the FIA response for with increasing concentration of glucose in phosphate buffer (pH 7) at an applied potential of +0.05 V at a flow rate of 2 ml/min. The calibration plot was linear up to 15 mM glucose with a high sensitivity and regression coefficient of 0.999 and shows curvature at higher concentration (Fig. 6(B)). Reproducibility of MWNT-Au nano-ME was evaluated by successive injection of glucose and an RSD value of 0.5% was observed. Thus, MWNT-Au nano-ME shows good reproducibility for repeated detection of glucose and offers to work with low working volumes of about 20 µl using FIA.

3.6. Influence of Interferences

Electrocatalysts based on metals such as Pt, lose their activity rapidly by the accumulation of chemisorbed intermediates, which block the electrocatalyst surface. Another disadvantage of these metal catalysts is that they lack the selectivity for glucose. Various organic substances can be simultaneously oxidized with glucose at these electrode surfaces and gives interfering electrochemical signal. It is to remember that MWNT-Au nano-ME has a flexible operating potential window (+0.05 to +0.60 V) for monitoring the glucose oxidation process (Fig. 5). MWNT-Au nano-ME shows significant current at +0.05 V (Fig. 5). And at such a low applied potential (+0.05 V), the current responses of common interferences are minimum and current from the reduction of oxygen is negligible.

Figure 7(i) and (ii) represent the amperometric response of MWNT-Au nano-ME with 1 mM glucose and with the addition of relevant physiological levels (0.5 mM) of ascorbic acid (AA), acetaminophen (AP), and dopamine (DA) measured at +0.05 V and +0.60 V, respectively. As it can be seen from the figure that MWNT-Au nano-ME shows current response to glucose at both applied potentials. The subsequent injection of relevant physiological levels of AA, AP, and DA did not show any additional signal or modify the current response at MWNT-Au nano-ME when the potential was held at +0.05 V (Fig. 7(i)). However, at +0.60 V, the glucose response is modulated or increased by anodic contribution of the electroactive species (AA, AP, and DA) (Fig. 7(ii)). No interfering electrochemical signals was observed at +0.05 V. It is reported that a large response signal from AA and DA is observed at MWNT electrode and Au nanoparticles. The accelerated electron transfer due to the combined presence of MWNT and Au nanoparticles allows glucose detection at a very low potential (+0.05 V). The low-potential detection leads to high selectivity with effective discrimination against coexisting electroactive species. Note that, a high selective response for glucose detection is obtained at MWNT-Au nano-ME (+0.05 V) in the absence of any external (permselective) coating.

Most of the electrochemical glucose sensors based on metals or alloys can easily lose their activity due to the poisoning of chloride ions. The amperometric glucose signal at Pt and Pt2Pb diminishes rapidly in the presence of 0.01 M NaCl and eventually almost disappears. In order to understand the effect of chloride ions on the glucose detection at MWNT-Au nano-ME, the amperometric response for glucose was examined by addition of 0.01 N NaCl to the electrolyte solution. A stable, linear response for glucose detection without change in the response current is observed at MWNT-Au nano-ME before and after addition of 0.01 N NaCl. This infers that the MWNT-Au nano-ME is not subjected to surface fouling by the action of chloride ions.

Generally, glucose oxidation at metal electrocatalysts suffers from poisoning resulting from the adsorption of gluconolactone. Gluconolactone is the intermediate formed during the oxidation of glucose in phosphate buffer at potential negative to Au oxide formation. Amperometric measurements were made at MWNT-Au nano-ME for glucose detection in order to study the poisoning effect by...
gluconolactone. No significant difference in response was observed for glucose detection at MWNT-Au nano-ME in the presence and absence of gluconolactone.

3.7. Stability of MWNT-Au nano-ME

The most common and serious problem in glucose biosensors based on enzyme immobilization is its insufficient stability. A gradual deterioration of the enzyme activity was observed. Further, these biosensors are susceptible to denaturing. Generally, such problems are not encountered in enzyme-less sensor electrodes. We have monitored the stability of MWNT-Au nano-ME by measuring the response from day to day during storage in phosphate buffer (pH 7) at room temperature for a period of 10 days. During the test period, the MWNT-Au nano-ME showed no apparent decrease in current response for the detection of glucose, which demonstrates the good stability of the electrode.

4. CONCLUSIONS

A new modified electrode based on gold nanoparticles decorated multiwall carbon nanotubes was fabricated. The MWNT-Au nano-ME shows high selective and sensitive response for glucose detection in the absence of any enzyme. Further, MWNT-Au nano-ME has a flexible operating potential window (+0.05 V to +0.60 V) for monitoring the glucose oxidation process. High selectivity and high sensitivity for glucose detection was observed at this electrode when the potential was held at +0.05 V and +0.60 V, respectively.

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References and Notes

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