A Comparison of a Nanostructured Enzymeless Au/Fe$_2$O$_3$/MWCNTs/GCE Electrode and a GOx Modified One in Electrocatalytic Detection of Glucose


Abstract: Acid functionalized multi-walled carbon nanotubes (f-MWCNTs) were decorated with Au and Fe$_2$O$_3$ nanoparticles (FeONPs) and deposited on glassy carbon electrode (GCE). The resulting hybrid Au/Fe$_2$O$_3$/f-MWCNTs/GCE electrode and the one further modified by glucose oxidase were compared for detection of glucose. FeONPs and Au were deposited on the f-MWCNTs by sonication-assisted precipitation and deposition-precipitation methods, respectively. The morphology and structure of the samples were characterized by transmission electron microscopy, scanning electron microscopy, X-ray diffraction and Raman spectroscopy. A uniform distribution of FeONPs with an average size of 5 nm increased the surface area of functionalized nanotubes from 39 to 50 m$^2$/g. The electrocatalytic glucose detection on the modified electrodes was evaluated using cyclic voltammetry and chronoamperometry in 0.1 M phosphate buffer solution at pH 7.0. The non-enzymatic and enzymatic electrodes show sensitivity of 512.4 and 921.4 mA/mM.cm$^2$ and detection limit of 1.7 and 0.9 mM, respectively. The enzymatic and enzymeless electrodes retained more than 70% and 80% of their cathodic faradic current after 70 days, respectively. The sensing mechanism of the non-enzymatic biosensor is described through the reaction of glucose with iron (III) ions, while in the case of enzymatic electrode, glucose is oxidized by glucose oxidase.

Keywords: Carbon nanotubes · Iron oxide · Gold · Glucose biosensors

1 Introduction

The development of easy and reliable methods for determination of glucose received considerable attention, mainly due to its importance in various fields such as medical diagnostics, environmental monitoring, food processing and biotechnology [1,2]. Among numerous glucose determination methods, electrochemical approaches include enzymatic and non-enzymatic electrodes are promising, owing to their fast response, simplicity and low cost [3].

Commonly applied electrochemical glucose biosensors are based on immobilization of glucose oxidase (GOx) and show high selectivity and sensitivity [4]. The main limitations of GOx-based biosensors are their instability and inefficient electron transfer [5,6]. Compared to enzyme-based biosensors, non-enzymatic ones have some advantages such as stability, simplicity and low cost [6]. Lack of selectivity is the most important restriction of enzyme-free glucose biosensors, which limit their practical applications [5]. Hybrid materials made from metallic nanomaterials and carbonaceous nanostructures such as graphene and carbon nanotubes (CNTs), have been utilized in the fabrication of glucose biosensors, in order to overcome the limitations [7,8,9].

CNTs, owing to their unique properties such as high surface to volume ratio, good electron transfer rate and suitable surface chemistry for adhering external molecules, have been used as electrochemical matrices [10]. The main drawback of CNTs for the solution phase applications is their hydrophobic nature, which hinder CNTs uniform and stable dispersion. To overcome this challenge and reinforce the surface properties, CNTs can be functionalized with surfactants [11], polymers [11], biomolecules [12] and metal/metal oxide nanoparticles (NPs) [11]. The hybrid of CNTs and different metallic NPs such as Pt [13], Au [14], ZnO [15,16], Cu$_2$O [17], MnO$_2$ [2] iron oxides [1,18] and bimetallic composites [9] have been used for glucose biosensors.

However, the Fe$_2$O$_3$ NPs, due to their good biocompatibility, direct electrocatalytic oxidation of glucose, chemical stability and easy preparation, are of the great interest for preparing CNT hybrids in electrochemical analysis of glucose [18,19]. A combination of hematite and Au NPs with facile bioconjugation and multiple oxidation states [5], could supply more binding sites for GOx, which provides suitable micro-environment for better electron transferring, as a result [20]. In this work, acid functionalized MWCNTs were decorated with FeONPs and Au NPs to investigate the performance of enzymatic and non-enzymatic glucose biosensors based on

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Au/Fe₂O₃/f-MWCNTs hybrid. To the best of our knowledge, the hybrid of MWCNTs, FeONPs and Au has neither been used as a modified enzymeless electrode nor as a GOx immobilization matrix in detection of glucose.

2 Experimental

2.1 Chemical Agents

MWCNTs (with density of 1.5–1.7 g/cm³) were purchased from BuckyUSA Co. All other agents were of analytical grade and obtained from Sigma-Aldrich and Merck. The deionized water was used for preparation of all the aqueous solutions.

2.2 Functionalization of MWCNTs

0.5 g MWCNTs was treated in 175.0 mL of HNO₃ (10.0 M) solution for 4 h under reflux conditions [6]. The nanotubes suspension was then centrifuged and washed with deionized water and finally dried at 80°C for 12 h. This sample is designated as f-MWCNTs.

2.3 Synthesis of Au/Fe₂O₃/f-MWCNTs Hybrid

Fe₂O₃/f-MWCNTs hybrid was prepared by sonication-assisted precipitation method. Using an ultrasonic homogenizer (250 UL, Helchier, 250 W), 50.0 mg of f-MWCNTs was dispersed in 0.4 L of deionized water and sonicated for 60 min and then 25.3 mg of Fe(NO₃)₃·9H₂O was added to the solution, while continuing sonicating for another hour. Na₂CO₃ solution was slowly added to the Fe³⁺ suspension (0.16 mM) to reach pH 11.0 at 7°C, to obtain FeONPs [21]:

\[
\begin{align*}
\text{Fe(NO}_3\text{)}_3 \cdot 9\text{H}_2\text{O} & \rightarrow \text{Fe}^{3+} + 3\text{NO}_3^- \\
2\text{Fe}^{3+} + 6\text{OH}^- & \rightarrow \text{Fe}_2\text{O}_3 + 3\text{H}_2\text{O}
\end{align*}
\]

The final suspension was centrifuged, washed with deionized water and dried. The prepared hybrid was subsequently calcinated at 200°C for 2 h and named as Fe₂O₃/f-MWCNTs.

The Au/Fe₂O₃/f-MWCNTs hybrid was synthesized by a deposition-precipitation approach [22]. 50.0 mg of Fe₂O₃/f-MWCNTs sample was suspended in an aqueous HAuCl₄ solution (2.5 mg/mL) and the aqueous NaHCO₃ solution was then added dropwise to the suspension and stirred for 1 h. The precipitate was washed, dried and then the prepared hybrid was calcinated at 170°C for 2 h. This hybrid hereafter is called Au/Fe₂O₃/f-MWCNTs.

2.4 Fabrication of Modified Electrodes

A PalmSens potentiostat/galvanostat workstation and standard three-electrode cell was employed for electrochemical studies. The electrode system consisting of a modified glassy carbon electrode (GCE, A = 0.07 cm²), a platinum wire as the auxiliary electrode and a KCl saturated Ag/AgCl reference electrode. Phosphate buffer solution (PBS, 0.1 M, pH = 7.0) was used as an electrolyte. The GCE was polished by aluminum slurry, washed with deionized water and sonicated in a mixture of water and ethanol to obtain cleaned surface. 15.0 µL of the MWCNTs hybrid suspension (5.0 mg/mL) was dropped on the bare GCE and dried at 60°C. To fabricate enzymatic electrode, 10.0 µL of the GOx (G7141-50KU) solution (14.0 mg/mL) was cast onto the surface of modified electrode and dried at 4°C for 24 h and was stored at 4°C, when it was not in use.

2.5 Characterization

The morphology and structure of f-MWCNTs and their hybrids were characterized by scanning electron microscopy (SEM, IROST, 15.0 kV) and transmission electron microscopy (TEM, Philips-CM30-250KV microscope) equipped with an energy-dispersive X-ray (EDX) detector. Raman (SENTERRA model at wavelength of 785 nm) and X-ray powder diffraction (XRD, XPert PW3040 using Cu Ka radiation) were used to identify composition and phase structure of nanotube hybrids. The BET specific surface area of the samples was determined, using a Quanta chrome CHEMBET-3000 apparatus, after degassing at 200°C for 2 h.

3 Results and Discussion

3.1 Characterization

3.1.1 Raman Spectra

Figure 1 shows the Raman spectra of the f-MWCNTs and Au/Fe₂O₃/f-MWCNTs samples. The strong peaks at 1292 and 1581 cm⁻¹ can be assigned to the D and G bands of MWCNTs, respectively. The ratio of these bands densities, i.e. I_D/I_G, is 1.96 and 1.59 for f-MWCNTs and Au/Fe₂O₃/f-MWCNTs samples, respectively, indicating that the decora-
The treatment of nanotubes with FeONPs and Au NPs increases the degree of crystallinity of nanotube hybrids [23,24]. Moreover, the Au/Fe$_2$O$_3$/f-MWCNTs sample shows two peaks at 221 and 285 cm$^{-1}$ attributed to the symmetric bend and symmetric stretch of oxygen atoms along Fe–O bonds [25].

3.1.2 Morphology by SEM and TEM

SEM image of the f-MWCNTs is presented in Figure 2a. The BET surface area of the acid functionalized f-MWCNTs is 38.9 m$^2$/g, comparable to 28.5 m$^2$/g for the
MWCNTs. This may be due to the MWCNTs breakup to some extent during the functionalization.

SEM, TEM and HR-TEM images of Au/Fe₂O₃/f-MWCNTs (Figure 2b, c and d) show the decoration of nanotubes surface by FeONPs with the average size of 5 nm (Figure 2f), while still there are plenty of unfilled spaces on the surface of the f-MWCNTs. Although Au NPs are negatively charged, they may aggregate [20] and form larger particles. SAED characterization (Figure 2e) displays a ring pattern, which is attributed to the crystalline surfaces of Au NPs. This crystalline structure could be indexed to face-centered cubic (FCC) phase [26]. EDX analysis of Au/Fe₂O₃/f-MWCNT hybrid approves the presence of 83.3, 8.6, 3.8 and 4.3 wt% of C, O, Fe and Au elements in the sample, respectively.

3.1.3 XRD Result

XRD pattern of Au/Fe₂O₃/f-MWCNTs hybrid (Figure 3) illustrates a sharp peak at 26.7° which corresponds to (002) plane of graphitic carbon. The dominant diffraction peaks at 38.7°, 44.9°, 65.1°, 78.0° and 82.2° corresponds to (111), (200), (220), (311) and (222) crystalline plane of Au NPs, respectively [11]. These characteristic peaks are indexed to the FCC structure of the gold, which is confirmed by the SAED data. The peaks at 37.2°, 53.2 and 63.1 are assigned to (222), (422) and (440) crystalline planes of FeONPs, respectively (JCPDS 25-1402). These peaks are relatively short and broad, possibly due to the small size of iron oxide in the sample. The results are in accordance with the Raman pattern (Figure 1) which shows that hybridization of MWCNTs with FeONPs and Au NPs increases the crystallinity of the sample.

3.2 Glucose Biosensor Performance

3.2.1 Effects of Fe₂O₃ Loading

Figure 4A illustrates the cyclic voltammograms of different electrodes containing various FeONPs loadings on f-MWCNTs/GC. The presence of FeONPs increases the cathodic current intensity of f-MWCNTs/GCE to 12, 52 and 7 mA for 0.08, 0.16 and 0.23 mM Fe³⁺ in the Fe(NO₃)₃ solution, respectively. This indicates that the 0.16 mM concentration is the optimum value which provides the maximum electroactive surface area. The higher amounts of iron oxide may lead to its aggregation on the nanotubes surface and blocking the electroactive surface of the modified electrode, as a result. The 0.16 mM concentration of Fe³⁺ was selected for fabricating Fe₂O₃/f-MWCNTs hybrid in this work.

Fig. 3. XRD pattern of Au/Fe₂O₃/f-MWCNT hybrid.

Fig. 4. A) Cyclic voltammograms of different electrodes containing various loadings of (a) 0.08, (b) 0.16 and (c) 0.23 mM Fe³⁺ in the solution. B) Cyclic voltammograms of (a) bare GCE, (b) f-MWCNTs, (c) Fe₂O₃/f-MWCNTs/GCE, (d) Au/Fe₂O₃/f-MWCNTs/GCE and (e) GOx/Au/Fe₂O₃/f-MWCNTs/GCE in PBS (pH = 7.0); scan rate = 50 mV/s.

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3.2.2 Non-Enzymatic and Enzymatic Electrodes

The cyclic voltammograms of different fabricated electrodes in PBS are given in Figure 4B. The cyclic voltammogram of the bare GCE (Figure 4B.a) shows small charging current with no electrochemical peaks. Addition of f-MWCNTs on the bare GCE enhances current intensity (Figure 4B.b), which could be related to the increases in the surface area and facilitation of electron transferring. The presence of FeONPs on the f-MWCNTs/GCE, improves charging current and generates a well-defined anodic and cathodic peaks at 0.05 V and −0.31 V, respectively (Figure 4B.c). This redox wave can be assigned to the Fe(III)/Fe(II) redox couple [6]:

\[
\text{Fe(III)} + \text{OH}^- \leftrightarrow \text{Fe(II)} \text{ OH} + e^- \tag{3}
\]

Compared to Fe₂O₃/f-MWCNT/GCE, the Au/Fe₂O₃/f-MWCNT/GCE (Figure 4B.d) has higher charging and faradic current which reveals that Au NPs increase the electroactive surface area of the modified electrode. Au NPs may act as electron-transfer mediators that receive electrons directly from GOx and expedite electron-transfer to the electrode surface [14].

The immobilization of GOx on the Au/Fe₂O₃/f-MWCNT/GCE (Figure 4B.e) creates a redox wave around −0.28 V, which is related to the redox center of GOx [27]:

\[
\text{GOx (FAD)} + 2e^- + 2\text{H}^+ \leftrightarrow \text{GOx (FADH}_2) \tag{4}
\]

The surface average concentration of GOx (Γ) can be calculated using the following equation [28]:

\[
\Gamma = \frac{q}{nFA}
\]

where q is the quantity of consumed charge (area under oxidation/reduction peaks), A is the surface area of the modified electrode, which is 7.07 × 10⁻² cm² corresponding to 430 times the bare GCE surface area, n is the number of electron transferred and F is Faraday constant (96485 C/mol). The calculated Γ is about 4.4 × 10⁻¹⁰ mol/cm² for GOx which is much larger than the theoretical value, i.e. 2.86 × 10⁻¹² mol/cm², [27] for the GOx monolayer. These results suggest that multilayer of GOx contributes to electron-transfer process. Au NPs can easily immobilize tight multilayers of GOx, due to their high surface area and free energy [29].

3.2.3 Effect of Scan Rate on the Modified Electrodes

Figure 5 and Figure 6 show cyclic voltammograms of the non-enzymatic and enzymatic electrodes at various scan rates. As shown, the current intensity and the peak-to-peak separation potential increases with the increase of scan rate. The cathodic and anodic peak currents of Au/Fe₂O₃/f-MWCNTs and GOx/Au/Fe₂O₃/f-MWCNTs electrodes have linear relation with scan rate in the range of 30–200 mV/s.

Fig. 5. (a) cyclic voltammograms of Au/Fe₂O₃/f-MWCNTs/GCE in PBS (pH = 7.0) at different scan rates: 30, 50, 80, 100, 130, 150, 170, 180, 200, 230, 250, 280, 300, 330, 350, 380, 400 and 430 mV/s.

Calibrated plot of the peak currents vs. (b) scan rate in the range of 30–200 mV/s and (c) square root of scan rate in the range of 180–430 mV/s.
(Figure 5b) and 30–210 mV/s (Figure 6b), respectively. These peak currents show linear dependency on the square root of scan rate in the range of 180–430 mV/s for enzyme-free (Figure 5c) and in the range of 240–360 mV/s for enzyme-based (Figure 6c) electrode. These results indicate that the electron transferring of non-enzymatic and enzymatic electrodes are semi-reversible, surface-control in low scan rates and diffusion-control in high scan rates.

3.2.4 Amperometric Response of Modified Electrodes

Figure 7 presents amperometric response of prepared electrodes to sequential addition of glucose in PBS at a potential of $-0.55 \text{ V}$. As expected, both electrodes show step-like increasing current responses. In the case of non-enzymatic electrode, this current increase could be justified through glucose oxidation reaction and electron generation [30]:

$$2\text{Fe}^{3+} + \text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2\text{Fe}^{2+} + \text{C}_6\text{H}_{10}\text{O}_6 + 2\text{H}^+$$  \hspace{1cm} (6)

$$\text{C}_6\text{H}_{10}\text{O}_6 + \text{H}_2\text{O} \rightarrow 2\text{H}^+ + \text{C}_6\text{H}_{10}\text{O}_7$$  \hspace{1cm} (7)

$$2\text{Fe}^{2+} \rightarrow 2\text{Fe}^{3+} + 2\text{e}$$  \hspace{1cm} (8)

The glucose oxidation for enzymatic electrode can be expressed as follows:

$$\text{GOx} (\text{FAD}) + \text{C}_6\text{H}_{12}\text{O}_6 \rightarrow \text{GOx} (\text{FADH}_2) + \text{C}_6\text{H}_{10}\text{O}_6$$  \hspace{1cm} (9)

The redox reaction of GOx is a two electrons – two protons process, in which GOx catalyze the glucose oxidation.
oxidation. The enzymeless electrode shows relatively high sensitivity of 512.4 μA/mM·cm² towards glucose detection which is determined through the slope of linear part of corresponding calibration curve (Figure 7 inset I) in the range of 0.01–0.08 mM. The detection limits of this electrode is estimated to be 1.7 μM (based on the signal to noise ratio of 3). Compared to non-enzymatic electrode, the enzymatic one shows linear behavior in the range of 0.01–0.06 mM (Figure 7 inset II) with higher sensitivity of 921.4 μA/mM·cm² and lower detection limit of 0.9 μM.

The diffusion coefficient of glucose in PBS can be determined using linear relationship of I vs. \( T^{1/2}/C_0 \) (Figure 8) which corresponds to the Cottrell equation [28]:

\[
I = \frac{nFAD^{1/2}C}{\pi^{1/2}D^{1/2}t^{1/2}} \tag{10}
\]

Where \( C \) and \( D \) are bulk concentration and diffusion coefficient of glucose, respectively and \( A \) is the surface area of the bare electrode. From the slope of this plot, diffusion coefficient of glucose in PBS is estimated to be 1.7 \( \times 10^{-8} \) cm²/s.

3.2.5 Selectivity of the Modified Electrodes

One of the important analytical features of the glucose biosensors is their ability to discriminate between glucose and interfering species such as ascorbic acid (AA) and uric acid (UA), which may donate non-glucose-derived electrons at working potential. The response of enzyme-free and enzyme-based electrode during successive addition of glucose, AA and UA are illustrated in Figure 9. Both electrodes show significant response to glucose solution (2.0 mM) despite of the negligible responses toward AA and UA solution (0.1 mM). The response deviation of AA and UA to glucose is 4.2 and 3.7 % for non-enzymatic electrode and 1.8 and 2.3 % for enzymatic one, respectively. These results demonstrate that AA and UA don’t disturb the detection of glucose at \(-0.55\) V, thus the fabricated electrodes are fairly selective.

3.2.6 Stability of the Modified Electrodes

The long-term stability of the Au/Fe₂O₃/f-MWCNTs/GCE and GOx/Au/Fe₂O₃/f-MWCNTs/GCE are examined by comparing the cathodic peak current of the modified electrodes every 7 days, as presented in Figure 10. The non-enzymatic and enzymatic electrodes retain more than...
80 and 70% of their initial cathodic peak current after 70 days, respectively, indicating a relatively high long-term stability in electrochemical activity of the constructed electrodes. The less stability of enzyme-based biosensor might be due to the inherent nature of GOx [5].

The analytical characteristics of the fabricated biosensors along with some other biosensors based on the iron oxide and Au NPs are summarized in Table 1. To compare the stability of the electrodes, the reduction percent of cathodic peak current is measured per day. Although the linear range of our proposed biosensors is not as wide as other biosensors, these electrodes are more stable with higher sensitivities and lower detection limits.

### 4 Conclusion

The enzymatic and non-enzymatic glucose biosensors based on the Au/Fe3O4/f-MWCNTs hybrid were fabricated and their performances in detection of glucose were investigated. The modified hybrid electrode surface area is enhanced by 430 times. In this case Fe3+/Fe2+ acts as the redox couple in the enzymeless electrode, and Au easily immobilize tight multilayers of GOx. Although the enzyme-based electrode has less stability, it shows a higher sensitivity and lower detection limit compared to the enzyme-free electrode. The attractive features such as easy preparation, high sensitivity, long-term stability and low detection limit make the Au/Fe3O4/f-MWCNTs hybrid as a good candidate for the construction of enzyme-based and enzymeless glucose biosensors.

### References

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