Fast and clean dielectric barrier discharge plasma functionalization of carbon nanotubes decorated by electrodeposited nickel oxide: Application to glucose biosensors

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Abstract

Multi walled carbon nanotubes (MWCNTs) have been functionalized through a dielectric-barrier-discharge plasma in presence of H₂O-saturated atmosphere at 70°C and atmospheric pressure. The functionalized MWCNTs (F-CNTs) were decorated with electrochemically deposited 10 nm NiO nanoparticles, followed by glucose oxidase (GOx) immobilization and the treated electrode has been used for glucose detection. TEM, FE-SEM, TPD and XPS techniques were used to characterize the NiO/F-CNTs samples. The maximum extent of oxygen containing functional groups including carbonyl, hydroxyl and carboxylic groups has been formed at the plasma contact time of 4 min. The optimum time for chronoamperometric deposition of NiO was 3 min.

The presence of GOx on the NiO/F-CNTs electrode displayed a quasi reversible and surface controlled reduction-oxidation wave at −0.52 V. The peak separation of this wave is 0.05 V. The GOx/NiO/F-CNTs electrode showed a linear performance in the glucose concentration of 0.2-3.8 mM with sensitivity of 2.16 μA/mM and detection limit of 93.0 μM.

Keywords: Plasma, Functional, Carbon nanotubes, Nickel, Biosensor
1. Introduction

Hybrids of carbon nanotubes (CNTs) and metal oxide nanoparticles (NPs) or polymeric materials have received particular attention for their potential application in various devices such as ion batteries [1], fuel cells [2], medical instrument [3, 4], tissue engineering [5] and biosensors [6]. Glucose biosensors regarding their significance in different applications including clinical detection, food industries and environmental protection have remained the most attractive [7,8]. Many approaches including fluorescent spectroscopy [9], colorimetry [10], conductometry [11] and electrochemical [12,13] methods, have been improved for glucose monitoring. These techniques mostly rely on the detection of hydrogen peroxide, which has been generated through reaction of glucose oxidase (GOx) and glucose [14]. The electrochemical biosensors regarding their high sensitivity and selectivity, simplicity as well as low cost have been frequently used [13,15].

In spite of the exceptional properties of CNTs, inherent hydrophobic surface hinders their dispersion in aqueous solutions. Functionalization of nanotubes with hydrophilic groups improves their interaction with solvent matrices and enhances their abilities to be used in different applications such as biological systems [16]. The connection of different functional species to the CNTs surface can be achieved using either wet chemical or dry oxidation approaches. The later method including plasma functionalization is a fast, flexible and non-
polluting technique, which can create new active sites for enhancing the NPs loadings and reducing their sizes on the surface of CNTs. Dielectric barrier discharge (DBD) is a low temperature and atmospheric pressure plasma, which can produce a uniform discharge atmosphere. The quantity of functional groups and surface defects may be controlled by power and exposure time of the plasma [16, 17].

Among the various metal (oxide) NPs utilized in combination with CNTs, NiO with small band gap [18] has acquired special interest, due to its ion exchange, molecular adsorption, environmental friendliness, catalytic and electrochemical properties [19,20]. In addition, the nickel can be combined with the other metals such as cobalt and copper to enhance the electrochemical and catalytic properties of the biosensor electrode [21, 22]. To the best of author’s knowledge, the composition of NiO and CNTs was rarely utilized for analytical glucose detection. One of the promising methods for fabrication of CNT-NiO hybrid is electrochemical deposition approach, in which the thickness of NiO film might be controlled through regulating parameters of the electrochemical process [23].

In the present work, multi walled carbon nanotubes (MWCNTs) have been functionalized through DBD plasma in H₂O-saturated atmosphere at 70°C for 4 min. Afterwards, the NiO-NPs have been decorated on the MWCNTs surface by an electrodeposition method. Finally, the glucose sensor has been
constructed using GOx immobilization on the surface of NiO/F-CNTs and utilized in the glucose detection experiments.

2. Materials and methods

2.1. Reagents

GOx (EC 1.1.3.4) and MWCNTs have been purchased from Sigma-Aldrich and Shenzhen Nanotech Co. Ltd., respectively. All other reagents are of analytical grade, which have been obtained from Merck. The phosphate buffer solution (PBS, KH$_2$PO$_4$ + K$_2$HPO$_4$) and the other solution samples have been made using deionized water at pH 7.0.

2.2. Equipment

The electrochemical studies have been performed through the typical three electrode cells which includes Pt as a counter electrode, a working glassy carbon electrode (GCE, A = 3.14 × 10$^{-2}$ cm$^2$) and Ag/AgCl as a reference electrode. The experiments have been carried out at ambient conditions. The structure, morphology and oxygenated groups of the nanotube hybrids have been examined through transmission electron microscopy (TEM) equipped with energy dispersive X-ray spectroscopy (EDS), field emission scanning electron microscopy (FE-SEM), X-ray photoelectron spectroscopy (XPS), and Fourier transformed infrared spectroscopy (FTIR). The temperature-programmed desorption (TPD) has been performed for quantitative analyses of the functional
groups of plasma-functionalized MWCNTs. This method was explained in other our work [12].

2.3. DBD plasma functionalization of MWCNTs

In order to remove surface groups possibly produced via synthesis or purification period, the purchased MWCNTs were annealed at high temperature (1000˚C) in He. The process of functionalization (Fig. 1a) has been done through DBD in saturated air. The zero air with flow rate of 40.0 sccm was humidified using H$_2$O at 70˚C and moved over the annealed MWCNTs inside a plasma reactor. The details of the reactor have been given elsewhere [16]. The nanotube samples were exposed to DBD plasma at various times (i.e. 1, 2, 4 and 6 min). In this text, the DBD functionalized nanotubes are called F-CNTs.

2.4. Preparation of electrodes

The unmodified electrode has been cleaned using polishing tissue and alumina slurry and sonicated in ethanol/water mixture. The F-CNTs have been dissolved in nafion solution (0.5 wt. %) and mixed for 10 min, using ultrasonic homogenizer (250 UL Hielscher) to obtain a uniform suspension (2.0 mg/ml). Then 10.0 µl of this mixture has been casted on the surface of GCE and dried at 25 ˚C. To fabricate the NiO/F-CNTs/GCE (Fig. 1b), the F-CNTs/GCE has been inserted in the solution of NiNO$_3$/NaNO$_3$ (0.005 M) at constant potential (i.e. –0.8 V), versus reference electrode, for 3 min and rinsed in water. Subsequently, 5.0 µL of the GOx solution with concentration of 10.0 mg/ml has been
immobilized on the-NiO/F-CNTs/GCE (Fig. 1c) surface and dried at 4°C overnight. A concentration range of 0.01-4.0 mM glucose was selected for glucose detection experiments. The GOx/NiO/F-CNTs/GCE was stored at 4°C in the refrigerator.

3. Results and discussion

3.1. Characterization

3.1.1. TPD analyses of F-CNTs

Figure 2 presents TPD profiles of F-CNTs samples functionalized in the humid air for different exposure times. The gas evolution profile starts from 160°C and shows three major peaks around 250, 400 and 800°C. These evolved gases, which mainly include H$_2$O, CO$_2$ and CO are the result of decomposition of oxygenated functional groups [24]. The area under the peaks of TPD spectrum (Fig. 2 inset) corresponds to total amount of the evolved gases. As the exposure time increases from 1 to 4 min, the amount of evolved gases enhances. An additional exposure time of 6 min results in a smaller quantity of functional groups. Since there is limited number of defect points on the surface of nanotubes, as primary site for functionalization, further plasma treatment leads to detachment of some of the functional groups [25]. Figure 2B shows the TPD profile of MWCNTs functionalized for 4 min, which is deconvoluted to 5 peaks corresponding to H$_2$O, CO$_2$ and CO evolution during the decomposition of
various oxygenated functional groups including carboxylic, anhydride, phenol, lactone, carbonyl and quinone groups.

In order to evaluate the emerged gases, the TPD instrument was coupled with an FTIR gas cell and the results of identified and quantified evolved gases at various temperatures are shown in Fig. 3. The major component in the low temperature region of 160-280°C is H₂O, which is originated either from water trapped in the micropores or from interaction of adjacent OH-containing groups [26]. Decomposition of these functional groups leads to the formation of carboxylic anhydrides and H₂O molecules [27].

The intermediate temperature region in the range of 260-600°C is mostly a mixture of H₂O and CO₂. These gases have evolved from dissociation of phenol and carboxylic/anhydride groups [27, 28]. The slight CO evolution around 600°C is from decomposition of anhydride and phenol groups [28]. The high temperature region of 600-1000°C is mainly attributed to the evolution of CO₂ and CO from lactone and carbonyl/quinone groups, respectively [28]. The existence of these oxygenated functional groups on the F-CNTs surface was confirmed by FTIR in our other work [12]. Another major source of CO₂ desorption in high temperature region is the secondary reactions between CO and oxygen surface species as the following:

\[ CO + C - O \rightarrow CO_2 + C_u \]  \hspace{1cm} (1)
where \( C_a \) is an adsorption site on the CNTs surface and C-O is oxygenated group attached to the nanotubes. During the TPD, \( C_a \) may also react with the desorbed H\(_2\)O, CO\(_2\) and CO and reform the oxygenated functional groups.

3.1.2. XPS results

To further study the surface chemical state of different F-CNTs samples, XPS survey spectra were employed. The XPS data for annealed sample and F-CNTs (Fig. 4a) indicates the presence of carbon and oxygen atoms. During DBD plasma treatment of MWCNTs, the photoelectron peak of O 1s at 533 eV increases from 1.5% to 5.6% for the annealed and functionalized samples, respectively. In order to examine the bond structure of the oxygen-containing species, the C 1s peak was deconvoluted and the results were presented in the Fig. 4a inset. The major component at 284.9 eV was attributed to C=C bond as well as the peaks at 286.3, 287.2 and 288.4 eV can be assigned to the hydroxyl, carbonyl and carboxyl groups, respectively [29]. These oxygenated species are created through interaction of the reactive components such as O, O\(_3\), H and OH with the surface of MWCNTs. These reactive species generated during DBD plasma in humid air atmosphere can split C=C bond and interact with open ends and defect points of CNTs [16]. The oxygenated groups have polar properties and make negative charge on the surface of CNTs and improve dispersion in aqueous solutions [12].
Figure 4b illustrates XPS spectra of NiO/F-CNT sample. Attachment of nickel oxide to the surface of F-CNTs, leads to the increase of oxygen atoms percentage to 11.3%. Two photoelectron peaks at 857.7 and 875.7 eV might be related to the Ni 2p\textsubscript{3/2} and Ni 2p\textsubscript{1/2}, respectively [30]. Moreover, the Ni atoms percentage in the NiO/F-CNT hybrid was calculated to be about 5.4%.

3.1.3. Morphology of F-CNTs and NiO/F-CNTs

FE-SEM micrograph obtained for F-CNTs (Fig. 5a) reveals a twisted morphology of MWCNTs with diameters of 20-30 nm while the length reaches to tens of micrometers. The SEM of NiO/F-CNTs sample (Fig. 5b) shows that NiO-NPs were deposited on the nanotubes surface and created a relatively thin film. TEM image of NiO/F-CNTs (Fig. 5c) further shows that NiO has uniformly and directly grown on the CNTs surface. From the HRTEM image of the NiO/F-CNTs (Fig. 5d) the particle size of NiO-NPs was determined to be approximately 10 nm. EDS analyses (data not shown here) confirms the presence of carbon, oxygen and nickel elements in the NiO/F-CNTs hybrid.

3.2. Biosensor performance

3.2.1. Optimum deposition time

Figure 6 presents cyclic voltammogram of NiO/F-CNTs electrodes synthesized by chronoamperometry at different deposition times. The formation of NiO-NPs (Equation 2-4) [20] on the F-CNTs/GCE surface increases current, indicating that the electroactive surface of NiO/F-CNTs/GC electrode is enhanced.
2OH⁻ + Ni²⁺ → Ni(OH)₂  \hspace{1cm} (2)

Ni(OH)₂ → H₂O + NiO  \hspace{1cm} (3)

2C = O + 2Ni²⁺ → 2C – NiO  \hspace{1cm} (4)

The charging current improvement is the greatest for optimum deposition time of 3 min and further increase of the deposition time may cause the agglomeration of NiO-NPs and reduction of the reactive surface of the modified working electrode.

3.2.2. Enzyme immobilization

The modified electrodes were investigated using cyclic voltammetry in the range of −0.8 - 0 V and the results were illustrated in Fig. 7. The anodic charging current has increased from 2.0 µA for the bare GCE (Fig. 7a) to 15.0 µA for F-CNTs/GCE (Fig. 7b), indicating that the functionalized nanotubes improve the reactive surface of treated electrodes. The GOx immobilization on the F-CNTs/GC electrode (Fig. 7c), has created a reduction-oxidation wave centered at −0.54 V with peak to peak potential separation (ΔE_p) of 0.08 V. Because of the existence of NiO nanoparticles on the F-CNTs/GCE (Fig. 7d), the reactive surface has further increased and enhanced the efficient points for adsorption of GOx. The GOx/NiO/F-CNTs/GCE (Fig. 7e) presents a reduction-oxidation wave at −0.52 V with ΔE_p of 0.05 V. The metal nanoparticles may act as effective electron conducting channels and decrease working potential and ΔE_p for the enzymatic electrode. The modified electrodes, in absence of GOx, show no faradic current as a result of oxidation-reduction process, suggesting
that reduction-oxidation activity of the modified electrodes is assigned to the immobilized GOx as follows:

$$GOx(FAD) + 2e + 2H^+ \leftrightarrow GOx(FADH_2)$$  \hspace{1cm} (5)

3.2.3. Effect of scan rate

The effect of various scan rates on the cyclic voltammograms of GOx/NiO/F-CNTs electrodes was presented in Fig. 8. The $\Delta E_p$ of GOx peaks has slightly increased with an increase in the scan rate. Moreover, the currents of cathodic and anodic peaks are closely equal and shows linear behavior ($R^2 = 0.99$) versus scan rate in the range of 10-250 mV/s. The results (Fig. 8b) illustrates that the reduction-oxidation process is quasi reversible and surface confined [31].

The plots of cathodic and anodic potential peak ($E_p$) versus log of scan rate is given in Fig. 8c. As shown, in the scan rate of 80 to 250 mV/s, two lines with the slopes of $\pm 2.3RT/\alpha nF$ are generated. In this equation, $\alpha$ is coefficient of charge transfer, $n$ is the number of transferred electrons as well as other parameters are constant ($T = 298$ K, $R = 8.314$ J/mol.K, $F = 96485$). Through the slope of plotted lines, the mean values of $\alpha$ and $n$ were approximated to be 0.39 and 2, respectively. In addition, the charge transfer rate constant ($k_s$) of the enzyme at the scan rate of 250 mV/s has been evaluated to be $1.12 \pm 0.1$ s$^{-1}$ based on Laviron’s equation [32]:

$$\log k_s = \alpha \log (1-\alpha) + (1-\alpha) \log \alpha - \log \left(\frac{RT}{nFV}\right) - \alpha(1-\alpha) \frac{nF\Delta E_p}{2.3RT}$$  \hspace{1cm} (6)
The average GOx concentration ($\Gamma$) was calculated through following equation:

$$I_p = \frac{n^2 F^2 A \Gamma}{4RT} \nu$$  \hspace{0.5cm} (7)

where $A$ denotes the surface area of the treated electrode (0.125 cm$^2$) and $I_p$ is the peak current. Through the slope of plot of peak currents versus scan rate (Fig. 8b), the GOx concentration was calculated to be $1.2 \times 10^{-9}$ mol/cm$^2$. In addition, the ratio of GOx/NiO was estimated to be about 5.4 and suggests that multilayered GOx involve in the process of electron transferring.

3.2.4. Glucose detection using GOx/NiO/F-CNTs/GCE

The response of GOx/NiO/F-CNTs/GCE toward addition of glucose has been studied using cyclic voltammetry [33, 34] and results have been shown in Fig. 9. The oxidation current decreases from 19.8 to 11.0 $\mu$A with sequential glucose adding due to the blocking effect of the modified electrode [28]. The reaction mechanism of glucose in the sensor matrix has been proposed to be [13]:

$$Glucose + GOx(FAD) \rightarrow Gluconolactone + GOx(FADH_2)$$ \hspace{0.5cm} (8)

$$2NiO + 2H_2O \rightarrow 2Ni(OH)_2 + O_2$$ \hspace{0.5cm} (9)

$$2OH^- + 2Ni(OH)_2 \leftrightarrow 2H_2O + 2NiO(OH) + 2e^-$$ \hspace{0.5cm} (10)

$$GOx(FADH_2) + 2NiO(OH) \rightarrow GOx(FAD) + 2NiO + 2H_2O$$ \hspace{0.5cm} (11)
Figure 9 inset presents the corresponding curve of calibration depending on anodic faradic current response. The current response in the range of 0.2-3.8 mM presents a linear performance with the equation of \( I (\mu A) = -2.16 \ C (\text{mM}) + 19.32 \) (\( R^2 = 0.99 \)). The modified electrode shows relatively high sensitivity of 2.16 \( \mu A/\text{mM} \) that may be assigned to the high surface area of F-CNTs and high electro-catalytic activity and adsorption ability of NiO-NPs. The detection limit of the fabricated electrode has been estimated to be 93.0 \( \mu M \) based on the signal-to-noise ratio of 3. Table 1 reports the analytical characteristics of the GOx/NiO/F-CNTs/GCE. This result is comparable with other works that have utilized nickel oxide in the medium of immobilization. It is noted that, the electrode preparation process in the present work is fairly faster and simpler than that of other reports.

4. Conclusions

GOx/NiO/F-CNTs modified electrodes for glucose detection were fabricated by treatment of nanotubes in the DBD plasma reactor in humid air, followed by electrodeposition of NiO and immobilization of GOx. The functionalization increases the oxygen-containing groups of MWCNTs from 1.5 to 5.6%, which in turn lead to an important improvement of the anodic charging current. The larger amount of oxygenated groups roll as active centers for uniform decoration of the F-CNTs with 10 nm NiO nanoparticles. This decoration improves the electroactive surface area, and enhance immobilization of GOx by
electrostatic attraction on the basic NiO. The presence of NiO nanoparticles improves electron transfer efficiency and reversibility of GOx reduction-oxidation wave, which is a quasi reversible and surface-confined process.

References


**Figure and Table captions**

**Figure 1.** The process steps of GOx/NiO/F-CNTs/GC electrode fabrication for analytical glucose detection.

**Figure 2.** A) TPD spectra of MWCNTs functionalized at power = 30.6 W and various exposure times of (a) 1, (b) 2, (c) 4 and (d) 6 min. Inset: the influence of contact time on total amounts of evolved gases. B) Deconvoluted peaks of TPD profile of MWCNTs functionalized for 4 min corresponding to different functional groups.

**Figure 3.** The relative amounts of evolved H\textsubscript{2}O, CO and CO\textsubscript{2} from the MWCNTs functionalized in the DBD plasma for 4 min at various temperatures during the TPD experiment.

**Figure 4.** (a) The XPS spectra of annealed sample and F-CNTs. Inset: deconvolution of C 1s XPS spectra of F-CNTs. (b) XPS spectra of NiO/F-CNTs. Inset: the magnified region of Ni 2p: the peaks at 857.7 and 875.7 eV were attributed to Ni 2p\textsubscript{3/2} and Ni 2p\textsubscript{1/2}, respectively.

**Figure 5.** The SEM micrographs of (a) F-CNTs and (b) NiO/F-CNTs. (c) TEM image and (d) HRTEM of NiO/F-CNTs hybrid.
**Figure 6.** The cyclic voltammograms of blank F-CNTs and NiO/F-CNTs electrodes synthesized by chronoamperometry method at different deposition times.

**Figure 7.** The cyclic voltammograms of (a) bare GCE, (b) F-CNTs/GCE, (c) GOx/F-CNTs/GCE, (d) NiO/F-CNTs/GCE and (e) GOx/NiO/F-CNTs/GCE.

**Figure 8.** (a) The cyclic voltammograms of GOx/NiO/F-CNTs/GCE in 0.1 M PBS at various scan rates of 10-250 mV/s, (b) the peak current plot versus scan rate, (c) the plot of $E_p$ versus log (scan rate).

**Figure 9.** Cyclic voltammograms of the GOx/NiO/F-CNTs/GCE in presence of 0.2 (outer) to 3.8 mM (inner) glucose. Inset: the curve of calibration for oxidation currents at different glucose concentrations.

**Table 1.** The comparison of GOx/NiO/F-CNTs/GCE and other similar electrodes.
Figure 1
Figure 2B
Figure 3
Figure 4a
Figure 4b
Figure 5b
Figure 6
Figure 7
Figure 8
Figure 9
<table>
<thead>
<tr>
<th>Electrode material</th>
<th>Formal potential (V)</th>
<th>Sensitivity (µA.mM$^{-1}$)</th>
<th>Detection limit (µM)</th>
<th>Linear range (mM)</th>
<th>Ref.</th>
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<td>GOx/NiO/GC</td>
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<td>0.44</td>
<td>24</td>
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<td>[34]</td>
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<td>CHIT$^a$/GOx/NiO/GC</td>
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<td>47</td>
<td>1.5-7</td>
<td>[19]</td>
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<tr>
<td>NiCFP$^b$ electrode</td>
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<td>3.3</td>
<td>1</td>
<td>0.002-2.5</td>
<td>[7]</td>
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<tr>
<td>GOx/NiO/F-CNTs/GCE</td>
<td>– 0.52</td>
<td>2.16</td>
<td>93</td>
<td>0.2-3.8</td>
<td>This work</td>
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$^a$ chitosan, $^b$ carbon nanofiber paste.

Table. 1
Technical biography

Abbas Ali Khodadadi received his MSc in Chemical Engineering from University of Tehran in 1986 and his PhD in Catalysis and Reaction Engineering from University of Waterloo, Canada, in 1994. His research interests include nanostructured materials synthesis, surface functionalization, characterization and use as (Bio/Photo/Electro) catalysts, adsorbents, membranes in energy, environmental, chemicals production, gas/bio-sensors applications.

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