Plasma thiol-functionalized carbon nanotubes decorated with gold nanoparticles for glucose biosensor

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For the first time, MWCNTs were thiold functionalized using dielectric barrier discharge plasma in the presence of H\textsubscript{2}S/He at room temperature and atmospheric pressure. At the optimum plasma conditions, maximum amount of 0.57 wt.% thiol groups was formed on the surface of MWCNTs. The deposition-precipitation method was used to decorate MWCNTs with Au-nanoparticles. TEM, FE-SEM, FTIR, Raman, Ellman reagent method, XRD and cyclic voltametry were used to characterize the samples.

A glucose biosensor was fabricated by immobilizing glucose oxidase (GOx) on the Au/MWCNTs hybrid. The redox process of the GOx/Au/MWCNTs hybrid electrode was shown to be reversible and surface-controlled process in 0.1 M phosphate buffer solution at pH 7.0. The charge transfer coefficient and charge transfer rate constant for electron transfer between GOx and the electrode surface were calculated as 0.48 and 1.13 s\textsuperscript{-1}, respectively. The GOx/Au/MWCNT-modified electrode exhibited a linear behavior in the range of 0.4–4.0 mM glucose with detection limit of 3.0 µM and sensitivity of 23.1 µA/mM cm\textsuperscript{2}. The modified electrode retained more than 58% of its initial faradic current value after 50 days.

1. Introduction

Glucose biosensors, due to their importance in clinical diagnosis, biology and food industry, have been an important issue in both academia and industries. Because of the high sensitivity and selectivity of glucose oxidase (GOx), the sensors based on this enzyme are used widely for glucose detection. The main limitations of GOx-based biosensors are their short-time stability and difficult electron transferring process between electroactive center of enzyme and surface of electrode. In order to overcome these barriers, the researchers in the field have tried to make use of the benefits associated with nanostructures such as carbon-based nanomaterials and metal nanoparticles (NPs) [1,2].

Carbon nanotubes (CNTs), owing to their chemical stability and inactivity, strong adsorption ability and high aspect ratio, are quite useful for the immobilization of glucose oxidase and electron transferring processes [3,4]. The attachment of metal NPs to the surface of CNTs results in the formation of hybrid nanostructure with synergistic properties. CNTs in combination with metal (oxide) NPs such as Pt [3], Ag [5], Au [6], zinc oxide [7], copper oxide [8] and bimetallics [9] have been utilized in glucose biosensors. Among these nanomaterials, Au-NPs, regarding their biocompatibility, high stability and catalytic activity for glucose oxidation in neutral medium, has attracted more attention [2,6,10].

In order to attach metal NPs to the surface of nanotubes, CNTs should be functionalized with functional groups such as carboxyl, amine and thiol groups, which have high affinity toward metallic NPs [11,12]. High interaction energy of thiol groups and Au-NPs leads to the self-assembly of gold atoms on the surface of the thiolated nanotubes [13]. Thiol-functionalization of CNTs have been carried out by multistep chemical approaches such as carboxyl chloride formation followed by thiolamination [14] and sulfur impregnation followed by Ar/H\textsubscript{2} plasma [15].

An alternative method for surface thiolation of CNTs is the use of discharge plasma in H\textsubscript{2}S/He atmosphere. Dielectric barrier discharge (DBD) plasma is an easy, one-step and flexible approach in which the functional groups are directly formed on the surface of nanotubes and the quantity of functional groups may be adjusted by changing the operating parameters, e.g. power and exposure time [16]. Helium as a diluents gas improves charge and energy...
transfer mechanism to reactive species and increases the amount of functional groups formed on the surface of CNTs [17].

Several approaches for preparing Au/CNTs hybrids may be classified as ex situ and in situ methods. In the ex situ approaches, Au-NPs are synthesized and modified in advance and then attached to the CNTs covalently or noncovalently. In contrast, the in situ approaches leads to direct formation of Au-NPs on the surface of CNTs and the film thickness and morphology of the deposited materials may be controlled. Various in situ synthesizing techniques such as chemical vapor deposition, Au precursor reduction, sol–gel, and electrochemical approach have been reported [18,19].

In this study, multi-walled carbon nanotubes (MWCNTs) were directly thiolated, in the absence of any long chain hydrocarbon groups, using H2S plasma. The Au-NPs were then attached to surface of the MWCNTs by reduction of the Au precursor. A glucose biosensor was fabricated by immobilization of GOx on the Au/MWCNTs hybrid and used in direct electrochemical detection of glucose.

2. Experimental

2.1. Chemicals

MWCNTs were purchased from Shenzhen Nanotech Port Co. Ltd. (China). GOx (EC 1.1.3.4) and 5,5-dithiobis (2-nitrobenzoic acid) (DTNB), as an Ellman’s reagent, were obtained from Sigma–Aldrich, St. Louis, USA. All other materials were purchased from Merk. Deionized water was used through all the experimental investigations.

2.2. Plasma functionalization of MWCNTs

MWCNTs were heated to 1000 °C in a flow of He to remove possible functional groups. The resulted sample is denoted as annealed MWCNTs. The annealed samples were functionalized using a DBD plasma reactor in an atmosphere containing 70% H2S and 30% He. The total gas flow rate for each 0.08 g of the annealed MWCNTs was set to 50.0 sccm by a UPCI-7300 mass flow controller. A function generator (PINEKG FG-32) connected to a high voltage amplifier (TREK 10/40A) was utilized to generate the DBD plasma. The amplifier employed a high voltage of 9.0 kV with an output frequency of 2.5 kHz. The DBD plasma reactor consists of a stainless steel rod – inner electrode, an aluminum thin sheet – external electrode and a quartz tube as a dielectric barrier. Details of the instrument settings and sample preparation are given elsewhere [16]. The plasma discharge was carried out at constant power of 27.7 W and different exposure times of 1–6 min. The plasma functionalized sample is denoted as MWCNTs-SH.

2.3. Preparation of Au/MWCNTs hybrid

Fig. 1 illustrates schematic of Au/MWCNT preparation. The Au/MWCNTs hybrid was prepared by a deposition–precipitation method. 70.0 mg of MWCNTs-SH was dispersed in 3 ml aqueous HAuCl4 solution (1 mg/ml). Aqueous NaBH4 was then added to the suspension slowly [13]. The hybrid of MWCNTs and Au-NPs was obtained after stirring for 1 h at room temperature. The precipitate was centrifuged, washed with deionized water for several times and then dried at 80 °C for 12 h.

2.4. Characterization

Morphology, structure and functional groups of MWCNTs and their hybrids with gold NPs were characterized using FE-SEM (JSM 6700F, JEOL, operating at 5.0 kV), TEM (Philips CM120, at 120.0 kV acceleration voltage), XRD (using Cu Kα radiation), Raman (SENN TERRA, using laser wavelengths at 785 nm) and diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy (Bruker Vector 22 spectrometer in transmission mode with a resolution of 5.0 cm⁻¹). The amount of thiol groups on the MWCNTs was determined using Ellman reagent [20] based on the reaction of DTNB and free thiol group to yield a mixed disulfide and 2-nitro-5-thiobenzoic acid (TNB). TNB has a specific absorbance at 412 nm. Thiol groups were estimated by comparison to a standard curve composed of known concentrations of a thiol-containing compound such as cysteine. This standard curve was generated by dissolving various concentrations of cysteine hydrochloride monohydrate in a buffer solution (0.1 M sodium phosphate, pH 8.0, containing 1 mM EDTA). To further characterize the Au/MWCNTs hybrids, cyclic voltammetry (CV) was taken with the modified electrode in a 0.5 M H2SO4, 0.1 M NaOH and buffer solution at a scan rate of 50 mV/s.

2.5. Fabrication of glucose biosensor

All electrochemical studies were performed using PalmSens potentiostat/galvanostat and a conventional three-electrode cell at room temperature. A working glassy carbon electrode (GCE, A = 3.14 × 10⁻² cm²), a saturated Ag/AgCl reference electrode, and a Pt wire counter electrode (1.85 cm²) were used. The bare GCE was polished with alumina slurry, sonicated in mixture of deionized water and ethanol to get a mirror surface. 10.0 μl of 0.5 wt.% nafion solution was added to the Au/MWCNTs aqueous solution and sonicated for 15 min to obtain a uniform suspension with concentration of 2.0 mg/ml. 5.0 μl of the Au/MWCNTs suspension was cast onto the surface of the GCE and dried. Afterwards a 5.0 μl of GOx solution (5.0 mg/ml) was cast onto the surface of the Au/MWNT/GCE and allowed to dry at 4 °C for 24 h. Phosphate buffer solution (PBS, 0.1 M, pH 7.0, KH2PO4 + K2HPO4) was used as an electrolyte. For deoxygenating PBS, it was saturated with N2 gas for 20 min before electrochemical tests. The GOx/Au/MWCNTs/GCE was stored in PBS at 4 °C, when it was not in use. Electrochemical detection of glucose was carried out in concentration range of 0.01–4.0 mM in the PBS at −0.55 V vs. Ag/AgCl. Selectivity of the fabricated electrode was measured in the presence of ascorbic acid (AA) and uric acid (UA).

3. Results and discussion

3.1. Characterization

3.1.1. DRIFT spectroscopy results

Fig. 2 shows DRIFT spectra of the annealed and functionalized MWCNT samples. The peak at 1576 cm⁻¹ (Fig. 2a and b) is assigned to the stretching bond of C=O [21]. The intensity of this peak is increased as a result of defect generation and functional group attachment during plasma functionalization (Fig. 2b). Both spectra show a weak peak around 1710 cm⁻¹ which is assigned to the carbonyl groups remained after oxidative purification [16,21]. The MWCNTs-SH spectrum has a peak at 2550 cm⁻¹, characteristic of thiol groups [22]. This sample also shows two peaks at 2860 and 2917 cm⁻¹ which are attributed to C–H stretching band [21]. The C–H bonds are formed as a result of reaction of hydrogen radical with surface of the CNTs during H2S dissociation.

As a result of dissociation of H2S molecules during plasma reactions, hydrogen-sulfide and hydrogen radicals are produced according to the following reactions [23]:

\[
\text{H}_2\text{S} \leftrightarrow \text{HS}^* + \text{H}^*
\]  

(1)

\[
\text{HS}^* + \text{e}^- \leftrightarrow \text{HS}^*\text{−} \text{(or HS}^{2−})
\]  

(2)

\[
\text{H}_2\text{S} + \text{e}^- \leftrightarrow \text{H}_2\text{S}^\text{−} \text{(or H}_2\text{S}^2\text{−})
\]  

(3)
These reactive species break sp²-carbon bonds and efficiently react with the defect sites and open ends of nanotubes to form C–SH and C–H bonds.

In the presence of He, as a diluents gas, the charge and energy are transferred from He reactive species, e.g. He⁺ and He²⁺ to H₂S through the following plasma reactions [17):

\[
\text{He} + \text{e}^- \rightarrow \text{He}^+ + 2\text{e}^- \quad (4)
\]

\[
\text{He}^+ + 2\text{He} \rightarrow \text{He}_2^+ + \text{He} \quad (5)
\]

\[
\text{He}^+(\text{or He}_2^+) + \text{H}_2\text{S} \rightarrow \text{He}(\text{or 2He}) + \text{H}_2\text{S}^+ \quad (6)
\]

### 3.1.2. Raman spectra

Raman spectra of the annealed and functionalized samples are presented in **Fig. 3**. The ratio of the intensity of G band at 1609 cm⁻¹ to that of D-band at 1340 cm⁻¹ is 1.38 and 1.16 for the annealed MWCNTs and MWCNTs-SH, respectively. The decrease in this ratio is due to creation of defects sites and functional groups on the surface of the nanotubes [24].

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>SH (wt.%)</td>
<td>0.32</td>
<td>0.39</td>
<td>0.45</td>
<td>0.57</td>
<td>0.32</td>
<td>0.16</td>
</tr>
</tbody>
</table>

The peak at 567 cm⁻¹ which has not been observed for the annealed MWCNTs is assigned to S–S vibration of the disulfide bond [25] which may be formed as a result of reaction of hydrogen-sulfide with free thiol group and reaction of two adjacent free thiol groups during plasma reactions [26]:

\[
\text{C–SH} + \text{HS}^+ \rightarrow \text{C–S–SH} + \text{H}^+ \quad (7)
\]

\[
\text{C–SH} + \text{C–SH} \rightarrow \text{C–S–S–C} + 2\text{H}^+ + 2\text{e}^- \quad (8)
\]

### 3.1.3. Quantification of thiol groups on MWCNTs

Dependence of the amount of thiol groups, determined by Ellman’s reagent method, on the DBD plasma exposure time is shown in **Table 1**. At 4 min exposure time the amount of thiol groups is maximum. However, longer exposures, lead to lower amount of thiol groups. It seems that longer treatment times remove some of the functional groups formed on the surface of nanotubes [16].
Therefore, the MWCNTs functionalized for 4 min is selected for preparation of the Au/MWCNTs hybrid.

3.1.4. FE-SEM and TEM

Fig. 4a and b shows FE-SEM micrographs of MWCNTs-SH and that of Au/MWCNTs, respectively. Fig. 4c and d shows the TEM images of Au/MWCNTs with different magnifications. Fig. 4a and c presents that nanotubes have entangled form with an average diameter of about 20 nm. FE-SEM of Au/MWCNTs hybrid (Fig. 4b) shows a relatively uniform layer of Au-NPs deposited on the surface of nanotubes. The formation of Au-NPs decorated MWCNTs is confirmed by the TEM images (Fig. 4c and d). Excluding a small number of aggregate particles, the size of Au-NPs coated on the nanotubes is in the range of 4–22 nm with an average of about 15 nm.

Due to the weak SH bond (dissociation energy of about 20 kcal/mol) [27], the thiol groups on the surface of MWCNTs-SH can be deprotonated as follows:

\[ \text{SH}_{\text{surface}} \rightarrow S^{-}_{\text{surface}} + H^{+} \]  

(9)

The sulfur anions owing to the good stability of the Au–S bond with dissociation energy of about 38 kcal/mol [28], may act as links for deposition and crystallization of Au-NPs. The Au-NPs are formed as AuCl4− is reduced with the electron source of BH4−.

3.1.5. XRD

Fig. 5 presents XRD patterns of MWCNTs-SH and Au/MWCNTs. The XRD pattern of MWCNTs-SH (Fig. 5a) shows a sharp peak at 26.23° which is assigned to graphitic carbon [7]. The major peaks at 38.05°, 44.14°, 64.47°, 77.50° and 81.42° (Fig. 5b) can be attributed to (1 1 1), (2 0 0), (2 2 0), (3 1 1) and (2 2 2) crystalline plane diffraction peaks of gold, respectively [7,9]. The XRD peaks confirm the presence of Au-NPs in the hybrid which are indexed as the face-centered cubic Au-NPs.

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Fig. 4. SEM micrographs of (a) MWCNTs-SH and (b) Au/MWCNTs. (c) and (d) are TEM images of Au/MWCNTs.

Fig. 5. XRD patterns of (a) MWCNTs-SH and (b) Au/MWCNTs hybrid.
3.1.6. Redox wave of Au-NPs

The cyclic voltammograms of the bare GCE, MWCNTs-SH- and the Au/MWCNTs-modified electrode in 0.5 M H₂SO₄ at 50 mV/s are shown in Fig. 6. The MWCNTs-SH-modified GC electrode has a higher charging current compared to the bare GCE due to the increase of the electrode surface area. No voltammetric peaks are observed in the cyclic voltammogram of MWCNTs-SH-modified electrode. Compared to this electrode, the Au/MWCNTs/GCE exhibits an increase in anodic current around 1.2 V which is assigned to the oxidation of Au-NPs deposited on MWCNTs and a typical peak at 0.9 V which is attributed to the reduction of the oxide formed on the Au-NPs [29].

The oxidation reaction of Au-NPs can be described by the following equation [30]:

\[ 2Au + 3H₂O → Au₂O₃ + 6H⁺ + 6e⁻ \]  \hspace{1cm} (10)

Fig. 6 inset shows cyclic voltammogram of Au/MWCNTs/GCE in 0.1 M PBS, pH 7.0 and 0.1 M NaOH. The redox wave of Au-NPs in PBS and NaOH is shifted to about 0.9 V and 0.7 V on the forward scan and 0.5 V and 0.2 V on the reverse scan, respectively. The shift of redox peaks of gold to lower potential with increasing pH is common for aqueous solution [30].

3.2. Glucose sensor performance

3.2.1. Enzyme immobilization

Fig. 7a shows cyclic voltammogram of the bare GC electrode. As a result of the presence of MWCNTs-SH on the modified electrode, an obvious increase in the charging current is observed (Fig. 7b). The GOx/MWCNTs-SH/GCE (Fig. 7c) presents a pair of well-defined and nearly symmetric redox anodic and cathodic peaks around –0.5 V. This redox wave is assigned to bioactivity of redox center in GOx [6]. The electrocatalytic process may be expressed as follows [31]:

\[ \text{GOx(FAD)} + 2e^- + 2H^+ \rightarrow \text{GOx(FADH₂)} \]  \hspace{1cm} (11)

Compared to MWCNTs-SH/GCE, the Au/MWCNTs/GCE shows higher charging current (Fig. 7d). This increase is related to the presence of Au-NPs. The GOx/Au/MWCNTs/GCE (Fig. 7e) shows an enhanced GOx redox wave around –0.47 V. The peak-to-peak potential separation (ΔEₚ) for the Au/MWCNTs/GCE and the MWCNTs-SH/GCE are 0.037 and 0.052 V. The quantity of charge consumed during oxidation reaction (the base-to-base area under the oxidation peak) of GOx/Au/MWCNTs/GCE is about 1.6 times higher than that of GOx/MWCNTs-SH/GCE. The Au-NPs decrease formal potential and ΔEₚ and increase charge transferring of redox wave of GOx. According to other studies, Au-NPs are able to accept electrons directly from glucose oxidase and facilitate electron transferring from GOx toward electrode [32,33].

3.2.2. Effect of scan rate

Fig. 8a shows CVs of the GOx/Au/MWCNTs-modified GCE at various scan rates. The potential and peak current depend on the scan rate. For the scan rate in the range of 10–250 mV/s, the linear relationship between the oxidation (R² = 0.991) and reduction (R² = 0.993) peak currents and the scan rates (Fig. 8b) indicates that the redox process of the hybrid is a reversible and

![Fig. 6. Cyclic voltammograms of (a) bare GCE, (b) MWCNTs-SH- and (c) Au/MWCNTs-modified electrode in 0.5 M H₂SO₄. Inset: Cyclic voltammograms of Au/MWCNTs/GCE in 0.1 M PBS, pH=7.0 (solid) and 0.1 M NaOH (dashed). Scan rate=50 mV/s.](image)

![Fig. 7. Cyclic voltammograms of (a) bare GCE, (b) MWCNTs-SH/GCE, (c) GOx/MWCNTs-SH/GCE, (d) Au/MWCNTs/GCE and (e) GOx/Au/MWCNTs/GCE in 0.1 M deoxygenated PBS at a scan rate of 50 mV/s.](image)

![Fig. 8. (a) Cyclic voltammogram of GOx/Au/MWCNTs/GCE in 0.1 M deoxygenated PBS at different scan rates in the range of 10–250 mV/s. (b) calibrated plot of the peak currents vs. scan rate, (c) plot of E_p vs. log v.](image)
The oxidation peak current is nearly equal to the reduction peak current at the same scan rate. With increasing the scan rate the oxidation peak potential is shifted to a more positive value and the reduction peak potential to a more negative value, whereas the $\Delta E_p$ increases gradually.

The charge transfer coefficient ($\alpha$) and charge transfer rate constant ($k_t$) of the GOx may be calculated using Laviron’s model [34]. When $\Delta E_p > 200$/mV, the linear sweep voltammetry response is expressed as follows:

$$E_{pa} = E^0 + \frac{RT}{\alpha_F nF} \ln \left[ \frac{\alpha_a}{m} \right]$$

$$E_{pc} = E^0 + \frac{RT}{\alpha_F nF} \ln \left[ \frac{\alpha_c}{m} \right]$$

$$\log k_t = \alpha \log(1 - \alpha) + (1 - \alpha) \log \alpha - \log \left( \frac{RT}{nF} \right) - \alpha(1 - \alpha)^2 nF \Delta E_p \frac{2.3RT}{E^0 - E_p}$$

where $m = (RT/\alpha_F k_t/nF)$, $n$ is the number of electrons transferred; $R$, $T$, and $F$ are constants ($R = 8.314$ J/mol K, $T = 298$ K, $F = 96.485$ C/mol) and $\nu$ is the scan rate.

From Fig. 8c, the plot of $E_p$ versus $\log \nu$ yields two straight lines with the slopes of $2.3RT/\alpha_a n$ and $-2.3RT/\alpha_a n$ for the anodic and cathodic peaks, respectively. Using the slopes of these plots, the values for $\alpha_a n$ and $\alpha_c n$ are calculated to be 0.96 and 0.94, respectively, with the average value of 0.95 for $\alpha$. Therefore, the values for $\alpha$ and $\alpha$ can be estimated as 2 and 0.48, respectively. Based on Eq. (14) and from the values of $\Delta E_p$ (for scan rates > 200 mV/s) the average value of $k_t$ is determined to be $1.13 \pm 0.1$ s$^{-1}$. These results reveal that Au/MWCNTs hybrid offers enhanced electrochemical transfer.

The diffusion coefficient of glucose in PBS and surface area of the modified electrode (A) can be estimated using linear relationships of $I$ vs. $t^{1/2}$ which corresponds to the Cottrell equation [35]:

$$I = \frac{n^2 F^2 A D}{RT} t^{-1/2}$$

where $D$ and $C$ are diffusion coefficient and bulk concentration of glucose, respectively. From the slope of the linear plot of $I$ vs. $t^{1/2}$ for the bare electrode and GOx/Au/MWCNTs/GCE, the diffusion coefficient and surface area are calculated to be $7.15 \times 10^{-9}$ cm$^2$/s and 1.63 cm$^2$, respectively.

For the reversible redox reaction of GOx, the surface average concentration of GOx ($\Gamma$) may be estimated as follows [36]:

$$I_p = \frac{n^2 F^2 A \Gamma}{4RT \nu}$$

where $I_p$ is peak current, $A$ is the surface area of the modified electrode. From the slope of peak currents vs. scan rate (Fig. 8b), the calculated concentration of GOx is $1.87 \times 10^{-10}$ mol/cm$^2$. This value is much larger than the theoretical value, i.e. $2.86 \times 10^{-12}$ mol/cm$^2$ [31] for the monolayer of GOx on the bare electrode surface, proposing that multilayers of GOx participate in the direct electron-transfer process.

3.2.3. Determination of glucose using GOx/Au/MWCNTs electrode

The typical amperometric responses of GOx/Au/MWCNTs/GCE electrode at an optimum potential of $-0.55$ V toward addition of glucose are shown in Fig. 9. The current for electrocatalytic oxidation of glucose increased upon successive addition of glucose and yield an increasing step–like amperometric response. In fact the H$_2$O$_2$ produced as result of oxidation of glucose in presence of GOx is detected. The average time to reach steady state current is found to be 12 s, indicating a relatively fast and stable response of the modified electrode. The inset of Fig. 9 shows the corresponding calibration curve based on the oxidation currents of glucose. The oxidation current shows a linear behavior in the range of 0.4–4.0 mM. The linear equation is represented by: $I(\mu A) = -1.63 C$ (mM)$^{-1}$–2.66 with a correlation coefficient of 0.986. The detection limit is estimated to be 3.0 µM (based on the signal to noise ratio of 3) with a relatively high sensitivity of 23.1 µA/mM cm$^2$. The high sensitivity of the modified electrode may be attributed to the good electrocatalytic activity of Au-NPs and high surface area of MWCNTs which improves the accessibility of glucose to the active sites of GOx.

Table 2 compares the analytical parameters of the GOx/Au/MWCNTs/GCE with the other glucose biosensors, which have used MWCNTs, Au-NPs and polymers as the immobilization matrix. GOx/Au/MWCNTs/GCE exhibits higher sensitivity and lower detection limit as is compared to the other electrodes, although the detection potential is higher. It should be emphasized that, the method of electrode fabrication in this work is quite easier and faster than that of other studies.

3.2.4. Investigation of selectivity of the modified electrode

The oxidizing species such as AA and UA usually co-exist with glucose in human blood. One of the important analytical features of the glucose biosensor is their ability to distinguish between these species [40]. Fig. 10 shows the response of the GOx/Au/MWCNTs electrode upon addition of 2.0 mM glucose and 0.1 mM AA and UA. A noticeable current for glucose oxidation is observed, while no response could be seen after addition of AA and UA. This demonstrates that AA and UA do not interfere with the detection of glucose at $-0.55$ V and thus the sensor is quite selective.

3.2.5. Stability of the modified electrode

Fig. 11 shows the long-term stability of the GOx/Au/MWCNTs/GCE generated by the measurements of the anodic peak currents of the modified electrode every 5 days. The faradaic components of these peaks are presented in Fig. 11 inset. Comparing these figures shows that the charging current of the modified electrode remains constant after 50 days. The absence of variation in the charging current suggests a good mechanical stability of GOx/Au/MWCNTs on the surface of bare electrode.
The modified electrode retains more than 58% of its initial faradic current value (18.6 μA) after 50 days, indicating a relatively good stability in redox activity of the fabricated sensor. This stability may be due to the presence of Au-NPs which improves immobilization of GOx on the surface of electrode. Since GOx contains six cysteine residues, some Au–S bonds facilitate the GOx/Au complex formation. Moreover, the attraction between hydrophobic layers that covered FAD in GOx and Au-NPs, which also shows hydrophobic nature, provides suitable microenvironment for GOx immobilization. [41].

4. Conclusion

MWCNTs were thiol-functionalized in a DBD plasma reactor under H2/He atmosphere and Au-NPs were decorated on the surface of MWCNTs-SH through the thiol linkages. The redox process of GOx on Au/MWCNTs hybrid was quasi-reversible and surface controlled. The GOx/Au/MWCNTs-modified electrode presents long-term stability which may be due to the minimum unfolding of immobilization approach. Amperometric features such as linear response to addition of glucose, high sensitivity, low detection limit and selectivity toward glucose in the presence of AA and UA show that Au/MWCNTs provide excellent environment for GOx to detect glucose.

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