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Amperometric glucose biosensor based on glucose oxidase dispersed in multiwalled carbon nanotubes/graphene oxide hybrid biocomposite

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ABSTRACT

An amperometric glucose biosensor based on enhanced and fast direct electron transfer (DET) of glucose oxidase (GOx) at enzyme dispersed multiwalled carbon nanotubes/graphene oxide (MWCNT/GO) hybrid biocomposite was developed. The fabricated hybrid biocomposite was characterized by transmission electron microscopy (TEM), Raman and infrared spectroscopy (IR). The TEM image of hybrid biocomposite reveals that a thin layer of GOx was covered on the surface of MWCNT/GO hybrid composite. IR results validate that the hybrid biocomposite was formed through the electrostatic interactions between GOx and MWCNT/GO hybrid composite. Further, MWCNT/GO hybrid composite has also been characterized by TEM and UV–visible spectroscopy. A pair of well-defined redox peak was observed for GOx immobilized at the hybrid biocomposite electrode than that immobilized at the MWCNT modified electrode. The electron transfer rate constant (K_s) of GOx at the hybrid biocomposite was calculated to be 11.22 s^{-1}. The higher K_s value revealed that fast DET of GOx occurred at the electrode surface. Moreover, fabricated biosensor showed a good sensitivity towards glucose oxidation over a linear range 0.05–23.2 mM. The limit of detection (LOD) was estimated to be 28 μM. The good features of the proposed biosensor could be used for the accurate detection of glucose in the biological samples.

1. Introduction

Nanotechnology has received a considerable attention over the past two decades in the field of nanoscience [1]. The unifying features of the nanomaterials increased their applications in the fields of electrochemical sensors [2], solar cells [3], and drug delivery [4]. Nowadays, nanomaterials have been extensively used in electrochemical biosensors to achieve good sensitivity and selectivity [5]. Among various electrochemical biosensors, glucose biosensor has received special attention due to its vital role in determining glucose levels in diabetic patients. In particular, amperometric glucose biosensors enable simple, rapid and continuous glucose monitoring [6]. But, there have been great challenges in the development of highly sensitive and sophisticated glucose biosensors, which includes the fabrication of novel multifunctional or homogenous nanofilms with high quality, detailed mechanisms explaining the behavior of these nanocomposites on the surface of electrodes, and enhancing the signal to noise ratio, transduction and amplification of the signals. Though metal nano particles have been widely used for achieving lower detection limits, large variations in the glucose detection during batch measurements occurred due to small variations in the density of metal nanoparticles or GOx [6,7]. Electrochemical methods have been employed for the fast and real time detection of biomolecules. Moreover, they have been used as potential alternatives to traditional methods like HPLC [8], calorimetry [9] and spectrophotometry [10], because electrochemical methods are very simple and rather less time consuming when compared with the existing traditional methods [11]. In most of the enzyme based glucose biosensors, glucose oxidase (GOx) was used as a common enzyme because of its high selectivity towards glucose oxidation. However, the direct immobilization of GOx on bare electrode is impossible, because it is very difficult to achieve the direct electron transfer as the redox active sites of GOx were deeply buried inside the enzyme [12]. Numerous nanomaterials have been applied as matrix for the immobilization of GOx in glucose biosensors by using several approaches that include cross-linking [13], physical adsorption [14], and covalent entrapment [15]. On the other hand, multiwalled carbon nanotubes (MWCNT) have been widely employed as a matrix for the immobilization of GOx [16]. In recent years, MWCNT based metal oxides [17], metal nanoparticles [18] and carbon nanomaterials [19] have been extensively used for electrochemical biosensors. Graphene oxide (GO) is one of the imperative nanomaterials from carbon family that has been used for many sensor applications [20]. Recently, GO has been extensively modified with other nanomaterials like MWCNT and single-walled carbon nanotubes to produce the synergetic effect [21]. The first enzyme dispersed biocomposite has been reported by Wang and Musameh. Their results revealed that enzyme dispersed nanomaterials increased the direct electron transfer of GOx significantly [22]. Recently, our group reported that fast and enhanced direct electron transfer of GOx occurred at MWCNT incorporated electrochemically reduced graphene oxide

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(ERGO) than only MWCNT [23]. Moreover, our previous studies confirmed that GOx could be easily absorbed onto GO nanosheets through electrostatic interaction by simple sonication [24]. At the same time, MWCNT/GO hybrid composite has better electrochemical sensing ability and more capacitance when compared with only MWCNT and GO [25]. However, it is very difficult to disperse MWCNT in aqueous solution without any functionalization. Hence, we dispersed MWCNT with the aqueous dispersion of GO to prepare highly stable MWCNT/GO hybrid composite through the interaction between MWCNT and GO. Further, we used the hybrid composite to disperse GOx because GOx can be easily dispersed in aqueous solution. Compared to MWCNT electrode, MWCNT/GO hybrid biocomposite modified electrode showed an enhanced direct electron transfer for GOx. To the best of our knowledge, enzyme dispersed MWCNT/GO hybrid composite has never been used for the fabrication of glucose biosensor.

Herein, we used a simple solution based method to fabricate a glucose biosensor based on dispersed GOx at MWCNT/GO hybrid composite. GOx was immobilized onto the surface of MWCNT/GO through the physical adsorption and due to the interaction between the positively charged free-NH_2 groups of GOx with the negatively charged functional groups anchored on the surface of GO. The fabricated hybrid biocomposite showed an enhanced direct electron transfer for GOx than other modified electrodes. Moreover, the hybrid biocomposite is highly stable, because no agglomeration was observed even when stored up to one month. The fabricated biosensor showed a good electrocatalytic activity towards glucose oxidation with good sensitivity and selectivity.

2. Experimental

2.1. Materials and method

MWCNTs with the lengths of 0.1–10 μm were purchased from Aldrich. Graphite powder with 98% purity was obtained from Sigma-Aldrich. Glucose oxidase, from Aspergillus niger was obtained from Sigma Aldrich and used as received. The supporting electrolyte used for all experiments was pH 7 phosphate buffer solution (PBS), which was prepared by using 0.05 M Na_2HPO_4 and NaH_2PO_4. The pH was adjusted with 0.5 M H_2SO4 and 2.0 M NaOH. All other chemicals used in this study were analytical grade and used without any further purification.

Electrochemical studies were performed by using a computer controlled CHI 750a work station. Transmission electron microscopy (TEM) study was performed using JEM 2007 model transmission electron microscope. An infrared spectrum (IR) was recorded using Hitachi U-3300 spectrophotometer. Raman spectrum was recorded using a Raman spectrometer (Dong Woo 500i, Korea) equipped with a 50× objective and a charge-coupled detector. Electrochemical impedance spectroscopy (EIS) studies were performed using IM6ex ZAHNER (Kroanch, Germany). Amperometric measurements were performed using CHI-750a potentiostat with analytical rotator AFMSRX (PINE instruments, USA). A modified glassy carbon electrode (GCE) was used as the working electrode (active surface area = 0.079 cm^2). Ag/AgCl electrode (Sat. KCl) was used as the reference electrode and a platinum wire with 0.5 mm diameter was used as the counter electrode for all electrochemical experiments. All measurements were carried out at ambient temperature and electrolyte cell solutions were purged with high purity nitrogen (N_2) prior to electrochemical analysis (except glucose catalysis).

2.2. Fabrication of enzyme dispersed hybrid biocomposite modified electrode

Graphite oxide was synthesized from graphite powder by Hummers method [26]. The as-prepared graphite oxide was well dispersed in water (0.5 mg/ml) and exfoliated by ultrasonication to produce GO. The MWCNT/GO hybrid composite was prepared as reported elsewhere [25]. A fresh GOx (1, V %, 6 mg/ml) was added in to the hybrid composite and then subjected for sonication, which leads to the homogeneous dispersion of hybrid composite and GOx. Finally, the hybrid biocomposite was centrifuged at 1500 rpm to remove the loosely bound MWCNT and GOx. After sonication and centrifugation, the supernatant was carefully collected and then analyzed by using UV–vis spectroscopy. We did not observe any significant absorption peak at 230 nm for GOx, revealing that no GOx was released from the composite surface upon subjecting it to sonication and centrifugation. This confirms that GOx has been strongly immobilized at the MWCNT-GO composite. The purified hybrid biocomposite was dried overnight at room temperature, and then redispersed in pH 7 and used for all experiments. We also checked the stability of the MWCNT-GO/GOx modified GCE before and after overnight drying by using CV. No notable decrease in the redox peak current and shift in the peak potential of GOx (FAD/FADH2) was observed in the cyclic voltammograms, suggesting that GOx is highly stable at the MWCNT-GO hybrid biocomposite.

Before modifying GCE surface, it was carefully polished with alumina slurry and then bath sonicated for 3 min in ethanol containing doubly distilled water and dried in an air oven. About 8 μl (optimum concentration) of the hybrid biocomposite was drop casted on the electrode surface and then dried at room temperature. The modified GCE was immersed in pH 7 solution and the potential cycles were performed between −0.7 to 0 V at inert atmosphere. The hybrid biocomposite modified GCE was used for further experiments and stored at 4 °C under dry condition when not in use.

3. Results and discussion

3.1. Characterization of hybrid biocomposite

The morphology of the hybrid biocomposite was examined by TEM. Fig. 1A shows the typical TEM image of MWCNT/GO/GOx hybrid biocomposite, revealing that a thin layer of GOx was covered on the surface of MWCNT/GO hybrid composite. The isoelectric point (pI) of GOx is 4.2, which reveals that GOx carries net negative charges above this pH [27,28]. Therefore GOx is negatively charged at pH 7. As reported previously, the zeta-potential of GO-MWCNT was above −35, revealing that the surface was negatively charged. [25] The negatively charged functional groups on the surfaces of GO interact with the positively charged free amino groups of GOx [29] resulting in GOx to be easily adsorbed onto the composite surface through the electrostatic interactions. Moreover, GOx adsorbed at the hybrid composite was further confirmed by IR spectroscopy and CV studies (Section 3.2). The electrochemical impedance behavior of different modified GCEs was investigated by EIS and the results are shown in Fig. 1B. EIS provides useful information about the impedance changes occurring at the electrode surface during each electrode modification step. The diameter of the semicircular part is equivalent to the electron transfer resistance. Fig. 1B shows the EIS analysis of bare GCE (a), MWCNT/GO (b), and MWCNT (c) modified GCEs in PBS containing 5 mM $Fe(CN)_6^{3-/4-}$. The linear part at lower frequencies corresponds to the diffusion process. Bare GCE exhibits a larger semicircle (a), but a depressed semicircle with smaller diameter was observed at MWCNT/GCE (c), revealing that faster electron transfer occurs at the MWCNT modified GCE than bare/GCE. The decreased electron transfer resistance observed at the MWCNT modified GCE could be due to the large surface area of MWCNT networks. When compared with bare GCE, a very small semicircle was observed at the MWCNT/GO film modified GCE in the same frequency range (b). However, the negatively charged functional groups on GO sheets repelled the negatively charged $Fe^{3-/4-}$ probe; as a result semicircle diameter was larger at MWCNT/GO film than that of MWCNT film. EIS results clearly validates that the hybrid composite has excellent conductivity and good electron transfer for GOx. Raman spectra were used to evaluate the degree of transformation of the D and G bands in graphitic systems. Fig. 1C shows the corresponding Raman spectrum of MWCNT (a), GO (b) and

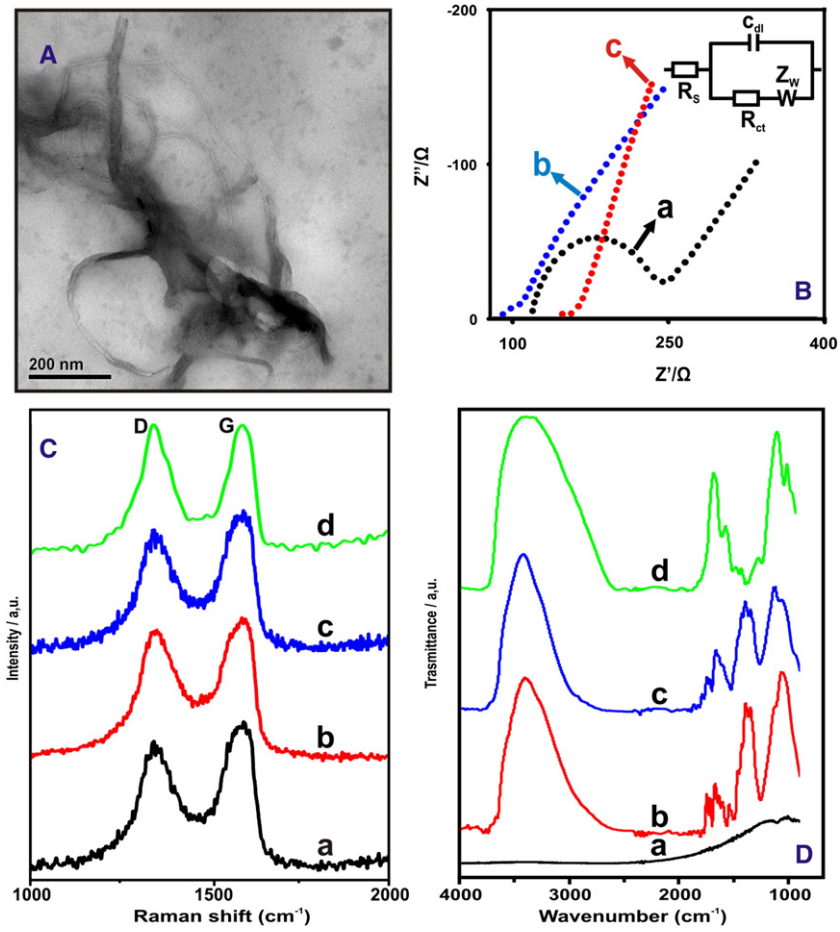

Fig. 1. (A) Typical TEM image of MWCNT/GO/GOx hybrid biocomposite. (B) EIS of bare (a), MWCNT (b) and MWCNT/GO hybrid biocomposite (c) modified GCEs in 5 mM $Fe(CN)_6^{3-/4-}$ containing 0.1 M KCl. Inset shows the Randles equivalent circuit model fitting of EIS data acquired in the frequency range 0.1 Hz to 100 kHz. (C) Raman spectrum and (D) IR spectrum of MWCNT (a), GO (b) and MWCNT/GO (c) and MWCNT/GO/GOx hybrid biocomposite (d).

MWCNT/GO/GOx hybrid biocomposite (c). As shown in Fig. 1C, well defined D and G bands appeared at 1335 and 1588 cm^{-1} for MWCNT and 1338 and 1585 cm^{-1} for GO, respectively. The enhanced D and G bands shifted slightly for MWCNT/GO/GOx hybrid biocomposite at 1336 and 1586 cm^{-1}, which were attributed to the strong interaction between GOx and MWCNT/GO composite. IR spectra were used to investigate the various functional groups existing in the graphitic system before and after the modification. Fig. 1D shows IR spectrum of pristine MWCNT (a), GO (b), MWCNT/GO (c) and MWCNT/GO/GOx (d). MWCNT does not show any peaks, validating that MWCNT belongs to the pure carbonic form of the graphitic system. Whereas, for GO, a strong and broad band appeared at 3402 cm^{-1} due to the presence of O–H stretching vibration of carboxylic groups. Moreover, two distinct peaks appeared at 1720 and 1665 cm^{-1} for C═O and 1380 cm^{-1} for ether functionalities, while the C–O epoxy or alkoxy groups were observed at 1062 cm^{-1}. MWCNT/GO hybrid composite displays IR spectrum similar to that of GO confirming that MWCNT does not react with the functional groups of GO. However, a new peak appeared at 1650 cm^{-1} for MWCNT/GO/GOx hybrid biocomposite, due to the strong interaction between the carboxylic groups of GO and amino groups of GOx, while another distinct peak appeared at 1555 cm^{-1} for the presence of zwitterions of GOx. IR result confirmed the formation of hybrid biocomposite through the physical absorption of GOx at hybrid composite.

Fig. 2 shows the typical TEM images of pristine MWCNT (A), GO (B), MWCNT/GO (C) and its corresponding UV–visible spectra (D). In Fig. 2A, hallow tubular structures of MWCNT with an average diameter of 30–50 nm were observed. Whereas, GO appears like ultra thin sheets with 50 nm diameter. The GO thin sheets were covered by the MWCNT networks, which confirmed the formation of the MWCNT/GO hybrid composite (C). The strong π–π interaction between hydrophilic GO sheets and MWCNT favored the formation of MWCNT/GO hybrid composite. The interaction between the MWCNT networks and GO sheet was further confirmed by UV–visible spectra (Fig. 2D). A sharp shoulder peak was appeared at 231 nm due to π–π transition between GO and MWCNT. Another peak at 300 nm was due to the π–π transition from the carbonyl groups of GO to MWCNT networks [25], confirming that the formation of hybrid composite occurred through the π–π interaction between GO sheets and MWCNT networks.

3.2. Direct electrochemistry of GOx

Cyclic voltammograms were performed to evaluate the electrochemical behavior of GOx at different modified electrodes. The cyclic voltammograms of bare/GOx (a), GO/GOx (b), MWCNT/GOx (c), and MWCNT/GO/GOx (d) modified GCEs are shown in Fig. 3A. The cyclic voltammograms were recorded in deoxygenated PBS in the potential range from −0.7 to 0 V at 50 mV scan rate. A pair of well-defined redox peaks appeared at MWCNT/GO/GOx modified electrode with a formal potential (E°′) of −0.420 V, which revealed the presence of redox active center ($FAD/FADH_2$) in the GOx [30]. Meanwhile, the peak to peak separation (ΔE_p) was calculated as ~36 mV. The smaller (ΔE_p) revealed the fast direct electron transfer occurring at the hybrid biocomposite modified electrode. ΔE_p at MWCNT/GOx (c) modified

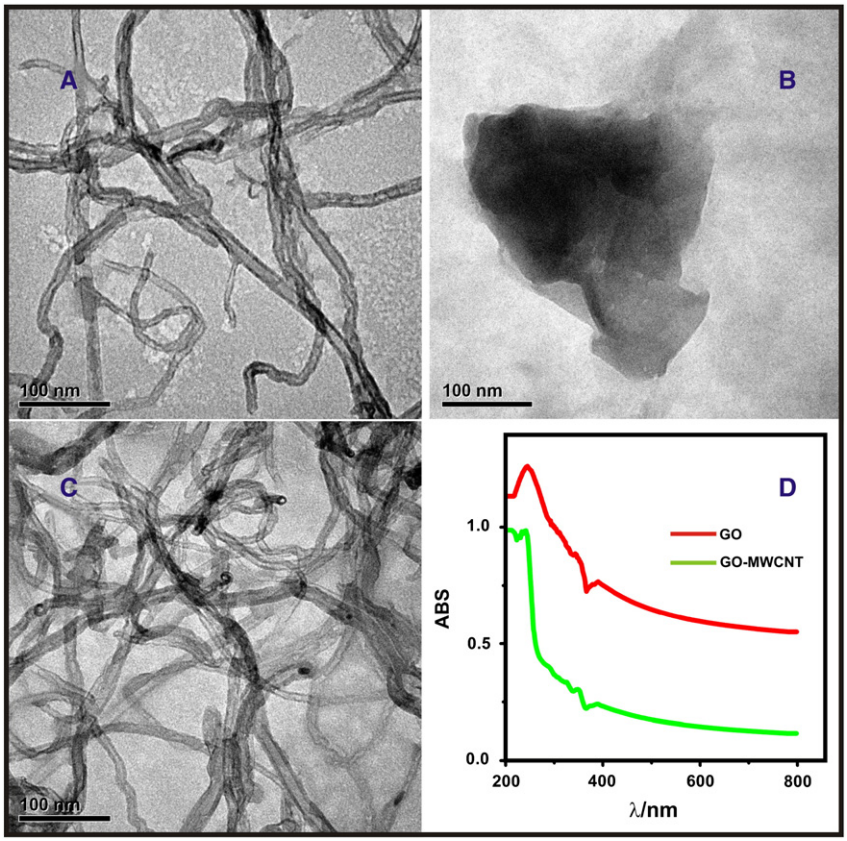

Fig. 2. TEM images of MWCNT (A), GO (B), MWCNT/GO hybrid composite (C) and UV visible spectrum of GO (green) and MWCNT/GO (red).

electrode was calculated to be ~41 mV and the current density of the anodic and cathodic peaks were found to be smaller than that of MWCNT/GO/GOx hybrid biocomposite modified electrode. Moreover, MWCNT/GO/GOx hybrid biocomposite modified electrode exhibits more background current (active surface area) than pristine MWCNT/GOx modified electrode while, bare GCE (a) and GO/GOx (b) modified electrode does not show any electrochemical signal for the GOx in the same potential window. In comparison with bare, GO/GOx and MWCNT/GOx GCEs, well-defined redox peaks appearing at the MWCNT/GO/GOx modified electrode revealed the fast direct electron transfer and higher surface area. GO plays a key role with MWCNT in producing the synergetic effect leading to the fast direct electron transfer of GOx at the electrode surface.

The different scan rate studies were performed at MWCNT/GO/GOx hybrid biocomposite modified electrode and the results are shown in Fig. 3B. Upon increasing the scan rates form 10 mV to 100 mV, the redox peak currents and peak-to-peak separation (ΔE_p) increased linearly. Meanwhile very small shifts in the anodic and cathodic peak currents were observed, while ΔE_p increased linearly at scan rates higher than 1.0 V. The anodic and cathodic peak currents increased linearly with increases in the scan rates from 10 to 100 mV s^{-1} (inset), reveling that the electrode reaction of GOx at the hybrid biocomposite is a typical surface-controlled reversible electron transfer process. The electron transfer rate constant (k_s) was calculated to be 11.22 s^{-1} using Eq. (1) [31].

$$\begin{aligned}\mathrm{Log}\, k_s = {} & \alpha\,\mathrm{Log}(1-\alpha) \\ & + (1-\alpha)\mathrm{Log}\,\alpha - \mathrm{Log}(RT/nF\nu) - \alpha(1-\alpha)nF\Delta E_p/2.3\,RT \end{aligned} \quad (1)$$

where, R is the gas constant (8.314 $Jmol^{-1}$ K^{-1}), T is the room temperature (298.15 K) and ΔE_p is the peak to peak separation of the FAD/$FADH_2$ redox couple. Here, α value was assumed as ≈0.5 and the number of electrons (n) transferred was considered as 2. The k_s value of the hybrid biocomposite was higher than that of MWCNT/ERGO/GOx (3.02 s^{-1}) [23], RGO/GOx (reduced graphene oxide (RGO), 4.8 s^{-1}) [24], RGO/PAMAM-silver nanoparticles/GOx (8.59 s^{-1}) [32] and gold nanoparticles/carbon nanotubes (2.2 s^{-1}) [33] modified electrodes. The good biocompatibility and high conductivity of the hybrid biocomposite provides the suitable microenvironment for the GOx that facilitated the direct electron transfer between the enzyme matrix and the electrode surface.

3.3. Different pH and electrocatalysis of glucose

pH is an important parameter that plays a strong impact on the enzyme based electrochemical biosensors in which the redox peaks are produced. The effect of the pH at the hybrid biocomposite modified electrode was investigated in pH 4.0–9.0. Fig. 4 shows the effect of different pH at MWCNT/GO/GOx modified electrode at the scan rate of 50 mV. The maximum peak current response was observed at pH 7.0; hence the same pH was used to evaluate the performance of the modified electrodes. Moreover, the $E^{0'}$ has linear dependence with pH 4.0–9.0 with a slope value of −56.1 mV/pH (0.999), while, anodic and cathodic potentials were shifted towards the negative direction upon increasing the pH 4.0–9.0. Furthermore, the slope value was found to be in close agreement with the theoretical value of −59 mV pH^{-1} [34]. This value corresponds to the redox reaction of enzyme involving an equal number of protons and electrons at ambient conditions. This result demonstrates that the redox reaction of GOx at MWCNT/GO/GOx modified electrode belongs to two protons ($2H^+$) and two electrons ($2e^-$) process, as supported by the following equation.

$$GOx-FAD + 2\,e^- + 2\,H^+ \leftrightarrow GOx-FADH_2 \quad (2)$$

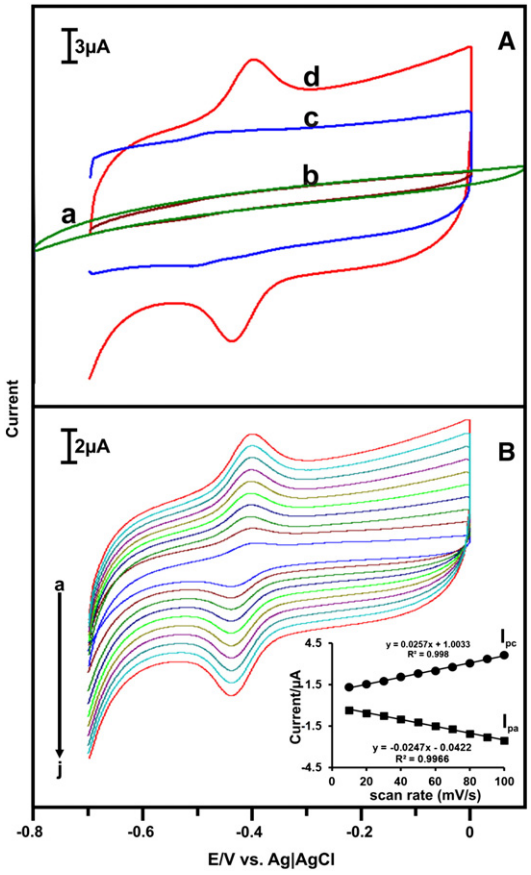

Fig. 3. (A) Cyclic voltammograms of bare GCE/GOx (a), GO/GOx (b), MWCNT/GOx (c) and MWCNT/GO/GOx (d) modified GCEs in deoxygenated PBS at 50 mV s^{-1} scan rate. (B) Cyclic voltammograms of MWCNT/GO/GOx modified GCE in deoxygenated PBS at different scan rates (10 to 100 mV s^{-1}). Inset shows the linear dependence of E_{pa} and E_{pc} on scan rate (10 to 100 mV s^{-1}).

The electrocatalytic property of the MWCNT/GO/GOx modified electrode towards the oxidation of glucose was studied in oxygen saturated PBS. Fig. 5 illustrates the electrocatalysis of glucose at the hybrid biocomposite modified electrode in the potential range 0 to −0.7 V at the scan rate of 50 mV. In the absence of glucose, a high oxidation current appeared, which gradually decreased upon each addition of glucose.

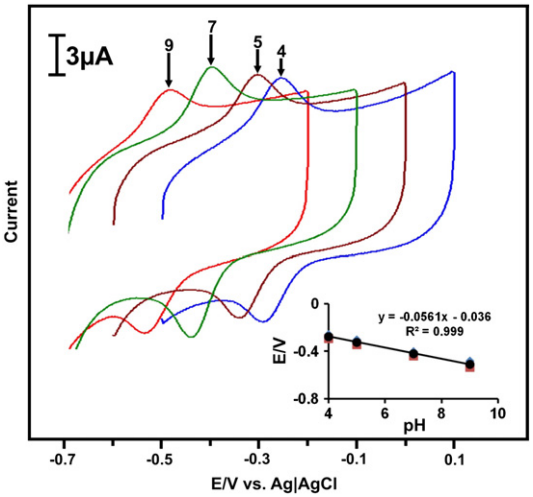

Fig. 4. Cyclic voltammogram of MWCNT/GO/GOx hybrid biocomposite modified electrode in deoxygenated different pH solutions at 50 mV s^{-1} scan rate.

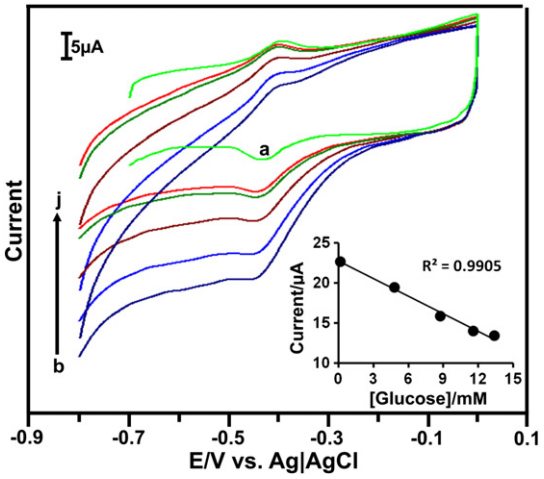

Fig. 5. Cyclic voltammograms of MWCNT/GO/GOx hybrid biocomposite modified electrode for the addition of 0.1–13.3 mM glucose (b–j) in oxygen saturated PBS at 50 mV s^{-1} scan rate. Inset plot shows the linear dependence of I_{pa} *vs.* [glucose].

This process is called as a typical electrocatalysis of glucose by the GOx in the presence of oxygen saturated PBS. Moreover, the electrocatalysis of glucose was favored with oxygen consumption in the hybrid biocomposite electrode leading to a linear relationship with the glucose concentration 0.1 mM–13.3 mM with a correlation coefficient (R) of 0.9905 (inset). The possible reaction mechanism of glucose oxidation and oxygen reduction at the composite electrode can be explained by Eqs. (3)–(4).

$$\mathrm{GOx\ (FAD)} + \mathrm{Glucose} \leftrightarrow \mathrm{GOx\ (FADH_2)} + \mathrm{Glucolactone} \tag{3}$$

$$\mathrm{GOx\ (FADH_2)} + \mathit{Glucolactone} + O_2 \rightarrow \mathrm{GOx\ (FAD)} + \mathrm{Gluconic\ acid} + H_2O_2 \tag{4}$$

3.4. Amperometric and selective determination of glucose at hybrid biocomposite modified electrode

Amperometric i–t technique is a reliable and sensitive method to evaluate the electrocatalytic activity of the electrochemical biosensors. Amperometric i–t results of MWCNT/GO/GOx hybrid biocomposite modified electrode are shown in Fig. 6A. For amperometric i–t measurements a rotating disk electrode (RDE, electrode active surface area = 0.24 cm^2) was used to investigate the electrocatalytic activity of glucose in oxygen saturated PBS. Once the background current was stable, we monitored the response current for the successive additions of glucose into the constantly stirred PBS, as the applied potential was held at −0.402 V. The applied potential was fixed from the electrocatalysis of glucose in CV analysis. Upon adding different concentrations of glucose into the electrolyte solution, the response current decreased gradually. Further the response current of the proposed biosensor decreased linearly with increase in glucose concentrations from 0.1 to 19.82 mM. The response time of the proposed sensor was calculated to be ~5 s. The sensitivity was calculated from the linear regression equation of I_{pc} to be 0.266 μA mM^{-1} with a limit of detection (LOD) of 0.028 mM (S/N = 3). The excellent conductivity and good biocompatibility of the MWCNT/GO/GOx modified electrode revealed its good electrocatalytic ability towards glucose oxidation. The performance of the proposed amperometric glucose sensor was compared with previously reported enzymatic glucose sensors as shown in Table 1. The k_s value at the MWCNT/GO/GOx modified electrode was higher than that of other glucose sensors mentioned in Table 1. Moreover, the linear range of glucose detection is comparable with that of other reported ones, which revealed the good performance of this glucose sensor.

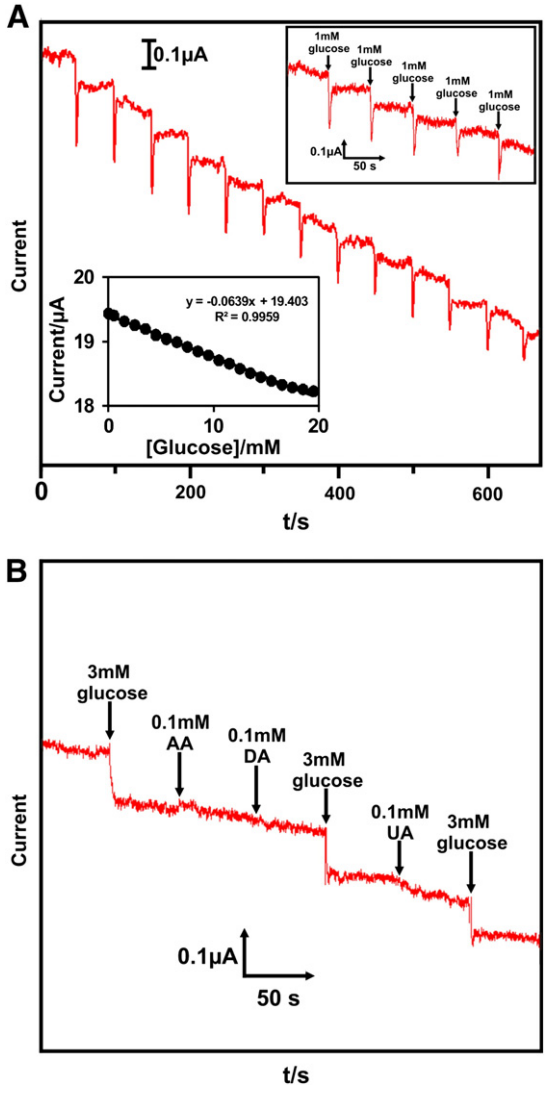

Fig. 6. (A). Amperometric i–t response of MWCNT/GO/GOx hybrid biocomposite modified rotating disk GCE upon successive additions of 0.1–19.82 mM glucose into continuously stirred oxygen saturated PBS (pH 7). Applied potential: −0.402 V; inset plot shows the calibration curve of [glucose] *vs.* current response (bottom) for each 1 mM glucose additions into the oxygen saturated PBS (top). (B) Amperometric i–t response of MWCNT/GO/GOx hybrid biocomposite modified rotating disk GCE towards 3 mM glucose, 100 μM ascorbic acid, 100 μM uric acid and 100 μM dopamine solutions added into continuously stirred oxygen saturated PBS (pH 7).

The selectivity of the proposed biosensor was evaluated by using amperometry in the presence of 3 mM glucose and 0.1 mM of other common interfering species like uric acid (UA), ascorbic acid (AA) and dopamine (DA). For selectivity studies, the interfering species were added into constantly stirred PBS at different intervals under the same working conditions and parameters as that of 6A. As shown in Fig. 6B, upon adding each interfering species no current response was observed, whereas a noteworthy response was observed for 3 mM glucose (Fig. 6B). It clearly validates that the interfering species like DA, UA and AA does not affect the response current of glucose, showing that the MWCNT/GO/GOx hybrid biocomposite modified electrode is highly selective for the determination of glucose. At higher negative potential (−0.402 V), the oxygen reduction becomes lessened, mainly because the common interference species are less active.

3.5. Stability, repeatability and reproducibility of the biosensor

To evaluate the stability of the proposed biosensor, fabricated electrode was stored in PBS at 4 °C when not in use. We monitored the background current response of the MWCNT/GO/GOx hybrid biocomposite modified electrode periodically over one month. The modified electrode retained about 82% of its initial response even after one month, revealing good stability of the proposed electrode. The repeatability of the biosensor was examined, showing that the relative standard deviation (RSD) of about 2.87% was obtained for 10 successive measurements using each 3 mM of glucose. The RSD at 3 different electrodes for the detection of each 3 mM of glucose was 4.43%. These results validate that the proposed biosensor has good repeatability and reproducibility. All experiments were performed using CV analysis under the experiment conditions same as that mentioned in Section 3.4.

Table 1
Comparison of electroanalytical performances of the proposed electrode with other GOx based glucose biosensors.

Electrode matrix	Ks (s^{-1})	Linear range	LOD[a] (μM)	Reference
ERGO[b]/MWCNT[c]/GOx[d]	3.02	0.01–6.5	10	[23]
RGO[e]/GOx	4.8	0.1–27.0	NA[p]	[24]
RGO/PAMAM[f]–Ag[g]/GOx	8.59	0.032–1.89 mM	4.5	[32]
AuNPs[h]/GOD–MWCNT–PVA[i]	2.2	0.5–8.0 mM	200	[33]
GMWCNT[j]/GOx	1.08	6.3–20.09 mM	NA	[34]
GOD[d]/MWCNT-ACS[k]	2.10	Up to 0.8 mM	17.5	[35]
GOx/CNx-MWNT[l]	4.6	Up to 1.02 mM	10	[36]
GOx-BSA[m]-MWCNT	NA	0.1–5.0 mM	NA	[37]
GNP[n]/MWCNT/GOx	NA	Up to 9.0 mM	128	[38]
MWCNT/GO[o]/GOx	11.22	0.1–19.82 mM	28	This work

[a] LOD — Limit of detection.
[b] ERGO — Electrochemically reduced graphene oxide.
[c] MWCNT — Multiwalled carbon nanotubes.
[d] GOx, GOD — Glucose oxidase.
[e] RGO — Reduced graphene oxide.
[f] PAMAM — Carboxyl terminated poly amido amine dendrimer.
[g] Ag — Silver nanoparticles.
[h] AuNPs — Gold nanoparticles.
[i] PVA — Polyvinyl alcohol.
[j] GMWCNT — Gelatin dispersed multiwalled carbon nanotubes.
[k] ACS — Alumina coated silica.
[l] CNx-MWNT — Nitrogen doped carbon nanotubes.
[m] BSA — Bovine serum albumin.
[n] GNP — Gold nanoparticles.
[o] GO — Graphene oxide.
[p] NA — Not available.

4. Conclusions

A highly selective amperometric glucose biosensor based on GOx dispersed at MWCNT/GO hybrid composite modified electrode was demonstrated. MWCNT/GO hybrid composite was prepared through the π–π interaction between the GO nano sheets and MWCNT networks. GOx was immobilized onto the surfaces of MWCNT/GO hybrid composite through the electrostatic interactions between the positively charged free amino groups of GOx with the negatively charged functional groups of GO at the hybrid composite. The direct electron transfer of GOx was greatly enhanced at the surface of hybrid composite. The proposed biosensor was highly selective and sensitive for glucose determination, with the results being repeatable and reproducible. The MWCNT/GO/GOx biosensor fabricated through a facile route with interesting characteristics of high selectivity and sensitivity has a great potential to be used in the determination of glucose from biological samples. This hybrid biocomposite also has a great potential for use in enzyme based glucose biofuel cells.

Novelty of this work

- For the first time, we are reporting, an amperometric determination of glucose at enzyme dispersed Multiwalled carbon nanotubes/graphene oxide hybrid biocomposite modified electrode.

- Fast and enhanced direct electron transfer of glucose oxidase was observed at hybrid biocomposite modified electrode than MWCNT modified electrode.
- The proposed method of electrode fabrication is very simple and eco-friendly.
- The fabricated sensor is highly sensitive and selective towards glucose determination.
- The high stability of the hybrid biocomposite could be used for monitoring of glucose in biological samples.
- The exceptional properties of this hybrid biocomposite could be used as the bioanode material at enzymatic biofuel cells in the near future.

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