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An electrochemical glucose biosensor exploiting a polyaniline grafted multiwalled carbon nanotube/perfluorosulfonate ionomer–silica nanocomposite

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ABSTRACT

A glucose biosensor was fabricated with loading of glucose oxidase (GOx) into a new organic–inorganic hybrid nanocomposite. The preparation involves formation of silica network into a Nafion (perfluorosulfonate ionomer) and subsequent loading of polyaniline grafted multiwalled carbon nanotubes (MWNT-g-PANI) onto Nafion–silica nanocomposite. Field emission scanning electron microscopy (FE-SEM) of Nafion–silica/MWNT-g-PANI composite reveals the presence of spherical silica particles (sizes in the range 250 nm–1 μm) and tubular MWNT-g-PANI particles. Chronoamperometry and cyclic voltammetry were used to evaluate the performance of biosensor towards glucose. The Nafion–silica/MWCNT-g-PANI/GOx biosensor exhibited a linear response to glucose in the concentration range of 1–10 mM with a correlation coefficient of 0.9972, good sensitivity (5.01 μA/mM), a low response time (~6 s), repeatability (R.S.D value of 2.2%) and along-term stability. The presence of silica network within Nafion and MWNT-g-PANI synergistically contributes to the performance of the biosensor towards the electrochemical detection of glucose.

1. Introduction

Enzymatic biosensors are attractive as they have many potential applications in various fields that include medical diagnosis, pharmaceutical and environmental controls. Electrochemical sensors hold much promise among the enzymatic biosensors. Sensitivity and stability are the key features for using the biosensors in practical analysis and commercial developments. Electrode modifying materials [1] and techniques for enzyme immobilization [2] have been developed to achieve these key features. A wide variety of electrodes modifying materials such as carbon nanotubes (CNTs) [3,4], polymers [5] and silica [6–8] have been used for the fabrication of biosensors.

For the electrochemical biosensor applications, the electrode modifying material is expected to possess several characteristics such as good electron transduction capability, physical or chemical environment for the stable immobilization of enzyme, bioactivity, easy accessibility towards the analyte and large surface area. Literature reveals that all these important characteristics cannot be inbuilt in a single material. Hence, there is always a demand for the development of composite materials, comprising two or more components, to achieve adequate sensitivity and stability for the biosensors [1,9,10].

Silica-based organic–inorganic composites are attractive materials because they combine both the properties of a rigid three-dimensional porous silica and the organic component [11]. The high specific surface area, mechanical stability, chemical inertness and excellent biocompatibility are the chief characteristics of silica towards fabrication of biosensors [12]. Silica-based materials provide suitable environment for biomolecule entrapment with enhanced electrochemical stability [13]. Besides, the porous and open frame works of silica in sol–gel material validate that the analyte into the active functionalities, which resulted in high sensitivity for the detection of analyte [14]. A simple approach for an efficient entrapment of enzyme into silica has been demonstrated [15]. Thionine doped silica composite was used as a mediator to construct biosensor with the immobilization of horse radish peroxiodase (HRP) [16]. An amperometric glucose sensor was fabricated by in-situ incorporation of glucose oxidase (GOx) within sol–gel silica film on a Prussian blue modified electrode [17]. Cholesterol oxidase was immobilized into chitosan–silica-multiwalled carbon nanotube (MWNT) composite [18]. HRP was

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immobilized onto a dendric Ag particles/silica nanocomposite to fabricate the hydrogen peroxide biosensor [19]. Silica gel/polyvinyl alcohol composite-based biosensor has been fabricated by immobilizing GOx [20]. Thus, literature reveals that sol–gel-derived silica can be combined with another component to prepare functional composites in the process of achieving combinational properties.

Nafion, a perfluorosulfonate ionomer, has been entrapped into sol–gel silica by sol–gel reactions to develop a new class of solid acid catalyst [21,22] and used for the fabrication of electrochemical biosensors [6–8,23,24]. The incorporation of Nafion into the sol–gel-derived silica film minimizes the brittleness of the pure sol–gel-derived silica and enhances the long-term stability of phenol biosensor [25]. GOx was immobilized into Nafion-ordered mesoporous silica composite for the fabrication of glucose biosensor [26]. However, reports on the use of Nafion–silica composites with the incorporation of carbon nanotubes (CNT) or a conducting polymer (CP) towards fabrication of electrochemical biosensor are scarce.

CNT have been extensively used in electrochemical and biosensing studies due to their advantageous properties [27–31]. Recently, CNT-based modified electrodes have been used for the sensitive detection of various analytes [3,4,9,32–34]. Electrochemical studies have clearly demonstrated that CNT-based modified electrodes could exhibit high sensitivity and selectivity for electrochemical detection of analytes [35,36]. The incorporation of CNT into CPs produce new type of composite materials with individual properties of each component as well their synergistic effect on the performance of biosensors [37]. Among CPs, polyaniline (PANI) has been extensively studied due to its high electrical conductivity and redox suitability. To date, several studies have been reported on the use of CNT/PANI or derivatives of PANI composites for the fabrication of biosensors [36,38,39]. The electrocatalytic role of PANI grafted CNT for the electron transfer reaction of biomolecules [32–34] has been well documented.

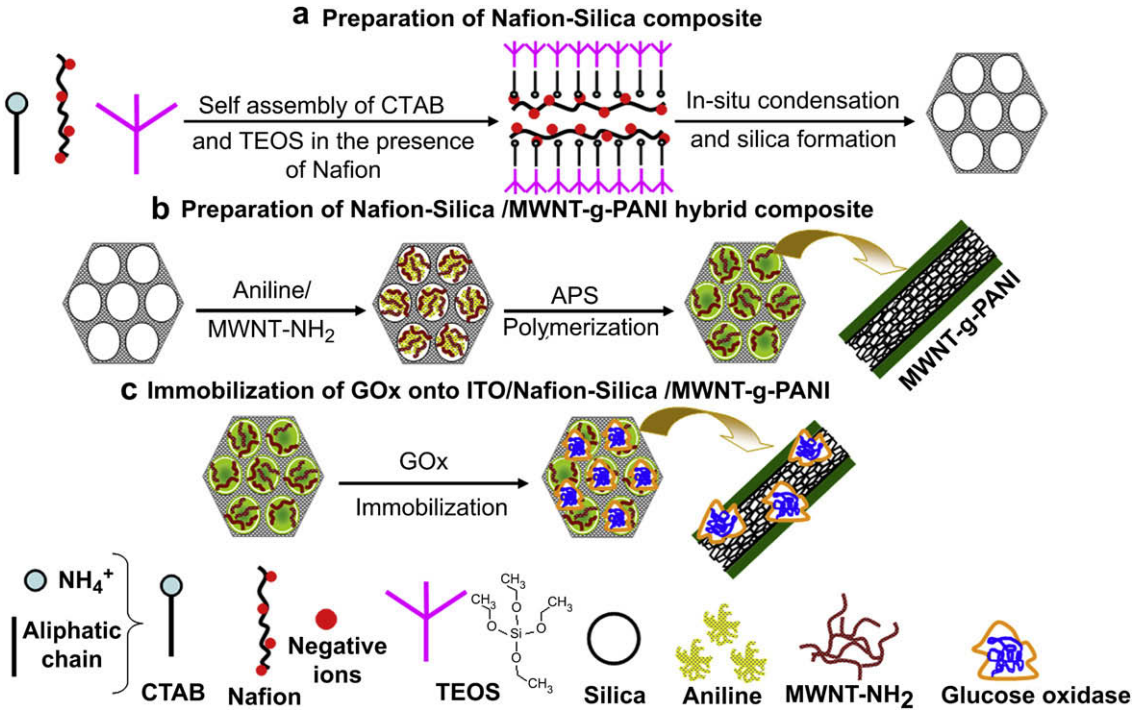
In the past two decades, glucose biosensors received great attention due to the importance in the detection of glucose for the growing population of diabetic patients [40,41]. The ultimate goal of the glucose electrochemical biosensors lies on designing high performance sensor with appropriate characteristics such as sensitivity, selectivity, response time, good linear concentration range, stability and reproducibility. Several approaches have been developed in the process of performance improvements which include development of efficient matrix for enzyme immobilization and preparation of electrocatalytic components [42]. Nafion, silica, MWNT, and conducting polymer have individually been known to improve the electrocatalytic activity [43], electron conduction path [44], sensitivity [45], stability of biosensors [46], respectively. Sol–gel silica, CNT, PANI and Nafion were used either alone or in binary combinations for the fabrication of glucose biosensor, silica gel/GOx [45], Nafion/GOx/CNTs [46], Silica sol–gel/GOx/CNTs [47], Sol–gel/GOx/copolymer [48]. To the best of our knowledge, a multi-component composite comprising of CNT, silica, Nafion and PANI has not been utilized for the fabrication of glucose biosensor.

Considering the advantageous properties of silica–Nafion composites and CNTs–PANI composites, we have developed an electrochemical biosensor-based on a multi-component composite comprising of MWNT, silica, Nafion and PANI. We have judiciously integrated the four components in the composite by employing the following strategy (Scheme 1). First, Nafion–silica composite was prepared by the condensation of silica precursors in a Nafion solution. In the subsequent step, MWNT-g-PANI was incorporated into silica–Nafion composite. The glucose biosensor was fabricated by the immobilization of GOx into Nafion–silica/MWNT-g-PANI composite. The glucose biosensor, Nafion–silica/MWNT-g-PANI/GOx exhibited electrochemical response to glucose over a wide concentration range with a good selectivity and sensitivity.

2. Experimental

2.1. Materials

MWNTs were obtained from CNT Co. Ltd. Incheon, Korea. GOx (256 U mg^{-1}; U: enzyme units) and D(+)-glucose were purchased from Sigma (South Korea). Nafion (5%) solution, aniline, ascorbic acid (AA), uric acid (UA), acetaminophen (AP),



Scheme 1. Fabrication of Nafion–silica/MWNT-g-PANI/GOx biosensor.

ammonium persulphate (APS), cetyltrimethyl ammonium bromide (CTAB), tetraethylorthosilicate (TEOS), *N,N′*-dimethylformamide (DMF) and hydrochloric acid (HCl) were of analytical grades and used as received. Glucose solutions were prepared in 0.1 M phosphate buffer solution (PBS, pH 7.0) fresh at the time of experiment. Serum sample was obtained from the Kyungpook National University Hospital, Daegu, South Korea. The serum was diluted using 0.1 M PBS (pH 7) and was analyzed without any pretreatment. Indium-doped tin oxide (ITO)-coated glass plate (specific surface resistance ~10 Ω) was purchased from Corning Inc., USA. The ITO plate was rinsed with acetone and washed with distilled water. Double distilled water was used throughout the experiment.

2.2. Fabrication of nafion–silica/MWNT-g-PANI/GOx biosensor

The fabrication of biosensor involves the following steps: (i) preparation of amine functionalized MWNTs (ii) preparation of Nafion/silica composite (iii) loading of MWCNT-g-PANI within the Nafion–silica composite and (iv) immobilization of GOx onto Nafion–silica/MWNT-g-PANI (Scheme 1).

2.2.1. Preparation of nafion–silica composite

Nafion–silica composite was prepared by in-situ condensation of TEOS in the presence of Nafion. 5 mL of 5% Nafion in ethanol was added to 195 mL of aqueous NaOH (1.73 g) and the mixture was stirred for 10 min. A solution of CTAB (10 mM) was then added to the above mixture and stirred for 30 min. TEOS (16.69 mL) was added dropwise to the mixture and stirred vigorously for 12 h. The white color residue (Nafion–silica composite) was filtered, washed with water and dried at 80 °C.

2.2.2. Loading of MWNT-g-PANI into the nafion–silica network

The amine functionalized MWNT (MWNT–NH_2) was prepared as detailed elsewhere [49]. 2 g of Nafion–silica composite was dispersed into a solution (50 mL) containing 100 mM of aniline and 10 mg of MWNT–NH_2. The mixture was stirred vigorously for 1 h. 20 mL (0.2 M) of ammonium persulphate (in 1 M HCl) was added dropwise to the mixture. The color of the mixture turned into green indicating the occurrence of polymerization of aniline. The green mass (Nafion–silica/MWNT-g-PANI composite) was centrifuged, washed with 1 M HCl and dried at 40 °C for 12 h.

0.2 g of Nafion–silica/MWNT-g-PANI composite was dispersed into 5 mL of DMF and the solution was evaporated into a paste. The paste was grounded well with a mortar (15 min). The black suspension was drop coated onto the surface of ITO and dried to obtain ITO/Nafion–silica/MWNT-g-PANI electrode. For a comparative study, ITO/Nafion–silica, ITO/Nafion–MWNT–NH_2 and ITO/Nafion–PANI electrodes were also fabricated.

2.2.3. Immobilization of GOx onto nafion–silica/MWNT-g-PANI

10.4 mg glucose oxidase (GOx) was dissolved in 1.0 mL buffer solution (pH 7.0). 8 μL of GOx was immobilized onto the surface of ITO/Nafion–silica/MWNT-g-PANI electrode to fabricate ITO/Nafion–silica/MWNT-g-PANI/GOx biosensor.

2.3. Instrumentation

The morphology of the electrodes was investigated using field emission scanning electron microscopy (FE-SEM) (TEOL JSM-5600LV instrument with the operation of 25 kV). Electrochemical measurements were performed using IVIUMSTAT electrochemical interface analyzer (Netherlands).

2.4. Electrochemical studies and determination of glucose

A conventional three electrode cell assembly was used for the electrochemical measurements. Nafion–silica/MWNT-g-PANI/GOx biosensor was used as the working electrode. Ag/AgCl and platinum wire were used as reference and counter electrodes, respectively. The electrocatalytic activity of Nafion–silica/MWNT-g-PANI/GOx was evaluated using cyclic voltammetry in 0.1 M PBS (pH 7). Hydrodynamic voltammograms were obtained by allowing the background current to reach a stable value (by 5–10 min) at each applied potential and recording the steady state current after the injection of glucose. A small magnetic bar provided the convective transport. Amperometric measurements at Nafion–silica/MWNT-g-PANI/GOx were carried out at an applied potential of +0.2 V (vs. Ag/AgCl) for various concentrations of glucose in an electrochemical cell. The current response was recorded as follows: after applying the potential (+0.2 V vs. Ag/AgCl), the current was allowed to reach a steady value (after 50 s). Subsequently, aliquot of glucose solution was added successively and the stable current response was recorded.

3. Results and discussion

3.1. Morphology

FESEM image of Nafion–silica composite (Fig. 1A) shows the existence of spherical silica particles (sizes in the range of 250 nm to 1 μm). Fig. 1B presents the FESEM image of Nafion–silica/MWNT-g-PANI composites. Randomly distributed tubular particles of MWNT-g-PANI are seen (Fig. 1B, inset).

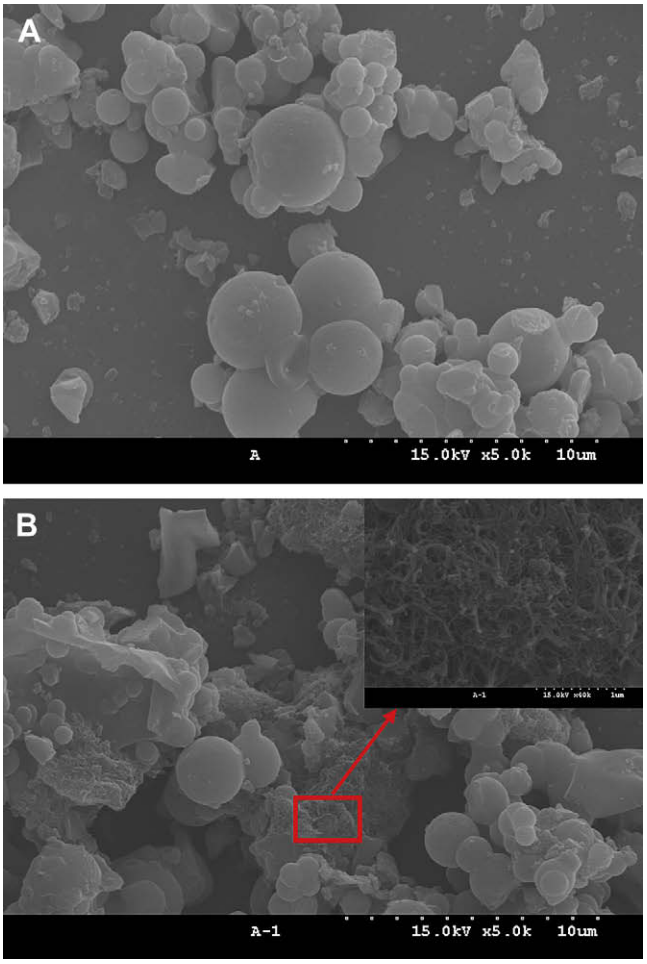


Fig. 1. FE-SEM images of ITO/Nafion–silica (a), (b) ITO/Nafion–silica/MWNT-g-PANI composites and MWNT-g-PANI (Fig. 1B, inset).

3.2. Electrochemical behavior of nafion–silica/MWNT-g-PANI/GOx modified electrode

The electrochemical behavior of the modified electrodes was investigated by cyclic voltammetry using $Fe(CN)_6^{3-}/^{4-}$ as the redox marker. Cyclic voltammograms (CVs) were obtained at bare ITO (Fig. 2, curve a), ITO/Nafion–silica (Fig. 2, curve b), ITO/Nafion–silica/MWNT (Fig. 2, curve c), ITO/Nafion–silica/PANI (Fig. 2, curve d) and ITO/Nafion–silica/MWNT-g-PANI (Fig. 2, curve e) in 0.1 M KCl with 1 mM of $Fe(CN)_6^{3-}/^{4-}$. A pair of one electron quasireversible redox wave (0.29 mV, anodic and 0.22 mV, cathodic) was observed at the bare (ITO) electrode with a peak separation between anodic and cathodic waves (ΔE_p) as ~70 mV (curve a). The ITO/Nafion–silica electrode showed a ΔE_p of 110 mV but with 2.8 times increased current than observed at ITO electrode. This observation is quite interesting. The existence of electrically insulating layer of Nafion is expected to show less current for the redox processes of $Fe(CN)_6^{3-}/^{4-}$. However, the presence of porous silica in Nafion–silica composite electrode provides adequate percolation of $Fe(CN)_6^{3-}/^{4-}$ ions to the electrode surface and facilitates electron transfer at the electrode. Thus, an enhanced electrocatalytic current (2.8 times higher than at ITO) was witnessed at ITO/Nafion–silica electrode. The redox waves of the $Fe(CN)_6^{3-}/^{4-}$ were also observed at ITO/Nafion–silica/MWNT (Fig. 2, curve c), ITO/Nafion–silica/PANI (Fig. 2,

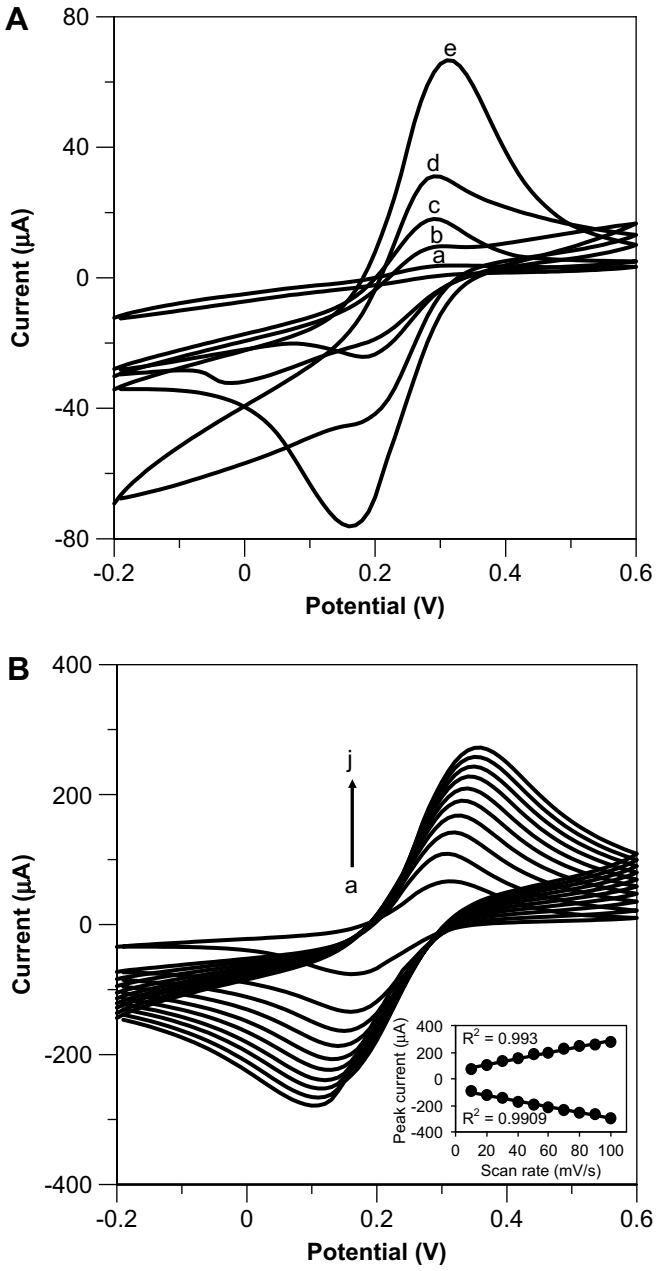


Fig. 2. (A) Cyclic voltammograms of different modified electrodes (a) bare ITO (b) ITO/Nafion–silica (c) ITO/Nafion–silica/MWNT (d) ITO/Nafion–silica/PANI and (d) ITO/Nafion–silica/MWNT-g-PANI/GOx-ME; supporting electrolyte 0.1 M KCl containing 1 mM of $Fe(CN)_6^{3-}/^{4-}$; Scan rate = 50 mV/s. (B) Cyclic voltammograms of ITO/Nafion–silica/MWNT-g-PANI/GOx-ME in 1 mM $Fe(CN)_6^{3-}/^{4-}$ (phosphate buffer, pH 7.0) for various scan rates: (a) 10 (b) 20 (c) 30 (d) 40 (e) 50 (f) 60 (g) 70 (h) 80 (i) 90 (j) 100 mV/s. Inset: Peak current vs. scan rates.

curve d) and ITO/Nafion–silica/MWNT-g-PANI (Fig. 2, curve e) electrodes. Larger peak separations and higher peak currents were observed at the other modified electrodes as compared to ITO (bare) electrode. ITO/Nafion–silica/MWNT and ITO/Nafion–silica/PANI modified electrodes (ME), showed 5.2 and 9.2 times increased current over ITO electrode for the anodic process of $Fe(CN)_6^{3-}/^{4-}$ redox reaction, with ΔE_p as 130 and 150 mV, respectively. Thus, it is clear that the inclusion of MWNT or PANI into Nafion–silica composite film improves the electrocatalytic activity. MWNT and PANI are known for their electrocatalytic action for organic/inorganic reactions [50–52].

In the case of Nafion–silica/MWNT-g-PANI electrode, a significantly high peak current (19 time higher) than ITO electrode was observed. Importantly, the peak current at ITO/Nafion–silica/MWNT-g-PANI electrode was 3.8 and 2.2 times higher than ITO/Nafion–silica/MWNT and ITO/Nafion–silica/PANI electrodes, respectively. Thus, when PANI chains were grafted onto MWNT and included into Nafion–silica composite, the electroactivity of the electrode was significantly improved. MWNT and PANI in MWNT-g-PANI, synergistically contributes to the electrocatalytic process. The above results clearly demonstrated that modification of ITO by Nafion–silica/MWNT-g-PANI provides adequate electron transfer path to enhance higher catalytic current.

CVs were recorded in a solution of 1 mM $Fe(CN)_6^{3-}/^{4-}$ at ITO/Nafion–silica/MWNT-g-PANI electrode for different scan rates (Fig. 2B). It can be seen that the potentials and peak currents of the $Fe(CN)_6^{3-}/^{4-}$ redox process (anodic as well as cathodic) are dependent on the scan rates. Larger peak-to-peak separations were observed with increase in scan rates. The anodic and cathodic peak current values increased linearly with the scan rates (correlation coefficient values of 0.993 and 0.9909 for anodic and cathodic peaks, respectively) in the scan rate range of 10–100 mV/s (Fig. 2B, inset). The linearity between peak current and scan rate suggests that the redox reaction is surface-confined.

3.3. Electrochemical performance towards glucose

3.3.1. Electroactivity of different modified electrodes

Fig. 3(a–d) compares the electrochemical response of glucose at ITO/Nafion–silica, ITO/Nafion–silica/MWNT, ITO/Nafion–silica/PANI and ITO/Nafion–silica/MWNT-g-PANI/GOx electrodes in the positive potential scans (−0.2 to 0.6 V) in 0.1 M PBS. Ill-defined voltammetric responses were obtained for the oxidation of glucose at ITO/Nafion–silica (Fig. 3, curve a), ITO/Nafion–silica/MWNT (Fig. 3, curve b), ITO/Nafion–silica/PANI (Fig. 3, curve c) electrodes, probably due to slow electron transfer process at the electrodes. As compared to the other electrodes, significant electro-oxidation of glucose occurred at ITO/Nafion–silica/MWNT-g-PANI/GOx biosensor.

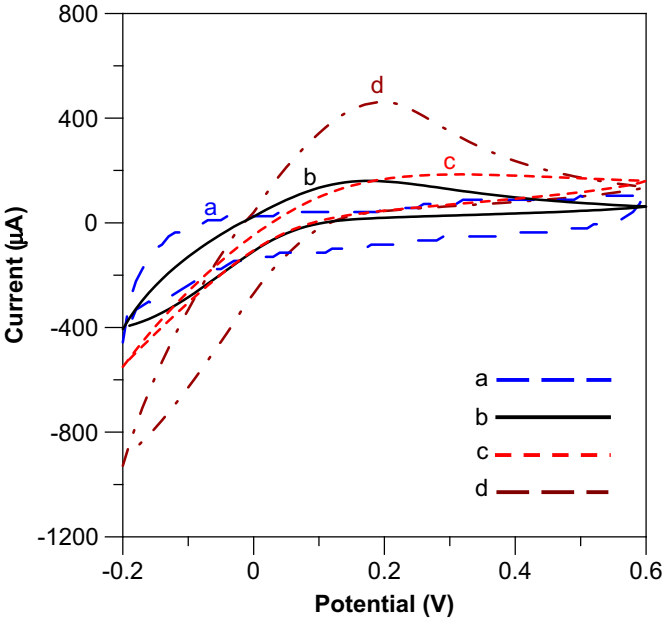


Fig. 3. CVs of 5 mM glucose at different modified electrodes, (a) ITO/Nafion–silica (b) ITO/Nafion–silica/MWNT (c) ITO/Nafion–silica/PANI and (d) ITO/Nafion–silica/MWNT-g-PANI/GOx in 0.1 M PBS (pH 7.0); scan rate = 50 mV/s.

A prominent anodic peak (+0.2 V) with significant peak current was observed for the oxidation of glucose at ITO/Nafion–silica/MWNT-g-PANI/GOx biosensor (Fig. 3, curve d). The higher peak current for glucose oxidation at Nafion–silica/MWNT-g-PANI/GOx is attributed to the influence of MWNT-g-PANI in the Nafion–silica film. Thus, PANI-g-MWNT biosensor exhibited enhanced glucose oxidation current as compared to the biosensor fabricated with the inclusion of either MWNT or PANI into Nafion–silica composite. Thus, MWNT-g-PANI synergistically contributes to the electro-oxidation of glucose.

3.3.2. Electrochemical glucose sensing characteristics at nafion–silica/MWNT-g-PANI/GOx biosensor

Knowing the superior performance of Nafion–silica/MWNT-g-PANI/GOx biosensor towards oxidation of glucose, we have performed electrochemical experiments with different concentrations of glucose. After the addition of glucose (1 mM), the anodic peak current significantly increased (Fig. 4b) as compared to the oxidation current in the absence of glucose (Fig. 4a). Fig. 4(b–e) illustrates that the successive addition of glucose concentration (1 mM) resulted a prominent and proportional increase in the peak current corresponding to oxidation of glucose. Thus, the remarkable enhancement in the peak current provides a clear evidence of the electrocatalytic effect of Nafion–silica/MWNT-g-PANI/GOx biosensor towards glucose oxidation.

3.3.3. Hydrodynamic voltammetry nafion–silica/MWNT-g-PANI/GOx biosensor

In order to fix the most appropriate working potential for the detection of glucose, hydrodynamic voltammetric measurements were performed at Nafion–silica/MWNT-g-PANI/GOx biosensor. Hydrodynamic voltammograms were recorded in the potentials between −0.2 V and 0.6 V for a solution of glucose (5 mM in PBS) at Nafion–silica/MWNT-g-PANI/GOx biosensor and the steady state current values were recorded (Fig. 4, inset). A substantial rise in current was observed at potentials higher than +0.2 V, presumably due the electrocatalytic oxidation of glucose at the biosensor matrix. Hence, +0.2 V was fixed as the working potential for the detection of glucose.

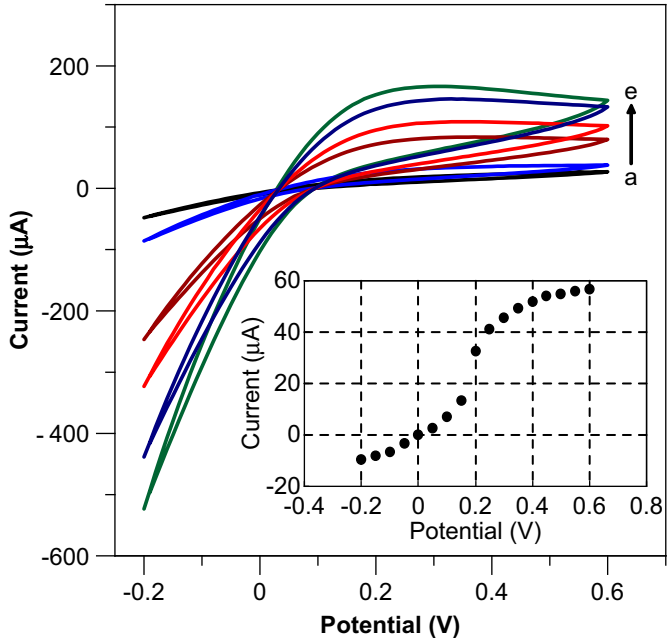


Fig. 4. Cyclic voltammograms of the modified electrodes ITO/Nafion–silica/MWNT-g-PANI/GOx-ME in phosphate buffer (pH 7.0) for different glucose concentrations a: no glucose, (b–d). Each addition increased the concentration of glucose by 1.0 mM (inner to outer). Inset shows the hydrodynamic voltammograms recorded at Nafion–silica/MWNT-g-PANI/GOx biosensor in PBS (pH 7.0) containing 5 mM glucose.

3.3.4. Amperometric response

Amperometric measurements were performed to evaluate and analyze the performance of Nafion–silica/MWNT-g-PANI/GOx biosensor towards increasing glucose concentrations in PBS (pH 7.0). Fig. 5A shows the current–time profile of the Nafion–silica/MWNT-g-PANI/GOx biosensor for successive additions of glucose concentration under the optimized experimental conditions ($E = +0.2$ V; rpm = 400). Upon each injection of glucose at regular intervals, rapid and prominent increase in currents was observed. The corresponding calibration curve is shown in Fig. 5A (inset). The current response was linear for glucose concentration in the range of 1–10 mM (correlation coefficient, $R = 0.9992$). The sensitivity of the Nafion–silica/MWNT-g-PANI/GOx biosensor (5.01 μA/mM) is

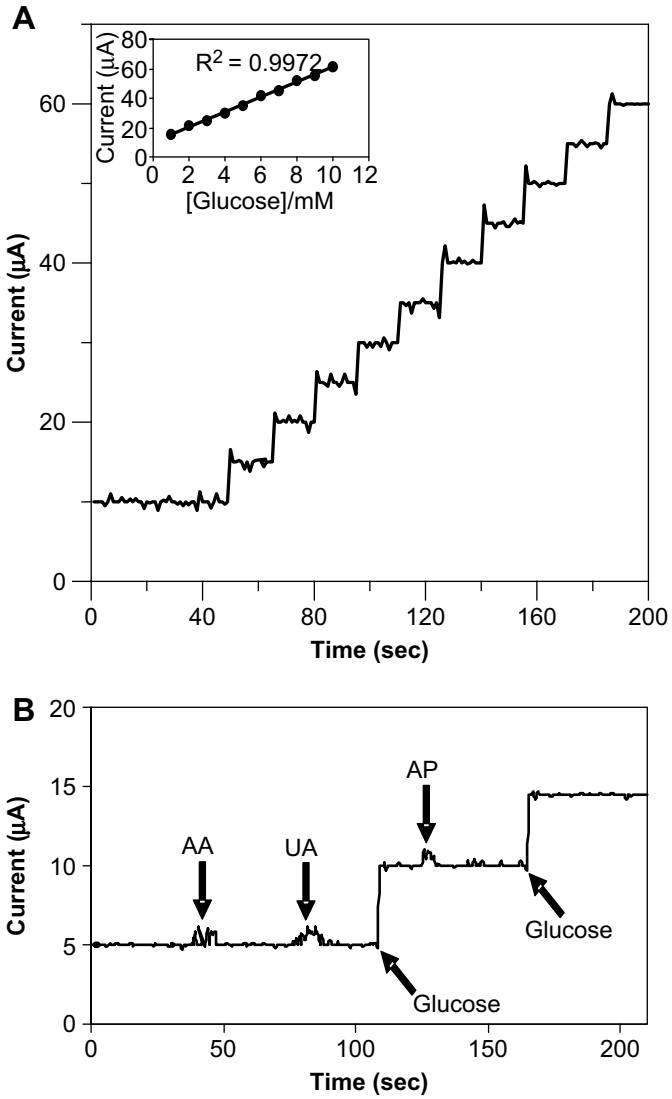


Fig. 5. (A) Chronoamperometric responses of Nafion–silica/MWNT-g-PANI/GOx biosensor for successive addition of 1 mM glucose solution at an applied potential of +0.2 V (vs. Ag/AgCl); Inset: calibration plot of the glucose concentration vs. current response. (B) Amperometric responses of Nafion–silica/MWNT-g-PANI/GOx biosensor to glucose (1 mM), ascorbic acid (1 mM), uric acid (1 mM) and acetaminophen (1 mM) in PBS (pH 7.0).

Table 1
Comparison of performances of some glucose biosensors-based on CNT or silica or Nafion or PANI as one of the component in the matrix for enzyme immobilization.

Modified electrode	Linear range (mM)	Sensitivity (μA/mM)	Detection limit (μM)	Reference
GOD-silica-modified Pt	0–10	3.53	10	[45]
TTF/hybrid-GOx/Nafion	1–10	0.6	–	[48]
Bppg/CNT/biocomposite	0.2–20	0.196	50	[47]
MWNT-Nafion-GOD	upto 2	0.33	4	[46]
GOx/Au-(SH)PANI-g-MWNT}$_n$	1–9	0.06	3.97	[42]
Nafion-silica/MWNT-g-PANI/GOx	1–10	5.01	0.1	This Work

superior than reported for the glucose biosensor fabricated with silica or Nafion or CNT or CNT–Nafion composites or PANI-g-MWNT as matrix for GOx immobilization (Table 1). Typically, Nafion/GOx/CNTs (0.33 μA/mM) [46], Silica sol–gel/GOx/CNTs (0.196 μA/mM) [47], Sol–gel/GOx/copolyme (0.6 μA/mM) [48], PEDOT/PB/MWNT (2.67 μA/mM) [53], GOx/Au/CS–IL–MWNT(SH) (4.10 μA/mM) [9] and MWNT/Au NPs/GOx (2.527 μA/mM) [54] biosensors show lesser sensitivity than Nafion–silica/MWNT-g-PANI/GOx biosensor. The superior sensitivity of Nafion–silica/MWNT-g-PANI/GOx arises from the judicious design for the fabrication. The biosensor was fabricated by forming a Nafion–silica composite in the first step. From the electroactivity results, it can be seen that ITO/silica–Nafion composite-based electrode (Fig. 2b) shows much higher electron transfer capability as compared to bare ITO (Fig. 2a). Thus, a good conductive film environment was generated for further loading of PANI-g-MWNT as an integrated unit into silica–Nafion composite film. CV results clearly documented that ITO/Nafion–silica/MWNT-g-PANI electrode (Fig. 2e) has higher electroactivity as compared to ITO/Nafion–silica/MWNT (Fig. 2c) and ITO/Nafion–silica/PANI (Fig. 2d). The higher sensitivity for Nafion–silica/MWNT-g-PANI/GOx biosensor electrode documents the importance of loading of MWNT-g-PANI within Nafion–silica network.

Also, Nafion–silica/MWNT-g-PANI/GOx shows a lower response time of ~6 s for the detection of glucose to reach a steady state current. The larger active surface area provided by the MWNT and augmented electron transduction at MWNT-g-PANI unit are the reasons for the effective electron mediation at Nafion–silica/MWNT-g-PANI/GOx biosensor to result a lower response time for glucose detection. Importantly, Nafion–silica/MWNT-g-PANI/GOx biosensor exhibited the combination of high sensitivity (5.01 μA/mM), wide linear concentration range (1–10 mM) low detection limit (0.1 μM) and lower response time (6 s) because of the combined presence of silica–Nafion and MWNT-g-PANI.

3.4. Interference study

The influence of electrochemical interferents on the current response of glucose was evaluated at Nafion–silica/MWNT-g-PANI/GOx biosensor. Fig. 5B represents the chronoamperometric responses of glucose (1 mM) in the presence of AA (1 mM), UA (1 mM) and AP in PBS (pH 7.0). The injection of AA, UA, and AP did not influence the current response of glucose. Electrochemical detection of glucose was possible at a lower potential (+0.2 V) due to the electrocatalytic influences of MWNT-g-PANI. Hence, interference from other electroactive components was negligible at the Nafion–silica/MWNT-g-PANI/GOx biosensor.

3.5. Real sample analysis, stability and reproducibility

The applicability of the Nafion–silica/MWNT-g-PANI/GOx biosensor towards detection of glucose in human serum and recovery tests to added glucose was evaluated. The glucose detections were carried out using standard addition method, with three times addition of a standard glucose solution. The results showed that RSD for three measurements were in the range of 2.9–4.1% and recoveries in the range of 98.0–105.5.

A reproducible current response (R.S.D = 2.2%) was noticed at Nafion–silica/MWNT-g-PANI/GOx biosensor for 10 measurements with a fabricated biosensor. The stability of the Nafion–silica/MWNT-g-PANI/GOx biosensor was determined by storing the biosensor at 4 °C in PBS (pH 7) for 20 days and monitoring the response current everyday for 1.0 mM of glucose at +0.20 V. The biosensor retained its 93% current response after 20 days. Hence, Nafion–silica/MWNT-g-PANI/GOx biosensor exhibits adequate stability and reproducibility. The minimum loss of current response (7% after 20 days) for the Nafion–silica/MWNT-g-PANI/GOx biosensor may arise from leaching of GOx to the buffer solution or the development of over potential at the electrode during the storage period and consequent loss of activity of GOx due to denaturation. There was no leaching of GOx to PBS solution. This was ensured by following the enzyme activity of electrolyte solution by dianisidine test [55,42]. The possible over potential may be minimized by applying a constant potential during the storage time.

4. Conclusion

We have developed an enzymatic glucose biosensor by loading MWNT-g-PANI within the Nafion–silica matrix. The Nafion–silica/MWNT-g-PANI/GOx biosensor exhibits high sensitivity (5.01 μA/mM) and good reproducibility (R.S.D = 2.2%) towards glucose detection. The Nafion–silica/MWNT-g-PANI biosensor showed linear response to glucose in the range of 1–10 mM with a shorter response time of ~6 s. In addition, the biosensor exhibited high selectivity to glucose detection, storage and stability. We anticipate that the methodology of including conductive polymer grafted MWNT into Nafion–silica matrix would be extended for the development of other biosensors.

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Appendix

Figures with essential colour discrimination. Certain figures in this article, in particular Figs. 1, 3 and 4 and Scheme 1, are difficult to interpret in black and white. The full colour images can be found in the on-line version, at doi:10.1016/j.biomaterials.2009.07.047.

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