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Glucose oxidase immobilized amine terminated multiwall carbon nanotubes/reduced graphene oxide/polyaniline/gold nanoparticles modified screen-printed carbon electrode for highly sensitive amperometric glucose detection

Debasis Maity[a], Minitha C.R.[b], Rajendra Kumar R.T.[a,b,*]

[a] DRDO - BU Center for Life Sciences, Bharathiar University, Coimbatore 641046, Tamil Nadu, India
[b] Advanced Materials and Devices Laboratory (AMDL), Department of Nanoscience and Technology, Bharathiar University, Coimbatore 641046, Tamil Nadu, India

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ABSTRACT

A novel amine terminated multiwall carbon nanotubes/polyaniline/reduced graphene oxide/gold nanoparticles modified screen-printed carbon electrode (SPCE) was fabricated. Followed by, glucose oxidase (GOx) was immobilized on SPCE for highly sensitive glucose biosensor. The synthesized nanomaterial and their composites were characterized using scanning electron microscope (SEM) and UV–Visible spectroscopy. The electrochemical analysis has been followed at different stages of glucose oxidase coating on modified SPCE using cyclic voltammetry. The reduction current has enhanced 13.43 times with the lowest working potential by the modified SPCE when compared to bare SPCE. The glucose biosensor exhibited good reproducibility (90.23%, $n = 7$), high stability (after 30 days 96% at −20 °C storage, 2 week 74.5% at −4 °C storage), wide linear range (1–10 mM), less K_M^{app} value (0.734), lowest detection limit (64 μM) and good sensitivity (246 μ $Acm^{-2}mM^{-1}$). The biosensor was validated for the detection of glucose level in human blood serum samples using the amperometric technique. As designed nanocomposite based SPCE has the potential for an efficient glucose sensor, which also enabled the platform for various biochemical sensors.

1. Introduction

Glucose is the most important carbohydrate in the human body. According to the world health organization (WHO), around 3.4 million died due to high blood sugar and currently, 143 million people in worldwide suffering from diabetes. It is necessary to estimate our body glucose level to control diabetes as well as in food processing control, biotechnology, and environmental protection [1]. Also, it is necessary to develop highly sensitive, cost-effective, portable, robust glucose biosensor to determine the glucose level in the current lifestyle. There are several methods available for glucose determination such as fluorescence-based analysis [2], colourimetric [3], flow injection analysis [4], luminescence [5] and optical methods [6]. The Electrochemical sensing, by advantage of its quick response, ease of use, reliability, good sensitivity, low cost, portability and one of the promising and extensively accepted technique for detection of glucose in the current scenario. The biosensor has been fabricated by immobilizing bio-recognition elements such as enzyme [7], antibody [8], DNA [9] along with different nanomaterial as a transducer.

Recognition element (enzymatic, non-enzymatic), electrochemical transducer (for signal processing) and user-friendly data representation was the major components of all type electrochemical sensing. The Glucose oxidase (enzymatic) based biosensors are highly selective, sensitive, fast and reversible. Glucose biosensor is the most common enzymatic biosensor, where Glucose oxidase acts as catalysis and convert glucose into gluconolactone and hydrogen peroxide. An efficient, sensitive, fast and reversible glucose biosensor with good selectivity can be fabricated by immobilizing Glucose oxidase with different electrochemical transducers. The drop casting method (Physical adsorption) is the simplest for all the immobilizing techniques to immobilize enzymes as transducers. Although there is a large amount of literature available based on glucose oxidase modified different electrodes for the selective detection of glucose, few attempts were taken by the research community to improve the sensitivity by using different materials (transducers). The higher sensitive sensor gives the opportunity to fabricate a low-cost device with more precise results. Since, enzymatic reaction

* Corresponding author at: Department of Nanoscience and Technology, Bharathiar University, Coimbatore 641046, Tamil Nadu, India.
E-mail address: rtrkumar@buc.edu.in (R.K. R.T.).

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was well-known nanomaterials (carbon based nanomaterial (CNT, GO, rGO), Metal nanoparticals (Ag, Au, Pt, Ru), conducting polymer (PPy, PANI)) modified electrode is used before enzyme immobilization to improve the analytic response to enhance the electrons transform rate between analyte and electrode [9–12]. The GOx could be easily absorbed onto rGO nanosheets through electrostatic interaction. The amine-terminated MWCNTs formed a bond through carboxyl group of GOx by hydrogen bonding. The NH_2-MWCNT/rGO hybrid composite has better electrochemical sensing ability, enhanced direct electron transfer and more capacitance when compared with simple NH_2-MWCNTs and rGO modified electrodes. The conduction polymers, polyaniline (PANI) was used as an immobilization platform to biomolecules such as DNA, enzyme and protein for excellent electrochemical activity in biosensing application [13]. The electrical conductivity and electrochemically activity of rGO/NH_2-MWCNT can be enhanced by modifying with conducting polymer PANI [14]. The PANI/rGO/NH_2-MWCNT composite modify electrodes was used without any binder in order to increase the electrochemical reaction rate, shorten ion diffusion path and to reduce the interfacial resistance. Another hand, the gold nanoparticals (AuNP) had been increasingly used in the fields of biosensing application owing to their excellent electrocatalytic activity. The citrate-stabilized gold nanoparticles (AuNP) could provide efficient negative charges for anionic doping. The AuNP/ PANI/rGO/NH_2-MWCNT has better electroactivity than PANI/rGO/NH_2-MWCNT. Due to the size effect, the gold nanoparticles (AuNP) in the hybrid material shows a good catalytic effect on the oxidation and reduction of H_2O_2. The AuNP/ PANI/rGO/NH_2-MWCNT was deposited on a screen printed carbon electrode (SPCE), and a glucose biosensor was fabricated by adsorption of glucose oxidase (GOx) on the hybrid material [15–21]. The sensitivity was achieved due to AuNP/PANI/rGO/NH_2-MWCNTs complex architecture and any single component or other combination will not provide this much high charge transformation between electrodes and analyte [22–24].

To the best of our knowledge, so far no reports are available on the applicability of AuNP/PANI/rGO/NH_2-MWCNTs composites for biosensors applications. The charge transfer based electrochemical properties and analytical performance of SPCE were analysed in the various stages of modification. The sensing platform was optimized by concerning pH, applied potential, nanocomposite layers and number of glucose oxidase layer. The amperometric technique was used to analyses different concentration of glucose. The proposed biosensor's stability, sensitive, reproducibility and interference influences were investigated. Based on the experimental results, it can be concluded that the proposed third generation glucose biosensor was cost-effective, portable, flexible, and highly sensitive with excellent reproducibility and paved the stable platform for glucose sensing.

2. Experimental

2.1. Materials and reagents

Ethylenediamine, Thionyl Chloride ($SOCl_2$), Tetrahydrofuran (THF), Polyaniline, Gold chloride trihydrate, Disodium citrate, Flavin adenine dinucleotide (FAD), Ascorbic acid (AA), Dopamine (DA), Uric acid (UA) and Glucose Oxidase from Aspergillus Niger were purchased from Sigma Aldrich (analytical-grade). Ultrapure water (18.2 MU) was used throughout the experiment.

2.2. Synthesis of nanomaterials

Carboxyl-functionalized MWCNTs were synthesized by a single step pyrolysis method using a tubular furnace at 870 °C. The characterization and properties were already reported in our previous articles [25,26]. Amine termination on carbon nanotubes was done by two-step chemical treatment, 50 mL of $SOCl_2$ and 50 mL of Tetrahydrofuran (THF) was added in carboxyl functionalized MWCNTs (1 mg/mL) solution. The suspension was dispersed in an ultrasonic bath for 10 min and stirred at 90 °C for 12 h to convert the surface-bound carboxyl groups into acyl chloride groups. The solid sample was collected by centrifuge followed by drying under vacuum at 60 °C for 6 h. Thus obtained solid sample was used to synthesis amine functionalization [27]. Acyl chloride functionalized carbon nanotubes (CNTs-COCl) was dispersed in *N*,*N*-dimethylformamide (50 mL) along with 50 mL of ethylenediamine was added and sonicated in an ultrasonic bath for 10 min followed by stirring at 100 °C for 24 h. Aliphatic multi-amines were grafted on MWCNTs through an amide linkage. Finally, amine-functionalized carbon nanotubes were dried in a hot air oven for overnight.

Synthesis and characterization details of rGO were given in our previous articles [28]. Polyaniline was synthesized by Chemical-oxidation process from aniline [29], 0.1 M ammonium di sulphate solution was dropped wise added in HCl and it was kept at room temperature. After 4 h of incubation, the deep green colour was obtained to indicate the formation of polyaniline. Then, the solution was purified by centrifugation at 8000 rpm for 10 min. Gold nanoparticles were synthesized from gold chloride trihydrate in the presence of disodium citrate as reducing agent [30]. 100 mL of $HAuCl_4$ aqueous solution was added into 400 mL distilled water contain flask under boiled condition. After 10 min, 50 mL 0.1 mM of disodium citrate solution was added quickly to the solution. The colour of the $HAuCl_4$ solution was changed immediately from yellow to black colour, the same conditions were maintained until the solution colour changes into wine red colour. The solution was freeze-dried to obtain powdered gold nanoparticles and the size was measured as 42 nm by using particle size analyzer.

2.3. Fabrication of GO_x/AuNP/PANI/rGO/NH_2-MWCNTs modified SPCE glucose biosensor

Amine-terminated carbon nanotubes (1 mg/mL), Polyaniline (10 mg/mL), reduced graphene oxide (1 mg/mL) and gold nanoparticles were taken in an equal concentration and sonicated for 30 min. SPCE was washed with 0.1 mM KOH solution to activate the surface. For a single layer of composite, five μL of AuNP/PANI/rGO/NH_2-MWCNTs solution was drop cast on SPCE (3 mm) and dried at room temperature for 4 h. Further, five μL of GOx (1 mg/2 mL) dropped cast on the SPCE and stored under a dehydrated condition at 4 °C overnight. GOx and GOx coated bioelectrode was stored at −20 °C for further applications. Schematic of GO_x/AuNP/PANI/rGO/NH_2-MWCNTs modified SPCE glucose biosensor and schematic for the synthesis of materials as illustrated in Fig. 1.

2.4. Instrumentation and analytical procedure

SEM images were taken using 30 kV beam under vacuum (FEI-QUANTA 200). Particle size was analysed by ZETASIZER MALVERN make. Absorbance spectra were taken using UV –Visible spectrophotometer (UV–VIS3600, Shimadzu). SPCE was purchase from CH Instruments (Zensor-TE100) where carbon was used as a working electrode and counter electrode as well. Ag/AgCl was used as a reference electrode. The working electrode area was filled with GO_x/AuNP/PANI/rGO/NH_2-MWCNTs nanocomposite. The electrochemical performance of variously modified electrodes was measured by cyclic voltammeter from 0.8 V to −0.6 V at a scan rate of 0.1 V/s in 0.1 M phosphate buffer solution (pH-7.4) containing $K_3Fe(CN)_6$/$K_4Fe(CN)_6$. Sensitivity studies were performed in an amperometric method at the set potential of 0.4 V by the addition of a standard glucose solution.

The possible interference (1 mM uric acid (UA), 1 mM ascorbic acid (AA), 1 mM dopamine (DA) for glucose detection in the biological sample was analysed with the addition of 1 mM Glucose. The recovery study for the detection of glucose in human serum sample was analysed bystander addition methods. The storage ability of the sample was tested for one month by storing in optimized temperature conditions

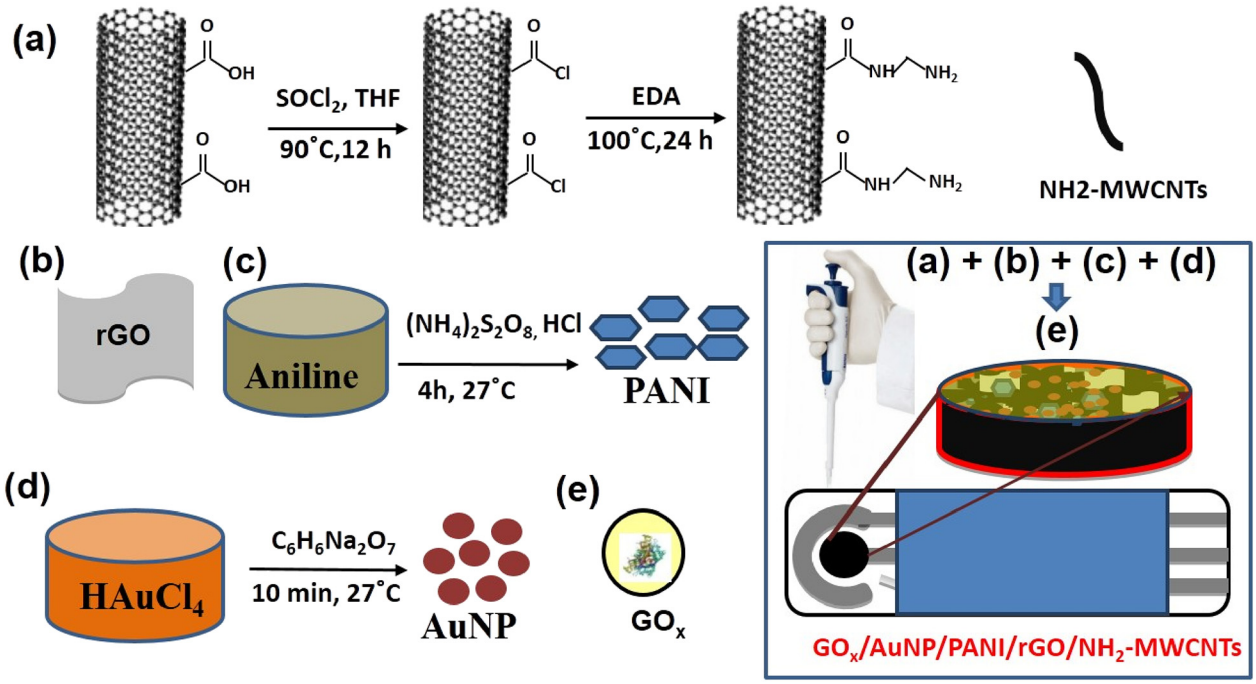

Fig. 1. Schematic explanation of nanoparticals synthesis and GO_x/AuNP/PANI/rGO/NH_2-MWCNTs modified SPCE glucose biosensor.

(−4 °C and −20 °C).

3. Results and discussion

3.1. Characterization of the synthesized nanocomposite

The analytical performance of nanoparticles has varied by synthesis technique, size and shape of nanoparticles. Therefore, it is necessary to characterize them before proceeding applications. Synthesized materials were characterized by SEM, UV- spectroscopy and X- ray diffraction which is described in following sections.

The AuNP/PANI/rGO/NH_2-MWCNTs coated nanocomposite was scrubbed from SPCE and follow by bath sonicated for HRTEM analysis. As shown in Fig. 2(a) AuNP were homogeneously distributed on the MWCNTs. This demonstrates a strong interaction between the NH_2-MWCNTs and AuNP (yellow line). Panels b–d show HRTEM image of AuNP/PANI/rGO/NH_2-MWCNTs nanocomposite. The rGO was hardly found in HRTEM image (blue line) due to present of few layer. PANI was strongly cover rGO surface and MWCNTs surface due to π–π stacking interaction (Fig. 2(b)). The present of AuNP in PANI matrix also observed in Fig. 2(c) (green line). The strong attachment of AuNP/PANI/rGO/NH_2-MWCNTs of probably contribute to the high electrochemical activity of the sensor (Fig. 2(d)).

The SEM image of different nanomaterials and their composite, which was deposited on the screen-printed electrode was provided in supplementary information (S1).

3.2. UV spectra of nanomaterial

The absorbance spectra of synthesized nanomaterials were shown in Fig. 3. From the results, it can be observed that the absorbance spectra of rGO have an absorbance maximum at 278 nm and it is associated with redshift (electronic conjugation) Π–Π* transition of aromatic C=C bonds of graphene oxide layer [31]. The absorption peak at 528 nm was confirmed the formation of gold nanoparticles [32]. PANI contains absorption peaks at 354 nm due to the Π–Π* transition of benzoic rings. The secondary absorbance bands at 420–440 nm are related to a doping level of PANI [33]. The UV–Vis spectrum of the MWCNTs shows a strong absorbance band at 273 nm which is due to the Π–Π* transition of aromatic C=C bonds of MWCNTs.

The X-ray diffraction (XRD) patterns of AuNP, MWCNTs, PANI and AuNP/PANI/rGO/NH_2-MWCNTs nanocomposite was provided in the supplementary information (S2).

The Bragg reflections were presented in XRD analysis, which can be indexed on the basis of the face-centred cubic fcc structure of gold. The diffraction peaks at 2 θ = 38° (111), 44° (200), 64° (220) and 77° (311) obtained are indistinguishable as those peaks are described for the standard gold metal (S2(a)). The PANI peak diffracted at an angle of 2θ = 25.60° shows low crystallinity of the conductive polymers due to the repetition of quinoid rings in PANI chains (S2(b)). The characteristic peak of MWCNTs for the crystalline plane of (002), (101), (004) and (110) was found at 26°, 42°, 54° and 78° respectively (S2(c)). The diffraction intensity value of AuNP/PANI/rGO/NH_2-MWCNTs nanocomposite for NH_2-MWCNTs, AuNP and rGO was reduced due to present of PANI (S2(d)). The X-ray diffraction was confirmed that the synthesis nanoparticals were well crystalline [[34–37,39]].

3.3. Electrocatalytic activities of fabricated biosensor electrodes

The Cyclic voltammograms were performed to estimate the electrochemical behaviour of GOx at a different stage of modified screen printing electrodes. The cyclic voltammograms of (a) GOx/SPCE, GOx/rGO, GOx/COOH-MWCNTs, GOx/NH_2-MWCNTs, GOx/AuNP, GOx/PANI (b) GOx/rGO/PANI, GOx/PANI/NH2-MWCNTs, GOx/rGO/NH_2-MWCNTs, GOx/PANI/rGO/NH_2-MWCNTs, GOx/AuNP/PANI/rGO/NH_2-MWCNTs on screen printing carbon electrode were shown in Fig. 4. The cyclic voltammograms were recorded in 0.1 M pH 7.0 PBS containing 1 mM $[Fe(CN)6]^{3-/4-}$ in the potential range from +0.8 V to −0.6 V at 0.1 V/s scan rate. The CV of different stage modified electrodes GOx /SPCE, GOx/rGO, GOx/COOH-MWCNTs, GOx/NH_2-MWCNTs, GOx/AuNP, GOx/PANI, GOx/rGO/PANI, GOx/PANI/NH2-MWCNTs, GOx/rGO/NH_2-MWCNTs, GOx/PANI/rGO/NH_2-MWCNTs, GOx/AuNP/PANI/rGO/NH_2-MWCNTs were demonstrated a pair of well-defined quasi-reversible redox peak with peak current value of 31, 148, 80,127, 55, 154, 95,154, 95, 154, 255, 381 μA with peak-to-peak separation (ΔE_p) of 0.60, 0.39, 0.81, 0.10, 0.13, 0.65, 0.26, 0.30, 0.46,

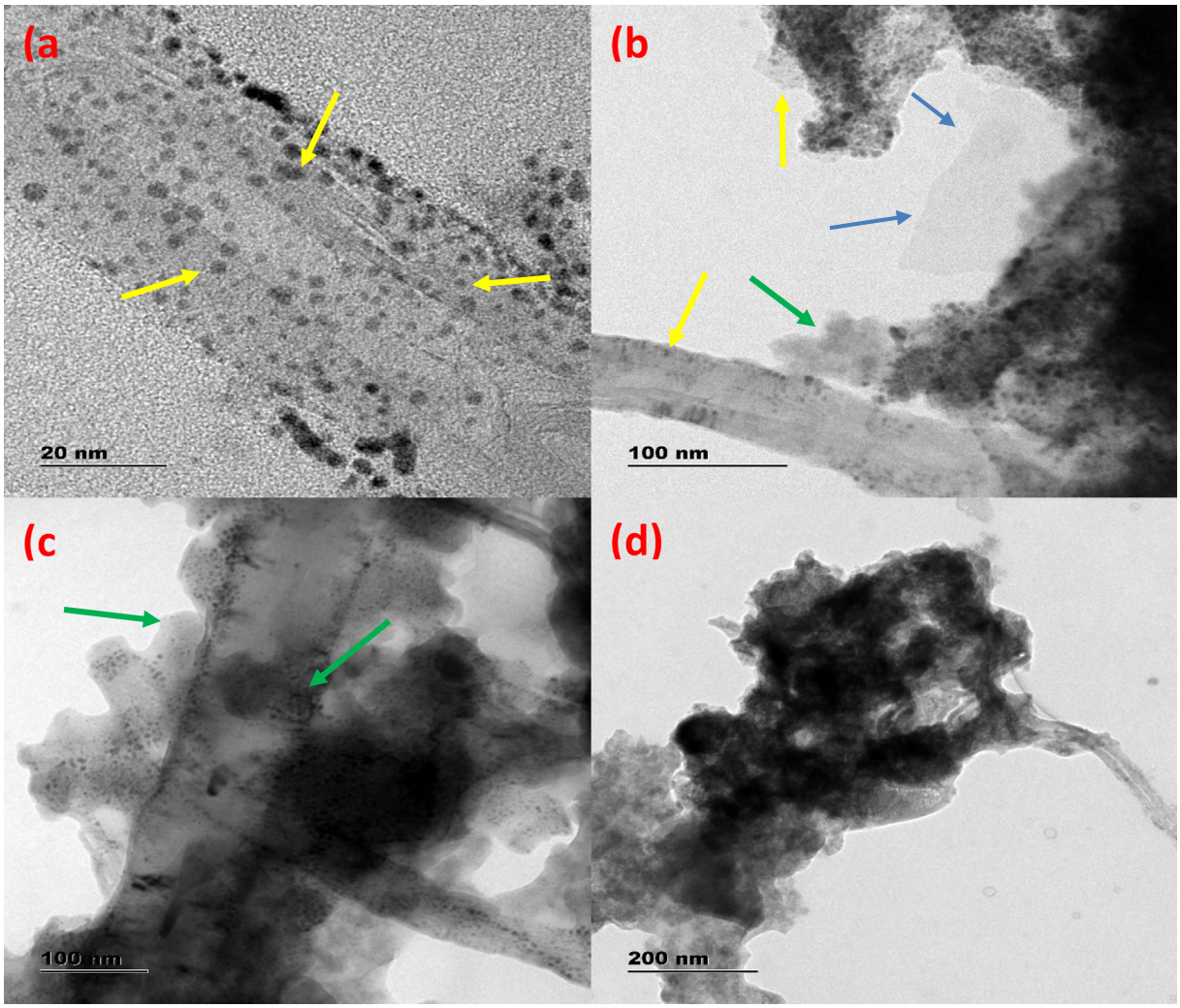

Fig. 2. HRTEM image of (a) AuNP/NH_2-MWCNTs and (b–d) AuNP/PANI/rGO/NH_2-MWCNTs nanocomposite different magnification.

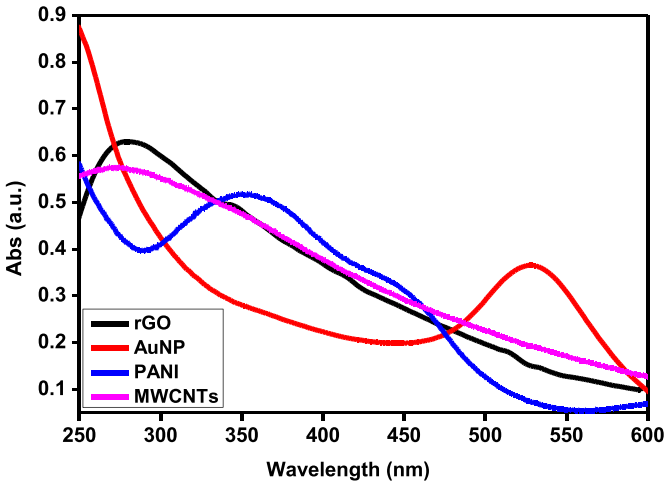

Fig. 3. UV–Vis absorbance spectrum of reduced graphene oxide (rGO), Gold nanoparticles (AuNP), Polyaniline (PANI) and amine terminated carbon nanotubes (MWCNTs). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

0.47 mV at formal potential ($E^{1/2} = (E_{pc} + E_{pa})/2$ of +0.3, +0.195, +0.040, +0.051, +0.064, + 0.325, +0.132, +154, +230, +237 V respectively. This result indicated that SPCE has poor charge transfer rate with high formal potential and high peak-to-peak separation potential value, which was improved extensively by nanoparticals modification. The oxidation was over potential and low oxidation potential was observed for MWCNTs and their composite with poor direct electron transfer ability. Other hands PANI, rGO and composite material have shown higher charge transport properties due to enhancement in electron transfer rate between electrode and analyte. The highly enhanced peak currents of GOx/AuNP/PANI/rGO/NH_2-MWCNTs modified electrode indicated the attainment of direct electron transfer between the electrode surface and enzyme. The amine group of carbon nanotube can bind with the carboxylic group of the GO_X enzyme, forming the amide bond. Therefore, both amine groups and electrostatic interaction have improved the immobilization and bonding capability between GO_X and AuNP/PANI/rGO/NH_2-MWCNTs composite. Where conducting polymer PANI work as a binder for composite on the surface of SPCE. The rGO was used as a better electron acceptor.

In other words, the GOx/AuNP/PANI/rGO/NH_2-MWCNTs modified electrode provides better immobilization environment through glucose oxidase for glucose biosensor due to the proper combination of AuNP, PANI, rGO and NH_2-MWCNTs that led to offer additional porosity, large surface area, extra sites for immunization of enzyme.

The influence of redox peaks current on scan rates was investigated in the range of 10–100 mV Figs. 5(a). Both cathode and anodic peak current were proportional to the scan rate as shown in Fig. 5(b) which indicates that electrochemical kinetics followed the surface controlled process. The redox peak currents (cathode and anodic) were linearly

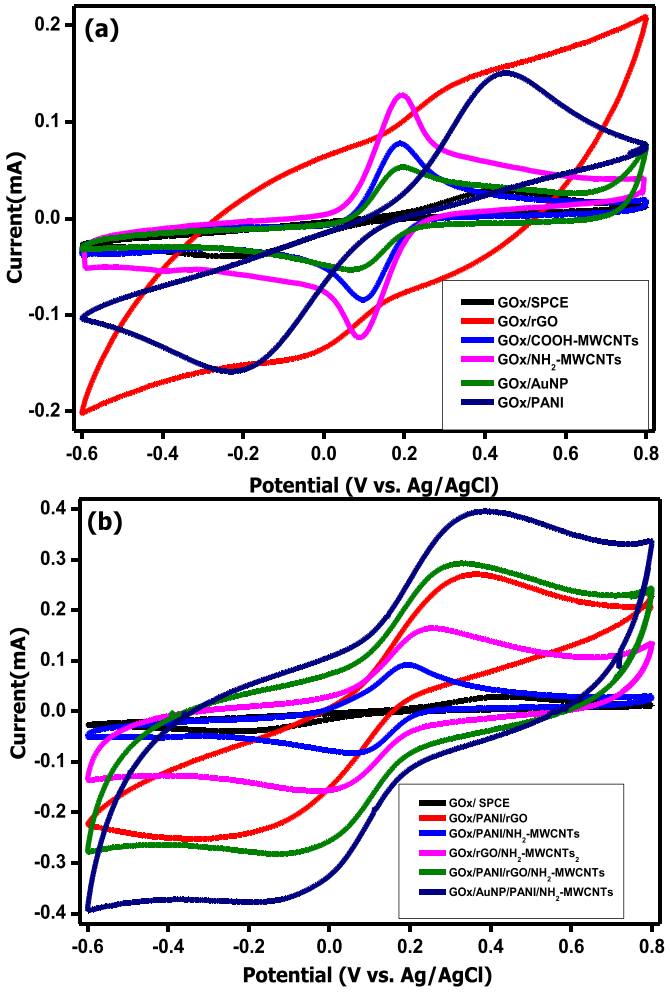

Fig. 4. Cyclic voltammetry profile of Glucose oxidase coated (a) SPCE, rGO, COOH-MWCNTs, NH_2-MWCNTs, AuNP, PANI nanomaterials (b) bare, rGO/PANI, PANI/NH2-MWCNTs, rGO/NH_2-MWCNTs, PANI/rGO/NH_2-MWCNTs, AuNP/PANI/rGO/NH_2-MWCNTs on screen printing electrode in 0.1 M pH 7.0 PBS containing 1 mM $[Fe(CN)6]^{3-/4-}$ with 0.1 V/s scan rate.

increased as the scan rate increased. The decent linearity between peak currents and the scan rates was demonstrated surface-controlled redox reaction of biosensors. The cathode peak potential (E_{pc}), anode potential peak (E_{pa}) and their difference ΔE_p varied with scan rate and determined charge transfer kinetics of bioelectrode. The peak-to-peak potential differences ΔE_p were 213, 268, 298, 336, 411, 438, 475, 504, 545 and 570 mV for GO_x/AuNP/PANI/rGO/NH_2-MWCNTs bioelectrode obtained for 10, 20, 30,40, 50,60,70,80,90 and 100 mV s^{-1}scan rate respectively. The cathode peak potential and anodic peak potential of GO_x/AuNP/PANI/rGO/NH_2-MWCNTs modified SPCE on logarithmic scan rate has shown in Fig. 5(c). The cathode and anode potential shifted linearly with logarithmic scan rate in the range of 30 to 100 mVs^{-1}. The linear regression equations for the anodic peak current (I_{pa}) and cathodic peak current (I_{pc}) of sensors with respect to scan rates were expressed as, $I_{pa}/A = 0.00302*X + 0.000103201$, $R^2 = 0.9885$; and $I_{pc}/A = Y = -0.00285*X + 0.000103656$, $R^2 = 0.99383$ respectively. Where X was the scan rate in V/s. The AuNP/PANI/rGO/NH_2-MWCNTs electrode charge values (Q) was calculated by exchanging the slope of scan rate vs peak currents versus in the on Laviron's equation [38].

$$I_P = \frac{n^2F^2\nu A\Gamma}{4RT} = \frac{nF}{4RT}Q\nu \quad (1)$$

where $n = 2$ is a number of an electron generated per reactant molecules. F, R, T,Q v and A are faraday current, gas constant, temperature, charge values, scan rate and area of screen-printed electrode respectively. By applying $R = 8.314$ $JK^{-1}mol^{-1}$, $T = 300$ K and $F = 96{,}485$ $Cmol^{-1}$ in Eq. (1), the $Q = 1.56 \times 10^{-06}$ C was obtained from slope of Fig. 5(b). It is extremely large when compared to other electrodes.

The GOx/AuNP/PANI/rGO/NH_2-MWCNTs matrix shows quick electrons movement between the reactive sites of glucose oxidase and the electrode surface, which facilitated the direct electron transfer. The CVs of bare SPCE at different scan rates (10–100 mV·s^{-1}) was shown in S4. To calculate the effective surface area of GO_x/AuNP/PANI/rGO/NH_2-MWCNTs modified SPCE, The Randles-Seveik equation was used [40].

$$I_P = 2.69 \times 10^5 n^{3/2} A D^{1/2} \nu^{1/2} C \quad (2)$$

where, D and C is the diffusion coefficient of the molecules in solution (7.6×10^{-6} $cm^{-2}s^{-1}$) and the bulk concentration of probe molecules (0.1 mM). The effective surface area (0.0620 cm^2) was almost same original surface area (0.0707 cm^2).

3.4. Optimization of biosensor

The effect of the number of nanocomposite layer coating, number of GOx coating layer, buffer solution pH and operating potential was optimized to improve the charge transfer rate of glucose biosensor. The GOx/AuNP/PANI/rGO/NH_2-MWCNTs nanocomposite layer was sequentially dropped cast (5 μL) on SPCE to determine the effect of nanomaterial loading on the response of the biosensor. It was observed from Fig. 6(a) current response was increased to the second layer of coating and increment of current was not adequate beyond the second layer. Hence, the double layer nanocomposite coating was used throughout the experiment. The different layer of enzyme coating was analysed to determine the effect of GOx enzyme loading on the current response (Fig. 6(b)). The current response was increased to the second layer of GOx enzyme loading and then decreased gradually. Therefore, the layer of the enzyme was used throughout the experiment. The more amount of GOx decreases the charge transport due to its resistive nature and activity of the enzyme was reduced because of agglomeration of GOx molecules that blocks the active sites of the enzyme. The enzyme is active in neutral solution, acid and alkali medium leads to the degradation easily. The amperometric response at different applied voltage was analysed in the presence of 0.1 M phosphate solution containing 5 mM glucose. It was observed that sensing response gradually increased with increase in applied voltage. The highest operating potential would lead to various electrochemical subtract as interference in glucose sensing signal (Fig. 6(c)). So the operating potential was maintained at 0.4 V throughout the sensing procedure. The pH of the buffer solution is an essential parameter for sensing studies. The pH-dependent studies were performed in the presence of 0.1 M phosphate solution containing 5 mM glucose in the wide range of pH (3 – 11) (Fig. 6(d)). The electron activity was decreased in both acidic and alkali medium and reached the maximum at pH 7.4. As-prepared GOx/AuNP/PANI/rGO/NH_2-MWCNTs were employed as glucose sensing anode materials (S5). The electrocatalytic activity of the GOx/AuNP/PANI/rGO/NH_2-MWCNTs towards the oxidation of glucose was investigated. Fig. S5 represents the CVs of the GOx/AuNP/PANI/rGO/NH_2-MWCNTs in the absence (a) and presence (b) of 1 mM glucose (0.1 M pH 7.0 PBS). In the presence of 1 mM glucose (b), a very large oxidation current was observed at 0.4 V during the cathodic scan, that represents pronounced glucose oxidation.

3.5. Amperometric detection of glucose

Amperometric detection by GOx/AuNP/PANI/rGO/NH_2-MWCNTs glucose biosensor with a successive drop casting of different concentration of glucose was shown in Fig. 7(a). The working potential was

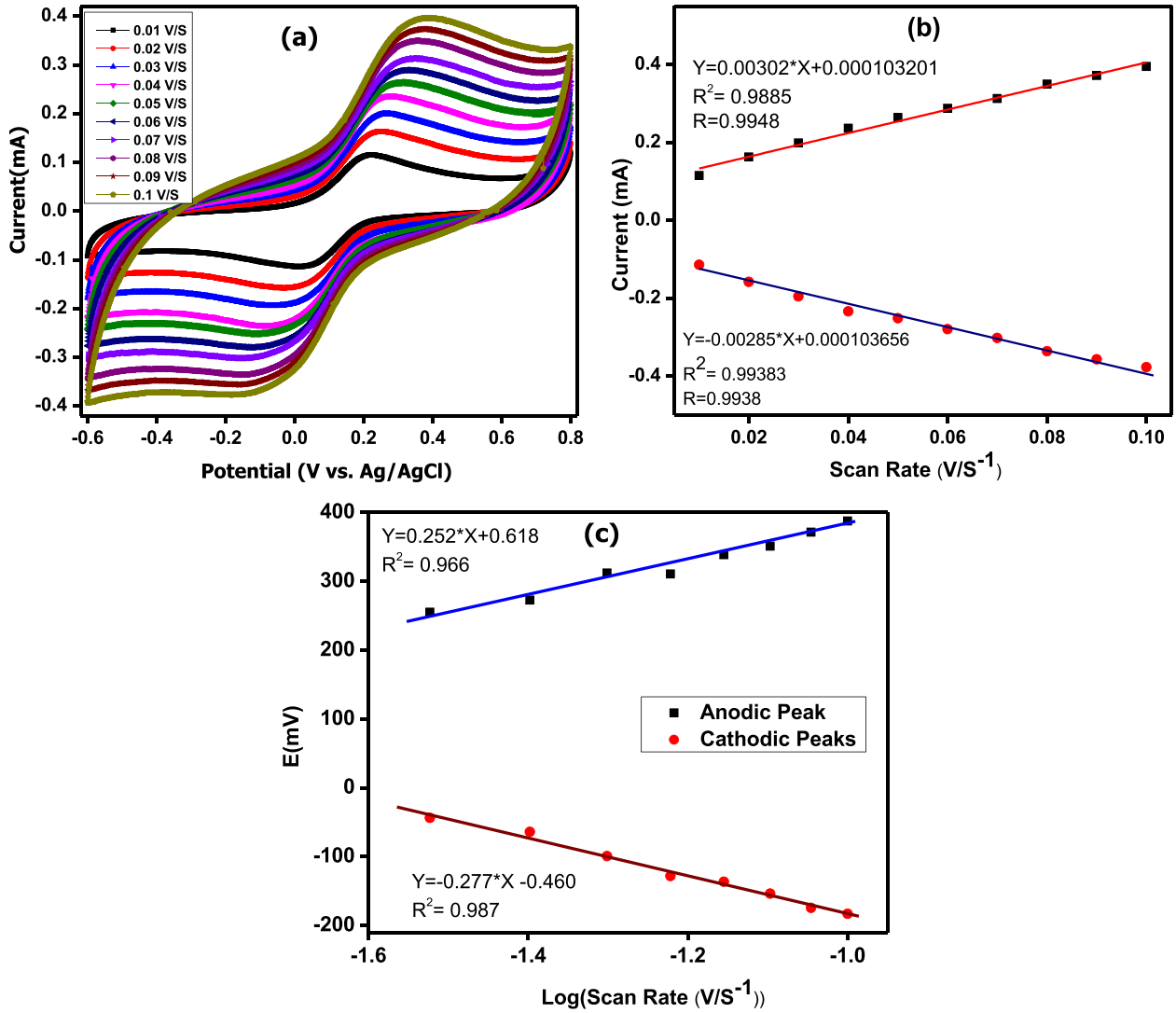

Fig. 5. (a) CVs of GO_x/AuNP/PANI/rGO/NH_2-MWCNTs at different scan rates (10–100 $mV{\cdot}s^{-1}$) (b) anodic and cathode peak currents vs Scan rate linear fit and (c) peak potentials vs logarithmic scan rates linear fit.

set as 0.4 V to avoid interference's effect with the maximum amperometric response. The current response increased linearly with glucose concentration in the range of 1 mM to 10 mM. The sensing response (R) defined by the difference in the current with the presence and absence of glucose respectively.where I_{max} is a current response in the presence of glucose (100 s) and I_0 was steady-state current value. The sensing response of glucose biosensor varies with glucose concentration linearly ($Y = -1.720 \times 10^{-5}x – 8.846 \times 10^{-6}$with $R^2 = 0.9959$). This indicated that many active sites are available in our fabricated glucose biosensor that may be useful in large-scale glucose sensing application. The sensitivity of the fabricated glucose sensor was 246 $\mu A\, mM^{-1}\, cm^{-2}$ with the lowest detection limit of 63 μM (S/N = 3).

The enzymatic affinity calculated from Linear-Burk Eq. (4) [41].

$$\frac{1}{I_{SS}} - \frac{1}{I_{max}} = \frac{K_M^{app}}{I_{max} C} \quad (4)$$

where K_M^{app} is Michaelis–Menten constant determined enzymatic affinity, I_{max} and I_{SS} are the maximum currents under the saturated substrate and steady-state current. C is the concentration of the substrate. The K_M^{app} value of the GOx/AuNP/PANI/rGO/NH_2-MWCNTs biosensor was calculated in the presence of 1 mM glucose is 0.734.

The possible interference for glucose in the biological sample was analysed such as 1 mM uric acid (UA), 1 mM ascorbic acid (AA), 1 mM dopamine (DA) after the addition of 1 mM Glucose. No significant effect was observed in the presence of interferences (Fig. 7(c)).

The mechanism of the electrochemical behaviour of GOx/AuNP/PANI/rGO/NH_2-MWCNTs bio-electrode is explained as below [42].

$$Glucose + GO_X(FAD) \rightarrow Gluconolactone + GO_X(FADH_2) \quad (5)$$

$$GO_X(FADH_2) + O_2 \rightarrow H_2O_2 + GO_X(FAD) \quad (6)$$

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^- \quad (7)$$

The glucose reacts with catalyzed enzyme reaction form of GO_x(FAD). In this reaction, Gluconolactone and $GO_X(FADH_2)$ was produced. Next, $GO_X(FADH_2)$ reacts with dissolved oxygen in the solution then forms H_2O_2 and GOx(FAD). The further H_2O_2 level was reduced due to the decomposition of H_2O_2 and increases the response current (Fig. 8).

Table 1 shows the comparison of developed glucose biosensor on SPCE's analytical parameters such as linear range, sensitivity, detection limit and stability in the present study with literature results. It is evident from the table that the GO_x/AuNP/PANI/rGO/NH_2-MWCNTs bioelectrode exhibits the highest sensitivity and another sensing parameter was quite satisfactory, suggestively improved sensing performance than other previously reported glucose biosensor. These results clearly reveal that developed new GO_x/AuNP/PANI/rGO/NH_2-

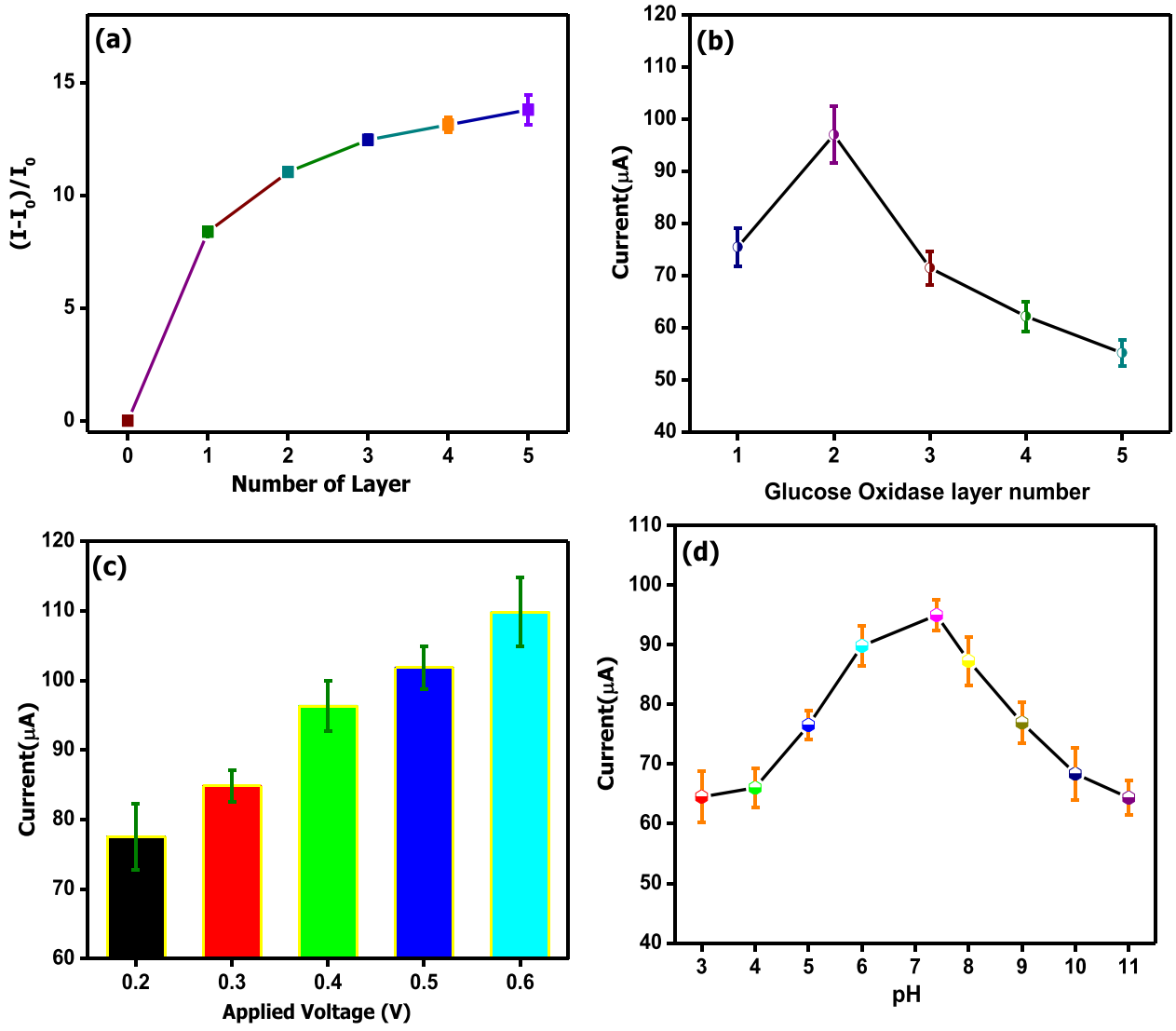

Fig. 6. Optimization of the modified SPCE on the effect of (a) number of nanocomposite layer (b) number of GOx layer (c) pH of buffer solution (d) operating potential.

MWCNTs material modified electrode is a more promising candidate for glucose detection.

The excellent analytical performance biosensor was maybe attributed for flowing reason. (i)The higher electrochemical performance of carbon-based nanostructure MWCNTs and rGO, (ii) AuNP to enhanced electrons transfer rate and higher catalytic activity (ii) conducting polymer PANI which act a conducting binder of nanocomposite and π–π stacking interaction between MWCNT and rGO (iv) selective interaction of enzyme (glucose oxidase) towards substrate (glucose resulting in a boosted the electrocatalytic activity towards glucose enhanced electrochemical activity.

The biosensor was validated for the detection of glucose level in human blood serum samples using the amperometric technique. The concentration of glucose level in human blood was 4–5 mM/L, therefore glucose level was predetermined by a commercial detector and concentration of serum was diluted with phosphate buffer for the real sample analysis. The experimental conditions were remaining constant as describe Fig. 7. The recovery study of glucose in human serum samples was designed using the standard addition method (Table 2). It can be seen that the GO_x/AuNP/PANI/rGO/NH2-MWCNTs sensor was showing a satisfactory recovery towards glucose in human serum.

3.6. Reproducibility, repeatability and stability study of the modify biosensor

The reproducibility, repeatability and stability study of the modified biosensor was characterized by measuring amperometric response current at 5 mM glucose concentration. Different bioelectrode reproducibility of the GO_x/AuNP/PANI/rGO/NH2-MWCNTs different biosensor was examined in the presence of 5 mM glucose. It was observed that our biosensor was highly reproducible with an average sensitivity and stander deviation of 0.91%.

The repeatability of the same biosensor was measured for continuous 6 cycles (after washing with water) in the presence of 5 mM glucose. High reproducibility and 96% of the initial value were maintained after 6 cycles. The sample was stored in refrigerators (4 °C) before and after usage. Our biosensors have shown 82.91% and 74.57% response of its original value even after 1st and the 2nd week from the production of the sensor. The sensor (store in −20 °C) shows 93.4% of initial response after two months. The study was demonstrated efficiently highly sensitive GO_x/AuNP/PANI/rGO/NH2-MWCNTs bioelectrode for portable glucose biosensor.

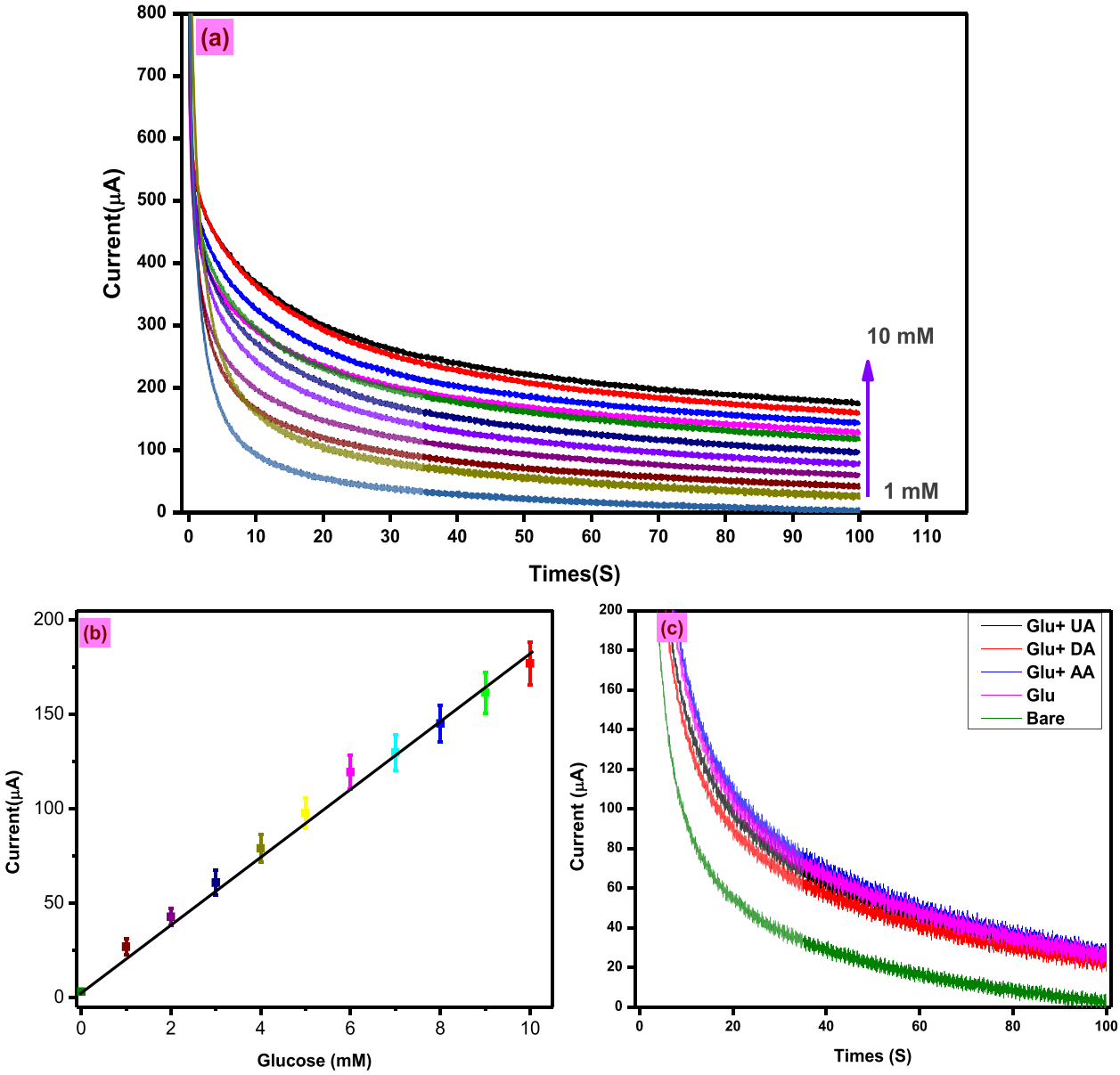

Fig. 7. (a) Amperometric response of GOx/AuNP/PANI/rGO/NH_2-MWCNTs biosensor (b) Calibration Curve (c) Interference for Glucose Sensing.

$$R(\mu A) = I_{max} - I_0 \quad (3)$$

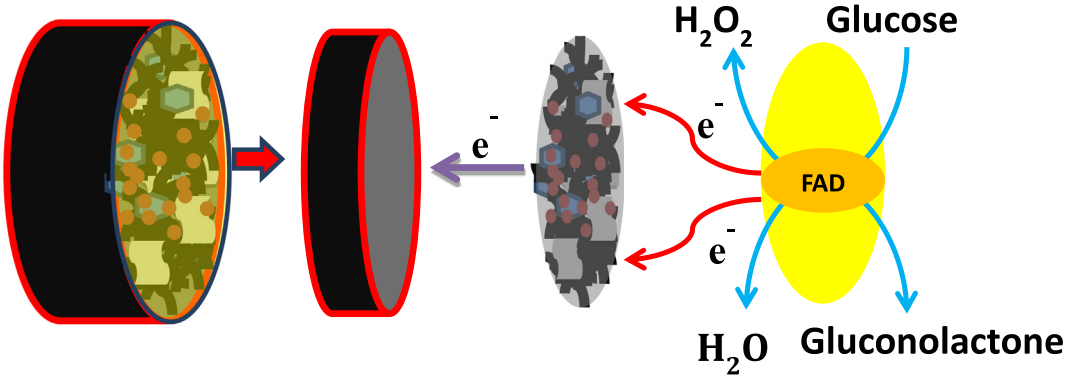

Fig. 8. Mechanism for glucose detection by GO_x/AuNP/PANI/rGO/NH_2-MWCNTs biosensor.

Table 1
Comparison of the analytical performances of the proposed glucose biosensor with other SPCE based biosensors.

Biosensor composition	Method	Linear range (mM)	Sensitivity ($\mu A\ mM^{-1}\ cm^{-2}$)	R^2/LOD (μM)	Stability	Ref
Cellulose-4-aminophenyl-boronic acid	A	0.05–100	–	0.992/860	–	2016 [43]
PSPCE	CV	1–8	–	0.989	15 days	2017 [44]
PBNCs/AgNWs	A	0.01–1.3	131.31	0.9997/5	30 days	2016 [45]
MnO_2-HBCs	A	0.16–1.94	–	0.995/38	–	2016 [46]
Pt-decorated graphite	A	0.033–0.9	–	0.992/10	–	2017 [47]
RGO-Fe_3O_4	A	0.05–1	5.9	0.993/0.1	30 days	2017 [48]
Pd-MWCNT	A	0.16–0.72	18.8	0.997/0.02	–	2017 [49]
rGO/Chitosan	CV	0.010–0.750	41.7	0.9849/5	–	2016 [50]
Graphene(nanoribbons)	cv	0.27–11.11	–	0.9925/11	–	2017 [51]
pencils	A	1–12	–	0.9987/50	–	2016 [52]
GOx/AuNP/PANI/rGO/NH_2-MWCNTs	A	1–10 mM	246	0.9959/63	60 Days	Our work

Table 2
Determination of glucose in human blood serum samples using GO_x/AuNP/PANI/rGO/NH_2-MWCNTs biosensor.

Sample	Added (mM)	Found (mM)	Recovery (%)	RSD
1	2	1.93	96.5	4.6
2	4	3.94	98.5	3.2
3	6	5.85	97.5	3.2
4	8	7.82	97.7	4.5

4. Conclusions

In summary, glucose oxidase immobilized amine terminated carbon nanotubes/ polyaniline/reduced graphene oxide/gold nanoparticles modified screen-printed electrode for preparation of highly sensitive glucose sensor. The SEM and UV–Vis spectroscopy studies have conformation synthesis of nanomaterial and there composite. The electrochemical performance of different stage of composite coated GOx and GO_x/AuNP/PANI/rGO/NH2-MWCNTs biosensor was analysed by cyclic voltammeter. Enzymatic catalyst reaction generates electrons and directly transferred between the analytic and nanocomposite. The novel nanocomposite enhances redox peak current value13 times also decrease the redox potential than bare SPCE. The fabricated glucose biosensor shows excellent sensitivity 246 $\mu A mM^{-1}\ cm^{-2}$ with a wide range of linearity (1–10 mM) and the lowest detection limit of 63 μM. The cost-efficient sensor with high reproducibility, repeatability, and stability has given the horizons for the fabrication of glucose sensing device. The fabricated high-performance sensing composite can be applied in other biosensing analysis.

Credit author statement

Dr. Debasis Maity involved in the design, execution of the work and wrote the first draft of the manuscript. Dr. C.R.Minitha involved in the synthesis and optimization of rGO. Dr. R T Rajendra Kumar contributed on concept, interpretation and supervised the work.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.msec.2019.110075.

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