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Silver nanoparticles/multiwalled carbon nanotube/polyaniline film for amperometric glutathione biosensor

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ABSTRACT

A new silver nanoparticles (AgNPs)/carboxylated multiwalled carbon nanotubes (c-MWCNT)/polyaniline (PANI) film has been synthesized on Au electrode using electrochemical techniques. The enzyme glutathione oxidase (GSHOx) (EC 1.8.3.3) was immobilized covalently on the surface of AgNPs/c-MWCNT/PANI/Au electrode to construct the glutathione biosensor. The modified electrode was characterized by scanning electron microscopy (SEM), cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and Fourier transform infrared (FTIR) spectrophotometry. The biosensor showed optimum response within 4 s at +0.4 V vs. Ag/AgCl, pH 6.0 and 35 °C, with a linear working range of 0.3–3500 μM and a detection limit of 0.3 μM. The glutathione biosensor was employed for measurement of glutathione content in hemolysated erythrocyte (RBC). The sensor was evaluated with 97.77% and 99.16% recovery of added glutathione in hemolysated RBC and 2.4% and 6.3% within and between batch coefficients of variation (CVs) respectively. The enzyme electrode lost 50% of its initial activity after 300 uses over a period of 3 months, when stored at 4 °C. The biosensor has the advantages over earlier biosensors in terms of greater stability, lower response time and no interference by a number of RBC hemolysate substances.

1. Introduction

Glutathione (γ-l-glutamyl-l-cysteinyl-glycine) (GSH) is the principal non-protein thiol compound present in most mammalian cells. The tripeptide acts as a major bio-reducing agent. It also plays important biological functions in the organisms such as protein and DNA synthesis, enzyme activity, metabolism and cell protection [1–3]. Measurement of GSH is useful marker in certain disorders such as leukemia [4,5], diabetes [6,7], DNA base damages [8,9] and in the investigation of some kinds of cancer [10–13]. Normally, the ratio of GSH to GSSG is used as an indicator of oxidative stress and carries great importance in pathology and clinical applications [1]. Altered levels of glutathione in plasma have been implicated in a number of pathological conditions, including Alzheimer's, Parkinson's diseases, diabetes, macular degeneration and HIV disease [2]. Hence, determination of GSH is very important. Methods available for glutathione measurement are high-performance liquid chromatography [14–17], spectrofluorimetry [18], spectrophotometry [19,20] and chemiluminescence [21]. All these methods/techniques are complicated, require time-consuming sample preparation, which involve the risk of oxidation of GSH, costly equipment and trained person to operate. Simplicity, rapidity, high sensitivity and low cost are the main advantages of electrochemical techniques for the analysis of biological compounds.

A number of electrochemical biosensors, mostly based on oxidation of GSH on unmodified [22,23] or chemically modified electrodes [24,25], have been reported, of which amperometric biosensors were based on two gold electrodes and two complementary oligonucleotides [26], pyrrolofullerene bis-adduct [27], molecularly imprinted polymer [28], silicon nanowires [29], sulfhydryl oxidase bi-enzymatic system [30] and self assembled monolayers (SAMs) [31]. In most of these electrodes, the enzyme was immobilized onto support by electrostatic interaction or adsorption, which allows leakage of enzyme, resulting in a low stability of the enzyme electrode. Covalent immobilization of enzyme not only overcomes this problem but also leads to better biomolecule activity and greater stability.

Nanoparticles (NPs) have been employed in biosensors as effective catalyst supports, due to their large surface areas and unique structural and electromechanical properties, good biocompatibility, easy preparation and renewal of their surface [32–39]. MWCNTs (multiwalled CNTs) consist of several concentric tubes of graphite inside one other. The oxidation of the array introduced —COOH groups at the opening ends of MWCNT have provided a stabilizing hydrophilic environment that allowed adsorption and insertion of the enzyme into the cavity of the nanotubes. The immobilization of enzyme within nanotubes may permit a mediated direct

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electron transfer to Pt electrode [39]. Polyaniline (PANI) is also an attractive conducting polymer, since it exhibits two redox couples facilitating efficient enzyme–polymer charge transfer [40,41]. Silver is the best conductor among metals and so silver nanoparticles (AgNPs) may facilitate more efficient electron transfer than gold nanoparticles in biosensors [42]. We describe herein the construction of amperometric glutathione biosensor based on covalent immobilization of glutathione oxidase (GSHOx) (EC 1.8.3.3) onto AgNPs/c-MWCNT/PANI/Au electrode in order to get its improved analytical performance.

2. Experimental

2.1. Sources of chemicals

Aniline from Sigma (Aldrich), glutathione, silver nitrate (>99%) and sodium borohydride ($NaBH_4$) (99%) from Sisco Research Laboratory, Mumbai, India, glutathione oxidase (glutathione sulfhydryl oxidase, EC 1.8.3.3) from Penicillium *sp.* 5.5 U mg^{-1} powder from Yamasa Shouy Co., Tokyo, Japan and carboxylated multiwalled carbon nanotubes (c-MWCNT) (functionalized MWCNT) (12 walls, length 15–30 mm, purity 90%, metal content: nil) from Intelligent Materials Pvt. Ltd., Panchkula (Haryana), India were used. All other chemicals were of analytical reagent (AR) grade. Double distilled water (DW) was used throughout the study.

2.2. Instruments and equipments

All electrochemical experiments, including cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and amperometric measurements were carried out in Potentiostat/Galvanostat (Autolab, Eco Chemie, The Netherlands; Model: AUT83785). Scanning electron microscopy (SEM) of Au electrode at different stages of its modification was carried out at AIRF, Jawaharlal Nehru University, New Delhi to confirm immobilization of GSHOx. Fourier transforms infrared (FTIR) spectroscopy of electrode before and after immobilization of GSHOx was done in FTIR spectrometer (mode iS10, Thermoelectron, USA). The morphological characterization of AgNPs was carried out by transmission electron microscope (TEM) at Department of Anatomy, All India Institute of Medical Sciences, New Delhi.

2.3. Preparation of silver nanoparticles (AgNPs)

An excess of sodium borohydride was used to both reduce the ionic silver and stabilize the AgNPs. A 10-ml solution of 1.0 mM $AgNO_3$ was added dropwise (about 1 drop/s) to 30 ml of 2.0 mM sodium borohydride ($NaBH_4$) solution already chilled in an ice bath under constant stirring. The solution turned light yellow after the addition of 2 ml of $AgNO_3$ and a brighter yellow solution obtained, when all of the $AgNO_3$ was added. The entire addition took about three min, after which the stirring was stopped and the stir bar removed. The clear solution of yellow colloidal AgNPs solution was dried and stored in a transparent vial [43].

2.4. Preparation of the AgNPs/c-MWCNT/PANI/Au electrode

The Au electrode (1.5 cm × 0.05 cm) was polished with alumina slurry and then immersed in piranha solution (a hot mixed solution of 30% H_2O_2 and conc. H_2SO_4, in 3:1 ratio (v/v)) for 10 min followed by ultrasonic cleaning with D.W. Aniline (50 μl) was added to 10.0 ml of 1 N HCl and electrodeposited it onto polished Au electrode through cyclic voltammetric technique (10 polymerization cycles at 0.0 to +1.5 V) using Potentiostat/Galvanostat [44]. One milligram of c-MWCNT was suspended in 1 ml mixture of concentrated H_2SO_4 and HNO_3 in 3:1 ratio (v/v) and ultrasonicated for 2 h to get a finally dispersed black colored solution. The c-MWCNT solution was washed with DW thoroughly to remove acid. The PANI coated Au electrode was dipped into this c-MWCNT solution for 24 h. The resulting c-MWCNT/PANI/Au electrode was washed thoroughly with DW to remove unbound matter and kept in dry Petri plate at 4 °C. The electrode was washed with DW thoroughly and then immersed into AgNPs solution for 12 h at 4 °C. The electrode was washed thoroughly with DW to remove unbound matter and kept in dry Petri plate at 4 °C.

2.5. Preparation of the enzyme electrode

The working electrode was prepared by placing drops of 20 μl of GSHOx solution (50 mg/ml) on the surface of AgNPs/c-MWCNT/PANI/Au electrode and keeping it at 4 °C for 24 h. After rinsing clearly, the enzyme electrode was dried and stored in the 0.05 M sodium phosphate (pH 7.0) buffer solution at 4 °C until use.

2.6. Scanning electron microscopy

Scanning electron microscopy (SEM) of bare Au electrode, AgNPs/c-MWCNT/PANI/Au electrode and GSHOx/AgNPs/c-MWCNT/PANI/Au electrode was carried out. The electrodes were cut into small pieces (1 cm × 1 cm) and placed on copper mount of 2 cm diameter. The gold particles were deposited on its surface using spray gun and their electron micrographs were taken.

2.7. Cyclic voltammetric response

Cyclic voltammetric studies were carried out using a three-electrode cell comprising of GSHOx/AgNPs/c-MWCNT/PANI/Au electrode as working electrode, Ag/AgCl as a reference electrode and Pt wire as auxiliary electrode. To test the functioning of the biosensor, three electrodes were connected to three terminal of Potentiostat/Galvanostat and then immersed into 15 ml 0.05 M sodium phosphate (pH 6.5) in a 50 ml beaker. The cyclic voltammograms of GSHOx/AgNPs/c-MWCNT/PANI/Au electrode were recorded in 0.1 M phosphate buffer, pH 6.5 containing substrate (GSH) concentration varying from 0.3 to 3500 μM, at 0 to +1.0 V with a scan rate of 50 mV s^{-1} in Potentiostat/Galvanostat.

2.8. Response measurement of GSHOx/AgNPs/c-MWCNT/PANI/Au electrode and its optimization

Cyclic voltammogram of GSHOx/AgNPs/c-MWCNT/PANI/Au electrode was recorded in Potentiostat/Galvanostat from −0.1 to +0.9 V vs. Ag/AgCl in a 0.05 M sodium phosphate (pH 6.5) containing 100 μM glutathione. The maximum response was observed at +0.4 V vs. Ag/AgCl and hence subsequent studies were carried out at this voltage. The reaction was started by adding glutathione solution and the current (mA) generated was recorded at +0.4 V vs. Ag/AgCl.

2.9. Optimization of glutathione biosensor

Various kinetic properties of glutathione biosensor such as optimum pH, incubation temperature, time for maximum response and effect of substrate concentration were studied amperometrically in order to optimize the working conditions of the biosensor.

2.10. Amperometric determination of glutathione content in human erythrocyte

The glutathione content in human erythrocytes was measured by the present biosensor as follows. Intravenous blood was collected from Hospital of Pt. BDS University of Health & Medical

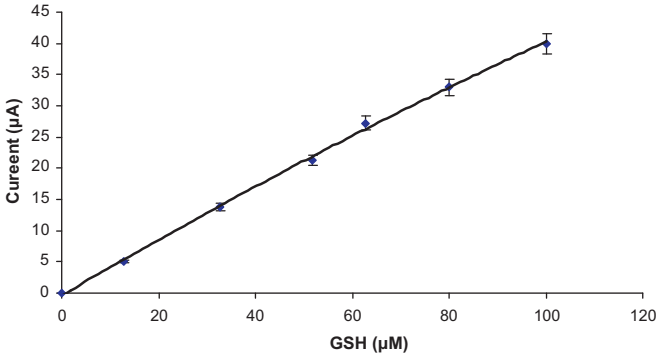


Fig. 1. Effect of glutathione concentration on the oxidation current response of GSHOx/AgNPs/c-MWCNT/PANI/Au electrode at lower concentration range (0.3–100 μM), linear equation being $y = 0.4074x + 0.2568$. Error bars represent the standard deviation for three independent measurements.

Science, Rohtak and stored at −20 °C until use. In venous blood, EDTA 1 mg/ml (final concentration in solution), was added as anti-coagulant. The blood was centrifuged at 3000 × *g* for 15 min. Packed erythrocytes were then collected, washed with a saline isotonic solution (0.9% NaCl) and stored at 4 °C as ready to use. The erythrocytes were hemolysated in 5 mM of EDTA solution and diluted in 1:l00 ratio with the working buffer for analysis. All experiments were carried out at 25 °C. Assay was carried out in the same manner as described earlier for testing of biosensor under its optimal working condition except that glutathione was replaced by hemolysated erythrocytes. The concentration of GSH in RBC was interpolated from standard curve between response current (in μA) vs. concentration of GSH (Fig. 1).

2.11. Evaluation of glutathione biosensor

The biosensor was evaluated by studying analytic recovery, precision and correlation. To study interference by metabolites, 0.1 ml of 1 mM aqueous solution of following important metabolite was added to the reaction mixture: methionine, cysteine, glutamic acid, glycine, ascorbic acid, uric acid and EDTA and the reaction buffer was reduced by 0.1 ml. Rest of the procedure was same as described above for response measurement.

3. Results and discussion

3.1. Characterization of AgNPs

AgNPs synthesized in this study were essentially very fine and monodisperse with diameter of ca. 3–9 nm as seen from TEM image (Fig. 2A).

3.2. FTIR characterization of c-MWCNT/PANI/Au and GSHOx/AgNPs/c-MWCNT/PANI/Au electrodes

Fig. 2B shows FTIR spectra for the PANI/Au electrode (Curve i), c-MWCNT/PANI/Au electrode (Curve ii) and GSHOx/AgNPs/c-MWCNT/PANI/Au electrode (Curve iii). Curve (i) shows peaks of quinoid and benzenoid structures of PANI at 1577 and 1485.11 cm^{-1} and bands at 1319.01 cm^{-1} assigned to —C—N stretching of 2° amines. It shows that the aniline was polymerized to form PANI chain linked through —NH— bonds at the Au electrode with two free $—NH_2$ groups at its ends. Curve (ii) shows the peak of —C—O bond of free —COOH groups present at 1294.99 and 1343.93 cm^{-1} and —C—N bonds of c-MWCNT immobilized on PANI at 1045.63 and 1164.13 cm^{-1}. It reveals that the —COOH group at one end of c-MWCNT gets attached to $—NH_2$ groups of PANI through

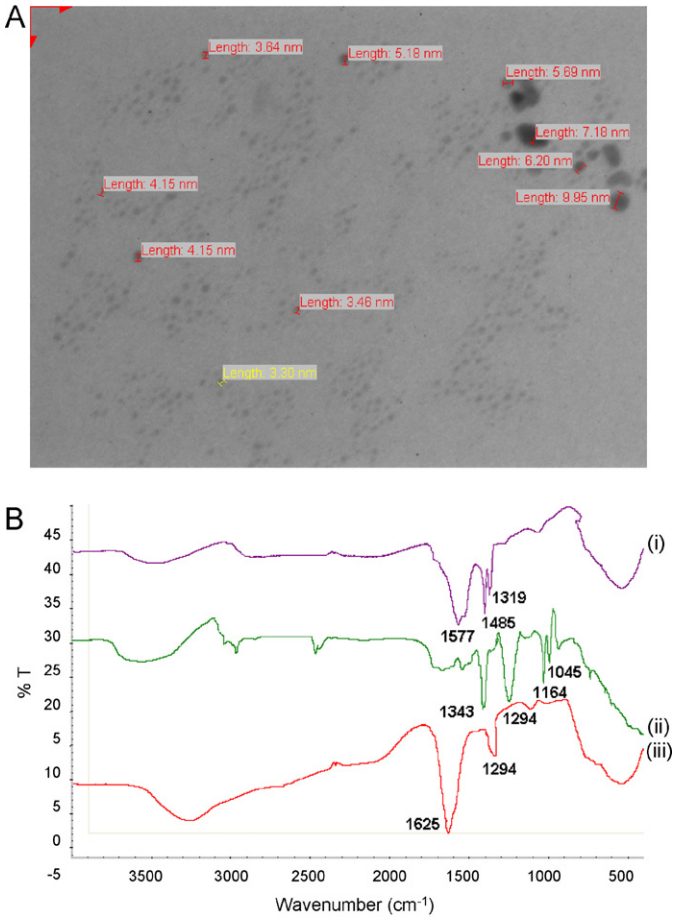


Fig. 2. (A) Transmission electron microscopy (TEM) image of AgNPs. (B) FTIR spectra of (i) PANI, (ii) c-MWCNT/PANI/Au electrode and (iii) GSHOx/AuNPs/c-MWCNT/PANI/Au electrode.

—CO—NH— bonds, while it is free at another end. Curve (iii) shows the peak of the C—N bond. These studies confirmed that the enzyme was immobilized covalently onto the free —COOH group of MWCNT through —CONH— bonds. The disappearance of the peak of free —COOH at 1294.00 cm^{-1} in this curve proves it. There was one more peak at wavelength 500.00 cm^{-1}.

3.3. Scanning electron microscopy (SEM) and electrochemical impedance spectroscopy (EIS)

The morphology of bare Au electrode, c-MWCNTs/PANI/Au electrode and GSHOx/AgNPs/c-MWCNT/PANI/Au electrode was characterized by SEM studies. SEM of the bare Au electrode (Fig. 3A) showed a smooth and featureless morphology, whereas some beaded globular structure (Fig. 3B and C) displayed a stepwise immobilization of PANI/c-MWCNT, AgNPs and GSHOx onto the Au electrode. Fig. 4 shows EIS of bare Au electrode, c-MWCNT/PANI/Au, AgNPs/c-MWCNT/PANI/Au and GSHOx/AgNPs/c-MWCNT/PANI/Au electrode. The charge transfer process in GSHOx/AgNPs/c-MWCNT/PANI/Au bioelectrode has been studied by monitoring charge transfer resistance (RCT) at the electrode and electrolyte interface. The RCT values for the bare Au electrode (Curve i), c-MWCNT/PANI/Au (Curve ii), AgNPs/c-MWCNT/PANI/Au (Curve iii) and GSHOx/AgNPs/c-MWCNT/PANI/Au electrode (Curve iv) have been obtained as 1.00×10^2, 1.40×10^2, 0.90×10^2 and 1.80×10^2 Ω, respectively. The electron transfer via redox couple is hindered by the presence of enzymes on electrode surface.

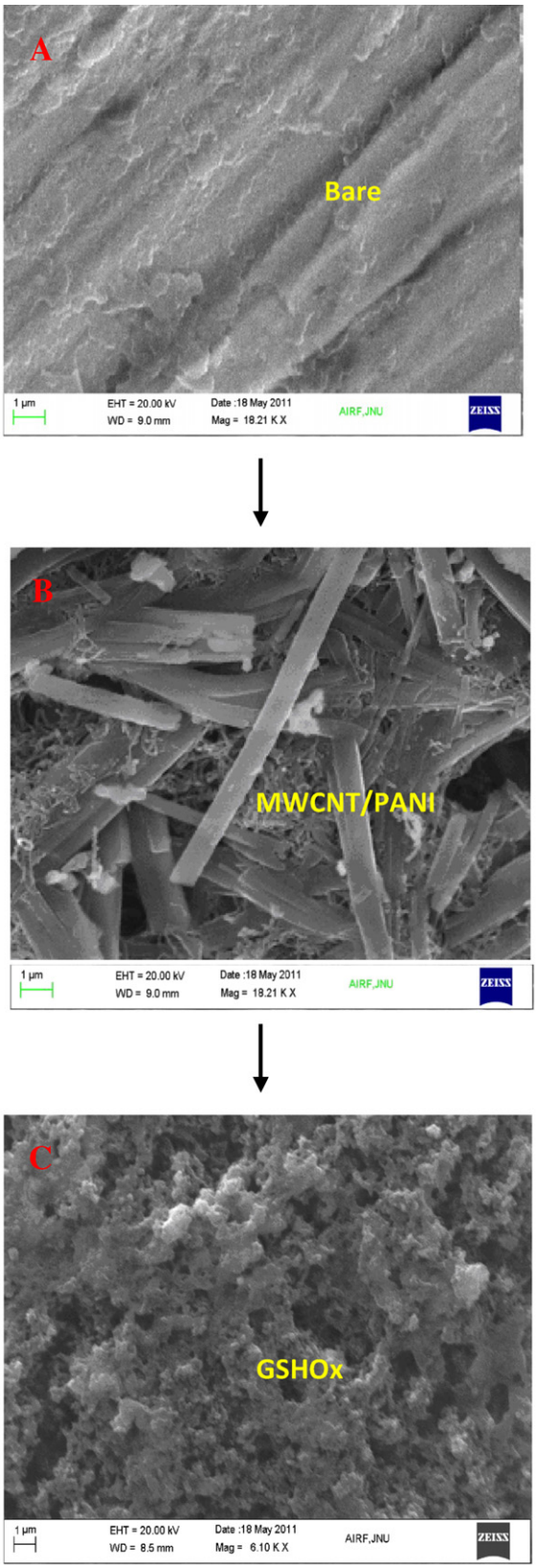


Fig. 3. (A) Scanning electron microscopic (SEM) of bare Au electrode, (B) electrodeposited c-MWCNT/PANI layer modified Au electrode and (C) GSHOx/AgNPs/c-MWCNT/PANI.

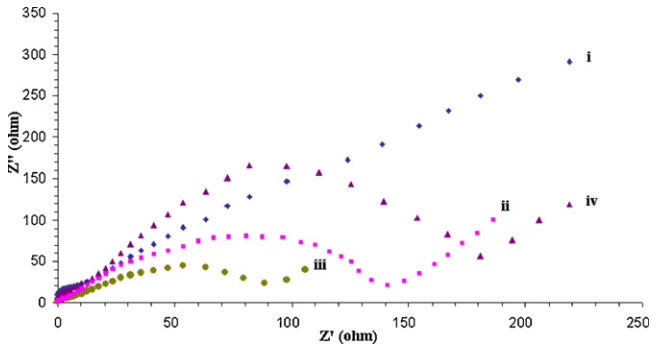


Fig. 4. Electrochemical impedance spectra of bare Au electrode (i), c-MWCNT/PANI (ii), AgNPs/c-MWCNT/PANI (iii) and GSHOx/AgNPs/c-MWCNT/PANI (iv) electrodes in unstirred 0.05 M sodium phosphate buffer (pH 6.5) containing 5 mM $[Fe(CN)_6]^{3-/4-}$. Frequency range: 0.01 Hz to 10 kHz.

The increased RCT value of GSHOx/AgNPs/c-MWCNT/PANI/Au electrode compared to other electrodes was due to the immobilization of enzymes. This increase in RCT is attributed to the fact that most biological molecules, including enzymes, are poor electrical conductors at low frequencies (at least <10 kHz) and cause hindrance to the electron transfer.

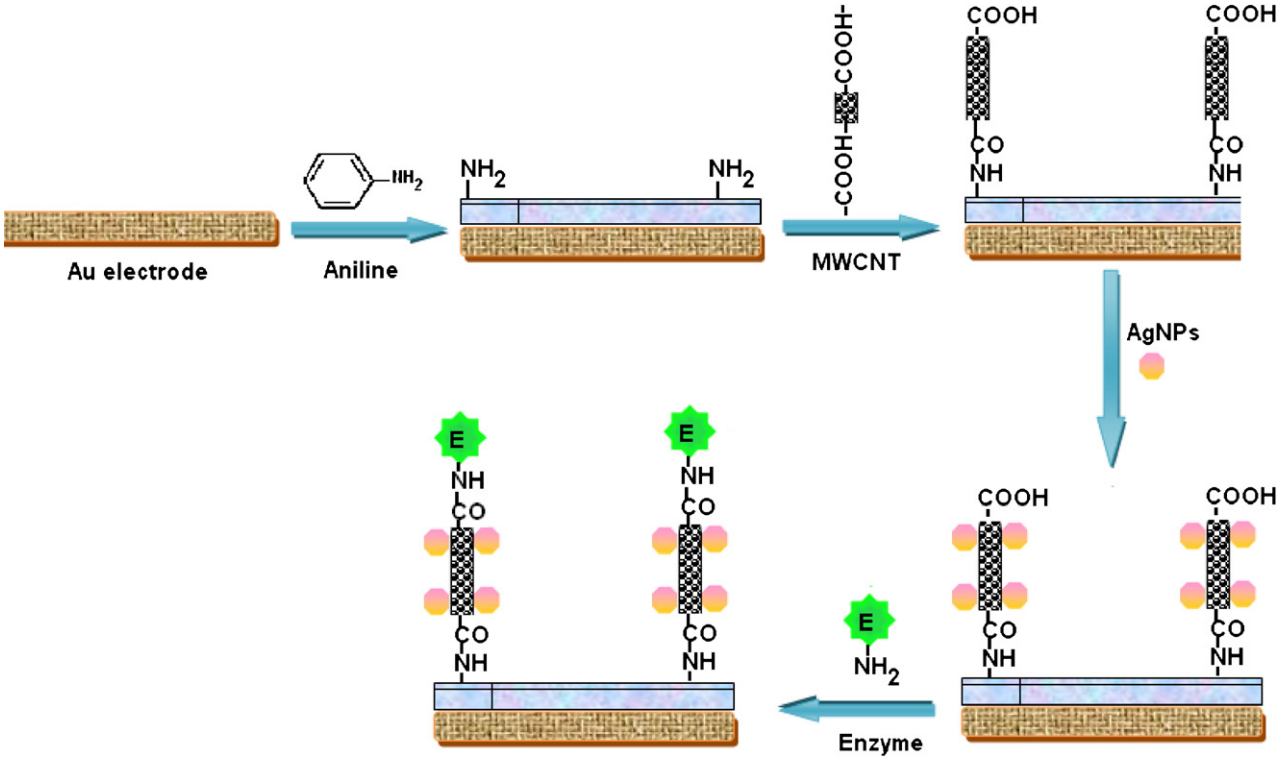
3.4. Characteristics of AgNPs/c-MWCNT/PANI/Au electrode by cyclic voltammetry

The fabrication of the glutathione biosensor based on GSHOx/AgNPs/c-MWCNT/PANI modified Au electrode is summarized in Scheme 1. Firstly PANI was electrodeposited onto Au electrode with free $-NH_2$ groups at its ends, which gets linked to —COOH groups of c-MWCNT through amide bond (—CO—NH—). Then, AgNPs are electrochemically deposited on the surface of PANI/c-MWCNT film. The electrodeposition method was selected to produce AgNPs on electrode surface, because this method is easy to be carried out and the layer thickness can be controlled. GSHOx was immobilized covalently, as $-NH_2$ groups on surface of enzyme forms amide bond (—CO—NH—) with free —COOH groups of c-MWCNT. Our results show that AgNPs and c-MWCNTs provided a remarkable synergistic effect toward the oxidation of glutathione.

To evaluate the charge-transfer properties on the surface of the modified electrodes, cyclic voltammetric technique was employed, using KCl as redox probe. Cyclic voltammograms recorded in 0.1 M KCl solution and 0.1 M acetate buffer (pH 5.0) is shown in Fig. 5A. The oxidation current values obtained for bare Au electrode (curve a), c-MWCNT/PANI/Au modified electrode (curve b) and AgNPs/c-MWCNT/PANI/Au (curve c) modified electrode were 0.050 mA, 0.100 mA and 0.200 mA respectively. CV of c-MWCNT/PANI/Au revealed two redox peaks for oxidation of aniline to radical cation and then to radical dication [45]. The electrodeposition of AgNPs onto c-MWCNT/PANI modified Au electrode surface leads to increase in current intensity, as a result of increase in electroactive area. The preservation of a quasi-reversible electrode process and the large increase in peak currents for the nanocomposite film modified electrode proved that AgNPs exerted an obvious improvement effect on c-MWCNT/PANI/Au electrode property.

3.5. Response toward glutathione at the AgNPs/c-MWCNT/PANI/Au electrode

To evaluate the catalytic activity of GSHOx at the AgNPs/c-MWCNT/PANI/Au electrode, the modified electrode was characterized by a cyclic voltammogram in the presence of glutathione in the



Scheme 1. A scheme showing the steps involved in the construction of glutathione biosensor based on covalent immobilization of GSHOx on AgNPs onto c-MWCNT/PANI/Au electrode.

potential range from 0.0 V to +0.6 V. Fig. 5B shows cyclic voltammograms of the AgNPs/c-MWCNT/PANI/Au electrode in an unstirred 0.1 N KCl solution and 0.1 M acetate buffer (pH 5.5) with (curve b) and without glutathione solution (curve a) at scan rate 50 mV s^{-1}. It was observed that with the addition of 100 μM glutathione, both the reduction current and oxidation current increased, which showed the improved catalytic properties of modified electrode to the oxidation of glutathione. The following electrochemical reactions occur during the response measurements:

$$2\,\text{glutathione} + O_2 \rightharpoonup \text{GSHOx glutathione disulfide} + H_2O_2$$

$$H_2O_2 \rightarrow O_2 + 2e^- + 2H^+$$

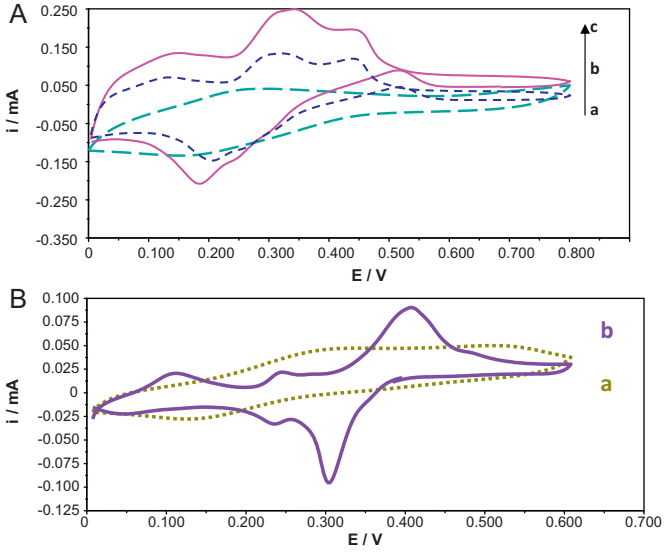


Fig. 5. (A) Cyclic voltammograms of (a) bare Au electrode, (b) c-MWCNT/PANI modified Au electrode and (c) AgNPs/c-MWCNT/PANI/Au electrode. Supporting electrolyte: 0.1 N KCl solution and 0.1 M acetate buffer (pH 5.5) [(1:1)]; scan rate: 50 mV s^{-1}. (B) Cyclic voltammograms (CV) of GSHOx/AgNPs/c-MWCNT/PANI Au electrode (a) with (100 μM) and (b) without substrate. Supporting electrolyte: 0.1 N KCl solution and 0.1 M acetate buffer (pH 5.5) [(1:1)]; scan rate: 50 mV s^{-1}.

3.6. Optimization of biosensor

The effect of pH of reaction buffer from 4.0 to 10.0 for 100 μM glutathione solutions was investigated. The current response resulting from the AgNPs/c-MWCNT/PANI/Au bound enzyme-catalyzed reaction achieved a maximum value at pH 6.5. The optimum temperature of biosensor/immobilized GSHOx was 35 °C. The increase in the optimum temperature compared to free enzyme (30 °C) might be due to the improvement in the enzyme rigidity upon immobilization through covalent binding. The biosensor showed optimum response within 4 s.

3.7. Effect of glutathione concentration on GSHOx/AgNPs/c-MWCNT/PANI/Au electrode response

There was a linear relationship between current (μA) and glutathione concentration ranging from 0.3 to 100 μM (lower concentration range) with linear equation being $y = 0.4074x + 0.2568$ (Fig. 1) and cyclic voltammogram of GSHOx/AgNPs/c-MWCNT/PANI/Au electrode response at higher glutathione concentrations ranging from 100 to 3500 μM were shown in Fig. 6. The sensitivity of the present method was 0.477 μA/μM.

The linear range of the present sensor was 0.3–3500 μM, which is better than that of earlier reported electrode based on, pyrolytic graphite-working electrode (19–140 μM) [46], glassy carbon

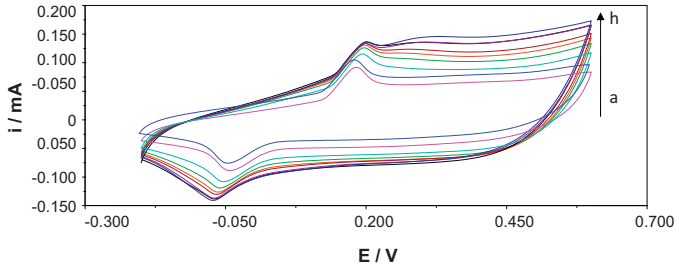


Fig. 6. Cyclic voltammogram of GSHOx/AgNPs/c-MWCNT/PANI/Au electrode obtained in the presence of higher concentration range of substrate (100–3500 μM) containing 0.05 M sodium phosphate buffer (pH 6.5). Scan rate: 50 mV s^{-1}.

Table 1
Analytical recovery of added glutathione in the hemolysated erythrocytes, as measured by GSHOx/AgNPs/c-MWCNT/PANI Au electrode.

Glutathione (μM)	GSH found (μM)	% Recovery
–	40	–
50	88	97.77 ± 1.7
200	238	99.16 ± 0.2

electrode plated with mercury thin film (1–300 μM) [47] and clark-type oxygen electrode (1–500 μM) [48].

3.8. Amperometric determination of glutathione by GSHOx/AgNPs/c-MWCNT/PANI/Au electrode

An amperometric method was developed for determination of glutathione in hemolysated RBC employing the present electrode. The detection limit of the present method was 0.3 μM (S/N = 3). The method was evaluated as follows.

3.8.1. Recovery

The analytic recovery of known amount of added glutathione into hemolysated erythrocytes was determined by the present biosensor. The mean analytic recoveries of added 50 μM and 200 μM (final conc. in reaction mixture) were 97.77 and 99.16% respectively (Table 1), which is comparable to earlier reports (93 and 105%) [48].

3.8.2. Precision

To test the reproducibility and reliability of the present glutathione biosensor glutathione content in six hemolysated erythrocytes samples was determined on the single day (within batch) five times again after storage at −20 °C for one week (between batch). The results showed that determinations were consistent and within and between coefficients of variation (CVs) were 2.4% and 6.3% respectively. The high precision indicated the reproducibility and consistency of the present method (Table 2).

Table 2
Within and between assay coefficients of variation (CVs) for determination of urinary glutathione in the hemolysated erythrocytes as measured by GSHOx/AgNPs/c-MWCNT/PANI/Au electrode.

n	GSH (μM)Mean ± SD	CV (%)
Within assay (5)	50.2 ± 1.21	2.4
Between assay (5	50.9 ± 3.25	6.3

Table 3
The mean ± SD values of glutathione level in the hemolysated erythrocytes samples, as measured by GSHOx/AgNPs/c-MWCNT/PANI Au electrode.

Sex/age	Erythrocytes glutathione levels ($\times 10^{-6}$ mol L^{-1}) (mean ± S.D.), $n = 5$
F/29	3.1 ± 0.07
F/33	3.6 ± 0.09
F/25	3.0 ± 0.02
F/41	7.2 ± 0.04
F/33	4.5 ± 0.03
M/37	4.7 ± 0.06
M/35	3.4 ± 0.01
M/30	5.1 ± 0.03
F/28	3.6 ± 0.07
F/27	5.8 ± 0.04
M/30	2.8 ± 0.12

3.8.3. Interference study

Among the important metabolite tested such as methionine, cysteine, glutamic acid, glycine, ascorbic acid uric acid and EDTA, none had significant effect.

3.9. Application of biosensor

The present biosensor was employed to estimate glutathione in hemolysated RBC in apparently healthy person. The mean value of erythrocytes glutathione was 4.25 μM ($n = 11$) (Table 3), which is in normal established range (2.8–7.2 μmol L^{-1}).

3.10. Stability and reusability

The biosensor was stored at 4 °C, when not in use. The enzyme electrode lost only 50% of its initial activity after 300 reuses over a period of 3 months, which is better than earlier reports (2 months) [48].

A comparison of various analytic properties of present glutathione biosensor with those of earlier amperometric glutathione biosensors is summarized in Table 4.

Table 4
A comparison of present GSH biosensor with earlier reported biosensors.

Matrix/method	Enzyme	Response time	Detection limit (μM)	Linearity (μM)	Stability	Reference
Clark-type oxygen electrode/DO metric biosensor	Glutathione oxidase	2 min	5	5–500	60 days	[48]
Pyrolytic graphite electrode	Glutathione peroxidase	8 s	NR	19–140	2 months	[47]
Gold nanoparticle modified silicon nanowires	Non-enzymatic	NR	0.33	NR	NR	[28]
Glassy carbon electrode plated with mercury thin film	Non-enzymatic	NR	NR	1–300	NR	[46]
Silver nanoparticles/carboxylated multiwalled carbon nanotubes/polyaniline film modified Au electrode	Glutathione oxidase	4 s	0.3	0.3–3500	3 months	Present work

NR = not reported.

4. Conclusion

The use of AgNPs decorated c-MWCNTs/PANI film electrodeposited on Au electrode in construction of an amperometric GSH biosensor has resulted into its improved analytic performance in terms of rapidity (4 s), detection limit (0.3 μM), sensitivity (0.477 μA/μM) and storage stability (3 months). The study also showed that AgNPs/c-MWCNT/PANI hybrid film could also be used for the improvement of other biosensor also.

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