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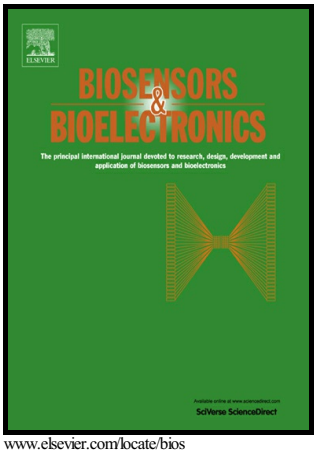

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Glycated hemoglobin detection with electrochemical sensing amplified by gold nanoparticles embedded N_2- doped graphene nanosheet

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Abstract

In the diabetic patients the level of glucose must be determined without any short term fluctuations. The level of Glycated haemoglobin (HbA1c) is accordingly examined for checking diabetes mellitus. HbA1c is considered one of the primarily factor to discern the concentration of average plasma glucose over a long-drawn-out period. In our work, we describe a construction of biosensor which is based on fructosyl amino-acid oxidase (FAO) immobilized nitrogen-doped graphene/gold nanoparticles (AuNPs)/fluorine doped tin oxide (FTO) glass electrode. This constructed biosensor exhibits a wide linear range of 0.3 to 2000μM in response to HbA1c at +0.2 V. Consequently, the detection limit of 0.2μM and good stability (4 months) were achieved. The electrocatalytic activity of this sensor was good as a result of synergistic effect of graphene and AuNPs (2D and 0D nanomaterials). The charge transfer resistance was decreased which was observed by electrochemical impedance spectroscopy (EIS) study. The graphene/AuNPs composites film reveals a distinguished electrochemical response to fructosyl valine (FV) which demonstrates a promising application for electrochemical detection of HbA1c in human blood samples.

Keywords: Fructosyl amino-acid oxidase; FTO electrode; graphene nanosheet; gold nanoparticles; glycated hemoglobin; whole blood

1. Introduction

Diabetes mellitus, a disease caused by deficiency of internal secretion within the body or the defect of duct gland for secretion (Alexander et al., 2006; Berg and Sacks, 2008; Nathan, 2009), is a chronic metabolic disorder. In this diabetic condition a high blood sugar level is

observed over a prolonged period which is analyzed by Glycaemic Index (GI). Presently, there are several assays available for measurement of GI including ion-exchange chromatography, affinity chromatography, high performance liquid chromatography (HPLC) (Davis et al., 1978), Raman (Barman et al., 2012), boronate affinity chromatography (Li et al., 2002), electrophoresis (Doelman et al., 1997), isoelectric focusing (Koval et al., 2011) and immunoassays (Qu et al., 2009). In spite of their prudent use and availability, they are lacking sensitivity. Moreover, they are very time consuming, require complex sample preparation methods with trained, intensive and expert handling and time consuming which leads to ascertain a fast analysis of glycated hemoglobin (HbA1c) for analyzing glucose level or GI.

HbA1c, a good marker for glycine level in blood, has shown consistency over a very long period of time (up to two to three months). Hence HbA1c has been mostly used and is indeed an established test for monitoring the glycaemic control in persons suffering from diabetes (Djamal et al., 2011; Goldstein et al., 1986; John, 2003). Further HbA1c assay has been seen a nearly correct detection marker as compared to other glucose assay. Now a days, HbA1c assay is used as primary methodology for detection of polygenic disorder by several leading medical organizations.

After comparing the conventional methods with our method, it is observed that the HbA1c detection using electrochemical biosensing technique is a specific, easy and rapid assay (Chauhan et al., 2015; Jain et al., 2015). HbA1c biosensors with easy operating principles substantiates other techniques including bioassay, ionophoresis, isoelectric focusing, ion exchange activity and HPLC (Ahn et al., 2016).

The development of biocompatible nanomaterials has recently emerged as a new direction for developing third-generation biosensors based on direct electron transfer between biomolecule and electrode (Chauhan and Pundir, 2014). Graphene nanosheets (GNs) as 2D nanomaterial, an effective biocompatible nanomaterial, has emerged as a new material specially for the application related to the field of biosensors, physics (classical electronics and bio electronics) and chemical process (Horiuchi et al., 1989; Liu et al., 2008; Shan et al., 2009; Lu et al., 2009; Shao et al., 2010). An accurate modification can be done by doping a carbon based material causing an effective change of its intrinsic properties for instance modification of structural framework of the material's surface and its electronic properties

(Usachov et al., 2011). Further these metal nanoparticles are designed for introducing interlayers of GNs to GNs-based hybrid materials which subsequently avoid them from aggregating. Thus due to the strong binding between metal nanoparticles and GNs, an effective chemical route was developed to attach directly and uniformly gold nanoparticles (AuNPs) with high density GNs causing an immobilization scaffold for the protein (Yang et al., 2013).

In our research work, we have fabricated the N_2-doped GNs with AuNPs by ethylene glycol reduction process and further used it for the biosensing of HbA1c. Characterization studies were carried out by X-ray diffraction, spectrophotometer, UV-vis analysis and Transmission electron microscopy (TEM). After enzyme immobilization, the scanning electron microscope (SEM), cyclic voltammetry and electrochemical impedance spectroscopy (EIS) studies were performed. Results obtained through this work demonstrated that AuNPs/GNs composites film provides an effective electrochemical response for the selective detection of HbA1c in human blood samples.

2. Materials and Methods

2.1 Chemicals and Reagents

Fructosyl-aminoacidoxidase (FAO, EC:1.5.3, extracted from recombinant *Escherichia coli*), Bovine serumalbumin (BSA), glutaraldehyde (GA) and fluorine-doped tin oxide (FTO) glass electrode (100mm×100mm×2.3mm) with usual resistance of approximately ~7 Ω/Sq. were bought from Sigma Aldrich, St. Louis, USA and L-valine, pyridine and detergents including Triton X-100 and SDS (Sodium dodecyl-sulfate), CHES (2-(Cyclohexylamino) ethanesulfonic acid), dessmartin periodinane, MES (4- Morpholinoethane sulfonic acid), ethylene glycol (EG), $HAuCl_4$, purified *Bacillus sp.* Proteases, graphite, 4-aminoantipyrine, urea, chitosan bought from Sisco Research Laboratory, Mumbai, India were used. Distilled water (DW) was always taken for the preparation of all solutions. Fresh whole blood samples of healthy persons and diabetes patients were collected from BiodiagnosticsLaboratory, Rohini, New Delhi and always stored at 4 ^{o}C when not in use.

Potentiostat/galvanostat (Autolab AUT83785, Ecochemie, The Netherlands) was employed for all electrochemical measurements. Scanning electron microscopy (SEM) of prepared modified electrodes was performed at AIARS, Amity University, Noida, Uttar

Pradesh. The ultra sonication was accomplished by MisonixUltrasonic Liquid Processors (mode XL-2000 series). The X-ray diffraction (XRD) of GNs studies using X-ray diffractometer (Make-Rigaku MiniFlex, AmericasCorp.) was conducted at Department of Physics, Guru Jambheshwar University of Science & Technology, Hisar.

2.2 Synthesis of graphene nanosheets (GNs)

Modified Hummers method is widely used for synthesis of GNs sheets by using expandable graphite flakes (Hummers and Offeman, 1958). To prepare GNs, graphite (2.0gm) was mixed with 50 mL concentrated sulfuricacid in a 250 mL conical flask with vigorous agitation at 25 ^{o}C. Furthermore, $KMnO_4$ (6.0gm) and $NaNO_2$ (2.0gm) were slowly and carefully poured into the conical flask and then mixed gently. This obtained mixture was then heated at 25^{o}C for 20h. 80 mL of DW was drop wise added to the solution and kept untouched for 15min. 20 mL of 30% H_2O_2 was poured into the reaction mixture and was then washed once with hydrochloric acid solution and 3 times with DW. The precipitate obtained by this process was suspended in DW through ultra sonication method for preparation of the aqueous dispersion of GNs having 5mg/mL concentration.

2.3 Preparation of the N_2-doped graphene nanosheets (GNs)

GNs with a high N_2 content were synthesized through a one pot procedure and added urea as the chemical dopant (Sun et al., 2012). 10 mL of GNs (50mg) dispersed in 25 mL of DW & then 2gm of urea was mixed with the GNs dispersion under sonication for 4h. This solution was potted in a 50 mL Teflonlined autoclave and kept at 150 ^{o}C for 5h. The solids (N_2-doped GNs) were filtered and washed with DW 3times. Eventually, this collected sample was dried in a vacuum oven at 55 ^{o}C providing N_2-doped GNs.

2.4 Synthesis of N_2-doped GNs embedded AuNPs (AuNPs/GNs)

In order to confirm the functionalization of AuNPs on the N_2-doped GNs, gold-precursor salts collectively with GNs in EG solution were added (Li et al., 2010; Deng et al., 2011). In short, 50mg N_2-doped GNs were infused in 50 mL aqueous solution containing 0.02mM $HAuCl_4$. The mixture was ultrasolicated for 2h or until a steady colloid is formed.

subsequently, 20 mL of EG was injected into the blend while continuous mixing for 2h. The product was then maintained at a 130^{o}C for 6h under continuous stirring. Consequently, AuNPs/N_2-GNs composite were collected after filtration and further washed with DW and dried in a vacuum desiccator (Scheme 1).

2.5 Enzyme immobilized onto AuNPs/N_2-dopped GNs modified FTO electrode

Firstly, the FTO electrode was thoroughly cleaned with acetone using ultrasonic bath for 10min and later with DW for further 10min. AuNPs/N_2-dopped GNs was electrochemically deposited in 3 electrodes while merged in electrochemical cell by chronoamperometryat -0.6 V with respect to an Ag/AgCl reference electrode. In these experiments, the working electrode was FTO electrode (area of 1cm^2) while the counter electrode was a Pt wire. A freshly coated film was used after drying at room temperature. Various enzyme concentrations were used to measure the optimum enzyme concentration including 5, 15, 25, and 35 IU.

Chitosan solution (1% w/v) was prepared by dissolving chitosan in 1% (v/v) acetic acid (Barsan et al., 2014) and ultrasonicated for 1h and vortexed. 4 μL was drop casted on the FTO electrode. This electrode was kept for drying for 12h at 30^{o}C. In order to prepare biosensors, an enzyme solution containing BSA was prepared by 1%FAO and 4%BSA (25U FAO per 100 μL enzyme solution. This enzyme solution was further mixed with 2.5% GA solution with a ratio of 2:1 FAO solution: GA and then 2 μL of this mixture was spread onto FTO electrode and kept drying for 4h at 4^{o}C.

2.6 Protocol FV synthesis

Since FV is not commercially available easily therefore it was synthesized in our laboratory (Rajkumar et al., 2007; Keil et al., 1985). 40 mL pyridine and 40 mL acetic acid were mixed together with L-valine (0.16M, 1.88gm) and then stirred for 30 min at 25 ^{o}C or RT. Then glucose (0.25 M, 4gm) was added and purged by using N_2 for 5 min and again stirred for 4 days at 25 ^{o}C. After filtering the dark colored product was then separated and the solvent left for evaporation. By the process of re-crystallization in methanol, a yellowish amorphous product was obtained.

2.7 Measuring response for AuNPs/N_2-doped GNs onto the FTO

FAO/AuNPs/GNs/FTO-coated glass plate was used for referring working electrode, a platinum wire considering a counter electrode and silver/silver chloride electrode indicating standard electrode in a conventional 3 electrode system. This electrode system was incorporated for electrochemical characterizations and analysis. CVs measurements were performed in 3 electrode cell having 20 mL electrolyte [2.5mM $K_3Fe(CN)_6/K_4Fe(CN)_6$(1:1)], 5 mL sodium phosphate buffer (0.1 M, pH 7.0) and 0.1 mL of FV.

2.8 Testing of real samples

The healthy individuals and diabetes patients provided blood samples which were collected with EDTA as anticoagulant in Biodiagnostics Laboratory, Rohini, New Delhi. In order to mix the blood cells, a gentle inversion was applied. The mixing of 30 µL of whole blood in 300 µL of lysis buffer was carried out to ascertain proper Hemolysis and then incubated for 10min. Furthermore, the cells were lysed by using Lysis buffer consisting 100mM CHES (pH 8.7), 0.45% SDS, 1% Triton X-100 and 0.5mM of dessmartin periodinane. A proteolytic digestion was performed of human blood samples by mixing of a solution including oxidizing agent (1mM), 4U/mL purified *Bacillus sp.* proteases and 5mM MES. This proteolytic digestion leads to the release of certain amino acids including glycated valines from the hemoglobin betachains which then serves as substrates for the FAO and consequently produces H_2O_2 by combining 0.1M sodium phosphate buffer (pH 7.5), 90U/mL peroxidase and 0.8mM of 4-aminoantipyrine which breaks N-terminal amino acids either histidine or valine (Liu et al., 2008). The numbers of e^- i.e. current produced for detection is principally based upon splitting of H_2O_2 released by the oxidation of substrate by immobilized enzyme (FAO) which is evidently proportional to the HbA1c concentration.

Fru-Val-His-Leu-Thr-Pro-Glu-Glu-Lys-Ser.... (N-terminal residue of β-chain in HbA_{1c})

↓ Protease

Fructosyl valine + $(amino\ acid)_{n-1}$

(FV) (His, Leu, Thr)

$FV + O_2 + H_2O$

↓ FAO

Valine + D-glucosone + H_2O_2

In this study, the method followed for HbA1c detection in whole blood samples was identical to response measurement of the electrode excluding the substrate which was replaced by whole blood sample. The HbA1c level is indicated by the ratio of glycosylated hemoglobin (mM) and total hemoglobin (mM).

$$\%\text{HbA1c} = \frac{\text{Glycosylated hemoglobin (mM)}}{\text{Total hemoglobin (mM)}} \times 100$$

Results obtained here were further interpreted by using various statistical tools and parameters. The standard immunoassay method was correlated with our method.

3. Results and Discussions

3.1 Characterization of nanomaterials

A sharp XRD peak of 2 theta at 13.8 degree considering a standard for graphene oxide was analyzed. By further heating and doping with nitrogen the peak is shifted towards right and forms a broad peak. This indicates the reduction of graphene oxide (GO) to graphene (Fig. 1A). The diffraction peaks from numerous planes and d-values are matched accurately with already reported JCPDS data of GNs (Kannan et al., 2013). The relative peak intensities of GNs and their peak positions were arranged accordingly with standard JCPDS and no impurities are noticed.

A pi-pi* transition of GO at 230nm in the UV-Vis spectrum was noticed. An absorption peak at 305nm corresponds to n-pi * transition of C–O bonds and embedded through exfoliation and inclusion on the graphene. The 275nm peak is observed by analyzing reduction of the graphene oxide into the graphene by the doping of N_2. The further peak at 535nm is caused by the AuNPs (Fig. 1B).

A uniformly dispersed AuNPs onto the GNs having good dispersion was achieved which is shown in high magnification TEM image of the AuNPs/GNs (Fig. 1C). These AuNPs were appeared in the form of dots on the GNs (bright dots). The spherical AuNPs have a small size dispersion of 15 ± 0.5 nm.

3.2 Electrode surface characterization by SEM and EIS

In order to check the morphology of bare FTO electrode, SEM images of N_2-doped GNs/FTO, N_2-doped GNs embedded AuNPs/FTO and FAO immobilized N_2-doped GNs embedded AuNPs modified FTO electrodes were taken. The Bare FTO electrode has a smooth surface (Fig. 2a). Fig. 2(b) reveals a random orientation of sheet like structure of GO. The sphere like nature of AuNPs is shown in Fig. 2(c). During analysis of nanocomposite (AuNPs/GNs) morphology, it is recognized that the GNs are obvious in lower magnification and the AuNPs were distributed sporadically on GNs. Furthermore, a confirmation of immobilization of FAO in N_2-doped GNs embedded AuNPs was shown in Fig. 2(d). Various enzymes having spherical morphology were also observed.

EIS is a convenient technique to investigate stepwise modification process when the change of interface properties of an electrode is occurred. A typical Nyquist plots for the different electrodes were obtained by using 0.1 M KCl containing 5 mM $[Fe(CN)_6]^{3/4}$ as the probe (Fig. 3). The electron transfer resistance (Rct) of bare FTO electrode was 1027 Ω. As shown in Fig 3, the value of Rct was decreased to 220 Ω and 110 Ω for GNs and AuNPs/GNs modified FTO electrodes (curve b and c). This indicates relatively fast electron transfer kinetics of nanocomposites between the electrolyte and electrode surface. In the FAO/AuNPs/GNs modified FTO electrode (curve d), Rct value was increased to 450 Ω. This increase was occurred by the formation of barrier layer of the FAO molecules resulting a pathway of electron transfer by enzyme was hindered.

3.3 Electrochemical analysis using cyclic voltammetry

The cyclic voltammetry technique is a suitable method for evaluating the charge-transfer properties on the surface of the modified electrodes. Cyclic voltamogramms recorded in 5.0mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ solution and 0.1 mL FV (0.1mM) are shown in Fig. 4. There is not any well-defined oxidation and reduction peaks for bare FTO electrode in forward and reverse scan [curve (a)]. Once the electrode is modified by N_2-doped GNs and AuNPs/GNs, the current response of CVs are increased [which is observed in curve (b and c)]. To confirm, the values of the peak separation considering the difference between anodic peak potential and cathodic peak potential, Ep were determined (Fig. 4).

The bare FTO electrode and AuNPs/GNs modified FTO electrode show current intensity of 12 and 22 μA. After electrodeposition of AuNPs/GNs onto FTO electrode surface, the electro active area was increased resulting in a vast increase of current intensity.

Because of a quasi reversible electrode process and a huge increase in peak currents of the nanocomposite film modified electrode demonstrated that the AuNPs/GNs modified FTO electrode led to an improvement on electric conductivity of enzyme electrode. Furthermore, the oxidation of FAO/AuNPs/GNs modified FTO electrode was elevated because of oxidation of FV once catalyzed by the immobilized FAO on the AuNPs/GNs modified FTO surface [curve d].

3.4 Evaluation of biosensor

The maximum response of biosensor was observed at 7.5 pH; hence, subsequent studies were done at the same pH. The optimum amount of enzyme for the preparation of working electrode was 25 IU. This electrode system has given a linear response in the range of 0.3–2000 μM in relation to the change in FV concentration (Fig. 4B). The detection limit (S/N = 3) of this biosensor electrode system is 0.2 μM. This detection limit is considered lower than that of iridium nano-particle incorporated in the carbon-based working electrode (Fang et al., 2009), core–shell magnetic bionanoparticles modified Au electrode (Chawla and Pundir, 2011) and zinc oxide nanoparticles/polypyrrole hybrid film deposited onto Au electrode (Chawla and Pundir, 2011). For studying analytical recovery, an exogenous FV of a known concentration was added to the serum sample. The concentrations of HbA1c were estimated before and after addition of exogenous FV in the blood. The % recovery of added FV in serum was calculated. The calculated analytical recoveries in serum of 5.0 mM and 10.0 mM FV were 96.0% and 97.5% indicating good efficiency of the biosensor. The between and within batch coefficient of variation (CV) for FV determination in blood by this biosensor were 4.42% and 3.62% revealing the reproducibility and reliability of the method. The effects of numerous possible interfering substances e.g. ascorbic acid, uric acid, bilirubin, urea, triglycerides and glucose on this biosensor were investigated at +0.2 V versus Ag/AgCl in sodium phosphate buffer (pH 7.5). The results obtained after comparing ascorbic acid, bilirubin, urea, uric acid, triglycerides and glucose indicated that there is no interference of these substances (Fig. 5A).

The accuracy of this method was evaluated by comparing HbA1c values (%) in 20 blood samples with our method (y) to those obtained by standard auto analyzer method (x). After comparing these value, it was noticed that both the methods showed a very good correlation $R^2 = 0.997$ with regression equation, $y = 0.9706x + 0.2108$ (Fig. 5B). These results indicate a

very good analytical performance of this constructed biosensor in biological (whole blood) samples.

3.5 Determination of HbA1c in whole blood using FAO/AuNPs/GNs modified FTO electrode

HbA1c levels measured in human whole blood samples of healthy individuals ranges from 4.3–5.8%. This is a normal established range (Supplementary Table 1). HbA1c values in whole blood of diabetic patients measured by our biosensor were in the range of 6.2–9.9 %, which is significantly higher than those of health persons (p value<0.001) (Supplementary Table 2). A higher glycation of b-chain of hemoglobin cause an elevated level of HbA_{1c} in diabetic patients resulting in a prolonged increased level of blood glucose (Agarwal et al., 2013).

3.6 Long-term stability of electrode

The activity of FAO/AuNPs/GNs modified FTO electrode during storage time for long-term storage stabilities was evaluated. The enzyme electrode was washed every time with reaction buffer 2–3 times before its reuse to avoid any possibility of plugging or masking of the electrode by soluble protein in blood. Only the initial activity of 50 % was lost after 100 regular uses over the period of 4 months indicating a higher activity than earlier reported FAO biosensors e.g. iridium nano-particle which was incorporated in the carbon-based working electrode (Fang et al., 2009) and core–shell magnetic bionanoparticles modified Au electrode (Chawla and Pundir, 2011). After careful examination, we observed that the covalent coupling between enzyme and nanocomposite material was a very efficient method for retaining the activity of immobilized FAO preventing it from leaking out from the working electrode.

4. Conclusion

In our research work, we have reported a sensitive electrochemical detection of HbA1c through a AuNPs/GNs/FTO interface. The cross-linking method was used for the immobilization of FAO, which provides the higher stability to the working electrode. This biosensor shows a linear dynamic range of HbA1c from 0.3 to 2000 μM. Normally the clinically important concentration range is considered in this extent and subsequently shows a negligible response to interfering species including ascorbic acid, uric acid, TG, bilirubin,

glucose and urea. These findings may open up new and vital research for the advancement of amperometric based sensors for HbA1c detection in biological samples.

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Captions to Figures

Scheme1 Schematic illustration of AuNPs/N_2-doped GNs showing the bonding of N_2 atoms in the GNs. Urea is added as the chemical dopant.

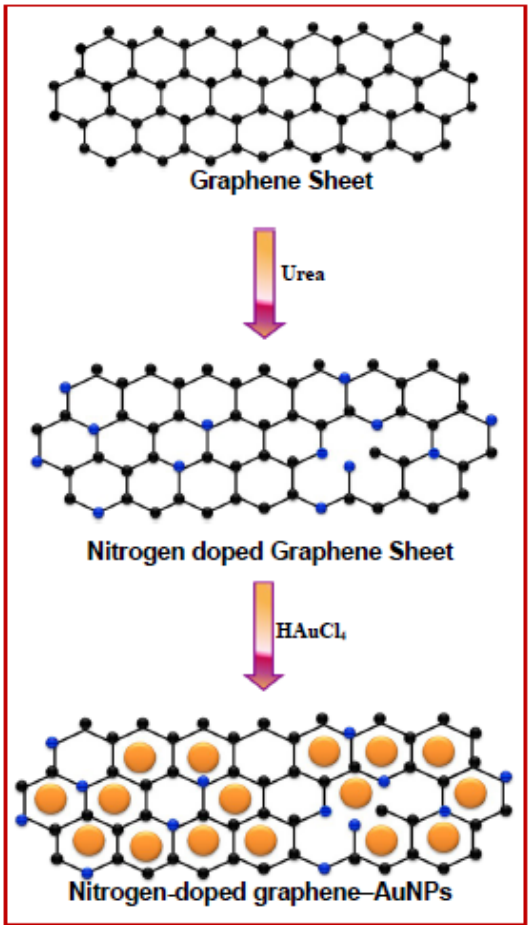
Fig. 1 **(A)** X-Ray diffraction (XRD) pattern of graphene oxide (a) and N_2-doped GNs (b). **(B)** Absorption spectra of graphene oxide (a) and AuNPs/N_2-doped GNs in aqueous solution (b). **(C)** Transmission electron microscopy image of AuNPs/N_2-doped GNs. The image shows a red color scale around 100 nm.

Fig. 2 Scanning electron microscopy image showing (a) bare FTO electrode, (b) N_2-doped GNs, (c) AuNPs/N_2-doped GNs and (d) FAO immobilized onto AuNPs/N_2-doped GNs modified FTO electrode.

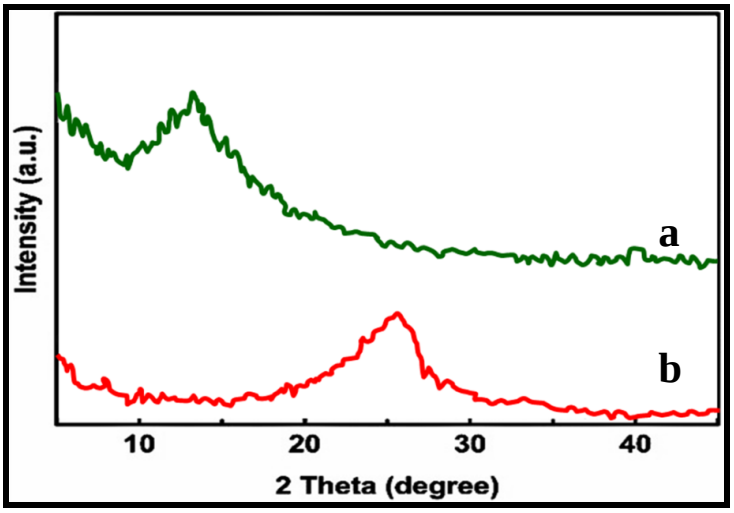
Fig. 3 EIS Nyquist plots of bare FTO electrode, (b) N_2-doped GNs, (c) AuNPs/N_2-doped GNs and (d) FAO immobilized onto AuNPs/N_2-doped GNs modified FTO electrode in 0.1 M KCl containing 5 mM $[Fe(CN)_6]^{3/4}$. The frequency varied from 0.1 Hz to 100 kHz, and 5 mV amplitude of sine voltage signal was used.

Fig. 4 **(A)** Cyclic voltammograms curve of bare FTO electrode, (b) N_2-doped GNs, (c) AuNPs/N_2-doped GNs and (d) FAO immobilized onto AuNPs/N_2-doped GNs modified FTO electrodes in 5.0 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ solution and 0.1 mL FV (0.1mM). Scan rate at 50 mV s^{-1}. (**B)** Cyclic voltammograms recorded at different concentration of substrate i.e. FV from (a) 0.3, (b) 1.0, (c) 10, (d) 100, (e) 500, (f) 1000 and (g) 2000 μM in 15 mL sodium phosphate buffer (0.1 M, pH 7.5). Scan rate, 50 mV s^{-1}.

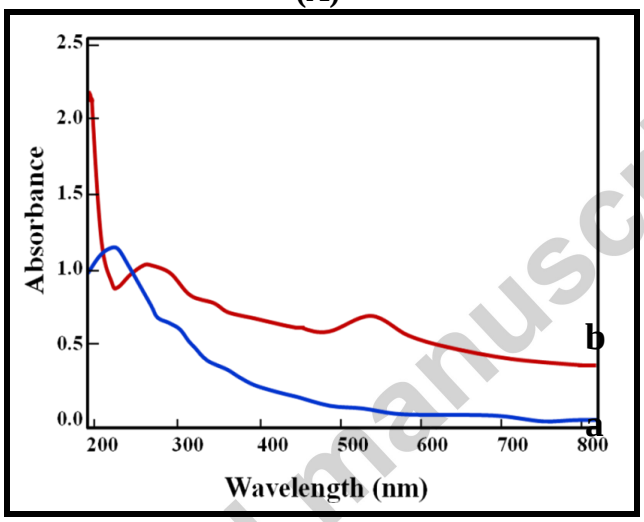
Fig. 5 **(A)** Effect of potential interferents on FAO/AuNPs/N_2-doped GNs modified FTO electrode with 0.1 mM FV in 0.1 M sodium phosphate buffer (pH 7.5). **(B)** Correlation between blood HbA1c values (20 samples) determined by standard method (x - axis) and FAO/AuNPs/N_2-doped GNs modified FTO electrode (y - axis). Error bars show the values of experiments run in triplicate.



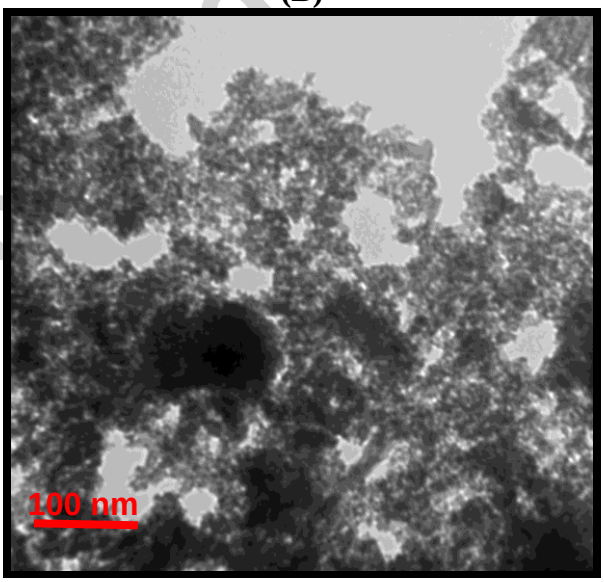
Scheme 1.



(A)

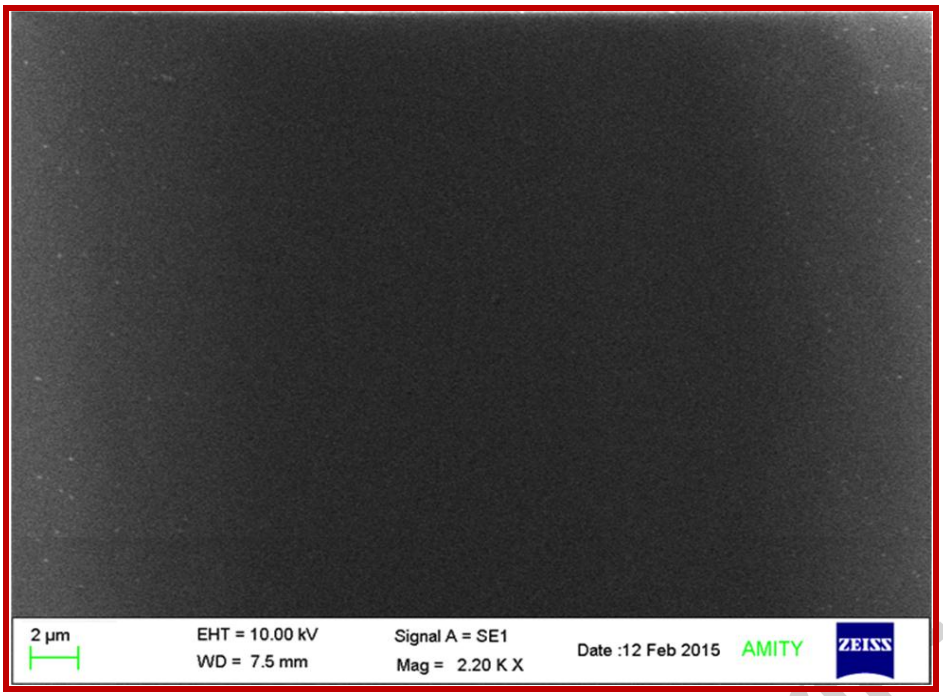


(B)

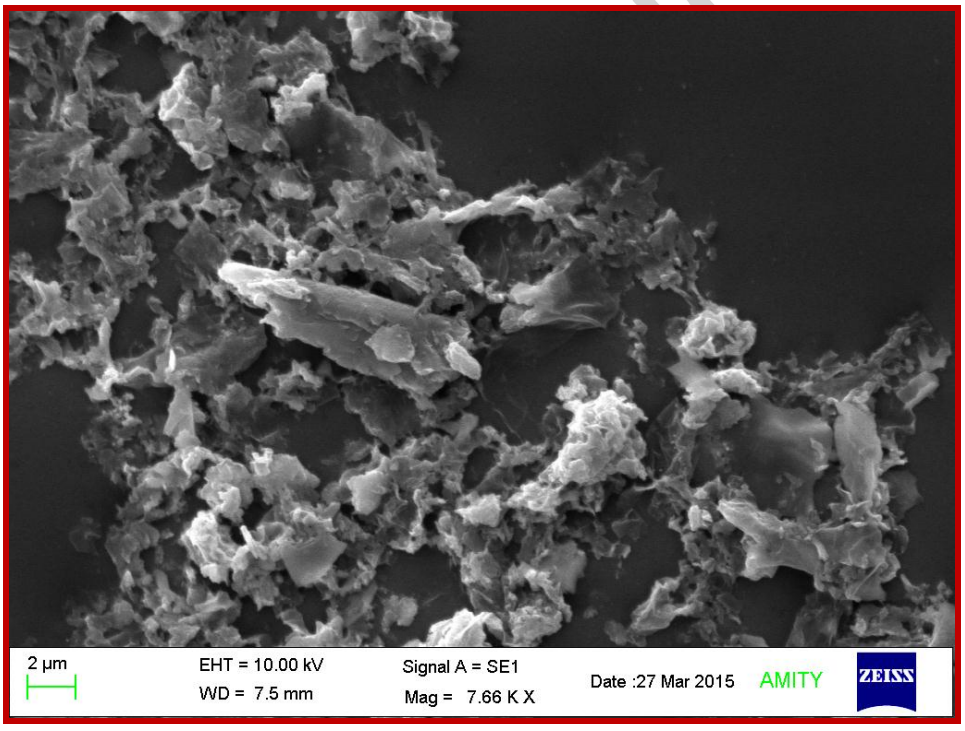


(C)

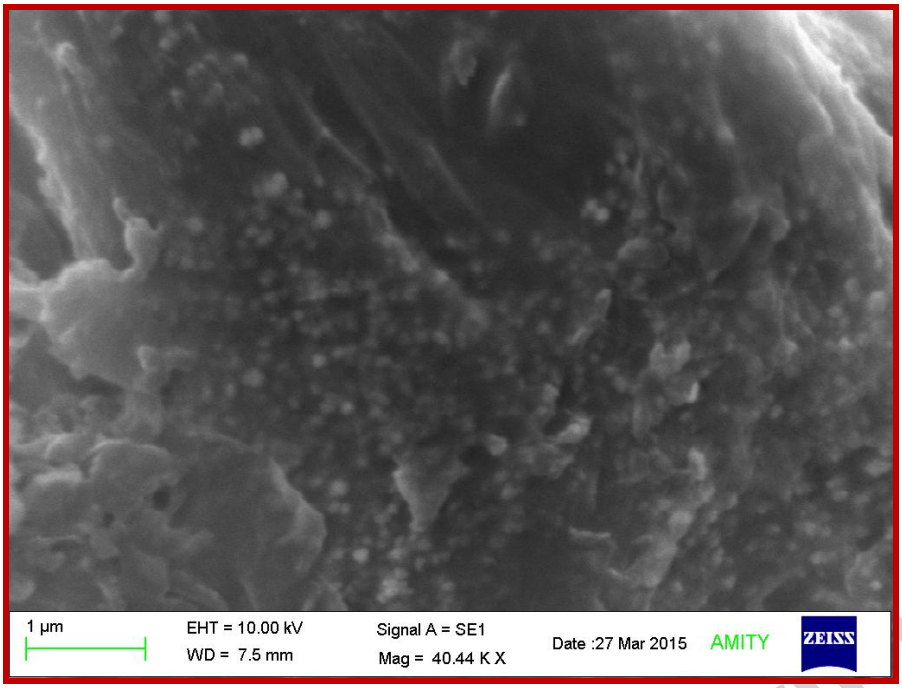
Fig. 1


2 µm
EHT = 10.00 kV
WD = 7.5 mm
Signal A = SE1
Mag = 2.20 K X
Date :12 Feb 2015
AMITY
ZEISS

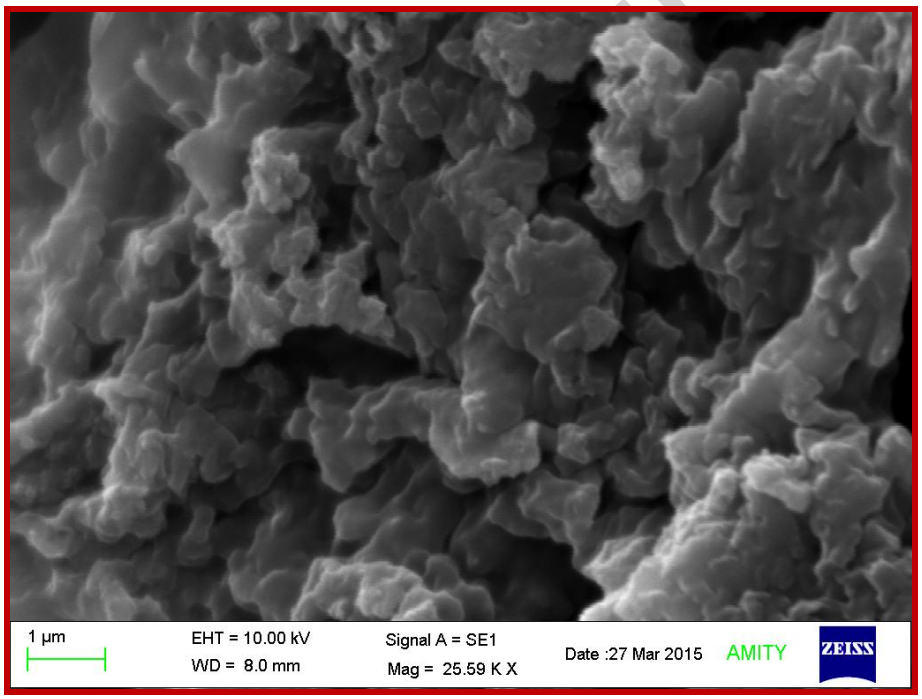
(a)


2 µm
EHT = 10.00 kV
WD = 7.5 mm
Signal A = SE1
Mag = 7.66 K X
Date :27 Mar 2015
AMITY
ZEISS

(b)


1 µm
EHT = 10.00 kV
WD = 7.5 mm
Signal A = SE1
Mag = 40.44 K X
Date :27 Mar 2015
AMITY
ZEISS

(c)


1 µm
EHT = 10.00 kV
WD = 8.0 mm
Signal A = SE1
Mag = 25.59 K X
Date :27 Mar 2015
AMITY
ZEISS

(d)

Fig. 2

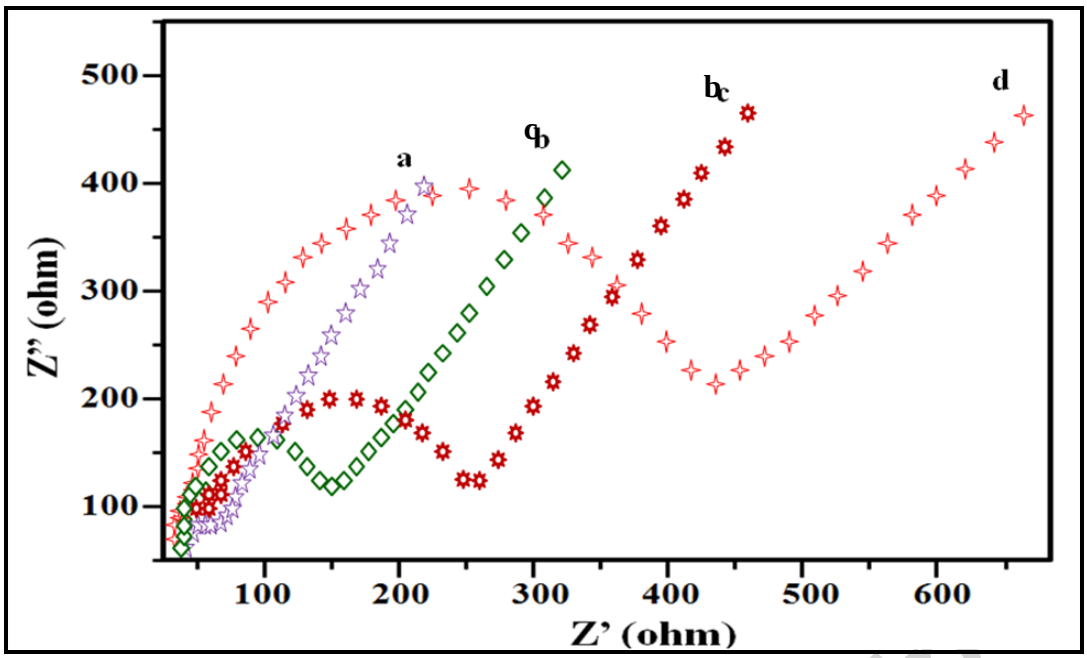
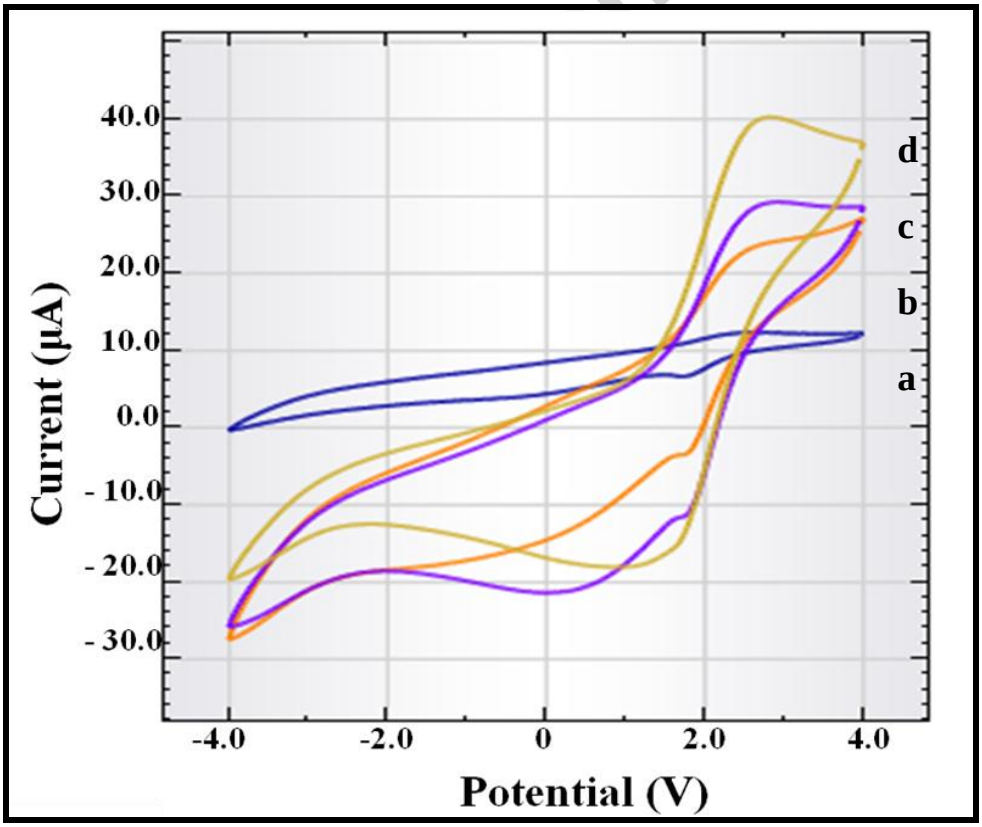
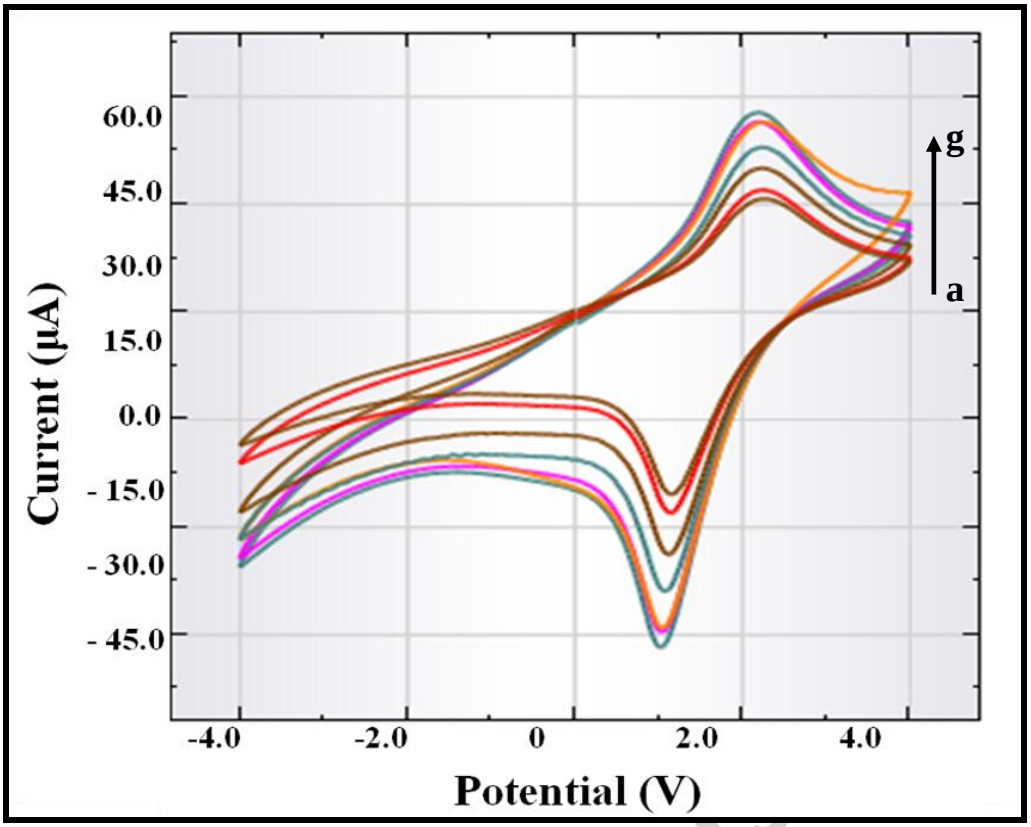

500
400
300
200
100
Z' (ohm)
100
200
300
400
500
600
Z' (ohm)
a
b
c
d

Fig. 3

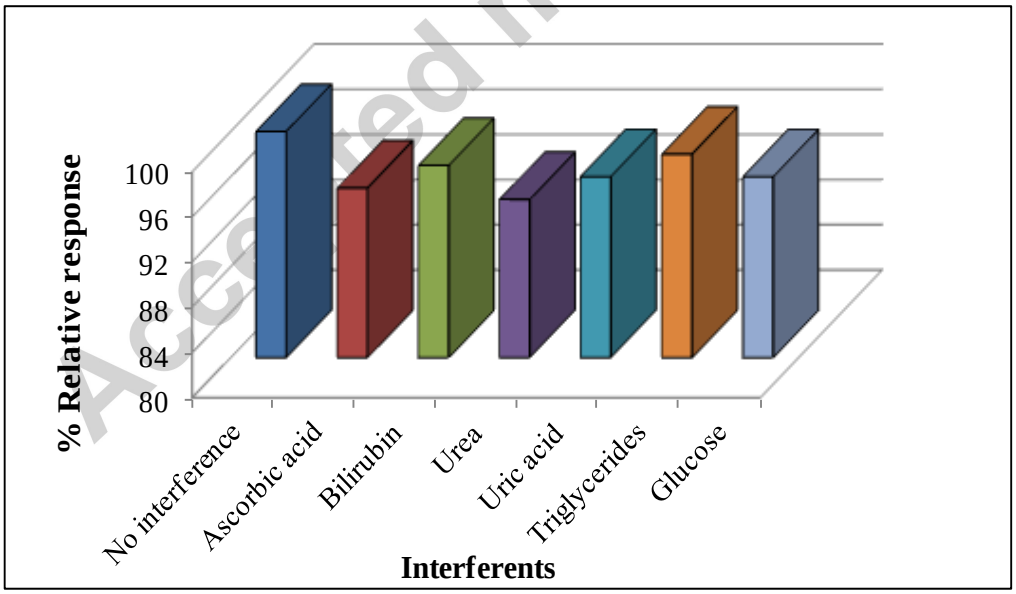

40.0
30.0
20.0
10.0
0.0
- 10.0
- 20.0
- 30.0
Current (μA)
-4.0
-2.0
0
2.0
4.0
Potential (V)
d
c
b
a

(A)

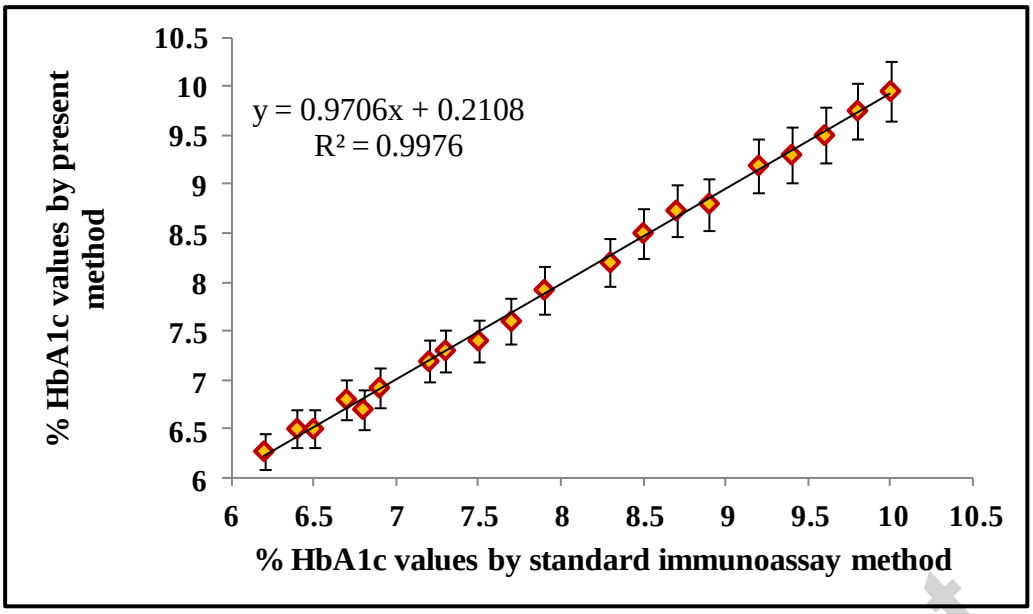


(B)

Fig. 4



(A)



(B)

Fig. 5

Highlights

- Synthesis of a signal amplified interface using AuNPs/N_2-doped graphene nanosheet/FTO electrode.
- Biosensor exhibited low detection limit (0.2 μM).
- The half life of enzyme electrode was 4 months.
- Biosensor was suitable for detection of glycated hemoglobin in human whole blood.