

C-MEMS Derived Glassy Carbon Glucose Sensor

Naresh Mandal¹ and Bidhan Pramanick^{1,2*}

¹ School of Electrical Sciences, Indian Institute of Technology Goa, Ponda 403401, India

² Centre of Excellence in Particulates Colloids and Interfaces, Indian Institute of Technology Goa, Ponda 403401, India

*e-mail: bidhan@iitgoa.ac.in

Abstract. Diabetes is one of the most severe health diseases in the world. More than 150 million people worldwide suffer from diabetes caused by an abnormal glucose concentration in their blood and tissues. The analysis of glucose levels in the body is significant, primarily due to diabetes mellitus. Therefore, accurately detecting blood glucose is crucial for diagnosing, treating, and managing diabetes. Diabetic patients can self-manage their condition by monitoring their glucose levels. The electrochemical glucose biosensor has several advantages, including recognizing glucose specifically, low price, ease of sensor manufacture, correctness, portability, and easy operation. We have developed glassy carbon electrodes (GCE) with conventional carbon-micro-electromechanical (C-MEMS) procedures for glucose sensing. SU-8 photoresist was used as the carbon precursor. The fabricated C-MEMS-derived GCE surface has been functionalized with chitosan and glucose oxidase. Glucose oxidase is a well-known enzyme for oxidizing glucose into gluconic acid and H₂O₂. This reaction kinetics has been recorded with increasing glucose concentration using electrochemical analysis. In summary, we have presented an electrochemical glucose biosensor in a single-step immobilization protocol of glucose oxidase on the glassy carbon electrode (GCE) surface and obtained satisfactory responses for 1 mM to 10 mM glucose solutions. © 2023 Journal of Biomedical Photonics & Engineering.

Keywords: C-MEMS; glassy carbon; biosensor; chitosan, glucose oxidase, impedance spectroscopy; electrochemical sensing; bio-functionalization.

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1 Introduction

In the 21st century, diabetes has become a significant disease around the globe, with more than 439 million cases of Diabetes Mellitus (DM) estimated in 2030 [1]. According to WHO, 422 million people worldwide suffer from diabetes mellitus, which is predicted to be the seventh deadliest disease by 2030 [2]. Long-term suffering from DM causes cardiovascular diseases, kidney failure, blindness etc. The only way to cure DM is to control glycaemia levels. To control the glucose level in the blood, a diabetic patient needs to monitor the blood glucose level regularly. Therefore, it is a need for the hour to develop a novel, quick-response, low-cost biosensor for glucose sensing. Nano glucose sensors are usually made using an oxidoreductase to oxidize the glucose on the electrode surface. In general, glucose

oxidase (GOx) is used for this purpose because it has excellent glucose selectivity, a high degree of stability, and a high activity level across a wide pH range [3]. According to this research, when GOx is used directly with bare electrode surfaces, it changes its structure, biological activity, and function [4]. The conventional finger-pricking method of measuring blood glucose levels causes pain, infection, and tissue damage [5]. The alternative way of finger pricking is without using blood samples in a non-invasive way [6], using bio fluids like tears, sweat, interstitial fluid (ISF), and saliva [7]. The non-invasive measurement techniques include the electromagnetic wave (EM) method, optical measuring and electrochemical sensing. M. Gourzi et al. developed a technique for measuring blood glucose from the skin using EM waves. However, this method is unreliable because measured glucose levels depend on temperature

and cholesterol and ionize the biomolecules [8]. The mid-infrared spectroscopy is better than near-infrared spectroscopy [9]; in both methods, the pressure, temperature, and humidity severely affect the measured glucose value from the skin [10]. The temperature changes influence the measured value of glucose from the skin by optical coherence tomography (OCT) [11]. The specificity of the optical polarimetry technique of glucose measurement from the eye is very low because of eye movement, motion artifact and the optically active composition of aqueous humor [12].

A great deal of interest has been shown in enzyme immobilization technology concerning designing, fabricating, and applying biosensors. Various immobilization techniques are available, such as physical methods (absorption or entrapment) and chemical methods (for example, covalent bonds). Detecting trace components of biological, clinical, industrial and environmental systems can be carried out relatively simply, sensitively, and selectively using electrochemistry. When preparing biosensors, immobilization on the solid matrix is vital to maintaining enzyme function. It is essential that the structure and thickness of the materials used to immobilize enzymes do not reduce their functionality severely.

In the case of chitosan (CS), this polysaccharide biopolymer is formed by the partial deacetylation of chitin. The biocompatibility of chitosan makes it a desirable structural material for enzymatic immobilization and enhances biosensors' performance by retaining enzyme activity. Using chitosan for immobilizing enzymes and cells has been demonstrated as a functional, structural material. Several papers have reported using chitosan-modified biosensors, for example, the modified glucose biosensor built using a sol-gel/chitosan membrane [13, 14]. Adhesion and non-toxicity are good characteristics of this material. Additionally, its structure contains amino and hydroxyl groups, allowing easy chemical modification [15].

The electrochemical method of sensing glucose has immensely made the process easy due to its quick response, less sample use and reduced cost of testing. It is possible due to the advancement of low-cost fabrication techniques of sensors [16]. There are two methods of electrochemically sensing glucose from biofluids such as saliva, sweat, and tears: enzymatic and non-enzymatic. Clark and Lyons first evolved the concept of enzymatic bio-modified electrode sensor [17]. There have been numerous attempts to improve electron transfer from glucose oxidase (GOx) to electrode surfaces. As a result, there has been a growing interest in using semiconductors, carbon materials, such as carbon nanotubes, graphene, glassy carbon (GC) etc., and conductive polymers for achieving this goal. A fabricated platinum electrode was bio-modified in the layer-by-layer structure of single-walled carbon nanotube (SWNT), chitosan, and gold nanoparticles to measure glucose from saliva, obtaining a low detection limit of 0.1 mg/dl [18]. H. Yao et al. developed a contact lens sensor system to measure enzymatically tear glucose

in vitro [19]. A study was carried out to measure glucose from ISF in tattoo based, noninvasive way [20]. Z. Temoçin implemented a sensor by modifying the surface of glassy carbon electrodes (GCE) with a blend nanofiber of poly (vinyl alcohol) and poly (ethylenimine), immobilized glucose oxidase (GOx). They obtained linear responses for blood glucose range, long storage stability, and high anti-interference for ascorbic and uric acid [21]. A GCE functionalized with high catalytic MnO₂ nanowires and coated with GOx showed high sensitivity [22]. Followed by modified GCE with MnO₂NPs and carbon nanofiber (CNF) nanocomposites, which showed higher performance in terms of sensitivity, selectivity, and response time [23]. The electron transfer rate was enhanced in a GCE modified by Co₃O₄ nanoparticles, multiwalled carbon nanotubes (MWCNTs) and immobilized GOx [24]. It was also experimentally verified that Ni/CoO-loaded carbon nanofiber deposited GCE showed a good catalytic effect on glucose [25]. To enhance the performance of glucose sensors, GCE was modified by polypyrrole, nafion functionalized Multiwalled carbon nanotubes (MWCNTs) nanocomposite, chitosan and glucose oxidase showed excellent catalytic activity [26]. GOx, encapsulated in liposome microreactors, was coated on a chitosan layer over GCE showing excellent repeatability and stability [27]. The non-covalently functionalization of MWCNTs and GOx was also deposited on GCE to detect glucose in milk [28]. The poly(p-phenylenediamine) (PPD) and Fe₃O₄ nanoparticles (Fe₃O₄ NPs) nanocomposites were developed as enzyme immobilizers on GCE. The modified electrode showed good affinity to glucose in clinical samples without interferences [29]. The GOx was immobilized on single-layer graphene doped with AuNPs over fabricated GCE. The sensor showed ultra-sensitive for low concentrations of glucose [30]. The 2D-RGO (Reduced graphene oxide) nanocomposites modified GCE, developed by G. Bharath et al., obtained higher electron transfer rate and sensitivity [31]. It has been demonstrated that palladium nanoparticle/chitosan-grafted graphene nanocomposites can be used as glucose biosensors [32]. To detect dopamine selectively, graphene/chitosan electrodes were developed [33]. In direct electrochemistry and glucose sensing, a glucose oxidase-graphene-chitosan electrode was developed [34].

Sol-gel techniques have been used to construct many of these very successful biosensors. However, according to our knowledge, the method for producing biosensors also has some disadvantages. For example, through the porous nature of the thin sol-gel matrix, the enzyme is slowly leached from the electrode surface. There are several issues with the biosensor, such as its slow response time, the procedures being more complicated, and a fragility that makes it difficult to use. It is important to note that during the sol-gel process, certain harsh conditions are exposed to the enzyme, namely partial denaturation by the catalyst (either basic or acidic) or the solvent (usually methanol).

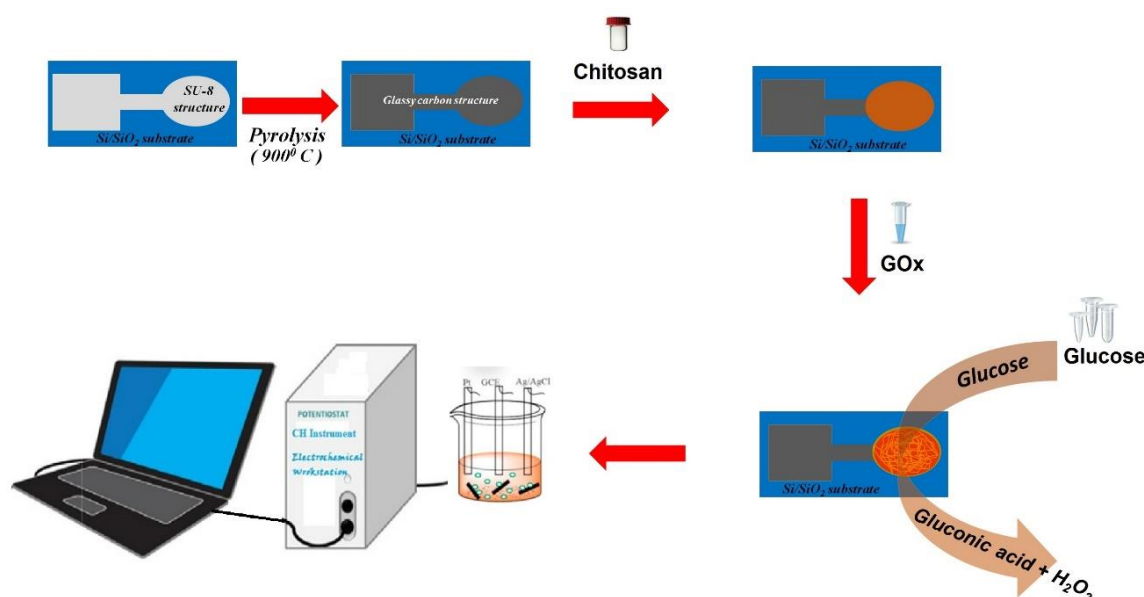


Fig. 1 General scheme of bio-modified glassy carbon electrode (GCE) sensor.

In this paper, a novel biosensor is presented to overcome these problems. We have developed a simple, cost-effective glucose biosensor based on the entrapment of glucose oxidase directly in chitosan on a GC electrode. Compared to the sol-gel/chitosan-modified Glucose Oxidase biosensor, this low-cost design simplifies the fabrication process to ensure a stable and sensitive biosensor. A significant advantage of such devices is that they offer faster, more straightforward methods of obtaining sequence-specific information than the traditional sol-gel techniques. An optimization study was conducted for the biosensor, and its analytical performance was evaluated. A schematic representation of the steps involved in the modification process of the glassy carbon electrode (GCE) for the glucose electrochemical sensing platform is shown in Fig. 1.

2 Materials and Methods

2.1 Reagents and Solutions Preparation

For bio-modifying GCE chitosan from shrimp shells, $\geq 75\%$ (deacetylated) Glucose Oxidase from *Aspergillus niger* with an activity of 100000–250000 units/g was purchased from Sigma Aldrich. Phosphate buffer saline (PBS) contains NaCl, KCl, Sodium dihydrogen phosphate (NaH₂PO₄), and Potassium dihydrogen phosphate (KH₂PO₄), which were purchased from Sigma Aldrich. Standard glucose solution is prepared from D-(+)-Glucose $\geq 99.5\%$ (GC) was purchased from Sigma Aldrich. Glacial acetic acid was purchased from Sigma Aldrich to prepare chitosan solution of chitosan in 0.1 M acetic acid. Milli-Q 0.5 water with a resistivity of 18 M Ω cm was taken from in house plant of IIT Goa for making PBS solutions and washing the electrodes. Chitosan solution has been prepared by dissolving (2 mg/mL) chitosan sample in 0.6 wt% glacial acetic acid at room temperature using magnetic stirring at 1000 rpm

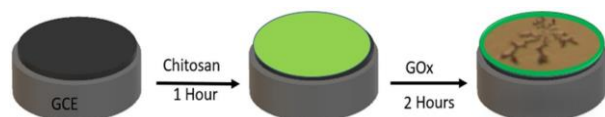
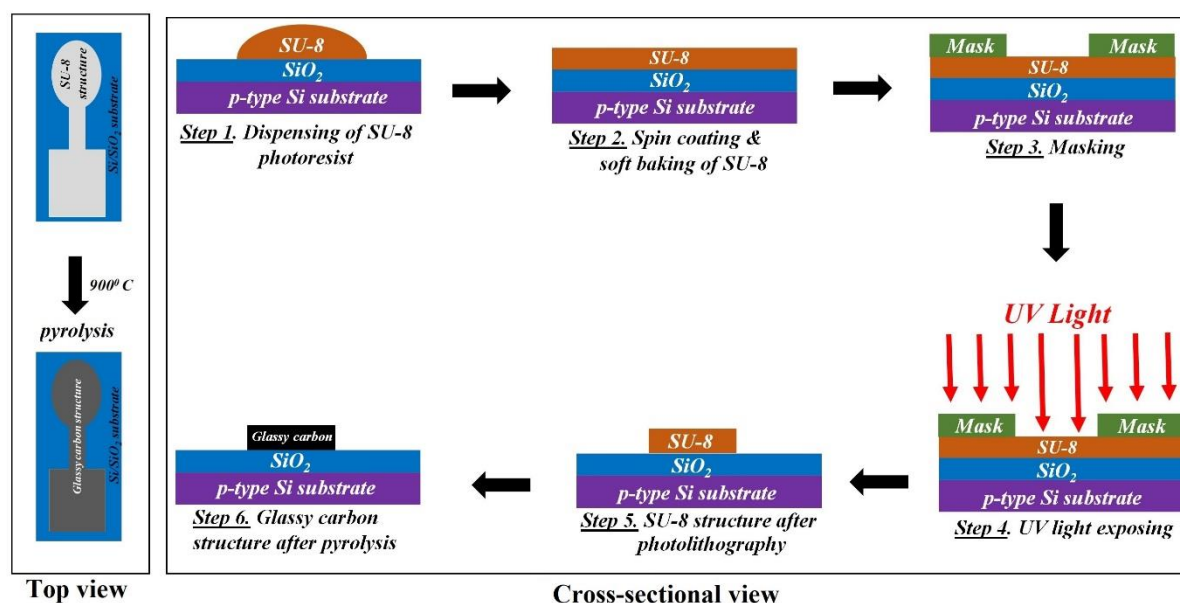
for 1 h. A glucose oxidase (GOx) concentration of 35 mg/mL has been prepared in 0.25 M PBS.

2.2 Fabrication of Electrodes

The conventional method of photolithography and pyrolysis was used to fabricate GCE (Fig. 2) on a 4-inch silicon wafer with a 1 μ m thermally-oxidized SiO₂ layer (NOEL Technologies, Campbell, CA, USA). SU-8 negative photoresist was spin-coated at 3000 rpm for the 30 s and then soft-baked on a hotplate at 95 °C for 3 min to get an even distribution of the layer on the substrate and evaporate the solvent. UV radiation was exposed on photoresist through a thin film photomask to a total energy of 120 mJ/cm² and baked again for 3.5 min at 95 °C to crosslink the exposed patterns. Then the samples were dipped in SU-8 PR developer solution for 2 min to dissolve the unexposed region [35]. The SU-8 structured pattern was pyrolyzed in an inert furnace to convert the SU-8 to glassy carbon (GC) structure. Initially, the SU-8 pattern was heated to 300 °C at a determined temperature ramp rate of 5 °C/min and kept at that temperature for 60 min for thermal stabilization. Then the temperature was increased to 900 °C at a rate of 5 °C/min, maintained the temperature for 1 h and cooled down the furnace at a rate of 10 °C/min. Thus, the SU-8 sensor structure was pyrolyzed to GC structure [36].

2.3 Electrode Surface Modification

The chitosan solution 0.6 wt% in 0.1 M acetic acid was prepared by magnetic stirring at 1000 rpm for 1 h. The GCE electrode was cleaned in Milli-Q water, dried using N₂ gas flow, and coated with chitosan solution by pipetting. Then it was kept at room temperature for 1 h till drying. Afterwards, the chitosan-coated GCE was pipetted with 35 mg/ml GOx solution in 0.25 M PBS and preserved at 4 °C temperature for 2 h before use (Fig. 3).



The bio-modified electrode was cleaned in PBS solution to remove unbound glucose oxidase enzyme before electrochemical testing. All electrochemical experiments were carried out using a potentiostat of CHI 760E electrochemical workstations. A three-electrode system of bio-modified GCE as the working electrode, Ag/AgCl as the reference electrode and Platinum wire as the counter electrode was used for electrochemical characterization.

2.4 Structural Characterization

The surface of the patterned SU-8 structure was shrunk due to pyrolysis. Scanning Electron Microscopy (SEM) (EVO, Zeiss, Germany) was used to characterize the surface morphology and to measure the shrinkage of the carbonized electrodes. The crystallinity of the fabricated carbon electrode was examined with Raman spectroscopy (inVia Qontor, Renishaw plc, UK) using a 514 nm Ar laser source. This work has been reported in the previously reported work [36].

2.5 FTIR Characterization

FTIR characterization has been performed using a SHIMADZU (IRAFFINITY 1S WL model) with a resolution of 4 cm^{-1} in the $400\text{--}4000\text{ cm}^{-1}$ region. This instrument is equipped with Specac attenuated total reflection (ATR) fitted with diamond crystal, scanned (transmittance vs wave numbers mode) at room temperature $25\text{ }^{\circ}\text{C}$ and humidity. Fourteen hundred scans

have been taken for accuracy, and we reported the average value.

2.6 Electrochemical Characterization

Electrochemical impedance spectroscopy (EIS) is an efficient electrochemical measurement technique to analyse the behaviour of the surface-modified electrodes for a particular analyte [1]. The EIS data is usually fitted with an equivalent electrical circuit model to get the electrical fingerprint of the electrochemical behaviour of the sample. EIS was carried out for glucose concentrations 1 mM to 10 mM in 0.25 M PBS, applied ac signal perturbation 5 mV and frequency range from 100 Hz to 1 MHz. Cyclic voltammetry (CV) was carried out to observe the redox peak of bio-modified GCE for glucose concentrations 1 mM, 3 mM, 5 mM, and 8 mM for an applied potential range of -0.1 V to 0.6 V vs Ag/AgCl electrode at a scan rate of 100 mV/sec . We recorded Chronoamperometry reading for 1 mM, 3 mM, 5 mM, 8 mM, and 10 mM glucose concentrations to quantify the glucose concentration. All the experiments were carried out for glucose solutions in 0.25 M Phosphate buffer saline (PBS) of pH 7.4 and room temperature $25\text{ }^{\circ}\text{C}$. All the readings were taken in triplets for each technique and the average has been plotted.

3 Results and Discussion

3.1 Morphologies of GCE, Chitosan, and Chitosan/ Glucose Oxidase / GCE

Micrographs of chitosan and chitosan/glucose oxidase have been characterized using SEM images, as shown in Fig. 4. Noticeably, Fig. 4(b) shows that the membrane of chitosan consists of a homogeneous arrangement, and its surface is flat and symmetrical.

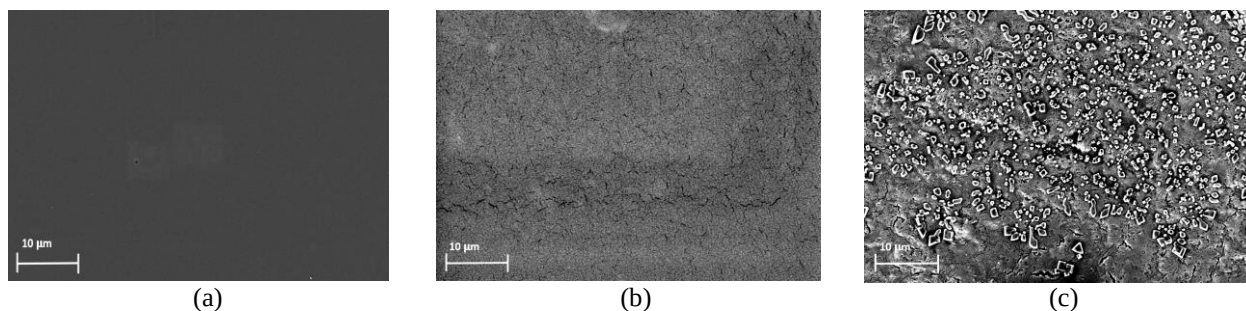


Fig. 4 Scanning electron microscopy (SEM) photomicrograph (images captured at the magnification of 1.63 K X) showing the morphology of (a) glassy carbon electrode (GCE), (b) chitosan/GCE, and (c) chitosan/ glucose oxidase /GCE.

A bump structure is visible on the chitosan/glucose oxidase/GCE compared to the chitosan/GCE (Fig. 4b). The bumps are uniform in size and shape, suggesting glucose oxidase is immobilized in the chitosan/GCE surface (Fig. 4c).

3.2 Characterization of the Sensor Electrode by FTIR

First, 2 mg/mL chitosan solution was pipetted out on the GCE and kept at room temperature for 1 h. After the treatment, FTIR measurement was carried out to estimate the binding of GC with chitosan. After thoroughly drying the surface of the active area of GCE, 10 µL of glucose oxidase solution was pipetted out and allowed to dry for 2 h at room temperature. Again FTIR measurement has been recorded. For each of the electrochemical processes, FTIR plot of transmittance vs wave numbers have been recorded, which is depicted in Fig. 5(a) glassy carbon electrode (GCE), (b) GCE/chitosan, and (c) GCE/chitosan/ glucose oxidase.

GCE, the bare electrode, does not show many FTIR features that suggest the glassy carbon nature of the electrode (Fig. 5a). After adding chitosan (2 mg/mL) to the GCE, a broad peak appears around 3235 cm^{-1} , corresponding to O-H and N-H stretching frequency. The broadness of the peak is due to substantial hydrogen bonding. The peaks around 2915 and 2872 cm^{-1} are due to C-H stretching frequency. Carbonyl stretching frequencies around 1631 and 1549 cm^{-1} are characteristics of amide bonds of chitosan (N-acetyl groups). The most substantial peak at 1061–1023 cm^{-1} is attributed to C-O stretching frequencies of polysaccharides (Fig. 5b). On adding glucose oxidase to the chitosan-modified GCE, the peak intensities are reduced substantially, suggesting surface modification. A broad peak around O-H and N-H stretching frequency regions ($\sim 3250 \text{ cm}^{-1}$) was observed. The amide peaks around 1680–1643 cm^{-1} and 1543–1502 cm^{-1} were also observed. The prominent peak at 1067 due to C-O stretching was observed for glucose oxidase-modified electrodes (Fig. 5c). Comparing the FTIR of surface-modified electrodes, it is evident that the surface was modified initially by chitosan and subsequently with glucose oxidase. No new peaks were observed as chitosan, and glucose oxidase interaction is primarily hydrogen bonding.

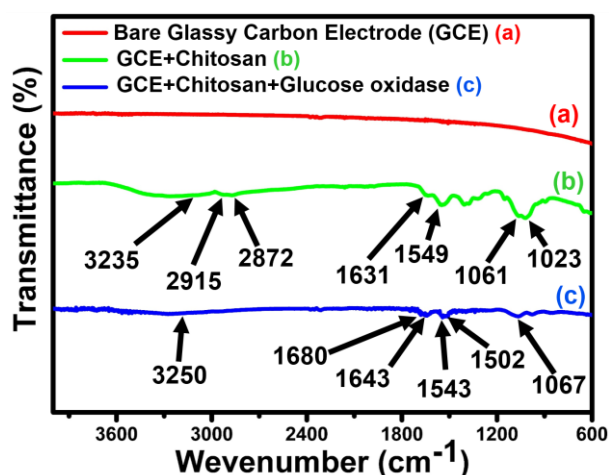
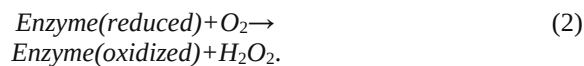
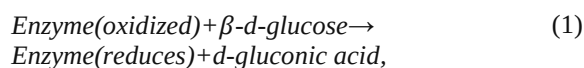


Fig. 5 FTIR spectrum of (a) glassy carbon electrode (GCE), (b) GCE/chitosan, and (c) GCE/chitosan/glucose oxidase.

3.3 Electrochemical Characterization Results

Electrochemical characterization of GCE has been carried out using 0.25 M Phosphate buffer saline (PBS) of pH 7.4 and room temperature 25 °C. A conventional three-electrode system (CHI Electrochemical work analyzer (CH 760E)) has been employed for all Electrochemical measurements. In this system, we have used the carbon electrode as the working electrode, a platinum wire as the counter electrode and an Ag/AgCl as a reference electrode. The Eqs. below show that glucose oxidase reacts with glucose when oxygen is used as an electron acceptor.



In the presence of an applied potential of +0.5 V, the following reaction occurs when H_2O_2 is brought closer to the surface of the anode.

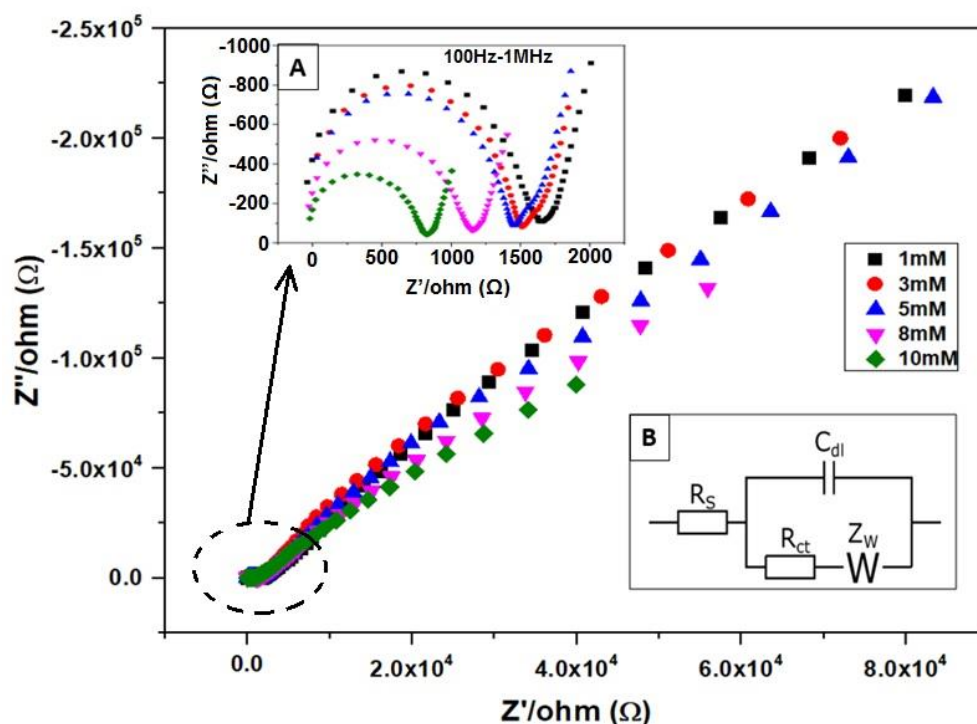
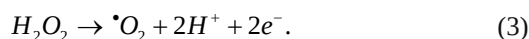


Fig. 6 EIS response of biomodified GCE for 1 mM–10 mM glucose concentrations, (A) zoom view of Nyquist plot for higher frequency ranges 100 Hz–1 MHz and (B) Randle's equivalent circuit model.



When the H_2O_2 redox reaction occurs near the carbon electrode sensor surface, the resulting current increases as glucose concentration increases.

3.3.1 Electrochemical Impedance Spectroscopy (EIS) Measurements

During EIS (Electrochemical Impedance Spectroscopy) measurements, a 3-electrode configuration and a PC interfaced a conventional three-electrode system (CHI Electrochemical work analyzer (CH 760E)) was used with an applied sinusoidal signal of 5 mV amplitude in the frequency range of 100 Hz to 1 MHz. The Nyquist impedance plots have been recorded after the GCE has been exposed to 0.25 M Phosphate buffer saline (PBS) of pH 7.4 and room temperature 25 °C. The Nyquist plots of the EIS data in Fig. 6A showed that with the increase in glucose concentration, the radius of the semi-circle was reduced, indicating the decrease in R_{ct} in Randle's circuit model Fig 6B.

The current through the electrode consists of faradaic current and double-layer capacitor charging current. Therefore, the electrical equivalent model can be defined as solution resistance (R_s) in series with the parallel combination of double-layer capacitance and in series with charge transfer resistance (R_{ct}) and warbag resistance (Z_w), responsible for mass transfer [37]. The warbag resistance (Z_w) is associated with diffusion, migration and convection of charges towards the

electrode [38]. The double layer capacitance (C_{dl}) is easily formed on GCE due to the porous surface [39]. The value of C_{dl} depends on the ionic concentration, pH, temperature, type of ions, oxide layers, and roughness of the electrode [40]. The impedance magnitude is decreased with the increase in glucose concentration, as the reactant will be more for higher concentrations. The concentrations measured in this study ranged from 1 mM to 10 mM, indicating that the whole process was controlled by the enzyme's catalytic kinetics. Increasing glucose concentration resulted in more hydrogen peroxide (H_2O_2) being released from enzyme base reactions and under air saturation conditions; as hydrogen peroxide (H_2O_2) concentration increases at the GC electrode surface, the oxidation current increases.

3.3.2 Cyclic Voltammetry (CV) Measurements

Cyclic voltammetry (CV) is a versatile electrochemical technique to study the redox kinetics on electrode surfaces with bio analytes. Cyclic voltammetry (CV) measurements of the GCE sensor have been performed in 0.25 M Phosphate buffer saline (PBS) of pH 7.4 at room temperature 25 °C. For Cyclic voltammetry (CV) measurements, a three-electrode system has been used in a similar manner. Using this system, we used the GCE sensor as a working electrode, the platinum wire as a counter electrode, and the Ag/AgCl as a reference electrode. Cyclic voltammetry (CV) was carried out with an electrode potential range of -0.1 V to 0.6 V vs Ag/AgCl electrode at a scan rate of 100 mV/sec.

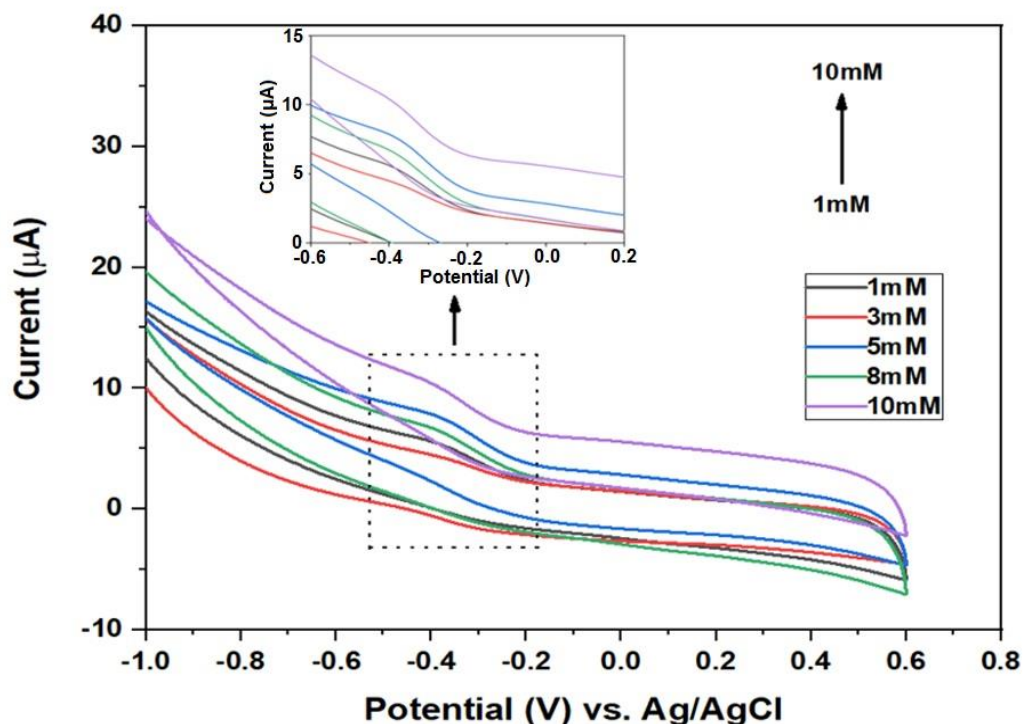


Fig. 7 Cyclic voltammogram of biomodified GCE for glucose concentrations 1 mM, 3 mM, 5 mM, 8 mM, and 10 mM.

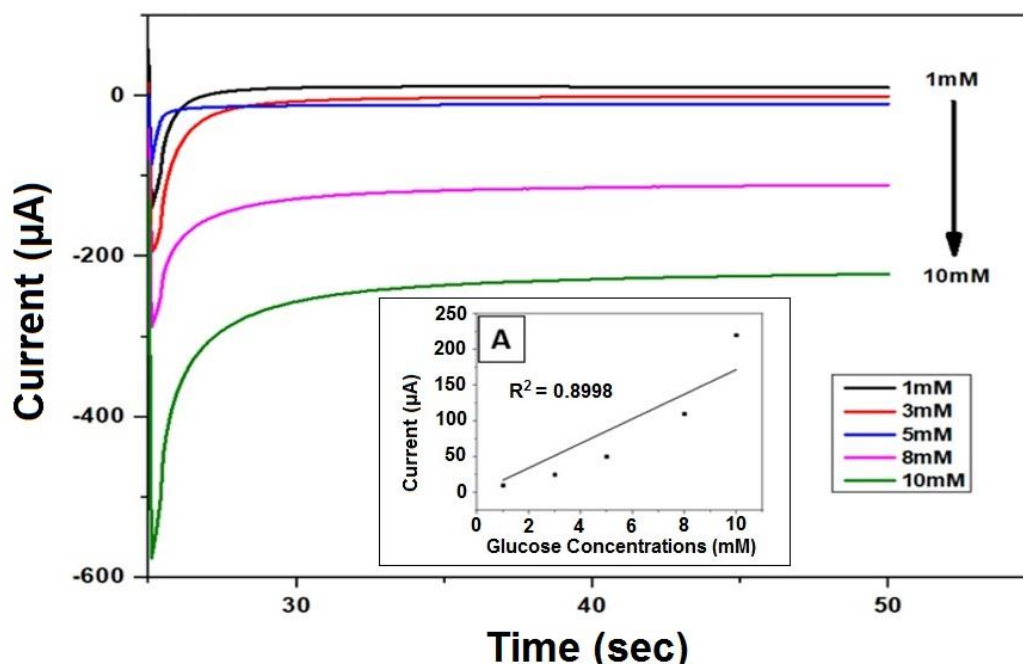


Fig. 8 Chronoamperometry response of bio-modified GCE. (A) The current change of different concentrations of glucose solution and the associated fitting curve.

The redox reaction on the top surface of the electrode occurs at a specific potential. It is related to the surface area, rate constant, interface with solution, pH of the solution, and temperature [41]. The peak current of CV responses was increased with the increase in glucose concentrations. The peak current is validated by Randles-Sevick Eqs. (4), peak current is directly proportional to the concentrations [42].

$$I_p = (2.69 \times 10^5) n^{\frac{3}{2}} A D^{\frac{1}{2}} C v^{\frac{1}{2}} \quad (4)$$

Here, C is the concentration, and other symbols have standard meanings. From the CV response, it is obtained that maximum oxidation occurs at 0.5 V. The change of peak current of CV response with the change of concentration is shown in Fig. 7.

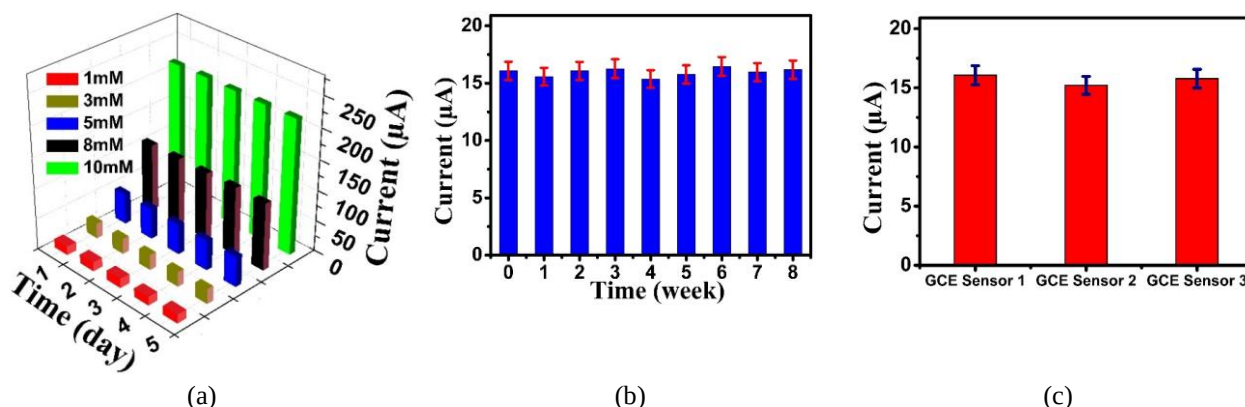


Fig. 9 (a) Variations of chronoamperometry measurements conducted for the glucose concentrations of 1, 3, 5, 8 and 10 mM for successive five days. (b) The measured current variation as a function of storage time for glucose concentration of 1 mM. (c) The reproducibility testing of three GCE sensors.

3.3.3 Chronoamperometry Measurements

The Michaelis-Menten constant is mainly utilized for the biological activity of immobilized glucose oxidase, and the Michaelis-Menten Eq. (5) can be used to calculate it.

$$I = I_{MAX} - K_s \left(\frac{I}{C} \right), \quad (5)$$

where I represents the steady-state catalytic current, I_{MAX} represents the maximum current (under saturated substrate conditions), C denotes the glucose concentration, and K_s represents the Michaelis-Menten constant of the system. As shown in Fig. 8, the glucose biosensor shows a typical current-time curve under optimized experimental settings. Chronoamperometry current was recorded for 1 mM–10 mM glucose solution by applying dc voltage 0.5 V. Fig. 8A shows that amperometric current increases with the increase of glucose concentration. The sensitivity obtained by linearizing the stable amperometric current with $R^2 = 0.8966$ curve is $17 \mu\text{A}/\text{mM}$. It was observed that when 0.5 V was applied, an amperometric response was well-defined, stable, and fast. There was a direct relationship between glucose concentration and the currents produced in the solution when glucose was added. It took about 10 s for the current value to reach a steady state. This study evaluated 1 mM to 10 mM, which demonstrated that the enzyme's catalytic kinetics governed the whole system.

3.3.4 Stability and Reproducibility Test of the Bio-Modified GCE Sensor

Stability is a crucial factor when evaluating the effectiveness of an electrochemical biosensor. The stability of the GCE was assessed using two different methods. First, the GCE was employed to measure glucose levels over five consecutive days, and the resulting chronoamperometric current responses were recorded to determine the stability. The measurements were taken using 1, 3, 5, 8, and 10 mM glucose

concentrations at pH 7.4. Followed by the measures, the GCE was stored in a refrigerator at 4.0°C . The current values in Fig. 9(a) demonstrate that the GCE exhibited excellent stability in accurately measuring various glucose concentrations throughout the five days.

Ensuring long-term stability in enzyme-based biosensors is a challenging task due to the inherent instability of enzymes. Therefore, the GCE was stored in a refrigerator for 56 days to evaluate its stability further. In the stability test, chronoamperometric current measurements were taken at specific time intervals using glucose solutions with a consistent concentration of 1 mM. Fig. 9(b) illustrates the current values obtained from these measurements over the storage period. It was observed that there was a maximum decrease of 4% and an increase of 3% compared to the initial value. These findings indicate that the GCE exhibits exceptional long-term stability, which can be attributed to the adequate protection of the GOx enzyme through the single-step immobilization process.

The reproducibility of the analytic signal is a crucial requirement for biosensors. Three separate GCE sensors were prepared and utilized to detect glucose in 1 mM glucose solutions to assess its stability. The chronoamperometric current was recorded for each sensor with 1 mM glucose solution. The reproducibility of the different glucose sensors was determined and presented in Fig. 9(c). By calculating the relative standard deviation of glucose concentrations through consecutive measurements, it was found to be 5%. This result indicates that the bio-modified GCE sensors yielded glucose analysis outcomes with relatively low variability, demonstrating a high level of reproducibility.

4 Conclusions

The research on the development of blood glucose measurement in a non-invasive way has received enormous attention in the past two decades as this has become an utmost priority to monitor patients' blood glucose levels regularly. This work presents a thorough study of the available glucose measurement techniques

and their limitations, followed by the development of a glassy carbon glucose sensor. GCE has been fabricated by a conventional photolithography process and carbonization of SU-8 negative photoresist by pyrolysis. The GCE was functionalized by chitosan and glucose oxidase to detect glucose. Glucose oxidase converts glucose into gluconic acid and H_2O_2 , and this reaction kinetics has been measured with an increasing glucose concentration of 1 mM to 10 mM glucose solutions. We have obtained a sensitivity of 17 $\mu A/mM$. We have presented and discussed the electrochemical glucose biosensor in this manuscript, which lies in a single-step immobilization protocol of glucose oxidase on the glassy carbon electrode (GCE) surface.

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Disclosures

The authors declare no conflict of interest.

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