CHAPTER 3

CuO Nanowire based Extended-Gate Field-Effect-Transistor (EGFET) for Enzyme-Free /Receptor-Free Glucose Sensing Applications

Contents

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CHAPTER 3

CuO Nanowire Based Extended-Gate Field-Effect-Transistor (FET) For Enzyme-Free /Receptor-Free Glucose Sensing Applications

3.1 Introduction

As discussed in the introduction chapter, the very importance of glucose sensors has inspired researchers to develop low-cost and high sensitive biosensors using new sensing electrodes and devices. The ion-sensitive field-effect transistors (IS-FETs) are widely used for detecting ions and molecules in biochemical environments [125], [126] owing to their internal gain mechanism. In general, IS-FETs can be viewed as the modified form of metal-oxide-semiconductor field-effect transistors (MOSFETs) where an ion-sensitive membrane, electrolyte solution, and reference electrode replace the metal gate of the MOSFET structure [125],[126]. These devices have drawn significant attention of the researchers for sensing applications because of their low-cost, well-established fabrication technology, ease of scaling of various geometrical dimensions, and compatibility with the well-matured CMOS technology for developing future-generation smart-sensing technology. Since then the development of the first Ion sensing FET in 1970 [82], a number of different methods and sensing membranes have been explored in the ISFETs for various biomedical sensing applications, including pH sensing, ion sensing, and glucose sensing in human blood [83], [84]. However, the major drawbacks of the ISFETs include higher noise levels due to their inherent internal gain mechanism and poor stability due to the fragile nature of the membrane deprivation

in extreme conditions. To overcome the shortcomings mentioned above, an extended-gate (EG) field-effect transistor (FET) device is used as an alternative to ISFET, where an externally developed sensing electrode is connected to the gate of a commercially available FET. In fact, EGFET can be thought of as an ISFET consisting of the interconnection of two main parts: the conventional FET device whose gate is connected to a separately fabricated sensing membrane electrode which is submerged into the solution containing the ions/molecules to be sensed /detected by the sensor [127], [128], [129]. Although the sensing mechanism in both the ISFET and EGFET is nearly the same, the EG-FET has some advantages over the conventional ISFET. The separately used transistor part from the sensing membrane can prevent the transistor from chemical damage. Further, the fabrication requirement of only the sensing-electrode part gives us the freedom of developing various sensing electrodes with different sensing materials by using different fabrication methodologies for different sensing applications [130]–[131].

Metal oxides such as NiO, ZnO, CuO, MnO2, SnO2, TiO2, Ag2O, and MWCNT-In2O3 have been widely used for glucose sensing, application [41], [64], [72], [87], [88], [89], [95], [96], [123],. Among them, copper oxide (CuO) is considered to be the best choice because of its low fabrication cost, abundance in nature, and electro-catalytic properties [41], [72], [123]. It is intrinsically a p-type semiconductor with a narrow bandgap of ~1.2 eV. The interesting properties of CuO have been explored widely for various sensing applications such as gas sensing [96], lithium-ion batteries [95], glucose sensing [64], and photoelectric devices [132]. CuO nanostructures on copper foils have been widely explored for glucose sensors with high sensitivity, fast

response, and stable detection due to their better catalytic property over other metal oxide nanostructures [39]. Ni et al.[72] and Mishra et al.[123] have reported CuO nanowires (NWs) based enzyme-free and template-free glucose-sensing techniques by growing CuO NWs directly on a copper foil using solution processing method. Singh et al. [89], Wang et al. [88], and Qi et al. [87] have used different sensing-electrodes in EG-FET for sensing both pH and glucose. While Singh et al.[89] have used a RuOx based electrode, Wang et al.[88] and Qi et al.[87] have used ZnO nanostructure-based electrodes in the EG-FET for pH and glucose sensing applications. Some researchers have also used CuO NWs electrodes in non-transistor-based sensor structures for various sensing applications such as gas sensing, lithium-ion battery, gas sensing, and pH sensing application[95], [96], [64]. Most of the popular glucose sensors use an enzyme (i.e., glucose oxidase) that binds glucose selectively in the sensing membrane of the electrode. In general, the enzymes are not stable for continuous long-term operations [133], enzyme-based glucose sensors are not suitable for continuous glucose monitoring systems. Therefore, efforts have been made to develop enzyme-free glucose sensors. In this direction, some researchers have used artificial receptors (e.g., lectins and saccharides) for glucose sensing [23], [134] - [136]. Recently, a synthetic receptor has been reported that binds glucose with better affinity and selectivity than that of the previously reported receptor-based glucose sensors [137]. However, poor selective binding of the synthetic receptors to glucose in water results in poor selectivity of such sensors [133]. However, no significant work has been reported on the CuO NWs electrode EGFET based glucose sensor without any enzyme/receptor.

In view of the above discussion, an attempt has been made in this chapter to report

enzyme-free/receptor-free glucose sensing by using a CuO NWs electrode-based EGFET sensor for the first time. The EGFET uses a CuO NWs electrode grown on a conducting Cu foil by a simple and facile route. The reported sensor is shown to be capable of sensing glucose in nearly a neutral medium with pH ~7.4 at room temperature, which is still a challenge for many reported sensors [72], [123]. The proposed CuO NWs electrode-based EGFET sensor is also shown to be capable of sensing glucose in human blood serum. The performance parameters of the proposed sensor have finally been compared with those of some of the commercially available. glucose sensors.

3.2 Experimental Details

3.2.1 Materials Used

Highly pure (99.9%) copper foil has been purchased from Alfa Aesar, Thermo Fisher Scientific (India). Ammonium persulfate [$(NH_4)_2S_2O_8$], sodium hydroxide (NaOH), hydrochloric acid (HCl), acetone, isopropanol, and Malt extract powder have been purchased from Merck Life Science Private Limited (India). Glucose, sucrose, and mannose have been purchased from Sisco Research Laboratories Private Limited (India). All the chemicals used here are of analytical grade and ultra-pure; hence no further purification is done. The ultra-pure deionized (DI) water (resistivity 18 MΩ-cm) is obtained from Merck Millipore system.

3.2.2 Electrode Preparation

3.2.2.1 Preparation of Cu $(OH)_2$ NWs Electrode

Small squares (5 cm × 5cm) of copper foils are used as the starting material for

the electrode preparation. These copper foils are cleaned ultrasonically in DI water, HCl, acetone, and isopropanol, respectively. Then the foils are immersed in a solution consisting of 80 µl of 10 M NaOH, 180 µl of H_2O, and 40 µl of 1M $(NH_4)_2S_2O_8$. After 30 minutes, the foils are taken out from the solution and kept for drying in the airflow. A deep blue film on the Cu foil confirms the presence of $Cu(OH)_2$ NWs onit.

$$Cu + 2NaOH + (NH_4)_2S_2O_8 \rightarrow Cu(OH)_2 + Na_2SO_4 + 2NH_3 + 2H_2O \quad (3.1)$$

3.2.2.2 *Preparation of CuO NWs*

The Cu (OH)2 NWs grown on Cu foils are kept on an alumina boat and then put inside the furnace with argon gas flow for 30 minutes. Then the argon gas flow is stopped, and the foils are heated to 120°C for three hours. Temperature is then raised to 180°C for heating the foils for 2 hours to promote crystallization. The blue film on the Cu foil is then converted into black CuO nanowire [72].

$$: Cu(OH)_2 \rightarrow CuO + H_2O \quad (3.2)$$

3.3 Results and Discussion

3.3.1 Electrode Characterization and Electrical Measurements

The surface morphology of CuO NWs based electrode on copper foil is investigated by Nova Nano SEM 450 from the FEI Company of USA (SEA.) PTE, LTD. The crystal structures of CuO NWs were investigated by X-ray diffraction (XRD) (RIGAKU-Smart XDMAX, PC-20, 18-kW Cu rotating anode, Rigaku, Tokyo). The microstructure of the CuO NWs film had also been studied by using high-resolution transmission Electron

Microscopy (HR-TEM) from Tecnai G2 20 TWIN, FEI Company of USA (SEA.) PTE, LTD. We have used two electrodes with CuO NWs based electrodes as working electrodes and an Ag/AgCl based electrode as the reference electrode for electrical measurements. The CuO NWs based working electrode is connected to the Gate of a commercially available MOSFET (CD-4007UB) to act as an EG-FET. The drain and source of the FET are connected to drain and source points of the Source Measuring Unit (SMU) of the Semiconductor Device Analyzer (Agilent TechnologyB1500A). A reference electrode was connected to the gate terminal of the SMU unit of the parameter analyzer. The details of the measurement setup are shown in figure 3. 1.

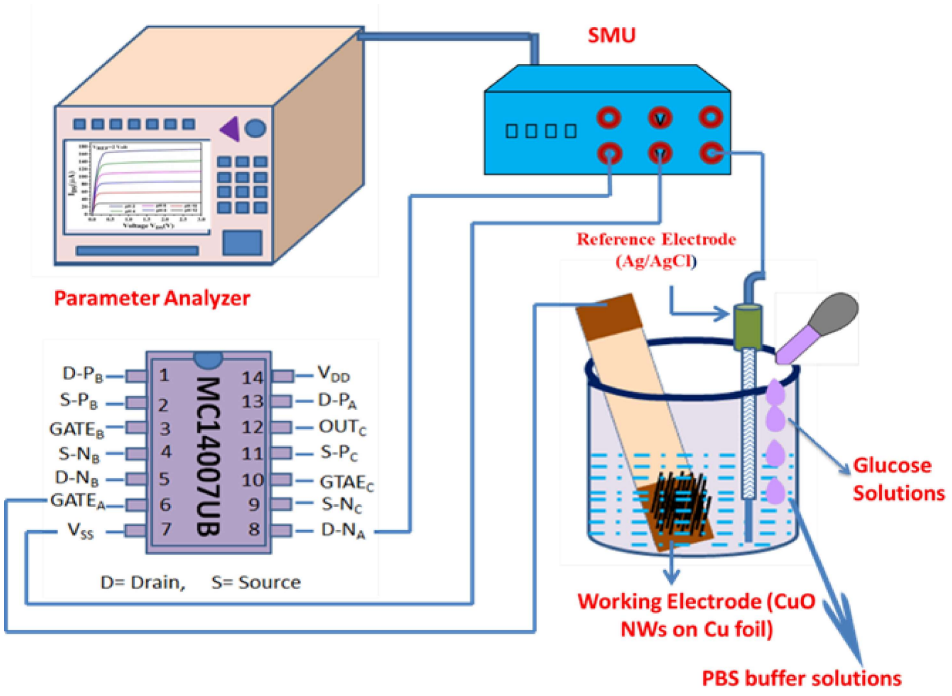


Figure. 3.1 Measurement set-up of the CuO NWs electrode based EG-FET Sensors for Detection of Glucose

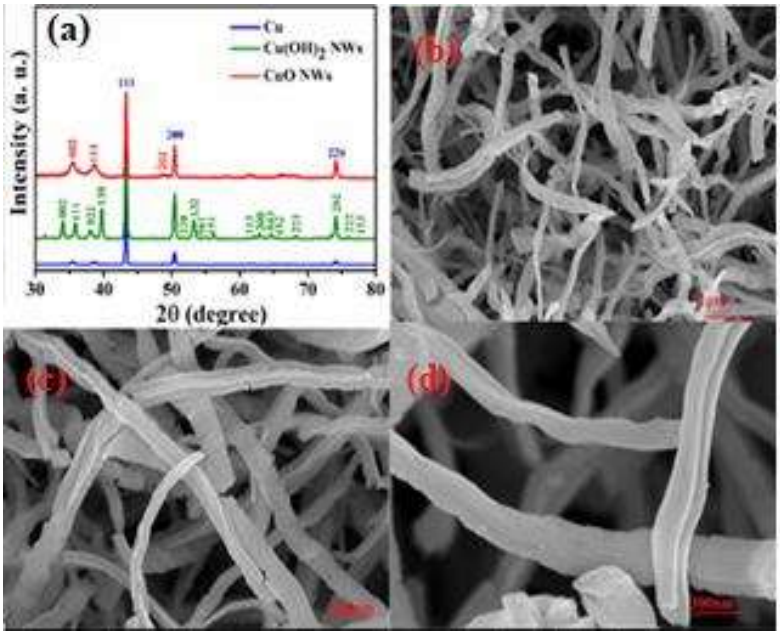


Figure3.2. (a) XRD of CuO NWs, (b) FESEM of CuO NWs at 1µm (c) Magnified version of CuO nano wires at 500nm (d) further magnified view for CuO NWs at 300nm.

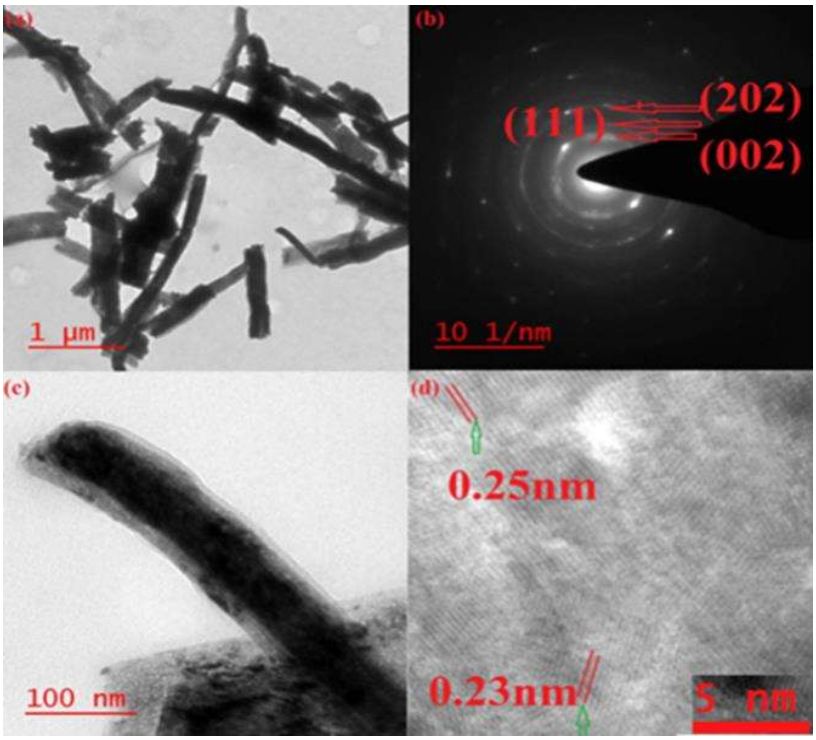


Figure 3.3. (a) The HRTEM images of free standing CuO NWs, (b) SAED patter of selected area,(c) Magnified version of single CuO NWs, (d) further magnified view for CuO NWs.

3.3.2 Characterization of CuO NWs Electrodes

The XRD patterns of Cu foil, Cu(OH)2 NWs, and CuO NWs have been compared in Fig.3. 2(a). Peaks have been determined by using standard JCPDS file numbers 04-0836, 35-0505, and 80-1917 of Cu, Cu(OH)2, and CuO respectively. The surface morphology obtained for CuO NWs grown on Cu foil was examined by FESEM as shown in Fig 3.2(b)-2(d). The FESEM images of different magnifications shown in fig 3.2(b)-2(d) confirm the consistent growth of CuO NWs. The surface morphology of CuO NWs understudy is further investigated by HRTEM analysis shown in Fig. 3.3(a). The average diameter of the CuO NWs is 100-300 nm, and the lengths of the CuO NWs are 1-2 μm. The diameters of the first three rings correspond to CuO (002), CuO (111), and CuO (202) with d-spacing of 2.5 Å, 2.32 Å, and 1.58 Å, as shown in figure 3.3(b). The Group of nanowires in the HRTEM image is shown in fig3. 3(a). A magnified version of the single nanowire is shown in fig3.3 (c), and a further magnified view is shown in fig 3.3(d). In this case, the HRTEM shows a lattice spacing of 2.5Å, which matches with CuO (002), and lattice spacing 2.32 Å matches with CuO(111).

3.3.3 Glucose Sensing of CuO NWs Electrode Based EGFET

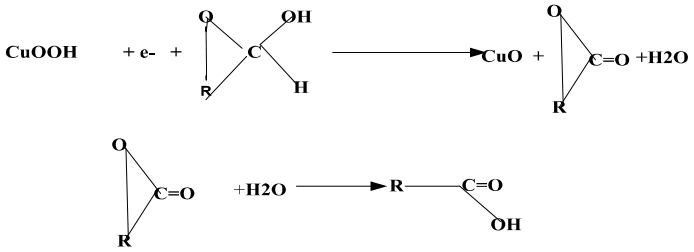
3.3.3.1 Sensing of glucose in chemical solution

We will now investigate glucose sensing in a slightly basic medium (pH=7.4) by using our proposed CuO NWs electrode-based EGFET. When glucose came in contact with CuO NWs, the following reaction took place[39]:

$$CuO + H_2O + 2OH^- \rightarrow Cu(OH)_4^- + e^-$$

Or,

$$CuO + H_2O \rightarrow CuOOH + e^- + H^+$$



$$Glucose \rightarrow Gluconolactone + e^-$$

$$Cu(III) + e^- \rightarrow Cu(II)$$

Cu(III) + Glucose⟶ Glucoconolactone + Cu(II)

$$Gluconolactone \rightarrow Gluconic\ Acid\ (Hydrolysis)$$

It is observed that oxidation of glucose and reduction of metal oxyhydroxide (CuOOH) take place when metal oxyhydroxide reacts with glucose [95],[138]. In this process, glucose is oxidized into gluconolactone, metal oxy-hydroxide is reduced into metal oxide, and free-electron is continuously produced and consumed. Since CuO NWs have a larger surface-to-volume ratio than the bulk CuO, a large number of direct electron transfers take place between the electrode surface and solution. The oxidation of glucose is increased with the increase in the concentration of glucose. This causes de-protonation, which isomerizes further to form enediol on the catalytically active sites of Cu(OOH). This ultimately changes into gluconolactone and CuO. The increase in OH- concentration due to hydrolysis raises the anodic potential of the electrode. The higher anodic potential enhances glucose oxidation by fast dissociation of water [95].

Table.3.1: A Comperation to Our CuONWs EGFET Based Glucose Sensor With Previously Reported EGFET Based Glucose Sensors

Sensing Membrane	**Fabrication mode**	**Sensitivity**	**Range**	**Linearity**
RuOx[89]	Sol-gel	6.89mV/mM	1-8 m	99.3
Al-doped ZnO nano structure[88]	Hydrothermal	60.5μA/mM	1-13.9mM	99.96
ZnO Nano array[87]	Sol-gel	0.39μV/mM	0.02-.1mM	0.967
Passivated ZnO[85]	Photo electrochemical	20.3 μA/mM	--	--
Sno_2/ITO[139]	RF-Sputtering	0.256mV	2.5-20 mM	0.95
Nb_2O_5[86]	LPCVD	5.62mV/M	2-7 mM	.880
ZnO nano rod deposited on the surface of gold interdigited electrode[140]	Hydrothermal	--	0.01-5 mM	--
A gold thin film with receptor[141]	Organic semiconductor	--	--	--
CuONWs(this work)	**Eaching and heating**	**3.03mV/mM**	**1-12 mM**	**0.9968**

Table3.1 compares the results of our proposed sensor with those of reported works. Our sensor has a reasonably good linear range of operation over 1mM-12 mM glucose concentration, excellent linearity of (99.68), and sound sensitivity of 3.03 mV/mM. Previously reported EGFET based glucose sensors shown in Table 1 are either enzyme or receptor-required Glucose sensors. This is the probably first EGFET based glucose sensors in which neither enzyme required, nor are receptors required.

Figure 3.4(a) shows the transfer characteristics of EGFET for different glucose concentrations. It is observed that the reference voltage is increased with glucose concentration for a fixed V_{DS}=100 mV and I_{DS}= 100 μA. In Figure 3.4(b), we have

plotted V_{REF} vs. time (min) graph for different glucose concentrations.

A large linear range from 1 mM to 12 mM confirms that the proposed sensor is not only capable of detecting the glucose level of a healthy person (with glucose range 3.6-6.6 mM) but capable of measuring the glucose level of a moderate diabetic person with the maximum sugar level of 12 mM (217 mg/dl).

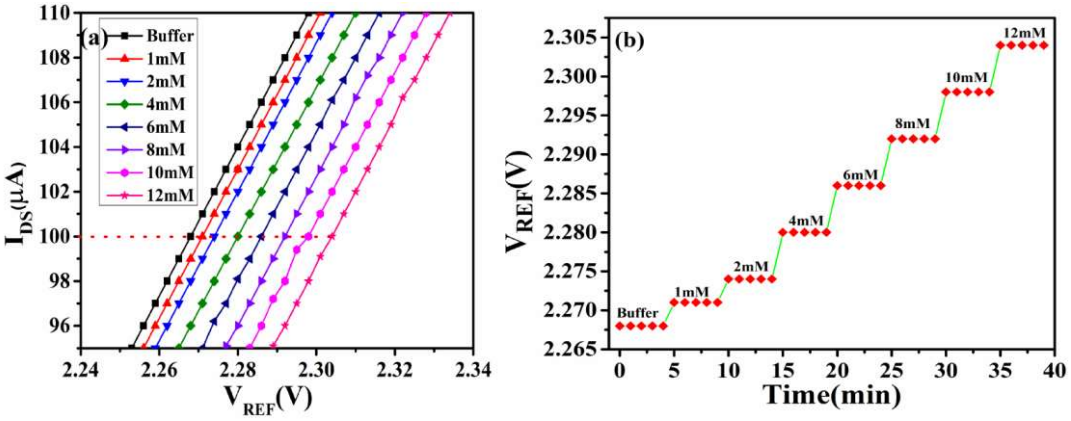


Figure.3.4.(a) Transfer characteristics (I_{DS}-V_{REF}) of CuO NWs based EGFET biosensors with V_{DS}=100 mV for detection of glucose in PBS (100 mM, pH=7.4). (b) Time dependent response (V_{REF}-Time) for step wise changes in the glucose (1–12 mM).

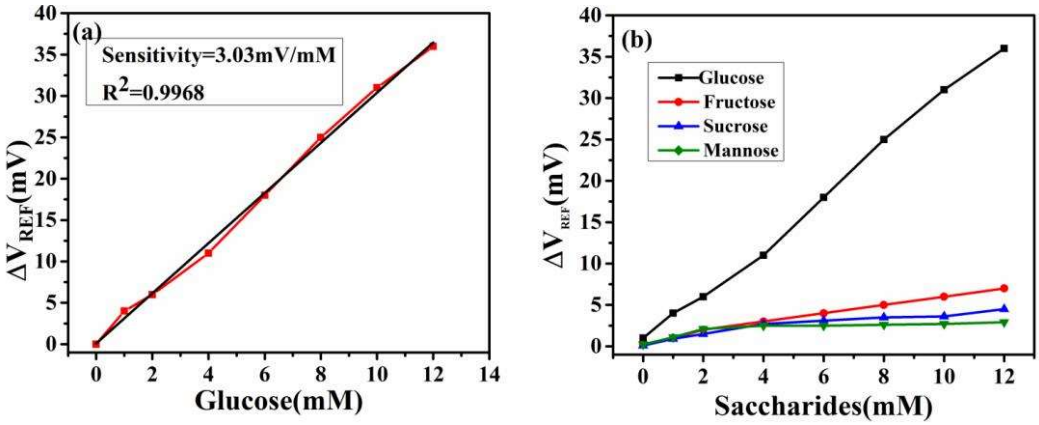


Figure.3.5.(a) V_{REF} shift of the CuO NWs based EGFET biosensor as a function of glucose concentration (1–12mM). Sensitivity have been found 3.03 mV/mM with R^2=0.9968 for EGFET biosensor. (b) Change inV_{REF} of the CuO NWs based EGFET biosensor with respect to various saccharide concentrations in PBS (100 mM, pH=7.4).

Figure 3.5(a) shows a highly linear and stable graph with a 3.03 mV/mM

sensitivity and regression coefficient R^2 = 0.9967. Figure3.5(b) investigates the selectivity of the proposed sensor with respect to various saccharides. It is observed that maximum response is observed for glucose with the maximum change of 36mV in the reference voltage at 12 mM concentration. Change in the reference voltage for other saccharides such as fructose, sucrose, and mannose is negligible with respect to that for the glucose.

3.3.4 Human Serum Samples Measurement

For the practical application purpose, this glucose sensor was used to measure the glucose level of human blood serum. Blood samples of our fellow researchers have been taken from the health center (Sir Sundar Lal Hospital, Institute of Medical Sciences, BHU, Varanasi, India) with tested blood sugar levels. Approximately 100µL of blood is added into 9.90ml of 0.1M PBS, and the voltage-time responses are measured at I_{DS}=100µA. The concentration of sugar in the blood serum sample is estimated from the calibrated curve shown in figure 3.5(a).

Table3.2:Detection of blood glucose sample in the blood

Sample	Spectrometric method mM (provided by health centre)	Praposed method (mM)	Recovery(%)
1	8.35 (150mg/dl)	8.15	97.60
2	5 (92mg/dl)	4.8	96
3	4.438	4.2	96.6

3.4. Conclusion

This work demonstrates the glucose-sensing capability of a CuO NWs based EGFET biosensor. The CuO NWs based electrode is grown on a conducting Cu foil by a facile and straightforward route. The enzyme-free/receptor-free glucose detection using the proposed EGFET shows good glucose sensitivity (~3.03mV/mM) and excellent linearity of 99.67%. The sensor can effectively measure the glucose concentration over a wide concentration range (1 mM-12mM), covering the sugar levels of normal healthy persons and moderate diabetic patients. This is the first time enzyme-free/receptor-free glucose detection based on EGFET has been found. Results are awe-inspiring as compared to that of other commercially available glucose sensors. The glucose sensing of blood serum has also been compared with the pathological reports obtained from the government hospital. A reasonably good matching between our measured data and pathological data confirms the suitability of our CuO NWs electrode-based EGFET sensor for practical applications of glucose sensing in solution as well as in human blood.