CHAPTER 4

CuO Nanoparticle Decorated ZnO Nanorods based Extended-Gate Field-Effect-Transistor (EGFET) Enzyme-Free Glucose Sensing Applications

Contents

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CHAPTER 4

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4.1 Introduction

This chapter evaluated CuO NPs decorated aligned ZnO nanorod arrays as anode for electro-catalytic oxidation of glucose. The sensor device is based on EGFET architecture, as discussed in Chapter3. The rationale for using these materials is to exploit the single-crystalline ZnO array's sturdy, aligned structure as an effective electron transport channel and excellent support for functionalizing with already-established highly electroactive CuO NPs.

There are many metal oxide such as ZnO ,NiO CuO, RuOx,SnO2,TiO2, Ag2O etc based sensors used glucose sensing [41], [79], [87-89], [116], [123], [142], [143] applications. In all the metal-oxide as mentioned above, zinc oxide is used as an ideal choice because of its abundance in nature, low fabrication cost, easy synthesis, and negligible toxicity [79], [87], [88], [116]. Moreover, ZnO nanostructure can provide a large surface for enzyme-free glucose detection [116]. ZnO nanostructure-based devices have enhanced catalytic activity because of the improved surface-to-volume ratio of vertically grown ZnO nanorods and ZnO's fast electron transfer capacity [144]-[146].

Qi et al. [88] and Wang et al. [79] have used ZnO nanostructure-based sensing-electrodes in EGFET for glucose sensing applications. More researchers have utilized

ZnO nanostructures with metal oxide-based nanostructures for glucose sensing applications. More researchers have utilized ZnO nanostructures with metal oxide-based nanostructures for glucose sensing applications. Qi et al. [88] and Jung et al. [87] have used ZnO nanorod decorated with Metal oxide-based nanostructure composite for sensing of glucose with the help of FET. Still, no reported article takes advantage of ZnO decorated with metal oxide-based nanostructure with EGFET. On the other hand, CuO nanostructures have also been well studied for several sensing applications. CuO nanostructured materials have low cost, high abundance, and high sensors sensitivity due to higher surface-to-volume ratio, high stability, fast response. which make the CuO nanostructure-based sensors more attractive as compared to other metal oxide-based sensors[41], [79], [87], [89], [116], [123], [145], [147]-[149]. The above mentioned interesting properties of CuO based sensors have been explored widely for various sensing applications such as pH sensing [89], [147], [148],glucose sensing [41], [79], [87], [88], [116], [123], [147], lithium-ion batteries [145], and gas sensing[149]. Because of the above discussion, an attempt has been made to take advantage of both ZnO nanostructure and CuO nanostructure. Therefore in this article, the vertically grown ZnO nanorod decorated with CuO nanoparticles (NPs) on FTO(Fluorine doped tin oxide) as electrode has been reported for enzyme-free EGFET based glucose sensor for the first time to the best of our knowledge. These reported sensors work at room temperature in a neutral medium, which is still a challenge for many published articles [133], [146]. This sensor is also used to sense the glucose in human blood serum. The proposed sensor has shown better performance in comparison with some commercially available glucose sensors.

4.2 Experimental Details

4.2.1 Materials Used

Fluorine doped tin oxide(FTO) has been used as a substrate. $Zn(NO_3)_2.6H_2O$ and Hexa-mythel-tetramine (HMTA; $C_6H_{12}N_4$), copper sulfate pentahydrate($CuSo_4.5H_2O$) has been purchased from Alfa Aesar, Thermo Fisher Scientific (India). Hydrochloric acid (HCl), sodium hydroxide (NaOH), acetone, isopropanol, and Malt extract powder have been purchased from Merck Life Science Private Limited (India). Glucose, sucrose, mannose have been purchased from Sigma Aldrich). All the chemicals used here are of analytical grade and ultra-pure; hence no further purification was required. All the necessary solutions have been prepared in ultra-pure deionized (DI) water (resistivity 18 MΩ-cm) which is obtained from the Merck Millipore system.

4.2.2 Electrode Prepration

4.2.2.1 Preparation of ZnO seed layer on FTO substrate

ZnO seed layer deposition on the FTO substrate is the first step for the fabrication of the electrode. 1.5cm*1.5 cm FTO has been used for the preparation of the working electrode. This electrode has been finally treated with oxygen plasma after cleaning with water, TCE, acetone, and 2-propanol, respectively. Just after the plasma cleaning, the ZnO solution was spin-coated at the speed of 2000 rpm for 30 sec. This process has been repeated 4 to 5 times for getting 60nm film thickness of ZnO. Finally, this film was annealed at 300ºC for 30 minutes.

4.2.2.2 Synthesis of ZnO nano rods

ZnO nanorod has been grown on the seed layer by putting the electrode in a

hydrothermal environment in 50 ml DI water solution with 0.1M Zn(NO3)2.6H2O and 0.1M hexamethylenetetramine (HMTA; C6H12N4). The resultant solutions were put in the autoclave for four hours at a temperature of 95ºC. ZnO nanorods grow vertically over the ZnO seed layer. After completion of the reaction, the substrate was rinsed and cleaned with DI water[116].

4.2.2.3 Modification of ZnO NR with CuO nano particle

In this step, ZnO nanorod on the FTO substrate had been modified with copper oxide nanoparticles. Metallic Cu nanoparticles were initially deposited on ZnO nanorods by sequence dipping the ZnO substrate in CuSO4 and sodium borohydride, respectively, and this process was repeated multiple times. After this, the electrode was rinsed with DI water and air-dried. Finally, the substrate was annealed at a temperature 500ºC for two hours to convert the Cu nanoparticle into CuO NPs[116].

4.3 Results and Discussions

4.3.1 Electrical Mesurement Setup

The surface morphology of ZnO nanorods(NRs) decorated with CuO nanoparticles(NPs) based electrode on FTO substrate was investigated by HR-SEM (model Nova Nano SEM 450 from the FEI Company of USA (S.E.A.) PTE, LTD). The structural investigation of ZnO nanorods(NRs) decorated by CuO nanoparticles(NPs) electrode is done by X-ray diffraction (XRD) (RIGAKU-Smart XDMAX, PC-20, 18-kW Cu rotating anode, Rigaku, Tokyo). Microstructural Characterization of the ZnO NRs decorated by CuO NPs has also been performed by high-resolution transmission Electron Microscopy (HR-TEM) from Tecnai G2 20 TWIN. FEI Company of USA

(S.E.A.) PTE, LTD. For EGFET based electrical measurement, copper oxide nanoparticle decorated ZnO NRs electrode was used as working electrode by connecting it to the gate of the available commercial MOSFET (CD-4007UB) to act as an EGFET.Ag/AgCl(3M KCL)(reference electrode) was connected to the gate of the source measurement unit(SMU) of the parameter analyzer (Agilent TechnologyB1500A). The Drain and source of the same EGFET were connected to drain and source points of the SMU of the parameter analyzer. The complete measurement setup is shown in Figure 4.1(a).

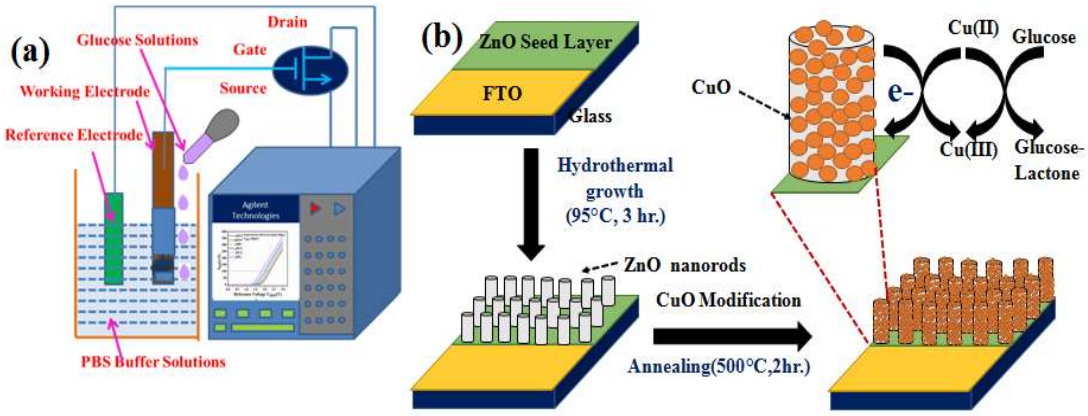


Fig 4.1: (a) Measurement set-up of theZnO NRs decorated with CuO NPs connected to the gate EGFET for glucose sensing applications.(b) Schematic diagram of non enzymatic decetion of glucose through step by step electrode fabrication.

4.3.2 Electrical Characterization

4.3.2.1 XRD of The Electrode

The XRD patterns of ZnO nanorod decorated with CuO NPs have been shown in figure 4.2. All the diffraction peaks confirm the hexagonal structure of the ZnO nanorod (JCPDS 36-1451) and other relevant peaks matched with phase and in accord with (JCPDS 48-1548).

4.3.2.2 FESEM of The Electrode

The surface morphology obtained of ZnO NRs grown on FTO were studied by FESEM, as shown in Fig4.3. Low magnification of the image of the Copper oxide NPs is shown in figure4. 3(a) to give the overall morphology of the electrode. A high-resolution image is shown in figure4.3(b), which clearly shows the decoration of the CuO NPs on ZnO NRs.The measured average length of the NRs varies from 0.75µm to 1.25 µm. The average diameter of the nanorods is approximately 200nm. The diameter of the CuO NPs clusters varies from 20 nm to 45 nm. The EDS spectra, as shown in figure 4.4, confirms the presence of Cu and Zn in the atomic ratio of Cu and Zn is 1:18.

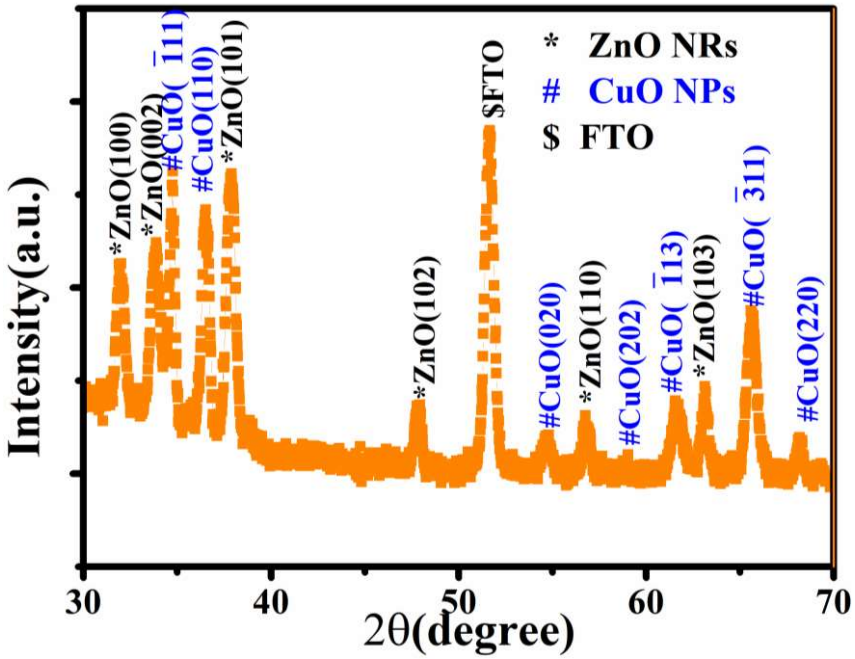


Figure 4.2: XRD patterns of CuO NPs decorated ZnO nanarods on FTO

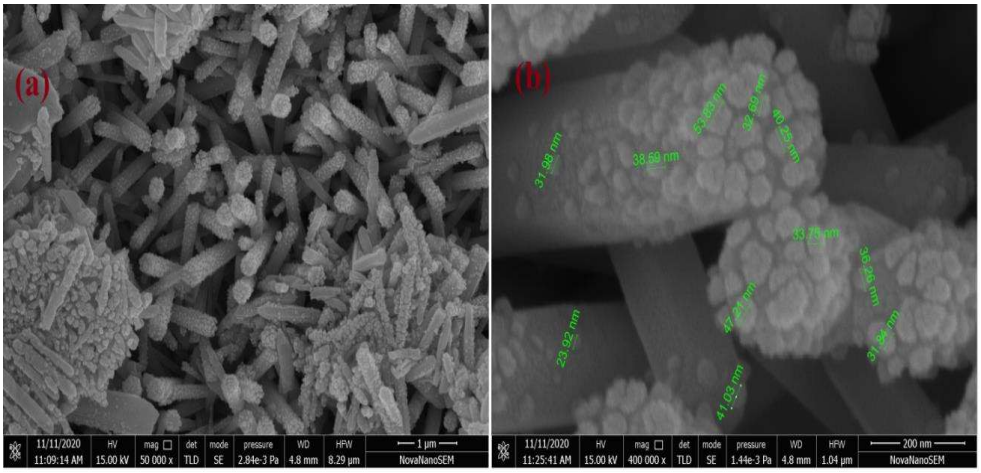
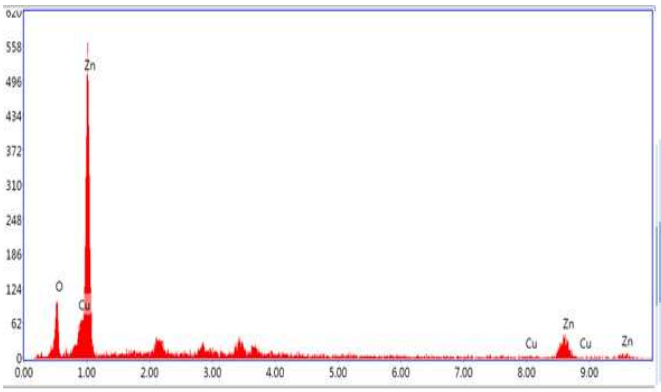


Figure 4.3: (a) Low resolutions FESEM Image (b) high resolutions FESEM Image of ZnO NRs with CuONPs



Element	Weight %	Atomic %	Net Int.	Error %
O K	13.69	39.29	20.59	13.52
CuK	4.44	3.21	1.78	65.12
ZnK	81.87	57.51	20.47	10.94

Figure 4.4 EDS spectra ZnO NRs decorated with CuO NPs

4.3.2.3 TEM Analysis of the Electrode

TEM image of free-standing ZnO nanorod decorated with CuP NPs is shown in figure 4.5(a). A single nanorod decorated with CuO nanoparticles is shown in figure 5(b). The diameter of the CuO NPs varies from 3nm to 8nm, as shown in figure 4.5(b). An even higher magnification image of a single nanorod is shown in figure4.5(c). The SAED pattern of the composite is shown in figure4.5(d); the SAED pattern can be indexed with copper oxide and Zinc oxide plain, proving the presence of CuO and ZnO.

The pattern is a mix of bright diffraction spots and diffused rings which corroborates well with the single crystalline nature of ZnO NRs nanocrystalline CuO nanoparticles.

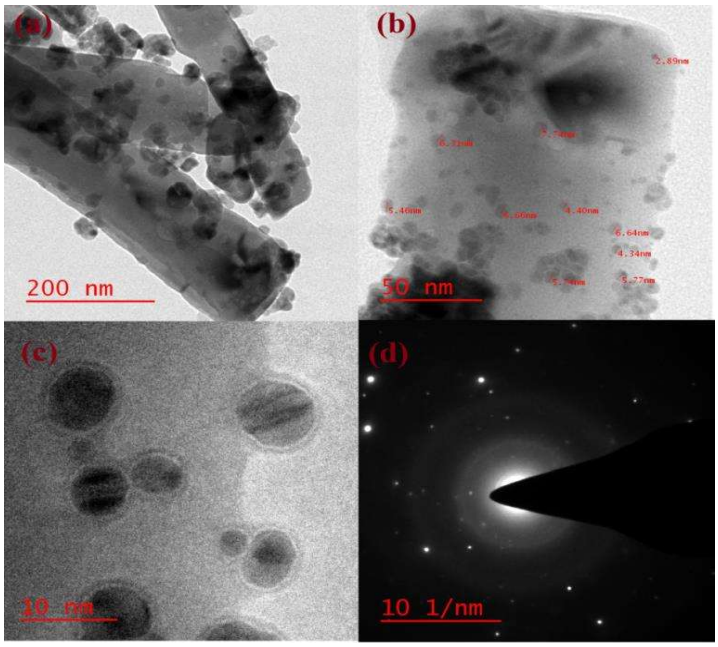


Figure 4.5. (a) TEM images of free standing CuONps on ZnO nano rods, (b) Magnified version of single ZnO NR with CuO NPs, (c) further magnified view for ZnO with CuO NPs (d) SAED patter of selected area,

4.3.2.4 XPS Analysis of the Electrode

The surface chemical composition of the composite was studied using X-ray photoelectron spectroscopy (XPS) and shown in figure6. Figure 4.6(a) shows the Survey spectra of the hybrid material. The presences of Cu 2, Zn2p, C, and O indicate the existence of CuO and ZnO. The high resolution of O1s spectra, as shown in figure 4.6(b), clearly features a splitting with the binding energy of

531.17 eVand 532.72 eV. The peaks around ~531 eV correspond to metal-oxygen bond, and 532 eV correspond to Ov, which corroborates well with the observed high conductivity of different oxygen ZnO[149]-[152]. High resolutions Zn 2p spectra are shown in figure 4.7a. Two peaks at 1021.6 eV and 1044.2 eV corresponds to Zn2p/3/2 and Zn 2p1/2 of Zn 2+. This proves the presences of ZnO peaks are slightly shifted

as compared to the reported values[116], suggesting different environments of the ZnO is CuO NPs –ZnO NRs composite.

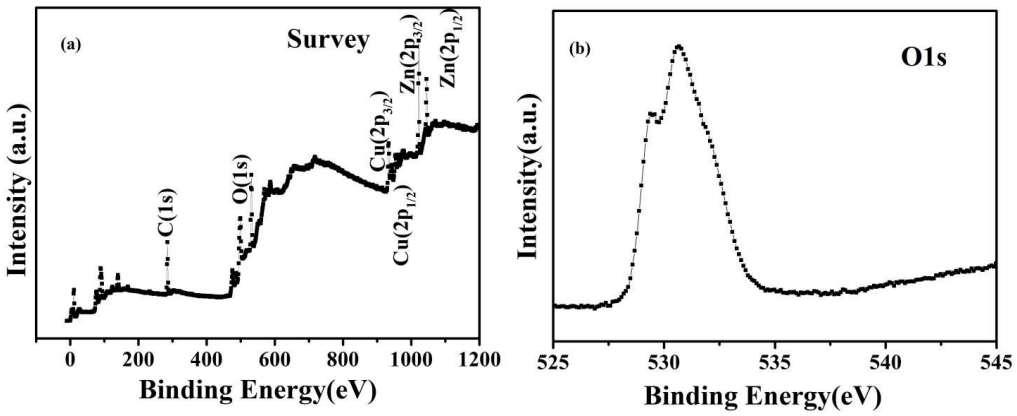


Figure 4.6.(a) XPS analysis XPS spectrum of CuO decorated ZnONR with full scan survey, and (b) corresoponding peaks in the high resolation spectra for O1s

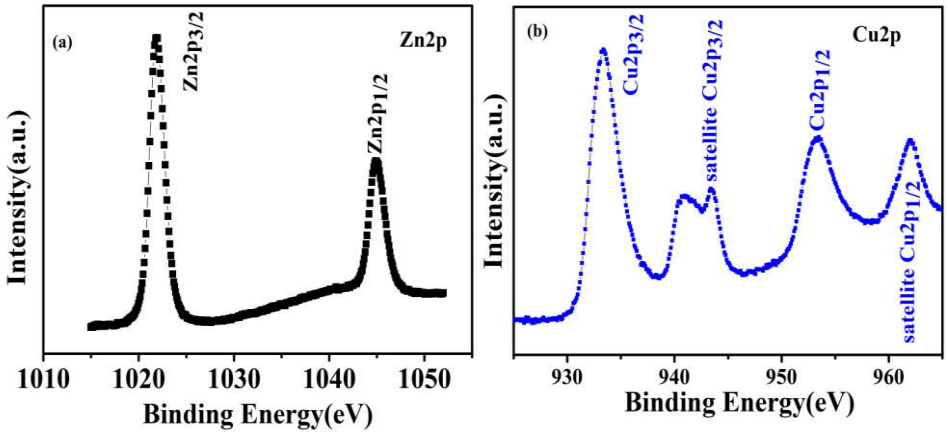


Figure 4.7: High resolution XPS spectra of CuO/ZnO sample (a) Zn 2p, (b) Cu2p

Figure 4.7(b) shows the high-resolution Cu-2p spectra. Peaks at 934.6 eV and 954.3eV correspond to Cu 2P3/2 and Cu 2P 1/2 respectively of CuO. Two additional satellite peaks of Cu 2P3/2 and Cu2P1/2 were observed at 943.2 eV, and 962.8 eV may correspond to partially filled d block Cu2+(3d9)[153] which suggest the formation of CuO on ZnO nanorod surface.

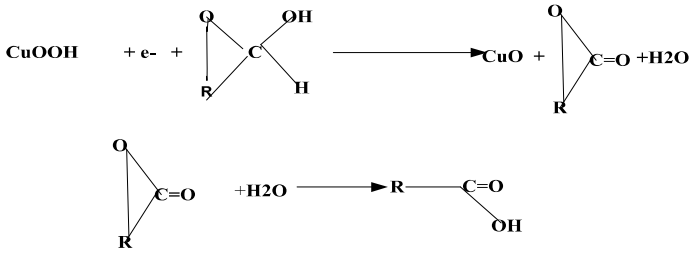
4.3.3 Sensing of the Glucose in Solution through EGFET

Glucose sensing through CuO NPs/ZnO NRs/FTO-based working electrode has been used in a slightly primary medium (pH=7.4). Growth of ZnO NRs on the ZnO seed layer, which is deposited on FTO substrate, gives a high surface area for CuO NPs decorations. This results in fast electron transfer during the glucose oxidation between the solution and the working electrode. When CuO NPs came in contact with electrolyte solutions, the following reaction took place in between the solutions and the CuO NPs [39].

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

Or,

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



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

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

$$Cu(III) + Glucose \rightarrow Gluconolactone + Cu(II)$$

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

Metal oxyhydroxide (CuOOH) reduces whereas oxidation of glucose takes place when metal oxyhydroxide (CuOOH) comes in contact with glucose to a slightly basic medium(with pH 7.4) [108], [110]. Glucose oxidized into gluconolactone and CuOOH reduces into CuO; the free electron is continuously consumed and produced by this process. Since ZnO NRs provide a high surface-to-volume ratio to CuO NPs, many direct electron transfers between the electrode (CuO NPs on ZnO nanorods) and PBS buffer solutions(pH=7.4). More oxidation of glucose took place as the amount of glucose increases in the solutions.

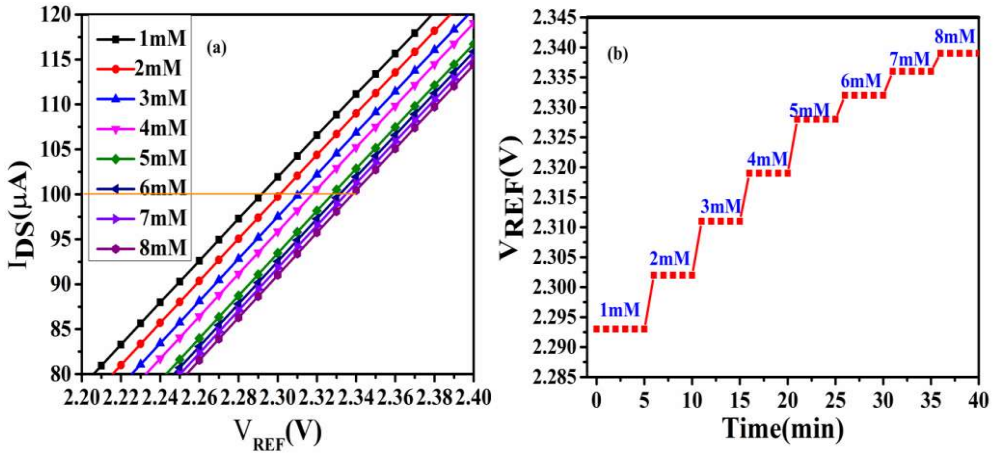


Figure. 4.8.(a) Transfer characteristics (I_{DS}-V_{REF}) of ZnO NRs withCuO NPs based EGFET biosensors with V_{DS}=0.1 V for glucose sensing in PBS (0.1 M, pH=7.4). (b) Time dependent response (V_{REF}-Time) for step wise changes in the glucose (1–8 mM).

This causes de-protonation, which isomerizes further to form enediol on the catalytically active sites of Cu(OOH). The products ultimately change into gluconolactone and CuO. In the process of hydrolysis, OH-concentrations increase, which further raises the anodic potentials of the electrode. This increased anodic potential caused more glucose oxidation by fast separation of water[110]. Voltage and current transfer characteristics of EGFET based glucose sensors with different glucose concentrations are shown in figure4.8(a). From figure 4.8(a), it has been observed that VREF voltages increase as the glucose concentration rises for a fixed voltageVds=0.1V and a fixed current of I_{DS}=100μA The graph between VREF and time are shown in figure 4.8(b), also confirms that as the glucose concentrations increase from 1mM to 8mM the reference voltage also increases in same proportion.

Table.4.1: A Comparison to Our CuO NPs Decorated On ZnO NRs EGFET Glucose Sensor With Previously Reported EGFET-Based Glucose Sensors

Sensing Membrane	Fabrication	Sensitivity	Range(mM)	Linearity
RuOx[89]	Sol-gel	6.89mV/mM	1-8	0.993
Al-doped ZnO nano structure [88]	Hydrothermal	60.5 μA/mM	1-13.9	0.9996
ZnO Nano array [87]	Sol-gel	0.39μ/mM	0.02-.1	0.967
CuO NWs [147]	Eaching and heating	3.03mV/mM	1-12	0.9968
Passivated ZnO [85]	Photo	20.3	--	--

	electrochemical	µA/mM		
SnO2/ITO [139]	RF-Sputtering	0.256mV	2.5-20	0.95
ZnO nano rod deposited on the surface of gold interdigited electrode [140]	Hydrothermal	--	0.01-5	--
Nb2O5 [86]	LPCVD	5.62 mV/M	2-7	.880
ZnO NRs decoated with CuONPs (this work)	Hydrothemal and heating	6.643 mV/mM	1-8	0.981

Table 4.1 compares the results of our proposed sensor with other reported sensors. Proposed sensor has high sensitivity (6.643mV/mM) with an excellent linear range (1mm-8mM) of glucose concentration and excellent linearity (R^2=0.981). Moreover, these proposed sensors have also been worked without the use of any enzyme and receptors. The graph between V_{REF} and glucose concentration is shown in figure4.9(a). A large linear range from 1mM to 8 mM confirms that this sensor can detect glucose levels of a healthy person (3.6mM-6.6mM) and measure glucose level up to 8mM, which is capable of detecting the preliminary stage of debiatic patient. The sensitivity of these sensors is 6.643mV/mM which is very much high with regression cofficent R^2=0.981. Selectivity of these sensors had been investigated in 4.9(b) with other saccharides like fructose, sucrose, mannose. The total change in voltage with respect to glucose concentration is 47 mV at the concentration range of(1 to 8mM). Change in reference voltage is negligible in other saccharides such as fructose, sucrose, and

mannose as comparison of glucose.

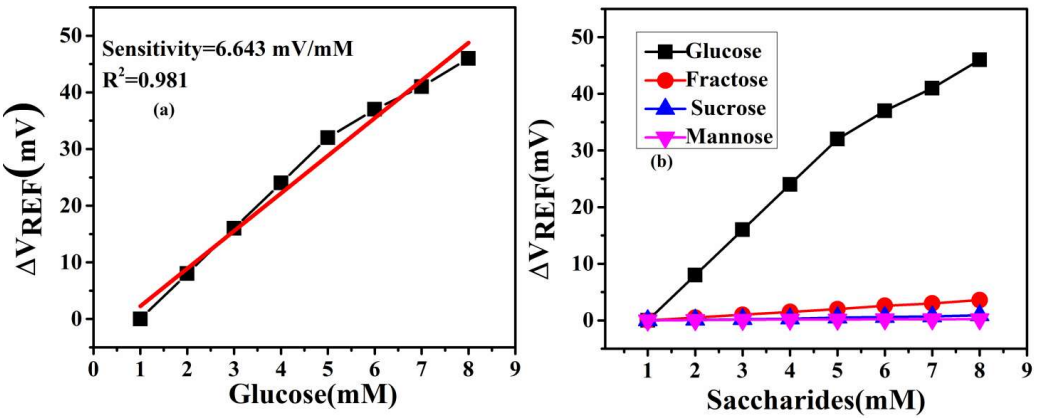


Figure 4.9.(a) V_{REF} shift of the ZnO NRs decorated with CuO NPs based EGFET glucose sensor as a function of glucose concentration (1–8mM),(b) Change in V_{REF} of the ZnO NRs/CuO NPs based EGFET biosensor with respect to various saccharide concentrations in PBS (100 mM, pH=7.4).

4.4 Sensors Performance-Repeatability, Reproducibility and Stability:

For repeatability test, one electrode (ZnO NRs decorated with CuO NPs) has been used in 1mM glucose with 0.1M PBS solutions. One electrode has been used ten times, and the resultants reading shows that standard deviation in all ten reading are less than 1%.and repeatability graph shown in figure 4.10(a).To find the reproducibility of the electrodes, six different electrodes of the same materials have been used, and their corresponding voltage at 1mM glucose concentration in 0.1M PBS solution has been shown in figure 4.10(b). The standard deviation in all six readings is less than 4%, indicating that this proposed device has excellent reproducibility. For the long-term stability, data of this sensor has been taken at the interval of 2 days total of 8 data has been taken in 14 days. Vds=0.1 Volt and 1mM of glucose concentrations the values of Current at 14th days is approximately 95% of the first days as shown in figure 4.11.

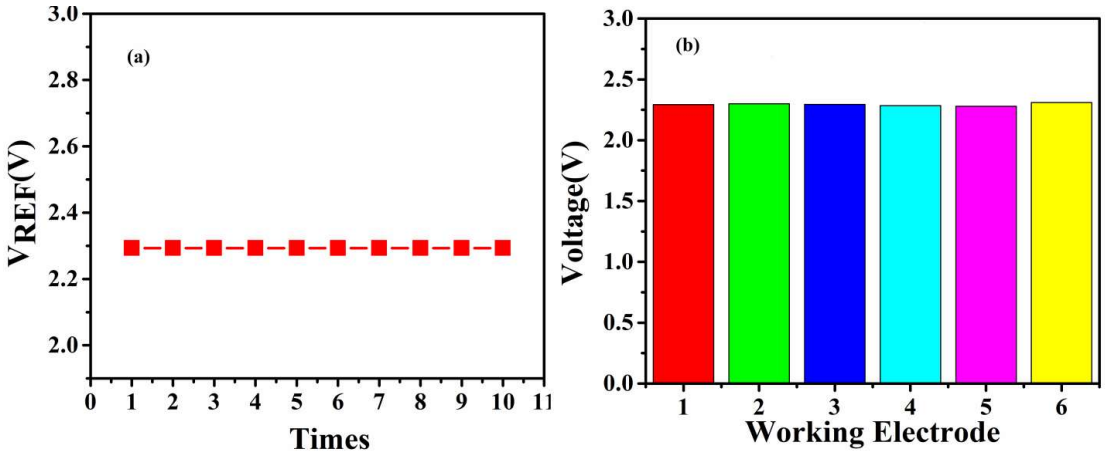


Figure 4.10.(a) Repeatibility graph of CuO NPs decorated on ZnO nano rods based working electorde for sensing 1mM glucose for ten times;(b) reproducibility graph of 6 working electrode(CuO NPs decorated on ZnO nano rods for detection of 1mM glucose

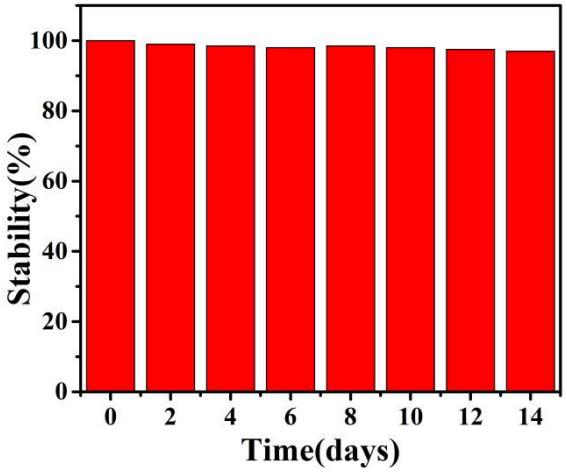


Figure 4.11: The stability measurement of ZnO nano rods decorated with CuO NPs based electorde for 14 day.

4.5 Human Serum Samples Measurement

For the practical application of this glucose sensor, this sensor has been used to detect the glucose level in human blood serum. Blood samples have been collected from our research group to test the blood glucose level in the blood. The health center has done this collection (Sir Sunder Lal Hospital, Institute of Medical Sciences, BHU, and Varanasi,

India). The report of this tested blood sugar level has been compared with the proposed technique of glucose sensing. Blood serum was separated out from the blood by removing the clotting part of the blood.100µl of this blood serum is added in 9.90ml of 0.1M PBS solution the corresponding voltage-time response is measured at 100µA.

The glucose concentration in human serum is calculated using the calibration curve of the obtain from ΔVREF to glucose concentrations graph at 100µA as shown in figure 4.9(a). These results have been compared with standard results provided by the Health Centre. Each sample is measured three times, and the average outcome has been shown in table 4.2.

Table 4.2 :Detection of blood glucose sample in the blood

Sample	Spectrometric method mM (provided by health centre)	Praposed Method (mM)	Recovery (%)
1	7.92 (142mg/dl)	7.83	98.9
2	5.27 (97mg/dl)	5.15	97.7
3	4.99 (90 mg/dl)	4.85	97.2

The results of the proposed sensors are closely matched with the expected results provided by the Health Centre. The closed matching between this reported data and the results provided by the health Centre shows that proposed sensors can be easily employed with sensing of glucose in human blood and in solutions.

4.6 Conclusion

In this article, glucose sensing performance has been done with the help of EGFET. ZnO NRs decorated with CuO NPs on FTO substrate have been used as a sensing electrode. In previously published articles, ZnO NRs modified with CuO NPs based electrodes have not been used as a glucose-sensing device with EGFET. The sensitivity of these proposed glucose sensing devices is 6.643mV/mM with a linearity range (1mM-8mM). This sensor's linearity range (1mM-8mM) covers the sugar level (3.6mM-6.6mM) of a healthy human. The performance of this sensor has been done without the use of enzymes and receptors. This result has also been compared with other reported glucose sensors. Results of this sensor are impressive as a comparison of other reported sensors. The proposed device is also used for detecting the blood sugar levels from the human blood serum and human blood. Results obtained from this sensor have also been compared with pathological reports collected from the Health Centre. Proposed results are very close to the results taken from the pathological data, which confirms the suitability of our proposed sensors. Moreover, these sensors show good stability, reproducibility, and repeatability, which affirms that this electrode is quite reliable for glucose sensing applications.