

CHAPTER 3

DEVELOPMENT OF A CARBON NANOTUBE BASED BIOELECTRONIC DEVICE FOR CHOLESTEROL SENSING

Chapter 3

Development of a carbon nanotube based bioelectronic device for cholesterol sensing

3.1. An Overview

Accurate measurement and detection of cholesterol in blood serum is very essential. Many analytical methods have been developed for detection of cholesterol; those are time consuming, complex and expensive [15]. Such problems can be overcome using enzyme field effect transistors (ENFETs) [16] due to low cost, mass production, fast response, and one chip integration to use in vivo system. But traditional ENFETs have many difficulties such as high threshold voltage, large internal contact resistance, small ON–OFF current ratio, scaling limitation of silicon material and complicated fabrication process for development of such ENFETs [79].

To overcome these drawbacks of traditional ENFETs, junctionless carbon nanotube field effect transistor (JLCNTFET), a new concept has been presented here [80]. A JLCNTFET does not contain of pn, n^+n and p^+p junctions in the source–channel–drain paths. It is a uniform resistor having either n -type or p -type material through which mobile carriers can be modulated by gate voltage. In the OFF–state (at zero gate bias), channel is depleted due to the difference of workfunction between semiconductor and gate electrode. In the ON–state (positive gate bias for n -type channel), there is large drain current due to heavily doped charge carriers. The gate modulates the resistance of the heavily doped semiconductor; hence the device behaves like a gated single resistor [81]. JLCNTFETs offer several advantages such as simple fabrication process, low electric field in the ON state and sharp sub–threshold slope [82].

In this chapter, based on these advantages, junctionless CNTFET has been fabricated for detection of cholesterol.

3.2. Experimental

3.2.1. Materials and Chemicals/Reagents

Cholesterol oxidase (ChOx) with ~24 U/mg activity and cholesterol powder has been purchased from Sigma Aldrich. Multiwall carbon nanotube (MWCNT) with carbon purity of ~ 98 %, diameter ~100–150 nm and length ~30 μm has been purchased from Sigma. Indium tin oxide (ITO) coated glass with sheet resistance of 15 Ω/sq has been obtained from Balzers, UK. Other chemicals like zinc acetate ($\text{Zn}(\text{CH}_3\text{COO})_2$), Zinc oxide (ZnO), polyaniline (PANI), solid zirconium tetrachloride (ZrCl_4), solid potassium hydroxide (KOH) are of analytical grade [39].

3.2.2. Preparation of solutions

Cholesterol stock solution (22.5 mM) has been prepared using Triton X-100 as surfactant. Phosphate buffer saline (PBS) of 50 mM, & pH 7 has been prepared using disodium phosphate (Na_2HPO_4) and monosodium phosphate (NaH_2PO_4). PBS has been used as mediator for measurement of cholesterol concentration. ChOx (1mg/ml) solution has been prepared using same PBS for immobilization purpose on sensing membrane of FET. A solution comprising of water (H_2O), hydrogen peroxide (H_2O_2) and ammonium hydroxide (NH_4OH) in the ratio of 5: 2: 2 have been used to clean ITO and other apparatus [39]. De-ionized water has been used for preparation of solutions. Prior to use, all glassware have been autoclaved.

3.2.3. Fabrication of cholesterol JLCNTFET

ITO coated glass has been used as a substrate. Prior to being used, ITO glass plate has been cleaned by using de-ionized water and special chemicals. On the top of the ITO glass with dimension ~5 mm \times 2 mm, a layer of zirconium dioxide (ZrO_2) has been deposited using electrochemical deposition (ECD) technique. The dimension of this layer is ~5 mm \times 2 mm \times 10 nm. This layer has been used to prevent leakage current from channel to ITO layer. For ZrO_2 layer, 5.0 mg solid zirconium tetrachloride (ZrCl_4) has been hydrolyzed in 5 ml water

and sonicated for several minutes [83]. The solution has then been kept in a glass beaker for deposition of ZrO_2 layer. For deposition, three electrode system is used: platinum (Pt) wire as counter electrode, Ag/AgCl as reference electrode and ITO as working electrode. The whole system has been heated at a temperature of 190 °C for thin dry ZrO_2 layer. On this ZrO_2 layer, potassium doped multiwall carbon nanotube has been deposited using ECD technique. For CNT solution, 5 mg CNT has been dispersed in 5 ml acetonitrile and sonicated for several minutes. This layer acts as source (S), drain (D) and as well as channel region of the FET. The dimension of source and drain are $\sim 5 \text{ mm} \times 2 \text{ mm} \times 100 \text{ nm}$ for each region and for channel it is $1 \text{ mm} \times 2 \text{ mm} \times 100 \text{ nm}$. Catalytic chemical vapor deposition (CVD) technique has been used for preparation of CNTs [29]. CNTs were functionalized using boiling acid treatment technique before use [84]. Since potassium oxidizes rapidly in air and very reactive with water, therefore, to dope potassium with CNT, a potassium solution has been first prepared as: 5 mg solid potassium hydroxide (KOH) has been dissolved in 5 ml water. KOH solution forms K^+ ions, OH^- ions and releases heat. At cathode, K^+ ions attach to carbon atoms. This makes CNTs as *n*-type material and increases electrical conductivity [85]. On the channel region, another layer of ZrO_2 with thickness 10 nm has been deposited as gate insulator of the FET using ECD technique. The thickness has been calculated using gravimetric analysis method as explained in chapter 2. The dimension of this layer is $\sim 1 \text{ mm} \times 2 \text{ mm} \times 50 \text{ nm}$. ZrO_2 ($\kappa \sim 25$) increases gate oxide capacitance and thus, enhances drain current by reducing direct tunneling leakage current [53]. Polyaniline (PANI) doped zinc oxide (ZnO) nanocomposite has been deposited on the top of the gate insulator using ECD technique and used as sensing membrane. The dimension of this layer is $\sim 1 \text{ mm} \times 2 \text{ mm} \times 50 \text{ nm}$. Nanostructured ZnO has unique properties such as high catalytic efficiency, large surface area, strong adsorption ability and high chemical stability [53]. For ZnO solution, 10 mg zinc acetate ($Zn(CH_3COO)_2$) has been dissolved in 10 ml distilled water and 2ml NH_4OH has been added and stirred at room temperature for several minutes. Ten microlitre of PANI (1M) in 10 ml of ZnO has been added. This increases biocompatibility of the device [86], [87]. Channel length and width of this FET have been chosen equal to be 1mm and 2 mm, respectively. To make contact with source and drain, Aluminum metal has been deposited using filament evaporation technique as shown in Fig. 2.11. Thickness of this layer has been calculated using Eq. (2.21) as mentioned in Chapter 2. Aluminium metal has been used due to its low resistivity, low melting point, and no contamination as reported in literature. The

contact is quasi-ohmic, where work function of Aluminium is less than work the function that of CNT (n -type) [88]. To make ohmic contact, fermi level of CNT matches with the fermi level of Aluminium. For this, CNT has been heavily doped with potassium that raises the fermi level towards conduction band of CNT. For passivation on the whole FET except on the sensing layer, polydimethylsilaxane (PDMS) has been coated at the time of cholesterol measurement [89]. Features of PDMS have been mentioned in the chapter 2. Thus, complete cholesterol JLCNTFET has been fabricated as shown in **Fig. 3.1**. An electrochemical mechanism of polyaniline (PANI)/zinc oxide (ZnO) has been proposed as shown in **Fig. 3.2**.

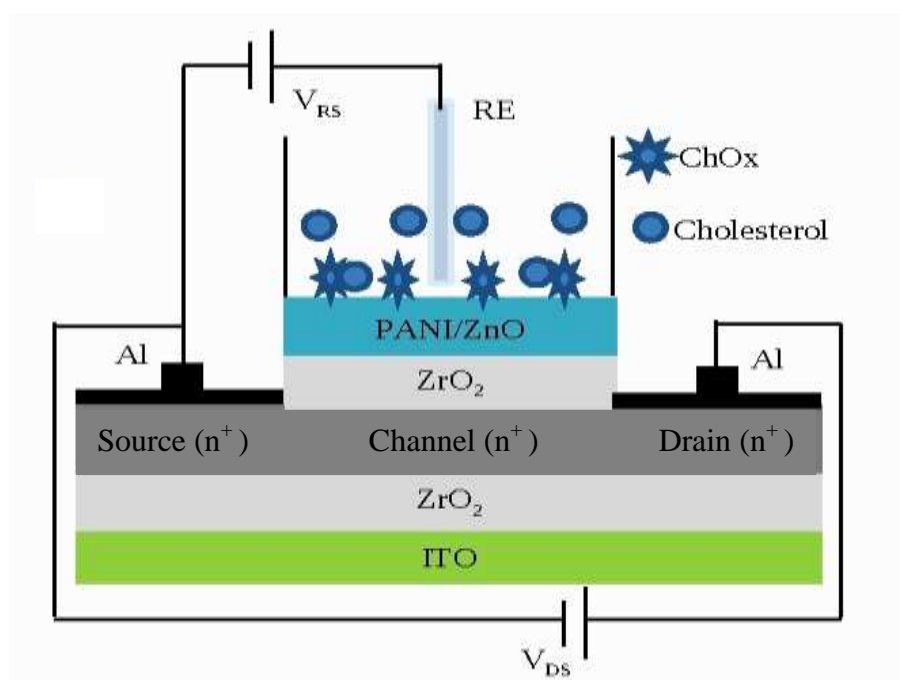


Fig. 3.1: Schematic of JLCNTFET for cholesterol detection

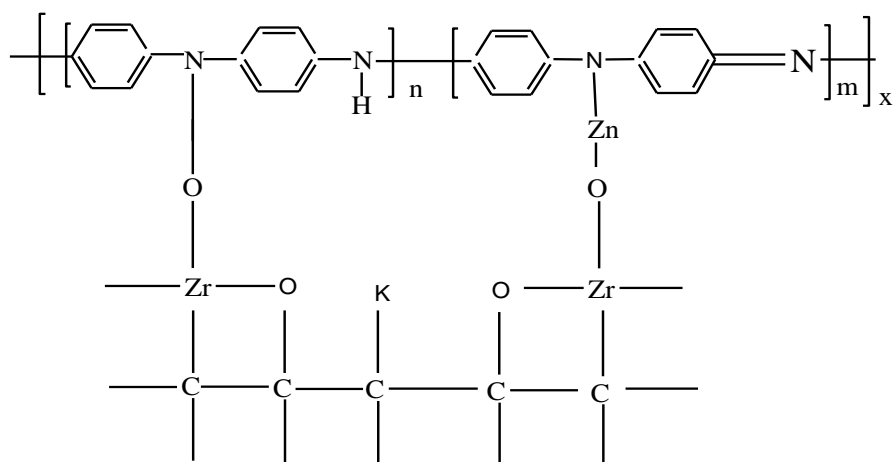


Fig. 3.2: Electrochemical mechanism of PANI/ZnO/K/CNT

Acceptable amount of potassium in CNTs has been determined by varying concentration of potassium from 1 to 30 % and depositing thin film using electrochemical deposition technique. The resistance of the thin film has been calculated using digital multimeter for each concentration of potassium and plotted as shown in **Fig. 3.3** with dotted line. Similarly, acceptable amount of PANI in ZnO has been determined by varying concentration of PANI from 1 to 30 % and depositing thin film using electrochemical deposition technique. The resistance of the thin film has been calculated using digital multimeter for each concentration of PANI and plotted as shown in **Fig. 3.3** with solid line. As the concentration of potassium increases, the resistance of the K/CNT composite decreases. When potassium concentration reaches to 10 %, the resistance of the composite does not change. Thus, the acceptable concentration of potassium has been found to be 10 %. Similarly, acceptable value of PANI in ZnO has been found to be 12–15 % at which resistance takes saturation and then slowly decreases.

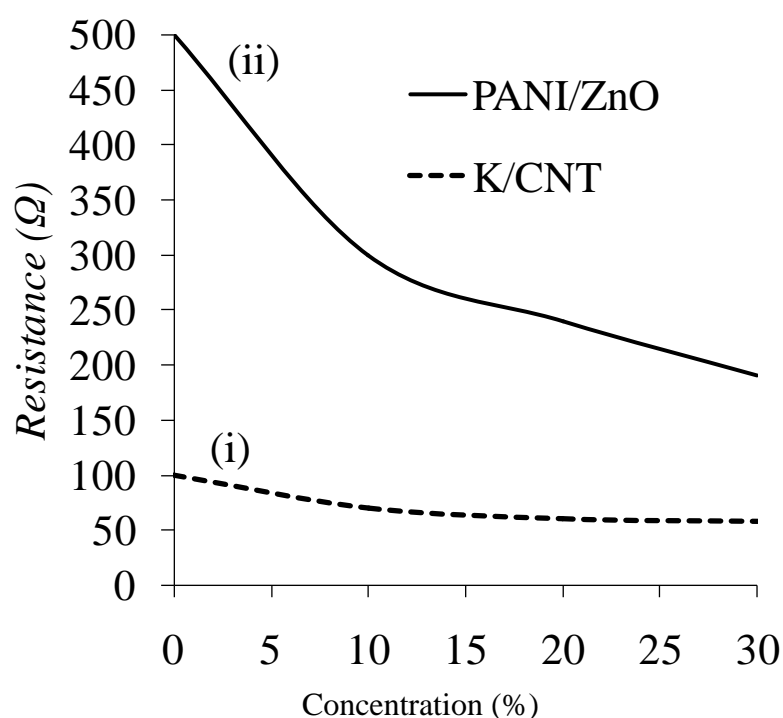


Fig. 3.3: Effect of potassium concentration in CNT and PANI concentration in ZnO

The values of parameters used for fabrication of this device have been summarized in **Table 3.1**.

Table 3.1: Value of parameters used for fabrication of JLCNTFET

Parameters	Value used
Dimension of gate oxide	1 mm × 2 mm × 10 nm
dimension of channel	1 mm × 2 mm × 100 nm
dimension of both Source and Drain	5 mm × 2 mm × 100 nm
Doping concentration of K in CNT	10 %
Doping concentration of PANI in ZnO	12–15 %
Dimension sensing membrane	1 mm × 2 mm × 50 nm
Drain voltage at which experiment was performed	0.3 V
Reference voltage at which maximum response obtained	0.6 V

3.2.4. Theory and working principle of cholesterol JLCNTFET

Theory of cholesterol JLCNTFET has been explained in Chapter 2. The Eq. (2.10) is the expression for total drain current of single gated cholesterol JLCNTFET. In this case, the term surface inversion potential ($2\phi_f$) does not arise because there is no need of minimum surface inversion potential that is n -channel already exists since CNT has been doped with potassium [85]. In n -channel FET, drain current increases with positively charged biomolecules, therefore, it is called as enhancement mode cholesterol CNTFET. The basic enzymatic reaction for cholesterol detection has been given by Eq. (2.12) in Chapter 2. In this equation, in presence of oxygen, the enzyme ChOx transforms cholesterol into cholest-4-en-3-one and H_2O_2 . At reference voltage of 0.6 V, H_2O_2 releases proton (H^+) to the electrolyte solution. The gate interface is affected by these protons and consequently affects the potential difference between the gate and the source and modulates the channel current.

3.3. Results and discussions

3.3.1. DC characteristics of JLCNTFET without cholesterol

This experiment has been performed to measure the intrinsic voltage gain and to show the behavior of this experiment outside liquid. For this, DC drain current has been recorded using digital multimeter (DMM) at different gate voltages before immobilization of ChOx on the surface of PANI/ZnO sensing membrane. Drain current has been plotted against drain voltage from 0 to 1 V, in step 0.2 V with applied gate voltages from 0 to 1 V, in step 0.2 V as shown in **Fig. 3.4**. Intrinsic voltage gain (A_V) has been calculated from the dc characteristics curves and has been found to be ~ 16 using the relation $A_V = g_m/g_{ds}$, where g_m is called transconductance and g_{ds} is called drain conductance. The value of A_V signifies that this CNT based JLFET has high performance for sensing purpose. This is the experiment for gate dependency of this device. This experiment shows that the fabricated CNTFET has MOSFET like characteristics. Since it is n -channel CNTFET, as gate voltage positively increases drain current also positively increases. Thus, it acts as an enhancement mode.

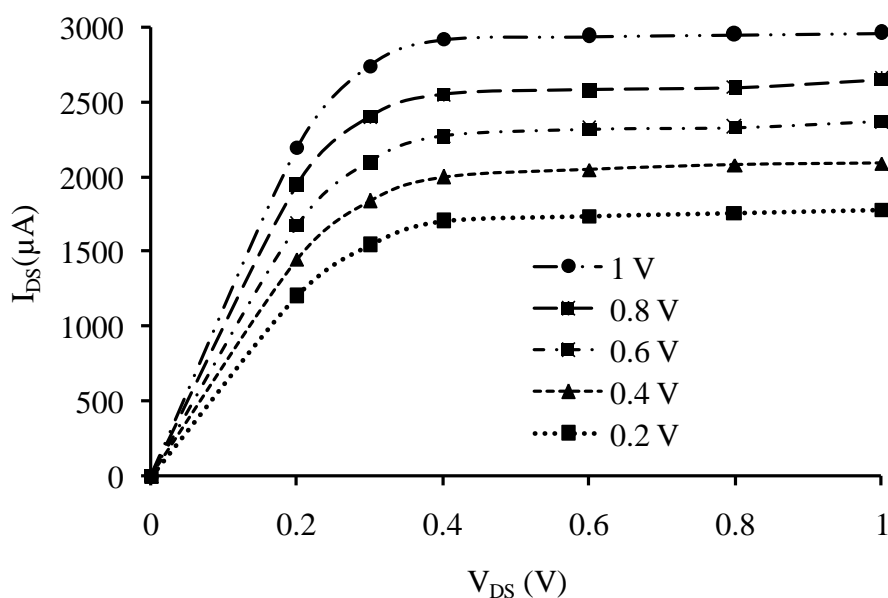


Fig. 3.4: DC characteristics of JLCNTFET without cholesterol for different V_{gs} .

3.3.2. Immobilization of ChOx on PANI/ZnO sensing layer

The surface of PANI/ZnO has been washed with deionized water. This was done for good attachment of enzyme. After that, 1 μ l of ChOx has been immobilized onto the PANI/ZnO surface using physical adsorption technique. Physical adsorption technique has advantages as mentioned in Chapter 2 [39]. One microlitre of ChOx has been chosen, because it is sufficient on the surface of the sensing membrane. Prior to being used, PANI/ZnO/JLCNTFET has been dried overnight under desiccated conditions and washed with PBS to remove any unbound ChOx and stored in a refrigerator at temperature of 4 °C when not in use.

3.3.3. Electrochemical response measuring apparatus for cholesterol detection

For measurement of cholesterol, a technique has been proposed. For this, the sealed device JLCNTFET has been inserted with a reference electrode of Ag/AgCl in a glass pot containing 20 ml of PBS with 50 mM, pH 7 as shown in **Fig. 3.5**. Supply voltage from 0 to 1 V in step 0.2 V has been applied between source and drain, where positive and negative supply has been connected to drain and source, respectively. Reference electrode voltage has been applied between reference electrode and source, where positive and negative supplies have been connected to reference electrode and source, respectively. A DMM has been connected to record the drain current (I_{DS}). Positive terminal of DMM has been connected to the drain side of JLCNTFET and negative terminal of DMM has been connected to the positive side of the drain voltage through a small resistance. The resistance has been used to reduce large drain current through the DMM. Initially, 10 μ l stock solution of cholesterol (10 mM) has been added by a micropipette to PBS in the pot by varying the reference voltage from 0 to 1 V in step 0.2 V and drain current has been recorded. For different reference voltages, different drain responses have been obtained and plotted as shown in **Fig. 3.6**. From this graph, it has been found that maximum drain response obtained at reference voltage 0.6 V. Therefore, for further experiments, reference voltage was kept constant at 0.6 V.

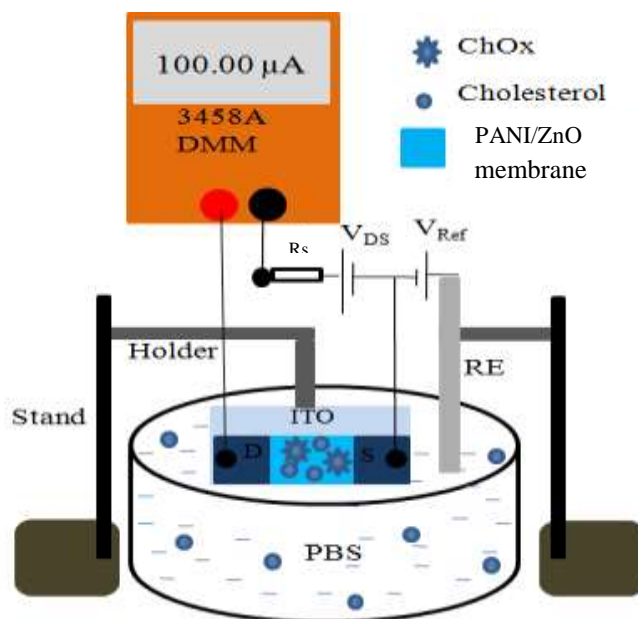


Fig. 3.5: Electrochemical response measuring apparatus

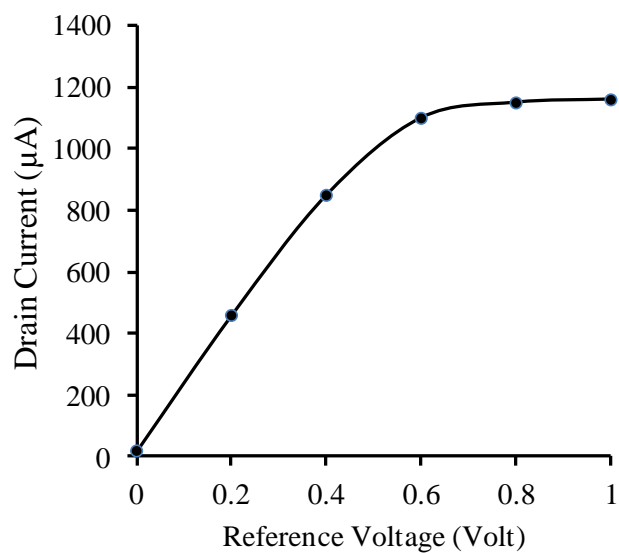


Fig. 3.6: Reference voltage Vs. Drain current at $V_{DS} = 0.3$ V

3.3.4. Electrochemical characterization of cholesterol JLCNTFET

The electrochemical characterization of cholesterol JLCNTFET has been performed in a glass pot containing 20 mM of PBS with 50 mM and pH 7 using the setup as shown in **Fig. 3.5**. DC drain current (I_{DS}) of the cholesterol JLCNTFET has been measured using DMM and plotted against drain voltage (V_{DS}) for cholesterol concentration from 0.5 to 22.2 mM at a fixed reference voltage of 0.6 V as shown in **Fig. 3.7**. This figure shows that drain current is linear upto drain voltage of 0.4 V and then saturates. From this linear range, experiments have been conducted at 0.3 V. Drain currents have been plotted against corresponding different cholesterol concentrations as shown in **Fig. 3.8**. This graph reveals that the device has linearity for cholesterol concentration from 0.5 to 16.6 mM. Therefore, the ENFET can be used for measurement of cholesterol concentration up to 16.6 mM.

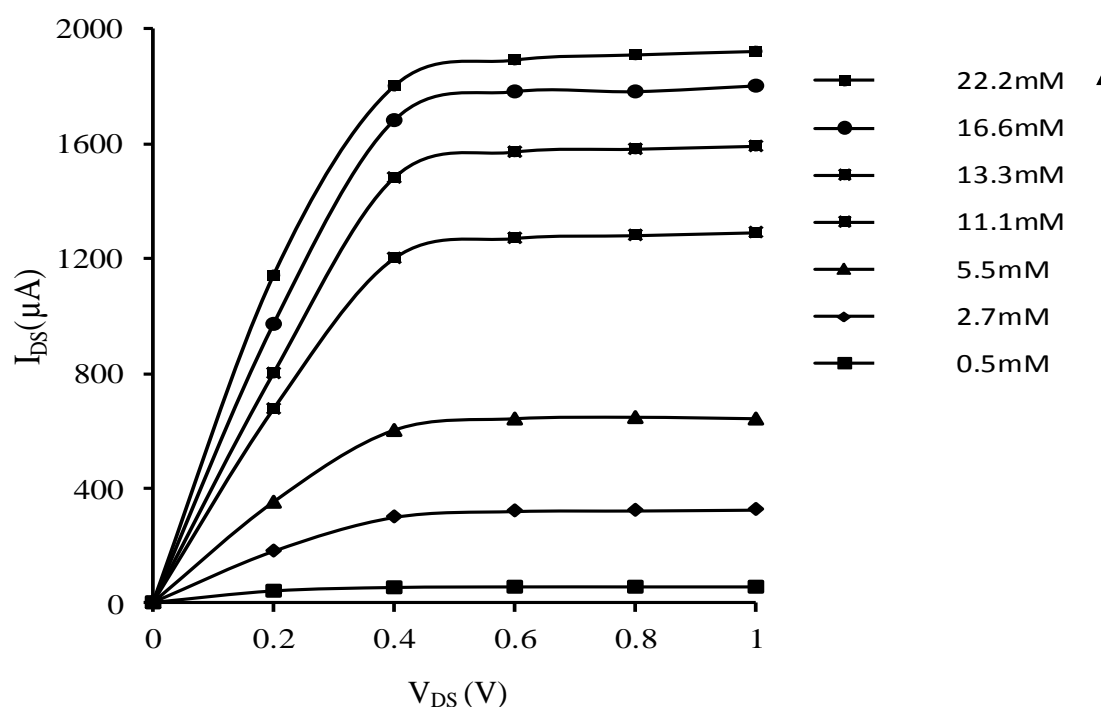


Fig.3.7: Drain characteristic of JLCNTFET for different cholesterol concentration

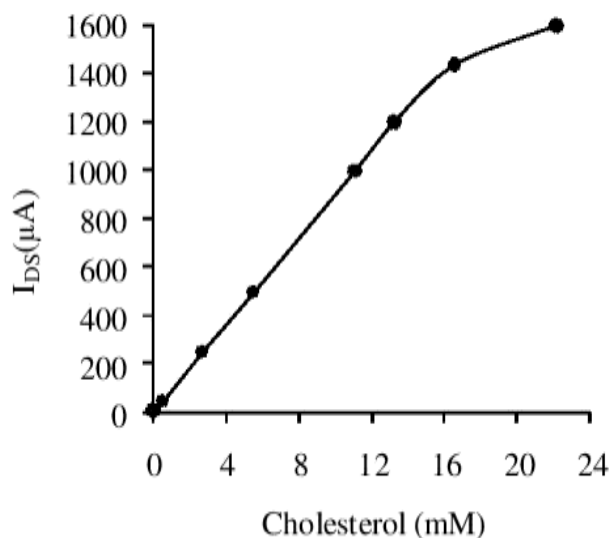


Fig. 3.8: Transfer characteristics at $V_{DS} = 0.3$ V.

3.3.5. Activity of ChOx on cholesterol JLCNTFET

Enzyme activity can be estimated from the Michaelis–Menten constant (K_m). The value of K_m has been calculated from the Lineweaver–Burk plot as shown in **Fig. 3.9**. Lineweaver–Burk Plot is a plot of reciprocal of concentration of analytes (cholesterol) Vs. reciprocal of response (drain current) as explained in Chapter 2. From this graph, the value of K_m has been found to be ~ 1.4 mM. This small value reveals that the enzyme ChOx has high activity for detection of cholesterol.

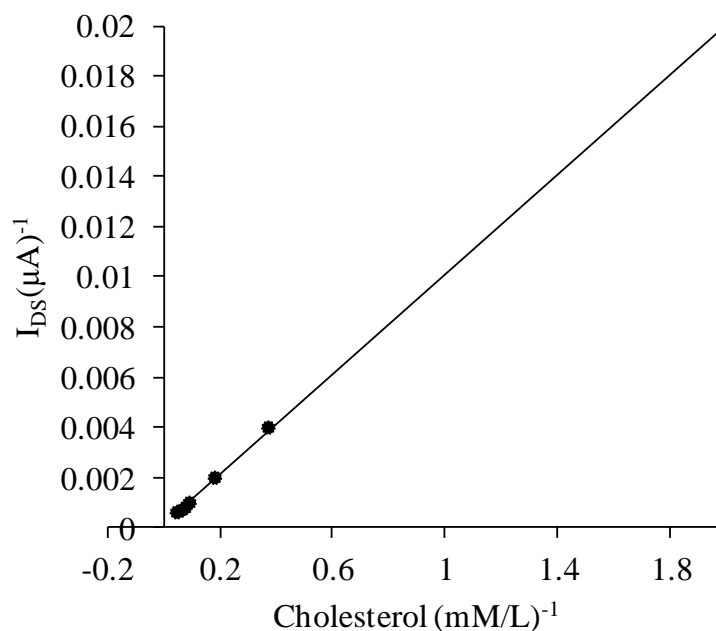


Fig. 3.9: Lineweaver–Burk Plot for cholesterol concentration

3.3.6. Interfacial potential and sensitivity of cholesterol JLCNTFET

The interfacial potential ($\Delta\psi_0$) developed between electrolyte solution (cholesterol) and oxide layer (sensing membrane) of this cholesterol ENFET has been calculated using the Eq. (2.14) as mentioned in Chapter 2. **Fig. 3.10** shows the plot of cholesterol concentration and interfacial potential at temperature 25 °C and pH 7. Sensitivity for this ENFET is the ratio of shift of the interfacial potential with respect to cholesterol concentration. The sensitivity of this ENFET has been calculated from this graph and found to be 60 mV/decade. Calculation of the interfacial potential has been explained by the Nernst Equation [14] as mentioned in Chapter 2.

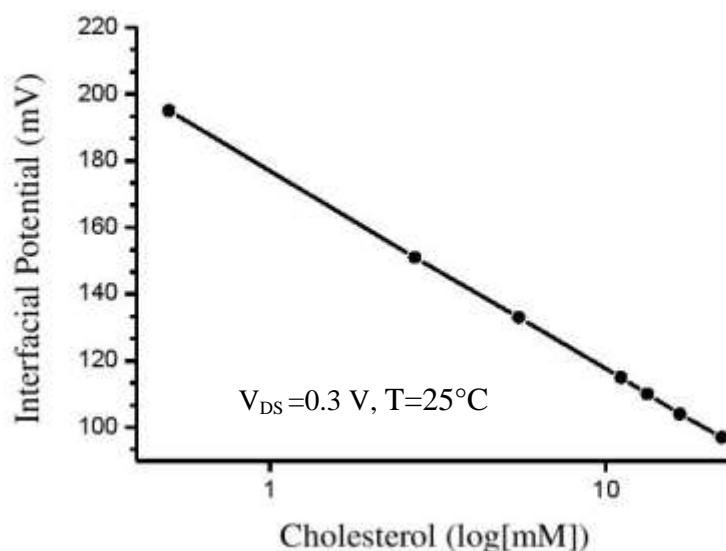


Fig. 3.10: Sensitivity calculation for cholesterol concentration.

3.3.7. Limit of detection and regression co-efficient calculation

The limit of detection (*LoD*) for this has been calculated using Eq. (2.18) in Chapter 2 and found to be 0.2 mM. This small value of *LoD* reveals that at least 0.2 mM of cholesterol is possible to detect using this ENFET. The regression coefficient has been calculated using Eq. (2.21) in Chapter 2 and found to be ~0.998. Here, the value of *R* signifies that there is a good correlation between enzyme and cholesterol with this device.

3.3.8. Effect of temperature and pH on cholesterol JLCNTFET

The effect of temperature on PANI/ZnO/JLCNTFET has been investigated by measuring drain current using the set up as shown in **Fig. 3.5**. The temperature of the set up has been maintained by inserting it in to a temperature booth. Thermometer has been to monitor the

temperature. Taking 11.1 mM of cholesterol solution and by varying the temperature from 15 to 50 °C, drain current has been recorded using DMM. This experiment has been performed in PBS of 50 mM and pH 7. The recorded drain current has been plotted against given temperature as shown in **Fig. 3.11**. The graph shows that drain response increases upto temperature 30 °C and then decreases beyond temperature 37 °C. In case of silicon FET, as temperature increases drain current decreases. But in case of CNTFET, drain current increases as temperature increases. Again, at higher temperature, activity of enzyme become low and hence, drains response decreases. The maximum response for cholesterol has been obtained in the temperature range of 30 to 35 °C. Therefore, this cholesterol ENFET can be used at this environment.

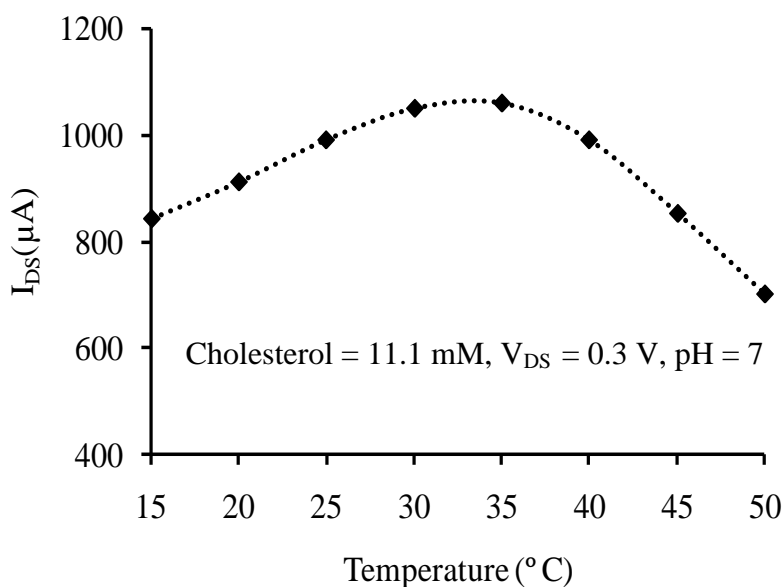


Fig. 3.11: Effect of temperature on cholesterol responses.

Similarly, the effect of pH on this device has been estimated taking 11.1 mM of cholesterol solution and by varying pH from 5 to 10 at temperature of 25 °C. Using the set up as shown in **Fig. 3.5**, drain current has been recorded using DMM at different pH and plotted as shown in **Fig. 3.12**. The graph shows that drain response increases up to pH 7 and then decreases pH 8 onwards because, at high value of pH, enzyme activity becomes slow. Therefore, this device is useful for pH 6.5–8.0.

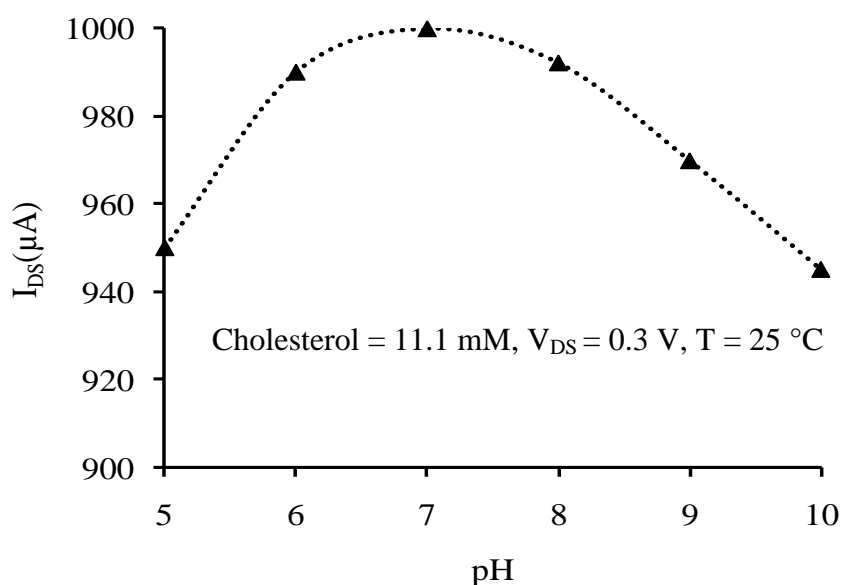


Fig. 3.12: Effect of pH on cholesterol response.

3.3.9. Interference and repeatability test

Interference on cholesterol has been estimated using the same procedure as explained above. For this, 13.3 mM cholesterol solution has been tested in the presence of urea (16.6 mM), glucose (13.3 mM), uric acid (11.1 mM), and ascorbic acid (10 mM) at pH 7 and temperature 25 °C. The drain current has been recorded using DMM and plotted against the concentration of mixtures as shown in **Fig. 3.13**. The graph reveals that cholesterol response has no affect due to presence of other biomolecules. The percentage of interference has been calculated using Eq. (2.15) in Chapter 2. It has been found that average percentage of interference of cholesterol with other biomolecules is ~2 %.

The repeatability of this cholesterol ENFET has been investigated taking 13.3 mM of cholesterol solution and repeating the experiment for 10 times at temperature 25 °C and pH 7. The drain current has been recorded using DMM and the graph has been plotted as shown in **Fig. 3.14**. It has been observed that the cholesterol response has no large variation after several repetitions.

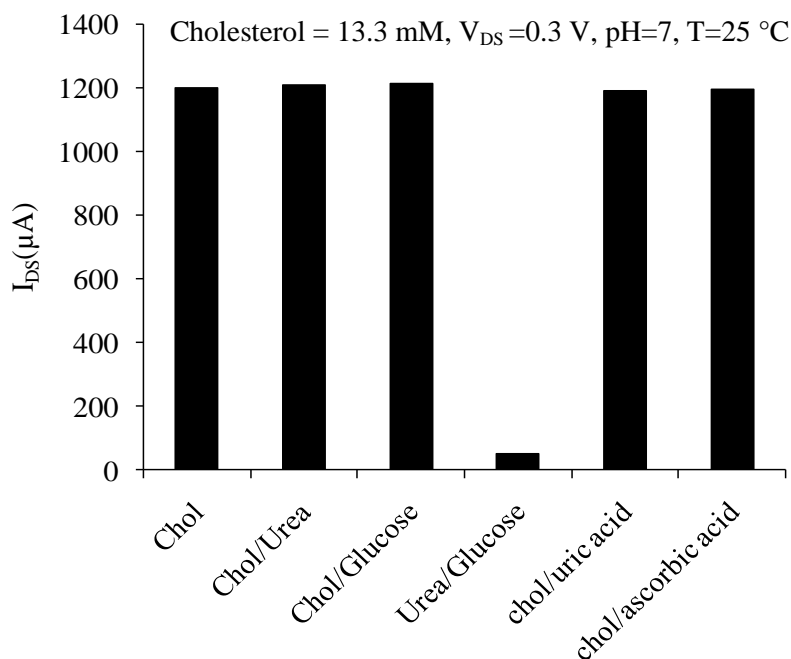


Fig. 3.13: Interference on cholesterol response.

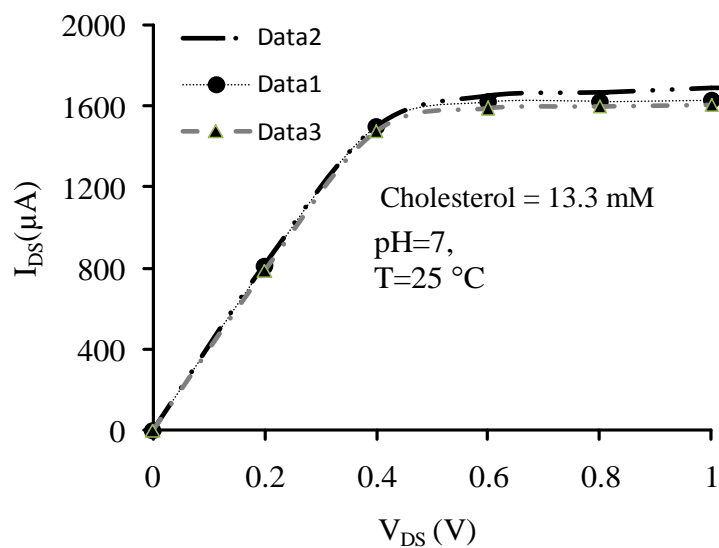


Fig. 3.14: Plot of three data for repeatability of the device.

3.3.10. Reproducibility and stability test

To test the reproducibility, experiments were conducted on two devices fabricated by using the same procedure. The reproducibility has been proven by taking 13.3 mM of

cholesterol solution and by performing the experiments at the same condition. Both the devices have produced almost same results for 13.3 mM of cholesterol solution (**Fig. 3.15**). The experimental results showed that the device has reproducibility. Similarly, to test the stability of the device, degradation of the device has been shown after every 1 month upto 5 months, taking 13.3 mM of cholesterol solution. The degradation plot shown in **Fig. 3.16** has shown average ~98% stability results after 5 months storage.

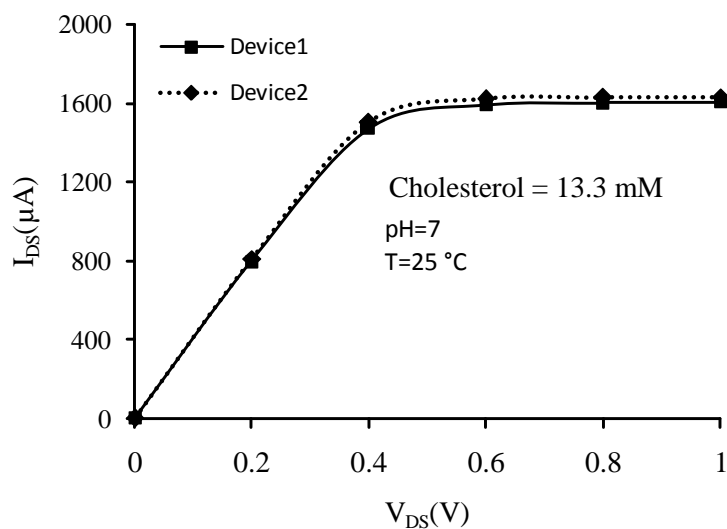


Fig. 3.15: Reproducibility plot of two devices

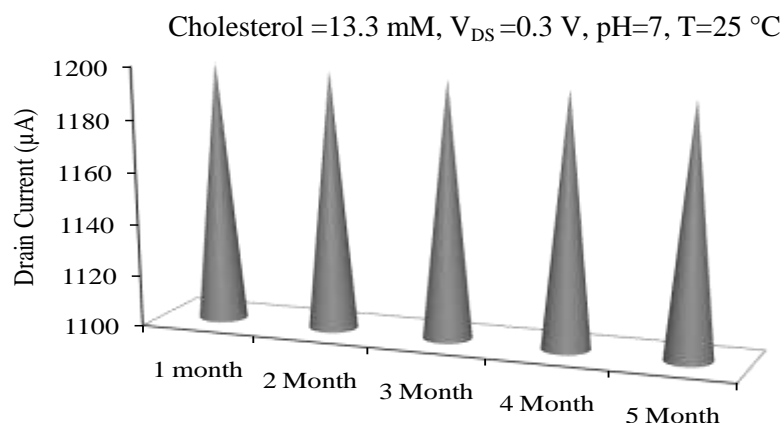


Fig. 3.16: Stability plot of the device.

The results obtained from this work have been summarized in **Table 3.2** and a comparison studies about sensitivity, LoD , and K_m with other reported works using FET based biosensors have been given in **Table 3.3**.

Table 3.2: Output results obtained from the cholesterol JLCNTFET

A_v	Linearity (mM)	Interference	Repeat-ability	Stability	Optimum temperature	Optimum pH	Response time (s)
16	0.5–16.6	2 %	10 times	150 days	30–37 °C	6.5–8.0	1

Table 3.3: Comparison of this work with other reported FET based cholesterol biosensors

Sensor type	Sensing Materials	Sensitivity (Interfacial potential)	Sensitivity (Drain current)	LoD /mM	K_m /mM	Ref.
Extended gated-FET	Ferrocenyl/Alkanethiol	57 mV/dec	–	–	–	[34]
Solution gated FET	ZnO	48.8 mV/dec	10 μ A /mM/mm ²	–	1 \times 10 ⁻⁴	[35]
JLCNT-FET	PANI/ZnO	60 mV/dec	100 μ A /mM/mm ²	0.2	1.4	[This work]

From this table, it can be concluded that this cholesterol ENFET is more advantageous than other cholesterol ENFETs as reported in the literature. It has long stability, small value of K_m , LoD and high sensitivity with fast response time. Therefore, this ENFET could be used for detection of other clinical parameters with reasonable advantages.

3.4. Summary

This work has described about fabrication and characterization of CNT based JLFET for detection of cholesterol. Fabrication process has significant merits such as low maintenance, low fabrication cost and user reliability. This integrated device has improved sensitivity of 60 mV/decade. Good reproducibility, repeatability and stability have been found in this work. The interference with other biomolecules has been found to be insignificant. This miniaturized device requires minimal instrumentation. Therefore, this nano-structured JLCNTFET can be used in the field of nano bioelectronics for estimation of other clinically parameters such as glucose, urea, acetylcholine etc.