Chapter 5

A COMPARATIVE STUDY BETWEEN GRAPHENE BASED AND CNT BASED ENZYME MODIFIED FIELD EFFECT TRANSISTOR FOR CHOLESTEROL SENSING

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A comparative study between graphene based and CNT based enzyme modified field effect transistor for cholesterol sensing

5.1. An overview

Many potential merits of enzyme junctionless enzyme CNTFETs have been discussed in Chapters 3 and 4 for detection of cholesterol and acetylcholine, respectively. But the extra ordinary activities of graphene such as tunable band gap property and one atom thick channel formation property have lead towards development of enzyme FET based devices [56], [95], [96]. Several graphene based biosensing devices have been developed but few work has been found on graphene based cholesterol FET.

In this chapter, graphene based traditional cholesterol enzyme FET cholesterol detection has been fabricated and characterized for comparing its results with the junctionless CNTFET for cholesterol detection as mentioned in Chapter 3.

5.2. Experimental

5.2.1. Materials and Chemicals/Reagents

Graphene nanosheet powder of size ~5–10 µm has been purchased from Alibaba. Other reagents such as ITO coated glass, cholesterol oxidase (ChOx) and cholesterol powder with the same parameters and units have been purchased as mentioned in Chapter 3. Multiwall carbon nanotube (MWCNT) with carbon purity ~98 %, length ~30 µm and diameter ~ 100–150 nm has been purchased from Sigma Aldrich. All other materials such as zirconium tetrachloride ($ZrCl_4$), bromine tribromide (BBr_3) Na_2HPO_4, NaH_2PO_4, KOH and polypyrrole are of analytical grade [39].

5.2.2. Preparation of solutions

Cholesterol stock solution of 25 mM concentration and phosphate buffer saline (PBS) of 50 mM, & pH 7 have been prepared using the procedure as mentioned in Chapter 3. ChOx (1 mg/ml) solution has been prepared using same PBS and kept at temperature of 4 ºC while not in use. De–ionized water has been used to clean ITO and other apparatus [39].

5.2.3. Fabrication of graphene based cholesterol FET

Graphene–FET (g–FET) has been fabricated on ITO using electrochemical deposition technique. On the ITO with dimension 11 mm × 5 mm, a layer of ZrO_2 has been deposited using ECD technique. This layer prevents the flow of channel current to the ITO. Solid zirconium tetrachloride ($ZrCl_4$) of 5 mg has been hydrolyzed in 5ml water and sonicated for several minutes [83] and deposited on ITO using ECD technique. The layer has been heated at a temperature of 190 ºC for getting thin layer of ZrO_2. Thickness of this layer does not matter, since it is not a gate insulator. On the ZrO_2 insulating layer, *p*–type graphene has been deposited using ECD technique and used as substrate. The dimension of the substrate is 11 mm × 5 mm × 100 nm. For *p*–type graphene, 2 µl of BBr_3 (1M) has been mixed in 100 µl of graphene (1M) and hydrogen gas has been blown through the solution [97], [98]. Similarly, nitrogen (N_2) gas has been blown by gas flow sputtering at normal temperature and pressure for making *n*–type source (S) and drain (D) [99], [100]. The gate width (W) and length (L) of channel have been chosen to be ~5 mm and ~1 mm, respectively. The large area of gate surface is suitable for immobilization of enzyme. 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 [54]. The thickness of the gate insulator has been calculated using gravimetric analysis method as explained in Chapter 2 [64]. The dimension of this layer is ~1 mm × 5 mm × 10 nm. This ZrO_2 layer increases gate capacitor and thus, device performance increases. To make contacts on both source and drain, aluminium metal has been deposited using filament evaporation technique as explained in Chapter 2 [88].

Potassium (K) doped CNT with polypyrrol (PPy) nanocomposite has been deposited as sensing membrane on the oxide layer using ECD technique. This layer has been used for immobilization of enzyme, ChOx [47], [101], [102]. For this, 5 mg CNT has been dispersed in 5 ml acetonitrile and sonicated for 10 minutes. For doping of potassium in CNTs, solid KOH tablet has been dissolved in pure water since potassium is unstable in air and very reactive with water. Then, 2 µl of KOH solution (1M) has been doped in 10 µl CNT solutions and mixed well. Ten microlitre of CNT with 8 µl pyrrole (1 mg/ml in formic acid) has been dispersed in 10 ml acetonitrile. Thus, a composite solution of K–doped CNT with PPy has been obtained. The dimension of this layer has been taken as ~1 mm × 5 mm × 50 nm. Thus, graphene based ENFET has been fabricated for detection of cholesterol as shown in **Fig. 5.1.** A mechanism has been proposed for electrochemical synthesis of PPy/K/CNT with ZrO_2 as shown in **Fig. 5.2.** Polydimethylsilaxane (PDMS) has been coated on the whole FET except channel region for passivation purpose at the time of cholesterol measurement [89]. PDMS has been used for coating purpose due to some features as mentioned in Chapter 2 [91].

Acceptable amount of potassium doping concentration in CNTs has been explained in Chapter 3. But acceptable doping concentration of PPy in K/CNT has been performed by varying doping concentration from 0 to 30 % as shown in **Fig. 5.3**. When PPy concentration increases, the resistance of the composites decreases. When its concentration reached to 15 %, the resistance of the composite does not change more. Therefore, acceptable concentration of PPy in K/CNT has been found to be 15 %. The values of parameters used for fabrication of this device have been summarized in **Table 5.1.**

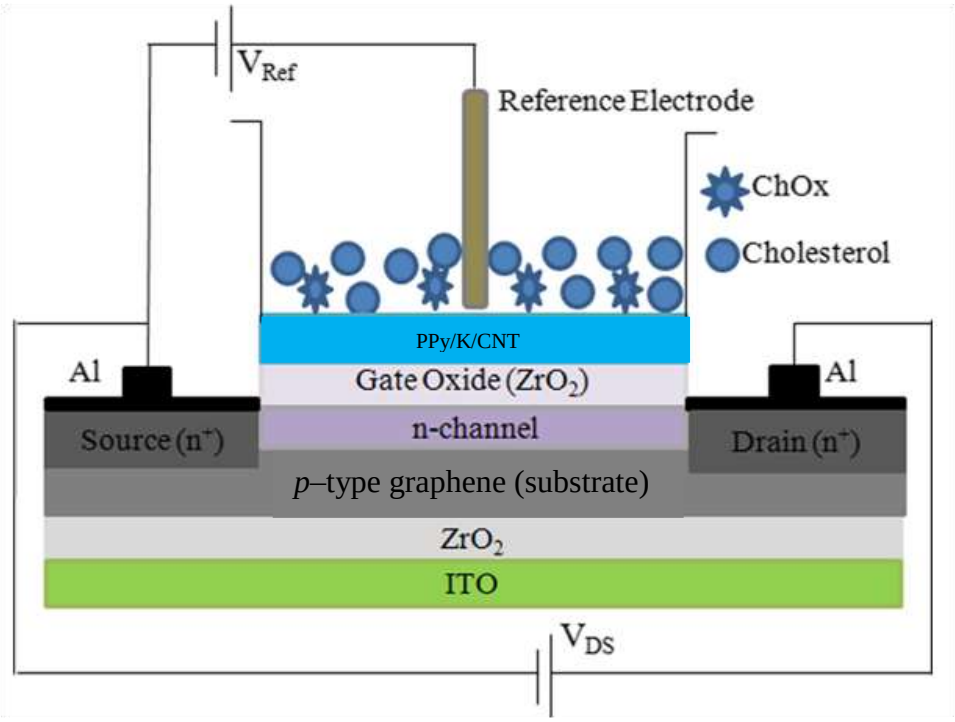

Fig. 5.1: Schematic of graphene based FET for cholesterol detection

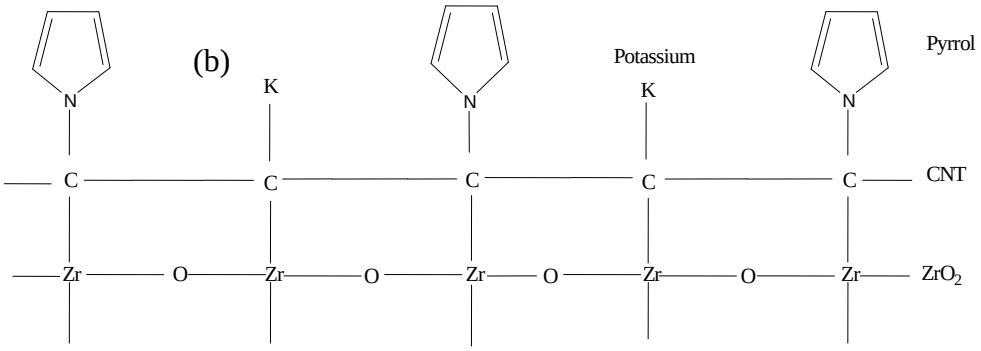

Fig. 5.2: Proposed electrochemical mechanism of PPy/K/CNT/ZrO_2

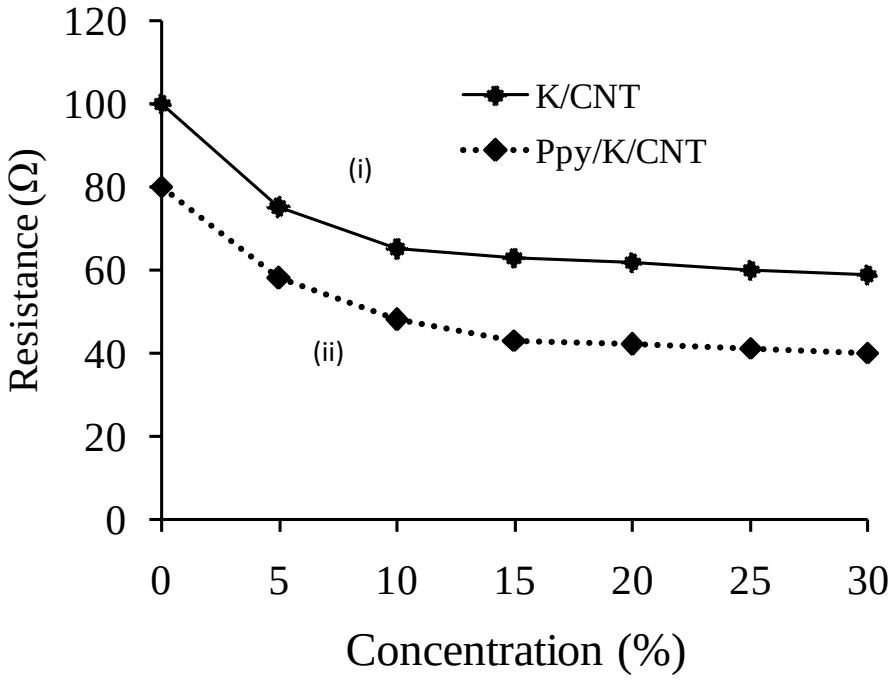

Fig. 5.3: Effect of PPy and potassium concentration in CNT

Table 5.1: Value of parameters used for fabrication of graphene FET

Parameters	Value used
dimension of gate oxide	1 mm × 5 mm × 10 nm
dimension of channel	Similar to oxide except thickness
dimension of Source and Drain	5 mm × 5 mm × 50 nm
Doping concentration of K in CNT	10 %
Doping concentration of PPy in K/CNT	15 %
Dimension of sensing membrane	1 mm × 5 mm × 50 nm
Darin voltages at which experiment was performed	0.2 V
Reference voltage at which maximum response obtained	0.6 V

5.2.4. Theory and working principle of graphene based cholesterol FET

Theory of cholesterol graphene FET has been explained in **Chapter 2**. Eq. (2.8) is the expression for total drain current of single gated cholesterol graphene based FET. In this case, the term surface inversion potential ($2\phi_f$) arises because; it is a traditional FET having junctions [87]. The drain current passes when this surface inversion potential is overcome. Since this is an *n*–channel *g*–FET, drain current increase with positively charged biomolecules. Therefore, it is called as enhancement mode cholesterol graphene FET. 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 some potential ~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.

5.3. Results and discussions

5.3.1. DC characteristics of graphene based FET without cholesterol

This experiment has been performed to calculate intrinsic voltage gain and to show the behavior of this FET device outside the liquid. For this, DC drain current has been recorded at different gate voltages using digital multimeter (DMM) before immobilization of ChOx on the surface of PPy/K/CNT sensing membrane. Drain currents have plotted against drain voltages from 0 to 1 V, in step 0.2 V with some applied gate voltages from 0 to 1 V, in step 0.2 V as shown in **Fig. 5.4.** Intrinsic voltage gain (A_V) has been calculated from the dc characteristics curves and has been found to be ~10 using the relation $A_V = g_m/g_{ds}$, where g_m is transconductance and g_{ds} is drain conductance. The value of A_V signifies that this graphene based FET has moderate performance for sensing purpose. This is the gate dependency experiment of this device. This experiment shows that the fabricated graphene FET has MOSFET like characteristics.

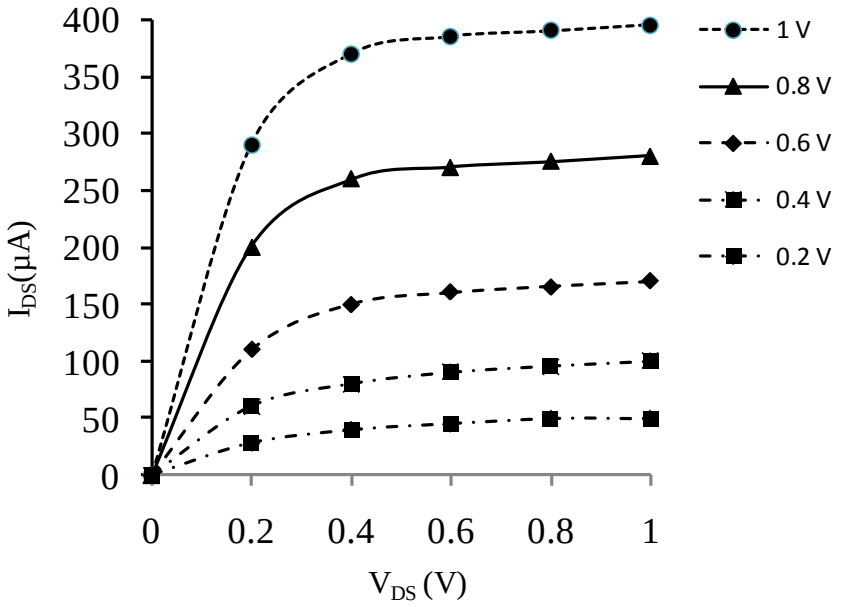

Fig. 5.4: DC characteristics of graphene FET outside liquid for different V_{gs}

5.3.2. Immobilization of ChOx on PPy/K/CNT sensing layer

The surface of PPy/K/CNT has been modified with glutaraldehyde by spreading 0.1 % of glutaraldehyde for several hours and washed with de-ionized water. One microliter of ChOx has been immobilized onto PPy/K/CNT surface using physical adsorption technique as mentioned in Chapter 2. Prior to being used, PPy/K/CNT/g–FET has been dried overnight under desiccated conditions and washed with PBS to remove any unbound ChOx and stored in a refrigerator at room temperature when not in use.

5.3.3. Electrochemical response measuring apparatus for cholesterol detection

Electrical response of graphene based cholesterol ENFET has been recorded using digital multimeter. For this purpose, the ENFET with reference electrode (Ag/AgCl) has been inserted in a glass pot containing 20 ml PBS (50 mM, pH7, 0.9 % NaCl). Drain voltage (0 to 0.4 V in step 0.1 V) has been applied between source and drain. The positive and negative supply has been connected to drain and source, respectively. Reference voltage of 0.6 V (the reason has been mentioned in Chapter 3) has been applied between reference electrode and

source. Positive and negative supply has been connected to reference electrode and source, respectively. Ten microliter stock solution of cholesterol from 0.5 to 25 mM have been added to the pot each time by micropipette and corresponding drain current against each cholesterol concentration have been recorded by digital multimeter as shown in **Fig. 5.5.**

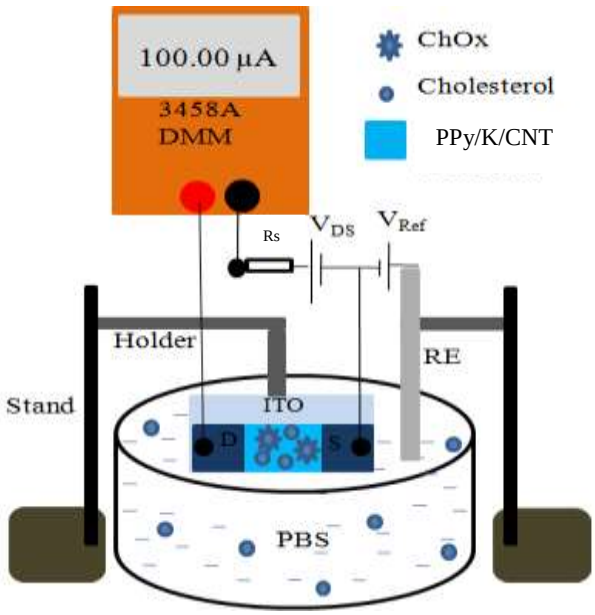

Fig. 5.5: Electrochemical response measurement set up

5.3.4. Electrochemical response of cholesterol graphene FET

The drain voltage (V_{DS}) versus drain current (I_{DS}) for cholesterol concentration from 0.5 to 25 mM have been plotted as shown in **Fig. 5.6.** The drain current is linear up to 0.2 V and then saturates like characteristic curve of a FET. **Fig. 5.7** shows the linearity of cholesterol concentration. It is the graph plotted for different cholesterol concentrations Vs. corresponding drain currents. This reveals that cholesterol concentrations from 0.5 to 20 mM have linearity. This ENFET can be used for this range of cholesterol concentration.

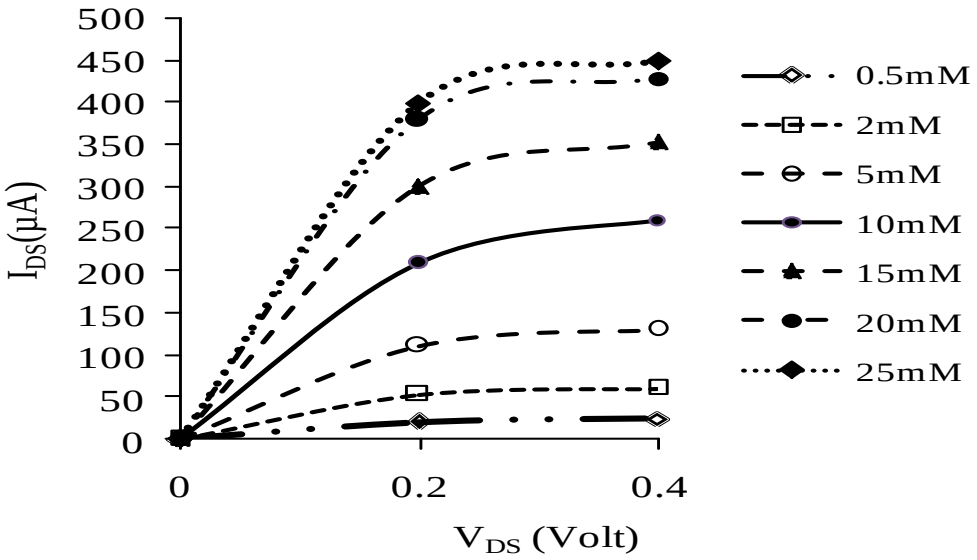

Fig. 5.6: Electrochemical response of graphene FET at temperature 25 °C.

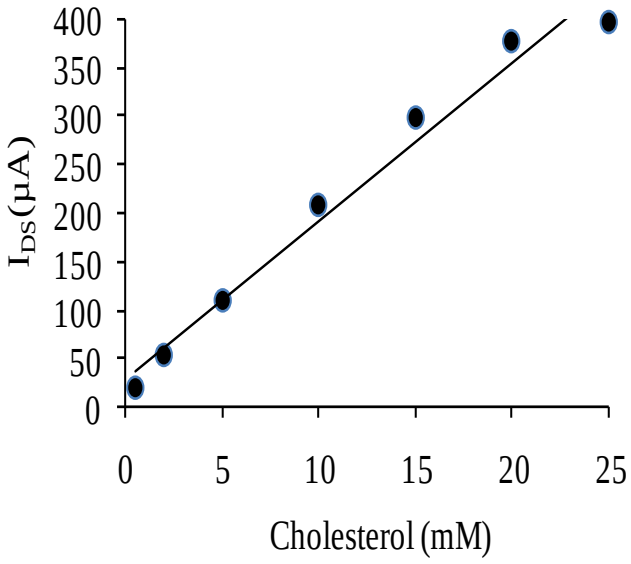

Fig. 5.7: Linearity of graphene FET for cholesterol detection at V_{DS} = 0.2 V

5.3.5. Activity of ChOx on graphene based cholesterol FET

Enzyme activity can be estimated from the Michaelis–Menten constant (K_m). The value of K_m has been calculated from the linweaver Burk plot as shown in **Fig. 5.8.** Lineweaver–Burk plot is the plot of reciprocal of drain current and reciprocal of cholesterol concentration as explained in Chapter 2. From this graph K_m has been found to be ~2.5 mM. This reveals that ChOx has moderate affinity with sensing membrane of graphene based ENFET.

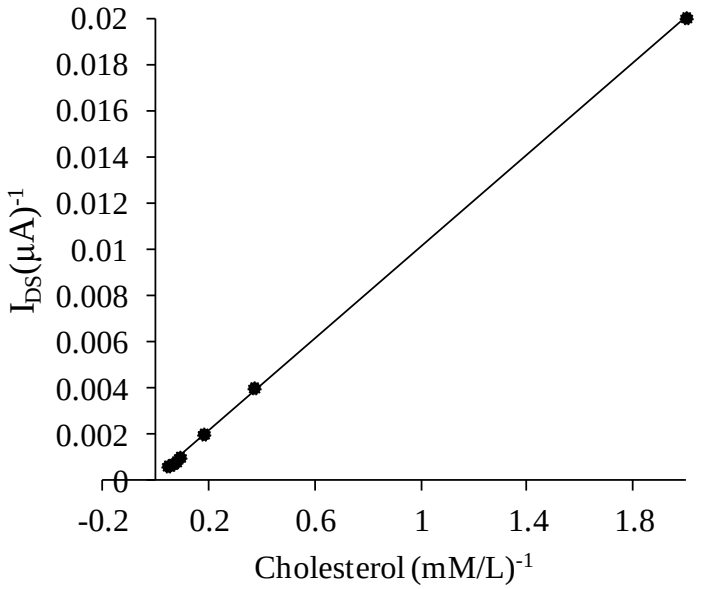

Fig. 5.8: Lineweaver-Burk Plot for cholesterol

5.3.6. Interfacial potential and sensitivity of cholesterol graphene FET

To calculate the interfacial potential ($\Delta\Psi_0$) developed at the interface between electrolyte solution (cholesterol) and oxide layer (sensing membrane) of this cholesterol ENFET, Eq. (2.14) as mentioned in Chapter 2 can be used. It has been calculated and plotted against cholesterol concentration at temperature of 25 °C as shown in **Fig. 5.9**. For this device, sensitivity is a ratio of shift in the interfacial potential with respect to cholesterol concentration. The sensitivity has been found to be 58 mV/decade. This mechanism can be explained by the Guoy–Chapman–Stern theory as mentioned in Chapter 2.

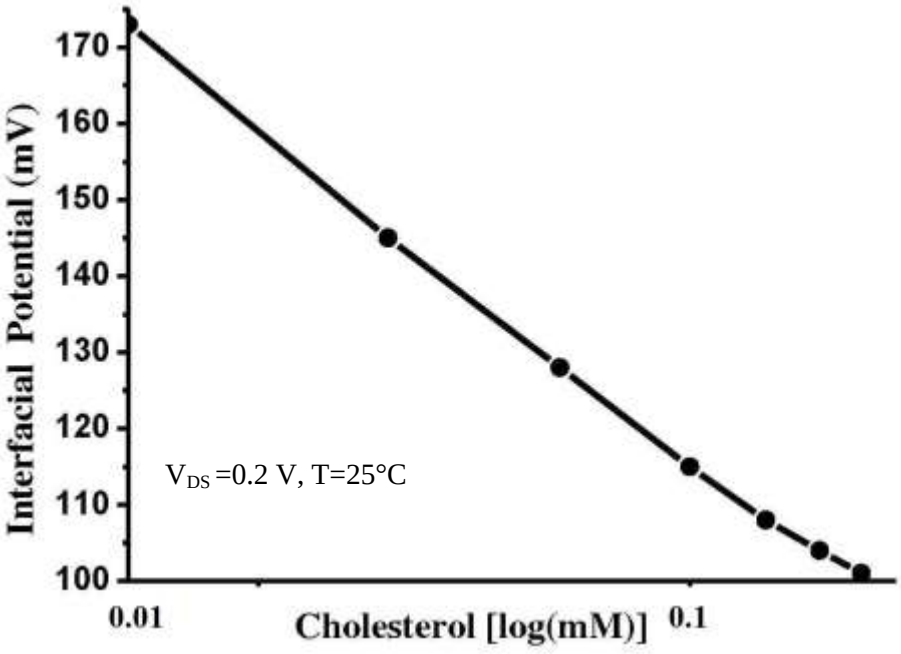

Fig. 5.9: Sensitivity calculation of graphene FET.

5.3.7. Limit of detection and regression co-efficient calculation

The limit of detection (*LoD*) has been calculated using the Eq. (2.18) in **Chapter 2** and found to be ~1.4 mM. This reveals that at least 1.4 mM of cholesterol can be detected using this ENFET. The regression coefficient can be calculated using Eq. (2.21) in **Chapter 2** and found to be ~0.998.

5.3.8. Effect of temperature and pH of graphene based cholesterol FET

The effect of temperature on PPy/K/CNT/g–FET has been investigated by measuring drain current using the set up as shown in **Fig. 5.5**. The temperature of the set up has been maintained by inserting it in to a temperature both and the temperature was monitored using a

thermometer. Taking 10 mM of cholesterol solution and varying the temperature from 15 to 50 ºC, drain current has been recorded using DMM. This experiment has been performed at pH 7 and using PBS of 50 mM. The recorded drain current has been plotted against given temperature as shown in **Fig. 5.10.** The graph shows that drain response has increased up to temperature 35 º C and then decreased. This is the contradiction of FET device because, in case of silicon FET as temperature increases, drain current decreases. This has happened because; in case of graphene FET, drain current increases as temperature increases. Again, at higher temperature, enzyme activities become low and hence, drain response decreases. The maximum response for cholesterol has been found in the temperature range of 35 ºC. From this graph, it can be concluded that this cholesterol ENFET can be used at other temperatures.

Similarly, the effect of pH on this device has been estimated taking 10 mM of cholesterol solution and by varying pH from 5 to 10 at temperature of 25 ºC. Using the set up as shown in **Fig. 5.5,** drain current has been recorded using DMM at different pH and plotted as shown in **Fig. 5.11.** The graph shows that drain response has increased up to pH 7–8 and then decreased again from pH 8 onwards. This mechanism has happened due to enzyme activity. Therefore, this device can be used at other pH.

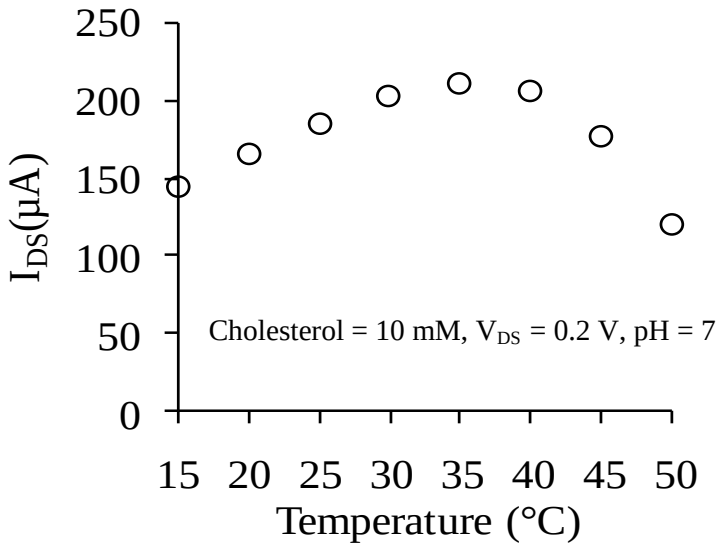

Fig. 5.10: Effect of temperature on graphene FET

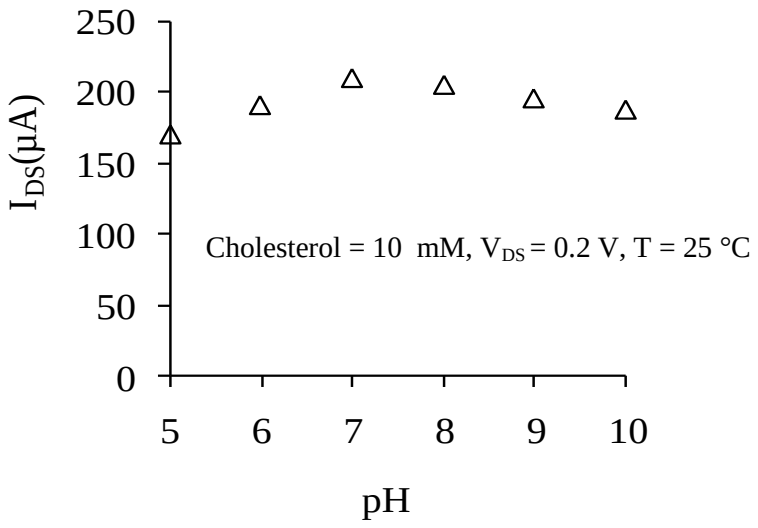

Fig. 5.11: Effect of pH on graphene FET

5.3.9. Interference and repeatability test

Interference on cholesterol has been estimated using the same procedure as explained above. For this, 10 mM cholesterol solution has been tested in the presence of urea (10 mM), glucose (3 mM), and uric acid (5 mM) at pH 7 and temperature 25 ºC. The drain current has been recorded using DMM and plotted against the concentration of mixtures as shown in **Fig. 5.12**. The graph reveals that cholesterol response has no affect due to presence of other biomolecules. The percentage of interference has been calculated using Eq. (2.20) given in Chapter 2. It has been found that average percentage of interference of cholesterol with other biomolecules is ~3.7 %.

The repeatability of this cholesterol ENFET has been investigated taking 10 mM of cholesterol solution and repeating the experiment for 10 times at temperature 25 ºC and pH 7. The drain current has been recorded using DMM and the graph has been plotted as shown in **Fig. 5.13.** It has been observed that the cholesterol response has no large variation after several repetitions.

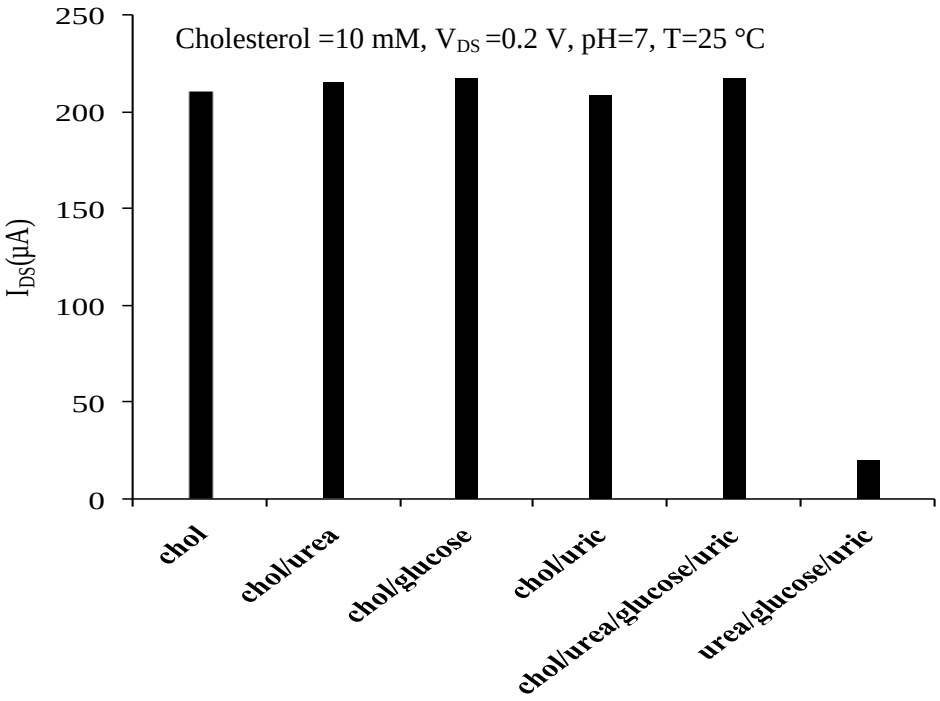

Fig. 5.12: Interference on cholesterol with other biomolecules

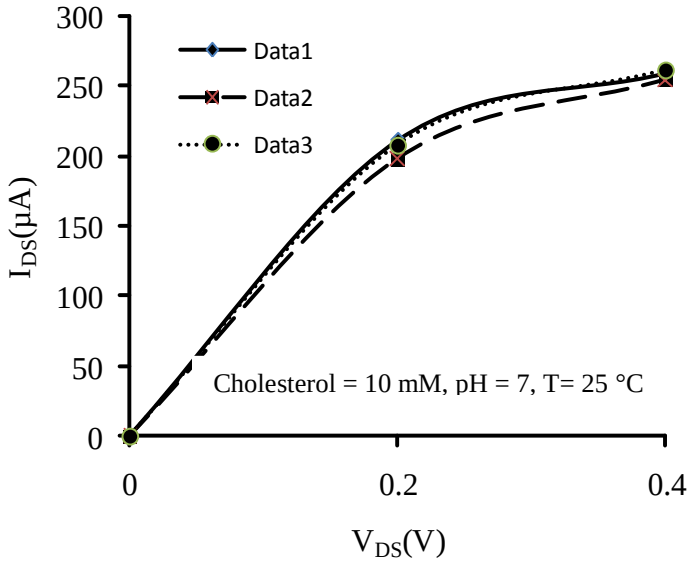

Fig. 5.13: Plot of three data for repeatability of the device.

5.3.10. 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 10 mM of

cholesterol solution and by performing the experiments at the same condition. Both the devices have produced almost same results for 10 mM of cholesterol solution (**Fig. 3.14**). 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 10 mM of cholesterol solution. The degradation plot shown in **Fig. 3.15** has shown average ~97% stability results after 6 months storage.

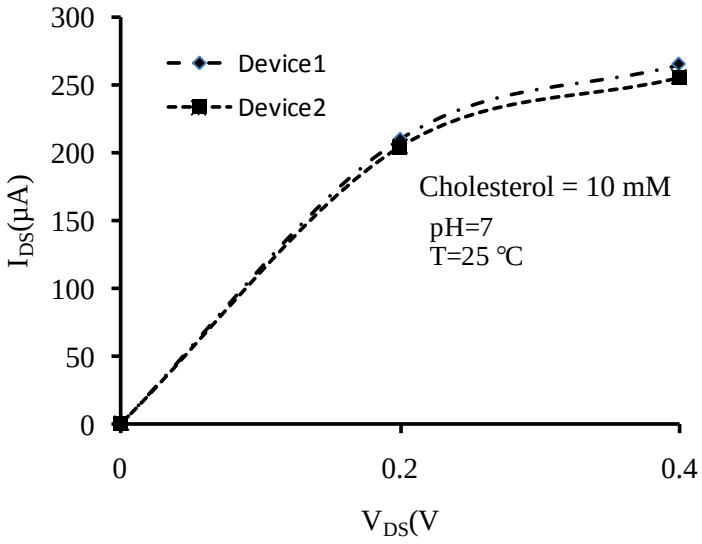

Fig. 5.14: Reproducibility plot of two devices

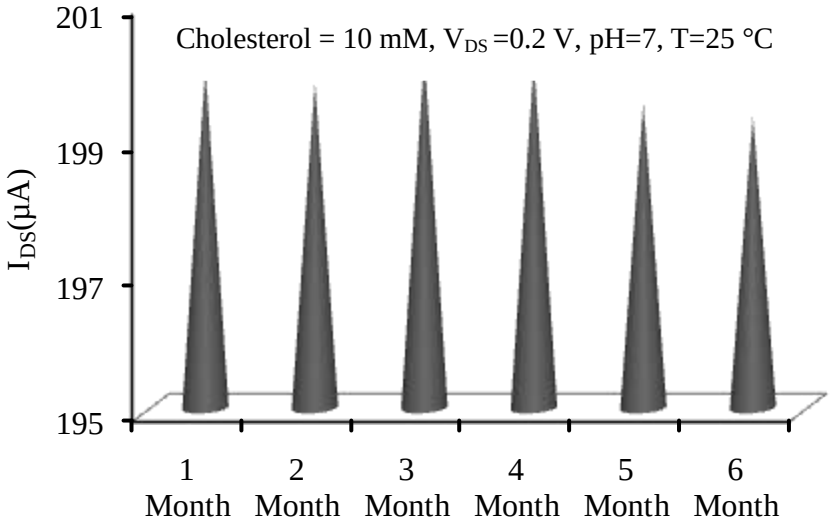

Fig. 5.15: Stability plot of the device.

The results obtained from this work have been summarized in **Table 5.2** and a comparison studies about sensitivity, *LoD*, and K_m with other reported works using FET based devices have been given in **Table 5.3.**

Table 5.2: Output results obtained from cholesterol graphene FET

A_v	Linearity mM)	Interference	Repeat-bility	Stability	Optimum temperature	Optimum pH	Response time (s)
16	0.5–20	3.7 %	10 times	180 days	30–36 ºC	7–8	>1

Table 5.3: Comparison of graphene based cholesterol FET with other FET based biosensor.

Sensor type	Sensing materials	Sensitivity (interfacial potential)	Sensitivity (Drain current)	*LoD* /mM	K_m /mM	Ref.
Extended gated–FET	Ferrocenyl/ Alkanethyol	57 mV/dec	–	–	–	[33]
JLCNT–FET	PANI/ ZnO	60 mV/dec	100 µA/ mM/mm^2	0.25	1.4	[Chap3]
DG–JL CNTFET	CH/ NiO	1.25 V/dec	500 µA/ mM/mm^2	37×10^{-8}	0.2	[Chap4]
Graphene FET	PPy/K /CNT	58 mV/dec	20 µA/ mM/mm^2	1.4	2.5	This work

It can be concluded from this table that dual gated FET has highest interfacial potential per decade for acetylcholine measurement as compared to other works presented in this Table.

5.4. Summary

This chapter has described about the fabrication of potentiometric graphene based ENFET using electrochemical deposition technique for detection of cholesterol. The extra ordinary property of graphene leads to high device performance fast response and high drain response. The complex nanocomposite of K–doped CNT with PPy has created new biosensing way. The interface of enzyme ChOx with graphene FET leads to the nano–engineering technique for detection of biomolecules. Reproducibility and repeatability and insignificant interference also have been observed. Due to these features, this could be used in similar biomedical analysis such as for detection of glucose, acetylcholine and urea etc.