CHAPTER 4

FABRICATION AND CHARACTERIZATION OF ENZYME MODIFIED CARBON NANOTUBE BASED FIELD EFFECT TRANSISTOR FOR ACETYLCHOLINE DETECTION

Chapter 4

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4.1. An overview

Detection of acetylcholine in the central and autonomous nervous system is important. Early method for detection of biomolecules as mentioned in the Chapter 3 has many demerits such as low sensitivity (Maximum sensitivity is 60 mV/decade), high power consumption, high threshold voltage and small ON–OFF current ratio as reported in the literature [25]. These problems can be overcome using dual-gated junctionless CNTFET (DG–JLCNTFET) [90]. In DG–JLCNTFET, channel potential and electric field distributions along the channel can be controlled by adjusting the work function of metal and semiconductor. In this chapter, a dual gated JLCNTFET has been reported for acetylcholine detection.

4.2. Experimental

4.2.1. Materials and Chemicals/Reagents

Acetylcholine esterase (AChE) with activity of 301 U/mg and acetylcholine (ACh) were obtained from Sigma. Single walled carbon nanotube (SWCNT) having carbon purity ~99 %, tube length ~20 µm and diameter ~100 nm was purchased from Alibaba. Indium tin oxide (ITO) coated glass (Sheet resistance is 15 Ω/sq) has been obtained from NANOCS. Other materials like Hafnium dioxide (HfO_2), nickel oxide (NiO), chitosan (CH), zinc oxide (ZnO) and other chemicals and materials are of analytical grade [39].

4.2.2. Preparation of solutions

One mole (1 M) of acetylcholine esterase (AChE) solution has been prepared by dissolving 1 mg of AChE powder in 1 ml of phosphate buffer saline (PBS) with molar concentration of 50 mM and pH 7. The prepared solution has been at temperature of 4 ºC while not in use. Acetylcholine stock solution of 0.2 mM (maximum) has been prepared using de–ionized water and from this solution, acetylcholine concentration from 0.01 to 0.18 mM has been prepared by dilution process. Sodium phosphate buffer saline and chemical solution to clean ITO and other apparatus have been prepared using the method as mentioned in the Chapter 3 (preparation of solution) [39].

4.2.3. Fabrication of acetylcholine DG–JLCNTFET

Dual gated junctionless carbon nanotube (DG–JLCNTFET) has been fabricated using solution process. Glass with dimension ~5 mm × 2 mm has been used as substrate on which ITO was coated and used as bottom gate material. On this ITO glass, a thin layer of intrinsic ZnO with dimension ~5 mm × 2 mm × 10 nm has been deposited to act as bottom gate insulator. Due to change in dielectric constant of ZnO, the drain current contributed by bottom gate can be varied as desired [53]. For ZnO solution, 10 mg zinc acetate ($Zn(CH_3COO)_2$) has been dissolved in 10 ml distilled water and 2 ml of NH_4OH has been added and stirred at room temperature for several minutes. The thickness of ZnO layer has been measured by the gravimetric analysis technique, as explained in Chapter 2 using the Eq. (2.11) and found to be 10 nm [64]. On the top of the ZnO layer, single walled carbon nanotube (SWCNT) uniformly doped with polyethylene imine (PEI) has been deposited using ECD technique. This layer acts as *n*–type source (S), drain (D) and channel regions since, it is junctionless FET. The dimension of source as well as drain is ~5 mm × 2 mm × 100 nm and dimension of channel is 1 mm × 2 mm × 100 nm [33], [34]. Prior to being used, CNT was prepared using catalytic chemical vapor deposition (CVD) technique and functionalized using boiling acid treatment technique [29], [84]. For CNT solution, 10 mg CNT has been dispersed in 10 ml methanol and sonicated for several minutes. Then, 5 ml solution of PEI has been added to this CNT solution. On the top of the channel region, a thin layer of HfO_2 with dimension ~1 mm × 2 mm × 10 nm has been deposited as top gate

insulator using ECD technique. The high–κ dielectric constant of HfO_2 increases capacitance and reduces direct tunneling leakage current [53]. This capacitance enhances drain current and thus, ON–OFF current ratio increases. For preparation of HfO_2 layer, 100 mg solid $HfCl_4$ has been dissolved in 10 ml de–ionized water and sonicated for several minutes [91], [92]. Solid $HfCl_4$ has been used here since HfO_2 is insoluble in water. Then, it was deposited on the top of the channel region by using solution process and heated at temperature of 180 ºC for getting dry layer of HfO_2 [82]. Thickness of HfO_2 layer has been measured by gravimetric analysis technique using Eq. (2.11) in Chapter 2 and found to be ~10 nm [63].

Chitosan doped NiO with dimension ~1 mm × 2 mm × 50 nm has been deposited on the top gate insulator using ECD technique and used as sensing membrane. For preparation of nanostructured NiO solution, 20 ml (100 mM) of $NiCl_2.6H_2O$ and 20 ml (100 mM) of NaOH have been dissolved in 20 ml of distilled water at room temperature and deposited using ECD technique. Then the layer has been heated up to temperature of 290 ºC for getting dry thin layer of NiO. Thus, a solid nanostructured NiO thin layer has been obtained. Biocompatibility of NiO has been improved by doping of chitosan in NiO solution [50]. For preparation of 0.5 mM chitosan solution, 50 mg of chitosan has been poured in 100 ml of acetate buffer and 10 µl of chitosan solution has been added to NiO solution. For the simplicity of immobilization of biomolecules, the channel length and width have been chosen equal to 1 mm and ~2 mm, respectively. Aluminum metal has been deposited on source and drain using filament evaporation technique as explained in Chapter 3 and used for contact purpose. Because of aluminum has low resistivity, low melting point, and no contamination [88] as mentioned in Chapter 3. The whole FET has been sealed with polydimethylsilaxane (PDMS) except the sensing region for passivation purpose at the time of acetylcholine measurement [89] as mentioned in Chapter 3. Thus, complete dual gated JLCNTFET has been fabricated shown in **Fig. 4.1**. Electrochemical mechanism of CH/NiO/HfO_2/PEI/CNT has been proposed for detection of acetylcholine as shown in **Fig. 4.2.**

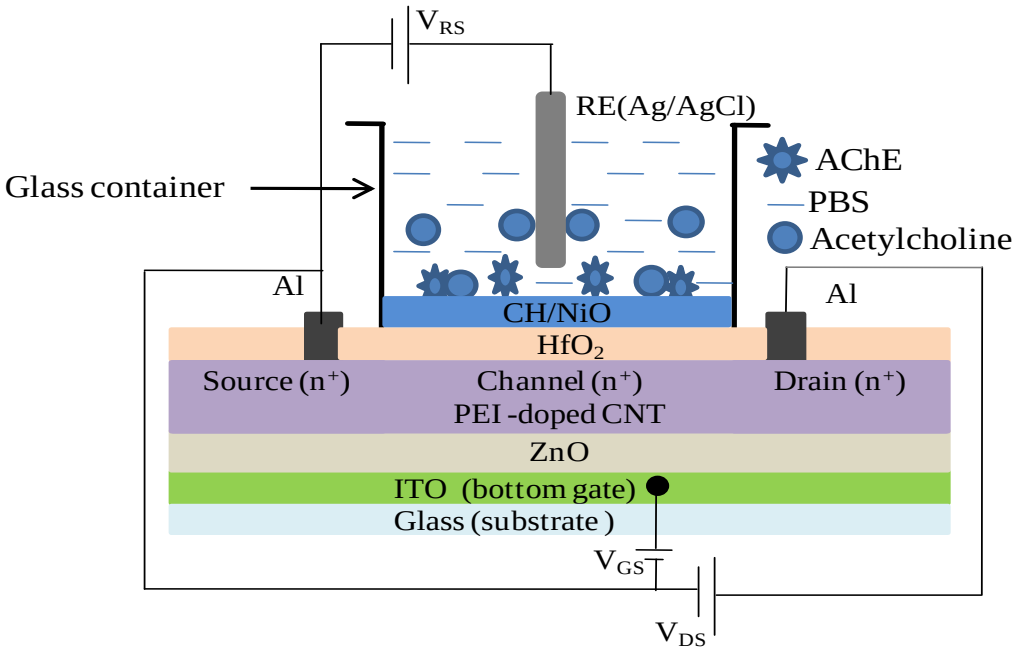

Fig. 4.1: Schematic of dual gated JLCNTFET for detection of acetylcholine

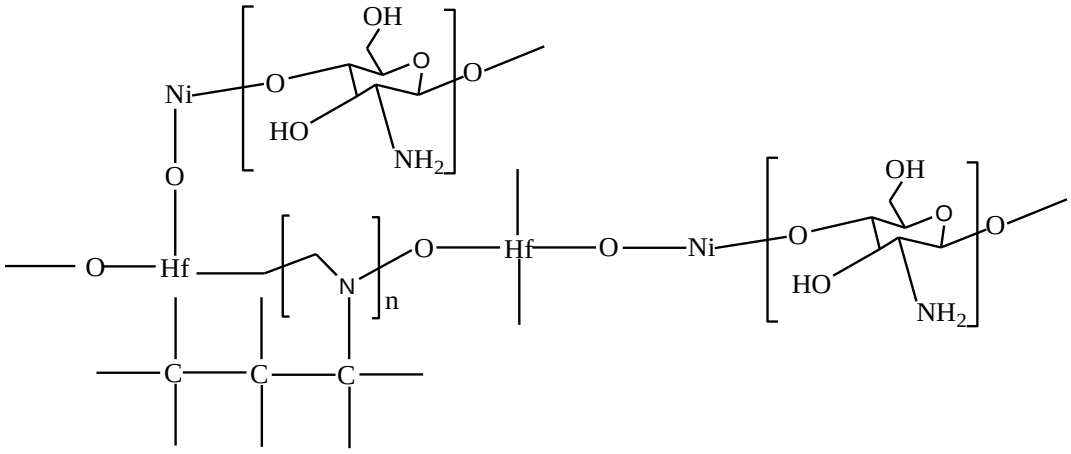

Fig. 4.2: Proposed electrochemical mechanism of CH/NiO/HfO_2/PEI/CNT

About ten FETs have been fabricated with different PEI and chitosan concentrations. Doping concentration of PEI in CNTs has been varied from 0 to 40 %. The resistance versus PEI concentration curves has been shown in Fig. 4.3 with dotted lines. It has been observed that the maximum response of the device was found at ~30 % of PEI concentration. Similarly, doping concentration of chitosan has been varied from 0 to 40 %. The resistance versus chitosan concentration curves has been shown in Fig. 4.3 with solid lines. It has been observed that the maximum response of the device was found at ~5 % of chitosan concentration.

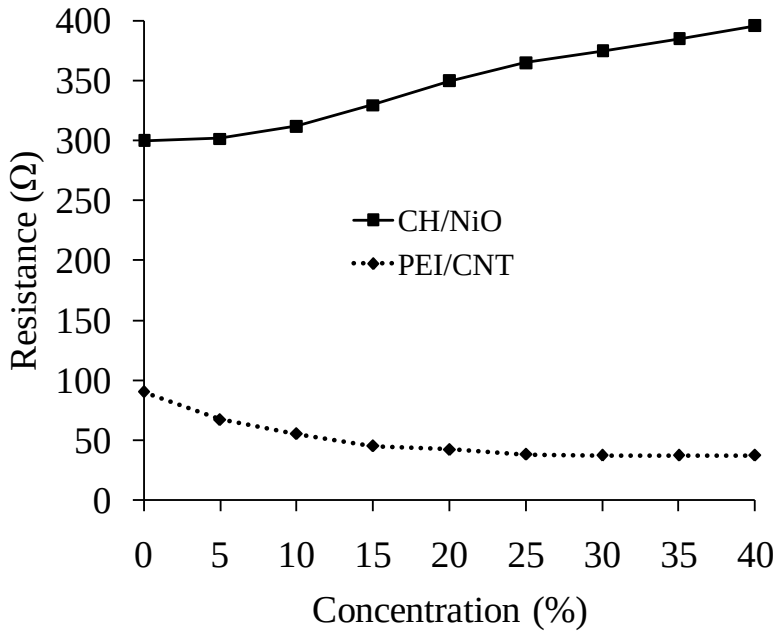

Fig. 4.3: Effect of chitosan concentration in NiO and PEI concentration in CNTs

The value of parameters used for fabrication of this device has been summarized in **Table 4.1.**

Table 4.1: Value of parameters used for fabrication of DG–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 Source and Drain	5 mm × 2 mm × 100 nm
Doping concentration of PEI in CNT	30 %
Doping concentration of CH in NiO	5 %
Dimension of sensing membrane	1 mm × 2 mm × 50 nm
Darin voltage at which experiment was performed	0.4 V
Reference voltage at which maximum response obtained	0.6 V

4.2.4. Theory and working principle of acetylcholine DG–JLCNTFET

Theory of acetylcholine DG–JLCNTFET has been explained in Chapter 2. Eq. (2.11) is the expression for total drain current of dual gated acetylcholine 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 means *n*–channel already exists for doping of PEI in CNT [85]. Since in *n*–channel FET, drain current increases with positively charged biomolecules, therefore, it is called as enhancement mode acetylcholine CNTFET. Basic enzymatic reaction for acetylcholine detection has been given by Eq. (2.13) in Chapter 2. In this Equation, enzyme AChE transforms acetylcholine in to choline and acetic acid by releasing protons (H^+) to the electrolyte solution. The enzyme reaction thus, generates or consumes protons, changing the charge at the gate surface of the FET, and consequently affects the potential difference between the gate and the source and modulates the channel current. This mechanism can be explained the site binding mechanism as mentioned in Chapter 2.

4.3. Results and discussions

4.3.1. DC characteristics of DG–JLCNTFET without acetylcholine

This experiment has been performed to measure the intrinsic voltage gain and to show the behavior of this FET outside the liquid. Before immobilization of AChE on the surface of the sensing membrane of CH/NiO, DC drain current has been recorded at different gate voltages using DMM. Drain currents have been plotted against drain voltages from 0 to 1 V, in step of 0.2 V with applied gate voltages from 0 to 1 V, in step of 0.2 V as shown in Fig. 4.4.

Intrinsic voltage gain (A_V) has been calculated from the dc characteristics curves and has been found to be ~20 using the relation $A_V = g_m/g_{ds}$, where g_m is called the transconductance and g_{ds} is called the drain conductance as mentioned in Chapter 3. The high value of A_V signifies that this dual–gated JLCNT FET can be used for amplification of small bio–signal for sensing purpose. This is the experiment for gate dependency of this device. This experiment shows that the fabricated device has MOSFET like characteristics. Since it is *n*–

channel CNTFET, therefore as gate voltage is positively increases, drain current also positively increases. Thus, it acts as an enhancement mode.

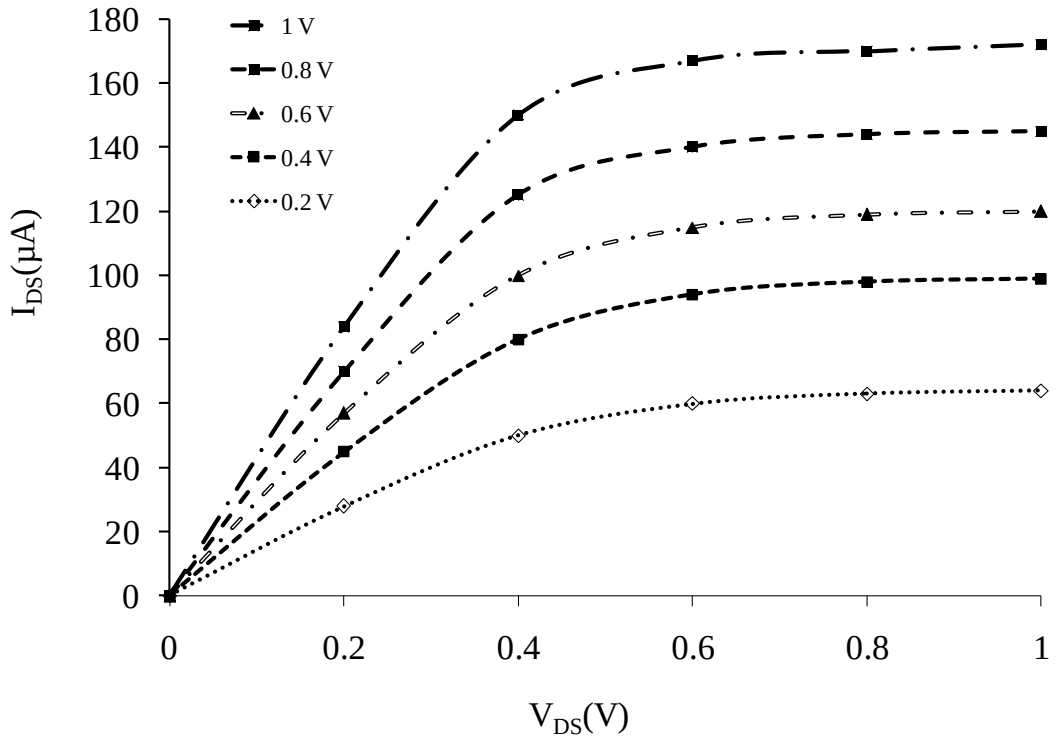

Fig. 4.4: DC curves of DG-JLCNTFET outside liquid at different Vgs.

4.3.2. Immobilization of AChE on CH/NiO sensing layer

The surface of CH/NiO has been washed with deionized water prior to being immobilized. After that, 1µl of AChE has been immobilized onto the CH/NiO surface using physical adsorption technique because, this technique has some advantages as mentioned in Chapter 2 [40]. One µl of ChOx has been chosen, because it is sufficient on the surface of the sensing membrane. Prior to being used, CH/NiO/DG–JLCNTFET has been dried overnight under desiccated conditions and washed with PBS to remove any unbound AChE and stored in a refrigerator at temperature of 4 ºC when not in use.

4.3.3. Electrochemical response measuring apparatus for acetylcholine detection

For measurement of acetylcholine, a new technique has been proposed. For this, the device DG–JLCNTFET has been inserted in a glass pot containing 20 ml of PBS (50 mM, pH 7) with a reference electrode of Ag/AgCl as shown in **Fig. 4. 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. The Positive supply has been connected to reference electrode and bottom gate (bottom gate connection is not shown) and negative supply has been connected to source. A DMM has been connected to record the drain current (I_{DS}). Positive terminal of DMM has been connected to the drain side of DG-JLCNTFET and negative terminal of DMM has been connected to the positive side of the drain voltage through a small resistance. This resistance has been used to reduce large drain current through the DMM. Initially, 100 µl stock solution of acetylcholine (0.01–0.2 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, drain response has been plotted as shown in **Fig. 4.6**. From this graph, maximum response has been obtained at reference voltage of 0.6 V. Therefore, in further experiments, reference voltage was kept constant at 0.6 V.

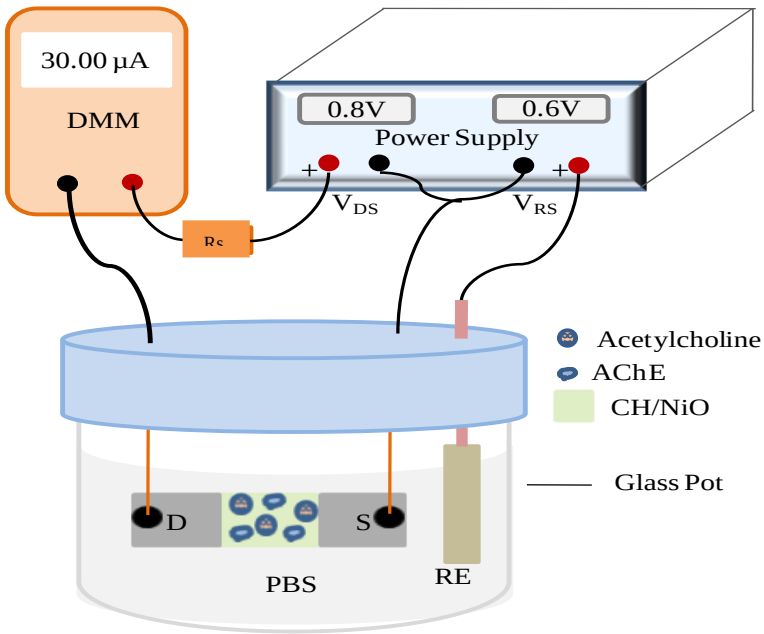

Fig. 4.5: Electrochemical response measuring apparatus for acetylcholine

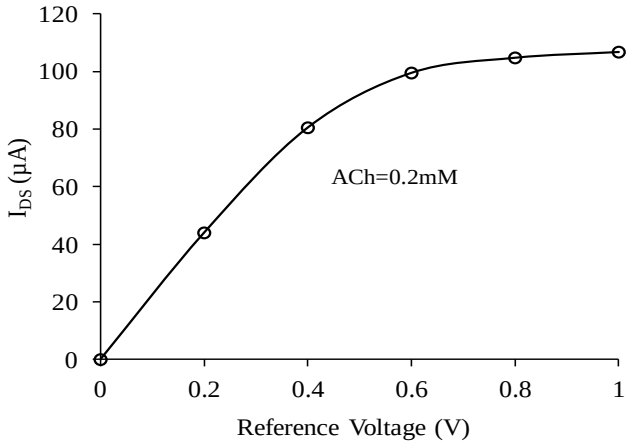

Fig.4.6: Reference voltage for acetylcholine concentration at V_{DS} = 0.4 V

4.3.4. Electrochemical characterization of acetylcholine DG–JLCNTFET

Drain characteristics of the acetylcholine ENFET has been obtained by plotted drain voltage (V_{DS}) Versus drain current for acetylcholine concentration from 0.01 to 0.2 mM at a fixed reference voltage of 0.6 V as shown in **Fig. 4.7**. It has been observed that when drain voltage reaches at 0.4 V, saturation occurs in drain current like characteristics of a FET.

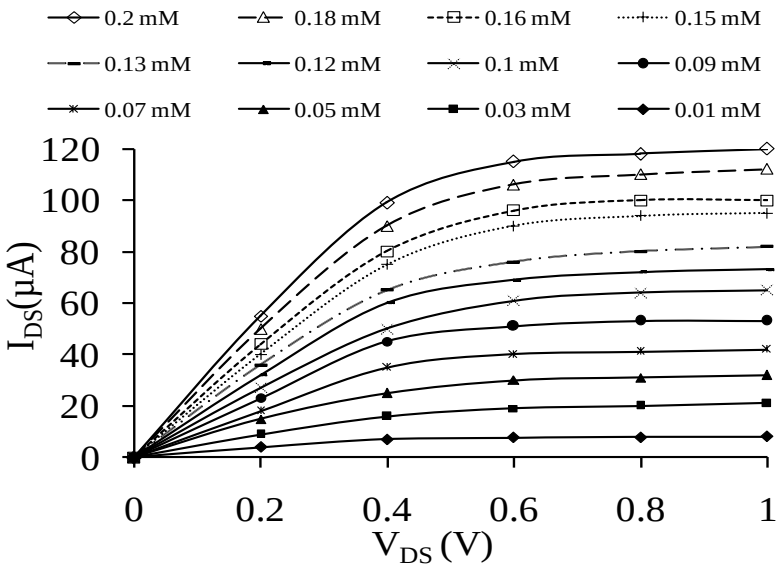

Fig. 4.7: Drain characteristics for different acetylcholine concentrations

Fig. 4.8 shows the linearity of the sensor from 0.01 to 0.2 mM corresponding to the drain voltage of 0.4 V. This experiment was performed at 25 ºC and pH 7. The experiments were repeated 10 times for every acetylcholine samples using same procedure and condition as mentioned and only slight variation of the response has been observed. The standard deviation and the regression coefficient for these samples have been found to be 60 µM and 0.999, respectively using basic relation.

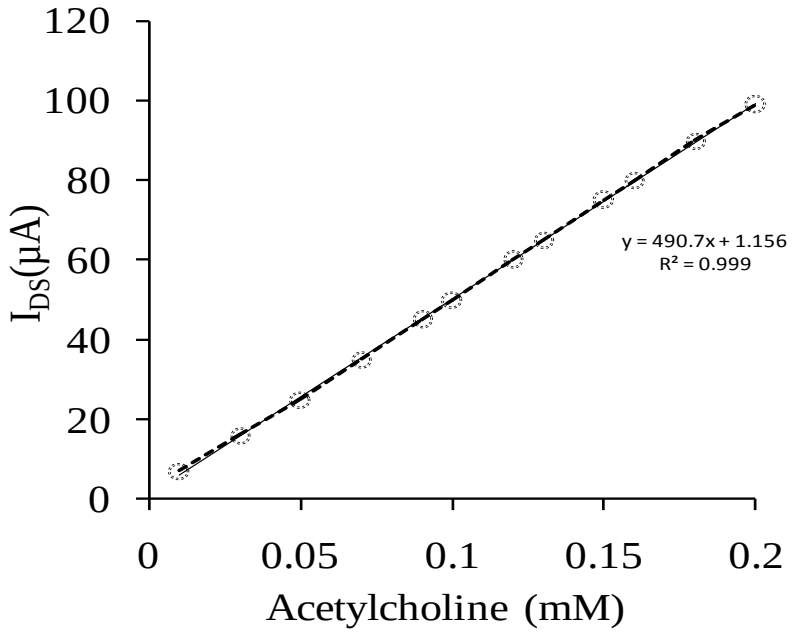

Fig.4.8: Linearity for different acetylcholine concentrations at V_{DS} = 0.4 V

4.3.5. Activity of AChE on acetylcholine DG–JLCNTFET

The electrochemical activity of enzyme AChE can be estimated from the Michaelis–Menten constant (K_m). The value of K_m has been calculated using Linweaver–Burk plot as shown in **Fig. 4.9.** The process of Linweaver–Burk Plot has been explained in Chapter 2. From this graph the value of K_m has been found to be 0.2 mM using Eq. (2.17). This small value of K_m reveals that enzyme AChE has high activity for acetylcholine detection.

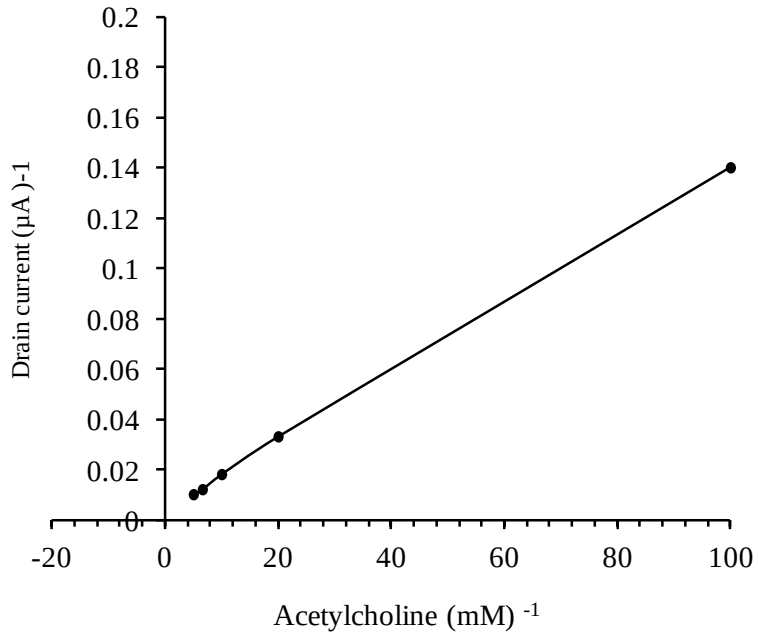

Fig. 4.9: Lineweaver–Burk Plot for acetylcholine concentration

4.3.6. Interfacial potential and sensitivity of acetylcholine DG–JLCNTFET

The interfacial potential ($\Delta\Psi_0$) developed between electrolyte solution (acetylcholine) and oxide layer (sensing membrane) has been calculated using Eq. (2.14) as mentioned in C**hapter 2.** The change in threshold voltage of dual–gated JLCNTFET is equal to multiplication of shift in the surface potential and ratio of top to bottom gate capacitors. Thus, change in threshold voltage of this dual–gated acetylcholine JLCNTFET can be calculated from Eq. (4.1) [24], [93].

$$\Delta V_{TH,\text{top}}(\text{EN}) = \frac{C_{top}}{C_{bottom}} \Delta\Psi_0 \tag{4.1}$$

where, C_{top} and C_{bottom} are the capacitances of top and bottom gate, respectively.

The thickness of top gate insulator has been kept constant ~10 nm while thickness of bottom gate insulator has been varied. Because of the large thickness of bottom gate insulator suppresses total drain current and acts as single gated FET. As a result, drain response corresponds to Nernstian response. Again low thickness of bottom gate insulator provides bottom gate leakage current that affects sensitivity of device. Thus, an identical thickness of bottom gate insulator is required. Identical thickness bottom gate insulator has been chosen in the range from 10 nm to 100 nm and corresponding to these variations, the threshold voltage has been found from 196 mV to 1.9 V, where drain current sufficiently flows without affecting sensitivity.

Fig. 4.10 shows the plot of acetylcholine concentration and threshold voltage at temperature 25 °C. Sensitivity of this ENFET has been obtained from this graph. For this device, sensitivity is a ratio of shift in the threshold voltage with respect to the concentration of acetylcholine. Sensitivity of this ENFET has been calculated and found to be 1.25 V/decade. Thus, using dual–gated junctionless CNTFET sensitivity can be enhanced beyond Nernstian response.

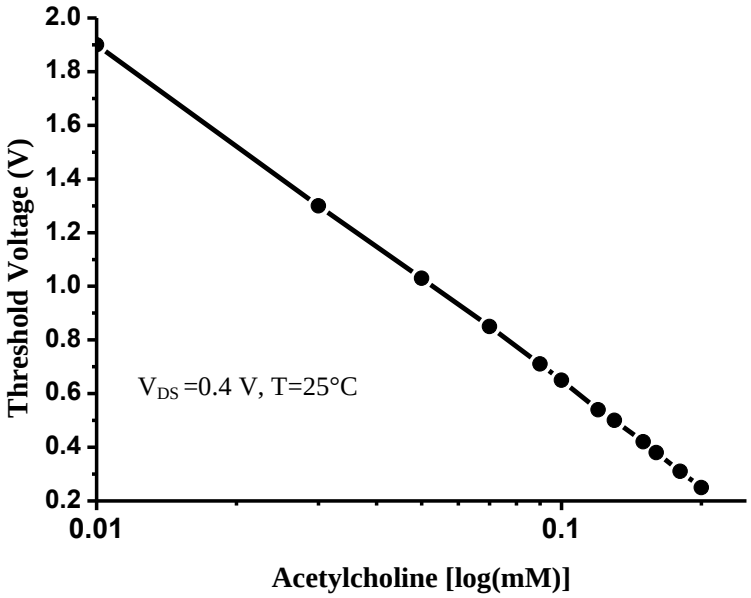

Fig. 4.10: Sensitivity calculation of acetylcholine concentration.

4.3.7. Limit of detection and regression co-efficient calculation

Limit of detection (*LoD*) has been calculated using Eq. (2.18) in **Chapter 2** and found to be 0.37 µM. This reveals that at least 0.37 µM of acetylcholine can be detected using this ENFET. The regression coefficient has been calculated using Eq. (2.21) in **Chapter 2** and found to be ~0.999. Here, the value signifies that there is a good correlation between enzyme and acetylcholine in this device.

4.3.8. Effect of temperature and pH on acetylcholine DG–JLCNTFET

The effect of temperature on CH/NiO/DG–JLCNTFET has been investigated by measuring drain current using the set up as shown in **Fig. 4.5**. The temperature of the set up has been maintained by inserting it in to a temperature booth. The temperature of this booth has been monitored using a thermometer. Taking 0.2 mM of acetylcholine solution and by varying the temperature from 15 to 45 ºC, drain current has been recorded using DMM. This experiment has been performed using PBS of pH 7 and 50 mM. The recorded drain current has been plotted against given temperature as shown in **Fig. 4.11.** The graph shows that drain response increases up to temperature of 30 ºC and then decreases beyond temperature of 35 ºC. In case of silicon FET, as temperature increases, the drain current decreases. But in case of CNTFET, drain current increases as temperature increases. Again, at higher temperature, activity of enzyme AChE becomes low and hence, drains response decreases. The maximum response for acetylcholine has been found in the temperature range of 30 to 35 ºC.

Similarly, the effect of pH on this device has been estimated taking 0.2 mM of acetylcholine solution and by varying pH from 5 to 9 at temperature of 25 ºC. Using the set up as shown in **Fig. 4.5,** drain current has been recorded using DMM at different pH and plotted as shown in **Fig. 4.12.** The graph shows that drain response has increased up to pH 7 and then decreased again from pH 8 onwards. This mechanism has happened due to enzyme activity. Therefore, this device can be used at pH 7–8.

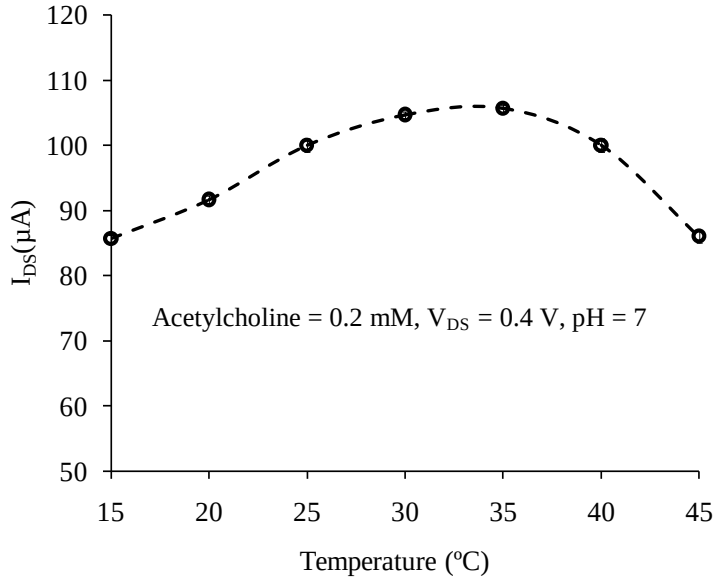

Fig.4.11: Effect of temperatures on acetylcholine response

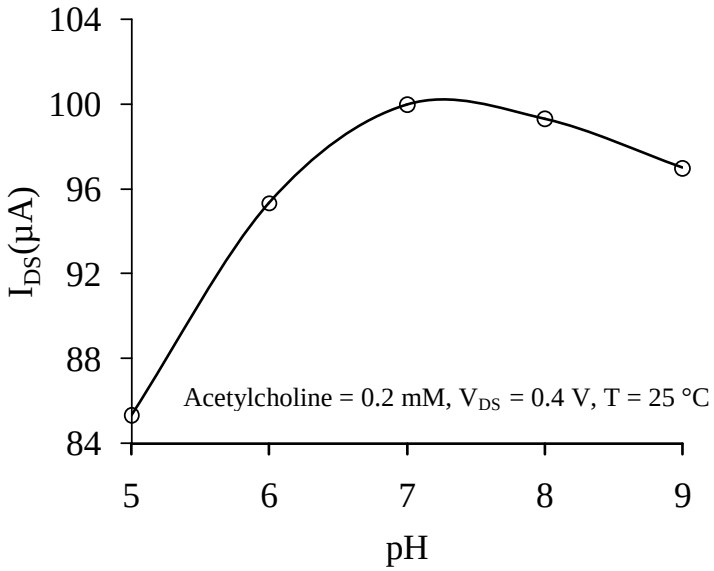

Fig. 4.12: Effect of pH on acetylcholine response

4.3.9. Interference and repeatability test

Interference on acetylcholine has been investigated using the same procedure and at the environment as explained above. For this, 0.2 mM solution of acetylcholine has been tested in the presence of urea (0.2 mM), glucose (0.2 mM), uric acid (0.2 mM), ascorbic acid (0.2 mM), dopamine (0.2 mM), lactic acid (0.2 mM) and heparin sodium (0.2 mM) at pH 7 and temperature of 25 ºC. The drain current has been recorded using DMM and plotted against the concentration of affordable mentioned biomolecules as shown in **Fig. 4.13**. The graph

reveals that acetylcholine response has no affect due to presence of other biomolecules. The percentage of interference has been calculated using Eq. (2.20) in C**hapter 2**. It has been found that average percentage of interference of acetylcholine with other biomolecules is ~1.5 %.

The repeatability of this acetylcholine ENFET has been investigated taking 0.2 mM of acetylcholine solution and experiment has been for repeated 10 times at temperature 25 ºC and pH 7. Drain current has been recorded using DMM and the graph has been plotted as shown in **Fig. 4.14.** It has been observed that the acetylcholine response has no large variation after several repetitions.

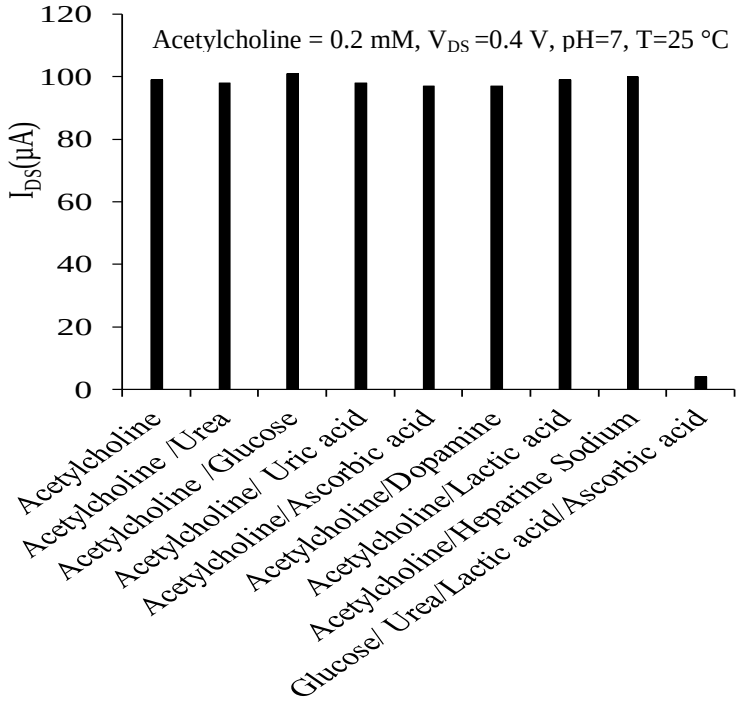

Fig. 4.13: Interference on acetylcholine with other biomolecules.

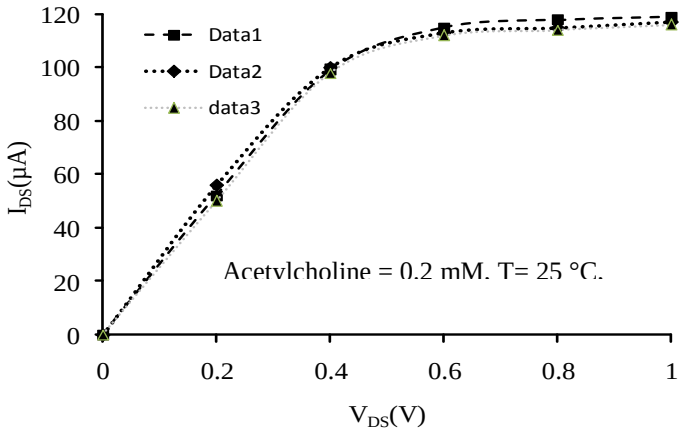

Fig. 4.14: Plot of three data for repeatability of the device.

4.3.10. 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 0.2 mM of acetylcholine solution and by performing the experiments at the same condition. Both the devices have produced almost same results for 0.2 mM of acetylcholine solution (**Fig. 4.15**). 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 2 month upto 8 months, taking 0.2 mM of acetylcholine solution. The degradation plot shown in **Fig. 4.16** has shown average ~99% stability results after 8 months storage.

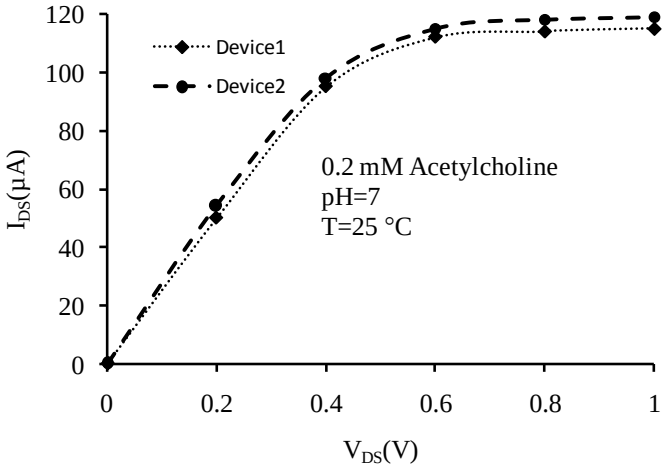

Fig. 4.15: Reproducibility plot of two devices

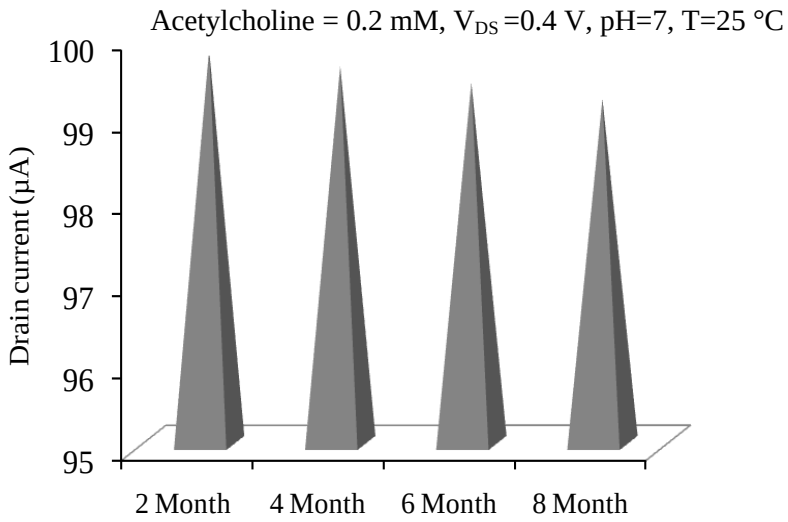

Fig. 4.16: Stability plot of the device.

The results obtained from this work have been summarized in **Table 4.2** and a comparison studies about sensitivity, *LoD*, and K_m with other reported works using FET based biosensors have been given in **Table 4.3.**

Table 4.2: Output results obtained from acetylcholine DG–JLCNTFET

A_v	Linearity mM)	Interference	Repeat–bility	Stability	Optimum temperature	Optimum pH	Response time (s)
20	0.01–0.2	1.5 %	10 times	240 days	30–35 ºC	7–8	1

Table 4.3: Comparison of acetylcholine DG–JLCNTFET with other reported ENFETs

Sensor type	Sensing Materials	Sensitivity (interfacial potential)	Sensitivity (Drain current)	LoD /mM	K_m /mM	Ref.
Si–FET	Al_2O_3	44.2 mV/dec	–	–	–	[22]
Si–FET	SnO_2	58 mV/pH	–	–	–	[94]
DGJL–CNTFET	CH/NiO	1.25 V/dec	500 µA/ mM/mm^2	37× 10^{-5}	0.2	[This work]

This table concludes that this acetylcholine ENFET is more advantageous than other acetylcholine ENFETs as reported in the literature. It has long stability, small value of K_m, *LoD* and high sensitivity with fast response time.

4.4. Summary

In this work, a sensitive integrated *n*–type CNT based dual–gated junctionless field effect transistor has been successfully fabricated for detection of acetylcholine. The sensitivity of this device has been found to be 1.25 V/decade with good linearity from 0.01 to 0.2 mM at temperature of 25 ºC and pH 7. This technique has overcome the limitation of Nernstian equation related to maximum sensitivity. The sensitivity depends on the ratio of coupling capacitor of top and bottom gates. It has been observed that this biosensor has reproducibility, repeatability and insignificant interference with other biological parameters. This FET based biosensor requires minimal instrumentation and can be easily fabricated. Thus, this nano–structured dual gated JLCNTFET can be used in the field of bioelectronics and biomedical applications.