Chapter 3

Fabrication and characterization of Acetylcholine sensitive field effect transistor (AchFET) to use as an analog in a neuron circuit.

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3.1 An overview

Acetylcholine (neurotransmitter) is a chemical secreted by neuron which is responsible for action potential generation and transmission of action potential from one neuron to another through synapse. Detection of acetylcholine is very important for generation of action potential and their propagation [3]. Acetylcholine is responsible for opening and closing of gates in a neuron to reproduce action potential. When acetylcholine is secreted in the synapse, it triggers the opening of gates in neuron membrane which in turn helps the ions move selectively inside the membrane and results in change of membrane potential. Unable to secrete acetylcholine by nerve axon may lead to Alzheimer's and Parkinson's disease. Here, a junctionless CNT (carbon nanotube) based AchFET (acetylcholine field effect transistor) was fabricated and characterized by electrochemical deposition technique (ECD) to use in an electronic circuit for reproduction of action potential. This circuit was named as NEUROAchFET circuit. CNT-AchFET was used because it is easy to synthesize CNT into nano form, high mobility of charge carriers, low internal contact resistance and high sensitivity [3]. AchFET is an ENFET (enzyme modified field effect transistor) where biomolecules are integrated with ISFET. ISFET (ion sensitive field effect transistor) is basically a MOSFET (metal oxide semiconductor field effect transistor) where gate terminal is modified for measuring ion concentration. The drain current changes with the change in ion concentration.

3.2 Experimental

3.2.1 Materials and Chemicals/ Reagents

Acetylcholine esterase (AChE) (activity~ 301 U/mg) and acetylcholine (Ach) was obtained from Sigma. Single walled carbon nanotube (SWCNT) was bought from Alibaba of purity nearly 99%. The length and diameter are ~20 μm and ~100 nm, respectively. Indium tin oxide (ITO) coated glass of sheet resistance ~15 Ω/sq was

purchased from NANOCS. Other chemicals and materials such as Hafnium dioxide (HfO_2), zinc oxide (ZnO), chitosan (CH) and nickel oxide (NiO), polyethylene imine (PEI) were of analytical grade.

3.2.2 Preparation of solutions

Acetylcholine stock solution (0.2 mM) was prepared in de-ionized water. Dilution process was used for preparation of 0.01 mM to 0.15 mM acetylcholine solution. For preparation of Acetylcholine esterase solution of 1 M, 1mg of powder Acetylcholine esterase (AchE) was mixed with 1ml of phosphate buffer saline (PBS) of 50 mM and pH 7. It was stored at 4^oC except when in use. ITO and all other apparatus was cleaned with a solution of water (H_2O), hydrogen peroxide (H_2O_2) and ammonium hydroxide (NH_4OH) in the ratio of 5:2:2. Phosphate buffer saline (50 mM, pH 7.0) was prepared using monosodiumphosphate (NaH_2PO_4) and disodium phosphate (Na_2HPO_4).

3.2.3. Fabrication of AchFET

A junctionless AchFET was fabricated on an indium tin oxide (ITO) coated glass as substrate of dimension ~5 mm × 2 mm. A thin layer of ZnO (Zinc oxide), a low-κ dielectric (κ~ 1.5) was deposited on ITO to act as an insulator to avoid leakage current from channel to ITO. ZnO solution was prepared by dissolving 10 mg Zinc acetate ($Zn(CH_3COO)_2$ and 2 ml ammonium hydroxide (NH_4OH) with 10 ml of distilled water. On top of it, PEI (polyethylene imine) doped CNT (carbon nanotube) was deposited, which acts as n-type source (S), drain (D) and channel region. The dimension of the layer was found to be ~5 mm × 2mm × 100 nm. PEI doping makes CNT an n-type semiconductor (~30% doping concentration). HfO_2 (Hafnium dioxide), a high-κ dielectric (κ~25) was deposited as gate insulator on the top of the channel region. This high-κ dielectric layer increases capacity and reduces direct tunneling leakage current.The thickness of HfO_2 was measured to be ~10 nm. HfO_2 solution was prepared by dissolving 100 mg solid $HfCl_4$ in 10 ml de-ionized water and then sonicated for several minutes. The solution was deposited and heated at temperature~180^o C for getting dry HfO_2 film.

An additional sensing membrane was deposited on top of the gate insulator (HfO_2) of the ENFET. Nickel oxide (NiO) and chitosan (CH) was deposited as the sensing membrane (dimension ~1 mm×2 mm×50 nm). The solution for NiO film was prepared

by dissolving 20 ml (100 mM) of each $NiCl_2.6H_2O$ and NaOH with distilled water and heated after deposition at 290ºC to get a solid NiO sensing material. Chitosan (10 µl) was mixed with the NiO solution to increase the biocompatibility of NiO. Chitosan of concentration 0.05 M was prepared by adding 50 mg in 100 ml of acetate buffer. Nickel oxide has many applications in biosensor due to its biocompatibility, high chemical stability, good mechanical strength, high electron transfer etc.

Aluminum was deposited to form source(S) and drain (D) contact using filament evaporation technique. It was chosen because of its advantages such as low melting point, low resistivity, no contamination and ease for deposition. The whole fabricated device was coated with PDMS (Polydimethysilxane) for passivation except the sensing area at the time of the detection of acetylcholine. A complete schematic of AchFET is shown in Fig.3.1. Fig. 3.2 shows the electrochemical mechanism of CH/NiO/HfO_2/PEI/CNT for acetylcholine detection.

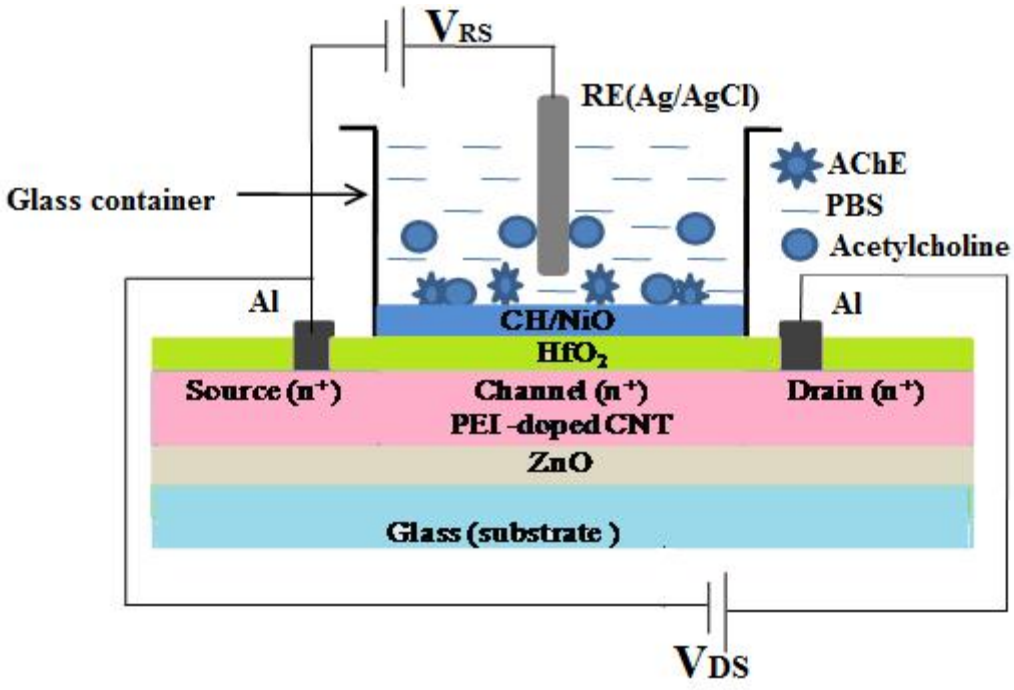

Fig.3.1: Schematic diagram of junctionless FET for acetylcholine detection (AchFET)

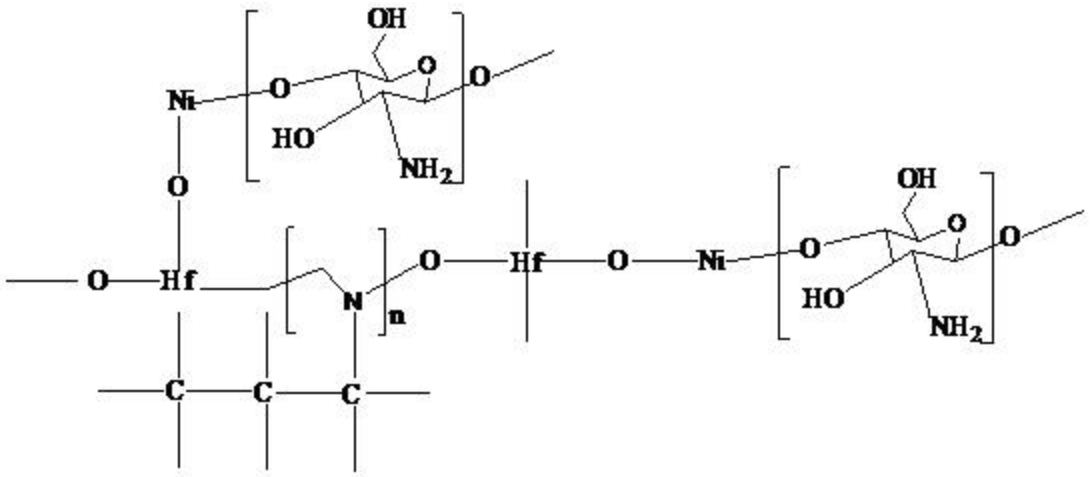

Fig.3.2: Electrochemical mechanism of CH/NiO/HfO_2/PEI/CNT

The doping concentration of PEI was varied in CNT from 0 to 40%. A curve was plotted between PEI concentration and resistance. The maximum response occurs at a concentration of 30% as shown in Fig. 3.3 .Similarly, Chitosan was doped in NiO from 0 to 40% and maximum response was observed at 5% of chitosan doping concentration. Fig.3.3 also shows resistance for each chitosan doping concentration in NiO. Table 3.1 summarizes the parameter values used in the fabrication of AchFET.

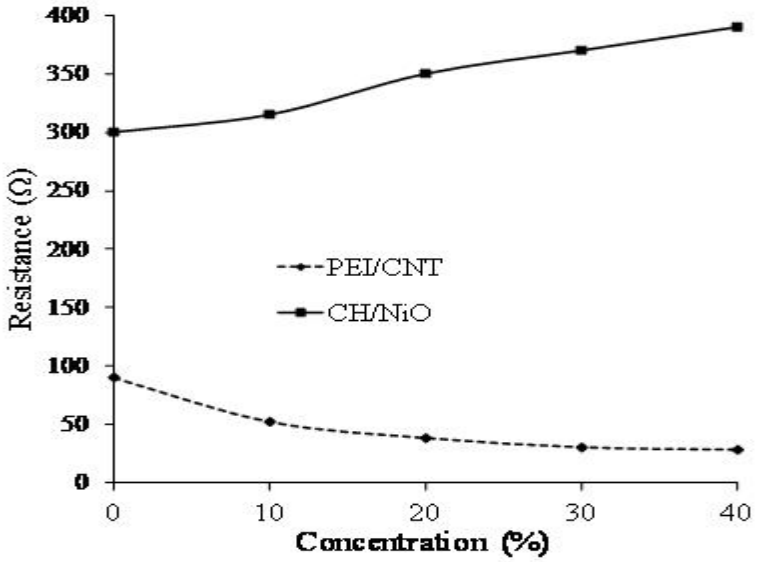

Fig. 3.3: Effect of chitosan concentration in NiO and PEI concentration in CNTs

Table 3.1: Value of parameters used for fabrication of AchFET

Parameters	**Value used**
Gate dimension	1mm×2mm×10 nm
Channel dimension	1mm×2mm×100 nm
S/D dimension	5mm×2mm×100nm
PEI-doping concentration in CNT	30 %
CH doping concentration in NiO	5 %
Sensing membrane dimension	1mm×2mm×50 nm
Drain voltages	0.4 V
Reference	0.6 V

3.2.4 Theory and Working principle of AchFET

Theory of CNTFET is described in Chapter 2 and the equation (2.47) is the drain current for AchFET. CNT was doped with PEI for making n channel AchFET. AchFET works in enhancement mode and so drain current increases with positive charged biomolecules. Acetylcholine esterase (AchE) is an enzyme which breaks down acetylcholine (biomolecule) into choline and acetic acid and releases protons to the solution.These enzymatic reaction generates protons and thus changes the gate potential according to site binding theory [25]. The potential difference between gate and the

source results in modulation of the drain current. Site binding mechanism is confined to surface i.e. with the insulating surface and sensing membrane and so the drain and source are not affected by the reaction as these are not connected to the sensing membrane [3]. The reference electrode used is non–polarizable making the drain and source unaffected.

3.3 Results and Discussion

3.3.1. DC characterization of AchFET without Acetylcholine

The behavior of the AchFET was studied before in-liquid measurements. For this, aluminum (Al) was deposited as metal gate using filament evaporation method. The Drain current (I_D) was measured using DMM at different drain voltages (V_{DS}) in the range from 0 to 1 V in steps of 0.2 V and at different gate voltages (V_{GS}) from 0 to 1 V in steps of 0.2 V. Fig.3.4 shows the drain characteristics of the device . The device shows good linearity up to 0.4 V and then drain current saturates just like MOSFET characteristic curves.

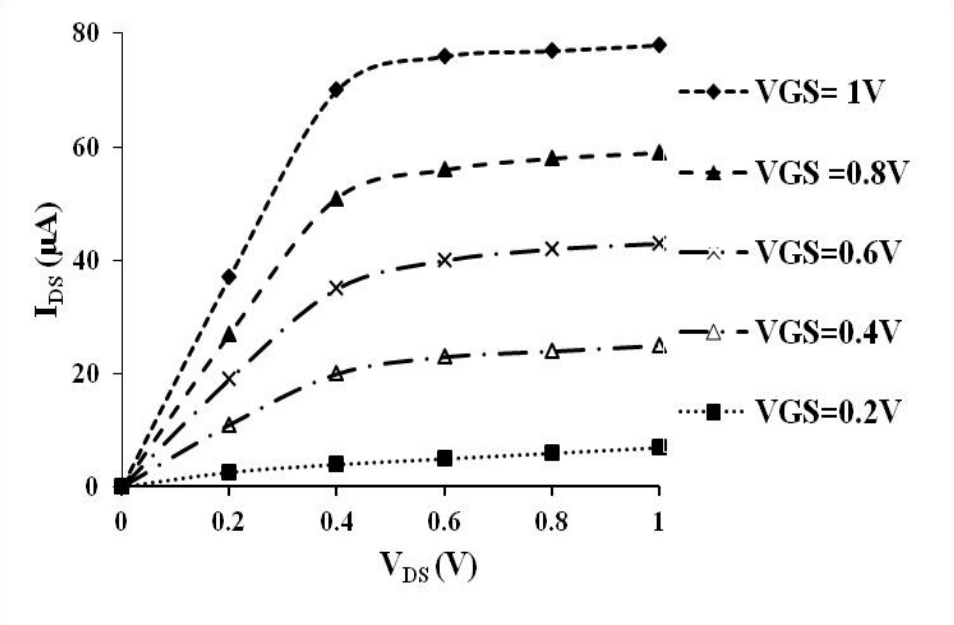

Fig.3.4: Drain characteristic curves for JLCNTFET outside liquid at different V_{GS}

3.3.2. Immobilization of AchE on CH/NiO sensing layer

The surface of CH/NiO sensing layer was washed before use. Glutaraldehyde (0.1%) was added to the surface of the AchFET for modification and then washed with deionized water. Immobilization of AchE (1 µl) was carried out by using physical adsorption technique on the sensing membrane [3]. Then the AchFET was dried for 12 hours prior to use. It was washed with PBS for any trace of AchE and then was stored at 4^0C except when in use.

3.3.3 Electrochemical response measuring apparatus for acetylcholine detection

Fig.3.5 shows the measurement set up for acetylcholine. For acetylcholine measurement, AchE was immobilized to the surface of the device before sealing it with PDMS. Acetylcholine was added by micropipette (100µl) to observe the change in drain current (I_D) for each time the acetylcholine was added to PBS in the glass pot. In presence of water, AchE breaks down ACh to choline and acetatic acid and releases H^+ions.These protons affect the potential of the gate of the device and in turn affect the gate to source potential modulating the drain current. A reference electrode of Ag/AgCl(Silver chloride) was used. Then the drain to source voltage was connected (V_{DS}) and changed in steps to observe the change in drain current. V_{GS} (gate source voltage) of fixed voltage 0.6 V was applied. Power supply was connected to drain and source terminals, where positive terminal of the battery was connected to drain and negative terminal of the battery was connected to source terminal. Reference electrode was connected to positive of the battery and source was connected to the negative terminal of the battery. In the next step, DMM (Digital Multimeter) was used to measure the drain current. The positive terminal of the DMM was connected to the positive node of the drain voltage through a resistance (R) and negative terminal of DMM was connected to the drain of the AchFET.

The drain current was measured for different concentration of acetylcholine (0.01-0.2 mM) by varying the reference voltage from 0 to 1 V in steps of 0.2 V shown in Fig. 3.6.Maximum response was found to be at 0.6V, so reference voltage of 0.6 V was fixed for further measurements.

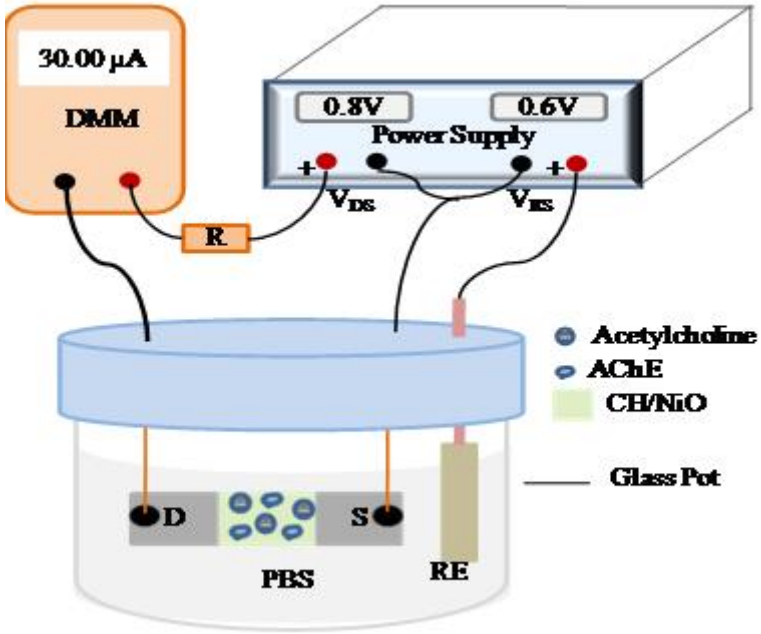

Fig. 3.5: Electrochemical response measuring apparatus for acetylcholine detection

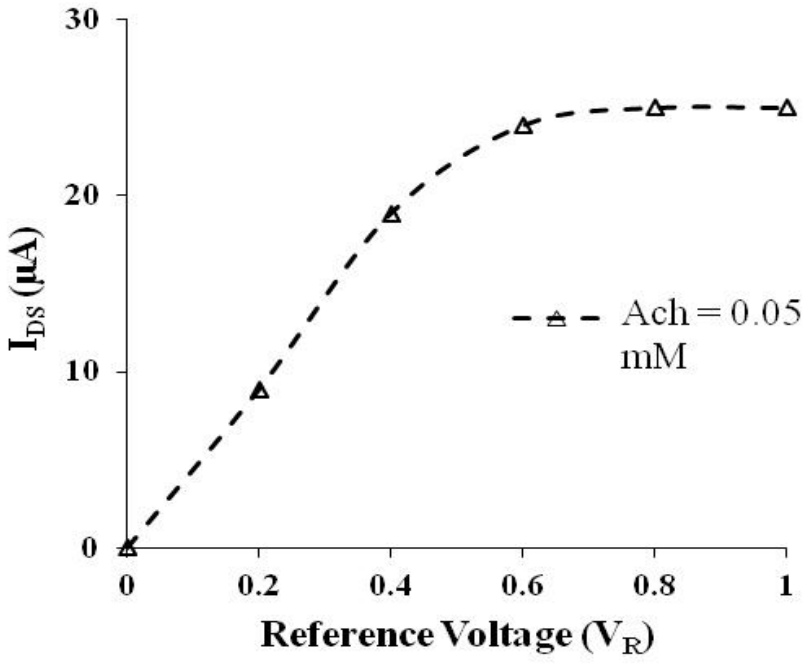

Fig.3.6: Reference voltage for acetylcholine concentration at V_{DS} = 0.4 V.

3.3.4 Electrochemical Characteristics

Characteristic curves for AchFET was plotted between drain current (I_D) and drain to source voltage (V_{DS}) for different concentration of acetylcholine (0.01-0.2 mM) as shown in Fig. 3.7. The reference voltage was kept constant at 0.6 V. From this figure, it is clear that saturation of drain current occurs as in case of MOSFET and at a drain to source voltage of 0.4 V. Hence, the drain to source voltage was fixed at 0.4 V for further experiments. Fig.3.8 shows the linearity of drain current with acetylcholine concentration at V_{DS} = 0.4 V. For this experiment, temperature was maintained at 25 ºC and at pH 7.0. This experiment was performed ten times for different concentration of Acetylcholine and very little variation in response was observed.

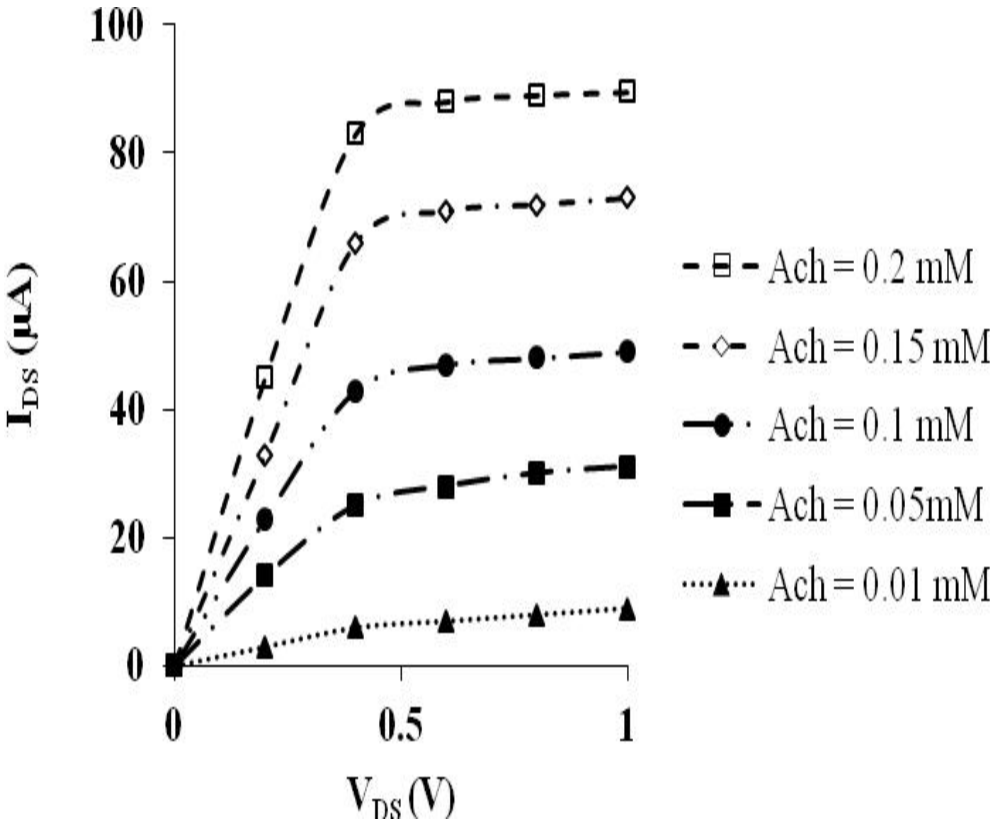

Fig.3.7: Drain characteristic curves for Acetylcholine concentration at 25 ˚C and pH 7.

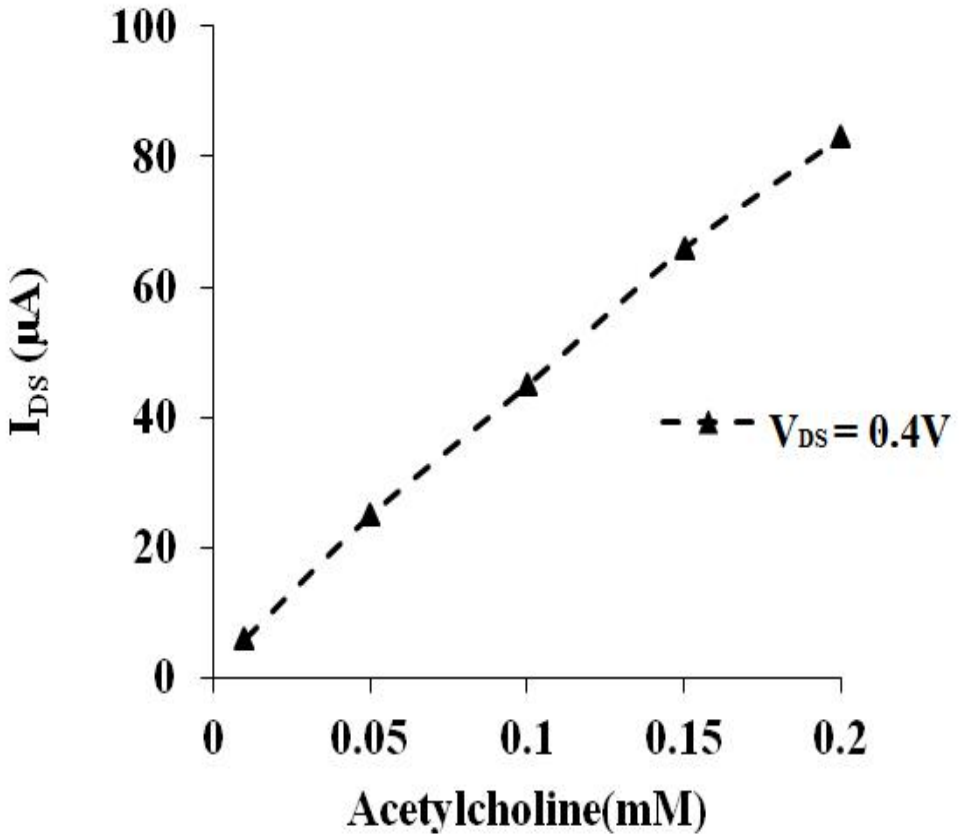

Fig.3.8: Drain current for different acetylcholine concentration

A graph was plotted between I_D versus V_{GS} for different concentration of acetylcholine at temperature of 25 ºC for determination of threshold voltage (V_{TH}) of the AchFET as shown in Fig. 3.9. Threshold voltage at various concentration was determined from the slope of the curves drawn by extrapolation in linear region method [25]. Then, a graph was drawn between V_{TH} and acetylcholine concentration as shown in Fig.3.10. From this graph, sensitivity (i.e. shift in threshold voltage to acetylcholine concentration) of the AchFET was found to be 57 mV/decade.

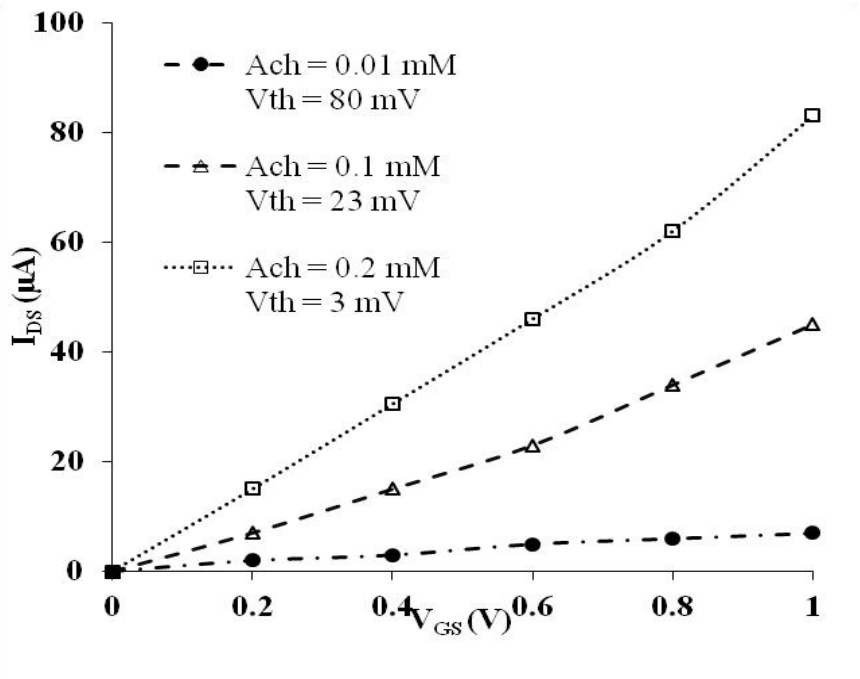

Fig.3.9: Drain current versus gate source voltage graph at V_{DS} = 0.4 V

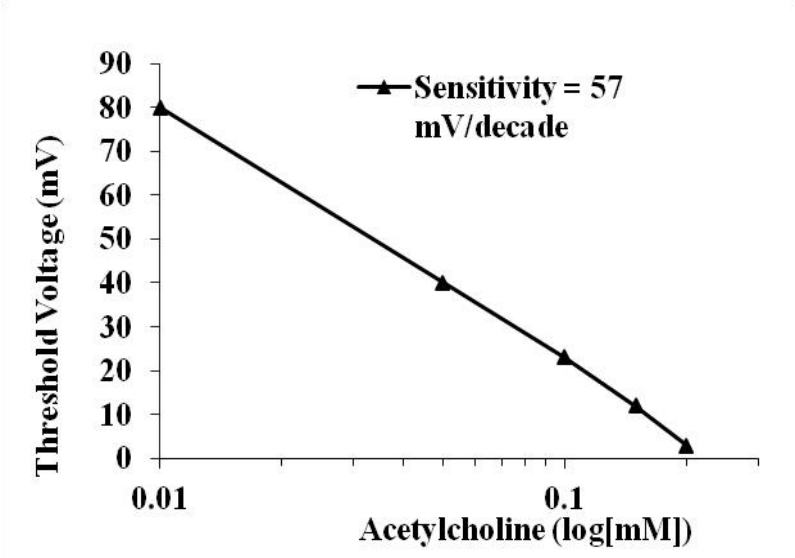

Fig. 3.10: Sensitivity for AchFET

3.3.5 Effect of temperature, pH and other biomolecules on AchFET

The set up shown in Fig.3.5 was used for measurement of temperature effect. The temperature of the set up was maintained constant by inserting it into temperature bath and monitored with the help of a thermometer. The drain current was recorded by varying the temperature in the range of 15 to 45 °C as shown in Fig.3.11. The variation of

drain current with respect to temperature was measured at 0.05 mM of Ach concentration. It was performed in PBS solution (50 mM) and at pH 7. It was observed that drain current increases with increase in temperature up to 35 ℃ and then decreases. This increase in drain current with increase in temperature is a characteristic of CNTFET [76]. However, the enzymatic activities become low at higher temperature and as a consequence drain current decreases. From the graph, it can be concluded that maximum response occurs at about 35 ℃. Thus, it is preferable to use AchFET in this range.

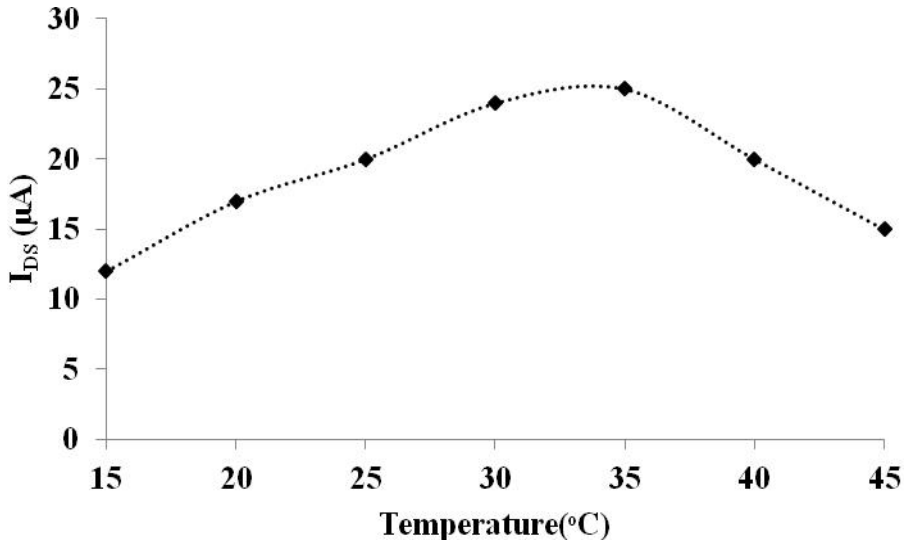

Fig. 3.11: Temperature effect on AchFET.

The effect of pH on the device was studied using the set up shown in Fig. 3.5. Fig. 3.12 shows the variation of drain current with pH in the range 5 to 9 at temperature 25 ℃ using DMM at acetylcholine concentration of 0.05 mM .The response of the device increases up to pH 7 and then decreases. This is due to enzyme activities occurring in the device. Thus, the AchFET can be used in the pH range 7 to 8.

Interference of urea, uric acid and glucose on the measurement of acetylcholine was studied. Fig. 3.13 shows the effects of urea (0.05 mM), uric acid (0.05 mM) and glucose (0.05 mM) on acetylcholine (0.05mM) at pH 7 and 25 ℃. Drain current was measured using DMM and found that there was very little or no effect on the device performance. The average interference percentage was found to be nearly 1.5%.

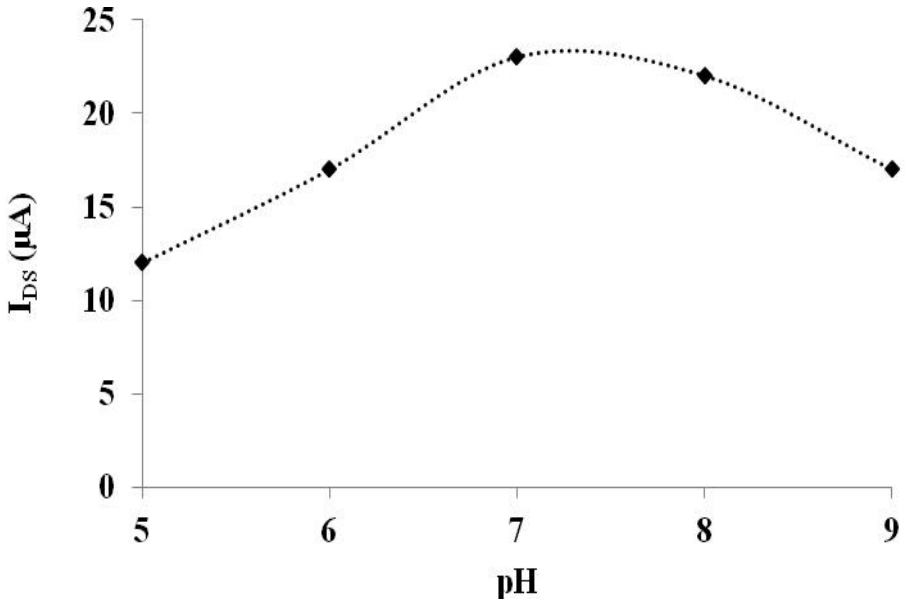

Fig.3.12: Effect of pH on AchFET.

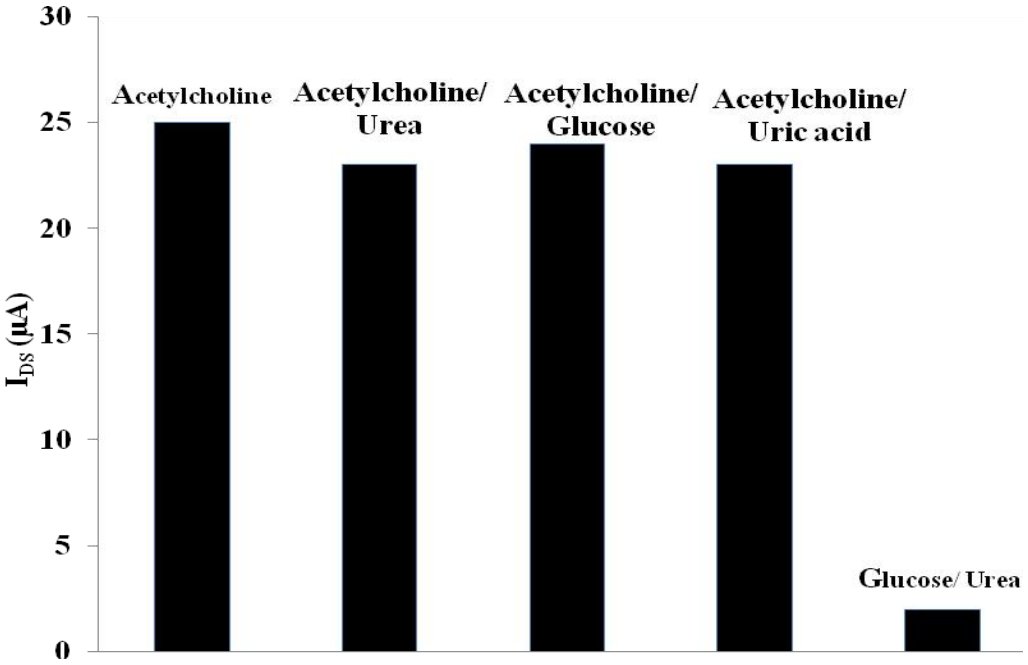

Fig.3.13: Effect of other biomolecules on AchFET.

3.3.6 Repeatability, Reproducibility and Stability Test

The repeatability test of the device was performed at acetylcholine concentration of 0.05 mM at 25 ℃ and pH 7. The drain current of the device was recorded using DMM by varying drain to source voltage. This procedure was repeated for 10 times and found little variation in device performance as shown in Fig. 3.14.

Two AchFETs was fabricated using the same procedure to study the reproducibility of the device. The devices was tested after one week under the same condition at acetylcholine concentration of 0.05 mM and same results were obtained as shown in Fig.3.15. For stability test, the drain current of the device was measured in every 2 months for 0.05 mM concentration of Acetylcholine at 25 ℃ and pH 7. The stability was found to be 99% as shown in Fig.3.16. It was kept in a refrigerator when not in use.

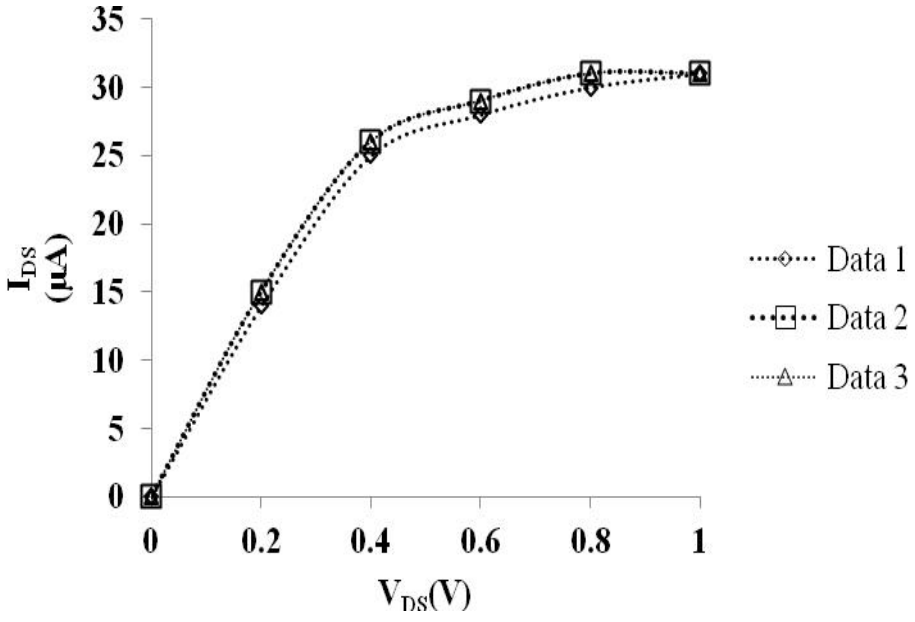

Fig.3.14 : Repeatibility test for AchFET of three data

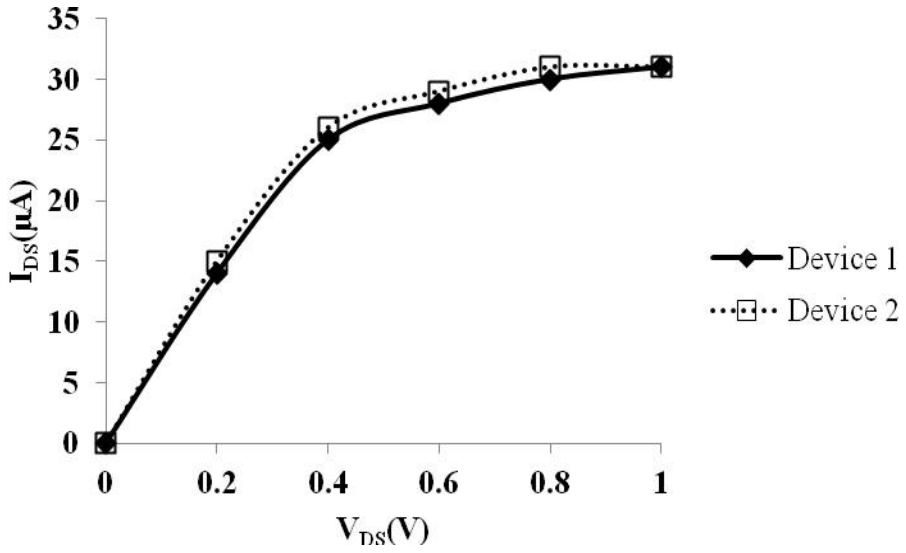

Fig.3.15: Reproducibility test for two AchFET

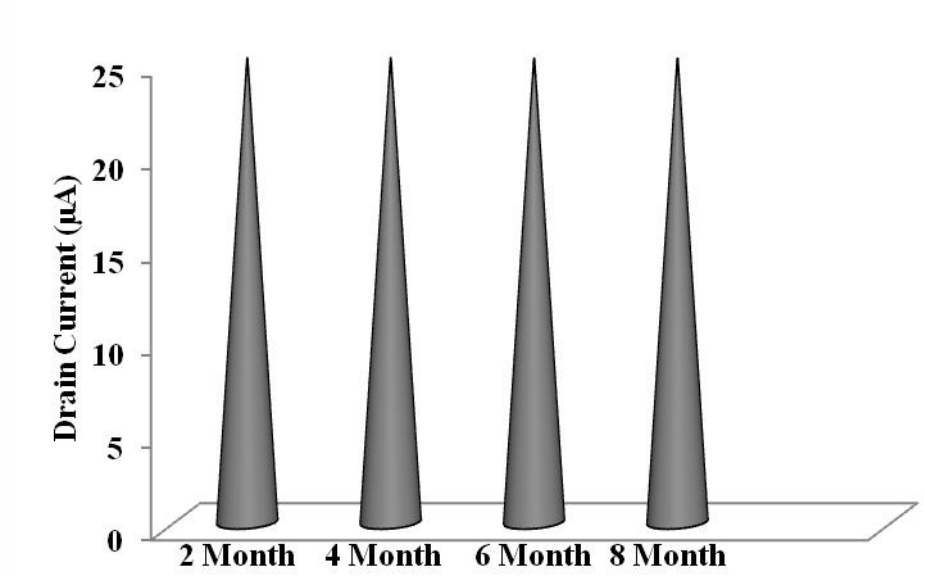

Fig.3.16: Stability of the AchFET

3.4 Summary

An AchFET was fabricated and tested for its performance and reliability. It was found to be highly suitable for acetylcholine detection with good linearity from 0.01 to 0.2 mM at 25^{o}C. The sensitivity of the device was found to be 57 mV/ decade. The device showed good repeatability, reproducibility, stability and little interference effect in presence of other biomolecules. This AchFET requires minimal instrumentation for fabrication and therefore can be fabricated easily in laboratory.