Chapter 4

Study on the development of an Electronic analog of excitable neuron membrane: The NEUROAchFET

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4.1 An overview

An electronic circuit that can reproduce the action potential of neuron is known as neuron circuit. Among all the electronic equivalent circuits of neurons, FET is the ideal electronic element to simulate the axon membrane conductances. It is because FET can be used as variable resistor at very low drain source voltage (V_{DS}) and it is a voltage controlled device (sodium and potassium conductances are voltage dependent). The drain source conductance (g_{ds}) can be varied by the gate to source voltage (V_{GS}). It is found that g_{ds} versus V_{GS} characteristic is similar to that of axon membrane conductances [83]. Based on this, Guy Roy had developed an electronic circuit model of neuron using Junction field effect transistor (JFET). In this chapter, using the same concept of Guy Roy, a simple electronic circuit model of neuron using AchFET was proposed as an analog of excitable membrane. This circuit was given the name “NEUROAchFET”. Its simulation and experimental results are presented in the following sections.

4.2 NEUROAchFET

4.2.1 PSPICE simulation

It was discussed in Chapter 3 that AchFET behaves like MOSFET (Fig.3.4 & Fig.3.7). Therefore, for simulation of the proposed circuit in PSPICE, MOSFET was used in place of AchFET. It is because AchFET device is not available in the PSPICE library. For this purpose, the characteristics of MOSFET (MBREAK N) were compared with the fabricated AchFET. For comparison, as shown in the Fig 3.7, the characteristic curves of MOSFET was plotted between drain current (I_D) and drain source voltage (V_{DS}) for different gate voltages as shown in Fig.4.1. Exactly like Fig.3.7, here also the drain to source voltage was varied from 0 to 1 V in steps of 0.2 V and gate voltage from 0 to 1 V in steps of 0.2 V. It was observed from Fig.3.7 and Fig. 4.1 that the drain current of the MOSFET at V_{GS} =1V is equivalent to the drain current of AchFET at 0.05 mM concentration of acetylcholine. Therefore, acetylcholine concentration was fixed at 0.05mM in further measurements.

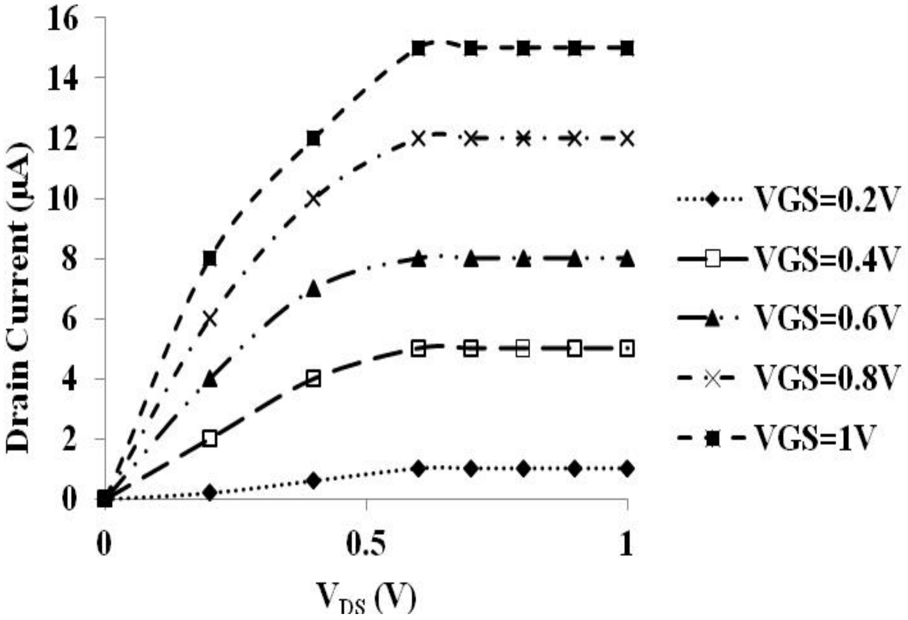

Fig.4.1: MOSFET characteristics curves

For simulation of sodium current, the circuit shown in Fig. 4.2 was used and connected in the workspace of PSPICE .The drain terminal of the MOSFET was connected to the inverting terminal of the opamp and source was connected to the non-inverting terminal of the opamp. V_{DS} (drain source voltage) was connected directly across it for different depolarization. The gate terminal was connected to the output of the opamp through resistor and capacitors as shown in Fig. 4.2. The sodium current was simulated as voltage across the resistor 39 k when V_{DS} was applied. Similarly for potassium current simulation, potassium circuit was connected and simulated across the 56 k when V_{DS} was applied as shown in Fig. 4.3. For simulation of action potential, the sodium conductance circuit, potassium conductance circuit are connected along with their batteries with membrane capacitance (0.0047 µF) and leakage conductance of 200k in parallel as shown in Fig. 4.4. The resting potential of sodium and potassium was maintained by connecting a voltage source (115mV for sodium and -12mV for potassium) connected in series with drain to source voltage (V_{DS}). The Pspice connection is shown in Fig. 4.5 for obtaining action potential. The working principle of the circuit is described below.

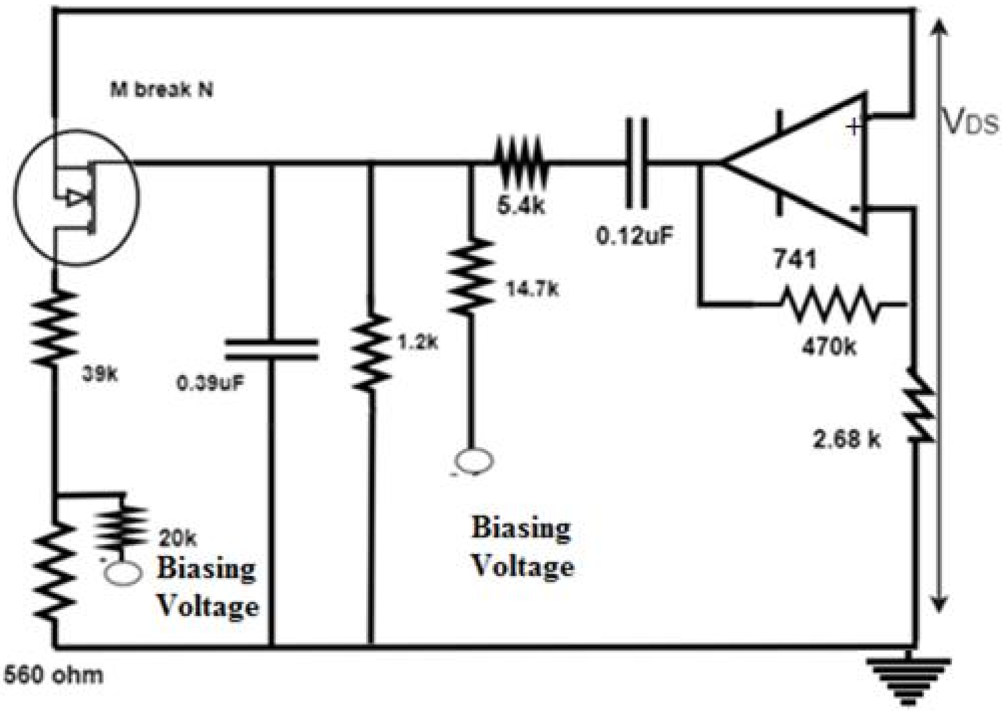

Fig. 4.2: Sodium current simulation circuit

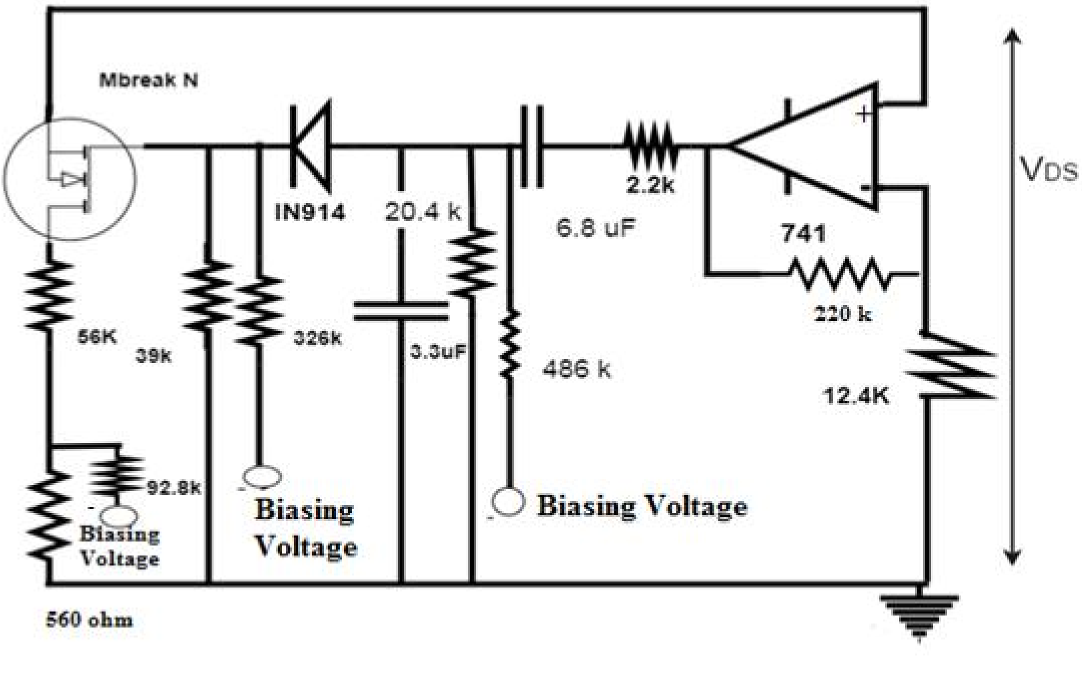

Fig. 4.3: Potassium current simulation circuit

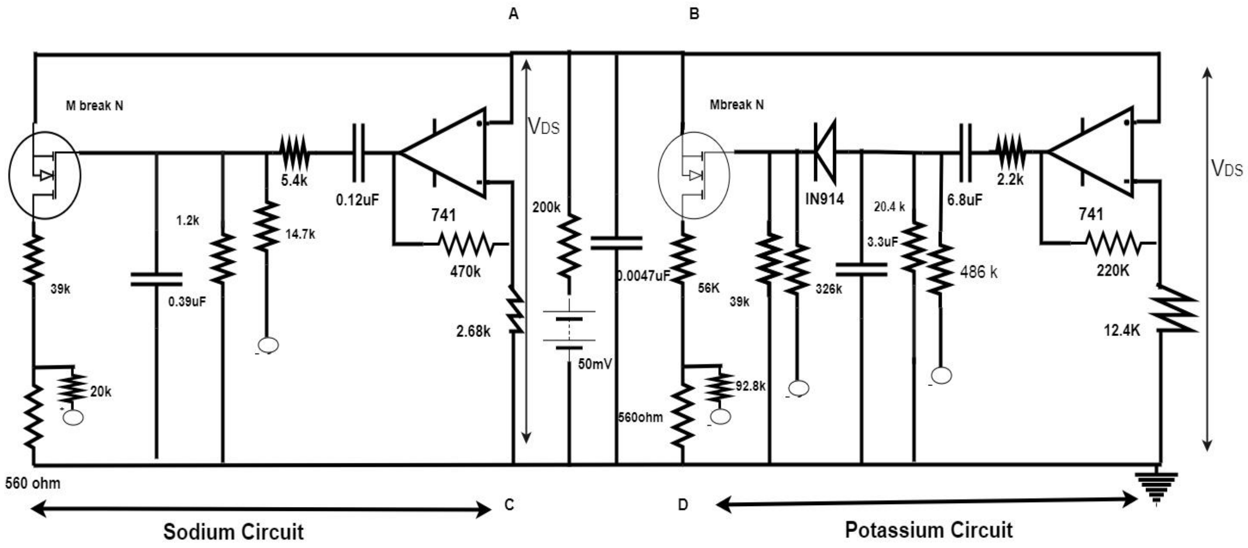

Fig. 4.4: Combined circuit representing H-H circuit for simulation of action potential

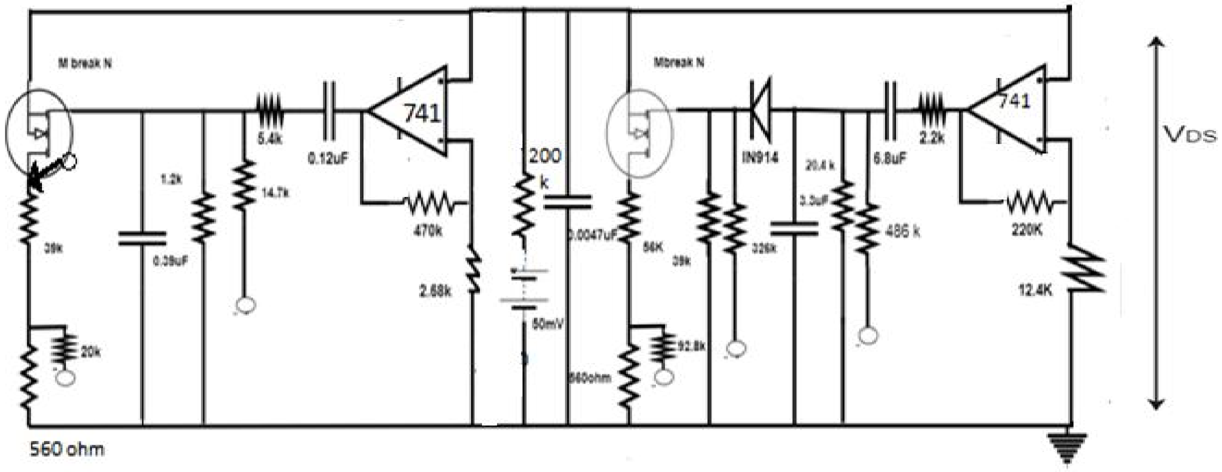

Fig. 4.5: Circuit built in Pspice for simulation of action potential

It was stated earlier that MOSFET can be used as a variable resistor when low V_{DS} are applied. When gate voltage is varied, it is observed that g_{ds} characteristic is similar to the axon membrane characteristics. g_{Na} and g_K was represented by g_{ds} of each MOSFET, the membrane current was represented by I_{DS} and voltage across neuron membrane was represented by V_{DS} of the MOSFET. It was explained by Hodgkin-Huxley that the sodium and potassium conductances maintain a low potential at resting state and so negative bias was applied to maintain this state. The amplitude of the conductances can be increased by applying external voltage (V_{DS}). The conductance increases with V_{DS} up to a certain time and after that it remains constant. When different

V_{DS} were applied to the MOSFET, the amplitude of the conductance was varied.V_{DS} was feedback to the gate of the MOSFET to provide required voltage dependence to the circuit. The time variance of the conductance was maintained by RC circuit. The amplifier circuit was connected for separation of the circuit with the MOSFET to determine time dependence of g_{ds} and amplify low V_{DS} to observe the change in g_{ds} .

Sodium conductance was observed for a short period of time but potassium conductance occurred for a longer time and it was of sigmoid in behavior. For the potassium conductance, a diode was introduced to get a delayed rise in the circuit. The negative voltage is adjusted so that the diode does not work when V_{DS} is 0. When V_{DS} is applied, the capacitor (3.3μF) becomes less negative; the diode starts conducting and brings a delayed rise for the potassium conductance. The prolong or short delay can be adjusted by proper biasing. A capacitor (6.8μF) was connected to oppose dc biases of the source of MOSFET and it resets to its initial value when V_{DS} is constantly applied to it. This circuit was used for potassium conductance for slow inactivation. The other half of the circuit was used for sodium conductance. In this circuit, there was no diode taken due to no delay in sodium conductance seen in the experimental curves [41]. g_{ds} variance with time was made more fast and inactivated faster by using capacitor of low value of 0.12 μF. Simulation results show that both the circuits can reproduce sodium and potassium conductance satisfactorily. The circuit for potassium and sodium when connected in parallel with membrane capacitance (0.00047 μF) and leakage conductance (200k) results in an action potential. Fig. 4.6 shows the simulated action potential obtained from the circuit. The current of sodium and potassium ions obtained from the circuit during action potential is shown in Fig. 4.7 and Fig. 4.8.

The PSpice Technology student release 9 of OrCAD was used for simulation. PSpice is open source software used for circuit simulation and to predict circuit behavior. Out of the many applications in Pspice, transient analysis settings were used for simulation of action potential, sodium ionic current and potassium ionic current of the developed circuit. Time was generally set from 0 to 3ms and the cursor was pointed towards the point of observation in the schematics. The values of voltage sources, resistors, capacitors etc. was changed by clicking on the component and editing the value of the device.

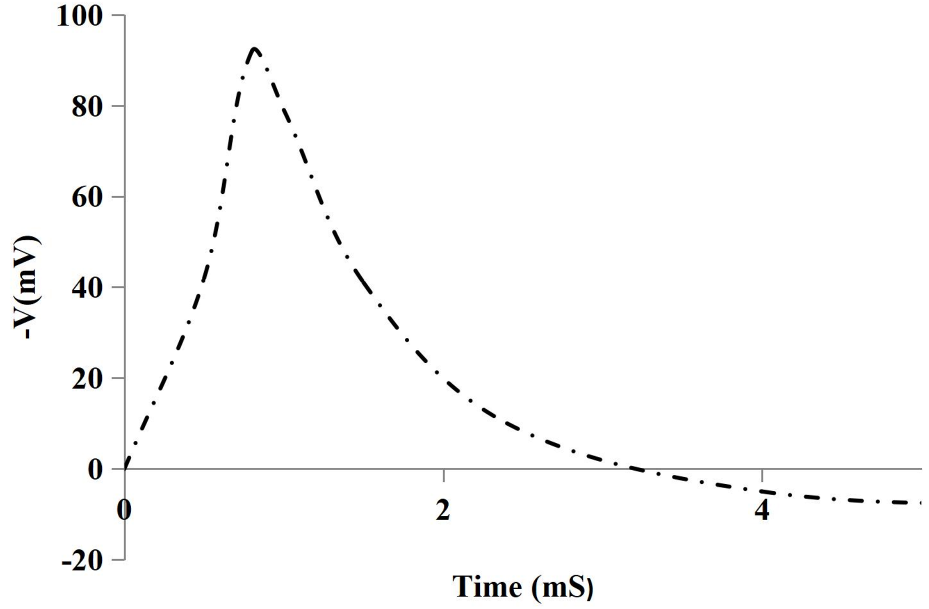

Fig. 4.6: Action potential simulated from the circuit in PSPICE

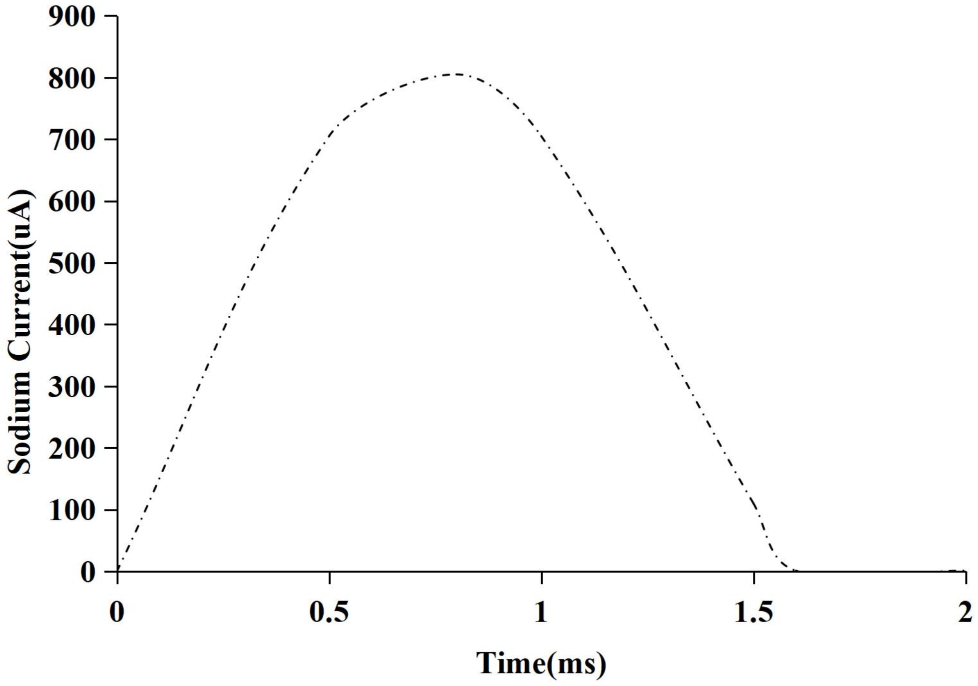

Fig. 4.7: Sodium current simulated in PSPICE

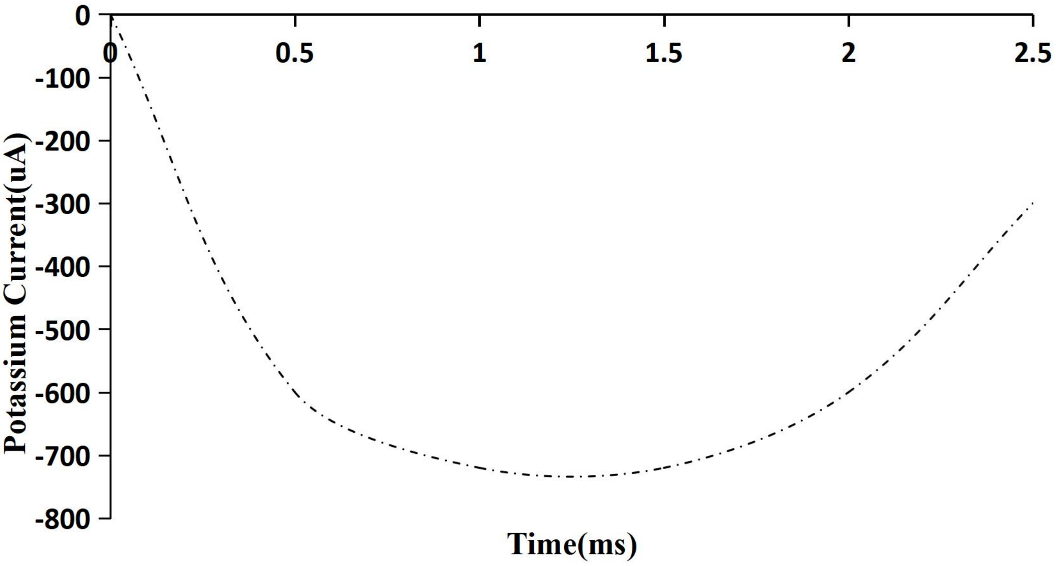

Fig. 4.8: Potassium current simulated in PSPICE

4.2.2 Experimental set up for the NEUROAchFET

For measurement of sodium (g_{Na}) conductance, the AchFET, resistors, capacitors, opamp (741), power supply were connected as shown in the circuit of Fig.4.9. The set up for obtaining sodium conductances is shown in Fig. 4.10. The AchFET was dipped in a glass pot containing phosphate buffer saline solution (15 ml of 50 mM, ph7) with reference electrode (Ag/ AgCl).The gate potential was applied by using the reference electrode. The output of the opamp was connected to the reference electrode through resistors and capacitors as shown in Fig. 4.9. The drain terminal of the AchFET was connected to the inverting terminal of the opamp and source terminal was connected to the non-inverting terminal of the opamp. Drain current was measured by adding 100 µl of acetylcholine solution (0.05 mM) using micropipette to phosphate buffer saline solution. Dual input power supply was used for the opamp power supply. The power supply V^+ was applied to pin number 7 and V^- was connected to pin number 6 of opamp. The depolarization voltage was connected similar to Pspice circuit. The output was measured with AD INSTRUMENTS by connecting the probe. The one terminal of the measuring probe was connected to AD INSTRUMENTS and the other terminal was connected across the sodium current output which was measured. The output of the sodium current was obtained across the voltage transients of resistor 39 k and the respective ground. The AD INSTRUMENTS was connected to computer via USB cable and output was observed in the screen of the computer. Similarly, for the potassium circuit, the electronic components were connected as shown in Fig.4.11. Fig. 4.12 shows the set up for obtaining potassium conductance. The output was measured using AD INSTRUMENTS across the resistor 56 k as described in sodium circuit. Sodium (I_{Na}) and potassium (I_k) currents with respect to time were calculated using a shunt resistor across the output. For obtaining an action potential, the sodium circuit, potassium circuit, membrane capacitance of 0.0047 µF and resistor of 200k ohm for leakage conductance were connected in parallel with their respective batteries and required V_{DS} as shown in Fig. 4.13. The corresponding set up for measurement of action potential is shown in Fig. 4.14.

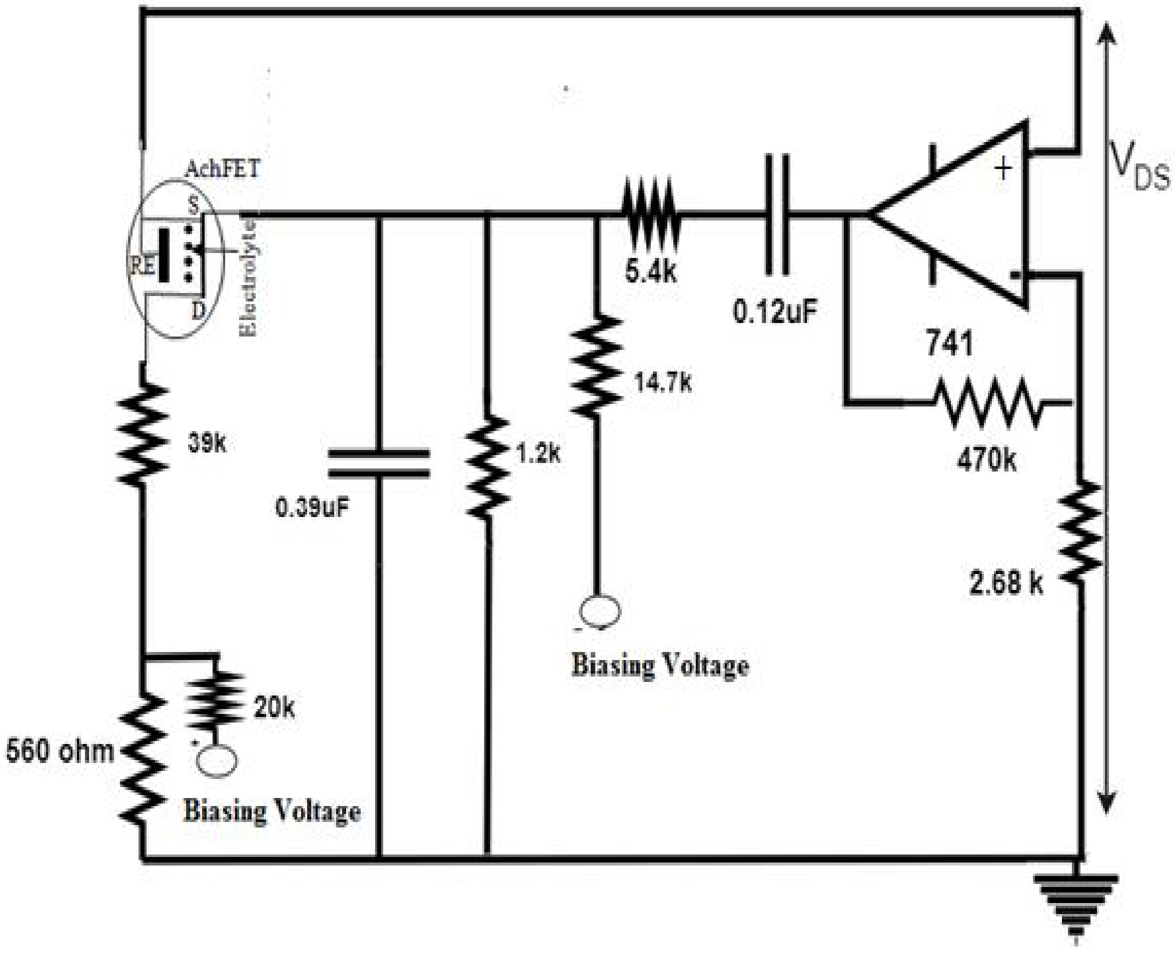

Fig.4.9: Circuit for obtaining sodium current using AchFET

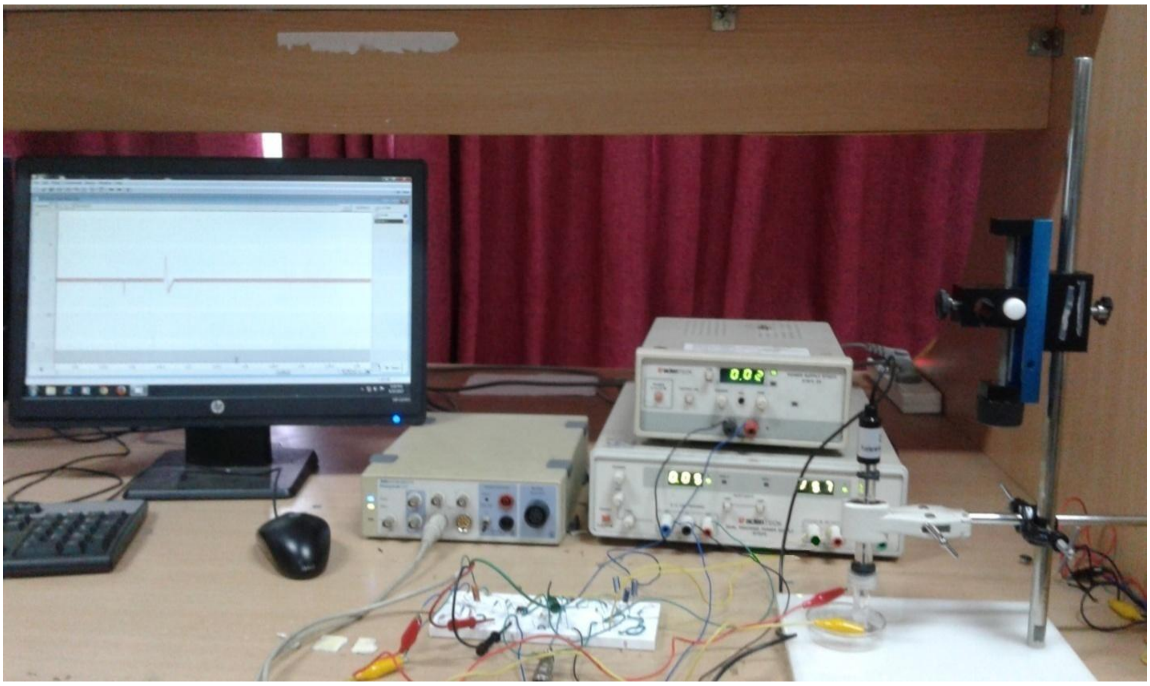

Fig.4.10: Set up for obtaining sodium conductance

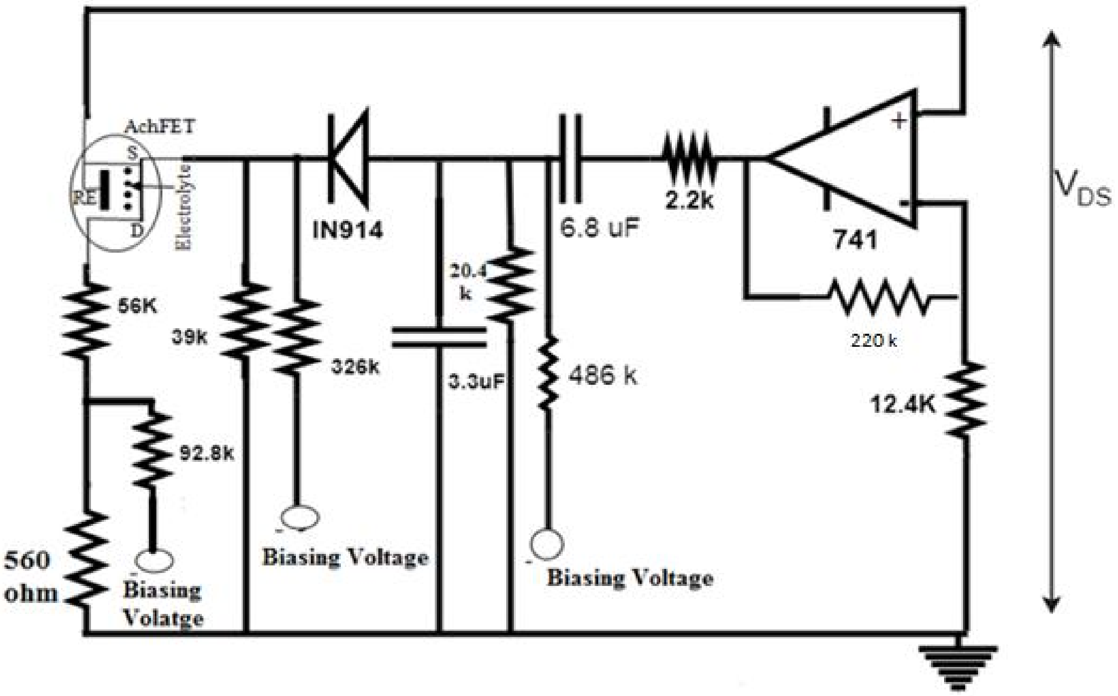

Fig. 4.11: Circuit for obtaining potassium current using AchFET.

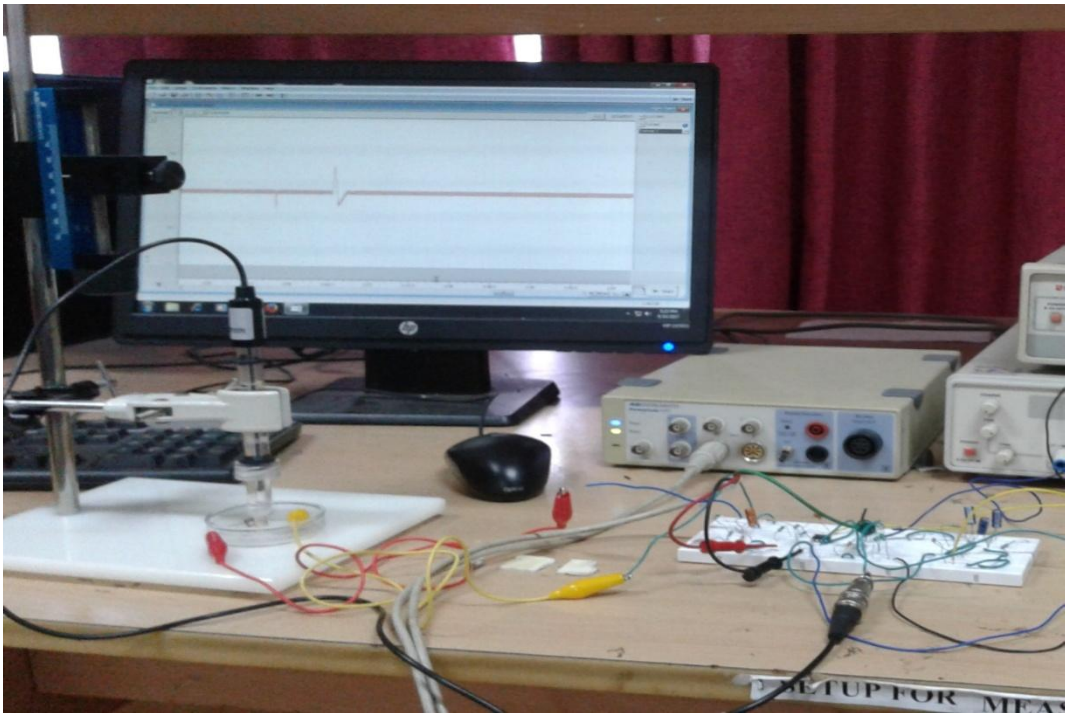

Fig.4.12: Set up for potassium conductance using AchFET

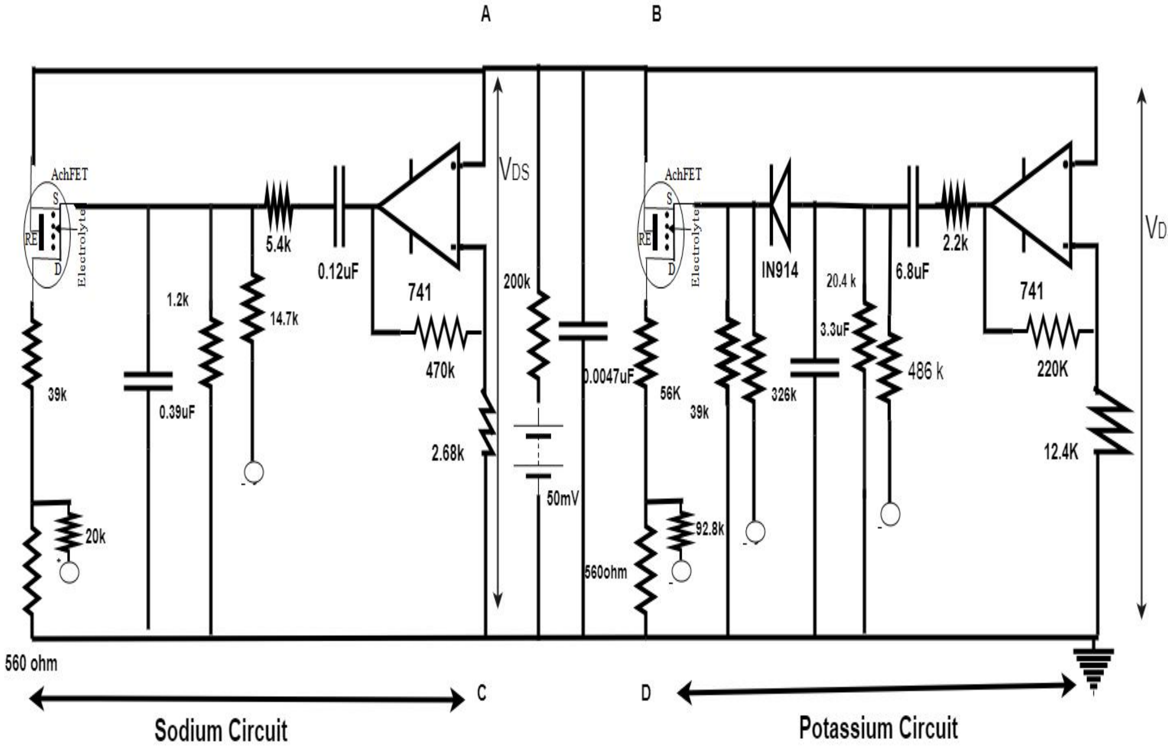

Fig. 4.13: Circuit for obtaining action potential using AchFET

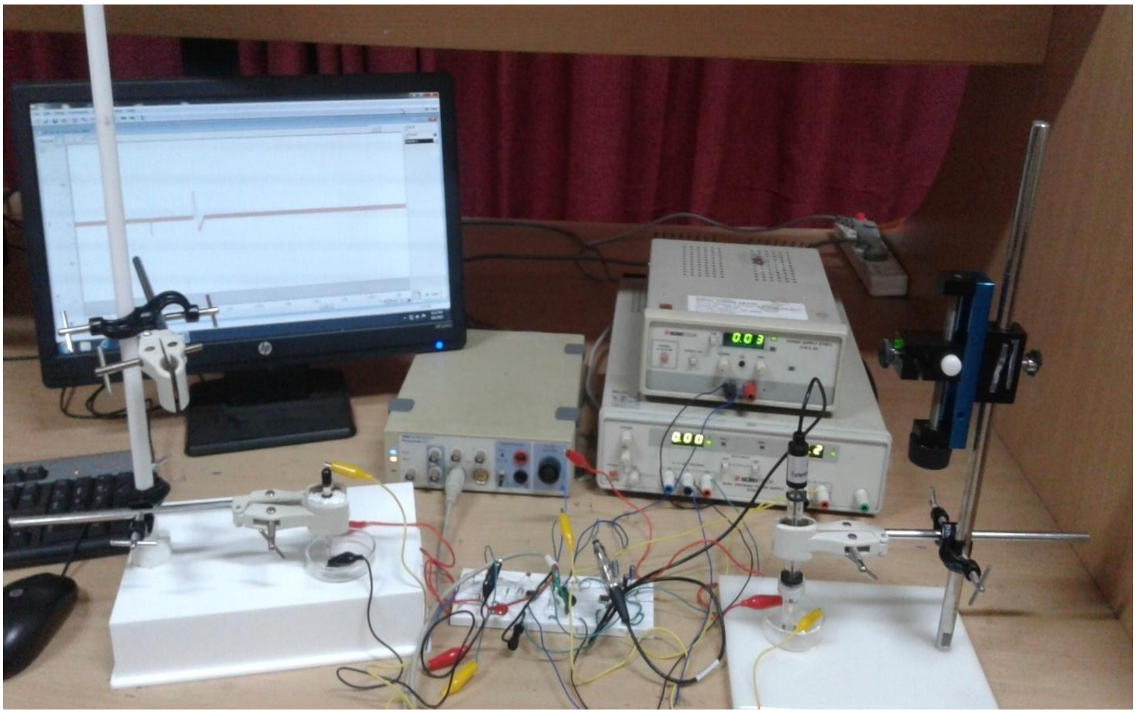

Fig.4.14: Set up for obtaining action potential by connecting sodium circuit, potassium circuit, and membrane capacitance and leakage conductance.

4.3 Results and Discussions

4.3.1 Sodium and Potassium conductance measurement

The sodium and potassium conductance maintain low potential in the resting state [41-45]. When an external energy (V_{DS}) was applied, the conductance of sodium and potassium were increased and remained in a constant state. Since the resting potential of membrane is at low voltage [41], negative bias was applied to maintain V_{DS} at low potential. The output of each circuit was obtained in the monitor using AD INSTRUMENTS as described in section 4.2.2. For sodium circuit and potassium circuit, the output was measured across the output of the respective resistor discussed above. The values of the output voltage (V_{Na} or V_K) with time were noted down and then the ionic currents and conductance were calculated at different V_{DS}. Table 4.1 shows the results obtained for sodium and potassium conductance variance with time and voltage in tabular form. Fig.4. 15 and Fig. 4.16 show the measurement taken for conductance of sodium and potassium with various V_{DS}. Fig. 4.17 and Fig.4.18 show the transient response of sodium and potassium conductance when V_{DS} was varied in steps.

Table 4.1: Experimental values for sodium conductance and potassium conductance with respect to time and V_{DS} (Shunt resistor (R) : 10Ω)

V_{DS} (mV)	V (Sodium Circuit) (mV)	V (Potassium Circuit) (mV)	Time (ms)	$I_{Na}= V_{Na}/R$ (µA)	$-I_k= V_k/R$ (µA)	$g_{Na}= I_{Na}/V_{DS}$ (m.mho)	$g_K= I_k/V_{DS}$ (m.mho)
20	0.8	0.42	0.5	80	42	4	2.1
20	1.4	0.6	0.8	140	60	7	3
20	0.8	1	1	80	100	4	5
20	0.4	1	1.5	40	100	2	5
20	0	0.6	2	0	60	0	3
20	0	0.2	2.5	0	20	0	1
40	6.8	6	0.5	680	600	17	15
40	10	6.6	0.8	1000	660	25	16.5
40	6.8	7.2	1	680	720	17	18
40	1.2	7.2	1.5	120	720	3	18
40	0	6.6	2	0	660	0	16.5
40	0	3	2.5	0	300	0	7.5

V_{DS} (mV)	V (Sodium Circuit) (mV)	V (Potassium Circuit) (mV)	Time (ms)	$I_{Na}= V_{Na}/R$ (μA)	$-I_k= V_k/R$ (μA)	$g_{Na}= I_{Na}/V_{DS}$ (m.mho)	$g_K= I_k/V_{DS}$ (m.mho)
60	15	9.6	0.5	1500	960	25	16
60	22	10.2	0.8	2200	1020	36.6	17
60	15	11.4	1	1500	1140	25	19
60	3	11.4	1.5	300	1140	5	19
60	0	10.8	2	0	1080	0	18
60	0	4.8	2.5	0	480	0	8
80	21.6	14.4	0.5	2160	1440	27	18
80	32	15.2	0.8	3200	1520	40	19
80	21.6	16	1	2160	1600	27	20
80	5.6	16	1.5	560	1600	7	20
80	0	15.2	2	0	1520	0	19
80	0	11.2	2.5	0	1120	0	14

V_{DS} (mV)	V (Sodium Circuit) (mV)	V (Potassium Circuit) (mV)	Time (ms)	$I_{Na}= V_{Na}/R$ (µA)	$-I_k= V_k/R$ (µA)	$g_{Na}= I_{Na}/V_{DS}$ (m.mho)	$g_K= I_k/V_{DS}$ (m.mho)
100	28	20	0.5	2800	2000	28	20
100	43	20.6	0.8	4300	2060	43	20.6
100	28	21	1	2800	2100	28	21
100	9	21	1.5	900	2100	9	21
100	0	20	2	0	2000	0	20
100	0	16	2.5	0	1600	0	16

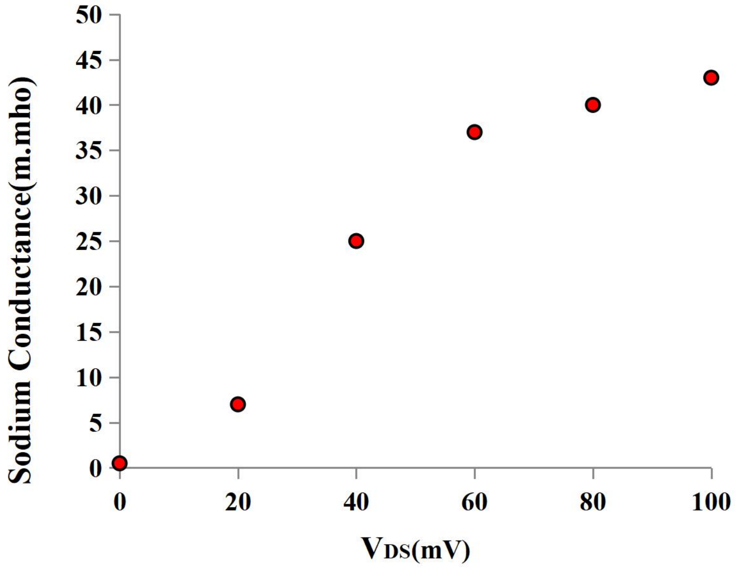

Fig.4. 15: Sodium conductance variance with applied voltage (V_{DS}).

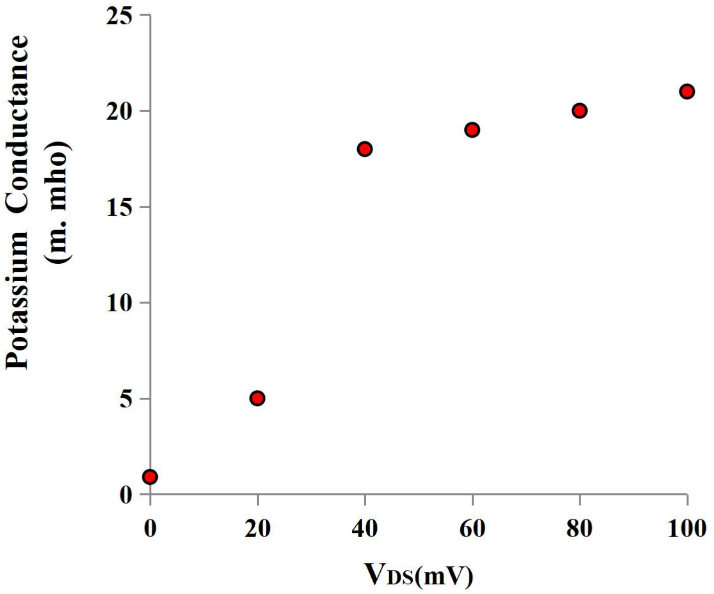

Fig.4.16: Potassium conductance versus applied voltage (V_{DS})

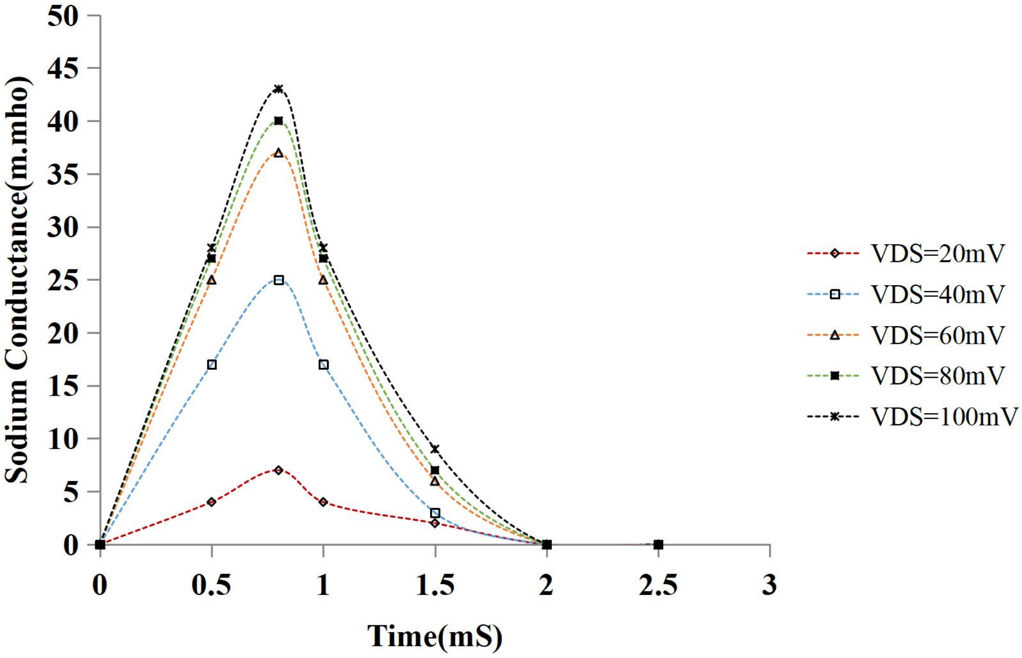

Fig. 4.17: Sodium conductance variance with time in NEUROAchFET model

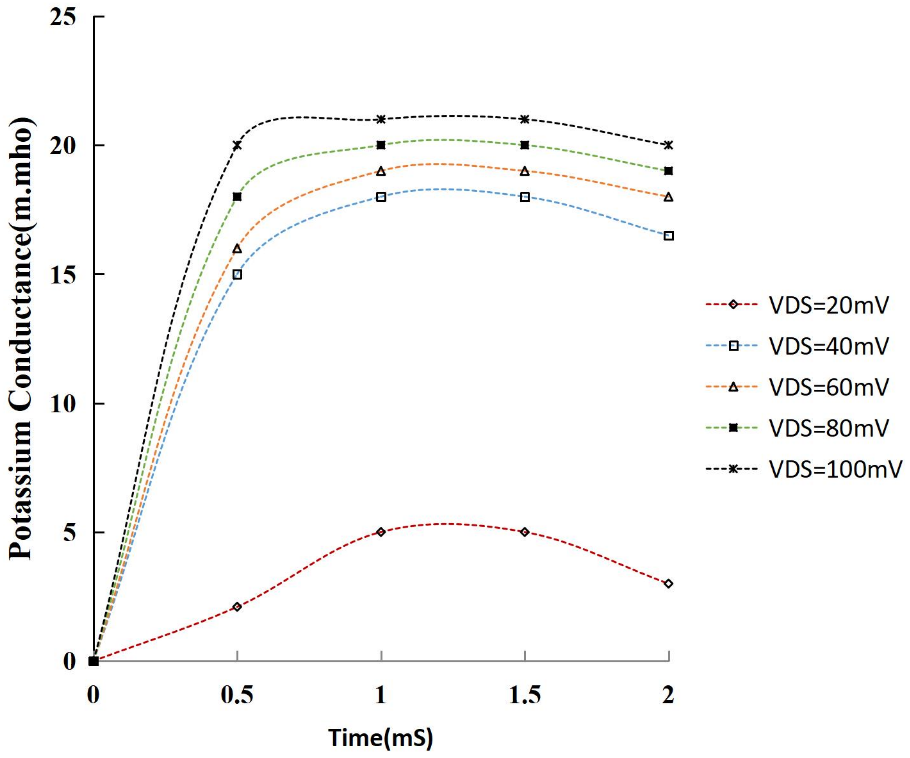

Fig. 4.18: Potassium conductance with respect to time obtained from NEUROAchFET circuit

4.3.2 Measurement of action potential and ionic current

After reproduction of sodium and potassium conductance, an action potential was generated by combining the sodium, potassium conductance circuit in parallel with leakage conductance of 200k and membrane capacitance of 0.0047µF as shown in Fig. 4.13. The action potential was measured at the output of the resistor (486 k) of sodium circuit shown in Fig. 4.19 and compares with the action potential obtained by Pspice simulation of the circuit. With threshold of larger than 10mV applied, an action potential was generated .V_{DS} was varied from 0 to 100 mV while gate voltage was varied from 0 to 1V to get the required coductance (g_{ds}). Currents due to sodium (I_{Na}) and potassium (I_K) ions was calculated from Table 4.1 and shown in Fig. 4.20 and Fig 4.21.

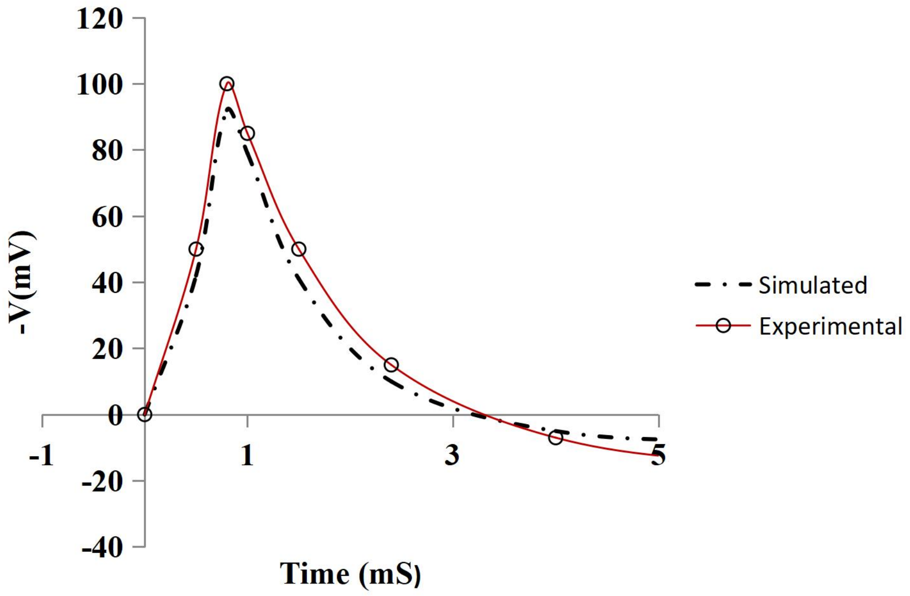

Fig.4.19: Action Potential generated from the NEUROAchFET

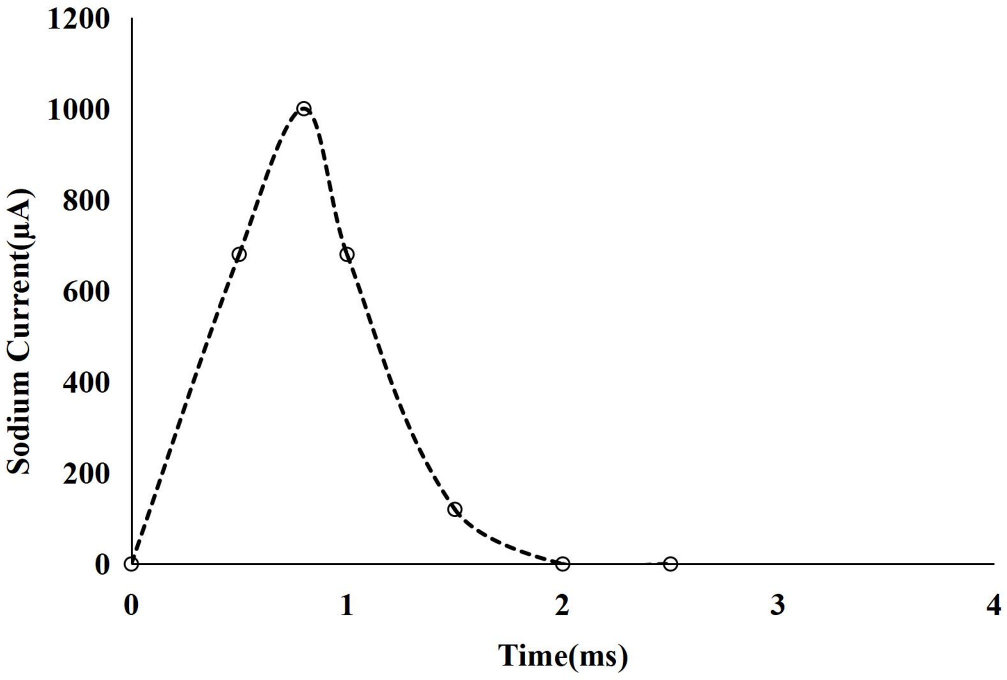

Fig. 4.20: Sodium current with respect to time obtained from the NEUROAchFET

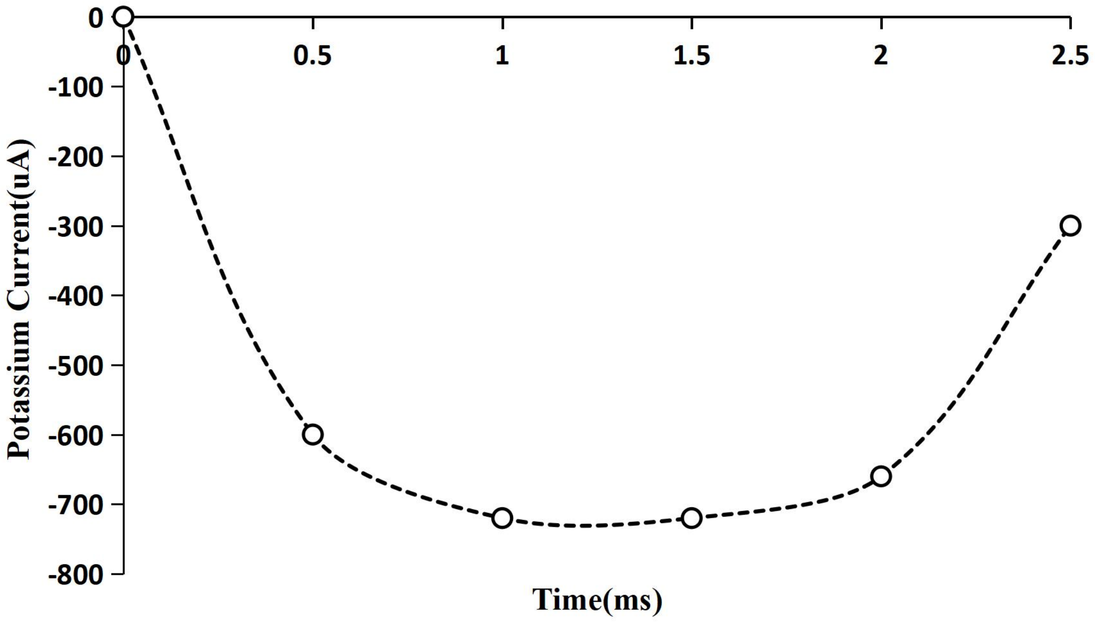

Fig. 4.21: Potassium current with respect to time obtained from NEUROAchFET circuit.

4.3.3 Effect of sodium and potassium conductance on action potential

It was explained in Chapter 2 how sodium and potassium conductance affect the membrane potential and ionic current in neuron. Increase of sodium conductance increases the membrane potential while decrease of sodium conductances decreases the membrane potential. But the action potential reduces when potassium concentration is increased and vice versa [41]. These activities were observed in NEUROAchFET. Conductances of sodium and potassium were varied by varying the value of V_{DS} of each AchFET. When V_{DS} was increased, conductance was increased. For each conductance change, effect on action potential was observed.

The circuit in Fig.4.13 was used to study the effects of sodium and potassium conductance on action potential. Fig. 4.22 shows the effect of sodium conductance on action potential when sodium conductance was increased. Sodium conductance can be increased by increasing V_{DS}. The increase of action potential is directly proportional to the increase of sodium conductance [41-45]. V_{DS} value was changed for increasing and decreasing of sodium conductance. Action potential reduces when sodium conductance decreases as shown in Fig. 4.23.

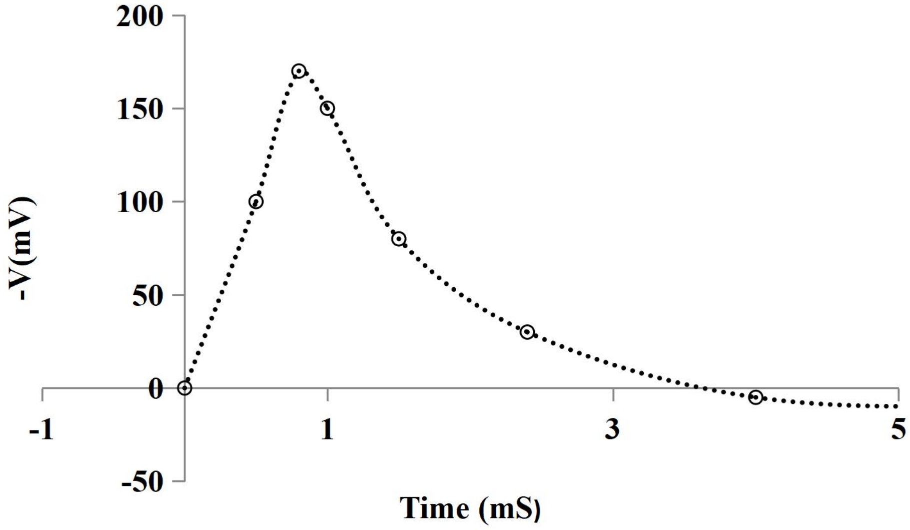

Fig. 4.22: Effect of increase in sodium conductance on action potential (V_{DS} = 0.1V)

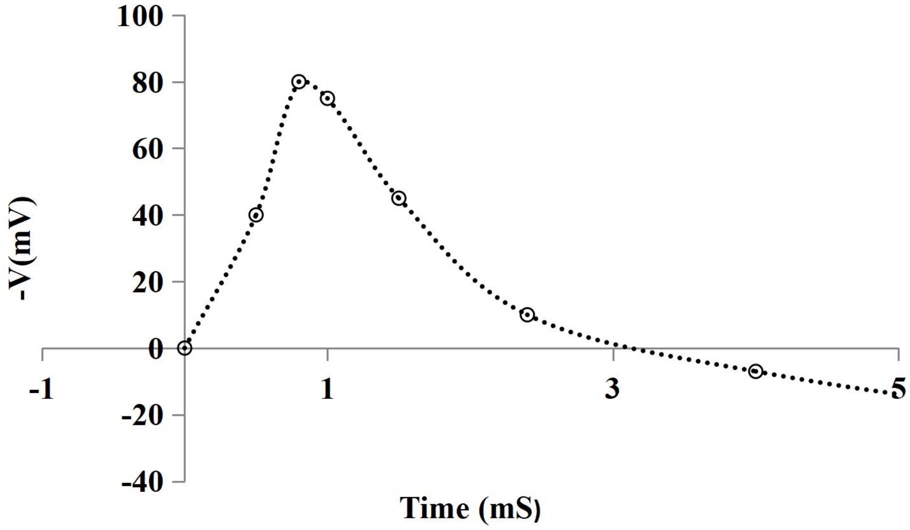

Fig. 4.23: Effect of decrease in sodium conductance on action potential (V_{DS} = 0.02V).

Fig. 4.24 shows the change in action potential when potassium conductance was lowered. It is evident from the figure that when potassium conductance was lowered, action potential increases. V_{DS} was changed in regular steps to observe the effects on action potential. Fig. 4.25 shows the effect on action potential due to increase in potassium conductance which decreases the action potential peak. Effects of potassium concentration was studied by Curtis and Cole (1942), Hodgkin and Katz (1949) and found to be similar with this study. Table 4.2 shows the variance of membrane potential with conductance of sodium and potassium.

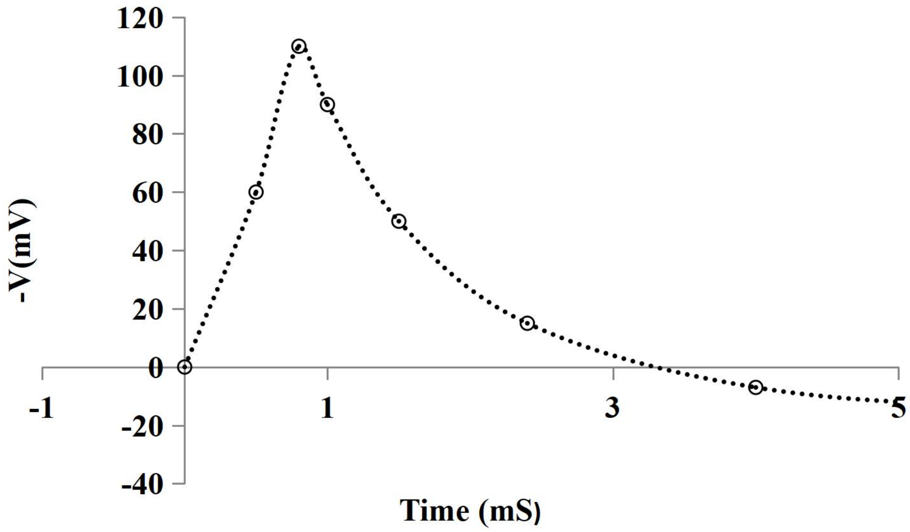

Fig. 4.24: Decrease of potassium conductance (V_{DS} = 0.02V) and the effect on action potential

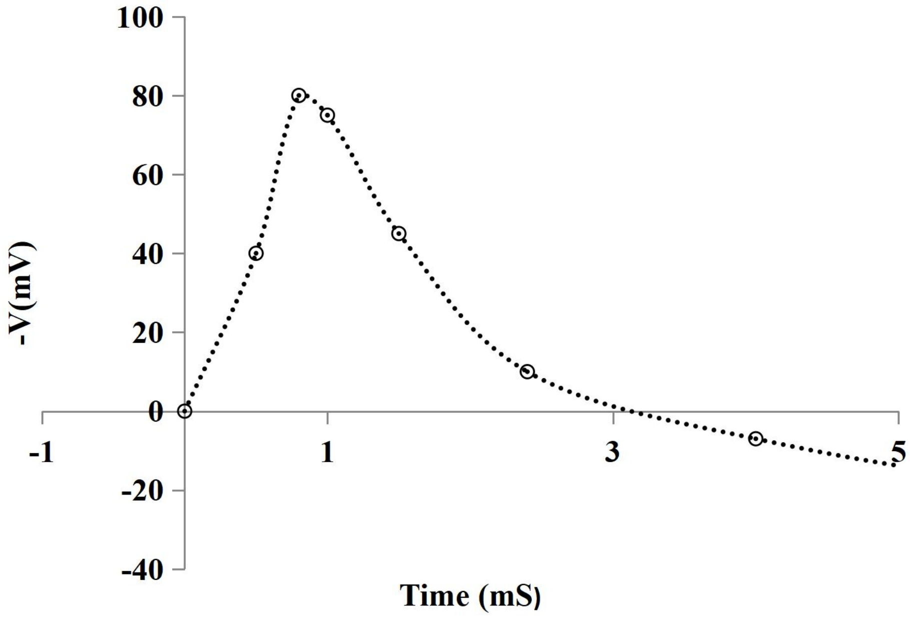

Fig. 4.25: Effect of increase of potassium conductance (V_{DS} = 0.1V) in action potential.

Table 4.2 : Effect on action potential with sodium and potassium conductance

V_{DS} for sodium circuit and potassium circuit(V)	Action Potential when V_{DS} for sodium circuit is varied (-V_M) (mV)	Action Potential when V_{DS} for potassium circuit is varied (-V_M) (mV)
0.02	80	110
0.04	100	100
0.06	110	98
0.08	120	90
0.1	170	80

Fig. 4.26 shows the variance of action potential with applied voltage V_{DS} (sodium conductance). It is evident from the Fig.4.26 that the result obtained for sodium conductance variance is in good agreement with the result available in literature [41-45]. Fig. 4.27 shows change in action potential with the change of potassium conductance. It can be seen that potassium conductance has little effect on action potential and action potential decreases with increase in potassium conductance.

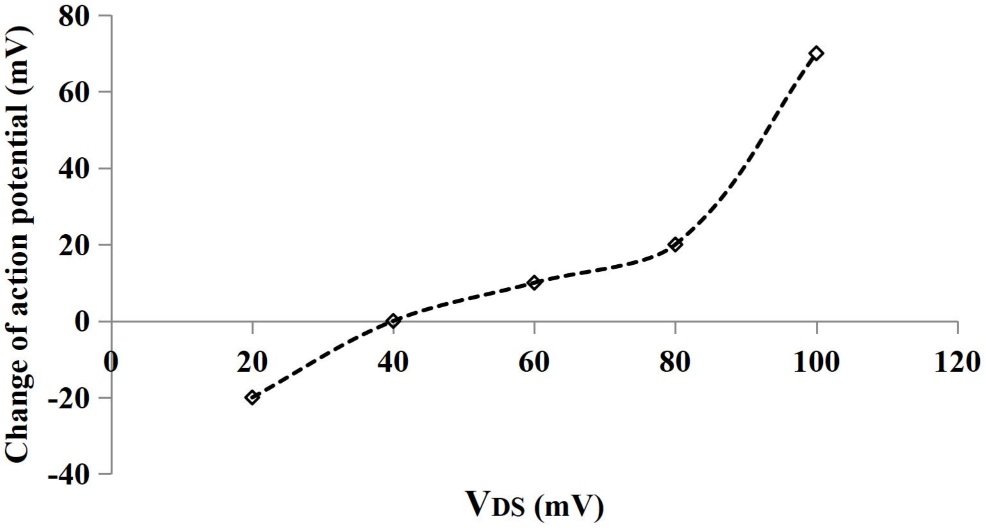

Fig.4.26: Change of action potential with change in sodium conductance by varying V_{DS}. Change of action potential was evaluated from the difference between action potential in normal conductance to the action potential due to change in conductance.

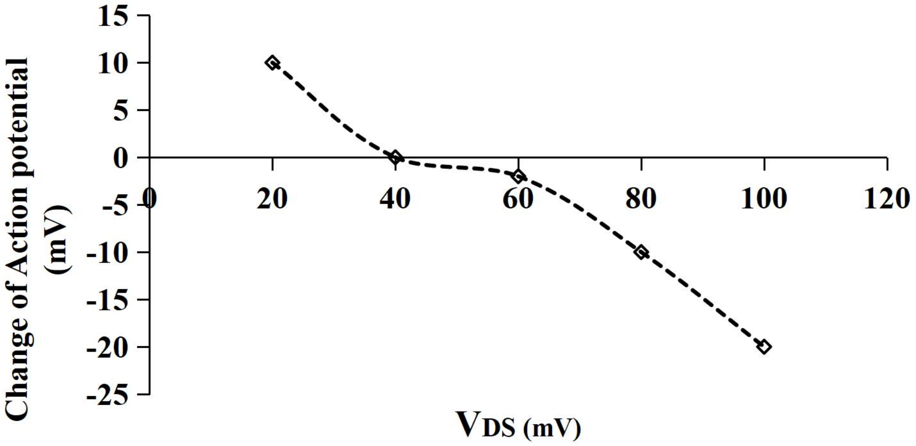

Fig. 4.27: Action potential change with potassium conductance. Change of action potential was evaluated from the difference between action potential in normal conductance to the action potential due to change in conductance.

4.3.4 Effect of sodium conductance and potassium conductance on ionic current

Ionic current depends on the conductance of ion channels. When sodium conductance is decreased, there is no inward current but outward current occurs [41-45]. Fig 4.28 and Fig. 4.29 show the effect of sodium conductance on sodium current. It was observed that the sodium current increases inward with increase of sodium conductance while inward current decreases with decrease of sodium conductance. The direction of potassium current is same whether the potassium conductance is increased or decreased i.e. outward .When potassium conductance was increased abruptly, ionic current of potassium increased and decreased when potassium conductance decreases. Fig. 4.30 and Fig. 4.31 show the effect of potassium concentration on potassium current. Table 4.3 shows the change of ionic current of sodium and potassium with change in V_{DS}.

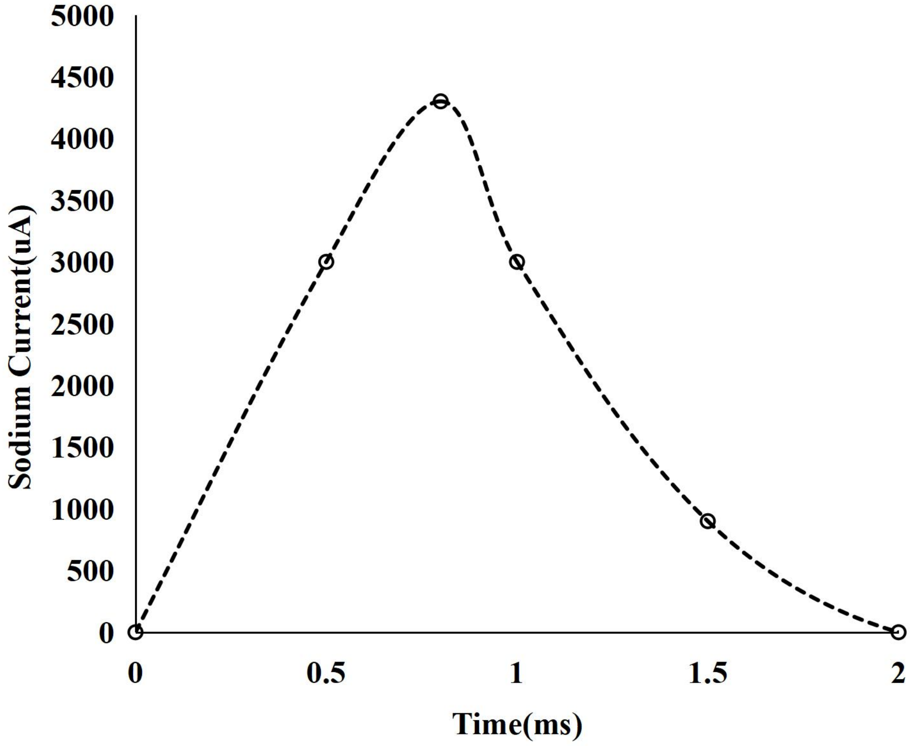

Fig. 4.28: Increase of sodium conductance(V_{DS} = 0.1V) and effect on sodium current

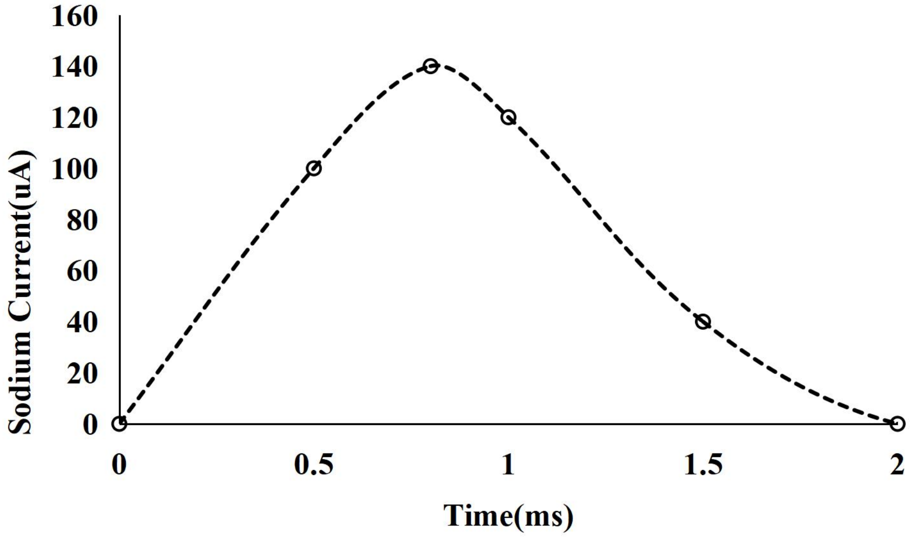

Fig. 4.29: Effect of decrease of sodium conductance(V_{DS} = 0.02V) on ionic current of sodium.

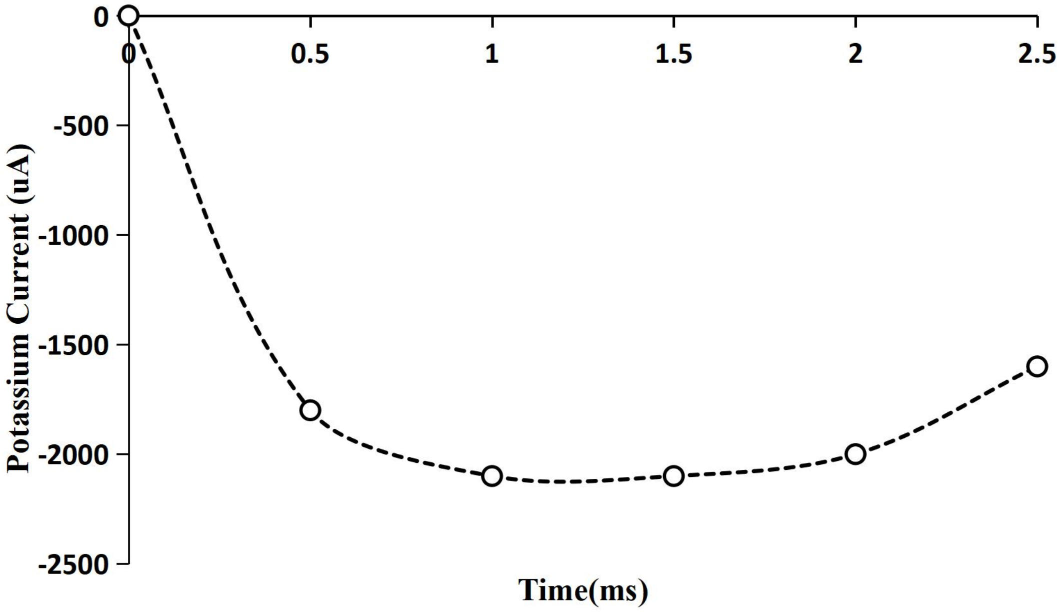

Fig. 4.30: Effect of increase of potassium conductance (V_{DS} = 0.1V) on potassium current

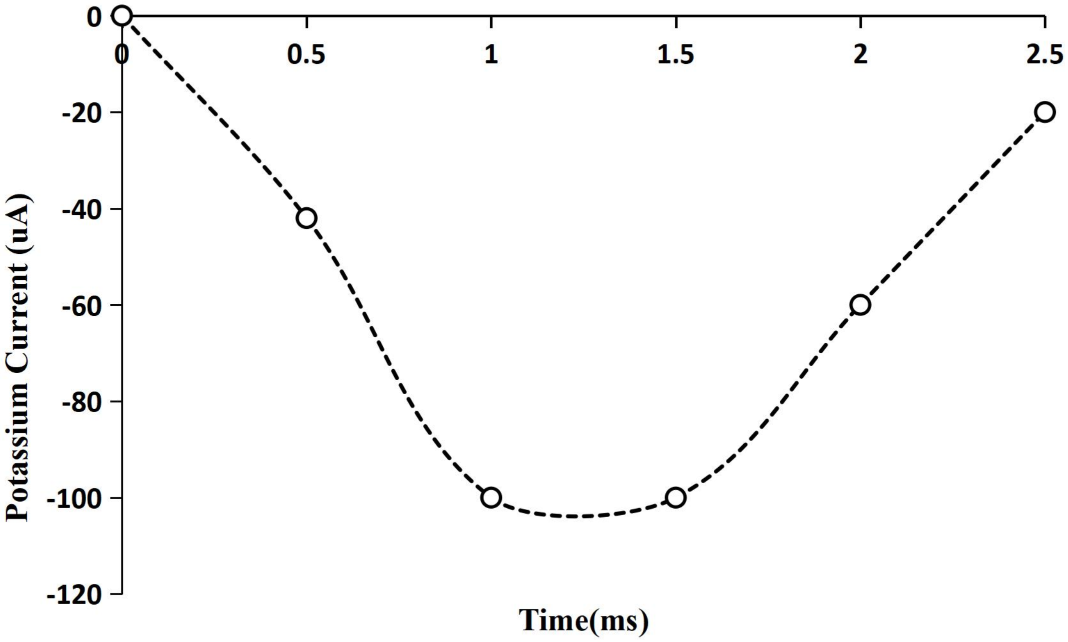

Fig.4.31: Decrease of potassium conductance (V_{DS} = 0.02V) and the effect on potassium current.

Table 4.3 : Change of ionic current of potassium and sodium with respect to their conductance.

V_{DS} (V)	I_{Na} (μA)	I_K(μA)
0.02	140	-100
0.04	1000	-720
0.06	2200	-1140
0.08	3200	-1600
0.1	4300	-2100

4.4 Summary

A simple electronic circuit model of neuron using AchFET was developed as an analog of excitable membrane and simulated in PSpice. The simulated results were found to be in good agreement with experimental results. This developed circuit was given the name "NEUROAchFET'. Experimental results indicated that this circuit can reproduce a wide variety of membrane conductances and different types of action potentials. Due to inclusion of AchFET in the circuit model, it may open the possibility of measurement of concentration of acetylcholine neuro-transmitters responsible for synaptic actions, i.e. excitatory and inhibitory postsynaptic potentials. It may be expected that such a NEUROAchFET will become a useful research unit in neurology for simulation of receptor function and electrical activity of neuron.