CHAPTER 5

RESULTS AND DISCUSSION

5.1 KINETICS OF MEMBRANE FORMATION

Fundamental studies on membrane electrical properties are important to ascertain the stability and suitability of the present experimental set-up for BLM studies and to know the changes in the electrical properties of BLMs due to drug membrane interaction. Model BLM systems are said to be alive in the sense they respond to electrical, mechanical and physicochemical stresses. The extent of changes produced by these stresses is indicative of the consequent structural changes in lipid membranes which are discussed later.

The impedance spectra for BLM systems were recorded after forming BLMs in various methods in order to represent and describe the changes in the electrical properties of BLMs during self assembly of lipid molecules into bilayer and study the kinetics of BLM formation. A stabilization period of 30 minutes was given for spontaneous self assembly of lipid molecules into bilayer in all methods to get a bilayer phospholipid membrane of stable electrical properties. From the Bode plot of impedance spectra for stable membrane system, the frequency at which the capacitive nature (Hydrocarbon core) of BLM dominated was identified. It was found that this frequency, showing capacitive nature of BLM, is different for different model membrane systems (Eliaz, 2011). At this frequency the electrical properties of BLM namely capacitance and Phase angle are measured with time. The electrical

properties with time at single frequency are measured using electrochemical impedance spectroscopy by superimposing AC sinusoidal voltage of small amplitude, 25 mV, at the open circuit potential.

5.2 CHANGES IN THE ELECTRICAL PROPERTIES OF BLACK OR PLANAR LIPID MEMBRANES

The dispersion of phase angle of black lipid membrane formed in 1.0 M, 0.1 M and 0.01 M NaCl solutions with frequency are shown in Figure 5.1. From this Figure it is seen that at the frequency 10.8 Hz the phase angle (φ) is the maximum and theφ values are reflective of the quality and capacitive nature of membranes.

Hence, after applying the lipid dispersion at the membrane supporting aperture, the thinning and stabilization processes of BLMs were monitored by following the time-dependent changes in capacitance and phase angle difference.

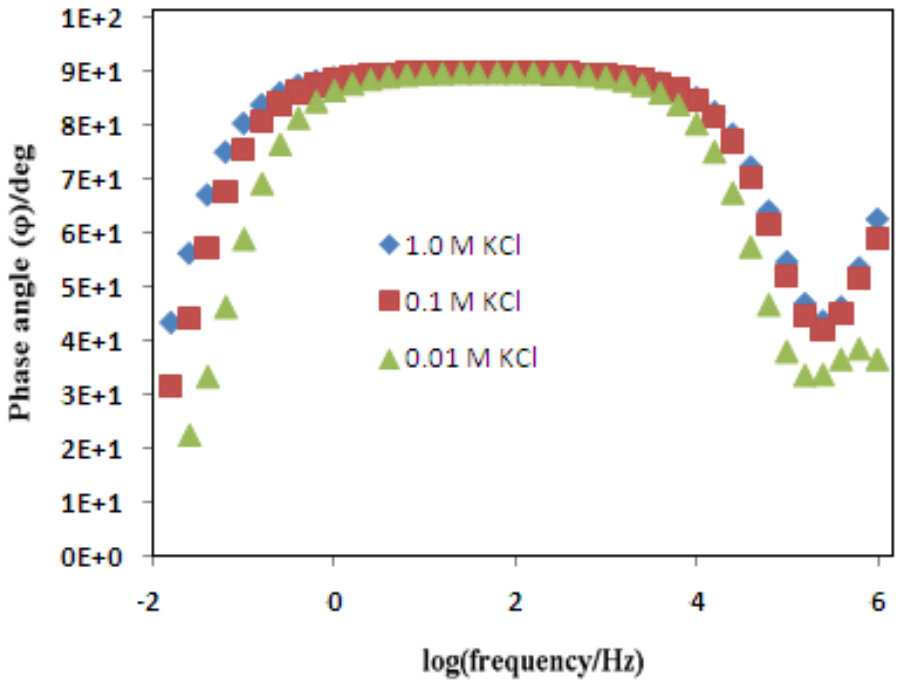


Figure 5.1 Bode Phase angle diagram for planar BLM in 1.0 M, 0.1 M and 0.01 M KCl bath solutions

The variation of phase angle and capacitance values with time is shown in Figure 5.2 and 5.3 respectively.

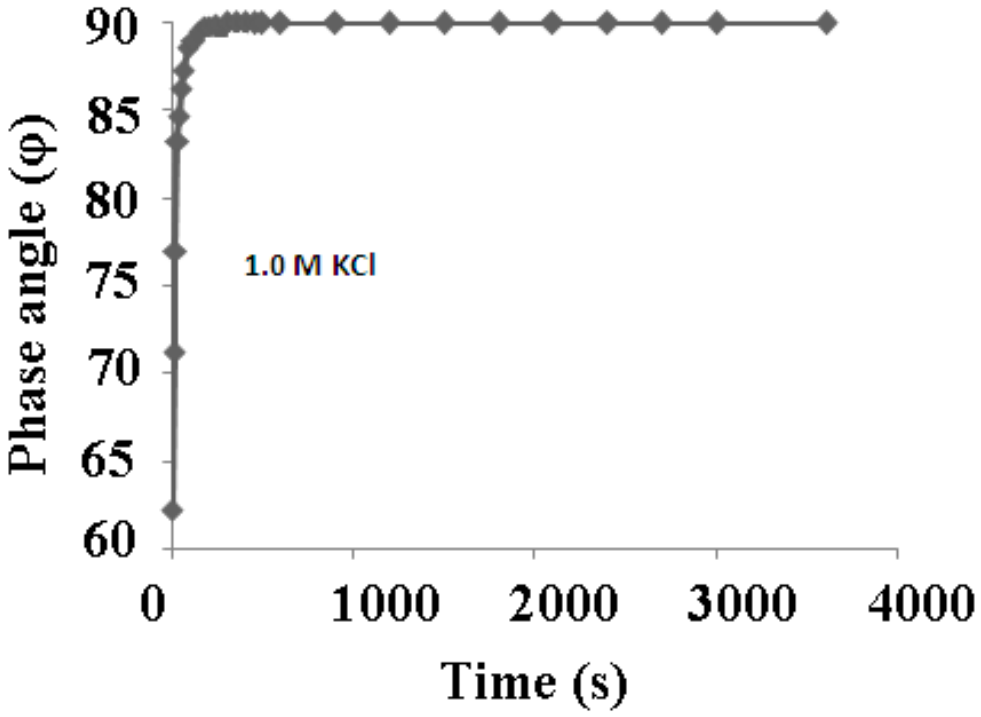


Figure 5.2 Bode Phase angle during formation of BLM with time in 1.0 M KCl bath solutions

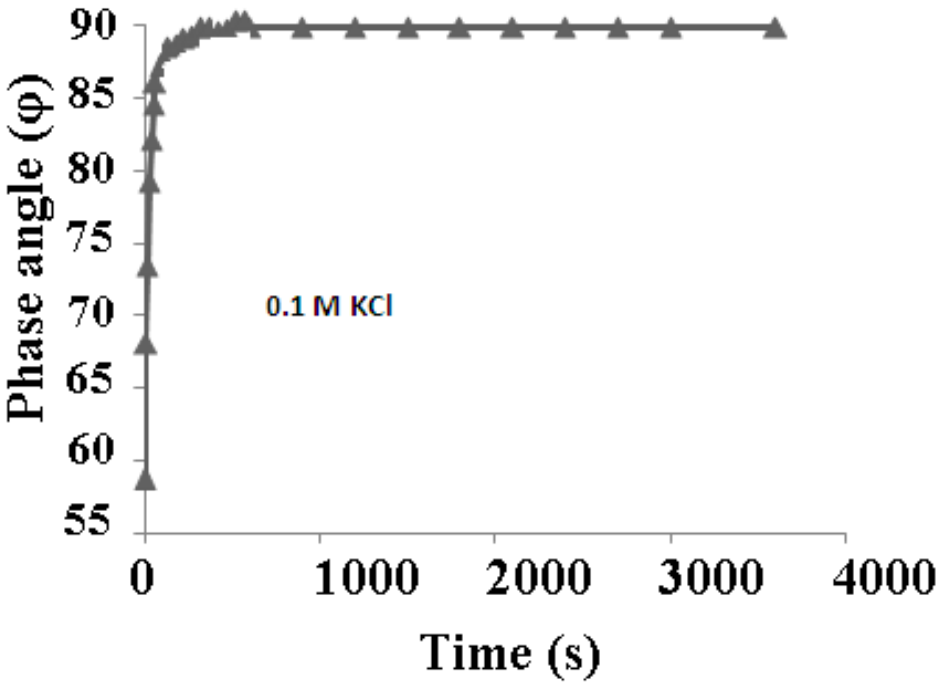


Figure 5.2 Variation of Bode Phase angle during formation of BLM with time in 0.1 M KCl bath solutions

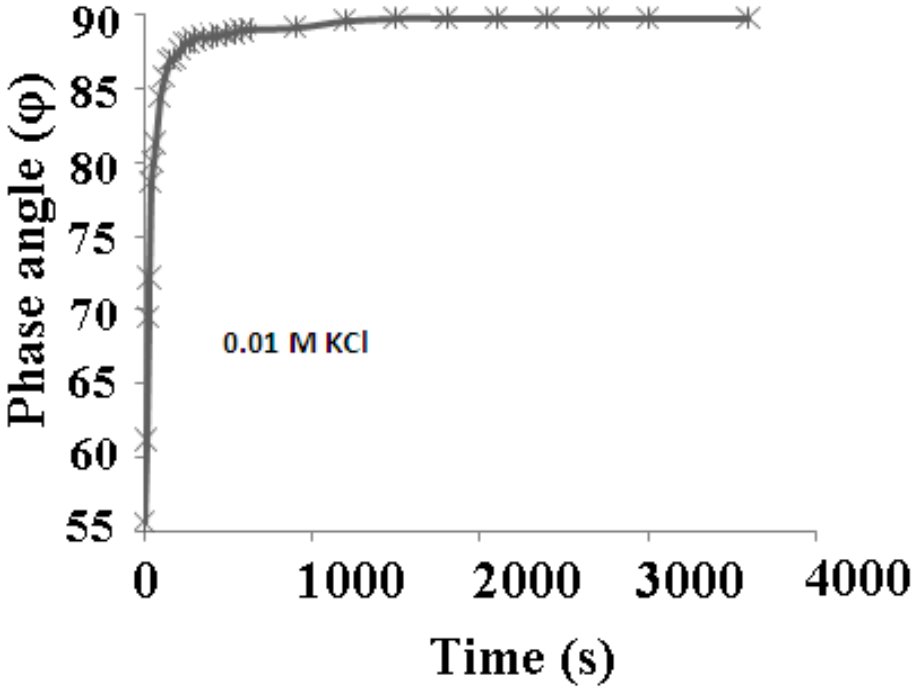


Figure 5.3 Variation of Bode Phase angle during formation of BLM with time in 0.01 M KCl bath solutions

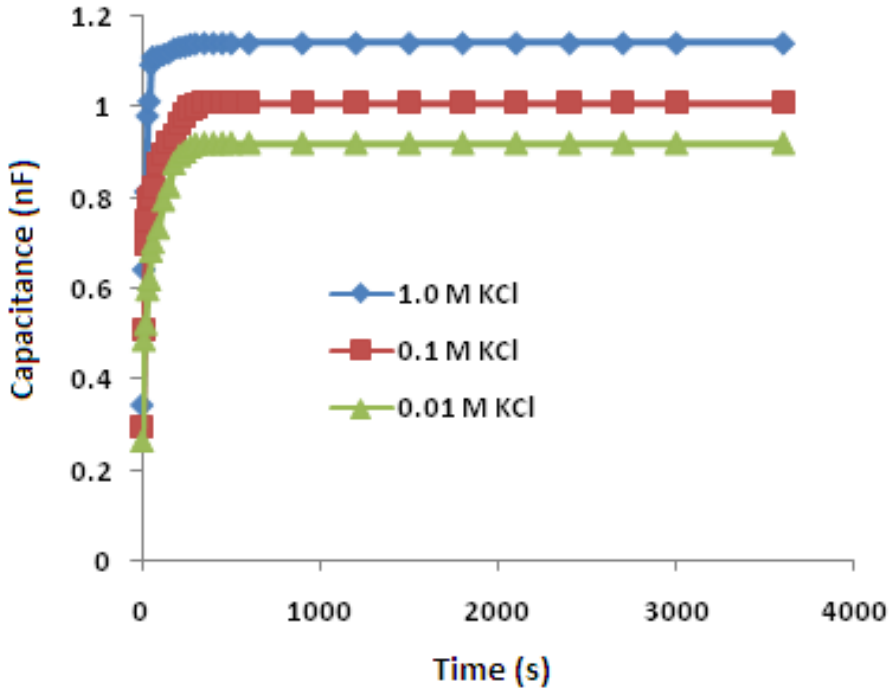


Figure 5.4 Variation of capacitance during formation of planar BLM with time in KCl bath solutions

The increasing trend shown in phase angle and capacitance values of BLM with time indicate the thinning and formation of membrane by self orientation of the phospholipid molecules into a bilayer with the exclusion of solvent molecules. Usually, the thinning of lipid films to form BLM begins after an incubation period. In the present study, an incubation period of only few seconds was observed, whereas up to 2 to 5 minutes have been reported by earlier workers (Sakmann and Neher, 1983). After this incubation period, there was a steep rise in capacitance indicating the formation of BLM. Capacitance of the membrane reached maximum and stable values in about 3 to 5 minutes. After three minutes, a marginal increase in capacitance of BLM was observed with time and the trend continued to remain so as seen in Figure 5.3.

When the increase in capacitance was less than 10pF per minute, planar BLM was considered as stable and used for further experiments. Though the incubation period was very short, a stabilization time of 30 minutes was invariably given in all the experiments to ensure the quality of the membrane and this stabilization time was recommended by earlier workers also. Some of the factors contribute to the stabilization process of membranes include slow exclusion of solvent molecules, increased intermolecular interactions developed between the lipid molecules and slow increase in the area of the membrane due to movement of P-G border towards the periphery of the aperture.

5.3 STABILITY AND AGING OF BLMS

Changes in phase angle and capacitance values of planar BLM with time are depicted in Figures 5.4 and 5.5, which indicate that black or planar BLMs are stable at least upto 10 - 15 hours. Though the capacitance of a bare membrane continued to rise even after 10 hours because of the above said factors, whereas the phase angle values started drifting down. This is indicative of the beginning of deterioration of the membrane after 10 to 15 hours. Aging of lipids and changes in the physico-chemical interactions between the medium and lipid molecules contribute to the deterioration of membranes.

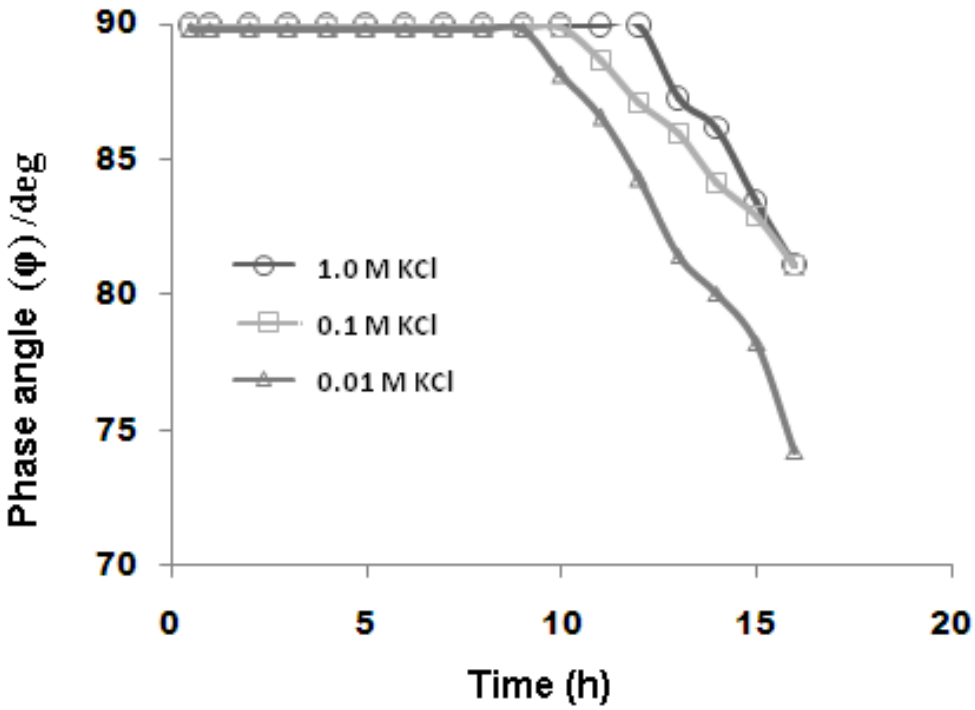


Figure 5.5 Aging of BLMs and changes in φ values

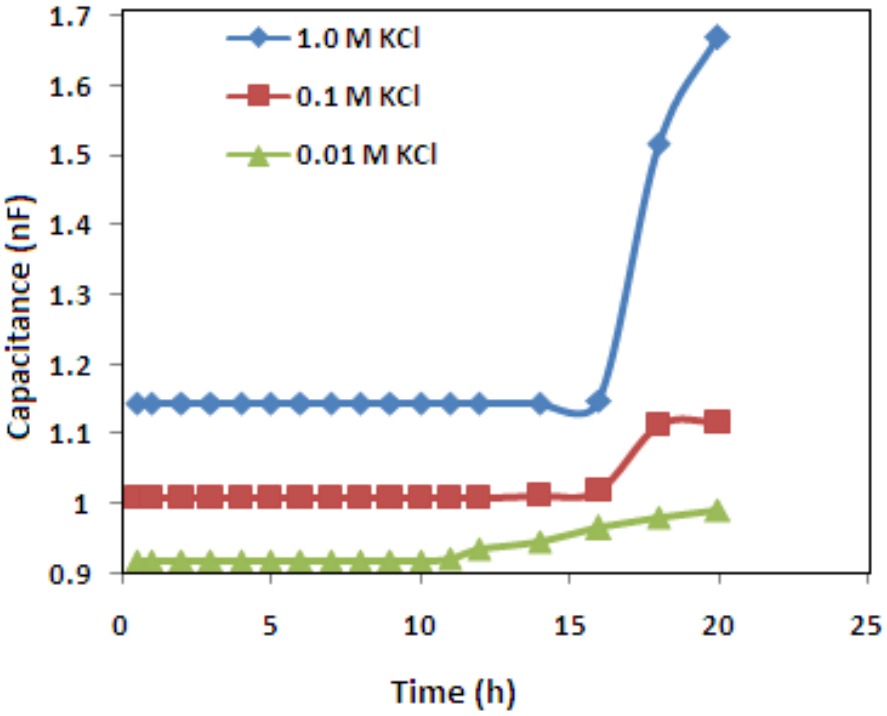


Figure 5.6 Capacitance profile of BLM with time

5.4 REPRODUCIBILITY OF PHASE ANGLE AND CAPACITANCE

From the very beginning of BLM studies, many workers have reported that the major drawback in BLM techniques is the irreproducibility of DC conductance from membrane to membrane (Corvington, 1979). But, the property of a particular membrane is consistent for a given period of time, where useful measurements are made. Earlier reports on AC studies showed that the capacitance values are somewhat reproducible under given set of conditions. Similar results were observed in the present study also as evident from the capacitance and phase angle values of BLMs in five trials as presented in Tables 5.1, 5.2 and 5.3.

Table 5.1

Reproducibility of capacitance values of Planar BLMs in 1.0 M KCl bath solution (5.0 Hz)

Trial No.	Phase angle (φ) deg	Deviation of phase angle from mean value	Capacitance (nF)	Deviation of capacitance from mean value
1	89.92	-0.002	1.144	0.0002
2	89.84	0.018	1.141	-0.0028
3	89.91	-0.012	1.147	0.0032
4	89.93	0.0008	1.144	0.0002
5	89.91	-0.012	1.143	-0.0008
mean	89.922	-	1.1438	-

Table 5.2

Reproducibility of capacitance values of Planar BLMs in 0.1 M KCl bath solution (5.0 Hz)

Trial No.	Phase angle (φ) deg	Deviation of phase angle from mean value	Capacitance (nF)	Deviation of capacitance from mean value
1	89.88	-0.002	1.009	-0.001
2	89.90	0.018	1.012	0.002
3	89.86	-0.022	1.005	-0.005
4	89.88	-0.002	1.014	0.004
5	89.89	0.008	1.010	0
mean	89.882	-	1.010	-

Table 5.3

Reproducibility of capacitance values of BLMs in 0.01 M KCl bath solution (5.0 Hz)

Trial No.	Phase angle (φ) deg	Deviation of phase angle from mean value	Capacitance (nF)	Deviation of capacitance from mean value
1	89.81	-0.018	0.919	0.001
2	89.83	0.002	0.917	-0.001
3	89.80	0.028	0.916	-0.002
4	89.82	0.008	0.920	0.002
5	89.78	-0.048	0.918	0
mean	89.828	-	0.918	-

5.5 CHANGES IN THE ELECTRICAL PROPERTIES OF SALT BRIDGE SUPPORTED BLM (sb-BLM)

The dispersion of phase angle of agar gel salt bridge supported bilayer lipid membrane formed in 1.0 M, 0.1 M and 0.01 M KCl solutions with frequency are shown in Figure 5.6. At the frequency 1000.2 Hz the phase angle values are 89.83, 88.95 and 78.92 in 1.0 M, 0.1 M and 0.01 M KCl bath solutions respectively. The specific membrane capacitance calculated at this frequency is very close to those values reported in the literature.

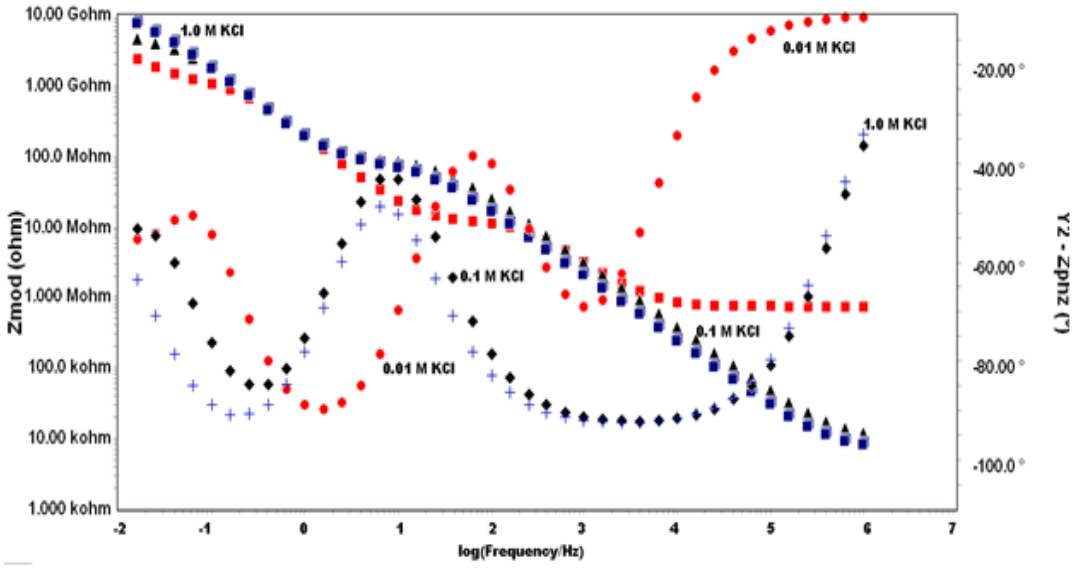
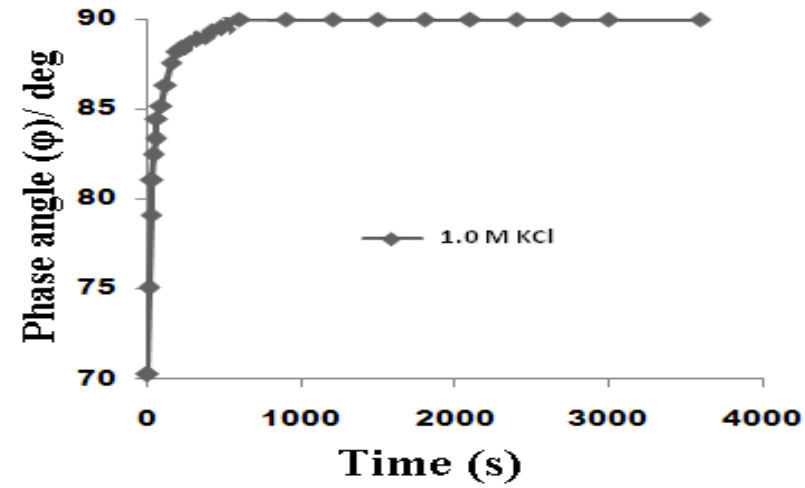
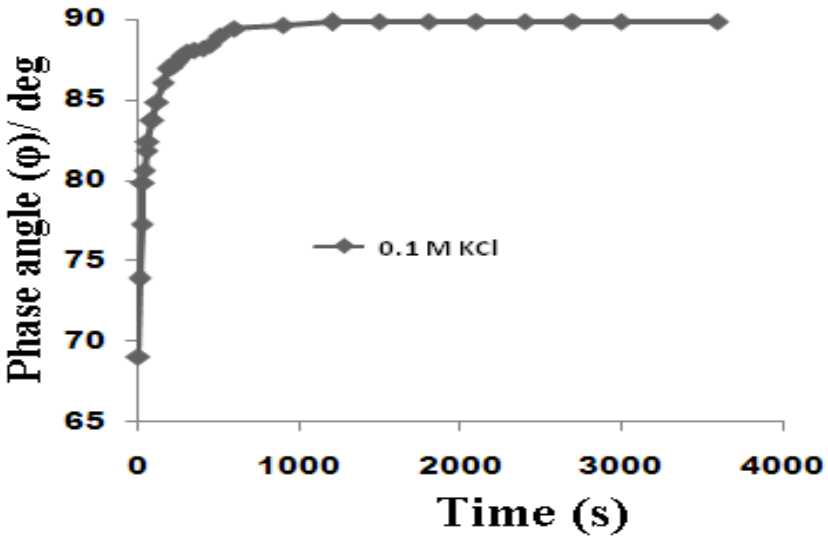


Figure 5.7 Dispersion of Bode Phase angle and IZI of sb-BLM with frequency in 1.0 M, 0.1 M and 0.01 M KCl Bath solutions.

The time dependent changes in the phase angle values of sb-BLM in 1.0 M, 0.1 M and 0.01 M KCl bath solutions are shown in Figure 5.7.





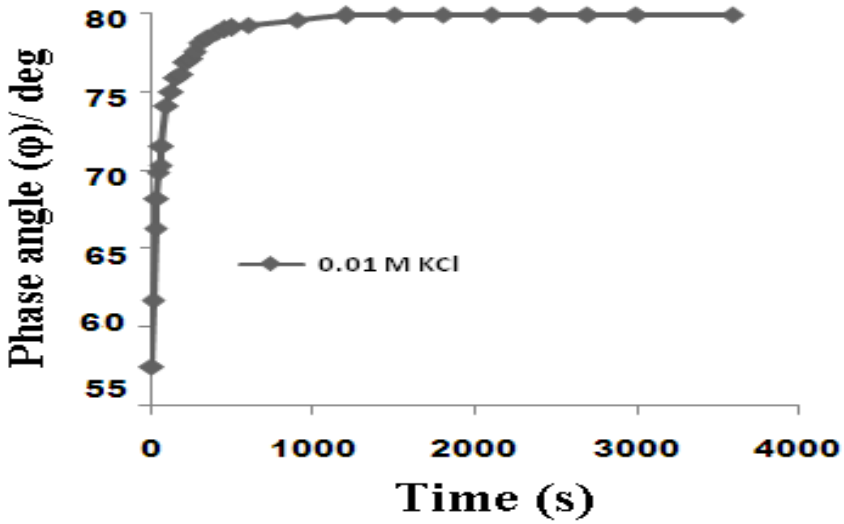


Figure 5.8 Variation of Bode Phase angle of sb-BLMs with time in KCl bath solutions.

The initial steep increase in phase angle value at 1002.3 Hz indicated that as soon as the lipid dispersion coated agar gel electrode is immersed in the bath solution the lipid molecules assembled spontaneously into a bilayer by eliminating solvent molecules.

Time dependent change in the capacitance of sb-BLM in 1.0 M, 0.1 M and 0.01 M KCl bath solutions are shown in Figure 5.8. Similar to phase angle, an increasing trend in capacitance was observed with time. A steep rise in capacitance was observed initially from 3 to 5 minutes. There after the capacitance increases slowly with time. In this time period the phase angle also becomes greater than 88 degree in 1.0 M and 0.1 M KCl bath solutions and it is greater than 75 in 0.01 M KCl bath, indicating formation of a stable membrane in short period. However, the phase angle and capacitance values become constant after 15 to 25 minutes. Hence, the electrical properties of sb-BLM were measured after 30 minutes in the drug membrane interaction studies.

The long time variation of phase angle and capacitance values with time is shown in Figure 5.9 and 5.10 respectively. From these Figures it is clear that the membrane formed exhibited stable phase angle and capacitance values for 40 to 55 hours. Hence, this type of model membrane system is suitable for studying long term drug- membrane interactions.

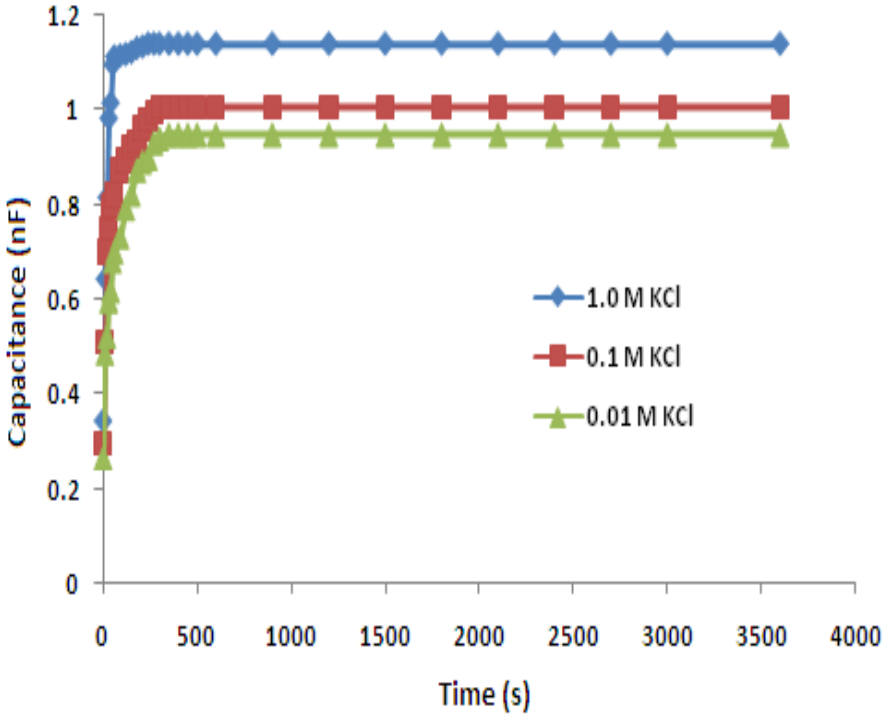


Figure 5.9 Variation of Capacitance of sb-BLM with timeStability and aging of sb-BLMs

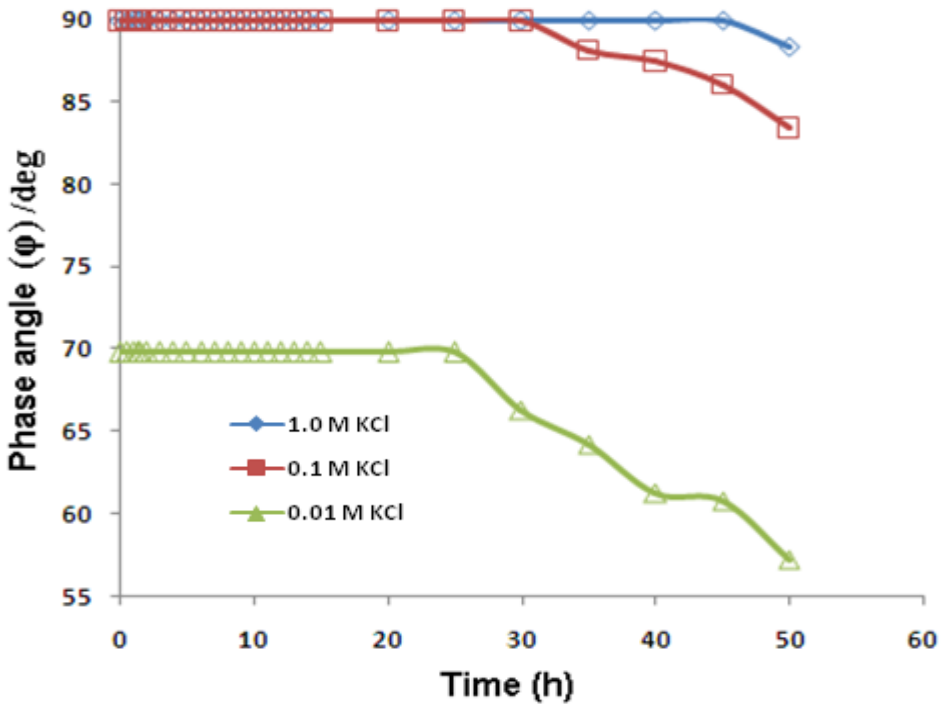


Figure 5.10 Time-dependent changes in phase angle (φ) values of sb-BLM

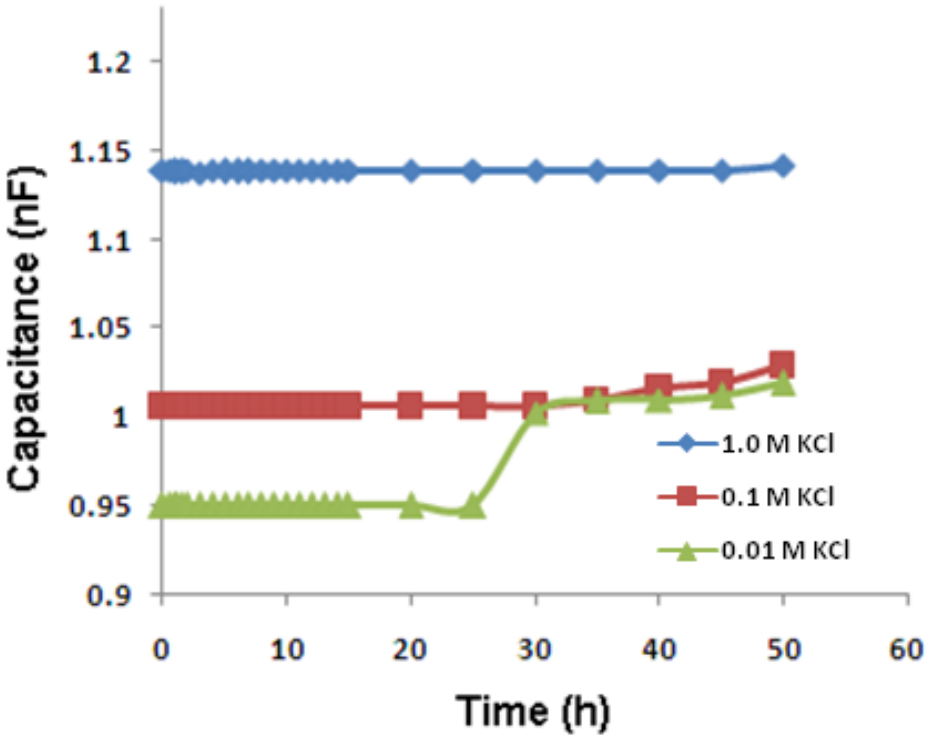


Figure 5.11 Capacitance Time profile of sb-BLM

5.6 REPRODUCIBILITY OF PHASE ANGLE AND CAPACITANCE

The phase angle and capacitance values of sb-BLM measured in five trials, in KCl bath solutions, are shown in Table 4.4, 4.5 and 4.6. The values measured in five trials are very close and the deviation from mean value is very low confirming the reproducibility of phase angle and capacitance.

Table 5.4

Reproducibility of capacitance values of sb-BLMs in 1.0 M KCl bath solution (1002.3 Hz)

Trial No.	Phase angle (φ) deg	Deviation of phase angle from mean value	Capacitance (nF)	Deviation of capacitance from mean value
1	89.92	0.032	1.139	0
2	89.91	0.022	1.142	0.003
3	89.90	0.012	1.137	-0.002
4	89.88	-0.008	1.141	0.002
5	89.83	-0.058	1.136	-0.003
mean	89.888	-	1.139	-

Table 5.5

Reproducibility of capacitance values of sb-BLMs in 0.1 M KCl bath solution (1002.3 Hz)

Trial No.	Phase angle (φ) deg	Deviation of phase angle from mean value	Capacitance (nF)	Deviation of capacitance from mean value
1	88.89	0.032	1.006	-0.001
2	88.86	0.002	1.009	0.002
3	88.84	-0.018	1.003	-0.004
4	88.87	0.012	1.011	0.004
5	88.83	-0.028	1.005	-0.002
mean	88.858	-	1.007	-

Table 5.6

Reproducibility of capacitance values of sb-BLMs in 0.01 M KCl bath solution (1002.3 Hz)

Trial No.	Phase angle (φ) deg	Deviation of phase angle from mean value	Capacitance (nF)	Deviation of capacitance from mean value
1	80.03	-0.104	0.952	-0.0002
2	79.92	-0.214	0.955	0.0028
3	80.11	-0.024	0.957	0.0048
4	79.55	-0.584	0.951	-0.0012
5	81.06	0.926	0.961	0.0058
mean	80.134	-	0.9552	-

5.7 ELECTRICAL PROPERTIES OF GLASSY CARBON SUPPORTED – BLM (s-BLM OR GCE-BLM)

The dispersion of phase angle of GCE-BLM formed in 1.0 M, 0.1 M and 0.01 M KCl solutions with frequency are shown in Figure 5.11. At the frequencies 6.1k Hz, 12.4 kHz and 15.4 kHz the phase angle values are found to be maximum (88.88, 82.15 and 70.12) in 1.0 M, 0.1 M and 0.01 M KCl bath solutions respectively. Normally s-BLMs show lower phase angle due to their higher ionic conductance (Zviman et al, 1991) (Laptkova et al, 2005).

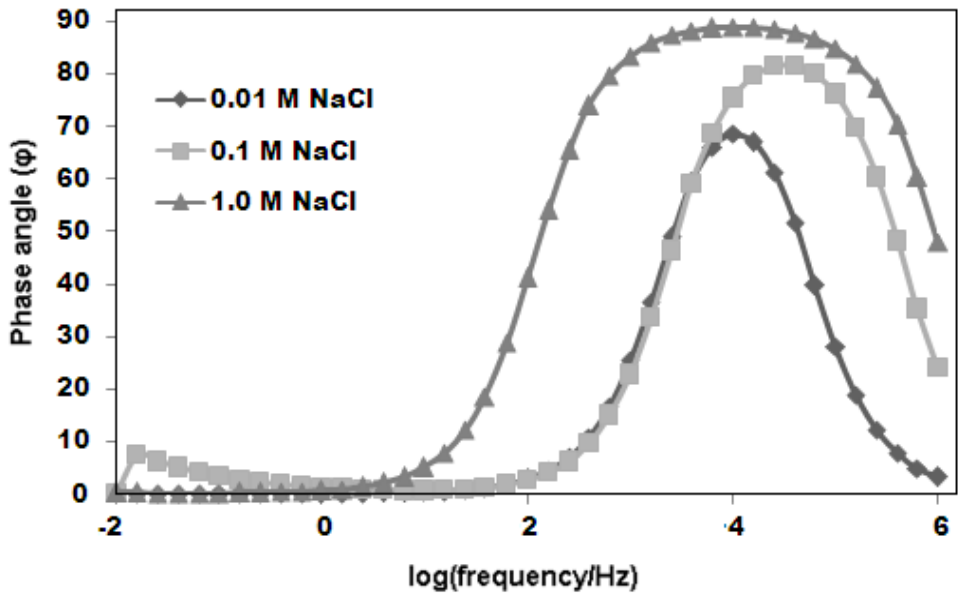


Figure 5.12 Dispersion of Bode Phase angle of glassy carbon supported BLM with frequency

The time dependent changes in the phase angle values of s-BLM in 1.0 M, 0.1 M and 0.01 M KCl bath solutions, in the above mentioned frequencies, are shown in Figure 5.12.

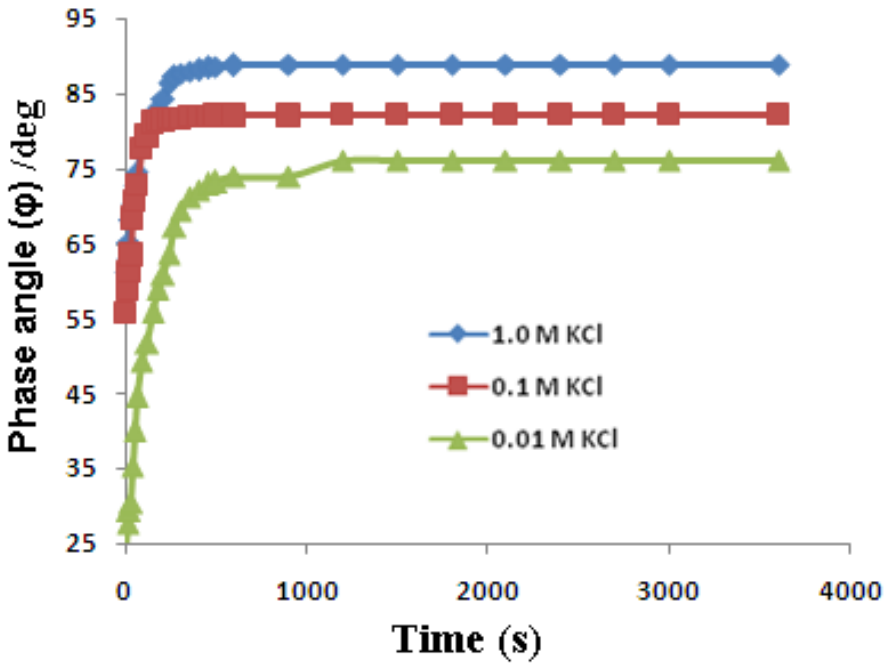


Figure 5.13 Variation of Phase angle of GCE-BLM with time in KCl bath solutions

The phase angle values are measured at the frequencies 6.1, 12.4 and 15.4 kHz in 1.0 M, 0.1 M and 0.01 M KCl bath solutions respectively, after applying the lipid dispersion on the electrochemically activated glassy carbon electrode surface and immersing in KCl bath solutions. Initially the phase angle values increased steeply like in the previous membrane systems and reached a value close to maximum in 5 minutes and then increased incrementally.

Variation of capacitance of GCE-BLM in KCl bath solutions with time at the above mentioned frequencies are represented in the Figure 5.13.

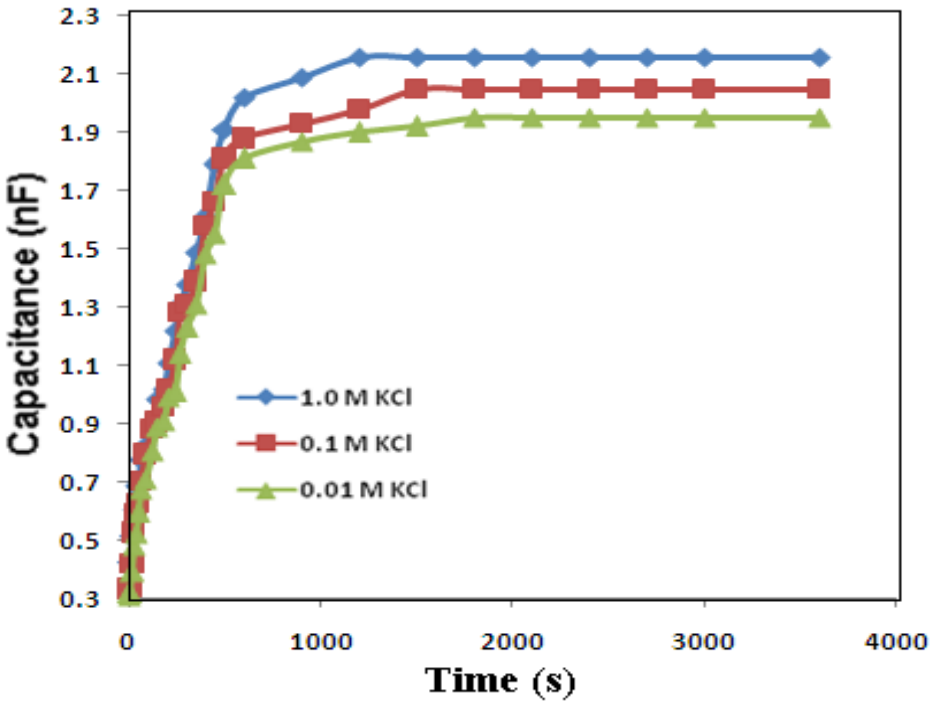


Figure 5.14 Capacitance Time profile of GCE-BLM

The capacitance values of GCE-BLM also initially increased steeply, reached the maximum value in 5 minutes, and then the increase became incremental.

In GCE-BLM the difference in phase angle values is much larger than those of the previously discussed model membrane systems. This is due to a fact that in GCE-BLMs, there exists relatively large sized pores and provides path for movement of smaller ions across it leading to a higher ionic conductance (Zviman et al, 1991) (Laptkova et al, 2005). Larger the size of the pores lower is the phase angle and hence, a decrease in NaCl concentration in the bath solution might have increased the pore size in GCE-BLM.

5.8. STABILITY AND AGING OF GCE-BLMS

The time dependent changes in the phase angle and capacitance values of GCE-BLMs in KCl bath solutions are shown in Figure 5.14 and 5.15 respectively. The membrane exhibited stable phase angle and capacitance values for 40 to 50 hours and hence, this type of model membrane system is suitable for studying long term drug- membrane interactions. Since pore size is relatively larger they can also be used to construct electrochemical biosensors, where the lipid soluble drugs can directly undergo the redox reaction or redox peak currents of marker ion varies with lipid soluble analyte concentration (Lu et al, 2008).

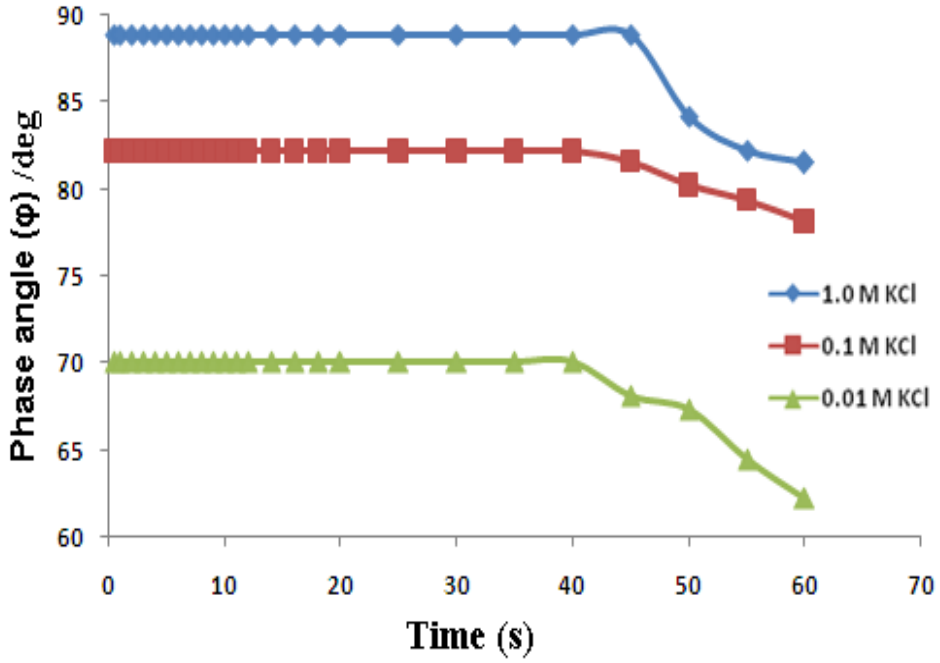


Figure 5.15 Time-dependent changes in phase angle (φ) values of GCE-BLM

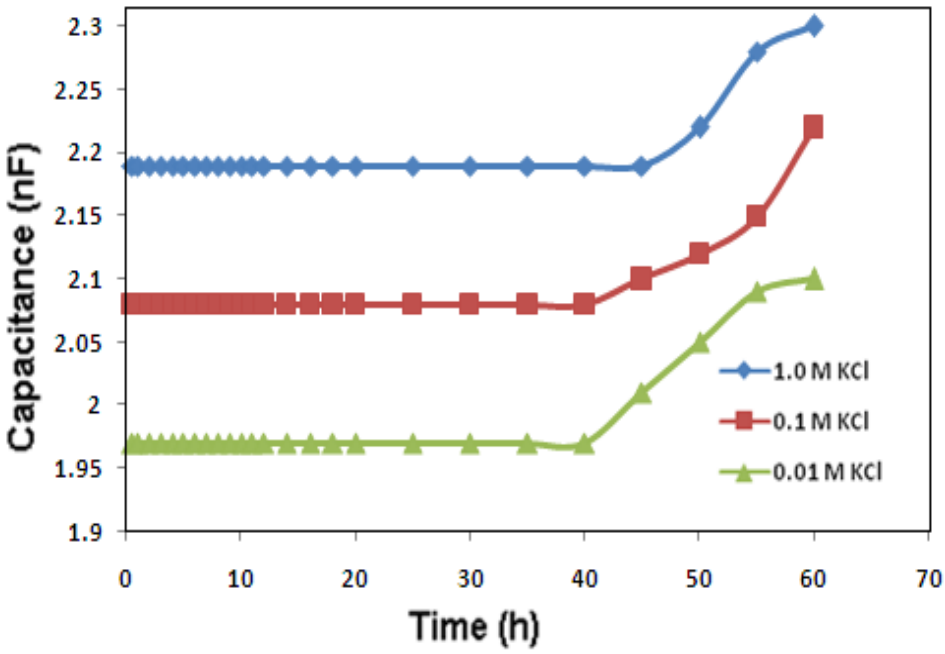


Figure 5.16 Capacitance profile of GCE-BLM with time

5.9. REPRODUCIBILITY OF PHASE ANGLE AND CAPACITANCE

The phase angle and capacitance values of GCE-BLM measured in five trials, in KCl bath solutions, are shown in Table 5.7, 5.8 and 5.9. The values measured in five trials are very close and the deviation from mean value is very low confirming the reproducibility of phase angle and capacitance.

Table 5.7

Reproducibility of capacitance values of GCE-BLMs in 1.0 M KCl bath solution (6.1 kHz)

Trial No.	Phase angle (φ) deg	Deviation of phase angle from mean value	Capacitance (nF)	Deviation of capacitance from mean value
1	88.85	0.002	2.191	0.001
2	88.83	-0.018	2.194	0.002
3	88.87	0.022	2.189	-0.001
4	88.81	-0.038	2.192	0
5	88.88	0.032	2.194	0.002
Mean	88.848	-	2.192	-

Table 5.8

Reproducibility of capacitance values of GCE-BLMs in 0.1 M KCl bath solution (12.4 kHz)

Trial No.	Phase angle (φ) deg	Deviation of phase angle from mean value	Capacitance (nF)	Deviation of capacitance from mean value
1	82.22	0.042	2.08	0.026
2	82.24	0.062	2.04	-0.014
3	82.15	-0.028	2.07	0.016
4	82.17	-0.008	2.05	-0.004
5	82.11	-0.046	2.03	-0.024
Mean	82.178	-	2.054	-

Table 5.9

Reproducibility of capacitance values of GCE-BLMs in 0.01 M KCl bath solution (15.4 kHz)

Trial No.	Phase angle (φ)	Deviation of phase angle from mean value	Capacitance (nF)	Deviation of phase angle from mean value
1	70.25	-0.154	1.962	0.0002
2	70.33	-0.074	1.959	-0.0028
3	70.51	0.106	1.964	0.0022
4	70.45	0.046	1.966	0.0042
5	70.48	0.076	1.958	-0.0038
Mean	70.404	-	1.9618	-

5.10 EFFECT OF KCl CONCENTRATION ON THE STABILITY AND KINETICS OF MEMBRANE FORMATION

The above discussions clearly indicated that the stability and membrane formation kinetics are strongly influenced by the KCl concentration in the bath solution. When the KCl concentration increases, the ionic pressure on the BLM surfaces also increases and this keeps the phospholipid molecules intact. Similarly due to high ionic pressure the solvent molecules (n-decane) are excluded from the dispersion and moved to the PG border, while the phospholipid molecules self assemble into a bilayer. This can be easily seen in the capacitance profile diagram of various model membrane systems with time.