Electrochemical Studies on Interaction of Ofloxacin with ITO-PANI Supported Bilayer Lipid Membrane

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Abstract: Bilayer phospholipid membrane was formed on the ITO-Pani surface in NaCl bath solution at different pH. Characterization and interaction of bilayer lipid membrane (BLM) with Ofloxacin molecules in 0.1 M NaCl solution was studied at different pH employing electrochemical impedance spectroscopy, cyclic voltammetry and chronoamperometric methods. The changes in the properties of BLM due to addition of Ofloxacin depends on the pH of bath solution. At low pH the membrane surface is positively charged and the interaction of drug molecules with the BLM surface takes place through chloride bridge. At alkaline pH the membrane surface is negatively charged and the drug molecules get attached through hydrogen bonding. The results of electrochemical impedance spectroscopy, cyclic voltammetry and chronoamperometry support each other.

Keywords: Bilayer lipid membrane; Drug-membrane interaction; EIS; cyclic voltammetry; Ofloxacin

1. INTRODUCTION

The field of drug – membrane interactions cover wide range of scientific discipline starting from chemical methods of synthesis of drug through biophysics to pharmacology [1]. The phospholipid molecules, which act as protecting layer for cells and cell organelles are involved in several pathologies including cancer [2]. The cell membranes protect the animal cells from the entering of unwanted materials into the cell and cell organelles. Protein molecules span the cell membrane and provide a path for materials transport across the cell membrane. Many proteins and lipid molecules gathered at the membrane and most of the cellular activities occur. Even though the drug molecules bind to the membrane protein and regulate their activities, the role of cell membrane is very crucial.

The development of any drug must take into account the activities of membrane. The action of drugs on the biological membranes and inside the cells controlled by biomembranes. For the action of drug molecules inside the cell, they must cross the cell membranes and reach the targets. Some properties of cell membranes change due to interaction of drug molecules with lipid proteins. When many drug molecules or foreign molecules enter the membrane, cell permeability, cell fusion, cell resistivity change and lead to changes in the conformation of receptor protein [3]. The lipid membrane may also change the conformation in preference to the drug molecules [3].

The unique properties of supported bilayer lipid membranes are fascinating and gained more attention in the fundamental and applied research works [4-8]. The model membrane systems are used to study the properties of cell membranes and the associated progressions such as molecular recognition, cell adhesion, enzymatic catalysis and cell fusion [4]. Moreover, the pharmacological activity of number of drugs depends on their interaction with biological membranes [5,9]. The perturbation of biomembranes by various drug molecules lead to changes in the membrane curvature or phase separation [9]. This can also cause change in the conformations of embedded protein molecules. Thus, drug membrane interaction becomes an important factor to be considered in the drug actions.

Urinary tract diseases, pneumonia, bronchitis, venereal sickness etc. are caused by microorganisms. These infections are treated using an antibiotic Ofloxacin [11]. Ofloxacin also exists in special forms for the treatment of eye and ear

infections [11]. . Gastrodigestive problems are the most widely recognized unfavorable occasions related to the utilization of ofloxacin, including sickness, looseness of the bowels, and stomach uneasiness. Normal CNS grievances incorporate cerebral pain, wooziness, and anxiety. Tipsiness and rest aggravations might be risky, especially at higher doses. Mind flights and maniacal responses have likewise been accounted for. Looseness of the bowels might be brought about by the disposal of useful microorganisms ordinarily tracked down in the colon. The indications of a dangerous response include wheezing; snugness in the chest; fever; tingling; terrible hack; blue skin tone; fits; expanding of the face, lips, tongue, or throat. Fig.1 shows the structure of Ofloxacin. The present study is aimed at to study the interaction of Ofloxacin with supported lipid bilayer. A bilayer lipid membrane was formed on the polymer coated ITO surface in 0.1 M NaCl solution. The drug – membrane interactions are characterized by electrochemical impedance spectroscopy, chronoamperometry and cyclic voltammetry.

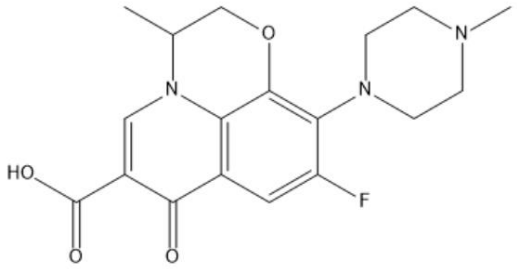

Figure.1 Structure of Ofloxacin

2. MATERIALS AND METHODS

2.1 Extraction of Phospholipid molecules from egg yellow yolk

Fresh hen eggs were purchased locally and used for the extraction of phospholipid. All the chemicals used were analytical grade and a triple distilled water was used for preparation solutions. The lipid extraction was done using chloroform and acetone purchased from Sigma Aldrich. The yellow yolks were isolated from three hen eggs. The isolated yellow yolks were dissolved in 500 mL of chloroform and acetone mixture in the ratio 3:2. The solution is subjected to vacuum evaporation at 0.1 atm pressure for 5h. Yellow colored oily liquid obtained was added to acetone, where a white colored solid of phospholipid separated out. The phospholipid was dried under nitrogen atmosphere and weighed. About 6 g phospholipid was obtained. It is dissolved in chloroform to have a stock solution of 5mg/mL and used for lipid bilayer formation. 100 µL of the stock solution was taken in the 2 mL screw cap bottle and dried under nitrogen atmosphere to form a film. This film was redissolved in n-decane (Merck, Germany) and used for the formation of bilayer lipid membrane. The ITO (indiumtinoxide) coated glass plates were purchased locally and used as support for bilayer lipid membrane formation. 1.0 M NaCl solution was prepared in triple distilled water and diluted to 0.1 M concentration using the same.A 3.5 mM Ofloxacin stock solution was prepared using triple distilled water and from this suitable dilutions were made. Sodium hydroxide and boric acid were used for preparing buffer solutions of pH 2, 5 and 10.

2.2 Electrochemical impedance spectroscopy

Interaction of Ofloxacin with the bilayer lipid membrane on ITO surface was studied using electrochemical impedance spectroscopy. A three-electrode set up was used for this study. Bare and BLM formed ITO acted as working electrode, saturated calomel electrode was used as reference electrode. A platinum foil served as counter electrode. The Nyquist plots were recorded for the bare and drug doped ITO supported BLM at various concentrations at the open circuit potential by superimposing an AC sinusoidal voltage of 10 mV in the frequency range 1MHz to 10mHz.

2.3 Cyclic voltammograms

Cyclic voltammograms were recorded for the bare and drug doped ITO supported BLM using the same cell set up employed for the electrochemical impedance studies. Cyclic voltammograms were recorded by sweeping the potential in the range 0.0 V to 1.8 V vs SCE at the scan rate 50 mV/s.

2.4 Chronoamperometric studies

A three-electrode set up employed for the previous two studies was used for chronoamperometric studies. The chronoamperograms were recorded with an initial delay of 300s. The potentials were steeped with an equilibration time of 5s. The potential of working electrode was stepped and the resulting current was recorded as a function of time.

3. RESULTS AND DISCUSSION

3.1 Electrochemical polymerization of aniline on ITO surface

Aniline was electrochemically polymerized on the ITO surface by cyclic voltammetric technique. 0.1 M aniline in 1.0 M HCl solution was used for electropolymerization. The potential of ITO electrode swept between -0.3 V to 1.3 V vs SCE at the scan rate of 100 mV/s. The various peaks characterizing the electropolymerization of aniline on ITO surface are presented in Fig.2. The deposition of polyaniline onto ITO surface is characterized by successive increase in the peak currents, which is presented in the Fig.3. During positive scanning three peaks were observed for oxidation. The first peak corresponds to conversion of leucoemeraldine(le) to emeraldine (em) [12] while the third peak corresponds to diradical cation formation [13]. The first and third peaks in the reduction half cycle are coupled reductive peaks of third and first oxidation peaks respectively [14]. Formation of orthocoupled polymers is represented by the second peak both in the oxidative and reductive peaks under experimental conditions (middle peak) and also the degradation of polyaniline formed to give soluble species such as benzoquinone and hydrobenzoquinone[15].

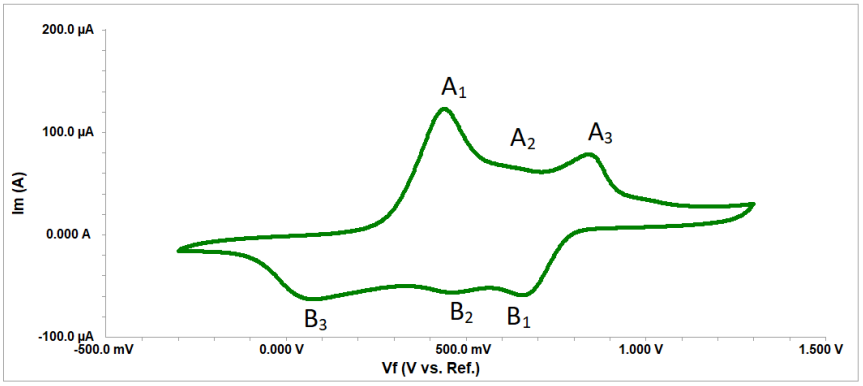

Figure.2 Characteristic peaks for electropolymerization of aniline on ITO surface.

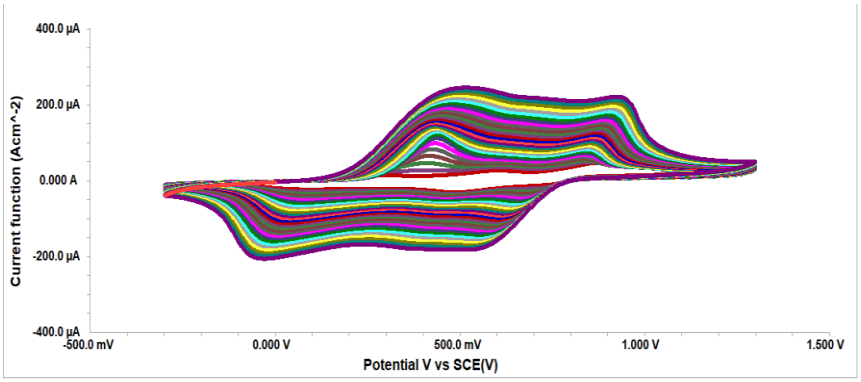

Figure.3 Electropolymerization of Aniline on ITO surface by cyclic voltammetry.

3.2 Electrochemical Impedance Spectroscopy

The formation of bilayer phospholipid membrane on the polyaniline coated ITO surface was analysed using electrochemical impedance spectroscopy. Fig.4 shows the Bode plots for BLM covered polyaniline surface in 0.1 M NaCl solution. Wide range of frequency corresponding to 90 degree phase angle implies formation of BLM phase over polyaniline coated ITO surface. The Nyquist plots recorded for the interaction of Ofloxacin with ITO supported BLM in 0.1 M NaCl solution at different pH are presented in the Fig.5,6 and 7 respectively. The Nyquist plots obtained

are not straight forward to obtain the electrochemical properties of any system. Equivalent circuits are employed to extract the electrochemical properties of the system under study [16-18].

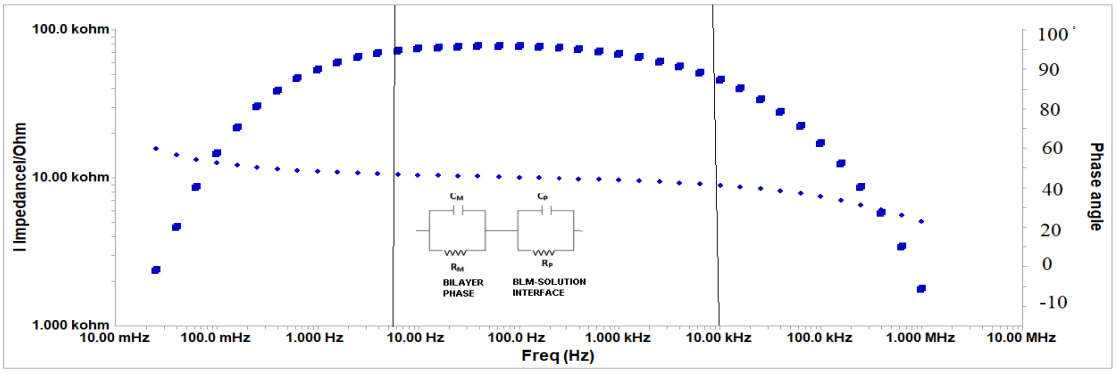

Figure.4 Bode plot for the BLM formation over the polyaniline deposited ITO surface.

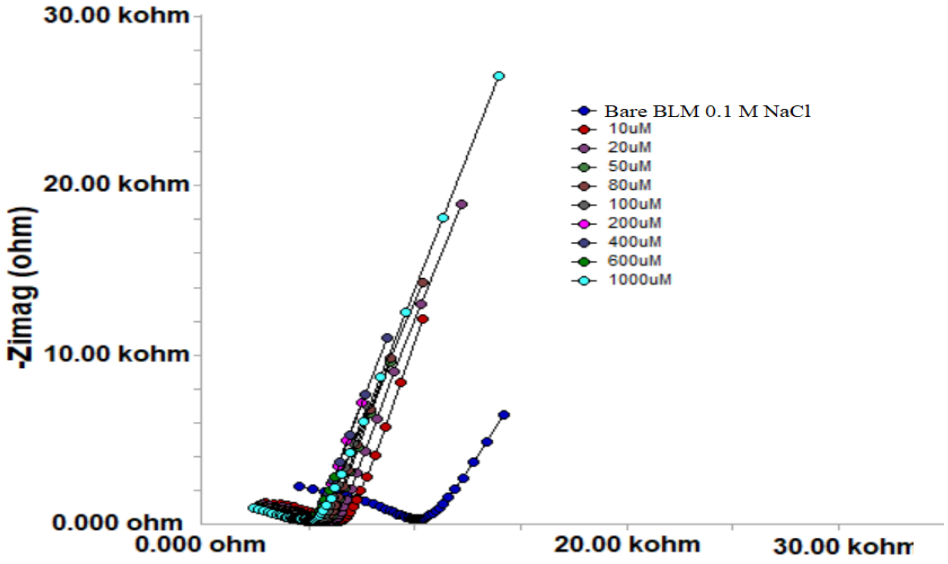

Figure.5 Nyquist plots for bare and drug doped ITO-Pani supported BLM in 0.1 M NaCl solution at pH 2

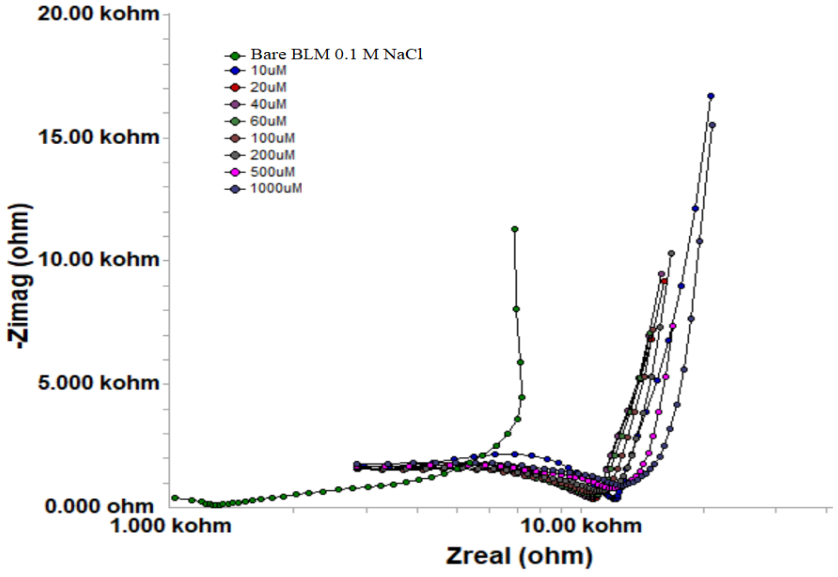

Figure.6 Nyquist plots for bare and drug doped ITO-Pani supported BLM in 0.1 M NaCl solution at pH 5

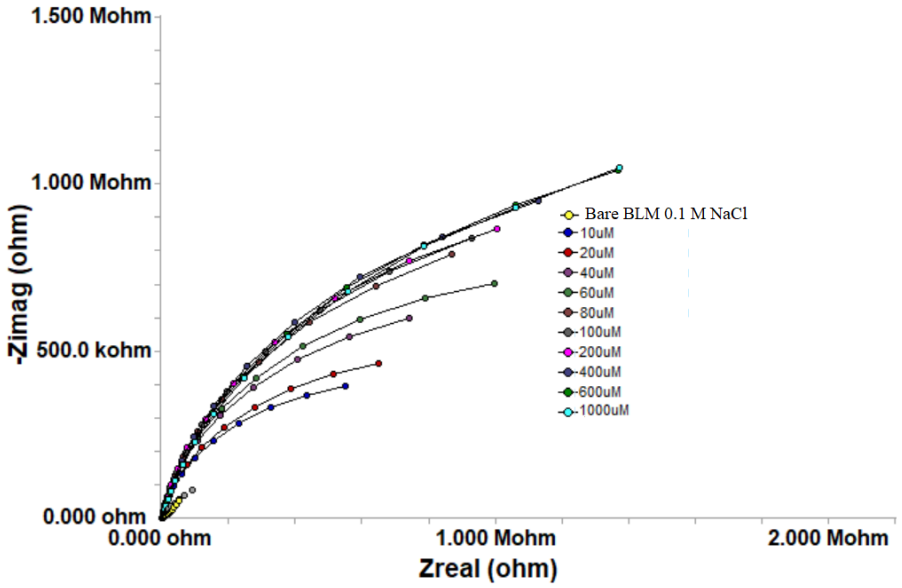

Figure.7 Nyquist plots for bare and drug doped ITO-Pani supported BLM in 0.1 M NaCl solution at pH 10

The electrochemical impedance parameters were obtained by fitting the experimental impedance curves with the equivalent circuit shown in the Fig.8. The electrochemical impedance parameters are presented in the Table 1,2 and 3. From these tables it is clear that in the acidic pH, the impedance of BLM phase decreases and the impedance of BLM phase increases with increase in concentration of Ofloxacin. This can be attributed to the change in surface charge of BLM with pH. In acidic pH the BLM surface is positively charged [19,20] and in alkaline pH the surface of BLM is negatively charged [21,22]. In acidic pH Ofloxaxin molecules are protonated and the positively charged drug molecules can't get attached with the positively charged BLM surface. The chloride ions can get attached to the positively charged BLM surface through electrostatic force and can also bind to the positively charged drug molecules. Thus, the chloride ions act as bridging ions and attach the positively charged drug molecules to the BLM surface which can further get partitioned into BLM phase and thereby increase the conductance of BLM phase (decrease in resistance of BLM phase). Similar type of anion interaction with the positively charged BLM surface were reported in the literature [23-26].

In alkaline pH the drug molecules mostly exist in neutral form. The drug molecules form hydrogen bonding with COOH proton of Ofloxacin with the negatively charged BLM surface. When the concentration of Ofloxacin increases, the surface attachment through hydrogen bonding with the negatively charged BLM surface also increases and hence the ion transport across BLM surface decreases.

Table.1 Electrochemical impedance parameters for the bare and drug doped ITO-Pani supported BLM in 0.1 M NaCl at pH 2.

S.NO	Concentration of Ofloxacin (µM)	R_1 (Ohm)	C_1 (nF)	R_2 (Ohm)	C_2 (nF)
1	Bare	2004	0.0419	9765	1.674
2	10	1426	0.0032	8907	1.688
3	20	1070	0.02923	8015	1.753
4	50	890	0.0618	7018	1.906
5	80	821	0.2955	6984	2.005
6	100	701	0.1168	5325	2.112
7	200	549	0.0252	5021	2.321
8.	400	504	0.0455	4212	2.763
9	600	473	0.0781	3735	2.915
10	1000	396	0.0220	2005	3.664

Table.2 Electrochemical impedance parameters for the bare and drug doped ITO-Pani supported BLM in 0.1 M NaCl at pH 5.

S.NO	Concentration of Ofloxacin (µM)	R_1 (k Ohm)	C_1 (nF)	R_2 (Ohm)	C_2 (nF)
1	Bare	3207	0.0026	11593	1.496
2	10	3115	0.0058	10919	1.501
3	20	3017	0.0099	7418	1.554
4	40	2876	0.0208	7060	1.673
5	60	2564	0.0209	6343	1.703
6	100	1997	0.0227	6033	1.765
7	200	1765	0.0341	4656	1.923
8.	500	1043	0.0453	4636	2.112
9	1000	827	0.1487	4506	2.443

Table.2 Electrochemical impedance parameters for the bare and drug doped ITO-Pani supported BLM in 0.1 M NaCl at pH 5.

S.NO	Concentration of Ofloxacin (µM)	R_1 (Ohm)	C_1 (nF)	R_2 (Ohm)	C_2 (nF)
1	Bare	1803	0.0237	298170	1.2898
2	10	1886	0.0258	310629	1.3369
3	20	1983	0.0299	362468	1.6172
4	40	2004	0.0356	465954	2.0031
5	60	2015	0.0398	555133	2.0558
6	80	2076	0.0421	608313	2.2693
7	100	2122	0.0643	646183	2.3057
8	200	2131	0.0753	670278	2.3263
9.	400	2196	0.0995	731561	2.6332
10	600	2214	0.1128	798728	2.9463
11	1000	2231	0.1765	798858	2.9847

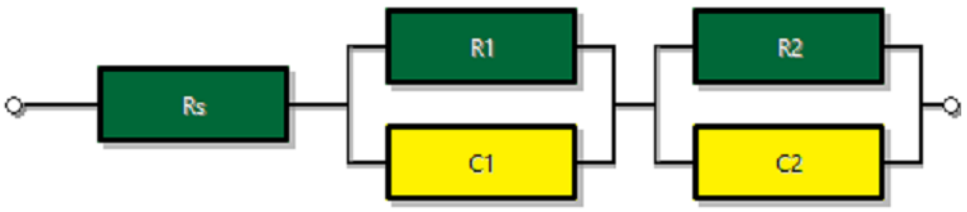

Figure.8 Equivalent circuit employed for the extraction of electrochemical impedance parameters

3.3 Cyclic voltammetric studies

The cyclic voltammograms recorded for the interaction of Ofloxacin with BLM in 0.1 M NaCl bath solution at the pH 2,5 and 10. The recorded cyclic voltammograms are presented in the Fig.9,10 and 11. From these cyclic voltammograms it is clear that the anodic and cathodic peak currents observed decrease with the concentration of Ofloxacin in both the acidic and alkaline bath solutions. This is attributed to the partition of Ofloxacin into the BLM phase and thereby obstruct the electron transfer reactions, namely redox reactions at ITO-Pani surface in the acidic pH and in the alkaline bath the electron transfer for oxygen evolution (oxidation) and reduction ($^{-}$OH formation) are obstructed by surface attachment of drug molecules.

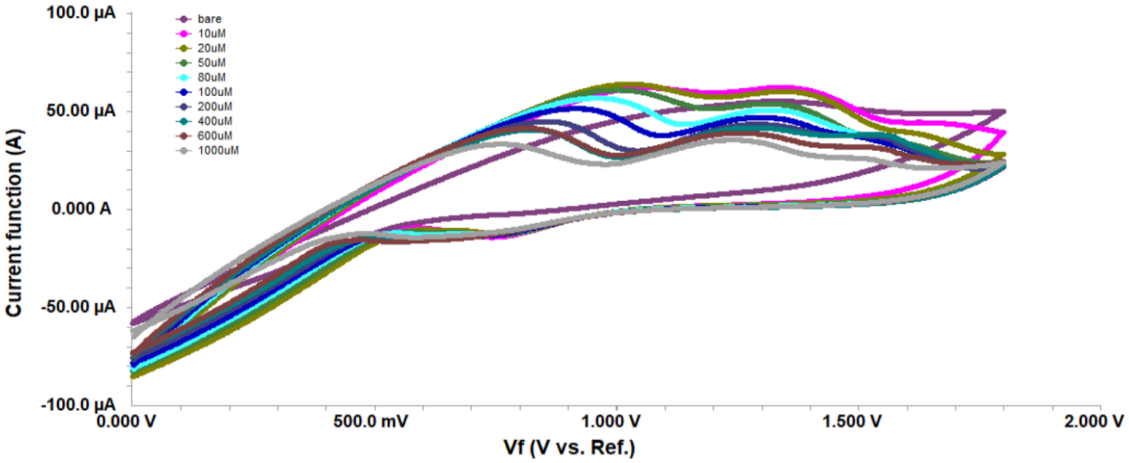

Figure.9 Cyclic voltammograms for bare and drug doped ITO-Pani supported BLM in 0.1 M NaCl solution at pH 2

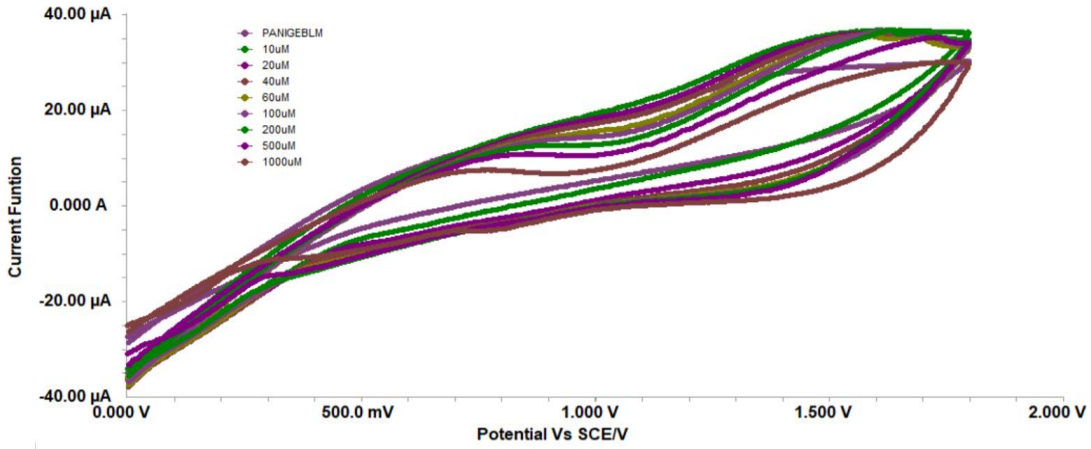

Figure.10 Cyclic voltammograms for bare and drug doped ITO-Pani supported BLM in 0.1 M NaCl solution at pH 5

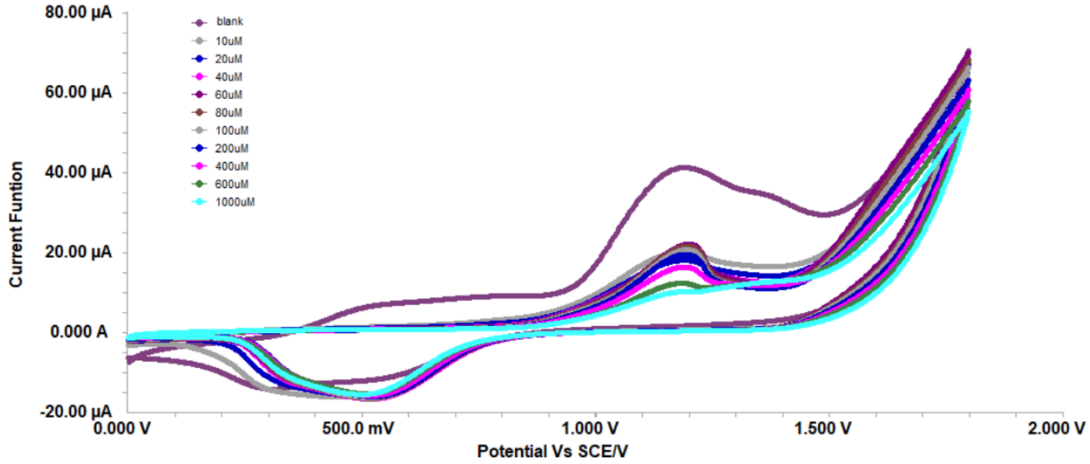

Figure.11 Cyclic voltammograms for bare and drug doped ITO-Pani supported BLM in 0.1 M NaCl solution at pH 10

3.4 Chronoamperometric measurements

The chronoamperograms recorded for the bare and drug doped ITO-Pani supported BLMs in 0.1 M NaCl bath solutions at different pH are shown in Fig.12,13 and 14. From these figures it is clear that the current in the cathodic and anodic directions decreased with the concentration of Ofloxacin in the bath solutions. This could be due to the partition of drug molecules into the BLM phase at low pH and increase in surface attachment of drug molecules at high pH. Thus, results of electrochemical impedance spectroscopy, cyclic voltammetry and chronoamperometry support each other and provide evidence for the obstruction of electron transfer reaction at the BLM coated ITO-Pani surface.

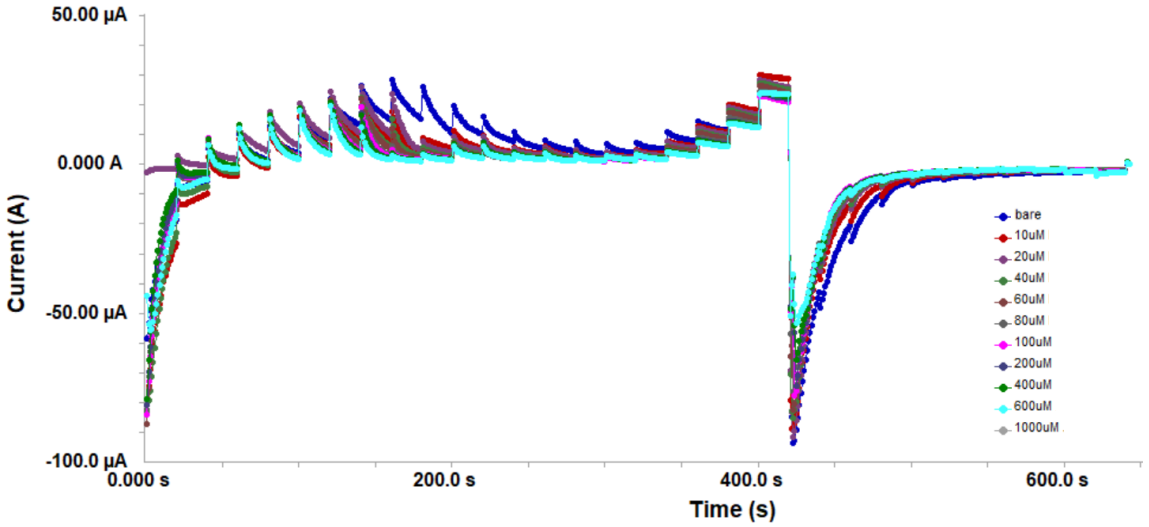

Figure.12 Chronoamperograms for the bare and drug doped ITO-Pani supported BLM in 0.1 M NaCl solution at pH 2

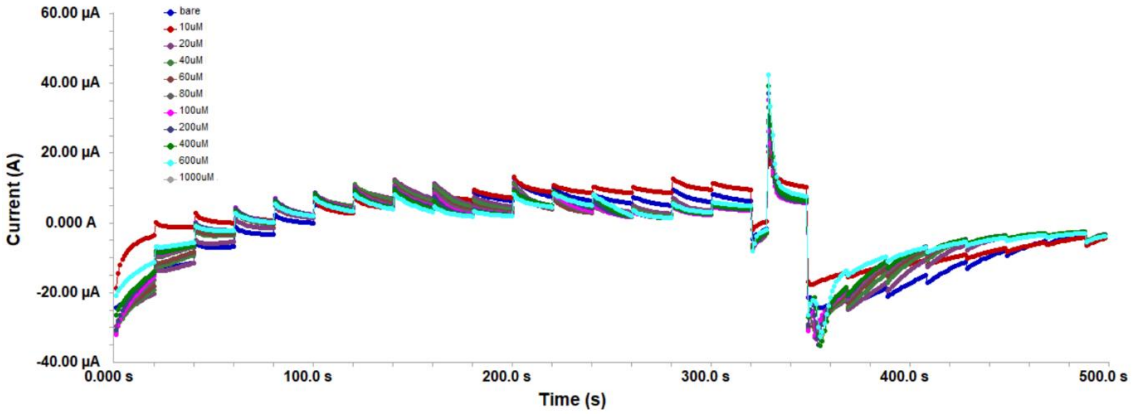

Figure.13 Chronoamperograms for the bare and drug doped ITO-Pani supported BLM in 0.1 M NaCl solution at pH 5

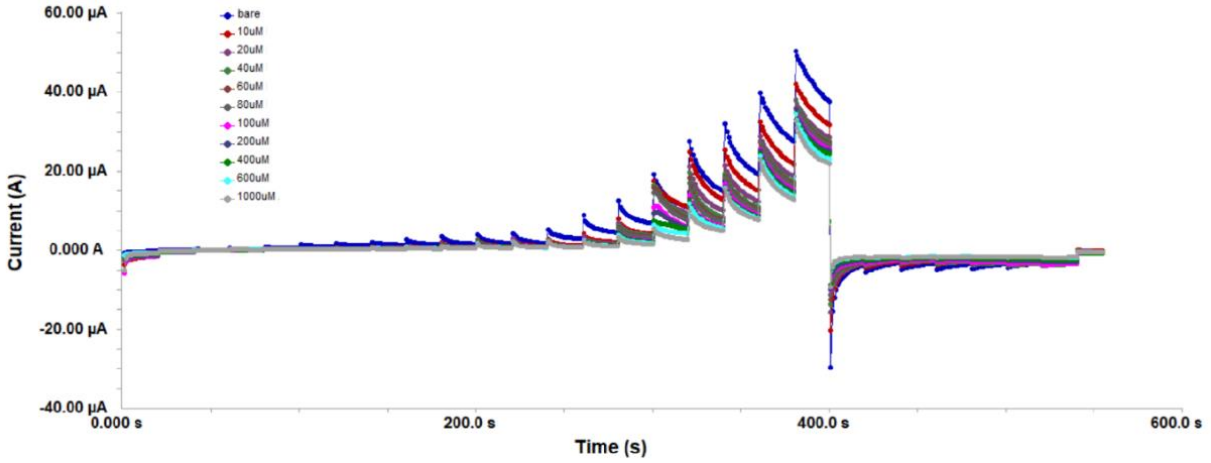

Figure.14 Chronoamperograms for the bare and drug doped ITO-Pani supported BLM in 0.1 M NaCl solution at pH 10

4. CONCLUSION

ITO-Pani supported bilayer lipid membrane was formed and its interaction with Ofloxacin was studied in 0.1 M NaCl bath solutions at different pH using electrochemical impedance spectroscopy, cyclic voltammetry and chronoamperometric studies.. The experimental results indicated that at low pH the drug molecules attached to the membrane surface and get partitioned into the membrane. At high pH the drug molecules get attached to the membrane surface via hydrogen bonding and provide a high resistance for electron transfer reactions at this interface.

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DOI: https://doi.org/10.15379/ijmst.v10i2.3355