Interaction Ciprofloxacin with Agar Gel Supported BLM

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***Abstract*: - Egg lecithin was extracted from egg yellow yolk and is made to self assemble into a bilayer on the freshly cut agar gel surface in KCl bath solutions. The electrical properties of this salt bridge supported bilayer lipid membrane (sb-BLM) were studied using electrochemical impedance spectroscopy. The interaction of ciprofloxacin with sb-BLM in KCl bath solutions was also studied using EIS. The effect of KCl concentration in the bath solutions on membrane conductance, capacitance and thickness was discussed. The effect of ciprofloxacin on the redox peak currents of the marker ions at sb-BLM electrode in 0.1 M KCl solution was discussed.**

***Key words*: sb-BLM; Ciprofloxacin; Electrochemical Impedance Spectroscopy; Cyclic Voltammetry; Self assembly; Surface coatings.**

I. INTRODUCTION

The cell is the basic structural and functional unit of all known living organisms. It is the smallest unit of life that is classified as a living thing (except virus, which consists only from DNA/RNA covered by protein and lipids), and is often called the building block of life. The cytoplasm of a cell is surrounded by a cell membrane or *plasma membrane*. The cell membrane is a biological membrane that surrounds the cytoplasm of living cells, physically separating the intracellular components from the extracellular environment. The cell membrane is selectively permeable to ions and organic molecules and controls the movement of substances in and out of cells. The basic function of the cell membrane is to protect the cell from its surroundings.The plasma membrane serves as an interface between the interior and exterior of the cell and has definite physical and chemical structure and has an inherent property of regulating the flow of material into and out of the cell. It consists of the lipid bilayer with embedded proteins. Cell membranes are involved in a variety of cellular processes such as cell adhesion, ion conductivity and cell signaling and serve as the attachment surface for several extracellular structures and intracellular cytoskeleton. The vital functions of membrane channels in biosystems are numerous, including conduction of nervous impulses [1,2], molecular transport [3], signal processing [4], energy transduction [5], and routes for drug delivery [6]. Additionally, the membrane channel may be regarded as a 'smart' sensor of nanometer dimensions [7].

Ciprofloxacin (INN) is a second-generation fluoroquinolone antibiotic. Its spectrum of activity includes most strains of bacterial pathogens responsible for respiratory, urinary tract, gastrointestinal, and abdominal infections, including Gram Negative bacterial infections. Ciprofloxacin and other fluoroquinolones are valued for this broad spectrum of activity, excellent tissue penetration, and for their availability in both oral and intravenous formulations. The mechanism of action of Ciprofloxacin drug, is different from that of other antimicrobial agents such as beta-lactams, macrolides, tetracyclines, or aminoglycosides; therefore, organisms resistant to these drugs may be susceptible to Ciprofloxacin drug. There is no known cross-resistance between ciprofloxacin HCl drug and other classes of antimicrobials.

The aim of present study is to analyze, understand and interpret the interactions between the drug (ciprofloxacin) and the Bilayer Lipid Membranes. It involves the extraction of phospholipids, formation and stabilization of the bio membrane within the experimental conditions and to study the characteristics of the bare and drug-interacted Bilayer Lipid Membranes.

II. MATERIALS AND METHODS

Commercially available ciprofloxacin in its hydrochloride form was purchased from medical shop and dissolved in hot water to prepare 10 µM stock solution. The insoluble materials are removed by filtration. Hen eggs were purchased locally for the extraction of phospholipids.The phospholipid was extracted using a procedure described by Ramesh et al [8]. An agar solution is formed by heating a mixture of 2-5% agar insaturated KCl solution and once gel is prepared it is immediately filled in cylindrical Teflon tubes of 1.52 mm diameter and 5 cm long. Agar filled tubes are then immediately placed in fresh saturated KCl solution to prevent shrinkage of the agar during cooling. All solutions used in this study were prepared triple distilled water. The membrane forming lipid was dissolved in n-decane (30 mg/ mL).

All electrochemical studies were carried out using a three electrode set up, where egg lecithin coated agar gel electrode was used as working electrode, while Ag/AgCl and Pt electrodes were used as reference and counter electrodes

respectively. The electrochemical measurements were carried out at room temperature (30±1 °C).

III. RESULTS AND DISCUSSION

3.1. Extraction of Phospholipid from egg yellow yolk.

The separation of phospholipids from the egg yellow yolk was done using a procedure described by Lundberg [9].The yolks of four eggs (20 g) were lyophilized overnight. The resulting cake was homogenized by mixing with 50 ml of diethyl ether in a waring blendor. The mixture was filtered and dried in a vacuum evaporator. The crude phospholipid was obtained by adding 50 mL of cold acetone. The precipitation was repeated by dissolving in diethyl ether (100 mL) and redissolving in cold acetone for three times. The undissolved materials in diethyl ether solution were removed by filtration. The precipitate from ice cold acetone was dried under nitrogen atmosphere at -20° C and purified by column chromatographic technique (The pharmacia type SR 25/45) using 1:3 methanol, chloroform mixture.

3.2. Formation of bilayer lipid membranes on the agar gel surface and its electrochemical characterization.

The spontaneous self assembly of egg lecithin molecules into a bilayer and its interaction with ciprofloxacin were monitored by electrochemical impedance spectroscopy. The Nyquist plots for bare and drug doped BLMs in 1.0 M, 0.1 M and 0.01 M KCl bath solutions are shown Figure.1a,1b and 1c. The impedance spectra obtained fit well with an equivalent circuit shown in Figure.2 [10].

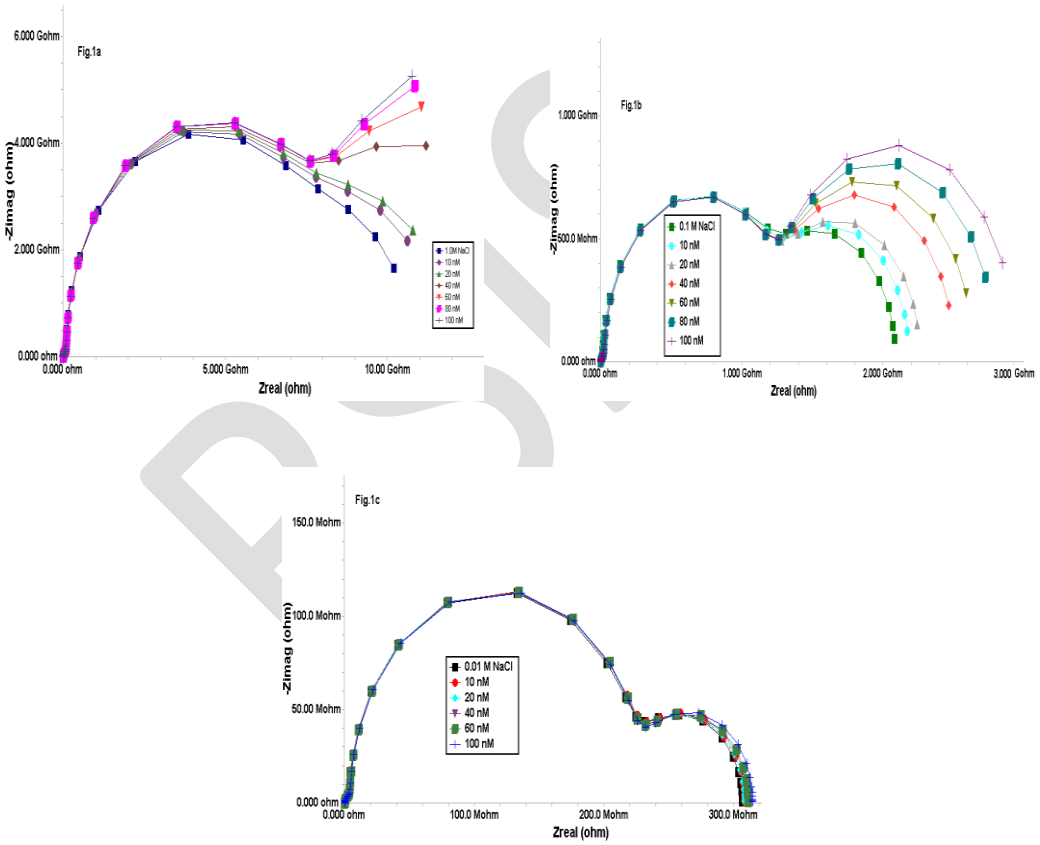

Fig.1a,1b and 1c. Nyquist plots for interaction of ciprofloxacin in (a) 1.0 M, (b) 0.1 M and (c) 0.01 M KCl bath solutions respectively.

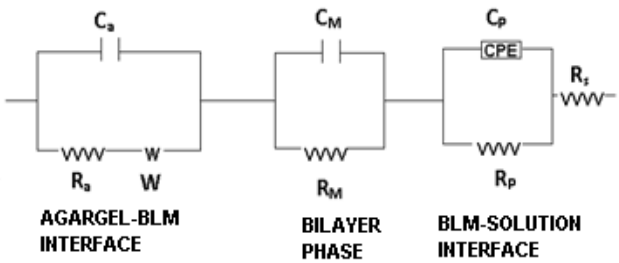

Fig.2 Equivalent electrical circuit for sb-BLM

In this circuit, R_s corresponds to the resistance of the electrolyte, connectors, wires etc and its value proportional to length of the gel column in the working electrode [10]. For long term investigation, the length of the agar column is very important and as recommended agar columns of length 3.0 to 4.0 cm were used for the studies. CPE is constant phase element which is used in place of a capacitor for bilayer lipid membrane -electrolytic solution interface and its impedance function is represented by the expression [11];

$$Z_{CPE} = \frac{1}{Y_0(i\omega z)^n} \quad (1)$$

Qualitative treatment of the observed impedance spectra of sb-BLMs can be made by considering slabs of different dielectric properties present in them [12] as in the case of black lipid membrane. The flow of ions in each slab gives rise to an ionic current while accumulation of ions at the boundary between contiguous dielectric slabs under AC conditions gives rise to a capacitive current [12]. Hence, each dielectric slab in the proposed equivalent circuit, shown in Fig.2, is simulated by a parallel combination of a resistor and a capacitor, namely by a RC mesh [12]. However, when the net impedance is also affected by mass transfer in a slab, Warburg impedance (W) is also used in the RC mesh.

Where Y_0 represents the admittance of the system, 'ω' is angular frequency and n is a constant (-1≤ n ≤1). The CPE represents pure resistor when n=0 and pure capacitor when n=+1. If n=-1 then the CPE represents an inductor [13]. The presence of CPE in the proposed equivalent circuit can be easily detected from the depressed nature of the semicircle in the impedance spectra, which indicates a non-ideal capacitive behavior of the interface [13]. In general the depression of the semicircles in the impedance spectra is attributed to the chemical inhomogeneities and anion adsorption [14]. R_p represents charge transfer resistance of the membrane-solution interface. C_M and R_M represent the capacitance and resistance of the phospholipid bilayer phase. C_a, R_a and W correspond to the capacitance, resistance and Warburg impedance of the agar gel- bilayer interface.

The corresponding electrochemical impedance parameters obtained using this equivalent circuit is shown in Table.1. The thickness of the bilayer lipid membrane was calculated from the capacitance values measured using impedance spectra in KCl bath solutions using the following equation [15].

$$C_M = \frac{\varepsilon_0 \varepsilon}{d} \quad (2)$$

Where ε_0 is the permittivity of free space (ε_0 =8.854*10^{-12} FM^{-1}], ε is dielectric constant of the lipid bilayer phase ε=2.05 [16].The calculated thickness of sb-BLM in 1.0 M, 0.1 M and 0.01 M KCl bath solutions were calculated to be 4.9, 5.7 and 5.8 nM respectively [17-19]. These values are close to twice the thickness of monolecithin layer, conforming the formation of bilayer.

TABLE.1

Electrochemical impedance parameters of bare and drug doped sb-BLM in 1.0 M, 0.1 M and 0.01 M KCl bath solutions.

S.No	Concentration of ciprofloxacin nM	1.0 M KCl				0.1 M KCl				0.01 M KCl			
		C_P pF	R_P Ω X10^7	C_M nF	R_M Ω X10^9	C_P pF	R_P Ω X10^6	C_M nF	R_M Ω X10^8	C_P pF	R_P Ω X10^6	C_M nF	R_M Ω X10^6
1	0	90.75	5.93	2.14	3.25	54.27	9.24	1.84	8.50	8.19	3.16	1.78	81.50
2	10	89.82	6.13	2.19	3.98	51.40	10.34	2.11	9.38	7.22	3.21	1.80	81.79
3	20	89.03	6.26	2.22	4.23	50.08	12.35	2.27	10.12	6.99	3.23	1.81	81.94
4	40	88.24	6.47	2.31	6.82	48.03	18.51	2.31	12.47	6.51	3.42	1.85	83.41
5	60	87.37	7.15	2.43	8.54	47.22	19.72	2.33	13.84	6.02	3.91	1.87	84.08
6	80	86.21	7.64	2.48	9.84	45.36	20.04	2.38	15.41	5.42	4.13	1.92	85.81
7	100	85.74	7.95	2.94	10.62	44.27	21.33	2.42	16.83	4.78	4.28	1.98	86.93

The capacitance value increases with decrease in KCl concentration in the bath solution. At neutral pH, the positive charges on the surface of lipid bilayer due to nitrogenous base of phospholipid molecules are covered by Cl^- ions from bath solution and hence the surface of BLM is negatively charged due to uncovered negatively charged phosphate groups [20]. However, this negative charge is partially neutralized by the adsorption of Na^+ ions from bath solution [20]. The surface negative charge of BLM decreases with increase in KCl concentration, due to the increased adsorption of Na^+ ions at the BLM surface [20] or in other words the excess surface negative charge of BLM increases as the bath concentration decreases.

The increase in adsorption of Cl^- ions on the lipid bilayer surface with KCl concentration as discussed brings in a tightening effect on the membrane and inturn a reduction in thickness of lipid bilayer phase. The capacitance of lipid bilayer phase (C_M), which is inversely proportional to thickness of the membrane.

From Table .1 it is clear that the membrane resistance increases with increase in ciprofloxacin concentration in the KCl bath solutions. This can be explained as follows. In solution ciprofloxacin exists in ion pair, neutral and ionized forms. The equilibria between these forms can be represented as:

$$CFHCL \leftrightharpoons CFH^+ + Cl^- \leftrightharpoons CFH^+Cl^- \quad (3)$$

The extent of ionized form of ciprofloxacin mainly depends on the concentration of Cl^- ions in the bath solution. In 1.0 M KCl bath solution, due to common ion effect of Cl^- ion it mostly exists in ion pair form. But, in 0.1 M and 0.01 M KCl bath solutions the ionized form exists relatively to large extent. Ciprofloxacin is not a liphophilic drug and hence the

neutral and ion pair forms of ciprofloxacin will not interact with BLM. However, in 0.1 M and 0.01 M KCl bath solutions the ionized form of ciprofloxacin interacts with the surface negative charges of BLM and shows a little more tightening effect on BLM surface in addition to Cl^- ions. Hence, the capacitance of BLM increases with drug concentration.

The extent of interaction between ionized form of ciprofloxacin and BLM increases with decrease in KCl concentration in the bath solution. This can be explained as follows. In 1.0 M KCl bath solution BLM surface is mostly covered by Cl^- ions and this will provide only less space for interaction, whereas in 0.1 M and 0.01 M KCl bath solutions the surface coverage by Cl^- ions is relatively less.

The membrane resistance decreases with increase in KCl concentration in the bath solution. When the KCl concentration is high in the bath solution the adsorption of Cl^- ion will be larger and will cover the maximum BLM surface and block the minute pores or holes on the BLM surface and thus provides resistance for the flow of smaller ions like Na^+, K^+ across the BLM phase. Hence, the membrane conductance (G) decreases or membrane resistance(R) increases with increase in KCl concentration in the bath solution. The interaction of ciprofloxacin with BLM partially closes the pores and hence resistance of BLM increases with ciprofloxacin concentration.

3.3. Cyclic Voltammetric Studies on interaction of ciprofloxacin with BLM in the presence of 2 mM $[Fe(CN)_6]^{-4}$/$[Fe(CN)_6]^{-3}$ ions.

The cyclic voltammetric studies were carried out in 0.1 M KCl bath solution in the presence of 1:1 2 mM $[Fe(CN)_6]^{-4}$/$[Fe(CN)_6]^{-3}$ ions (marker ions). Since in 1.0 M KCl bath solution surface coverage by Cl^- ions is larger the chance for surface interaction of ferro-ferricyanide ions is less and due to lower concentration of KCl in 0.01 M KCl bath solution, the cyclic voltammograms shows more contribution from double layer charging current, no clear peaks are observed. In 0.1 M KCl bath solution the marker ions can relatively reach the BLM surface and shows redox peaks.

The redox peaks observed for marker ions on the surface of sb-BLM in 0.1 M KCl solution is shown in Figure.3.

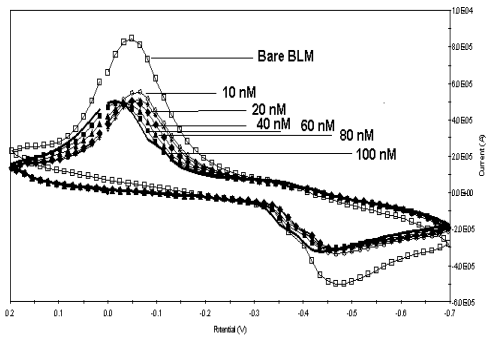

Fig.3 Cyclic Voltammogram of 2 mM ferro-ferricyanide ions at sb-BLM electrode in 0.1 M KCl bath solution

From the cyclic voltammograms it is clear that the peak currents decreases with increase in ciprofloxacin concentration, whose ionized form get attached to the sb_BLM surface and provides resistance for interaction of marker ions.

IV. CONCLUSIONS

The egg lecithin extracted from egg yellow yolk was successfully used for formation of BLM on the agar surface. The electrical properties of sb-BLM in 1.0 M, 0.1 M and 0.001 M KCl bath solutions were evaluated by electrochemical impedance spectroscopy technique. The interaction of ciprofloxacin with sb-BLM depends on the concentration of KCl in the bath solution. Cyclic voltammetric response of the marker ions using sb-BLM electrode was recorded in the presence and absence of ciprofloxacin and the cyclic voltammograms show with increase in ciprofloxacin concentration peak currents decrease.

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